Abbreviated Title: COXes in MS

NIH IRB #: 00483

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Title: PET Imaging of Cyclooxygenases in Multiple Sclerosis

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Name of the Coordinating Center: National Institutes of Mental Health (NIMH) will be the Coordinating Center for this multi-institutional study.

Investigational Agent:

Drug Name:	[^{11}C]PS13	[^{11}C]MC1
IND Number:	136241	142872
Sponsor:	NIMH IRP	NIMH IRP
Manufacturer:	NIMH IRP	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; an IRB 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:	PET Imaging of Cyclooxygenases in Multiple Sclerosis
Study Description:	This study will examine whether cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) are elevated in the brain of individuals with Multiple Sclerosis (MS)
Objectives:	To determine whether COX-1 and COX-2 are detectable in the brains of individuals with MS.
Endpoints:	Primary endpoint: Calculation of COX-1 and COX-2 densities from [^{11}C]PS13 and [^{11}C]MC1 PET scans, respectively, using baseline scans and scans after blockade with ketoprofen and celecoxib, respectively. Secondary endpoint: 1) Comparison of [^{11}C]PS13 and [^{11}C]MC1 specific uptake in different types of MS lesions (active, chronic active, inactive) and in normal white matter. 2) Comparison of [^{11}C]PS13 and [^{11}C]MC1 specific uptake in the brain lesions of the same subjects
Study Population:	Sixteen (16) participants with MS will be recruited. Participants may be male or female, 18 years or older and enrolled in the HP-00079860 protocol at the University of Maryland in Baltimore (UMB).
Phase:	2
Description of Sites/Facilities Enrolling Participants:	This is a multisite protocol that will enroll patients from University of Maryland and screening and PET imaging will be performed at the NIH Clinical Center.
Description of Study Intervention:	Participants will be intravenously injected with up to 20 mCi of [^{11}C]PS13 and [^{11}C]MC1 twice, once at baseline and once after blockade by ketoprofen and celecoxib, respectively.
Study Duration:	36 months.
Participant Duration:	2-5 days.

1.2 SCHEMA

Visit 1
Time Point

Obtain informed consent. General medical history and examination, and laboratory testing)

Visit 2-3
Time Point

Day hospital visits:
1 IV, pre-lab EKG
PET scan #1 at baseline with one tracer
PET scan #2 after blockade
Post-labs
Follow-up phone call

Visit 3-5
Time Point

Day hospital visits:
1 IV, pre-lab EKG
PET scan #1 at baseline with the other tracer
PET scan #2 after blockade
Post-labs
Follow-up phone call

1.3 SCHEDULE OF ACTIVITIES (SOA)

This study will require one to five visits, including the initial evaluation (1 day) and four PET scans (1-4 days); two PET scans for each radioligand. The screening evaluations during the initial visit will take 4-6 hours and will typically be performed in one day. Those who agree to join the study on an outpatient basis will adhere to the following schedule: during the first visit, the participant will give informed consent and complete the screening, which includes a history and physical exam. The screening evaluation will include screening labs and EKG and will typically be done on the same day. The two PET scans with each radioligand will typically occur on the same day, but they may be done on separate days.

These patients enrolled will have already been recruited under protocol HP-00079860 (In vivo assessment of meningeal inflammation and its clinical impact in multiple sclerosis by 7 Tesla MRI) at the University of Maryland, Baltimore (UMB; PI: Daniel Harrison). Participants will have already undergone the study activities involved in UMB protocol HP-00079860, which includes a 7T MRI of the brain. The 7T MRI acquired under the UMB protocol will later be used for co-registration of PET, so MRI does not need to be repeated under this protocol.

Table 1

	1st Visit / Screen	2nd Visit	3rd Visit	4th Visit	5th Visit
Informed consent	X				
History and physical exam	X				
Screening lab tests*	X				
EKG	X	On each visit with PET scan			
Brain PET scans		Up to two at each visit, no more than four total			
Safety tests for PET scans**		On each visit with PET scan			

'If a blocker is given, the following set of PET scans with the other blocker must not be done earlier than two days after. The PET scans should be completed within a timeframe of two months.

**The screening lab tests are described in Section 5.2.*

*** Safety tests for each PET scan are described in Section 8.3.*

2 INTRODUCTION

2.1 STUDY RATIONALE

Our laboratory developed two novel radioligands, [^{11}C]PS13 and [^{11}C]MC1 for PET imaging of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), respectively. COX-1 and COX-2 are two isoforms of COX enzyme which play a key rate limiting step in the conversion of arachidonic acid (AA) into pro-inflammatory lipid metabolites called prostaglandins (PGs). COX-1 is constitutively expressed in most brain tissues whereas COX-2 is induced by inflammatory stimuli. We recently found that COX-2 was elevated and measurable in the currently symptomatic joints of patients with rheumatoid arthritis [1]. We now seek to determine whether COX-2 is measurable in the brains of participants with MS, in particular in the MS plaques.

Although MS provides the technical advantage of local areas of active inflammation, it also entails the technical disadvantage of a breakdown of the blood-brain barrier surrounding active plaques. Thus, the uptake could reflect both parent radioligand binding to COX-1 and COX-2 as well as inactive, polar radiometabolites that can spill into the compromised tissue. To address this problem, a COX-1 and COX-2 blocking agent, ketoprofen and celecoxib, will be given. Radioligand specific uptake will be blocked by ketoprofen and celecoxib, whereas the activity due to radiometabolites won't be affected.

In this way, the outcome measure will be a comparison of brain lesions at baseline and after blockade, normalized to a reference region such as the contralateral mirror image of the lesion, and thus does not require absolute quantitation with arterial plasma samples. Of course, this ratio would not determine whether COX-1 or COX-2 are globally increased in MS brain, in addition to any local increases associated with active plaques. However, arterial blood sampling seems excessively burdensome for an exploratory study. Expressed in other terms, if COX-1 or COX-2 do not detect any specific binding in MS lesions, then it's unlikely to detect a global increase compared to healthy subjects.

A reference region will be selected based on the PET scan blocked by celecoxib or ketoprofen. A true reference region has no specific/blockable binding, and a pseudo-reference region has a small amount of specific binding. The cerebellum might not be the most appropriate choice because the cerebellum is affected by diffuse inflammation in MS. This is however a problem common to all regions, since MS affects the whole brain. A more accurate reference region might be the mirror image of the ROI in the opposite hemisphere because, even if affected by generalized inflammation, the contralateral ROI would more accurately reflect the amount of nonspecific binding of the target region. A comparison with the contralateral region would track the change in COX-1 and COX-2 binding after blockade with sufficient accuracy, without the need of an arterial input function.

