1. TITLE PAGE

Protocol Title: Assessment of [^{11}C]ER176 to image translocator protein in brain and whole body of healthy subjects

Short title: [^{11}C]ER176 in healthy subjects

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Total requested accrual (47)
47 Healthy volunteers

Project Uses Ionizing Radiation: ☐ No ☒ Yes (attach *RSC/RDSC documentation)*
☐ Medically-indicated only
☒ Research-related only
☐ Both

IND/IDE ☐ No ☒ Yes *(attach FDA documentation)*
Drug/Device/# IND not yet submitted
Sponsor: Robert Innis
Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☒ No ☐ Yes
Institution_____ FWA #_____
Date of IRB approval_____ *(attach IRB documentation)*
Data and Safety Monitoring Board ☒ No ☐ Yes
Technology Transfer Agreement ☒ No ☐ Yes
Agreement type and number _____Expiration Date_____
Confidential Disclosure Agreement ☒ No ☐ Yes
Samples are being stored ☒ No ☐ Yes

Reading level for consent forms: 8.1 (from “Purpose” to “Conflicts of Interest”)

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ABBREVIATIONS:

HABs: High-affinity Binders
LABs: Low-affinity Binders
MABs: Medium-affinity Binders
MRI: Magnetic Resonance Imaging
PET: Positron Emission Tomography
SNP: Single Nucleotide Polymorphism
SUV: Standardized Uptake Value
TSPO: Translocator Protein
V_T: Distribution volume

Table of Contents

2. PRECIS

i. Objective

Translocator protein 18 kDa (TSPO) is highly expressed in activated microglia and reactive astrocytes in brain, and it may, thereby, be a useful biomarker of neuroinflammation. We developed [^{11}C]PBR28 as a positron emission tomographic (PET) radioligand to bind to TSPO and measure its density. Although [^{11}C]PBR28 has high in vivo specific signal, it is very sensitive to the high and low affinity states of TSPO, which are caused by a single nucleotide polymorphism (SNP) in the fourth exon of the TSPO gene. This co-dominant mutation yields three genetic groups: HH, HL, and LL, where H is the high-affinity form and L is the low affinity form. The frequency of the L allele is ~30%; thus, the frequency of the LL homozygote is ~9%. The affinity of PBR28 to H and L forms differs about 50 fold; thus, LL carriers provide no measureable signal in brain from [^{11}C]PBR28. We recently developed a new TSPO ligand ER176, the affinity of which differs by only 1.2 fold and therefore LL carriers should provide measureable brain uptake. The purpose of this study is to assess the potential of [^{11}C]ER176 to image TSPO in brain, characterize its binding sensitivity in lung of healthy subjects from all three genetic groups, and to do whole-body imaging for biodistribution and estimation of radiation dosimetry in humans.

The present protocol will use a new PET ligand—[^{11}C]ER176 —to 1) perform an initial whole-body scan after [^{11}C]ER176 injection in a single healthy volunteer to confirm wide-spread distribution of radioactivity to different body organs (Phase 0); 2) perform kinetic brain scans in healthy volunteers of 3 different genotypes, with about half of these volunteers undergoing lung scans in the same session (Phase 1), and; 3) perform whole-body imaging in healthy volunteers (Phase 2).

This study will assess the relative robustness of absolute quantitation of TSPO in the brain of healthy subjects, using an arterial input function and pharmacokinetic modeling. In addition, lung imaging will provide in vivo binding sensitivity of [^{11}C]ER176 to TSPO genotype. Furthermore, the whole-body imaging would estimate the radiation-absorbed doses for future use of [^{11}C]ER176 in clinical studies.

ii. Study Population

We will select up to 36 healthy adult female and male volunteers (age 18 and older) of 3 different TSPO genotypes for brain imaging, and up to 11 additional healthy volunteers for whole body dosimetry analysis.

iii. Design

For absolute quantification of TSPO, up to 36 healthy controls (up to 12 each of three TSPO genotypes) will have brain PET imaging using [^{11}C]ER176 and these subjects will have the arterial line and a brain MRI scan. In about half of those subjects from each genotype group, lungs will be scanned in the same session. For radiation dosimetry of [^{11}C]ER176, up to 11 subjects will have whole-body PET imaging. These subjects will not have arterial line and MRI scans.

iv. Outcome Measures

The primary outcome measures are: (a) To assess absolute quantitation of TSPO with [^{11}C]ER176, we will determine the identifiability and time stability of distribution volume in the brain calculated with compartmental modeling. The difference in mean distribution

volumes among subjects with different genotypes would be used to evaluate the genotype sensitivity of [^{11}C]ER176. (b) To assess whole-body biodistribution and dosimetry of [^{11}C]ER176 we will use the organ time-activity curves.

As secondary outcome measure, we will examine the effect of polymorphism on [^{11}C]ER176 binding in lungs because lungs have much higher density of TSPO and may be more effective to show whether ER176 is sensitive to the SNP.

3. SCIENTIFIC RATIONALE

PET Imaging to measure brain inflammation

Brain inflammation activates microglia and causes them to over-express the translocator protein (TSPO) (Stephenson *et al* 1995). Measurement of microglia activation, defined by the number of available TSPO binding sites, is used as a surrogate bio-marker of active brain inflammation (Banati *et al* 1997). Positron emission tomography (PET) imaging can quantify TSPO density *in vivo* using radioligands that cross the blood-brain barrier and bind to TSPO sites. One TSPO-selective radioligand, [^{11}C]PK 11195, has been used to identify areas of brain inflammation in several neurological diseases, including Alzheimer's disease (Cagnin *et al* 2001), stroke (Pappata *et al* 2000), multiple sclerosis (Banati *et al* 2000), amyotrophic lateral sclerosis (Turner *et al* 2004), HIV dementia (Hammoud *et al* 2005), Huntington's disease (Pavese *et al* 2006), and Parkinson's disease (Gerhard *et al* 2006).

Unfortunately, [^{11}C]PK 11195 has limitations as a radioligand. First, [^{11}C]PK 11195 has relatively low brain uptake, causing poor signal-to-noise in image analysis. Second, [^{11}C]PK 11195 binds to other compounds besides TSPO. Less than 50% of [^{11}C]PK 11195 is specifically bound to TSPO in human myocardium (Charbonneau *et al* 1986). These amounts of specific binding are too low for stable quantitative analysis. Therefore, the results of studies using [^{11}C]PK 11195 are difficult to interpret and imaging studies with an improved radioligand are warranted.

[^{11}C]ER176, a novel radioligand for brain inflammation

After synthesizing and screening more than 100 candidates, we developed a new ligand [^{11}C]ER176 (*O-methyl*-^{11}C](*R*)-*N-sec*-butyl-4-(2-chlorophenyl)-*N*-methylquinazoline-2-carboxamide)(Fig. 1), which has similarly high affinity to HH and LL. Ki values of ER176 measured in human leucocytes and [^{3}H]PK 11195 was 1.4 and 1.6 nM for HH and LL, respectively. ER176 also has lipophilicity of clog D = 3.80, which is appropriate for brain imaging.

In vivo monkey data

We performed a pair of baseline and pre-blocked PET scans of [^{11}C]ER176 in two rhesus monkeys (a total of two baseline and two pre-blocked scans) and confirmed that [^{11}C]ER176 has high levels of specific binding. In both monkeys, binding blockade was measured using brain activity. In addition, in one monkey, a more complete measurement of binding was performed by measuring total distribution volume, V_T, using metabolite-corrected arterial input function, i.e., [^{11}C]ER176 levels in arterial plasma. For the pre-blocked scans, PK 11195 (5 mg/kg) was used as the blocking agent. In the baseline scans, the mass dose of ER176 was 0.111 μg/kg on average. The scans were performed under

isoflurane anesthesia with monitoring blood pressure, heart rate, and ECG. These mass doses did not cause changes in vital signs.

Based on brain activity at late time points (60 - 120 min), 60% of brain activity in baseline scans was from specific binding (Fig. 1). However, these measurements did not take into account changes in blood data. V_T measured with arterial input function and Logan plot was 11 – 17 mL/cm^3 in baseline and 1.6 – 2.5 mL/cm^3 in pre-blocked scans. Therefore, based on the complete measurements, ~85% of activity of the baseline scan was specific binding.

