**Simvastatin: Proof of Concept for Prevention of Neurodegeneration in Mild TBI**
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**SPECIFIC AIMS**

Mild traumatic brain injury (mTBI) secondary to exposure to repetitive blast has been termed the "signature injury" of the wars in Iraq and Afghanistan. Recent autopsy case reports of the neuropathology of chronic traumatic encephalopathy (CTE) in five Iraq and Afghanistan Veterans with repetitive mTBI[1,2] and demonstration of widespread tau pathology following a single blast exposure in a mouse model of blast mTBI[1] increase the urgency of finding effective treatment to prevent neurodegeneration and dementia in Veterans with mTBI. In addition, head trauma is the clearest environmental risk factor for Alzheimer's disease (AD), the common late-life neurodegenerative disorder. Finding a therapy to prevent CTE and reduce risk of AD in Veterans with TBI is a high priority.

Because the pathologic processes of CTE and AD start years to decades prior to clinical dementia, the challenge in prevention trials are: 1) developing tools to assess disease-modifying effects prior to clinical dementia; 2) recruiting and retaining a large cohort over a prolonged follow-up period (owing to the low incidence of CTE and AD in young age through early aging); and 3) assuring the safety of a potential disease-modifying drug being administered to large numbers of healthy subjects of which only a minority may be at risk of developing CTE or AD. For example, toxicity and side effects resulted in the cessation of a trial of naproxen and celecoxib for AD (the Alzheimer's Disease Anti-inflammatory Prevention Trial).

The feasibility of primary prevention trials for neurodegenerative dementia may be improved by employing biological markers related to neurodegenerative processes in CTE and/or AD[3-5] as surrogate outcomes. Cerebrospinal fluid (CSF) biomarkers are attractive surrogate end-points for prevention trials as they have proven sensitivity for the prediction of disease onset[6,7] and, in addition, have the potential for providing information regarding the mechanisms of the preventive treatment. Using CSF biomarkers in a pilot study, we found that 14 weeks of simvastatin (a statin with high central nervous system [CNS] penetration) reduced CSF levels of total tau (t-tau), tau protein phosphorylated at threonine 181 (p-tau$_{181}$) and amyloid $\beta_{1-42}$ (A$\beta_{42}$), and showed a trend for increasing brain-derived neurotrophic factor (BDNF, a growth factor which promotes neuronal survival, growth and synaptic plasticity[8]) in cognitively normal hypercholesterolemic middle-aged subjects compared to the treatment with pravastatin (a statin with low CNS penetration). **This pilot study suggests that the innovative use of CSF biomarkers as surrogate outcomes in a prevention trial is feasible.**

Statins are attractive therapeutic agents for neurodegenerative dementia primary prevention trials because: 1) they have demonstrated safety and tolerability in healthy subjects at risk for cardiovascular disease[9], including those with normal cholesterol levels;[10] 2) epidemiologic studies from our laboratory and others indicate that statin use is associated with reduced risk of clinical dementia and AD as well as reduced neurofibrillary tangle (NFT) burden at autopsy;[11] 3) neuroprotective effects of statins have been shown in TBI animal models[12-14]and in observational studies of functional recovery in older TBI victims; and 4) our pilot study of simvastatin demonstrated reduction in CSF biomarkers related to tau pathology that could be detected after as little as 14 weeks of treatment.

We propose a 12-month, double-blind, randomized, placebo-controlled trial to establish *proof-of-concept* for use of simvastatin (40 mg/d) for decreasing CSF biomarkers of neurodegeneration and increasing CSF neurotrophins in 120 Iraq and Afghanistan Veterans with repetitive blast mTBI. Specific Aims are:

**Specific Aim 1: To examine the effects of 12 months of treatment with simvastatin 40 mg/day on CSF concentrations of tau biomarkers (t-tau, p-tau$_{181}$, and t-tau:p-tau$_{181}$ ratio) and BDNF in Iraq and Afghanistan Veterans with repetitive blast concussion mTBI.**

Hypothesis 1: Compared to placebo, simvastatin will reduce levels of CSF t-tau, p-tau$_{181}$, and p-tau$_{181}$:t-tau ratio; and will increase level of CSF BDNF.

**Specific Aim 2: To explore the effects of 12 months of treatment with simvastatin 40mg/day on CSF A$\beta_{42}$, and biomarkers of oxidative stress and neuroinflammation in Iraq and Afghanistan Veterans with repetitive blast concussion mTBI.**

Hypothesis 2: Compared to placebo, simvastatin will reduce levels of CSF A$\beta_{42}$, and biomarkers of oxidative stress ($F_2$-isoprostanes) and neuroinflammation (interleukin [IF]-6, IL-8, and S100β).

Because of concerns regarding potential adverse effects of statins, we will administer a neurocognitive test battery to monitor memory and other cognitive functions during the study period. We will also monitor potential effects of simvastatin treatment on persistent postconcussive symptoms (PPCS), posttraumatic stress disorder (PTSD), depression, alcohol use, functional status, and health-related quality of life. **The findings of the proposed study may provide, in a relatively short period of time, proof-of-concept in support of larger scale primary prevention trials to prevent neurodegeneration and dementia in Iraq and Afghanistan Veterans and service members with repetitive mTBI.**

**RESEARCH DESIGN AND METHODS**

Overview: The proposed study is a 12-month, double blind, randomized placebo-controlled trial of simvastatin (40 mg/day) vs. placebo in Iraq and Afghanistan Veterans with repetitive blast mTBI with PPCS). We will use National Institute of Neurological Disorders and Stroke Common Data Elements (NINCDS CDE) Core and selected Supplemental measures relevant to mTBI from the as well as additional assessments. The study definition of mTBI will follow ACRM criteria, defined as a traumatically induced physiologic disruption of brain function manifested by at least one of the following: loss or alteration of consciousness lasting less than 30 minutes; any loss of memory for events immediately before or after the accident with any posttraumatic amnesia lasting not more than 24 hours; initial Glasgow Coma Scale score ≥13. Although most participants will have multiple exposures, they will be considered to have mTBI if any single exposure meets these criteria. Because Glasgow Coma Scale scores in the combat setting will be unavailable, this criterion will not be required if other criteria for mTBI are met.

Primary outcomes are change in concentration of CSF t-tau, p-tau$_{181}$, p-tau$_{181}$:t-tau ratio, and BDNF. The secondary outcomes include change in concentration of CSF biomarkers of amyloid deposition: A$\beta_{42}$; oxidative stress: $F_2$-isoprostanes; and inflammation: IL-6, IL-8, and S100β. Potential cognitive adverse effects of statin treatment will be assessed by psychometric measures of simple and sustained attention, working and declarative memory, and complex reasoning. Primary and secondary CSF biomarker outcomes and neuropsychological tests will be measured at baseline and 12-months of treatment. Customary clinical monitoring for clinical response and for adverse effects (including CPK and hemoglobin A1C) will be monitored at 6-weeks and at 3-, 6-, 9-, and12-months of treatment. Compliance will be monitored with pill counts at each visit.

Participants:120 Iraq and Afghanistan Veterans who meet study inclusion/exclusion criteria will be enrolled. Persons of all races and ethnicities and both genders will be eligible. Participants will be naïve for statins, and will have either normal LDL cholesterol or mildly elevated LDL cholesterol which does not require drug therapy per National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATP-III) guidelines.

*Inclusion Criteria for all participants:*

- Males and females ages 21-50 years.
- Documented hazardous duty in Iraq and or Afghanistan with the U.S. Armed Forces.
- Exposure to one or more blast trauma events resulting in mTBI according to ACRM criteria as defined above.
- More than 6 months since last blast trauma exposure
- Ability to complete psychometric and other clinical assessments in English (i.e., adequate English language skills, vision and hearing).
- Normal or mildly elevated cholesterol which does not require drug therapy based on NCEP/ATP-III guidelines (i.e., fasting LDL <190 for those with ≤ 1 cardiovascular risk factor and < 160 for those with 2+ risk factors).
- No use of statins during the previous year and no recent (past 4 weeks) use of other lipid-lowering drugs (e.g., fibrates, niacin > 500mg/d, or high dose omega-3 fatty acids) preceding randomization.
- No clinically significant laboratory abnormalities (electrolytes, glucose, carbon dioxide, BUN, creatinine, vitamin $B_{12}$, folate, albumin, thyroid stimulating hormone).
- Platelet count > 100,000/mm$^2$.
- Body Mass Index (BMI) between 18 and 36 inclusive (BMI outside this range would affect biomarker measurements or make LP difficult to perform).

*Exclusion criteria for all Participants:*

- History of head trauma with LOC>30 minutes, or with a penetrating head wound, or with moderate to severe memory or other cognitive impairment.
- Neurological disorders: multiple sclerosis, epilepsy, stroke, PD, other degenerative CNS disorders, or neuropathy with radicular involvement.
- Acute or chronic major psychiatric disorders: schizophrenia, bipolar disorder or severe major depressive disorder, or severe anxiety disorder except PTSD and panic disorder (PTSD and depressive symptoms are common co-morbid conditions for combat mTBI and a subset of these patients have symptoms consistent with panic disorder as well).
- Use of illegal drugs; alcohol abuse within the past 6 months.
- Poorly controlled hypertension, heart failure, coronary heart disease, peripheral artery disease, carotid artery disease, diabetes mellitus, pulmonary disease with hypoxia or hypercapnia,

significant hepatic disease or hepatitis C seropositivity, renal failure, treatment for cancer, HIV positive, active infectious disease or presence of abdominal aortic aneurysm.

