IMAGING INFLAMMATION IN ALZHEIMER'S DISEASE WITH ^{11}C-ER176

Regulatory Sponsor: *William Charles Kreisl, MD*
Taub Institute
622 W. 168th St, PH 19, New York, NY 10032
212-305-9194

Funding Sponsor: *Taub Institute*
622 W. 168th St, PH 19, New York, NY 10032
212-305-9194

Study Product: *^{11}C-ER176; ^{18}F-Florbetaben*

Protocol Number: *AAAR6570*

IND Number: *139712*

Date: October 18, 2019
Amended:
Administrative Change:

CONFIDENTIAL

Table of Contents

List of Abbreviations

PET	Positron emission tomography
^{11}C-ER176	*O-methyl*-^{11}C](*R*)-*N-sec*-butyl-4-(2-chlorophenyl)-*N*-methylquinazoline-2-carboxamide (a radioligand for inflammation)
CSF	Cerebrospinal fluid
MRI	Magnetic resonance imaging
AD	Alzheimer's disease
MCI	Mild cognitive impairment
EKG	Electrocardiogram
TSPO	18 kDa translocator protein
SNP	Single nucleotide polymorphism
AD	Alzheimer's disease
SUVR	Standardized uptake value ratio
HAB	High affinity binder
MAB	Mixed affinity binder
LAB	Low affinity binder

Study Summary

Title	Imaging inflammation in Alzheimer's disease with ^{11}C-ER176
Short Title	Imaging inflammation in Alzheimer's disease with ^{11}C-ER176
Protocol Number	Pending
Phase	II
Methodology	This is a single center PET study. After a screening period which includes brain MRI, neurological and neuropsychological examination, and determination of clinical and biomarker evidence of Alzheimer's disease pathology (presence or absence of cognitive impairment and amyloid positivity on ^{18}F-florbetaben PET scan). Subjects will be diagnosed as Alzheimer's disease (AD) or mild cognitive impairment (MCI) or cognitively normal. Subjects will then have one PET scan with ^{11}C-ER176.
Study Duration	4 years
Study Center(s)	Taub Institute, CUMC
Objectives	The primary objective is to determine the ability of ^{11}C-ER176 to detect increased TSPO (a marker of inflammation) in AD.
Number of Subjects	Up to 50 subjects will be screened and scanned with florbetaben (25 subjects with MCI or AD and 25 controls. From the screened subjects, up to 30 subjects in total (15 with MCI or AD, 15 cognitively normal) will receive ^{11}C-ER176 PET.

Diagnosis and Main Inclusion Criteria	Inclusion criteria: 1. Age 50 and older 2. Meet criteria for either a) amnestic mild cognitive impairment (single or mixed domain) or Alzheimer's disease, or b) have no cognitive impairment, based on history, exam, and neuropsychological testing. 3. Subjects unable to provide informed consent must have a surrogate decision maker and be able to verbally assent to the study procedures. 4. Written and oral fluency in English 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. 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. Such serious medical conditions include uncontrolled epilepsy and multiple serious injuries. 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. Participation in the last year in a clinical trial for a disease modifying drug for AD. 7. Inability to have a catheter in subject's vein for the injection of radioligand. 8. Inability to have blood drawn from subject's veins. 9. Taking anticoagulant medication (e.g., warfarin).
Study Product, Dose, Route, Regimen	^{18}F-florbetaben, up to 8.1 mCi (300 MBq), IV, total of one injection. ^{11}C-ER176, up to 20 mCi (740 MBq), IV, total of one injection. Four control subjects will be used in test-retest study who will receive ^{11}C-ER176, up to 20 mCi (740 MBq), IV, total of two injections.
Duration of administration	A single dose of radioligand will be injected over 1 minute for the ^{18}F-florbetaben scan. A single dose of radioligand will be injected over 3 minute for the ^{11}C-ER176 scan.
Reference therapy	N/A

Statistical Methodology	*Comparing PET imaging among groups* PET images will be analyzed using 1) the two-tissue kinetic model to calculate total distribution volume, corrected for free fraction of radioligand in plasma (V_T/f_P), and 2) the cerebellum as a pseudo-reference region to calculate standardized uptake value ratios (SUVR). Effect size will be calculated for different regions as the difference in means (AD vs. controls) divided by the standard deviation. *Secondary outcomes* Coefficient of variation (%COV = standard deviation / mean) will be calculated for controls and patients with AD for both V_T/f_P and SUVR data. SUVR values will be calculated using cerebellum as reference regions. Additional reference regions will be explored. Correlation coefficients will be computed to look for correlation between ^{11}C-ER176 binding and clinical severity. We will also look for correlations between ^{11}C-ER176 binding and atrophy, as determined using ROI volumes derived from MRI data. We will also calculate test-retest reproducibility for ^{11}C-ER176 using data from the 4 controls who have two ^{11}C-ER176 scans. *Sample size determination* This is a Phase 2 pilot study to determine feasibility and preliminary data for a larger study. Therefore, the proposed sample size is not necessarily expected to provide enough statistical power to see significant group differences.

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.

^{11}C-ER176 is a novel TSPO radioligand that was developed because of its relative insensitivity to the rs6971 polymorphism. The ratio of binding affinity in HABs to that in LABs is only 1.3 to 1 for ER176, whereas the comparable ratio was 55 to 1 for PBR28 [19]. ^{11}C-ER176 also performed well as a PET radioligand for TSPO in monkey brain, showing more than 80% specific (i.e., displaceable) binding. Human blocking studies show that ^{11}C-ER176 has higher

specific-to-nonspecific binding than both ^{11}C-(R)-PK 11195 and ^{11}C-PBR28 [20]. While there exists a small amount of in vivo sensitivity to the rs6971 polymorphism, ^{11}C-ER176 binding can be stably quantified even in low affinity binders.

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.

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.

1.3 Preclinical Data

1.3.1. Preclinical Data for ER176

1.3.1.1. Pharmacology of ER176

Because TSPO is highly expressed in microglia and reactive astrocytes, it has been widely used over the last three decades as an in vivo biomarker to detect neuroinflammation using PET [9]. In recent years, a large number of clinical studies have been performed using new PET ligands, including [^{11}C]PBR28, which has been used extensively by our group. Although these new PET ligands have higher levels of specific binding than the classic ligand [^{11}C]PK 11195 [22], they are confounded by the fact that the binding affinity of most new ligands is affected by the rs6971 polymorphism on the TSPO gene [17]. As noted above, this polymorphism determine whether human subjects are high- (HAB), mixed- (MAB), or low-affinity (LAB) binders. PET results may thus be affected by genotype in addition to pathological changes in TSPO density [23].

To cope with the confounding factor of polymorphism, a new ligand has been developed, [^{11}C]ER176, with similarly high affinity for all three genotypes. Ki values of ER176 and [H-3]PK 11195 measured in human leukocytes were 1.4 and 1.6 nM for HAB and LAB, respectively. ER176 also has lipophilicity of clog D = 3.80, which is appropriate for brain imaging.