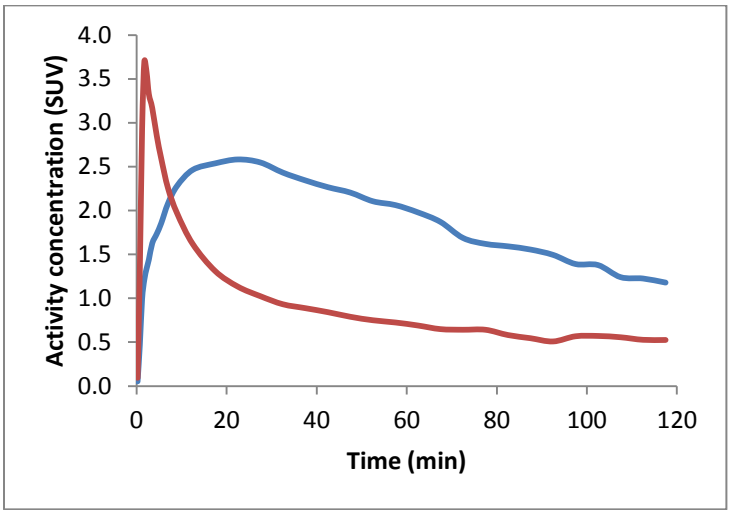

Fig. 1 Mean time activity curves at baseline (red) and pre-blocked with PK 11195 (blue) scans

Absorption, Distribution, and Metabolism

In the baseline and the pre-blocked scans of one monkey described in section 2, we measured [^{11}C]ER176 levels in arterial plasma (Fig. 2). The pre-blocked scan showed about five times higher [^{11}C]ER176 levels in arterial plasma. TSPO exists in several peripheral organs such as lungs, heart, and kidneys. With another ligand [^{11}C]PBR28, by performing whole body imaging and arterial blood sampling at the same time, we confirmed that the increase of [^{11}C]PBR28 in arterial plasma was caused by binding blockade to TSPO in peripheral organs (Imaizumi *et al* 2008). Therefore, the increase of [^{11}C]ER176 levels in arterial plasma in the pre-blocked scan is consistent with the presence of specific binding to TSPO in peripheral organs.

The arterial data in the two monkey scans showed fast clearance of [^{11}C]ER176, the concentration of which decreased to a half of the peak in 3 min after injection and to < 10% in 30 min. Clearance was 526 and 195 mL/min for the baseline and blocked scan and the terminal half life by tri-exponential fitting was 24 and 49 minutes, respectively.

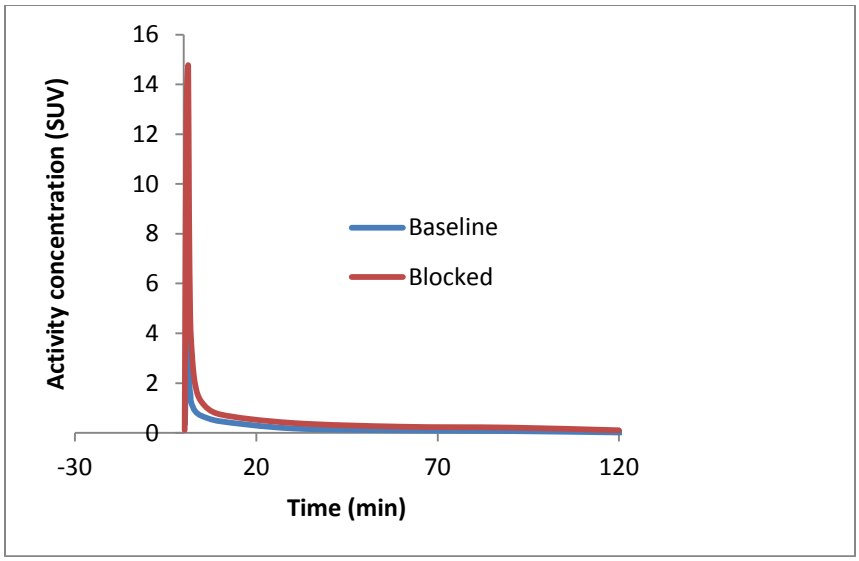

Fig. 2. [^{11}C]ER176 levels in arterial plasma in baseline (blue) and pre-blocked (red) scans.

4. STUDY OBJECTIVES OR HYPOTHESIS

A. Statement of hypotheses

The objective of this study is the comprehensive evaluation of [^{11}C]ER176 as an inflammation tracer in healthy volunteers, with regards to distribution volume (V_T) values, V_T time stability and differential uptake across binders, mixed-affinity binders and non-binders.

For the dosimetry study, we expect an overall effective dose similar to that of other ^{11}C-labeled radioligands (~5 µSv/MBq) (Zanotti-Fregonara and Innis 2012).

B. Primary and secondary goals

The first goal of this study is to study the brain uptake of [^{11}C]ER176 and perform kinetic modeling of [^{11}C]ER176 in healthy subjects of three different TSPO genotypes. The primary outcome measures will be the distribution volume of the radioligand and its stability over time (i.e. whether the distribution volume is constant for scans of different duration). Distribution volume is proportional to the density of receptors and is equal to the ratio at equilibrium of uptake in brain to the concentration of parent radiotracer in plasma. As the genotype sensitivity is best visualized in lungs, we will do lung imaging in about half the subjects during brain scans to study [^{11}C]ER176 binding sensitivity to.TSPO polymorphism. This polymorphism is due to the non-conservative amino-acid substitution at position 147 from alanine to threonine (Ala147Thr) in the fifth transmembrane domain of the TSPO protein (Owen *et al* 2012). This polymorphism has categorized the TSPO binding in humans by [11C]PBR28, a highly genotype-sensitive TSPO ligand, into high affinity binders (HABs), medium affinity binders (MABs) and low-affinity binders (LABs) whose TSPO genotype match to HH, HL, and LL, respectively.

Another goal of this study is to study the biodistribution and dosimetry of [^{11}C]ER176.

5. SUBJECTS

A. Description of study populations

- Up to 36 healthy volunteers for brain and/or lung imaging.
- Up to 11 healthy volunteers for dosimetry.

B. Inclusion criteria

- Age 18 or older.
- Able to give written informed consent.
- Healthy based on medical history, physical examination and laboratory testing.

C. Exclusion criteria

- Any current Axis I diagnosis.
- Clinically significant laboratory abnormalities.
- Positive test for HIV.
- Unable to have a MRI scan.
- History of neurologic illness or injury with the potential to affect study data interpretation.
- History of seizures, other than in childhood and related to fever.
- Recent exposure to radiation (i.e., PET from other research) which when combined with this study would be above the allowable limits.
- Inability to lie flat on camera bed for at least two hours.
- Pregnancy or breast feeding.
- Able to get pregnant but does not use birth control.
- Drug/alcohol abuse or dependence
- Are taking prescription or over-the-counter medications for pain, fever or other inflammation. Such medications include prednisolone (Orapred), aspirin (Ecotrin), ibuprofen (Advil), acetaminophen (Tylenol) and many others. We will review your medications to see if you can be in the study. If you are taking any medication that is not permitted, you may be in the study if the medication can be safely stopped for three weeks. We will work with you and your doctors to stop a medication if necessary. We need your written permission to contact your doctor.

Please note that exclusion criteria for the dosimetry subjects are the same reported above, except for MRI contraindications, because an MRI will not be performed in these subjects.

6. STUDY DESIGN AND METHODS

i. Study Overview

This is a single center study in healthy volunteers and entail the following: initial evaluation, brain MRI and [^{11}C]ER176 PET scans for brain imaging with about half of the volunteers undergoing lung scans in the same session, and for whole-body dosimetry imaging. Healthy volunteers will be screened under screening protocol 01-M-0254. The participants must be documented to meet inclusion and exclusion criteria and sign an informed consent document. There are three phases in this protocol that follows the suggested pathway for first-in-human trial of any new PET radiotracer (Zanotti-Fregonara and Innis 2012).

Phase 0: A whole-body PET scan after [^{11}C]ER176 injection in a single healthy volunteer to confirm the radioactivity distributes across different organs and the radiation dose is safe and below the theoretical maximum for any organ in the body. This single subject will be a high-affinity binder and recruited using the consent of whole-body dosimetry.

