Gulf War Illness Inflammation Reduction Trial

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Project Narrative

Background: During Desert Shield and Desert Storm (August 2, 1990 to July 31, 1991) 696,841 United States military personnel were deployed to the Kuwaiti Theater of Operations. Today approximately one-third of those veterans are suffering from Gulf War Illness (GWI), an unexplained chronic multisymptom illness (1, 2).

In 1995 the Department of Veterans Affairs (VA) initiated a retrospective cohort survey of 15,000 deployed and 15,000 non-deployed Gulf War era veterans (3). A second study reexamined the health status of the veterans who participated in the 1995 baseline survey (4). This work identified the prevalence of multisymptom illnesses as the most significant difference between the deployed and non-deployed veterans (36.5% vs. 11.7%, adjusted risk ratio = 3.05). Thus, GWI became the signature health-related outcome of the Gulf War.

The underlying pathophysiology of GWI is not understood. Therefore, we performed a pilot study comparing blood samples from Gulf War veterans with and without multiple symptoms of pain, fatigue, and cognitive dysfunction. The objective of the study was to determine if there are quantifiable differences in blood that could be used to identify potential therapeutic targets for the treatment of GWI. The blood analysis included a complete blood count (CBC), plasma proteomics, platelet function studies, and the measurement of multiple coagulation parameters.

The pilot study results provide strong evidence of chronic inflammation in veterans with GWI. This entirely new and provocative line of evidence presents an exciting opportunity to test an intervention that has the potential to both reduce symptoms and further define the pathophysiology of GWI.

The goal of this proof-of-principal trial is to determine if reducing inflammation is an effective treatment for GWI. A successful trial with improved clinical outcomes and reduced biomarkers of inflammation would establish a new paradigm for the diagnosis and treatment of GWI. The testing of other therapeutic interventions designed to reduce inflammation and minimize toxicity could produce additional improvements in GWI treatment beyond those achieved in this trial. The immediate and long-term positive consequences for the health and well-being of veterans with GWI would be significant.

Table 1. Plasma Protein Analysis: GWI^{positive} vs. GWI^{negative}

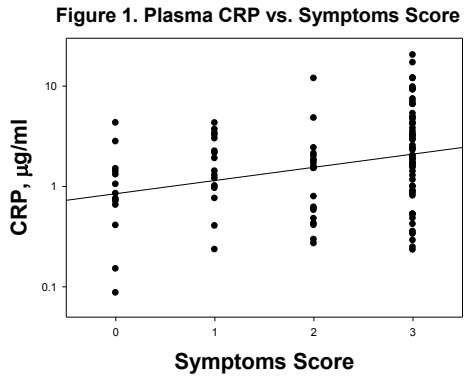
Analyte	GWI Status	n	Mean ± SD	Median	Median Ratio	P value*
C-Reactive Protein (CRP), μg/ml	+	57	3.4 ± 3.9	2.1	1.75	0.032
	-	27	1.6 ± 1.2	1.2		
Leptin, ng/ml	+	57	10.9 ± 8.0	9.0	1.67	0.047
	-	28	8.7 ± 10.2	5.4		
Interleukin 1 beta (IL-1β), pg/ml	+	14	1.0 ± 0.61	0.83	1.66	0.022
	-	11	0.60 ± 0.27	0.50		
Brain-Derived Neurotrophic Factor (BDNF), ng/ml	+	57	1.5 ± 0.92	1.3	1.59	0.015
	-	28	1.4 ± 1.6	0.82		
Matrix Metalloproteinase-9 (MMP-9), ng/ml	+	57	115 ± 62.2	103.0	1.14	0.034
	-	28	94.5 ± 43.7	90.5		
Matrix Metalloproteinase-2 (MMP-2), μg/ml	+	57	2.3 ± 0.59	2.1	0.88	0.005
	-	28	2.7 ± 0.74	2.4		
Fatty Acid-Binding Protein, heart (FABP, heart), ng/ml	+	53	2.4 ± 1.7	1.8	0.64	0.020
	-	25	3.4 ± 2.6	2.8		

*Since the data summarized in this table were not normally distributed the Mann-Whitney rank sum test was employed.

The pilot study was funded by research awards from the VA Office of Research and Development (ORD) and the Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) Gulf War Illness Research Program (GWIRP).

The CDC-10 survey instrument was used in the pilot study for symptoms assessment (5). Veterans with multiple symptoms were designated GWI^{positive}. All other subjects were designate GWI^{negative}. Plasma levels of 90 proteins were quantified using Multi-Analyte Profiling (MAP) technology. Univariate analysis identified 7 statistically significant differences ($p<0.05$) between the two groups, Table 1. These data strongly suggest that chronic inflammation is present the majority of subjects with GWI.

The presence of C-reactive protein (CRP) in Table 1 is particularly noteworthy. The physiologic role of CRP is to initiate the complement cascade on the surface of damaged cells and invading microbes. CRP is also an acute-phase protein produced by the liver. Hepatocyte synthesis of CRP is regulated by Interleukin 6 (IL-6). Plasma CRP is stable and easy to measure. Furthermore, unlike IL-6, it is not subject to diurnal variations. Therefore, CRP is employed as a biomarker of IL-6-mediated inflammation (6). Linear regression analysis, Figure 1, revealed further evidence of a strong positive correlation (p=0.002) between plasma CRP and the number of GWI symptoms from the CDC-10.



Chronic inflammation can trigger GWI-like symptoms:

- Plasma CRP levels are frequently elevated in chronic inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic lupus erythematosus (SLE). Increasing symptoms of pain, fatigue, and cognitive dysfunction often correlate with increasing CRP, and decreases in CRP are associated with corresponding decreases in symptoms (7-12).
- It has been demonstrated that IL-6 produced in the periphery can induce microglial-mediated inflammation in the brain; and this activation of microglial cells is associated with fatigue, depression, sleep deprivation, hyperalgesia, and cognitive impairment (13).
- Leptin is an adipokine that not only serves as a regulator of body weight and appetite, but also as an essential link between energy utilization and the immune system (14, 15). Both IL-6 and leptin play central roles in the symptomology of the acute-phase response to bacterial infections (16). Also, fatigue is associated with circulating leptin levels in irritable bowel syndrome (17).
- Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin with functions related to the survival and proliferation of nerve cells and neuroinflammation. In addition, BDNF enhances inflammation-related pain (18, 19). Increases in blood levels of BDNF have been correlated with increased pain perception in both RA and fibromyalgia (18, 20).
- Thus, the symptoms of pain, fatigue, and cognitive dysfunction experienced by $GWI^{positive}$ veterans may be due in part to increased levels of CRP/IL-6, leptin, and BDNF.

Interleukin 1 beta (IL-1β) was also identified in our pilot study as significantly higher in the $GWI^{positive}$ group. IL-1β is a key pro-inflammatory cytokine and a primary driver of the innate immune response. The strength of this observation is tempered by the fact that a majority of subjects in both groups had levels of the cytokine below the detectable limit of the assay.

In the second phase of the proteomic analysis bivariate linear regression was used to look for associations between CRP and the other plasma proteins. Twelve statistically significant linear correlations with R values>0.30 and P values<0.01 were observed. We refer to this set of proteins as the CRP Proteome, Table 2. The correlations with CRP suggest that IL-6-mediated inflammation plays some role in regulating the plasma levels of these proteins. Thus, some members of the proteome may be useful as biomarkers of a treatment-induced inflammation reduction. If the anticipated changes correlate with improved HRQOL and reduced symptoms, then this information may contribute to our understanding of GWI pathophysiology.

Table 2. The CRP Proteome

Analyte (vs. CRP)	R value	P value
Serum Amyloid P-Component	0.594	<.001
Fibrinogen	0.469	<.001
Leptin	0.459	<.001
Plasminogen Activator Inhibitor 1	0.433	<.001
Adiponectin	0.359	<.001
Complement C3	0.341	<.001
Insulin	0.335	<.001
Apolipoprotein C-III	0.322	<.001
Interleukin 6	0.316	0.007
Thyroxine-Binding Globulin	0.311	<.001
Interleukin-1 receptor antagonist	0.310	<.001
EN-RAGE	0.301	<.001

The presence of IL-6 in the proteome is not surprising given the coupling of IL-6 to CRP production. The proteome also contains other ligands for cell-surface receptors with important inflammation-related signaling functions, acute-phase proteins, complement and coagulation proteins, regulators of energy intake and utilization, etc. Their functions are consistent with a wound healing response involving activation of the coagulation and complement cascades, cell recruitment, extracellular matrix remodeling, and angiogenesis. Chronic inflammation is frequently accompanied by tissue destruction and repair, i.e., a state of chronic wound healing (21, 22). Therefore, the results presented in Table 2 support the conclusion that chronic inflammation is part of the underlying pathophysiology of GWI.

A previous study observed evidence of platelet activation in a majority of $GWI^{positive}$ subjects (23). Therefore, as part of our pilot study platelet functions were studied in $GWI^{positive}$ and $GWI^{negative}$ subjects (24). Studies performed included platelet count, immature platelet fraction (IPF), plasma thrombopoietin (TPO), CRP, platelet aggregation and ATP secretion in response to six agonists, and spontaneous aggregation. Platelet counts and CRP were significantly elevated in $GWI^{positive}$ compared to $GWI^{negative}$ subjects without elevation in IPF or TPO. Platelet aggregation did not differ between groups except for spontaneous aggregation that was significantly greater in $GWI^{positive}$ subjects. Platelet ATP secretion was similar in the two groups except the response to a thrombin receptor activating peptide, 50 μM TRAP 6, was significantly greater in $GWI^{positive}$ subjects. When platelet aggregation was analyzed in relation to CRP the response to a thromboxane receptor agonist, 0.5 μM U46619, was significantly greater in subjects whose CRP was ≥2 μg/ml. Therefore, $GWI^{positive}$ subjects had elevated platelet counts, spontaneous aggregation, TRAP 6-induced secretion, and CRP, but no impairment of platelet function. The increased platelet counts and U46619-induced aggregation appear to be consequences of an underlying inflammatory state in GWI.

Thrombin-antithrombin (TAT) is the inhibited end-product of thrombin generation and is rapidly cleared from the blood (t½ ~5min). Therefore, high levels of TAT indicate ongoing activation of the coagulation cascade. Median plasma TAT levels for the $GWI^{positive}$ and $GWI^{negative}$ groups were 16.9 ng/ml and 8.9 ng/ml, respectively. The difference is statistically significant (p=0.006, Mann-Whitney rank sum test). Coagulation is an integral part of the innate immune response (25-27). Therefore, the activation of plasmatic coagulation in $GWI^{positive}$ subjects is consistent with the chronic inflammation hypothesis.

The plasma proteomics, platelet, and coagulation evidence presented above support the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. Therefore, chronic inflammation was selected as the therapeutic target for this clinical trial.

Objectives/Specific Aims/Hypotheses: The hypothesis on which this proposal is based is the following: Chronic inflammation is part of the underlying pathophysiology of GWI. Furthermore, products of inflammation contribute directly to GWI-associated symptoms of pain, fatigue, and cognitive dysfunction. Therefore, an intervention that reduces this inflammation may alleviate symptoms of the disorder and improve the health-related quality of life (HRQOL) of veterans with GWI.

