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TITLE: Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military
(BALSAM)

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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. The Durham VA and Univ. of Cincinnati IRB protocols have also been approved. Durham VA IRB began consenting the VA ALS registry members for this study. Model system 2-D gel and mass spectrometry studies have been conducted to develop improved techniques for biomarker identification.					
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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 in Investigators:

None during this year.

Note that AFRL/HEPB is now known as AFRL/RHPB

2. Changes to Methods:

None have been made since last IRB approval (29 December 2006)

3. Changes to Schedule:

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

4. Identification of Cases

Because diagnosing ALS is challenging, and often done by exclusion, the study team felt it was important to utilize confirmed ALS. The study team identified the Epidemiologic Research and Information Center (ERIC) at the Durham VA Medical Center, which maintains a national registry for veterans diagnosed with ALS. In late 2006, this research team began negotiations with the Durham VA to obtain personal identifying information for ALS registry members. Durham agreed and obtained an amendment to their IRB protocol in January 2007. The Durham began obtaining consent from Gulf War Veterans or their next of kin if the veteran was deceased, to provide Social Security Numbers to this research team. By July 2007, the Durham VA had recruited 51 Gulf War Veterans, and there was a signed data transfer agreement between the Durham VA and Dr. Wells, a co-investigator on this study. Because the protocol had called for a total of 75 cases, Durham VA agreed to seek additional amendments to their IRB protocols to contact Gulf War Era Veterans with ALS for permission to provide social security numbers to this research team. During July 2007 – Dec 2007, less progress was made as key Durham VA personnel were not available and the remaining Durham VA personnel were busy conducting routine work. Recently, the Durham VA is back to being fully staffed, and have begun consenting Gulf War Era ALS cases.

5. Technical Efforts

Development of new biomarker identification methods was a focus of the past year's efforts in preparation for receiving serum samples. A summary of this effort is provided below:

a. Background

Given the delay in acquiring samples for the BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS IN ACTIVE DUTY MILITARY (BALSAM) project and the technical challenges in the approaches to be used to evaluate these samples, the UC Proteomics Laboratory personnel have been using this time to develop optimized protocols for sample preparation and analysis. The goal is to have all the protocols in place so that when the samples are received, we can avoid some of the technical delays in sample preparation and analysis.

Early in calendar year 2007, we completed the optimization of the protocol for the scale-up of immuno-depletion of abundant serum proteins on the Agilent MARs columns and for the GE Health Care Differential Gel Electrophoresis (DIGE) profiling of immuno-depleted serum samples. The scale-up of the immuno-depletion was targeted at minimizing the number of FPLC runs needed per sample to generate sufficient material for both the LC-MS/MS approach (AFRL) and the 2D DIGE approach. We had previously optimized the 2D DIGE methodology on Liver extracts, but have now completed analysis using immuno-depleted serum samples. In addition, we have gone on to evaluate the use of isotope-tagging approaches comparative proteomics quantitation without need for 2D gels, as an alternative approach for the ALS serum profiling in the event that the 2D gel approach is not fruitful in detecting biomarkers. The following provides an overview of the optimization of the 2D-DIGE of serum samples and a summary of the progress made on the Isotope tagging approach.

b. 2D DIGE Optimization

As described above, the 2D DIGE approach offers the ability to directly compare two samples on the same gel by differentially-labeling each sample with a fluorescent tag. The primary advantage is that the direct comparison eliminates the need for manipulation of the 2D gel image due to the inconsistencies typically seen from gel to gel. As a result, better quantitative comparisons are achieved with significantly less image analysis. In our previous report, we demonstrated the optimal conditions and protein concentrations needed for labeling and detecting proteins by 2D DIGE using liver tissue lysates.

We began investigating use of DIGE for 2D gels in October 2006 for several projects using liver tissue homogenates. Initially, we had significant issues with getting different signal intensity and spillover from channel to channel when labeling the same samples with one of the 3 CyDyes. However, these issues were almost exclusively related to the scan filters and the scan setting being improperly tuned on the FujiFilm Fluorescent scanner when we received the instrument. After changing the scan filters and having the instrument serviced by the Fujifilm technical personnel, the system is now working properly. To test the comparative profiling of samples labeled with CyDyes, 50 µg (the amount recommended by the manufacturer) of liver homogenate was labeled with either Cy 2, Cy 3 or Cy 5, then

mixed together and run on a 2D gel. The gels were then scanned and images captured for each of the 3 dyes (Figure 1). Subsequently, the gels were stained with silver to evaluate overall resolution of the separation and the sensitivity of the CyDyes. Note that the silver-stain detects the mixture of all 3 labeled samples for a total of 150 μ g while each CyDye only represents 50 μ g of protein. Clearly, the overall sensitivity of the silver-stain is better (many more proteins are visible), but importantly, the 3 CyDyes produce comparable images. Finally, to try to increase the sensitivity of the CyDye labeling, we evaluated 60 μ g or 70 μ g of homogenate labeled with each CyDye for a total of 180 or 210 μ g of protein on the gel. As expected a greater number of labeled proteins were detected at the higher concentrations (lower panels) with no significant effect on spot resolution.

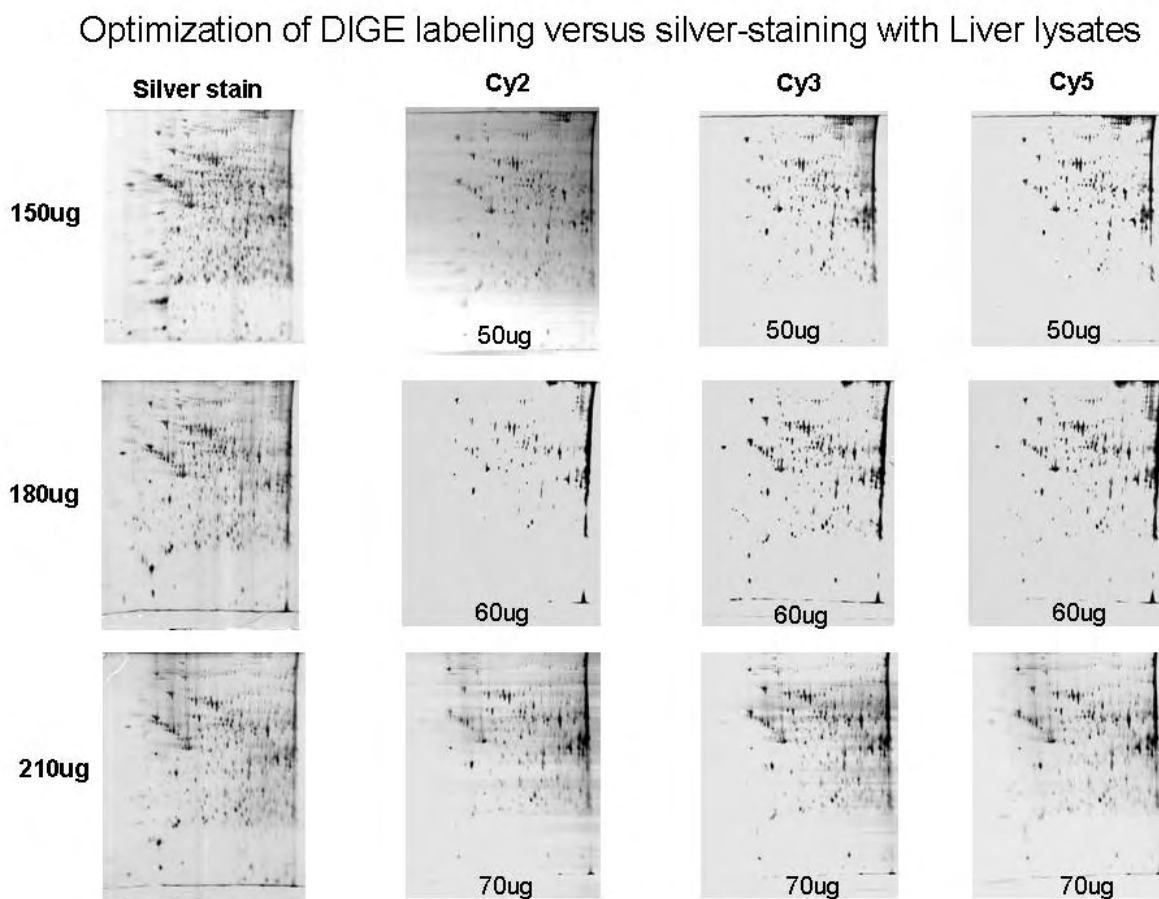


Figure 1. 50 μ g of liver homogenate was labeled with either Cy 2, Cy 3 or Cy 5, then mixed together and run on a 2D gel. The gels were then scanned and images captured for each of the 3 dyes.

We have now extended this optimization to include control serum samples after immunodepletion to confirm that the techniques developed for the liver homogenates were also acceptable for serum samples (**Fig. 2**). Given these results we are now confident that we have optimized both the 2D DIGE approach and the Serum Immuno-depletion methodology so that we may expedite and analyses of the ALS samples when they become available.

