Imaging Inflammation in Elders With Different Clinical and Biomarker Profiles of Alzheimer's Disease

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

1.1.1. Background on Alzheimer's disease

While amyloid plaques are a pathological hallmark of Alzheimer's disease (AD), whether β-amyloid is the causal agent of neurodegeneration in AD remains unclear. Evidence supporting the hypothesis that amyloid is a primary contributor to AD pathogenesis (the amyloid-first hypothesis) include the finding that genetic mutations responsible for inherited forms of AD cause increased amyloid production. Studies using positron emission tomography (PET) to measure amyloid plaque burden in vivo have demonstrated amyloid positivity before obvious atrophy or cognitive decline in healthy older controls. While tau positive neurofibrillary tangles represent the second hallmark of AD, tau mutations are seen in patients with variants of frontotemporal dementia, not AD. The propagation of tau pathology is thought to follow amyloidosis, suggesting amyloid in some way induces tau mediated degeneration. However, the link between amyloid and tau not clear as there are spatial and temporal distinctions. For example, bulk of amyloid burden in medial parietal cortex and frontal cortex early on, while tau burden begins in medial temporal cortex.

While β-amyloid has been shown to have direct neurotoxic effects in vitro, these effects are seen at concentrations of β-amyloid much higher than found in human brain[1].Therefore, amyloid may not confer significant direct toxicity. Rather, amyloid may induce secondary injury via activation of immune response. At physiologic concentrations, β-amyloid activates microglia[2], triggering a pro-inflammatory cascade as these resident neuroimmune cells attempt to clear β-amyloid from the brain[3]. This inflammatory response may be more toxic than β-amyloid itself, as the resulting release of cytokines, activation of complement, and phagocytosis cause loss of neurons[4]. This inflammatory response may be the link between amyloid and tau, as inflammation can result in hyper-phosphorylation of tau, leading to the destabilization of tau filaments and aggregation of the neurofibrillary tangles that make up the second pathological hallmark of AD[5]. In turn, extracellular tau activates microglia[6], which could create a positive feedback loop of inflammation and tau pathology that propagates, independent of amyloid burden. The inflammation/tau relationship could also occur independently of the presence of amyloid. Indeed, autopsy findings of pre-tangle pathology occurring in midlife prior to amyloidosis[7] suggest that tauopathy may begin prior and independent to significant amyloid plaque deposition. Since amyloid burden correlates poorly with cognition[8], inflammation may be a more important mediator of neurodegeneration in AD.

Measuring inflammation in vivo may also have an important role in determining prognosis and evaluating progression of disease in AD patients. Up to 30% of cognitively normal elders are amyloid positive on PET. Determining predictors of future cognitive decline among amyloid-positive elders would allow us to distinguish incidental amyloid-positivity from that due to incipient Alzheimer's disease in an elderly population. Furthermore, identifying the relative impact of inflammation on cognition may justify a more strategic approach for the development of anti-inflammatory therapeutics for AD.

1.1.2. Background on PET imaging of inflammation

Inflammation can be quantified in vivo using PET imaging with radioligands that bind to the 18 kDa translocator protein (TSPO, formerly called the peripheral benzodiazepine receptor). Brain inflammation activates microglia and causes them to over-express TSPO[9]. Measurement of microglia activation, defined by the number of available TSPO binding sites, is used as a surrogate bio-marker of active brain inflammation[10]. PET imaging can quantify TSPO density *in vivo* using radioligands that cross the blood-brain barrier and bind to TSPO sites. ^{11}C-*(R)*-PK 11195, the prototypical TSPO radioligand, has been used to measure neuroinflammatory changes in several diseases, including Alzheimer's disease[10] and ^{11}C-*(R)*-PK 11195 binding has been shown to correlate with Mini-Mental State Examination score in patients with Alzheimer's disease[11]. However, there have been conflicting reports about whether ^{11}C-*(R)*-PK 11195 binding is increased in Alzheimer's disease or not[10, 12, 13]. Whether ^{11}C-*(R)*-PK 11195 binding is increased in MCI appears even less conclusive. For instance, [14] found a small increase in ^{11}C-*(R)*-PK 11195 in PIB-positive patients with MCI relative to controls, but other studies found no increase in patients with MCI, even those who later progressed to dementia[12, 13].

