Protocol:
Impact of Icosapent Ethyl on Alzheimers Disease Biomarkers in Preclinical Adults: "Brain Amyloid and Vascular Effects of Eicosapentaenoic Acid (BRAVE-EPA)" Study

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A) PROJECT SUMMARY

Over five million Americans have Alzheimer's disease (AD), and this number is expected to triple by 2050 unless effective preventive strategies are identified. Veterans are at an even higher risk for AD than the general population, possibly due to their increased exposure to factors that accelerate AD pathology, including vascular risk factors, traumatic brain injury, and post-traumatic stress disorder. AD pathology occurs decades before cognitive symptoms occur and is characterized by amyloid plaques, neurofibrillary tangles, and reduced regional cerebral blood flow in areas of the brain related to memory and learning. While cerebral arterial dysfunction occurs early in the development of AD pathology and decades before symptoms begin, the effects of treating such early vascular dysfunction in the brain are poorly understood. The omega-3 fatty acid eicosapentaenoic acid (EPA) improves arterial function and cerebral blood flow, attenuates adverse brain changes related to β-amyloid protein, and improves cognition in animals – changes that could all potentially protect against AD. However, it is not clear whether EPA beneficially affects these processes or cognitive performance in cognitively-healthy adults at increased risk for AD. In 2012, the Food and Drug Administration approved the first high-dose prescription EPA-only medication to treat hypertriglyceridemia, called icosapent ethyl (available as Vascepa® in the United States). This agent is readily available for use and has a good safety profile, making it a favorable agent to consider for AD prevention.

The proposed study is a proof-of-concept, randomized, placebo-controlled, double-blind, parallel-group clinical trial assessing the efficacy of 18 months of icosapent ethyl (IPE) therapy on magnetic resonance imaging (MRI), cerebrospinal fluid (CSF), and cognitive biomarkers for AD in 150 cognitively-healthy Veterans ages 50–75 years. Since Vascepa® has a daily dose recommendation of 4 grams daily, participants will be randomized to either 4 gram daily IPE (Vascepa®) or matching placebo. The overarching goal of this trial is to assess whether IPE beneficially affects intermediate physiological measures associated with the onset of AD in order to evaluate whether larger, multi-site, longer-duration Alzheimer's prevention trials are warranted to assess more definitive clinical outcomes.

B) BACKGROUND AND SIGNIFICANCE

AD is a devastating illness leading to progressive neurodegenerative changes and cognitive decline. Current therapies to treat AD are limited in their efficacy and work primarily to delay the inevitable decline in function. Disease-modifying and preventive therapies have not yet been identified. The projected tripling of the number of persons with AD in the United States in the next 40 years has led to an urgent need to rapidly identify AD preventive strategies. Veterans are at even higher risk of developing AD than the general population due to a variety of factors including increased prevalence of vascular risk factors, traumatic brain injury (TBI), post-traumatic stress disorder (PTSD), and depression. While longitudinal studies such as the groundbreaking Department of Defense Alzheimer's Disease Neuroimaging Initiative (DOD-ADNI) are seeking to better understand how TBI, PTSD, and depression contribute to AD pathology in Vietnam Veterans, some earlier data suggest that these conditions may mediate AD risk in part through vascular mechanisms. Depression and AD both share common vascular risk factors and Veterans treated for PTSD are more than twice as likely to have dyslipidemia or hypertension compared to Veterans without PTSD. Within the Veteran population, persons who are eligible for and use care through the Veterans Affairs (VA) system have an even greater risk for AD than non-VA Veterans due to their aggregation of risk factors related to VA eligibility – on average, they have lower income, are less likely to be employed, have fewer years of education, and are more likely to have

service-related medical or psychiatric conditions. In addition, compared to non-VA Veterans, VA Veterans are more likely to be African American – a population with increased incidence rates of vascular risk factors and twice the prevalence of AD compared to white adults. Based on available data, experts anticipate 423,000 new cases of AD in Veterans by 2020, including 140,000 cases directly attributable to military service. Thus, finding effective therapies to prevent or delay the onset of AD in Veterans is critical. The VA is poised to lead cutting-edge AD prevention research that includes Americans with a high likelihood of developing AD – namely Veterans within the VA healthcare system.

Evidence supports that AD neurodegenerative changes likely begin decades before clinically-apparent disease. The NIA-Alzheimer's Association (AA)-sponsored workgroup defined research criteria for the preclinical stages of AD and identified factors that best predict risk of progression from "normal" cognition to mild cognitive impairment (MCI) and AD. Our investigative team at the NIH-funded, jointly-sponsored University of Wisconsin (UW)/ Madison VA Wisconsin Alzheimer's Disease Research Center (ADRC) has focused our studies on early identification and treatment of such preclinical, high-risk individuals – in particular, asymptomatic middle-aged adults with parental history of AD. While previous studies have demonstrated that having a first-degree relative with AD increases the risk of developing AD, independent of the risk conveyed through the genetic risk factor apolipoprotein E ε4 (*APOE4*) allele, more recent research supports that biomarker elevations, but not *APOE* or parental history status, are associated with risk of cognitive decline in cognitively unimpaired adults.[1] Our Wisconsin ADRC investigators have found that asymptomatic middle-aged adults with parental history of AD show early neurobiological changes on CSF biomarkers, MRI biomarkers including measures of regional cerebral blood flow (rCBF), and cognitive biomarkers for AD while demonstrating normal global cognitive and clinical function. In a group of 218 cognitively healthy adults ages 40-65 years with parental history of AD in our Wisconsin ADRC-related studies, we found that 129 (60%) are either carriers of the APOE4 allele or have CSF β-amyloid-42 (Aβ42) or tau biomarker levels suggesting preclinical disease (preliminary data, unpublished). Thus, these high-risk individuals are ideal to study in that they are clinically asymptomatic, yet have neurobiological evidence supporting preclinical AD-related changes in their brains. For the proposed clinical trial, we will focus on cognitively healthy Veterans ages 50-75 years with or without parental history of AD. Participants will have Veteran-specific risk factors related to increased prevalence of atherogenic vascular risk profiles, TBI, PTSD, and depression. In addition, we will gather information on their parental history of AD to account for potential risk variation given the conflicting findings on the importance of parental history of AD in the development of this common form of dementia.

Hypothesis:
In middle-aged and older cognitively-healthy Veterans (ages 50–75), 18 months of treatment with icosapent ethyl (IPE) will beneficially modify preclinical AD biomarkers, including regional cerebral blood flow (rCBF), cerebrospinal fluid (CSF) markers of AD pathology, and cognitive performance.

Specific Aims:
(a) To investigate the effects of 18 months of IPE 4 g daily vs. placebo on arterial spin-labeling (ASL) MRI regional cerebral blood flow (rCBF) in a pre-defined statistically-identified region of interest (ROI) affected early in preclinical AD.

(b) To determine the impact of 18 months of IPE vs. placebo on CSF biomarkers of preclinical AD pathology (CSF β-amyloid-42, total tau, and phosphorylated tau181).

(c) To evaluate the effects of 18 months of icosapent ethyl vs. placebo on a composite measure designed to assess preclinical cognitive changes — the Alzheimer's Disease Cooperative Study (ADCS) Preclinical Alzheimer's Cognitive Composite (PACC).

Description of the primary endpoint: The primary endpoint will be the change in rCBF in a pre-defined statistical ROI at 18 months in participants randomized to IPE vs. placebo. Animal and human studies show that regionally reduced CBF (related to both neuronal hypometabolism and systemic vascular disease) in areas of the brain important for memory and learning is a very early change in AD pathogenesis and, thus, therapies known to beneficially modify arterial function may improve CBF and lead to early neuroprotection and maintenance of cognitive function. The pre-defined statistical ROI that will be used for the proposed trial was developed using longitudinal ASL-MRI CBF data from 133 adults (ages 43–73) in the NIH-funded Wisconsin Registry for Alzheimer's Prevention (WRAP) cohort, which is enriched for parental history of AD. Areas of the brain with greatest CBF decline over two years were identified using the Alzheimer's Disease Neuroimaging Initiative (ADNI)-derived analytic method developed by Chen et al. The statistically-defined region is consistent with those affected early by AD pathology. Using anatomical ROIs that aligned with the statistically-defined ROI, treatment effect on 18 month rCBF was examined hierarchically in a priori selected regions of interest in the following order: bilateral posterior cingulate, bilateral precuneus, bilateral medial temporal lobe. Analyses of ROIs were discontinued if the group difference in 18 month rCBF in the previous ROI was not significant. Secondary endpoints will be 18-month changes in CSF biomarkers of AD pathology (Aβ42, t-tau, p-tau) and changes in ADCS-PACC cognitive score.