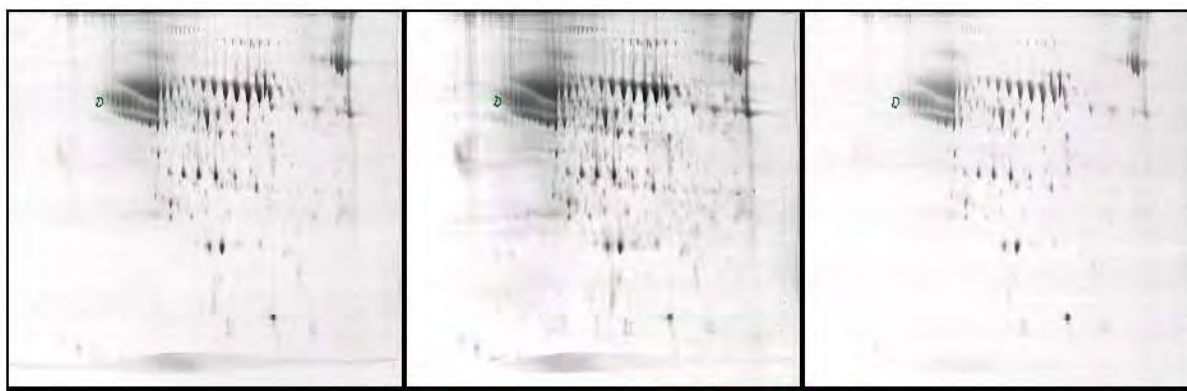


Fig. 2 The figure above shows the Cy2, Cy3 and Cy5 images from an immuno-depleted serum sample all run on the same gel.

c. Immuno-depletion of serum samples

One of the primary challenges in evaluating serum samples for biomarkers is the wide dynamic range of protein concentrations in whole serum. The normal protein concentration in whole serum is about 50-60 mg/mL; however greater than 90% of this protein concentration is accounted for by the 6 most abundant proteins (albumin, IgG, antitrypsin, IgA, transferrin, and haptoglobin). These high abundance proteins typically mask the detection and comparative quantitation of the lesser abundant proteins that may represent disease biomarkers. Thus Agilent has developed various Multiple Affinity Removal System (MARS) columns that selectively remove these proteins from serum samples. Over the past few years the UC proteomics laboratory has used this for small scale sample sets in preparation of 2D gel electrophoresis. These samples were prepared using a 4.6x 50mm HS6 column which required 3-4 separation runs per serum sample that could be pooled to get sufficient protein to run triplicate 2D gels. For large sample sets this approach is too inefficient and thus we investigated using a newer generation Agilent MARS column (4.6 x100mm HS-7). The HS-7 means it is targeted for the top 7 proteins in human serum. The additional protein depleted on this column compared to the HS-6 is fibrinogen which makes this column also appropriate for plasma samples as well as serum samples. In addition, the larger column size provides better capacity to help minimize the number of separations needed to get sufficient immuno-depleted sample for subsequent 2D DIGE and LC-MS/MS approaches. Optimization of the method for the HS-7 column was targeted at the maximum amount of serum that could be loaded per run while still getting an efficient depletion of the high abundance proteins. For this optimization, 50, 55, 60, 65 and 70 μ L equivalence of fresh serum were diluted 1:4 as recommended by the Agilent and loaded onto the column. The flow through and the eluted fraction were monitored by UV and collected. After a protein assay, 150 μ g of each fraction was run on a 2D gel and stained with silver (**Fig. 3**). Note that none of the depleted proteins were detected in the flow-through indicating the efficient removal of the high abundance proteins was achieved. Importantly, many new protein spots were visible in the depleted sample (middle panel) that could not be detected in the whole serum (left panel). At the higher loads, some of the albumin began to appear in the flow through (specifically for the 70 μ L sample, not shown), thus 60 μ L was selected as the optimized level. Multiple collections of the flow through at the 60 μ L load demonstrated a

recovery of about 300 µg of protein, thus 2 runs on the MARS column should provide sufficient protein for duplicate 2D DIGE gels and the sample needed for the LC-MS/MS profiles to be done at AFRL.

Immuno-depletion to remove the 7 most abundant proteins for serum

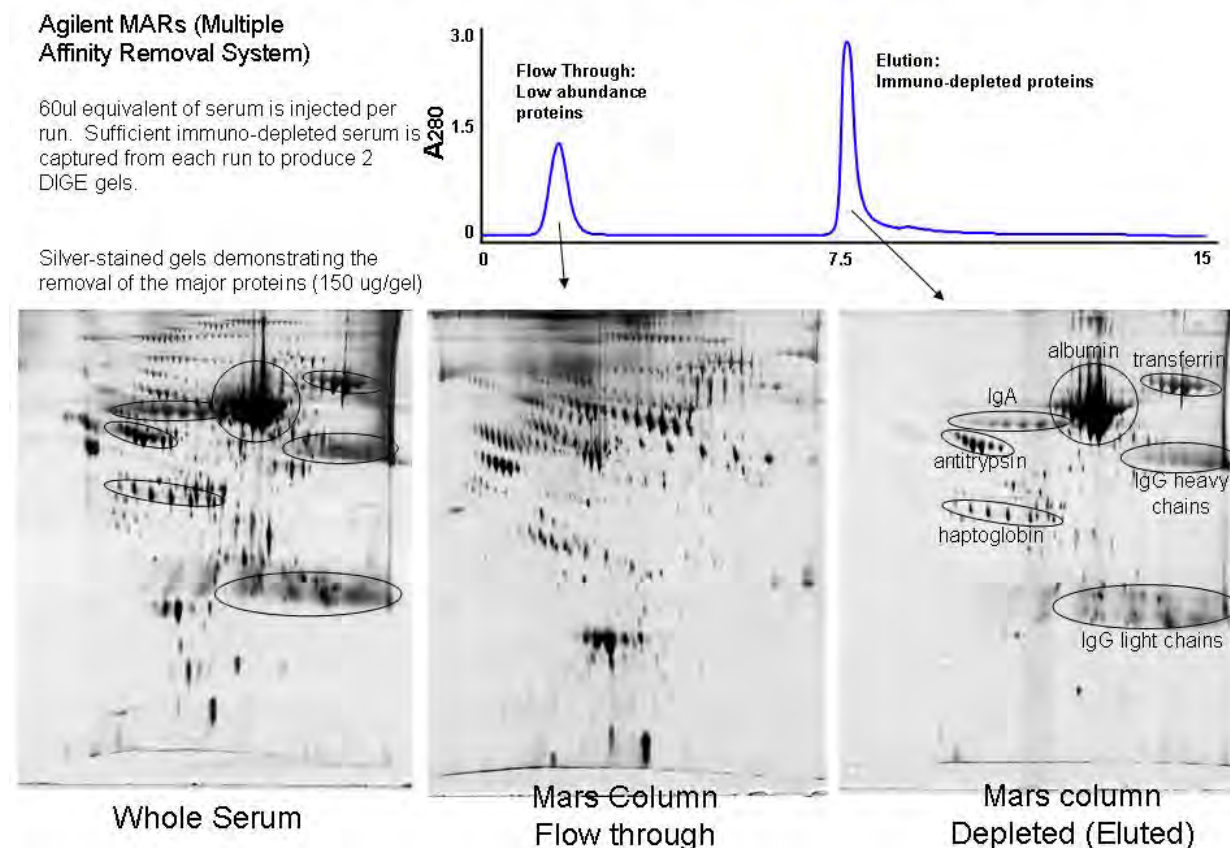


Figure 3 Upper panel shows the MARS column UV profile for the 60 µg equivalence run. The lower panels show the resulting silver-stained gels when 150 µg of total protein was loaded to 2D gels.

d. Isotope Labeling and Quantitation by Mass Spectrometry Using iTRAQ Reagents:

iTRAQ is a newly developed technique for quantitative comparison among four sample groups which uses MS directly, thus bypassing the traditional separation on 2-D gel electrophoresis (11;12). Tryptic digests of each protein sample are labeled with one of 4 iTRAQ mass tags (114, 115, 116, 117), which react with the N-terminus of every peptide and the side chain of lysine. All four tagged samples are mixed together and then collective pool of peptides is separated on capillary LC and collected onto a MALDI target plate with 4-HCCA matrix is added. Each fraction is then evaluated on an Applied Biosystems 4800 TOF/TOF instrument both in a reflector mode for intact peptide masses and in an MS/MS mode for peptide sequencing. Fragmentation of each iTRAQ-tagged peptide produces sequence information that identifies the peptide and also releases a low molecular weight reporter ion that is unique to the specific tag. Comparison of reporter ion intensities provides

the relative quantitation of the peptides among the four treatment groups. To validate the technique, four tubes of a protein mixture (BSA, α -lactalbumin, α -galactosidase, apotransferrin, lysozyme, and β -lactoglobulin) were prepared either at equal concentrations or at four 1/2-fold dilutions. **Fig 4** illustrates how iTRAQ-protein tags can be used to identify and quantify proteins.

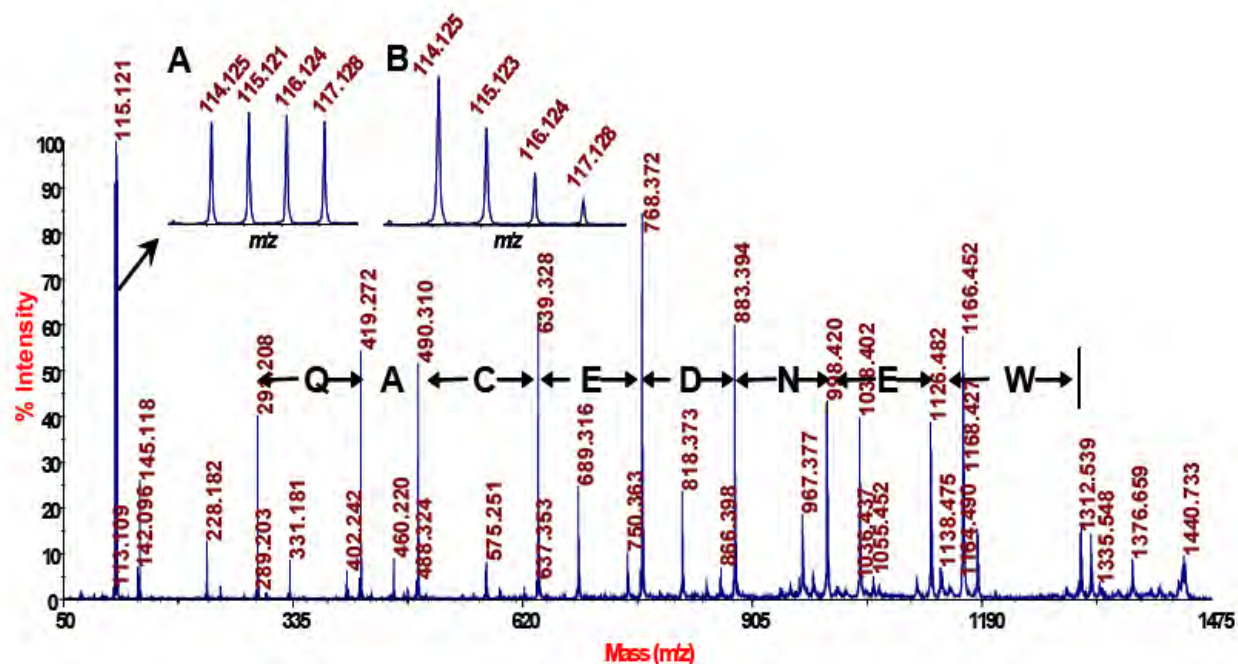


Fig 4: Representative MS/MS fragmentation spectrum from one of the tagged peptide from α -lactalbumin. The sequence derived from the spectrum (WENDECAQ) was used to identify the protein from a database search. Fragmented mass tags are detected in the low mass region of the spectrum. **Inset A** shows the relative quantitative profile from the tagged mixtures at equal amount. **Inset B** shows the quantitative profiles from the mixtures at 1/2-fold dilutions.

e. Next Steps:

We will continue to investigate the iTRAQ approach for quantitative comparison of immuno-depleted serum samples as an alternative means to detect and identify biomarker of ALS development. Thus in the event that biomarkers are not detected in the 2D gel profiling of serum sample, we will already have techniques available to continue to expand our search for common biomarkers among the ALS patient group.

