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YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Researchi
100 FR1 (2015-2)

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: PBR28 brain PET imaging with lipopolysaccharide challenge for the study of microglia function in Alzheimer's disease			
Principal Investigator: Adam Mecca, MD, PhD		**Yale Academic Appointment:** Assistant professor	
Department: Psychiatry			
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Protocol Correspondent Name & Address (*if different than PI*):			
Campus Phone:	**Fax:**	**E-mail:**	
Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*):			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax**	**E-mail**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☒ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI

APPROVED BY THE YALE UNIVERSITY IRB 11/1/2021

☐ Yes x No

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?

☐ Yes x No

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as co-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

☒ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Clinical Trials Office (CTO)
☐ Yale Cancer Center/Smilow
☐ Yale-New Haven Hospital
☐ Cancer Data Repository/Tumor Registry
☒ Yale University PET Center
☐ YCCI/Church Street Research Unit (CSRU)
☐ YCCI/Hospital Research Unit (HRU)
☐ YCCI/Keck Laboratories
☐ Yale-New Haven Hospital—Saint Raphael Campus
☒ Specify Other Yale Location: Alzheimer's disease research center.

b. External Location[s]:

☐ APT Foundation, Inc.
☐ Connecticut Mental Health Center
☐ Clinical Neuroscience Research Unit (CNRU)
☐ Haskins Laboratories
☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven

☐ Other Locations, Specify:

☐ International Research Site
(Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☐ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:
☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☒ *Radioactive Drug Research Committee (RDRC) Approval Date: Pending
☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☒ Yale University RSC (YU-RSC) Approval Date: Pending
☒ Magnetic5Resonance Research Center PRC (MRRC-PRC) Approval Date: 3/1/2017
☐ *Nursing Research Committee Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx)

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. **5 Years**

3. **Research Type/Phase: (Check all that apply)**
   a. **Study Type**
   ☒ Single Center Study
   ☐ Multi-Center Study
   Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
   ☐ Coordinating Center/Data Management
   ☐ Other:

   b. **Study Phase** ☒ **N/A**
   ☐ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
   ☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:

☐ Clinical Research: Patient-Oriented
☐ Clinical Research: Epidemiologic and Behavioral
☐ Translational Research #1 ("Bench-to-Bedside")
☐ Translational Research #2 ("Bedside-to-Community")
☐ Clinical Research: Outcomes and Health Services
☒ Interdisciplinary Research
☐ Community-Based Research

5. Is this study a clinical trial? Yes ☐ No ☒

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events"

If yes, where is it registered?

Clinical Trials.gov registry ☐

Other (*Specify*)

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

If this study is registered on clinicaltrials.gov, there is new language in the consent form and compound authorization that should be used.

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)? Yes ☐ No☐

7. Will this study have a billable service? *A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects*.

Yes ☐ No☒

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

8.. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No _X__ *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? NO
b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

c. Will a novel approach using existing equipment be applied?

If you answered "no" to question 8a, or "yes" to question 8b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. ***By signing this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH****.*

SECTION III: FUNDING, RESEARCH TEAM AND TRAINING

Funding Source: See IRES-IRB

Research Team: See IRES-IRB

SECTION IV: PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.

Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)

☒ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC)

☒ No

I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

David Hafler — Chair Name (PRINT) and Signature

10/31/16 — Date

Neurology — Department

YNHH Human Subjects Protection Administrator Assurance Statement

Required when the study is conducted solely at YNHH by YNHH health care providers.

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and has the support of the hospital for this research project.

YNHH HSPA Name (PRINT) and Signature

Date

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

We have developed a functional test of brain microglial response that involves administering LPS (0.4 ng/kg) intravenously and measuring 11C-PBR28 binding (measured as volume of distribution or VT) at baseline and 180

minutes post LPS. We define microglia activation reserve index (MARI) as the proportional increase in binding of PBR28, 180 minutes after the administration of the LPS: MARI = [(VT(LPS)-VT(baseline))/ VT(baseline))] x 100. In a previous study, we demonstrated the feasibility and robustness of this technique by applying this methodology to 8 healthy volunteers showing 30-60% increase in PBR28 binding after LPS administration (10). We predict increased MARI in patients with early AD compared to cognitively normal aged matched individuals. Given that we also have data from young healthy individuals from our earlier study, we can explore whether the microglia activation reserve index is increased in microglia with aging (which we predict to be the case). We can gauge also whether the changes in MARI in AD represent incremental increases compared to normal aging or that a significant dysregulation of innate immunity occurs in AD. With this experiment we aim to:

Aim - Examine the differences in the capacity to activate microglia in patients with AD compared to age-comparable cognitively normal subjects and younger healthy controls.

Hypothesis: In early AD the innate immune response becomes more reactive to PAMPs and DAMPs compared to those with normal cognition.

Our primary outcome is MARI (calculated in the parietal ROIs) in AD patients compared to elderly controls. We will use 7 AD and 7 comparably aged cognitively normal successfully scanned subjects to calculate the microglial activation reserve index. We expect significant differences between the two groups suggesting increased reactivity of the microglia in AD. The reverse would suggest a burn-out phenomenon which we expect to occur in a later stage of the disease but not in our study cohort. This result would be an exciting one suggesting at least one mechanism by which some patients with significant amyloid load have progressive dementia while comparable others are either cognitively normal or have stable MCI. It will also suggest avenues for intervention in amyloid positive MCIs to prevent progression to Alzheimer's dementia. Our exploratory analysis will include:

a) Effects of aging on MARI: We will evaluate the effects of aging on MARI. Comparisons will be made for MARI between cognitively normal elderly and the 8 healthy individual to whom we applied this protocol in an earlier study. The reactivity of microglia is reported to increase with age (11) and so we expect MARI to increase in the cognitively normal elderly. However, we expect the differences between the young and elderly cognitively normal subjects to be small relative to the comparison of AD and elderly normal controls.

b) Effects of amyloid on MARI: We will look at the relationship of regional and global amyloid load to MARI. The presence of a relationship suggests that one of the reasons for increased microglial reactivity might be interactions of microglia with pathologic amyloid.

c) Effects of MARI on cognition: We will explore whether MARI would correlate with measure of disease stage, with higher MARIs associated with worse neuropsychological score and more severe disease. We will look for correlations between MARI and the individual neuropsychological scores. This would determine if disease severity is correlated with microglial activation reserve.

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

Microglia and its interactions with amyloid plaques play an important role in the pathogenesis of Alzheimer's disease (AD), but current methodologies, including animal models, CSF proteomics, histopathology of necropsy tissue and epidemiological approaches, have failed to elucidate the exact role of microglia in the progression of disease (1–4). It is possible that microglia have differing roles in different stages of AD: they may be protective against amyloid accumulation but cause disease progression in later disease. The discovery of translocator protein (TSPO), a mitochondrial protein upregulated with microglial activation, and positron emission tomography (PET) ligands for imaging TSPO present opportunities for better in vivo study of neuro-inflammation (5). Amongst these radio-ligands 11C-PBR28, a newer agent, appears to be superior to earlier ligands for the purposes of in vivo imaging of TSPO (6). Using PBR28, a recent NIMH (7) study showed significantly greater microglial activation in early AD compared to normal controls and those with mild cognitive impairment (MCI). Interestingly, MCI subjects did not differ significantly from normal controls in terms of microglial activation. Given that amyloid status in MCI more closely resembles AD than cognitively normal individuals (8), this finding cannot be accounted for by increases in amyloid

load alone. More recently, a longitudinal study using 11C-(R)-PK11195 PET showed increases in neuro-inflammation in spite of stable amyloid levels over the period of the study (9). The ideal next step would be to assess whether in AD, microglia are more reactive to pathogen and danger associated molecular patterns (PAMPs and DAMPs), which include constituents of both lipopolysaccharide (LPS) and amyloid plaques.

Neuro-inflammation is a hallmark of Alzheimer's disease pathology as evidenced by CSF studies of inflammatory cytokines, necropsy tissue histopathology and most convincingly by genetic studies (12). Microglia, as long lived cells which replenish themselves by division, are vulnerable to the effects of aging and senescence. Even with normal aging the phenotype of microglia appears to become more pro-inflammatory (i.e. reactive microglia). It also seems that compared to young healthy individuals their reaction to immunogens is amplified and prolonged, either as a compensatory mechanism for inefficiencies of aged microglia or due to a loss of regulatory control over these cells (11). For this reason, inflammation was once thought to be an epiphenomenon and a result of over-sensitivity of aging microglia to amyloid plaque constituents. But there is now strong evidence linking neuro-inflammation with pathophysiology of sporadic Alzheimer's disease. In humans, genome wide association studies in patient with sporadic AD show mutations in several genes involved with the innate immune system of the brain (13–27) namely TREM2, CD33, CR1, ABCA7, SHIP1 and APOE.

Neuro-inflammation seen in AD consists of chronic microgliosis and to a lesser extent reactive astrogliosis (28,29). Microglia can be activated either by cytokines (IFN-γ) or when it comes into contact with danger and pathogen associated molecular patterns (PAMPs and DAMPs) (30) such as lipid constituents of the amyloid plaque (31) or lipopolysaccharides (32). In AD the interaction between microglia and the amyloid deposits in the brain is thought to initiate neuro-inflammation in the brain. This inflammatory response was initially thought to be damaging but contrary evidence also exists. Anti-inflammatory strategies in several clinical trials, based on the assumption that neuro-inflammation accelerated AD pathology, not only failed to slow the progression of disease, but in some cases may have accelerated it (33–45). In fact, there is evidence that neuro-inflammation may be protective in certain circumstances. TREM2 gene codes for a receptor that senses the presence of certain lipids within the amyloid plaques and leads to activation of the microglia. It appears that pathogenic mutations in the TREM2 gene (for example R47H) cause a loss of function in TREM2 receptor and that this loss of function leads to diminished protection by neuro-inflammation (1). The problem is that currently little is known about the timing of neuro-inflammation in the course of AD, its relationship to peripheral inflammation, and its exact role in AD pathogenesis. Physiologically microglia appears to have contradictory roles in the progression of Alzheimer's disease: on the one hand it appears to phagocytose amyloid (46) and form a barrier around the plaque which protects adjacent neurites from synaptic loss (47); on the other it may accelerate disease progression especially in the later stages of the disease (12).

The study of neuro-inflammation in Alzheimer's disease has been challenging, partly because the underlying mechanisms are still incompletely understood but also because there is contradictory clinical evidence and a seeming unreliability of mouse models where well designed experiments appear to demonstrate contradictory results. In one study TREM2 knockout reduced pro-inflammatory polarization of microglia and accelerated AD pathology in the 5XFAD mouse models (1). Another group using the APPPS1 mouse model found the elimination of TREM2 positive 'macrophages' and resolution of AD pathology (2). Additionally, the deletion of genes responsible for inflammation in mouse models of AD lead to accelerated progression of the disease suggesting a possible protective role for neuro-inflammation (48–54). Similarly, blocking immunosuppressive cytokines such as TGF-β1, IL-4 and IL-10 improve AD pathology in mouse models (55–61). Conversely, in a study carried out in very young wild type mice using the viral mimic polyriboinosinic-polyribocytidilic acid to drive inflammation, there was development of A-beta plaques, tau aggregation, microglia activation and reactive gliosis (62). Additionally, several studies demonstrate a link between the presence systemic inflammation or increased activation of innate immunity, and accelerated AD progression (63–71). There is also evidence for a detrimental role for local inflammation in that A-beta may bind to CD36, TLR4, and TLR6 resulting in inflammation which may lead to intracellular accumulation of amyloid and activation of inflammasomes (50,72,73).

In vivo physiological studies in humans have also been difficult to perform. Although the brain and peripheral immune systems influence each other, the relationship is not a direct one and the central nervous system represents a partially privileged site immunologically. This necessitates the measurement of biomarkers of inflammation in the CSF. However even this method has yielded contradictory results suggesting a heterogeneous phenotypic picture or

technical difficulty (3). Histopathological studies are similarly inconclusive because activation of microglia is not always discernable from morphology of the microglia (4).

The transporter protein imaging therefore represents a major potential advancement in the study of neuro-inflammation in AD. TSPO, previously known as the peripheral benzodiazepine receptor (PBR), is an 18 kDa protein located in the outer mitochondrial membrane which is claimed (sometimes controversially) to sub serve a number of physiological functions including steroidogenesis and regulation of the immune response. For the study of inflammation, the most significant property of the molecule is its up regulation during neuro-inflammation in microglia and astroglia (in reactive astrogliosis) (29). Various PET ligands have been developed but until recently these ligands appear to have suffered from a number of problems.

The first and most commonly used TSPO radio-ligand has been PK11195, however it suffers from a number of important shortcomings: 1- It has poor pharmacokinetics and washes out too quickly (74). 2- It has relatively weak binding to TSPO and is lipophilic so that signal to noise ratio is relatively poor (75). 3- There are technical difficulties with calculating binding because metabolite-corrected arterial plasma input curve is impractical due to ligand binding to plastic tubing and the simplified reference tissue model is complicated by a lack of such a reference region in neurodegenerative diseases (76,77). There are 15 reported human studies of PK11195 in AD. These studies have yielded inconsistent results: One study (78) showed increased inflammation in the cortex of AD patients, four studies did not report a statistical difference between the case and control groups (79–82). Another four studies reported the absence of statistical differences between the two groups (83–86). A further two showed equivocal results with one showing a difference between controls and MCI and not AD (87) and the other showing no difference with region of interest analysis but small areas of inflammation in the occipital lobe on voxel-wise statistical parametric mapping (88). The remaining four smaller studies show widespread inflammation in the cortex and subcortex including temporal (89–92), parietal (89,91,92), frontal (89–92), occipital (89,91) and cingulate (89,91) cortices as well as cerebellum (89,91), amygdala (89,91), putamen (89) and pallidum (89).

