

**AWARD NUMBER: W81XWH-13-2-0085**

**TITLE: Understanding Gulf War Illness: An Integrative Modeling Approach**

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> The goal of the GWI consortium is to develop a better understanding of GWI and identify specific disease targets to find treatments that will address the cause of the disease. The consortium will integrate our clinical understanding of the disease process with basic research efforts using a novel mathematical model. The computational biology approach will enable the consortium to quickly identify targets of dysfunction and find treatments that will address the causes of the disease. The project will combine animal models of GWI with focus on the immune, cardiovascular and autonomic systems.					
<b>15. SUBJECT TERMS</b>  NONE LISTED					
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The underlying mechanisms of GWI remain unknown and treatment has been palliative, symptom-driven and physician-directed. The purpose of this multidisciplinary consortium project is to investigate animal GWI models with the goal of testing chemical treatments. The immune and autonomic biomarkers will be tested using a computational modeling approach allowing for a critical analysis and an accurate selection of test agents. The idea is to combine animal and human studies – a translational approach. Animal studies will be followed by clinical trials with agents thought to be most efficacious.

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

GWI, Inflammation, Computational Biology, Cytokines, Homeostasis.

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

**What were the major goals of the project?**

	<b>Timeline (Months)</b>	<b>Percentage Complete</b>
<b>Major Task 1: Setup the administrative structure required for the conduct of the animal and human studies</b>		
<b>Subtask 1: Prepare Regulatory Documents and Research Protocols for Study 1</b>		
Prepare, submit and receive approval for animal protocols	1-4	100%
Refine experimental protocols via conduct of preliminary experiments.	4-12	100%
Refine eligibility criteria, exclusion criteria, screening protocol	3-12	100%
Finalize consent form & human subjects protocol	3-12	100%
Submit amendments, adverse events and protocol deviations as needed	As Needed	100%
Coordinate with Sites for annual IRB** report for continuing review	Annually	100%
<b>Subtask 2: Establishment of administrative structure including coordinating center and database system</b>		
Recruit, hire and train key personnel, students, staff and faculty	1-6	100%
Setup the coordinating center including database setup	1-12	100%
Setup administrative including committee appointments and scheduling of key review meeting	1-5	100%
Development of reporting procedures – minimum of updates every 6 months.	3-12	100%

Finalize consent form & human subjects protocol , receive approval	24-36	95%
Annual meeting with the consortium members and the external advisory committee – live and via internet	As scheduled	100%
Meetings in the DC region with DoD staff and representatives of the groups – twice per year	As scheduled	100%
	<b>Timeline (Months)</b>	<b>Percentage Complete</b>
<b>Major Task 2: Refinement and enhancement of animal models for GWI.</b>		
<b>Sub task 1: Establish the model of autonomic dysfunction as a surrogate for GWI.</b>		
Train staff and students in specialized surgical methods used to setup for monitoring autonomic function.	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function – predict long term deficits	4-15	80%
Employ spectral analytical methods for examination of sympathetic and parasympathetic balance	Begin 4 continue	100%
Conduct wheel running acute and chronic exercise tests to simulate the exercise model in humans	4-12	100%
Combine tests of acute and chronic exercise in the GWI chemical toxin model, providing an excellent preclinical comparison.	12 continue	70%
Submit animal protocol amendments as required	As needed	100%
Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function	24 continue	35%
Extend preliminary analysis to transcriptional level. Filter and normalize data using accepted best practices and perform traditional analysis of expression profiles at the level of individual genes	6-18 continue	30%
Successful use of data coordination/statistical analysis center bringing together large amounts of data from multiple systems	5 continue	35%
<b>Subtask 2: Establish the model of DFP/CORT as a surrogate for GWI.</b>		
Train staff and students in conduct of model	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of immune markers in brain and periphery	4-15	100%
Employ analytical methods for examination of immunological balance	Begin 4 -16	100%
Establish the minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the sarin surrogate, DFP	4-12	100%
Evaluate stress regimens to establish protocols required to exacerbate proinflammatory response to sarin surrogate, DFP	5-15	100%