KEY RESEARCH ACCOMPLISHMENTS

1. USAMRMC approved Protocol on 24 January 2007.
2. All local IRB approvals were secured by 9 Feb 2007. Continuing Reviews were conducted as required.
3. Case ascertainment began approximately 15 Apr 2007. By mid December 2007, 51 cases had been consented (VA Durham).
4. Optimization of the protocol for the scale-up of immuno-depletion of abundant serum proteins based on prior work on liver homogenates (UC/GRI).
5. Optimize Differential Gel Electrophoresis (DIGE) profiling of immuno-depleted serum samples. The scale-up of the immuno-depletion was targeted at minimizing the number of FPLC runs needed per sample to generate sufficient material for both the LC-MS/MS approach (AFRL) and the 2D DIGE approach (UC/GRI).
6. Evaluate the use of isotope-tagging approaches comparative proteomics quantitation without need for 2D gels (UC/GRI).
7. Data Transfer Agreement signed between Durham VA Medical Center and Dr. Tim Wells, 3 Jul 2007.
8. Timeline revised several times in accordance with late start and unexpected delays in Case Consenting.

REPORTABLE OUTCOMES

There are no reportable outcomes at this time.

CONCLUSIONS

None as yet.

Appendix A

-----Original Message-----

From: Duchesneau, Caryn L Ms USAMRMC [mailto:Caryn.Duchesneau@us.army.mil]
Sent: Saturday, February 24, 2007 2:13 PM
To: millhorn@tennessee.edu; Horner, Ronnie (hornerrrd); Wells, Timothy S LtCol USAF AFRL/HEPA; oddon001@mc.duke.edu
Cc: Campbell-Rosen, Holly Dr USAMRMC; Burns, Julie L Ms USAMRAA; Simon, Irwin (simonib); tana.housh@uc.edu; Carter, Louise A CIV USAF AFRL/HEPA; Stanley, Amber L Ms AZIMUTH; ronnie.horner@uc.edu; Brosch, Laura R COL USAMRMC; Duchesneau, Caryn L Ms USAMRMC; Bennett, Jodi H Ms USAMRMC; Collins, Jo A Ms AMDEX; Dominic, Mary R CTR USAF AFRL/HEPB
Subject: A-13569.a, Approval Memo (Proposal Log Number PR054256, Award Number W81XWH-06-2-0016)

SUBJECT: Protocol, "Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military - BALSAM," Submitted by David E. Millhorn, Ph.D., Genome Research Institute, University of Cincinnati, Cincinnati, Ohio, Proposal Log Number PR054256, Award Number W81XWH-06-2-0016, HRPO Log Number A-13569.a (University of Cincinnati Study Site)

1. The subject protocol for the University of Cincinnati study site has been reviewed and found to comply with applicable Federal, DOD, U.S. Army, and U.S. Army Medical Research and Materiel Command (USAMRMC) human subjects protection regulations. Documentation of approval by the University of Cincinnati (UC) Institutional Review Board (IRB) on 9 February 2007 was received 23 February 2007.

2. This no greater than minimal risk study is approved for the use of 150 serum samples donated from military personnel who served in the Gulf War during 1990 - 1991.

3. On 1 September 2006 the USAMRMC implemented a change to the human subjects protection review process. Due to the change, the review, approval, and oversight responsibility for your protocol has been transferred from the USAMRMC Human Subjects Research Review Board (HSRRB) to the USAMRMC Office of Research Protections, Human Research Protections Office (ORP HRPO). Your protocol/award currently states that the protocol and all amendments must be approved by the HSRRB prior to implementation. This HRPO approval is the document that meets the requirement for HSRRB approval.

Please note that our requirements for approval of amendments have changed. If your protocol requires subsequent amendments or at the time of continuing review at your institution, the following statement may be introduced into your protocol to replace the current language regarding HSRRB approval of amendments:

**Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC Office of Research Protections (ORP), Human Research Protections Office (HRPO) for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

4. Please note the following reporting obligations:

a. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments must be submitted to the ORP HRPO for acceptance with the continuing review report.

b. All unanticipated problems involving risks to subjects or others, serious adverse events related to study participation, and deaths related to study participation must be reported promptly to the ORP HRPO.

c. Any deviation to the subject protocol that affects the safety or rights of the subject and/or integrity of the study data must be reported promptly to the ORP HRPO.

d. All modifications, deviations, unanticipated problems, adverse events, and deaths must also be reported at the time of continuing review of the protocol.

e. A copy of the continuing review report approved by the UC IRB should be submitted to the ORP HRPO as soon as possible after receipt of approval. It appears that the next continuing review is due no later than 30 June 2007.

f. In addition, the current version of the protocol and consent form (if applicable) should be submitted along with the continuing review report and the UC IRB approval notice for continuation of the protocol.

g. When available, the final study report submitted to the UC IRB, including a copy of any IRB acknowledgement documentation and any supporting documents, must be submitted to the ORP.

4. The ORP HRPO point of contact for this study is Jo A. Collins, MSA, Human Subjects Protection Scientist, at 301-619-2380.

CARYN L. DUCHESNEAU, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
U.S. Army Medical Research and Materiel Command

Note: The official copy of this approval is housed with the protocol file at the Office of Research Protections, Human Research Protection Office, 504 Scott Street, Fort Detrick, MD 21702. Signed copies will be provided upon request.

Note: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds.

Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans
F-WR-2006-0054-H

SPONSOR: The United States Department of Defense

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3. Medical Consultant or Monitor

None

4. Facility/Contractor

Non

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

University of Cincinnati: 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).

Air Force Research Laboratory: 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

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.

Appendix B

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:
 - 335.xx "Anterior horn cell disease"
 - 335.20 "ALS"
 - 335.2 "Motor Neuron Disease"
 - 335.8 "Other anterior horn cell disease"
 - 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.

Control Ascertainment

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 ($\pm$ 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

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CM 320-389). Our selection of these parameters is based on their potential role in the development of ALS. 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.

Serum Samples

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. Protein assay post affinity removal	30 µl
2. Two-dimensional gel electrophoresis	120 µl
3. LC-MS/MS	50 µl
4. MALDI-TOF	50 µl
Total	250 µl/0.25 ml

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)

- c. Duration of the study: Three years from date of US Army Medical Research and Materiel Command (USAMRMC) approvals obtained.
- d. Description of experiment, data collection, and analysis:

Study design and data collection were covered above under subjects.

Statistical Analyses:

Aim 1: 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.

Aim 2: 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.

Sample Size Estimate:

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.

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.

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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

e. 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

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:

1. Personal identifying information (i.e., SSNs) will be restricted to Dr. Wells and Dr. Oddone.
2. Investigators who will have access to volunteers' study data, identifying information and/or who will be involved with volunteers' serum samples will:
 - a. Sign an Investigator Assurance Agreement.
 - b. Carry data, other paper records, and computer disks from one location to another in locked briefcases.
 - c. 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.

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- d. 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.
 - e. Password protect all sectors of the study database containing personal identifiers.
3. 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.
4. 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.
5. 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 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:

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).

(1) 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.

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(2) 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.

(3) 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.

(4) 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.

(5) 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.

(6) 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

1) 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.

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

3) 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.

4) 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

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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.

5) Pavel Shiyonov, Ph.D. – Research Associate – Dr. Shiyonov 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.

6.) 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/universal 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

1. Horner RD, Kamins KG, Feussner JR, al. e. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology*. 2003;61:743-749.
2. 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.

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Attachment A. INVESTIGATOR ASSURANCE AGREEMENT(s)

INVESTIGATOR ASSURANCE AGREEMENT

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 Gulf War Veterans" 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 all applicable laws and regulations, and I agree that in all cases, the most 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
Co-Investigator

____/____/____

Institutional Review Board
Durham VAMC Research (151)
508 Fulton St. • Durham, NC 27705 • 919-286-6926 • Fax: 919-286-6824

MISCELLANEOUS IRB SUBMISSION FORM 304 DEC 19 AM 11:48 [07/17/2001]

Date: 12/15/06

ID: 01025 Prom#: 0026

Principal Investigator: **Oddone, Eugene Z., M.D.**Protocol Title: **Biomarkers for ALS among Active Duty Military**Research Coordinator: Kelli Allen / Barbara Norman Phone: 7090 Pager: _____

Check one or more of the following:

☒ **Consent Form Change** not related to SAE or Protocol Amendment
(attach copy of old consent form and new consent form with highlighted changes)

Old Version Date: _____ New Version Date: _____

☐ **Advertisement(s) / Recruitment Letter(s)**

Include:

Investigator's Name, Purpose of Research, Criteria for Eligibility, Brief List of Benefits (e.g., no-cost health examination), Location of Research and Contact Person

☐ **Safety Update Summaries**

Update Number: _____ Update Date: _____

Are consent form changes required? ☐ Yes ☐ NoList Commonalities noted (e.g., renal failure - 5, chest pain - 3):

☐ **PI Change**

Specify (include letter from sponsor acknowledging change, if applicable):

☐ **Investigator Brochure Update** (attach update summary and copy of consent form)

☒ **Other**

Specify: Change in protocol format to comply with DoD
requirementsSignature of PI: [Signature]Date: 12/13/06Reviewer: [Signature]Approved: ☐ Yes ☐ NoDate: 1/11/07

Comments: _____

The Durham VAMC VAMC IRB is not connected with, has no authority over, and is not responsible for human research conducted at any other institution. Separate consent forms, initial reviews, continuing reviews, amendments, and reporting of serious adverse events are required if the same study is conducted at multiple institutions.

