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TITLE: Testing the Model: A Phase I/II Randomized Double Blind Placebo Control Trial of Therapeutics: Liposomal Glutathione and Curcumin

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

Richard Deth; Nancy G Klimas

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

VA Medical Center, Miami, FL
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VA Medical Center 111-I
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Miami, FL 33125

Nova Southeastern University
College of Pharmacy
3301 College Avenue
Fort Lauderdale, FL 33314

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

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We propose to perform a phase I/II study comparing two nutraceuticals and placebo that target mediators identified in our prior dynamic modeling study of Gulf War Illness (GWI). We will repeat the dynamic modeling before treatment and on therapy to assess our modeling and the impact of the interventions on the homeostatic networks we have identified, with an added focus on the glutathione/redox system.

In our prior study "Dynamic Modeling in GWI" we used an exercise stress model (rest, peak VO2, and 7 follow-up sampling points) to measure the mediators of relapse in the context of their interactive homeostatic networks. We surveyed the response of genes and blood-borne biomarkers in order to interrogate and map regulation of neuro-endocrine-autonomic- immune function in these subjects as compared to GW era sedentary healthy controls. We applied an integrative-systems- based approach rooted in computational biology connecting gene expression and biomarkers to pathways and to symptoms in order to identify potential therapeutic targets as well as optimal strategies for manipulation of these targets. Using this data, we have developed a virtual model of the illness, which we have used to identify potential therapeutic targets. The proposed project is directed at the validation of pharmacologic mediation of illness targets for GWI, identified using an integrative systems-based approach to the study of molecular and cellular markers.

15. SUBJECT TERMS

GWI
Comprehensive Molecular Profiling

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

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INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Within months after their return from Operation Desert Storm an alarming number of Gulf War veterans began to report a variety of symptoms, including fatigue, musculoskeletal discomfort, skin rashes, and cognitive dysfunction. During deployment, these troops were subjected to a number of potentially hazardous conditions and multiple hypotheses as to the etiology of Gulf War Illness (GWI) have been considered. The symptoms of (GWI) that are most consistently reported include those which are often reported in Chronic Fatigue Syndrome (CFS). The objective of this study is to improve our understanding of GWI pathogenesis in two ways; by integration across several of the body's regulatory systems of data and knowledge collected from disparate sources, and by mapping of the coordinated interactions between these physiologic systems and the potential for altered "wiring" of these signaling networks in GWI. Using comprehensive molecular profiling, network and control theory the overarching objective of this proposal is to define the precise nature of these irregularities in immune and neuroendocrine signaling as well as the altered activation states of the corresponding cells such that treatment courses can be designed to redirect the system as a whole to normal pattern of coordinated activity.

KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Gulf War Illness
Comprehensive Molecular Profiling

ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

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.

Please see inserted chart below

Statement of Work	Timeline	MVAMC	NSU INIM	NSU Phar	NSU Stats
1) **Major Task 1:** Perform a randomized phase I/II study comparing curcumin BCM-85, 400 mg twice a day to liposomal glutathione 630 mg bid 3 Months month intervention and assess safety, efficacy and biomarker response to therapy					
Sub-task 1: Prepare Regulatory Documents and Research Protocol for Study					
Request pre-IND meeting to determine exemption if necessary, submit Investigational New Drug	1 1 TO 4	NK	Completed		
Refine eligibility criteria, exclusion criteria, screening protocol	1 TO 2	NK	Completed		
Finalize consent form & human subjects protocol	2 TO 4	NK	Completed		
IRB protocol submission MVAMC	2 TO 3	NK	Completed		
IRB protocol submission NSU (with MVAMC revisions if suggested)	2 TO 5	NK	Completed		
Military 2nd level IRB review (ORP/HRPO)	4 TO 5	NK	Completed		
Submit amendments, adverse events and protocol deviations (As Needed)		NK	Completed As Needed		
Submit annual IRB report for continuing review	(Annually)	NK	Completed		
Milestone Achieved: Local IRB approval at MVAMC	4	NK	Completed		
Milestone Achieved: HRPO approval for all protocols and local IRB approvals	6	NK	Completed		
Sub-task 2: preparation for initiation of clinical trial (staff/space/platform)					
Study Staff definition of duties, cross training			Completed		

Coordinate with study staff and research pharmacy a flow chart for all study steps, web data and database requirements	3 to 5 collection Completed	NK	MF	RD	GB
Mock run of virtual subject through all time Review platform for HIPAA, permissions, and issues	5 5 data retrieval Completed	NK NK			GB GB
Milestone Achieved: Research staff trained Completed	1 to 5	NK			GB
Completed					
Milestone Achieved: 1st participant consented, screened and enrolled	*7 to 8M* Completed	NK			
Milestone Achieved: Study begins Completed	7 to 8	NK			
Sub-task 3: Initiation of randomized control clinical trial					
Recruitment of subjects, screening, informed process	7 to 30 consent	NK	Completed		
Completion of exercise challenge and specimen collection before and after 12 weeks of treatment	10 to 33 condition	NK	Underway		
Monitor and report adverse events to IRB, Data monitoring board chair	7 to 33	NK/GD	Ongoing Underway		
Milestone achieved: Clinical trial underway, meeting recruitment goals:	12,24	NK			
Milestone achieved: last subject (subject 75) initiates study condition	30	NK			
Milestone achieved last subject completes	33	NK	Ongoing		
Milestone Achieved: Study begins	7 to 8 m	NK	Achieved		
Sub-task 4: Biomarkers/lab studies					
Review established protocols and establish accession, barcoding, and sample flow	1 to 3 m		MF Completed		