- Contraindications to LP (e.g., spinal cord injury; deformity, severe disease or infection in the region of the lumbosacral spine; bleeding tendency, use of anticoagulant medications, or platelet count <100,000/$mm^2$).
- Receiving medication in an investigational drug study.
- Exclusionary medications (used in the 4 weeks prior to screening):
  - Fibrates and niacin due to increased risk for myopathy in combination with statins;
  - Potential drug-drug interactions with statins via effects on CYP3A4: itraconazole, ketoconazole, erythromycin, clarithromycin, HIV protease inhibitors, nefazodone, amiodarone, cyclosporine, isoniazid, quinidine, or large quantities of grapefruit juice (>1 quart daily);
  - Selected CNS-acting medications: antipsychotics, anti-Parkinson's disease medications and CNS stimulants
  - Other medications affecting coagulation and/or inflammation: coumadin, potent anti-inflammatory medications (hydrocortisone, methotrexate or other potent immune-modulating medications), and anti-HIV medications.
- All female subjects of childbearing potential will undergo a urine pregnancy test at every subject visit; subjects with positive pregnancy test results will be excluded. In addition, all female subjects of childbearing potential will be required to use a reliable method of contraception throughout the duration of the study.

Description of Recruitment Process: Participants will be recruited from the MIRECC Mild TBI/Behavioral Specialty Clinic; Deployment Health Clinic, Polytrauma Clinic, General Internal Medicine Clinic, and Mental Health Clinics at VA Puget Sound; the Seattle downtown Vet Center; community-based outpatient clinics (CBOCs), and from outreach to National Guard and Reserve units. Recruitment will be assisted by posters, flyers, brochures, announcements in the VA daily bulletin, and print and radio media advertising. Prospective participants will have their charts reviewed by referring providers to screen for exclusionary medical conditions and medications.

Potentially eligible persons will be referred to the study team for obtaining informed consent. Written informed consent will be obtained from participants by the PI, study Co-Investigator, or Study Coordinator. If a potential participant's primary mental health care provider is a study investigator, another investigator will conduct the consent process. Care will be taken to assure participants that research study participation is voluntary and their current treatment and future services and benefits are not contingent on participation in this or any research study.

Participants will receive compensation for their time and inconvenience as follows: $50 for the screening visit, $50 for each cognitive testing day, $200 for each LP day, and $25 for each safety visit. The total each subject will receive if all study visits are completed is $650. Subjects who withdraw early or are withdrawn from the study will receive pro-rated payments based on the number of study visits completed.

Inclusion of Women and Minorities: Because of the requirement that blast trauma group participants have experienced at least one blast trauma exposure, the majority of participants in this group will come from Military Occupational Specialties (MOSs) with high blast trauma exposure: Infantry, Combat Engineers, Explosive Ordnance Disposal, and Military Police (MP). Infantry, Combat Engineers, and Explosive Ordnance Division are all male combat MOSs. It is estimated that fewer than 25% of the MP MOS participants will be women based on the gender composition of the Military Police who served in Iraq and Afghanistan. Although it is not anticipated that we will have sufficient numbers of women subjects to have adequate power to detect gender effects on study outcomes, gender will be used as a stratification variable during the randomization to ensure that women subjects have an equal chance of receiving active drug. Persons of all races and ethnic backgrounds will be eligible. The veteran patient population at VA Puget Sound is comprised of 75% Caucasians, 14% African Americans, 4% Native Americans, 3% Hispanic Americans, 2% Asian Americans, and 2% "other". No differences based on racial/ethnic differences are expected.

**Study procedures:**

There will be a total of nine study visits over a period of 12 months, including the screening visit, four visits for monitoring adverse effects and compliance, and four visits for measuring study outcomes (two at Baseline and two at 12 months). Table 3 below outlines all study visits.

Screening visit: Screening for eligibility will include a brief cognitive screen with the Montreal Cognitive Assessment (MoCA). Detailed medical and psychiatric history, current medications, and medications used over

past 6 months will be reviewed. Potential participants will be administered the Structured Clinical Interview for DSM-IV--Patient Edition (SCID-IV) to diagnose exclusionary psychiatric disorders.

Table 3. Detailed Schedule of Study Visits

| | Screen Visit | Baseline Visits | | Safety Visits | | | | 12 mo Outcome | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 6 wk | 3 mo | 6 mo | 9 mo | 1st | 2nd |
| Demographics | X | | | | | | | | |
| Medical/psychiatric/military history | X | | | | | | | | |
| Physical/neurological examination | X | | | | | | | X | |
| CBC, Chem 7, TSH, B12, folate, albumin, routine urinalysis | X | | | | | | | | |
| fasting lipid panel (HDL, LDL, triglycerides) | X | X | | | | | | X | |
| Plasma homocysteine & C-reactive protein (CRP) | | X | | | | | | X | |
| LFTs, bilirubin, CPK, HbA1c | X | | | X | X | X | X | X | |
| Urine pregnancy test | X | X | X | X | X | X | X | X | X |
| APOE genotype | X | | | | | | | | |
| LP – CSF biomarkers | | X | | | | | | X | |
| SCID-IV | X | | | | | | | | |
| CAPS, SWLS | | | X | | | | | | X |
| PCL-M, PHQ-9, AUDIT-C | | | X | X | X | X | X | | X |
| MoCA | X | | X | X | X | X | X | X | |
| Neuropsychological test battery | | | X | | | | | | X |
| Vital signs | X | X | X | X | X | X | X | X | X |
| Review of concurrent medications | X | X | | X | X | X | X | X | |
| Assess adverse events | | | X | X | X | X | X | X | X |
| Pill count for med compliance | | | | X | X | X | X | X | |
| Dispense study medications | | | X | X | X | X | X | | |

A complete physical and neurological examination will be performed. Screening laboratory tests will be obtained, including complete blood count (CBC), chemistry panel, thyroid stimulating hormone (TSH), vitamin B12, folate, SGOT, SGPT, SGGT, bilirubin, alkaline phosphatase, CPK, albumin, and fasting lipid panel (total cholesterol, HDL, LDL, and triglycerides), urinalysis, and (in female subjects of childbearing potential) a urine pregnancy test. Blood samples will also be collected for apolipoprotein E (APOE) genotyping (described below). A urine sample will be obtained for routine urinalysis and (in female subjects of childbearing potential) a urine pregnancy test.

Baseline: The Baseline visit will be scheduled within 4 weeks of the Screening visit. The Baseline visit consists of 2 separate appointments within a week of each other. During the first visit, vital signs will be obtained, a brief physical examination will be performed, concurrent medications will be recorded, a blood draw will be performed for measurement of plasma lipids and inflammatory markers, and an LP will be performed as described in detail below.

During the second baseline visit, the neurocognitive test battery will be administered and behavioral assessments will be performed. Comorbid PTSD will be assessed with the CAPS. A detailed description of all study instruments is below.

Randomization, titration, and continued study treatment: Upon completion of all baseline procedures, participants will be randomized to receive simvastatin or placebo. Stratification factors will include age (<40 vs ≥ 40 years), gender, and number of blast trauma exposures (<5 and ≥5). Permuted block randomization will be used within each stratum. Varying the block size reduces the predictability of treatment assignment. Separate blocked sequences will be generated for each of the three strata and provided directly to the Research Pharmacist at the VA Puget Sound, who will prepare study drug for dispensing.

Simvastatin will be started at 20 mg qhs and titrated over 2 weeks to a final dose of 40 mg qhs. Placebo will be titrated at week 2 correspondingly. We assume a drop-out rate of ten percent, yielding a total sample size of 50 subjects per group at the end of the study.

Safety and compliance visits: Four additional visits will be scheduled at 6 weeks and at 3- 6- and 9-months of treatment to monitor adverse effects, assess compliance with study medications, and dispense study medication. Each visit will include measurement of vital signs, update of concurrent medications, and any self-report of adverse effects. Pill counts will be used to assess compliance. Safety assessments as described below will be performed.

Outcome assessment: Outcome assessments will be obtained in two visits at the end of 12 months of treatment. Procedures for outcome assessments duplicate the procedures performed during the Baseline visits as described above. Pill counts will be used to assess compliance with medication use.

Monitoring safety of participants**:** Simvastatin is used widely for lowering cholesterol and for primary prevention of cardiovascular and cerebrovascular disease at the doses proposed in this application. It is generally safe and well tolerated. The rate of adverse reactions necessitating withdrawal from statin therapy is <1% in clinical practice.[15] The most common adverse effects in clinical use are elevated liver transaminases, myalgias, and elevated CPK. In our pilot study,[16] all subjects tolerated a final simvastatin dose of 40mg/day. Of 24 subjects recruited, only two had significant adverse effects in response to statin therapy. One experienced significant myalgias requiring discontinuation of therapy despite a non-significant elevation in CPK. Another exhibited a slight increase in CPK with statin therapy. Mean serum CPK concentrations increased marginally over the course of the study. Mean SGOT and SGPT values increased equivalently. Only one subject developed a significant, though asymptomatic, elevation in SGPT. None of the subjects in our pilot study nor in our current placebo-controlled trial of simvastatin for primary prevention of AD experienced adverse cognitive changes or psychiatric symptoms.

Subjects will be advised of potential drug-related side effects such as muscle pain and weakness and nausea or vomiting at both the Screen (consent process) and Baseline visits. In addition, subjects will be monitored at 6 weeks and at 3-, 6-, 9-, and 12 months of treatment for potential adverse events. At each visit, we will review symptoms of anticipated adverse effects (such as muscle pain, weakness, and nausea), measure LFTs, CPK, and HbA1c, and (for female subjects of childbearing potential) conduct urine pregnancy tests.

