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IMAGING INFLAMMATION IN PATIENTS WITH DIFFUSE LEWY BODY DISEASE

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-PBR28*

Protocol Number: *AAAQ0756*

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List of Abbreviations

PET	Positron emission tomography
^{11}C-PBR28	^{11}C-[O-methyl-^{11}C-*N*-acetyl-*N*-(2-methoxybenzyl)-2-phenoxy-5pyridinamine (a radioligand for inflammation)
MRI	Magnetic resonance imaging
DLBD	Diffuse Lewy Body Disease
AD	Alzheimer's disease
PD	Parkinson's disease
DLB	Dementia with Lewy bodies
EKG	Electrocardiogram
TSPO	18 kDa translocator protein
SNP	Single nucleotide polymorphism
MCI	Mild cognitive impairment
SUVR	Standardized uptake value ratio
HAB	High affinity binder
MAB	Mixed affinity binder
LAB	Low affinity binder

Study Summary

Title	Imaging inflammation in patients with diffuse Lewy body disease
Short Title	Imaging inflammation in DLBD
Protocol Number	AAAQ0756
Phase	II
Methodology	This is a single center PET study. Screening will include history, physical examination, routine laboratory studies, neurological examination, genetic testing for 18 kDa translocator protein (TSPO) status, and brain MRI. Clinical evalution will include Montreal Cognitive Assessment (MoCA) and Unified Parkinson's disease rating scale (UPDRS). Subjects determined to have dementia with Lewy bodies or Parkinson's disease dementia (collectively considered as diffuse Lewy body disease) or cognitively normal by consensus diagnosis will be included. Subjects will then have one PET scan with ^{11}C-PBR28. Subjects who have no previously had genetic testing for glucocerebrosidase (GBA) or ApoE will have these tests performed.
Study Duration	1 year
Study Center(s)	Taub Institute, CUMC
Objectives	The primary objective is to generate pilot data in patients with DLBD. The aims are: 1. Determine feasibility of performing ^{11}C-PBR28 PET in patients with DLBD. 2. Determine effect size of ^{11}C-PBR28 binding in patients with DLBD vs. controls. The exploratory aims are: 1. Identify potential reference regions for ^{11}C-PBR28 binding in patients with DLBD. 2. Determine if ^{11}C-PBR28 binding correlates with cognitive impairment and Parkinsonism in patients with DLBD.
Number of Subjects	Target enrollment is 8 patients and 8 controls. Target accrual is 4 patients and 4 controls.

Diagnosis and Main Inclusion Criteria	Inclusion criteria for patients: 1. Age 60 and older 2. Meet criteria for either a) dementia with Lewy bodies, or b) Parkinson's disease dementia 3. Able to provide informed consent 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. Inclusion criteria for controls: 1. Age 60 and older 2. Normal cognitive and motor function based on neurological examination 3. Written and oral fluency in English 4. Able to participate in all scheduled evaluations and to complete all required tests and procedures. 5. 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 DLB or PDD for patient participants). 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. Low affinity binding on TSPO genetic screen 7. Currently taking anticoagulant drugs (e.g., warfarin). 8. Women who are able to become pregnant.
Study Product, Dose, Route, Regimen	^{11}C-PBR28, up to 20 mCi (740 MBq), IV, total of one injection.
Duration of administration	A single dose of radioligand will be injected over 1 minute for the PET scan.

Reference therapy	N/A
Statistical Methodology	*Primary outcome* 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 (DLBD vs. controls) divided by the standard deviation. *Secondary outcomes* Coefficient of variation (%COV = standard deviation / mean) will be calculated for controls and patients with DLBD 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-PBR28 binding (V_T/f_P and SUVR) and clinical severity. Severity will be measured using CDR-sum of boxes, MoCA score, and UPDRS. We will also look for correlations between ^{11}C-PBR28 binding and atrophy, as determined using ROI volumes derived from MRI data. *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 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

Neuroinflammation is a proposed pathogenic contributor to diffuse Lewy body disease (DLBD), which includes Parkinson's disease dementia and dementia with Lewy bodies. DLBD is defined by aggregated α-synuclein inclusions (i.e., Lewy bodies), which activate microglia in vitro and stimulate release of proinflammatory mediators[1]. Activated microglia are found proximal to Lewy bodies at autopsy[2-4]. The amount of activated microglia is proportional to both the number of Lewy bodies found at autopsy[3] and the amount of α-synuclein and loss of dopaminergic neurons measured in transgenic mice[5]. However, the exact role of inflammation in the progression of DLBD remains poorly understood. Activated microglia surrounding degenerating Lewy body-containing neurons could simply be a response to upstream cellular damage[6]. Yet, α-synuclein activates microglia prior to loss of Lewy body-containing neurons[7], suggesting an early role of inflammation in pathogenesis. Microglia may play a protective role by producing antioxidant enzymes proximal to Lewy body-containing neurons in response to oxidative stress[4]. However, microglia produce reactive oxygen species in response to α-synuclein[1], and transgenic mouse studies have shown that 1) α-synuclein-induced neurodegeneration does not occurs in the absence of chronic inflammation, and 2) inhibiting oxidative enzymes produced by microglia ameliorates this neurodegeneration[5]. Therefore, dysfunctional glial response to Lewy body pathology may accelerate neuronal loss, and attenuating the neuroimmune response to α-synuclein aggregation may have therapeutic benefit.