The primary objective of this proposed clinical trial is to determine if treatment with an anti-inflammatory drug improves HRQOL and reduces symptoms of veterans with GWI. This trial also provides a significant opportunity to advance scientific knowledge by testing the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. Improved clinical outcomes combined with objective evidence of reduced proinflammatory biomarkers would confirm that chronic inflammation plays an important role in GWI.

This clinical trial may contribute to the advancement of clinical practice. A successful trial would validate the strategy of treating GWI-associated chronic inflammation. This new paradigm would change the standard of care by focusing on treatments that reduce inflammation. It is likely that other therapeutic interventions designed to reduce inflammation and minimize toxicity will emerge from this new perspective. These treatments for GWI could become part of a comprehensive patient-centered therapeutic program tailored to the specific needs of individual Gulf War veterans (28).

Standardized self-report measures and proteomics will be used to evaluate treatment-related changes by performing the assessments at the beginning and the end of the treatment period and again eight weeks after the end of treatment. The specific aims to this study are the following:

- Specific Aim 1: To measure the effects of the treatment on physical and mental functioning (Veterans Short Form 36-Item Health Survey).

- Specific Aim 2: To assess intervention-related changes in symptoms: pain (McGill Pain Questionnaire-Short Form), fatigue (Multidimensional Fatigue Inventory), and cognitive dysfunction (Cognitive Failures Questionnaire).
- Specific Aim 3: To quantify changes in biomarkers of inflammation in response to the treatment (plasma proteomics).

Study Design: This is a randomized, two-group, placebo-controlled, double-blind, clinical trial with the treatment group receiving a low dose (10mg qD) of delayed-release (DR)-prednisone for 8 weeks versus the placebo group receiving matching placebo for 8 weeks. The study will determine if treating $GWI^{positive}$ veterans for 8 weeks with DR-prednisone improves HRQOL, alleviates symptoms, and reduces inflammation parameters compared to $GWI^{positive}$ veterans receiving placebo. Horizon Pharma, Inc., the developer of (DR)-prednisone (Rayos®), will supply the drug and the matching placebo for this study at no cost per the approved CRADA.

Intervention Chronic inflammation has been identified as the therapeutic target. There is no direct evidence to guide the selection of the medication for this trial. Therefore, information relating to drug efficacy in established diseases with similar symptoms and biomarker profiles has been used to provide therapeutic guidance.

Pain, fatigue, and cognitive dysfunction are the predominant symptoms of GWI. These symptoms also occur with high frequency in chronic inflammatory diseases such as RA, MS, Crohn's disease, and SLE. For these diseases, increases in proinflammatory cytokines are often associated with increases in symptoms. Likewise, anti-inflammatory drugs that decrease proinflammatory cytokine levels have established efficacy for reducing symptoms and improving HRQOL (7-12).

Four classes of anti-inflammatory drugs with established efficacy for the treatment of chronic inflammatory diseases were considered:

- Biologic Response Modifiers (BRMs)
- Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)
- Disease-Modifying Anti-Rheumatic Drugs (DMARDs)
- Glucocorticoids (GCs)

There are no published studies in veterans with GWI utilizing drugs from any of these categories. Therefore, results of treatment of the aforementioned chronic inflammatory diseases were carefully reviewed to provide insight into the choice of a therapeutic modality. We first considered the mechanisms of action of the four drug classes. We then selected the mechanism with greatest likelihood of reducing GWI-associated inflammation. Following the selection of an appropriate mechanism, the available drugs were reviewed for potential efficacy and severity of side effects.

BRMs (e.g. etanercept, infliximab, rituxan) target specific elements of the immune system. For example, the BRM etanercept is an inhibitor of tumor necrosis factor. Since the information that would guide the choice of a BRM for this study is limited, there is a high likelihood of choosing incorrectly. Also, many BRMs have serious side effects, they are expensive, and the mode of delivery (injection) would likely reduce recruitment and compliance. Thus, BRMs were eliminated from further consideration.

NSAIDs (e.g. aspirin, naproxen, indomethacin, piroxicam) are cyclooxygenase (COX) inhibitors. The COX enzymes, COX1 and COX2, catalyze essential reactions in prostaglandin (PG) synthetic pathways. PGs are oxygenated metabolites of arachidonic acid. They are rapidly generated in response to perturbations such as physical trauma and infection. PGs are important signaling molecules with a multitude of paracrine and autocrine functions. NSAIDs are used primarily to treat pain and fever. The analgesic and antipyretic effects of NSAIDs are primarily the result of decreased production of PGs.

One argument against the use of NSAIDs is related to safety. The potential toxicity of NSAIDs at the doses that may be required to achieve anti-inflammatory effects is a concern. The FDA has imposed new labeling requirements for both prescription and over-the-counter NSAIDs. The black box warnings highlight the potential for rare but serious cardiovascular events (COX 2) and gastrointestinal bleeding (COX 1) associated with their use. Another argument against the use of NSAIDs is the limited potential for efficacy. The primary mechanism of action of NSAIDs is the suppression of proinflammatory PG production. This is a relatively narrow downstream target, and there is no direct evidence linking PGs to the symptoms of GWI. Therefore, NSAIDs were rejected.

DMARDs (e.g. hydroxychloroquine, cyclosporine, methotrexate) are a category of otherwise unrelated drugs defined by their ability to suppress the immune system and reduce symptoms of rheumatic diseases. DMARDs are attractive with respect to their pleiotropic anti-inflammatory effects. However, DMARD immunosuppression is achieved by multiple mechanisms and in most cases the mechanism of action remains obscure. This lack of information regarding mechanism makes it difficult to judge the potential efficacy of DMARDs for treating GWI. In addition, the potential toxicity of these drugs is substantial. Therefore, DMARDs were rejected.

GCs are steroid hormones made in the adrenal gland (cortisol, cortisone, etc.) as well as numerous synthetic derivatives (dexamethasone, prednisone, etc.). The mechanism of GC action begins with passage of the molecule across the plasma membrane, binding to the GC receptor (GCR) in the cytosol, and translocation of the complex into the nucleus. The GCR is a ubiquitous ligand-activated transcription factor present in most cells from vertebrate animals. Changes in transcription are mediated by the binding of the GC-GCR complex to GC response elements in the suppressor or promoter sites of target genes. The multiple effects induced by this ligand-receptor interaction include stimulation of gluconeogenesis and immune system suppression (29).

Some anti-inflammatory effects of GCs are achieved via the inhibition of proinflammatory transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein-1. In particular, the blockage of NF-κB activity may be a key to understanding the pleotropic

anti-inflammatory effect of GCs (30-32). The suppression of NF-κB activity is achieved, at least in part, by the GC-GCR-induced synthesis of the NF-κB inhibitor (IκB). I-κB traps NF-κB in inactive cytoplasmic complexes resulting in the suppression of pro-inflammatory gene expression.

The efficacy of GCs as anti-inflammatory and immunosuppressive drugs is clearly established. Their pleiotropic effects on immune system functions make them attractive as potential treatments for the inflammation associated with GWI. Prednisone is an effective and widely prescribed synthetic GC. Its consideration for use in this trial has been enhanced by the evidence that a new formulation of the drug (Rayos®- delayed-release (DR)-prednisone) improves efficacy and safety.

One of the limitations of prednisone treatment is related to the time of administration. Patients normally take the drug in the morning (~8 AM) which is not optimal with respect to achieving the anti-inflammatory effects. It has been demonstrated that prednisone chronotherapy, i.e., administration of the drug at the diurnal peak of proinflammatory cytokines levels (~2 AM), increases both efficacy and safety (33-37). DR-prednisone is designed to optimize the time of delivery without requiring patients to wake in the middle of the night to take the medication. It is a tablet that releases the drug approximately 4h after ingestion. Therefore, DR-prednisone can be taken at bedtime.

The efficacy and safety of DR-prednisone chronotherapy have been established in recent clinical trials. In the CAPRA-1 trial DR-prednisone was compare to immediate-release prednisone (average dose ~6mg qD) in the treatment of RA (38). The superiority of DR-prednisone was demonstrated after 12 weeks for the primary outcome (morning stiffness) and the reduction of IL-6, a key circadian proinflammatory cytokine. In the CAPRA-2 trial RA patients who were inadequate responders to DMARD therapy were randomized to either DR-prednisone (5 mg qD) or placebo in addition to existing therapy (39). After 12 weeks the subjects were assessed for signs and symptoms of RA. DR-prednisone was significantly better than placebo in 11 of 13 clinical measures and 2 of 3 HRQOL assessments. Adverse event rates for the two arms of CAPRA 1 and CAPRA 2 were similar. In a longer term RA treatment study the effects on symptoms and the favorable safety profile of DR-prednisone persisted for 12 months, and no changes in hypothalamic-pituitary-adrenal axis function were detected (35). This experience provides confidence that significant toxicity will not be observed with the short-term administration of prednisone in the proposed trial.

The clinical trial results presented above have established the efficacy and safety of DR-prednisone. Based on this evidence and the well-established pleiotropic anti-inflammatory effects of the drug, DR-prednisone has been chosen for the proposed trial. It is worthy of emphasis that a successful outcome of the clinical trial will provide support for additional study of anti-inflammatory agents, but it will not imply that prednisone would be the drug of choice for chronic management of GWI. Long-term toxicity of corticosteroid therapy may preclude its use; however, a positive outcome would justify further research.

Measurement of Study Variables The primary and secondary outcome measures, summarized in Table 3, were selected to examine the range of GWI-related symptomatology and biomarkers of inflammation. The self-report instruments will be administered and blood will be collected at the beginning and the end of the 8 week treatment period and at the end of the 8 week post-treatment period.

The SF-36V is a modification of the well-established Medical Outcomes Study Short Form Health Survey (SF-36) for use with ambulatory Veterans Health Administration patients. It surveys eight concepts of health: physical functioning, role limitations because of physical problems, bodily pain, general health perceptions, energy/vitality, social functioning, role limitations due to emotional problems and mental health (40-42). From these concepts, two summary component scores are derived: a Physical Component Summary (PCS) and a Mental Component Summary (MCS). Scores are standardized and range from 0 to 100, with a US population mean of 50 points and a SD of 10 points. The PCS and the MCS have been demonstrated to have excellent psychometric properties (40, 41) and have been used extensively in GWI studies and throughout VA settings (5, 40-44). The subscale items largely address the diverse array of symptoms reported as a part of GWI. SF-36V PCS is a measure of HRQOL with respect to physical functioning and symptoms.

Table 3. Primary and Secondary Outcome Measures

Primary Outcome Measure	Secondary Outcome Measure	Name of Measure	Description
x		Veterans Short Form 36-Item Health Survey Physical Component Summary (SF-36V-PCS)	HRQOL/ Physical Health Functioning
	x	McGill Pain Questionnaire- Short Form (MPQ)	Sensory pain, affective pain, pain now, and typical pain
	x	Multidimensional Fatigue Inventory (MFI)	General fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue
	x	Cognitive Failures Questionnaire (CFQ)	Self-report of cognitive symptoms, such as attention, concentration, and memory
	x	Veterans Short Form 36-Item Health Survey Mental Component Summary (SF-36V-MCS)	HRQOL/ Mental Health Functioning
	x	HumanMAP®-v2.0/Antigens, hs-CRP assay,TAT ELISA	Quantitative analysis of plasma biomarker levels

The primary outcome measure will be the presence or absence of a positive response on the SF-36V PCS following treatment. A positive response is defined as a 7-point or greater increase in the SF-36V PCS at 8 weeks versus the baseline score. This definition of a positive response to

an intervention in veterans with GWI is based upon the psychometric properties of the SF-36V PCS and the distribution of scores observed in the population and comes from VA Cooperative Study Program (CSP) Study #470 (43, 44). Based on the results of the Veterans Health Study (40, 41), a 7 point change in the SF-36V PCS score is expected to represent an effect size of 0.75.

