^{11}C-ACETATE PET/CT IMAGING AS A MARKER OF AMYLOID-INDUCED NEUROINFLAMMATION

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IND Sponsor: The Department of Radiology, University of Pennsylvania, School of Medicine

Study Product: [^{11}C]acetate

Protocol Number: *IRB # 824822*

IND Number: *119, 179*

Current Protocol Version Date: October 27, 2017
Previous Protocol Version date: August 31, 2017

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Study Summary

Title	^{11}C-Acetate PET/CT Imaging as a Marker of Amyloid-Induced Neuroinflammation
Short Title	^{11}C-Acetate PET/CT in neuroinflammation
Protocol Number	IRB #824822
Study Design	Subjects will be part of one of the following cohorts: – Cohort 1: Patients with Mild Cognitive Impairment (MCI) (up to 14) will be recruited primarily from the Penn Memory Center (PMC). Clinical diagnostic criteria (described in further detail in Study Procedures section) will be used to identify subjects who are meet criteria for amnestic MCI. – Cohort 2: Normal control subjects in the same age range (up to 6) will be recruited from a current ongoing study investigating neuroinflammation in late life depression and healthy controls, from the control group in which all subjects receive MRI and LP (IRB 819654) and from the PMC research cohort. – Cohort 3: Cognitively normal subjects with a positive Amyloid PET (up to 5) may be recruited through other ongoing studies or PMC research cohort Screening assessments may take place over several days and may include demographic information, neurocognitive testing, with focus on language and visuospatial/ visuoperceptual measures; if available within 6 months of the study entry these tests may not be repeated. Amyloid brain PET/CT scan (required for Cohort 1 and Cohort 3 only) will be completed within 6 months prior to the ^{11}C-Acetate PET/CT, these scans may be done as part of this research protocol or they may be completed as part of another research study or clinical care, the images and reports for these scans will be reviewed. If the Amyloid brain PET/CT is done as part of this research study it will be done according to standard clinical procedure, using the FDA approved amyloid radiotracer, ^{18}F-Florbetaben (Neuraceq). If the subject does not have a Brain MRI that is deemed acceptable for use for this study within 6 months of ^{11}C-Acetate PET/CT they will be asked to undergo a research Brain MRI after they have consented for this study. Positron emission tomography/Computed Tomography (PET/CT) imaging will be used to evaluate the brain using the investigational radiotracer ^{11}C-Acetate. All patients will undergo a dynamic brain PET/CT scan approximately 60 minutes starting at the time of injection of ^{11}C-Acetate. Some subjects may be asked to undergo an optional cerebrospinal fluid (CSF) collection by lumbar puncture (LP) for additional research testing. Subjects have already undergone CSF collection clinically or as part of another study will not be asked to repeat these procedures.
Study Center(s)	University of Pennsylvania
Objectives	**Primary Objective** • Evaluate whether ^{11}C-acetate uptake is increased in amyloid positive Mild Cognitive Impairment (MCI) subjects compared to amyloid negative healthy controls

	Secondary Objectives • Correlate ^{11}C-acetate uptake to areas of greater amyloid plaque burden quantified on Amyloid PET/CT • Compare ^{11}C-acetate in areas of mildly elevated PET SUVR to areas with high or sub-threshold amyloid burden • Correlate ^{11}C-acetate uptake to levels of inflammatory markers in CSF
Number of Subjects	Up to 30 subjects may be enrolled, however, if a subject in the MCI cohort undergoes an Amyloid brain PET/CT scan as part of this study and it is deemed negative that subject will be considered non-evaluable and will be withdrawn from the study and replaced in the enrollment. The target enrollment is 25 evaluable subjects.
Diagnosis and Main Inclusion Criteria	**Inclusion Criteria for Cohort 1: (amnestic MCI)** 1. Participants will be at least 55 years of age An Amyloid brain PET/CT is required. If a Amyloid brain PET/CT has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a Amyloid brain PET/CT will undergo an Amyloid brain PET/CT as a part of this study 2. Mini-mental status examination (MMSE) score ≥ 24 at screening visit 3. A brain MRI is required. If a brain MRI has been performed within 6 months of ^{11}C-Acetate PET/CT and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study 4. Participants must identify a study partner who is willing to accompany the patient to study visits 5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures. If the patient is unable to provide informed consent, the patient's legal representative may consent on behalf of the patient but the patient will be asked to confirm assent. **Inclusion Criteria for Cohort 2 (Normal Control)** 1. Participants will be at least 55 years of age 2. History of negative brain amyloid PET/CT scan within 6 months of study screening OR negative CSF analysis for AD biomarkers within 6 months of study screening 3. Mini-mental status examination (MMSE) > 27 at screening visit 4. A brain MRI is required. If a brain MRI has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study 5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures. **Inclusion Criteria for Cohort 3 (Cognitively normal Amyloid positive)** 1. Participants will be at least 55 years of age 2. History of positive brain amyloid PET/CT scan within 6 months of study screening 3. Mini-mental status examination (MMSE) > 27 at screening visit

	4. A brain MRI is required. If a brain MRI has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study 5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures. **Exclusion Criteria (for both cohorts)** 1. Inability to tolerate or contraindication to imaging procedures (PET/CT or MRI) in the opinion of an investigator or treating physician 2. History of stroke or other neurological disease that in the opinion of the investigator might interfere with evaluation of the ^{11}C-Acetate scan 3. Any medical or psychological conditions that, in the opinion of the investigator, would compromise the subject's safety or successful participation in the study.
Study Product	**[^{11}C]Acetate**
Statistical Methodology	Several methods for acetate binding quantification will be tested and compared; optimal quantification will be the simplest model that shows strong correlations between acetate binding and amyloid SUVR. ^{11}C-Acetate binding and amyloid SUVR will be analyzed using PMOD for modeling and static uptake estimates and Statistical Parametric Mapping using T1 MRI for co-registration and template-based regional segmentation. We will compare and correlate amyloid and acetate quantification using both Pearson's correlation and non-parametric Spearman's correlation. The expected positive correlation between acetate binding and CSF cytokine levels will be evaluated. Global, regional and voxel based correlations will be performed between acetate uptake and amyloid PET SUVR from MCI subjects. Acetate binding in voxels with amyloid SUVR below, between 100-125%, or above 125% of previously validated thresholds[19,20] will also be compared.

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. Background

1.1. Investigational Agent

^{11}C-Acetate is a radiolabeled imaging agent that has been used for studying tumor cells, myocardial oxidative metabolism and also has been shown to quantify astrocyte activation with positron emission tomography (PET/CT). Acetate metabolism has long been linked to astrocyte activation[1,2] brain metabolism is highly compartmentalized and acetate is actively taken up by astrocytes and used for energy metabolism and lipid synthesis. The latter mechanism accounts for trapping of radiolabel in ^{11}C-Acetate

PET/CT[3] which can be quantified by both static uptake measures and kinetic analysis. This approach has been used to demonstrate astrocyte activation in multiple sclerosis [4] and alcoholism [5]. Acetate PET/CT allows quantification of astrocyte activation without perturbing brain metabolism due to the tracer doses used for PET/CT, operating well below physiologic substrate concentrations.