To monitor for potential cognitive side effects, we will perform the MoCA (a brief but sensitive global cognitive test) at all safety visits. If a subject reports problems with memory or cognitive function after initiation of study medication sufficient to interfere with daily function or MoCA score decline of one standard deviation or greater compared to Baseline, we will perform a thorough neurological and psychiatric examination and will administer the entire neuropsychological battery. If a decline of one standard deviation or greater compared to Baseline is present on two neuropsychometric measures, the subject will be terminated from the study.

To monitor for emergence or worsening of comorbid depression, the PHQ-9, a self-report measure of depression will be administered at all visits. The PHQ-9 score can range from 0 to 27. Scores of 5, 10, 15 and 20 represent thresholds demarcating the lower limits of mild, moderate, moderately severe and severe depression, respectively. If the PHQ-9 score is increased by 10 (i.e., an increase of two severity categories) compared to baseline, the subject will be terminated from the study. To monitor alcohol use and symptoms of comorbid PTSD, we will administer the AUDIT-C and the PCL-M at all visits.

If at any time during the study, a participant complains of symptoms which may indicate a potential adverse event, additional safety visit(s) as necessary will be performed. Procedures to be performed at additional visits will depend on the symptoms the participant is experiencing. If the participant meets any criterion for study removal (as described below), an early discontinuation visit will be performed.

Early treatment discontinuation procedures: The primary reasons for treatment discontinuation anticipated during the study are adverse effects, protocol violation, noncompliance, and withdrawal of consent. We will make every reasonable effort to keep each subject in the study. Every effort will be made to schedule a discontinuation visit as soon as possible following discontinuation of study medication to allow collection of outcome and safety measures.

Criteria for termination from study: Subjects will be removed from the study if they: 1) develop a significant adverse medication reaction, including psychiatric adverse effects; 2) exhibit >1 standard deviation decline on more than one component of the neuropsychological test battery; 3) develop a significant elevation in serum transaminases (i.e., > 3 X upper limit of normal for SGOT and/or SGPT) or CPK (i.e., > 10 X upper limit of normal); 4) have a positive urine pregnancy test; or 5) meet NCEP/ATP-III criteria for initiation of lipid-lowering therapy at the 3- or 6-month safety visits.

Compliance with study medications: Subjects will be given a 6-week supply of medication at Baseline and at the 6-week visit, and subjects will be given 3-month supplies of medication at the 3-, 6- and 9-month

visits. Pill counts to assess medication compliance will be performed at each safety visit. In our pilot study of simvastatin and pravastatin, 23 (96%) of 24 subjects completed the 3-month study and the completers had taken 94–100% of scheduled doses of medication as assessed by pill counts. The compliance rate in a longer (one year) study might be expected to be lower. However, the 5-year compliance rate was fairly high in the Heart Protection Study.[9] In this study, the rate of compliance (> 80% of scheduled tablets) was 89% at the end of the first year of follow-up and 85% at the end of the second year among participants allocated to take 40 mg simvastatin daily. In this proposed study, we will make a 6-week interim phone contact, in addition to the scheduled study visits, to ensure adequate compliance with study medication. We will make interim phone contact between months 3 and 6, 6 and 9 and 9 and 12, in addition to the scheduled study visits, to ensure adequate compliance with study medication. We expect a compliance rate of 90% over the course of the 1-year study.

**Biomarkers:**

Overview: The primary outcome measures are biomarkers in CSF: t-tau, p-tau$_{181}$, p-tau$_{181}$:t-tau ratio, and BDNF. The secondary outcomes are additional CSF biomarkers of amyloid dysregulation: A$\beta_{42}$; oxidative stress: $F_2$-isoprostanes; and neuroinflammation: IL-6, IL-8, and S100β. All subjects will also undergo APOE genotyping. For each substance measured in CSF or blood, all study samples (i.e., from the baseline and from the 12 month follow-up visit) from any one subject and equal numbers of samples from subjects in the statin and placebo groups will be assayed within a single assay to reduce inter-assay variability. All samples will be measured blind to treatment assignment.

Blood collection for DNA and plasma and serum biomarkers: On the day of LP at baseline and at the 12 month follow-up visit, subjects will undergo blood collection for plasma and serum for biomarkers. Thirty five ml of blood will be collected into pre-chilled tubes containing EDTA and placed on ice. These samples will be cold centrifuged within one hour of collection, aliquoted into polypropylene tubes and frozen at -70 degrees C until assayed. Twenty ml of blood will be collected into plain glass tubes and sent to the VA Puget Sound clinical laboratory for the measurement of total cholesterol, HDL, LDL, homocysteine, and C-reactive protein (CRP).

At the screen visit only, 30 mls of blood will be collected into unchilled tubes containing EDTA and sent to Dr. Chen-En Yu's laboratory at VA Puget Sound for DNA preparation and APOE genotyping. Clinical lab analysis will take place at VA Puget Sound. All plasma and serum samples for biomarker analysis will also be accessioned, inventoried, and stored at -70 degrees C at VA Puget Sound S until assayed.

APOE genotyping will be performed by the UW ADRC Genetics Core under the direction of Dr. Chang-En Yu. APOE genotyping will be performed using previously described PCR conditions[17] and the HhaI restriction digest method.[18]

Lumbar puncture for CSF collection: Following blood collection, CSF will be obtained using procedures developed and characterized in our laboratory as minimally invasive and well-tolerated by research participants (post-LP headache incidence <1%).[19] Subjects will be placed in the lateral decubitus position. The L3-4 or L4-5 interspace will be infiltrated with 1% lidocaine to provide local anesthesia. A 24g Sprotte atraumatic spinal needle will be used to obtain 28 ml of CSF, which will be collected under negative pressure into sequential 6ml polypropylene syringes. The first three ml of CSF will be sent to the hospital laboratory for cell count and measurement of protein and glucose concentration. The remaining 25 ml of CSF will be divided into 0.5 ml aliquots into polypropylene tubes, frozen immediately on dry ice at the bedside, and then stored at -80 degrees C until assayed. Subjects will remain at bed rest for one hour after lumbar puncture and will be instructed to avoid any exertion for 48 hours.

Detailed methods for measuring CSF biomarkers: All CSF biomarkers will be measured in the laboratory of Dr. Thomas Montine at the University of Washington. Frozen samples will be taken from VA Puget Sound to Dr. Montine's laboratory using approved leak-proof containers by personnel using universal precautions. Primary outcome measures will be change in concentration pre- to post-treatment of CSF tau, p-tau$_{181}$, and BDNF. CSF A$\beta_{42}$, $F_2$-isoprostanes, IL-6, IL-8, and S100β will be secondary measures. For each CSF protein measured, pre- and post-treatment samples from any one subject and equal numbers of samples from subjects in each treatment group will be assayed within a single assay to reduce inter-assay variability. All samples will be measured blind to subject medication assignment.

Analyses of CSF BDNF, t-tau and p-tau$_{181}$ proteins, concentrations of $F_2$-isoprostanes, IL-6, IL-8, S100β, and A$\beta_{42}$ on unthawed samples will be performed using immunobead-based multiplex assays. Panels of capture antibody-coated beads and labeled detection antibodies are purchased from two sources: Biosource Division of Invitrogen, Camarillo, CA, and Linco Research Inc., St. Charles, MO. The assay systems are run according to the instructions provided by each manufacturer and data is collected using a LiquiChip

Workstation from Qiagen (Valencia, CA). Standard curves for each protein are generated using the reference proteins supplied in these kits, and CSF concentrations interpolated from these standard curves.

CSF concentrations of $F_2$-isoprostanes will be measured using a stable isotope dilution method with gas chromatography (GC) and mass spectrometry (MS) with selective ion monitoring (SIM) and [$^2H_4$]15-F2t-isoprostane as an internal standard, as previously described.[20] This oxidized and cyclized lipid product of arachidonic acid is an excellent quantitative *in vivo* biomarker of oxidative damage.

CSF will be banked at VA Puget Sound for future research, such as proteomic analysis before and after statin treatment. Proteomics, similar to genomics, is an unbiased method that simultaneously assesses thousands of proteins. Although there are several CSF biomarkers that reflect particular facets of neurodegeneration, it is unlikely that statins affect only a single or a small group of molecules. Thus, conducting a large scale survey of potential CSF biomarkers before and after statin treatment will allow us to define an ensemble of biomarkers that may discriminate treatment effects and provide insight into as yet unknown mechanisms of action. In addition, samples will be made available to other investigators nationally and internationally for research in neurodegenerative disorders. Samples will only be shared after appropriate Materials Transfer Agreements are executed. No identifying information will be included with any shared samples without subject consent.

**Neurocognitive and Behavioral Measures** (*Note: NINDS CDE and all other cognitive and behavioral measures are commonly used in similar studies; because of space limitations for references, the measures are described but not referenced*)**:**

**Behavioral, functional and quality of life measures:** The SCID-IV will be administered at screening only to rule out exclusionary psychiatric conditions. Comorbid PTSD will be assessed using the CAPS at baseline and after 12 months of treatment and the PCL-M at all study visits. Current mTBI symptoms, functional status, and health-related quality of life will be quantified using the Brief Symptom Inventory (BSI), the Rivermead Postconcussive Questionnaire (RPQ), the Neurobehavioral Symptom Inventory (NSI), Satisfaction with Life Scale (SWLS) and the Craig Handicap and Assessment Reporting Technique (CHART-DF). The PHQ-9 and AUDIT-C will be administered at all study visits to assess depressive symptoms and alcohol use.