Several second generation TSPO radio-ligands have been developed including DAA1106, vincopectine and PBR28. These agents have much higher affinity for TSPO and better pharmacokinetics. However, some of these ligands have yet to live up to their original promise. Two studies (93,94) with 11CDAA1106 showed widespread uptake including in some parts of the brain such as the striatum and cerebellum which are not commonly thought to be affected by disease. Part of the problem may have been the relative slow washout of DAA1106 compared to the short half-life of C11, confounding accurate measurement of binding. 18FEEDAA1106, a higher binding related compound, was used in another study and no increase in inflammation was reported. Similarly, a study using 11C-vincopectine showed no effect (95).

For this study we choose PBR28. Amongst TSPO ligands PBR28 seems to have the most desirable characteristics for inflammatory imaging in AD: It has very high affinity for TSPO and an excellent signal-to-noise ratio (96). The main problem with this ligand, shared by all TSPO ligands except PK11195, is the variable specific binding of PBR28 due to TSPO gene polymorphism (rs6971) (97,98). The method used for adjusting for this fact involves genotyping TSPO (or using a competitive binding assay) and excluding low affinity binders. For the mixed (heterozygous for the polymorphism) and high affinity binders compensatory changes can be made in calculating binding (6,99–101). Four studies thus far have used PBR28 in Alzheimer's disease but only one attempted to directly compare AD patients with MCI and cognitively normal patients. This particular study was conducted at the molecular imaging branch of National Institute of Mental Health7, which compared 19 AD, 10 MCI and 13 amyloid negative controls for TSPO binding. Patients with AD showed increased binding especially in the temporal and parietal lobes. Additionally, there were correlations between TSPO binding and neuropsychological as well as volumetric parameters. Another group (102) performed a genome wide association study of longitudinal accumulation of amyloid independent of APOE status. They found carriers of IL1RAP rs12053868 had faster progression and worse atrophy compared to non-carriers. In a different cohort the same gene polymorphism was associated with lower cortical binding of 11C-PBR28 suggesting a protective role for inflammation. In yet another study (103), the same group showed a relationship between PBR28 binding and PPAR1 polymorphism, and they inversely correlated PBR28 and cortical atrophy.

Innate immunity, mediated by microglia, plays an important role in the pathogenesis of Alzheimer's disease. As discussed above, several genes implicated in microglial function are risk factors for late onset AD. TREM2 one of the genes with the highest odds ratios for AD risk, codes for a membrane protein which is involved in the interaction of

the microglia with constituents of the amyloid plaques. The NIMH study (7) of PBR28 binding in patients with AD, MCI and normal cognition showed increased binding in the AD group compared to the other two groups. Equally as important was the finding that there was no significant difference in the PBR28 binding of MCI patients compared to normal controls. Most of amyloid deposition occurs before the onset of symptoms and the rate of amyloid increase plateaus in MCI, so that patients in early AD on average have a relatively small increment in their amyloid deposition compared to MCI (8). The difference in immune activation in AD and MCI may represent differing microglial sensitivity to molecular patterns that activate them for example amyloid plaques. To test this hypothesis, we need to know both the state of activation of microglia and the microglia capacity to activate (activation reserve) which we have defined as reactivity of microglia to a given stimulus. Activation status of microglia depends on a variety of factors including the presence of peripheral inflammation, age and the amount of amyloid present in the brain, whereas microglial activation reserve is more likely influenced by genetics, senescence and regulation of microglia.

LPS activates the same immune mechanisms which are involved in the immunological responses to amyloid deposition (104,105). We earlier showed the activation of microglia in the nervous system in response to peripheral administration of LPS in non-human primates (106). We then went on to apply this to healthy human volunteers who showed a robust response to LPS administration (10). The imaging protocol involves a baseline PBR28 scan which will be compared to a post-LPS scan. Percentage change is likely to be the most meaningful measure (MARI=[(VT(LPS)-VT(baseline))/ VT(baseline))] x %100). Our previous study in baboon showed the predominant binding of TSPO immunoreactivity is to microglia and not to astroglia, the other kind of cell also capable of expressing TSPO. Furthermore, the use of 0.1 mg/kg and 1 ng/kg of LPS in baboon and humans respectively showed similar magnitude of change in the activation of microglia. This is important as much higher doses of LPS is likely to have detrimental effects on the health of subjects and may be symptomatically more burdensome. For this experiment our calculations will be made for the parietal region of interest as this was the area most activated by LPS administration in our healthy controls and showed the greatest amount of baseline activation amongst AD subjects in the NIMH study.

Preliminary Results and Demonstration of Plausibility

To calculate the test retest reliability, we obtained two PBR28 scans (107), on average 1.4 weeks apart, on 4 clinically stable relapsing-remitting MS subjects (age 41 ± 7 years, two men/two women) and four healthy control (age 42 ± 8 years, 2 two men/two women), matched for translocator protein genotype for test. Mean test VT values (ml cm^3) were 3.9 ± 1.4 in the whole brain gray matter (GM), 3.6 ± 1.2 in the whole brain white matter (WM) or normal-appearing white matter (NAWM), and 3.3 ± 0.6 in MS WM lesions; mean retest VT values were 3.7 ± 1.0 in GM, 3.3 ± 0.9 in WM/NAWM, and 3.3 ± 0.7 in MS lesions. Test-retest results showed a mean absolute TRV ranging from 7 to 9 % across GM, WM/NAWM, and MS lesions. Another MS subject (age 41 years, male) with clinical and radiological activity was studied for lesion detectability.

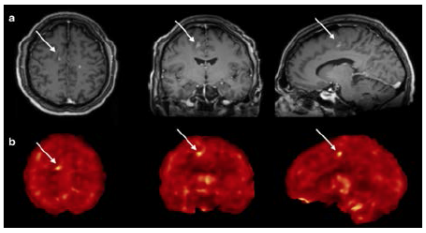


Figure 1 – The top row contains three slices of a gadolinium enhanced T1, showing a white matter lesion in the right mesial frontal subcortex. The bottom row shows the same three sections with PBR28 spatially corresponding to the exacerbating lesion.

Dynamic data were acquired over 120 min after injection of 634 ± 101 MBq (11)C-PBR28. Focal (11)C-PBR28 uptake was detected in two enhancing lesions of the active MS patient (Figure-1). We thus showed that PBR28 scanning has good test-retest reproducibility in humans and can localize focal inflammation. To show its utility in imaging more diffuse inflammation, we employed the primate endotoxemia model (108). Six female baboons (Papio anubis) were scanned before and at 1h and/or 4h and/or 22 h after intravenous administration of E. coli lipopolysaccharide (LPS; 0.1mg/kg), which induces systemic inflammation (figure 2). LPS administration increased [(11)C]PBR28 binding ($F(3,6)=5.1$, $p=.043$) with a 29 ± 16% increase at 1h (n=4) and a 62

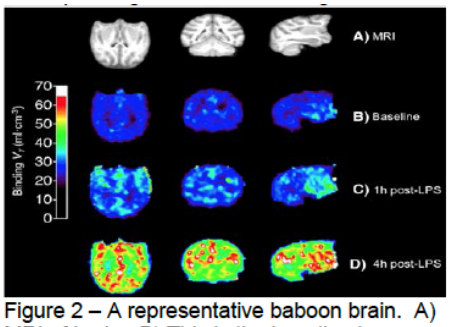


Figure 2 – A representative baboon brain. A) MRI of brain. B) This is the baseline image showing sparse inflammation. C) Increased inflammation 1 hour post injection of LPS. D) It reaches a maximum 4 hours post injection.

± 34% increase at 4h (n=3) post-LPS106. Significantly immunohistochemistry from one baboon showed immunoreactivity exclusively in microglia and not in astrocytes.

Next we applied the same paradigm to healthy young volunteers using a reduced dose of LPS (1ng/kg). We recruited eight (109) healthy non-smoking consented males (24.9±5.5 years old, 87.5±12.3 kg) and imaged them pre and post LPS administration. Eight healthy male subjects each had two 120-min [(11)C]PBR28 PET scans in 1 d, before and after an LPS challenge. LPS (1.0 ng/kg, i.v) was administered 180 min before the second [(11)C]PBR28 scan. LPS administration significantly increased [(11)C]PBR28 binding 30-60%, demonstrating microglial activation throughout the brain (Figure 3). This increase was accompanied by an increase in blood levels of inflammatory cytokines, vital sign changes, and sickness symptoms, well-established consequences of LPS administration. To our knowledge, this was the first demonstration in humans that a systemic LPS challenge induces robust increases in microglial activation in the brain.

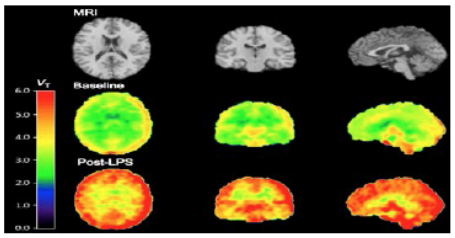


Figure 3A- Average VT images with the magnetic resonance image (MRI) shown for anatomical reference (Top row). Significance level was *P < 0.01 (linear mixed models) of the difference between baseline and post-LPS challenge scans.

Figure 3A demonstrates the changes in mean PBR28 binding of 8 healthy subjects. There is heterogeneity in the increases in the absolute values of the increases in binding Figure 3A- Average VT images with the magnetic resonance image (MRI) shown for anatomical reference (Top row). Significance level was *P < 0.01 (linear mixed models) of the difference between baseline and post-LPS challenge scans. after the administration of LPS in this young healthy cohort perhaps reflecting variability in microglial reactivity reserve. It is also notable in Figure 3B that regional microglial reactivity reserve indices vary from the lowest in the thalamus to the highest in the parietal lobes. This ties in well with the NIMH study showing the greatest difference in the level of baseline activation can be found by comparing parietal lobes between patients with Alzheimer's disease and normal controls.

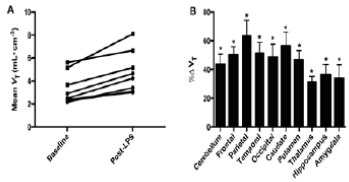


Figure 3B- LPS administration significantly increases [11C]PBR28 binding (VT) from baseline in healthy control subjects (n = 8) shown in A, regional percent increase in VT averaged across subjects (error bars are SEM). (B) VT for each subject averaged across regions examined in high-affinity (squares) and mixed-affinity binders (circles).

The magnitude of the post LPS signal prompted us to think of the human endotoxemia paradigm as a physiological test of the innate immune system, especially microglial activation in response to molecular pattern associated with PAMPs and DAMPs (see significance). The microglia activation reserve index would be defined by the proportional increase in the baseline level of PBR28 binding in response to LPS.

3. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

Aim- To examine the differences in the capacity to activate microglia in patients with AD compared to age-comparable cognitively normal subjects and younger healthy controls.
Hypothesis: In early AD the innate immune response becomes more reactive to PAMPs and DAMPs compared to those with normal cognition.

Rationale
Amyloid deposition plateaus before the onset of symptoms. As such MCI resembles AD more than cognitively normal subjects in terms of amyloid status. Yet the NIMH study showed a significant difference in microglial activation in AD and

not MCI or cognitively normal subjects. One possible explanation may be increased reactivity of microglia to ambient amyloid in AD. If true, this may be the mechanism that drives disease progression after amyloid deposition has plateaued. The use of PBR28 in patients with Alzheimer's disease presents significant advantages over the previous modes of study of neuroinflammation in vivo. Using this ligand, we aim to demonstrate dysregulation of the immune system in early AD compared to normal aging.

Presently amyloid specific therapies are the only avenue sought for treatment of Alzheimer's disease. The study has the potential to revolutionize our approach to therapy.

Procedure

Screening and selection procedures:

Screen/Baseline visit to ADRU: Each subject will have an initial screening visit that will include:
Screening: Demographics, history, examination, GDS, EKG, Labs, MOCA, logical memory I/II, CDR and Informed consent.
Baseline: WAISIII – picture completion, digit symbol, symbol search, arithmetic, digit span, information, letter number sequencing, RAVLT, Trails A/B, Boston naming, Stroop, FAS, Rey complex figure, ADCS-ADL, CAM and NPI. In certain cases, this can take more than 4 hours and we may need to split the day into two where screening and baseline are administered on separate days.

Possible pre-imaging visit to MRRC: Many of the patients who will enroll will already have clinical MRI. For those who do not we will organize one for this research.

Imaging visit to PET center:
On the day of the scan there are two steps performed:
Step 1:
1. arterial line placement (there is no suitable reference region)
2. 1st scan – 120-min emission
Step2:
3. LPS injection 3 hours prior to 2nd scan, fasting for 8 h prior to this injection
4. 2nd scan – 120-min emission5. Optional blood draw during the PET center visit If the participant agrees, 10-20mls of blood will be taken at 0,1,3 and 6 hours and banked at 300 George Street in Hafler Laboratory. These samples will only be stored in Hafler laboratory; they will not be added to one of Dr. Hafler's existing repositories. The samples will be used by only the investigators on this project; they will not be made available to outside researchers.
In certain cases, the length of this day may not be tolerated by the subject on the day so that we divide the day into two where Steps 1 and 2 are administered on separate days.

Note: White coat hypertension is a common phenomenon in the elderly population. During the visit to the PET center, the unfamiliarity of the environment, the placement of the arterial line, and other cannulae can have an additive effect to the white coat phenomenon. Subject's will be asked to bring any prescribed hypertension medication to the scanning day. High blood pressure does not predictably increase the risk during endotoxin administration as the latter often reduces blood pressure. Furthmore, patients with unstable hypertension will be excluded from the protocol. However, in the interest of patient safety and ethical consideration of ignoring a possibly significant clinical finding, Dr. Mecca will implement the following protocol:

1) If blood pressure is raised (SBP > 180 or DBP > 100) and patient is symptomatic:
a) If the symptoms raise concerns of end organ damage (chest pain, SOB, focal neurological sign, thunderclap headache, etc) the patient will be referred to the ED via ambulance.
b) If headache is the only symptom and the headache is more severe or different character than the patients habitual headaches, then the experiment will be stopped. The patient's primary care physician will be contacted or the patient will be referred to urgent care.