This proposed protocol will test [11]C-Acetate PET/CT as a marker for astrocyte activation associated with pathologic amyloid deposition in AD. Validating neuroinflammation markers in AD ultimately may guide therapeutic modulation of beneficial and damaging inflammatory responses to slow disease progression, as well as providing new insights into AD pathophysiology.

1.2. Rationale

Alzheimer disease (AD), the most common neurodegenerative cause of dementia, affects millions of Americans with prevalence that will dramatically increase as the population ages. AD is associated with two abnormal protein aggregates: amyloid plaques and tau neurofibrillary tangles, which spread across the brain as the disease progresses, however the precise mechanism of pathogenesis remains unknown. A likely precipitant or accelerant of AD neurodegeneration is neuroinflammation[6]. Glia, including astrocytes, which have myriad functions to support neuron and brain function, are mediators of neuroinflammation. Prior studies suggest that inflammation may have beneficial and detrimental effects on the brain. Astrocytes were noted to be increased in AD in the original observations by Alois Alzheimer, and subsequently demonstrated to be both associated with amyloid plaque and displaying ability to degrade them[7,8]. Thus, reactive astrocytosis may be in part beneficial. However, chemokine-induced inflammation, which induces activation of microglia, the macrophages of the CNS, also causes neuronal damage.

Efforts to image neuroinflammation in AD have recently intensified[9 10,11]. Based on the precedent of amyloid PET/CT, which profoundly increased our understanding of the biology of AD, and the emergence of tau PET/CT, such efforts are likely to provide critical understanding of the link between proteinopathy and neuroinflammation in AD pathophysiology. Much effort thus far has investigated the Translocator protein (TSPO), expressed by microglia[9,10 11,12], including showing an inflammatory response the pre-dementia Mild Cognitive Impairment (MCI) phase of AD[13]. However, targeting microglia does not investigate the full spectrum of neuroinflammation, particularly the potentially beneficial astrocyte response.

Our proposed study aims to use [11]C-acetate PET/CT to preliminarily test and validate methods for imaging astrocyte activation as an early indicator of neuroinflammation in AD. [11]C-Acetate PET/CT has been shown to quantify astrocyte activation *in vivo*, but no reports have evaluated its potential in AD. We propose to test [11]C-Acetate PET/CT as a marker for astrocyte activation associated with pathologic amyloid deposition in AD. We will compare binding between subjects with early stage AD and cognitively normal subjects with positive Amyloid PET and healthy controls. Further, we will investigate the correlation between amyloid and acetate binding. If we find increased astrocyte activation in response to cerebral amyloid by showing a group difference in brain acetate uptake between disease and controls or a strong correlation between acetate and amyloid PET/CT binding. Validating neuroinflammation markers in AD ultimately may

guide therapeutic modulation of beneficial and damaging neuroinflammation to slow disease progression, as well as providing new insights into AD pathophysiology.

1.3. Risk/Benefit assessment

^{11}C-acetate is an investigational imaging drug, which has been extensively used in oncologic, cardiac and brain PET/CT imaging with no evidence of pharmacological effect. No adverse events have been reported in a number of published studies of human experience for ^{11}C-acetate at doses similar to what will be used for this study. The prescribed dose of ^{11}C-acetate will be approximately 20 mCi, with an expected range of the injected dose for most studies of 15 - 25 mCi at the time of injection. In the unlikely circumstance that a participant has an AE, the principal investigator will determine the severity of the AE and the relationship of the event to radiotracer administration and decide the course of action for the study subject, usually with the aid of treating physicians.

Risk/Benefit

^{11}C-Acetate

Participants will undergo one ^{11}C-acetate scan in this study. ^{11}C-acetate is a positron emitting radiopharmaceutical. As such, it poses an intrinsic radiation exposure risk. However, when administered in low tracer doses as a PET imaging agent, as described in this protocol, this risk is felt to be small. The organ and total body doses associated with ^{11}C-acetate PET/CT imaging are felt to be comparable to those associated with other widely used clinical nuclear medicine procedures.

To date approximately 3,450 humans have been studied with ^{11}C-acetate in published literature. There have been no reported AEs associated with the administration of ^{11}C-acetate and no adverse reactions are expected as a result of the IV injection of ^{11}C-acetate. We will continue to monitor this information as the study progresses, including reviewing current literature and our in-house monitoring of this study.

There is potential with intravenous injections, including ^{11}C-Acetate, for allergic reactions. The dose will be delivered intravenously by skilled clinical professionals, and subjects will be monitored for any signs or symptoms of allergic reaction by trained personnel during the PET procedure. Symptoms of an allergic reaction could include itching, rashes, shortness of breath, low blood pressure or wheezing.

[^{18}F]florbetaben

[^{18}F]florbetaben injection (Neuraceq) is an FDA approved radiopharmaceutical for imaging individuals with cognitive impairment and this agent has been used extensively in humans with no evidence of pharmacological effect. In this study we will administer approximately 8.1 mCi (± 20%) of [^{18}F]florbetaben according to the standard procedures used for clinical amyloid PET/CT scans at the University of Pennsylvania.

IV Placement

Venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. It is possible that bruising, dizziness or fainting could occur in some subjects. There is a risk of phlebitis or infection, which is very remote.

PET/CT scan procedure

The PET/CT scan takes place in a small, enclosed space and therefore can be uncomfortable for some people with claustrophobia or musculoskeletal disorders (such as arthritis). Subjects will be made as comfortable as possible and staff will be available throughout the imaging to address any discomfort.

A standard clinical report for ^{11}C-acetate PET/CT scans will not be part of the subject's medical record, the images will be reviewed by trained radiology readers designated by the study PI for the purposes of this study. If there is a potentially clinically important finding that is incidentally discovered on this exam, it will be reported to a treating physician and may result in additional testing that will not be covered by the study budget. Images from the research scan will be stored on PACS.

Brain MRI

Subjects may undergo a research brain MRI if they have not undergone one within 6 months prior to study enrollment. The greatest risk for MRI is a magnetic object flying through the air toward the magnet and hitting the subject. Some of the pulse sequences and/or RF coils are not FDA approved but are considered to pose no more than minimal risk. There is no known health risk associated with exposure to magnetic fields during an MRI. There are minimal risks from the loud noise associated with the MRI scanner and from discomfort of lying on a hard surface. Implanted medical devices and metallic foreign fragments inside the body may pose a risk is subjects were to enter the MRI magnet room. Potential participants will be screened to be sure they do not have an medical contraindications for MRI.

There is a slight risk of anxiety due to claustrophobia. Any participant who experiences anxiety when placed on the MRI scanner will be removed from the scanner, offered reassurance from the technologist or study staff and offered the option of continuing or terminating the scan. If the participant decides that the anxiety associated with the MRI is uncomfortable for them and they wish to terminate the exam at any point, then the examination will be ended at that point. There will be no attempt to coerce the participants to complete exams that they are not comfortable with.