Phase 1: Kinetic brain PET scans will be performed in healthy volunteers (n = up to 36) to quantify [^{11}C]ER176 binding parameters in brain. In this phase, we will recruit up to 12 subjects for each group of HABs, MABs and LABs. These subjects will have arterial lines placed for accurate measurement of input function and have a brain MRI for anatomical localization. In about half of the subjects for each group, we plan to image both brain and lungs in a single scan session lasting 120 minutes. The rationale for lung imaging is as follows.

Although the brain has a relatively low density of TSPO, it can demonstrate genotype sensitivity of binding for radioligands that have high in vivo specific binding, like [^{11}C]PBR28. However, in genotype-sensitive ligands with low in vivo specific binding, like [^{11}C]PK 11195, the sensitivity is only seen in peripheral organs that have relatively high TSPO density (Kreisl *et al* 2010). In contrast to [^{11}C]PBR28, which showed significantly lower uptake in five target organs known to express TSPO (namely brain, heart, lung, spleen and kidney), [^{11}C]PK 11195 showed significantly lower uptake only in lungs and heart of low-affinity binders (Kreisl *et al* 2010). In vitro results suggest that the newly developed TSPO ligand ER176 is not sensitive to genotype. In order to effectively evaluate the genotype sensitivity of ER176 in vivo, measuring the lung uptake will be very useful.

Uninterrupted dynamic brain scans accompanied by intermittent measurement of arterial input function are best for quantifying radioligand binding in brain. Nevertheless, some interruptions in brain scan acquisition lasting for a few minutes in the middle of a scan session do not interfere with binding quantification, given that early and late time point acquisitions are most important for binding quantification in brain, and that full quantification is possible by pharmacokinetic modeling. In fact, brain quantification was successfully performed when rhesus monkeys underwent whole-body imaging for radiation dosimetry that involved intermittent scan acquisition for all organs including brain (Kimura *et al* 2011). Evidence from previous whole-body scans performed in healthy human volunteers using [^{11}C]PK 11195 and [^{11}C]PBR28 suggest that the difference in lung uptake between binders and non-binders is best determined between five and 20 minutes post-injection of radiotracers (Kreisl *et al* 2010). Unlike brain uptake, which requires full quantification by kinetic modeling along with arterial sampling, uptake in the lung will simply be simply measured as area under the time-activity curve (Kreisl *et al* 2010).

In the current study, for subjects undergoing brain and lung scans in a single session, the brain will be scanned for approximately the first 10 minutes, the lungs for roughly the next 10 minutes, and the brain for the rest of the scan duration. Thus, uptake in the lung, which will be measured as area under the time-activity curve, will be only minimally influenced by blood flow at early time points or by any possible contamination by radiometabolites at later time points. Please note that this proposed scanning schedule is based on the time-activity curves in lung of [^{11}C]PK 11195 and [^{11}C]PBR28. Because

[^{11}C]ER176 may differ from these two radioligands, we wish to maintain flexibility. Thus, approximately half of the subjects will have both brain and lung imaging, but this percentage may vary depending on the fidelity of the results for each organ. That is, if we find that intermediate lung imaging compromises the quality of brain data, we will acquire brain scans only for all other subjects. In addition, we intend to scan the lung for about 10 minutes, but retain the flexibility to scan it for more or less time, if necessary.

Phase 2: Whole-body PET scans in healthy volunteers (n = up to 11) for estimation of biodistribution and radiation dosimetry of [^{11}C]ER176. These subjects will not have MRI scan and arterial sampling, but may have venous sampling to measure [^{11}C]ER176 levels.

PET scans will be followed by safety monitoring with laboratory blood and urine tests. The MRI for Phase 1 will be obtained within 12 months of the PET scans. MRI and PET will take approximately 1 h, and 4 h, respectively.

For participation in this study, 2 visits will be necessary as below.

Number of visits and time commitment of participants
1st visit: Pregnancy test and MRI (2 hours)
2nd visit: Pregnancy test and PET, blood and urine tests (4 hours)

If the scan is cancelled or incomplete due to synthesis failure of a radioligand or camera problems and the subject did not receive any radioactive material, a 3rd visit may be necessary. Participants can have visits on consecutive days. If the MRI can be performed on the same day as one of the PET scans, the number of visits can be reduced to 1. For dosimetry, only 1 visit may be sufficient, because there will be no brain MRI.

Subjects will be outpatients for all visits, as none of the procedures requires hospitalization.

All procedures performed under this protocol are for research purposes only. Radiation for this study is for research purposes only.

ii. Recruitment

Healthy volunteers will be recruited by the Molecular Imaging Branch (MIB) NIMH or CES NINDS and from the community and NIH through IRB-approved advertisements in newspapers and newsletters, private physicians, social service agencies, and the e-mail list of NIH IRTA students.

iii. Screening

Healthy volunteers will be screened under protocol 01-M-0254 "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate) to have basic screening such as physical exams, blood and urine tests and a medical and psychiatric history.

To determine binding status, blood will be drawn under the screening protocol. Eligibility for the current protocol will depend on genotype, because LABs represent only

10% of the population. Thus, we will need to screen a relatively large number of subjects in order to obtain equal numbers of HABs, MABs, and LABs.

Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures.

iv. Study procedures

MRI

Participants will have a brain MRI scan for anatomical localization. MRI scanning will be done on 3 Tesla scanner located at the NIH Clinical Center and will take about one hour. Participants who have had an adequate brain MRI performed at the NIH within 12 months of the dedicated brain PET scan will be exempt from this part of the protocol and their earlier MRI will be used for anatomical localization. Participants undergoing dosimetry scan will not have an MRI scan.

PET procedures

1) Radioligand

[^{11}C]ER176 will be administered via an indwelling intravenous catheter over ~3 minutes, within 120 minutes after synthesis of the radioligand. The radioligands is stable during this period.

2) PET acquisition for brain and lung imaging

PET scans will be performed using the GE Advance or the PET/CT. The choice of the scanner for a particular study will depend on scanner availability. Participants will be placed on the scanner bed with their head held firmly in place with a thermoplastic mask fixed to the bed. A ^{68}Ge transmission scan will be performed before the PET scan to measure and correct for attenuation. If we use the PET/CT machine, transmission scans will be obtained with the CT scan. Tracer infusions will be performed in the nuclear medicine department, when the subject is already on the scanner bed.

After an intravenous bolus of about 20 mCi, arterial blood samples will be drawn from the arterial catheter during the PET scan. We will collect about 23-25 arterial samples. First, blood samples will be drawn every 15 seconds after injection for about 3 to 5 minutes, and then they will be collected every 5 or 10 minutes. The total amount of arterial blood will be about 100 mL. PET images will be acquired in three-dimensional mode with increased length of frame for a total of 120 minutes. For scans that involve both brain and lung imaging, we will scan the brain for approximately the first 10 minutes. The scanner bed will then be moved to the chest region in order to scan the lungs for about 10 minutes. The bed will then be moved back to the head region for the remainder of the scan.

At the end of the scans, the arterial and venous lines will be removed and the subject will be instructed to void frequently to minimize radiation exposure.

3) PET acquisition for dosimetry

Whole body dosimetry scans will be performed with the GE Advance or the PET/CT. The choice of the scanner for a particular study will depend on scanner availability. No arterial sampling will be done for dosimetry studies. Both a pre-injection transmission scan and a series of dynamic emission scans will be acquired. Each subject will be imaged in

contiguous segments from the top of the head to a point below the gonads. To minimize extraneous motion, all subjects will wear a head-holding mask and will have their arms and abdomen wrapped with body-restraining sheets. This may add some additional discomfort. Before injection of the radioligand, a transmission scan will be acquired for subsequent attenuation correction. Then, an initial set of dynamic emission scans will be acquired following the intravenous injection of [^{11}C]ER176. The acquisition of each cycle will begin with an emission scan at the first bed position (*i.e.*, the head), and continue by moving the bed distally to the next body segment. Venous samples up to 30 mL may be obtained to measure [^{11}C]ER176 levels in the blood.

If the PET scans detailed above are cancelled or incomplete due to synthesis failure of a radioligand or camera problems and the subject did not receive any radioactive material, the scan will be repeated on the same or another day. The transmission scan may also be repeated.