A potential complication in studying inflammation in dementia with Lewy bodies is the frequent co-existence of Alzheimer's disease (AD) pathology [8-11], as β-amyloid is also a proposed trigger for neuroinflammation[12]. However, autopsy studies have shown microglial activation in patients with "pure" dementia with Lewy bodies without AD pathology[3]. Glucocerebrosidase (GBA) gene mutations are more prevalent in patients with "pure" dementia with Lewy body patients than in those with AD co-pathology [13,14], while patients with dementia with Lewy bodies who are ApoE4 non-carriers have lower Braak stage [15,16] and less amyloid on PET[17] than ApoE4 carriers. Therefore, selecting DLBD patients with either GBA mutations or ApoE4 non-carrier status would reduce the incidence of AD co-pathology and facilitate investigation of the relationship between inflammation and synuclein in the absence of amyloidopathy.

Activated microglia over-express the 18 kDa translocator protein (TSPO) in response to cellular injury[18], and TSPO density can be quantified in vivo using positron emission tomography (PET). Only two previous PET studies have measured TSPO in DLBD. In the first, patients with Parkinson's disease dementia showed greater TSPO binding with ^{11}C-(R)-PK 11195 than controls in anterior and posterior cingulate, striatum, frontal, temporal, parietal, and occipital cortical regions, while non-demented Parkinson's disease patients showed greater binding in temporal, parietal, and occipital regions[19]. In the second, patient's with dementia with Lewy bodies and patients with Parkinson's disease showed greater binding than controls in substantia nigra and putamen, while patients with dementia with Lewy bodies also showed

greater binding in several associative cortices and cerebellum[20]. While studies have shown cerebellar atrophy in dementia with Lewy bodies[21], and some studies have shown α-synuclein deposits in cerebellum[22], others have shown decreased[23] or infrequent α-synuclein in this region[24]. Therefore, whether TSPO density is increased in cerebellum in living DLBD patients has not been conclusively established.

^{11}C-(*R*)-PK 11195 has low specific-to-nonspecific binding[25] which could confound accurate quantification of TSPO density. The second generation TSPO radioligand ^{11}C-PBR28 has greater specific-to-nonspecific binding than ^{11}C-(*R*)-PK 11195 in monkey brain[25] and has been used to detect neuroimmune activation in stroke, schizophrenia, and epilepsy[26-28]. Therefore, ^{11}C-PBR28 PET imaging is expected to detect inflammatory changes in DLBD and may be useful for monitoring disease progression and response to novel treatments. Because the relationship between inflammation and the clinical symptoms exhibited in DLDB remains unknown, there is a critical need to determine the relationship between inflammation and DLBD to increase knowledge about the mechanisms underlying the disease and help develop antiinflammatory treatments as disease-modifying therapy for this presently incurable disorder.

One limitation of ^{11}C-PBR28, shared by all tested second generation TSPO radioligands, is differential affinity caused by the rs6971 single nucleotide polymorphism on the TSPO gene[29]. Statistical correction for TSPO genotype allows inclusion of individual heterozygous for the polymorphism; however, homozygotes have negligible binding with ^{11}C-PBR28 and must be excluded from analysis[27].

The primary research objective is to generate pilot data in patients with DLBD to more effectively design an R01 application to comprehensively study the relationship between inflammation and DLBD. The aims are to:

1. Determine feasibility of performing ^{11}C-PBR28 PET in patients with DLBD.
2. Determine effect size of ^{11}C-PBR28 binding in patients with DLBD vs. controls. The exploratory aims are:

1. Identify potential reference regions for ^{11}C-PBR28 binding in patients with DLBD.
2. Determine if ^{11}C-PBR28 binding correlates with cognitive impairment and Parkinsonism in patients with DLBD.

1.2 Investigational Agent

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

This radioligand will be administered in tracer doses. ^{11}C-PBR28 will be administered at activity of up to 20 mCi per injection.