Limitations of ^{11}C-*(R)*-PK 11195 have led to development of second generation TSPO radioligands. One such radioligand is PBR28, which has high in vitro affinity (~ 1 nM) for TSPO. In nonhuman primates, [^{11}C]PBR28 has high brain uptake and fast washout[15], which allows reliable PET quantification. In addition, more than 80% of brain uptake is displaceable by nonradioactive TSPO ligands in nonhuman primates[15]. This means that over 80% of uptake is specifically bound to TSPO. [^{11}C]PBR28 has high enough signal-to-noise ratio to localize inflammation to relatively small areas in humans with lacunar stroke[16]. Animal toxicology studies and early clinical PET studies have shown safety and tolerability of [^{11}C]PBR28. In addition, the radiation exposure after injection of activity needed for reliable PET measurement (10-20 mCi) is well within limits for human research studies.

The main limitation of ^{11}C-PBR28, shared by all tested second generation TSPO radioligands, is differential affinity for the target protein[17]. This differential affinity is caused by the rs6971 polymorphism on the *TSPO* gene that causes a non-conservative amino acid substitution, resulting in three patterns of TSPO binding. Subjects without the polymorphism have high affinity binding for PBR28 and are referred to as high affinity binders (HABs). Homozygotes have low affinity binding and are referred to as low affinity binders (LABs). Heterozygotes express both high and low affinity TSPO and are referred to as mixed affinity binders (MABs). LABs are easily identified by PET due to negligible ^{11}C-PBR28 binding *in vivo*; however, PET cannot easily resolve the difference between HABs and MABs, and MABs have, on average, 22% less total ^{11}C-PBR28 binding than HABs[18]. Previous work from our laboratory demonstrated that correcting *in vitro* binding data for rs6971 genotype improves the ability of ^{3}H-PBR28 to detect differences in TSPO density in schizophrenia and control brain tissue[18]. This strategy of *TSPO* genotype correction can be applied to PET imaging to remove confounding effects of differences in TSPO affinity.

We recently demonstrated that ^{11}C-PBR28 binding is greater in AD patients than in MCI patients or older controls[19]. Areas with greatest binding included parietal and temporal cortices, regions with high amyloid burden. No difference in binding was seen in cerebellum, a region typically spared of pathology in AD. However, ^{11}C-PBR28 binding did not correlate with PIB binding, suggesting a disassociation between inflammation and amyloid burden, particularly

in more severe patients. Instead, ^{11}C-PBR28 binding correlated with brain atrophy and lower cognitive scores, suggesting inflammation is associated with neurodegeneration. This study used arterial sampling to calculate ^{11}C-PBR28 binding using kinetic modeling and required a relatively large sample size (19 AD patients, 10 MCI patients, and 13 controls) due to relatively high variance of the PET data to see the reported effect. While kinetic modeling is considered the gold standard method of PET quantification, a less invasive method with less variance would be preferred to improve subject tolerability and increase statistical power.

We developed a simplified ratio method using the cerebellum as a pseudo-reference region[20]. This method calculates standardized uptake value ratios (SUVR) similar to methods used for other radioligands such as PIB. This method removes the need for arterial sampling and increases sensitivity for detecting differences in ^{11}C-PBR28 binding among AD and MCI patients and healthy controls by reducing variance. For example, using the SUVR method we were able to detect significantly increased ^{11}C-PBR28 binding in the hippocampus in MCI patients, while this difference was only at the trend level using the gold standard method.