2) If blood pressure is raised (SBP > 180 or DBP > 100) and patient is asymptomatic (except for regular headaches):

A) If the patient has their hypertension medication as instructed, if not otherwise indicated, the patient will be given an extra dose of their prescribed hypertension medication. Their BP will be retaken before administration of the endotoxin. If their BP is still >180/100 or if the patient is hypotensive, the

administration of the endotoxin will be delayed or cancelled. If the BP is optimal, we will proceed with endotoxin administration.

B) If the patient is prescribed hypertension medication but did not bring it to the scanning day, Dr. Mecca will order one dose of the prescribed medication unless there is a contraindication. The medication will be ordered through EPIC, sent to the CVS at 800 Howard Avenue in New Haven, and picked up by the patient's scan day companion. The cost of the study medication will be covered by the study. Their BP will be retaken before administration of the endotoxin. If their BP is still >180/100 or if the patient is hypotensive, the administration of the endotoxin will be delayed or cancelled. If the BP is optimal, we will proceed with endotoxin administration.

C) If the patient is not prescribed hypertension medication, Dr. Meccawill order amlodipine through EPIC. The medication will be sent to the pharmacy and picked up by the patient's scan day companion. The medication will be given in 2.5mg or 5mg doses at 30 minute intervals. If the patient permits, this intervention will be communicated with their PCP. The medication will be provided at no cost to the patient. Their BP will be retaken before administration of the endotoxin. If their BP is still >180/100 or if the patient is hypotensive, the administration of the endotoxin will be delayed or cancelled. If the BP is optimal, we will proceed with endotoxin administration.

If a subject becomes hypotensive after the administration of anti-hypertensive medication, Dr. Mecca will implement the following protocol.

A) If the subject is asymptomatic, they will be monitored at the PET center until their blood pressure returns to a normal level.

B) If the subject has minor symptoms, such as slight nausea or dizziness, they will receive IV fluids to increase their blood pressure. If this does not work, they will be referred to the emergency department at Yale New Haven Hospital.

C) If the subject has severe symptoms such as vomiting and fainting, they will be referred to the emergency department at Yale New Haven Hospital.

Safety Visits:

- 48 hours after the administration of the endotoxin there will be a phone screening using CAM to check for delirium. If there is ongoing delirium then the subject will be directed to immediately go to the emergency room for evaluations and then a follow-up visit can be scheduled with the study doctor
- One week visit: RAVLT, Trail A/B, digit symbol, CAM, ADCS-ADL, MMSE/MOCA, stroop. 1- 1.5 hrs.

Optional additional visit for CSF draw

In volunteers who agree to do so, one 20mls of CSF Sample 0-7days after LPS will be taken at Hospital research unit (HRU) of Yale new haven hospital and and banked at 300 George Street in Hafler Laboratory. The samples will only be stored in Hafler laboratory; they will not be added to one of Dr. Hafler's existing repositories. The samples will be used by only the investigators on this project; they will not be made available to outside researchers.

Optional additional visits to assess Cognitive Measures with Cog-state Ipad testing

We may obtain these measures at baseline during the week before LPS injection and the following one during the week after LPS injection. These patients will be separately consented and we will not exclude anyone if they do not accept to do these tasks.

1. Cogstate Battery (30 minutes) – This computerized test battery will assess memory and cognition. The tasks may include:

a. International Shopping List Task – a computerized task to assess verbal learning and memory.
b. Groton Maze Learning Task – a computerized task to assess executive function and spatial problem solving.
c. Detection Task – a computerized task to assess psychomotor function and speed of processing.
d. Identification Task – a computerized task to assess visual attention and vigilance.
e. One Card Learning Task – a computerized task to assess visual learning and memory.
f. One Back/Two Back Tasks – computerized tasks to assess attention and working memory

6 month Visits:
The patient will have MOCA, GDS, CDR, and other neuropsych similar to screening and baseline.

PET for amyloid.

Most participants will who enroll in this study will have previous biomarker evidence of AD from either a brain amyloid PET scan or or CSF amyloid β measurement. If needed, a PiB PET scan will be performed prior to ^{11}C-PBR28 imaging. We expect we will need do up to 10 PiB PET scans under this protocol.

Subjects in the MCI cohort who have a PiB-PET scan will learn their results. The following will be discussed with them:

> "Amyloid" or "beta-amyloid" forms plaques in the brains of people with Alzheimer's disease. Scientists believe that the buildup of amyloid in the brain may play a key role in the eventual development of Alzheimer's disease-related memory loss. It is expected that about 60-70% of people with symptoms of mild cognitive impairment will have elevated brain amyloid. Research studies suggest that individuals with mild cognitive impairment who have elevated brain amyloid are more likely to have cognitive decline caused by Alzheimer's disease. In addition individuals with mild cognitive impairment who have elevated brain amyloid are more likely to progress to Alzheimer's dementia during their lifetime than those who do not have elevated brain amyloid.
>
> An experienced clinician will discuss the meaning of participants amyloid scan results with them by reviewing the information above and addressing any questions or concerns that they have.

If an MCI has a negative PiB-PET scan, they will not have the ^{11}C-PBR28 scans.

PiB PET scan results will not be disclosed to participants in the CN cohort They will have the ^{11}C-PBR28 scans regardless of their amyloid status.

TSPO gene polymorphism:

We will genotype the patient with regards to rs6971 polymorphism on the TSPO gene to exclude those who are homozygous (low affinity binders). For this we will use 50 ng of genomic DNA, amplified with PCR reaction. Control plasmids encoding for high affinity binders (C/C) and low affinity binding (T/T) will be used as controls. For the heterozygote i.e. mixed affinity binders a 1:1 mixture of both will be used.

MRI:

A screening MRI of the brain will be obtained using a 3.0T magnet. The MRI will ensure that patients do not meet exclusion criteria by showing evidence of infection, infarction, or other focal brain lesions. Subjects with multiple lacunes will be excluded. The MRI scan will be used for PET image registration. 11C-PIB PET: A screening PET scan for amyloid positivity will be obtained. Only those with a positive scan in the AD groups will be enrolled for the study.

Cognition:

See above.

PET Imaging of TSPO in AD

We will compare PBR28 binding site availability in brain regions of 7 living human subjects who have AD with that in 7 age-matched healthy controls by using the ligand 11C-PBR28 and PET. Dose of LPS (see below) will be administered intravenously and the subject will be reimaged at 180 minutes after the injection. Considering possible failures in scans, a total of 16 patients will be studied in 2 groups, cognitive normal AND amyloid negative versus clinical criteria for mild AD and amyloid positive to reach our goal of 7 successfully scanned subject for each group. The detailed protocol may be found

in our previous publication (109). We expect that 20-30% of cognitively normal participants who receive amyloid scans under this protocol will be amyloid positive. Therefore, we will enroll up to 10 participants in the cognitively normal cohort to attain the goal of 7 participants who are cognitively normal AND amyloid negative.

Primary Outcome:

The 16 enrolled subjects will have 11C-PBR28 using the bolus/infusion method. Measurements will be made at baseline and 180 minutes after the injection of LPS. We will measure Total Volume of Distribution (VT) for 12 brain regions defined by MRI scan, as described (111). All primary outcomes will use the parietal ROI for calculations of both baseline activation and microglial activation reserve. For our primary outcomes we will choose the microglia activation reserve index defined as MARI = [(VT(LPS)-VT(baseline))/ VT(baseline))] ×100. In the NIMH study the greatest difference in the regional PBR28 binding between AD and cognitively normal subjects was found in the parietal lobe (7). We also found the greatest microglia activation reserve index in the parietal lobe when we applied our paradigm to young healthy controls (109). Other ROIs will be used in secondary analyses.

Statistical Considerations and Sample Size:

Several comparisons of 11C-PBR28 VT brain level and microglia activation reserve index in the parietal lobe will be made using Student T-test to test for a difference between group means: young healthy group vs. elderly cognitively normal, as well as AD vs. elderly cognitively normal.

Although this is intended as a pilot study we have performed some basic power analyses. For the comparison of baseline activation in AD vs. aged matched cognitively normal subjects based on standard deviations derived from Kreisel (7) and our own previous studies (107,109,111), using an alpha of 0.05 and power of 0.80, our study is powered to detect an effect size of > 1.6 which is not far off from the findings in the NIMH study. For the comparison of percentage post-LPS change from baseline in AD and NC groups we are powered to detect an effect size of more than 2.

Endotoxin Administration (Dr. Cosgrove's protocol.)

Endotoxin administration Subjects will receive intravenous administration of open-label endotoxin at a dose of 0.4 ng/kg body weight. This will take place at the Yale PET Center. All subjects will fast for at least 8 hours before endotoxin administration to reduce the risk of nausea, but they will be encouraged to hydrate well. They will be asked not to use aspirin, NSAIDs, COX-2 inhibitors, or acetaminophen for at least 3 days prior to each study day. Clinical Center Reference Endotoxin (CCRE) will be provided by the NIH Clinical Center and will be stored and prepared by the YNHH IDS pharmacy. CCRE is an investigational drug, and investigator Kelly Cosgrove has obtained an Investigational New Drug (IND) for its use in humans from the Food and Drug Administration (FDA). CCRE vials will be stored at 2-8°C in a refrigerator in the locked YNHH IDS pharmacy. Preparation of CCRE will be performed by the YNHH IDS research pharmacist. As recommended by NIH, the pharmacist will dissolve the lyophilized CCRE in Sterile Water for Injection USP under a laminar flow hood on the evening before each endotoxin administration. The dissolved CCRE will be stored at 4°C until the following morning. Dissolved CCRE is stable for at least 24 hours at this temperature.

On the day of endotoxin administration, subjects will rest on a stretcher in the PET Center. An IV catheter will be placed to allow for hydration with normal saline, endotoxin infusion, and optional blood draws. In the case of bradycardia or hypotension, this catheter will be used for rapid bolus infusion of normal saline. To reduce the already low risk of adverse cardiovascular events, subjects will be hydrated with 500 mL of saline prior to endotoxin administration, and they will receive approximately 100 mL/hr thereafter for the duration of the endotoxin administration day.

After endotoxin administration, vital signs (blood pressure, heart rate and temperature) will be recorded every 15 minutes for the first two hours. If vital signs change significantly, monitoring will continue at least every 30 minutes, or more frequently as determined by the study MD, until they have returned to within 10% of the baseline values. All subjects will be connected to continuous two-lead EKG monitoring for the duration of the challenge. All subjects will be carefully monitored at all times by a research nurse and by a study physician, who will be present until 3 hours after endotoxin administration. Any subject experiencing prolonged effects of endotoxin or any other adverse event may be transferred to the Yale New Haven Hospital emergency room if appropriate.

There is no evidence that experimental endotoxin administration causes hypoxemia in humans and therefore there is no literature to support monitoring of this parameter (as opposed to parameters such as heart rate and blood pressure, which do change and which will be monitored closely). Furthermore, pulse oximetry can be unreliable when there is significant peripheral vasoconstriction, as occurs during e.g. experimental endotoxemia, sympathetic activation, and fever.

If an individual experiences bothersome symptoms after endotoxin administration and wants to stop the study, flu-like symptoms may be treated with acetaminophen (650 mg every 6 hrs- no NSAIDs will be used because of the risk of antiplatelet effects on the arterial line bleed), and anxiety may be treated with lorazepam (0.5 mg every 2 hrs for a maximum dose of 6 mg every 24 hrs). During screening each subject will be asked about medication allergies, and no medication to which a subject may be allergic will be administered. If a subject request so, the study will be stopped and these medications may be administered. In addition, if any of the following symptoms occurs, if of a severity such that it prevents normal activity, the study in that individual subject will be stopped: Chills, headache, malaise, myalgias, arthralgias, fatigue, nausea and vomiting. If a subject's body temperature exceeds 39.2°C the study will be halted and the subject will receive acetaminophen as described above. If these medications are insufficient to lower body temperature, subjects may be transferred to a higher level of care. Subjects will be discharged at the end of the study day, provided that all of the following have occurred: their blood pressure is within 10% of baseline AND their heart rate is within 20% of baseline AND their mental status is normal AND they do not feel physically or mentally unable to return home. The determination will be made by the PI and/or the study physician.

Monitoring of depressive symptoms Subjects will be rated for depressive symptoms using Geriatric Depression Scale before each endotoxin administration and at several time points afterwards. Subjects will also be rated for fatigue and pain with relevant rating scales.

4. Genetic Testing N/A ☐

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned

We will use 10 mL of blood to test for Ala147Thr polymorphism to identify and exclude PBR28 non-binder. Polymorphism in this gene has not clinical significance except for PBR28 testing.

ii. the plan for the collection of material or the conditions under which material will be received

See above.

iii. the types of information about the donor/individual contributors that will be entered into a database

The genetic polymorphism results will be entered. The results of this testing will be confidential, will not be entered into the subject's medical record, and will not be made available to the subject.

iv. the methods to uphold confidentiality

The results of genetic testing in locked file cabinets and by separating the personal identifying information of the subjects from the genetic information.

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?

No sharing is planned. Samples drawn for genetic testing will be sent to an outside lab and disposed of after testing is complete.

C. Is widespread sharing of materials planned?

No.

D. When and under what conditions will materials be stripped of all identifiers?

It will not.

E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?

Yes.

F. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

Subjects will be informed that their material has been anonymized.

G. Describe the provisions for protection of participant privacy

Risks associated with genetic testing will be minimized by keeping the results of genetic testing in locked file cabinets and by separating the personal identifying information of the subjects from the genetic information.

5. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

The subject sample of the PET study will contain both men (~50%) and women (~50%) aged 55-90. The sex ratio is expected to reflect that of other and control samples. The sample is expected to reflect the racial diversity of the state of Connecticut. Based on previous experience with similar studies in the Yale ADRU, we expect the sample to comprise mostly Caucasians, African-Americans, and Hispanics, with a relatively smaller number of Asian participants. The patients who are frail, morbidly obese, have liver disease or have multiple co-morbidities especially immunologically related.