1.3 Preclinical Data

1.3.1. Preclinical Data for PBR28

1.3.1.1. Pharmacology of PBR28

1.3.1.1.1. General pharmacology

Formerly called the peripheral benzodiazepine receptor, the 18 kDa translocator protein (TSPO) is located both in the central nervous system (CNS) and many peripheral organs, including endocrine tissues, kidney, heart, liver and blood cells. In the CNS, TSPO exists mostly but not exclusively in glial cells. In most of these organs, TSPO is located in the outer membrane

of mitochondria. In some organs, TSPO shows other subcellular localizations. For example, TSPO has been localized in mitochondrial inner membrane of guinea pig lung[30]. In rat liver, TSPO has been localized in two sites: a mitochondrial and an unidentified non-mitochondrial location[31]. In heart, TSPO is located in the plasma membrane, where it is reported to be coupled to calcium channels[32]. TSPO has been proposed to be involved in cellular proliferation, calcium channel activity, immune responses, transport of porphyrin and anion and regulation of steroid biosynthesis[33].

PBR28 is a selective aryloxyanilide ligand for TSPO. PBR28 affinity was measured in using [^{3}H]PK11195 as the radioligand and membranes prepared from rat brain, following a published procedure[34]. The K_i value was 0.2 nM in rat brain[35].

1.3.1.1.2. In vitro receptor binding

PBR28 showed a K_i of >10 μM at several central benzodiazepine (GABAA) receptors ($\alpha_1\beta_1\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$, and $\alpha_6\beta_2\gamma_2$ subtypes) and at 10 μM concentration was found to cause less than 50% displacement of reference ligand 5-HT$_{1A,1B,1D,1E,2A-C,3,5A,6,7}$, $\alpha_{1A,1B,2A-C}$, β_{1-3}, D_{1-4}, H_{1-4}, $M_{2,5}$, DAT, NET, and SERT sites. A full displacement study revealed a K_i of 2.2 μM at κ opiate receptors[35].

1.3.1.2. Animal toxicology for PBR28

1.3.1.2.1. Rat study

Male and female Sprague-Dawley rats (10/sex/group) were given a single intravenous (IV) dose of PBR28 at 440.5 μg/kg (2643 μg/m2, 500 times the human dose, Group 2) or at 88.1 μg/kg (528.6 μg/m2, 100 times the human dose, Group 3) on Day 1 or a daily IV dose on Days 1–5 of PBR28 at 88.1 μg/kg/day (528.6 μg/m2/day, 100 times the human dose, total dose 440.5 μg/kg). A control group (10/sex), Group 1, was given a single IV dose of vehicle, 5% ethanol in sterile saline, at an equivalent volume on Day 1. Dose administration in the repeat dose group (Group 4) was initiated 4 days prior to dosing of the single dose animals (Groups 1−3) so that necropsies occurred on the same calendar day for all groups. This schedule permitted control clinical pathology and necropsy data to be shared between the two dose regimens. Animals were sacrificed on Day 3 or Day 15 (interim and terminal necropsy, respectively) for the single dose groups (Groups 1–3) or on Day 7 or Day 19 (interim and terminal necropsy, respectively) for the repeat dose group (Group 4).

The following parameters were evaluated: mortality/morbidity, clinical observations, body weights, food consumption, clinical pathology (hematology and serum chemistry), organ weights, macroscopic observation at necropsy and microscopic histopathology.

All animals survived until their scheduled necropsy. No drug-related effects were found for clinical observations, body weights, food consumption, clinical pathology, organ weights, macroscopic or histopathologic evaluations.

In conclusion, IV administration of PBR28 to male and female Sprague-Dawley rats at 440.5 μg/kg (2643 μg/m2; 500× human dose) for a single day or 88.1 μg/kg (528.6 μg/m2, 100× human dose) for a single day or 5 days did not result in overt clinical adverse effects. The noobserved-adverse-effect (NOAEL) is considered to be at least 440.5 μg/kg (2643 μg/m2) for a single IV dose administration and at least 88.1 μg/kg/day (528.6 μg/m2/day) once each day for 5 daily IV dose administrations. Because no significant adverse effects were seen at the highest dose evaluated, the maximum tolerated dose (MTD) could not be determined, but it is considered

to be greater than 440.5 µg/kg (2643 µg/m2) when given in a single IV injection and greater than 88.1 µg/kg/day (528.6 µg/m2/day) when given in a daily IV injection for 5 consecutive days.

1.3.1.2.2. Dog study

This study involved three (Days 1, 8, and 15) dose administrations of PBR28 separated by one week intervals. Body weights were collected to determine dose calculations for each dose administration. Cardiovascular (CV) baseline data were collected prior to each dose administration including electrocardiogram (ECG), heart rate, respiratory rate, and blood pressure (indirect). Body temperature was also collected. Following administration of PBR28, each
CV parameter was collected at 2, 30, 60, 120, 240 min, and 24 hr post dose administration. Clinical observations were made immediately post dose and daily thereafter for the duration of the study.