Incidental Findings: It is possible that during the course of the research study, the research staff may notice an unexpected finding(s). Should this occur, the finding(s) will be considered by the appropriate personnel and the PI will determine if the patient should be informed. These possible finding(s) may or may not be significant and may lead to anxiety about a condition and to further work-up by the subject's physician.

Lumbar Puncture (Optional)

Lumbar puncture (LP) may be associated with pain during the performance of the procedure. This is usually temporary and confined to the lower back. Headache may occur in about 5% of elderly people who undergo a LP. Less commonly, in about 1-4% of participants, a persistent low pressure headache may develop, probably due to a leakage of CSF. Lower rates of post-LP headaches have been noted in elderly patients. If a post-LP headaches persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed. Potential but rare risks of lumbar puncture include

infection, damage to nerves in the back, and bleeding into the CSF space. The risk of these is much less than 1%.

No psychological, social or legal risk is expected. While loss of confidentiality is possible, it is felt to be very unlikely due to the small number of professionals involved in the study with knowledge of this information. All clinicians and research staff involved are well trained in HIPAA practices.

There is no anticipated benefit to study subjects as a result of their participation in this study. There is a potential benefit to general society if ^{11}C-acetate PET/CT proves to be a useful imaging agent for evaluating amyloid-induced neuroinflammation.

2. Study Objectives

2.1. Primary Objective

- Evaluate whether ^{11}C-acetate uptake is increased in amyloid positive Mild Cognitive Impairment (MCI) subjects compared to amyloid negative healthy controls

2.2. Secondary Objectives

- Correlate ^{11}C-acetate uptake to areas of greater amyloid plaque burden quantified on Amyloid PET/CT
- Compare ^{11}C-acetate in areas of mildly elevated PET SUVR to areas with high or sub-threshold amyloid burden
- Correlate ^{11}C-acetate uptake to levels of inflammatory markers in CSF

3. Study Design

Subjects will be part of one of the following cohorts:

- Cohort 1:Patients with Mild Cognitive Impairment (MCI) (up to 14) will be recruited primarily from the Penn Memory Center (PMC). Clinical diagnostic criteria (described in further detail in Study Procedures section) will be used to identify subjects who are meet criteria for amnestic MCI.
- Cohort 2: Normal control subjects in the same age range (up to 6) will be recruited from a current ongoing study investigating neuroinflammation in late life depression and healthy controls, from the control group in which all subjects receive MRI and LP (IRB 819654) and from the PMC research cohort.
- Cohort 3: Cognitively normal subjects with a positive Amyloid PET (up to 5) may be recruited through other ongoing studies or PMC research cohort

Screening assessments may take place over several days and may include demographic information, neurocognitive testing, with focus on language and visuospatial/ visuoperceptual measures; if available within 6 months of the study entry these tests may not be repeated. Amyloid brain PET/CT scan isrequired for Cohort 1 and Cohort 3 only, subjects in Cohort 2 may have an Amyloid PET that has been done as part of standard care or another study but this is not required for entry into Cohort 2- In order to reduce costs, subjects may be recruited from other studies that perform amyloid PET/CT (typically with ^{18}F-florbetaben or ^{18}F-florbetapir) or have this test performed clinically. The CMS coverage

with evidence development IDEAS amyloid PET/CT study starts recruitment February 2016, providing Medicare reimbursement for clinical amyloid PET/CT scans for the first time; Penn will be a site for this study and we anticipate that many MCI patients being considered for this experimental ^{11}C-Acetate imaging protocol will qualify to undergo clinical Amyloid PET/CT as part of the IDEAS trial (IRB # 824052). Amyloid PET/CT scans should be completed within 6 months prior to the ^{11}C-Acetate PET/CT. If a subject has not undergone an Amyloid brain PET/CT as part of IDEAS or clinically then they will be asked to undergo a research Amyloid brain scan after they have consented for this study. If it is done as part of this research study the FDA approved radiotracer [^{18}F]Florbetaben (Neuraceq) will be used according to the standard Penn imaging procedure. When available, an Amyloid brain PET/CT scan will be reviewed by an investigator or designee prior to scheduling the ^{11}C-Acetate PET/CT scan. For Cohort 1, if the Amyloid PET/CT scan is deemed to be negative then the subject will be considered non-evaluable and will be withdrawn from the study. Cognitively normal subjects will be assigned to Cohort 2 (negative amyloid PET) or 3 (positive amyloid PET); cognitively normal subjects can also be included in Cohort 2 with negative CSF biomarkers. Non-evaluable subjects will be replaced in the study enrollment total.

If the subject has undergone a brain MRI within 6 months of ^{11}C-Acetate PET/CT it may meet the study requirements for MRI imaging; the scan will be reviewed by a study physician to determine whether it can be used for the purposes of this study. If the subject does not have a Brain MRI that is deemed acceptable within 6 months of ^{11}C-Acetate PET/CT they will be asked to undergo a research Brain MRI after they have consented for this study.

Subjects who qualify for the study will undergo an ^{11}C-Acetate PET/CT imaging session after completing the screening requirements. Patients that meet the eligibility criteria will be approached about study participation regardless of race or ethnic background.

Positron emission tomography/Computed Tomography (PET/CT) imaging will be used to evaluate the brain using the investigational radiotracer ^{11}C-Acetate. Imaging will be performed using a dedicated PET/CT scanner (Philips Medical Systems, Netherlands). The protocol will be performed under the regulatory approval of the IRB and FDA IND that is held by the University of Pennsylvania, Department of Radiology. All patients will undergo a dynamic brain PET/CT scan including approximately 60 minutes of imaging starting at the time of injection of ^{11}C-Acetate. All images will be reconstructed using standard reconstruction techniques. Standardized uptake value ratio (SUVr), the ratio of regional to reference uptake, will be calculated with cerebellum as reference. The cerebellum does not show amyloid accumulation until very advanced stages of AD.

Some subjects may be asked to undergo an optional cerebrospinal fluid (CSF) collection by lumbar puncture (LP) for additional research testing. Subjects have already undergone CSF collection clinically or as part of another study will not be asked to repeat these procedures.

We anticipate enrolling up to 25 participants total (14 in Cohort 1 MCI cohort, 6 in Cohort 2 normal control cohort, 5 in Cohort 3 cognitively normal amyloid positive) who meet eligibility requirements for this study. Accrual will likely occur over approximately 2 years.

4. Participant Selection

4.1. Inclusion Criteria for Cohort 1: Amnestic MCI

4.1.1. Participants will be at least 55 years of age

4.1.2. An Amyloid brain PET/CT is required. If a Amyloid brain PET/CT has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a Amyloid brain PET/CT will undergo an Amyloid brain PET/CT as a part of this study

4.1.3. Mini-mental status examination (MMSE) score ≥ 24 at screening visit

4.1.4. A brain MRI is required. If a brain MRI has been performed within 6 months of ^{11}C-Acetate PET/CT and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study

4.1.5. Participants must identify a study partner who is willing to accompany the patient to study visits

4.1.6. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures. If the patient is unable to provide informed consent, the patient's legal representative may consent on behalf of the patient but the patient will be asked to confirm assent.

