Abbreviated Title: [18F]SF12051 in Healthy Subjects

NIH IRB: 000674

Version Date: 12/05/23

Title: Assessment of [18F]SF12051 for Imaging the 18-kDa Translocator Protein (TSPO) in Brain and Whole Body of Healthy Subjects

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Drug Name:	[18F]SF12051
IND Number:	162310
Sponsor:	NIMH IRP
Manufacturer:	NIMH IRP

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

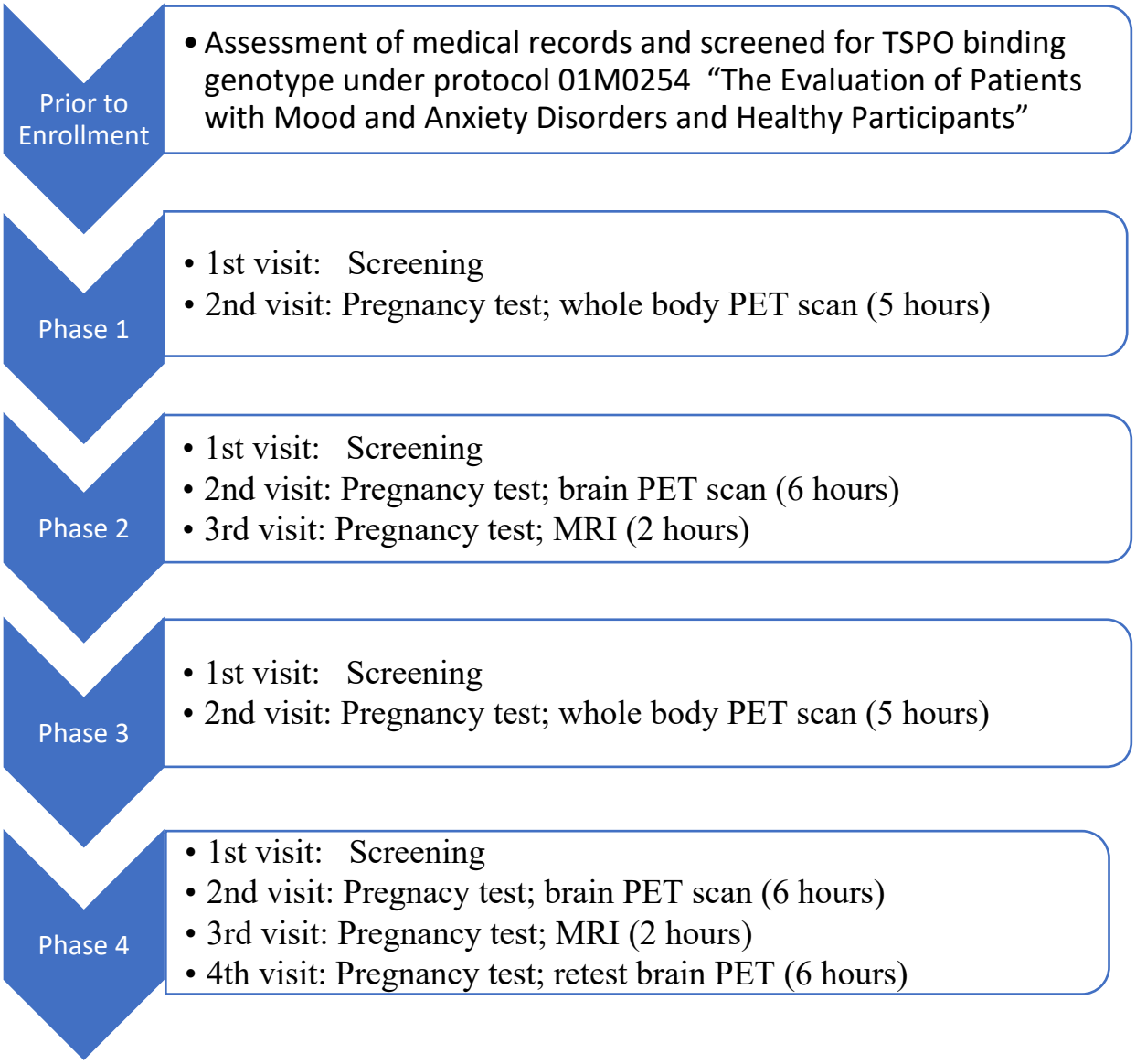
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Assessment of [^{18}F]SF12051 for Imaging the 18-kDa Translocator Protein (TSPO) in Brain and Whole Body of Healthy Subjects
Study Description:	This study is intended to provide information on the novel [^{18}F]SF12051 radioligand and its ability to localize and measure TSPO in the brain and body of healthy individuals.
Objectives:	Primary Objective: To study the brain uptake of [^{18}F]SF12051 and perform kinetic modeling of the [^{18}F]SF12051 in the three different TSPO genotypes. Secondary Objectives: To study the brain retest characteristics, biodistribution and dosimetry of [^{18}F]SF12051 in healthy subjects.
Endpoints:	Primary Endpoint: The distribution volume of the radioligand and stability over time calculated with compartmental modeling, attention paid to differences in mean distribution volumes between TSPO genotypes for determining genotype sensitivity. Secondary Endpoints: Retest variability and reliability and organ-time-activity curves to determine biodistribution and dosimetry.
Study Population:	Up to forty-five (45) healthy male and female volunteers (18 years and older) of the three different TSPO genotypes for brain and whole-body imaging.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	Screening and PET imaging will be performed at the NIH Clinical Center.
Description of Study Intervention:	Arterial line, PET scan, and brain MRI scan for absolute quantification of TSPO. Blood drawn from all subjects to determine TSPO genotype.
Study Duration:	3 years
Participant Duration:	1-4 visits, 2-6 hours per visit.

1.2 SCHEMA


Prior to Enrollment
• Assessment of medical records and screened for TSPO binding genotype under protocol 01M0254 "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Participants"
Phase 1
• 1st visit: Screening
• 2nd visit: Pregnancy test; whole body PET scan (5 hours)
Phase 2
• 1st visit: Screening
• 2nd visit: Pregnancy test; brain PET scan (6 hours)
• 3rd visit: Pregnancy test; MRI (2 hours)
Phase 3
• 1st visit: Screening
• 2nd visit: Pregnancy test; whole body PET scan (5 hours)
Phase 4
• 1st visit: Screening
• 2nd visit: Pregnacy test; brain PET scan (6 hours)
• 3rd visit: Pregnancy test; MRI (2 hours)
• 4th visit: Pregnancy test; retest brain PET (6 hours)

Schedule of Activities (SOA)

This study will require 1-4 visits for each participant depending on the phase in which they are in. An initial blood sample to determine TSPO binding genotype will be acquired under protocol 01-M-0254 "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Participants". The first visit for each phase will encompass a screening evaluation that includes H&P, lab tests and EKG. Subsequent visits will include an MRI and/or a PET scan, in order of earliest availability, and arterial line for the volunteers in the primary study with brain PET scans. If possible, MRI and PET scans can be done on the same day to limit to two or three total visits. The MRI and PET visits will take approximately 2 and 6 hours, respectively. For dosimetry, two total visits are sufficient due to lack of MRI.