Submit animal protocol amendments as required	As needed	100%
<b>Subtask 3: Characterize the molecular and cellular phenotypes of GWI mouse models with the idea of using them to test treatments.</b>		
Use transcriptional analysis to study the immunological basis for the brain and blood changes in the GWI models	12 -24	90%
Use bioinformatic method to estimate pathway activation from gene expression and conduct comparisons between mouse and humans.	6-18	80%
Use molecular modeling to identify and develop networks of expression allowing for robust comparisons between GWI and animal models. Test under baseline and stimulated (stress hormones or exercise)	12-30	90%
<b>Major Task 3: Identification of Illness specific networks with focus on human and mouse comparisons</b>		
<b>Subtask 1: Conduct network analysis for humans and animal models</b>		
Apply biological modeling techniques to pathway activation computed in task 2 sub 3 to render pathway networks	6-12	85%
Integrate with other levels of biology then identify and compare functional modules at various resolutions across groups	12 -18	80%
Conduct detailed analysis of network topology applying measures of network structure and information flow to identify critical information-processing modules	12-24	90%
Conduct an analysis of the alternate steady states available to the regulatory networks identified in human and mouse models.	12-30	85%
Inform pathway-specific genomic panel based on the key network regulatory pathways	12-30	95%
<b>Major Task4: Large-scale simulation of treatment.</b>		
<b>Subtask 1: Conduct in silico sensitivity analysis and rank candidate target nodes</b>		
Use simulation experiments to assess and rank the impact of introducing an in silico equivalent standardized treatment pulse or pulse train at each node in turn throughout the model network	18-30	80%
Rank the candidate target nodes in terms of their relative contribution to shifting the structure of the network recovered under treatment and the network presented in healthy control subjects	18-30	80%
<b>Major Task 5: Define and deploy large-scale optimization.</b>		
<b>Subtask 1: Evaluate and select the best global search algorithm for targeting intervention possibilities</b>		
Review latest developments in evolutionary programming techniques as well as hybrid gradient-based techniques to determine the most suitable search algorithm. Acquire or develop code and deploy.	12-18	80%
Configure simulation-based optimization scheme that	18-24	75%

evaluates the fitness of candidate interventions by repeatedly launching short network simulation runs in search of the most robust treatment course		
<b>Major Task 6: Identify candidate treatment courses for GWI</b>		
<b>Subtask 1: Using task 5 launch optimization runs from multiple initial conditions of endocrine-immune status</b>		
Identify and describe mathematically the immune and endocrine descriptors that can be effectively and safely changed and over what range they may be changed.	24-30	95%
Using drug databases and bioinformatic techniques identify drugs currently available for repurposing to treat GWI	12-30	95%
Search for novel treatment courses. Launch repeated searches for optimal treatments using the set of candidate cytokine, hormone/autonomic and immune markers isolated in task 5	24-36	95%
<b>Major Task 7: Identify candidate treatment courses for GWI</b>		
<b>Subtask 1: Select and test pharmacological therapies on basis of data from computational models in animals</b>		
Use previous data to select best animal models based on immunological and autonomic biomarkers	24-36	95%
Develop computer/mathematical paradigms for evaluation of treatment strategies	12-30	95%
Develop pilot clinical trials on basis of animal studies	24-36	95%
<b>Major Task 8: Verify treatment effectiveness in human subjects</b>		
<b>Subtask 1: Studies of treatment effectiveness in humans</b>		
Design assessment platform for use in human translational studies using the RedCAP platform as a foundation	18-24	100%
Complete the IRB process for selected study drugs, using the Miami VAMC IRB with OCMR review.	24-30	98%
Recruit and perform assessments of GWI subjects on intervention(s) in the phase 1 translational studies.	30-40	95%
Evaluate change in network interactions from interventions suggested Study 3 and 4. Inform the model with the human study data and refine as necessary	32-48	40%

**What was accomplished under these goals?**

**Phase I Proof of Concept Trial:**

Open Label study of safety and feasibility of two repurposed drugs, etanercept and mifepristone in the setting of GWI. Additional data was collected to look at the impact of treatment on homeostatic networks in n=20. Despite COVID pandemic related high incidences in South Florida, we were able to complete recruitment. We needed 3 additional participants to be recruited early 2020 which was not possible due to COVID related closures and IRB determinations. In late this year, we could open back up and we were able to recruit the remaining 3. All participants are currently on study drug. The completed data set on 9 participants shows encouraging results which are provided below:

Preliminary results from 9 males GWI subjects undergoing Enbrel-Mifepristone with assessments made at baseline, immediately after Enbrel-Mifepristone (8 weeks), and follow-up (16 weeks) showed improved vitality subscore up to 16 weeks. The data also reflected overall emotional wellbeing (Figure 1 appendix). Additionally, the hormones and IL1a, IL2, IL4, IL8, and TNF were seen to move closer to control data whereas, IL10, TNFb moved away from HC across study (Figure 2 appendix).

The multidimensional projection of homeostatic state space compared Hormone-Immune profiles of GWI over trial to controls based on healthy and hypercortisolic, hypoadrenergic and Th1 state. We found that all timepoints of trial remain in orbit of hypercortisolic, hypoadrenergic and Th1 state. However, proximity of timepoints move closer to health across study which indicates improvement, but not homeostatic reset (Figure 3 appendix).

**What opportunities for training and professional development has the project provided?**

Nothing to Report

**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?**

Completion of last three participants and laboratory data which will feed the computational modeling work.

**4. IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

The preliminary data from this study encouraged the development of Phase I study under a separate CDMRP award with combination therapy using Enbrel/Mifepristone with different duration and dosages to allow to find the safest and most efficacious dosing strategy which will be moved to Phase II study.

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

**5. CHANGES/PROBLEMS:**

COVID19 infection has caused disruptions in all aspects of research. The impact consisted of disruptions in workflow, delays in review timeline due to reassigned priorities and thus unavailability, institutional hiring freezes and layoffs, pause on HRPO approved studies and disruption in scheduling of participants for screening, etc.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

**Changes that had a significant impact on expenditures**

N/A

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

**Significant changes in use or care of human subjects**

Nothing to report

**Significant changes in use or care of vertebrate animals**

Nothing to report

**Significant changes in use of biohazards and/or select agents**

Nothing to report

**6. PRODUCTS:**

- **Publications, conference papers, and presentations**

**Journal publications.**

N/A

**Books or other non-periodical, one-time publications.**

Nothing to report

**Other publications, conference papers and presentations.**

- **Website(s) or other Internet site(s)**

N/A

- **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:* Nancy Klimas  
*Project Role:* Principal Investigator  
*Researcher Identifier:* eCommons: nklimas  
*Nearest person month worked:* 0.6  
*Contribution to Project:* Overseeing the clinical trial.

*Name:* Fanny Collado  
*Project Role:* Coordinator  
*Researcher Identifier:*  
*Nearest person month worked:* 2.4  
*Contribution to Project:* Clinical trial assessments.

**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?**

Organization Name: South Florida VA Foundation for Research and Education

Location of Organization: Miami VA

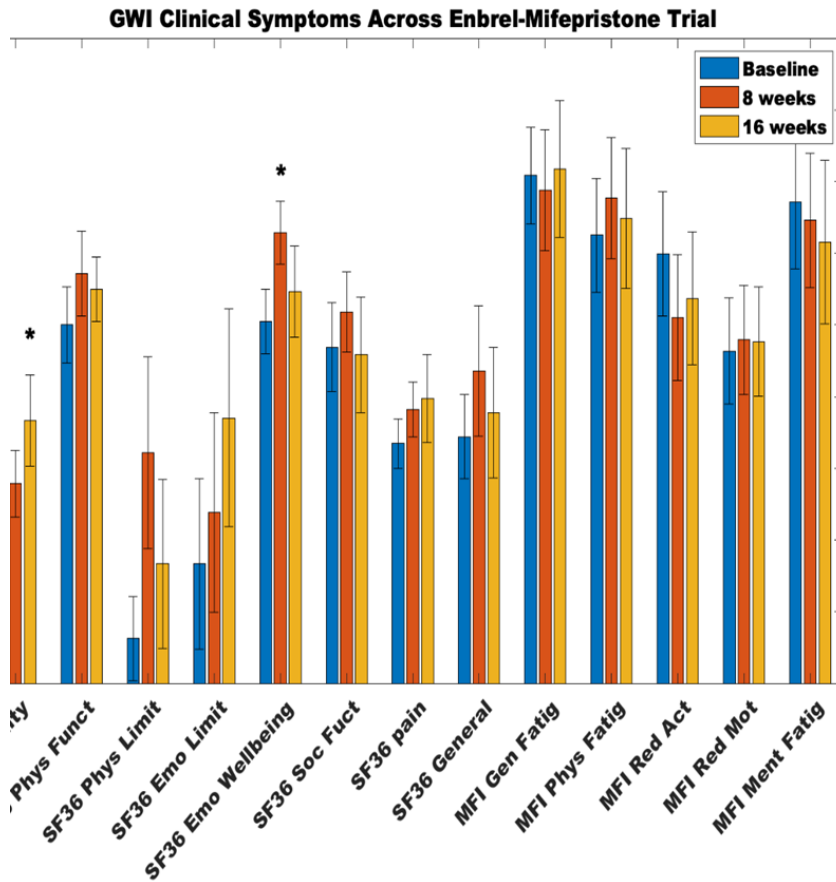
Partner's contribution to the project: Collaborator (Clinical Site)