4.2. Inclusion Criteria for Cohort 2: Normal Control

4.2.1. Participants will be at least 55 years of age

4.2.2. History of negative brain amyloid PET/CT scan within 6 months of study screening OR negative CSF analysis within 6 months of study screening

4.2.3. Mini-mental status examination (MMSE) > 27 at screening visit

4.2.4. A brain MRI is required. If a brain MRI has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study

4.2.5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures.

4.3. Inclusion Criteria for Cohort 3 (Cognitively normal Amyloid positive)

4.3.1. Participants will be at least 55 years of age

4.3.2. History of positive brain amyloid PET/CT scan within 6 months of study screening

4.3.3. Mini-mental status examination (MMSE) > 27 at screening visit

4.3.4. A brain MRI is required. If a brain MRI has been performed within 6 months of enrollment to this study and of adequate quality that scan may be used for the study analysis, subjects who do not have a brain MRI will undergo a brain MRI as a part of this study

4.3.5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures.

4.4. Exclusion Criteria (for both cohorts)

4.4.1. Inability to tolerate or contraindication to imaging procedures (PET/CT or MRI) in the opinion of an investigator or treating physician

4.4.2. History of stroke or other neurological disease that in the opinion of the investigator might interfere with evaluation of the ^{11}C-Acetate scan

4.4.3. Any medical or psychological conditions that, in the opinion of the investigator, would compromise the subject's safety or successful participation in the study.

All individuals will be told that their choice regarding study participation will in no way change their access to clinical care. This should negate any undue influence or coercion.

4.5. Subject Recruitment and Screening

For the amnestic MCI cohort, potential subjects, or their legally authorized representative (as appropriate), will be allowed to read a written informed consent form. The principal investigator or designee will explain all study procedures, risks, and alternative therapies. The subject and legally authorized representative will have an opportunity to have all questions answered by a physician. The subject will then sign and date the informed consent form, indicating willingness to participate in the study.

Subjects with neurodegenerative diseases are potentially a vulnerable population with compromised mental capacity. Investigators should take extra care to evaluate a patient's ability to give consent. If the subject is capable of giving informed consent then the subject should sign on the consent line of the informed consent form. The designated study partner should sign as well, indicating that they have witnessed the subject's consent, and further agree to participate as a study partner.

If the subject is not capable of giving consent, consent may be given by a legally authorized representative. However, it is expected that all subjects entering this study should at least have the capacity to understand that they are engaging in a research study and should affirm that they do not object to participating, by signing on the Subject Assent line of the consent form. If the legally authorized representative is also the designated study partner they should sign the study partner line of the consent form as well, indicating that they have witnessed the subject's assent, and further agree to participate as a study partner.

All informed consent forms must be approved by the appropriate Institutional Review Board (IRB). In some cases a verbal consent discussion will be carried out by phone prior to scheduling of Amyloid or ^{11}C-Acetate PET/CT scans. Healthy volunteers will be contacted by phone to discuss the study. For candidates for the MCI cohort a verbal consent discussion by phone will only occur if both the patient and the study partner are available for the discussion. No study related procedures shall be performed prior to completion of the informed consent process, and signing of the consent form. A copy of the signed informed consent should be given to the patient and/or their legally authorized representative for their records.

Patients may be receiving care at the Penn Memory Center, Frontotemporal Lobar Degeneration Center, or another clinic of the University of Pennsylvania Health System.

Age matched normal control cohort candidates will be recruited from a current ongoing study investigating neuroinflammation in late life depression and healthy controls, in which all subjects receive MRI and LP (IRB 819654) and from the PMC research cohort. Patients who meet the study inclusion criteria for this imaging protocol will be approached by study personnel for recruitment into this study. The patient will be consented by a physician or research personnel.

All patients being considered for the study and eligible for screening must sign an informed consent for the study prior to any study specific procedures. Following completion of the pretreatment assessments and confirmation of eligibility, patients may undergo the ^{11}C-Acetate PET/CT scan.

A two-part consent process will be used for this study and the consent for administration of the investigational drugs will be discussed again with the patient on the day of the PET/CT scan and documented on a supplemental consent form by a delegated Nuclear Medicine Authorized User prior to administration of any study drugs.

4.6. Subject Withdrawal

The criteria for enrollment must be followed explicitly. If a subject who does not meet enrollment criteria is inadvertently enrolled, that subject should be discontinued from the study. A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. If a subject is prematurely discontinued from participation in the study for any reason, at any time, at either the investigator's discretion or the subject's request, an effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study. The primary reason for discontinuing participation in the study may include, but is not limited to, one of the following:

- Withdrawal of consent for imaging protocol by patient
- Noncompliance with protocol, e.g., the patient fails to appear at one or more imaging procedures
- Development of an intercurrent illness, injury, or medical condition likely to interfere with subject safety, the overall assessment, or the required administration of study medication
- Development of any condition for which the investigator feels study withdrawal is justified.
- Termination of the study

Follow –up information may be obtained for subjects who discontinue the study if possible.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

5. Investigational Agent

5.1. Description

[11]C-Acetate is a radiolabeled imaging agent that has been used for studying tumor cells, myocardial oxidative metabolism and also has been shown to quantify astrocyte activation with positron emission tomography (PET/CT). Acetate metabolism has long been linked to astrocyte activation [1,2]; brain metabolism is highly compartmentalized and acetate is actively taken up by astrocytes and used for energy metabolism and lipid synthesis. The latter mechanism accounts for trapping of radiolabel in [11]C-Acetate PET/CT[3] which can be quantified by both static uptake measures and kinetic analysis. This approach has been used to demonstrate astrocyte activation in multiple sclerosis [4] and alcoholism[5]. [11]C-Acetate PET/CT allows quantification of astrocyte activation without perturbing brain metabolism due to the tracer doses used for PET/CT, operating well below physiologic substrate concentrations.

This proposed protocol will test [11]C-Acetate PET/CT as a marker for astrocyte activation associated with pathologic amyloid deposition in AD. Validating neuroinflammation markers in AD ultimately may guide therapeutic modulation of beneficial and damaging inflammatory responses to slow disease progression, as well as providing new insights into AD pathophysiology.

5.2. Preparation of Study Drug

The manufacturing of [11]C-acetate will occur in the Cyclotron Facility of the Department of Radiology at the University of Pennsylvania. This facility manufactures USP compliant radiolabeled compounds for human use on a daily basis. The drug manufacturing will be fully documented and controlled by a set of Standard Operating Procedures (SOPs) prepared and maintained by the University of Pennsylvania Cyclotron.