This protocol is a first-in-human evaluation of [18F]SF12051 and has four phases:

a) Phase 1: whole body PET imaging of one [1] subject with a low injection activity of ~2 mCi to confirm that no organ has prominently high uptake of [18F]SF12051;
b) Phase 2: kinetic brain PET imaging in ten [10] subjects with up to 5 mCi injection to quantify TSPO in brain relative to concurrent measurement of the parent radioligand in arterial plasma;
c) Phase 3: if [18F]SF12051 is successful in Phase 2 in the first couple of subjects, we will estimate the radiation-absorbed doses by performing whole body PET imaging on up to ten [10] subjects, with 5 mCi injection;
d) Phase 4: test-retest brain PET analysis in up to twenty-four [24] subjects, of brain binding relative to concurrent measurement of the parent radioligand in arterial plasma (5 mCi per scan).
   Thus, a total of 11 participants for whole body imaging and 34 participants for brain imaging.

Table 1 for Phases 1 and 3

	1st Visit / Screen	2nd Visit
Informed Consent	X	
History and physical exam	X	
Screening lab tests*	X	
EKG	X	X
Pregnancy test (female subjects)	x	X
Whole-body PET with venous sampling		X
Safety tests for PET scans**		X
Follow-up phone call		X

* *The screening lab tests are described in Section 8.1.2.*
** Safety tests for each PET scan are described in Section 8.3

Table 2 for Phases 2 and 4

	1st Visit / Screen	2nd Visit	3rd Visit	4th Visit
Informed Consent	X			
History and physical exam	X			
Screening lab tests*	X			
Pregnancy test (female subjects)	X	X	X	X
EKG	X	X		X
Brain MRI			X	
Brain PET with arterial sampling		X		X
Safety tests for PET scans**		X		X
Follow-up phone call		X		X

* The screening lab tests are described in Section 8.1.2.
** Safety tests for each PET scan are described in Section 8.3
If the MRI can be performed on the day of a PET scan, the number of visits will be reduced.

2 INTRODUCTION

2.1 STUDY RATIONALE

The mitochondrial protein 18-kDa translocator protein (TSPO) is highly expressed in phagocytic inflammatory cells, including activated microglia and reactive astrocytes in the brain and macrophages in the periphery (1,2). Although numerous positron emission tomography (PET) radioligands have been developed to image TSPO, several limitations have restricted their clinical utility for quantifying inflammation in the brain. Of these radioligands, the first-generation TSPO radioligand ^{11}C-(R)-PK11195 has been the most extensively studied. ^{11}C-(R)-PK11195 has high affinity to TSPO; however, its utility is limited by low ratio of specific-to-nondisplaceable uptake (i.e., nondisplaceable binding potential, BP_{ND}) in the brain as well as the relatively short half-life of ^{11}C (20 minutes) (3,4). Second-generation radioligands, such as ^{11}C-PBR28, offer a higher in vivo TSPO-specific signal but suffer from sensitivity to the single nucleotide polymorphism (SNP) rs6971 (5,6), which splits the human population into high-, mixed-, and low-affinity binders (HAB, MAB, LAB). LAB have too little TSPO binding to measure. This SNP sensitivity both complicates the interpretation of results and requires genotyping of study participants to exclude LABs before imaging.

Recently, we synthesized a new TSPO ligand, [^{11}C]ER176, which, although still sensitive to genotype differences, has enough specific binding to allow the imaging of low binders as well (7). This has important practical advantages, because low binders would not need to be excluded from clinical protocols and genotyping could be performed at the end of the protocol for all subjects at once, rather than case-by-case before scanning takes place. However, ER176 is labeled with ^{11}C, and therefore would not be available to centers without a cyclotron on site. The rationale of this study is to determine whether a novel ^{18}F-labeled radioligand, [^{18}F]SF12051,

whose structure is similar to that of [11C]ER-176, has comparable or improved efficacy for imaging TSPO and/or dependence on TSPO genotype.

We predict that the new 18F-radioligand will be like [11C]ER176 and, therefore, that it *will* be sensitive to genotype. Even though [11C]ER176 is sensitive to genotype, it provides time-stable values of receptor binding. Thus for [11C]ER176 and presumably for [18F]SF12051, LAB subjects need not be excluded a priori, but the results must be corrected a posteriori. If [18F]SF12051 values of receptor binding are unstable in LABs, it would still be an improvement over current 11C-rdaioligands and can be used at more sites because of the longer half-life of 18F compared to 11C."

2.2 BACKGROUND

2.2.1 Scope of Study

The 18 kDa translocator protein (TSPO) is found on the membrane of mitochondria, and in the brain is primarily located in microglia and astrocytes, cell types typically activated during inflammation. Inflammation has been suggested to be a facet of many neurological disorders, giving the possibility of in vivo measurements significance in terms of furthering understanding the physiology of and diagnosing these diseases. Upon activation, microglial expression of TSPO expression increases significantly, implicating it in the process of neuroinflammation (8). Due to the phenomenon, TSPO has been suggested as a good in vivo biomarker for measuring neuroinflammation on the assumption that increased TSPO binding sites would indicate an increase in activated microglia, and thus in inflammation (9). A key method to do this measurement is to use radiotracers during positron emission tomography (PET) scans that can cross the blood brain barrier and bind to TSPO. The first radiotracer developed for TSPO, [11C]PK11195, has issues of high non-specific binding and poor signal-to-noise ratio resulting in a lack of stable quantitative analysis, driving research to look for an alternative.

A significant factor to consider in developing and employing TSPO radioligands is the presence of a single nucleotide polymorphism in the TSPO gene. This polymorphism divides populations into three genetic groups: high-affinity binders (HABs), mixed-affinity binders (MABs), and low-affinity binders (LABs). The development of the most recent third generation TSPO radioligand, [11C]ER176 sought to overcome these challenges to provide better in vivo imaging data. While an in-human study showed consistently quantifiable distribution volume for TSPO with [11C]ER176 than its predecessor in all three genetic groups, there was still some genetic preference for HABs as compared to MABs or LABs (7). Despite this residual sensitivity to genotype, this new ligand allows for the inclusion of all three genetic types into future TSPO studies.

