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TITLE: Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military

PRINCIPAL INVESTIGATOR: Dr. David Milhorn
                          Dr. John Schlager

CONTRACTING ORGANIZATION: University of Cincinnati
                           Cincinnati OH 45267-0553

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               Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: To compare serum samples from individuals diagnosed with amyotrophic lateral sclerosis (ALS) to serum samples from matched individuals who did not develop ALS. In this study we aim to identify candidate serum biomarkers that are unique for ALS and identify a subset of diagnostic serum biomarkers for early detection of ALS prior to the appearance of overt symptoms.
Scope: The significance of a positive identification of protein biomarkers for ALS is indisputably great. However, to date no validated clinically relevant biomarkers have been found to allow a more specific diagnosis of ALS at an earlier stage. Previous efforts to identify ALS associated biomarkers have often focused on the identification of genes and proteins characteristic for familial ALS, yet validated biomarkers for sporadic ALS, which accounts for as much as 90-95% of all ALS cases, have yet to be identified.
Major Findings: None at this time.
Progress: This study received USAMRMC HSSRB approval on 15 Jan 2007. Changes are being made to the Durham VA and Univ. of Cincinnati IRB protocols at this time. As soon as the Durham VA IRB approves the protocol, they will begin consenting the VA ALS registry members for this study.

15. SUBJECT TERMS
Amyotrophic Lateral Sclerosis (ALS), Serum, protein biomarkers, early detection, DoD Serum Repository

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

Following their service in the first Gulf War, veterans were faced with an increased incidence of ALS. Most recently, concern has been raised that military service per se could result in an elevated risk for ALS. ALS is a rapidly progressing, invariably fatal neurodegenerative disease, and despite exhaustive studies, the possible genetic or environmental causes remain largely unknown. Early symptoms of the disease are often similar to those of other, less severe neurodegenerative diseases, which can complicate and delay the diagnosis. No validated clinically relevant biomarkers exist to assist the physician in a more precise diagnosis of the disease. No therapies exist to cure the disease, and the mean time of survival after diagnosis of ALS is four to five years. In light of this problem, there is a pressing need for the identification of new ALS biomarkers that allow an early, specific diagnosis and that have the potential for the development of future therapies.

Objectives:

Comparing serum samples from individuals diagnosed with amyotrophic lateral sclerosis (ALS) to serum samples from matched individuals who did not develop ALS, we aim to:

Objective 1: Identify candidate serum biomarkers that are unique for ALS.
Objective 2: Identify a subset of diagnostic serum biomarkers for early detection of ALS prior to the appearance of overt symptoms.

BODY OF REPORT

1. Changes with Investigators:

    a. Deletions: Detlef Schumann, Michael Wyder, Anne Maclachlan, John O'Lear

    b. Additions: Kenneth Greis, Pavel Shiyanov, Eugene Z. Oddone

2. Changes to Methods:

Methods have been modified as described in the attached protocol (Appendix B). In summary, changes include adding Dr. E. Oddone from the Durham VA, National ALS Registry, as a co-investigator. The 66 ALS cases previously described as being under the control of Dr. Horner are actually maintained in the National ALS Registry. Dr. Oddone will contact Registry members in order to obtain consent to participate in this study. If sufficient Registry cases meet study requirements, then Dr. Wells will not need to access the Military Health System for additional cases.

3. Results will be reported following completion of data collection and analyses.

KEY RESEARCH ACCOMPLISHMENTS

- o Study was approved by the HSRRB on 15 Jan 2007, which is not consistent with original Statement of Work.

- o Anticipate all local IRB by 15Feb07 and expect to begin case ascertainment approximately 01Mar07.

o   Made revisions to statement of work based on protocol revisions and revised timeline.

REPORTABLE OUTCOMES

There are no reportable outcomes at this time.

CONCLUSIONS

None as yet.