5.3. Receipt of Study Drug [11]C-acetate

[11]C-acetate will be delivered to the Nuclear Medicine Division of the University of Pennsylvania Medical Center by a trained Cyclotron team member in single dose vials according to the standard procedures outlined by the Cyclotron Facility. Once the drug has been delivered to the Nuclear Medicine Division all standard hospital procedures will apply for handling, processing, and destruction of any residual amounts if applicable. As [11]C-acetate is a short-lived radiotracer with a half-life of approximately 20 minutes it will be synthesized for same day use; [11]C-acetate will not be stored. The remaining activity in the vials that exists will be stored only until the activity decays to an undetectable level. All vials will be disposed of according to the standard procedures set forth by the hospital and overseen by Nuclear Medicine staff.

5.4. Study Drug Administration: [11]C-acetate

The [11]C-acetate dose will be drawn and measured by the dose calibrator in the imaging facility and administered by IV injection to the patient under the direct supervision of a Nuclear Medicine Authorized User. The prescribed dose of [11]C-acetate will be approximately 20 mCi, with an expected range of the injected dose for most

studies of 15 - 25 mCi at the time of injection, a lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User complete imaging data could be generated. In the dose of ^{11}C-acetate, only a small fraction of the acetate molecules are radioactive. ^{11}C-acetate is administered to subjects by intravenous injection. The injection or imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain. For additional study drug information refer to the Investigator's Brochure for ^{11}C-acetate.

6. Study Procedures and Study Calendar

STUDY CALENDAR

	Screening Assessments[8]	Baseline Assessments	Day of ^{11}C-Acetate PET/CT scan
Informed Consent	X		
Demographics	X		
Brain MRI[1]	X		
Amyloid PET/CT scan[2]	X		
MMSE[3]	X		
Lumbar Puncture[4]	X	X	
Neuropsychological test battery[5]		X	
Pfeffer Functional Activities Questionnaire (FAQ)		X	
^{11}C-acetate PET/CT scan			X
Venous blood sampling[6]			X
Adverse Event Evaluation[7]			X

[1] Patients that have a Brain MRI scan previously performed within 6 months of study enrollment may not need to repeat this scan, images will be reviewed by a physician to determine if the MRI can be used for the purposes of the study, this may occur prior to consent and enrollment in this study.

[2] Many patients will be recruited from other studies that perform Amyloid PET/CT scans (typically using ^{18}F-florbetaben or ^{18}F-florbetapir) the amyloid PET/CT scan should be performed within 6 months of study enrollment. If a subject has not undergone an Amyloid brain PET/CT as part of IDEAS or clinically then they will be asked to undergo a research Amyloid brain scan after they have consented for this study. If it is done as part of this research study the FDA approved radiotracer [^{18}F]Florbetaben (Neuraceq) will be used according to the standard Penn imaging procedure. Only Cohort 1 and Cohort 3 patients will be required to have an Amyloid PET/CT scan. Scan results will be reviewed prior to scheduling the ^{11}C-Acetate PET/CT scan.

[3] Mini Mental State Exam

[4] Optional: Patients who have previously had CSF stored or tested will not be asked to repeat these procedures. Timing of this will vary depending on if the patient has already had this done as part of clinical care or another study. If it is being performed prior to the imaging, lumbar puncture should occur a minimum of 48 hours prior to the ^{11}C-Acetate PET/CT scan.

[5] Neuropsychological test battery will include the following tests:

a. Trail Making Test A and B
b. Category Fluency
c. 30-item Boston Naming Test
d. Rey Auditory Verbal Learning Test (AVLT) - for episodic memory
e. Digit-symbol – for processing speed, executive functioning
f. Lexical fluency (F words in 1 min).
g. Pfeffer Functional Activities Questionnaire (FAQ)
h. Geriatric Depression Scale (GDS)
i. Optional: ADAS-cog, if time permits

[6] Venous blood samples will be taken during the [11]C-Acetate PET/CT imaging session in order to measure radioactive counts in the blood. If a second IV line is not able to be successfully accessed or an investigator does not think the patient can tolerate a second IV for blood draws this may be omitted.
[7] Follow up may occur by telephone or in person, depending on the subject's schedule. Follow up should take place the next available business day (i.e. not a weekend or a holiday). The AE monitoring period is 10 hours following [11]C-acetate injection
[8] Screening assessments may be performed over multiple days

6.1. Initial screening and baseline visits prior to [11]C-acetate PET/CT scan

The investigator will provide for the protection of the subjects by following all applicable regulations. These regulations are available upon request from the sponsor. The sponsor and IRB must review the informed consent form used during the informed consent process, and it must be available for inspection.

Before any procedures specified in this protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent.
- Be given time to ask questions and time to consider the decision to participate.
- Voluntarily agree to participate in the study.
- Sign and date an IRB/IEC-approved informed consent form.

Potential subjects for Cohort 1 will be identified by treating physicians at Penn Memory Center or their referral clinics within the University of Pennsylvania Health System. Potential subjects will be approached by their treating physician about participation in the study. If the patient is interested in participation they will be contacted by one of the investigators and/or study staff for recruitment. The research team will review the study and obtain consent after subjects have adequate opportunity to discuss the study and have all questions answered.

Potential subjects for Cohort 2 and Cohort 3 may be recruited from the subject population of a current study investigating neuroinflammation in late life depression and health controls (IRB 819654) from the healthy control group and from the PMC research cohort. Subjects in that ongoing study all receive MRI and LP and would be evaluated for additional eligibility criteria for this imaging protocol and will be approached by study personnel about participation.

Screening may take place over several days. All screening assessments should be performed prior to the [11]C-Acetate PET/CT imaging session.

Screening assessments include:

1. Demographics (age, gender, race, ethnicity)
2. Medical history (may be taken from medical record review)
3. Concomitant medications recorded
4. Mini Mental State Exam (MMSE)
5. Brain MRI including standard clinical sequences and volumetric MRI. Patients that have a Brain MRI scan that was performed within 6 months of study enrollment may not need to repeat this scan; images will be reviewed by a

physician to determine if the MRI can be used for the purposes of the study, this may occur prior to consent and enrollment in this study.

6. Amyloid brain PET/CT scan (required for Cohort 1 and Cohort 3) - In order to reduce costs, subjects may be recruited from other studies that perform amyloid PET/CT (typically using ^{18}F-florbetaben or ^{18}F-florbetapir. The CMS coverage with evidence development IDEAS amyloid PET/CT study starts recruitment February 2016, providing Medicare reimbursement for clinical amyloid PET/CT scans for the first time; Penn will be a site for this study and we anticipate that many patients being considered for this experimental ^{11}C-Acetate imaging protocol will qualify to undergo clinical Amyloid PET/CT as part of the IDEAS registration trial (IRB 824052). Amyloid PET/CT scans should be completed within 6 months of study enrollment. If a subject has not undergone an Amyloid brain PET/CT as part of IDEAS or clinically then they will be asked to undergo a research Amyloid brain scan after they have consented for this study. If it is done as part of this research study the FDA approved radiotracer [^{18}F]florbetaben (Neuraceq) will be used according to the standard Penn imaging procedure. For Cohort 1 and Cohort 3, the Amyloid brain PET/CT scan will be reviewed by an investigator or designee prior to scheduling the ^{11}C-Acetate PET/CT scan, for subjects in Cohort 2 if an Amyloid PET is available it will be reviewed, however, it is not required for participation in Cohort 2.. If the Amyloid PET/CT scan is deemed to be negative then the subject will be considered non-evaluable and will be withdrawn from the study. Non-evaluable subjects will be replaced in the study enrollment total.