In contrast to carbon-11, fluorine-18 has several beneficial characteristics for clinical PET imaging. Its relatively longer half-life (~110 versus ~20 minutes) allows imaging for a longer period of time and broader distribution of the radioligand from a central radiopharmacy, giving 18F-labeled radioligands greater flexibility for widespread use. Unfortunately, ER176 does not contain a fluorine atom and cannot be easily labeled with fluorine-18. To address this issue, our laboratory synthesized six new fluorine-containing analogs of ER176 as candidate compounds for 18F-radiolabeling—three isomers with a fluoro group and three with a trifluoromethyl group at each of three positions (ortho, meta, and para) of the pendant aryl ring (Figure 1). In vitro studies demonstrated that all six analogs had high affinity for human TSPO

(K_i = 1.2−7.0 nM) and could be successfully labeled with carbon-11 with good yield (66–81%) and excellent chemical (>95%) and radiochemical purities (> 99%). Of these six, the most promising for ^{18}F-labeling was SF12051, which was then evaluated in mice and monkeys (See Section 2.2.2).

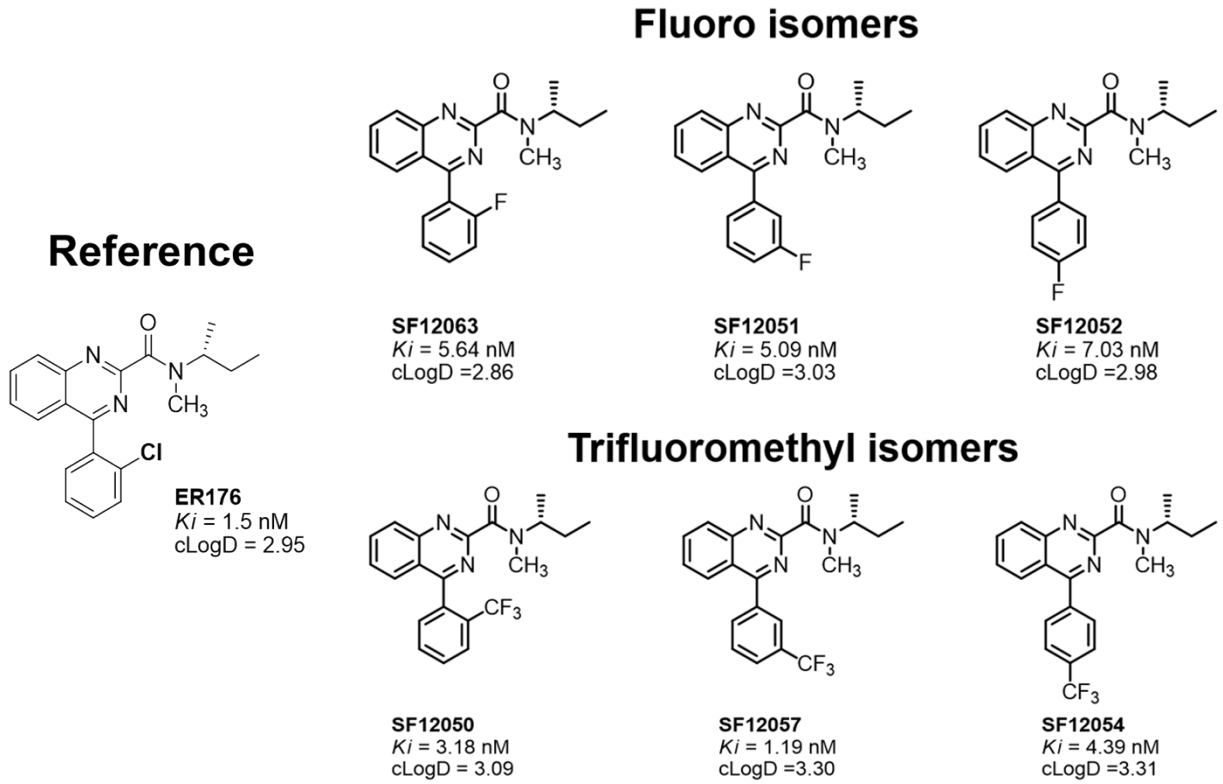


Figure 1. *Chemical structures of ER176 and six fluorine-containing analogs.*

The objective of this first-in-human study is evaluation of [^{18}F]SF12051 as a TSPO radioligand, specifically looking at its distribution volume values, stability over time, and how uptake varies between the three genetic binding affinities. The expectation for this study is that the [^{18}F]SF12051 radioligand will exhibit characteristics like [^{11}C]ER176. If found to be successful, the [^{18}F]SF12051 radioligand would be a more widely useful tracer for TSPO in vivo studies

2.2.2 [^{18}F]SF12051 as a TSPO Radioligand in Mice and Rhesus Monkeys

To warrant this human study, first research was done to evaluate the effectiveness of [^{18}F]SF12051 in mice and rhesus monkeys. Use of [^{18}F]SF12051 in mice showed high brain uptake and subsequent strong signal blockade by PK11195, a TSPO-specific blocking agent, suggesting that the radioligand was binding specifically to TSPO in the mouse brain.

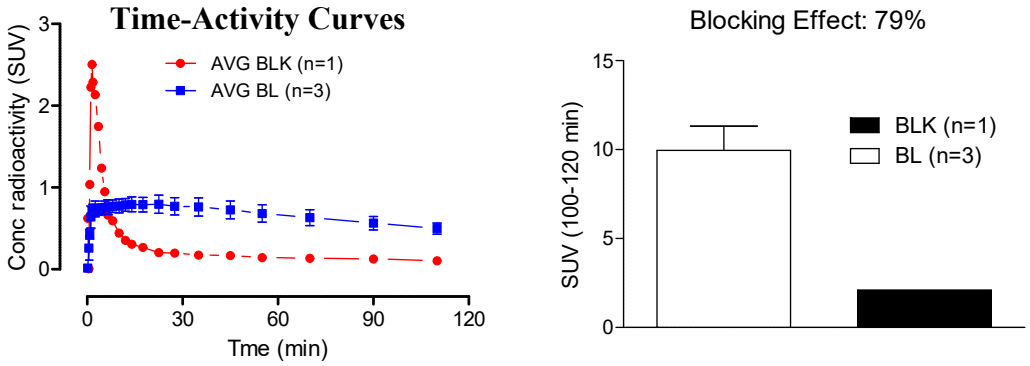


Figure 2. *Left panel: Baseline (blue) and blocked (red) curves of [18F]SF12051 in rat. Right panel: administration of PK11195 blocked 79% of the signal in the brain.*

Following its success in mice, [18F]SF12051 was then evaluated in a rhesus macaque. Results showed typical time-activity curves for baseline and blocked scans and good time-stability of the ligand after sixty minutes of scanning. The uptake of the radioligand was significantly displaced by administration of PK11195, suggesting specific TSPO binding in monkey brain. These results suggest that [18F]SF12051 will be able to specifically bind to TSPO in human brain and warrants further investigation.