The MS lesions will be identified anatomically by a 7T MRI, acquired under a specific protocol of UMB (PI: Daniel Harrison). The 7T MRI images include the following brain sequences: MP2RAGE (magnetization prepared 2 rapid acquisition gradient echo), MPRAGE (magnetization prepared rapid acquisition gradient echo), FLAIR (fluid attenuated inversion recovery), and a ME-GRE (multi-echo gradient recalled echo). All are 3D acquisitions and acquired both pre- and post-gadolinium contrast. All 7T MRI images will be co-registered to the pre-contrast MP2RAGE T1-weighted image at 0.5 mm^3 resolution. MRI images will undergo both manual processing for MS lesion identification and classification, along with use of

automated and semi-automated tools for brain structure/tissue segmentation. PET images will also be co-registered to the pre-contrast T1-weighted MP2RAGE image, allowing interrogation of PET signal in various lesion types and brain structures. Every effort will be made to have PET study visits occur as close to the date of the 7T MRI as possible. In order to account for logistical problems, PET will be performed within 180 days before or after MRI. Similarly, the baseline/blocked scans will be performed within two months of each other. An interval of at least two days will be respected between the administration of the two blockers (ketoprofen and celecoxib). Lesions will be identified on MRI independently of the PET scans and by investigators blinded to the PET scans.

As a secondary endpoint, we will explore whether different types of MS lesions display different amounts of COX-1 and COX-2 binding. In particular, active lesions will be identified as those that show contrast enhancement with administration of gadolinium. Chronic-active lesions will be identified as those who have a paramagnetic outer rim identified on ME-GRE images that have been processed for quantitative susceptibility maps (QSM). Inactive lesions will be white matter lesions that do not meet criteria for active or chronic-active. Normal appearing white matter will be all white matter minus lesioned white matter, as determined by automated segmentation. We hypothesize that COX-1 and COX-2 binding positively correlates with the activity of the lesion.

Finally, we will determine whether the expressions of COX-1 and COX-2 are correlated by analyzing the uptake of the two tracers within the lesions of the same subjects.

2.2 BACKGROUND

Multiple sclerosis (MS) is an autoimmune disease whose pathogenesis is characterized by neuroinflammation. The socioeconomic impact of MS is considerable in terms of healthcare costs and individual disability. The clinical presentation of MS is highly heterogeneous. Typically, the onset of the disease is characterized by acute worsening of neurological symptoms followed by a full or partial recovery. On average 10 years after disease, secondary progressive MS leads to irreversible and progressive disability [2]

Although the progression of the disease can be slowed down to some extent with disease-modifying therapies, there is no curative treatment, especially to prevent the cumulative disability in the more progressive forms of the disease. Thus, more research is clearly needed to understand the pathophysiology, to develop alternative biomarkers for the evaluation of disease activity, and to seek alternative pathways for therapeutic development in the treatment of progressive MS.

Neuroinflammation and axonal loss occur during all stages of MS [3]. Thus, by studying inflammation, one may gain important insights about the physiopathological course of the disease, especially for its degenerative component.

MRI and MS

Magnetic resonance imaging (MRI) is the main diagnostic and prognostic tool used in the study and treatment of patients with multiple sclerosis (MS). Visualization of MS pathology by MRI has brought about substantial advances in MS care, including earlier and more accurate

diagnoses and the ability to monitor the effects of treatment and visualize subclinical disease activity. MS, especially after contrast enhancement, can assess the activity of lesions. Active lesions are those that show contrast enhancement with administration of gadolinium. Chronic-active lesions are those who have a paramagnetic outer rim, and inactive lesions are white matter lesions that do not meet criteria for active or chronic-active.

Despite these diagnostic assets, standard clinical MRI fails to visualize much of MS-related pathology.[4, 5] Although current MRI performs well as a tool to measure white matter (WM) inflammation in MS, it has been less successful as a tool to quantify gray matter (GM) pathology, meningeal inflammation, neurodegeneration, chronic inflammatory changes, and myelin tissue content. For this reason, researchers continue to strive towards development of new imaging technologies – hoping to bring us closer to *in vivo* quantification of MS disease processes akin to postmortem histopathology.

PET and MS

Contrarily to MRI, positron emission tomography (PET) imaging enables molecular and neuroreceptor imaging in vivo. Particularly relevant for the study of MS is PET imaging of activated microglia. Traditionally, imaging of microglia has been achieved with radioligands binding to the 18 kDa translocator protein (TSPO)—a protein structure expressed on the outer mitochondrial membrane of activated, but not resting microglia [6].

The first TSPO-PET studies in MS patients were done with the prototypical radioligand [^{11}C] (R)-PK11195. These early studies were able to show for example that TSPO was increased in acute lesions but was low in chronic lesions [7] or that increased TSPO was associated to areas related to demyelinating lesions in MRI [8]. [^{11}C] (R)-PK11195, however, has issues of high non-specific binding and poor signal-to-noise ratio. Newly developed TSPO radioligands, such as ^{11}C-PBR28 [9] and ^{11}C-ER176 [10], have much higher specific binding, but are more sensitive to different binding affinities in 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). While an in-human study showed consistently quantifiable distribution volume for TSPO with [^{11}C]ER-176 than its predecessor in all three genetic groups, there was still some genetic preference for HABs as compared to MABs or LABs [11].

Given the limitations of TSPO radioligands, PET radioligands aimed at different targets have been tried. These include Purinoreceptor P2X7, which has been linked to microglia-mediated neuroprotection via activation of the receptors by ATP [12], the cannabinoid receptor 2, which is also expressed in activated microglia [80], and [11C]TMSX-PET, a tracer for adenosine receptors, which has been used to study the pathologic alterations in the white matter associated with progressive MS disease [13]

The role of COX in MS

Cyclo-oxygenase receptors are involved in neuroinflammation and appear as interesting targets for PET imaging of MS. This enzyme plays a key role in the conversion of arachidonic acid to essential cell-signaling eicosanoids which is accompanied by the production of reactive oxygen species. It is believed that oxidative stress might be responsible for brain inflammatory disorders causing deleterious effects during CNS pathogenesis. What is more, oxidative stress

can activate several intracellular signaling cascades that can have deleterious effects on the cellular homeostasis [14].

COX-1 is almost exclusively localized in microglia which play a complex role in MS pathophysiology [2, 18]. Microglia produce proinflammatory mediators in activated states and play a key role in neuroinflammation. COX-1 expression is elevated in activated microglia and facilitates oxidative stress and proinflammatory processes that ultimately leads to neuronal loss and neurodegeneration.

The role of COX-1 in the neuroinflammatory pathway has been postulated primarily using genetic modulation of COX-1 in rodents as well as neuroinflammation models in animals. For instance, genetic knockout of COX-1 in mice reduced oxidative stress and neuronal damage compared to wild-type mice. In a neuroinflammation model in monkeys, COX-1 expression was increased globally in activated microglia as well as macrophages [19]. Taken together, the evidence suggests that COX-1 is a promising biomarker for studying neuroinflammatory processes.

COX-2 is extensively expressed in MS lesions. COX-2 was found near regions showing active demyelination suggesting that these inducible enzymes could contribute to the pathology of MS [15]. In addition, evidence from genetic and pharmacological studies indicate that increased COX-2 expression and activity contributes to neuronal excitotoxic cell death. Therefore, COX-2 could contribute to excitotoxic death of oligodendrocytes and damage of axons leading to disease progression in MS [15].