1.3.1.2. Animal toxicology for ER176

SRI International conducted an extended acute toxicity study in rats. The results of this toxicity study have been provided by Dr. Robert Innis of NIMH and is publically available at https://pdsp.unc.edu/databases/snidd/IND/CER176.php. The executive summary is provided below. Please note that the toxicity study was designed around the assumption that the maximal mass dose would be 10 µg in a human subject. After receiving the report, and to be extra cautious, Dr. Innis's group reduced the maximal mass dose to 5 µg for the first-in-human studies.

Thus, the report and Dr. Innis's IND differ two-fold when referring to the projected human dose. For example, in the report, the actual dose of 22.0 µg/kg in a rat is referred to as 25X human equivalent dose (HED), whereas Dr. Innis's IND refers to it as 50X HED. Under this present IND, we plan to use the same conservative mass dose limit of 5 µg per injection of ^{11}C-ER176.

Executive Summary

The objective of this study was to determine potential toxic effects and to identify potential target organs of toxicity, if possible, for the toxicity endpoints examined following a single intravenous (iv) bolus administration of ER176 given to Sprague Dawley rats. Additionally, the maximum tolerated dose (MTD) and no observed adverse effect level (NOAEL) of ER176 in rats may be established. Information from this study may be used to determine the suitability of the proposed human dose.

Two groups of 10 male and 10 female rats were administered a single dose of either the vehicle control (Group 1) or ER176 at 88.1 µg/kg (528.6 µg/m^2; 100 times the human dose; Group 2) on Day 1 by iv bolus injection into the tail vein. The vehicle was 10% ethanol and 90% saline. In Groups 1 and 2 five males and five females in each group were sacrificed on Day 3, while the remaining animals were sacrificed on Day 15. In-life evaluations of mortality, morbidity and clinical observations were conducted daily. Body weights were collected on Days 1, 3 and 15. Food consumption was evaluated twice weekly. Clinical pathology samples were collected on the day the animals were euthanized. All animals survived to their scheduled sacrifices. There were no test article–related effects seen in the animals treated with 88.1 µg/kg ER176 on body weights, food consumption, clinical pathology, gross necropsy, organ weights or histopathology, compared with the controls. However, slight ataxia was observed immediately post dose administration in 10 of 10 males and 6 of 10 females in the ER176 treated group. The effect was slight and transient, and the animals recovered quickly (within 2 hr post dose administration). All animals in the control group were normal. Due to this clinical finding and in an attempt to establish the NOAEL, additional animals were evaluated for clinical observations after consultation with the Sponsor.

Three groups of 10 male and 10 female rats were administered ER176, by iv tail vein injection, at 22.0 µg/kg (132.5 µg/m^2; 25 times the human dose; Group 3), 44.1 µg/kg (264.3 µg/m^2; 50 times the human dose; Group 4), or 88.1 µg/kg (528.6 µg/m^2; 100 times the human dose; Group 5). In-life evaluations of mortality, morbidity and clinical observations were conducted daily for 3 days, and animals were sacrificed on Day 3. No test article–related effects were observed in the animals treated with 22.0 or 88.1 µg/kg of ER176 (Groups 3 and 5). However, a slight and transient ataxia was observed in one male rat immediately following dosing in the 44.1 µg/kg dose group (Group 4). It is not clear why animals in the high dose groups (100 times the human dose; 88.1 µg/kg) responded differently to ER176 in Groups 2 and 5 in clinical observations immediately post dose administration. It may be due to animal-to-animal variations in different shipments.

In conclusion, a single dose of ER176 (22.0 µg/kg, 132.5 µg/m^2; 25 times the projected human dose) administered intravenously was well tolerated in rats and produced no significant treatment–related effects. Animals in the 44.1 and 88.1 µg/kg treatment groups also tolerated the single dose administration, except a slight and transient ataxia was observed. The NOAEL is considered to be 22.0 µg/kg (132.5 µg/m^2; 25 times the projected human dose). The MTD is considered to be greater than 88.1 µg/kg (528.6 µg/m^2; 100 times the projected human dose).

Requested Mass Dose

We request permission to inject a maximal mass dose of 5 µg, which is two-fold less than the 10 µg that was considered the maximal dose for toxicity in the study described above.

1.3.1.3. Monkey imaging studies with ^{11}C-ER176

Baseline and pre-blocked PET scans of [^{11}C]ER176 were performed in two rhesus monkeys (a total of two baseline and two pre-blocked scans) and confirmed that [^{11}C]ER176 has high levels of specific binding. In both monkeys, binding blockade was measured using brain activity. In addition, in one monkey, a more complete measurement of binding was performed by measuring total V_T using metabolite-corrected arterial input function, i.e., [^{11}C]ER176 levels in arterial plasma. For the pre-blocked scans, PK 11195 (5 mg/kg) was used as the blocking agent. In the baseline scans, the mass dose of ER176 was 0.111 µg/kg on average. The scans were performed under isoflurane anesthesia; blood pressure, heart rate, and ECG were monitored. These mass doses caused no changes in vital signs.

Based on brain activity at late time points (60 - 120 minutes), 60% of brain activity in baseline scans was from specific binding (Fig. 2). However, these measurements did not take into account changes in blood data. V_T measured with arterial input function and Logan plot was 11 – 17 mL/cm^3 in baseline and 1.6 – 2.5 mL/cm^3 in pre-blocked scans. The complete measurements suggest that ~85% of activity of the baseline scan was specific binding.

In the baseline and pre-blocked scans of one monkey described in section 8.2.2.1., [^{11}C]ER176 levels were measured in arterial plasma. The pre-blocked scan showed about five times higher [^{11}C]ER176 levels in arterial plasma. TSPO exists in several peripheral organs such as lungs, heart, and kidneys. Whole body imaging and arterial blood sampling were performed at the same time with another ligand ([^{11}C]PBR28), and confirmed that [^{11}C]PBR28 increases in arterial plasma were caused by binding blockade to TSPO in peripheral organs [24]. Therefore, increased [^{11}C]ER176 levels in arterial plasma in the pre-blocked scan is consistent with the presence of specific binding to TSPO in peripheral organs.

The arterial data from the two monkey scans showed fast clearance of [^{11}C]ER176, the concentration of which decreased to a half of the peak within three minutes post-injection and to < 10% in 30 minutes. Clearance was 526 and 195 mL/min for the baseline and blocked scan, and the terminal half life by tri-exponential fitting was 24 and 49 minutes, respectively.

1.3.2. Preclinical Data for ^{18}F-florbetaben

1.3.2.1. Pharmacology of ^{18}F-florbetaben

^{18}F-florbetaben is a ^{18}F-labeled stilbene derivative, which binds to β-amyloid plaques in the brain. The F-18 isotope produces a positron signal that is detected by a PET scanner. 3H florbetaben in vitro binding experiments reveal two binding sites (Kd of 16 nM and 135 nM) in frontal cortex homogenates from patients with AD. Binding of ^{18}F-florbetaben to β-amyloid plaques in post-mortem brain sections from patients with AD using autoradiography correlates with both immunohistochemical and Bielschowsky silver stains. ^{18}F-florbetaben does not bind to tau or α-synuclein in tissue from patients with AD. Neither Neuraceq nor non-radioactive florbetaben F-18 bind to AT8 positive tau deposits in brain tissue from patients with frontotemporal dementia (FTD), using autoradiography and immunohistochemistry, respectively.