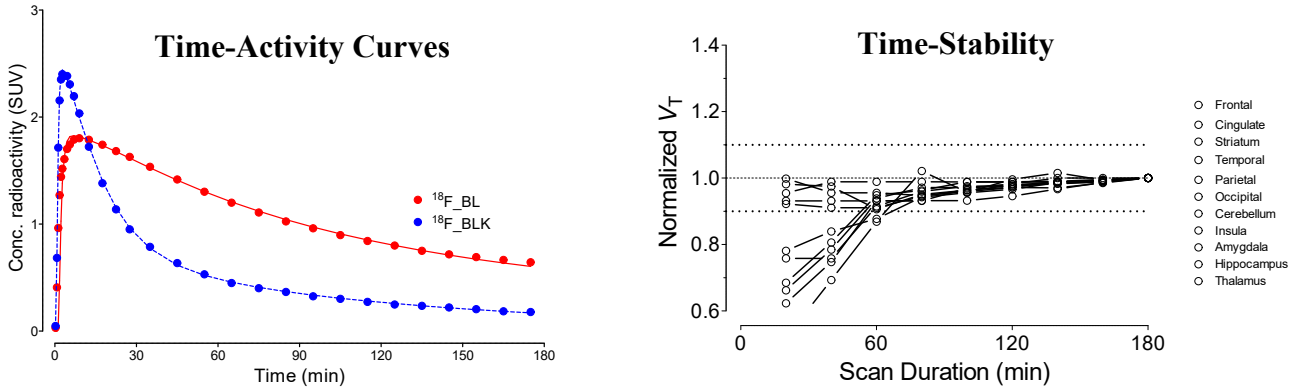


Figure 3. *Left panel: Baseline (blue) and blocked (red) curves of [18F]SF12051 in monkey. Right panel: time-stability analysis, which shows that stable V_T is reached at about 60 minutes.*

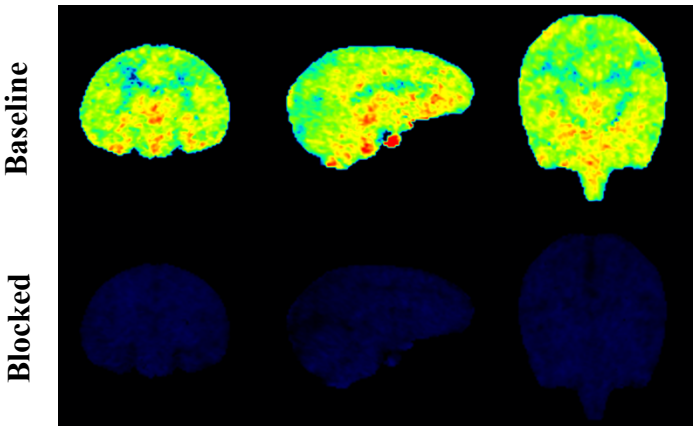


Figure 4. *Baseline (top) and blocked (bottom) images show uptake of [18F]SF12051 in rhesus macaque brain before and after administration of PK11195.*

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

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) PET scanning, d) placement of arterial or venous lines, e) blood sampling, f) MRI, and g) ECG.

2.3.1.1 Medical Examinations

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.

2.3.1.2 Radiation Exposure

Radiation exposure in this protocol will be from [^{18}F]SF12051 and the associated transmission scans. With our PET/CT scan, the effective doses for one head and one whole-body transmission scans 0.02 and 0.6 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 effective dose from two head CT scans is 0.04 rem (and from four CT scans in case of test-retest acquisition is 0.08 rem) and from two whole body scans is 1.2 rem. The radiation exposure from [^{18}F]SF12051 is unknown, but ^{18}F-labeled compounds generally show very similar dosimetric values of about 20 µSv/MBq (0.074 rem/mCi) (10) or about 0.37 rem for a 5 mCi injection.

Please note that no pharmacological effects are expected from [^{18}F]SF12051. Therefore, only radiation risks are relevant to this tracer.

2.3.1.3 PET Scanning

PET scans, which detect injected radioactivity within the body, are not associated with any known physical hazards to the participant lying on the table. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. 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.

2.3.1.4 Arterial and/or Venous 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.

Arterial catheterization is a generally safe and reliable method of obtaining arterial blood samples (11). Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. Over 3,000 arterial catheters have been placed to date in the PET Department. Of these, only two complications requiring physician's care arose. 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.

2.3.1.5 Blood Sampling

Participants may have arterial or venous blood sampling. The total amount of blood drawn shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Blood sampling may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the participant. 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 within eight weeks before the study or for eight weeks following the study.

2.3.1.6 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, an 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. Participants will be screened again for these conditions before having any scan and will not receive an MRI scan if they have any contraindications.

It is not known whether MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a urine 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. Participants will be

asked to complete an MRI screening form for each MRI scan they have. There are no known long-term risks associated with MRI scans.

2.3.1.7 EKG

The patient may feel uncomfortable while the electrodes are attached to the chest. The conductive gel sometimes causes some mild irritation.

2.3.2 Known Potential Benefits

This study offers no direct benefits to participants. Information obtained from this protocol study may improve our understanding of the pharmacokinetics and usefulness of a new TSPO tracer.

2.3.3 Assessment of Potential Risks and Benefits

This study will determine the viability of [^{18}F]SF12051 as a prominent radiotracer for TSPO in vivo imaging. If successful, this radiotracer can be a more stable alternative for future TSPO imaging studies as compared to the currently used ^{11}C counterpart. The risks are reasonable in relation to anticipated benefit.

The ways in which the study design aims to minimize risks, please reference each risk detailed above (see Section 2.3.1).

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the brain uptake of [^{18}F]SF12051 in healthy volunteers and perform kinetic modeling of the [^{18}F]SF12051 in the three different TSPO genotypes	Receptor density measured as distribution volume (V_T)	V_T is the 'gold standard' method to measure receptor density.
Secondary		
To study retest variability and reliability of [^{18}F]SF12051	Receptor density measured as distribution volume (V_T)	The two key parameters for retest studies are variability and reliability.
To study the biodistribution and dosimetry of [^{18}F]SF12051 in healthy subjects	Radiation burden to organs of the body	We follow the FDA standards for calculating radiation burden.