Appendix A – Approval of Amended Protocol - 2007 – Wright-Site IRB

DEPARTMENT OF THE AIR FORCE
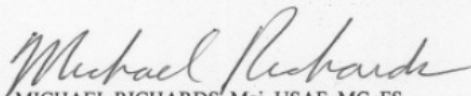AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE OHIO

29 December 2006

MEMORANDUM FOR: AFRL/HEPA (DR DAVID MILLHORN)

FROM: AFRL/Wright Site Institutional Review Board

SUBJECT: IRB approval for the use of human volunteers in research

1. Protocol title: Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military

2. Protocol number: F-WR-2006-0054-H

3. Type of review: Amendment

4. The amendment to the above protocol has been reviewed and **approved** by the Wright Site IRB via **expedited** review procedures. This protocol meets the criteria for expedited review under 32 CFR 219.110 (b) (2): Minor changes in previously approved research during the period (of one year or less) for which approval is authorized.

5. Any serious adverse event or issues resulting from this study should be reported immediately to the IRB. Amendments to protocols and/or revisions to informed consent documents must have IRB approval prior to implementation. Please retain both hard copy and electronic copy of the final approved protocol and informed consent document.

6. All inquiries and correspondence concerning this protocol should include the protocol number and name of the primary investigator. Please ensure the timely submission of all required progress and final reports and use the templates provided on the Wright Site IRB web site https://www.he-internal.afrl.af.mil/org/IRB/index.htm.

7. For questions or concerns, please contact the IRB administrator, Helen Jennings at (937) 904-8094 or helen.jennings@wpafb.af.mil OR Lt. Douglas Grafel at douglas.grafel@wpafb.af.mil or (937) 656-5437.

MICHAEL RICHARDS, Maj, USAF, MC, FS
Vice Chair, AFRL/Wright Site IRB

Appendix B –Amended Protocol - 2007 – Wright-Site IRB

Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans
F-WR-2006-0000-H

1. **Principal Investigator**
   a. David E. Millhorn PhD. University of Cincinnati, 513-558-5473, david.millhorn@uc.edu
   b. John J. Schlager PhD. Chief Applied Biotechnology Branch AFRL/HEPB, john.schlager@wpafb.af.mil

2. **Associate Investigators**
   a. Ronnie Horner, PhD. University of Cincinnati, 513-558-2756, ronnie.horner@uc.edu
   b. Kenneth Greis, PhD., University of Cincinnati, 513-558-7102, ken.greis@uc.educ.
   c. Michael Wyder, University of Cincinnati, 513-558-4057, wyderma@email.uc.edu
   d. Michael Wagner, PhD. Cincinnati Children's Hospital, 513-636-2935, Michael.wagner@cchmc.org
   e. Timothy S. Wells Lt Col, Chief Epidemiologist AFRL/HEPA, 937-255-3931, timothy.wells@wpafb.af.mil
   f. Pavel Shiyanov, AFRL/HEPB, 937-904-9499, pavel.shiyanov@wpafb.af.mil
   g. Claude C. Grigsby. AFRL/HEPB, 937-904-9579, claude.grigsby@wpafb.af.mil
   h. Deirdre A. Mahle, AFRL/HEPB, 937-904-9496, deirdre.mahle@wpafb.af.mil
   i. Eugene Z. Oddone, Director, Health Services Research and Development, 919-286-6936, oddon00@mc.duke.edu

3. **Medical Consultant or Monitor**
   None

4. **Facility/Contractor**
   None

5. **Objective**
   Comparing serum samples from individuals diagnosed with amyotrophic lateral sclerosis (ALS) to serum samples from matched individuals who did not develop ALS, we aim to:

   Aim 1: Identify candidate serum biomarkers that are unique for ALS (supports hypothesis 1)
   Aim 2: Identify a subset of diagnostic serum biomarkers for early detection of ALS prior to the appearance of overt symptoms (supports hypothesis 2)

   The significance of a positive identification of protein biomarkers for ALS is indisputably great. However, to date no validated clinically relevant biomarkers have been found to allow a more specific diagnosis of ALS at an earlier stage. Previous efforts to identify ALS associated biomarkers have often focused on the identification of genes and proteins characteristic for familial ALS, yet validated biomarkers for sporadic ALS, which accounts for as much as 90-95% of all ALS cases, have yet to be identified. The discovery of biomarkers that are characteristic for ALS will yield fundamental insights into the biology of this disease, it will provide new targets for the development of more accurate diagnostic tools and most importantly, it will provide new targets for the development of drugs for therapeutic efforts.