Arterial Line Placement

A radial artery catheter will be inserted by the NIH Clinical Center Anesthesiology Department, after the presence of adequate ulnar collateral flow has been established. The skin at the site of the puncture will be anesthetized with 1% lidocaine. The catheter will be perfused (3 mL/hr) with heparin solution (600 units/L) during the procedure. The amount of heparin infused will be much smaller than the amount necessary to produce any systemic anticoagulant effect.

v. End of Participation

Participants remain under the care of their own physicians during and after participation in this protocol.
We will notify participants and, with their written permission, their doctors of any clinically significant results from any procedure done under this protocol.

7. ADDITIONAL CONSIDERATIONS

This study with [^{11}C]ER176 will be performed under an IND, which has not yet been submitted to the FDA.

8. STORAGE OF DATA AND SAMPLES

We will follow NIH guidelines to prevent identification of study participants and other violations of subject confidentiality. Information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.) will be used that could allow direct linking of database information to individual subjects. Secure e-mail will be used for all electronic communications of subject information between investigators.
Blood samples will be discarded at the end of the study. Demographic and clinical data will be archived on a password-protected server.

All data will be stored on the NIMH server under password protected accounts accessible only to the principal investigator and directly involved study personnel to preserve subject privacy. All data is regularly backed up, either by the NIMH system administrator, or by NIMH CIT personnel.

9. RISKS/DISCOMFORTS

Potential risks from this study include those associated with: a) medical examinations including phlebotomy for blood and urine analysis, b) radiation exposure from the PET and transmission scans, c) Pharmacological effects, d) PET scanning, e) placement of arterial, f) placement of venous lines, g) blood sampling, h) MRI, i) ECG.

9A. Medical examination and laboratory testing

Medical examinations, including phlebotomy for blood analysis are not associated with more than minimal risks. There is usually some discomfort when the needle is inserted for phlebotomy.

9B. Radiation exposure risks

Radiation exposure in this protocol will be from [^{11}C]ER176 and the associated transmission scans. The radiation exposure from [^{11}C]ER176 is unknown, but [^{11}C]-labeled compound generally show very similar dosimetric values of about 5 µSv/MBq (Zanotti-Fregonara and Innis 2012) or about 0.38 rem for a 20 mCi injection. We wish to maintain flexibility in the choice of PET cameras, which differ in the dose from the transmission scan. Among the various PET cameras, the PET/CT has the highest dose from the transmission scan. The effective doses for one head, one chest and one whole-body transmission scans from a PET camera that uses a ^{68}Ge transmission source are 0.01, 0.01, and 0.05 rem respectively. The effective doses for one head, one chest and one whole-body transmission scans from a PET/CT camera that uses CT transmission source are 0.06, 0.21, and 0.66 rem respectively. In addition, we routinely include the dose from two transmission scans in the event that it must be repeated in a subject. The total effective dose including emission and transmission scans for any individual subject in Phase 1 is about 0.92 rem and in Phase 0 or Phase 2 is about 1.7 rem – which are well below the NIH RSC guideline of 5 rem.

All subjects will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, does not exceed an effective dose of 5 rem per 12 months.

9C. Pharmacological effects

We inject PET radioligands at such low mass doses at the tracer level (typically less than 10 µg) that no pharmacological effects are expected. To ensure safety, new ligands are first tested in animals (e.g. rodents) typically at 100X the human equivalent dose (HED). Ligands are considered safe for administration in humans if the no observed adverse effect level (NOAEL) is at least 100X HED. We performed an acute toxicity study in male and female rats at 100X HED considering a maximal human dose of 10 µg per subject. However, in this study, slight ataxia was observed immediately after intravenous administration in 10 of 10 males and 6 of 10 females in the ER176 treated group. The ataxia was transient and noted within a few minutes after injection. The animals were not examined again until about two hours after injection, at which time the ataxia was gone.

To better understand this adverse effect, we commissioned another study using lower doses and observation at more frequent time points. Three groups of 10 male and 10 female

rats were intravenously administered ER176 at 100X, 50X, and 25X HED. On this repeat study, none of the animals at 100X HED and only one of the animals at 50X HED showed ataxia. Please note that we used the same batch and formulation of the test compound. Thus, this behavioral finding was not robust enough to be reproducible at the 100X HED dose, and may depend on the somewhat variable condition of the test animals. Nevertheless, because one of the 20 animals at 50X HED showed ataxia, the NOAEL for ER176 was 25X HED.

In view of these results in rats, we propose the following safety measures for using [^{11}C]ER176 in humans.

(a) Decrease the maximal mass dose: For all of our other INDs for radiopharmaceuticals, the maximal mass dose is 10 µg, whereas for ER176, we will limit the maximal mass dose to 5 µg.

(b) Decrease the infusion rate: Because the ataxia in rats was transient and ended within a few minutes of its bolus injection, it was likely related to transient high plasma concentrations of ER176. To decrease the plasma concentration in human subjects, we will infuse [^{11}C]ER176 over three minutes, rather than the 0.5 to 1.0 minute commonly used in PET studies. Thus, the combination of injecting half the maximal dose over a three-times-longer time period would be expected to decrease the peak plasma concentrations by about six-fold.

(c) Extra monitoring of subjects: We will, of course, inform subjects of these behavioral findings in rats as part of the consent process. We will also mention that the effects may be transient and occur within the first few minutes of the study and ask them to be particularly vigilant of any subjective effects during the early portion of the study. At the end of the study, we will ask the subject to gradually stand up. We will be there to assist them as needed and ask if they have any difficulty standing or walking. We will release subject only after we, and they, feel entirely normal and are walking normally.

The toxicology report states the NOAEL is 25X HED based on a maximal human dose of 10 µg per subject. However, we will inject a maximal dose of 5 µg per subject. Therefore, although the NOAEL is 25X HED based on a 10 µg dose, it is 50X HED based on a 5 µg dose.

9D. PET scanning

PET scanning, which detects injected radioactivity within the body, is associated with no known physical hazards to the subject lying on the table. We routinely use a series of procedures to minimize the risk for discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom participants will have ready access should they experience any problems. Participants can communicate with the trained health professionals while in the scanner and can be removed from the scanner and withdraw from the study at any time if they wish to do so.
Participants can also request that the operator stop the scan.

9E. Arterial line placement

Arterial catheterization has been shown to be a generally safe and reliable method of obtaining arterial blood samples (Lockwood 1985). Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. In the past, over 3,000 arterial catheters have been placed in the PET department. Of these, two complications requiring physician's care were reported. In the first case, a small radial artery aneurysm developed several months later, which was successfully repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically.

9F. Intravenous line placement

Venous catheter insertion can be associated with discomfort, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

In case of tracer extravasation, we will stop the study, remove the venous line from the arm, and apply cold to the site.

9G. Blood sampling

Participants will have arterial and venous blood sampling of about 250 mL during the entire course of the study. The amount of blood drawn will not exceed 400 mL. Some of the blood will be collected via phlebotomy and some through venous and arterial line. Blood sampling may lead to the formation of a small subcutaneous hematoma caused by blood leaking from a punctured blood vessel. This hematoma causes only minor discomfort. It is not dangerous and requires no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. We will ask participants not to donate blood for a period of eight weeks after participation in this study.

9H. MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if they have any, they will not receive an MRI scan.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.
People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. Subjects will be asked to complete an MRI screening form for each MRI scan you have. There are no known long-term risks of MRI scans.

9I. ECG

There is no risk associated with having an ECG. The patient may feel uncomfortable while the electrodes are attached to the chest. The conductive gel sometimes causes some mild irritation.

10. SUBJECT SAFETY MONITORING

Participants will be carefully monitored by clinical staff throughout the study. Participants will be asked about the presence of adverse events throughout the procedure and also for at least an hour following the PET procedure. In view of the transient ataxia observed in acute toxicity studies in rats at 50X HED, and as those effects occur at first few minutes of the study, we will ask the participants to be particularly vigilant of any subjective effects during the early portion of the study. At the end of the study, we will ask the person to gradually stand up. We will be there to assist as needed and ask if they have any difficulty standing or walking. We will release subject only after we and they feel entirely normal and are walking normally. Participants will be monitored during the PET procedure. The site of the catheter insertion will be carefully monitored for signs of bleeding.

10A. Parameters to be monitored

Pulse rate, temperature, blood pressure, respiratory rate, and ECG will be recorded within 3 hours before tracer injection for all the subjects of the study, and again at about 15, 30, 90 and 120 minutes (end of the scan) after tracer injection. Monitoring will be performed by the credentialed clinician. Laboratory tests (CBC, chemistry panel, thyroid panel, urine analysis, pregnancy test) will be performed ~3 hrs before the tracer injection and will be repeated (excluding pregnancy test) ~24 hours after tracer injection. If the subject will not be available at the above-mentioned times, blood and urine samples will be taken shortly after the PET procedure.