Validated and standardized methods for the assessment of symptoms were selected due their wide use in GWI studies, sound psychometric properties, and demonstrated sensitivity to clinically meaningful changes in GWI symptoms following treatment:

- The MPQ is a self-report survey which measures the quality of pain by asking patients to rate the intensity of 15 verbal descriptors of pain on a 0 to 3 rating scale with lower scores indicative of lower pain levels (45). The scale yields subscale scores in the following domains: Sensory pain, affective pain, pain now, and typical pain. The MPQ is a commonly used pain measure with considerable documentation of its reliability, sensitivity to change, and concurrent and predictive validity as a measure of pain quality and intensity (46).
- The MFI is a 20-item self-report instrument designed to measure fatigue (47). The MFI covers the following dimensions: general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue. Scores on each dimension range from 4 to 20, with lower scores indicative of higher functioning. The instrument has demonstrated good internal consistency, with an average Cronbach's alpha coefficient of 0.84.
- The CFQ is a 25-item self-report measure designed to assess the frequency of failures in perception/attention (e.g. ``do you fail to notice signposts on the road?''), memory (e.g. ``do you forget appointments?'') and action (e.g. ``do you bump into people?'') (48). Patients are asked to indicate on a 5-point scale (0= never to 5=very often) how often they have experienced each cognitive failure in the past months. Studies have demonstrated the CFQ to have adequate internal consistency and test-retest reliability.
- The MCS is one of two summary component scores derived from SF-36V (40-42). It is a clinically relevant measure that has been well-validated in the general population as well as a variety of disease states. The MCS measures changes in mental health-related functioning and has been used in other studies of GWI (5, 43, 44).

Intervention-related changes in biomarkers of inflammation will also be assessed. At each study visit a blood sample will be drawn and fractionated. The platelet-free EDTA plasma samples will be stored at -80^{o}C prior to assay. Biomarker levels will be determined at the beginning and the end of the 8 week treatment period and at the 16 week follow-up visit. Preparation and storage of the plasma samples will take place in Dr. Bach’s lab. Established immunoassay procedures utilizing MAP technology will be employed to quantify the concentrations of 85 plasma proteins (HumanMAP®-v2.0/Antigens). The assays will be performed on platelet-free

EDTA plasmas by Myriad RBM, Inc., Austin, TX. Myriad RBM validates all assays to clinical laboratory standards. The Myriad RBM laboratory is accredited by the College of American Pathologists. Its CLIA number is 45D1037483.

Clinical laboratories generally use what is known as the high-sensitivity CRP (hs-CRP) assay to detect plasma CRP. The hs-CRP assay measures CRP using laser nephelometry. In our pilot study CRP levels were determined by the MAP assay described above. CRP levels will be measured by both methods in the proposed trial. The hs-CRP assays will be performed by the clinical laboratory of the Minneapolis VA Health Care System (MVAHCS). Using both procedures to determine plasma CRP levels will permit comparison with published data from the study of other inflammatory states.

Plasma TAT levels will be quantified by enzyme-linked immunosorbent assay (ELISA) using the AssayMax Human TAT Complexes ELISA Kit from AssayPro®. The TAT ELISAs will be performed in Dr. Bach's laboratory. All personal protective equipment appropriate for the handling of blood and blood products are used. Study personnel are trained in proper handling techniques for biohazards and hazardous chemicals.
Other clinical laboratory tests will be performed in order to establish that certain exclusion criteria are not met. The tests will include a CBC, creatinine, blood glucose, hemoglobin A1C, and hs-CRP. Also, the CBC, hemoglobin A1C, blood glucose, and hs-CRP assay will be performed at each study visit. These tests will be will be conducted by the clinical laboratory of the MVAHCS. The laboratory has met all applicable standards for accreditation and is accredited by the College of American Pathologists. Its CLIA number is 24D0988147.

Recruitment methods The primary method for recruiting potential subjects will be an institutional review board (IRB)-approved recruitment letter sent to Gulf War veterans identified through registries and prior studies. Gulf War veterans who participated in our pilot study will be invited to participate in the trial. Additional names of potential subjects will come from MVAHCS Gulf War Registry. This list currently includes 1,418 names. Any veteran deployed to the Gulf with an honorable discharge during Operation Desert Shield, Operation Desert Storm, and Operation Iraqi Freedom is eligible to have the Gulf War Physical and be included on this local registry. The addresses for the registrants are found in the Computerized Patient Record System (CPRS) of the MVAHCS. Some of the addresses in CPRS will be no longer be current, as some people have moved since having the registry physical and have not updated their medical records with the facility, but the names and addresses Gulf War veterans are also available from other lists described below.

Addresses of Gulf War veterans will also be obtained from lists generated by the DoD Data Manpower Data Center (DMDC) and the VA Decision Support Service (DSS). These lists, whose constituents overlap, have 4,371 and 1,871 names, respectively. The DMDC list is made up of US Armed Forces veterans who served in the Gulf War (1990-1991) with their most current DoD addresses located in Minnesota or Western Wisconsin within the VISN 23 boundary. The DSS list consists of addresses for Gulf War who have medical charts in the MVAHCS CPRS, live within 250 miles of the MVAHCS, and do not have a diagnosis of hepatitis, cancer, diabetes,

terminal illness, or condition that would prevent informed consent or survey completion, which would render them ineligible to participate in the study. In sum, we have access to names and addresses of more than 5,000 Gulf War veterans who are potentially eligible to participate in this study.

Another method for reaching potential study subjects is a recently added feature of the MVAHCS in-house daily email newsletter. The newsletter now contains a posting section for active research studies. With IRB approval, the trial will be posted there. With veterans composing 26% of our MVAHCS workforce, this newsletter has the possibility of reaching many potential subjects. In addition, posters, fliers, and announcements in local media may also be used to further enhance recruitment. Contact with local Veterans' Service Organizations (VSOs) and County Veteran Service Officers (CVSOs) and advertising for participation via their venues may also be employed.

Human subject-to-group assignment process Block randomization will be used to assign participants to comparison groups. For each consecutive pair of enrolled subjects, one member will be assigned to the treatment condition, the other member to the placebo condition. The pseudo-random number generator in Microsoft Excel will be used to generate random numbers to permit the treatment assignment within each pair (block) of subjects. Treatment assignment codes will be generated and kept by the biostatistician. These codes will be stored on the secure VA Server behind VA firewalls.

Case Definition of GWI Eligible participants for the proposed study are Gulf War veterans who were deployed in the Kuwaiti Theatre of Operations between August 2, 1990 and July 31, 1991 and who have GWI. The Kansas GWI case definition questionnaire will be administered as a screening tool in order to determine eligibility to participate in the study. It has both exclusionary and inclusionary components. The Kansas GWI case definition was developed by Dr. Lea Steele from her population-based survey of over 2,000 Kansas Gulf War veterans (49). It is a more comprehensive analysis of eligibility than the Fukuda case definition (CDC-10) employed in the pilot study (5). To be considered a "case" of GWI requires:

- Veterans endorse moderately severe and/or multiple symptoms in at least 3 of 6 symptom domains: fatigue/sleep problems, pain symptoms, neurological/cognitive/mood symptoms, gastrointestinal symptoms, respiratory symptoms, and skin symptoms.
- The symptoms first became a problem during or after the Gulf War.

Statistical Plan and Data Analysis:

Power analysis The power calculation is based results from CSP #470 (Cognitive Behavioral Therapy and Aerobic Exercise for Gulf War Veterans' Illnesses: A Randomized Controlled Trial) (44). The study population, $GWI^{positive}$ Gulf War veterans, and the primary outcome measure, SF-36V PCS, are the same in CS #470 and this proposed trial.

Based on an estimate of the variability of change in SF-36V PCS, i.e., variability associated with change in SF-36V PCS in response to a 3-month exercise intervention, we calculated that n=40 subjects per group will be needed to detect the difference between a 7-point change in SF36V PCS in response to medication (versus no change in placebo response) with 80% power at the p=0.05 significance level (nQuery Advisor v 7.0).

A 7-point increase in the SF-36V PCS in $GWI^{positive}$ veterans is believed to be clinically meaningful. The support for this assertion is presented in the design paper for CSP #470 (43). Based on the sample size estimate and a maximum projected attrition rate of 20%, a recruitment goal of 100 participants (50 per group) has been set for the Gulf War Illness Inflammation Reduction Trial.

Data analysis The analysis of the data will first focus on the primary outcome measure of physical functioning (SF-36V PCS). Study subjects will be classified after the 8 week treatment phase as "improved/not improved" relative to baseline using the criteria of CSP #470 for a positive response of a 7-point or greater increase in the SF-36V PCS (43, 44). This outcome variable will be analyzed using logistic regression. Treatment condition will be the primary predictor variable in this model with age, gender, medications, complete blood count (CBC), body mass index, and baseline PCS included as adjustment covariates.

The first round of data analysis will be followed by the analysis of differences in the outcome measures. The first group includes measures of symptoms and mental health-related functioning: MPQ, MFI, CFQ, and SF-36V MCS. The second group includes the biomarkers of inflammation. Differences will be assessed using separate analyses of covariance. Post-treatment scores will be the outcome variables. Baseline measurements of the same outcome variable will be included as an adjustment covariate along with age, gender, medications, CBC, and body mass index.

An intent-to-treat analysis will be used for the primary and secondary outcome measures. Participants without calculable PCS scores at baseline will be excluded from the analysis. Participants who withdraw from the study or miss the final study visit will be classified as not improved. Missing values at the final assessment due to patient dropout will be set to equal baseline scores (last-observation-carried-forward strategy). Parallel analyses will be conducted on intent-to-treat (all patients) and per protocol (completers only) datasets. All outcome variables will be assessed for distributional normality. Non-normally distributed data will be transformed, if possible, to achieve a normal distribution. All primary and secondary outcome measures will be administered three times (0 weeks-8 weeks-16 weeks).