6. **Subject classification:** Check off all classifications of subjects that will be <u>specifically recruited for enrollment</u> in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐ Children	☐ Healthy	☐ Fetal material, placenta, or dead fetus
☐ Non-English Speaking	☐ Prisoners	☐ Economically disadvantaged persons
☒ Cognitively Impaired	☐ Employees	☐ Pregnant women and/or fetuses
☐ Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ Yes ☒ No (If yes, see Instructions section VII #4 for further requirements)

7. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

Inclusion Criteria:

a- Mild AD Subjects:

1. NIA-Alzheimer's Association core clinical criteria for probable AD
2. Age between 55 and 90 (inclusive)
3. Score on the MOCA greater than or equal to 17
4. Presence of a responsible caregiver who will accompany AD subjects to all procedures.
5. Biomarker evidence of Alzheimer's disease via an amyloid PET scan or CSF amyloid β measurement.
6. The patient should have a capacity to consent.
7. CDR global score greater than 0.

b- Cognitively normal elderly Subjects:

1. Absence of NIA-Alzheimer's Association core clinical criteria for probable AD
2. Objective memory scores within normal range for age (do not meet MCI Subjects criterion 2)
3. Age between 55 and 90 (inclusive)
4. CDR global score of 0.0

Exclusion Criteria:

1. Any significant neurologic disease (other than probable AD in the AD Subjects group), such as stroke, Parkinson's disease, brain tumor, seizure disorder, multiple sclerosis, or history of significant head trauma followed by persistent neurologic deficits.
2. Any significant systemic disease including hepatic failure, heart failure, renal failure, COPD, active infection and autoimmune disease.
3. Screening/baseline MRI scan with evidence of infection, infarction, or other focal lesions. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.
4. Any significant systemic illness or unstable medical condition, including: uncontrolled or insulin-dependent diabetes mellitus, uncorrected hypothyroidism or hyperthyroidism,coagulopathy, or systemic cancer.
5. Current or regular use of over-the-counter medication that may affect the immune system (e.g., ibuprofen), including corticosteroids or immunosuppressant drugs; no use in 3 weeks prior to the PET scan
6. Investigational agents are prohibited 4 weeks prior to entry and for the duration of the study. Previous treatment with an investigational small molecule with anti-amyloid properties or passive immunization against amyloid within 1 year of study entry. Previous treatment with an active immunization against amyloid.
7. History of schizophrenia or other major psychiatric disorder (DSM IV criteria).
8. History of alcohol or substance abuse or dependence (DSM IV criteria) within the past 2 years.
9. Clinically significant abnormalities on screening laboratory tests (B12, TFTs, hematology, chemistry, urinalysis, ECG).
10. Pregnancy, as determined by screening pregnancy tests for pre-menopausal females
11. Impairment of visual or auditory acuity sufficient to interfere with study procedures.
12. Education level < 6 years.
13. Evidence of current depression as defined by a score of a score of ≥ 5 on the Geriatric Depression Scale.
14. Presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body. The presence of claustrophobia, precluding MRI.
15. Current or recent participation in any procedures involving radioactive agents such that the total radiation dose exposure to the subject in any given year would exceed the limits of annual and total dose

commitment set forth in the US Code of Federal Regulations (CFR) Title 21 Section 361.1.

16. Vaccination in the last month.
17. Drink more than 5 alcoholic drinks per week or any heavy drinking days in the last 30 days2
18. BMI > 35 or < 19
19. Women who are pregnant or nursing, or fail to use one of the following methods of birth control unless she or partner is surgically sterile or she is postmenopausal (hormone contraceptives [oral, implant, injection, patch, or ring], contraceptive sponge, double barrier [diaphragm or condom plus spermicide], or IUD
20. Individuals who are classified as "low binders" for the rs6971 polymorphism (<10% of the population)
21. Patients on antiplatelet and anticoagulant medications will be excluded.
22. Any patient without capacity to consent will be excluded.
23. Unstable hypertension. If blood pressure is greater than 160/100, we will contact the subject's primary care physician to manage their blood pressure. If their blood pressure is not reduced to be consistently below 160/100 by the scanning day the subject will be excluded from the protocol.
24. Patients with contraindications for lumbar puncture procedure can be still involved and will be excluded from the portion of the study with the lumbar puncture. These include existing intracranial space–occupying lesion with mass effect, posterior fossa mass, risk of cerebral herniation by increased CSF pressure or Arnold chiari malformation, as well as anticoagulant medication, coagulopathies and uncorrected bleeding diathesis, congenital spine abnormalities, and local skin infection at the puncture site.

Collaborating Sites. The Yale Memory Clinic and Yale Alzheimer's Disease Research Unit (ADRU) will serve as the recruitment and assessment centers in this study.

b- How will **eligibility** be determined, and by whom?

Eligibility to participate will be determined by the PI of this study after completion of the medical and psychiatric evaluation of the potential participant. The PI has both neurology and internal medicine training.

c- **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

Risks associated with screening and evaluation.
During the screening interview, we will ask about psychiatric and medical history. Certain questions may make subjects uncomfortable or anxious. Only trained and experienced research assistants will perform these interviews, which will be done in a sensitive and gentle manner.

Risks associated with worsening of Alzheimer's Symptoms:
Signs and symptoms of Alzheimer's dementia might be exacerbated as a consequence of study participation either due to the fatigue, pain, or disruption of normal routine related to study procedures or due to the direct effects of endotoxin administration.

The presence of acute on chronic deterioration will belong to one of the following scenarios:
1- There are changes in cognition but no changes in ADLs: the patient will be seen in the clinic by Dr. Mecca within two weeks, a cognitive neurologist, and appropriate medical interventions and follow-up arrangements will be made.
2- There are changes in cognition, there are changes in ADLs, but there is no change to the level of care or the ability of the carer to continue to care for the subject: the patient will be seen emergently in the clinic (within 48 hours) and appropriate arrangements will be made. Follow-up will track resolution.
3- There are changes in the level of care or the ability of the carer to care for the subject: we will admit the patient into the hospital and provide appropriate treatments and case management.

Risks associated with confidentiality.
Although all information collected about each subject in this study will be protected by HIPAA and stored in locked cabinets and in password-protected computers, it is possible that information could be accessed by individuals who are not part of the study team. Such illegitimate access could have deleterious consequences for the subjects with regards to employment, access to health insurance, and stigma. The protection of subject information is not absolute.

It does not apply to any State requirement to report certain communicable diseases or to report cases of physical or sexual abuse. Such duty to report will be explained to each subject during the consent process and if reporting becomes necessary, we will inform the subject. We may release information that identifies subjects in some circumstances, without their permission. For example, we may disclose medical information in the event of an emergency. We may take steps, including notifying the authorities, to protect the subject or someone else from serious harm (including child abuse and elder abuse).

Risks associated with blood drawing and with IV and radial catheter placement.
Blood drawing and venous catheter insertion can be associated with mild pain, bruising, infection, or clot formation. These risks are minimized by the use of proper technique. No more than a total of 150 mL of blood will be drawn during the entire study. This amount of blood loss over a period of 10 or more days should have no deleterious consequences in a non-anemic individual. On the morning of the PET scan, a radial artery catheter will be inserted by an experienced healthcare provider. The site will be anesthetized with lidocaine prior to arterial line insertion.

The arterial line will remain in place for the duration of the [11C]PBR28 scans, after which it will be removed. Arterial sampling may be associated with mild-to-moderate pain or bruising at the puncture site. In rare instances, blocking of the artery, poor healing, hematoma, inflammation, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during arterial catheter placement.

Risks of radial artery cannulation are minimized by having the procedure performed by an experienced health care provider. The health care provider would be either a physician or an advanced practice registered nurse (APRN) with experience in critical care and placement of arterial catheters, as is the practice at Yale-New Haven Hospital. For an APRN to place the arterial line at the Yale PET Center, they must meet the following criteria:
1.) Be currently credentialed at Yale-New Haven Hospital or similar institute and
2.) Perform 3 arterial line procedures supervised by a currently privileged PET Center physician
The 3 supervised arterial line placements will be documented and signed off by both the APRN and supervising physician. The completed document must be on file at the Yale PET Center prior to an APRN performing any arterial line catherizations independently.

Risks associated with optional CSF Collection

-Post-lumbar puncture headache. Up to 25 percent of people who have undergone a lumbar puncture develop a headache afterward due to a leak of fluid into nearby tissues. The headache typically starts several hours up to two days after the procedure and may be accompanied by nausea, vomiting and dizziness. The headaches are usually present when sitting or standing and resolve after lying down. Post-lumbar puncture headaches can last from a few hours to a week or more. See below for mitigation.

-Back discomfort or pain. The patient may feel pain or tenderness in his/her lower back after the procedure. The pain might radiate down the back of his/her legs.

-Bleeding. Bleeding may occur near the puncture site or, rarely, into the epidural space.

-Brainstem herniation. Increased pressure within the skull (intracranial), due to a brain tumor or other space-occupying lesion, can lead to compression of the brainstem after a sample of cerebrospinal fluid is removed.

Risks associated with radiation.

The Yale University Radioactive Drug Research Committee (RDRC) will review the use of radiation in this research study, and no subjects will be enrolled until RSC approval is obtained. This research study involves exposure to radiation from two 20 mCi doses of [11C]PBR28 and from the transmission PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only. The amount of radiation subjects will receive in this study is below the dose guidelines established by the federal government and adhered to by the Yale University Radioactive Drug Research Committee (YU RDRC) for research subjects. This guideline is an effective dose of 5 roentgen equivalents in man (rem) per scan and 15 rem/year. The targeted amount of radiation an individual subject will receive in this study is from two injections of 20 mCi of [11C]PBR28, for a total of 40mCi of [11C]PBR28. Although each organ will receive a different dose, the targeted amount of radiation exposure subjects will receive

from this study is equal to an effective dose equivalent of 1.628 rem. This calculated value is used to relate the dose received by each organ to a single value.

In addition to the values given above, subjects who have an ^{11}C-PIB-PET scan under this protocol may be exposed to radiation from one 15mCi injection of 11C-PIB. This radiation exposure is not necessary for medical care and is for research purposes only. Subjects who undergo the ^{11}C-PIB scan will receive a total of 15mCi ^{11}C-PIB and 40mCi ^{11}C-PBR. Although each organ will receive a different dose, the targeted amount of radiation exposure subjects will receive from this study is equal to an effective dose equivalent of 1.888 rem. The amount of radiation subjects will receive in this study is below the dose guidelines established by the federal government and adhered to by the Yale University Radioactive Drug Research Committee (YU RDRC) for research subjects. This guideline is an effective dose of 5 roentgen equivalents in man (rem) per scan and 15 rem/year

In situations where a PET scan is not successful following the radiotracer infusion (e.g., problems with the PET camera) the subject may receive an additional radiotracer infusion(s), if deemed appropriate. If an additional injection is completed and the ^{11}C-PIB-PET scan ***WAS NOT*** performed, subjects would receive a total radiation dose of 2.44 rem. If an additional injection is completed and the ^{11}C-PIB-PET scan ***WAS*** performed, subjects would receive a total radiation dose of 2.70 rem.

The effects of radiation exposure on humans have been studied for over 60 years and no harmful effect to humans has been observed from the levels of radiation subjects will receive while taking part in this research study. However, scientists disagree on whether radiation doses at these levels are harmful. It is unclear whether already low doses of radiation could cause cancer. Even though no effects have been observed, some scientists believe that radiation can be harmful at any dose - even low doses such as those received during this research study.

Subjects will be asked to tell the research team if they have taken part in other research studies or received any medical care at any hospitals or any other place that used radiation. This is done to confirm that subjects will not receive excessive radiation. Examples of the types of radiation exposure considered include x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into their body. However, the possibility exists for a rare reaction to any of the substances or procedures to which a subject is exposed.

Risks of MRI:
Magnetic resonance (MR) imaging carries a risk for subjects who are claustrophobic or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. MR is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines. Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches.

These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them. There are some risks with an MR study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a subject. To reduce this risk, we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet. We want subjects to read and answer very carefully the questions on the MR Safety Questionnaire related to their personal safety. We will be sure that subjects have read the MR Safety Questionnaire and tell us any information they think might be important. This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The PI's, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the PI or consulting physician will

contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure.

The decision for additional examination or treatment would lie solely with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that a subject receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

Pregnancy and Breast Feeding:
Since the acceptable levels of radioactivity are lower for pregnant individuals, women will be excluded from participating in this study if they are currently pregnant or might become pregnant during the study. They will be tested for pregnancy as part of the routine laboratory tests. If the test is positive, they will not be included in the study. Before starting the study, we will ask women to avoid becoming pregnant and what precautions they plan to take. We ask women to tell us immediately if they change their minds about becoming pregnant. If the woman is currently breast-feeding, she will be excluded from the study. Women will be given one pregnancy test during the initial physical evaluation, and in addition on day of
the PET scans, prior to radiotracer injection.

Lipopolysaccharide Administration:
Lipopolysaccharide (LPS, also called endotoxin) is a large molecule found on the outer membrane of gram-negative bacteria that has been widely used to evoke a robust immune response. We propose to use the same dose and protocol of LPS that we have successfully used in our recent study (4): 0.4 ng/kg, IV bolus. Specifically, on the day of the [11C]PBR28 PET scans, LPS (0.4 ng/kg, IV) will be administered 3 hours prior to the second PET scan. Vital signs (systolic and diastolic blood pressure, heart rate and respiration rate) and sickness and psychiatric symptoms will be monitored before drug administration and every 15 to 30 min up to 360-min after LPS administration. Because of the risk of nausea and emesis, subjects are required to fast for the day of the study until after the scan. In our experience to date (18 subjects total 57 have received LPS at the dose of 1.0 ng/kg), no subjects have reported nausea that was significant enough to require stopping the study. If significant nausea or other adverse events occur, the study will be stopped.