Baseline assessments: may be performed the same day as screening assessments or on a separate day within 90 days of the ^{11}C-Acetate PET/CT, including the day of that study.

Amyloid brain PET/CT scan (if performed as a research scan for this study)

The following procedures will be done on the day of the [^{18}F]florbetaben PET/CT scan

- Weight and height will be recorded
- Injection of [^{18}F]florbetaben

The patient will be made comfortable in a preparatory room, one intravenous line (IV) will be placed, usually one in the arm or hand. Patients will receive approximately 8.1 mCi (± 20%) of [^{18}F]florbetaben intravenously by trained nuclear medicine staff, a lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User complete imaging data could be generated.

Patients will be escorted to the scanner room and positioned in the PET/CT scanner with arms by the side. According to standard procedures used for clinical amyloid PET/CT scans at the Hospital of the University of Pennsylvania, an approximately 15 minute static brain PET scan will be obtained starting at approximately 90 minutes post-injection. A low-dose brain CT scan will be acquired according to standard PET/CT imaging procedures; this CT is used for attenuation correction in PET scan processing. The CT can be performed either before or after the PET transmission scan. There are no separate diagnostic CT scans performed as part of this research.

Brain MRI

If the subject has undergone a brain MRI within 6 months of study entry it may meet the study requirements for MRI imaging; the scan will be reviewed by a study physician to determine whether it can be used for the purposes of this study. If the subject does not have a Brain MRI that is deemed acceptable within 6 months of study enrollment they will be asked to undergo a research Brain MRI after they have consented for this study. Prior to the brain MRI they will be asked to complete an MRI safety form which is a standard assessment form, created by the Department of Radiology at the Hospital of the University of Pennsylvania to assess history of specific prosthesis, surgical implants, and other MRI contraindications. The greatest risk is a magnetic object flying through the air toward the magnet and hitting the subject. Some of the pulse sequences and/or RF coils are not FDA approved but are considered to pose no more than minimal risk. There is no known health risk associated with exposure to magnetic fields during an MRI. There are minimal risks from the loud noise associated with the MRI scanner and from discomfort of lying on a hard surface. Implanted medical devices and metallic foreign fragments inside the body may pose a risk is subjects were to enter the MRI magnet room.

Incidental Findings: It is possible that during the course of the research study, the research staff may notice an unexpected finding(s). Should this occur, the finding(s) will be considered by the appropriate personnel and the PI will determine if the patient should be informed of these findings. These possible finding(s) may or may not be significant and may lead to anxiety about a condition and to further work-up by the subject's physician.

Neuropsychological testing

Neuropsychological test battery will include standardly used tests including:

j. Trail Making Test A and B
k. Category Fluency
l. 30-item Boston Naming Test
m. Rey Auditory Verbal Learning Test (AVLT) - for episodic memory
n. Digit-symbol – for processing speed, executive functioning
o. Lexical fluency (F words in 1 min).
p. Pfeffer Functional Activities Questionnaire (FAQ)

q. Geriatric Depression Scale (GDS)
r. Optional: ADAS-cog, if time permits

***Optional:* Cerebrospinal fluid (CSF) by Lumbar Puncture (LP).**

Some subjects may be asked to undergo an optional lumbar puncture to collect CSF. If subjects agree to the LP it will be performed by a qualified physician who is experienced in performing the procedure. Subjects, or their designated decision maker, will call the investigator to report any adverse events associated with the LP procedure. Patients who have previously had CSF collected and stored or tested will not be asked to repeat these procedures. If it is being performed prior to the imaging, lumbar puncture should occur a minimum of 48 hours prior to the ^{11}C-Acetate PET/CT scan.

The following additional patient data may be self-reported by the patient or obtained from medical chart review: demographics (including gender, date of birth and race).

6.2. Day of ^{11}C-acetate PET/CT scan

Consent for administration of the investigational drugs will be discussed again with the patient on the day of the PET/CT scan and documented on a supplemental consent form by a delegated Nuclear Medicine Authorized User prior to administration of any study drugs.

The following procedures will be done on the day of the ^{11}C-acetate PET/CT scan

- Weight and height will be recorded
- Injection of ^{11}C-acetate.

The patient will be made comfortable in a preparatory room, two intravenous lines (IVs) will be placed, usually one in each arm or hand. The location of the IV used for injection will be documented. Patients will be escorted to the scanner room and positioned in the PET/CT scanner with arms by the side. Patients will receive approximately 20 mCi of ^{11}C-acetate intravenously under the direct supervision of a Nuclear Medicine Authorized User (AU).The prescribed dose of ^{11}C-acetate will be approximately 20 mCi, with an expected range of the injected dose for most studies of 15 to 25 mCi at the time of injection, a lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User complete imaging data could be generated.

Subjects will undergo a 60 minute dynamic brain PET/CT scan starting at approximately the same time as the injection of ^{11}C-Acetate. A low-dose brain CT scan will be acquired according to standard PET/CT imaging procedures; this CT is used for attenuation correction in PET scan processing. The CT can be performed either before or after the PET transmission scan. There are no separate diagnostic CT scans performed as part of this research.

Venous Blood Sampling: Subjects will have two intravenous (IV) lines placed prior to start of scanning, these will generally be placed one in each upper extremity, one IV will be used for injection of the tracer, the other IV will be used for collecting venous blood samples during the course of the scanning protocol. The side of the body used for injection will be documented.

Venous Blood Sampling: Venous blood samples will be collected at approximately 2, 5, 10, 20, 40 and 60 minutes post-injection to measure radioactive counts in the blood. The actual time of each blood draw will be recorded. Sampling may be reduced to fewer time points if experience from the first subjects indicates fewer time points will be sufficient for analysis. The total amount of blood drawn for venous sampling will be approximately 12 mL (about 3 teaspoons). If a second IV line is not able to be successfully accessed or an investigator does not think the patient can tolerate a second IV for blood draws this may be omitted at the discretion of an investigator, this will be recorded in the CRF and will not be considered a protocol deviation.

Adverse events that are grade 3 or higher will be recorded for the period up to 10 hours post injection of the radiotracer. Research personnel will conduct follow up by telephone

or in person, depending on the subject's schedule. Follow up should take place the next available business day (i.e. not a weekend or a holiday).