If IPE beneficially modifies AD biomarkers in at-risk Veterans, the results from the proposed trial would be used to develop multi-site, longer-duration clinical trials using the VA Cooperative Studies Program to assess the efficacy of icosapent ethyl on not only AD biomarkers, but also on more clinically definitive outcomes, such as rate of cognitive decline and conversion to mild cognitive impairment (MCI). If future studies show that icosapent ethyl delays the onset of AD by even an average of 5 years, estimates suggest that this could reduce the prevalence of AD by about 50%.

C) RESEARCH DESIGN AND METHODS

Trial Design Summary

The proposed proof-of-concept study will use an 18-month randomized, placebo-controlled, double-blind, parallel-group clinical trial design to investigate the effects of IPE (available as Vascepa® in the United States) on rCBF, CSF AD biomarkers, and cognition in 150 cognitively-healthy Veterans ages 50-75 years at increased risk for AD due to their Veteran-related risk factors including high vascular risk profiles, TBI, and PTSD. The primary aim of the trial (**Specific Aim 1**) is to assess whether IPE increases 18-month rCBF in a statistically-defined region of interest (sROI) in a population of middle-aged veterans compared to placebo. Key secondary aims within this proof-of-concept pilot RCT are: (**Specific Aim 2**) to assess whether IPE decreases CSF Aβ42; and (**Specific Aim 3**) to assess whether IPE improves cognitive performance on the ADCS-PACC battery compared to placebo. Data collected within this clinical trial will also allow exploratory analyses of the effects of IPE on measures of axonal and neuronal health, including CSF biomarkers (total tau, phosphorylated tau [p-tau], neurofilament light [NFL] protein, soluble amyloid precursor protein α and β [sAPP-α and sAPP-β], monocyte chemotactic protein 1 [MCP1], YKL-40, and other CSF measures) and neuroimaging biomarkers (diffusion

weighted imaging, volumetric changes, 4D flow measures, and white matter hyperintensity measures among others). In addition, data collection from this trial will allow for exploration of the impact of previous TBI and/or PTSD on response to therapy. We hypothesize that in this population, IPE will beneficially affect mechanisms central to AD pathology by: 1) increasing rCBF within the sROI; 2) reducing CSF Aβ42; and that these neurobiological changes will be associated with 3) an increased ADCS-PACC cognitive composite score. While recognizing that the proposed trial is not addressing all potential effects of IPE, such as changes in measures of inflammation or oxidative stress, we will store neuroimages and blood and CSF samples for future analyses of other potential mechanisms.

Table 2. Study Design

VA BRAVE-EPA STUDY	**Visit Number/Month (+/- 8 weeks)**								
	Pre-Study V0 M-1	**Bse V1 M0**	**V2 M1**	**V3 M3***	**V4 M6***	**V5 M9**	**V6 M12***	**V7 M15***	**Final (Or Early Termination) Visit V8 M18**
Eligibility review	X								
Informed consent	X								
Past medical history & medication review	X	X	X	X	X	X	X	X	X
Side effect review			X	X	X	X	X	X	X
Randomization		X							
Medication dispensing		X	X	X	X	X	X	X	X
Pill count			X	X	X	X	X	X	X
Participant honorarium given		X				X			X
Screening cognitive tests									
MoCA, CERAD List Learning	X								
Dietary Food Frequency Questionnaire		X				X			X
Screening questions									
Deployment history, TBI, PTSD		X							
Hamilton Depression Rating Scale		X	X	X		X			X
MINI International Neuropsychiatric Interview (subset)		X							
Vitals, anthropometrics	X	X	X	X		X			X
Physical and neurologic exam		X				X			X
ADCS-PACC cognitive test battery		X				X			X
Lumbar puncture		X				X			X
MRI (ASL, VIPR, FLAIR, DWI)		X				X			X
Screening/safety labs									
Fasting lipid profile (TC, TG, HDL, LDL)	X	X				X			X
glucose	X	X				X			X
High sensitivity c-reactive protein		X				X			X
AST, ALT	X	X	X	(X)**	(X)**	X	(X)**	(X)**	X
creatinine	X	X				X			X
Urine pregnancy (if of childbearing potential)	X	X	X	X	X	X	X	X	X
Complete blood count	X								
CSF cell count with differential		X				X			X
***APOE* genotyping**[***]	X								

CSF Aβ42, tau, p-tau-181, Aβ40, NFL (Zetterberg lab)		**X**				**X**			**X**

*Month 3, 6, 12, and 15 visits may be done remotely over the phone with labs drawn and a pregnancy test completed locally if participant travels from a distance or as required/requested during COVID. Medications will be shipped to participants and any remaining pills returned at their next in-person visit for pill counts.

**Participants with baseline or month 1 AST or ALT between 1.5 upper limit of normal (ULN) and ≤2 x ULN at the preceding visit will have repeat AST and ALT values drawn at 3-, 6-, 12- and 15-month visits as well.

***After final visit, whole blood collected at the Pre-Study visit will be used for APOE genotyping.

To test this hypothesis, we will enroll 150 cognitively healthy male and female Veterans and randomize them in a 1:1 ratio to IPE 4 g daily or matching placebo. All participants will undergo informed consent and will be screened for eligibility via a pre-study screening visit (Table 2). At baseline and months 9 and 18, all participants will undergo MRI, lumbar puncture (LP) for CSF collection, a blood draw, cognitive testing, vascular risk assessment, and will complete questionnaires including a food frequency questionnaire (FFQ) targeting omega-3 fatty acid intake and screening questions assessing prior combat and non-combat deployment, TBI, PTSD, and related sequelae. At months 1, 3, 6, 12, and 15, participants will have an assessment of medical/medication changes and potential side effects, interim lab assessments as indicated (aspartate transaminase [AST], alanine transaminase [ALT], urine pregnancy as needed) (Table 2), have vitals and body measurements collected, and be evaluated for depression using the Hamilton Rating Scale for Depression (HAM-D) test. As detailed in the Statistical Analysis section, using a two-sided test at the 0.05 level and anticipating a conservative 90% retention rate, a sample size of 150 participants will provide 90% power to detect significant changes in our primary and key secondary outcomes. Specifics of the above procedures are outlined below.

We will aim to schedule study visits within 8 weeks of the target visit time, but it is possible that the actual visit will be outside of this window. If the final visit is delayed, we may ask the participant to remain on the study medication for no more than 6 additional months based on statistical analysis, resources, funding, or other factors. This will ensure the study team to collect accurate results at the participant's final biomarker visit.

If the participant does not bring their remaining study medication to their final visit, we will provide them with a postage-paid envelope to return the medication to the study team for disposal. If this envelope was not provided at the visit, it will be sent with a letter requesting the return of the study medication. If we do not receive the remaining study medication, the study team will create a note to file documenting that the participant completed the study, but any remaining study medication was not returned.

If we are having trouble contacting the participant to schedule their visit within the window, we will send a follow-up letter asking them to call us by a certain date if they wish to continue participating. If we do not hear from the participant by the end of the day on the date specified in the letter, we will assume they are no longer interested and withdraw them from the study. The letter will include a request that the participant return any remaining study medication in the enclosed postage-paid envelope. The study team will properly dispose of any remaining medication after completing a final pill count. If we do not receive the remaining study medication,

the study team will create a note to file documenting that the participant was withdrawn from the study, but any remaining study medication was not returned.