The first IV administration of PBR28 was 26.43 µg/kg (528.6 µg/m2), 100 times the proposed human dose level. No adverse signs were observed for 7 days following administration. On Day 8, dose administration was escalated to 500 times the proposed human dose level (132.15 µg/kg; 2643 µg/m2). Again, no adverse clinical observations were observed for the week following administration. On Day 15, 1 naïve male and female dog were administered 132.15 µg/kg PBR28 to confirm the NOAEL. Body weights and body temperatures were normal for all dogs for the duration of the study except for a minor body temperature decrease at the 24 hour time point for one dog administered 26.43 µg/kg PBR28 on Day 1. The minor change of body temperature for this dog is considered to be a sporadic variation and not test article-related.

There were no test article related CV effects, including ST segment abnormalities, pronounced U waves, nor T wave changes such as changes in polarity, increased amplitude, or flattening seen in any of the dogs after a single intravenous injection of 100x or 500x the human dose level of PBR28 for the duration of the study, except for dog #4, which was administered 132.15 µg/kg PBR28, had mild ST segment elevation on Day 15 at time points of 2 min and 4 hour. Also in dog #4, there was a change in T wave polarity from pre-dose positive T wave to 2 min negative T wave, to time 4 hour positive T wave, returning to negative T waves at time 24 hour. Dog #3 had increased QRS amplitude at baseline, pre-dose and throughout the study; and this was not due to a treatment effect.

The ST segment elevation changes seen in dog #4 administered 132.15 µg/kg PBR28 on Day 15 were transient, which may represent regional myocardial hypoxia and may have normalized at 24 hour post-dose. However, dog #3, also administered 132.15 µg/kg PBR28on Day 15, had no adverse test article related CV effects. In addition, Dogs #1 and #2 were administered 132.15 µg/kg PBR28 on Day 8, with no test article related CV effects; therefore, the changes seen in dog #4 were not definitively due to a treatment related adverse effect.

Several dogs (#s 1, 2 and 4) had left and/or right mean electrical axis (MEA) deviations at the baseline, pre-dose and post dose administration. These changes can be affected by many extra-cardiac factors and are not considered to be test article-related.

One dog (#2) had trivial hypotension at 4 hour post dose on Day 1. Animals with only reduced diastolic pressures were not considered to be hypotensive, as the diastolic pressure may be more likely to be erroneously low with oscillometric techniques; therefore, this effect is not considered to be test article related.

In conclusion, the study results confirmed that there were no overt cardiovascular or toxicity effects of PBR28 after a single IV injection of 100 times the proposed human dose level

of PBR28 in Beagle dogs. Effects at 500 times the proposed human dose (132.15 µg/kg, 2643 µg/m2) are inconclusive, with minor, transient effects on ST segment elevation seen in a single dog out of 4 evaluated at this dose level. The NOAEL for PBR28 is considered to be greater than 26.43 µg/kg (528.6 µg/m2) and possibly less than 132.15 µg/kg (2643 µg/m2) via a single intravenous administration to male and female Beagle dogs. The maximum tolerated dose (MTD) was not determined, but is considered to be greater than 132.15 µg/kg (2643 µg/m2).

1.3.1.3. Imaging studies with ^{11}C-PBR28

1.3.1.3.1. Whole body studies in monkey

Male rhesus monkeys were scanned with ^{11}C-PBR28. A preblocking scan was performed on 1 monkey after its baseline scan, in which nonradioactive PK 11195 was injected 3 min before the radioligand. Injection of ^{11}C-PBR28 caused no change in ECG, heart, or respiration rates. Gallbladder, lungs, spleen, heart, brain, kidneys, liver, and urinary bladder were visually identified as organs with moderate-to-high activity. Uptake of ^{11}C-PBR28 was highest in the lungs, with a peak of 50% injected activity occurring during the first frame (0–1.15 min). Peak uptake in kidneys, heart, liver, brain, and urinary bladder were 19.5%, 7%, 10%, 4.2%, and 0.8% standardized uptake value, respectively. The human effective dose estimated from monkey whole-body imaging was 10.3 µSv/MBq. The 3 organs with the highest radiation burden (µSv/MBq) were lungs (70.5), kidneys (43.1), and brain (19.5). Compared to baseline scans, the blocking experiment decreased areas under the curve in organs with PBRs (brain, lungs, kidneys, and heart) and increased areas under the curve in organs involved with metabolism and excretion (liver and bladder).

1.3.1.3.1. Brain studies in monkey

Rhesus monkeys had brain imaging with ^{11}C-PBR28. A blocking experiment was performed by administering DAA1106 (3 mg/kg IV) with the radiotracer. In all brain scans, arterial blood sampling was performed to measure [^{11}C]PBR28 and the radiometabolite levels in plasma.