Following intravenous administration, ^{18}F-florbetaben crosses the blood brain barrier and shows differential retention in brain regions that contain β-amyloid deposits. Differences in

signal intensity between brain regions showing specific and nonspecific ^{18}F-florbetaben uptake form the basis for the image interpretation method.

Results from the first in-human study of ^{18}F-florbetaben are as follows (see Package Insert). Ten minutes after intravenous bolus injection of 300 MBq of 18F-florbetaben in human volunteers, approximately 6% of the injected radioactivity was distributed to the brain. 18F-florbetaben plasma concentrations declined by approximately 75% at 20 minutes post injection, and by approximately 90% at 50 minutes. The F-18 in circulation during the 45- 130 minute imaging window was principally associated with polar metabolites of florbetaben. ^{18}F-florbetaben was 8.5% bound to plasma proteins and was eliminated from plasma primarily via the hepatobiliary route with a mean biological half-life of approximately 1 hour. In vitro studies show that metabolism of florbetaben is predominantly catalyzed by CYP2J2 and CYP4F2. At 12 hours post-administration, approximately 30% of the injected radioactivity had been excreted in urine. Almost all F-18 radioactivity in urine was excreted as polar metabolites of ^{18}F-florbetaben and only trace amounts of ^{18}F-florbetaben were detected. In in vitro studies using human liver microsomes, florbetaben did not inhibit cytochrome P450 enzymes at concentrations present in vivo.

1.3.2.2. Nonclinical Toxicology of florbetaben

Animal studies have not been performed to evaluate the carcinogenic potential of florbetaben. Florbetaben did not demonstrate mutagenic potential in an in vitro bacterial mutation assay (Ames test) using five strains of Salmonella typhimurium and one strain of Escherichia coli or in an in vitro chromosomal aberration assay using human peripheral lymphocytes in the absence and presence of a metabolic activator. No study on impairment of male or female fertility and reproductive performance was conducted in animals.

1.4 Clinical Data to Date

1.4.1. Human Experience with ^{11}C-ER176

1.4.1.1. Safety data for ^{11}C-ER176

Nine healthy volunteers had ^{11}C-ER176 injected for whole-body PET scans (1 man and 2 women; mean age ± SD, 30 ± 10 y) [20]. Eight separate healthy volunteers had ^{11}C-ER176 injected for brain PET imaging (1 man and 2 women; 32 ± 9 y old). ^{11}C-ER176 was intravenously injected over 3 min. Specific activity was 121 ± 64 GBq/μmol at the time of injection.

No adverse or clinically detectable pharmacologic effects were observed with ^{11}C-ER176 for any of the 17 subjects. No significant changes in vital signs or electrocardiograms were observed during the PET scan or in the results of laboratory tests repeated after the scan.

1.4.1.2. Whole body imaging with ^{11}C-ER176 in human subjects

Nine healthy volunteers participated in the whole-body PET scans (1 man and 2 women each with HAB, MAB, and LAB status; mean age ± SD, 30 ± 10 y)[20]. TSPO affinity type was determined by in vitro receptor binding to TSPO on leukocyte membranes or genetic analysis. Time–activity curves were derived from volumes of interest for each source organ (i.e., brain, heart, lungs, liver, spleen, kidneys, and thyroid) delineated on the whole-body images using PMOD. For whole-body images, the measured radioactivity in each organ was converted to SUV. Regional SUV averaged from 60 to 120 min after injection (SUV60–120 min) was used to

measure radioligand uptake in each organ. This period was used because it better reflects receptor binding than the early period, which is strongly affected by blood flow. Differences in SUV60–120 min for each organ were compared between genotypes by Kruskal–Wallis testing.

Whole-body imaging clearly showed that the in vivo binding of ^{11}C-ER176 was sensitive to rs6971. Uptake for brain and lung was lower in LABs than in HABs at all time-points, with intermediate values for MABs. SUV60–120 min showed a significant difference between genotypes in heart ($P < 0.05$). The same tendency was observed in brain, lung, and spleen (Table 1).

Table 1. Organ Uptake of ^{11}C-ER176 for Each Genotype[20]

	Organ uptake			
Organ	HABs (n = 3)	MABs (n = 3)	LABs (n = 3)	P
Brain	1.3 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.05
Thyroid	2.8 ± 1.2	1.4 ± 0.4	1.3 ± 0.2	0.11
Lung	2.2 ± 0.3	1.5 ± 0.5	0.9 ± 0.1	0.06
Heart	6.8 ± 0.3	5.0 ± 0.6	3.5 ± 0.9	0.04
Liver	3.0 ± 0.7	3.3 ± 0.3	2.8 ± 0.1	0.25
Spleen	4.3 ± 0.1	3.7 ± 0.8	2.7 ± 0.3	0.06
Kidney	3.9 ± 0.4	3.6 ± 0.3	2.5 ± 0.2	0.10

Data are mean $SUV_{60–120\ min}$ ± SD. P values were derived from Kruskal–Wallis test between genotypes.

Residence times were calculated directly using the “Residence Times” model implemented in PMOD. Absorbed radiation doses were calculated by entering the residence times for each source organ into OLINDA/EXM, version 1.1, using the model for a 70-kg adult man. The residence time of organs was calculated for the 9 subjects. The effective dose was 4.1 ± 0.4 μSv/MBq, which is similar to that of other ^{11}C-labeled radioligands.

1.4.1.3. Brain imaging with ^{11}C-ER176 in human subjects

Eight separate healthy volunteers with either HAB, MAB, or LAB status had brain PET scans with ^{11}C-ER176 (1 man and 2 women each for HAB and MAB status and 2 men with LAB status; 32 ± 9 y old) [20]. TSPO affinity type was determined by in vitro receptor binding to TSPO on leukocyte membranes or genetic analysis. The 3 HABs who participated in the brain PET scans received an oral dose of 90 mg of the TSPO agonist N-benzyl-N-ethyl-2-(7-methyl-8-oxo-2-phenylpurin-9-yl)acetamide (XBD173) as a partial blockade about 1.75 h before the second injection of ^{11}C-ER176.

For brain images, total volume of distribution (V_T), an index of receptor density that equals the ratio at equilibrium of the concentration of radioligand in tissue to that in plasma, was calculated with 1- and unconstrained 2-tissue compartment models (2TCM and 1TCM, respectively) using the radiometabolite-corrected plasma input function. To determine the minimal scan length for reliable measurements and also to indirectly assess whether ^{11}C-ER176

radiometabolites enter the brain, the time stability of V_T was examined by increasingly truncating the 90-min scan by 10-min increments to the shortest length of 0–50 min.

A modification of the Lassen plot was used to estimate V_{ND} of ^{11}C-ER176 in brain in 2 ways: with an occupancy plot using the difference in V_T at baseline and after partial blockade with XBD173 in HABs, and with a polymorphism plot using the difference in V_T between 2 genetic groups (i.e., between HABs and MABs, between HABs and LABs, and between MABs and LABs).