6. **Background**
   a. Hypothesis or question to be answered:

      1. We hypothesize that specific protein- or peptide-based biomarkers are present in sera of individuals diagnosed with ALS that are not present in the serum of individuals not affected with the disease.
      2. We hypothesize that a subset of these protein- or peptide based biomarkers are present in the sera of ALS patients prior to their diagnosis with ALS and prior to the manifestation of overt symptoms of the disease.

   b. Scientific rationale
      The Department of Defense Serum Repository (DoDSR) provides a rare and unique source for a thorough investigative effort to identify novel biomarkers for ALS. To date, more than 36 million serum samples have been collected, thus providing us with a sample size that is sufficiently large to apply the crucial cross-validation techniques to assess the statistical significance of the identified biomarkers. The annual collection of sera provides a unique time series of each individual that open the possibility for an unparalleled analysis for ALS biomarkers.

Taking full advantage of this opportunity, we will be able to a) analyze serum samples of individuals after a diagnosis with ALS in efforts to identify characteristic disease biomarkers, and b) analyze pre-diagnosis serum samples from the same individuals in an effort to monitor the presence of these biomarkers prior to any clinical diagnosis. Not only will we have access to a large pool of matched controls, but the time series allows each individual to serve as their own control.

Proteomics provides the potential to screen large, highly complex protein mixtures for the presence of characteristic markers that can distinguish between different sample groups. However, as of yet no single methodology has emerged which has proven to be uniformly superior for proteomic biomarker identification. In light of this fact, we propose a multi-pronged approach using a combination of established (2-D gel electrophoresis) and developing (mass spectrometry) protein profiling methods for the identification of ALS specific biomarkers. With this approach we will be able to perform a non-biased, in-depth analysis of the peptidome and proteome of these samples using the strengths of complementary, non-overlapping methods.

   c. Air Force Relevance

Following their service in the first Gulf War, veterans were faced with an increased incidence of ALS.[1] Most recently, concern has been raised that military service per se could result in an elevated risk for ALS.[2] ALS is a rapidly progressing, invariably fatal neurodegenerative disease, and despite exhaustive studies, the possible genetic or environmental causes remain largely unknown. Early symptoms of the disease are often similar to those of other, less severe neurodegenerative diseases, which can complicate and delay the diagnosis. No validated clinically relevant biomarkers exist to assist the physician in a more precise diagnosis of the disease. No therapies exist to cure the disease, and the mean time of survival after diagnosis of ALS is four to five years. In light of this problem, there is a pressing need for the identification of new ALS biomarkers that allow an early, specific diagnosis and that have the potential for the development of future therapies.

7. Impact

As previously stated, ALS is not a highly prevalent illness, a query submitted to the Defense Medical Epidemiology Database identified a total of 237 Air Force members diagnosed with ALS during the period of January 1, 1995 through December 31, 2004. However, ALS is rapidly progressive and highly fatal, so the consequences associated with being diagnosed with ALS are enormous. One legacy following the 1990-1991 Gulf War was a concern of increased risk of ALS among Gulf War veterans, and a recent study has found a two-fold increase in ALS risk among all Gulf War veterans, and a nearly three-fold increase in risk among Air Force personnel.[1] Further study to more rapidly diagnose ALS, and better understand the epidemiology of ALS demonstrates resolve to care for those who volunteer to stand in harm's way.

8. Experimental Plan
   a. Equipment and facilities
      i. <u>University of Cincinnati</u>: The Genome Research Institute, located at the University of Cincinnati has equipment and expertise necessary to conduct the two-dimensional gel electrophoresis and biomarker analysis using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).
      ii. <u>Air Force Research Laboratory</u>: The Applied Biotechnology Section has the expertise and equipment to utilize the Agilent Multiple Affinity Removal System (Agilent, CA), and perform one-dimensional liquid chromatography mass spectrometry (LC-MS/MS).

   b. Subjects
      i. *Case Ascertainment:*
        (1) The study design calls for a total of 75 cases. Dr. Horner has verified 66 cases among 1990-1991 Gulf War Veterans during his previous research.[1] These, and other ALS cases comprising US service members who are no longer serving on active duty, are maintained in the National Registry of Veterans with ALS, with Dr. Eugene Z. Oddone acting as the principal investigator. Dr. Oddone will obtain consent for ALS Registry cases from individuals who served during the 1990-1991 Gulf War era. Dr. Oddone will Fedex the SSNs of those consenting ALS Registry cases to Dr. Wells via a password protected diskette. Upon receipt, Dr. Wells will call Dr. Oddone to obtain the password. Dr. Wells will maintain the diskette in a locked file cabinet. See item 9, Medical Risk Analysis for further details.