Recruitment

Potential subjects will be recruited from the William S. Middleton Memorial Veterans Hospital (Madison VA), surrounding VA Community-based Outpatient Clinics (CBOCs) (Baraboo, Beaver Dam, Janesville, WI and Rockford and Freeport, IL) through VA primary care and specialty care clinics, physician referrals, electronic message boards in VA waiting rooms, letters, brochures, emails, newsletters, newspaper and web-based advertisements, educational and outreach events, including our Madison VA's Annual Research Day, and from the ADRC Clinical Core or other ADRC-affiliated studies through similar methods. Our partners and affiliates may use their websites, newsletters, and emails to announce open enrollment into our study or educational and outreach events being led by us. Veterans' records will be flagged in the VA's VistA and Computerized Patient Record System to obtain release of information from participants to 1) review their medical records for eligibility in the study and 2) safety measures to place a note in Veterans' records to indicate the Veteran is in our study and to clarify the participant is on medication that could be a contraindication for medical procedures or other health care procedures the patient may have to undergo. Additionally, clinic providers will provide descriptive study materials to potential enrollees.

The first steps for screening potential participants will be to 1) provide an explanation of the goals, scope and nature of the study, 2) complete a Screening Checklist, 3) obtain permission for study personnel to keep their contact information and Screening Checklist for the duration of the screening process, and 4) to exclude individuals who fall into the exclusion criterion. If the potential participant does not desire or is ineligible to be part of this study, any information collected during the screening interview, including contact information will be retained in order to avoid re-contact in the future. During the telephone screening process, participants will be asked a series of questions to screen out people who do not meet the inclusion criteria (age (50-75 years), and Veteran status criteria). We will also ask if they have a parental history of AD to include in final analyses. In addition, we will exclude participants who meet any of our exclusion criteria which include being allergic to fish and/or shellfish, currently using anticoagulants or are taking medications that adversely interfere with IPE, are not MRI compatible, pregnant, etc. The reason for this telephone screening is to prevent the inconvenience to potential subjects of attending a screening visit who are unable to participate in the study because they are not eligible for MRI, LP, or randomization to IPE study medication. If no exclusions arise and the patient indicates desire to participate in the study, they will be scheduled for a screening visit. Some potential participants may have the screening occur in person, if they are at the Madison VA Hospital as part of their normal health care routine and express interest in participating in the study. In general, the study team will resort to phone screening participants. The study team will attempt to reach the participant at varying times of the day up to three times then will leave a voicemail message for the participant, stating that "This is [research team member's name] from the Madison VA leaving a message for [participant name] regarding their interest in our VA Research Study. I would be happy to discuss this study in more detail with you. I may be reached at [phone number]. I look forward to talking to you soon and answering what questions you may have." If someone other than the participant answers the phone, our team will make the same comment and emphasize that we cannot disclose the type of study without permission of the potential participant. Contact information will be left with the household member for the potential participant to call our team. If no response from the potential participant is received after leaving a message with another family member, another attempt will be made just in case the participant's family member forgot to relay the message. If no response is received following that contact, a follow-up letter with contact information will be mailed as a final contact initiation. After that, it will be assumed the potential participant is no longer interested in the study. In accordance with VA rules,

the participant's contact information will be kept in a file in a locked drawer, in a locked office, of a restricted-access section (the VA GRECC) of the VA.

Wisconsin has 436,630 total living Veterans (93% non-Hispanic white, 3.6% African American, 1.3% Hispanic or Latino, 8.6% women) and there are 191,500 Wisconsin Veterans between the study eligibility ages of 50-75 years (44% of total Wisconsin Veterans). The Madison VA Hospital serves 130,000 Veterans living in 15 counties in south-central Wisconsin and in five counties in northwestern Illinois. Assuming that approximately 44% of these Veterans are in the 50-75 year age range (57,200) and that approximately 13% will have a parent with AD (based on AD prevalence estimates for Wisconsin), we estimate that we will have a recruitment pool of approximately 7500 Veterans between the ages of 50-75 years with a parent with AD. Based on the gender and race/ethnicity of Veterans in Wisconsin, we assume that approximately 580 (7.8%) will be women, 270 (3.6%) African American, and 98 (1.3%) Hispanic/Latino.

As our ADRC is jointly sponsored by the University of Wisconsin and our VA, many of our ADRC-related outreach events include Veterans and occur within our VA hospital. While the pool of participants from whom we draw for the proposed VA study is somewhat different than our ADRC participant pool, our ADRC team has a strong track record of outreach and recruitment and has established networks and infrastructure on which to build for the proposed VA-based study. As of September 1, 2014, the Wisconsin ADRC had enrolled 251 cognitively-healthy participants with parental history of AD who fall within the proposed age range (50-75 years). Each month, the ADRC recruits on average 10 new participants and has a high number of cognitively-healthy middle-aged and older adults interested in study participation. Thus, we anticipate a high level of interest in this age group for the proposed study as well. In addition, the PI and her colleagues have successfully recruited participants from the community prior to the funding of the Wisconsin ADRC in 2009. As Leader of the ADRC Clinical Core, Dr. Carlsson is integrally involved in the recruitment and retention of participants, including persons from communities of color. The Wisconsin ADRC has a Minority Recruitment Satellite Program which works closely with African American communities in south central Wisconsin and northern Illinois. While Wisconsin is approximately 6.5% African American, 11% of Wisconsin ADRC participants are African American. Adult children of persons with AD are very motivated to participate in research and have had a very high retention rate (94-97%) in our prior trials. Thus, a recruitment rate of 4-5 new participants per month for the proposed trial is very feasible and should allow us to successfully complete recruitment over a 38-month period. Retention efforts will include post-visit feedback forms, interim safety telephone calls, and birthday and thank-you cards, newsletters from our VA GRECC and ADRC, and invitations to our ADRC's Annual November Lecture on Alzheimer's disease.

Inclusion/Exclusion Criteria

Study inclusion and exclusion criteria are outlined in **Table 3.** Participants must be 50-75 years old, and have served in the active military, naval, or air service. Veterans with dementia or MCI will be excluded as will those with contraindication to IPE therapy, LP, or MRI. Individuals lacking consent capacity, constituting prisoners, or those unable to read consent materials will also be prohibited from inclusion in this study. Veterans with ethical contraindication to placebo therapy (i.e. persons who should be on lipid-lowering therapy, such as Veterans with diabetes mellitus or high atherosclerotic cardiovascular disease [ASCVD] risk) will be encouraged to discuss lipid-lowering therapy with their primary care provider. These people may enroll if their primary care provider decides not to put them on a cholesterol-lowering medication. The person may also re-screen after taking a stable statin dose for 3 months. Persons with significant medical or psychiatric conditions or medications that may affect interpretation of study results will also be excluded at the discretion of the PI. Those who enroll in the study and start an anticoagulant

medication following enrollment will be given the option to continue in the study for observation purposes. They will not have any further LP's completed, and they will immediately stop study medication for safety purposes. All other study procedures will remain constant. Participants who attempt baseline MRI, but cannot complete the procedure due to claustrophobia may continue in the study if they are able to successfully complete baseline lumbar puncture with CSF obtained. No further MRIs will be attempted if the participant has claustrophobia. Participants who attempt baseline lumbar puncture, but our clinician team cannot successfully obtain CSF, will be allowed to continue with the study if they successfully complete the baseline MRI procedures. No further lumbar punctures will be attempted if baseline CSF is not obtained for comparison.

Study Protocol (Table 2)

Following review of eligibility criteria with potential participants over the phone, all participants will undergo a pre-study visit where trained study personnel will obtain informed consent, review medical and medication history, and collect a fasting blood sample to assess lipid profile, glucose, AST/ALT levels, creatinine, and a complete blood count (**Tables 2 and 3**).

One vial of the fasting blood sample will be stored and sent at the end of the study to assess APOE genotype. Individuals who are diabetic or have questions or concerns with fasting will be identified through the screening process and contacted by a clinician. All individuals who consent to the pre-study fasting visit will receive a fasting tip sheet in the mail with visit reminder letters, and instructed to stop the fast if they experience any of the symptoms listed on the sheet.