If an individual experiences bothersome symptoms after LPS administration and wants to stop the study, flulike symptoms may be treated with acetaminophen (650 mg every 6 hrs), or ibuprofen (400 mg every 6 hrs), and anxiety may be treated with lorazepam (0.5 mg every 2 hrs for a maximum dose of 6 mg every 24 hrs). During screening each subject will be asked about medication allergies, and no medication to which a subject may be allergic will be administered. If a subject requests the study will be stopped and these medications may be administered. If a subject's body temperature exceeds 39.2°C the study will be halted and the subject will receive ibuprofen or acetaminophen as described above. If these medications are insufficient to lower body temperature, subjects may be transferred to a higher level of care.

At the end of the PET scan day, subjects will be assessed (physical and vital signs) by the study physician. Subjects will be discharged when vital signs are within normal limits and when behavioral changes (if any) are found to be not clinically significant by the MD attending to the subject at the PET Center. If subjects experience any adverse events, they will be treated until they become asymptomatic, prior to discharge. In the proposed study and in our ongoing studies, psychiatric patients who are administered a challenge drug may be admitted to an inpatient unit if psychiatric symptoms worsen or become dangerous on the PET scan day. If required the patients will be assessed by Dr. Adam Mecca in the neurology clinic and may be sent to emergency department, Yale New Haven Hospital, for admission if necessary. All subjects are followed for 1 week (in person the following day and by phone thereafter) following LPS administration or until potential symptoms (self-report and by assessment) resolve.

Background and potential side effects of LPS administration:
LPS administration in human subjects is a generally safe experimental procedure that has been used in at least 2,200 subjects (116). Intravenous LPS administration at doses from 2 to 4 ng/kg causes flu-like symptoms such as fever, chills, rigors, malaise, and increased heart rate (117-119). These effects peak at around 90 minutes, and completely disappear within 6-8 hours or less. Of the 2,200 subjects who have received LPS, only four severe adverse events have been reported, all involving bradycardia or sinus arrest (5). This occurred in subjects who either had a history of vasovagal syncope and/or who participated in a protocol in which they were not adequately hydrated. In large cohorts

of subjects who received adequate hydration, no such effects occurred (116). We have administered LPS (0.8-1.0 ng/kg) to >30 subjects, including both healthy controls and individuals with psychiatric disorders, at Yale with no unexpected or serious adverse effects. A small study (119) detected gender differences in peripheral cytokine levels after LPS administration, with females having a more pronounced response than males; 50% of our sample will be women so we will be able to explore sex differences. We have not noted particular behavioral differences (e.g., sickness behavior) between men and women receiving LPS to date. A review of the literature published in 2007 (120) describes all the studies wherein LPS was given to human subjects. All articles were reviewed for any potential adverse effects, morbidity or mortality; however, no long-term morbidity or mortality was reported in these more than 1000 healthy volunteers. At a 5 ng/kg dose of EC-5, subjects experienced nausea, vomiting and fever. Consequently, doses of ≤ 4 ng/kg have been subsequently used. We are giving 1/10 of that dose. LPS given at 2 ng/mg is not associated with known longterm sequelae or serious adverse events in healthy populations. Subjects (up to 4 total) who did have side effects at doses as low as 2 ng/kg were found to have underlying medical issues. When side effects have occurred, they are remarkably similar. Typically, within 50-90 min following LPS, subjects describe flu-like symptoms such as fatigue, malaise, arthralgias, myalgias, headache, nausea, and often chills that resolve within 3-4 h. Fever, however, is the hallmark of LPS infusion. After a latent period up to 120 min, there is a monophasic increase in fever which peaks at 1-2°C above baseline. The fever may last up to 3-4 h (depending on dose) and resolves without residual effect.

This critical review of all the cases of LPS administration in human subjects concludes as follows, "for well over a century, endotoxin has been intentionally administered to humans for therapeutic purposes, for the evaluation of anti-inflammatory reagents as well as to address basic scientific questions of endotoxin biology. Much has been learned from the use of this model, and it has proved to be remarkably safe. Well over 1,000 subjects have been studied, yet we were able to find documentation of only five serious adverse events, and these were in subjects who were later shown to have vagal hypersensitivity," (120). Thus, all the available data support that LPS administration in healthy human subjects is safe.

Although there are no studies applying the lipopolysaccharide to Alzheimer's disease population, endotoxin has been used in the elderly population with age distributions that approximate our cohort. In one study eight healthy young volunteers (median age 24 yr) and nine healthy elderly volunteers (median age 66 years) were compared for their blood pressure, heart rate, core temperature and hourly plasma catecholamine concentration after the administration of 2ng/kg of endotoxin. At baseline the elderly had a higher mean arterial pressure (MAP) of about 10 mm Hg, after the administration of the endotoxin there was a reduction of about 20 mm Hg in the MAP. In almost all cases the maximal response occurred 4-7 hours after the administration of the endotoxin and was associated with fever. In no cases there were hemodynamically critical changes to the blood pressure (128). The same cohort also showed the presence of overactive initial response and prolonged elevation of the inflammatory markers compared to people in their third decade of life (129). The difference between our experimental design and theirs is: 1- we use 1/5 the dose they used as a bolus. 2- We will have a low threshold for using antipyretics. The changes to MAP seem to have most closely followed the temperatures.

Lipopolysaccharide, or Clinical Center Reference Endotoxin (CCRE) will be provided by the NIH Clinical Center and will be stored and prepared by the YNHH IDS pharmacy. CCRE is an investigational drug, Dr. Kelly Cosgrove has obtained an Investigational New Drug (IND) for its use in humans from the Food and Drug Administration (FDA). CCRE vials will be stored at 2-8°C in a refrigerator in the locked YNHH IDS pharmacy. Preparation of CCRE will be performed by the YNHH IDS research pharmacist. As recommended by NIH, the pharmacist will dissolve the lyophilized CCRE in Sterile Water for Injection USP under a laminar flow hood on the evening before each endotoxin administration. The dissolved CCRE will be stored at 4°C until the following morning. Dissolved CCRE is stable for at least 24 hours at this temperature.

Fatigue associated with cognitive testing and the discomfort during the PET scanning:
The entire duration of cognitive testing will be approximately 3 hours, raising the potential risk of fatigue. Fatigue will be addressed by providing appropriate breaks as needed. Also even though the PET and MRI studies are considered safe procedures and do not cause the subject any pain, they are somewhat uncomfortable as the subject must be motionless during the scans.

Minimizing Risks: **Describe the manner in which the above-mentioned risks will be minimized.**

To minimize risks associated with endotoxin

- Using 1/10 of the maximum safe dose
- Medically evaluating subjects and including only those with no history of cardiovascular disease, liver failure, atopy, morbid obesity (BMI>35), underweight (BMI<19), vasovagal syncope, acute or chronic inflammatory conditions.
- Adequately hydrating with saline,
- NPO to reduce the risk of vomiting.
- Close monitoring of blood pressure, heart rhythm, and subjective symptoms, and by the continuous presence of two trained medical professionals.

To Minimize the risk of liver damage

- We will recruit only non-obese patients with laboratory results with normal LFTs, bilirubin, and PT ranges.
- We will exclude any subject with clinical or laboratory signs of liver damage. Those who are vulnerable to liver damage as a result of the endotoxin exposure will not be studied, making this group very similar to the groups of HC who had tolerated the endotoxin in the past, without problems.
- We will measure LFTs, bilirubin and PT after endotoxin administration to ensure these values do not go up significantly."

To minimize the risk of an exaggerated systemic inflammatory response in the Elderly- We are using a lower dose.

To minimize risks associated with blood drawing Only experienced staff and proper technique will be used tominimize risks associated with use of an arterial catheter. Individuals on anti-coagulation and anti-platelet therapy are excluded from the study.

To minimize risks associated with lumbar punctures:

Related to CSF Leak

- Post-lumbar puncture headache. We ask the patients to remain recumbent for one hour and to remain hydrated for the 48 hours following the procedure, during which they should refrain from lifting heavy weights or bending over for as long as possible. If the headache persists more than 24 hours the patients are instructed to contact us. If persistent headache exists the patient would be encouraged to try bedrest and hydration for a further 3 days. If that is insufficient we may bring them into the headache clinic for hydration and/or caffeine administration or refer them for a blood patch.

- Brainstem herniation. Increased pressure within the skull (intracranial), due to a brain tumor or other space-occupying lesion, can lead to compression of the brainstem after a sample of cerebrospinal fluid is removed. The patients all have MRI imaging that precludes space occupying lesion, making this a very rare adverse event. Brain herniation when it very rarely happens, occurs at the time of the procedure and is a medical emergency and internal emergency mechanism will be activated.

Related to other AE

- Back discomfort or pain. The participant may feel pain or tenderness in his/her lower back after the procedure. The pain might radiate down the back of his/her legs. If the pain persists for more than 72 hours the patients are instructed to contact us. If the pain remains after simple analgesia then we will refer the patients to the pain clinic.

- Bleeding. Bleeding may occur near the puncture site or, rarely, into the epidural space. Bleeding into epidural space is a rare event. The patients are instructed to contact us if there is any weakness, numbness in the legs or there is urinary/bowel incontinence. This would be a medical emergency.

Risks of radial artery cannulation These are minimized by having the procedure performed by an experienced healthcare provider. Pain is minimized by local anesthesia. Bleeding is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again following catheter removal. Also, subjects will be asked to abstain from using aspirin and other NSAIDs for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24-hour physician telephone number (2037854085) to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

To minimize the risks associated with radiation We will ensure that the radiation exposure in each subject does not exceed Federal guidelines. Information about [11C]PBR28 dosimetry was obtained from published data as described above. The dose of radiation will be submitted for approval to the Yale University Radioactive Drug Research Committee (YU RDRC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the Yale University PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and execution of PET scans will be performed by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radionuclides. Subjects will be asked about their previous radiation exposure, and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study.

To minimize risks associated with MRI, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and change into scrubs immediately prior to the study and pass through the metal detector in the MRRC before entering the MRI room. If the subject has any metallic prostheses/implants they will be excluded from the study. If a subject becomes anxious during the scan they can request that the MRI scan be stopped.

To minimize risks associated with pregnancy and breastfeeding we will not enroll women who are pregnant or breast-feeding.

Data and Safety Monitoring Plan: Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.

a. What is the investigator's assessment of the overall risk level for subjects participating in this study?

This study is associated with moderate risk. The PI, Dr. Adam Mecca will meet with Dr. Christopher van Dyck to review adverse event data no less than one time per year before IRB renewal, and ad hoc any time an unforeseen adverse event occurs.

b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?

No children are involved.

c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://www.yale.edu/hrpp/forms-templates/biomedical.html for
   i. Minimal risk
   ii. Greater than minimal

1. We do not view the risks associated with endotoxin administration as minimal.
2. We do not view the risks associated with the combination of older subjects and endotoxin as minimal.
3. Given the now established safety and validity of the current protocols in our prior work, we do not view the proposed studies as high risk.
4. Given our rigorous screening of patients we do not consider the combination of age and endotoxin as high risk.

2. Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

Greater Than Minimal Risk Data Safety Monitoring Plan

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance. He will achieve this by convening a **safety committe**e consisting of:

- Adam Mecca (PI- geriatric psyc)
- Christopher Van Dyck (Director of Alzheimer's disease research unit (ADRU) and Yale ADRC – geriatric psychiatrist)
- Kelly Cosgrove (Associate Professor, Department of psychiatry)
- Martha MacAvoy (Neuroscientist at ADRU)
- Susan Good (Advanced nurse practitioner at ADRU)

We will review safety data **every 4 months** or **after every 4 patients** whichever happens first (including when re-approval of the protocol is sought). During the review process, the principal investigator and the safety monitoring evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator, the IRB or the FDA have the authority to stop or suspend the study or require modifications.

A start-up meeting is scheduled for Jan 12 to address this risk of extra scans within a one-year period, and an effective monitoring plan established. The meeting would be attended by Arash Salardini, David Matuskey (Medical Director PET Center), Ming-Kai Chen

(Medical Director PET Center), Richard Carson (PET Center Director), Shannon Henry (Administrator) and some other people.

2. The risks associated with the current study are deemed greater than minimal for the following reasons:

1. We do not view the risks associated with endotoxin administration as minimal.
2. We do not view the risks associated with the combination of older subjects and endotoxin as minimal.
3. Given the now established safety and validity of the current protocols in our prior work, we do not view the proposed studies as high risk.
4. Given our rigorous screening of patients we do not consider the combination of age and endotoxin as high risk.
5. We do not view the risks associated with the lumbar puncture as minimal risk.
6. We do not view the risks associated potential future genetic testing as minimal risks. The risks are due to concerns with privacy.
7. Given the now established safety and validity of the current genetic testing and lumbar puncture in our prior work, we do not view the proposed studies as high risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator (*Adam Mecca*) according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Expected adverse effects:

We divide the adverse effects into expected and unexpected. For expected adverse effects related to LPS, based on the previous studies of human experimental endotoxemia:

a) Flu-like symptoms: chills, headache, myalgias and arthralgias, nausea and photophobia can seems to peak about one hour after the administration of the LPS. The phenomenon has a lot of inter- individual variation but within an individual it is dose dependent. The incidence of flu-like symptoms below the dose of 2ng/kg is unrecorded in the literature [1,2].

b) CNS-related adverse effects: in young healthy individuals at 0.8 ng/ml of LPS (2 x the dose we are using), there was some effects on memory, with anxiety and depression emotions. At 0.1 ng/ml there was an improvement in memory recall. Also from clinical experience, which cause much higher levels of inflammation, we know that there is at least a theoretical risk of acute delirium. The course of possible CNS effects follows TNF-alpha which peaks 90 minutes after the administration of the LPS[3].

c) CVS-related adverse effects: the most common side effect in the healthy population is tachycardia where there is an increment of roughly 20 beat/min. In a previously pre-disposed individual the administration of LPS can theoretically precipitate arrhythmias [4].

d) Pulmonary related adverse effects: There is an association between high dose administration of LPS and increases in the respiratory rate and possibly dyspnea [2].

e) Liver and gastrointestinal organs: The administration of high dose (4ng/ml) may change the metabolism of other drugs. Liver is also the main site of removal of LPS from the blood stream. We will exclude patients with evidence of liver damage both semiologically and baseline laboratory values. [3]

The expected adverse effects from lumbar puncture:

Though lumbar punctures are generally recognized as safe, they do carry some risks. These include:

a) Post-Lumbar Puncture headache: Headache, which occurs in 10 to 30 percent of patients, is one of the most common complications following LP. Post-LP headache is caused by leakage of cerebrospinal fluid (CSF) from the dura and traction on pain-sensitive structures. Patients characteristically present with frontal or occipital headache within 24 to 48 hours of the procedure, which is exacerbated in an upright position and improved in the supine position. Associated symptoms may include nausea, vomiting, dizziness, tinnitus, and visual changes.
b) Infection: Infections are rare after LP; in typical patients no techniques beyond usual aseptic technique are required.
c) Bleeding: The CSF is normally acellular, although up to five red blood cells (RBCs) are considered normal after LP due to incidental trauma to a capillary or venule. A higher number of RBCs is seen in some patients in whom calculation of the white blood cell (WBC)-to-RBC ratio and the presence or absence of xanthochromia may differentiate LP-induced from true central nervous system (CNS) bleeding. Serious bleeding that results in spinal cord compromise is rare in the absence of bleeding risk.
d) Cerebral herniation: Increased pressure within the skull (intracranial), due to a brain tumor or other space-occupying lesion, can lead to compression of the brainstem after a sample of cerebrospinal fluid is removed.The most serious complication of LP is cerebral herniation. Suspected increased intracranial pressure (ICP) is a relative contraindication to performance of an LP and also requires independent assessment and treatment.
e) Radicular symptoms and low back pain: It is not uncommon for patients to experience transient electrical-type pain in one leg during the procedure. However, more sustained

radicular symptoms or radicular injury appear to be rare. Up to one-third of patients complain of localized back pain after LP; this may persist for several days, but rarely beyond.