Development of PET Radioligands Selective for COX-1 and COX-2

Over the past years, our laboratory developed the first radioligands to image COX-1 and COX-2. These radioligands—[^{11}C]PS13 for COX-1 and [^{11}C]MC1 for COX-2—are potent and selective for each isoform and have shown promising results in monkeys (Fig. 1) and humans (Fig 2). We found that COX-1 is expressed constitutively (i.e., under healthy conditions) in several organs, including brain, spleen, kidney, and GI tract. The binding of the COX-1 radioligand was selective for its targeted isozyme, as it could be blocked by COX-1-selective inhibitors in both monkeys and humans, but not by COX-2-selective inhibitors.

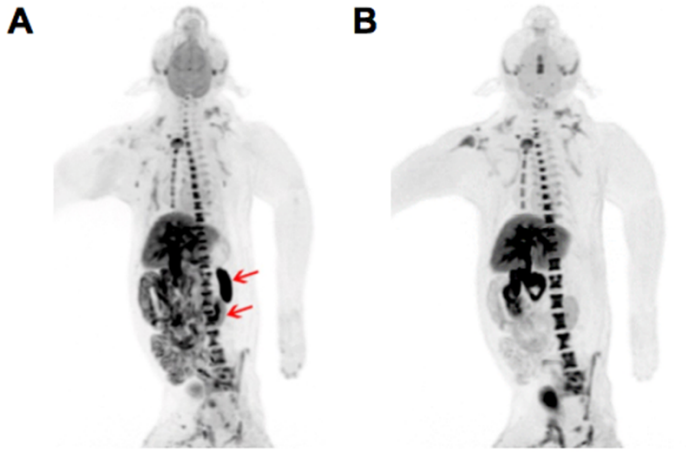


Figure 1. The maximum intensity projection images in rhesus macaque after injection of [^{11}C]PS13 under baseline conditions (A) and after injection of PS13 (0.3 mg/kg) (B). High uptake was observed in the brain, spleen (upper arrow), gastrointestinal tract, and kidney medulla (lower arrow) at baseline. Uptake was blocked by pharmacological doses of PS13 [16].

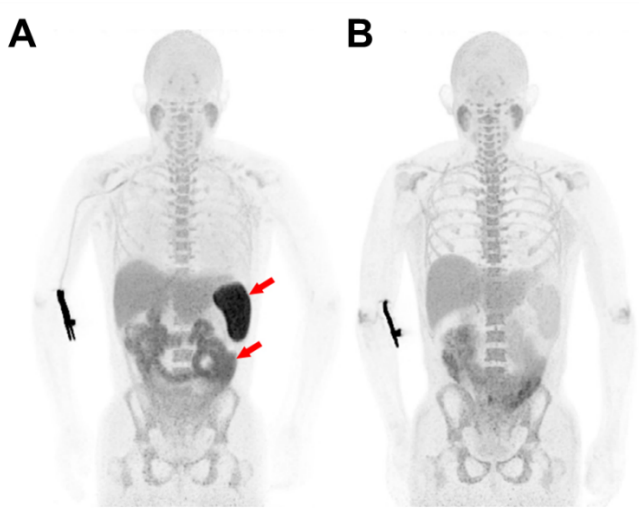


Figure 2. The whole-body maximum intensity projection images with [^{11}C]PS13 under baseline conditions (A) and after oral administration of ketoprofen 75 mg (B) in a healthy volunteer. Ketoprofen, a selective COX-1 inhibitor, blocked 50−80% of uptake in spleen (upper arrow) and gastrointestinal tract (lower arrow) [17]

COX-1 was found in all areas of brain, but was particularly high in the pericentral cortex, occipital cortex, and hippocampus (Fig. 3). This regional distribution of COX-1 protein measured with PET in brain is consistent with that of the mRNA for *PTGS1*, the gene that encodes the COX-2 protein (Allen Brain Atlas).

Development of a radioligand selective for COX-2 was more challenging because COX-2 is expressed at very low concentrations under healthy conditions. To evaluate [^{11}C]MC1, we first had to upregulate COX-2 in monkey brain using intracerebral injection of the inflammogen lipopolysaccharide (LPS). Under baseline/healthy conditions, [^{11}C]MC1 detected no COX-2 in brain. However, after LPS injection, [^{11}C]MC1 clearly visualized areas of elevated COX-2 and showed appropriate subtype selectivity (Fig. 4). Our interpretation was that [^{11}C]MC1 lacked the sensitivity to measure low baseline concentrations of COX-2 but could detect the enzyme after upregulation, which can be a 10- to 20-fold effect. To determine whether [^{11}C]MC1 can detect COX-2 in human participants, we studied individuals with rheumatoid arthritis (RA), as COX-2 is upregulated in affected joints. In the four participants studied to date, symptomatic joints had

increased uptake of [^{11}C]MC1 that was blocked by celecoxib (Fig. 5) [1]. These preliminary results confirmed that [^{11}C]MC1 is capable of imaging COX-2 in humans if COX-2 concentrations are adequately elevated by peripheral inflammation.

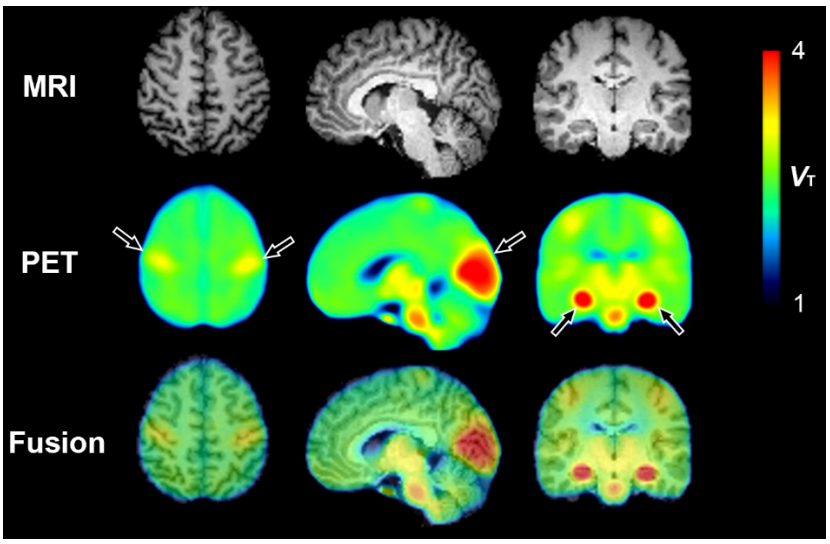


Figure 3. Parametric total distribution volume (V_T) images calculated by the Logan graphical analysis method. MRI is from a representative participant, and PET images are averaged from 20 scans in 10 participants. Arrows represent notable [^{11}C]PS13 binding in the pericentral cortex, occipital cortex, and hippocampus [17].