In the baseline experiments, [^{11}C]PBR28 showed high peak uptake in brain (~ 300% SUV) and was widely distributed, with greater activity in gray than white matter. The choroid plexus in the 4th ventricle had the highest radioactivity at late times, consistent with the known distribution of TSPO.

In blocking experiments, brain activity at early times was increased due to elevated plasma concentrations of radiotracer. For example, the peak uptake of [^{11}C]PBR28 was ~ 300% SUV at baseline and ~ 500% SUV after receptor blockade. Nevertheless, receptor blockade in brain was evident from the faster washout compared to baseline. That is, receptors were blocked by nonradioactive ligand, and the radioligand was not significantly retained in brain. For example, under baseline conditions, peak brain uptake occurred at about 40 min and decreased only 1.3% in the 15 min period thereafter. In contrast, for the receptor-blocked condition, brain uptake peaked at about 4 min and decreased by 62% in the subsequent 15 min.

1.3.1.3.2. Rat stroke

The ability of [^{11}C]PBR28 PET to localize TSPO in a rat permanent middle cerebral artery occlusion (MCAO) model of neuroinflammation was determined. [^{11}C]PBR28 was intravenously administered to rats at 4 and 7 days after permanent MCAO. In all experiments,

arterial blood was sampled for compartmental modeling of regional distribution volumes, and rat brains were sampled after imaging for *in vitro* [^{3}H]PK 11195 autoradiography and histological evaluation. [^{11}C]PBR28 PET and [^{3}H]PK 11195 autoradiography showed similar areas of increased TSPO, especially in the peri-ischemic core. Results from these in vivo and in vitro methods were strongly correlated.

1.4 *Clinical Data to Date*

1.4.1. Human experience with PBR28

Dr. Kreisl has 8 years experience using ^{11}C-PBR28 in clinical studies at the National Institute of Mental Health Intramural Research Program. Over 100 subjects received injection of ^{11}C-PBR28 in clinical protocols on which he was either PI or sub-Investigator No AEs related to PBR28 administration have been reported.

1.4.1.1. Whole body studies

Whole-body images were acquired after intravenous bolus administration of ^{11}C-PBR28 in 7 healthy humans (651 +/- 111 MBq). For typical subjects, the 3 organs with highest exposure were those with the high TSPO densities (kidneys, spleen, and lungs), and the effective dose was 6.6 microSv/MBq[36].

1.4.1.2. Brain studies in controls

Results from the first in-human brain study of ^{11}C-PBR28 are as follows: Twelve healthy volunteers (1 female and 11 males, 25 ± 5 years of age, 81 ± 15 kg body weigh) underwent brain imaging with ^{11}C-PBR28 with arterial catheterization[37]. Injected activity was 650 ± 92 MBq (17.6 ± 2.5 mCi). After injection of ^{11}C-PBR28, 10 of 12 subjects showed moderate levels of activity in brain that washed out gradually. The peak uptake occurred at 5 min and was ~ 200% SUV. Brain activity decreased to 50% of the peak by 70 min and to 40% of peak by 120 min. As expected from known distribution of TSPO in human brain, the distribution of activity was widespread and fairly uniform in gray matter of cerebral cortices and cerebellum, basal ganglia, and thalamus. The unconstrained two-tissue compartment model provided significantly better fit than the one-tissue compartment model, consistent with the presence of significant amounts of both specific and nonspecific binding in human brain.

1.4.1.3. Differential affinity

In the initial human whole-body and brain studies, two subjects were found to have a strikingly different time course of radioactivity in brain and periphery, including markedly faster washout[36, 37]. These two unusual subjects showed a higher peak radioactivity in brain at an earlier time. Activity washed quickly from brain and was at half of the peak concentration within only 6–7 min, compared to the other 10 subjects. In addition, brain activity remained almost constant after ~ 20 min.

In the whole body images, these unusual subjects had decreased activity in organs with high TSPO density—that is, brain, lungs, heart, spleen, and kidneys. In fact, spleen, lungs, and kidneys could not be identified visually. Furthermore, the activities in liver and gallbladder of these unusual subjects were much higher than in the other subjects.

One of the two subjects reported taking ibuprofen (1000 mg per day for several days/weeks) prior to the first scan. To test whether the ibuprofen may have blocked radioligand binding in brain, after ~ 100 days off ibuprofen and 114 days after the first PET scan, we repeated the brain scan in this subject. The second scan was almost identical to the first in terms of peak uptake and washout rate.

