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TITLE: Identifying Immune Drivers of Gulf War Illness Using a Novel Daily Sampling Approach

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT</b>  Since the last annual report, we have initiated further participant screening, participant enrollment and the study protocol.  All start up subtasks have been completed. To date 329 potential participants have filled out the online screening questionnaire, 279 of those have been screened by telephone, and 19 have completed the protocol, 1 participant is currently running through the protocol and 1 participant withdrew. We expect to meet our end goal number during the next reporting period. After review of exclusionary and inclusionary criteria and the fact that our population is aging we decided to include participants with comorbid diabetes. We have been IRB approved to enroll participants with A1c>9%  Screening and enrollment will continue as we work toward completing this study.									
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## 1. INTRODUCTION:

The major aim of this research project is to identify aspects of the immune system that are dysregulated in veterans with Gulf War Illness. A second aim is to determine whether identified immune system dysregulations are similar to those found in men with fibromyalgia. To accomplish those aims at UAB, we are recruiting 29 male veterans diagnosed with Gulf War Illness (GWI), as well as 2 healthy veteran controls, and 4 males with Fibromyalgia/Chronic Fatigue Syndrome (FM/CFS). Participants will complete 25 consecutive days of blood draws and provide daily reports of symptom severity. Analyses will then be conducted to identify immune system factors that correlate with day-to-day symptom fluctuations in the participants. Ultimately, this information may be used to develop new treatments that specifically target the pathophysiological mechanisms of Gulf War Illness.

(Note: A complete analyzed data set will consist of the data collected from the 22 participants at Stanford University and the 35 participants at UAB).

## 2. KEYWORDS:

Gulf War Illness, cytokines, microglia, daily, immune, phlebotomy, fibromyalgia

## 3. OVERALL PROJECT SUMMARY:

Current Objectives:

- Continue recruitment and data collection at UAB
- Complete the protocol on 35 participants.

Results, Progress and Accomplishments

**Task 1:** Team review and progress meetings

75% Completed.

(Note: The analysis planning meeting in the revised SOW has been rescheduled to allow time to complete data collection from the 35 participants that will be enrolled at UAB).

**Task 2:** Submission of Documents for Regulatory Approvals

100% Completed.

(Note: UAB IRB approval was received April 27, 2015 and USAMRMC ORP HRPO final approval was received May 28, 2015).

**Task 3:** Start up Machine/Personnel

100% Completed

**Task 4: Advertisement**

75% Completed. Recruitment tools have been launched and are actively on-going.

A recruiting event was held from October 8-11, 2015 at the Birmingham Jefferson Convention Complex (BJCC) in Birmingham, Alabama. Advertisements via the UAB campus/local newspaper and website are on-going.

Radio advertisements (subtask 1) were aired May 2, 2016-May 27, 2016 and August 8, 2016-September 8, 2016, and a third round May 22, 2017-June 29<sup>th</sup> 2017. The recruitment webpage (subtask 2) has been published, and 329 individuals have filled out the online screening questionnaire linked from the webpage.

*Milestone: 279 potential participants screened by telephone—100% Completed.*

(Note: This reported percentage is a representation of the entire study including advertising that took place at Stanford University).

**Task 5: Enroll GWI Participants for Study**

65% Completed. We are actively recruiting participants and screening potential participants. Recruitment for enrollment is actively on-going. There have been 40 males to complete our in-person screening sessions of which 15 participants were determined to be eligible and have enrolled. 1 participant is currently running through protocol.

(Note: The reported percentage in the previous annual report was a representation of the entire study and included the enrollment of 22 participants at Stanford University. The percentages reported in this current annual report are a representation of the revised SOW at UAB).

**Task 6: Recruit control groups**

50% Completed. Will continue recruitment in January 2017, 3 of the 2 healthy controls have completed the protocol and 2 of the 4 Fibromyalgia participants have completed the protocol and 1 participant was put on, the remaining will be enrolled in 2017.

**Task 7: Run Protocol**

50% Completed. Further progress is dependent on completing Tasks 5 and 6.

(Note: The reported percentage in the previous annual report was a representation of the entire study and included the blood samples and self-reported symptom data collected from the 22 participants at Stanford University).

**Task 8: Assays**

0% Completed. Further progress is dependent on completing Task 6.

(Note: The reported percentage in the previous annual report was a representation of the entire study and included the biochemical quantification of the blood samples collected from the 22 participants at Stanford University).