All women of childbearing potential will complete a urine pregnancy test at pre-study and all subsequent visits until are considered no longer of childbearing potential. They will be asked to sign a separate consent section outlining the risk of IPE use in pregnancy (Pregnancy Category C) and agreeing to use of birth control (as appropriate) or another method of contraception during the course of the study. Women will be considered past childbearing potential if they have had a hysterectomy or are 1 year postmenopausal.

Table 3. Inclusion/Exclusion Criteria for Proposed Trial.

INCLUSION CRITERIA:	
Veteran status	Age 50-75 years
EXCLUSION CRITERIA:	
Dementia or MCI on screening evaluation	Current use of other investigational drug
Current use of fish oil supplements	Persons not taking statins AND with high ASCVD risk as defined by clinical ASCVD or DM or LDL-C ≥ 190 mg/dL or ≥7.5% estimated 10-year ASCVD risk* (will refer to PCP to discuss statin use – may enroll if PCP decides not to use statin or may rescreen after taking stable statin dose for 3 months)
Active liver disease with AST or ALT > 2 x ULN	LDL-C <80 mg/dL
Elevated creatine kinase >2 times ULN	Creatinine > 1.8 mg/dL
Prior adverse reaction to fish oil	Previous lumbar surgery w/ contraindication to LP
Pregnant, nursing, or pregnancy planned w/in 12 mo	Claustrophobia requiring sedation for MRI
Use of medications that interact with IPE	Pacemaker or other contraindication to MRI
Current use of anticoagulants	Known hypersensitivity/allergy to fish and/or shellfish

• According to the 2013 American College of Cardiology/American Heart Association Guideline on the Treatment of Blood Cholesterol with estimated 10-year ASCVD risk calculated as their risk calculator. AD=Alzheimer's disease; ALT=alanine transaminase; ASCVD=atherosclerotic cardiovascular disease (including coronary heart disease, stroke, and peripheral arterial disease); AST=aspartate transaminase; DM=diabetes mellitus; FAs= fatty acids; IPE=icosapent ethyl; LDL-C=low-density lipoprotein cholesterol; LP=lumbar puncture; MCI=mild cognitive impairment; ULN=upper limit of normal.

All potential participants will be screened for a cognitive disorder such as MCI through questioning about memory concerns and administration of the Montreal Cognitive Assessment (MoCA) and CERAD List Learning task. Scores falling 1.5 SD below the mean will trigger a referral for a detailed clinical evaluation. Trial enrollment will be deferred until a cognitive disorder can be ruled out. As has been done for the Wisconsin ADRC, the Wisconsin Registry for Alzheimer's Prevention cohort, and Dr. Carlsson's prior clinical trials, parental history of AD will be confirmed by chart review and/or through use of the validated Dementia Questionnaire[67] using NIA-AA criteria at the weekly ADRC Consensus Conference for the purpose of analysis.

At baseline (month 0), participants will be asked to complete a short questionnaire about their past medical history, prescription and non-prescription medication/supplement use, lifestyle practices (e.g. exercise, tobacco use), combat and non-combat deployment history, exposure to

blast-related and other injuries, associated short-term and long-term symptoms following any injury,[68] and PTSD symptoms with updates reviewed at each visit.

At baseline and months 9 and 18, all participants will undergo MRI, LP for CSF collection, cognitive testing with the ADCS-PACC battery (with the alternate version given at month 9), fasting serum lipid profiles, and will complete a FFQ targeting omega-3 fatty acid intake.[69]

At every visit (depending on previous lab results), participants will have interim drug safety assessments (AST, ALT, urine pregnancy as indicated) and an assessment of medical/medication changes and potential drug or procedural side effects. Participants with baseline or month 1 AST or ALT between 1.5 upper limit of normal (ULN) and ≤2 x ULN at the preceding visit will have repeat AST and ALT values drawn at 3-, 6-, 12- and 15-month visits as well. The proposed study drug monitoring plan exceeds that required by the FDA for IPE (Vascepa) which states that patients with hepatic impairment should have ALT and AST levels monitored periodically during therapy.

All research visits will occur at the William S. Middleton Memorial Veterans Hospital in Madison, Wisconsin with the exception of visits that can occur remotely over the phone (3-, 6-, 12- and 15-). These remote phone visits will depend on AST and ALT values as noted above and if they need a urine pregnancy test. If baseline or month 1 AST or ALT do not fall between 1.5 upper limit of normal (ULN) and ≤2 xULN and they are not of childbearing potential, these visits can occur by phone. In this case, the phone visit will be scheduled at the preceding in-person visit, or by a coordinator 1 – 3 weeks prior to phone visit date. The study team will attempt contact with the participant 5 times, at different times of the day/evening over a 2-week period, as well as leave at least two voicemails. If contact for phone visit is still unsuccessful at this point, the visit will be marked as incomplete.

All neuroimaging will occur in the UW Wisconsin Institutes for Medical Research (WIMR) (a 10-minute walk from the physically-adjacent Madison VA Hospital). All MR images will be reviewed before participants begin treatment to ensure high quality data were obtained.

Subjects who successfully complete the baseline visit will be randomized in a 1:1 ratio to IPE 4 g (four 1-g gel caps) daily or matching IPE gel cap placebos (four 1-g mineral oil gel caps). Vascepa (IPE) 1 g gel caps will have logos de-inked to match the mineral oil placebo gel caps. Participants will be randomized to either four 1-g Vascepa gel caps daily or four matching 1-g mineral oil gel caps daily. The participant will take 2 capsules (2-g) by mouth twice daily. Amarin will provide all study medication and matching mineral oil gel cap placebos and is responsible for all study drug quality testing, packaging, and distribution of study drugs to the Madison VA Pharmacy research team. Randomization will be conducted by the Madison VA Pharmacy using a forced block design and will be stratified based on gender. Participants, investigators, and other study personnel will be blinded to the identity of the study medications and to lipid-lowering effects of the study drugs.

A clinician not directly involved in data analysis and interpretation will review possible side effects related to IPE at each visit, including liver enzyme monitoring. If adverse lab changes occur, participants will have appropriate safety interventions performed (see Protection of Human Subjects). The VA Psychiatric, Behavioral Health and Neurologic Disorders (PBN) Central Data Monitoring Committee (DMC), VA Hines Hospital, Hines, IL will oversee participant safety monitoring. Potential study drug adverse effects will be collected at each study visit via a questionnaire.

Lumbar puncture adverse effects include post-lumbar puncture (LP) headache at a rate of 5-10% based on clinical data from the literature. Our team has a post-LP headache rate at around 2%. Data on rates of post-LP headaches will be collected to ensure our rates are within the expected range.

Incidental abnormal MRI findings have occurred in our prior studies; thus, a neuroradiologist will review all baseline and month 18 MRI scans for incidental findings of clinical significance. MRI scans will be reviewed by the neuroradiologist within 1 month of the scan.

Cognitive performance will also be evaluated. If participants test 1.5 standard deviations below the normal range, their results will be reviewed with a neuropsychologist to evaluate whether the findings are of clinical significance.

Laboratory data including liver enzymes (AST, ALT), creatinine, complete blood count, glucose, hs-CRP, and urine pregnancy (as indicated) will also be collected and evaluated by the PI within a week of their collection (these data do not need to be blinded). In addition, cholesterol results will be reviewed within a week of collection by the study clinician who will notify the participant of any clinically-important findings (PI will be kept blinded to cholesterol results). Cerebrospinal fluid (CSF) cell counts will be done immediately after each lumbar puncture. The PI will review those results within a week. Any clinically relevant abnormalities on blood, CSF cell count, MRI, or cognition will be discussed with the participant via phone and a letter sent summarizing the clinically relevant abnormalities.

Complete CSF biomarker levels (including CSF beta-amyloid and tau) will not be analyzed until study completion (to reduce laboratory variability). These biomarkers are for research purposes only, are not clinically useful, and so will not be shared with participants. The analyzing laboratory will not receive any identifiable information on the research participants as the CSF samples will be coded with their unique subject ID and date, and he will not have access to the key. Baseline analysis of CSF samples will be performed at the WI Alzheimer's Disease Research Center - Biospecimen Lab. This analysis will be for specific measures of beta-amyloid and tau. The UW Biospecimen laboratory will not receive any identifiable information on the research participants as the CSF samples will be coded with their unique subject ID and date, and they will not have access to the key.