Brain time–activity curves fitted better with 2TCM than 1TCM. The unconstrained 2TCM fitting converged in brain time–activity curves from all regions, in all scans, and in all genotypes, both at baseline and after blockade with XBD173 in HABs (Fig. 4A). Compared with the 1TCM, the 2TCM showed lower mean Akaike information criterion scores (130 vs. 242) and higher mean model selection criterion scores (6.4 vs. 2.9). An F test also showed that the goodness of fit was significantly better with the 2TCM than with the 1TCM in all 154 fittings over a total of 11 scans, indicating the presence of significant amounts of both specific and nonspecific binding in human brain. The 2TCM identified V_T well, with an average SE of 2.4% across brain regions. Regional V_T values (mL·cm−3) were consistent with the known distribution of TSPO, showing high levels in the brain stem (4.3) and low levels in the putamen (3.2) at baseline for HABs.

After receiving XBD173, all 3 HABs showed marked blocking effects, both in brain and in plasma. That is, receptor blockade decreased peak uptake in brain, increased washout, and decreased total uptake over the 90-min scan (Fig. 1A). In plasma, XBD173 significantly increased the concentration of ^{11}C-ER176, consistent with its blocking the distribution of the radioligand to organs of the body (Fig. 1B)

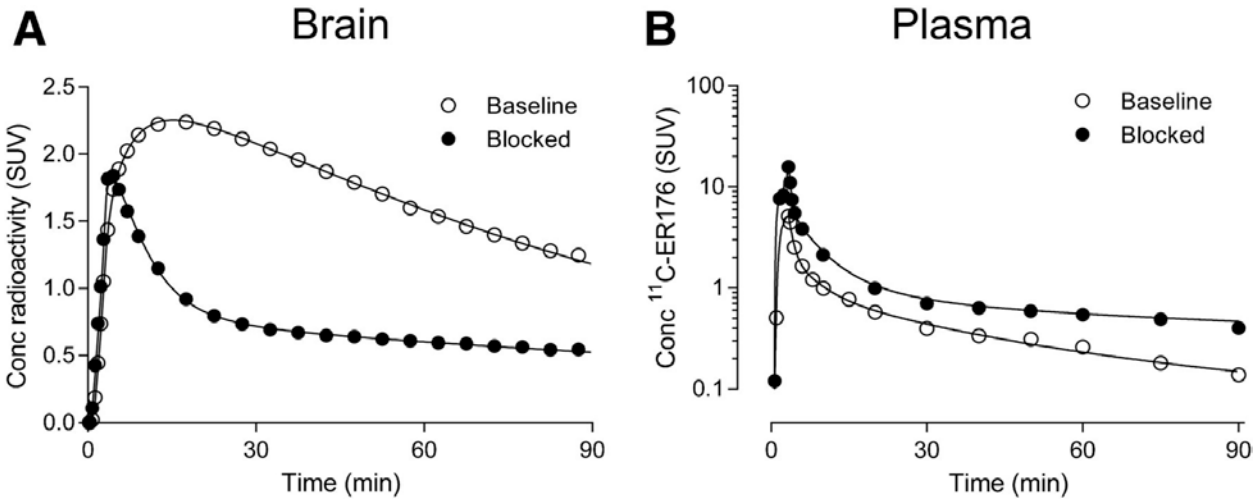

Fig. 1. Brain uptake and plasma radioactivity concentrations (conc) of ^{11}C-ER176 in representative HAB at baseline (○) and after blockade with 90 mg of XBD173 (●).

V_T values were stable from 60 to 90 min and were well identified (SE < 10%) in all 3 genotypes with the exception of 1 MAB and in the 3 HABs after blockade, which had low specific binding like that in LABs, indicating negligible accumulation of radiometabolites regardless of the genotype.

V_T values were higher for HABs than for LABs in all regions of interest, with intermediate values for MABs. The whole-brain V_T in HABs (3.3 ± 0.9 mL·cm−3) was about 1.2-fold higher than that in MABs (2.9 ± 0.9 mL·cm−3) and about 2.2-fold higher than that in LABs (1.6 ± 0.5 mL·cm−3). V_T for ^{11}C-ER176 was well identified by the 2TCM in all genotypes.

The receptor blocking study using XBD173 found that ^{11}C-ER176 had adequately high BP_{ND} for all genotypes. The whole brain BP_{ND}—without correcting for f_P—was 4.2 ± 1.3 and 1.4 ± 0.8 for HABs and LABs, respectively. The high BP_{ND} and good identifiability in all genotypes, including LABs, suggests that this new radioligand would likely have better sensitivity in detecting abnormalities in patients and that LABs may not need to be excluded as they are for ^{11}C-PBR28.

In summary, although ^{11}C-ER176 does not show sensitivity to the rs6971 SNP in vitro (see Section 8.1.1.), this radioligand does show a small degree of sensitivity to the SNP in vivo. This in vivo sensitivity is seen in both brain and peripheral organs (see Section 9.2.). However, the difference in binding between HABs and LABs is much lower for ^{11}C-ER176 than for ^{11}C-PBR28. Importantly, V_T and BP_{ND} may be reliably quantified in LABs with ^{11}C-ER176.

1.4.2. Human Experience with ^{18}F-florbetaben

Human brain studies with ^{18}F-florbeteben have been performed (see Package Insert). The results are summarized as follows. ^{18}F-florbetaben was evaluated in three single arm clinical studies (Study A-C) that examined images from adults with a range of cognitive function, including some end-of-life patients who had agreed to participate in a post-mortem brain donation program. Subjects underwent ^{18}F-florbetaben injection and scan, then had images interpreted by independent readers masked to all clinical information. The Standard of Truth (SoT) was based on the histopathologic examination using Bielschowsky silver staining (BSS) of six brain regions assessed by a Pathology Consensus Panel masked to all clinical information (including PET scan results). ^{18}F-florbetaben PET imaging results (negative or positive) corresponded to a histopathology derived plaque score based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria using neuritic plaque counts. For the subject level SoT, if in any of the six regions β amyloid neuritic plaques were more than sparse, the subject was classified as positive; if in none of the regions the β-amyloid neuritic plaques were assessed as being more than sparse, the subject was classified as negative.

Study A evaluated ^{18}F-florbetaben PET images from 205 subjects and compared the results to postmortem truth standard assessments of brain β-amyloid neuritic plaque density in subjects who died during the study. The median age was 79 years (range 48 to 98 years) and 52% of the subjects were male. By medical history 137 study participants had AD, 31 had other non-AD dementia, 5 had dementia with Lewy Bodies (DLB), and 32 had no clinical evidence of dementia. Interpretation of images from 82 autopsied subjects was compared to the subject level histopathology SoT. Three readers, after undergoing in-person tutoring, interpreted images using a clinically applicable image interpretation methodology. At autopsy, the subject level brain β amyloid neuritic plaque density category was: frequent (n = 31); moderate (n = 21); sparse (n = 17); or none (n = 13).

In Study B five independent, blinded readers underwent the Electronic Media Training in the clinically applicable image interpretation methodology and assessed images from the same 82 end-of-life subjects who enrolled in Study A. The time interval between the 18F-florbetaben

scan and death was less than one year for 45 patients, between one and two years for 23 patients and more than two years for 14 patients.