We already have preliminary data suggesting that ^{11}C-PBR28 binding increases with AD progression. Eleven patients (7 AD, 4 MCI) and 7 older controls had ^{11}C-PBR28 binding at baseline and then returned for repeat imaging after mean follow up of 2 years. The patients showed greater binding in the entorhinal cortex at follow up, while there was no difference between baseline and follow up scan in the controls. Since the entorhinal cortex is a region with high tau burden, these data suggest that inflammation may be increasing in parallel with neurodegeneration in the progression of AD.

1.1.3. Background of PET imaging of amyloid.

While post-mortem examination is required to definitively demonstrate the presence of amyloid plaques in suspected cases of AD, PET imaging allows measurement of amyloid burden in vivo. ^{18}F-florbetaben (FBB, formerly known as BAY94-9172) binds to fibrillar amyloid plaques in vivo[21]. FDA approved for commercial use to exclude AD. Dosimetry studies have shown that radiation exposure from this radioligand is similar to that from other 18F radioligand commonly used in human PET studies and clinical practice.

Amyloid PET imaging allows in vivo detection of amyloid plaques to improve risk assessment and diagnosis of AD. Therefore, clinical evaluation using current neuroimaging techniques can be used to identify the following four groups:

1) Amyloid-positive with cognitive impairment (AD). Amyloid-positive patients who already have cognitive impairment are most likely to be clinically expressing AD pathology[22, 23], either in the prodromal stage (MCI) or dementia stage (AD dementia). Patients with MCI who are amyloid-positive on PET convert to AD dementia at reported rates as high as 80% within 3 years[24, 25].

2) Amyloid-positive without impairment (preclinical AD). Cognitively normal subjects who are amyloid-positive show greater rates of memory decline than those who are amyloid-negative[26]. These subjects represent a preclinical stage of AD[27].

3) Amyloid-negative without impairment (normal aging). Cognitively normal subjects who are amyloid-negative on PET, and lack signs of neurodegeneration from other biomarkers, have no discernible evidence of AD pathology[28]. While such subjects may develop pathological changes in the future, at the time of investigation they represent normal aging.

4) Amyloid-negative with cognitive impairment (impairment due to suspected non-AD pathophysiology). Notably, 29% of elders with MCI in a recent population-based study had

evidence of neurodegeneration without amyloidosis[29], and longitudinal PET studies have shown that amyloid-negative MCI patients progress to dementia at rates ranging from 0 - 23% per year[8, 24, 30, 31]. Evidence of neurodegeneration (i.e., hippocampal atrophy on MRI or hypometabolism on FDG PET) without amyloidosis has been termed "suspected non-AD pathophysiology"[32], and subjects with this biomarker profile are thought to mostly likely represent patients with non-Alzheimer's diagnoses[28].

Taken together, these four groups may be considered different categories of disease along the aging spectrum.

1.2 Investigational Agent

^{11}C-PBR28 is a PET radioligand that binds to the 18 kDa translocator protein (TSPO), a marker of inflammation. ^{11}C-PBR28 has previously been administered in humans.

^{18}F-Florbetaben (Neuraceq) has FDA approval for human use in evaluation of Alzheimer's disease. We will use 18F-Florbetaben in this study for research purposes.

Both radioligands will be administered in tracer doses. ^{11}C-PBR28 will be administered at activity of up to 20 mCi per injection. ^{18}F-Florbetaben will be administered at activity up to 8.1 mCi per injection.

1.5 Dose Rationale and Risk/Benefits

Based on prior human experience and dosimetry data, we expect the proposed injected activity of ^{11}C-PBR28 (up to 20 mCi) to be safe without expected toxicity. ^{18}F-florbetaben is an FDA-approved radiopharmaceutical used here for research purposes at injected activity recommended by the manufacturer (up to 8.1 mCi).

2 Study Objectives

2.1. The primary objective is to determine the extent and spatial distribution of inflammation at different stages along the cognitive spectrum of aging.

2.2. Secondary objectives are to determine how ^{11}C-PBR28 binding in brain relates to cognitive performance, amyloid binding, atrophy on MRI, and CSF concentrations of markers of inflammation and neurodegeneration.