Support

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List of Abbreviations, Acronyms, and Symbols:

BDNF	Brain-Derived Neurotrophic Factor
BRM	Biologic Response Modifiers
CBC	Complete Blood Count
CDC-10	Centers for Disease Control 10 Question Symptoms Assessment
CDMRP	Congressionally Directed Medical Research Programs
CFQ	Cognitive Failures Questionnaire
CITI	Collaborative Institutional Training Initiative
COX	Cyclooxygenase
CMI	Chronic Multisymptom Illness
CPRS	Computerized Patient Record System
CRP	C-Reactive Protein
CRADA	Cooperative Research and Development Agreement
CSP	Cooperative Studies Program
CVSO	County Veteran Service Officer
DMARD	Disease-Modifying Anti-Rheumatic Drug
DMDC	Data Manpower Data Center
DoD	Department of Defense
DR	Delayed Release
DRS	Data Request System
DSMB	Data and Safety Monitoring Board
DSS	Decision Support Service
ELISA	Enzyme-Linked Immunosorbent Assay
GC	Glucocorticoid
GCP	Good Clinical Practices
GCR	Glucocorticoid Receptor
GRECC	Geriatric Research, Education & Clinical Center
GWI	Gulf War Illness
GWIRP	Gulf War Illness Research Program
HIPAA	Health Insurance Portability and Accountability Act

HRPO	Human Research Protection Office
HRQOL	Health-Related Quality of Life
hs-CRP	high-sensitivity CRP
IκB	Nuclear Factor-kappa B Inhibitor
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IPF	Immature Platelet Fraction
IRB	Institutional Review Board
ISO	Information Security Officer
MAP	Multi-Analyte Profiling
MCS	Mental Component Summary
MFI	Multidimensional Fatigue Inventory
MPQ	McGill Pain Questionnaire- Short Form
MS	Multiple Sclerosis
MVAHCS	Minneapolis VA Health Care System
NDA	Non-Disclosure Agreement
NF-κB	Nuclear Factor-kappa B
NSAID	Nonsteroidal Anti-Inflammatory Drug
OHRP	Office of Human Research Protections
OIG	Office of the Inspector General
ORO	Office of Research Oversight
ORP	Office of Research Protections
ORD	Office of Research and Development
PCS	Physical Component Summary
PHI	Protected Health Information
PI	Principal Investigator
PO	Privacy Officer
RA	Rheumatoid Arthritis
RIPS	Research Information Protection Subcommittee
SDTU	Special Diagnostic Treatment Unit
SF-36V	Veterans Short Form 36-Item Health Survey
SLE	Systemic Lupus Erythematosus
TAT	Thrombin-Antithrombin Complex
TPO	Thrombopoietin

TF	Tissue Factor
TRAP	Thrombin Receptor Activating Peptide
UDS	Upload-Download System
UPS	United Parcel Service
USAMRMC	US Army Medical Research and Materiel Command
VA	Department of Veterans Affairs
VHA	Veterans Health Administration
VSO	Veterans' Service Organization

Facilities, Existing Equipment, and Other Resources: The Minneapolis VAHCS is a large medical facility with excellent research space and resources. The VAHCS is only 10 minute by light rail connection from the main terminal of the Minneapolis/St. Paul International Airport. Dr. Bach's and Dr. Johnson's laboratories are located in two adjacent rooms. Their main labs are 500 square feet each and Dr. Bach's second lab directly across the hall is 250 square feet.

The Research Service of the Minneapolis VAHCS provides the infrastructure necessary to carry out this research. The Special Diagnostic Testing Unit (SDTU) located in the MVAHCS is the location where the physical exams and blood draws will take place. A Registered Nurse will perform the blood draws in a quiet and private setting. The SDTU intake room will be the designated meeting room for the study visits.

Staff members are in place to manage budgets, place orders, and meet all regulatory requirements. Research oversight is provided by the Research and Development Committee, the entity within Research Service overseeing two IRB committees, the subcommittee on research safety, and the subcommittee on animal care and use. In addition to laboratory space there are rooms in the research wing containing common equipment for use by all VA investigators and meeting rooms with the requisite audiovisual equipment for scientific presentations.

The VISN23 Gulf War Registry at the Minneapolis VAHCS contains the names of approximately 1,400 veterans. This Gulf War Registry is one starting point for our recruitment of individuals to participate in this study.

The Investigational Pharmacy at the MVAHCS will be the site for the management of the study drug. The Investigational Pharmacist will provide documentation of drug receipt, storage, dispensing, documentation of dispensing, monitoring expiration date of study drugs, documentation of study drug return, and study drug destruction.

Equipment: Dr. Bach's laboratory includes the following major equipment: Nuaire model NU-425-400 laminar flow hood, Napco model 5410 CO2 incubator, Dynatech Ultrawash II microtiter plate washer, Molecular Diagnostics SpectraMax M5 microtiter plate reader, Rainin Liquidator 96 manual benchtop pipetting system, Coag-A-Mate XM coagulation instruments (2), Savant Speed Vac, IEC Centra-CL2 centrifuges (4), Eppendorf model 5415C microfuges (2), Eppendorf model 5242 microfuge, Beckman Microfuge 11 (2), Nikon TMS inverted microscope, Beckman Coulter Ac●T diff 2TM hematology analyzers(2), Beckman Coulter Z2 cell and particle counter.

There are two Dell desktop computers in the lab connected to the VA network. In addition to the standard Microsoft Office Suite package provided by VA, both computers have additional statistics, graphics, and curve fitting and statistics software and for data handling (SigmaPlot® 11). The laboratory also contains -20oC and -80oC freezers, a refrigerator/freezer combination, Dewar liquid N2 cell storage, a fume hood, Rainin manual and automatic pipettes, electrophoresis power supplies and equipment, analytical and top loading balances, pH meter, stirrers, water baths, temperature blocks, fraction collectors, etc.

Common Service equipment in Minneapolis VAHCS Research Service includes spectrofluorimeters, spectrophotometers, preparative and ultracentrifuges, confocal microscope, an imaging center, and walk-in cold rooms.

Intellectual Property:

Background and Proprietary Information Elements relating to proprietary information and intellectual property are addressed in the CRADA. Horizon Pharma, Inc. has agreed to the terms of the CRADA.

Intellectual and Material Property Plan The Principal Investigator, Dr. Ronald R. Bach is a full-time VA employee with an appointment as an Assistant Professor at the University of Minnesota. The VA and the University of Minnesota have a Cooperative Technology Administration Agreement (CTAA) which governs intellectual property rights where VA staff develop an invention and both VA and the University assert an interest in the invention VA and the University have experience using the CTAA when dual-appointed personnel develop inventions.

Commercialization Strategy Does not apply.

Data and Research Resources Sharing Plan **Data and research resources generated by this clinical trial will be made available to the research community and to the public at large. Data sharing will occur in a timely fashion. The expectation is that this will occur no later than the acceptance for publication of the main findings from the final dataset. The informed consent documents for this clinical trial will take the Data and Research Resources Sharing Plan into consideration and will address any potential risks for study subjects.**

D**ata** sharing will be under the auspices of the PI. A data-sharing agreement that imposes appropriate limitations on user will be prepared. This agreement will ensure that the dataset will not be misused or misinterpreted. Approvals for this agreement will be obtained from the MVAHCS IRB, Privacy Officer (PO), and Information Security Office (ISO) as well as the US Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO). Data sharing will be performed in accord with VA and USAMRMC policies and procedures.

Central to the data sharing process is protection of study subject privacy. Shared data will contain no PHI. The agreement will specify the criteria for access to the de-identified data, conditions for research use, privacy and confidentiality standards to ensure data security at the recipient site, and prohibitions for manipulating data for the purposes of identifying subjects. The agreement will also contain elements safeguarding confidential and proprietary data, and third-party intellectual property.

Technical Abstract

Background: Today approximately one-third of the U.S. military personnel who served in the 1990-1991 Gulf War are suffering from Gulf War Illness (GWI), an unexplained chronic multisymptom illness. The absence of information regarding the underlying pathophysiology of GWI has hindered efforts to develop effective treatments. Therefore, we performed a pilot study comparing blood samples from Gulf War veterans with and without multiple symptoms of pain, fatigue, and cognitive dysfunction. The goal of the pilot study was to identify a potential therapeutic target for the treatment of GWI. Proteomic analysis revealed a biomarker signature of innate immune system activation in veterans with GWI. Thus, chronic inflammation was identified as a potential therapeutic target.
Objective/Hypothesis: The objective is to find an evidence-based treatment for GWI. This proposal is based on the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. The elevated biomarkers of inflammation observed in veterans with GWI in our pilot study are associated with increased symptoms of pain, fatigue, and cognitive dysfunction in chronic inflammatory diseases. Therefore, an intervention that reduces this GWI-associated inflammation may alleviate some symptoms of the disorder and significantly improve the health-related quality of life (HRQOL) of veterans with GWI.
Specific Aims: Standardized self-report measures and plasma proteomics will be used to evaluate treatment-related changes. The assessments will be performed at the beginning and the end of the eight week treatment period and again eight weeks after the end of treatment. The specific aims of this study are as follows:

- Specific Aim 1: To measure the effects of the treatment on physical and mental functioning (Veterans Short Form 36-Item Health Survey).
- Specific Aim 2: To assess intervention-related changes in symptoms: pain (McGill Pain Questionnaire-Short Form), fatigue (Multidimensional Fatigue Inventory), and cognitive dysfunction (Cognitive Failures Questionnaire).
- Specific Aim 3: To quantify changes in biomarkers of inflammation in response to the treatment (plasma proteomics).

Study Design: The proposed study is a randomized, two-group, double-blind, placebo-controlled clinical trial of delayed-release prednisone versus matching placebo. A total of 100 veterans with GWI will be enrolled in the trial. Prednisone was chosen as the study drug because of its well-established pleiotropic anti-inflammatory properties.
Impact: A successful trial with improved clinical outcomes and reduced proinflammatory biomarkers would validate the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. A new paradigm for the diagnosis and treatment of GWI would be established. The potential impact of this new paradigm on the health and well-being of veterans with GWI is significant.

Lay Abstract

The goal of this clinical trial is to determine if reducing inflammation is an effective treatment for Gulf War Illness (GWI). In our pilot study blood samples from Gulf War veterans with and without GWI were compared. There was strong evidence of chronic inflammation in veterans with GWI. Thus, chronic inflammation was identified as a potential target for treatment.

Only veterans with GWI will be enrolled in this study. An established anti-inflammatory drug known as prednisone will be the medication. Prednisone is widely used for the treatment of certain chronic inflammatory diseases. A new form of the drug known as delayed-release prednisone will be used in this study. It is designed to release the medication into the blood stream at ~2 AM when prednisone is most effective at reducing inflammation.

The dose of the drug and the length of the treatment were chosen to maximize the benefits of the treatment while minimizing the risks involved in taking the drug. Half the volunteers will take a pill containing the drug (treatment group) and half will take a pill that contains no medication (control group). The two pills will appear to be identical. Tests that measure quality of life, GWI symptoms, and inflammation will be performed three times during the study: at the beginning of the treatment period, at the end of the eight week treatment period, and eight weeks following the end of treatment. The trial will be completed in four years.

This clinical trial presents the exciting opportunity to determine if treating inflammation in veterans with GWI reduces their symptoms and improves their quality of life. If the results of the trial are positive, then a new point of view regarding the diagnosis and treatment of GWI would be established.

Using biomarkers of inflammation may improve diagnosis. Focusing on treatments designed to reduce inflammation and minimize toxicity could lead to additional improvements in the care of veterans with GWI. The impact of these changes on the health and well-being of veterans with GWI would be significant.

Statement of Work

September 12, 2013
Proposed Start Date: August 15, 2014

Specific Aims:
The first specific aim is to measure the effects of the treatment on physical and mental functioning (Veterans Short Form 36-Item Health Survey). The second specific aim is to assess intervention-related changes in symptoms: pain (McGill Pain Questionnaire-Short Form), fatigue (Multidimensional Fatigue Inventory), and cognitive dysfunction (Cognitive Failures Questionnaire). The third specific aim is to quantify changes in biomarkers of inflammation (plasma proteomics) in response to the treatment.