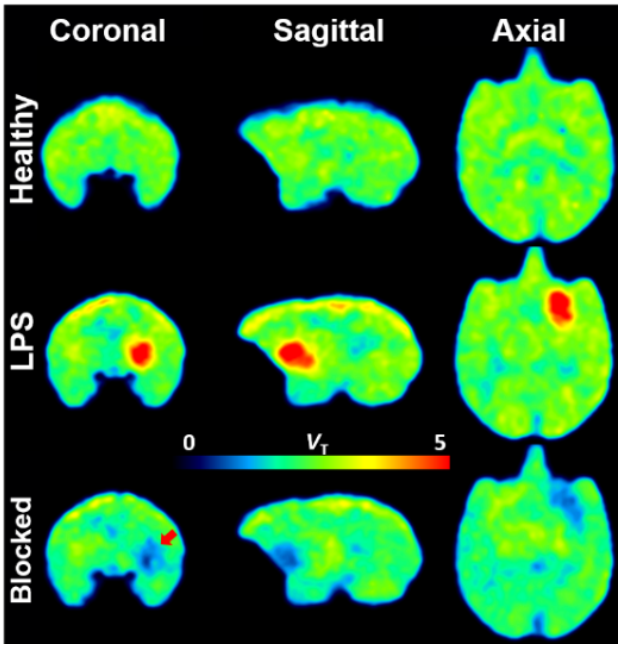


Figure 4. Parametric distribution volume (V_T) images of [^{11}C]MC1 uptake on Day 1 after the second LPS injection in a monkey. *Upper row:* [^{11}C]MC1 uptake was markedly increased surrounding the injection area in the right putamen. *Bottom row:* MC1 blocked radioligand binding to COX-2 at the injection site (marked by red arrow) to a level lower than that in the remainder of the brain [1].

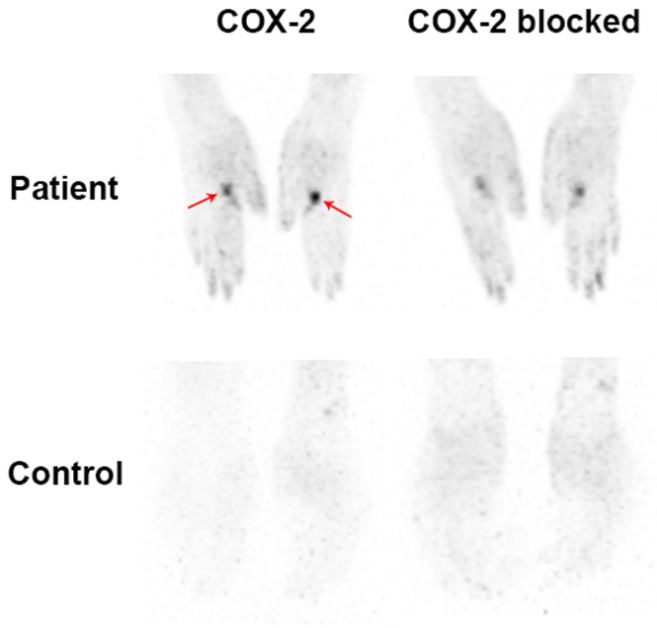


Figure 5. Human PET images of COX-2 in an individual with rheumatoid arthritis and a healthy control. Increased [^{11}C]MC1 uptake in the bilateral hand joints reflected increased COX-2 binding in a participant with rheumatoid arthritis compared to the healthy volunteer. The increased [^{11}C]MC1 uptake in the individual with rheumatoid arthritis was partially blocked by celecoxib (400 mg) but may be completely blocked by higher doses [1].

We scanned six new healthy participants under protocol 19-M-0079 (Evaluation of novel PET radioligands as inflammatory biomarkers in rheumatoid arthritis and myositis). The brain scans were fully quantitative and had an arterial input function, acquired at baseline and after blockade by 600 mg of celecoxib, a preferential COX-2 inhibitor. All subjects showed a small amount (10-20%) of displaceable COX-2 signal in the brain, measured as total distribution volume corrected for plasma free fraction of the parent radioligand. This measure of enzyme density (V_T/fp) was 95 ± 9.5 mL/cm^{-3} at baseline and 76 ± 4.7 mL/cm^{-3} after blockade (Fig 6).

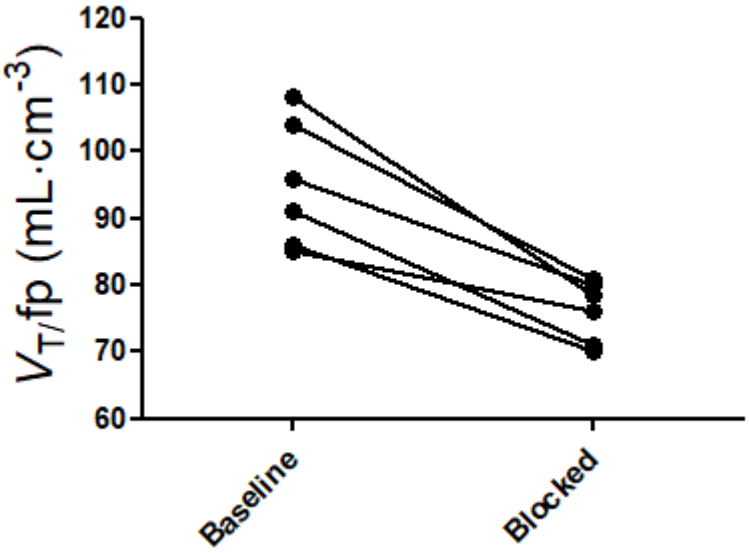


Figure 6: VT/fp at baseline and after blockade with 600mg celecoxib in 6 healthy subjects. A small displaceable binding (10-20%) is seen in each subject.

These quantitative brain studies demonstrate that our COX-2 radioligand, [^{11}C]MC1, has the sensitivity necessary to measure the low densities of COX-2 present in healthy human brain. These studies in healthy participants complement our prior finding that [^{11}C]MC1 can detect elevated COX-2 in symptomatic, but not asymptomatic, joints in participants with rheumatoid arthritis (Shrestha et al. 2020).

Given that the same target, COX-2, is expressed in MS lesions, we expect that [^{11}C]MC1 will be able to detect an increase of COX-2 concentrations also in this population of subjects.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks include those associated with: a) medical examinations including laboratory testing that may reveal previously undiagnosed medical disorders, b) placement of a venous line, c) venous blood sampling, d) radiation exposure from ^{11}C-PS13 and ^{11}C-MC1 and the transmission scans, e) PET scanning, f) celecoxib administration, and g) ketoprofen administration.

a. Examination and laboratory testing

Medical examinations are associated with minimal risks. We will first explain and familiarize the subjects with the laboratory testing to minimize discomfort, if any, during testing. In the present protocol, all subjects have already been diagnosed under the UMB protocol, and the only lab tests done under this protocol are the safety lab tests.

b. Venous catheterization

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

c. Venous sampling

Subjects will have venous blood sampling of no more than 150 mL during the entire course of the study. This includes approximately 75 mL for laboratory tests. 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.

d. Radiation exposure risks

Whole-body imaging with [^{11}C]MC1 in one male and one female healthy participant under protocol 19-M-0079 showed that the radiation exposure (rem) from a 20 mCi injection of [^{11}C]MC1 had an effective dose of 0.34. Liver (1.33), gallbladder wall (1.26), and kidneys (0.50) were the three organs with the highest exposures.

We calculated the radiation exposure of [^{11}C]PS13 from whole-body imaging in 15 healthy subjects. The radiation exposure (rem) from a 20 mCi injection, which is a typical dose for brain imaging, was: effective dose (0.34). Spleen (2.06), liver (0.74), and lungs (0.53) were the three organs with the highest levels.