Follow-up phone calls to participants will be made at 24 hours post-injection.

All adverse events (AEs) will be recorded on the case report form (appended to the present protocol). All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be evaluated until satisfactory resolution.

10B. Criteria for individual subject withdrawal

Subjects will be removed from the study if they become pregnant, are unable to continue to cooperate, or have an alteration in clinical status needing medical intervention. Subjects may withdraw from the trial at any time at their own request. The Investigators may withdraw a subject for safety or behavioral reasons.

11. OUTCOME MEASURES

Primary outcome measures:

1. [^{11}C]ER176 binding, with regards to identifiability and stability over time of distribution volume calculated with compartmental modeling.
2. Biodistribution and dosimetry of [^{11}C]ER176.

Secondary outcome measures:

1. The effect of polymorphisms of TSPO that may explain the differences in [^{11}C]ER176 binding. TSPOs are present in both brain and lung, and the SNP affects

binding in both organs. Compared to brain, lungs have much higher density of TSPO and may be more effective in assessing whether ER176 is sensitive to the SNP.

12. STATISTICAL ANALYSIS

Brain imaging data will be analyzed with compartmental modeling using a nonlinear least squares analysis, graphical analysis of integration plot, and multilinear regression analyses. PET and MRI scans will be coregistered for anatomic definition of regions of interest. Percent SUV will be calculated in various brain regions. Parametric images will be created using PMOD software (PMOD Technologies Ltd., Zurich, Switzerland).The kinetic modeling will give us the [^{11}C]ER176 binding values, and their identifiability and stability over time. Because of inter-subject variability in pharmacokinetics and metabolism of PET radioligands, sample sizes of approximately 15 per group are required in such studies (Fujita *et al* 1999; Ichise *et al* 2003; Koeppe *et al* 1999).

In order to evaluate genotype sensitivity of [^{11}C]ER176 in brain we calculated the percent difference in binding values necessary to differentiate between HABs, MABs and LABs. This was based on our previous results with [^{11}C]PBR28 brain scans in healthy volunteers of different genotypes (Kreisl *et al* 2012). The mean V_T of HH subjects (HABs; n = 9) for whole brain was 4.5 mL/cm^3 with a standard deviation of 1.4 mL/cm^3. In addition, the V_T of HH subjects was 40% higher than the V_T of HL subjects. Assuming similar SD/Mean for [^{11}C]PBR28 and [^{11}C]ER176, we can calculate how much of a difference in mean V_T can be detected between genotypes. Power analysis based on V_T of HH subjects from [^{11}C]PBR28 scans showed that with a sample size of 10 for each binder group, a power of 80% and alpha of 0.05 the percent difference to be detected from the mean value of a HAB is 40%. That is, the difference in mean V_T between HH and other genotypes should be >40% for it to reach a statistical significance with a sample size of 10. Therefore, we require a recruitment of 12 subjects for each group (36 in total for HABs, MABs and LABs) under Phase 1 considering some withdrawals.

Similar to brain, we calculated the percent difference in lung uptake (measured as area under time-activity curve) necessary to differentiate between HABs, MABs and LABs based on our previous results with [^{11}C]PK 11195 in healthy volunteers (Kreisl *et al* 2010). The mean lung uptake for [^{11}C]PK 11195 in HABs (n = 5) was 25.6 SUV • min with a standard deviation of 3.7 SUV • min. In addition, lung uptake was significantly higher (by 27%) in HABs than in LABs. Assuming similar SD/Mean for [^{11}C]PK 11195 and [^{11}C]ER176, we can calculate how much of a difference in mean lung uptake can be detected between genotypes. Power analysis based on [^{11}C]PK 11195 lung uptake in HABs showed that with a sample size of six for each binder group, a power of 80%, and alpha of 0.05, the percent difference to be detected from the mean value of an HAB is 23%. That is, the difference in mean lung uptake between HH and other genotypes should be >23% for it to reach statistical significance with a sample size of six.

In whole body imaging studies, organ time-activity curves will be obtained from the dynamic PET images, and used to calculate organ residence times. Residence time of the urinary bladder may be calculated using a dynamic bladder model (Cloutier *et al* 1973). The residence times will be used in OLINDA/EXM (http://www.doseinfo-radar.com/OLINDA.html) to obtain radiation-absorbed dose estimates. To take into account inter-subject variability, radiation absorbed doses are usually estimated from approximately 6-8 subjects (Fujita *et al* 2002; Seibyl *et al* 1994; van Dyck *et al* 1996). To also take into account possible gender differences, we propose to study approximately an equal number of

male and female subjects in the whole body imaging study. Currently we do not plan to match the genotype for subject recruitment under Phase 2 dosimetry scans but if we do find any unexpected differences between different binders from Phase 1 study, we will do at least 3 subjects for each binder group (9 subjects in total). Considering the requirement of one subject for the initial Phase 0 and any possible withdrawals, we seek a total of 11 subjects for Phase 2.

13. HUMAN SUBJECTS PROTECTION

13A Subject selection

Participants will be selected based upon their meeting the study's eligibility criteria. Participants will be admitted to the protocol regardless of their age, gender, race or ethnicity.

13B Justification for exclusion of children

Since this protocol has more than minimal risk from radiation exposure without possibility of direct benefit, inclusion of children is not appropriate.

13C Justification for the exclusion of vulnerable subjects

Pregnant women will be excluded because this protocol involves exposure to ionizing radiation.

Lactating women will be excluded because radioisotopes may be excreted in milk.

13D Justification for the exclusion of persons with HIV/AIDS or brain disease

This protocol studies only healthy volunteers. Persons with brain disease and HIV infection are excluded because such diseases are likely to be associated with a disruption of the blood-brain barrier or a viral infection of the brain which can alter the levels of translocator protein, the biomarker imaged in the current study.

13E Safeguards for Vulnerable Populations

Pregnancy testing will be performed before PET and MRI scanning for any participants of child-bearing potential.

13F Qualifications of investigators

Dr. Robert Innis, MD, PhD, has more than 10 years experience using nuclear imaging. Dr. Innis is a clinical authorized user (CAU) of radioisotopes and a credentialed physician. His responsibilities include study design, recruiting healthy subjects, consenting subjects, participating in PET scanning procedures, data analyses, and interpretation and publication of study results.

Talakad G. Lohith, MD, PhD will be the associate investigator for this study. He completed his MD program from India and a PhD program in PET molecular imaging in Japan. His responsibilities include but are not limited to study design, participating in PET scanning protocols, data analyses, and interpretation and publication of study results. He will not obtain informed consent.

Ms. Araneta and Ms. Rallis-Frutos RN, CRNP are credentialed nurse practitioners. Their responsibilities include recruiting healthy subjects, patient evaluation and care, consenting subjects, participating in PET scanning procedures.

Emily Page, RN, FNP-BC has been a nurse practitioner for 2 years and a nurse for 3 years prior to that. She is a credentialed clinician at the NIH Clinical Center and has patient care credentials including prescriptive authority from the NIH Clinical Center and knowledge of the research protocol under which the scan is being performed. She will participate in recruitment and evaluation of healthy subjects, and PET scanning. She will obtain informed consent.

Dr. William C. Kreisl, MD, is a credentialed neurologist. His responsibilities include study design, recruiting healthy subjects, consenting subjects, participating in PET scanning procedures, data analyses, and interpretation and publication of study results.

Gerald Hodges, RN – Nurse Specialist; Molecular Imaging Branch, NIMH/NIH; has more than 14 years experience in administrative and clinical research in human subjects. He will participate in recruitment and evaluation of healthy subjects and PET scanning.

14. BENEFITS

Participants participating in this protocol receive no direct benefit. Information obtained from this protocol study may improve our understanding of the pharmacokinetics of a new TSPO tracer.

15. SUMMARY/CLASSIFICATION OF RISK

This study entails more than minimal risk with no prospect of direct benefit to participants. The risks are reasonable in relation to anticipated benefit.

16. CONSENT DOCUMENTS AND PROCESS

The consent forms contain all required elements. There are two different consent forms submitted with the present protocol to scan healthy subjects: for brain imaging with arterial line, and for dosimetry.