The VA PBN DMC will review all blood and CSF cell count lab abnormalities, side effect rates, post-LP headache rates, clinical follow-up of MRI abnormalities, and clinical follow-up of any cognitive concerns during their regularly-scheduled meetings. The VA PBN DMC will teleconference regularly to ensure subject safety, research integrity, and compliance with federal regulations. Adverse events will be monitored on a regular basis.

Adverse events possibly associated with the study drug, IPE, including arthralgias, increased bleeding, jaundice, gastric upset, adverse liver effects and LDL-C changes, will be evaluated. All serious adverse events (SAEs) related and unrelated will be reported to the VA DMC. Compliance will be assessed by pill count.

Subjects who discontinue early from the study will be encouraged to have an early termination visit at the point of discontinuation, with procedures performed as shown in Table 2 (Study Design) for Visit 8.

We anticipate that the proposed trial will be successfully completed within the 5-year grant period. We anticipate a 6-month start-up period, recruitment at 4-5 participants per month over 38 months, 18 months of intervention, plus 4 months of data cleaning and analyses. As IPE (Vascepa®) is already FDA-approved for treating hypertriglyceridemia, we do not anticipate delays in obtaining study drug.

COVID-19

In response to the COVID-19 outbreak, all in-person study visits were halted effective March 16, 2020. Because of this, study visits transitioned to phone visits temporarily while participants are unable to come into the Madison VA for regularly scheduled visits. This included all portions of visits that can be completed over the phone, and the rest of study visit activities were completed when in-person visits resumed. We continued to monitor study medication side effects and track compliancy, as well as have a clinician administer the Ham-D one-on-one over the phone with participants. Another consequence of halting the in-person study visit is that several participants missed their final in-person visit (V8) and the corresponding measure of outcomes including the MRI scan and lumbar puncture. These participants were sent additional study medication to last them until they can come to the Madison VA for their final measure of

outcomes. This extension of study medication was only done after receiving verbal consent from each participant.

MRI Acquisition

All MRI acquisition and interpretation will be performed under the oversight of a VA- and NIH-funded expert in neuroimaging in preclinical AD. Participants will be screened for contraindications to MRI using standard questionnaires. Additionally, Veterans with a history of metal fragments in their body will have a plain radiographic scan performed on them as a safety measure, prior to MRI scans. At baseline and months 9 and 18, all participants will undergo ASL-MRI to assess CBF within an MRI protocol that also includes sequences capturing ancillary data on volumetrics, white matter hyperintensities (FLAIR), white matter tract changes (DWI), and macrovascular CBF (VIPR). All images will be acquired after a 12-hour fast in the morning prior to administration of any medications, caffeine, or nicotine, and prior to LP. Although all images and scan results will be void of identifiable patient health information and will use the participant's Study ID number to identify each individual, if there are any abnormal findings, the subject's identity may be revealed to contact the subject about the safety concern and to inform them about any observed abnormalities. Baseline and month 18 MR images will be reviewed by a neuroradiologist and any clinically-relevant findings will be communicated to the PI for clinical follow-up. The MR scanning will take place on a 3T GE x750 scanner with echo planar and asset capability using an 32-channel head coil (GE, Milwaukee, WI). The protocol will include: 1) a 3-plane localizer for slice selection; 2) a 3D T2-FLAIR (TR 6000, TE 120, TI 1870, 256 x 256 matrix, FOV 256 mm) acquired over 88 sections (2mm, no gap); 3) a 3D T1-weighted inversion-recovery prepared fast spin echo high resolution anatomic scan (TR 8.2, TE 4.0, TI 450, matrix 256 x 256, FOV 256 mm) acquired over 156 sections (1mm, no gap). For quantitative CBF assessment, ASL-MRI will be performed using a technique developed by Alsop et al.[70] with a background suppressed pseudo-continuous arterial spin labeling (pcASL) sequence. Scan parameters include TE/TR=10.5ms/4.9s, slice thickness=4mm (no gap) 128x128 matrix, FOV=240, NEX=3, and labeling RF amplitude=0.24mG. Multi-slice spin labeling will be implemented using a single coil that eliminates off-resonance errors. The post-labeling delay is 2025 ms. The sequence also includes a fluid-suppressed proton density (PD) acquisition, with the same imaging sequence/image slab location as the pcASL but without the RF labeling preparation, for CBF flow quantitation and image registration. The pcASL sequence (all 3 excitations plus PD scan) is acquired in 4.5 minutes. Sufficient time is allotted for each session that rescanning can occur if motion or other artifacts are observed. We have previously reported excellent test-retest reliability (intraclass correlation coefficient >.95) of this pcASL procedure.

Examples of the images sequences we plan to collect are listed below.

Arterial Spin Labeling (ASL-MRI) for computation of cerebral blood flow; network abnormalities can be measured using cerebral blood flow imaging.

T2 fluid-sensitive inversion recovery (FLAIR) will be used for quantification of macro structural white matter hyperintensities. The volume or number of hyperintensities is an important covariate in the analysis of DTI.

Diffusion weighted imaging (DWI) will be used for analysis of white matter microstructural integrity with *FLAIR*.

T1-weighted high-resolution images will be used to provide anatomic information for volumetric analysis, selection of regions of interest, and radiologist's interpretation.

VIPR will be used to measure cerebral blood velocity and cardiac-gate pulsatile flow.

The MRI Scanner Utilizes Investigational Devices of Non-Significant Risk:

The MRI scanner to be used in the study, which is being supplied by GE utilizes investigational software and hardware to determine MRI scan sequences. The sequences will help capture the MR images, assess cerebral blood flow, gather microstructural information and estimate white matter connections, and suppress signal from fluid. Although the MRI investigational software to be used is considered an "investigational device," the Magnetic Resonance Imaging (MRI) device is a FDA cleared device for safely and non-invasively imaging the interior of the human body. The scanner restricts research software from exceeding FDA safety levels. Thus, the scanner with the investigational software and equipment fully engaged operates, from a technical design and functional standpoint, as a non-significant risk device in accordance with 21 CFR 812. Please see the attached Non-Significant Risk documents regarding the 32-channel headcoil, DWI, Cube T2 FLAIR, MPnRAGE, and pcVIPR sequences and software packages for further information.

MRI Quality Control

The quality control of the scanner is done by the UW Department of Medical Physics in conjunction with GE field engineers. A MAGPHAN Quantitative Imaging phantom has been consistently scanned at least monthly since April 2009; the scanner has been highly stable over time. The imaging protocol is saved on the scanner and administered by trained and certified MR technologists in a standardized fashion. Scans are downloaded to the ADRC Neuroimaging Core lab immediately after the session and the post-processing procedures are implemented including an additional quality check by the study team. Based on our experience, we anticipate technical problems will be <2%. If it is determined that rescanning is necessary, subjects will wait 7-10 days after the LP procedure before returning for imaging. If re-imaging is necessary at baseline, initiation of study drug will be delayed until repeat images are obtained.

MRI Data Backup

Multiple backups are performed to prevent technical loss of data. At the scan console the exam is backed up to DVD and kept at the MR facility. The exam is also uploaded to a PACs server and permanently archived. A complete copy is downloaded to the ADRC to a 40TB RAID6 server and weekly backups (to a separately located ADRC server) are made of processed and incremental data.

Image Processing

Images are pre-processed prior to statistical analysis using GE-specific 32-bit binary code and well established tools and pipelines including SPM8 and AFNI. The pipelines are scripted and extendable such that improved methods developed in the software or in our local methods can easily be applied/reapplied to all data in batch. The pipeline steps include: 1) conversion of ASL flow maps and respective perfusion density (PD) maps to NIFTI file format for broad compatibility between software packages; 2) transformation into Montreal Neurological Institute (MNI) canonical atlas space by first warping PD maps to the PD template in SPM8 and then applying the transformation matrix to the respective ASL flow map; 3) spatial smoothing of the data with a Gaussian kernel of 8mm FWHM to accommodate individual variability and comply with assumptions of random field theory necessary for statistical analysis of the images; 4) conversion of quantitative ASL-CBF values to relative ASL-CBF values by normalizing each map to its global CBF mean value, in effect controlling for inter-individual variations in global CBF; 5) insertion of the exam into the ADRC imaging SQL database.