4 STUDY DESIGN

4.1 OVERALL DESIGN

- **Site:** This single-site study will be conducted at the NIH Clinical Center.
- **Hypothesis:** We hypothesize that [^{18}F]SF12051 will have satisfactory distribution regardless of genotype, acceptable retest variability and reliability, and dosimetry typical of ^{18}F-labeled radioligands.
- **Phase:** Phase 1
- **Type of Design:** Study is neither randomized nor placebo-controlled; exploratory study.
- **Study Groups:** Healthy volunteers
- **Study intervention:** Brain and body PET scans with [^{18}F]SF12051 and brain MRI.
- **Interim Analysis:** None
- **Stratifications:** None

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study is to evaluate the efficacy of a novel radioligand and could provide useful information for other studies investigating TSPO in the future.

4.3 JUSTIFICATION FOR DOSE

Studies with novel ^{18}F-radioligands typically inject 5 mCi i.v., which is enough to measure both radioactivity in brain with PET and the concentration of parent radioligand in arterial plasma.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Aged 18 years or older.
2. Healthy based on medical history, physical examination, and laboratory testing.
3. Able to provide informed consent.
4. Willing and able to complete all study procedures.
5. Have been screened for TSPO genotype under 01-M-0254 “The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Participants”.
6. Have their radial artery pulse checked for the presence of adequate ulnar collateral flow and the absence of any metal or foreign objects in both wrists*.
7. Agree to adhere to the lifestyle considerations (see Section 5.4)

*Does not apply to Phase 1 and 3 participants as they will not have an arterial line.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Clinically significant abnormalities on EKG or laboratory testing. This includes CBC; acute care panel (Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen); hepatic panel (alkaline phosphatase, ALT, AST, bilirubin total, and bilirubin direct); mineral panel (albumin, calcium, magnesium, phosphorus); prothrombin and partial prothrombin tests.
2. Participants should not have taken NSAIDs or willow bark tea for two weeks prior to the PET scan.
3. Any current Axis I diagnosis.
4. Positive test for HIV.
5. Unable to have an MRI scan*.
6. History of neurologic illness or injury with the potential to affect study data interpretation.
7. History of seizures, other than in childhood and related to fever.
8. Recent exposure to radiation (i.e., PET from other research) which when combined with this study would be above the allowable limits.
9. Inability to lie flat on camera bed for at least two hours, including claustrophobia and weight greater than the maximum for the scanner (500 lbs).
10. Pregnancy or breast feeding.
11. Able to get pregnant but does not use birth control.
12. Participants must not have substance use disorder or alcohol use disorder. However, alcohol or cannabis use by themselves are not exclusion criteria, unless that use affects the function of daily life.
13. Unable to travel to NIH.
14. NIMH staff or an NIH employee who is a subordinate/relative/co-worker of the investigators.

**Phase 1 and 3 participants will not get an MRI.*

5.2.1 Exclusion of Children

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

5.2.2 Exclusion of Pregnant or Breastfeeding Women

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.

5.2.3 Exclusion of Participants who are HIV Positive

Persons with HIV infection are excluded because HIV infection itself may change cAMP signaling.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Participation of NIH Staff or family members of study team members

NIH staff may be enrolled in this study as the population meets the study entry criteria and NIH Policy 404 will be followed. There will be no direct solicitation of NIH employees, nor will they be directly recruited by or through their supervisors or co-workers to participate in this study. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. NIH employees must have permission from their supervisor and/or take leave in order to receive compensation if participation is during work hours. Prior to enrollment, the NIH employee will be requested to review the Annual Leave Policy for NIH Employees. Participant confidentiality and privacy will strictly be held in trust by the participating investigators, their staff, and the sponsor(s).

Per NIMH Policy, NIMH employees, staff and their immediate family members will be excluded from the study due to ethical concerns about confidentiality and conflict of interest. A copy of the ""NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research" will be made available for NIH employees who wish to participate in this study.

5.4 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Abstain from strenuous exercise and from lifting more than 10 pounds using the affected arm for 48 hours after removal of the arterial line.
- Abstain from taking NSAID pain medication for 2 weeks prior to the PET scan visit(s). If subject is experiencing pain, they can take Tylenol and still be eligible for the study.

5.5 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

Healthy volunteers will be recruited from protocol # 01M0254.

Flyers (with tear offs) may be posted on NIH bulletin boards, at coffee shops, grocery stores, community centers, bookstores, NIH Clinical Center, libraries or placed in doctor's office waiting rooms, retail establishments as well as on public transportation vehicles and/or bus shelters/train stations may be used (all with permission and according to the guidelines of the facility) or sent electronically (without tear offs) to those who request a copy.

Recruitment will also include use of Research Match, Listservs, Craig's List, advocacy websites, resource listings or other website postings and Study-specific page on the NIMH-IRP:

Join A Study Website with URL (placeholder). Short text descriptions of this study will also appear on Facebook. Facebook posts will be made only via official NIMH accounts.

Brochures and other IRB-approved recruitment materials or study information may be given directly to individuals interested in our research in both hard copy and electronic formats, depending on request. Associate Investigators and members of the NIMH Marketing & Community Relations Unit will distribute recruitment materials to individuals/groups, such as mental health clinics, hospitals, self-help and advocacy groups, and local clinicians. Clinicians who are contacted will be provided with information to disseminate to patients as they see fit. We will explain to them that individuals interested in participating in our studies will need to initiate contact with our group and that we will not make this initial contact.

The protocol and contact information to obtain further details will be listed on www.clinicaltrials.gov.

5.6.1 Costs

We expect participants to incur no costs for participating in this study.

5.6.2 Compensation

Participants 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 complete whether or not they opt for early withdrawal from participation. Without incomplete study procedures or unanticipated inconvenience, the total possible compensation for procedures is $220 for Phases 1 and 3, $390 for Phase 2, and $650 for Phase 4. These compensation amounts do not account for time spent at the NIH, for which the first hour of each visit is compensated $20 and every subsequent hour is compensated $10. Payment will be submitted after completion of each visit to NIH through direct deposit. If the investigators need to delay study procedures or if additional time is need for completion, participants may receive additional compensation in accordance with NIH guidelines.

Due to last-minute cancellations, appointments may be scheduled within 72 hours' notice. Participants who are scheduled for appointments on short notice may be eligible for a bonus payment of $250 if they are scheduled and complete a visit with less than 72 hours' notice.

Outpatient participants will receive support for travel, meals, and lodging according to NIH travel policy guidelines. Lodging may be provided directly or reimbursed according to NIH guidelines. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Subjects undergoing Dosimetry Whole-body Scan (Phases 1 and 3)

Visit 1 to NIH	
Screening	$20
Pregnancy test	$10
Visit 2 to NIH	
PET scanning	$150

Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$230*

Subjects undergoing Brain Scan (Phase 2)

Visit 1 to NIH	
Screening	$20
Pregnancy test	$10
Visit 2 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 3 to NIH	
Brain MRI	$100
Pregnancy test	$10
Total	$400*

Subjects undergoing Test-Retest Brain Scans (Phase 4)

Visit 1 to NIH	
Screening	$20
Pregnancy test	$10
Visit 2 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 3 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 4 to NIH	
Brain MRI	$100
Pregnancy Test	$10
Total	$660*

* Participants are eligible for a $250 bonus payment if they were scheduled and completed a PET visit within 72 hours' notice.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

Each participant will receive an injection of [^{18}F]SF12051 during each PET scan.