Abbreviations: MVAHCS = Minneapolis Veterans Affairs Health Care System, GWV= Gulf War Veteran, GWI= Gulf War Illness HRQOL = Health-Related Quality of Life, RCT = Randomized Controlled Trial, MVMREF= Minnesota Veterans' Medical Research and Education Foundation, HP= Horizon Pharma, SRS= Subcommittee for Research Safety, USAMRMC= U.S. Army Medical Research & Materiel Command, HRPO= Human Research Protection Office, CITI= Collaborative Institutional Training Initiative SDTU= Special Diagnostic Testing Unit, CRADA = Cooperative Research and Development Agreement, IRB = Institutional Review Board, DSMB= Data and Safety Monitoring Board ACR= Authorization to Conduct Research

	Timeline (months)	**MVAHCS**
Major Task 1:Preparation for Initiation of Randomized Controlled Trial	1-6	
1a.Coordinate with HP for nondisclosure agreements (NDAs)	completed	RRB
1b. Submission and exemption of an Investigational New Drug (IND) application	completed	BCSS
1c. Coordinate with HP for CRADA submission	submitted	RRB
1d. Prepare Request for Waiver of Consent for Screening	1-6	BCSS
1e. Prepare IRB Initial Application, including consent form, HIPAA & human subjects protocol	1-3	BCSS
1f. Prepare SRS Safety Checklist and Hazard Assessment	1-6	BCSS
1g. Design job descriptions, advertise, interview, and hire project related staff	1-6	RRB/GJJ
1h. Create flow chart for all study steps, data collection and database requirements	4-6	BCSS
1i. Complete Project Training = CITI, Chemical Safety, Information Safety and Security	1-6	RRB/GJJ/BCSS
1j.Arrange for Memorandum of Understanding with VA Clinical Laboratory Service	1-6	RRB
1k. Arrange for Blood Draws and Appointment Scheduling for Screening Physicals on SDTU	1-6	GJJ/BCSS
1l. Set up documentation with MVMREF for Disbursement Authorization for Subject Payments	1-6	BCSS
1m. Finalize arrangements with HP for delivery of study drug and placebo	1-6	BCSS
1n. Meet with Research Pharmacist to set-up plan for study drug management	1-6	BCSS
1o. Finalize Randomization plan with Study Statistician	6	BCSS/MK
1p. Submit IRB approved ACR, Consent and HIPAA forms, Regulatory Documents to USAMRMC	6	RRB/BCSS
Milestone Achieved: *Treatment protocol, Research Pharmacy, Statistician and SDTU plans finalized*	6	RRB/GJJ/HK
Milestone Achieved: *Local IRB, SRS approval*	6	RRB/GJJ
Milestone Achieved: *at MVAHCS*	3	RRB/GJJ
Milestone Achieved: *Authorization to Conduct Research granted at MVAHCS*	5	RRB/GJJ
Milestone Achieved: *Regulatory and Administrative Approval at USAMRMC ORP and HPRO*	6	RRB/GJJ
Milestone Achieved: *New research staff hired*	1-3	RRB/GJJ
Milestone Achieved: *Research staff completes training*	1-6	RRB/GJJ

Human Subjects Procedures

Study Population: Gulf War veterans with Gulf War Illness (as defined by the Kansas case definition) are the focus of this study. All will be veterans of the first Gulf War (August 1, 1990 to July 31, 1991). The subjects, who may be males and females of all races and ethnic origins, are expected to be between 38 years and 70 years old. There are no targeted populations for this study. Vulnerable populations will not be targeted. There will be no children participating in the study.

Inclusion/Exclusion Criteria: After the initial medication questions, the veteran will be asked to answer the Kansas Gulf War Illness (GWI) Case definition questionnaire. Waivers of Consent and HIPAA will have been requested and obtained from the IRB in order to screen using the Kansas GWI Case definition questionnaire. The Kansas GWI Case definition questionnaire includes both inclusionary and exclusionary conditions. Only those having "moderate/severe" scores in 3 out of 6 categories listed in the Kansas GWI Case definition will meet inclusion criteria. In addition to the Kansas Criteria, subjects must also be willing to make several trips to the Minneapolis VAHCS for study visits and to take the study drug as directed. Subjects will be informed of the potential for drug interactions for medicines prescribed during the course of the study. Information regarding the use of the study drug will be given to each study subject (see Instructions for Study Medication attachment). Subject will be instructed to inform their personal physicians that they are participating in a research study involving treatment. Veterans, who are hypersensitive to any of the ingredients in prednisone, have active liver or kidney disease, untreated hypertension, diabetes or are pregnant or nursing, will not be permitted to participate. Additionally, those with inflammatory arthritis (RA, psoriatic arthritis, spondylitis, reactive arthritis, or IBD associated arthritis) will be excluded. Any other major inflammatory disease like active acute or chronic infections, ulcerative colitis, Crohn's disease, inflammatory lung diseases, pericarditis, vasculitis, will all also be excluded. People meeting other inclusion criteria but with active infections could be rescreened when their infections clear.

Veterans with diagnosed chronic conditions (e.g. Lupus, Stroke, Multiple Sclerosis, and fibromyalgia) that can produce symptoms, such as fatigue, cognitive impairment, and pain will be excluded based on the Kansas GWI Case definition questionnaire. Also, veterans who have conditions that might interfere with the ability to accurately report symptoms, such as severe psychiatric problems (e.g. Schizophrenia, bipolar disorder, or alcohol/drug dependence requiring hospitalization), or administration of mind-altering substances such as tranquilizers, will be excluded.

Inclusion of Women and Minorities in Study Subjects participating in this study may be males and females of all races and ethnic origins.

Description of Recruitment Process: The primary method for recruiting potential subjects will be to send an IRB-approved recruitment letter to veterans listed on the Persian Gulf War Registry from the MVAHCS. This letter is carefully written at the 8th-grade reading level, in order to accommodate a wide range of reading abilities. Following IRB-approval and the receipt of the Authorization to Conduct Research from the Research and Development Committee, the Study Coordinator will obtain the registry from the local registrar on behalf of the study and will prepare the mailing labels and envelopes. This list currently includes 1,418

names. Any veteran deployed to the Gulf with an honorable discharge during Operation Desert Shield, Operation Desert Storm, and Operation Iraqi Freedom is eligible to have the Gulf War Physical and be included on this local registry. The addresses for the registrants are found in the CPRS of the MVAHCS. Some of the addresses in CPRS will be no longer be current, as some people have moved since having the registry physical and have not updated their medical records with the facility. Gulf War veterans' names and addresses are also available from lists generated by the DMDC and the DSS. These lists will be generated following the placement of online requests using the following secure websites: **https://www.dmdc.osd.mil/drs** and **http://vaww.visn23.med.va.gov/VISN23/Applications/Projects/ReportRequests/index.cfm**.

These lists, whose constituents overlap, have 4,371 and 1,871 names, respectively. The DMDC list is made up of US Armed Forces veterans who served in the First Gulf War (1990-1991) whose most current DoD addresses are located in Minnesota or Western Wisconsin within the VISN 23 boundary. Therefore, it is likely that the DMDC list may have a veteran's actual current address. The DSS list consists of addresses for veterans of the Persian Gulf War (August 2, 1990 and July 31, 1991) who have medical charts in the MVAHCS CPRS, live within 250 miles of the Mpls VA, and do not have a diagnosis of hepatitis, cancer, or diabetes, which would exclude them from participating in the study.

Because it is important to consider undue inducement in the enrollment of subjects into a study, subjects' compensation will be limited to $100.00 per visit ($400.00 for the completed study). This amount is chosen because it will at least partially compensate the subject for missing at least a partial day of work. Because transportation to the VA would likely be outside their normal traveling distance for many subjects, especially those who would be traveling from outside the Twin Cities Metropolitan area, subjects will also be reimbursed for travel expenses at the rate of $0.415 per mile. In addition, subjects will be fasting when they arrive for each visit. Following each study visit, subjects will be encouraged to eat something before leaving the building. Subjects will be given a $10 food voucher for use in the MVAHCS cafeteria.

We will be requesting approval for access to DMDC DRS for the purpose of conducting a clinical trial at the Minneapolis Veterans Affairs Health Care System. In order to receive approval for access, we must submit an IRB-approved protocol and Authorization to Conduct Research from the Research Service at the Minneapolis VA Health Care System. In addition to this, we will fill out the DRS required documentation accurately and completely. After the approval of these documents, the Principal Investigator/Study Coordinator of the proposed study, Gulf War Illness Inflammation Reduction Trial (Congressionally Directed Medical Research Programs (CDMRP) - Gulf War Illness Research Program- Clinical Trial W81XWH-13-GWIRP-CTA), will be permitted to request access to a listing of Gulf War veterans' addresses for the purpose of conducting the above mentioned clinical study.

Description of Informed Consent Process: *Draft of Informed Consent provided at the end of this attachment*. Because recruitment is being carried out using the local Persian Gulf War Registry, the research could not practicably be carried out without a waiver or alteration of consent for recruiting. A request for a waiver or alteration of consent for recruiting will be made. Participation in the study will be initiated by an IRB-approved recruitment letter. When an interested veteran responds to the recruitment letter (by calling the included study hotline telephone number) he or she will be asked what medications are currently being taken.

If the potential participant is taking a medication that would trigger exclusion, such as current oral corticosteroid or diabetic therapy, the coordinator will stop the interview and let the person know that participation in this study will not be permitted. Those taking

aspirin, ibuprofen, or other similar over-the-counter medications will not be excluded. Data on other drug use will be collected and presumably the randomization process will put users of other non-excluded medications equally into the two treatment groups.
Once the veteran has met the pre-screening inclusion criteria, an initial appointment will be scheduled. Appointments will be conducted in the morning hours, initiated most often between the hours of 8:00am and 10:00am. At this appointment, the Study Coordinator will initiate the Informed Consent/HIPAA process. This appointment includes the screening physical conducted by a VA physician and clinical laboratory blood tests. The process of obtaining Informed Consent is guided by Good Clinical Practices (GCP) and begins at the initial appointment. The potential study participant will meet the Study Coordinator in the SDTU Intake Room. In this quiet, private room, they will discuss the consent and HIPAA forms. A description of the study, the purpose of the study, the benefits and risks of participation, research subject's rights, disclosure of protected health information and the subject's right to confidentiality are among the items covered in the consenting process. If the subject would like to discuss the study with a family member or other accompanying person, he or she will be given the opportunity to do so at this time.
Subjects who are unable to answer study questions, for any reason, will not be eligible to participate in the study. Any subject who meets any of the exclusionary criteria in the Kansas case definition will not be eligible to participate in the study. If a subject's decision making capacity is questioned in any way, a modified Dysken tool will be used as an assessment tool to assess capacity.
A potential study participant will have the opportunity to ask questions about the study and have questions answered by the Study Coordinator. The subject will be asked several open ended questions to assess the level of understanding just prior to signing the consent document. If further explanation and discussion of the study is necessary to improve the subject's understanding of the study's purpose and/or the subject's involvement, it will be done at this time. If the veteran agrees to the content of the consent and HIPAA forms, he or she will voluntarily sign them. It will be stressed several times throughout the process that involvement in the study is strictly voluntary and the subject may withdraw at any time by notifying Dr. Bach or Dr. Johnson in writing. It will also be stressed that the records kept by the study team are not for clinical purposes, but rather for research purposes only and will not become part of their medical record. A witness to the signature will be present. Study subjects will receive a copy of the consent form. The consent forms will contain pertinent names and telephone numbers. Because informed consent is a process, rather than a onetime event, the study subject will be encouraged to ask questions as they arise throughout the course of the study.
With the proper IRB authorizations for screening and/or recruitment and by virtue of being VA researchers, Dr. Bach and Dr. Johnson have access to the Gulf War registries and therefore access to the local Gulf War population.
Legally Authorized Representative and Assent There is no provision for obtaining consent via an individual's Legally Authorized Representative. Inclusion will not be made by assent.
Screening Procedures: Following the consenting process, the subject will have a screening appointment under the oversight of the Study Physician including a physical exam, Review of Systems, and blood tests. Subsequent visits will be formatted to provide an experience that will be predictable. By using the same appointment time and location, as often as possible, consistency of conditions will be met.
Personal questions, including a list of current medications, will be asked on the screening questionnaire. Upon inclusion in the study, HRQOL will be measured by the SF-36V. A survey pertaining to fatigue, Multidimensional Fatigue Questionnaire, will also be

administered. Pain will be assessed using the McGill Pain Questionnaire- Short Form. Cognition will be addressed using the Cognitive Failures Questionnaire. Subjects will answer the questions in a quiet and private room.