Study Visit Procedures

Lumbar Puncture (LP) Procedure (Cerebrospinal Fluid [CSF] Collection)

All CSF samples will be collected in the morning by a clinician after a 12-hour overnight fast by the participant. During the fast we will advise participants to drink plenty of water and avoid food and drink except water. Subjects will also be asked to withhold taking their normally prescribed medications with food during the fast. Prescription medications should be taken with water only. Participants will receive a meal as soon as all procedures requiring a fast are complete and resume taking their prescribed medications as they would normally. Over the last 10 years, our team has successfully performed over 2000 LPs for research with a post-LP headache rate of <2%. For the LP, participants are positioned by sitting on the clinical hospital bed, and maximally flexing their knees, hips, back, and neck. The skin is then prepped and draped in a sterile manner over L3/4-L4/5. After positioning, prepping, and draping the participant, a Sprotte 25- or 24-gauge needle is used to inject 1% lidocaine as a local anesthetic, followed by insertion of a Sprotte 25- or 24- gauge spinal needle with introducer into the L3/4-L4/5 interspace using sterile technique. CSF samples will be collected with a Sprotte 25- or 24-gauge spinal needle at L3/4 or L4/5 using gentle extraction into polypropylene collection syringes in a manner consistent with the updated 2014 "NIA Biospecimens Best Practice Guidelines for the Alzheimer's Disease Centers." Twenty-two mL of CSF will be collected into sterile 5-mL polypropylene syringes, then combined, gently mixed, and centrifuged at 2000g for 10 minutes. Supernatants will be frozen in 0.5 mL aliquots in polypropylene tubes and stored in a −80° C freezer within the VA GRECC with backup alarm and emergency power. Participants will be contacted 24 hours after the LP to inquire about any adverse effects. All specimen collections will be coded and will include a study ID number, visit collection date, and visit number. A note will be placed in the participant's VA chart indicating a lumbar puncture was performed.

Laboratory Evaluation

Blood samples will be collected after an overnight fast. At baseline and months 9 and 18 (key outcome visits), fasting lipid profiles, glucose, AST, ALT, hs-CRP, and creatinine will be measured using standard enzymatic techniques at the Madison VA Clinical Laboratory. APOE genotyping will be determined from coded specimens by resources from the UW ADRC, the UW Biotechnology Center and/or LGC genomics for a fee for service. Genotyping will occur for all participants at the end of the study using blood samples collected and stored from the pre-study visit. This APOE genotype information will be used in analysis. Baseline analysis of CSF samples will be performed at the WI Alzheimer's Disease Research Center - Biospecimen Lab. This analysis will be for specific measures of beta-amyloid and tau. The UW Biospecimen laboratory will not receive any identifiable information on the research participants as the CSF samples will be coded with their unique subject ID and date, and they will not have access to the key. Coded biospecimen data, including DNA samples will be analyzed to determine various SNP (single-nucleotide polymorphism) sequences. CSF Aβ42, Aβ40, T-tau, and p-tau-181 will be measured with Roche elecsys in the UW ADRC Biomarker Laboratory. Pre- and post-therapy samples will all be run simultaneously at the conclusion of the study to reduce laboratory variability. All biospecimen data will be stored in a freezer, within the secured Madison VA's Research Freezer until clinical analyses occur. Access to the freezer is protected by VA access control, and equipped with backup power and a telephone alarm system. The research freezer space can only be accessed by authorized personnel. Participants will be asked for permission to store their blood, DNA and CSF samples for future research purposes, this will be optional. Samples will be stored for banking purposes specified in the BRAVE Repository Protocol (IRB 2017-0013) and used with consistent approval of the banking repository protocol. Samples will be coded. Specifically, vials containing samples will be labeled with subject ID numbers (###). The label will also include the study name, collection site number, study visit number, contents of each collection vial, and the date of collection. Participants will be made aware, through informed consent, that their samples will be stored for future use.

Cognitive Battery (Table 4): At the pre-study visit, participants will be screened for cognitive dysfunction using the CERAD List Learning Task, questions regarding memory concerns, and an assessment of global cognitive function with the Montreal Cognitive Assessment (MoCA). CERAD scores falling 1.5 SD below the mean will trigger a referral for a detailed clinical evaluation and trial enrollment will be deferred until a cognitive disorder can be ruled out. At baseline and months 9 and 18, the ADCS-PACC battery will be administered to all participants following MRI, LP, blood tests, and a light breakfast. As is done in the A4 trial, the same battery will be used at baseline and month 18 and an alternate battery at month 9 to allow cognitive data collection at three time points while reducing practice effect. All cognitive testing will be recorded and stored on an audio recorder within the VA's protected environment until tests are scored. Upon scoring tests, the recorded audio will be deleted. Participants will be screened for depression with the Center for Epidemiologic Studies Depression Scale (CES-D) at baseline and months 9 and 18. The cognitive test battery will be administered by a trained technician in accordance with the ADCS-PACC guidelines. All psychometricians will be trained by an experienced VA neuropsychologist. As the Wisconsin ADRC is a site for the A4 trial, several psychometricians have already been trained and certified in ADCS-PACC administration. Scoring will be completed strictly according to ADCS-PACC guidelines. Changes in the ADCS-PACC composite scores of the intervention group will be compared to changes in the placebo group after adjusting for practice effects, thus, maximizing internal validity and interpretability of cognitive performance across time. The ADCS-PACC includes: 1) the Total Recall score from the Free and Cued Selective Reminding Test (FCSRT) (0-48 words), 2) the Delayed Recall score on the Logical Memory IIa subtest from the Wechsler Memory Scale (0-25 story units), 3) the Digit Symbol Substitution Test score from the Wechsler Adult Intelligence Scale–Revised (0-93 symbols), and 4) the MMSE total score (0-30 points). The composite score is determined from its components using an established normalization method. Each of the four component change scores is divided by the baseline sample standard deviation of that component, to form standardized z scores. These z scores are summed to form the composite.[74]

ADCS-PACC Battery Tests

Free and Cued Selective Reminding Test (FCSRT): Verbal memory is being measured during this test.

Immediate and Delayed Logical Memory Test: This test measures one's ability to learn and recall verbal information.

Wechsler Adult Intelligence Scale-Revised: During this test, executive function will be measured.

Mini-Mental Status Examination (MMSE): This 30-point examiner-administered questionnaire measures global cognitive function and evaluates orientation, language, reading, attention/concentration, memory, and constructional ability. Although widely used, it is not sensitive to mild cognitive impairment. This test will be administered to evaluate and identify enrollees with significant cognitive impairment. Subjects with significant cognitive impairment will not complete the remainder of the visits.

Questionnaires

At baseline and months 9 and 18, a 20-minute, validated 152-item FFQ will be administered to estimate omega-3 fatty acid intake from all food sources. The questionnaire was validated in a Midwestern population and included seafood items that were available in the

Midwestern food supply and, thus, will be similar to food patterns in our population. The questionnaire asks participants about their usual dietary intake over the past month, breaking it down into eight frequency responses. This FFQ has a high reliability (alpha coefficient = 0.83), demonstrating that volunteers are able to estimate and give the same information on intake over time. To evaluate for depression, the Hamilton Depression Rating Scale will be assessed at baseline, 1, 3, 9, and 18 months in all participants. To assess TBI exposure, specific questions from the Department of Defense's "Survey of Health Related Behaviors Among Active Duty Military Personnel" will be used to assess combat and noncombat deployments, exposure to blast-related and other injuries, and associated short-term and long-term symptoms following any injury (i.e. duration of loss of consciousness, amnesia, headache, etc.). In addition, PTSD symptoms will be evaluated using specific items from the same questionnaire assessing traumatic experiences during deployments and sequelae from those experiences. Additional information on depression, dysthymia, anxiety, PTSD, alcohol abuse, and other psychiatric symptoms will be assessed through administration of different portions of the Mini International Neuropsychiatric Interview (MINI) 6.0 at baseline. Drug dependence/abuse will not be assessed in this modified version of the MINI that will be administered to Veterans.