3 Study Design

3.1 General Design

3.1.1. Subject recruitment and screening procedures

Men and women age 50 and older will be recruited from the CUMC Washington Heights-Inwood Community Aging Project (known locally as WHICAP, R01 AG037212, PI Richard Mayeux), the CUMC Alzheimer's Disease Research Center (ADRC), and Dr. Davangere Devanand's Questionable Dementia 2 (QD2) cohort, and CUMC clinics. Subjects may also be co-enrolled in Columbia University Medical Center protocols AAAO9758 (PI Adam Brickman), AAAI2752 (PI Yaakov Stern), AAAQ8249 (PI Yaakov Stern), AAAQ8096 (PI Yaakov Stern), AAAR4352 (PI James Noble) and AAAR6570 (PI Edward Huey). Informed consent will be obtained prior to study enrollment. This protocol will encourage data sharing to reduce repeat procedures and thus participant burden. De-identified neuroimaging data may be shared with IRB-approved collaborators.

PET Imaging has ended for this study. Please note that anywhere in the protocol that makes mention of PET imaging, and its corresponding drugs/biologics (18F-florbetaben and 11C-PBR28) is only in reference to previous study design. PET imaging is no longer a part of the protocol but is kept in for study design comprehension.

For subjects with cognitive impairment, prior to obtaining informed consent, a licensed physician who is not listed as personnel on this protocol will determine whether the subjects has capacity to provide their own consent. If the subject lacks consent, a legally authorized representative will be required to provide consent for the subject.

Up to 150 subjects will undergo screening including history, physical examination, routine laboratory studies, neuropsychological testing, brain MRI, and amyloid PET imaging. If any of

the screening procedures have been performed with in one year of the ^{11}C-PBR 28 scan visit, then those procedures will not need to be repeated under this protocol. Rather the previously obtained results will be used to reduce participant burden. Subjects will be categorized as either amyloid-positive or amyloid-negative on PET. Subjects will be identified as either cognitively normal or cognitively impaired in consensus conference. Subjects must have Clinical Dementia Rating scale score of 0.5 or 1 and meet clinical criteria for either amnestic MCI (single or multiple-domain)[22] or Alzheimer's disease[23] to be included in the cognitively impaired category. Therefore, subjects who fulfill screening criteria will be stratified into four categories: 1) amyloid-positive with cognitive impairment, 2) amyloid-positive without impairment, 3) amyloid-negative with cognitive impairment, and 4) amyloid-negative without cognitive impairment. Hippocampal atrophy on MRI will be inclusionary criteria for group 3 and exclusionary for group 4, as neurodegeneration is a prerequisite for suspected non-AD pathophysiology and inconsistent with normal aging. Screening will include genetic analysis to exclude subjects homozygous for the rs6971 (Ala147Thr) SNP on the TSPO gene. Homozygous subjects have negligible binding on ^{11}C-PBR28 PET imaging and must be excluded; however, heterozygotes may be included[18]. We request to perform screening procedures, including 18F-florbeteben PET, on up to 150 subjects in anticipation of33% screen failure/dropout rate.

Target sample size for completers is 25 amyloid-positive elders with impairment, 25 amyloid-positive elders without impairment, 25 amyloid-negative elders without impairment, and 25 amyloid-negative elders with impairment (100 subjects total). Results from a population-based study showed that 43% of MCI patients are amyloid-negative on PET[29], and in a phase 3 bapineuzimab trial in mild-to-moderate AD patients, 36% of ApoE4 non-carriers were negative on baseline amyloid imaging[44]. Therefore, significant oversampling is not anticipated to be necessary for the impaired amyloid-negative group. Our enrollment goal is 25 subjects per group to account for a 30% general screen failure/drop-out rate and a 9% prevalence of low affinity binders. Using subjects from WHICAP with pre-determined TSPO affinity status from GWAS will reduce the screen failure rate.