Study C evaluated the reliability and reproducibility of the clinically applicable image interpretation methodology using the Electronic Media Training; 461 images from previous clinical studies were included from subjects with a range of diagnoses. Five new readers assessed randomly provided images from subjects with a truth standard (54 subjects who underwent an autopsy) and without a truth standard (51 subjects with mild cognitive impairment, 182 subjects with AD, 35 subjects with other dementias, 5 subjects with Parkinson's Disease and 188 healthy volunteers). Among the 461 subjects, the median age was 72 years (range 22 to 98), 197 were females, and 359 were Caucasian. Interreader agreement across all 5 readers had a kappa coefficient of 0.79 (95% CI 0.77, 0.83). The performance characteristics in 54 subjects with SoT were similar to those measured in Studies A and B. Additionally, intra-reader reproducibility was assessed from 46 images (10%); the percentage of intra- reader agreement for the 5 readers ranged from 91% to 98%.

1.5 Dose Rationale and Risk/Benefits

Based on prior human experience and dosimetry data, we expect the proposed injected activity of ^{11}C-ER176 (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

This is a proof-of-concept study to determine if ^{11}C-ER176, a novel and improved radioligand for TSPO, detects increased TSPO density in patients with Alzheimer's disease. ^{18}F-florbetaben will be used to confirm Alzheimer's disease pathophysiology in the patient group. We also seek to determine the test-retest reliability of ^{11}C-ER176 in humans.

We propose to inject ^{18}F-florbetaben in 50 human subjects, each as a one-time dose, as part of the screening process. Twenty-five subjects will be cognitively normal. Twenty-five subjects will have either mild cognitive impairment or Alzheimer's disease.

From the screened subjects, we propose to inject ^{11}C-ER176 in 30 human subjects, each as a one-time dose, as part of the screening process. Fifteen subjects will be cognitively normal. Fifteen subjects will have either mild cognitive impairment or Alzheimer's disease.

In four of the control subjects, we will perform test-retest ^{11}C-ER176 imaging. Each subject will have two injections of ^{11}C-ER176 for the test-retest study.

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 Columbia Doctors Aging and Dementia clinic, the CUMC Alzheimer's Disease Research Center (ADRC), Dr. Davangere Devanand's Questionable Dementia 2 (QD2) cohort, and the CUMC Washington Heights-

Inwood Community Aging Project (known locally as WHICAP, R01 AG037212, PI Richard Mayeux). Participants may also co-enroll from the cognitive aging studies of Dr. Adam Brickman (protocol AAAO9758) and Dr. Yaakov Stern (protocols AAAI2752, AAAQ8249, and AAAQ8096), and from CUMC protocols AAAO1151, AAAQ7868, and AAAR4352 (PI = Kreisl). Participants may also self-refer as this study will be registered on clinicaltrials.gov. Informed consent will be obtained prior to study enrollment.

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. Only subjects who can provide an understanding of the study procedures, particularly of the risk of pain associated with the arterial catheter placement, by providing verbal assent, may be enrolled via the surrogate consent process. Subjects who are unable to provide meaningful assent will not be enrolled into the study.

Up to 50 subjects will undergo screening including history, physical examination, routine laboratory studies, neuropsychological testing, brain MRI, and amyloid PET imaging.

If any of the above screening procedures have been performed within one year of the ^{11}C-ER176 PET scan visit, then those procedures will not need to be repeated under this protocol. Rather the previously obtained results will be used.

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 above and meet clinical criteria for either amnestic MCI (single or multiple-domain)[25] or Alzheimer's disease[26] to be included in the cognitively impaired category. Of the screened subjects, only amyloid-positive patients and amyloid-negative controls will undergo ^{11}C-ER176 PET.

Subjects will have genetic analysis to determine the presence of the rs6971 (Ala147Thr) SNP on the TSPO gene.

Target sample size for completers is 15 amyloid-positive patients and 15 amyloid-negative controls (30 subjects total). Our enrollment goal of 25 subjects per group will allow a 40% general screen failure/drop-out rate. Subjects who are coenrolled in other CUMC studies will not need to have brain MRI under this protocol if done within the last 12 months. Coenrolled subjects will not need to have ^{18}F-florbetaben PET under this protocol if 1) they are controls and have had a negative ^{18}F-florbetaben or other amyloid PET scan within the previous 12 months, or 2) they are patients and have ever had a positive amyloid PET scan using any amyloid radioligand (^{18}F-florbetaben, ^{18}F-florbetapir, ^{18}F-flutemetamol, or ^{11}C-Pittsburgh Compound B).

3.1.2 Neuropsychological testing

The diagnosis of MCI or AD will be based on updated criteria[25, 26]. The neuropsychological test battery will include the following tests: the Mini-Mental State Examination, Selective Reminding Test, Benson Complex Figure Copy and Recall, MINT, Rosen Drawing Test, single word and sentence repetition from the NACC FTD module,

Geriatric Depression Scale (15 items), Trail Making Tests A and B, and Verbal Fluency (CFL, and Animals). Raw scores and demographically corrected T-scores (age, years of education, sex, and ethnicity) will be determined.

3.1.3. Imaging procedures

One brain MRI will be performed on each subject using a 3T 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 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[27]. Subjects will have one PET scan with ^{11}C-ER176 (injected activity 10-20 mCi), except for 4 controls who will have two ^{11}C-ER176 scans for test-retest imaging. ^{11}C-ER176 imaging will be performed with arterial sampling. ^{11}C-ER176 will be synthesized by the CUMC PET Department Radiochemistry Laboratory. ^{18}F-Florbetaben will be purchased from Piramal Imaging. Subjects will be informed if a clinically important abnormality is detected on PET imaging (e.g., brain tumor).

Prior to ^{11}C-ER176 PET imaging, one arterial catheter and one intravenous catheter will be placed. After a low-dose CT scan for attenuation correction, up to 20 mCi ^{11}C-ER176 will be injected intravenously over 3 minutes. Emission scan will begin at the start of the radioligand infusion according to the following frame schedule: 30 seconds x 6, 1 minute x 3, 2 minutes x 2, and 5 minutes x 16 for total scan time of 90 min. During the emission scan, arterial samples will be drawn at 10 second intervals for the first 4 min using an automated blood sampler. Thereafter, manual arterial samples will be drawn at 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, and 90 minutes. Alternatively, manually samples may be drawn at 15 sec intervals for the first 2.5 min, then at 3, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, and 90 minutes. Radioactivity in plasma will be quantified by a γ-counter and analyzed by reverse-phase chromatography to separate parent radioligand from radiometabolites[43] by the PET center radiometabolite laboratory. Free fraction of ^{11}C-PBR28 in plasma (f_P) will be measured by ultrafiltration and normalized using a standard derived from pooled donor plasma[44]. Because one aim is to determine if ^{11}C-ER176 may be useful in AD studies using simplified analysis, some subjects may have ^{11}C-ER176 imaging without arterial catheterization .

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 ^{18}F-florbetaben image acquired 50-70 min post-injection will be read by a trained investigator blinded to the subject's diagnosis (Kreisl). 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.

^{11}C-ER176 PET images will be analyzed using Freesurfer. ROIs will be applied to coregistered PET images to generate time-activity curves. The metabolite-corrected plasma activity curve will be used to estimate rate constants using the one- and two-tissue compartment models using PMOD software. The total distribution volume will be calculated from the resulting rate constants.