The investigators designated in section #13 above will obtain informed consent. Each subject will receive an oral and written explanation of the purposes, procedures, and risks of this study in a language appropriate for the individual's level of understanding. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing the document. A member of the protocol team will be available to answer questions about the study to be performed. A copy of the signed consent form will be placed in the medical record, and a copy will be given to each study participant.

If a non-English speaking participant is unexpectedly eligible for enrollment, the CC standard short written consent form in the appropriate language and a written summary of what the Investigator will say to the participant will be used as part of an oral consent process. The IRB approved English written consent form will serve as the written summary if the short form process is used. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters will be found through the Department of Social Work. The interpreters will translate the current IRB-approved English version of the consent verbatim and facilitate discussion between the participant and investigator.

The written summary will be signed by the investigator obtaining consent and a witness to the oral presentation. The short written consent form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of the signed form will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter. Further, all instances of use of the short form process will be reported to the IRB at the time of annual review.

17. QUALITY ASSURANCE

1. QA Monitor: Robert Innis

The PI will ensure that:

- the protocol is being correctly followed
- changes to the protocol have been approved by the IRB
- accurate, complete, and current records are being maintained and are secure
- subject withdrawal or study failure is noted in the records and
- informed consent has been correctly documented

The QA audit committee will conduct periodic monitoring of the protocol.

2. QA Plan:

This protocol will undergo periodic review by the QA audit committee as outlined in the NIMH QA SOPs.

18. DATA AND SAFETY MONITORING

1.Data and safety monitor

The PI is responsible for data and safety monitoring for this protocol and will review the data every 6 months.

2.Data and safety monitoring plan

The PI will review the ongoing investigation every 6 months. The PI will monitor safety aspects of the study as a whole to make sure that the frequency of adverse events including serious adverse events and unanticipated problems are within expectations.

3.Criteria for stopping the study or suspending enrollment or procedures

In the event of a serious adverse event related to the research, or if new data shed light on the danger of any procedures used, the study team, including the PI, will suspend further testing, until the IRB and investigators have reviewed the safety information and make a determination about whether to continue the study.

19. ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and

deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 14 days after the PI first learns of the event.
Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.
All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

20. ALTERNATIVES TO PARTICIPATION

Subjects participating in this protocol will not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

21. CONFIDENTIALITY

1. Medical Records

Protocol consents and other medical information will be maintained in the participant's NIH medical record.

2. Research Data

All medical information collected from study participants will be kept in a locked file at the NIH Clinical Center, or password protected computers in secured rooms. Unique identifiers without personally identifying information will be used to label all data. Strict standards of confidentiality will be upheld at all times.

3. Stored Samples

Blood samples will be discarded at the end of the study. All data will be stored under password-protected accounts accessible only to the principle investigator and directly involved study personnel to preserve subject privacy.

22. CONFLICT OF INTEREST

A use-patent application will be submitted for the use of [^{11}C]ER176. The possibility does exist for the principle investigator, the medically responsible investigator and the NIMH to benefit financially from the results of this protocol.

23. TECHNOLOGY TRANSFER

There is no technology transfer agreement for this study.

24. RESEARCH AND TRAVEL COMPENSATION

Volunteers will be compensated for time and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project.

Participants will be paid for each portion of the study they have completed whether or not they opt for early withdrawal from participation. The total compensation will be up to $370

	Total Pay
Visit 1 to NIH (outpatient) (For Brain imaging i.e. Phase 1 only)	
MRI	$100
Pregnancy test	$10
Visit 2 to NIH (outpatient)	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$370

For dosimetry studies, the MRI and its relative pregnancy test, and the arterial catheter placement will not be done. Therefore only two visits to the NIH will be necessary and the total compensation will be $200.

NIH will cover travel expenses to the Clinical Center for all participants in accord with NIH guidelines.

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26. ATTACHMENTS

1. Consent forms
2. NIH 88-23a

I. APPENDIX

Eligibility checklist:

Inclusion/Exclusion Criteria

Subject Number:___ ___ ___Subject Initials:___ ___ ___ Date:___/___/_____

Inclusion Criteria

- Age 18 or older.
- Able to give written informed consent.
- No prior diagnosis of drug or alcohol abuse or dependence.

Exclusion Criteria

- Any current Axis I diagnosis.
- Clinically significant laboratory abnormalities.
- Positive test for HIV.
- Unable to have a MRI scan.
- History of neurologic illness or injury with the potential to affect study data interpretation.
- History of seizures, other than in childhood and related to fever.
- Recent exposure to radiation (i.e., PET from other research) which when combined with this study would be above the allowable limits.
- Inability to lie flat on camera bed for at least two hours.
- Pregnancy or breast feeding.
- Able to get pregnant but does not use birth control.
- Drug/alcohol abuse or dependence
- Are taking prescription or over-the-counter medications for pain, fever or other inflammation. Such medications include prednisolone (Orapred), aspirin (Ecotrin), ibuprofen (Advil), acetaminophen (Tylenol) and many others. We will review your medications to see if you can be in the study. If you are taking any medication that is not permitted, you may be in the study if the medication can be safely stopped for 3 weeks. We will work with you and your doctors to stop a medication if necessary. We need your written permission to contact your doctor.

Please note that exclusion criteria for the dosimetry subjects are the same reported above, except for MRI contraindications, because an MRI will not be performed in these subjects.

INSTITUTE: National Institute of Neurological Disorders and Stroke

STUDY NUMBER: T-M-1330 PRINCIPAL INVESTIGATOR: Dr. Robert Innis, MD PhD

STUDY TITLE: Assessment of [^{11}C]ER176 to image translocator protein in brain and whole-body of healthy subjects

Initial Review Approved by the IRB on Date Posted to Web:
Brain Imaging

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Purpose

The purpose of this study is to test the ability of a new drug to image a protein. This protein acts as a marker of inflammation in the brain.

Background

Some studies suggest that a protein called translocator protein may be associated with inflammation in the brain. This study will use magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning to study whether a new drug can image this protein in the brain. The translocator protein is known to be present in the lungs at higher levels than in the brain. To measure how the drug binds to the protein, we may also do a PET scan of your chest when we scan your brain.

You are taking part in this study as a healthy volunteer. You will have one PET or PET-CT scan and one MRI scan in this study. Before the PET scan, we will inject you with a drug, [^{11}C]ER176. [^{11}C]ER176 will help us identify inflammation in the body.

This is the first time that [^{11}C]ER176 is being used in humans as an Investigational New Drug.

Study Population
Thirty-six healthy volunteers will take part in this part of the study. Forty-seven healthy volunteers will take part in the entire protocol.

Who Can Be In This Study
You may be eligible for this research study if you:
- are healthy.
- are 18 years of age or older.
- are able to give informed consent.

Who May Not Be In This Study
You may not be eligible for this research study if you:
- have previous radiation exposure (for example, from X-rays or other PET scans) that, together with the radiation exposure from this study, would cause you to exceed the NIH research limits.
- have metal in your body that would make having an MRI scan unsafe, such as pacemakers, medication pumps, aneurysm clips, metallic prostheses (including metal pins and rods, heart valves or cochlear implants), shrapnel fragments, permanent eye liner or small metal fragments in the eye that welders and other metal workers may have.
- are not comfortable in small closed spaces (have claustrophobia) so that you would feel uncomfortable in the MRI machine.
- cannot lie comfortably flat on your back for up to two hours in the MRI scanner and during the PET scan.
- have a brain disease.
- have a serious illness or a neurologic illness based on the history and physical exam that we will perform.
- are pregnant or breastfeeding. Women able to have children must agree to use an effective form of birth control until one week after all PET scans are done.
- have drug or alcohol abuse or dependence.
- have a positive test for illegal drugs on a urine test we will do.
- are HIV positive.
- are taking prescription or over-the-counter medications for pain, fever, or other inflammation. Such medications include prednisolone (Orapred), aspirin (Ecotrin), ibuprofen (Advil), acetaminophen (Tylenol), and many others. We will review your medications to see if you can be in the study. If you are taking any medication that is not permitted, you may be in the study if the medication can be safely stopped for three weeks. We will work with you and your doctors to stop a medication if necessary. We need your written permission to contact your doctor.