3.1.2 Neuropsychological data analysis

Selected neuropsychological tests scores will be combined into four composite scores (memory, language, executive/speed, and visuospatial)[45]. Memory testing will include Selective Reminding Test and Benton Visual Retention Test (BVRT), Recognition Memory Multiple Choice version. Z-scores for each cognitive measure will be calculated and averaged to create a composite z-score for each domain. These factor domain scores will be subsequently averaged to produce a composite cognitive z-score to indicate mean cognition. Higher z-score indicates better cognitive performance.

3.1.3. Imaging procedures

One brain MRI will be performed on each subject using a 3 T Philips scanner. Sequences performed will include 3D T1 (MPRAGE, 180 slice 1 mm resolution, 256 x 256 voxel count) for volumetric analysis and clinical sequences to exclude subjects with visible evidence of cerebral amyloid angiopathy (microbleeds on GRE), more than moderate white matter disease on T2, or other intracranial pathology unrelated to AD. PET scans will take place on a Biograph mCT PET scanner (Siemens Healthcare) at the CUMC Kreitchman PET Center. Subjects will have one ^{18}F-florbetaben (Neuraceq) PET scan (up to 8.1 mCi) to determine amyloid status[46]. Subjects will have one PET scan with ^{11}C-PBR28 (injected activity 10-20 mCi). PET imaging will be

performed without arterial sampling. ^{11}C-PBR28 will be synthesized by the CUMC PET Department Radiochemistry Laboratory. ^{18}F-florbetaben will be purchased from Piramal Imaging. Cognitively normal subjects will not be informed of ^{18}F-florbetaben PET scan results, as we do not know the clinical significance of a positive amyloid scan in cognitively normal people. Subjects will not be informed of ^{11}CPBR28 PET scan results, as this scan is used only in research and have not yet been validated for clinical use. Subjects will be informed if a clinically important abnormality is detected on PET imaging (e.g., brain tumor).

Subjects will be allowed to take low dose lorazepam (0.5 - 1 mg orally one time dose) or equivalent dose of alternative benzodiazepine prior to the MRI, Florbetaben PET scan and/or PBR28 PET scan in the case that the subject is claustrophobic or otherwise unable to lay still without pre-medication. Low-dose one-time administration of anxiolytic medication is commonly done in clinical practice when patients have anxiety about undergoing the MRI and/or PET scan. Subjects will be informed of the risks associated with one-time administration of low-dose benzodiazepine, including sedation, lightheadedness, motor incoordination, difficulties with balance, and confusion. Subjects who receive benzodiazepine pre-medication will be accompanied during the appointment by a member of the study staff and the PI will be available in the case of an adverse event. Subjects who receive benzodiazepine pre-medication will be instructed not to drive, handle machinery, or drink alcohol until the next day, and will be required to bring a companion to escort them home. Standard doses of benzodiazepines, particularly lorazepam, are not expected to affect ^{11}C-PBR28 binding to TSPO[47].

3.1.4 Image processing

FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), the MRI software package comprising a suite of automated tools for segmentation, reconstruction, and derivation of regional volumes and surface-based rendering, will be used for derivation of regions-of-interest (ROIs). Eleven ROIs will be extracted from the structural T1 image: entorhinal cortex, hippocampus, inferior temporal cortex, combined superior and middle temporal cortex, superior parietal lobule, inferior parietal lobule, precuneus, occipital cortex, prefrontal cortex, striatum, and thalamus. Age-adjusted hippocampal volumes will be used to define the presence or absence of neurodegeneration[28]. A single static 18F-florbetaben image acquired 50-70 or 90-110 min post-injection will be read by a trained investigator blinded to the subject's diagnosis. A visual binary read (positive or negative) will be determined according to criteria established by Piramal imaging to stratify subjects as amyloid-positive or amyloid-negative. Semi-quantitative measurement of 1^{8}F-florbetaben binding will be used as an outcome measure for baseline amyloid burden (see below).