With regard to exposure from the transmission scan, the PET Department recently implemented Dr. Innis's suggestion to decrease the current (amperage) and, thus, overall radiation from the CT scan. We do not need a high resolution (high current) image for attenuation correction; a low-resolution scan, like that obtained from a line source, is perfectly adequate to correct attenuation in the PET emission scan. With the lowered current, the exposure to the lens of the eye is now 0.26 rem, about one-third of the previous value.

We routinely include the dose from two transmission scans in the event that it must be repeated in any given participant. The effective doses for two head transmission scans from a PET/CT are ~ 0.04 rem. Thus, the total effective dose in each [^{11}C]MC1 or [^{11}C]PS13 PET scan is 0.38 rem. Because each participant may undergo up to two [^{11}C]PS13 and two [^{11}C]MC1 PET scans under this protocol, the total effective dose is estimated as 1.52 rem. The estimated annual effective dose of 1.52 is well below the limit of 5 rem per year established by the NIH's Radiation Safety Committee. All participants will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, will not exceed an effective dose of 5 rem per year.

e. PET scan

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 subjects will have ready access, should they experience any problems. Subjects can communicate with the trained health professionals while in the scanner and can withdraw from the study at any time if they wish to do so. The head of the subjects may be constrained by a head-holder or a thermoplastic mask, which are usually well-tolerated.

Occasionally subjects become anxious during the scan. In that case, subjects can request the operator of the PET to stop the scan.

f. Celecoxib administration

Participants will receive a single dose of celecoxib (up to 600 mg) before the second PET scan. This dose is beyond the normal therapeutic range (100- 200 mg BID), but we feel it is safe based on prior experience. Celecoxib will be administered only short-term – i.e., one dose.

Prior Experience

a) Although the typical dose of celecoxib is 100-200 mg BID, the FDA-approved package insert (page 1) allows 400 mg BID for diseases like acute pain or primary dysmenorrhea. (https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/020998s050lbl.pdf)
b) Doses up to 2,400 mg/day for up to 10 days in 12 participants did not result in serious toxicity. See page 9 of celecoxib's package insert, which has been uploaded in iRIS.
c) In one large clinical trial of 8,059 individuals with RA, those enrolled in the celecoxib arm took celecoxib (400 mg BID) for six months (participants in the other two arms took either ibuprofen or diclofenac) [18]. This study found a decrease in GI toxicity among participants using celecoxib but no increase in cardiovascular events [18].
d) Our collaborator (James Katz, MD, NIAMS) has used up to 600 mg as a single dose in patients with RA on another protocol (19-M-0079) and observed no untoward effects.
e) We have been using a dose of 600 mg for our protocol 19-M-0079, in order to block ^{11}C-MC1 scans in a manner similar to the one envisioned in this protocol, without any untoward effects. To date, about ten subjects have received this blocking dose under this protocol.

Short-Term Use

Few studies have examined whether increased cardiac risk is associated with short-term use of celecoxib. In two randomized, double-blind studies, participants received 200 mg of celecoxib daily for six weeks without a significant increase in cardiovascular adverse events [19, 20]. Another study found that celecoxib had a superior safety and tolerability profile compared to diclofenac [19]. A seven-day trial of 200 mg celecoxib twice daily actually improved outcomes in myocardial injury in elective percutaneous intervention for stable angina [21]. To more thoroughly investigate this, a retrospective analysis conducted using the Veterans Integrated Service Network (VISN) 17 database found that although long-term celecoxib use was associated with increased cardiovascular risk, short-term use did not carry this risk [22].

Common reactions to repeated doses of celecoxib are: headache, upset stomach, upper respiratory infection, diarrhea, abdominal pain, nausea, vomiting, rash, gas, swelling in the legs, dizziness, elevated liver enzymes, impaired kidney function, and sensitivity to sunlight. In addition, the Food and Drug Administration (FDA) has placed a "Black Box Warning" for celecoxib that the drug might rarely lead to death, heart attack, or stroke. The "Black Box Warning" also describes that all non-steroidal anti-inflammatory drugs (NSAIDs), which includes celecoxib, are associated with an increased risk of ulcers or tear in the lining of the stomach or intestines, which may cause bleeding or increased risk of infection. We feel that the risk of serious reactions is low as we are giving a single dose of celecoxib. Participants will be monitored for adverse reactions. Rarely, a participant will have an allergic reaction to their very first dose. Because there are possible teratogenic or nonteratogenic effects of celecoxib on pregnancy, participants of child-bearing potential will be required to use contraception from the time they are enrolled until three days after the last administration of celecoxib. Urine pregnancy testing will be also done before each administration of celecoxib. This drug has been approved

by the U.S. Food and Drug Administration (FDA); it is not FDA approved for the off-label use in this study. However, the FDA is allowing its use in this study.

g. Ketoprofen administration

Participants will receive a single dose of ketoprofen of 75 mg before the second COX-1 PET scan. This dosing range is within the normal therapeutic dosing range for ketoprofen administration and is below the maximum therapeutic dose of ketoprofen which is 300 mg/day. Common reactions to ketoprofen administration include: dyspepsia, nausea, abdominal pain, constipation, headache, dizziness, somnolence, rash, AST/ALT elevation, peripheral edema, fluid retention, tinnitus, ecchymosis, photosensitivity, and delayed ovulation. Rare but serious reactions associated with ketoprofen administration include: gastrointestinal bleeding, perforation, or ulcer, myocardial infarction, stroke, thromboembolism, hypertension, congestive heart failure, renal papillary necrosis, nephrotoxicity, hepatotoxicity, anaphylaxis, bronchospasm, exfoliative dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiform, anemia, blood dyscrasias, and prolonged bleeding time (https://online.epocrates.com/rxmain accessed 12/8/2017). We feel that the risks of serious reactions are low as we are giving a single dose of ketoprofen within the recommended dose range in healthy volunteers. Subjects will be monitored for adverse reactions. Rarely, a subject will have an allergic reaction to their very first dose. This drug has been approved by the U.S. Food and Drug Administration (FDA); it is not FDA approved for the off-label use in this study. However, the FDA is allowing its use in this study.

Prior Experience

a) The maximum therapeutic dose of ketoprofen is 300 mg/day, and previous clinical trials in pain control with single dose of 150-225 mg reported of no serious adverse events. Most of the reported adverse effects were nausea, vomiting, and GI discomfort, but otherwise were well-tolerated (Gaskell *et al*, 2017; Sunshine and Olson, 1988; Turek and Baird, 1988).
b) Under protocol 17-M-0179, we administered blocking doses of ketoprofen in 8 subjects, with doses up to 75 mg. Despite evidence of substantial blockade of the organ uptake, especially in the spleen, no pharmacological side effects were noted.

2.3.2 Known Potential Benefits

This study offers no direct benefit to individual subjects but will lead to generalizable knowledge regarding the usefulness of COX-1 and COX-2 as potential biomarker of MS.