6.1.2 Dosing and Administration

Each participant will be intravenously injected with about 5 mCi of [^{18}F]SF12051 for each PET scan. The yield of the radioligand synthesis varies such that the available dose may be < 5 mCi. By prior agreement with NIH's Radiation Safety Committee, the clinician covering the scan can approve a dose as low as half of the prescribed dose. We expect that a dose of 2.5-5 mCi will likely be adequate for all participants. The maximal dose will provide the best accuracy to measure radioactivity in brain with PET and the parent radioligand in plasma with radioHPLC.

6.1.2.1 Dose Escalation

None.

6.1.2.2 Dose Limiting Toxicity

As described in Section 2.3.1, toxicity from this radiolabeled drug comes from radioactive emissions and the mass dose of the nonradioactive carrier. Both the injected radioactivity and mass dose are many-fold lower than that required to cause toxicity.

6.1.2.3 Dose Modifications

The dose will not be modified by toxicity. Instead, the dose may be less than that prescribed, based on the PI's judgment of the magnitude of noise that will be acceptable in the brain and blood measurements.

6.1.2.4 Drug Administration

The radioligand will be injected intravenously and must be administered within four hours of its preparation.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

The radioligands are prepared by the NIMH radiochemistry laboratory and handled by the PET department radiopharmacy according to the associated INDs and SOPs. The radioligands have minimal storage because they must be injected within one hour of their preparation.

6.2.1 Acquisition and Accountability

The radioligand will be delivered by the NIMH radiochemistry laboratory to the PET Department's radiopharmacy. Acquisition and accountability are the responsibility of the NIMH radiochemistry laboratory.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Each product will be formulated in sterile, apyrogenic saline containing 10% dehydrated alcohol in a single-use vial and labeled according to requirements for PET drug products described in USP Chapter <823>. The following label will be affixed directly to the vials prior to filling:

[18F]SF12051 Injection
Batch#: SF12051-[YYMMDD0X]
Sterile, apyrogenic saline solution for intravenous administration (contains 10% dehydrated alcohol)
Caution: New drug limited by Federal law to investigational use only

Two additional labels are placed on the outer lead shielding used to transport the vial to the radiopharmacy:

NIMH PET Radiopharmaceutical Sciences Section
Contents: Sterile, apyrogenic saline solution for i.v. administration
Caution: New drug limited by Federal law to investigational use only
Do not use if cloudy or contains particulate matter

[18F]SF12051 Injection
Batch #: SF12051-_____ Activity:_____mCi
Calibration Date: _____ Time:_____
Sterile, apyrogenic saline solution for i.v. administration
(contains 10% dehydrated alcohol)
Expires 4 h after calibration time Half-life of ^{18}F is 110 min

6.2.3 Product Storage and Stability

Products are stable at controlled room temperature for the four-hour expiration period and have no additional storage requirements. Product vials are expected to be single-use, but if the seal is broken and additional product must later be withdrawn, the same storage conditions and original expiration time would apply.

6.2.4 Preparation

Products are provided as sterile, directly injectable solution in a multi-dose vial. The PET radiopharmacist will aseptically remove from the multi-dose vial only the volume required for a participant dose. If necessary, this volume will be diluted with 0.9% normal saline, USP to 12 mL.

6.3 Measures to Minimize Bias: Randomization and Blinding

This study is neither randomized nor blinded.

6.4 Study Intervention Compliance

The injected dose is documented in CRIS.

6.5 Concomitant Therapy

This study has no concomitant therapies.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation instituted by the investigator has been rare in our PET studies but would likely occur during one of the scans (i.e., MRI, two PETs). Examples include a participant experiencing such anxiety that the investigator recommends study discontinuation, even though the participant is willing to proceed, or a post-scan safety measurement that indicates a clinically significant abnormality that must be investigated and rectified before they can proceed.

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- A clinically significant AE, laboratory abnormality, or other medical condition or situation suggesting that continued participation in the study would not be in the best interest of the participant.
- Screen failure, including positive pregnancy test.
- Investigator discretion – e.g., excessive anxiety of the participant.

Any new clinically relevant finding will be reported as an AE, and the cause of discontinuation will be recorded in CRIS. In the event of any discontinuation, we will seek to obtain the next safety measure – i.e., post-scan safety labs or EKG.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study follow-up period
- Death
- Screen Failure

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if they fail to return for a scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit as soon as practicable and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

8.1.1 Screening activities performed prior to obtaining performed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person, or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images

8.1.2 Screening activities performed after a consent for screening has been signed

Prior to consenting to this study, participants will have undergone an initial blood sample to determine TSPO genotype under protocol 01M0254 "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers", which allows for the sharing of collected data.

With the first visit under this protocol, all subjects will have a physical exam, EKG, and blood labs: CBC; acute care panel (Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen); hepatic panel (alkaline phosphatase, ALT, AST, bilirubin total, and bilirubin direct); mineral panel (albumin, calcium, magnesium, phosphorus); prothrombin and partial prothrombin tests. Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures. Results of these tests will identify participants who should be excluded because of active medical problems or make participation in the protocol unsafe.

Subjects who successfully screen and are found eligible will be asked about their availability for scheduling of the PET scan(s) appointment. Occasionally, we have a last-minute cancelation due to scheduling conflicts, COVID exposure, etc. Given the complexities of arranging for the PET scan slot and synthesis of the ligand, we will inquire whether candidates have flexibility in their schedules to allow them to participate with less than 72 hours' notice. We will maintain a list of those who agree to be available on short notice so that we may contact them if we have a scheduled subject that needs to cancel. In the event that we schedule a participant with a72-hour notice, we will provide an additional payment of $250 if they come in and complete the PET scan.

8.2 STUDY EVALUATIONS & PROCEDURES

In addition to the screening procedures described in Section 8.1, all participants will undergo:

- *Brain MRI* (phase 2 and 4 only) A brain MRI will be obtained for anatomic localization and will be performed on a 3 Tesla scanner located at the NIH Clinical

Center (Bethesda, Maryland). The MRI will take about one hour. Participants will undergo safety screening prior to the MRI to rule out contraindications such as cardiac pacemaker. If a suitable MRI is already available under another protocol or for clinical reasons, it will not be repeated if performed within one year.