5. Expected severity of adverse events:

In our study we will use low dose (0.4 ng/kg) in an older population. Since there are no previous studies which use low dose endotoxin in the elderly, we have reviewed the use of low dose endotoxin in the young population and one study of high dose LPS in the elderly. We have also commented below on the AD specific adverse effects for endotoxin challenge:

a) **Based on previous studies of low dose LPS studies in healthy young individuals:** have reviewed the effects of low dose endotoxemia on human subjects, in this case 0.4-0.8ng/kg. The inflammatory response to 0.4 ng/kg of endotoxin is relatively muted: in one study the administration of LPS caused an peak increase in IL-6 of 150 and TNF-alpha of 50 pg/ml [5]. Compare this with patients with sepsis with IL-6 levels of 42,154 +/- 181,966 and TNF-alpha levels of 273 +/- 648 pg/ml.[6] The following two studies are representative of the effects of low dose experimental endotoxemia:

- Eisenberger and others [7] used E-Coli (O:113) endotoxin to study mood changes in response to 0.8 ng/kg of iv LPS, in 39 healthy adults. Profile of Mood States (POMS), which is not a commonly used scale, is scored between 0 (no depression) to 4. The scale was used in this study. It was found there was an increase of 0.4 in the POMS scale in the endotoxin group and not in the saline group.

- In another study Grigoleit and others [5] administered LPS at doses of 0.4 ng/kg and 0.8 ng/kg to 18 and 16 healthy individuals respectively. In both groups there was an increase in the temperature (1 degree) and heart rate (~15 bpm) peaking around 3 hours. The authors used Multidimensionaler Befindlichkeitsfragebogen (MDBF) for measurement of neurobehavioral parameters. The results were dose dependent. Changes in mood, calmness, alertness and state anxiety were 6,3,5 and 9 for the 0.8 ng/kg group vs. 2, 2, 2 and 3 for the 0.4 ng/kg group. 2-back task was used to measure working memory: there were no changes in the accuracy of the task while there was improvement in reaction time in the 0.8 ng/kg group. For the 24 hour memory test 72 random pictures from the International Affective Picture System were used, half of which elicited high arousal (i.e. emotional content). There was slightly reduced memory accuracy for emotionally arousing pictures for the low LPS group.

b) **Based on previous studies of high dose LPS study in healthy older individuals**: There is one experiment which involves elderly patients receiving endotoxin (published in two papers) [8-9]. Nine elderly patients (median 66 years of age, 61-69) were compared to eight young healthy controls. Administering 2ng/kg intravenously of LPS caused an increase in temperature which was not significantly different between the two groups, but the normalization of the temperature was marginally slower in the elderly group compared to controls. For example at 8 hours there was a 0.7 degree difference between the two

groups. There was a greater TNF-alpha increase in the elderly which had a small delay in termination compared to the control group. They also looked at reduction in mean arterial pressure. The reduction peaked in the young (magnitude 10 mm Hg on average) in the third hour after the administration of the LPS. For the elderly the trough of mean arterial pressure (20 mm Hg on average from a larger baseline MAP) was 6 hours after the LPS.

c) **Alzheimer specific adverse events:** the effect endotoxin administration to subjects with Alzheimer's disease is unknown but two theoretical vulnerabilities may be surmised:

- Vulnerability to Delirium- delirium is a subacute neuropsychiatric syndrome characterized by disturbance and fluctuation of attention, changes in the level and content of consciousness, perceptual disturbance, circadian disruption and other cognitive problems. The relationship between inflammation and delirium is related to the high incidence of delirium in patients with inflammatory, often infectious ailments and higher than normal inflammatory markers [10]. Additionally, delirium is more common in patients who are older and have baseline cognitive impairment. Although we do not anticipate 0.4 ng/kg of endotoxin to cause delirium even in this vulnerable population, we will monitor the incidence of delirium in our study (please see under cognitive safety).

- Potential effects of inflammation on Alzheimer's pathology: As discussed in detail in the research plan, there is an association between Alzheimer's pathology and inflammation but whether it is beneficial or detrimental is still not determine. Again although we do not anticipate significant changes to the course of early AD after LPS challenge (as many other inflammatory events occur in the course of the year which are likely more prolonged and severe than 0.4 ng/kg LPS administration), we will monitor long term effects (please see under cognitive safety).

Expected severity of Lumbar puncture related adverse effects:

LP is a relatively safe procedure, but minor and major complications can occur even when standard infection control measures and good technique are used. The common complications include Post-LP headache, bleeding, cerebral herniation, minor neurologic symptoms such as radicular pain or numbness and back pain. In addition, an LP is the easiest procedure to perform a CSF pressure measurement. Given the use of CSF analysis for diagnosis, LPs are currently often performed to perform research to discover novel diagnostic biomarkers and understand brain pathology. A recent large international, multicenter study on LP feasibility by Duits et al. that included 3868 patients in a memory clinic setting, similar to our study population showed that LPs can be safely performed [11].

Post-lumbar puncture headache (PLPH): Up to 25 percent of people who have undergone a lumbar puncture develop a headache afterward due to a leak of fluid into nearby tissues. ounger age is the most important and well-known risk factor for PLPH and post-LP back pain that is not the case with our study. PLPH typically begins within three days after the procedure in most

patients. If a patient develops typical PLPH, bed rest, adequate hydration, and simple analgesics should be started. The headaches are usually present when sitting or standing and resolve after lying down. Post-lumbar puncture headaches can last from a few hours to a week or more [12,13]. More than 85% of PLPH will resolve without any other specific treatment. The only evidence-based treatment for typical severe PLPH (severe usually frontal headache possibly accompanied by nausea and vomiting which is relieved by recumbent posture) is an epidural blood patch. This procedure has a success rate of 70%–98% if carried out more than 24 hours after the LP. If a first epidural blood patch fails to resolve the headache, repeating the procedure has a similar success rate [14].

Back discomfort or pain: The patient may feel pain or tenderness in your lower back after the procedure. The pain might radiate down the back of legs. In the review of subjects in memory clinic, number of LP attempts was the only procedure-related risk factor for occurrence of local back pain that we would try to minimize. It is recommended to use 25G atraumatic needles. A total of four LP attempts is an acceptable maximum, active CSF withdrawals should be avoided. It is advised to perform LP in the lateral recumbent position, and collection up to 30-mL CSF is well tolerated and safe. There is no recommendation to apply local anesthesia and bed rest after the LP to reduce postpuncture complaints [11].

Bleeding: Bleeding may occur near the puncture site or, rarely, into the epidural space. Serious bleeding that results in spinal cord compromise is rare in the absence of bleeding risk. Patients who have thrombocytopenia or other bleeding disorders or those who received anticoagulant therapy prior to or immediately after undergoing LP have an increased risk of bleeding. In one series, spinal hematoma developed in 7 of 342 patients (2 percent) who received anticoagulant therapy after undergoing LP; five of these patients developed paraparesis. In one literature review, 47 percent of 21 published cases of spinal hematoma following LP occurred in patients with a coagulopathy [15,16].

Brainstem herniation: The most important contraindication for LP is an intracranial space–occupying lesion with mass effect as well as a posterior fossa mass because it can lead to herniation of the cerebellar tonsils, regardless of the volume of CSF that is sampled. Other contraindications for LP include a risk for cerebral herniation caused by abnormal intracranial pressure due to increased CSF pressure or Arnold-Chiari malformation [17]. Based on our protocol, We perform Brain imaging for our study before CSF collection and we would notice any existing incidental finding that might be a contraindication for lumbar puncture. We would exclude patients with contraindications from LP portion of the study.

6. Plan for Grading Adverse Events:

For grading the severity of adverse events noted during the study we will adopt a general scheme from FDA publication *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, Center for Biologics Evaluation and Research, September 2007)*[18]*:*

Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

1- For most of the anticipated adverse effects we will use this guidance and parameters set:

For arterial cannulation:

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 - 5 cm	5.1 - 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

For vital signs:

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) **	38.0 – 38.4	38.5 – 38.9	39.0 – 40	> 40
(°F) *	100.4 – 101.1	101.2 – 102.0	102.1 – 104	> 104

Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

For systemic symptoms:

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 - 3 loose stools or < 400 gms/24 hours	4 - 5 stools or 400 - 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

For laboratory values:

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23-26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK– mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

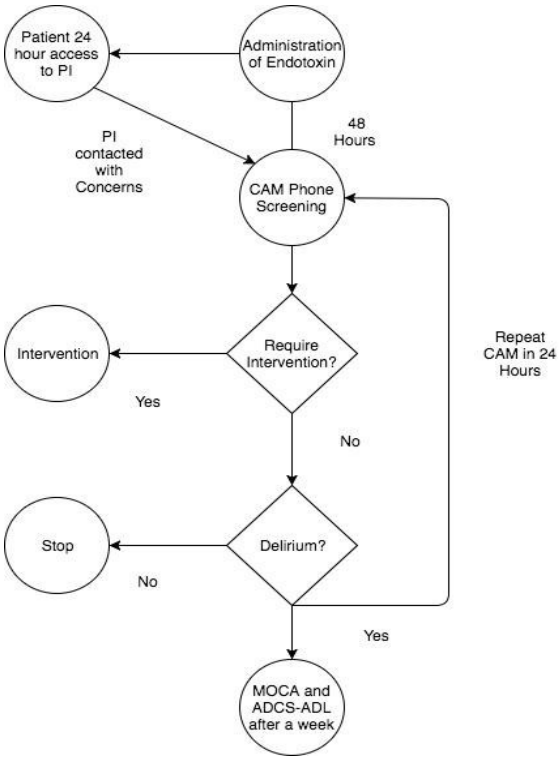
For Lumbar Puncture :

Post-Lumbar Puncture symptoms	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Cerebrospinal fluid leakage	postural care indicated	persistent moderate symptoms; blood patch indicated	Severe symptoms; medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Post-lumbar Puncture Headache	Mild pain, No interference with activity, transient. Postural Care Indicated	Moderate pain; limiting instrumental ADL, persistent moderate symptoms; blood patch indicated	Severe pain; limiting self care ADL; medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Back Pain	Mild pain, No interference with activity	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care AD	
Cerebral Herniation				Life-threatening consequences; urgent intervention indicated

7. Plan for surveillance of cognitive adverse effects

Cognitive adverse effects can occur because of the effect of endotoxin administration (see section 5c) and because of disrupting patient's daily routine and fatigue. Imaging studies which are common in Alzheimer's research, and often in more cognitively impaired individuals, often follow more rigorous procedures of testing. We anticipate that the latter is less likely to be a factor in the cognitive adverse effects. For our purposes we classify cognitive adverse effects as acute and chronic. We following the conventional practice of measuring global cognitive changes by monitoring functional status[19] (ADCS-ADL in this case) and multi-domain screening instruments (MOCA in this case) [20].

7.1 Protocol for the monitoring of acute cognitive changes for a one-time or test-dose administration of agents



- The patient's study partner has the PI's cell phone whom s/he can contact with any concerns
- If called, then patient screened with CAM over the phone[21]
- If not called, then the patient is screened with CAM after 48 hours
- If intervention is required that will be prioritized
- If not, then depending on the latest possible interval for late effects there is re-screening. Otherwise Stop.
- If there is delirium, then CAM is repeated every 24 hours until resolution AND ADCS- ADL will be performed in a week to gauge the severity of the adverse effects for reporting.
- MOCA will be performed in a week regardless; and weekly using alternative versions until resolution if ≥ 2 change in score.
-

8. Plan for Grading Cognitive Adverse Events:

The FDA guidance does not contain guidance regarding grading of cognitive adverse effects. We use the general scheme from the FDA guidance as the starting point. Grading of cognitive function in the elderly is often based on functional abilities. This approach appears to be in harmony with the general intent of FDA's guidance.

Assessment of cognitive safety is the assessment of "the impact of clinical treatments on the ability to perceive, process, understand, and store information, make decisions and produce appropriate responses" [22]. As with many systemic adverse effects of medications the severity is often expressed in terms of functional capacity. This is particularly apt for cognition for three basic reasons:

1- There is no ecologically valid single indicator of "cognition" which may be accepted as reliable surrogate for the overall brain cognitive ability, say when compared to the role of ejection fraction in cardiac ischemic complications.