These unusual subjects were initially referred to as "non-binders," reflecting the apparently lack of interaction between ^{11}C-PBR28 and the target protein. In vitro binding assays using peripheral leukocytes isolated from blood samples taken from non-binders showed that these subjects expressed TSPO with ~50-fold lower affinity for ^{11}C-PBR28 than binders[38]. Later binding studies showed that there exist three patterns of ^{11}C-PBR28 binding in human subjects[39, 40]. High affinity binders (HABs) behave as though they express only TSPO that binds with high affinity, low affinity binders (LABs) behave as though they express only TSPO that binds with low affinity, and mixed affinity binders (MABs) behave as though they express both a high- and low-affinity version of TSPO. Genetic studies determined there is a strong association between TSPO binding status and the rs6971 (Ala147Thr) single nucleotide polymorphism on the TSPO gene[29]. HABs lack this polymorphism, LABs are homozygous, and MABs are heterozygous. As the allelic frequency of the polymorphism is 30% based on population studies, 9% are expected to be LABs. Caucasians and African Americans have highest prevalence of the polymorphism while it is rare in Han Chinese and Japanese. Our ex vivo study showed that statistical correction for TSPO genotype improves the ability of ^{3}H-PBR28 to detect differences in TSPO density between controls and patients with schizophrenia in the dorsolateral prefrontal cortex[27]. Therefore, clinical studies have since employed the strategy of excluding LABs and performing statistical correction to remove the effect of systematic physiological differences in ^{11}C-PBR28 binding between HABs and MABs.

1.4.1.4. ^{11}C-PBR28 in Alzheimer's disease

In our first AD PET study using ^{11}C-PBR28, we found that AD patients had greater binding than MCI patients or controls[41]. The largest differences were seen in parietal and temporal regions. ^{11}C-PBR28 binding correlated with clinical severity, brain atrophy, and earlier age of symptom onset. 11C-PBR28 binding did not conclusively correlate with amyloid burden as measured with ^{11}C-Pittsburgh Compound B. This study used arterial blood sampling to calculate total distribution volume using the two-tissue compartment model, with correction for free fraction of radioligand. Even after TSPO genotype correction, variance of the PET data was relatively large (coefficient of variance = 34 - 73%).

We later used a simplified ratio method to measure ^{11}C-PBR28 binding in a larger sample (25 AD patients, 11 MCI patients, and 21 controls)[42]. By defining ^{11}C-PBR28 binding as uptake in a target region divided by that in cerebellum (standardized uptake value ratio, SUVR), we were able to detect differences between AD patients and controls in more regions with larger effect sizes. Variance of the PET data was much lower. Cerebellum was chosen because it is a large brain region relatively spared in AD (particularly early in disease). This simplified ratio method removes the need for arterial sampling and reduces the amount of time needed on the PET scanner.

1.5 Dose Rationale and Risk/Benefits

Based on prior human experience and human dosimetry data, we expect the proposed injected activity of ^{11}C-PBR28 (up to 20 mCi) to be safe without expected toxicity.

2 Study Objectives

Primary objectives

1). Determine feasibility of performing ^{11}C-PBR28 PET in patients with DLBD.

2). Determine effect size of ^{11}C-PBR28 binding in patients with DLBD vs. controls.

Our hypothesis is that ^{11}C-PBR28 binding will be greater in DLBD patients than controls, with largest differences in temporal and limbic regions.

Secondary objectives

1). Identify potential reference regions for ^{11}C-PBR28 binding in patients with DLBD.

Our hypothesis is that correcting cortical ^{11}C-PBR28 binding to that in the cerebellum, a region typically spared from pathology early in DLBD, will reduce intra-subject variance, avoid need for arterial catheterization, and improve sensitivity for detecting differences between DLBD patients and controls. We will also explore other potential reference regions such as cortical white matter.

2). Determine if ^{11}C-PBR28 binding correlates with cognitive impairment and Parkinsonism in patients with DLBD.

We postulate that ^{11}C-PBR28 binding will correlate with clinical severity (cognitive impairment and Parkinsonism) and the amount of atrophy on MRI.

3 Study Design

3.1 General Design

Subject selection. Patients and controls will be recruited from the ADRC, CUMC clinics, and direct referrals. Target accrual is n = 4 completers in each group (DLBD and controls). ADRC subjects who have already undergone GBA and ApoE genotyping through ADRC participation will be preferentially enrolled. If possible, only previously-determined GBA mutation carriers will be included. However, if not enough GBA mutation carriers are recruited to meet target enrollment we will also enroll GBA non-carriers who are also known ApoE4 noncarriers. This strategy is designed to reduce the inclusion of patients with co-existent AD-related tangle pathology, as GBA mutation and ApoE4 non-carrier status are both associated with pure DLBD pathology[14-16]. If we are still unable to meet target enrollment then we will include patients of unknown GBA and ApoE genotype. However, these patients will have ApoE genotyping performed in this study, and we will perform post-hoc analysis to see if ApoE4 status affects ^{11}C-PBR28 binding. ApoE testing will be done for research purposes only and results will not be disclosed to subjects. Subjects must have capacity to provide informed consent and consent will be obtained prior to study procedures.