**Task 9: Quantification of bio-chemicals in blood samples**

0% Completed. Further progress is dependent on completing Task 7.

**Task 9: Analyses**

0% Completed. Further progress is dependent on completing Tasks 6 & 7.

(Note: The reported percentage in the previous annual report was a representation of the entire study and analysis of the samples collected from the 22 participants at Stanford University).

**Task 10: Preparation of final report and publications**

40% Completed. The completion of this task is dependent on completing of Tasks 6, 7, & 8.

## Key methodology:

We have used the methodology outlined in other submitted documents. We are actively recruiting participants from a variety of sources, including local advertisements, referrals from the local VA Hospital and connecting with VA support group leaders. We will perform the study protocol exactly as described in the revised SOW. A total of 22 participants have completed the protocol at Stanford University. A total of 35 participants will complete the protocol at UAB.

## Research conclusions:

Because we have not completed the data collection, we have not performed only preliminary analyses. We have completed interim analysis from a total of 22 participants at Stanford University. The results and conclusions from that data set are reported in Section 4 (Key Research Accomplishments).

## Actual or anticipated problems or delays:

We encountered a significant problem after transferring to UAB from Stanford University. The freezer where our collected samples were stored failed and the samples were lost. Fortunately, we had already assayed the samples and obtained data from those samples, so the loss was not complete. The University Provost, School of Medicine and Department of Psychology compensated us for the sample loss. We selected and ordered a new freezer that will be used to store the project samples. To insure this problem does not occur in the future we will split all collected samples between two freezers that will be in two separate storage locations. One storage space has been designated in Campbell Hall on the 2<sup>nd</sup> floor where Dr. Younger's lab is located. The additional storage space has been designated in the UAB Clinical Research Unit (CRU) in Jefferson Towers on the 15<sup>th</sup> floor. All freezers will be alarmed and monitored. We do not anticipate delays in the future.

## Changes to approach:

Nothing to report.

## KEY RESEARCH ACCOMPLISHMENTS:

(Note: Results are from interim analysis and preliminary from the Stanford University data set).

- a. Individuals with GWI showed greater day-to-day fluctuation of serum levels of the pro-inflammatory cytokine Eotaxin-1 than healthy veterans of the Gulf War;
- b. Higher fatigue severity days were associated with higher serum levels of the pro-inflammatory cytokines IL-1 $\beta$  and IL-15 in individuals with GWI;
- c. Although these results are preliminary, these differences in cytokine expression suggest a role for inflammation in GWI.

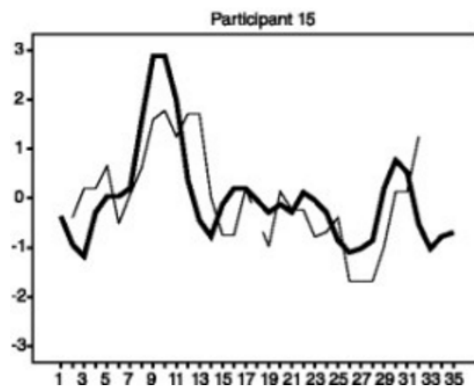


Figure: Plot showing the serum concentration of IL-15 (thin line) and fatigue severity (thick line) [on the y-axis] over time [on the x-axis] in one example individual. The close relationship between IL-15 and fatigue provides a visual representation of the temporal relationship that was determined statistically.

### 3. CONCLUSION:

We have only performed preliminary analyses at this time because the entire sample for each group has not yet been collected. Therefore, the conclusions are tentative at this time. When the UAB sample set has been collected, we will complete all analyses for the final conclusions. However, we can tentatively say that we have identified very interesting relationships between the immune system and GWI symptoms. First, GWI individuals show abnormally large day-to-day swings in inflammatory cytokines. These cytokines (Eotaxin-1 and IL-1beta) are closely associated with microglia activation and may therefore be a proxy measure of microglia activity. We also found that the microglial activating cytokine IL-15 was associated with fatigue symptoms. The data suggest that GWI symptoms are being driven by inflammatory factors. Our goal from this point is to finish our target recruitment numbers and perform the final analyses which will be inclusive of the data sets from Stanford University and UAB.

**PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:**

Nothing to report.

**1. INVENTIONS, PATENTS AND LICENSES:**

Nothing to report.

**2. REPORTABLE OUTCOMES:**

Nothing to report.

**3. OTHER ACHIEVEMENTS:**

Nothing to report.

**4. REFERENCES:**

No references.

**5. APPENDICES:**

Nothing to report.