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS**

VERBAL INFORMED CONSENT SCRIPT - PROXY

PATIENT ID: _____

NAME: _____

(Veteran's Name) previously participated in our research study: An Epidemiologic Investigation into the Occurrence of amyotrophic lateral sclerosis (ALS) in Gulf War Veterans. We are calling to ask your permission to use their information as part of another research study related to ALS in veterans. This new research study: Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans is sponsored by the United States Department of Defense. The purpose of this study is to identify substances called biomarkers that are associated with getting ALS. Biomarkers are proteins and other similar substances that are found in blood or other body fluids. By identifying biomarkers that are related to ALS, researchers may be able to identify people early who are at risk for getting ALS. These biomarkers may also help researchers to understand more about what causes ALS.

This study is being conducted by researchers at the University of Cincinnati in Cincinnati, Ohio, and at the U.S. Air Force Research Laboratory at the Wright-Patterson Air Force Base in Dayton, Ohio. Researchers at the Durham VA Medical Center are also participating in this study by helping to identify veterans with ALS who were part of the prior Gulf War ALS study. The person in charge of this study at the Durham VAMC is Dr. Eugene Oddone. Up to 150 military personnel and veterans will participate in this study of whom half will have been diagnosed with ALS.

The study will last about three years. Your participation in the study is limited to giving us permission to give the veteran's social security number to the researchers at the U.S. Air Force Research Laboratory. These researchers will be accessing the Department of Defense Serum Repository. This repository contains blood samples from about 36 million people who were in military service from 1989 and later. If the veteran was in the military during this time period, his/her blood sample may be a part of this collection. The blood samples are linked to the social security numbers of people who contributed the samples. When we give the researchers at the U.S. Air Force Research Laboratory the veteran's social security number, they will be able to find out whether the veteran's blood sample is included in the repository. If it is included, the researchers will analyze the veteran's blood sample for biomarkers. They will be comparing biomarkers in the blood samples of veterans who have ALS to veterans who do not have ALS. The researchers will destroy any unused part of the veteran's blood sample at the end of the study.

We will send the veteran's social security number to the researchers at the U.S. Air Force Research Laboratory in a password protected data file. Once they have received the veteran's social security number, they will also store it in a secure, password protected file that can only be accessed by key study personnel. The veteran's social security number will be stored at the U.S. Air Force Research Laboratory by Lt. Col. Timothy Wells, who is in charge of sending the social security numbers to the Department of Defense Serum Repository.

If you agree to the veteran participating in this study, we will only be asking you to give permission for us to make the veteran's social security number known to the U.S. Air Force Research Laboratory researchers. We will not ask you or the veteran to perform any other activities. Participation in this study is not believed to have any risks for the veteran's health. The veteran's social security number will be sent and stored in a secure fashion to minimize any risk of loss of confidentiality. Taking part in this study may not personally help the veteran, but the veteran's participation may lead to knowledge that will help others.

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS**

VERBAL INFORMED CONSENT SCRIPT - PROXY

As part of this study we are asking you to authorize Dr. Oddone and his research team to access the following information about the veteran: social security number and diagnosis of ALS. This is information the veteran provided to us previously as part of the study of ALS in Gulf War Veterans.

We may disclose the veteran's information to the U.S. Air Force Research Laboratory, Department of Defense Serum Repository, and University of Cincinnati researchers involved in this study, the Institutional Review Boards that oversee this project (University of Cincinnati, Wright-Patterson Air Force Base, and Durham, NC VAMC), and government agencies as required by law. We will not share any information with any groups outside the VHA unless they agree to keep the information confidential and use it only for the purposes related to the study. Any information shared with these groups may no longer be protected under federal law.

You do not have to authorize the use of this information. If you decide not to authorize the use of this information, it will not affect the veteran's regular medical care or your rights as a VHA patient (if you are a patient), but the veteran will not be allowed to participate in the study. This authorization has no expiration date. You can withdraw this authorization at any time by writing to Dr. Oddone at HSR&D Service (152), VA Medical Center, 508 Fulton Street, Durham, NC 27710. If you withdraw this authorization, Dr. Oddone and his research team can continue to use information about the veteran that has been collected, but no additional information will be collected. The VHA complies with the requirements of the Health Insurance Portability and Accountability Act of 1996 and its privacy regulations and all other applicable laws that protect the veteran's privacy. We will protect the veteran's information according to these laws.

You need to be aware that:

- You are not required to authorize the veteran's participation in this study; your authorization of the veteran to participate is strictly voluntary.
- You can refuse to authorize the veteran's participation now or you can withdraw authorization for participation in the study at any time after giving your verbal consent. These actions will not interfere with either the veteran's regular medical treatment or your regular medical treatment, if you are a patient.
- Eligibility for medical care is based upon the usual VA eligibility policy and is not guaranteed by participation in this or any other research study.
- The investigators will let you and the veteran's physician know of any important discoveries made during this study which may affect the veteran, the veteran's health, or your willingness to authorize the veteran's participation in this study.
- There will be no costs to the veteran for being part of this research study nor will the veteran be paid for taking part in this study. Some veterans are required to pay co-payments for medical care and services provided by VA. These co-payments requirements will continue to apply to medical care and services provided by VA that are not part of this study.
- If results of this study are reported in medical journals or at meetings, the veteran will not be identified by name, by recognizable photograph, or by any other means without your specific consent. The veteran's medical records and other study-related records will be maintained according to this medical center's requirements. To protect the confidentiality of the veteran's information, all study records will be maintained in a manner such that only the researchers with a need to know will have access to them. There is a possibility that the U.S. Army Medical Research and Material Command

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS**

VERBAL INFORMED CONSENT SCRIPT - PROXY

as part of their responsibility to protect human subjects in research, the Office for Human Research Protections (OHRP), the Office of Research Oversight (ORO), or the Institutional Review Board (IRB) may inspect the records.

- In case of adverse (bad) effects or physical injury resulting from this study, eligible veterans are entitled to medical care and treatment. The Durham VAMC has not set aside compensation payable in the event of physical injury or illness resulting from participation in this study. In case of research related injury resulting from this study you should contact the principal investigator at (919) 286-6936 during the day, and at (919) 401-4403 after hours. Further information about compensation and medical treatment may be obtained from the medical administration service at 286-6957 at this VA medical center. Non-eligible veterans are entitled only to medical emergency care and treatment on a humanitarian basis.
- If you have questions about the research or the veteran's rights as a research subject, you may contact the administrative officer of the research service at (919) 286-0411 ext. 7632.
- If you would like to speak to the lead researchers of this study, you may contact them during normal business hours of 8 am to 5 pm, Monday through Friday at the following telephone numbers:
 - University of Cincinnati: Dr. Millhorn at (513) 558-9971 or Dr. Homer at (513) 558-2756
 - U.S. Air Force Research Laboratory: Dr. John Schlager at (937) 674-9570 or Lt Col Timothy Wells at (937) 255-3931.
 - Durham VA Medical Center: Dr. Oddone at (919) 286-6936.
- If you agree to the veteran participating in this study, you are not waiving any legal rights to which you or the veteran are otherwise entitled.
- A copy of this consent form will be provided to you to keep for your personal record.

Are you willing for the veteran to participate in this study and authorize the use of the veteran's information as described?

YES

NO

I certify that the proxy consented verbally to participate in the study and permit use of the veteran's personal health information:

Name of Person Obtaining Verbal Telephone Consent
And HIPAA Authorization

Date

Name of Legally Authorized representative of the Veteran

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS****VERBAL INFORMED CONSENT SCRIPT**

PATIENT ID: _____

NAME: _____

You previously participated in our research study: An Epidemiologic Investigation into the Occurrence of amyotrophic lateral sclerosis (ALS) in Gulf War Veterans. We thank you again for participating in this study. We are calling to ask you to participate in another research study related to ALS in veterans. This new study: Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans is sponsored by the United States Department of Defense. The purpose of this study is to identify substances called biomarkers that are associated with getting ALS. Biomarkers are proteins and other similar substances that are found in blood or other body fluids. By identifying biomarkers that are related to ALS, researchers may be able to identify people early who are at risk for getting ALS. These biomarkers may also help researchers to understand more about what causes ALS.

This study is being conducted by researchers at the University of Cincinnati in Cincinnati, Ohio, and at the U.S. Air Force Research Laboratory at the Wright-Patterson Air Force Base in Dayton, Ohio. Researchers at the Durham VA Medical Center are also participating in this study by helping to identify veterans with ALS who were part of the prior Gulf War ALS study. The person in charge of this study at the Durham VAMC is Dr. Eugene Oddone. Up to 150 military personnel and veterans will participate in this study of whom half will have been diagnosed with ALS.

The study will last about three years. However, your participation in the study is limited to giving us permission to give your social security number to the researchers at the U.S. Air Force Research Laboratory. These researchers will be accessing the Department of Defense Serum Repository. This repository contains blood samples from about 36 million people who were in military service from 1989 and later. If you were in the military during this time period, your blood sample may be a part of this collection. The blood samples are linked to the social security numbers of people who contributed the samples. When we give the researchers at the U.S. Air Force Research Laboratory your social security number, they will be able to find out whether your blood sample is included in the repository. If it is included, the researchers will analyze your blood sample for biomarkers. They will be comparing biomarkers in the blood samples of veterans who have ALS to veterans who do not have ALS. The researchers will destroy any unused part of your blood sample at the end of the study.

We will send your social security number to the researchers at the U.S. Air Force Research Laboratory in a password protected data file. Once they have received your social security number, they will also store it in a secure, password protected file that can only be accessed by key study personnel. Your social security number will be stored at the U.S. Air Force Research Laboratory by Lt. Col. Timothy Wells, who is in charge of sending social security numbers to the Department of Defense Serum Repository.

If you agree to participate in this study, we will only be asking you to give permission for us to make your social security number known to the U.S. Air Force Research Laboratory researchers. We will not ask you to give a new blood sample or perform any other activities. Participation in this study is not believed to have any risks for your health. Your social security number will be sent and stored in a secure fashion to minimize any risk of loss of confidentiality. Taking part in this study may not personally help you, but your participation may lead to knowledge that will help others.