Risks/Benefits Assessment:

Foreseeable Risks Research risks to subjects include possible physical side effects from having blood drawn. They include faintness, inflammation of the vein, pain, bruising, or bleeding at the site of the puncture. There is also a slight possibility of infection. There may be other unknown side effects that could occur. This risk is no greater than minimal risk.

If the study subject meets inclusion criteria, additional blood samples will be obtained. These samples will be used for biomarker and safety analyses. The blood samples obtained from the study subjects will be strictly for purpose of the study and involve minimal risk.

There is the slight possibility of psychological risk if answering the questions causes a subject to become uncomfortable. These questions pose minimal social and legal risk.

Because the CPRS will be accessed for recruitment purposes and for the writing of Research Participant Progress Notes, there is a minimal risk that private personal information can be accessed for fraudulent purposes.

There is the potential for economic harm that subjects may experience by participating in the study. There may be transportation costs to travel to the facility and possible lost wages due to time missed from work. To minimize potential economic harm, each subject will be compensated $100.00 for each visit relating to the study (3 total), and reimbursement for travel expenses ($0.415 per mile). Because the subject will be required to fast for the required blood tests, each subject will also receive a $10 food voucher at the end of each visit to the MVAHCS.

There are physical risks involved with the administration of the study drug. Common adverse reactions to GCs include fluid retention, alteration in glucose tolerance, elevation in blood pressure, behavioral and mood changes, increased appetite and weight gain.

Subjects will be informed of possible drug interactions and contraindications. Each subject will receive a medication information sheet listing important safety information, including drug interactions and contraindications, as well as side effects that may be of concern. Important telephone numbers are included in the consent documents and the study hotline is listed on the medication information sheet.

Possible drug interactions include:

- Anticoagulant agents: The study drug may enhance or diminish the effects of anticoagulant.
- CYP 3A4 inducers and inhibitors: These agents may respectively either increase or decrease clearance of GCs.
- NSAIDs including aspirin and salicylates: The study drug may increase the risk of NSAID gastrointestinal side effects. Subjects will be instructed to take medications with food. To avoid additional NSAID side effects subjects will be advised to consider taking an over-the-counter proton pump inhibitor.

The consent and HIPAA forms will be copied and sent to the IRB office. These copies will be the scanned into the subject's CPRS record. A Research Participant Note will be written in the subject's medical record and a flag created to notify other CPRS users that the subject is enrolled in an interventional study at the Minneapolis VA Health Care System. This flag will warn of participation in an

interventional study and act to warn of possible dual enrollment, thereby protecting the subject from experiencing the risks involved in participating in two studies concurrently.

Risk Management and Emergency Response The subject will sign a HIPAA form giving the study staff permission to access his or her medical record. All study staff realize the importance of keeping private personal information secure and are trained annually by the VA to minimize this risk. All VA sensitive research data for this protocol recorded on paper records, especially participant PHI, will be stored in locked file cabinets, in a locked lab, inside a security card controlled research wing of the hospital. It will be available and used only by approved study personnel. Before the investigators initiate the study, the privacy officer (PO) and information security officer (ISO) will work with the investigators to ensure the proposed research is in compliance with relevant privacy and confidentiality requirements, and information security requirements, respectively. They will make recommendations to the investigators of options available to correct any deficiencies. The PO and ISO review the proposed study protocol and any other relevant materials when they are submitted in the IRB application. During the course of the study, the PO and ISO will conduct assessments to ensure that all applicable local, VA and other Federal requirements for privacy and confidentiality, and for information security have been met.

Because the MVAHCS Research Service is committed to protecting the integrity of the VA computer system and the security and privacy of data and PHI of study subjects, as well as that of employees, the Research Information Protection Subcommittee (RIPS) was formed. RIPS conducts annual audits of all Research areas to identify security and privacy vulnerabilities, assure the correction of these findings, educate the Research Staff in the principles of data and computer security, implement policy and procedures, and serve as a resource for researchers who have special data management needs. RIPS offers another layer of protection against risk for study subject data. To minimize risk, all VA sensitive research data for this protocol, including subject data and PHI, will be stored on a secure VA server behind the necessary VA firewalls.

Because mailing lists will be generated using secure websites, the use of these private records will pose minimal risk to the subject. Data will be retrieved from the Data Request System (DRS) website for the DMDC report. The spreadsheets will be uploaded from the DRS secure website and will be linked to our DRS account through the Upload-Download System (UDS) and saved to a secure drive behind the firewall at the VAMHCS. The DSS report will be posted on the DSS secure drive and then moved onto a secure drive at the VAMHCS.

Risk will be minimized by careful pre-screening of potential study subjects. Female subjects with child-bearing potential will agree to use acceptable, effective forms of contraception for the duration of the treatment portion of the study. Regular, careful monitoring of consented subjects is instituted to prevent or minimize potential risks. Following informed consent, a panel of blood tests will be performed in order to check for possible undiagnosed liver disease, diabetes or other exclusions. Subjects with results not falling within accepted ranges will not be permitted to continue their participation in the study and will be withdrawn from participation.

To minimize blood drawing risks, the blood will be drawn by skilled and experienced phlebotomists using accepted aseptic technique.

To minimize economic risk the subjects will be compensated $100.00 per visit ($400.00 for the completed study). They will also be reimbursed for travel expenses at the rate of $0.415 per mile. The subjects will be fasting when they arrive for each visit. To

encourage them to break the fast before leaving the Medical Center, the study subjects will be given a $10 food voucher for use in the MVAHCS cafeteria.

Subjects will be informed that if an adverse event occurs, depending on the severity of the effect, they need to inform the study coordinator, first seeking care from their own health care providers if the effect is severe. All adverse events will be monitored until stabilization or resolution occurs. Medical or professional intervention will be available, as necessary. Pertinent contact telephone numbers will be included in consent documentation and on the study medication information sheet. All serious adverse events, adverse events and unanticipated problems will be reported to the VA-IRB according to their current recommendations and regulations.

Reports of aggregate safety data will be sent to the Data and Safety Monitoring Board (DSMB) on a quarterly basis. A DSMB composed of members who do not have a conflict of interest with the research project will be appointed. The DSMB will determine the frequency of its meetings, but these meetings will occur at least quarterly to coincide with the receipt of the quarterly report. A written report of the meeting will be forwarded to the PI, the IRB, and R&D Committee within 14 days of the each meeting.

The DSMB will be composed of two physicians with relationships with the MVAHCS. One is a pulmonologist from the Primary Care Service with extensive clinical trial experience. The other recently retired MVAHCS physician was a psychiatrist from the Psychiatry Service with extensive expertise in psychometrics who also served as IRB committee chair. From the University of Minnesota Medical School, a Hematologist/Oncologist with extensive clinical research trial expertise will be appointed. All three appointees have extensive clinical expertise, as well as IRB and DSMB experience. The DMSB will determine the continued safety of research subjects based on the data submitted to the board. As the study progresses, aggregate safety data, including serious adverse events, adverse events and unanticipated problems, will be sent quarterly to the DSMB.

Unanticipated problems involving risks to subjects or others will be reported to the MVAHCS IRB within 5 working days after the investigator becomes aware of the problem. The Unanticipated Problem Report form, located on the Research SharePoint website on the IRB Forms page, is used for this purpose. Any supporting documentation (de-identified) will be attached to the form. If the PI recognizes that the event/problem involves risk to subjects or others and a modification to the IRB consent and/or protocol is required, he will also submit revised copies of the consent and/or protocol, as well as a Full Committee Review Amendment form.

Treatment assignment codes will be kept by the Biomedical Research Statistician, as well as the Research Pharmacist. This code will only be broken in the case of an adverse event when it is necessary for the Principal Investigators to know which treatment the patient is receiving before the participant can be treated or if someone not in the study uses the investigational agent. For example, if a child in the participant's household takes a study medication, the blind may be broken to determine treatment for the child.

When it is necessary to break the blind, the researcher must notify the IRB. If the code is broken for a participant, this must be documented on the Adverse Event forms together with the reasons for breaking the code. The reason for breaking the code should also be written on the code document. The reason for premature un-blinding of the investigational product should be given, e.g. due to serious adverse event.

The expected or unexpected, related or possibly related and serious or more prevalent than expected adverse event will be reported to the IRB no later than 5 working days after the investigator first learns of the event. An Internal Adverse Event form, located on the

Research SharePoint website on the IRB Forms page, must be completed and submitted to the IRB for each internal adverse event. The DMSB will determine the continued safety of research subjects based on the data submitted to the board.

Emergency Care or Treatment for Adverse Event If a subject is injured or becomes ill as a result of participation in this research study, treatment will be available, including first aid, emergency treatment and follow-up care, as needed, through the VA Health Care System. In the event a subject cannot reach a VA facility, the VA will consider payment for necessary medical care for any injury or illness directly related to participation in the research study. If a subject receives this type of medical care, he or she must contact the Research Investigator for this study. Contact information for the physicians associated with the study is located in the section of the consent entitled "Compensation for Any Injuries". Subjects are instructed to immediately report any injuries resulting from participation in this study to Dr. Bach at (612) 467-4418 or Dr. Johnson at (612)467-4134 during the daytime hours. If a VA physician needs to be contacted during the evenings or week-ends, subjects will be instructed to call the VA operator at (612) 725-2000 and ask to have the Hematology physician-on-call paged. Subjects will be told to tell the operator that they are a research study participants. Those not living in the Twin Cities metropolitan area will be instructed to call the toll-free number: 1-866-414-5058.

Special Precautions Because subjects will be instructed to fast from midnight prior to their study appointments, vouchers for the VA Canteen Service will be provided to each subject following each study appointment. This will be done to encourage subjects to eat and drink something before leaving the building after their appointments.

Subjects will also be instructed to take the study medicine with food each evening at bedtime, at approximately 10:00pm.

Potential Benefits: This trial has the potential both in the short-term and in the long-term to improve the health and well-being of veterans with GWI. A successful trial with improved clinical outcomes and reduced biomarkers of inflammation would establish a new paradigm for the diagnosis and treatment of GWI. A change in clinical practice that focuses on inflammation could be implemented immediately. The testing of other therapeutic interventions designed to reduce inflammation and minimize toxicity could produce additional improvements in GWI treatment beyond those achieved in this trial.