Coordinate with study coordinator and lab staff on scheduling, courier to lab during assessments and exercise challenge	3 to 4	NK	MF Completed		
Determine batch assays schedule, data entry plan, work with lab information system for direct data entry to REDCap platform	1 to 6 Completed	NK	MF		GB
Maintain quality control for all assays review with MF weekly	7 to 34 Ongoing				
Run assays as described in protocol, maintaining quality and timely data entry. Stats core to run monthly data status for all investigators	7 to 34 Ongoing		MF		GB GB
Milestone achieved: lab information system platform link to REDCap	6	Completed			
Milestone achieved completion of all assays with data entered into system	34	Yr 3	MF		GB
Projected enrollment					
Enrollment MVAMC 111 enrolled, 50 completed, 44 terminations, 17 in progress, 7 pending consent return, 1 pending					Goal 75

Major Task 2 (Specific Aim 2): Perform dynamic modeling studies before and after 3 months o therapy, repeating the method used previously in order to compare the response to exercise across groups and better quantify the degree of recovery in treated subjects using an exercise challenge and 9 point in time blood and saliva collections over				
Sub-Task 1: Apply previously developed computational modeling to before/after intervention comparisons	1 to 36	Ongoing		GB
Adapt computational modeling platform for intervention before/after analyses	1 to 12	completed		GB

Perform dynamic modeling utilizing group A/B/C designation for interim analyses Break blind Completion of final analyses Final publications and translation plan	24 34 36 34-36	RD Ongoing		GB GB GB
Milestone achieved: Interim analyses data used in modeling data *Milestone Achieved: Report results computational modeling*	34-36			GB
Specific Aim 3: Assessment of antioxidant and methylation-related metabolic status prior to, during and after acute exercise in GWI subjects before and after antioxidant	**Timeline**	**Site 1** (Initiating PI)	**Site 2** (Partnering PI)	
Major Task 1: Measurement of antioxidant and methylation pathway metabolites and vitamin B12 status	Months			
Subtask 1: Thiol and thioether metabolite assays: HPLC assay of redox and methylation pathway thiols and thioethers in plasma (GSH, GSSG, cysteine, cysteine, methionine, homocysteine, homocystine, cystathionine, S-adenosylmethionine, S-adenosylhomocysteine) • HPLC assay of redox and methylation pathway thiols and thioethers in HPLC assay of redox and methylation pathway	1to 36m		Underway RD	
Subtask 2: Measurement of vitamin B12 status: HPLC assay of vitamin B12 (cobalamin) species in PBMCs (cyanocobalamin, glutathioneylcobalamin, hydroxocobalamin, methylcobalamin, adenosylcobalamin	1 to 36		Underway RD	
Major Task 2: Evaluation of DNA methylation status				
Subtask 1: Global DNA methylation status: Pyrosequencing-based assay of LINE-1 nuclear DNA methylation status	1 to 36		Underway RD	
Subtask 2: Mitochondrial DNA methylation status: Pyrosequencing-based assay of MT-ATP6 mitochondrial DNA	1 to 36		Underway RD	
Milestone #1: Co-author manuscript on redox and methylation status in GWI	24-36	NK	RD	

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.

We have the 8 screenings scheduled to complete our recruitment numbers being that we had 44 terminations either due to withdrawals, exclusion or delayed time in phlebotomy company contact.

Unfortunately, we have had many issues that have delayed the completion of this study including COVID, delayed in study medication delivery and phlebotomy company setbacks but we are working hard to complete the study by April 2023.

Dr. Richard Deth is the partnering PI at NSU for the companion project addressing Specific Aim 3. Specific aim 3 involves assessment of antioxidant and methylation-related metabolic status prior to, during and after acute exercise in GWI subjects before and after antioxidant interventions. These measurements will be carried out with batched plasma and PBMC samples. Dr. Deth has been provided over 200 plasma and PBMC samples and is currently processing the latest batch of 150 samples. A research technician has been hired whose effort is dedicated to this project. In support of these assays a new HPLC system was purchased by Nova Southeastern University and methods for analysis of antioxidant and methylation pathway metabolites as well as vitamin B12 (cobalamin species) have been developed for the new instrument. He has also been provided with samples form a large cross- sectional biorepository, and has been working with Dr. Shungu to run the samples from his NAC study in GWI. We will wait until we have all samples to finish the Thiol and mitochondria DNA methylation assay, as this will decrease the variability within the assay.