3.1.5. Blood sampling and analysis
Subjects will undergo venipuncture to collect 70 mL of blood. Collected blood will be used for screening laboratories, and for research tests. Research tests include determination of *TSPO* genotype, determined by SNP analysis using polymerase chain reaction with a pre-existing Taqman assay[17, 18, 29] in the Biomarkers Shared Resource of CUMC. Blood will also be collected for genetic and non-genetic tests related to inflammation (e.g., TREM2, use of peripheral blood mononuclear cells to create monocyte-derived microglia-like cells). Results from research tests will not be released to participants. Approximately 20 mL of blood sampled will be used for screening tests and approximately 50 mL of blood will be used for research tests.Blood samples will be stored prior to research use in a secure freezer. Stored samples will be labeled with subject ID number and no personal identifying information will be included on the stored sample label. Because the first eight participants to enter this study only had blood drawn for screening tests and *TSPO* genotyping, these subjects will be asked to return for another study visit to have 50 mL of blood drawn for research tests. Such returning participants will be re-consented prior to having the 50 mL blood draw.

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.

Comparing PET imaging among groups
PET images will be analyzed using 1) the two-tissue kinetic model to calculate total distribution volume, corrected for free fraction of radioligand in plasma (V_T/f_P), and 2) the cerebellum as a pseudo-reference region to calculate standardized uptake value ratios (SUVR). Effect size will be calculated for different regions as the difference in means (AD vs. controls) divided by the standard deviation.

Secondary outcomes

3.3 Secondary Study Endpoints

Coefficient of variation (%COV = standard deviation / mean) will be calculated for controls and patients with AD for both V_T/f_P and SUVR data. SUVR values will be calculated using cerebellum as reference regions. Additional reference regions will be explored. Correlation coefficients will be computed to look for correlation between ^{11}C-ER176 binding and clinical severity. We will also look for correlations between ^{11}C-ER176 binding and atrophy, as

determined using ROI volumes derived from MRI data. We will also calculate test-retest reproducibility for ^{11}C-ER176 using data from the 4 controls who have two ^{11}C-ER176 scans.

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 Alzheimer's disease, or b) have no cognitive impairment, based on history, exam, and neuropsychological testing.
3. Subjects unable to provide informed consent must have a surrogate decision maker
4. Written and oral fluency in English
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. Such serious medical conditions include uncontrolled epilepsy and multiple serious injuries.
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. Participation in the last year in a clinical trial for a disease modifying drug for AD.
7. Inability to have a catheter in subject's vein for the injection of radioligand.
8. Inability to have blood drawn from subject's veins.
9. Taking anticoagulant medication (e.g., warfarin).

4.3 Subject Recruitment and Screening

Men and women age 50 and older will be recruited from the Columbia Doctors Aging and Dementia clinic, the CUMC Alzheimer's Disease Research Center (ADRC), Dr. Davangere Devanand's Questionable Dementia 2 (QD2) cohort, and the CUMC Washington Heights-Inwood Community Aging Project (known locally as WHICAP, R01 AG037212, PI Richard Mayeux). Participants may also co-enroll from the cognitive aging studies of Dr. Adam Brickman (protocol AAAO9758) and Dr. Yaakov Stern (protocols AAAI2752, AAAQ8249, and

AAAQ8096), and from CUMC protocols AAAO1151, AAAQ7868, and AAAR4352 (PI = Kreisl). Participants may also self-refer as this study will be registered on clinicaltrials.gov.

Informed consent will be obtained by a qualified investigator prior to study enrollment. Subjects will undergo screening including history, physical examination, routine laboratory studies, neuropsychological testing, brain MRI, and amyloid PET imaging to identify cohorts who are amyloid-positive with impairment (target n = 15) or amyloid-negative with normal cognition (n = 15). Approximately 40% of screened subjects are expected to fail screening procedures or withdraw from the study. We therefore plan to screen 50 subjects to ensure a total of 30 completers. Subjects who are co-enrolled in other CUMC studies will not need to have brain MRI or screening laboratories under this protocol if done within the last 12 months. Co-enrolled subjects will not need to have ^{18}F-florbetaben PET under this protocol if 1) they are controls and have had a negative ^{18}F-florbetaben or other amyloid PET scan within the previous 12 months, or 2) they are patients and have ever had a positive amyloid PET scan using any amyloid radioligand (^{18}F-florbetaben, ^{18}F-florbetapir, ^{18}F-flutemetamol, or ^{11}C-Pittsburgh Compound B).

During screening, participants will be administered a neuropsychological test battery and a medical interview.

If any of the above screening procedures have been performed within one year of the ^{11}C-ER176 PET scan visit, then those procedures will not need to be repeated under this protocol. Rather the previously obtained results will be used.

The diagnosis of MCI or AD will be based on updated criteria[25, 26]. The neuropsychological test battery will include the following tests: the Mini-Mental State Examination, Selective Reminding Test, Benson Complex Figure Copy and Recall, MINT, Rosen Drawing Test, single word and sentence repetition from the NACC FTD module, Geriatric Depression Scale (15 items), Trail Making Tests A and B, and Verbal Fluency (CFL, and Animals). Raw scores and demographically corrected T-scores (age, years of education, sex, and ethnicity) will be determined.

Subjects will be categorized by cognitive status, as impaired or unimpaired, based on a) history of cognitive impairment, b) neurological exam, c) neuropsychological testing, and d) a study team consensus diagnosis.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects will be withdrawn if they develop serious medical illness during the study, defined as an event determined to be grade 3 or higher, i.e., severe or life-threatening. The exception is a single event of syncope if felt by the PI to be vasovagal in etiology and related to catheter placement or venipuncture. Vasovagal syncope in response to blood drawing and IV placement is common and does not warrant study withdrawal if the subject's loss of consciousness is brief (i.e., less than 5 minutes) and does not require further medical attention.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Data collected prior to withdrawal will be analyzed if possible. No data collection will take place after withdrawal in subjects who drop out of the study.

5 Study Drug

5.1 Description

5.1.1. ^{11}C-ER176 is a PET radioligand that binds to the 18 kDa translocator protein. ^{11}C-ER176 will be administered at tracer doses and is not expected to have a pharmacological effect.

5.1.2. ^{18}F-Florbeteben is a PET radioligand that binds to amyloid plaques. ^{18}F-Florbetaben will be administered at tracer doses and is not expected to have a pharmacological effect.

5.2 Treatment Regimen

N/A

5.3 Method for Assigning Subjects to Treatment Groups

N/A

5.4 Preparation and Administration of Study Drug

^{11}C-ER176 will be synthesized and administered by the CUMC PET Department. Injection will be administered at a target imaging dose of up to 20 mCi (740 MBq).

^{18}F-florbetaben will be synthesized by an off-site commercial manufacturer and delivered to the CUMC PET Department as a clear solution containing ^{18}F-florbeteben (drug substance) formulated for intravenous bolus administration. 18F-florbetaben injection will be administered at a target imaging dose of up to 8.1 mCi (300 MBq).

Calculation of activity to be injected will be performed by a qualified radiopharmacist in the CUMC PET department.

5.5 Subject Compliance Monitoring

N/A

5.6 Prior and Concomitant Therapy

Subjects will not be included in the study if they have participated in the last year in a clinical trial for a disease modifying drug for AD.