Intervention

Complete Name and Composition: RAYOS is a delayed-release prednisone tablet. It consists of a prednisone-containing core tablet in an inactive shell, which delays the onset of in vitro drug dissolution by approximately 4 hours. The active ingredient in RAYOS is prednisone, a corticosteroid. Prednisone is a synthetic adrenocortical steroid drug with predominantly corticosteroid properties. The molecular formula for prednisone is $C_{21}H_{26}O_5$. The chemical name for prednisone is 17,21-dihydroxypregna-1,4-diene-3,11,20-trione.

Prednisone is a white to practically white, odorless, crystalline powder and has a molecular weight of 358.43. Prednisone is very slightly soluble in water; slightly soluble in alcohol, chloroform, dioxane, and methanol.
Each tablet contains 5 mg of prednisone, with the following inactive ingredients: dibasic calcium phosphate dihydrate, colloidal silicon dioxide, croscarmellose sodium, glycerol dibehenate, lactose monohydrate, magnesium stearate, povidone, yellow ferric oxide, and red ferric oxide.

Study Procedures:

Screening Appointment with Physical Exam (Week -1 or 0) Once the veteran has met the pre-screening inclusion criteria, an appointment will be made by the study team for the initial appointment, which will include a discussion of Informed Consent and HIPAA, and a screening physical exam and blood tests. The process of obtaining Informed Consent is guided by Good Clinical Practices (GCP) and begins at the initial appointment. Study staff will have completed Collaborative Institutional Training Initiative coursework (CITI) for the protection of human research subjects and will follow best practices as described by the IRB of record. The potential study participant will meet the Study Coordinator in the SDTU Intake Room. In this quiet, private room, they will sit to discuss the consent and HIPAA forms. A description of the study, the purpose of the study, the benefits and risks of participation, research subject's rights, disclosure of protected health information and the subject's right to confidentiality are among the items covered in the consenting process.
A potential study participant will have the opportunity to ask questions about the study and have questions answered. He or she will be asked several open ended questions to assess the potential subject's level of understanding. The questions will be related to the subject's understanding of the study's purpose, the side effects of the study medication, and what it means to receive a placebo. If the veteran agrees to the content of the consent and HIPAA forms, he or she will voluntarily sign them. A witness to the signature will be present. Study subjects will receive a copy of the consent form. Because informed consent is a process, rather than a onetime event, the study subject will be encouraged to ask questions that may arise throughout the course of the study.
The signed consent and HIPAA forms will be copied and sent to the IRB office. These copies will be the scanned into the subject's clinical record. The original will be retained in the subject's study file in a locked cabinet in the locked laboratory. A Research Participant Note will be written in the subject's medical record and a flag created to notify other CPRS users that the subject is enrolled in an interventional study at the Minneapolis VA Health Care System. This flag will warn of participation in an interventional

study and act to warn of possible dual enrollment, thereby protecting the subject from experiencing the risks involved in participating in two studies concurrently.

Following the signing of the consent and Health Insurance Portability and Accountability Act (HIPAA) forms, the subject will continue the evaluation process by filling out a detailed demographics questionnaire including details of location and duration of service during the First Gulf War, questions regarding exposures, tobacco and alcohol use. At this first visit, considered to be the screening visit, participants will complete a structured clinical interview, a screening physical exam, and blood tests to rule out pre-existing conditions that would preclude participation in the trial. The structured clinical interview is designed to obtain information regarding demographic variables and personal data, specifically age, gender, education level, ethnicity, marital status, employment status, service connection status, military branch, current and past mental and medical treatment, etc. There will also be a blood draw at this visit.

In order to establish that certain exclusion criteria are not met, and to provide baseline data the following parameters will be checked: CBC, creatinine, blood glucose, hemoglobin A1C, hs-CRP. To minimize blood drawing risks, the blood will be drawn by skilled and experienced phlebotomists using accepted aseptic technique. At the first, second and final study appointments blood samples will also be drawn for plasma proteomic analysis. A CBC will also be drawn at these appointments. The proteomic data and CBC data will serve as potential additional covariates to be used in making correlations during data analysis. The study statistician will receive the individual, uniquely coded, MAP assay results directly from the outside laboratory and also from the in-house clinical laboratory, thereby preserving the blind.

Those subjects who do not meet inclusion criteria following the screening physical will be notified via letter and thanked for their willingness to participate in the study. They will not be permitted to continue participating in the study. In order to proceed to the active phase of the study, all eligible subjects will meet inclusion criteria, and have screening blood tests that are within acceptable ranges. Consented subjects who have met inclusion criteria will be informed by phone call from the study staff.

First Study Visit (Week 1): At this time, the first study visit will be scheduled and the subject will be block randomized to treatment or placebo groups by the Study Statistician. (Data on drug use will be collected and presumably the randomization process will put users of other non-excluded medications equally into the two treatment groups.) The appointment for the second (8 week) study visit also made at this time. It will be as close to 56 days after the first study visit as possible, keeping the variation between subjects to a minimum. At the time of the 8 week visit, the appointment for the 16 week visit will be made. The SDTU scheduler makes every effort to schedule appointments for the day and time requested, with excellent results.

When the subject returns to the MVAHCS for the first study visit, the Study Coordinator will meet the subject at the SDTU Intake Room. The self-report instruments will be administered by the coordinator and the subject given ample time to answer the questions. Following this, the blood draw for CBC and biomarkers will take place in the same room. Upon completing the blood draw, the Study Coordinator will walk with the subject to meet with the Research Pharmacist in the pharmacy. The Research Pharmacist will dispense study drug or placebo to consented and enrolled study subjects using a pre-determined randomization plan generated by the Study Statistician. This is done to ensure careful dispensing of the study drug and to see that the study is not compromised by revelation of treatment assignments. The Rayos® DR-prednisone tablet and placebo will be identical in appearance.

The Study Coordinator will make Early-study (Week 2) phone calls to the subjects. The purpose of the phone call will be to assess how each subject is progressing with the study, to answer any questions they might have, and to encourage maintenance of the treatment log.

To facilitate the management of study drug or placebo administration, each study subject will receive an IRB-approved drug administration log. It will be much like a calendar on which a subject will record tablet self- administration. This log will be turned in at the conclusion of each subject's participation in the active phase of the study. The log will both improve compliance (by making it easier to remember if a tablet has been taken on any given day) and demonstrate apparent compliance or lack thereof (by reflecting the subject's record of tablet self-administration).

Second Study Visit (as soon as possible after completing Week 8): At the second study visit, the Study Coordinator will once again meet the subject in the SDTU Intake Room. The subject will surrender any unused Study Drug which will be returned to the Research Pharmacist. The Pharmacist will dispose of the drug in a manner consistent with the VA pharmacy's drug disposal policies. The self-report instruments will be administered by the coordinator and the subject given ample time to answer the questions. Following this, the blood draw for glucose, hemoglobin A1C, CBC and biomarkers will take place in the same room. At this point, the Study physician will conduct the Physical Exam and Review of Systems, followed by the blood draw. At this point the subject's participation in the Treatment Phase of the study will be concluded. A return appointment to occur 8 weeks later will be scheduled.

Final Study Visit (16 weeks): The subject will return at 16 weeks for the final visit of the study. The subject will meet the coordinator in the SDTU Intake Room for a final round of the self-report measures intended to detect any change in symptoms and a blood draw for the analysis of biomarkers, glucose, hemoglobin A1C, and CBC. This will conclude the subject's participation in the study. After this final study visit, there is no planned follow-up.

Good Clinical Practice The conduct of the study will be done in accordance with the IRB-approved protocol and changes to the protocol will only be made with approval from the VA-IRB after submitting a request for amendment, as needed. All serious adverse events, adverse events and unanticipated problems will be reported to the VA-IRB according to their current recommendations and regulations. Reports of aggregate safety data will be sent to the DSMB on a quarterly basis. Informed consent and HIPAA authorization will be obtained directly from the subject and documented in writing prior to the start of any study-related procedures, including screening tests and exams done solely to determine their eligibility for the study. Subjects, those taking the study drug and those taking placebo, will be informed that the drugs being used in the study are being used for investigational purposes. Adequate and accurate records will be maintained and will be made available for inspection, as required. The delegation of responsibilities by the investigator will only be made to individuals who are appropriately qualified by training and experience.

Study Participation for Individual Subject

Contact	Approximate Duration	Event	Procedure	Administered/ Reviewed by
Initial Contact	15 minutes	Contact with Veteran	Pre-Screening Phone Call, including Kansas Case Definition Questionnaire	Study Coordinator
Screening Visit (Week -1 or -2)	60 minutes	Informed Consent Process	Study Consent Form, HIPAA, Participant Information, including demographics	Study Coordinator
	30 minutes	Screening for Exclusion Criteria	History, Physical Exam, and Review of Systems	Physician
	15 minutes	Screening for Exclusion Criteria	Screening/ Baseline Blood Tests	Nursing Staff
First Study Visit (Week 0)	15 minutes	Initiation of Active Phase	SF-36V, McGill Pain Questionnaire-Short form, Multidimensional Fatigue Inventory, Cognitive Failures Questionnaire	Research Assistant
	10 minutes	Treatment Distribution	Subject Receives Study Drug or Placebo	Research Pharmacist
	15 minutes	Collection of Blood	Blood Draw for Biomarker Analysis and CBC, glucose, hemoglobin A1C	Nursing Staff
Early-Study (Week 2)	10 minutes	Telephone Contact with Subject	Phone call to assess study participation, check for possible toxicity, answer questions, and encourage maintenance of treatment log	Study Coordinator
Second Study Visit (Week 8)	15 minutes	Conclusion of Active Phase	SF-36V, McGill Pain Questionnaire-Short Form, Multidimensional Fatigue	Study Coordinator/ Research Assistant

			Inventory, Cognitive Failures Questionnaire	
	10 minutes	Collection of Blood	Blood Draw for Biomarker Analysis and CBC, Glucose, and hemoglobin A1C	Nursing Staff
	30 minutes	Active Phase Completed	Physical Exam and Review of Systems	Physician
Final Study Visit (Week 16)	15 minutes	Evaluation of Possible Change in Symptoms	SF-36V, McGill Pain Questionnaire-Short Form, Multidimensional Fatigue Inventory, Cognitive Failures Questionnaire	Study Coordinator/ Research Assistant
	10 minutes	Collection of Blood	Blood Draw for Biomarker Analysis and CBC, Glucose, and hemoglobin A1C	Nursing Staff

Data Management

Identifiers. Treatment assignment codes will be generated and kept by the Biomedical Research Statistician. These codes will be stored on the secure VA Server behind VA firewalls. These codes must be shared with the VA Research Pharmacist, who will prepare the medications. This code will only be broken in the case of an adverse event when it is necessary to know which treatment the patient is receiving before the participant can be treated or if someone not in the study uses the investigational agent. Plasma samples that are collected and stored prior to assay will be labeled with unique identifiers. The key that links the unique identifiers to the study subjects will be maintained by the study coordinator on a secure VA Server behind VA firewalls.

Confidentiality. Before the investigators initiate the study, the PO and the ISO will work with the investigators to ensure the proposed research is in compliance with relevant privacy and confidentiality requirements, and information security requirements, respectively. They will make recommendations to the investigators of options available to correct any deficiencies. The PO and ISO review the

proposed study protocol and any other relevant materials when they are submitted in the IRB application. During the course of the study, the PO and ISO will conduct assessments to ensure that all applicable local, VA and other Federal requirements for privacy and confidentiality, and for information security have been met.