Procedures
Overview
This study requires two visits to the NIH Clinical Center. You may only need to have one visit if the study machines are available and we can do all the procedures on the same day. The visits will take place over a maximum period of one year. The visits will be outpatient visits and will last from one to four hours. In one visit you will have one PET scan of your brain that may be combined with a lung scan in the same session. In another visit you will have one MRI scan of your brain if you have not had one at the NIH in the past year.

1) Screening
The screening procedures for taking part in this study will be done under another protocol (01-M-0254). You will have a history and physical exam, and blood and urine tests. We will use those results to see if you can take part in this study.

If you can take part in this study, you will be scheduled for the following procedures. The procedures may be done in a different order than is listed below. Women will have a pregnancy test before the PET scan and the MRI.

2) PET Scan with [^{11}C]ER176:
For the PET scan, a small amount of a radioactive chemical that can be detected by the PET scanner will be injected through an intravenous (IV) tube. A needle will be used to guide a thin plastic tube (catheter) into one of your arm veins. The needle will be removed, leaving only the catheter in the vein. The catheter will be taped to the skin to hold it in place.

The PET scanner is shaped like a doughnut. You will lie on a bed that slides in and out of the scanner. You will be asked to lie quietly without sleeping. To support your head and prevent it from moving during the scan, we will custom mold a plastic mask to your face and head. If your chest is being scanned, to prevent it from moving during the scan, we will wrap your body with restraining sheets. Most people do not find the mask or sheets uncomfortable. We will start the scan by taking an X-ray-like picture (transmission scan). We will then inject the radioactive drug, [^{11}C]ER176, and begin PET scanning. After injecting the radioactive drug, we will ask you to stay aware of how you feel and let us know if you are uncomfortable. During the scan, we may move the scan bed so that we can scan you from head to chest. The scan will last about 120 minutes. While you are in the PET scanner, we will monitor your pulse, breathing, blood pressure, and ECG several times during the scan. At the end of the scan, you will be asked to stand up slowly. After the scan, you will receive instructions about drinking fluids and voiding (urinating) to limit your exposure to radioactivity.

For the [^{11}C]ER176 PET scan, a thin plastic tube (catheter) will also be put into an artery at your wrist or elbow crease area. Before inserting a catheter into the wrist, a simple test will be done to make sure that both of the arteries in your wrist are working. We will inject a local anesthetic to numb the skin over the artery. Then, the catheter will be placed in the artery with a needle. The needle will be removed, leaving only the thin catheter in the artery. The catheter will be fastened to the skin with tape. During the time the catheter is in place, you should try not to move your arm. A physician or nurse will be available at all times and you should tell them right away if you have pain or discomfort. The catheter will be used to draw blood during the study. A total of about 100 ml (20 tsp) of blood will be drawn during the PET.

If we cannot do the PET scan because of technical problems and you had no other radiation exposure, we may ask you to have another PET scan on the same or another day. We may also repeat the transmission scan.

We will do blood and urine tests before and after the PET scan. Blood will be drawn through a vein in your arm. We will draw no more than 4 teaspoons (20 mL) of blood at any one time and no more than 50 teaspoons (250 mL) during the entire testing period. The blood will be used to look at the effects of the study drug on blood chemistry.

When available, we may use PET-CT instead of a PET scanner. The PET-CT uses built-in CT for the initial transmission scan; otherwise it is the same as the PET scanner.

3) MRI
MRI uses a strong magnetic field and radio waves to take pictures of your brain. The MRI scanner is a metal cylinder surrounded by a strong magnetic field. During the MRI, you will lie on a table that can slide in and out of the cylinder. You will be in the scanner about 30 minutes. While in the scanner you will hear loud knocking noises, and you will be fitted with earplugs or earmuffs to muffle the sound. You will be able to communicate with MRI staff at all times during your scan, and you may ask to be moved out of the machine at any time.

Risks, Inconveniences, and Discomforts

1. Radiation Exposure Risks

The PET scan involves exposure to radiation from 20 millicuries of [^{11}C]ER176 and the transmission scan. This radiation exposure is not required for your medical care and is for research purposes only. The amount of radiation you will receive in this study is about 0.92 rem, which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. If you would like more information about radiation, please ask the investigator for a copy of the pamphlet "An Introduction to Radiation for NIH Research Subjects".

While there is no direct evidence that the amount of exposure received from participating in this study is harmful, there is indirect evidence it may not be completely safe. There may be a very slight increase in the risk of cancer.

Please tell your doctor if you have had any radiation exposure in the past year, either from other research studies or from medical tests or care, so we can make sure that you will not receive too much radiation. Radiation exposure includes x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into your body.

If you are pregnant you will not be permitted to participate in this research study. If you are breastfeeding and the protocol involves injecting radioactive material you will not be able to take part in the study. It is best to avoid radiation exposure to unborn or nursing infants since they are more sensitive to radiation than adults.

2. Drug Exposure Risks

[^{11}C]ER176 is a research drug that is being used in humans for the first time. Based on studies in animals, we expect that this dose of the drug will not affect you. That is, this drug at 50 times the human dose had no effect on rats. However, a small percentage of rats studied at 100 times the human dose became unsteady on their feet for a few minutes right after injection. Thus, you may become unsteady on your feet, but you will be lying down for two hours. We will ask you to let us know if you have any discomfort after [^{11}C]ER176 injection. We will carefully watch your vital signs and ECG before and after the injection. In addition, we will make sure that you get up slowly after the PET scan. We will be there to help you as needed and will ask you if you have any trouble standing or walking. We will release you only after we are sure that you feel entirely normal and are walking normally.

3. PET Scanning

There are no medical risks from the PET scan other than the radiation exposure. The mask that helps hold your head may be somewhat uncomfortable. You may also be uncomfortable staying still during the scan itself. You can stop the scan at any time and ask to be removed from the PET scanner if you are uncomfortable.

4. MRI Scanning

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. You will be screened for these conditions before having any scan, and if you have any, you will not receive an MRI scan. If you have a question about any metal objects being present in your body, you should inform the staff. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, you should let us know right away. Please tell the investigators if you have hearing or ear problems. You will be asked to complete an MRI screening form for each MRI scan you have. There are no known long-term risks of MRI scans.

5. Intravenous catheter and blood sampling
The risks of an intravenous catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling. These will be treated if they occur.

6. Arterial Line Placement and blood sampling
There is usually some discomfort when the needle is put in. Bruising or swelling where the tube enters the skin occurs in five to 20 percent of patients, but does not last long. Fainting is uncommon, but possible. The tube can block the artery, but that is unlikely because the tube is left in for only a short time. There have been two delayed complications from wrist arterial catheters at the NIH. One person developed a small radial artery aneurysm two years after arterial catheterization. A second person developed a blocked artery a few days after arterial catheterization. Both required surgical repair.

You should not donate blood for eight weeks after the study is over.

7. ECG
There is no risk associated with having an ECG. You may feel uncomfortable while the electrodes are attached to your chest. The conductive gel sometimes causes some mild irritation. You may not like the smell of the paste or the glue remover, but they are not harmful.

Anticipated Benefits
There is no direct benefit to you from taking part in this research study. Your participation will help us to learn more about brain inflammation.

Right of Withdrawal and Conditions for Early Withdrawal
You may withdraw from the study at any time and for any reason without loss of benefits or privileges to which you are otherwise entitled. The investigator can remove you from the study at any time if she or he believes that continuation is not in your best medical interest or if you are unable to comply with the requirements of the study.

Results From this Study
We will share with you any new information that may relate to your willingness to continue to participate in this study.

Alternatives to Participation
The study does not provide treatment. You do not have to forego any treatment in order to take part in the study. The alternative to taking part in this study is not to take part.

Compensation

You will be paid for research-related discomfort and inconveniences as set out by NIH guidelines. If you are unable to finish the study, you will be paid for those parts you finished. Payment will be made when the study ends.
The total possible compensation is $370.

MRI	$100
PET scan and labs	$150
Antecubital venous catheters	$30
Arterial catheter	$60
Pregnancy test	$10 x 2
Movement restriction	$10

Conflict of Interest

The NIH and members of the research team have submitted a patent application for the use of this medication as an imaging agent for inflammation. This means that it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of this patent.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact Dr. Robert Innis; Room 10/B1D43; Tel: 301-594-1368.