D) REPORTING STUDY FINDINGS

Reporting Findings

If any abnormal clinically-relevant findings are noted on MRI, labs (blood and/or CSF), or cognitive testing, the participant will receive a phone call from the study team and the clinically-relevant abnormal findings will be sent to them in a letter. If the participant has consented and signed a release of information form, then we will contact their primary care physician with these results and send them to the PCP as well. Baseline and month 18 MRI scans will be reviewed by a neuroradiologist within a month of the scan, labs within a week of their collection, and all cognitive tests within a month of evaluation. Cognitive test results 1.5 standard deviations below normal will be reviewed by a staff neuropsychologist. CSF and blood tests will not be placed in the participant's chart as it could affect the study blind. MRI scans are research scans and not meant for clinical interpretation, so will not be placed in the VA chart. Participants will be informed of any abnormal cognitive findings and referred for further clinical evaluation of cognitive changes. Thus, while we will not put the cognitive scores in their VA chart, with the participant's authorization we will notify their PCP of the clinical concerns raised by the research cognitive testing and that provider will likely put that in their VA-based note. The following results will be released to Veteran participants following their baseline visits: 1) Lipid panel (total cholesterol, triglycerides, high and low density lipoprotein cholesterol) 2) High-sensitivity c-reactive protein 3) basic metabolic profile (blood glucose, serum creatinine, AST, and ALT) 4) Spinal fluid red blood cell and white blood cell counts 5) Results from urine pregnancy tests. These results will be summarized in a signed letter from one of our clinicians indicating participants' baseline laboratory results and normal/abnormal ranges. They will be instructed to contact their PCP if they have any concerns accompanied with the printed out results. Results for genetic tests will not be released to participants. If a participant is hospitalized or experiences adverse events, a VA clinician will place a note to the PI in the VA CPRS notification system. Study wide results (randomization arm, primary outcome analysis, complete study-wide laboratory values at each blood draw (if participant consented, if not these will be omitted), and any change in cognition over the course of the study) will be communicated with study participants through cover letters and newsletters. Participants will be directed to follow up with their primary care provider if they have any questions about their laboratory values or if they have noticed any memory concerns.

Potential Risks: Physical

Icosapent ethyl (IPE) or Vascepa (R) use: Individuals with known allergy to fish oil, fish, or shellfish may experience anaphylactic shock. The most common side effects of icosapent ethyl include constipation, gout, mouth, joint or muscle pain, and swelling of the hands, legs or feet. Some people also have an increased risk of heart rhythm problems, including atrial fibrillation or flutter, requiring hospitalization, especially if they have a history of these problems.

Fasting (12 hours for blood draw and/or lumbar puncture): shakiness, nervousness, sweating, dizziness or light-headedness, sleepiness, confusion, difficulty speaking, anxiety, and weakness.

Blood Draw: Phlebotomy can be associated with minor ecchymoses, pain, and a small risk of infection. Fainting or light-headedness may also occur.

MRI: An MRI presents virtually no risk to healthy individuals without metallic implants. Some patients may experience claustrophobia. The MRI protocol used in the proposed study requires lying on a hard surface for 30 - 60 minutes, which may cause some discomfort. Moreover the scanner is loud. The radio frequency waves used in the MRI have produced burns in about one in a million exams (most of those minor). Other risks of injury due to MRI include damage to implanted electronic devices (such as pacemakers), bleeding if aneurysm clips are present, and trauma if iron-bearing objects are brought too close to the scanner. Risks posed to pregnant women and fetuses from MRI scans are at this time unknown.

X-Ray Exam: If an x-ray is done prior to the MRI, the amount of radiation received is small(similar to the amount of background radiation received from living on this planet for about 6 months).

Lumbar Puncture: The most common complication of lumbar puncture is post-dural puncture headache. Bacterial meningitis is a very rare complication of lumbar puncture, occurring in fewer than 0.2% in one series. Uncal or tonsillar herniation is a very uncommon complication, occurring in <1% of very high-risk subjects with known primary or metastatic neoplasms who underwent a lumbar puncture. In rare cases, subjects may visit an emergency room after their visit to have a blood patch performed to alleviate the LP headache. A blood patch is an injection of some of the subject's blood into the LP site to patch a spinal fluid leak. This is only done in rare circumstances. As with the blood draw, there is a small risk of infection with a blood patch. There is also a risk of bleeding and lower back discomfort. Lidocaine is used to numb the lower back before the collection of CSF. In rare cases, allergic reactions to lidocaine may occur, which can cause redness and swelling of the skin.

Cognitive Testing: Mental fatigue from the cognitive tests is the most common or frequent physical risk expected related to this procedure.

Study Drug Formulation, Packaging, Labeling, and Storage

The manufacturing of Vascepa® and matching placebo capsules, along with packaging, bottling, and all the technical and functional activities required at the level of integration to complete these activities will be performed by Amarin Pharma, Inc. under a Material Transfer Cooperative Research and Development Agreement (MT CRADA) established between Amarin Pharma, Inc. and the William S. Middleton Memorial Veterans Hospital, in Bedminster, NJ and Madison, WI respectively. Under an active Investigational New Drug (IND) application all icosapent ethyl and mineral oil placebo capsules for this study have met stability and other product

testing (including dimensional analysis, weight checks of capsules fill color and shell composition, shell characterization, microbial limits, disintegration, and appearance on all active and inactive study medications) sufficient to meet the Food and Drug Administration (FDA) requirements. Amarin Pharma, Inc. the manufacturer of Vascepa®, will transfer all bottled and packaged clinical study medication to the William S. Middleton Memorial Veteran's Hospital Pharmacy department; the latter entity will store the study medication until study medication distribution visits and will be responsible for the study drug randomization of participants. Since Amarin Pharma, Inc. will blind and code study medication bottles, Amarin Pharma, Inc. will provide this coded list to the Madison VA Pharmacy who will keep this information confidential from the PI and study team members until the end of the study when the code is finally disclosed to the study team. Using the code provided by Amarin Pharma Inc., the Madison VA Pharmacy will randomize enrolled participants to either active drug (icosapent ethyl) or placebo (inert mineral oil) medication and dispense study medication at distributed time points outlined in Table 1: Study Design. At the completion of the study, the Madison VA Hospital Pharmacy Department will destroy all unused and leftover medication from the study.

D) DATA MANAGEMENT, ANALYSIS, AND STATISTICAL CONSIDERATIONS

Data Management

Personnel will be trained to monitor adherence to the study protocol. All CSF, blood, MRI, and cognitive data will be double entered into a password-protected database on a secure VA server by trained study personnel. Extreme or inconsistent values or data discrepancies will be reviewed by a study biostatistician and an investigator and data will be verified accordingly. Subjects' identifiable information will be stored on paper and in electronic format. The mature, secure web application, known as REDCap will be used as our electronic data management system. This database will be configured by the UW DOM IT to match the VA's version and then transferred to the VA server where some study data will be stored. Therefore, data entered into REDCap will be on a VA server and under VA purview. Database support for REDCap will be provided by the VA IRMS with appropriate cybersecurity measures adhered to. In the event that VA sensitive data is transmitted electronically, information will only be transmitted using VA approved solutions such as FIPS 140-2 or PKI encryption.