Screening procedures. Subjects will undergo screening including history, physical examination, routine laboratory studies, neurological examination and brain MRI. Patients meeting clinical criteria for either dementia with Lewy bodies or Parkinson's disease dementia (collectively considered as DLBD) will be included. Diagnosis will be made via consensus conference using clinical criteria[42]. Screening will also 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[27, 41]. Clinical evaluation will include Montreal Cognitive Assessment (MoCA), Clinical Dementia Rating scale – sum of boxes (CDR-SB), and Unified Parkinson's Disease Rating Scale (UPDRS).

PET imaging procedures. Subjects will undergo ^{11}C-PBR28 PET in the CUMC Kreitchman PET center on a Siemens Biograph mCT. Prior to 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-PBR28 will be injected intravenously over 60 seconds. 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. Radioactivity in plasma will 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].

Image analysis. PET images will be analyzed using two methods. First, kinetic analysis will be performed using PMOD software (PMOD Technologies). Subject MR images will be coregisered to native space PET images and then segmented to define gray matter regions of interest (ROIs): prefrontal cortex, combined superior and inferior parietal lobule, occipital cortex, inferior temporal cortex, combined middle and superior temporal cortex, hippocampus, parahippocampal gyrus, anterior cingulate, amygdala, insula, and cerebellum. These regions have been selected based on where the largest number of Lewy bodies have been previously reported[45]. The two-tissue kinetic model using time-activity curves from each ROI and the metabolite-corrected arterial input function will be used to calculate total distribution volume, corrected for free fraction of radioligand in plasma (V_T/f_P). Second, the cerebellum will be used as a pseudo-reference region to calculate standardized uptake value ratios (SUVR) for each target ROI. To determine the best time interval for non-invasive measurement of TSPO binding in DLBD, we will calculate SUVR using different time intervals and compute correlation coefficients with the gold-standard measurement V_T/f_P. The time intervals that correspond to SUVR values that have strongest correlation with V_T/f_P will be used to calculate SUVR for the group comparisons between patients with DLBD and controls.

Statistical analysis.

Primary outcome

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 (DLBD vs. controls) divided by the standard deviation.

Secondary outcomes
Coefficient of variation (%COV = standard deviation / mean) will be calculated for controls and patients with DLBD 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-PBR28 binding (V_T/f_P and SUVR) and clinical severity. Severity will be measured using CDR-sum of boxes, MoCA score, and UPDRS. We will also look for correlations between ^{11}C-PBR28 binding and atrophy, as determined using ROI volumes derived from MRI data.

3.2 Primary Study Endpoints

Because the drug used in this study is a radioligand given at tracer doses, there are no clinical endpoints of the study.

The primary outcome measures are ^{11}C-PBR28 binding (using both V_T/f_P and SUVR). We expect binding will be greater in DLBD patients than in controls, with greatest difference in occipital, temporal, and parietal regions. We expect that ^{11}C-PBR28 binding in cerebellum will not differ between DLBDpatients and controls and that this region can be used as a pseudo-reference region for non-invasive measurement of TSPO binding in DLBD. If we find that ^{11}C-PBR28 binding is different between patients and controls, we will explore other possible methods of non-invasive measurement. These methods include a cluster-based approach[46] or use of other potential pseudo-reference regions, such as white matter or whole gray matter.

3.3 Secondary Study Endpoints
N/A

3.4 Primary Safety Endpoints
N/A

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Inclusion criteria for patients:

1. Age 60 and older
2. Meet criteria for either a) dementia with Lewy bodies, or b) Parkinson's disease dementia
3. Able to provide informed consent
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.

Inclusion criteria for controls:

1. Age 60 and older
2. Normal cognitive and motor function based on neurological examination
3. Written and oral fluency in English
4. Able to participate in all scheduled evaluations and to complete all required tests and procedures.
5. 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.

Non-English speaking subjects will be excluded. The rationale is that because of the small scope of the study, we expect to reach accrual target with English speakers only. Also, because of the small scope we may not have resources for providing Spanish-speaking staff at all study visits.

4.2 Exclusion Criteria

1. Past or present history of certain brain disorders (other than DLB or PDD in the case of patient participants).
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. Low affinity binding on TSPO genetic screen
7. Currently taking anticoagulant drugs (e.g., warfarin)
8. Women who are able to become pregnant

4.3 Subject Recruitment and Screening

Patients and controls will be recruited from the ADRC, CUMC clinics, and direct referrals. Approximately 50% of enrolled subjects are expected to fail screening procedures or withdraw from the study. We therefore plan to screen 8 subjects in each group to ensure at least 4 in each group complete study procedures. Screening will also include genetic analysis to exclude subjects homozygous for the rs6971 (Ala147Thr) single nucleotide polymorphism on the TSPO gene.