**8. SPECIAL REPORTING REQUIREMENTS**

N/A

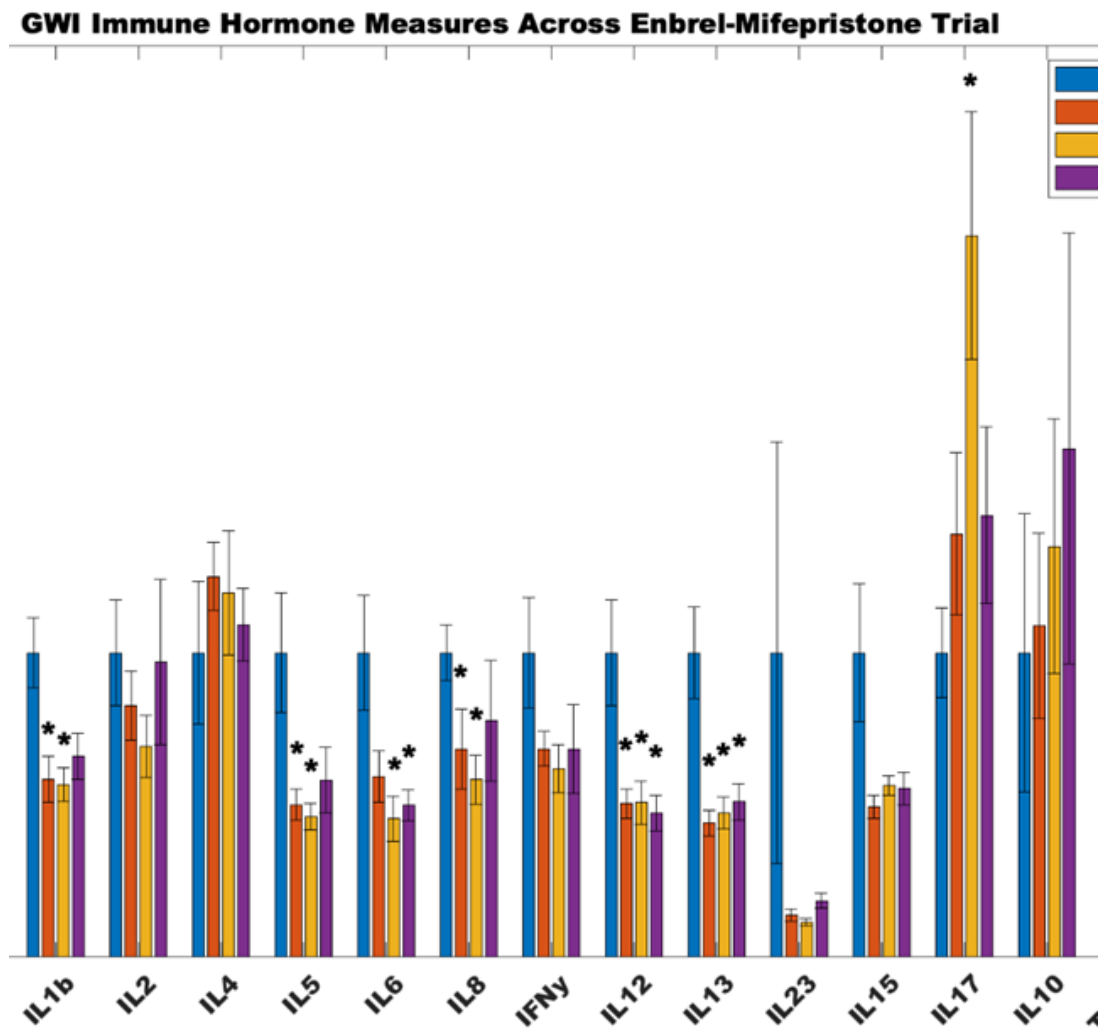
**9. APPENDICES**

Figure1:



\* p < 0.05 compared to HC