2.3.3 Assessment of Potential Risks and Benefits

This study will determine whether COX-1 and COX-2 are detectable in the brains of subjects with MS. If the results are positive, such PET imaging may be used to explore the pathophysiological role of inflammation in MS. This valuable information may then help guide future trials with anti-inflammatory drugs, a benefit to future patients that justifies the risks to the current participants.

With regard to how the study design sought to minimize risks, please see each risk listed above (Section 2.3).

3 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Justification for Endpoints
Primary		
To determine whether COX-1 and COX-2 are detectable in the brains of participants with MS.	PET scans with [^{11}C]PS13 and [^{11}C]MC1 with and without blockade by ketoprofen and celecoxib.	We previously demonstrated that these radioligands are selective for COX-1 and COX-2.
Secondary		
To determine whether COX-1 and COX-2 differ in various types of MS lesions (active, chronic active, inactive) compared to normal white matter.	PET scans with [^{11}C]PS13 and [^{11}C]MC1 with and without blockade by ketoprofen and celecoxib.	We previously demonstrated that these radioligands are selective for COX-1 and COX-2.
To determine whether the expressions of COX-1 and COX-2 are correlated in the lesions of the same subjects	PET scans with [^{11}C]PS13 and [^{11}C]MC1 with and without blockade by ketoprofen and celecoxib.	We previously demonstrated that these radioligands are selective for COX-1 and COX-2.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Subjects will undergo medical history and general medical examination, ^{11}C-PS13 and ^{11}C-MC1 PET scans at baseline and after blockade by ketoprofen and celecoxib, and laboratory testing.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

To determine whether COX-1 and COX-2 is detectable in the brains of individuals with MS, whether COX-1 and COX-2 binding is correlated with the activity of the lesion as measured by MRI, and whether COX-1 and COX-2 are correlated in the brain lesions of the same individuals.

4.3 JUSTIFICATION FOR DOSE

The FDA has allowed a dose up to 20 mCi of ^{11}C-PS13 and ^{11}C-MC1 to be used for research purposes.

Celecoxib 600 mg PO.

Ketoprofen 75 mg PO.

5 STUDY POPULATION

Patients with MS will be recruited by referral from the PI at UMB, with subjects being those who are currently enrolled under UMB protocol HP-00079860 and who have signed a clause in the UMB consent document allowing contact for future research.

Healthy subjects won't be needed for this study because MS subjects scanned at baseline will be their own controls for the scans after blockade.

5.1 INCLUSION CRITERIA

1. Aged 18 years and older.
2. Female participants of childbearing potential must be using a medically acceptable means of contraception.
3. Able to provide informed consent.
4. In good general health as evidenced by medical history and physical examination.
5. Enrolled under UMB protocol HP-00079860 (In vivo assessment of meningeal inflammation and its clinical impact in multiple sclerosis by 7 Tesla MRI), P.I. Daniel Harrison and have agreed to contact for future research.

5.2 EXCLUSION CRITERIA

1. Any medical contraindication to the procedures performed in the study, or any current severe medical or psychiatric illness other than MS. This includes contraindications to Celecoxib, such as aspirin sensitive asthma, and contraindications to ketoprofen, such as hypersensitivity to ketoprofen or history of upper or lower gastrointestinal bleeding.
2. Behavioral symptoms that would preclude the gathering of data for the study, or advanced disease such that subjects cannot provide assent.
3. Clinically significant abnormalities on EKG or safety labs. This includes CBC; acute care panel (Na, K, Cl, CO2, creatinine, glucose, urea nitrogen); hepatic panel (alkaline phosphatase, ALT, AST, bilirubin total, and bilirubin direct); mineral panel (albumin, calcium, magnesium, phosphorus).
4. MRI performed >180 days before or after the PET scan
5. Have taken NSAIDs for two weeks prior to the PET scan. Have taken aspirin, corticosteroids (except for topical creams), or immunosuppressants (except for FDA-approved disease-modifying therapy for MS) in the prior month.
6. Have other major neurological or medical diseases that may cause cognitive dysfunction, such as structural brain diseases, metabolic diseases, paraneoplastic syndromes, infectious diseases, or other significant neurological abnormalities.
7. Have an unstable medical condition that, in the opinion of the investigators, makes participation unsafe (e.g., an active infection or untreated malignancy).
8. Are unable to travel to the NIH.
9. Have recent exposure to radiation related to research (e.g., PET from other research) that, when combined with this study, would be above the allowable limits.
10. Have an inability to lie flat and/or lie still on the camera bed for at least two hours, including claustrophobia, overweight greater than the maximum for the scanner, and

uncontrollable behavioral symptoms, which will be screened by an interview with the patient and/or caregiver during the screening visit.

11. Pregnancy
12. HIV infection
13. Be NIMH staff or an NIH employee who is a subordinate/relative/co-worker of the investigators.

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 cause neuroinflammation, and we wish to specifically study the effect of MS on neuroinflammation.

5.2.4 Exclusion of Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may not be enrolled in this study.

5.3 Inclusion of Vulnerable Participant

None

5.4 Lifestyle Considerations

None

5.5 Screen Failures

Screen failures are defined as participants who consent/assent 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 demographic characteristics, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an exclusion criterion that may resolve by time of treatment may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.6 Strategies for Recruitment and Retention

Patients will be referred here after recruitment under protocol HP-00079860 (In vivo assessment of meningeal inflammation and its clinical impact in multiple sclerosis by 7 Tesla MRI), PI Daniel Harrison at University of Maryland, Baltimore. Potential participants will have signed a clause in that study's consent document allowing contact for future research and will

have given Dr. Harrison permission to provide the NIH study team with their contact information. These potential participants will then be contacted by the NIH study team, informed of the study, and a screening visit will be arranged with their permission. This process and subsequent study procedures will be governed by an IRB reliance agreement in which the UMB IRB will approve reliance upon the NIH IRB.

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

5.6.1 Costs

We expect that patients will incur no costs for participating in this study.

5.6.2 Compensation

Subjects will be compensated for time- and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. Volunteers will be paid for each portion of the study they complete whether or not they opt for early withdrawal from participation. Without any delay of study procedures or unanticipated inconvenience, the total possible compensation is $960. If the investigators need to delay study procedures or if additional time is need for completion, subjects may receive additional compensation in accordance with NIH guidelines.

Visit 1 to NIH	
Screening	$50
Pregnancy test	$10
Escort fee	$20
Visit 2 to NIH	
Pregnancy test	$10
PET scanning	$150
Antecubital venous catheter	$30
Movement restriction	$10
Escort fee	$20
Visit 3 to NIH	
Pregnancy test	$10
PET scanning	$150
Antecubital venous catheters	$30
Movement restriction	$10
Escort fee	$20
Visit 4 to NIH	
Pregnancy test	$10
PET scanning	$150
Antecubital venous catheter	$30
Movement restriction	$10
Escort fee	$20
Visit 5 to NIH	
Pregnancy test	$10
PET scanning	$150
Antecubital venous catheter	$30

Movement restriction	$10
Escort fee	$20
Total	$960

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

Each participant will receive an injection of [^{11}C]PS13 and [^{11}C]MC1 before and after blockade by ketoprofen and celecoxib, respectively.