7. Image Interpretation

7.1. ^{11}C-acetate PET/CT Image Analysis

The low dose CT from the ^{11}C-Acetate PET/CT scans will be reviewed by a trained radiology reader designated by the principal investigator. Any findings that may affect the clinical course of the subject will be reported to the patient's physician. The ^{11}C-Acetate PET/CT data is not validated for clinical use; as such, results will not be reported.

^{11}C-Acetate PET/CT, Amyloid PET/CT and MRI images will be coregistered to allow template-based determination of regions of interest (ROI) on PET. We will obtain venous blood samples to calibrate an image-based arterial input function for the kinetic analysis.

Standardized uptake value ratio (SUVr), the ratio of regional to reference uptake, will be calculated with cerebellum as reference for both the ^{11}C-Acetate and the amyloid PET/CT images. To account for atrophy, partial volume correction will be applied. Regional quantification will be performed using analysis software, such as Scenium (Siemens Medical Systems), PMOD (PMOD Technologies), or SPM (Wellcome Trust). Voxel based comparisons of PET/CT and MRI data may also be performed. We may also use graphical (Patlak) analysis, and with kinetic analysis using a two compartment model, which we have tested on other applications such as brain tumors [14 15 16]. Plasma CO_2 will be estimated from venous samples, which will each be divided into two aliquots, one into acidic and the other into basic solution. Radioactivity in each aliquot will be counted with the difference in counts between acidic and basic used to calculate plasma CO_2, which will be used in kinetic analysis of acetate binding.

Optimal quantification of acetate binding will be the simplest model that shows strong correlations between acetate binding and amyloid SUVR. Acetate binding and amyloid SUVR will be analyzed using PMOD[17] for modeling and static uptake estimates and Statistical Parametric Mapping[18] using T1 MRI for coregistration and template-based regional segmentation. We will compare and correlate amyloid and acetate quantification using both Pearson's correlation and non-parametric Spearman's correlation.

7.2. Measurement of Effect

This protocol is designed to develop relationships between parameters determined from an ^{11}C-Acetate PET/CT imaging biomarker study and ones derived from amyloid PET/CT, MRI, laboratory biomarker assays, and neurocognitive testing.

The primary analysis variables for the study are:
- ^{11}C-Acetate PET/CT SUVR regional quantification
- Amyloid PET/CT SUVR
- Statistical Parametric Mapping segmentation of MRI

Secondary analysis variables are:
- Levels of IL-6, IL-1 and TNFα in CSF

- Neurocognitive test results (tests detailed elsewhere in this document)

8. Statistical Plan

8.1. Sample Size Determination

The primary objective will be to evaluate whether [11]C-acetate uptake is increased in amyloid positive Mild Cognitive Impairment (MCI) subjects compared to amyloid negative healthy controls. With a sample size of 14 MCI and 6 normal controls, assuming 20% precision, we estimate we could detect a difference of 15%; with a smaller sample of 8 MCI subjects and 4 controls, we estimate that we could detect a difference of 30%, However, this is a pilot study which will allow us to determine feasibility and estimate sample size for a future larger study.

8.2. Statistical Methods

Optimal quantification will be the simplest model that shows strong correlations between acetate binding and amyloid SUVR. [11]C-Acetate binding and amyloid SUVR will be analyzed using PMOD for modeling and static uptake estimates and Statistical Parametric Mapping using T1 MRI for coregistration and template-based regional segmentation. We will compare and correlate amyloid and acetate quantification using both Pearson's correlation and non-parametric Spearman's correlation.

The expected positive correlation between acetate binding and CSF cytokine levels will be evaluated. Global, regional and voxel based correlations will be performed between acetate uptake and amyloid PET SUVR from MCI subjects and cognitively normal subjects with a positive Amyloid scan. Acetate binding in voxels with amyloid SUVR below, between 100-125%, or above 125% of previously validated thresholds[19,20] will also be compared.

8.3. Subject Population(s) for Analysis

The primary analysis population will consist of age matched cohorts of amnestic MCI patients and normal controls. Patients will be recruited from the clinical practices of Penn Memory Center, Frontotemporal Lobar Degeneration Center, or other clinics of the University of Pennsylvania Health System. All subjects with an [11]C-acetate PET/CT will contribute to the primary analysis.

9. Adverse Events

9.1. Definitions

Adverse Events are classified as serious or non-serious.
Adverse Events that do not meet the established criteria for Serious Adverse Events (see below) should be regarded as ***non-serious adverse events***.

Adverse Event
An ***adverse event*** (AE) is any untoward medical occurrence in a participant that does not necessarily have a causal relationship with the study procedure. It is any symptom, sign, illness or experience that develops or worsens in severity during the course of the

study procedure. 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

A ***Serious Adverse Event*** (SAE) is any Adverse Event 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

Expected and Unexpected Adverse Events

AEs may be **expected** or **unexpected**:

- An **expected AE** is one that is described in the protocol, the ICF, or the investigator's brochure.
- An **unexpected AE** is one that has not been described in the protocol, the ICF, or the investigator's brochure.

Adverse Event Grading

Grade denotes the severity of the AE.

1 – Mild
2 – Moderate
3 – Severe
4 – Life-threatening or disabling
5 – Fatal

Adverse Event Attribution

Attribution is used to determine whether an AE is related to a study treatment or procedure.

Attribution categories are:

Definite: The AE is **clearly related** to a treatment or procedure
Probable: The AE is **likely related** to a treatment or procedure
Possible: The AE **may be related** to a treatment or procedure
Unlikely: The AE is **likely unrelated** to a treatment or procedure
Unrelated: The AE is **clearly not related** to a treatment or procedure

Important Medical Events

Important Medical Events are those events that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject's health and may require medical intervention.

Preexisting Condition
A preexisting condition is a condition 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 event is resolved or the subject is lost to follow-up.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if the Principal Investigator believes the abnormality is related to participation in the study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be recorded as a serious adverse event. The event should be reported if the Principal Investigator believes the hospitalization is related to participation in the study.

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 treatment follow-up is defined as 10 hours following the administration of the study radiotracer,^{11}C-acetate.

9.2. Recording of Adverse Events

At each study visit session, the Principal Investigator or designee, must seek information from the subject in reference to any adverse events specific to imaging procedures or injection of ^{11}C-acetate that may have occurred by specific questioning and as appropriate by examination. Information on all adverse events should be recorded in as much detail as possible on a source document or the medical record and entered on the Adverse Event Log.

All adverse events occurring during the AE reporting period of 10 hours post ^{11}C-acetate injection will 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 imaging or study participation should be recorded and reported immediately.

The PI should be contacted immediately to determine the reporting requirements for the event as outlined in the protocol.

The clinical course of each event should be followed until resolution, stabilization or until it has been determined that the investigational agent or study procedure is not the cause of the event.

9.3. Reporting of Adverse Events and Serious Adverse Events

It is the responsibility of the Principal Investigator to determine the grade, attribution and expectedness of the event and if the event is reportable according to the requirements outlined in the protocol.