^{18}F-Florbetaben and ^{11}C-PBR28 PET images will be analyzed according to previously published methods for ^{11}C-PBR28[19]. In brief, Freesurfer based ROIs will be applied to coregistered PET images. Correction for partial volume effects using a region-based voxel-wise method[48] will be applied. Regional time-activity curves using 50-70 or 90-110 min of scan data for ^{18}F-florbetaben and 60-90 min for ^{11}C-PBR28 will be extracted from the PET scans, including cerebellum which will be used as a reference region. Standardized uptake value ratio (SUVR) values will be calculated by dividing SUV values for each target region by that of the cerebellum

3.1.5. Blood and cerebrospinal fluid sampling and analysis

Subjects not recruited from WHICAP will undergo venipuncture to collect blood to test TSPO genotype, determined by SNP analysis using polymerase chain reaction with a pre-existing Taqman assay[17-19] in the Biomarkers Shared Resource of CUMC. Subjects will have the option of having one lumbar puncture (LP) for CSF collection. Subjects may refuse the LP and still participate in the other study procedures. CSF analysis will be performed by Dr. Honig to determine CSF concentrations of total tau, phospho-tau, β-amyloid, and markers of inflammation such as IL-1β, TNF-α, glial fibrillary acidic protein, S100B, and YLK-40.

3.1.6. Procedures performed in WHICAP
Procedures that are performed under WHICAP include screening interview, history and physical examination, neurological examination, neuropsychological testing, genome-wide association study and diagnosis made at consensus conference. To date, over 500 WHICAP subjects have had brain MRI and 30 have had ^{18}F-florbetaben PET. GWAS data will be interrogated to screen out subjects homozygous for the rs6971 SNP.

3.2 Primary Study Endpoints

Because the drugs used in this study are radioligands given at tracer doses, there are no clinical endpoints of the study.

The primary outcome measures are:

1. Amount of ^{11}C-PBR28 binding in each of the four groups.

Secondary outcome measures are:
1. CSF concentration of inflammatory markers
2. CSF concentration of tau and phospho-tau

3.3 Secondary Study Endpoints
N/A

3.4 Primary Safety Endpoints
N/A

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria
1. Age 50 and older
2. Meet criteria for either a) amnestic mild cognitive impairment (single or mixed domain) or mild Alzheimer's disease, or b) have no cognitive impairment based on history, exam, neuropsychological testing, and consensus diagnosis. MCI and mild AD patients must have Clinical Dementia Rating scale score of 0.5 or 1. Unimpaired subjects must have Clinical Dementia Rating scale score of 0.
3. Subjects unable to provide informed consent must have a surrogate decision maker

4. Written and oral fluency in English or Spanish
5. Able to participate in all scheduled evaluations and to complete all required tests and procedures.
6. In the opinion of the investigator, the subject must be considered likely to comply with the study protocol and to have a high probability of completing the study.

4.2 Exclusion Criteria

1. Past or present history of certain brain disorders other than MCI or AD.
2. Certain significant medical conditions, which make study procedures of the current study unsafe.
3. Contraindication to MRI scanning
4. Conditions precluding entry into the scanners (e.g., morbid obesity, claustrophobia, etc.).
5. Exposure to research related radiation in the past year that, when combined with this study, would place subjects above the allowable limits.
6. Low affinity binding on TSPO genetic screen
7. Participation in the last year in a clinical trial for a disease modifying drug for AD.
8. Inability to have a catheter in subject's vein for the injection of radioligand.
9. Inability to have blood drawn from subject's veins.

6 Study Procedures

This study will involve up to 5 outpatient visits. See schedule below.

At the first screening visit, subjects will sign informed consent, undergo history and physical and neurological examination, neuropsychological testing, and have blood drawn routine safety laboratories and TSPO genotyping. Additional screening visit is required for brain MRI. Flexibility is allowed in screening procedures such that procedures may be performed in any order and may be performed on the same day if schedule allows for subject convenience. Subjects who already had screening procedures performed under WHICAP, or other CUMC studies listed, do not need to have them repeated unless performed more than 12 months prior to inclusion in this study. Up to 3 screening visits are anticipated. However, additional visits may be necessary if certain tests must be scheduled on different days due to scheduling.