(2) Dr. Wells will Fedex the diskette containing the ALS Registry cases to AMSA. Once AMSA has received the disk, they will call Dr. Wells to obtain the password. AMSA will determine how many of the ALS Registry cases have sera available in the DoD Serum Repository. If more than 75 ALS Registry cases exist with sera, then AMSA will do a simple random selection of 75 of the available cases.

(3) If there are less than 75 ALS Registry cases with sera, Dr. Wells will search the Military Health System (Standard Inpatient Data Record, Standard Ambulatory Data Record, Health Care Service Record) for additional ICD-9-CM coded cases using the following codes:

    (a) 335.xx   "Anterior horn cell disease"
    (b) 335.20   "ALS"
    (c) 335.2   "Motor Neuron Disease"
    (d) 335.8   "Other anterior horn cell disease"
    (e) 335.9   "Anterior horn cell disease, unspecified."

(4) The Social Security Numbers (SSNs) of the Military Health System (MHS) cases will be sent by FedEx by Dr. Wells to AMSA on a password protected diskette. Upon receipt, AMSA will telephone Dr. Wells to obtain the password. AMSA will determine the presence and temporal distribution of available serum samples for each MHS case.

(5) After determining the availability of sera for the potential MHS cases, AMSA will Fedex a listing of all SSNs meeting ALS case criteria back to Dr. Wells on a password protected diskette. Upon receipt, Dr. Wells will telephone AMSA to obtain the password. Dr. Wells will coordinate with the medical records repository in St. Louis Missouri to determine availability of medical records. A random sample of the MHS cases with sera, and having an available record will be selected to achieve a total of 75 cases. Once the MHS cases have been selected, copies of the individual medical records will be obtained from St Louis by Dr. Wells. Dr. Wells will de-identify each medical record and forward to Dr. Kasarkis at the University of Kentucky. Dr. Kasarkis is a neurologist with expertise in ALS who will perform case confirmation.

(6) Once all MHS cases have been confirmed, Dr. Wells will Fedex a password protected diskette to AMSA containing the SSNs of the MHS cases to be included in the study. Upon receipt, AMSA will telephone Dr. Wells to obtain the password.

    ii.   *Control Ascertainment*

(1) Only AMSA will provide controls for this study based upon the criteria listed below. For each case, one control will be randomly selected from among individuals who started military duty within one year of the case. Controls will be matched to cases by the AMSA for racial and ethnic group (white non-Hispanic, black, Hispanic, Asian, and other), for sex, for age (+/- 1 year), length of military service (± 2 years if over 5 years), branch of service, deployment history, on active duty during the same period as the case (+/- 1 year), date of serum specimens (+/- 30 days), and previous deployment to Operation Enduring Freedom or Iraqi Freedom. AMSA will ensure controls have not previously been diagnosed with a disease of the nervous system or sense organs (ICD-9 CM 320-389). Our selection of these parameters is based on their potential role in the development of ALS.

(2) If strict adherence to these selection criteria should result in difficulties in the selection of appropriate control samples, we will consider relaxing them in terms of age and/or length of military service or previous deployment history.

c. Serum Samples

    i.   For the proposed study 0.5 ml serum each will be requested from 75 case subjects and 75 matched controls. For the identification of pre-disease serum biomarkers, we will also request the complete history series of serum samples for all case subjects prior to their diagnosis with ALS. All samples will be sent in one shipment to the Air Force Research Laboratory, Applied Biotechnology Section (AFRL/HEPB) at Wright Patterson AFB. Serum samples received from the DoDSR will be stored at -80°C until accessed for biomarker studies. The total amount of serum necessary for the proposed experiments is approximately 50% of the amount provided by the DoDSR:

    (1) 1. Protein assay post affinity removal   30 µl

(2) 2. Two-dimensional gel electrophoresis   120 µl
(3) 3. LC-MS/MS   50 µl
(4) 4. MALDI-TOF   50 µl
(5) Total   250 µl/0.25 ml

    ii. Procedures for procuring samples from the DoDSR will be as follows. AMSA will identify all eligible controls, based upon the matching and exclusion criteria, and match 1 control per case. AMSA will then prepare an electronic database containing one record for each study subject. Each record will include the subject's ethnicity, age, length of service, branch of service, date of entry into active duty, deployment status, date of serum specimens, and subject identification linkage for matched cases and controls. Each record will also contain a randomly generated subject identifier that will also be found on each serum specimen. **No personal identifiers will be supplied by AMSA, and the linkage between the random subject identifier and the personal identity of the subject will be irreversibly destroyed by AMSA.** AMSA will send the electronic database to the principal investigator, Dr. Millhorn. AMSA will send all samples to AFRL/HEPB, Dr. John Schlager who will forward the samples to UC-GRI under the care of Dr. Kenneth Greis. (See item 12 Specimen Shipping and Storage for addition information)

d. Duration of the study: Three years from date of US Army Medical Research and Materiel Command (USAMRMC) approvals obtained.

e. Description of experiment, data collection, and analysis:

    i. Study design and data collection were covered above under subjects.

f. Statistical Analyses:

    i. <u>Aim 1</u>: The diagnostic utility of each identified biomarker will be assessed using the standard clinical epidemiological measures, notably sensitivity and specificity. If we find more than one potential biomarker, we will assess the improvement in diagnostic accuracy of using these biomarkers as a set. This will be done under parallel testing and serial testing scenarios. If the biomarkers are viewed as indicators of disease process, a serial testing approach will improve our specificity, allowing us to rule-in disease with a positive result. We would begin with the biomarker having the greatest specificity. Given the emotional impact of a positive diagnosis for ALS, we would be able to minimize the false positives. If the biomarkers are viewed as prognosticators of disease, we would wish to minimize false negatives. A parallel testing approach to the biomarkers will allow us to maximize sensitivity and rule-out those least likely to be at risk.

    ii. <u>Aim 2</u>: Under our second aim, the objective is to determine when highly sensitive or highly specific biomarkers are manifest in sera prior to symptom onset. Obviously, if a biomarker is present several years prior to onset, earlier intervention in the disease process is possible. To address this aim, we can graphically present the time dimension by arraying serum samples by time to disease onset (1 year increments) and showing proportion of serum samples with a specific biomarker present. A more sophisticated analytic approach could involve proportional hazards modeling to estimate the risk of a biomarker being present in a serum sample in the years prior to disease onset. This modeling would also allow adjustment for a limited number of patient characteristics.

    iii. <u>Sample Size Estimate</u>:

      (1) In this study, the number of cases and controls desired is dependent upon the desired width of the confidence interval, where the width is a function of the number of cases and controls, and the resulting sensitivity and specificity calculated from the study. Table 1 shows calculated confidence intervals based upon samples of 50, 75, and 100 cases and controls in each group, with sensitivity and specificity values ranging from 0.50 - 0.99.

      (2) As observed below, the width of the confidence interval for this study is influenced most by the desired precision for sensitivity or specificity rather than increasing the number of cases or controls. A useful biomarker will have a sensitivity or specificity of 0.90 or better and, as shown in the table, there is

minimal gain in statistical precision associated with using 100 versus 75 cases. For this reason, and to keep costs reasonable, 75 cases and 75 controls will be utilized in this study.

Table 1. Confidence Interval Estimation Based Upon Desired Precision, Number of Cases, or Controls.

Number*	Desired Precision[†]	Confidence Interval	Width
50	0.50	(0.36, 0.64)	0.28
50	0.75	(0.64, 0.88)	0.24
50	0.90	(0.82, 0.98)	0.16
50	0.95	(0.89, 1.00)	0.11
50	0.99	(0.94, 1.00)	0.06
75	0.50	(0.39, 0.61)	0.22
75	0.75	(0.65, 0.85)	0.20
75	0.90	(0.83, 0.97)	0.14
75	0.95	(0.90, 1.00)	0.09
75	0.99	(0.97, 1.00)	0.03
100	0.50	(0.40, 0.60)	0.20
100	0.75	(0.67, 0.85)	0.16
100	0.90	(0.84, 0.96)	0.12
100	0.95	(0.91, 0.99)	0.08
100	0.99	(0.97, 1.00)	0.03

*Applicable for either number of cases or controls
[†]Precision can be either sensitivity or specificity

g. On-site monitoring: No interaction with human subjects will occur during this study. Monitoring will be performed by the Principle Investigators, Dr. Millhorn and Dr. Schlager to ensure the quality control of all aspects of this study.

