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

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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

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Introduction

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.

Body

As noted above, this project involves a clinical trial that serves as both a proof of concept study, using nutraceuticals that target nfKB, identified in our modeling studies, performing before an after dynamic modeling studies of the study volunteers, and refining our modeling work as a result. It should be noted that we have a number of funded studies that aim to improve the modeling that would lead to clinical trials, and all of the data collected in this study serves as data used by our computational modeling staff to test both the utility of intervening at nfKb (in the case of this study) and to provide an increasingly rich and complex data set to inform the other aspects of their studies.

In this study we have a SOW. It should be noted that the start time of the recruitment was delayed by a prolonged HRPO process submitted on our notice of approval in Sept 2015 after a HRPO pre-review and IRB approval; it was HRPO approved April 2016, thus recruitment began shortly afterwards. We have reset our recruitment projections accordingly, still with the intent to complete this study in the 3 year window without the need for carry over funding. We have doubled our recruitment efforts to achieve numbers. To help with recruitment the CSP #585, "Gulf War Era Cohort and Biorepository (GWECB) group has mailed our flyers to thousands of their previous Gulf War survey participants. We are also looking into ways of possibly increasing payments for enrollment to see if this will help with retaining subjects.

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 As deviations as needed	Needed	NK			
Submit annual IRB report for continuing review	annual	NK	Completed		
<i>Milestone Achieved: Local IRB approval at MVAMC,</i>	4	NK	Completed		
<i>Milestone Achieved: HRPO approval for all protocols and local IRB approvals</i>	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 to customize web based assessment platform (REDCap)	1 to 3	NK	MF	RD	GB
Coordinate with study staff and research pharmacy a flow chart for all study steps, web data collection and database requirements	3 to 5	NK	MF	RD	GB
Mock run of virtual subject through all time	5	NK			GB
Review platform for HIPAA, permissions, and data retrieval issues	5	NK			GB
<i>Milestone Achieved: Research staff trained</i>	Completed 1 to 5	NK			GB
<i>Milestone Achieved: 1st participant consented, screened and enrolled</i>	7 to 8M	NK			
<i>Milestone Achieved: Study begins</i>	Completed 7 to 8	NK			

Sub-task 3: Initiation of randomized control clinical trial					
Recruitment of subjects, screening, informed consent process	7 to 30	NK	Completed		
Completion of exercise challenge and specimen collection before and after 12 weeks of treatment condition	10 to 33	NK	Underway		
Monitor and report adverse events to IRB, Data monitoring board chair	7 to 33	NK/GD	Ongoing Underway		
<i>Milestone achieved: Clinical trial underway, meeting recruitment goals:</i>	12,24	NK			
<i>Milestone achieved: last subject (subject 75) initiates study condition</i>	30	NK			
<i>Milestone achieved last subject completes intervention and final assessment</i>	33	NK	Yr 3		
<i>Milestone Achieved: Study begins</i>	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		M		G
<i>Milestone achieved: lab information system platform link to REDCap</i>	6	Completed	F		B
<i>Milestone achieved completion of all assays with data entered into system</i>	34	Yr 3	MF		GB
Projected enrollment					
Enrollment MVAMC 27 enrolled, 17 completed, 9 terminations, 1 in progress, 5 scheduled for screening					Goal 75 <input type="checkbox"/>

Major Task 2 (Specific Aim 2): Perform dynamic modeling studies before and after 3 months of 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 24 hours with genomic, cytokine, neuropeptide and cell population studies.				
Sub-Task 1: Apply previously developed computational modeling to before/after intervention comparisons	1 to 36	Yr 3		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	24			GB
Break blind	34	RP		G
Completion of final analyses	36	Yr 3		B
Final publications and translation plan	34-36			GB
<i>Milestone achieved: Interim analyses data used in modeling data</i>				G
<i>Milestone Achieved: Report results computational modeling</i>	34-36			B
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 interventions	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, 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 thiols and thioethers in PBMC mitochondria	1 to 36m		Underway RD	
Subtask 2: Measurement of vitamin B12 status: HPLC assay of vitamin B12 (cobalamin) species in PBMCs (cyanocobalamin, glutathionylcobalamin, 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 methylation status	1 to 36		Underway RD	
Milestone #1: Co-author manuscript on redox and methylation status in GWI	24-36	NK	RD	

Key Research Accomplishments

A 3rd no cost extension was approved in the beginning of the September 2020. We are now fully recruited, in the process of completing our last 3 exercises and 40 virtual participants. Thus, the randomized clinical trial is underway.