**Neurocognitive Battery:** We will use measures from the NINDS CDE as well as additional measures for assessment of neurocognitive function. The neurocognitive test battery includes measures of learning and memory, attention and information processing speed, and executive function. In addition, we will employ a measure of test effort (the Test of Memory Malingering [TOMM]) to provide an estimate of the validity of subject's test scores. If a subject's score on the TOMM suggests inadequate test effort, the rest of neurocognitive test scores will be considered invalid. The estimated time to complete the entire test battery is two hours. The complete neurocognitive battery will be administered at baseline and after 12 months of treatment with simvastatin or placebo. A brief global cognitive screening measure (the MoCA) will be administered at screen and at all subsequent study visits to ensure there is no cognitive decline during the study. The study psychometrist will be trained by Co-Investigator, Kathleen Pagulayan, Ph.D., MIRECC neuropsychologist, and she will conduct periodic checks for adherence to the standardized neuropsychological test battery administration protocol.

**Description of study instruments (those marked with † are included in the NINDS CDE)**

- Structured Clinical Interview for DSM-IV (SCID-IV): A structured diagnostic interview scheduled designed to measure the presence/absence of the full range of DSM-IV Axis I mental disorders. The SCID-IV is regarded as the "gold standard" for assessing mental disorders, having been used in over 100 published studies as of 1992.
- Clinician-Administered PTSD Scale for DSM-IV (CAPS): A structured clinical interview designed to assess the 17 symptoms of PTSD outlined in the DSM-IV. The CAPS allows the interviewer to make current (past month) and lifetime DSM-IV diagnoses of PTSD. The frequency and intensity of each symptom on the CAPS is rated on separate 5-point scales, yielding both dichotomous and continuous scores (range = 0 to 136) for each symptom and for the disorder as a whole. Test-retest reliability studies have consistently yielded coefficients ranging from .90 to .98, and internal consistency has ranged from .85 to .87 for symptom clusters to .94 for the entire scale. The 17-item CAPS has become the standard for psychopharmacologic outcome trials in PTSD.
- †PTSD Checklist-military version(PCL-M): A self-report inventory of the 17 DSM-IV symptoms that define the disorder PTSD. Symptoms are rated on 5-point Likert scales, yielding a score ranging from 17 to 85. The PCL-M has been shown to be a reliable and valid measure of military trauma-

related PTSD symptoms in a variety of special populations. The PCL-M is useful as a continuous measure of PTSD symptom distress in war-zone exposed Veterans. A score of 50 or greater has high diagnostic sensitivity (.81) and specificity (.83). Test-retest reliability (.96) and internal consistency (alpha=.93) for the PCL-M are exceptional.

- †Patient Health Questionnaire-9 (PHQ-9): The 9-item depression module of the Patient Health Questionnaire, a self-report version of the PRIME-MD used to diagnose major mental disorders. The PHQ-9 items correspond with DSM-IV criteria for depression, with each item scored from “not at all” to “nearly every day.” Items of the PHQ-9 are internally consistent (alpha = 0.86-0.89) and the questionnaire exhibits high test-retest reliability coefficients. Agreement between the PHQ-9 and clinician-based interview diagnosis of major depression is high, with a sensitivity of 88% and a specificity of 88% using a PHQ-9 score ≥ 10.
- †Alcohol Use Disorders Identification Test-Consumption (AUDIT-C): A 3-item modification of the AUDIT questionnaire that inquires about frequency and quantity of typical alcohol consumption, and the frequency with which the respondents report an episode of heavy drinking in the past year (defined as 6 drinks per one occasion in men and 4 drinks per one occasion in women). The AUDIT-C demonstrates excellent sensitivity and specificity for detection of heavy drinking and is used as a standard screening tool for alcohol problems in VA primary care settings.
- †Brief Symptom Inventory (BSI): This shortened version of the SCL-90 is self-report measure with 18-items rated on a 5-point rating scale. The BSI includes 3 clinical scales: Depression, Anxiety and Somatization) and an overall Global Severity Index. This questionnaire takes approximately 10 minutes to complete
- †Rivermead Postconcussive Questionnaire (RPQ) - A 16-item self-report measure of presence and severity of the 16 most commonly reported postconcussive symptoms. Items are reported on a 0-4 scale ranging from "not experienced at all" to "severe".
- †Neurobehavioral Symptom Inventory (NSI). The NSI is a 22 item questionnaire designed to assess the presence and severity of common cognitive, emotional, sensory, and somatic symptoms that can occur after traumatic brain injury. Patients rate on a 5-point scale the extent to which each symptom has affected them (rated from “absent” to “very severe”). Anchor descriptions are provided for level of severity. It is widely used in VAs across the country for the TBI Second Level Evaluation.
- †Satisfaction with Life Scale (SWLS) - includes 5 items answered on a 7-point Likert scale ranging from 1=strongly disagree to 7=strongly agree. Total score ranges from 7-35 with higher scores indicating better satisfaction with life.
- †Craig Handicap and Assessment Reporting Technique (CHART-DF): provides an objective measure of the degree to which impairments and disabilities result in long-term handicaps.
- Montreal Cognitive Assessment (MoCA): A cognitive assessment instrument designed to detect cognitive impairment and provides a brief assessment of global cognitive function, including short-term memory recall, executive function, sustained attention, calculation, language, and orientation. Scores range from 0-30 with lower scores indicating greater impairment. Test-retest reliability is high with less than one point variation. Internal consistency is good (Cronbach alpha=0.83). To correct for education, 1 point is added for those with less than 12 years of education.
- Wechsler Test of Adult Reading (WTAR): A neuropsychological test designed to provide estimates of premorbid intellectual functioning. This estimate is derived from the ability to read a list of irregular words, as single word reading recognition ability has been shown to be resilient to the effects of TBI.
- †WAIS-III Letter-Number Sequencing Subtest. The examiner reads a combination of numbers and letters that the subject is then asked to reproduce orally with the numbers first in ascending order, followed by the letters in alphabetical order. As the test progresses, items presented to the subject contain more numbers and letters. This test requires concentration, attention, and an ability to store and organize information in memory for a short duration (5-10 min.)
- †Grooved Pegboard: This is a test of fine motor coordination and speed. In this test, subjects are required to place 25 small metal pegs into holes on a 3" x 3" metal board. First the dominant hand is tested, and subjects are asked to place the pegs in the holes as fast as they can. This is repeated with the nondominant hand, and the total time for each hand is recorded. This test has been shown to be sensitive to statin manipulations.

- Auditory Consonant Trigrams: This is an adaptation of the Brown-Peterson technique. The task assesses short-term retention and due to the different lengths if interference activity, performance can be assessed at several difficulty levels. Three letters are read to the participant followed by a number. The subject begins to subtract from the number by threes until zero, three, nine, or 18 seconds have passed at which time the examiner asks the participant to recall the three letters. The test discriminates controls from persons with cognitive impairment.
- Simple and Choice Reaction Time, Continuous Visual Memory Test, Digit Vigilance Test: These tests are part of a battery of computerized tests that was developed and validated in all adult age groups by the U.S. Department of the Navy to rapidly measure cognitive processing efficiency. In the simple reaction time task, symbols are presented on a computer screen and subjects are asked to strike a particular key on the keyboard as soon as a symbol appears. For the choice reaction time task, two different symbols appear at random on the screen and subjects are asked to respond to only one of the symbols. For the continuous performance test, numerous symbols appear on the screen and subjects are asked to respond to only one symbol when it is preceded by another particular symbol. For all subtests, this process is repeated ten times and the average of ten trials is used. Several studies have shown the battery to be sensitive to the effects of medications, including amphetamines and nicotine, and to mild head injury. It has good test-retest reliability and validity when compared to other well established tests.
- †Rey Auditory Verbal Learning Test (RAVLT): This is a brief verbal learning and memory instrument, consisting of a 15-item word list presented to the subject orally in five consecutive trials followed by a second list. The number of words recalled is recorded after each presentation of the list and the interference or second list. A delayed recall of the list is given followed by a yes/no recognition. It has excellent test-rest reliability (.51 - .70) and validity as demonstrated by a good correlation with other memory tests (.50 - .56).
- †Processing Speed Index from the WAIS-III: Based on 2 subtests of the WAIS-III: Digit Symbol and Symbol Search. For Digit Symbol, examinee must accurately fill in symbols according to matched number-symbol pairs in a key. For Symbol Search, examinee determines whether either of 2 target symbols match any of the symbols in a search group.
- †Digit Span of WAIS-III: A test of attention and memory from the WAIS-III.
- †Controlled Oral Word Association Test (COWAT): assesses the ability to quickly generate words beginning with certain letters. This test has been shown to be sensitive to the effects of frontal lobe dysfunction and to the effects of TBI: Total number of words generated will be used in this study.
- †Trail Making Test: A measure of scanning, divided attention, and cognitive flexibility (a type of executive functioning). Total score is based on time to complete the task, and individuals with mild TBI tend to be slower than healthy controls on this task.
- Test of Memory Malingering (TOMM): A widely used symptom validity test that will be used to detect poor effort, and thus invalid results, on neuropsychological tests. The TOMM is face valid and performance appears to be unaffected by mild cognitive or neurological impairment.