9. **Medical Risk Analysis**

a. This protocol makes use of computerized data that have been obtained as part of standard medical practice. The study therefore should pose no physical risk to participants. The data of interest may be of a highly sensitive nature however. For this reason strict confidentiality procedures will be applied. These procedures will minimize any risk of mental or emotional discomfort for participants:

b. Personal identifying information (i.e., SSNs) will be restricted to Dr. Wells and Dr. Oddone.

c. Investigators who will have access to volunteers' study data, identifying information and/or who will be involved with volunteers' serum samples will:
   i.   Sign an Investigator Assurance Agreement.
   ii. Carry data, other paper records, and computer disks from one location to another in locked briefcases.
   iii. Store records in locked metal file cabinets within their personal offices. All data entered on portable computers will be saved on removable disks, which will in turn be stored in locked file cabinets. They will not store computer disks in the portable computer carrying case.
   iv. Personally carry out any necessary photocopying of medical records, rather than using copying services. Any copies created will be destroyed following entry into the computer system.
   v.   Password protect all sectors of the study database containing personal identifiers.

d. To ensure that Dr. Oddone and his research staff will be able to verify identification of Registry participants and/or the relationship of the legally authorized persons when requesting verbal consent over the telephone, participants (or their authorized representative) in the VA National ALS Registry are contacted every 6 months to update contact information and to obtain functional status. Six interviewers (5 in Durham,1 in Lexington, KY) make these contacts. All interviewers access a common database with the contact information and interviewers follow their assigned participants over time. A given interviewer, then, becomes familiar with not only their assigned veterans but also their family members / authorized representatives. Thus, there is assurance that verbal permission to release information is being obtained from the appropriate person.

e. Lt Col Wells will receive the diskette containing SSNs of ALS cases from Dr. Oddone. This disk will be stored in a locked file cabinet in Dr. Wells' office. Only Dr. Wells will have access to the disk. Dr. Wells will add additional cases, if necessary, to achieve a total of 75 cases. This disk will be sent via Fedex to AMSA. Once AMSA has downloaded the data, the disk will be destroyed in accordance with AMSA privacy act procedures.

f. Sera remaining after the study is complete will be destroyed. At the University of Cincinnati, all remaining samples at the end of the study will we be decontaminated by bleaching followed by autoclave sterilization then discarded through the University biohazardous waste vendor. At AFRL/HEPB, remaining sera will be disposed by placing in an infectious waste container that is properly labeled, securely sealed, checked for leaks, and then picked up by a hazardous waste vendor for incineration.

10. USAMRMC HSRRB Requirements:

a. The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command's Human Subjects Research Review Board (USAMRMC HSRRB), at the USAMRMC Office of Research Protections (ORP), Human Research Protection Office (HRPO).

  i. The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC, HSRRB and will not be initiated until written notification of approval of the research project by the USAMRMC, HSRRB is issued.

  ii. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

  iii. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and all subject deaths should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to USAMRMC, Human Subjects Research Review Board (HSRRB). A complete written report should follow the initial notification. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

  iv. Any deviation to the protocol that may have an effect on the safety of the subject or the integrity of the study must be reported to the USAMRMC, HSRRB, at the Human Research Protection Office (HRPO) as soon as the deviation is identified.

  v. Modifications to the research protocol must be submitted to the USAMRMC, HRPO after review and approval by the local IRB, but before implementation for HSRRB review and approval.

  vi. A copy of the approved continuing review report and the local IRB approval notification must be submitted to the USAMRMC, HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification must be submitted to the USAMRMC, HRPO as soon as these documents become available.