The MVAHCS Research Service is committed to protecting the integrity of the VA computer system and the security and privacy of data and PHI of study subjects, as well as that of employees. Therefore, the Research Information Protection Subcommittee (RIPS) was formed. RIPS conducts annual audits of all Research areas to identify security and privacy vulnerabilities, assure the correction of these findings, educate the Research staff in the principles of data and computer security, implement policy and procedures, and serve as a resource for researchers who have special data management needs. RIPS offers another layer of protection against risk for study subject data.
The study records will be available to and used by approved study personnel as needed for the conduct of the study. All study staff receive annual VA privacy and information security training. Federal agencies including, but not limited to, the Food and Drug Administration (FDA), the Office for Human Research Protections (OHRP), the VA Office of Research Oversight (ORO), the VA Office of the Inspector General (OIG), and representatives of USAMRMC may have access to the records.
If sensitive information is collected in the conduct of this study for which there is a mandatory reporting requirement, then MVAHCS policies will be followed in the reporting of this information to state and local authorities.
Disposition of data. All electronic VA sensitive research data for this protocol, including subject data and PHI, will be stored on a secure VA server behind the necessary VA firewalls. All hard copies of VA sensitive research data, especially participant PHI, will be stored in locked file cabinets, in a locked laboratory, inside a security card controlled research wing in Building 70 of the MVAHCS. Federal regulations for the destruction of federal records indicate that all study records generated during the course of VA research must be retained indefinitely.

Sharing study results. Laboratory results from the screening visit will be made available to the subject and to his/her physician if requested by the subject. If persistent increases in blood glucose/hemoglobin A1C are detected during the treatment phase of the study the subject will be notified and advised to consult with his/her physician.

Laboratory Evaluations:
Specimens to be collected, schedule, and amount. The volume of blood collected for the purpose of screening will be ~12 ml. The study will begin within two weeks of the screening visit. The volume of blood collected at each study visit will be ~20 ml. There will be a total of three study visits occurring at 8 week intervals. The total amount of blood collect from each study subject will be ~72 ml

Evaluations to be made. Blood tests performed for the purpose of screening will include a CBC, creatinine, blood glucose, hemoglobin A1C, and hs-CRP. Lab tests performed for each study visit will include a CBC, blood glucose, hemoglobin A1C, hs-CRP, TAT, and MAP plasma protein analysis (HumanMAP®-v2.0/Antigens). The screening assays are to establish that certain exclusion

criteria are not met. Tests associated with the study visits will quantify intervention-related effects on biomarkers of inflammation. The objective biomarker information is required to determine if DR-prednisone reduces chronic inflammation in veterans with GWI. The CBC results will be used as adjustment covariates in the data analysis. The CBC may also be used to evaluate the possibility of an intercurrent infection. For the purpose of subject safety blood glucose and hemoglobin A1C will be measured at each study visit.

Storage. Preparation and storage of the plasma samples will take place in Dr. Bach's laboratory in Building 70 of the MVAHCS. The platelet-free EDTA plasma samples will be stored in a locked ultralow freezer at -80^{o}C prior to assay. Labeling of the samples with unique identifiers and maintaining the key will performed as described above (see Identifiers). Samples will be destroyed at the completion of the study after all protocol-defined analyses have been performed and abstracts, manuscripts, or primary publications already exists or are under review. Sample destruction will be performed according the VA policies and procedures.

Labs performing evaluations and special precautions. Established immunoassay procedures utilizing MAP technology will be employed to quantify the concentrations plasma proteins (HumanMAP®-v2.0/Antigens). The assays will be performed on platelet-free EDTA plasmas by Myriad RBM, Inc., Austin, TX. Coded sample will be sent to Myriad RBM on dry ice via the VA-approved overnight shipping service, United Parcel Service (UPS). Myriad RBM validates all assays to clinical laboratory standards. The Myriad RBM laboratory is accredited by the College of American Pathologists. Its CLIA number is 45D1037483.

Lab tests performed for the purpose of screening will be conducted by the clinical laboratory of the MVAHCS. This lab will also perform the CBC, hemoglobin A1C, and hs-CRP assays at each study visit, The MVAHCS clinical laboratory has met all applicable standards for accreditation and is accredited by the College of American Pathologists. Its CLIA number is 24D0988147.
Plasma TAT levels will be quantified by ELISA using the AssayMax Human TAT Complexes ELISA Kit from AssayPro®. The TAT ELISAs will be performed on previously frozen platelet-free EDTA plasmas in Dr. Bach's laboratory. All personal protective equipment appropriate for the handling of blood and blood products are used. Study personnel are trained in proper handling techniques for biohazards and hazardous chemicals and in the shipping of specimens.

Surveys

The primary and secondary outcome measures are summarized in Table 1.

Table 1. Primary and Secondary Outcome Measures

Primary Outcome Measure	Secondary Outcome Measure	Name of Measure	Description
x		Veterans Short Form 36-Item Health Survey Physical Component Summary (SF-36V-PCS)	HRQOL/ Physical Health Functioning
	x	McGill Pain Questionnaire- Short Form (MPQ)	Sensory pain, affective pain, pain now, and typical pain
	x	Multidimensional Fatigue Inventory (MFI)	General fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue
	x	Cognitive Failures Questionnaire (CFQ)	Self-report of cognitive symptoms, such as attention, concentration, and memory
	x	Veterans Short Form 36-Item Health Survey Mental Component Summary (SF-36V-MCS)	HRQOL/ Mental Health Functioning
	x	HumanMAP®-v2.0/Antigens, hs-CRP assay,TAT ELISA	Quantitative analysis of plasma biomarker levels

The self-report instruments used to assess primary and secondary outcomes will be administered at the beginning and the end of the 8 week treatment period and at the end of the 8 week post-treatment period. These validated and standardized methods were employed in VA Cooperative Study Program (CSP) Study #470 (43, 44). In addition, the collection of a blood sample, plasma isolation, and storage of the plasma sample for proteomics analysis will be performed at each of study visits.

The primary outcome measure will be the presence or absence of a positive response on the SF-36V PCS following treatment. A positive response is defined as a 7-point or greater increase in the SF-36V PCS at 8 weeks versus the baseline score. This definition of a positive response to an intervention in veterans with GWI is based upon the psychometric properties of the SF-36V PCS and the distribution of scores observed in the population and comes from VA Cooperative

Study Program (CSP) Study #470 (43, 44). Based on the results of the Veterans Health Study (40, 41), a 7 point change in the SF-36V PCS score is expected to represent an effect size of 0.75.

Specialized rating scales and validation of the measurements. The SF-36V is a modification of the well-established Medical Outcomes Study Short Form Health Survey (SF-36) for use with ambulatory Veterans Health Administration patients. It surveys eight concepts of health: physical functioning, role limitations because of physical problems, bodily pain, general health perceptions, energy/vitality, social functioning, role limitations due to emotional problems and mental health (40-42). From these concepts, two summary component scores are derived: a Physical Component Summary (PCS) and a Mental Component Summary (MCS). Scores are standardized and range from 0 to 100, with a US population mean of 50 points and a SD of 10 points. The PCS and the MCS have been demonstrated to have excellent psychometric properties (40, 41) and have been used extensively in GWI studies and throughout VA settings (5, 40-44). The subscale items largely address the diverse array of symptoms reported as a part of GWI. SF-36V PCS is a measure of HRQOL with respect to physical functioning and symptoms.

McGill Pain Questionnaire-Short Form (MPQ). The MPQ is a self-report survey which measures the quality of pain by asking patients to rate the intensity of 15 verbal descriptors of pain on a 0 to 3 rating scale with lower scores indicative of lower pain levels (45). The scale yields subscale scores in the following domains: Sensory pain, affective pain, pain now, and typical pain. The MPQ is a commonly used pain measure with considerable documentation of its reliability, sensitivity to change, and concurrent and predictive validity as a measure of pain quality and intensity (46).

Multidimensional Fatigue Inventory (MFI). The MFI is a 20-item self-report instrument designed to measure fatigue (47). The MFI covers the following dimensions: general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue. Scores on each dimension range from 4 to 20, with lower scores indicative of higher functioning. The instrument has demonstrated good internal consistency, with an average Cronbach's alpha coefficient of 0.84.

Cognitive Failures Questionnaire (CFQ). The CFQ is a 25-item self-report measure designed to assess the frequency of failures in perception/attention (e.g. ``do you fail to notice signposts on the road?''), memory (e.g. ``do you forget appointments?'') and action (e.g. ``do you bump into people?'') (48). Patients are asked to indicate on a 5-point scale (0= never to 5=very often) how often they have experienced each cognitive failure in the past months. Studies have demonstrated the CFQ to have adequate internal consistency and test-retest reliability.

Impact

Participants: Gulf War veterans with GWI (Kansas Case Definition) will be the participants in this proposed trial. The potential impacts of this trial on veterans with GWI include reduced symptoms and improved HRQOL. In addition, a successful trial will fundamentally change the way GWI is diagnosed and treated.
Short-term impact: A successful trial with improved clinical outcomes and reduced inflammation would validate the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. Thus, a new paradigm for diagnosis and treatment would be established. A change in clinical practice that focuses on inflammation could be implemented immediately.

Long-term impact: The paradigm shift to GWI-associated chronic inflammation will have a long-term impact on GWI diagnosis and treatment. Testing other therapeutic interventions with the focus on reducing inflammation and minimizing toxicity could lead to new treatments for GWI. Biomarkers of inflammation may become objective measures of GWI. Thus, evidence-based methods for the diagnosis and treatment of GWI will continue to evolve and improve the HRQOL of veterans with GWI.

Transition

This work will transition to the next phase after the successful completion of the GWIRP CTA. The details of the path that will be pursued will be determined by the specific results of the Gulf War Illness Inflammation Reduction Trial.

Possibilities for future studies:

- A multi-site trial of more targeted anti-inflammatory drugs for the treatment of GWI. The choice of the intervention(s) would be guided by the evidence from the current trial. The biomarker evidence may reveal new details of the underlying chronic inflammatory state that will help to identify specific targets for the next phase intervention.
- GWI biomarker studies. The objective is to develop biomarkers of inflammation as objective measures for the diagnosis of GWI and for the evaluation of treatment efficacy. This study will continue the work initiated in the pilot study and continued the Gulf War Illness Inflammation Reduction Trial. The relationship of GWI symptoms patterns with specific biomarker signatures will be a focus. This study would be a component of the next phase intervention trial.

Potential sources of funding:

- VA ORD
- VA CSP
- CDMRP GWIRP CTA
- CDMRP GWIRP CTDA

Additional sources of funding for the continuity of development and future studies will be explored. Collaborations such as those described in The VA/DoD Collaboration Guidebook for Healthcare Research will be considered.

Milestones:

- Obtain funding for continuity of development (year 3).
- Successfully complete the Gulf War Illness Inflammation Reduction Trial (year 4).
- Identify and develop next phase trial (year 4/5).
- Obtain funding for next phase trial (year 5).
- Initiate next phase trial (year 5/6).

Issues of intellectual property and proprietary information, as addressed in Attachment 2 (Intellectual Property), CRADA, and NDA, will be similarly resolved in the next phase study.

The increased cost of a multi-site trial will require expanded sources of funding, as outlined above. Schedule and sustainability are significant challenges for the next phase trial. Utilizing VA CSP infrastructure and resources is a potential solution to these issues. Manufacturability will be an important consideration in choosing the next phase intervention(s). However, this is unlikely to be a significant barrier given the wide use of FDA approved anti-inflammatory drugs.