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

^{11}C-PBR28 is a PET radioligand that binds to the 18 kDa translocator protein. ^{11}C-PBR28 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-PBR28 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).

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 if they are currently taking anticoagulants such as warfarin. Antiplatelet drugs such as aspirin are allowed.

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

^{11}C-PBR28 will be administered the same day of synthesis.

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 3 outpatient visits. See schedule below.

At the first screening visit, subjects will sign informed consent, undergo history and physical and neurological examination, and have blood drawn for routine safety laboratories. Menopause (defined as absence of menses for at least one year) or history of hysterectomy and/or tubal ligation will be confirmed by medical history to exclude women of child-bearing potential. Blood will also be drawn for TSPO, ApoE and GBA genotyping if not previously performed in another study.

Brain MRI will take place at the Neurological Institute Neuro MRI Center on 3T Philips scanner. MRI will be performed to rule out intracranial disease other than PDD or DLB, and for coregistration with the PET images for analysis. An additional screening visit is required for brain MRI; however, this may be performed on the same day as the first screening visit is scheduling allows. Up to 2 screening visits are therefore anticipated. However, additional visits may be necessary if certain tests must be scheduled on different days due to scheduling. If subjects require a sedative for the MRI (e.g., because of claustrophobia), they may receive a onetime dose of lorazepam (0.5 - 1 mg orally) or equivalent dose of an alternative benzodiazepine prior to the MRI. Side effects of anti-anxiety medications may include prolonged sedation (usually less than 8 hours), lightheadedness, motor incoordination, difficulties with balance, or confusion. These effects are temporary and go away after several hours. Subjects will be instructed not to drive, handle machinery, or drink alcohol until the next day. Subjects who receive an anti-anxiety medication will be instructed to bring a companion to the MRI study

visit, and will be accompanied by a member of the study staff during the MRI. The PI will be available in the event of an adverse event during the MRI.

One study visit will be required for the ^{11}C-PBR28 PET scan. At the PET scan visit, one IV catheter will be placed by a qualified clinician for purposes of radioligand injection. After performing an Allen test to ensure ulnar perfusion to the hand, a catheter will be placed in the radial artery by a qualified clinician. After a CT attenuation scan, up to 20 mCi ^{11}C-PBR28 will be injected via the IV. The emission scan will last 90 minutes, during which members of the study team will draw arterial blood samples.

^{11}C-PBR28 PET scans must be completed within 12 months of the brain MRI. Other screening procedures must be done within 60 days prior to the PET scan.

Study Procedures	**Screen [a]**	**Study [b]**
	Day -60 to 1	**Visit 1**
Informed consent	X	
Medical history	X	
Inclusion/exclusion criteria	X	X
Blood draw for genetic tests	X	
MRI	X	
^{11}C-PBR28 PET		X
Laboratory determinations	X	
Neurological examination	X	
Vital signs	X	
Height and weight	X	
Adverse events	X	X

7 Statistical Plan

7.1 Sample Size Determination

This study is intended to generate pilot data as ^{11}C-PBR28 PET imaging has not previously been performed in patients with diffuse Lewy body disease. Therefore, preliminary data to accurately power this study does not exist. However, our previous study using ^{11}C-PBR28 in patients with Alzheimer's disease (a different neurodegenerative disease) showed effect size of 1.5 in brain regions with largest difference between patients and controls. If a similar effect size is seen in patients with diffuse Lewy body disease then we would expect statistically significant differences with sample size of n = 8.

7.2 Statistical Methods

Primary outcome

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 (DLBD vs. controls) divided by the standard deviation.

Secondary outcomes

Coefficient of variation (%COV = standard deviation / mean) will be calculated for controls and patients with DLBD 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-PBR28 binding (V_T/f_P and SUVR) and clinical severity. Severity will be measured using CDR-sum of boxes, MoCA score, and UPDRS. We will also look for correlations between ^{11}C-PBR28 binding and atrophy, as determined using ROI volumes derived from MRI data.

7.3 Subject Population(s) for Analysis

Subjects will be age 60 or older. Healthy controls and subjects who meet clinical criteria for DLB or PDD will be included.

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
- 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 followup. For this study, the study drug follow-up is defined as 24 hours following the last administration of study drug.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should 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 should 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 must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should 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 should 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 should notify the study sponsor 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. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following 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 should 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 must seek information on adverse events (AEs) by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should 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 must 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 should 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 (UP):

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. The PI will be monitoring the study. 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.

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 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 and a Pilot Grant Award from the Alzheimer's Disease Research Center (ADRC) of the Taub Institute for Research on Alzheimer's Disease and the Aging Brain (PI = 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 Committeesanctioned 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 receive compensation commensurate to other studies at CUMC. Subjects will receive payment of $50 for the screening procedures and $200 for the PET scan ($250 total).

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.

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