11. Titles, Roles and Responsibilities

a. Wendy D. Dominick is a Postdoctoral Fellow in the Proteomics Laboratory of the University of Cincinnati Genome Research Institute. She will participate in conducting the mass spectrometry based profiling experiments which will seek to identify biomarkers associated with increased prevalence of ALS.

b. Louise Carter is the Chief of the Biomechanics Branch and is responsible to provide supervisory and technical support to Lt Col Wells.

c. Rachana Jain is a Ph.D. student in the bioinformatics program at the University of Cincinnati working under Michael Wagner's supervision. She will be responsible for running the computational analyses that correlate mass spectral information obtained from serum samples with the incidence of ALS.

d. Dr. Eugene Oddone is the Principal Investigator, National Registry of Veterans with ALS and will be responsible for obtaining consent from registry participants and forwarding a listing of consentee's social security numbers to

Lt Col Wells for inclusion in the study. He will also be responsible to ensure study withdrawal requests from veterans and/or proxies who consented on behalf of veterans are processed in a timely manner for researchers involved in this research study to discontinue any future use of study data and/or serum samples.

e. Pavel Shiyanov, Ph.D. – Research Associate – Dr. Shiyanov is a senior chemist at the AFRL. He has more than 10 years experience in electrospray mass spectrometry of organic molecules and proteins. He will conduct all liquid chromatography mass spectrometry based protein profiling experiments in collaboration with Mr. Claude C. Grigsby. He will be responsible for the acquisition and the final quality assessment of the generated mass spectrometry data.

f. Kenneth Greis, PhD., is the Director of the Proteomics Core Facility at the University of Cincinnati Genome Research Institute. He will supervise the protein separation and protein identification by mass spectrometry experiments which will seek to identify biomarkers associated with increased prevalence of ALS. He will perform the data analysis associated with this work.

12. Specimen Shipping and Storage

Sera shipments will follow AMSA's standard method of shipping. This method is to package the requested aliquots in boxes of 150 specimens each. These boxes are then shipped within larger styrofoam containers packed with dry ice. All of the samples in the DoD Repository that are available for this study have previously tested negative for HIV-1 (thus packaging and shipping methods for infectious substances are not used). Even though we are assured all samples received are HIV-1 negative, each will be handled in accordance with current blood-borne pathogen/univeral precaution standards. AFRL/HEPB will receive and ship the samples as packaged the same day to UC-GRI via commercial carrier (FedEx) after making and retaining a copy of the shipping log. Upon receiving the samples at UC-GRI they will be immediately transferred to -80 ˚C storage and their receipt and temperature recorded.

13. References

a. Horner RD, Kamins KG, Feussner JR, al. e. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology.* 2003;61:743-749.

b. Weisskopf MG, O'Reilly EJ, M.L. M, et al. Prospective study of military service and mortality from ALS. *Neurology.* 2005;64:32-37.

14. Attachments

a. Investigator Assurance Agreement.

Attachment A.   INVESTIGATOR ASSURANCE AGREEMENT(s)

<u>INVESTIGATOR ASSURANCE AGREEMENT</u>

I, the Department Head, Principal Investigator or Co-Investigator, cited as responsible for performing and monitoring the research under the protocol titled, "Biomarkers For Amyotrophic Lateral Sclerosis In Active Duty Military" have read and understand the provisions of Title 32 Code of Federal Regulations Part 219 (Protection of Human Subjects), Title 45 code of Federal Regulations Part 46, Protection of Human Subjects, Department of Defense (DoD) Directive 3216.2 (Protection of Human Subjects in DoD-Supported Research), AFI 40-402, Protection of Human Subjects, AFRLI 40-402, Using Human Subjects in Research, Development, Test, and Evaluation, Title 21 Code of Federal Regulations Part 50 if applicable (clinical investigations regulated by the FDA) and all relevant local instructions. I will abide by <u>all</u> applicable laws and regulations, and I agree that in all cases, the <u>most</u> restrictive regulation related to a given aspect of research involving protection of research volunteers will be followed. In the event that I have a question regarding my obligations during the conduct of this project, I have ready access to each of these regulations, as either my personal copy or available on file from the Chairperson of the Institutional Review Board. I understand that my immediate resource for clarification of any issues related to the protection of research volunteers is the Chairperson of the Institutional Review Board.

Signatures and dates: (DD/MM/YY)

_____   ___/___/___
Louise Carter
Program Manager/Co-investigator

_____   ___/___/___
Timothy S. Wells, LtCol, USAF, BSC
Co- Investigator

_____   ___/___/___
Dr. Ronald Horner, University of Cincinnati

_____   ___/___/___
Dr. Eugene Z. Oddone, Durham VA Medical Center