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS**

VERBAL INFORMED CONSENT SCRIPT

As part of this study we are asking you to authorize Dr. Oddone and his research team to access the following information about you: your social security number and diagnosis of ALS. This is information you provided us previously as part of the study of ALS in Gulf War Veterans.

We may disclose your information to the U.S. Air Force Research Laboratory, Department of Defense Serum Repository, and University of Cincinnati researchers involved in this study, the Institutional Review Boards that oversee this project (University of Cincinnati, Wright-Patterson Air Force Base, and Durham, NC VAMC), and government agencies as required by law. We will not share any information with any groups outside the VHA unless they agree to keep the information confidential and use it only for the purposes related to the study. Any information shared with these groups may no longer be protected under federal law.

- You do not have to authorize the use of this information. If you decide not to authorize the use of this information, it will not affect your regular medical care or your rights as a VHA patient, but you will not be allowed to participate in the study. This authorization has no expiration date. You can withdraw this authorization at any time by writing to Dr. Oddone at HSR&D Service (152), VA Medical Center, 508 Fulton Street, Durham, NC 27710. If you withdraw this authorization, Dr. Oddone and his research team can continue to use information about you that has been collected, but no additional information will be collected. The VHA complies with the requirements of the Health Insurance Portability and Accountability Act of 1996 and its privacy regulations and all other applicable laws that protect your privacy. We will protect your information according to these laws.

You need to be aware that:

- You are not required to participate in this study; your participation is strictly voluntary.
- You can refuse to participate now or you can withdraw from the study at any time after giving your verbal consent. These actions will not interfere with your regular medical treatment, if you are a patient. Eligibility for medical care is based upon the usual VA eligibility policy and is not guaranteed by participation in this or any other research study.
- The investigators will let you and your physician know of any important discoveries made during this study which may affect you, your condition, or your willingness to participate in this study.
- There will be no costs to you for being part of this research study nor will you be paid for taking part in this study. Some veterans are required to pay co-payments for medical care and services provided by VA. These co-payments requirements will continue to apply to medical care and services provided by VA that are not part of this study.
- If results of this study are reported in medical journals or at meetings, you will not be identified by name, by recognizable photograph, or by any other means without your specific consent. Your medical records and other study-related records will be maintained according to this medical center's requirements. To protect the confidentiality of your information, all study records will be maintained in a manner such that only the researchers with a need to know will have access to them. There is a possibility that the U.S. Army Medical Research and Materiel Command as part of their responsibility to protect human subjects in research, the Office for Human Research Protections (OHRP), the Office of Research Oversight (ORO), or the Institutional Review Board (IRB) may inspect the records.
- In case of adverse (bad) effects or physical injury resulting from this study, eligible veterans are entitled to medical care and treatment. The Durham VAMC has not set aside compensation payable in

**BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS AMONG GULF WAR
VETERANS**

VERBAL INFORMED CONSENT SCRIPT

the event of physical injury or illness resulting from participation in this study. In case of research related injury resulting from this study you should contact the principal investigator at (919) 286-6936 during the day, and at (919) 401-4403 after hours. Further information about compensation and medical treatment may be obtained from the medical administration service at 286-6957 at this VA medical center. Non-eligible veterans are entitled only to medical emergency care and treatment on a humanitarian basis.

- If you have questions about the research or your rights as a research subject, you may contact the administrative officer of the research service at (919) 286-0411 ext. 7632.
- If you would like to speak to the lead researchers of this study, you may contact them during normal business hours of 8 am to 5 pm, Monday through Friday at the following telephone numbers:
 - University of Cincinnati: Dr. Millhorn at (513) 558-9971 or Dr. Horner at (513) 558-2756
 - U.S. Air Force Research Laboratory: Dr. John Schlager at (937) 674-9570 or Lt Col. Timothy Wells at (937) 255-3931
 - Durham VA Medical Center: Dr. Oddone at (919) 286-6936.
- If you agree to participate in this study, you are not waiving any legal rights to which you are otherwise entitled.
- A copy of this consent form will be provided to you to keep for your personal record.

Are you willing to participate in this study and authorize the use of your information as described?

YES

NO

I certify that the veteran consented verbally to participate in the study and permit use of their personal health information:

Name of Person Obtaining Verbal Telephone Consent
And HIPAA Authorization

Date



**Department of Genome Science
Genome Research Institute**
2180 E. Galbraith Road
Cincinnati, OH 45237

Phone: (513) 558-5473
Fax: (513) 558-5061

Tim Wells, LtCol, USAF, BSC
AFRL/HEPA
Bldg 824 Rm 206, 2800 Q Street
Wright-Patterson AFB, OH 45433

Lt. Col. Wells,

Attached is the response from the University of Cincinnati with regards to the protocol and work associated with the "Biomarkers of ALS in Gulf War Veterans" project. The IRB has determined that with regards to the work to be undertaken at the University of Cincinnati, it is "not research involving human subjects." The basis for this decision, as communicated to me is that the University of Cincinnati will not have any direct connection to or information from patients and that samples will come to the University of Cincinnati from AFRL without any patient related identification.

I am passing this information to you as you have been coordinating the submissions to the Army Medical Research and Materiel Command. Please send this information to them for their review.

Sincerely,

A handwritten signature in cursive script that reads 'David E. Millhorn'.

David E. Millhorn
Professor
Principal Investigator: BALSAM Project



Institutional Review Board

Medical Center

University of Cincinnati

PO Box 670567

Cincinnati OH 45267-0567

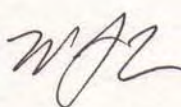
G-08 Wherry Hall

Eden Avenue and Albert Sabin Way

Phone (513) 558-5259

Fax (513) 558-4111

TO: David Millhorn, PhD
Department of Genome Science
ML 0505

FROM: Mike Linke, Ph.D., Chairman 
University of Cincinnati
Institutional Review Board #1

DATE: February 9, 2007

RE: 07-01-31-01 - Biomarkers for Amyotrophic Lateral Schlerosis in Gulf War Veterans.

Please be advised that I have reviewed the study referenced above as outlined in your January 25, 2007 correspondence, and have determined that the work described in this project is not research involving human subjects as described in 45CFR46.102(d, e, f).

Thank you for your continued compliance with the Board's requirements with regard to your research activities.

 UNIVERSITY OF Cincinnati	Determining Whether a Proposed Activity is Human Research According to DHHS or FDA Regulatory Definitions Investigators: Please complete this form and checklist; submit in hard copy to the IRB Office, ML 0567 (G-08 Wherry Hall). You will be notified in writing of the IRB's determination.
PERSON REQUESTING DETERMINATION AND CONTACT INFORMATION:	David Millhorn, PhD Department of Genome Science Phone: 558-5473 / Mail Location: 0505 email: david.millhorn@uc.edu
TITLE OF STUDY:	Biomarkers for Amyotrophic Lateral Sclerosis in gulf War Veterans
DESCRIPTION OF RESEARCH, INCLUDING THE PURPOSE:	See attached protocol

☐ The activity involves research because all of the following are true:

☐ The activity is a systematic investigation, including research development, testing and evaluation

☐ Either of the following is true:

☐ The activity is designed to develop generalizable knowledge.

☐ The activity is designed to contribute to generalizable knowledge.

☐ The activity involves human participants because both of the following are true:

☐ The data the investigator is planning to obtain are about living individuals

☐ Either or both of the following is true

☐ The investigator plans to obtain the data through one or more of the following:

☐ Physical procedures performed on those individuals

☐ Manipulation of those individuals

☐ Manipulation of those individuals' environments

☐ Communication with those individuals

☐ Interpersonal contact with those individuals

☐ The information to be obtained is both:

☐ Private, because either of the following is true

☐ The information is about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place

☐ The individual has provided the information for specific purposes and can reasonably expect that the information will not be made public (for example, a medical record)

☐ Individually identifiable, because either of the following is true:

☐ The identity of the participant is or may readily be ascertained by the investigator

☐ The identity of the participant is or may readily be associated with the information

Research for which FDA regulations may apply

- ☐ The activity involves an FDA regulated test article because one or more of the following is true:
- ☐ The activity involves the use of a drug, other than the use of an marketed drug in the course of medical practice:
- ☐ The activity will involve the use of a drug, meaning one of the following:
- ☐ An article recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them
 - ☐ An article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans or other animals
 - ☐ An article (other than food) intended to affect the structure or any function of the body of humans or other animals
 - ☐ An article intended for use as a component of any article specified in the above items
- ☐ Either of the following is true:
- ☐ The drug is **NOT** approved by the FDA for marketing
 - ☐ The drug is **NOT** being used in the course of medical practice
- ☐ The activity involves the use of a medical device, other than the use of an marketed medical device in the course of medical practice:
- ☐ The activity will involve the use of a medical device, meaning one of the following:
- ☐ Recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them
 - ☐ Intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in humans or other animals
 - ☐ Intended to affect the structure or any function of the body of humans or other animals, and which does not achieve any of it's primary intended purposes through chemical action within or on the body of humans or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes
- ☐ Either of the following is true:
- ☐ The medical device is **NOT** approved by the FDA for marketing
 - ☐ The medical device is **NOT** being used in the course of medical practice
- ☐ The activity is otherwise subject to FDA regulation:
- ☐ Data from the activity will be submitted to, or held for inspection by, the FDA.
 - ☐ The activity involves an FDA-regulated article one or more of the following:

☐ Food or dietary supplement that bears a nutrient content or a health claim ☐ Food or color additive for human consumption	☐ Infant formula ☐ Biological product for human use ☐ Electronic product for human use ☐ Other article subject to the FD&C Act
--	---
- ☐ The activity involves human participants because one or more of following is true:
- ☐ The test article will be used on one or more humans
 - ☐ All of the following are true:
 - ☐ The test article is a medical device
 - ☐ The medical device will be used on human specimens
 - ☐ The activity is being done to determine the safety or effectiveness of the device
 - ☐ Data from the activity will be submitted to, or held for inspection by, the FDA.

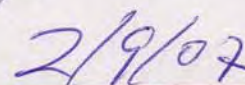
For IRB Office Use Only

Determined to be human research ☐

Determined to **NOT** be human research ☒



Signature of IRB Chair or Designee



Dated

Appendix E

Request for Continued Approval
VAMC Durham, NC

Principal Investigator: Oddone, Eugene Z., M.D.