Unfortunately, recruitment, screening and scheduling of participants were halted in November 2019 due to the unavailability of obtaining the study placebo drug. Then on March 2020, all research activities were halted due to COVID-19. We followed our IRB guidance and completed the proper documents which were sent to the appropriate HRPO contact. Mid July 2020 we received approval to resume research activities, as well as received the placebo drug. At this same time our clinic team was put on mandatory quarantine. On August 10, 2020 we resumed with all research activities once again. We received the placebo study drug and we are approved to virtually complete a portion of the group size.

Our goal now that we are recruited is to complete the study by March 2021.

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, he has been provided the samples of the subjects that have completed treatment. 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 from a large cross-sectional biorepository, and has been working with Dr. Shungu to run the samples from his NAC study in GWI.

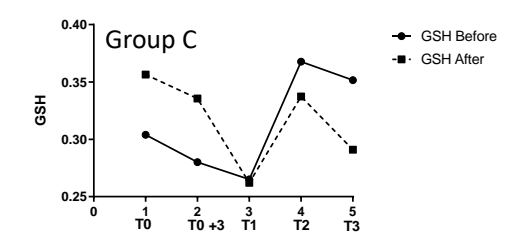
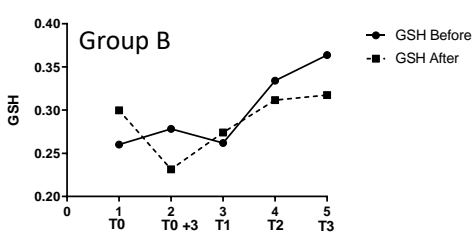
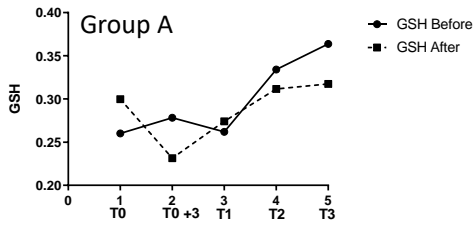
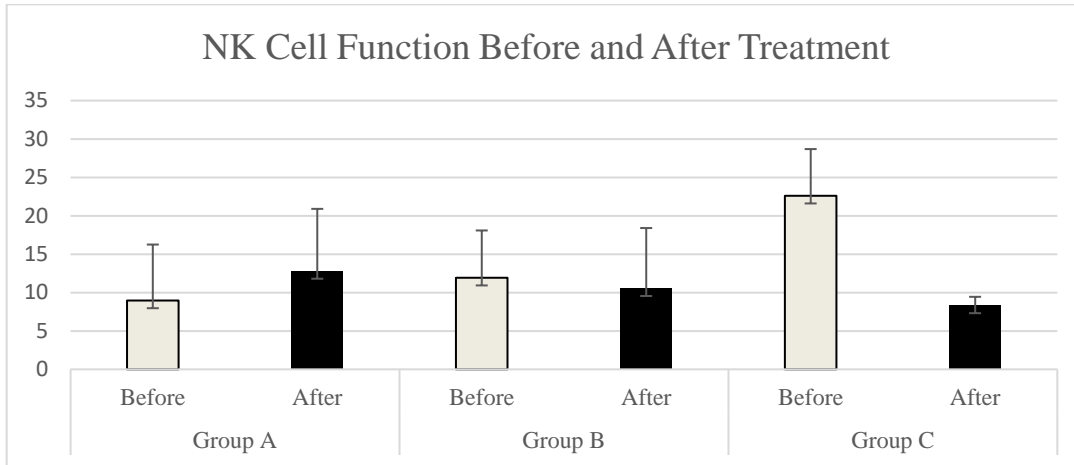
The immunology and screening labs are also managed through the Deth partnered project with the INIM Immunology labs, these samples are processed as they are received and the data sets of immune, cell subtype and cell function have been performed on a rolling basis.

Reportable Outcomes

A total of 9 participants have been completed the study in Group A, 4 in Group B and 3 in Group C. Preliminary data has been captured on Flow cytometry and NK cell function on each participant. Additional assays (Multiplex Cytokine Analysis, Catecholamines, Hormones, Cortisol and Nanostring gene expression analysis) will be run in equal groups of 4. Initial review of NK cell function suggests an increase in NK cell function at T0 after treatment in group A (Fig 1). A decrease in NK cell function is suggested at T0 after treatment in groups B and C. However, an increase in the number participants in each group are need for stronger analysis.

CONCLUSIONS

Preliminary measures of NK cell function and glutathione (GSH) levels show changes during testing (See below). Final testing will be performed when all subjects have completed the study and groups are unblinded.



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 .

APPENDICES

Curriculum Vitae for Key Personnel