2- The most useful indicators of organ system integrity rely on measuring surrogates of broad functions of those organ system. For example, renal side effects may be detected by measuring creatinine clearance and proteinuria. It is possible and in fact in some cases

necessary to look at renal parameters in greater detail including size, filtration rate, active transport mechanism and endocrine function. Especially in patients whose creatinine is shown to be affected However, these are often unnecessary in a majority of cases. Activities of daily living may provide a similar overall surrogate for cognitive function if and only if all other factors (chiefly mobility) remain the same.

3- The Dementia research community defines stages of dementia in terms of their impact on activities of daily living. Several scales including the Clinical Dementia Rating[23] are based on this idea.

8.1 Protocol for stratification of acute cognitive adverse effects for reporting

No adequate guidelines at present exist for grading of serious adverse effects of administered agents pertaining to acute cognitive changes. We will adopt the following scheme:

We use CTCAE 4.02 for guidance[24]: DL= dependence level (see below)

Grade	Definitions	Maximal daily DL change in first week	Change of DL in 1 week
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.	2	1
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.	3	2
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting selfcare ADL.	4	3
4	Life-threatening consequences; urgent intervention indicated.	N/A	N/A
5	Death related to AE.	N/A	N/A

The DL is determined using a published algorithm [25]:

DL	Types of Impairment	Care Needs	Scoring Algorithm versus Care Needs
0	No IADL/BADL impairment	None	No impairments in ADCS-ADL
1	Some supervision needed on isolated	Independent living with check-ins	Level 2 on any 1 item from the following clusters: Household activities, Communication and Engagement, Outside Activities

2	Supervision on multiple IADLs or loss of at least 1 Household Activity	Limited/inf or mal home care services	Level 2 on items from at least 2 of the following clusters: Household activities, Communication and Engagement, Outside Activities, OR Level 1 on any item from the following clusters: Household activities, Communications and Engagement, Outside Activities
3	Supervision on all types of IADLs or home- bound	Extensive home care services w/ supervisio n OR	Level 2 for all items from the following clusters: Household activities, Communications and Engagement, Outside Activities, OR Level 0 on 1 item of Outside Activities, OR Level 2 for either item: Eating (Q1), Bathing (Q4)
4	Supervision on some BADLs	Assisted living + nursing	Score <2 for item Dressing (Q6B), OR Level 1 or 0 or for any items: Grooming (Q5), Bathing (Q4), OR Level 2 for item Toileting (Q3), OR Level 0 for
5	Impaired transfer OR complete incontinenc	Nursing home	Level 1 for Walking (Q2), OR Level 0 for Toileting (Q3)

9. Plan for Determining Seriousness of Adverse Events:

In addition to grading the adverse event, the committee will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

8. Stopping criteria of the study in response to Adverse Events:
Any of the following will be sufficient reason for cessation of the experiment. Items i-ix were added with the guidance of FDA:

i. One Serious Adverse Event (SAE) that is possibly or probably study-related;
ii. One Grade 3 bradycardia or two Grade 2 bradycardias that are considered to be possibly or probably related to endotoxin administration;
iii. One episode of sinus pause;

iv. One Grade 2 or 3 tachyarrhythmia (e.g., atrial, supraventricular, etc.);
v. One Grade 3 or two Grade 2 systolic hypotension episodes that persist and require interventions, other than passive leg raising:
vi. Death of an enrolled subject unless unequivocally not attributable to study drug (e.g., traumatic injury);
vii. Occurrence of a life-threatening allergic/hypersensitivity reaction (anaphylaxis) requiring hemodynamic support with vasoactive medications or mechanical ventilation, the signs/symptoms of which include any of the following: bronchospasm, dyspnea, wheezing, stridor, hypoxemia urticaria, angioedema, hives, and facial or oropharyngeal edema;
viii. Grade 4 toxicity in a major organ system (liver, kidney, lungs, heart, etc.) in one subject; OR
ix. Any Grade 3 toxicity in the same major organ system (liver, kidney, lungs, heart, etc.) in two subjects. Grade 3 abnormalities of multiple laboratory parameters that indicate malfunction of a single organ system (e.g., aspartate aminotransferase, alanine aminotransferase, and bilirubin for liver function; blood urea nitrogen and creatinine for renal function) in a subject will be regarded as a single toxicity for purposes of this stopping criterion.
x. One grade 3 cognitive adverse effect which persists beyond 1 week or two grade 2 cognitive side effects which persist after a week.

10. Plan for expedited reporting of UPIRSOs and Serious Adverse Events to the IRB and the FDA

The principal investigator will report the following types of events to the IRB and FDA:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in

summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

12. Plan for expedited reporting adverse events to FDA, co-investigators on the study, DSMBs, study sponsor (NIA).

Written reporting to FDA will be completed in a time period equal or fewer than five calendar days for all UPIRSO's and serious adverse effects.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified:

- ✓ All Co-Investigators listed on the protocol.
- ✓ Food and Drug Administration (Physician-Sponsored IND #_____)
- ✓ NIA
- ✓ Our safety committee

The principal investigator (*Adam Mecca)* will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

Statistical Considerations: Describe the statistical analyses that support the study design.

Primary Outcome:
The 16 enrolled subjects will have 11C-PBR28 using the bolus/infusion method. Measurements will be made at baseline and 180 minutes after the injection of LPS. We will measure Total Volume of Distribution (VT) for 12 brain regions defined by MRI scan, as described (111). All primary outcomes will use the parietal ROI for calculations of both baseline activation and microglial activation reserve. For our primary outcomes we will choose the microglia activation reserve index defined as MARI = [(VT(LPS)-VT(baseline))/ VT(baseline))] X %100. In the NIMH study the greatest difference in the regional PBR28 binding between AD and cognitively normal subjects was found in the parietal lobe (7). We also found the greatest microglia activation reserve index in the parietal lobe when we applied our paradigm to young healthy controls (109). Other ROIs will be used in secondary analyses.

Statistical Considerations and Sample Size: Several comparisons of 11C-PBR28 VT brain level and microglia activation reserve index in the parietal lobe will be made using Student T-test to test for a difference between group means: young healthy group vs. elderly cognitively normal, as well as AD vs. elderly cognitively normal. Although this is intended as a pilot study we have performed some basic power analyses. For the comparison of baseline activation in AD vs. aged matched cognitively normal subjects based on standard deviations derived from Kreisel (7) and our own previous studies (107,109,111), using an alpha of 0.05 and power of 0.80, our study is powered to detect an effect size of > 1.6 which is not far off from the findings in the NIMH study. For the comparison of percentage post-LPS change from baseline in AD and NC groups we are powered to detect an effect size of more than 2.

Expected Result
1- Baseline binding: We expect increases in baseline binding (i.e. volume of distribution) of PBR28 in the parietal

lobe. This is both where most of the amyloid is deposited in early disease and also the location of the strongest positive findings in the NIMH study.
2- Microglia activation reserve index: We also expect to have significant up-regulation of the microglia activation reserve index. The calculations will be done using the parietal lobe ROI, this is the region that shows the most inflammatory response in our previous study of normal patient with LPS and also where most marked change in the PBR28 binding was found in the NIMH study. However we expect increases in the inflammatory response in the brain diffusely especially affecting the parts which were shown to have increased inflammation at baseline such as precuneus and mesial temporal areas.

Interpretation
We know of the presence of increased inflammation in AD and not MCI or cognitively normal individuals. This jump in neuroinflammation from MCI to AD may represent increased sensitivity of microglia to stimulus, most specifically constituents of the amyloid plaque. Microglia are activated when they come into contact with molecular patterns which indicate the presence of cell damage or pathogens. If MARI is increased in patients with AD, one may reasonably surmise that the response of the microglia to amyloid may also be stronger compared to subjects with normal cognition or MCI. This transition to a higher microglial reactivity may turn out to be an important step in the progression of AD.

Pitfalls and Limitations.
1- *Small subject numbers*: The considerable resources required for applying this paradigm to each patient means that the number of patients that can be enrolled in this study will necessarily be small. However, this grant will serve to collate preliminary data for a larger project which may include larger numbers as well as more longitudinal data, as well as inclusion of patients with mild cognitive impairment.
2- *Uptake may represent differing inflammatory phenotype:* PBR28 imaging does not differentiate between the different phenotypes of microglial or astrocytic activation (113,114). As such we cannot know with certainty whether the inflammation we are measuring in one patient is directly comparable with another (however to think that LPS will give two different phenotypes of microglial activation seems implausible). For example, it is known that astroglia also express TSPO in the event of inflammation and reactive astrogliosis. However, having done immunohistochemistry on a baboon brain after the administration of LPS we co-localized TSPO like immunoreactivity with morphologically amoeboid (inflammatory) CD68 positive cells and not with GFAP positive astroglia or resting microglia (106).
3- *Activation by LPS is not supramaximal*: For a quantitative assay a supramaximal stimulation of the immune system with LPS may seem desirable. However, using 100,000x more potent doses of LPS in baboons than in humans (109), we had similar immune responses of 30-60%.
4- *Selection bias due to LPS side effects:* The application of LPS to elderly patient will prompt us to exclude more frail elderly from our experiment for fear of complications. This would introduce a selection bias which will be partially addressed with having similar selection processes for the normal controls also.

Secondary Analyses:

a- Effects of aging on MARI: Senescent microglia increase their propensity to up-regulate in response to the presence of DAMPs and PAMPs and takes longer to deactivate after these influences are removed (66). In the elderly there appears to be an up-regulation of pro-inflammatory cytokines and MHC Class II expression increasing the propensity of microglia to be activated (112). We aim to demonstrate the efficacy of LPS stimulated PBR28 imaging in showing age related changes in immunity.

b- Effects of amyloid on MARI: Additionally, we will have the opportunity to explore whether exposure to amyloid increases the reactivity of the microglia to stimulation. This may be achieved by correlating the microglia activation reserve index with the amyloid regional SUVRs. If there is a correlation and we suspect that there will be then this may suggest a mechanism for the dysregulation of the innate immunity in AD.

c- Effects of MARI on cognition: we will compare the result of the neuropsychological testing with MARI and baseline inflammation. If inflammation is one of the determinants of neuronal damage, then one may expect to find worse cognition in areas with higher inflammation. This will likely reflect the observe inflammation pre-LPS rather than the capacity of the microglia to react. We will also make two composite scores: an executive and a memory composite. These will be formed by averaging the Z scores of the individual test pertaining to executive or memory function. We will then correlate these with frontal and mesial temporal inflammation.

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, state N/A and delete the rest of the section.

A. DRUGS, BIOLOGICS and RADIOTRACERS

1. **Identification of Drug, Biologic or Radiotracer:** What is (are) the **name(s)** of the drug(s) biologic(s) or radiotracer(s) being used? Identify whether FDA approval has been granted and for what indication(s).

- **[^{11}C]PBR28** or [O-methyl-11C]N-acetyl-N-(2-methoxybenzyl)-2-phenoxy-5-pyridinamine. This radiotracer is not approved by the FDA. It will be used under the auspices of the RDRC.
- **Endotoxin** (lipopolysaccharide) is an experimental drug used under IND # 13598.
- ^{11}C-PIB - Use of this radiotracer is approved by the Yale University RDRC

All protocols which utilize a drug, biologic or radiotracer **not** approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?
   - Endotoxin: BB IND 13598

b. Who holds the IND?
   - Dr Kelly Cosgrove

c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: _____

Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____X____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step) Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1

The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No

ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and

the intention of the investigation is NOT to support a significant change in the advertising for the product. ☐ Yes ☐ No

iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

Dosimetry studies of [11C]PBR28 in humans

Studies to date suggest that [11C]PBR28 binds to TSPO specifically and with high affinity and has the potential to serve as a ligand for in vivo imaging of microglial activation. PBR28 has been used in humans and have been shown to be safe and well tolerated after administration to healthy subjects (125,126). According to Brown et al. (127) "Intravenous injection of 11C-PBR28 (carrier PBR28, 0.019 ± 0.006 μg/kg, n = 5) produced no clinically observable effects. Blood pressure, pulse, and respiratory rate during the 2 h after injection as well as blood and urine tests on the next day had clinically insignificant changes. After injection of 11C-PBR28, brain, lungs, liver, heart, kidneys, spleen,

and urinary bladder were visually identified as source organs with moderate-to-high activity in most subjects. Lungs had the highest uptake, with a peak value of 35% injected activity (n = 6) during the first frame acquisition (0–2 min). Peak values of the percentage injected activity in liver, kidneys, brain, spleen, heart, and gallbladder were 15%, 14%, 5%, 4.5%, 3.7%, and 1%, respectively, all occurring within 10 min. The residence times of organs were calculated from 2D planar images. The effective dose was 6.6 μSv/MBq, and the 3 organs with highest radiation-absorbed doses were kidneys (53 μSv/MBq), spleen (26 μSv/MBq), and lungs (22 μSv/MBq).

Based on these numbers, the critical organ is the kidney (52.6 mSv/MBq, i.e., 194.7 mrem/mCi), and we estimate that the maximum allowable injection dose for [11C]PBR28 is 25.6 mCi per single injection. The proposed radioactivity dose is 20 mCi per single administration for this study, which is below the permissible single study radiation dosage of 25.6 mCi based on dosimetry calculations.

PET Tracer ^{11}C-PIB. Human experience to date

To date, over 250 human PET measurements have been conducted with ^{11}C-PIB in CN, AD, and MCI groups. There have been no adverse events associated with the administration of the radiopharmaceutical.**Endotoxin**

In 1976, endotoxin derived from E. coli (group O 113:H10:K negative) was processed to serve as a national standard for experimental studies of inflammation in humans. When this lot was depleted in 1998, the NIH Clinical Center in conjunction with FDA processed, under good-manufacturing-practice guidelines, endotoxin derived from the original bulk material extracted from E. coli O:113. This Clinical Center Reference Endotoxin (CCRE) has been used in multiple human research studies since then. CCRE is an investigational drug, and the PI has been allowed by FDA to use it in humans under an IND (BB IND#13598). The finished injectable dosage forms of CCRE are manufactured by the Bureau of Biologics and vialed by MARP/NCI-FCRDC, Frederick, MD. Each 5 cc vial contains 10,000 unites of lyophilized endotoxin (approximately 1 microgram), 10 mg of lactose and 1 mg of polyethylene glycol. Vials should be stored refrigerated and reconstituted in 5 cc of Sterile Water for Injection at the time of use. CCRE is administered intravenously or by lung instillation through bronchoscopy. The most commonly used dose is 2-4 ng/kg body weight by IV bolus. Preparation of the standardized CCRE will be performed by our clinical research pharmacist.