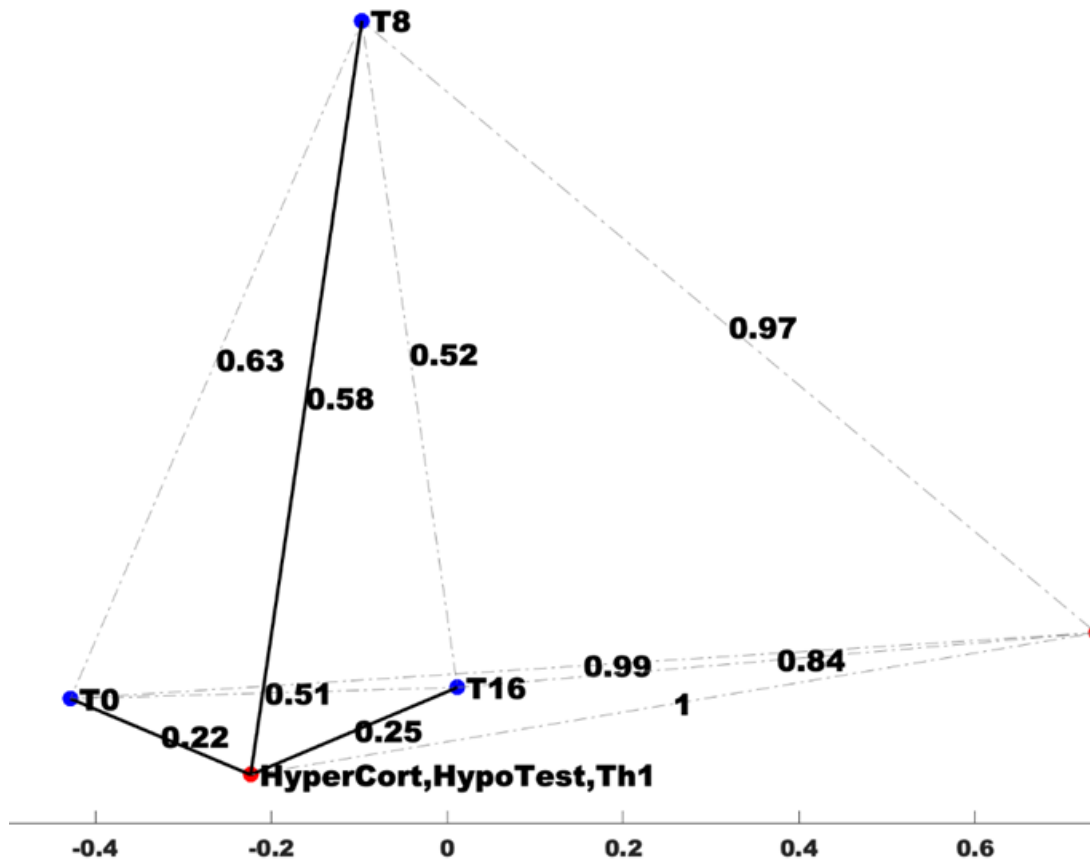
Figure 2:



\* p < 0.05 compared to HC  
All values normalized to HC

Figure 2:

### Multidimensional Projection of Homestatic State Space



# Understanding Gulf War Illness: An Integrative Modeling Approach

**Award Number:** GW120045 / W81XWH-13-2-0085

**PI:** Dr. Mariana Morris

**Org:** Nova Southeastern University

**Award Amount:** \$4,102,527



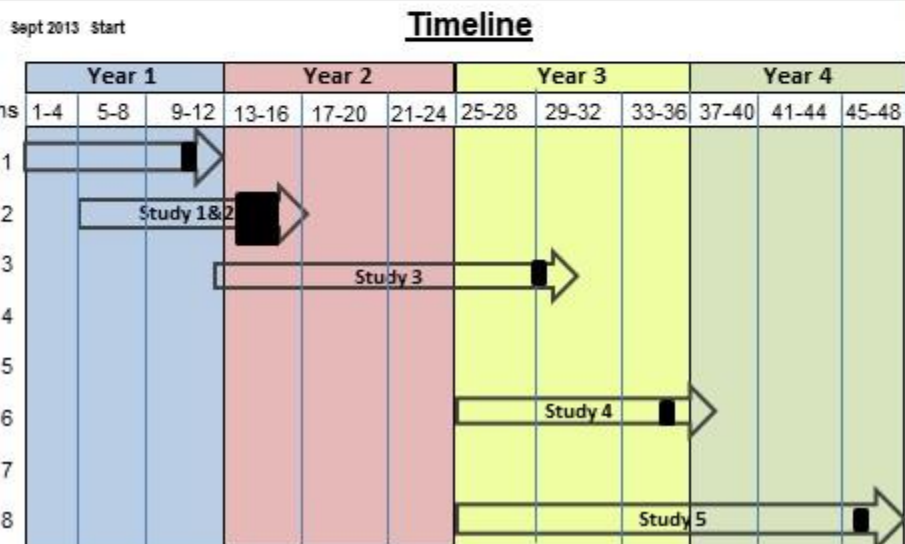
**Approach** To develop a translational model of GWI for rapid identification of molecular targets and prediction of effective therapeutic interventions. The effectiveness of candidate treatment in terms of system abatement and recovery of regulatory network configuration will be assessed in GWI subjects in phase 1 translational studies.

- ❑ **Study 1:** Characterize the autonomic neural/adrenal dysfunction in a mouse model of GWI using validation and direction from computational biology (Task 2).
- ❑ **Study 2:** Characterize the molecular and cellular phenotype of GWI in a mouse model to evaluate the role of stress response in persistence of the illness (Task 2).
- ❑ **Study 3:** Integrate human (previously completed) and animal studies using computational biology to identify mediators of deregulated balance and test putative therapeutics (Task 3-5)
- ❑ **Study 4:** Evaluate therapeutics suggested by computational model in GWI animal models. Two or three most favorable will move on to human testing (Task 6-7).
- ❑ **Study 5:** Perform translational human clinical trials to evaluate homeostasis "reset" as well as preliminary safety and efficacy (Task 8).



## Accomplishments to date

- 1-The consortium presented GWI research findings at GWI State of Science Meeting in Oct, 2020.
2. Despite COVID19 related lockdowns, we were able to fully recruit the study and continue the study procedures for participants.



## Goals/Milestones

### FY13 Goal – Administrative structure for animal/human studies (Task 1)

- ☑ Kick-off meetings with GWIRP staff and study PIs
- ☑ Protocol preparation and initiation of approvals for animal/human use
- ☑ Coordinating center database set-up

### FY14 Goal – Studies 1-3 - Refinement and enhancement of models for GWI

- ☑ Establish model of autonomic dysfunction as a surrogate for GWI (Task 2)
- ☑ Identification of illness specific networks with focus on human and mouse comparisons (Task 3)
- ☑ Large-scale simulation of treatment. (Task 4)
- ☑ Define/deploy optimization and target intervention possibilities (Task 5)

### FY15 Goal – Study 4 - Candidate treatment courses

- ☑ Identify candidate treatment courses for GWI (Task 6)
- ☑ Select and test therapies in animals (Task 7)

### FY16 Goal – Study 5 - Perform translational human clinical trials

- ☐ Verify treatment effectiveness in human subjects n=20 (Task 8)