6.1.2 Dosing and Administration

Each participant will be intravenously injected with up to 20 mCi of [^{11}C]PS13 and [^{11}C]MC1 for each PET scan. The yield of the radioligand synthesis varies such that the available dose may be < 20 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 (i.e., 10 - 20 mCi). We expect that a dose of 10-20 mCi will likely be adequate for all participants. The maximal dose will provide the best accuracy to measure radioactivity in brain with PET. Please note that the half-life of ^{11}C is only 20 minutes; thus, the scan will last several half-lives.

6.1.2.1 Dose Escalation

None

6.1.2.2 Dose Limiting Toxicity

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 Drug 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 one hour of its preparation.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

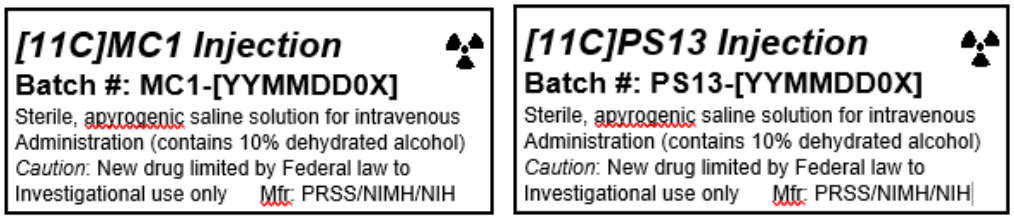
The radioligands are prepared by the NIMH radiochemistry laboratory and handled by the PET Department radiopharmacy according to the associated INDs and SOPs. The radioligands have minimal storage because they must be injected within one hour of their preparation.

6.2.1 Acquisition and Accountability

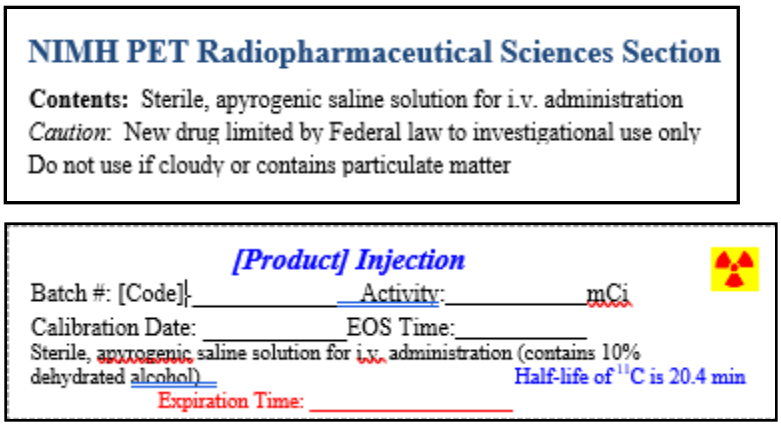
The radioligand will be delivered by the NIMH radiochemistry laboratory to the PET Department's radiopharmacy. Acquisition and accountability are the responsibility of the NIMH radiochemistry laboratory.

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:



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



6.2.3 Product Storage and Stability

Products are stable at controlled room temperature for the one-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 patient 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 PET scans. Examples include a subject 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 s/he can proceed. More specifically, the PI may discontinue or withdraw a participant from the study for the following reasons:

- Disease progression that requires discontinuation of the study intervention, which is unlikely for this relatively short study.
- A clinically significant adverse event (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 addition to discontinuation instituted by the PI, the subject may discontinue (i.e., withdraw) from the study at any point for any reason.

In the event of any discontinuation, we will seek to obtain the next safety measure – i.e., post-scan labs or EKG.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants may withdraw from the study at any point for any reason upon request.

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

- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Screen failure

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if s/he fails 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. Concomitantly, we will ascertain whether the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the PI or their 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 will be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she 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 informed consent

The following activities 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.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.
- Obtain medical release form

Patients will be evaluated for their ability and willingness to tolerate study procedures such as lying still in the PET scanner; their caregivers will be also interviewed to provide information about tolerability.

Some clinical data will be taken from protocol HP-00079860, including patient demographic characteristics, details on multiple sclerosis history and treatment history, disability scales which may include ((EDSS (Expanded Disability Status Scale), MSFC (multiple sclerosis functional composite), low contrast visual acuity, MFIS (modified fatigue impact scale), and the BICAMS (Brief international cognitive assessment for MS) cognitive battery.)

Clinical ratings will be obtained from UMB for the MS volunteers to describe the patient population in future publications. However, we will not correlate current rating scales with the PET findings, because current dysfunction represents the sum of prior plaques that are now quiescent and new plaques(s) with active neuroinflammation.

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

The following activities will be performed only after the subject has signed the consent this study. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

All screening tests and procedures must be performed within 28 days prior to enrollment unless a time period is specifically mentioned.

If the following screening tests have been performed up to 28 days before enrollment they won't be repeated under this protocol:

- General medical history,
- physical examination,
- four vital signs,
- EKG,

Screening labs (done within 28 days) Patients will be evaluated for their ability and willingness to tolerate study procedures such as lying still in the PET scanner; their caregivers will be also interviewed to provide information about tolerability.

8.2 EFFICACY ASSESSMENTS

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

- ***Physical examination*** Subjects will undergo a comprehensive medical history and examination by a credentialed investigator. The purpose of the medical history and examination is to rule out a medical condition as the cause of the subject's signs and symptoms, and to determine if subjects are eligible for enrollment in other protocols.
- ***Electrocardiograms (EKGs)****:* EKG is for screening purposes only. Screening EKGs are taken in the Heart Station Department and results are directly reported in CRIS.
- ***Pregnancy Tests.*** For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any PET scan. If the pregnancy test is positive, the PET will not be done, and the participant will be removed from the protocol.
-

8.2.1 PET Procedures

- *Radioligand*. [^{11}C]PS13 and [^{11}C]MC1 will be prepared according to the IND and administered via an indwelling intravenous catheter over approximately one to three minutes.
- *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 scans*. The two baseline/blocked scans for COX-1 and COX-2 each may occur on the same or on different days. Brain PET imaging will be performed using a PET or PET/CT scanner for up to two hours. Participants will be placed on the scanner bed. The participant's head may be secured with a head holder or a thermoplastic mask to prevent excessive motion, but the mask can be removed if the participant finds it uncomfortable. A CT transmission scan will be performed to measure and correct for attenuation. Tracer infusions will be performed when the participant is already on the scanner bed. The total amount of blood withdrawal is described in Section 8.2.5. When the scan is completed, the venous line will be removed, and the participant will be instructed to void frequently to minimize radiation exposure.
- *Administration of celecoxib.* In these scans, specific binding of [^{11}C]MC1 to COX-2 in the brain will be verified by a pre-blocking study using celecoxib (600 mg PO). Participants will be encouraged to have a light meal prior to PET studies with blockade by celecoxib. After oral administration, the plasma concentration peaks at about two hours [23]. Thus, we will administer celecoxib about two hours before the second PET scan. Participants will be monitored by study clinicians after drug administration to assess drug associated adverse events. To allow the drug's effect to

resolve, we will wait for at least two days to perform the second set of PET scans with ketoprofen.