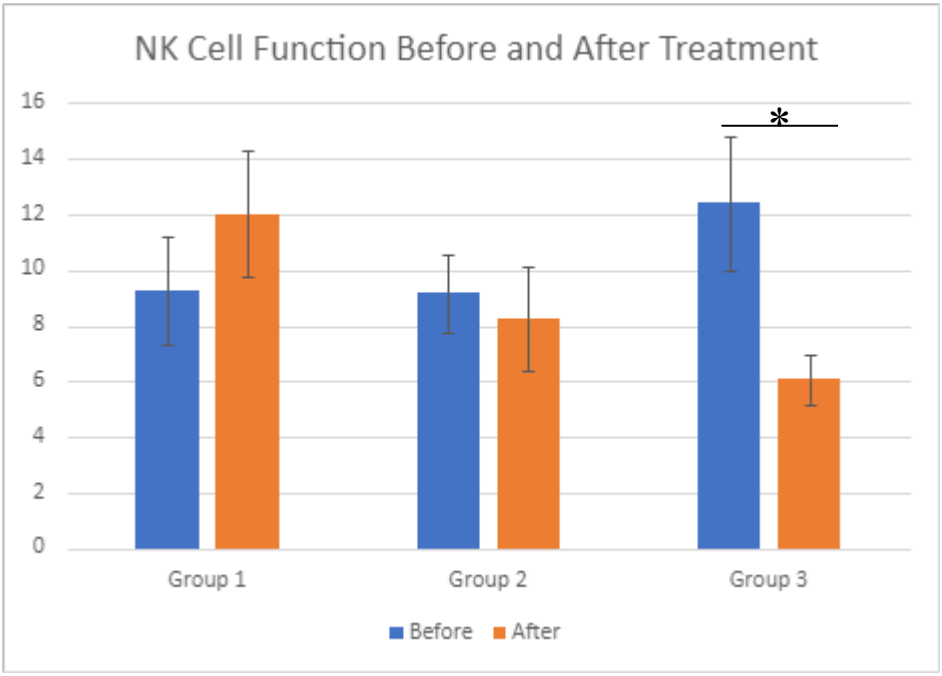

Fig 1. Analysis of NK Cell function before and after treatment of either Glutathione, Curcumin or Placebo

References

Publications: our computational group is using data from this project in their ongoing work and resultant publications including the following:

High-fidelity discrete modeling of the HPA axis: a study of regulatory plasticity in biology. Sedghamiz H, Morris M, Craddock TJA, Whitley D, Broderick G.
BMC Syst Biol. 2018 Jul 17;12(1):76. doi: 10.1186/s12918-018-0599-1.

Broderick G, Fletcher MA, Gallagher M, Barnes Z, Vernon SD, Klimas NG. Exploring the Diagnostic Potential of Immune Biomarker Co-expression in Gulf War Illness. Methods Mol Biol. 2018;1781:101-120. doi: 10.1007/978-1-4939-7828-1_7. PubMed PMID: 29705845.

We will not be publishing the clinical trial results until the end of the continuation year currently the subject conditions remain blinded.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

"Nothing to Report"

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

"Nothing to Report"

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

IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
transfer of results to entities in government or industry;
instances where the research has led to the initiation of a start-up company; or
adoption of new practices.

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
improving public knowledge, attitudes, skills, and abilities;
changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or
improving social, economic, civic, or environmental conditions.

Nothing to Report

CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No changes were made in approach during this reporting period

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.

As stated in the previous quarterly reports we have had delays due to different reasons, from COVID putting a pause on the study to delays in obtaining medication. We have had delays in recruitment due to pending contracts and issues with the phlebotomy company. We have finally crossed all of these hurdles and should be completed within time.

Changes that had a significant impact on expenditures
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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

There have been no changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

There have been no changes in use or care of vertebrate animals this study only involve humans.

Significant changes in use of biohazards and/or select agents

There have been no significant changes in use of biohazards and/or select agents.

Publications, conference papers, and presentations
Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

"Nothing to Report"

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

"Nothing to Report"

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

"Nothing to Report"

Website(s) or other Internet site(s)
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

"Nothing to Report"

Technologies or techniques
Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

"Nothing to Report"

Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

"Nothing to Report"

Other Products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
data or databases;
biospecimen collections;
audio or video products;
software;
models;
educational aids or curricula;
instruments or equipment;
research material (e.g., Germplasm; cell lines, DNA probes, animal models);
clinical interventions;
new business creation; and
other.

"Nothing to Report"

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."

and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

"Nothing To Report"

organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)
Financial support;
In-kind support (e.g., partner makes software, computers, equipment, etc.,

available to project staff);
Facilities (e.g., project staff use the partner's facilities for project activities);
Collaboration (e.g., partner's staff work with project staff on the project);
Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
Other.

"Nothing to Report"

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.