5.7 Packaging

N/A

5.8 Blinding of Study Drug

N/A

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, the CUMC PET Center staff will perform an inventory and a drug receipt log will be filled out and signed by the person accepting the shipment. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

5.9.2 Storage

N/A

5.9.3 Dispensing of Study Drug

Both ^{11}C-ER176 and ^{18}F-florbetaben will be administered the same day of synthesis or delivery to the CUMC PET Department.

5.9.4 Return or Destruction of Study Drug

Any unused radiopharmaceutical will be on site and documented in the study files.

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 and urine collected for routine safety laboratories and TSPO genotyping. Subjects who have previously had TSPO genotyping will not need to repeat this blood test regardless of when they were last genotyped for TSPO. Additional screening visits are required for brain MRI and ^{18}F-florbetaben PET scan. 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 brain MRI or safety laboratories performed under a different CUMC protocol do not need to have them repeated unless performed more than 12 months prior to inclusion in this study. Coenrolled subjects will not need to have ^{18}F-florbetaben PET under this protocol if 1) they are controls and have had a negative ^{18}F-florbetaben PET scan within the previous 12 months, or 2) they are patients and have ever had a positive amyloid PET scan using any amyloid radioligand (^{18}F-florbetaben, ^{18}F-florbetapir, ^{18}F-flutemetamol, or ^{11}C-Pittsburgh Compound B).

Up to 3 screening visits are anticipated. However, additional visits may be necessary <u>if</u> certain tests must be scheduled on different days due to scheduling.

Screening procedures may be performed in any order and may be performed on the same day as the ^{11}C-ER176 PET scan if schedule allows for subject convenience. Subjects who already had brain MRI or safety laboratories performed under a different CUMC protocol do not need to have them repeated unless performed more than 12 months prior to inclusion in this study. If any of the above screening procedures have been performed within one year of the ^{11}C-ER176 PET scan visit, then those procedures will not need to be repeated under this protocol. Rather the previously obtained results will be used. The ^{11}C-ER176 PET scan will be performed on a different day after screening procedures are completed. For the 4 subjects having test-retest imaging, two ^{11}C-ER176 PET scans will be performed. These may be performed on the same day or on separate days.

Subjects will have a total of 70 mL of venous blood drawn at time of screening to be used for screening laboratories, *TSPO* genotyping, and other research tests. Blood samples will be stored prior to research use in a secure freezer. Stored samples will be labeled with subject ID number and no personal identifying information will be included on the stored sample label. Results will not be released to participants.

Study Schedule

Study Procedures	Screen [a]	Study [b]	
	≤12 months	Visit 1 (within 12 months of first screening visit)	Visit 2 (within 12 months of first screening visit)
Informed consent	X		
Medical/psychiatric history	X		
Inclusion/exclusion criteria	X		
Blood draw	X		
Neuropsychological testing	X		
MRI	X		
^{18}F-florbetaben PET scan	X		
^{11}C-ER176 PET		X	X [c]
Laboratory determinations [d]	X		
Neurological examination	X		
Vital signs	X		
Height and weight	X	X	
Adverse events [e]	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 brain MRI or safety laboratories performed under a different CUMC protocol do not need to have them repeated unless performed more than 12 months prior to inclusion in this study. If any of the above screening procedures have been performed within one year of the ^{11}C-ER176 PET scan visit, then those procedures will not need to be repeated under this protocol. Rather the previously obtained results will be used. Coenrolled subjects will not need to have ^{18}F-florbetaben PET under this protocol if 1) they are controls and have had a negative ^{18}F-florbetaben or other amyloid PET scan within the previous 12 months, or 2) they are patients and have ever had a positive amyloid PET scan using any amyloid radioligand (^{18}F-florbetaben, ^{18}F-florbetapir, ^{18}F-flutemetamol, or ^{11}C-Pittsburgh Compound B). 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. Screening procedures may be performed on the same day as the ^{11}C-ER176 PET scan for subject convenience if schedule allows.

c. Four controls subjects will undergo 2 ^{11}C-ER176 PET scans, which may be performed on the same day or on different days.

d. Laboratory determinations here include complete blood count, basic metabolic panel, liver functions tests, thyroid stimulating hormone, and urinalysis.

e. Adverse event reporting on Visit 2 to be performed after the ^{11}C-ER176 PET scan is completed. Subject will be called by telephone 24 hours after both the ^{18}F-florbetaben and ^{11}C-ER176 PET scans for follow up adverse event reporting. Follow up adverse event reporting for ^{18}F-florbetaben is not needed if the amyloid PET scan is performed under a separate protocol.

7 Statistical Plan

7.1 Sample Size Determination

This is a Phase 2 pilot study to determine feasibility and preliminary data for a larger study. Therefore, the proposed sample size is not necessarily expected to provide enough statistical power to see significant group differences.

7.2 Statistical Methods

PET images will be analyzed using 1) the two-tissue kinetic model to calculate total distribution volume, corrected for free fraction of radioligand in plasma (V_T/f_P), and 2) the cerebellum as a pseudo-reference region to calculate standardized uptake value ratios (SUVR). Effect size will be calculated for different regions as the difference in means (AD vs. controls) divided by the standard deviation.

We will also calculate test-retest reproducibility for ^{11}C-ER176 using data from the 4 controls who have two ^{11}C-ER176 scans.

7.3 Subject Population(s) for Analysis

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

8 Safety and Adverse Events

8.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study drug follow-up is defined as 24 hours following the last administration of study drug. Follow up adverse event reporting for ^{18}F-florbetaben is not needed if this scan is performed under a separate protocol.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify IRB and FDA of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

Abnormal Laboratory Values

A clinical laboratory abnormality will be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality

- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events (AEs) by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document. All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

As per CUMC IRB's Policy, Unanticipated Problems will be defined as follows:

Unanticipated Problem (UP) is any incident, experience or outcome involving risk to subjects or others in any human subjects research that meets all of the following criteria:

• Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document and (b) the characteristics of the subject population being studied;

• Related or possibly related to participation in such research (i.e., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
• Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized (HHS IRB Guidance, Section I).

The following AEs will be considered Unexpected Problems:

• A single occurrence of a serious, unexpected event that is uncommon and strongly associated with drug exposure
• A single occurrence, or a small number of occurrences, of a serious, unexpected event that is not commonly associated with drug exposure
• Multiple occurrences of an AE that, based on an aggregate analysis, are determined not to be isolated occurrences and involve risk to human subjects
• An AE that is described or addressed in the investigator's brochure, protocol or informed consent documents (a Described AE), but occurs at a specificity or severity that is inconsistent with prior observations
• A serious Described AE, but for which the rate of occurrence represents a clinically significant increase in the expected rate of occurrence
• Any other AE or safety finding that would cause the sponsor to modify the investigator's brochure, study protocol or informed consent documents or would prompt other action by the IRB to ensure the protection of human subjects (2009 FDA Guidance, Section III (A)).

8.3 Reporting of Serious Adverse Events

8.3.1 IRB Notification by Investigator

At the time of the Occurrence of an Unanticipated Problem:

Each UP will be reported to the IRB, whether or not (a) it is serious or non-serious or (b) it occurs at a site at which the PI is conducting the research.