One visit will be for the optional lumbar puncture. Subjects may decline the lumbar puncture and still participate in other parts of the study. LP must be completed within 12 months of the brain MRI.

Study Schedule

Study Procedures	Screen [a]	Study [b]	
	Day -60 to -1	Visit 1	Visit 2
Informed consent	X		
Medical/psychiatric history	X		
Inclusion/exclusion criteria	X	X	X
Blood draw for TSPO genotyping	X		
Neuropsychological testing	X		
MRI	X		
Lumbar puncture [c, d]			X
Laboratory determinations [e]	X		
Neurological examination	X		
Vital signs	X		
Height and weight	X	X	
Adverse events [f]	X	X	X

a. Screening procedures may be performed in any order and may be performed on the same day if schedule allows for subject convenience. Subjects who already had screening procedures performed under a different protocol do not need to have them repeated unless performed more than 12 months prior to inclusion in this study. Up to 3 screening visits are anticipated. However, additional visits may be necessary if certain tests must be scheduled on different days due to scheduling.
b. Study procedures must be completed within 12 months of MRI.
c. Lumbar puncture is optional.
d. Laboratory determinations here include complete blood count, basic metabolic panel, liver functions tests, thyroid stimulating hormone, and urinalysis.
e. ^{18}F-florbetaben and ^{11}C-PBR28 PET Scans have concluded for this study.

7 Statistical Plan

7.1 Sample Size Determination

Power analysis was performed using preliminary data from a study using ^{11}C-PBR28 PET in patients with AD and MCI and healthy controls.

For the main effects, the minimal detectable effect size is Cohen d=0.74 with the sample size N=30 per group, 80% of power and a two-sided test at the 5% significance level. Based on my preliminary data using 11C-PBR28, the effect size for the group difference between MCI and controls is d=1.3 (hippocampal SUVR mean (SD) of MCI: 0.98 (0.18); HC: 0.82 (0.06)), and it is similar (d=1.3) after adjusting for TSPO genotype. Although these preliminary results were not controlled for amyloid, with the assumption of additive effect of amyloid I would be able to

detect the group difference between amyloid-positive MCI and amyloid-positive without impairment with more than enough power. For the interaction between diagnosis and amyloid, if the mean differences between positive/negative amyloid among the subjects with and without impairment differ by d=1.06, I would be able to detect such effect size with 80% power at 5% significance level for a two-sided test.

We also plan to compare ^{11}C-PBR28 binding with the amount and distribution of binding with ^{18}F-MK-6240, a tau radioligand that will be used under a separate IND (#135,864). Because some subjects who have ^{11}C-PBR28 PET may not wish to also undergo ^{18}F-MK-6240 PET, we need to increase the number of subjects so that a total of 60 subject receive both ^{11}C-PBR28 and ^{18}F-MK-6240 PET.

7.2 Statistical Methods

Subjects will be stratified using a two factor design based on diagnosis (impaired vs. normal) and amyloid status (positive vs. negative). Target sample size is 25 subjects per group (100 subjects total). ^{11}C-PBR28 binding will be the primary outcome measures. Between-group differences in SUVR values from 11C-PBR28 PET images will be compared in each of the 11 ROIs. Analysis will be performed using a two-way MANCOVA with diagnosis (MCI vs. normal) and amyloid status (positive vs. negative) as independent variables and TSPO genotype status (high affinity vs. mixed affinity binder) as covariate. I will additionally test the interaction between diagnosis and amyloid status. This model will be fitted using PROC GLM in SAS.

Regression models will be fit for ^{11}C-PBR28 binding as dependent variable and cognitive scores, voxel count on MRI, ^{18}F-florbetaben binding, and CSF concentrations of markers of inflammation and neurodegeneration as independent variables.

7.3 Subject Population(s) for Analysis

Subjects will be elders age 50 and older with normal cognition, mild cognitive impairment, or mild AD.

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