Participant contact activity will be recorded in the Contact Registry for Recruitment, Innovation and Education (CoRRIE) database and will be utilized by the study team throughout the course of time that a participant is actively enrolled in the study. CoRRIE is a University of Wisconsin Department of Medicine web-based application based on the Ruby on Rails web development framework. For licensing details see : https://github.com/rails/rails/blob/master/MIT-LICENSE. Access to CoRRIE is further limited to individuals both approved by the CoRRIE dataset custodians (or delegated authorities), and having completed all applicable SMPH HIPAA training. All communication between web clients (internet browsers) and the CoRRIE application takes place via Hypertext Transfer Protocol over Secure Socket Layer (SSL) or HTTPS. HTTPS provide the ability for normal web based communication over an encrypted SSL connection. This ensures that data passing between the client and CoRRIE is protected. The server hosting CoRRIE is located behind the UW-Madison SMPH firewall. The SMPH firewall does not allow outside (of SMPH network) access to the server itself. Outside access to the CoRRIE application is allowed through a combination of firewall rules, reverse proxy access, and directory level user authentication. BRAVE identifications will be further coded in CoRRIE and the key to re-identify participants will be stored on the VA server. No PHI/PII will be added to CoRRIE for BRAVE participants.

All participant paper documents will be filed in a locked storage cabinet, inside the secured VA GRECC office, a VA protected environment. Participants will be identified by their study ID numbers and only study team members will have access to the source document linking participants to their personal health information. All specimen collected during the course of this study will be labeled with coded study ID numbers unique for each participant. Only coded data will be transmitted securely to others as part of the study process. Coded data will be disclosed to the WI Alzheimer's Disease Research Center – Biospecimen Lab for CSF analyses, the Wisconsin Institute for Medical Research (WIMR) and UW Biotech. In the event of a suspected or actual data breach, the ISO and PO and ACOS for Research will be notified within one hour of any actual or suspected data breach. This includes the unauthorized use, disclosure, transmission, removal, theft, loss or destruction of VA research-related PHI, individually identifiable private information, or confidential information. Research data is not intended to be removed from the VA protected environment. Additionally, data access will be terminated for study personnel when they are no longer part of the research team. Lastly, if research data or information is transmitted electronically, it will be encrypted with FIPS 140-2 validated encryption.

Randomization

Subjects who successfully complete all baseline data collection will be randomized by the Madison VA Pharmacy in a 1:1 ratio as previously described, using a forced block design and stratifying based on sex to force balance in this factor.

Analysis Population

All analyses will be performed on an intent-to-treat basis as far as follow-up allows. All randomized patients will be encouraged to continue to be assessed and, if they consent, included in the analysis regardless of compliance with study medication. Follow-up rates in similar studies conducted by the PI are between 94-97% (see **Preliminary Studies**). We therefore conservatively predict 90% follow-up in this 18-month trial. For patients missing final values (month 18) but having intermediate measurements of outcomes (month 9), final outcomes will be imputed using expectations from longitudinal data analyses conditional on available data, with random noise added using standard errors taken from the residuals of these analyses. Patients missing all post-baseline values will be omitted. Sensitivity analyses will be conducted to quantify potential biasing effects of dropout on estimated treatment effects and multiple imputation used to quantify the additional variation. There will be no formal interim analyses for efficacy as this is a proof-of-concept study and the rCBF and CSF biomarker changes do not have any clearly known clinical consequences at this time.

Analyses

For imaging analyses, the statistical models prescribed by our ADRC biostatistician can be effected in SPM (including SPM5, SPM8, and SPM12) or AFNI. Analyses will use an empirically predefined statistical region of interest (sROI) to characterize the treatment effect with optimal power and freedom from multiple comparisons as described by Chen and Reiman. The sROI was defined using a training dataset, i.e., an independent longitudinal dataset (baseline and follow-up without treatment) of ASL-CBF maps collected in asymptomatic, middle-aged adults with a parental history of AD. Using a paired-samples voxel-wise t-test in SPM, the training dataset was analyzed for regions of statistically significant CBF declines. The candidate regions for the sROI were selected based on those that best characterized CBF declines in this group in regions implicated in AD pathology. The sROI will then be applied to ASL-CBF maps in the current study, and averaged values from the sROI will be extracted for statistical analysis. The predefined sROI facilitates correction for multiple comparisons and reduces our exposure to Type 1 error by greatly

reducing the number of voxels in the search region that are evaluated. Anatomical ROIs that aligned with the statistically-defined ROI will be examined hierarchically in a priori selected regions of interest in the following order: bilateral posterior cingulate, bilateral precuneus, bilateral medial temporal lobe. Analyses of ROIs will be discontinued if the group difference in 18 month rCBF in the previous ROI is not significant.

The outcomes - rCBF and CSF Aβ42 – were seen to be right skewed based on pilot data; thus, analyses for each specific aim will be conducted on the logarithmic scale so that "mean" refers to the geometric mean outcome. Specific Aim 1 (IPE effects on rCBF) will be addressed with an analysis of covariance on all subjects with 18 month rCBF of the sROI as the outcome, IPE use as a factor, and baseline rCBF as a continuous predictor, with the main hypothesis tested using the coefficient of IPE's effect on mean rCBF. Baseline rCBF is included as a flexible generalization of modeling change in rCBF. The models for Aim 2 (IPE effects on CSF) will take the same form as in Aim 1 except that the endpoints will be CSF Aβ42 and that we expect the coefficients of IPE to be negative if the alternative hypotheses are true. For exploratory and descriptive purposes, joint longitudinal analyses of baseline, 9, and 18-month outcomes will be conducted using the same model formulations as above and the method of Laird and Ware. Aim 3 is exploratory and will investigate the impact of IPE on 18-month cognitive performance using the constructs proposed for CBF and CSF analyses. The composite ADCS-PACC scores allow for assessment of overall cognitive functioning and a higher level of reliability than is possible with any given individual measure. A linear regression model will be fitted to the raw scores using age, gender, and education as covariates. Using the regression coefficients from this analysis we will calculate predicted scores for each experimental and control subject. The difference between the predicted and observed scores will then be determined for each subject. Relevant statistical graphics will be used to examine all scientific questions.

Covariates

Important potential confounding factors for changes in outcome measures will be assessed, including age, sex, APOE4 carrier status (positive/negative), parental history of AD, systolic blood pressure, use of antihypertensive agents, plasma LDL-C and HDL-C levels, body-mass index, waist-hip ratio, exercise frequency, Hamilton Depression Rating Scale score, history of moderate or severe TBI, PTSD, and DHA/EPA intake. In addition, all analyses for cognitive effects will be adjusted for age, sex, and education.

Power Calculations

For Aim 1, using ASL-MRI CBF data from our team's longitudinal WRAP cohort of adults at risk for AD (n=133, ages 43–73) and an interventional statin pilot study of similarly aged adults,16 we anticipate that CBF in the statistical ROI will decline by 15% in the placebo group and 5% in the treatment group over 18 months. With an effect size of 0.57 and a two-tailed α=0.05, 134 participants (67 per arm) will provide 90% power (1-β) to detect a difference between the two treatment groups. With a conservative 10% drop-out rate (our prior 9-18-month studies with n=88–100 had 94–95% retention rates), 150 participants (presumed 134 [90%] retained at month 18) should provide 90% power to detect a statistically significant difference. Using our team's preliminary baseline data from the SHARP study (Table 1), for Aim 2 we use a baseline CSF Aβ42 mean ± SD of 778.2±173.2 ng/L (INNOTEST) and a projected IPE-induced decline of approximately 13% in the treatment group and 0% in the placebo group over 18 months. With an effect size of 0.59 and a two-tailed α=0.05, 124 participants (62 per arm) will provide 90% power (1-β) to detect group differences in CSF biomarkers. As the ADCS-PACC battery (Aim 3) has been principally used in cognitively-healthy, preclinical older adults in their 70s-80s, Aim 3 will be used to understand the performance of this established preclinical cognitive battery in a middle-

aged cohort and to correlate cognitive changes with those noted in primary MRI and CSF biomarker changes.

E) REFERENCES

1. Johnson SC, Koscik RL, Jonaitis EM, Clark LR, Mueller KD, Berman SE, et al. The Wisconsin Registry for Alzheimer's Prevention: A review of findings and current directions. Alzheimers Dement (Amst). 2018;10:130-42. doi: 10.1016/j.dadm.2017.11.007. PubMed PMID: 29322089; PubMed Central PMCID: PMCPMC5755749.