*Note: Three of the NINDS-CDE assessments: the Functional Independence Measure – Cognitive and Motor Subscales, and the Glasgow Outcome Scale – Extended, will not be utilized because of their inappropriateness for mTBI. The remainder of the Core NINDS-CDE assessments and selected supplemental measures will be utilized and are described above.*

**Data management:**

A master database will be set up using Microsoft SQL2000 server software at VA Puget Sound by the Data Manager. Data will be double-data entered weekly through a web based data center application. The web application and backend processes will aid in checking data integrity. The Data Manager will generate weekly reports of data discrepancies. The Study Coordinator will be responsible for rectifying data entry errors. Database maintenance includes weekly backup to tapes, which are stored at a safe location, and SQL server routine maintenance. SQL server security will include sign-on authentication and access to the database will be password protected. Laboratory data (e.g., CSF t-tau, p-tau$_{181}$, and APOE genotype) will be generated at several different laboratories. These laboratories will enter data into their own databases and will export them as an Excel file (or similar easily readable file) to the Database Manager. All files sent to the Database Manager will be encrypted. These data will be linked via unique study identification numbers assigned to each study subject.

Confidentiality: All study information will be recorded on standardized case report forms (CRFs). CRFs

will be labeled only with the unique study identification number assigned to each study subject. Source documents will be kept separately from CRFs in locked filing cabinets in locked offices. Study records will be kept separate from the subject's medical record. Coded information from the CRFs will be entered into the study database. The database will reside in a restricted access folder on a VA server. Only personnel having the correct user name, password, and signing on from a computer with the appropriate IP address will have access. The link between study code and identifying information will be kept by the PI in a locked file or on a computer with password protection and will not be available to anyone other than members of the research team.

Only the investigators and the research staff will have access to the original research data. Appropriate regulatory agencies (Office for Human Research Protections [OHRP], Department of Veterans Affairs [DVA], local Institutional Review Boards [IRBs]) may view records that contain identifying information. Data will not be revealed to insurance companies or other individuals or organizations.

We plan to obtain a Federal Certificate of Confidentiality to protect subjects from compelled disclosure of sensitive information and genetic testing results. However, the Certificate will not prevent the research team from taking steps, including reporting to authorities, to prevent serious harm to the subject or others. We are not collecting any health information that is reportable to local health agencies.

Because a Records Retention Schedule approved by National Archives and Records Administration is required to destroy Federal records, and because at this time there is not such a schedule for VA research records, we must retain data pending approval of such a schedule. Therefore, all study data, including the link between subject identities and study code numbers will be maintained under secure conditions (locked cabinets and/or password-protected computers) until we have permission to destroy them or until data analysis is complete (whichever comes later). At that time, the link will be destroyed and all study data will become anonymous. Anonymous study data will be kept indefinitely.

Sharing Study Results: Results of diagnostic tests performed as part of the screening procedures (laboratory results) may be shared with study participants and/or their primary care providers if desired by the study participants. Individual results of the assessment instruments will not be shared, but if the participant wishes, a summary of the results will be provided to the participant and/or his/her mental health care provider after the participant has completed the study. A release of information form will be obtained before any results are shared with anyone but the participant. Results of CSF biomarkers and APOE genotype will not be shared.

**Data Analysis:**

**Specific Aim 1: To examine the effects of 12 months of treatment with simvastatin 40 mg/day on CSF concentrations of tau biomarkers (t-tau, p-tau$_{181}$, and t-tau:p-tau$_{181}$ ratio) and brain-derived neurotrophic factor (BDNF) in Iraq and Afghanistan Veterans with repetitive blast concussion mTBI.**

Hypothesis 1: Compared to placebo, simvastatin will reduce levels of CSF t-tau, p-tau$_{181}$, and p-tau$_{181}$:t-tau ratio; and will increase level of CSF BDNF.

**Specific Aim 2: To explore the effects of 12 months of treatment with simvastatin 40mg/day on CSF A$\beta_{42}$, and biomarkers of oxidative stress and neuroinflammation in Iraq and Afghanistan Veterans with repetitive blast concussion mTBI.**

Hypothesis 2: Compared to placebo, simvastatin will reduce levels of CSF A$\beta_{42}$, and biomarkers of oxidative stress ($F_2$-isoprostanes) and neuroinflammation (interleukin-6 [IL-6], IL-8, and S100β).

**Sample Size, Effect Size, and Power** are based on the change from baseline in the CSF t-tau, p-tau$_{181}$, and BDNF (Specific Aim 1). We will recruit 120 subjects for this study. Assuming a drop-out rate of about 17%, there will be approximately 50 subjects per treatment group (simvastatin vs. placebo). Using an overall Type I error rate of 5% and a Bonferroni adjustment based on four primary endpoints: CSF t-tau, p-tau$_{181}$, p- tau$_{181}$:t-tau ratio, and BDNF (i.e., setting $\alpha = 0.05/4 = 0.0125$), 50 subjects per treatment group yields 80% power to detect an effect size (i.e., | Mean | / SD) of 68% and 90% power to detect an effect size of 77%. For the preliminary study of statin treatment described in Preliminary Findings, change from baseline in t-tau was -107 pg/ml (SD = 129 pg/ml), yielding an observed effect size of 83%.

We believe that this is a conservative estimate of statistical power, because: 1) the estimated t-tau changes are based on only 14 weeks of statin treatment (the duration of the preliminary study). We expect a larger difference in change from baseline between the two treatment groups with one year of treatment, as proposed in this study; and 2) in our pilot data the variability in p- tau$_{181}$:t-tau ratio in the mTBI subjects was low and the age group is relatively young (21-50), which should reduce variation of CSF t- tau levels, since t-tau is correlated with age. Also, we will stratify during randomization by two factors that potentially influence t-tau

levels: gender and number of blast trauma exposures. The stratification should decrease the variability in the estimate of treatment effect and thus increase statistical power to detect a smaller change from baseline.

**Statistical analysis.**

**Primary analysis plan**: All CSF biomarkers, neuropsychological test scores, behavioral assessments, and functional and quality of life measures are continuous variables. Gender, APOE genotype, and number of mTBIs (≥5 or <5) are categorical. Analysis of covariance (ANCOVA) will be used to assess the change from the baseline in all biomarkers as well as neuropsychological test scores. All outcomes are continuous variables. The distribution of model residuals will be assessed and outcome variables may be transformed to conform to assumptions of the statistical tests (e.g., for non-normality). The response variables will be biomarker concentrations at the end of the study (12-month visit), and predictor variables will include biomarker concentrations at the beginning of the study (baseline visit), treatment arm assignment (simvastatin vs. placebo), as well as the variables that were used for stratification of treatment assignment (i.e., gender and number of blast exposures). To examine effects of statin treatment on memory and cognition, the response variables will be test scores at the end of the study (12-month visit). The predictor variables will include test scores at the beginning of the study (baseline visit), treatment arm assignment (simvastatin vs. placebo), as well as the variables that were used for stratification of treatment assignment (i.e., gender and number of blast exposures). Significance of treatment arm effect will be adjusted for multiple tests (i.e., the four primary endpoints) using the method of Holm (1979).[21]

The primary analysis will include all subjects who were randomized and completed the study. A secondary analysis will use a linear mixed-effects model to include all subjects who were randomized and had a baseline visit, including subjects with just a baseline visit as well as subjects with a baseline visit and an early termination visit. Baseline differences, if any, between study completers and non-completers will be summarized.

**Secondary analysis plan:** In addition to the above primary analysis, secondary analyses will include the following:

- Correlation analyses to assess relationships among CSF biomarkers and how they change over time relative to each other.
- Exploring what other covariates (such as serum cholesterol levels) may be important in predicting changes from baseline in CSF AD biomarkers
- An assessment of the nature of missing observations (i.e., random vs. systematic).
- One-sample tests assessing change from baseline within each treatment group, using parametric and/or nonparametric tests.
- Fisher's exact test comparing the proportion of values of change from baseline that are less than zero between the two treatment groups, because *consistent* subtle declines associated with simvastatin treatment could be very meaningful in terms of elucidating prevention mechanisms.

**Expected results and interpretation**

Our hypothesis is that 1-year of treatment with simvastatin, a CNS permeable statin, will reduce CSF levels of t-tau, p-tau$_{181}$, and p-tau$_{181}$:t-tau ratio; and increase CSF level of BDNF in Veterans with blast concussion mTBI and PPCS compared to treatment with placebo. We speculate that simvastatin may produce neuroprotective benefits through effects on multiple biochemical pathways, including, but not limited to, reduction of tau phosphorylation, altered Aβ production, increased production of brain neurotrophic factors, and reduction of brain antioxidant and anti-inflammatory effects. We expect to replicate our pilot study findings of reductions in CSF concentrations of t-tau, p-tau$_{181}$ and increase in CSF BDNF associated with simvastatin treatment. If this finding is confirmed, we will further examine how t-tau, p-tau$_{181}$, and p-tau$_{181}$:t-tau ratio are related to changes in other CSF AD markers (i.e., changes in levels of CSF BDNF, A$\beta_4$, IL-6, IL-8, S100β, and/or $F_2$-isoprostanes) in an attempt to further understand the mechanisms by which statins act to reduce tau phosphorylation and/or A$\beta_{42}$ turnover.