Proj. No: 0026/MIRB No: 1025

Project Title: Biomarkers for ALS among Active Duty Military

Last Review: 7/5/06

Approval Period: 7/5/07

through: 7/4/08

6/7/07

6/6/08

Funding/Administration (Identify the current funding source):

1. Project Status: (Check one)

- ☐ Project is Active but there was no enrollment during this review cycle.
- ☒ Project is Active and Human Subjects are involved.
- ☐ Project is active but closed to accrual. Subjects are being followed.
- ☐ Project is active, no subjects are being enrolled or are in follow-up, but data is being analyzed.
- ☐ Project is completed and there is no further data analysis, close the study. (Append overview of study.)

2. A signed consent form is in my files for each subject entered into this study and is also in the medical record for each VA subject entered into this study. ☐ Yes ☒ No

3. Total number of subjects entered into study. 51

Total number of subjects entered into study since last continuation report. 51

4. One or more subjects have claimed injury from participating in this study. ☐ Yes ☒ No

5. Adverse events that did not require immediate IRB notification have been appended. ☐ Yes ☒ No

If item 4 or 5 is yes, attach a detailed explanation citing dates, subjects, and circumstances, and state if a report was filed with the Human Studies Subcommittee (IRB) or an agency such as the NIH or FDA.

I am aware that all research projects using Human subjects must receive prior approval by the Human Studies Subcommittee (IRB), that any change in human use requires prior approval by the Subcommittee, that a signed consent form must be obtained from each subject before entry into the study, that continued human use approval requires annual review, that human use in projects not receiving favorable review must be discontinued, and that a copy of all consent forms and such other related matters as correspondents must be obtained by the Principal Investigator for three (3) years after the study is terminated. This form, together with any requested additional information, is submitted with compliance with these regulations.

E. Oddone
Principal Investigator Signature

5/1/07
Date

☒ Approve ☐ Disapprove

[Signature] 6/7/2007
Chairman, Institutional Review Board Date

☒ Approve ☐ Disapprove

[Signature] 6-8-07
Chairman, Bio-Safety Subcommittee Date

☒ Approve ☐ Disapprove

[Signature]
Chairman, Research & Development Committee

6/23/07
Date

PROTOCOL CONTINUING REVIEW STATUS

Your response to this request must be received before: **26 August 2007**

The protocol referenced below is within the 30-day window for expiration.

- a. CDO Number:** CDO-062022
- b. Vendor Protocol Number:** F-WR-2006-0054-H
- c. Title:** Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military
- d. Principal Investigator:** Dr. David Millhorn
- e. Government Project Manager:** Dr. John Schlager

1. Do you wish to continue this data collection/study? Yes ☒ No ☐

2. If you answered "YES" to question #1, please respond to all of the following questions. If you answered "NO" to question #1, please insert "n/a" in question (a) below and continue answering the remaining questions.

a. What is the reason for continuation of the data collection/study: e.g. equipment problems etc? The study is planned for three years, beginning on October 1, 2005.

b. Status of Data Collection: How many subjects have you obtained data on (include gender breakout). The staff of the Veteran's Administration ALS Registry are in the process of obtaining consent from Registry members at this time.

c. Adverse Events: (Did anyone get hurt?) None.

d. Do you have any preliminary results? None, see 2.b. above.

e. If subjects have dropped out of the project, what is the reason given for dropping out? N/A

f. Publication Prospects? Yes

g. How many signed consent forms have you collected? Please note it is your responsibility to maintain signed consent forms. The total number consented to date is 51, but recruitment continues at this time.

Completed by:



Date Completed:

8/3/07

8/3/2007



DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

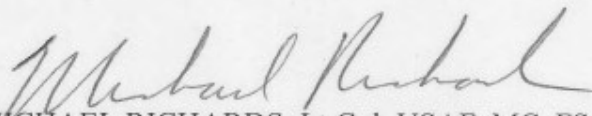
AUG 14 2007

MEMORANDUM FOR AFRL/HEPB (DAVID MILLHORN, JOHN SCHLAGER)

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 Gulf War Veterans
2. Protocol number: F-WR-2006-0054-H
3. Risk: Minimal
4. Approval date: 27 July 2007
5. Expiration date: 27 July 2008
6. Scheduled renewal date: 27 June 2008
7. Type of review: Continuing
8. 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 established by the U.S. Department of Health and Human Services per category (7): Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.
9. This approval applies to human use research (as defined in 32 CFR 219 and AFI 40-402) portions of this project only. Attitude and opinion surveys associated with this research must be conducted IAW AFI 36-2601.
10. 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.
11. 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>.
12. 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, Lt Col, USAF, MC, FS
AFRL/Wright Site IRB



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

22 June 2007

MEMORANDUM FOR AFRL/HEH (Wright Site Institutional Review Board)

FROM: AFRL/HEPB

SUBJECT: Progress report for Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans, F-WR-2006-00554-H

1. Status of Study: On 24 February 2007, approval was received from USAMRMC for the study protocol which was amended several times in 1Q FY07 in order to meet their criteria. In March, Dr. Eugene Oddone's group drafted a consent letter and began soliciting approvals from Gulf War (GW) veterans or their next of kin from the original Dr. Horner ALS study. By early May, 49 of these veteran cases had consented to have their serum involved in the study. In order to obtain at least 75 cases, other veterans from the National Registry of Veterans with ALS must be solicited for involvement. To enroll these veterans, an amendment must be made to the Durham VAMC IRB, which is in progress. The next step is to identify how many of these cases have serum samples in the DoD Serum Repository and available medical records. After selection of 75 positive ALS subjects, AMSA will select at least 75 appropriate control samples.

No SSN information has been transferred to Col. Timothy Wells at this time, and is pending completion of case ascertainment at Durham.

Some experimental work has been conducted on the equipment at The Genome Research institute (UC) to work out the sample handling protocols.

2. Summary of Subjects: A total of 49 veteran serum samples have been consented for involvement in this study. All are samples from Dr. Horner's original ALS study. Solicitation of other Gulf War veterans in the National Registry of Veterans with ALS will begin as soon as the Durham VAMC IRB is amended.

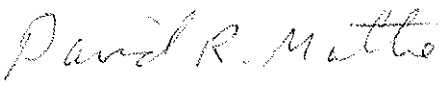
Summary of Resources: Considerable man-hours were spent in protocol revisions necessary to meet USAMRMC IRB requirements since the last review. Current man-hours and resources are being spent to identify ALS registry members who are willing to participate in the study but this is not a cost being charged to the study. Three months of man-hours were spent at UC/GRI optimizing the immunodepletion and the 2D-DIGE methods for serum samples.

3. Objective: This study is at the very early stages of selecting cases. It will be a number of months before samples are delivered to either of the labs involved. The final objective is to analyze serum samples to identify disease markers and to analyze pre-diagnosis serum samples in an effort to identify the presence of biomarkers prior to clinical diagnosis. The project is moving forward at last, but more slowly than anticipated.

Publications or Presentations: None

5. Adverse events: N/A

6. Amendments to Protocol: None at this time, protocol was recently amended on 29 December 2006.


for John J. Schlager, Ph.D., DABT
Principal Investigator

Attachments:

1. Updated CITI certificates
2. Original signed informed consent documents (This is a requirement)

OASD (HA) TMA HPA&E

27 AUG 2007

MEMORANDUM FOR: Col Timothy Wells, USAF AFRL/HEPA

SUBJECT: Protocol Approval - Continuing Review

1. This is to advise that the TMA IRB Program Office concurs with the continuing review approved by AFRL/Wright Site IRB

Protocol Number: CDO-06-2022

Primary IRB Number: F-WR-2006-0054-H

Protocol Title: Biomarkers for Amyotrophic Lateral Sclerosis in Gulf War Veterans

Principal Investigator (PI): David E. Millhorn, PhD; John J. Schlager, PhD, Air Force Research Laboratory, Wright Patterson AFB

2. You are reminded that you must notify this office if the project is altered in any way (e.g. changes in location, investigators, the number of subjects, age of subjects, changes to the informed consent form or any change methodology). You are further advised that it is your responsibility to ensure you and your associate investigators adhere to the guidelines of the protocol. Additionally you are also required to report any adverse events and actions taken to mitigate the events to the undersigned within 24 hours. **This protocol approval expires on 27 JULY 2008.**

3. Please note that this approval does not exempt this survey from any licensing requirements that may apply. You may still need to submit your survey to Washington Headquarters Services (WHS) for approval and licensing under DoDD 8910.1 and/or to another agency (i.e. Office of Management and Budget) for approval. You should contact these agencies for additional information prior to starting your study."

4. Should you have any questions, please feel free to contact the undersigned at 703-681-3636



Lorraine A. Babeu

LTC, MS

Exempt Determination Official for

HA/TMA Human Subject Protection Program

Appendix I

HRPO Log No. A-13569 USAMRMC Human Research Protection Office Continuing Review Submission Checklist

CONTINUING REVIEW OR TERMINATION DOCUMENTS TO SEND TO THE HRPO (If Applicable)

x The continuing review summary report that was submitted to your IRB – [Attachment 1](#)

x Local IRB approval letter with next expiration date – [Attachment 2 \(Expiration date: 27July08\)](#)

x Current copy of Protocol. Please list or track all Amendments that have occurred since the last time the protocol was submitted to HRPO.