You may also call the Clinical Center Patient Representative at 301-496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE
FROM THROUGH.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____

INSTITUTE: National Institute of Neurological Disorders and Stroke

STUDY NUMBER: T-M-1330 PRINCIPAL INVESTIGATOR: Dr. Robert Innis, MD PhD

STUDY TITLE: Assessment of [^{11}C]ER176 to image translocator protein in brain and whole-body of healthy subjects

Initial Review Approved by the IRB on Date Posted to Web:
Whole-body Imaging

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Purpose

The purpose of this study is to test how a new radioactive chemical is distributed in the body.

Background

Some studies suggest that a protein called translocator protein may be associated with inflammation in the brain. This study will use positron emission tomography (PET) scanning to study whether a new drug can image this protein in the brain. In some subjects, we will also look at how radioactivity from [^{11}C]ER176 is distributed in the body.

You are taking part in this study as a healthy volunteer. You will have one PET or PET-CT scan in this study. Before the PET scan, we will inject you with a drug, [^{11}C]ER176. [^{11}C]ER176 will help us identify inflammation in the body.

This is the first time that [^{11}C]ER176 is being used in humans as an Investigational New Drug.

Study Population
Eleven healthy volunteers will take part in this part of the study. Forty-seven healthy volunteers will take part in the entire protocol.

Who Can Be In This Study
You may be eligible for this research study if you:
- are healthy.
- are 18 years of age or older.
- are able to give informed consent.

Who May Not Be In This Study
You may not be eligible for this research study if you:
- have a previous radiation exposure (for example, from X-rays or other PET scans) that, together with the radiation exposure from this study would cause you to exceed the NIH research limits.
- cannot lie comfortably flat on your back for up to two hours during the PET scan.
- have a brain disease.
- have a serious illness or a neurologic illness based on the history and physical exam that we will perform
- are pregnant or breastfeeding. Women able to have children must agree to use an effective form of birth control until one week after all PET scans are done.
- have drug or alcohol abuse or dependence.
- have a positive test for illegal drugs on a urine test we will do.
- are HIV positive.
- are taking prescription or over-the-counter medications for pain, fever, or other inflammation. Such medications include prednisolone (Orapred), aspirin (Ecotrin), ibuprofen (Advil), acetaminophen (Tylenol) and many others. We will review your medications to see if you can be in the study. If you are taking any medication that is not permitted, you may be in the study if the medication can be safely stopped for three weeks. We will work with you and your doctors to stop a medication if necessary. We need your written permission to contact your doctor.

Procedures
Overview
This study requires one visit to the NIH Clinical Center. The visit will be an outpatient visit and will last from three to four hours. You will have one PET scan.

1) Screening
The screening procedures for taking part in this study will be done under another protocol (01-M-0254). You will have a history and physical exam, and blood and urine tests. We will use those results to see if you can take part in this study.

If you can take part in this study, you will be scheduled for the PET scan. Women will have a pregnancy test before the PET scan.

2) PET Scan with [^{11}C]ER176:
For the PET scan, a small amount of a radioactive chemical that can be detected by the PET scanner will be injected through an intravenous (IV) tube. A needle will be used to guide a thin plastic tube (catheter) into one to two of your arm veins. The needle will be removed, leaving only the catheter in the vein. The catheter will be taped to the skin to hold it in place.

The PET scanner is shaped like a doughnut. You will lie on a bed that slides in and out of the scanner. You will be asked to lie quietly without sleeping. To support your head and prevent it from moving during the scan, we will custom mold a plastic mask to your face and head. To support your body and prevent it from moving during the scan, we will wrap your body with restraining sheets. Most people do not find the mask or sheets uncomfortable. We will start the scan by initially taking an X-ray like picture (transmission scan). We will then inject the radioactive drug, [^{11}C]ER176, and begin PET scanning. After injecting the radioactive drug, we will ask you to stay aware of how you feel and let us know if you are uncomfortable. During the scan, we will move the scan bed so that we can scan you from head to upper thighs. The scan will last about 120 minutes. While you are in the PET scanner, we will monitor your pulse, breathing, blood pressure, and ECG several times during the scan. In addition, we may take serial blood samples from the venous catheter. A total of 6 teaspoons (30 mL) of blood will be removed. At the end of the scan, you will be asked to stand up slowly. After the scan, you will receive instructions about drinking fluids and voiding (urinating) to limit your exposure to radioactivity.

If we cannot do the PET scan because of technical problems and you had no other radiation exposure, we may ask you to have another PET scan on the same or another day. We may also repeat the transmission scan.

We will do blood and urine tests before and after the PET scan. Blood will be drawn through a vein in your arm. We will draw no more than 4 teaspoons (20 mL) of blood at any one time and no more than 50 teaspoons (250 mL) during the entire testing period. The blood will be used to look at the effects of the study drug on blood chemistry.

When available, we may use PET-CT instead of a PET scanner. The PET-CT uses built-in CT for the initial transmission scan; otherwise it is the same as the PET scanner.

Risks, Inconveniences and Discomforts

1. Radiation Exposure Risks

The PET scan involves exposure to radiation from 20 millicuries of [^{11}C]ER176 and the transmission scan. This radiation exposure is not required for your medical care and is for research purposes only. The amount of radiation you will receive in this study is about 1.7 rem, which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. If you would like more information about radiation, please ask the investigator for a copy of the pamphlet "An Introduction to Radiation for NIH Research Subjects".

While there is no direct evidence that the amount of exposure received from participating in this study is harmful, there is indirect evidence it may not be completely safe. There may be a very slight increase in the risk of cancer.

Please tell your doctor if you have had any radiation exposure in the past year, either from other research studies or from medical tests or care, so we can make sure that you will not receive too much radiation. Radiation exposure includes x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into your body.

If you are pregnant you will not be permitted to participate in this research study. If you are breastfeeding and the protocol involves injecting radioactive material you will not be able to take part in the study. It is best to avoid radiation exposure to unborn or nursing infants since they are more sensitive to radiation than adults.

2. Drug Exposure Risks
[^{11}C]ER176 is a research drug that is being used in humans for the first time. Based on studies in animals, we expect that this dose of the drug will not affect you. That is, this drug at 50 times the human dose had no effect on rats. However, a small percentage of rats studied at 100 times the human dose became unsteady on their feet for a few minutes right after injection. Thus, you may become unsteady on your feet, but you will be lying down for two hours. We will ask you to let us know if you have any discomfort after [^{11}C]ER176 injection. We will carefully watch your vital signs and ECG before and after the injection. In addition, we will make sure that you get up slowly after the PET scan. We will be there to help you as needed and will ask you if you have any trouble standing or walking. We will release you only after we are sure that you feel entirely normal and are walking normally.

3. PET Scanning
There are no medical risks from the PET scan other than the radiation exposure. The mask that helps hold your head may be somewhat uncomfortable. You may also be uncomfortable staying still during the scan itself. You can stop the scan at any time and ask to be removed from the PET scanner if you are uncomfortable.

4. Intravenous catheter and blood sampling
The risks of an intravenous catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling. These will be treated if they occur. You should not donate blood for eight weeks after the study is over.

5. ECG
There is no risk associated with having an ECG. You may feel uncomfortable while the electrodes are attached to your chest. The conductive gel sometimes causes some mild irritation. You may not like the smell of the paste or the glue remover, but they are not harmful.

Anticipated Benefits
There is no direct benefit to you from taking part in this research study. Your participation will help us to learn more about brain inflammation.

Right of Withdrawal and Conditions for Early Withdrawal
You may withdraw from the study at any time and for any reason without loss of benefits or privileges to which you are otherwise entitled. The investigator can remove you from the study at any time if she or he believes that continuation is not in your best medical interest or if you are unable to comply with the requirements of the study.

Results From this Study
We will share with you any new information that may relate to your willingness to continue to participate in this study.

Alternatives to Participation
The study does not provide treatment. You do not have to forego any treatment in order to take part in the study. The alternative to taking part in this study is not to take part.

Compensation
You will be paid for research-related discomfort and inconveniences as set out by NIH guidelines. If you are unable to finish the study, you will be paid for those parts you finished. Payment will be made when the study ends.
The total possible compensation is about $200.

PET scan and labs	$150

Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10

Conflict of Interest

The NIH and members of the research team have submitted a patent application for the use of this medication as an imaging agent for inflammation. This means that it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of this patent.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact Dr. Robert Innis; Room 10/B1D43; Tel: 301-594-1368.

You may also call the Clinical Center Patient Representative at 301-496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE
FROM THROUGH.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____