Based on our preliminary findings (*see Preliminary Studies*), we also expect a significant reduction in CSF A$\beta_{42}$ after one year of treatment with simvastatin compared to placebo. However, a caveat is that reduction in CSF A$\beta_{42}$ after statin treatment is difficult to interpret in a straightforward manner, as it could imply either reduction in brain A$\beta_{42}$ production (which potentially could prevent formation of neuritic plaques) or potentially sequestration of CSF A$\beta_{42}$ into neuritic plaques in the brain. However, cognitively normal persons in the proposed age range of 21-50 are extremely unlikely to exhibit changes CSF A$\beta_{42}$ over the course of a year unless these changes are due to the treatment intervention. To aid in interpretation of CSF A$\beta_{42}$ findings,

presence or absence of the APOE*4 allele (which is associated with lower CSF $A\beta_{42}$ in cognitively normal persons - likely reflecting incipient amyloid pathology) as well as pretreatment ratios of CSF t-tau/$A\beta_{42}$ will be used as covariates during the analyses.

**PROTECTION OF HUMAN SUBJECTS**

**(1) Risk to Subjects**

(a) Human Subjects Involvement and Characteristics: Participants will be 120 Iraq and Afghanistan Veterans between the ages of 21 and 50 who are in good general health and who meet the specific study inclusion/exclusion criteria as described in detail in the "Research Design and Methods" section above. Persons of all races and ethnicities and both genders will be eligible. Participants will be naïve for statins, and will have either normal LDL cholesterol or mildly elevated LDL cholesterol which does not require drug therapy per NCEP/ATP-III guidelines.

Specific inclusion criteria include documented hazardous duty in Iraq and/or Afghanistan with the U.S. Armed Forces and exposure to blast concussion (with >6 months since last blast exposure) sufficient to meet ACRM criteria for mTBI (*described in detail above*). Participants will have a BMI between 18 and 36 (BMI outside this range may affect the biomarker measurements and increase difficulty of LP).

Exclusion criteria for all participants will include: penetrating head wound; seizure disorder; insulin-dependent diabetes; alcohol abuse or other substance abuse; DSM-IV diagnosis of schizophrenia or other psychotic disorder, bipolar disorder, or dementia; use of exclusionary medications (used in the 4 weeks prior to screening) or receiving any investigational medication; contraindication to LP (spinal deformity, severe lumbar spine disease or infection at LP site, bleeding tendency or on anticoagulant medication); platelet count less than 100,000 at time of LP.

Women of childbearing potential must have a negative pregnancy test at screen and must agree to use an acceptable form of birth control for the duration of the study. We suggest that women continue to use birth control for one full menstrual cycle after completing or withdrawing from the study.

It is likely that economically disadvantaged subjects will participate in this study. This may be considered a vulnerable population. While subjects will receive up to $650 for completion of the study, it is not felt that degree of remuneration will represent any form of economic coercion to those that are economically disadvantaged, considering the potentially significant time commitment we are asking the subjects to make. Prisoners and institutionalized individuals will not be involved in this study.

(b) Sources of Materials: Research materials from living human subjects will be results from clinical/neuropsychiatric assessments; blood samples collected for screening labs, DNA, and biomarker analysis; and CSF collected for biomarker analysis. These procedures will be done specifically for research purposes. In addition, existing medical records will be reviewed to confirm eligibility for participation. Consent will be obtained before reviewing existing medical records.

(c) Potential Risks: Risk to subjects is modest. All risks are research risks, none are therapeutic risks. They include possible stress from undergoing clinical/neuropsychiatric assessment, risks of blood draw/i.v. placement, LP, risks of taking simvastatin, and potential loss of confidentiality.

*Risks of Simvastatin:* Simvastatin is used widely for lowering cholesterol and for primary prevention of cardiovascular and cerebrovascular disease at the doses proposed in this application. It is generally safe and well tolerated. The most common adverse effects in clinical use are elevated liver transaminases, myalgias, and elevated CPK. However, the literature suggests that the rate of adverse reactions necessitating withdrawal from statin therapy is less than one percent in clinical practice.[15] Simvastatin is classified by the US FDA as Pregnancy Category X and is contraindicated in women who are pregnant or nursing. All female subjects of child bearing potential will have urine pregnancy tests at every study visit, will be required to be using a reliable form of contraception, and will be told of the risks of becoming pregnant while participating in this study. We also advise women of childbearing potential that they wait one full menstrual cycle after stopping the medications offered in this study before becoming pregnant.

Simvastatin is highly lipophilic and shows good ability to cross the BBB. Because brain cholesterol is synthesized *in situ* within the brain,[22] the ability of a statin to penetrate the BBB may be important in proving neuroprotection. However, some concerns regarding potential adverse effect of brain penetrating statins have been raised in recent years because adequate brain cholesterol bio-availability is essential for the proper functioning and physical integrity of brain cells. It has been hypothesized that long-term reduction of cholesterol in the brain with statins that readily cross the BBB may adversely affect cognitive functioning. Two case reports of single individuals reporting memory impairment in association with simvastatin use have appeared in the literature.[23,24] However, other reports indicate that these events are extremely rare. Wagstaff et al[25] reviewed

over 11,000 adverse events reported to the Food and Drug Administration's MedWatch program between November, 1997, and February, 2002, for simvastatin and pravastatin and were able to identify only 37 case reports of cognitive adverse effects (e.g., short-term memory loss, amnesia, confusion), none of which was documented with formal cognitive testing.

Available prospective, randomized, double-blind, placebo-controlled trials have provided scant evidence of statin-induced cognitive impairment as assessed by formal neuropsychological testing. In a study of 209 hypercholesterolemic adult subjects treated with lovastatin 20 mg/d or placebo for six months, Muldoon et al.[26] found that the statin-treated subjects showed slightly less improvement over time in comparison to placebo-treated subjects on tests of attention and psychomotor speed, but did not differ on tests of mental flexibility, working memory or memory retrieval. In a follow-up study comparing placebo, simvastatin 10 mg/d, and simvastatin 40 mg/d,[27] the same pattern of lesser improvement in simvastatin vs. placebo-treated subjects was seen only on some tests of attention and psychomotor speed but not on others. However, four independent studies did not find any differences in performance on a broad range of neuropsychological tests between those treated with BBB crossing statins and those treated with non-BBB crossing statins.[28-30] Finally, Gibellato et al.[31] studied 136 middle-aged military aircrew personnel treated with placebo, pravastatin 40 mg/d, or simvastatin 40 mg/d for 28 days and found no effects on performance on a battery of computerized tests demonstrated to be sensitive to detecting subtle impairments in complex cognitive processes involved in piloting aircraft (the Federal Aviation Administration CogScreen Aeromedical Edition).

The prospective trials of statin effects on neuropsychological measures of cognition all had small sample sizes and may therefore have been underpowered to detect changes in cognitive function. However, secondary analyses of data from the Heart and Estrogen/progestin Replacement Study of 1037 postmenopausal women found that declines in MMSE scores over four years of follow up were less in statin users versus non-users.[32] The larger Cardiovascular Health Study of 3,334 patients found a trend towards lesser declines in Modified Mini-Mental State Examination (3MS) scores in statin users versus non-users with hypercholesterolemia.[33] Of note, the overwhelming majority of subjects in these studies were elderly at the time of enrollment and therefore at elevated risk of experiencing cognitive adverse effects of statins. That these elderly subjects did not demonstrate cognitive adverse effects of statins argues strongly for their safety in other vulnerable populations, such as Iraq and Afghanistan Veterans with repetitive mTBI.

The absence of short-term changes in cognitive function in these large N studies argues persuasively that the effect sizes of any potential adverse cognitive effects must be exceedingly small. As cogently stated in a recent review of statin safety, "These measurements in large numbers of participants in randomized trials establish beyond doubt that statins cause no perceptible decline in cognitive function".[34] Consistent with this low rate of adverse events, simvastatin was approved for over-the-counter sale in the United Kingdom in 2004.[35] In addition, a US Neurology Expert Panel charged with the task of reviewing the scientific evidence related to adverse effects of statins recently concluded that statins do not impair memory or cognitive function;[36] however, this expert panel also recommended that "investigation of statins should include secondary outcome measures that address potential adverse events," we will monitor subjects in our study for changes in cognitive function by means of a brief global cognitive scale at the 6 week and 3- 6- 9- and 12-month visits.

*Risks of venipuncture:* Removal of blood by a needle and Vacutainer or syringe poses a small risk of pain or bruising at the site of the needle stick. Infrequently, subjects may experience fainting or dizziness and there is also a slight risk of infection at the site of the needle stick.

*Risks of Lumbar Puncture:* The risk of serious effects from LP (infection, nerve root damage) is extremely low. Rarely, patients may experience transient local pain either at the site of the puncture or in the distribution of the sciatic nerve if the nerve roots of the *cauda equina* are stimulated. Because the LP is performed well below the termination of the spinal cord and because the nerve roots readily move aside from the spinal needle, the possibility of permanent damage to a nerve root is very remote. Subjects with blood clotting abnormalities, on anticoagulant medications, or with inadequate platelet function will be excluded. A previously common (approximately 20% in young persons) adverse effect was post-LP headache. The incidence of headache is a function of the diameter and type of spinal needle used and <u>not</u> the amount of fluid removed. Prior to the past 8 years, our method of using a very fine 25g Quincke (cutting) spinal needle reduced the incidence of post-LP headache to less than 5%. Over the past 8 years, we have instituted use of the 24g Sprotte atraumatic spinal needle. The Sprotte needle has been recommended in recently published guidelines for reducing risk of post-LP headache. We have performed over 300 LPs since we began using the Sprotte spinal needle and in our hands, risk of post-LP headache is now less than 1%, similar to that reported in the literature for the 24g Sprotte needle.