The UP will be reported promptly, but not later than one week following the occurrence of the UP or the PI's acquiring knowledge of the UP.

The PI will make the determination as to whether an incident, experience or outcome constitutes a UP.

Each Unanticipated Problem will be reported to the IRB using the Unanticipated Problem Report module in Rascal.

The investigator must conclude in the Unanticipated Problem Report whether the protocol and/or consent form(s) should be modified as the result of the UP. If the protocol and/or consent document(s) requires a revision, a modification must be submitted in Rascal.

At the Time of Continuing Review of a Protocol:

At the time of continuing review of a protocol, the PI will submit a summary of all UPs that occurred during the review period and since the beginning of the study. The summary for each UP should include:

• The number of subjects who experienced the UP;
• The investigator's determination as to whether or not the UP was serious;
• The investigator's determination as to the UP's relationship to the study procedures (e.g., definitely related, probably related or possibly related).

8.3.2 FDA Notification by Investigator

The principal investigator shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the investigator's original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the principal investigator will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

8.4 Unblinding Procedures

N/A

8.5 Stopping Rules

N/A

8.6 Medical Monitoring

We will not have a specific Data and Safety Monitoring Board. Adverse events will be documented in the patient's chart. Notification of UPs to the IRB and FDA will take place as described in Section 8.3. We will also report all adverse events to Piramal Imaging who is monitoring safety data for ^{18}F-florbetaben.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by the Principal Investigator. Dr. Kreisl will submit annual progress reports to the FDA within 60 days of the anniversary of the date that the IND became active (the date clinical studies were permitted to begin) in accordance with 21 CFR 312.33.

11 Ethical Considerations

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.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will

be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See attached copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is funded by a Rose and Boris Katz Assistant Professorship endowed to Dr. Kreisl. Additional funding is being sought through application to the Alzheimer's Drug Discovery Foundation (PI = Dr. Kreisl).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Columbia University Medical Center investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Subjects will be compensated commensurate to similar studies at CUMC. We wish to reduce the burden of travel and inconvenience of study procedures on patients and caregivers. Subjects will be given $100 for the screening visit, $100 for the MRI, and $100 for each PET scan.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 References

1. McGeer, P.L. and E.G. McGeer, *The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy.* Acta Neuropathol, 2013. **126**(4): p. 479-97.

2. Maezawa, I., et al., *Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity.* J Biol Chem, 2011. **286**(5): p. 3693-706.
3. Serrano-Pozo, A., et al., *Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease.* Am J Pathol, 2011. **179**(3): p. 1373-84.
4. Neniskyte, U., J.J. Neher, and G.C. Brown, *Neuronal death induced by nanomolar amyloid beta is mediated by primary phagocytosis of neurons by microglia.* J Biol Chem, 2011. **286**(46): p. 39904-13.
5. Ghosh, S., et al., *Sustained interleukin-1beta overexpression exacerbates tau pathology despite reduced amyloid burden in an Alzheimer's mouse model.* J Neurosci, 2013. **33**(11): p. 5053-64.
6. Morales, I., et al., *Tau oligomers and fibrils induce activation of microglial cells.* J Alzheimers Dis, 2013. **37**(4): p. 849-56.
7. Braak, H., et al., *Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years.* J Neuropathol Exp Neurol, 2011. **70**(11): p. 960-9.
8. Ossenkoppele, R., et al., *Long-term effects of amyloid, hypometabolism, and atrophy on neuropsychological functions.* Neurology, 2014. **82**(20): p. 1768-75.
9. Stephenson, D.T., et al., *Peripheral benzodiazepine receptors are colocalized with activated microglia following transient global forebrain ischemia in the rat.* J Neurosci, 1995. **15**(7 Pt 2): p. 5263-74.
10. Cagnin, A., et al., *In-vivo measurement of activated microglia in dementia.* Lancet, 2001. **358**(9280): p. 461-7.
11. Edison, P., et al., *Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study.* Neurobiol Dis, 2008. **32**(3): p. 412-9.
12. Wiley, C.A., et al., *Carbon 11-labeled Pittsburgh Compound B and carbon 11-labeled (R)-PK11195 positron emission tomographic imaging in Alzheimer disease.* Arch Neurol, 2009. **66**(1): p. 60-7.
13. Schuitemaker, A., et al., *Microglial activation in Alzheimer's disease: an (R)-[(1)(1)C]PK11195 positron emission tomography study.* Neurobiol Aging, 2013. **34**(1): p. 128-36.
14. Okello, A., et al., *Microglial activation and amyloid deposition in mild cognitive impairment: a PET study.* Neurology, 2009. **72**(1): p. 56-62.
15. Imaizumi, M., et al., *Brain and whole-body imaging in nonhuman primates of [11C]PBR28, a promising PET radioligand for peripheral benzodiazepine receptors.* Neuroimage, 2008. **39**(3): p. 1289-98.
16. Kreisl, W.C., et al., *Stroke incidentally identified using improved positron emission tomography for microglial activation.* Arch Neurol, 2009. **66**(10): p. 1288-9.
17. Owen, D.R., et al., *An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28.* J Cereb Blood Flow Metab, 2012. **32**(1): p. 1-5.
18. Kreisl, W.C., et al., *A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation.* J Cereb Blood Flow Metab, 2013. **33**(1): p. 53-8.
19. Zanotti-Fregonara, P., et al., *Synthesis and evaluation of translocator 18 kDa protein (TSPO) positron emission tomography (PET) radioligands with low binding sensitivity to*

human single nucleotide polymorphism rs6971. ACS Chem Neurosci, 2014. **5**(10): p. 963-71.
20. Ikawa, M., et al., *11C-ER176, a Radioligand for 18-kDa Translocator Protein, Has Adequate Sensitivity to Robustly Image All Three Affinity Genotypes in Human Brain.* J Nucl Med, 2017. **58**(2): p. 320-325.
21. Ong, K.T., et al., *Abeta imaging with 18F-florbetaben in prodromal Alzheimer's disease: a prospective outcome study.* J Neurol Neurosurg Psychiatry, 2014.
22. Chauveau, F., et al., *Nuclear imaging of neuroinflammation: a comprehensive review of [^{11}C]PK11195 challengers.* Eur J Nucl Med Mol Imaging, 2008. **35**(12): p. 2304-19.
23. Kreisl, W.C., et al., *A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation.* Journal of Cerebral Blood Flow and Metabolism, 2012.
24. Imaizumi, M., et al., *Brain and whole-body imaging in nonhuman primates of [^{11}C]PBR28, a promising PET radioligand for peripheral benzodiazepine receptors.* Neuroimage, 2008. **39**: p. 1289-98.
25. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 270-9.
26. McKhann, G.M., et al., *The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 263-9.
27. Rowe, C.C., et al., *Imaging of amyloid beta in Alzheimer's disease with 18F-BAY94-9172, a novel PET tracer: proof of mechanism.* Lancet Neurol, 2008. **7**(2): p. 129-35.
28. Caroli, A., et al., *Mild cognitive impairment with suspected nonamyloid pathology (SNAP): Prediction of progression.* Neurology, 2015. **84**(5): p. 508-15.
29. Kreisl, W.C., et al., *In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease.* Brain, 2013. **136**(Pt 7): p. 2228-38.