3. **Source:** a) Identify the source of the drug or biologic to be used.

   b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No
   If yes, by whom?

Radiotracer will be synthesized and radiolabeled by the Yale University PET Center under the supervision of Dr. Henry Huang.

Endotoxin will be provided by the NIH Clinical Center.

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Check applicable Investigational Drug Service utilized:

- ☒ **YNHH IDS**
- ☐ **YNHH IDS Pharmacy**
- ☒ **PET Center**
- ☐ **Other:**
- ☐ **Yale Cancer Center**
- ☐ **West Haven VA**
- ☐ **None**

Note: If the YNHH IDS (or comparable service at YNHH IDS or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

Due to the short half-life, PET drugs are prepared and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored at room temperature and are stable for at least 60 min after preparation.

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a 0.22 micron membrane filter during the last step in the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility after administration for further confirmation.

The level of endotoxin in each batch of the final PET drug product is determined quantitatively prior to release for administration using the FDA approved Charles River Laboratory's Portable Testing System (Endosafe®-PTS).

[11C]PBR28

[11C]PBR28 will be prepared at the Yale PET Center in accordance with procedures and quality specifications described in local Drug Master File (DMF) approved by the Yale University Radioactive Drug Research Committee (YU RDRC) and the Yale University Radiation Safety Committee (YURSC). Briefly, [11C]PBR28 is radiolabeled by O-methylation of the phenolic group of the O-desmethyl-precursor with [11C]methyl iodide or [11C]methyl triflate. The PET drug is purified first by semi-preparative HPLC, and then followed by solid-phase extraction to remove the HPLC buffer mixture. Finally the PET drug is formulated in <10% ethanolic saline solution, and the resulting PET drug product is finally passed through a 0.22 micron membrane filter for terminal sterilization.

Endotoxin

Preparation and storage of CCRE will be done by the YNHH IDS pharmacy, according to the recommendations by the NIH Clinical Center. CCRE is inherently pyrogenic and will therefore not be tested for pyrogenicity.

5. **Use of Placebo: ☒ Not applicable to this research project**

If use of a placebo is planned, provide a justification which addresses the following:

1. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
b. State the maximum total length of time a participant may receive placebo while on the study.
c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.
d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**

Will this research project involve the use of controlled substances in human subjects?
☐ Yes ☐ No *See HIC Application Instructions to view controlled substance listings*.

If yes, is the use of the controlled substance considered:
☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.

☐ Non-Therapeutic: *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.*

7. **Continuation of Drug Therapy After Study Closure** ☒ **Not applicable to this project**
Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?
☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES

1. Are there any investigational devices used or investigational procedures performed at Yale-New Haven Hospital (YNHH) (e.g., in the YNHH Operating Room or YNHH Heart and Vascular Center)? ☐Yes ☐No *If Yes, please be aware of the following requirements*:

a. A YNHH New Product/Trial Request Form must be completed via EPIC: **Pull down the Tools tab in the EPIC Banner, Click on Lawson, Click on "Add new" under the New Technology Request Summary and fill out the forms requested including the "Initial Request Form," "Clinical Evidence Summary, " and attach any other pertinent documents. Then select "save and submit" to submit your request**; and

   a. Your request must be reviewed and approved **in writing** by the appropriate YNHH committee before patients/subjects may be scheduled to receive the investigational device or investigational procedure.

3. What is the name of the device to be studied in this protocol?

Has this device been FDA approved? ☐ Yes ☐ No
If yes, state for what indication.

4. **Background Information:** Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.

5. **Source:**
   a) Identify the source of the device to be used.

   b) Is the device provided free of charge to subjects? ☐ Yes ☐ No

6. What is the PI's assessment of risk level (significant or non-significant) associated with the use of the device?

☐ **Significant Risk (SR) Device Study:** A study of a device that presents a potential for serious risk to the health, safety, or welfare of a participant and 1) is intended as an implant; 2) is used in supporting or sustaining human life; or otherwise prevents impairment of human health; 3) is of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise prevents impairment of human health; or 4) otherwise presents a potential for serious risk to the health, safety, or welfare of a participant.

Significant Risk Devices require an Investigational Device Exemption (IDE) issued by the FDA.

What is the **IDE number** assigned by the FDA?

Did the FDA approve this IDE as **Category A** (experimental/investigational) or as **Category B** (non-experimental/investigational)?

Who holds the IDE?

☐ **Non-Significant Risk (NSR) Device Study:** A study of a device that does not meet the definition for a significant risk device and does not present a potential for serious risk to the health, safety, or welfare of participants. Note that if the HIC concurs with this determination, an IDE is not required.

7. Abbreviated IDE or Exempt IDE: There are abbreviated requirements for an IDE and there also are exemptions to the requirement for an IDE. *See the criteria in the HIC Application Instructions,* Section VI.B.4 at http://www.yale.edu/hrpp/resources/docs/100FR1aHICProtocol_Application_Instructions5-25-11.pdf *to determine if these pertain to this study.*

☐ **Abbreviated IDE or Exempt IDE** – *If criteria set forth in the HIC Application Instructions are met, copy and paste the completed relevant section from the Instructions into this application.*

8. Investigational device accountability:

a. State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

Maintains appropriate records, including receipt of shipment, inventory at the site, dispensation or use by each participant, and final disposition and/or the return of the investigational device (or other disposal if applicable):

Documents pertinent information assigned to the investigational device (e.g., date, quantity, batch or serial number, expiration date if applicable, and unique code number):

Stores the investigational device according to the manufacturer's recommendations with respect to temperature, humidity, lighting, and other environmental considerations:

Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements:

Distributes the investigational device to subjects enrolled in the IRB-approved protocol:

SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

a. targeted for enrollment at Yale for this protocol up to 10 cognitively normal subjects and 10 MCI subjects. This is larger than the 16 subjects discussed above because we may need to enroll up to 3 additional subjects in each cohort to identify 7 cognitively normal AND amyloid negative and two additional to identify 7 MCI and amyloid positive subjects (total 9) due to possible failures in completing the study.

b. If this is a multi-site study, give the total number of subjects targeted across all sites___

2. Indicate recruitment methods below. Attach copies of any recruitment materials that will be used.

☐ Flyers	☐ Internet/Web Postings	☐ Radio
☐ Posters	☐ Mass E-mail Solicitation	☐ Telephone
☐ Letter	☒ Departmental/Center Website	☐ Television
☐ Medical Record Review	☒ Departmental/Center Research Boards	☐ Newspaper
☐ Departmental/Center Newsletters	☐ Web-Based Clinical Trial Registries	
☒ YCCI Recruitment database	☐ Clinicaltrials.gov Registry (do not send materials to HIC)	
☐ Other (describe):		

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.

The subjects will be recruited through Alzheimer's Disease Research Unit which has an extensive referral base in the community. These patients come under two categories:

- Patients that are originally clinical patients of mine referred to the ADRU.
- Patients who contact the ADRU to see what trials are available.

Additionally, Volunteer Patients will be recruited through YCCI's recruitment database.

b. Describe how potential subjects are contacted.

The referred subjects are contacted by research assistants on the phone. The volunteers will be reaching us in responding to YCCI's email.

c. Who is recruiting potential subjects? See IRES-IRB

4. Screening Procedures

a. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

description of symptoms, diagnosis and treatment, MR/CT date, result, other medical problems, meds, psychiatric problems, drug alcohol and substance use, family history.

HIPAA identifiers:

☒ Names

☐ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

☒ Telephone numbers

☐ Fax numbers

☐ E-mail addresses

☐ Social Security numbers

☐ Medical record numbers

☐ Health plan beneficiary numbers

☐ Account numbers

☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older

☐ Certificate/license numbers

☐ Vehicle identifiers and serial numbers, including license plate numbers

☐ Device identifiers and serial numbers

☐ Web Universal Resource Locators (URLs)

☐ Internet Protocol (IP) address numbers

☐ Biometric identifiers, including finger and voice prints

☐ Full face photographic images and any comparable images

☐ Any other unique identifying numbers, characteristics, or codes

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects

☒ Yes, some of the subjects

☐ No

If yes, describe the nature of this relationship.

The PI is one of the sources of referral to ADRU.

6. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:

☐ For entire study

☒ For recruitment purposes only
☐ For inclusion of non-English speaking subject if short form is being used

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;

N/A – requesting waiver of **signed** authorization only. We will obtain verbal authorization for use/disclosure of data.

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data; for telephone screening

We will conduct phone screens for recruitment purposes. Subjects will contact the ADRU about their interest in the study and we will complete a phone screen. At the beginning of the phone call, we will ask permission to ask questions about their health to evaluate their eligibility for the study. Because these screens will not be conducted in person, it is impracticable to obtain the subject's signed authorization. At the end of a phone screen, individuals are read a paragraph for authorization for storing screening information (see HIC protocol 0307025374). If authorization is denied, all screening information is destroyed.

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   ☐ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

8. **Consent Personnel:** List the names of all members of the research team who will be obtaining consent/assent: See IRES-IRB

9. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

The patient will be met at the ADRU, 1 Church St., where they will be screened for eligibility. The PI intends to consent most patient himself unless clinical schedule conflicts interfere. Otherwise this task may be performed by one of the above named individuals. Since the cutoff is MOCA of 17, the patient will be able to consent for themselves in all cases.

10. E**valuation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

The **Consent Personnel** obtaining informed consent will make a judgment about whether the subject is providing informed consent. This judgment will be based on an overall impression of the subject's ability to comprehend relevant information and make reasoned decisions. Participants who are not capable of providing informed consent will be excluded from the study.

11. Documentation of Consent/Assent: Specify the documents that will e ubsed during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

PBR-Consent.docx

Adult Consent/Authorization form will be used. The study will not include assenting subjects. Copies of the signed consent forms will be given to subjects. We will document that the consent process occurred on the screening clinic note in the subject's chart.

12. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

As we do not have the capacity to perform neuropsychometry in any language except English we will not enroll non-English speakers.

12(a) As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment?

YES ☐ NO ☒

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are found on our website at: http://www.yale.edu/hrpp/forms-templates/biomedical.html. If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via amendment prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

13. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☒ **Not Requesting a consent waiver**
☒ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)

☒ **Requesting a waiver of signed consent for Recruitment/Screening only**

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☒ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☒ No

☐ **Requesting a waiver of signed consent for the Entire Study (**Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **Full waiver** of consent: (No consent from subjects will be obtained for the activity.)

☐ **Requesting a waiver of consent for Recruitment/Screening only**

a. Does the research activity pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

☐ **Requesting a full waiver of consent for the <u>Entire Study</u> (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.***
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

Required private identifiable information about individuals, such as their medical history, current medications, psychiatric problems, and family history, will be collected by research staff and be used for research purposes and charting after consent is obtained.

b. How will the research data be collected, recorded and stored?

The data are collected and recorded by trained research personnel. The data will be recorded on Excel spreadsheets that will be saved onto a server or will be in the form of questionnaires that are filled out by the subject or the researcher. These paper research materials containing confidential information are stored in locked filing cabinets. Additional brain data is collected during the brain imaging scans by trained technologists and is stored on password-protected and encrypted computers with identifying information carefully in compliance with HIPAA regulations.

c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☒ Secured Server ☐ Laptop Computer ☐ Desktop Computer ☐ Other

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
Do all portable devices contain encryption software? ☐ Yes ☐ No

If no, see http://hipaa.yale.edu/guidance/policy.html

All staff members that come into contact with the data are fully trained to the current HIPAA regulations and are informed as to the proper use of all data.

Identifiable paper information is kept in locked file drawers and password protected computer files. Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PE Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only. Direct identifiers belonging to subjects who withdraw from the study, will be stripped from the key.

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

The data will be stored in locked filing cabinets and on the password-protected secure database on the internal Yale University PET Center Network for at least 7 years, accessed only by authorized personnel.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data)

The investigator and research staff (e.g., PET center nuclear technologists, recruiters) will have access to the PHI only on as needed to know basis. The FDA may also have access to the PHI.

g. If appropriate, has a Certificate of Confidentiality been obtained?

NA

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview - incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

Yes, a positive test result for HIV, hepatitis B or C will be reported to the Connecticut Department of Public Health per Connecticut law.

SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

This study offers no direct individual benefit to participating subjects with AD. All participants in this study may derive subjective benefit from volunteering to take part in a study for the advancement of scientific knowledge. Subjects with AD could benefit from future treatments developed based on the results of this study. The potential future benefits for AD subjects as a population are large and clearly outweigh the individual risks of the subjects who choose to participate in this study.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

   The alternative to participation in this research protocol is to not participate. Subjects will be informed that they are free to choose not to participate and, if they do agree to become a subject, they will be free to withdraw from the study at any time during its course. They will also be informed that if they choose not to participate or if they withdraw, it will not adversely affect their relationship with their doctors or the hospital (see attached Consent Form).

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

   The following payments will be offered to the patients:

   Screening 25
   Baseline assessments 50
   One week follow up 25
   50 for MRI
   50 for A-line
   350 for each [^{11}C] PRB 28 scan=700 total
   250 for each [^{11}C] PiB Scan
   Lipopolysaccharide 150
   6- month follow up 150
   Cogstate testing (before and after LPS) 50
   CSF Draw 350
   Total: $1850

3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

There will be no costs to subjects related to participation in this research intervention.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?
   e. How will the medical treatment be accessed by subjects?

Medical treatment will be offered to the subjects for any physical injuries that they receive as a result of participating in this research. However, the subject or his/her insurance company is responsible for the cost. Federal regulations require that subjects be told that if they are physically injured, no additional financial compensation is available.

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