- *Pregnancy Tests.* For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the participant will be removed from the protocol.

8.2.1 PET Procedures

Radioligand

[^{18}F]SF12051 will be administered via an indwelling intravenous catheter over ~1 minute, within four hours after synthesis of the radioligand. The radioligands is stable during this period.

Insertion of the intravenous line

An intravenous line will be placed in the arm. The venous line will be used to inject the radioligand and will be removed at the end of the day. The IV line may be replaced if it fails to remain patent.

PET acquisition for brain and/or lung imaging

PET scans will be performed using a PET/CT or the HRRT. 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 or a head holder fixed to the bed. A transmission scan will be performed before the PET scan to measure and correct for attenuation. Tracer infusions will be performed in the nuclear medicine department, while the subject is on the scanner bed.

After an intravenous bolus of about 5 mCi, arterial blood samples will be drawn from the arterial catheter during the PET scan. We will collect about 20-25 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, 10, 15, or 30 minutes. The total amount of arterial blood will be about 100 mL. PET images of the brain will be acquired in three-dimensional mode with increased length of frame for a total of 120 minutes. The duration may be shortened on the basis of the results of time stability analysis.

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.

PET acquisition for dosimetry

Whole body dosimetry scans will be performed with a 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 may wear a head-holding mask and will have their arms and abdomen wrapped with body-restraining sheets. This may add some additional discomfort. Venous samples up to 30 mL may be obtained

to measure [^{18}F]SF12051 levels. 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 [^{18}F]SF12051. 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. Then, a second transmission scan, identical to the first, will be performed followed by a second set of dynamic emission scans.

Arterial Line Placement

For brain scans, 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/h) 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.

8.2.2 Biospecimen Evaluations

After injections of the radioligand during brain scans, arterial sampling will be performed continuously at early time points and discretely at later time points, but this plan can be modified if required to improve quality of data. The arterial blood samples will be processed for total radioactivity though a gamma counter and will processed through high performance liquid chromatography (HPLC) to measure metabolites. Correlation between arterial radioactivity and brain activity via the PET scan will allow for pharmacokinetic modeling and accurate quantification of the signal.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

See Section **Error! Reference source not found.**.

8.2.4 Samples for Genetic/Genomic Analysis

Blood for TSPO screening will be acquired only under protocol 01M0254.

8.2.4.1 Management of Results

Participants will have access to “clinical” medical results via the patient portal (e.g., laboratory tests, EKG, and MRI). In addition, we will notify them if any of these results are abnormal. They will not have access to investigational results from the PET scan, as they have no normal ranges.

8.2.4.2 Genetic counseling

None.

8.3 Safety and Other Assessments

Safety Monitoring of PET Scans

Data for safety monitoring will be recorded at three timepoints: no more than three hours before injection, about the middle of the PET scan, and after the PET scan. Recorded data include: blood pressure, pulse, respiratory rate, and EKG (either 3- or 12-lead). The following laboratory tests will also be obtained, but only at two timepoints (before and after the PET scan: CBC, acute care panel (Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen), hepatic panel

(alkaline phosphatase, ALT, AST, bilirubin total, and bilirubin direct), mineral panel (albumin, calcium, magnesium, phosphorus).

Pregnancy Tests

For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the participant will be removed from the protocol.

Follow-up Procedures

Participants will be contacted one to three days after each PET scan to determine whether they have had any untoward sequelae.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

8.4.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.4.3.3 Expectedness

The PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Credentialed clinicians will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

Non-SAEs will be reported to the ISM (annually), to the IRB at the time of Continuing Review and to the Sponsor at regular intervals per request.

8.4.6 Serious Adverse Event Reporting

It is both the Principal Investigator's (PI) and the Sponsor's responsibility to ensure the safety of those on the clinical trial. The PI is responsible for tracking adverse events during the study and providing adverse events lists to the Sponsor at regular intervals per request. These may be requested quarterly and will be requested no less than once a year at the time of IND annual report to the FDA. ALL AEs that are collected, as determined by the written protocol, should be tracked in the ORSC RSS' template AE Tracker or similar document. If the sponsor determines that adverse events are occurring more frequently or more severely than the written protocol had expected and/or anticipated, this should be submitted in an IND Safety Report, as described below. In addition, the PI is responsible for updating the Sponsor about known risks from the drug, as discovered from literature searches or other means.

In accordance with the requirements of 21 CFR 312.32, the PI or designee will report all SAEs, whether or not these are considered related to the investigational drug or study intervention, that occur throughout the study to the Sponsor, including those events listed in the protocol or Investigator's Brochure as anticipated to occur, as follows:

Deaths: within 24 hours of the investigator's awareness*

All other SAEs: within 48 hours of the investigator's* awareness

All AEs will be sent to the Sponsor quarterly, unless requested more or less frequently, for submission to the FDA in the IND Annual Report.

*"Investigator's awareness" includes awareness by anyone on the study team

The PI will immediately report all deaths and SAEs to the Sponsor by disclosing all event-related information in a completed MedWatch Form 3500A. This form should include the IND number, protocol number, PI name, and an assessment on the reasonable possibility of a relationship between the event and the study drug or intervention. **MRNs should NOT be included on this form**. The completed MedWatch Form 3500A will be sent **ENCRYPTED** to the Clinical Director/CEO and/or designated medical monitor with a copy to the NIH Office of Research Support & Compliance (ORSC) Regulatory Support Section.

The Clinical Director/CEO and/or designated medical monitor will be responsible for determining whether the event is reportable to the FDA as an IND Safety Report if it is a serious, unexpected, and suspected adverse reaction (SUSAR). If the sponsor determines the SAE meets the criteria of a SUSAR, the ORSC will submit an Initial IND Safety Report to the FDA no later than 15 calendar days after the PI's notification of the event to the Sponsor. Deaths or life-threatening events will be reported to the FDA no later than 7 calendar days after the PI's notification of the event to the Sponsor. The Sponsor will submit any relevant additional information in a Follow-up IND Safety Report no later than 15 calendar days after receiving the information. All SAEs will be monitored until satisfactory resolution. All AEs and SAEs will be documented on appropriate study records.

8.4.7 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem or is new information that might affect the willingness of subjects on the NIH study to enroll or remain in the study will need to be reported to the NIH Intramural IRB.

8.4.8 Events of Special Interest

None.

8.4.9 Reporting of Pregnancy

Participants will be excluded if they are or may become pregnant.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

Primary Endpoint(s): We hypothesize that [^{18}F]SF12051 will show strong binding affinity for TSPO regardless of genotype.

Secondary Endpoint(s): We hypothesize that the retest variability and reliability of [^{18}F]SF12051 will allow its use in clinical protocols.