- [16Aug06 – original IRB Approval \(Attachment 3\)](#)
- [29Dec06 - approval after amendments required by USAMRMC \(Attachment 4\)](#)

x Current consent form, if applicable. List or track all revisions that have occurred since the last time the consent form was submitted to HRPO. [No new revisions, Attachment 5](#)

THE FOLLOWING CHECKLIST IS PROVIDED AS A GUIDANCE REFERENCE REGARDING THE REQUIRED ELEMENTS TO BE INCLUDED IN A CONTINUING REVIEW REPORT, PLEASE ENSURE THAT APPLICABLE ITEMS ARE ADDRESSED IN THE CONTINUING REVIEW REPORT OR ATTACHED IN A SEPARATE DOCUMENT:

x Total number of subjects enrolled in the study (i.e., number recruited, enrolled, withdrawn by PI, discontinuation by subject, disenrolled [deaths, other])

- [51 of 53 solicited \(or their families if deceased\) agreed to the use of their sera for this study.](#)

x Breakdown of participants by demographics as appropriate (e.g., groups/cohorts, gender, age, ethnicity, special populations)

[Although recruitment is ongoing, below describes the demographics to date:](#)

- [Cohort – Enrolled Veterans from the Durham VA ALS Registry](#)
- [Gender – 51 male, 0 female](#)
- [Age – average is 46.5, range is 39-59. Only 24 out of 51 ages are reported](#)
- [Ethnicity – 39 white, 6 black, 3 Hispanic, 3 not specified](#)
- [Special Populations - none](#)

na Summary of SAEs, adverse events and unanticipated problems involving risks to subjects or others

na Summary of withdrawals that have occurred, with reasons for withdrawal

na Summary of complaints received

na Summary of deviations that have occurred

na Report includes a summary of research progress, including results obtained to date

Appendix I

[na](#) Documentation of literature review update, including databases searched, dates of searches, key words and subject areas searched. Risk/benefit assessment or other protocol activities updated as necessary based on review of literature. Measures included to reduce or minimize any newly identified risks

[see above](#) Summary of any amendments, addendums, or modifications that have been made to the protocol since the initial approval (administrative, minor and major changes)

Name of individual to contact with questions regarding this report [Col. Timothy Wells](#)

Contact information (include email and phone number) [Col Timothy Wells](#)

2800 Q Street, Bldg 824
Wright-Patterson AFB, OH 45433-
timothy.wells@wpafb.af.mil, 937-255-3931

Date (day month year) [28Aug07](#)

Appendix J

ALS Cases Identified & Consented for Balsam Study

ID	Gender	Hispanic Yes / No	White	Black	Dead	Age
1001MG	1:Male	No	Yes	0	Dead	
1005GG	1:Male	No	Yes	0	Dead	
1009DH	1:Male	No	Yes	0	Dead	
1010RT	1:Male	No	Yes	0	Dead	
1011EH	1:Male	No	Yes	0	Dead	
1012FH	1:Male	No	Yes	0	Dead	
1013DA	1:Male	No	Yes	0	Dead	
1016JC	1:Male	Yes	0	Yes		50
1018TO	1:Male	No	Yes	0		50
1019WG	1:Male	No		Yes	Dead	
1020KN	1:Male	No	Yes	0		39
1037AS	1:Male	No	Yes	0		54
1040MD	1:Male	No	Yes	0	Dead	
1046RS	1:Male	No	Yes	0		41
1057GI	1:Male	No	Yes	0		55
1071PJ	1:Male			0	Dead	
1079SP	1:Male	No	Yes	0		59
1094RH	1:Male	No	Yes	0		45
1135RS	1:Male	No	Yes	0		48
1137TK	1:Male	No	Yes	0	Dead	
1140CL	1:Male	No	Yes	0		53
1164JQ	1:Male	Yes	Yes	0		43
1175AE	1:Male	No	Yes	0		42
1184EC	1:Male			0	Dead	
1201PG	1:Male			0	Dead	
1202PS	1:Male	Yes	Yes	0		41
1206MA	1:Male	No	Yes	0		36
1232PS	1:Male	No	Yes	0		56
1237JB	1:Male	No	Yes	0	Dead	
1306SR	1:Male	No	Yes	0		40
1308DA	1:Male	No	0	Yes		53
1310JT	1:Male	No	Yes	0		39
1320PL	1:Male	No	Yes	0		43
1322JS	1:Male	No	Yes	0		48
1326TM	1:Male	No	0	Yes		45
1327MC	1:Male	No	Yes	0	Dead	
1333JH	1:Male	No	Yes	0	Dead	
1334RB	1:Male	No	Yes	0	Dead	
1337BD	1:Male			0	Dead	
1359TL	1:Male			0	Dead	
1360JA	1:Male	No	0	Yes		46
1361BW	1:Male	No	Yes	0		47
1365CS	1:Male	No	Yes	0	Dead	

Appendix J

ALS Cases Identified & Consented for Balsam Study

1383HN	1:Male	No	Yes	0	Dead	
1384RW	1:Male	No	Yes	0	Dead	
1385DM	1:Male	No	Yes	0		43
1388MW	1:Male			0	Dead	
1389ME	1:Male	No	Yes	0	Dead	
1393JL	1:Male	No	Yes	0	Dead	
1394MF	1:Male	No		Yes	Dead	
2096TS	1:Male	No	Yes	0	Dead	
		3 hispanic	39 white	6 black	27 dead	46.5 avg age
		42 non hispanic	4 nonwhite	45 nonblack		24 ages reported
		6 unknown	8 unknown			



OFFICE OF THE ASSISTANT SECRETARY OF DEFENSE
HEALTH AFFAIRS

SKYLINE FIVE, SUITE 810, 5111 LEESBURG PIKE
FALLS CHURCH, VIRGINIA 22041-3206

TRICARE
MANAGEMENT
ACTIVITY

LtCol Timothy Woodruff
AFRL/HEP
Bldg 33, Rm 325
2215 1st Street.
Wright-Patterson AFB, OH 45433-7901

SEP -5 2007

Dear LtCol Woodruff,

The renewal request for Data Use Agreement (DUA) #06-347 submitted for the project titled, *Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military* has been approved for Protected Health Information (PHI). The files mentioned below are to be used only for the purpose(s) cited in this request, and will not be released to other organizations without prior DoD approval. The information related to this DUA unless renewed, may be retained until 30 September 2008.

Approved is the following data system and files via access:

Data System	File	Year(s)	Data Elements
As stated in renewal request.	As stated in renewal request.	As stated in renewal request.	As stated in renewal request.

Please note you will take reasonable steps to implement appropriate procedural, administrative, technical and physical safeguards to prevent unauthorized use or loss, theft, or compromise of Protected Health Information (PHI) or Personally Identifiable Information (PII). The Department of Health and Human Services HIPAA Privacy Rule promulgated under Health Insurance Portability and Accountability Act of 1996 and is applicable to uses and releases of data on or after April 14, 2003. All uses and releases of data are subject to the requirements of that rule, and DoD 6025.18-R, "DoD Health Information Privacy Regulation," January 24, 2003, which implements that Final Privacy Rule within DoD. Your Requestor must abide by the terms as outlined in the enclosed Business Associate Agreement. You will convey the responsibility to protect information under the agreement to all contractors, subcontractors and business associates who will have access to this data on your behalf.

Additionally, please provide the Health Affairs/TRICARE Management Activity (HA/TMA) Gatekeeper (HATMAIRB@tma.osd.mil) with an electronic copy of each peer reviewed journal article, publication, or report of findings, at the time of submission.

All future additional data requests in support of this project must be submitted in writing to: Privacy Officer, TMA Privacy Office. TMA Privacy Office has the authorization to review and approve all requests.

Enclosed are a copy of the signed Data Use Agreement (DUA), a copy of the Business Associate Agreement for your records and the approved Data Elements. Please reference these documents for information regarding use of the data, and/or the data access privileges that your Requestor(s) has received from DoD. The Project Study name and DUA #06-347A number will be necessary in all future correspondence with TMA Privacy Office.

If you have any questions about this DUA or the use of DoD data, you may contact Heather Goodwin at (703) 575-6545x 280.

Sincerely,



Leslie V. Shaffer
Privacy Officer
TRICARE Management Activity

Enclosures:
As Stated

cc:
LtCol Timothy Wells
AFRL/HEPA
Bldg 824, Room 206
2800 Q Street
Wright-Patterson AFB, OH 45433

July 2005

DUA Addendum/HIPAA Business Associate Agreement Clause

PRIVACY OF PROTECTED HEALTH INFORMATION

Introduction

IAW DoD 6025.18-R "Department of Defense Health Information Privacy Regulation" the Contractor meets the definition of Business Associate. Therefore, a Business Associate Agreement is required to comply with both the Health Insurance Portability and Accountability Act (HIPAA) Privacy and Security regulations. This clause serves as that agreement whereby the Contractor agrees to abide by all applicable HIPAA Privacy and Security requirements regarding health information as defined in this clause, and DoD 6025.18-R, as amended. Additional requirements will be addressed when implemented.

(a) *Definitions.* As used in this clause generally refer to the Code of Federal Regulations (CFR) definition unless a more specific provision exists in DoD 6025.18-R

Individual has the same meaning as the term "individual" in 45 CFR 164.501 and shall include a person who qualifies as a personal representative in accordance with 45 CFR 164.502(g).

Privacy Rule means the Standards for Privacy of Individually Identifiable Health Information at 45 CFR part 160 and part 164, subparts A and E.

Protected Health Information has the same meaning as the term "protected health information" in 45 CFR 164.501, limited to the information created or received by the Requesting Organization, hereafter referred to as Requestor, from or on behalf of The Government.

Electronic Protected Health Information has the same meaning as the term "electronic protected health information" in 45 CFR 160.103.

Required by Law has the same meaning as the term "required by law" in 45 CFR 164.501 and 164.103.

Secretary means the Secretary of the Department of Health and Human Services or his/her designee.

Security Rule means the Health Insurance Reform: Security Standard at 45 CFR part 160, 162, and part 164, subpart C.

Terms used, but not otherwise defined, in this Agreement shall have the same meaning as those terms in 45 CFR 160.103, 164.501, and 164.304.

(b) The Requestor agrees to not use or further disclose Protected Health Information other than as permitted or required by the DUA or as Required by Law.

(c) The Requestor agrees to use appropriate safeguards to prevent use or disclosure of the Protected Health Information other than as provided for by this DUA.

(d) The Requestor agrees to use administrative, physical, and technical safeguards that reasonably and appropriately protect the confidentiality, integrity, and availability of the electronic protected health information that it creates, receives, maintains, or transmits in the execution of this DUA.

(e) The Requestor agrees to mitigate, to the extent practicable, any harmful effect that is known to the Requestor of a use or disclosure of Protected Health Information by the Requestor in violation of the requirements of this DUA.

(f) The Requestor agrees to report to the Government any security incident involving protected health information of which it becomes aware.

(g) The Requestor agrees to report to the Government any use or disclosure of the Protected Health Information not provided for by this DUA of which it becomes aware.