In addition, it should be noted that removal of up to 30 cc of CSF does not increase the incidence of headache since CSF is rapidly produced. CSF is produced normally at the rate of 0.35cc/5 minutes; it is estimated that the amount of CSF that we will draw will be replaced in about 6-7 hours.[37,38] The post-LP headache is thought to occur due to a persistent leak of CSF from the hole left in the dura by the larger bore Quincke spinal needle used in commercial LP kits which routinely contain an 18 or 20 g Quincke spinal needle, rather than the amount of CSF removed acutely. This leads to a depletion of the normal CSF hydraulic "cushion" and leads to a headache the day after the procedure. We reduce the risk of CSF leak by having the subject lie in bed for one hour after the procedure, having them increase their fluid intake, and by instructing subjects not to engage in any strenuous activity for the 24 hours following the procedure. All investigators performing LPs are very experienced in performing this procedure in these subject populations.

Headaches following LP, if they occur, are usually mild to moderate and may last for 24-48 hours; such headaches are successfully managed with acetaminophen and caffeine (in the form of a soft drink, Mountain Dew). In unusual cases, typical post-LP headache, which is posturally sensitive, may occur; these may be fairly severe and can last as long as a week with conservative management. Our approach to treatment of severe typical post LP headache is the epidural blood patch, performed on the first morning of severe post-LP headache (usually the day following LP). Epidural blood patch consists of the injection of 30 cc's of the subject's own blood into the epidural space over the lumbar puncture site. This procedure is done by an anesthesiologist and usually provides immediate relief of post-LP headache. The Anesthesiology Service at VA Puget Sound Health Care System has agreed to perform these procedures if necessary and has quite graciously and successfully performed them in the past.

We measured stress hormone responses to LP in subjects with AD and young and older normal subjects. Stress hormone responses (cortisol, norepinephrine and epinephrine) in plasma and CSF were reduced in AD subjects compared to young or older normal controls. These stress hormone responses were consistent with the lack of behavioral arousal observed in the AD patients in response to LP. More recently, we have analyzed data from 428 research LPs performed on 342 subjects (67 with AD or MCI, and 275 cognitively normal adults). The frequency of any adverse event (11.7%), clinically significant adverse events (3.97%), and typical post-lumbar puncture headache (PLPHA) (0.93%) was low. Risk of PLPHA was unrelated to age, gender, position during LP, mls CSF collected, or minutes of recumbent rest following LP.[19]

*Risks of Genetic Studies:* We will be testing for APOE genotype. The APOE-$\varepsilon$4 allele has been implicated as a risk factor for development of AD in later life. However, APOE type should not be considered a genetic test for AD since the results will not conclusively determine whether a person or any of his/her relatives have or might develop AD. Subjects are informed that results of APOE testing will be used for research purposes only and that subjects will not be informed of their APOE genotype. Subjects will be told that if they wish to know their APOE genotype, that they can have the testing done at a commercial lab at their own expense.

The Genetic Nondiscrimination Act of 2008 protects subjects against risks to employability and access to health insurance due to participation in genetic studies. However, it is theoretically possible that participation in genetics studies may jeopardize access to life insurance, disability insurance, or long-term care insurance if involvement and/or results of the study become part of the medical record. Procedures to minimize such risk are described below.

*Risks of Clinical Assessment and Neuropsychological Testing:* Risks due to clinical assessment procedures are modest. Subjects may experience stress similar to that of a standard clinical evaluation for memory complaints or dementia. Subjects may also receive information that they are cognitively impaired. Such information may be distressing to a subject. The neuropsychological assessment battery may cause fatigue and/or unpleasant affective states due to the large number of individual tests. Some of the clinician and self-administered assessment instruments may cause participants to focus their attention on traumatic combat-related experiences and as a result may produce transient subjective distress. However, these combat experiences are routinely assessed and discussed as part of standard clinical care.

*Risks to Confidentiality:* There is a risk of loss of confidentiality. Procedures to address protection against loss of confidentiality are addressed below.

**(2) Adequacy of Protection from Risks**

(a) Recruitment and Informed Consent: Participants will be recruited from the MIRECC Mild TBI/Behavioral Specialty Clinic; Deployment Health Clinic, Polytrauma Clinic, General Internal Medicine Clinic, and Mental Health Clinics at the VA Puget Sound; the Seattle downtown Vet Center; CBOCs, and from outreach to National Guard and Reserve units. Recruitment will be assisted by posters, flyers, brochures,

announcements in the VA daily bulletin, and print and radio media advertising. Prospective participants will have their charts reviewed by referring providers to screen for exclusionary medical conditions and medications.

The study will be explained to eligible subjects by one of the clinician (PhD, MD, RN) investigators in a private setting. They will be informed of both the risks and benefits of the study. Potential subjects will be given ample opportunity to read the consent form and will be encouraged to ask questions. If a potential subject wishes to take the consent home to further consider participation, he or she will have that opportunity. Written informed consent will be obtained from subjects. The signed consent form will be placed in the subject's research record and a note documenting consent will be placed in the subject's computerized medical record.

If a potential subject's primary mental health care provider is a study investigator, another investigator will conduct the consent process. Care will be taken to assure subjects that their current treatment and future services and benefits are not contingent on participation in this or any research study. The voluntary nature of research study participation will be emphasized. Subjects will be informed that participation is voluntary and that their decision to participate or not will not effect their treatment at VA Puget Sound.

Participants will receive compensation for their time and inconvenience as follows: $50 for the screening visit, $50 for each cognitive testing day, $200 for each LP day, and $25 for each safety visit. The total each subject will receive if all study visits are completed is $650. Subjects who withdraw early or are withdrawn from the study will receive pro-rated payments based on the number of study visits completed.

(b) Protection Against Risk:

*Reduction of risks related to simvastatin:* All study participants will have either normal or mildly elevated cholesterol not meeting current NCEP guidelines for treatment with cholesterol lowering medications. Simvastatin has been widely used for primary or secondary prevention of cardiovascular diseases. Although we will treat subjects with non-elevated cholesterol, adverse effects related to excessively low cholesterol levels are not anticipated, as recent trials with high doses of potent statins producing very low levels of LDL-cholesterol have shown that such low LDL-cholesterol serum levels are generally safe and, in fact, are associated with improved clinical outcomes in terms of coronary artery disease.[10]

Simvastatin is classified by the US FDA as Pregnancy Category X and is contraindicated in women who are pregnant or nursing. All female subjects of child bearing potential will have urine pregnancy tests at every study visit and will be told of the risks of becoming pregnant while participating in this study. We consulted with pharmacist at VA Puget Sound whether a woman should wait for a certain period after ceasing statin drug before becoming pregnant and he suggested 16 days (the elimination half-life of radioactive simvastatin plus metabolites in humans is 4.5 hours. Assuming 95% of the drug and metabolites are eliminated after 5 half-lives, then 16 days could be considered safe). We are erring on the safe side and suggesting that women of childbearing potential wait one full menstrual cycle after stopping the medications offered in this study before becoming pregnant. Contraception is not required for male participants.

At randomization, subjects will be advised of potential drug-related side effects from simvastatin such as muscle pain and weakness and nausea or vomiting. They will be counseled on the importance of letting the study personnel know which medications they are currently taking and that they should inform the study staff before initiating any new medication treatment. Subjects will be told that many medications should not be taken with simvastatin and that they should not drink large quantities of grapefruit juice while in the study (as intake of large quantities of grapefruit juice may result in elevated simvastatin serum levels). Subjecst will also be advised to avoid drinking excessive amounts of alcohol.

Subjects will come to the research clinic for regular monitoring. At each visit, a qualified licensed clinician will ask subjects if they have experienced any adverse events, specifically if they have experienced any unexplained muscle pain, or weakness. Any adverse event will be reported to the PI, or if the PI is not available, an alternative MD investigator. Subjects will be encouraged to call a study clinician at any time if they are experiencing any side effects. Subjects will also be provided with 24-hour emergency contact information.

LFTs, CPK, and HbA1c levels will be measured at 6 weeks and after 3-, 6-, 9-, and 12- months of treatment. The PI will review the laboratory results for every subject within 24 hours of the study visit. If the PI is not available, an alternative MD practitioner will review the laboratory results. If SGOT or SGPT values are ≥ 3 times the upper limit of normal, or if CPK levels are ≥ 10 times the upper limit of normal, study drug will be discontinued and subjects will be followed until laboratory values return to normal.

To monitor for potential cognitive side effects, we will perform the MoCA (a brief but sensitive global cognitive test) at all safety visits. If a subject reports problems with memory or cognitive function after initiation of study medication sufficient to interfere with daily function or MoCA score decline of one standard deviation or greater compared to Baseline, we will perform a thorough neurological and psychiatric examination and will

administer the entire neuropsychological battery. If a decline of one standard deviation or greater compared to Baseline is present on any test, the subject will be terminated from the study.

To monitor for emergence or worsening of comorbid depression, the PHQ-9 will be administered at all visits. The PHQ-9 score can range from 0 to 27. Scores of 5, 10, 15 and 20 represent thresholds demarcating the lower limits of mild, moderate, moderately severe and severe depression, respectively. If the PHQ-9 score is increased by 10 (i.e., an increase of two severity categories) compared to baseline or if the subject develops suicidal ideation with intent, the subject will be terminated from the study. To monitor alcohol use and symptoms of comorbid PTSD, we will administer the AUDIT-C and the PCL-M at all visits.

Subjects who are discontinued for adverse events will be referred back to their primary medical provider for further assessment, treatment, and follow-up.