For this investigational agent the following criteria should be met to consider an adverse event reportable:

- The event occurred within 10 hours after the injection of the investigational agent, ^{11}C-acetate.
- All unexpected events regardless of grade or attribution.
- Expected events that are grade 3 or higher.
- Expected events that are possibly, probably or defiantly related to the investigational agent.

When reporting please supply a narrative with as much description of the event as possible (chart note is acceptable) along with the completed AE Log or CRF signed and dated by the Principal Investigator.

The narrative should include:

Protocol number and name
Subject number
Date of onset
A detailed description of the event
If the study procedure was discontinued

9.3.1. Investigator Reporting: Notifying the Study Sponsor

All reportable Adverse Events and Serious Adverse Events must be reported to the study sponsor (The Department of Radiology, IND Support Office) by email within 24 hours of the event. Please include the AE Log and narrative as mentioned above.

If the event is considered serious the Principle Investigator must complete the Serious Adverse Event (SAE) form and fax or email to the study sponsor within 24 hours. All documentation will be kept on file at the study site.

Significant new information on ongoing adverse events should be provided promptly to the study sponsor

9.3.2. Investigator Reporting: Notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB)

requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm.

The Penn IRB requires Principal Investigators to submit AE and SAE reports within 10 working days from the time the investigator becomes aware of the event.

The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below:

- The event occurred within 10 hours after the injection of the investigational agent.
- All unexpected events regardless of grade or attribution.
- Expected events that are grade 3 or higher.
- Expected events that are possibly, probably or defiantly related to the investigational agent.

Penn IRB Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: “Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events” or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria and follow-up/resolution).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

Other Reportable Events to Penn IRB:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.

- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.3.3. Sponsor Reporting

Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND Safety Reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - Associated with the use of the study drug.
  - Unexpected.
  - Fatal or life-threatening.

- ***Within 15 calendar days***
  Any study event that is:
  1. associated with the use of the study drug,
  2. unexpected, and
  3. serious, but not fatal or life-threatening

  -OR-

  – A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - Suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Notifying Participating Investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Additional Sponsor Reporting Requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

9.4. Medical Monitoring

It is the responsibility of the Principal Investigator, Dr. Nasrallah, to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 11 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

10. Data Handling and Record Keeping

10.1. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (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).

All research personnel associated with this study have completed the University of Pennsylvania human subjects training as well as HIPAA Compliance Training. Trained staff will assess eligibility, introduce the study rationale, procedures, study risks, and collect the combined informed consent/HIPAA authorization form. The study team will work to uphold the privacy of the participants in several ways. Communications made among study staff regarding participants will use ID numbers whenever possible and minimize the use of patient name or other identifying information except when necessary for conduct of the study. Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Whenever feasible, identifiers will be removed from study-related information. In data analysis sets, we will use ID numbers and/or patient initials only.

Precautions will be applied to protecting subject privacy and the protected health information detailed below:

1. Name

2. Address
3. Electronic mail addresses
4. Telephone Number
5. Date of Birth
6. Medical Record Number
7. Health Plan ID numbers
8. Social Security Number
9. Any other unique identifying number, characteristic or code
10. Current and past medications or therapies
11. Information from the tests and procedures described earlier in this document
12. Previous procedure, diagnosis and treatment information from institutions other than Penn if it relates to study eligibility
13. Emergency contact number, name, and relationship

Data will be accessible to the study investigators, all study staff, Department of Radiology IND office representatives, Radiation Research Safety Committee members, UPenn IRB and Office of Clinical Research, and the FDA (if desired).

10.2. 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.

10.3. Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

11. Study Monitoring, Auditing, and Inspecting

11.1. Study Monitoring Definition

Study Monitoring is the oversight of the progress of a research study and verifying that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, Good Clinical Practice (GCP), and federal regulations.

Monitoring oversees protocol and regulatory compliance, participant's welfare and safety. Monitoring is a recurring observation of a study as it progresses.

11.2. Study Monitoring

The Study Sponsor is responsible for assigning staff to monitor the research study. The Study Monitor will conduct all monitoring activities in accordance with the Study Monitoring Plan. Study Monitors will review all required regulatory documents, subject charts and Case Report Forms (CRFs) for completion and accuracy.

Study Monitoring Plan

The Study Monitoring Plan will be prepared prior to activation of the study and will be implemented after accrual to the study. The frequency and intensity of monitoring activities depend on several factors, including risk to study subjects, complexity of the study protocol, and implications of the research findings. The higher the risk to subjects, the more frequently monitoring will be conducted. Other factors that may increase monitoring frequency include the projected enrollment number, the/ anticipated rate of subject accrual and protocol compliance.

Study Monitoring Visits

The Principal Investigator will allocate adequate time and space for monitoring activities. The Principal Investigator will also ensure that the Study Monitor or other Compliance or Quality Assurance Reviewer is given access to all requested study-related documents and facilities if necessary.

Monitoring visits usually take place where the study is being conducted. There will be occasions where monitoring will take place in the IND Support office.

Monitoring Visits are usually composed of Study Initiation Visit, Interim Visits and the Study Close-out Visit. Principal Investigators will be informed of upcoming Visits and will be given adequate time to prepare for a Monitoring Visit.

Frequency of Visits

Enrollment will be complete when up to 25 subjects are enrolled in the study. Monitoring visits will be conducted periodically throughout the study as described below:

- The **first monitoring visit** will occur after the first subject from each cohort has completed their research imaging visit.
- A **second monitoring visit** will be conducted when approximately 5 subjects cohort 1 and 5 subjects from the Cohorts 2 and 3 have completed their research imaging visit. 3 subjects form Cohort 1 will be evaluated and 2 subjects from Cohorts 2 and 3 will be evaluated.

- A **third monitoring visit** will be conducted after all subjects have completed their research imaging visits. At this time 3 additional subjects from the Cohort 1 will be evaluated.
  This visit may also serve as the close-out monitoring visit.

Data Review

The study Regulatory Binder, subject charts and Case Report Forms (CRFs) will be reviewed at each monitoring visit.

11.3. Documentation of the Monitoring Visit

All monitoring visit findings will be documented on the Monitor's Report and a copy will be sent to the Principle Investigator. Any findings from the visit will require resolution within 30 working days

11.4. Auditing and Inspecting

The Principal Investigator will also permit study-related audits and inspections required by any University Compliance and/or Quality Assurance group, the EC/IRB and any government regulatory body including the FDA and according to their timeline.

12. Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

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

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. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator or investigator-designated research professional obtaining the consent.

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.

13. Subject Stipends or Payments

Subjects will receive $75 for completing the [11]C-Acetate PET/CT scan appointment. If the subject obtains an LP for the purpose of this study, they will receive an additional $50. If the patient completes the MRI as part of this study then they will receive an additional $50. The compensation will be mailed from the University's Accounts Payable Department. All necessary information (e.g. W9 form) required by Accounts Payable will be collected at the study visits. We will provide parking vouchers for the day of the [11]C-Acetate PET/CT scan appointment, the day of the Amyloid brain PET/CT scan (if completed for this study only) and the day of the Brain MRI (if completed only for this study).

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