(h) The Requestor agrees to ensure that any agent, including a subcontractor, to whom it provides Protected Health Information received from, or created or received by the Requestor on behalf of the Government, agrees to the same restrictions and conditions that apply through this DUA to the Requestor with respect to such information.

(i) The Requestor agrees to ensure that any agent, including a subcontractor, to whom it provides electronic Protected Health Information, agrees to implement reasonable and appropriate safeguards to protect it.

(j) The Requestor agrees to provide access, at the request of the Government, and in the time and manner designated by the Government to Protected Health Information in a Designated Record Set, to the Government or, as directed by the Government, to an Individual in order to meet the requirements under 45 CFR 164.524.

(k) The Requestor agrees to make any amendment(s) to Protected Health Information in a Designated Record Set that the Government directs or agrees to pursuant to 45 CFR 164.526 at the request of the Government or an Individual, and in the time and manner designated by the Government.

(l) The Requestor agrees to make internal practices, books, and records relating to the use and disclosure of Protected Health Information received from, or created or received by the Requestor on behalf of, the Government, available to the Government, or at the request of the Government to the Secretary, in a time and manner designated by the Government or the Secretary, for purposes of the Secretary determining the Government's compliance with the Privacy Rule.

(m) The Requestor agrees to document such disclosures of Protected Health Information and information related to such disclosures as would be required for the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

(n) The Requestor agrees to provide to the Government or an Individual, in time and manner designated by the Government, information collected in accordance with this Clause of the DUA, to permit the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

General Use and Disclosure Provisions

Except as otherwise limited in this Agreement, the Requestor may use or disclose Protected Health Information on behalf of, or to provide services to, the Government for the purpose stated in the DUA, if such use or disclosure of Protected Health Information would not violate the Privacy Rule or DoD 6025.18-R if done by the Government.

Specific Use and Disclosure Provisions

(a) Except as otherwise limited in this Agreement, the Requestor may use Protected Health Information for the proper management and administration of the Requestor or to carry out the legal responsibilities of the Requestor.

(b) Except as otherwise limited in this Agreement, the Requestor may disclose Protected Health Information for the proper management and administration of the Requestor, provided that disclosures are required by law, or the Requestor obtains reasonable assurances from the person to whom the information is disclosed that it will remain confidential and used or further disclosed only as required by law or for the purpose for which it was disclosed to the person, and the person notifies the Requestor of any instances of which it is aware in which the confidentiality of the information has been breached.

July 2005

DUA Addendum/HIPAA Business Associate Agreement Clause

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Introduction

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(d) The Requestor agrees to use administrative, physical, and technical safeguards that reasonably and appropriately protect the confidentiality, integrity, and availability of the electronic protected health information that it creates, receives, maintains, or transmits in the execution of this DUA.

(e) The Requestor agrees to mitigate, to the extent practicable, any harmful effect that is known to the Requestor of a use or disclosure of Protected Health Information by the Requestor in violation of the requirements of this DUA.

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(g) The Requestor agrees to report to the Government any use or disclosure of the Protected Health Information not provided for by this DUA of which it becomes aware.

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(k) The Requestor agrees to make any amendment(s) to Protected Health Information in a Designated Record Set that the Government directs or agrees to pursuant to 45 CFR 164.526 at the request of the Government or an Individual, and in the time and manner designated by the Government.

(l) The Requestor agrees to make internal practices, books, and records relating to the use and disclosure of Protected Health Information received from, or created or received by the Requestor on behalf of, the Government, available to the Government, or at the request of the Government to the Secretary, in a time and manner designated by the Government or the Secretary, for purposes of the Secretary determining the Government's compliance with the Privacy Rule.

(m) The Requestor agrees to document such disclosures of Protected Health Information and information related to such disclosures as would be required for the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

(n) The Requestor agrees to provide to the Government or an Individual, in time and manner designated by the Government, information collected in accordance with this Clause of the DUA, to permit the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

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Specific Use and Disclosure Provisions

(a) Except as otherwise limited in this Agreement, the Requestor may use Protected Health Information for the proper management and administration of the Requestor or to carry out the legal responsibilities of the Requestor.

(b) Except as otherwise limited in this Agreement, the Requestor may disclose Protected Health Information for the proper management and administration of the Requestor, provided that disclosures are required by law, or the Requestor obtains reasonable assurances from the person to whom the information is disclosed that it will remain confidential and used or further disclosed only as required by law or for the purpose for which it was disclosed to the person, and the person notifies the Requestor of any instances of which it is aware in which the confidentiality of the information has been breached.

(c) Except as otherwise limited in this Agreement, the Requestor may use Protected Health Information to provide Data Aggregation services to the Government as permitted by 45 CFR 164.504(e)(2)(i)(B).

(d) Requestor may use Protected Health Information to report violations of law to appropriate Federal and State authorities, consistent with 45 CFR 164.502(j) (1).

Obligations of the Government

Provisions for the Government to Inform the Requestor of Privacy Practices and Restrictions

(a) The Government shall provide at, <http://www.tricare.osd.mil/tmaprivacy/hipaa/hipaacompliance/index.htm>, the Requestor with the notice of privacy practices that the Government produces in accordance with 45 CFR 164.520, as well as any changes to such notice.

(b) The Government shall provide the Requestor with any changes in, or revocation of, permission by Individual to use or disclose Protected Health Information, if such changes affect the Requestor's permitted or required uses and disclosures.

(c) The Government shall notify the Requestor of any restriction to the use or disclosure of Protected Health Information that the Government has agreed to in accordance with 45 CFR 164.522.

Permissible Requests by the Government

The Government shall not request the Requestor to use or disclose Protected Health Information in any manner that would not be permissible under the Privacy Rule if done by the Government, except for providing Data Aggregation services to the Government and for management and administrative activities of the Requestor as otherwise permitted by this clause.

Termination

(a) Termination. A breach by the Requestor of this clause, may subject the Requestor to termination under any applicable default or termination provision of this DUA.

(b) Effect of Termination.

(1) If this DUA has records management requirements, the records subject to the Clause should be handled in accordance with the records management requirements. If this DUA does not have records management requirements, the records should be handled in accordance with paragraphs (2) and (3) below

(2) If this DUA does not have records management requirements, except as provided in paragraph (3) of this section, upon termination of this DUA, for any reason, the Requestor shall destroy all Protected Health Information received from the Government, or created or received by the Requestor on behalf of the Government, unless expressly requested to return the information to the Government. This provision shall apply to Protected Health Information that is in the possession of subcontractors or agents of the Requestor. The Requestor shall retain no copies of the Protected Health Information and shall submit in writing to the Government certification that the Protected Health Information has been destroyed within 30 days of the DUA end date.

(3) If this DUA does not have records management provisions and the Requestor determines that returning or destroying the Protected Health Information is infeasible, the Requestor shall provide to the Government notification of the conditions that make return or destruction infeasible. Upon mutual agreement of the Government and the Requestor that return or destruction of Protected Health Information is infeasible, the Requestor shall extend the

protections of this DUA to such Protected Health Information and limit further uses and disclosures of such Protected Health Information to those purposes that make the return or destruction infeasible, for so long as the Requestor maintains such Protected Health Information.

Miscellaneous

(a) Regulatory References. A reference in this Clause to a section in DoD 6025.18-R, Privacy Rule or Security Rule means the section as in effect or as amended, and for which compliance is required.

(b) Survival. The respective rights and obligations of Business Associate under the "Effect of Termination" provision of this Clause shall survive the termination of this DUA.

(c) Interpretation. Any ambiguity in this Clause shall be resolved in favor of a meaning that permits the Government to comply with DoD 6025.18-R, Privacy Rule or Security Rule.

Please note the language contained in this agreement cannot be altered in any form.



DEPARTMENT OF THE AIR FORCE

 AIR FORCE RESEARCH LABORATORY
 WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

DUA #06-347A

2 July 2007

 TMA Privacy Office
 Skyline Five, Suite 810
 5111 Leesburg Pike
 Falls Church, VA 22041

Received

JUL 17 2007

TMA Privacy Office

Dear Mr. Jenkins:

I am requesting permission to reuse data files listed below for the project or study originally titled **Biomarkers for Amyotrophic Lateral Sclerosis in Active Duty Military**. I am also requesting the renewal of DUA #06-347 in which these files were originally used for and given to me until 30 September 2007 to complete the research.

Data system (e.g. M2, MDR)	File (e.g. DEERS, PITE, etc.)	Year(s)	Data Elements (Be specific, i.e., name, SSN, address).
M2	SIDR	Oct 88 - Present	Admission date, Age, Ben Cat Common, DDS, Diagnosis 1 – 8, FMP, Gender, Race, Sponsor ID, Sponsor rank group, Sponsor service
	SADR	Oct 98- Present	Age, Ben Cat Common, DDS, Diagnosis 1 – 4, FMP, Gender, Race, Service date, Sponsor ID, Sponsor rank group, Sponsor service
	HCSR I	Oct 93 - Present	Admission date, Age, Ben Cat Common, DDS, Primary diagnosis, Sec diagnosis 1 – 8, Gender, Service date, Sponsor ID, Sponsor rank group, Sponsor service
	HCSR NI	Oct 98 - Present	Age, Begin date of care, Ben Cat Common, DDS, Primary diagnosis, Sec diagnosis 1 – 4, Gender, Service date, Sponsor ID, Sponsor rank group, Sponsor service

Revised 05 May 2006

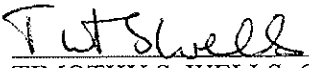
Appendix K

I would like to continue using the above listed DUA #06-347 until 30 September 2008. I understand that the renewal and reuse of the above data files and DUA cannot exceed my contract end date or 1 year, whichever ever comes first.

A copy of my contract renewal or modification for the above DUA is attached as required by the TMA Privacy Office to verify the dates on the contract.

Thank you in advance for your attention to this data request. If you have any questions, please contact **Col Timothy S. Wells** at (937) 255-3931.

Sincerely


TIMOTHY S. WELLS, Col
Chief Epidemiologist


TIMOTHY S. WOODRUFF, LtCol USAF, BSC
Chief, Biosciences and Protection

Enclosure(s): Note: There are no contractors working on this study. I have attached a copy of the most current IRB-approved protocol dated 29 December 2006.