We hypothesize that the dosimetry and biodistribution of [^{18}F]SF12051 will be similar to that of other ^{18}F tracers.

9.2 SAMPLE SIZE DETERMINATION

To evaluate genotype sensitivity of [^{18}F]SF12051 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]ER176 in healthy volunteers of different genotypes (Ikawa et al 2016). 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 the same SD for [^{11}C]ER176 and [^{18}F]SF12051, 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 reach a statistical significance with a sample size of 10. Therefore, we require a recruitment of 34 in total for HABs, MABs and LABs under Phase 2 and 4 considering some withdrawals.

We wish to balance the number of subjects in the three TSPO genotypes: HAB, MAB, and LAB. Although all genotypes will be accepted during the initial scans, we will likely require specific genotypes toward the end of the scan, especially for the rare LABs. For this reason, we may select individuals based on TSPO genotype in order to have roughly equal number of subjects in the three groups.

9.3 POPULATIONS FOR ANALYSES

All subjects are healthy volunteers.

9.3.1 Evaluable for toxicity

No toxicity is expected from the radioligand; nevertheless, safety will be monitored as described in Section 8.3.

9.3.2 Evaluable for objective response

No therapeutic response is expected from the radioligand as we are testing a diagnostic agent at sub-pharmacological doses.

9.3.3 Evaluable Non-Target Disease Response

Not Applicable.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

Brain imaging data will be analyzed with kinetic modeling. PET and MRI scans will be coregistered for anatomic definition of regions of interest. 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 [^{18}F]SF12051 binding values, and their identifiability and stability over time.

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 (12). The residence times will be used in OLINDA/EXM (http://www.doseinfo-radar.com/OLINDA.html) to obtain radiation-absorbed dose estimates.

To consider inter-subject variability, radiation absorbed doses are usually estimated from approximately 6-8 subjects (13-15). 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. In addition, we plan at least 3 subjects for each binder group (9 subjects in total). Considering the requirement of one subject for the initial Phase 1 and any possible withdrawals, we seek a total of 10 subjects for Phase 3.

9.4.2 Analysis of the Primary Endpoints

The outcome measure for the primary endpoint will be the total distribution volume and summarized as mean ± SD.

9.4.3 Analysis of the Secondary Endpoint(s)

The outcome measure for secondary endpoints will be the total distribution volume and the absorbed dose. Retest variability will be assessed as absolute variability and reliability will be assessed as interclass correlation coefficient (ICC). Dosimetry will be measured as absorbed dose and summarized as mean ± SD for organs.

9.4.4 Safety Analyses

The safety measures will be recorded but not analyzed statistically.

9.4.5 Baseline Descriptive Statistics

Mean +/- SD for age, weight, etc.

9.4.6 Planned Interim Analyses

None.

9.4.7 Sub-Group Analyses

The different genotype groups will be analyzed separately.

9.4.7.1 Tabulation of individual Participant Data

Individual participant data will be listed by measure and timepoint.

9.4.8 Exploratory Analyses

None.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Only the study investigators designated to obtain consent will be allowed to do so. All study investigators obtaining informed consent must have completed the NIMH HSPU training "Elements of Successful Informed Consent".

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the

investigator and participant below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the following electronic platform to obtain the required signatures:

- iMedConsent platform (which is 21 CFR Part 11 compliant)

Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse. Electronic signatures (i.e., the "signature" and a timestamp are digitally generated) will not be used.

The consent process will be documented in CRIS. A copy of the signed consent form will be given will be placed in the medical record, to the participant and also uploaded in CRIS. .

10.1.2 Consent for minors when they reach the age of majority

Not applicable.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH employees will be obtained as described in Section 5.3.1.

10.1.4 Consent of Subjects who are, or become, decisionally impaired

Adults unable to provide consent are excluded from enrolling in the protocol. For those subjects that become incapacitated, they will be removed from the study.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the NIH for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

In the consent form, we ask permission of the participant (yes/no response) to share imaging and other research data during or after completion of the study with collaborating laboratories at the NIH or outside of the NIH and/or submitted to open-access repositories for secondary research that may or may not involve a collaboration with the NIMH. Such open access repositories (e.g., OpenNeuro, sponsored by the NIMH) allows anyone to access the data for any purpose. Data will be stripped of all identifiers, including name, address, contact information, and medical record number prior to sharing. In addition, the face will be removed from MRI images. The data may be coded, but the key to the code will not be provided to any collaborator or party external to the NIH. After the study is completed, the de-identified data and the code may be indefinitely maintained at the NIH and used for secondary analyses. In contrast to data, no biological samples will be shared.

10.5 SAFETY OVERSIGHT

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial complies with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Monitoring for this study will be performed by an independent safety monitor (ISM), William Regenold, M.D.C.M, who is a board-certified psychiatrist with full clinical privileges at the NIH Clinical Center.

The PI will prepare a report on data and safety parameters for the ISM approximately every 12 months. The ISM will provide a written monitoring report to be submitted to the IRB at the time of continuing reviews.

10.6 CLINICAL MONITORING

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored. Monitoring for the NIH site will be conducted according to the "NIMH Intramural Program Guidelines for Monitoring of Clinical Trials". Monitors under contract to the NIMH OCD ORO will visit the NIH site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information from clinical databases (e.g. CTDB) with individual subjects' records and source documents

(subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, clinical database records and pertinent hospital/sources or clinical records readily available for inspection by the local IRB, FDA, the site monitors, and the NIMH staff for confirmation of the study data.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Principal Investigator (PI). The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, eligibility, and primary outcome data) will be entered into the Clinical Trials Database (CTDB) at NIH. CTDB complies with the Federal Information Security Management Act of 2002 and 21 CFR Part 11. The data system includes audit trail, password protection, and control staff access level to the application and data. Edit checks implemented at the eCRF include: data type validation and numeric range checks. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, or as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 PROTOCOL DEVIATIONS AND NON-COMPLIANCE

It is the responsibility of the PI to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to the NIMH Program Official and the IND sponsor; Dr. Maryland Pao, Clinical Director, holds both of these positions. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.10.2 Genomic Data Sharing Compliance

The only genomic data analyzed will be TSPO binding affinity, which will be shared under the conditions set in 10.10.1.

10.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HABs	High-affinity Binders
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LABs	Low-affinity Binders
MABs	Medium-affinity Binders
MRI	Magnetic Resonance Imaging
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
PET	Positron Emission Tomography
PI	Principal Investigator
SAE	Serious Adverse Event
SNP	Single Nucleotide Polymorphism
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SUV	Standardized Uptake Value
TSPO	18-kDa Translocator Protein
UP	Unanticipated Problem
US	United States
V_T	Total (specific plus non-displaceable) distribution volume

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