*Reduction of risks due to Lumbar Puncture:* Women of childbearing potential will have a negative urine pregnancy test before undergoing any LP procedures. Pain at the needle insertion site will be minimized by adequate local anesthesia with subcutaneous injection of 1% lidocaine. We will use an atraumatic 24g Sprotte spinal needle which is associated with a reduced risk of adverse events, including post-LP headache. The Sprotte needle has been recommended in published guidelines for reducing risk of post-LP headache. We have performed over 800 LPs since we began using the Sprotte spinal needle and in our hands, risk of post-LP headache is <1%.[19]

We reduce the risk of CSF leak by having the subject lie in bed for one hour after the procedure, having them increase their fluid intake, and by instructing subjects not to engage in any strenuous activity for the 48 hours following the procedure. All investigators performing LPs are very experienced in performing this procedure in these subject populations.

Lastly, if the subject wishes, the subject's spouse, other family member, or friend may be present in the room during the LP, and is able to help keep the subject relaxed and comfortable.

*Reduction of risk due to venipuncture and i.v. placement:* All such procedures will be performed by experienced study staff using sterile technique.

*Reduction of risk due to loss of confidentiality:* We plan on obtaining a Federal Certificate of Confidentiality to protect against compelled disclosure of answers to the questionnaires. We will keep study records and data in a secure location, either in locked filing cabinets in locked rooms or on restricted-access, password-protected computers which meet all VA-mandated data security standards. Identifying information will not be stored with study data.

*Reduction of risks due to questionnaires and assessments:* To prevent the development of fatigue and/or unpleasant affective states due to the subject matter and the large number of individual tests, subjects will be able to take breaks between individual tests. Subjects are free to refuse to answer any question.

*Safety procedures specific to the war zone population:* include exclusion of participants with severe psychiatric instability or severe situational life crises, including evidence of being actively suicidal or homicidal, or any behavior that poses an immediate danger to patient or others. For participants enrolled in the study, requiring psychiatric interventions to manage deterioration of clinical status will result in early study termination, and appropriate crisis management by a study clinician, in conjunction with the participant's primary mental health care provider and referral to outpatient or inpatient psychiatric care at each study site. The PI (or back-up) will be on call at all times to receive calls from participants regarding any adverse events; participants will be provided with 24-hour emergency phone numbers.

In addition, due to the increased scrutiny surrounding studies of persons with comorbid depression, we will specifically ask about suicidal ideation at each study visit. Subjects who endorse suicidal ideation will be assessed as to their level of risk and the level of care required to keep themselves or others from harm. If a patient requires inpatient treatment in order to prevent harm but is unwilling to accept that care, referral will be made to the county-designated Mental Health Professional (MHP) and the patient will be supervised in a safe setting in the VA Puget Sound emergency room until evaluated by the MHP and appropriate disposition made. In a similar fashion, patients that are found to have symptoms of disorganized thought processes secondary to psychosis would be referred for an emergency psychiatric evaluation at VA Puget Sound.

*Treatment of Adverse events:* All treatment for adverse events related to the study will be provided free of charge. DVA authorizes treatment for any study approved by a VA R&D Committee. Subjects who seek treatment for study-related adverse events in a non- VA facility will be reimbursed for treatment costs. VA Puget Sound has adequate personnel and equipment to respond to expected and unexpected adverse events.

**(3) Potential Benefit of the Proposed Research to the Subject and Others**

All study participants will have a history of blast mTBI, which is now a known risk factor for

neurodegeneration. If study results show that simvastatin decreases the risk of processes related to neurodegeneration and promotes neuronal survival and growth, subjects who were treated may benefit by having the development of neurodegeneration either halted or delayed. Given the potential neuroprotective effects of simvastatin, subjects may benefit from improved cognitive function and reduction of PPCS (such as headache, irritability, sleep problems). Subjects will not benefit from the LPs or neurocognitive testing. However, we expect a very minimal incidence of adverse events from these procedures and the risks are reasonable when weighed against the importance of the knowledge to be gained.

**(4) Importance of the Knowledge to be Gained**

Combat-related repetitive blast mTBI is a new and unfortunate phenomenon of the current wars in Iraq and Afghanistan. Although blast trauma mTBI is likely similar to sports-related repetitive mild TBI and recent autopsy studies suggest common pathology of chronic traumatic encephalopathy, it is unknown whether it will present a similar increased risk for neurodegenerative changes culminating in dementia. If so, the consequences will present a major public health and Veterans Health Administration challenge. Over 2.4 million soldiers and Marines have been deployed to Iraq and Afghanistan; approximately 9-18% return with symptomatic mTBI. Over the course of the wars, soldiers and Marines were re-deployed to additional tours of duty with increased exposure to blast trauma likely not yet reflected in the estimates of prevalence. The information gained from this study will enhance our ability to improve health care, appropriately allocate limited health care and social resources, and form the evidence base for pharmacotherapeutics for ameliorating current symptoms and preventing progression to neurodegenerative dementing disorders. The importance of gaining this information outweigh the risks of study participation.

**(5) Data Safety and Monitoring Plan**

(a) Monitoring of Subject Safety: Participant safety will be monitored as described above. Participants will be given 24 hour emergency contact information and will be instructed to call if they experience any AE during the study. In addition, subjects will called by a study clinician within 24 hours after lumbar puncture to enquire about any adverse events, specifically lumbar puncture headache.

All AEs and serious AEs (SAEs) will be recorded at each visit and communicated to the study PI. Unanticipated AEs (physical complaints that are not present at baseline) and AEs which are more serious than anticipated, but are not SAEs will be reported to the IRB at the time of the annual report. SAEs (defined as any untoward medical occurrence at any dose that results in subject death, is life threatening, requires hospitalization, or results in persistent or significant disability/incapacity) will be reported to the IRB within 5 business days of being reported to the PI.

We recognize that a majority of these Veterans with mTBI will also have comorbid PTSD and/or depression; thus there is the possibility of worsening of their mental health status during study participation. Any participant who develops dangerousness toward self or others or emergence of acute symptoms necessitating hospitalization will be discontinued from study participation if these events occur during the period of data collection and then reevaluated for appropriateness for continuing in the study following reestablishment of psychiatric stability.

All participants will receive follow-up per their primary mental health care provider or will be referred for appropriate follow-up if they do not have a primary mental health care provider at study entry.

(b) Accuracy and Integrity of Study Data: All study information will be recorded on standardized CRFs. CRFs will be labeled with study code number only. A master database will be set up using Microsoft SQL2000 server software at VA Puget Sound by the Data Manager. Data will be double-data entered weekly through a web based data center application. The web application and backend processes will aid in checking data integrity. The Data Manager will generate weekly reports of data discrepancies. The Study Coordinator will be responsible for rectifying data entry errors. Database maintenance includes weekly backup to tapes, which are stored at a safe location, and SQL server routine maintenance. SQL server security will include sign-on authentication and access to the database will be password protected. Laboratory data (e.g., CSF t-tau, p-tau$_{181}$, and APOE genotype) will be generated at several different laboratories. These laboratories will enter data into their own databases and will export them as an Excel file (or similar easily readable file) to the Database Manager. All files sent to the Database Manager will be encrypted. These data will be linked via unique study identification numbers assigned to each study subject.

(c) Safety Monitoring Board (DSMB). A DSMB will be established for this study. The DSMB will consist of two expert geriatricians with many years of experience in clinical and research use of statins and membership on DSMBs: William Hazzard, MD, and David Gruenewald, MD. Dr. Gruenewald will be DSMB

Chair. They comprise the DSMB for Dr. Li's clinical trial of simvastatin for primary prevention of AD. Data will be prepared for the committee by the Dr. Millard and Ms. Xiang and sent to the committee coded by arm. The committee will meet annually to review the safety profile of the study and, as requested, to review interim analyses for efficacy. Annual reports will be provided by the DSMB Chair to the PI, Dr. Peskind, and the IRB. The DSMB will: 1) review unmasked data as needed and appropriate during the trial; 2) identify problems relating to safety during the study; 3) identify needs for additional data relevant to safety issues, and request these data from the study investigators; 4) propose appropriate analyses and periodically review developing data on safety and endpoints; and 5) consider the rationale for continuation of the study, with respect to progress of randomization, retention, protocol adherence, data management, safety issues, and outcome data (if relevant) and make a recommendation for or against the trial's continuation.

**(6) Inclusion of Women and Minorities:**

Because of the requirement that blast trauma group participants have experienced at least one blast trauma exposure, the majority of participants in this group will come from MOSs with high blast trauma exposure: Infantry, Combat Engineers, Explosive Ordnance Disposal, and MP. Infantry, Combat Engineers, and Explosive Ordnance Division are all male combat MOSs. It is estimated that fewer than 25% of the MP MOS participants will be women based on the gender composition of the Military Police who served in Iraq and Afghanistan. Although it is not anticipated that we will have sufficient numbers of women subjects to have adequate power to detect gender effects on study outcomes, gender will be used as stratification variable during randomization to ensure that women subjects have an equal chance of receiving active drug. Gender will be used as a covariate in the analysis and results may generate hypotheses for future gender-targeted studies of mTBI. Persons of all races and ethnic backgrounds will be eligible. The veteran patient population at VAPSHCS is comprised of 75% Caucasians, 14% African Americans, 4% Native Americans, 3% Hispanic Americans, 2% Asian Americans, and 2% “other”. No differences based on racial/ethnic differences are expected. Ethnic and minority composition of participants in Dr. Peskind's ongoing VA Rehab R&D Merit Review: "Mild TBI and Biomarkers of Neurodegeneration" has been comparable to the overall Veteran population.


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