- *Administration of ketoprofen.* In these scans, specific binding of [^{11}C]PS13 to COX-1 in the brain will be verified by a pre-blocking study using ketoprofen (75 mg PO). Participants will be encouraged to have a light meal prior to PET studies with blockade by ketoprofen. Similar to protocol 17-M-0179, which showed peripheral organ blockade, we will administer ketoprofen about two hours before the second PET scan. Participants will be monitored by study clinicians after drug administration to assess drug associated adverse events. To allow the drug's effect to resolve, we will wait for at least two days to perform the second set of PET scans with celecoxib.

8.2.2 Biospecimen Evaluations

Safety labs will include chemistry panel and complete blood count. Women age of child-bearing potential (age 55 or younger without history of sterilization) will be tested for pregnancy. Women older than 55 years will also be tested for pregnancy unless they have not had menses for one year.

The amount of blood that will be drawn for research purposes, including screening, will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

Not applicable.

8.2.4 Samples for Genetic/Genomic Analysis

Not applicable.

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).
- *Pregnancy Tests.* For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any PET scan. If the pregnancy test is positive, PET will not be done, and the subject will be taken off the protocol.
- *Follow-up Procedures*. Subjects will be contacted one to three business 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 Events (AE) are defined as any untoward medical occurrence associated with the use of an intervention in humans, whether or not the AE is considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An 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 AE; 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 do not result in death, are not life-threatening, or do not 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.

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of all adverse events and shall report the results of such evaluation to the NIH Institutional Review Board (IRB) as per Policy 801."

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

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. Notably, 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 will examine and evaluate 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 within 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 provide 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 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 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 during the 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 seven (for non-SAEs) 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 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 Events of Special Interest

None.

8.4.8 Reporting of Pregnancy

Subjects 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.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

- Primary Endpoint: We hypothesize that administration of ketoprofen or celecoxib will reduce COX-1 or COX-2 levels (measured as SUVR between a lesion and a reference region such as the contralateral mirror image of the lesion) in MS lesions. Please note that detectable or specific binding is defined as that which can be displaced by a COX selective inhibitor. Thus, the primary objective "detectable binding" will be assessed with the primary endpoint "displaceable binding."
- Secondary Endpoint: 1) We hypothesize that more active MS lesions will have higher levels of COX-1 and COX-2 specific binding than less active ones, and that MS lesions will have higher binding than normal white matter. Active lesions will be identified as those that show contrast enhancement with administration of gadolinium. Chronic-active lesions will be identified as those who have a paramagnetic outer rim identified on ME-GRE images that have been processed for quantitative susceptibility maps (QSM). Inactive lesions will be white matter

lesions that do not meet criteria for active or chronic-active. Normal appearing white matter will be all white matter minus lesioned white matter. 2) We hypothesize that the expressions of COX-1 and COX-2 are correlated within the same individuals.

9.2 SAMPLE SIZE DETERMINATION

Using novel radioligands, this pilot study will attempt to measure the density of COX-1 and COX-2. When the standard deviation and the effect size are unknown, the resource equation method provides an alternative to the power analysis for calculating sample size [24-27]. This method is based on the law of diminishing returns and is normally used in animal experiments to minimize the number of animals committed to an exploratory study. The minimum and maximum sample sizes calculated using the equation are:

minimum n = 10/k + 1
maximum n = 20/k + 1
where k = number of groups and n = number of samples per group

For example, if there are two groups to compare (e.g., baseline vs blocked studies in the same participants), the minimum n = 10/2 + 1 = 6 and the maximum n = 20/2 + 1 = 11. Expressed in other terms, using fewer than six participants per group would have little value because of a high rate of false negatives. On the other hand, using more than 11 participants per group would have diminishing return of true positives.

Following this statistical approach, we request to scan 11 participants per group. Because of the failure rate for completing all scans is 30-40%, we request permission to accrue 16 participants per group.

9.3 POPULATIONS FOR ANALYSES

Using a within-subject comparison, the baseline scan of each MS participant will be compared to that after blockade

9.3.1 Evaluable for toxicity

No toxicity is expected from the radioligand; nevertheless, safety will be monitored as described in Section 8.3. Regarding the two blockers, participants will be asked if they have any discomfort or side effects after its administration and within one to three days after the PET scan via telephone.

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

Two-tailed paired: t-tests will be used to assess whether there are statistically significant differences between PET scans at baseline and after blockade.

ANOVA will be used to assess whether COX-1 and COX-2 binding differs in active, chronic-active, and inactive lesions, and normal appearing white matter.

Correlation analyses will be used to correlate the uptake of COX-1 and COX-2 in the same lesions of the same subjects.

9.4.2 Analysis of the Primary Endpoints

The outcome measure for the primary endpoint will be SUVR between the lesion and a reference region such as the contralateral mirror image of the lesion.

9.4.3 Analysis of the Secondary Endpoint(s)

The outcome measure for the secondary endpoint will be SUVR between the lesion and a reference region such as the contralateral mirror image of the lesion, and between the SUVR in the same lesions imaged with both tracers.

9.4.4 Safety Analyses

The safety measurements will be recorded but not analyzed statistically.

9.4.5 Baseline Descriptive Statistics

None.

9.4.6 Planned Interim Analyses

None.

9.4.7 Sub-Group Analyses

None.

9.4.8 Tabulation of individual Participant Data

Individual participant data will be listed by measure and timepoint.

9.4.9 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:

- iMed Consent 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 consent form will be given 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

NIH staff and family members of study team members may not be enrolled in this study.

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

Adults unable to give consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may lose the capacity to consent for themselves. In the event this occurs, the subjects 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 PI will promptly inform study participants, the IRB, and the Sponsor. Study participants will be contacted, as applicable, and be informed of changes to the 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 with protocol requirements.
- Data that are not sufficiently complete and/or evaluable.

- Determination that the primary endpoint has been met.
- Determination of futility.

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

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy are 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 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 (COC) has been issued by the 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, COCs help achieve the research objectives and promote participation in studies by helping ensure confidentiality and privacy to participants.

See Section 10.4 for information regarding sharing of research data during or after completion of the study.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Genetic testing will not be performed in this protocol. No samples will be stored or shared.

In the consent form, we ask permission of the subject (yes/no response) to share imaging and other research 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) allow 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 analysis. In contrast to data, no biological samples that you provide will be shared.

10.5 SAFETY OVERSIGHT

Monitoring for this study will be performed by the independent safety monitor (ISM) for this study, Michael Gregory, MD, who is 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 review.

10.6 CLINICAL MONITORING

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).

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.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

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 PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will 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 will 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 will be retained for a minimum of two years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least two 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

The PI will use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations will 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 will be 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 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 also 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. The 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 Plan

No genomic data will be acquired during this study.

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

Transfers that are associated with correlative studies conducted under an approved protocol: Investigators in the NIH intramural program may participate in multi-site clinical trials (either as a site or as the coordinating center) under which data will be transferred from the intramural program to another site for correlative studies that are part of the approved protocol. In such a situation, the protocol clearly documents the tests conducted under the correlative studies, and each institution participating in the clinical study is bound by the terms of their Protocol and their obligations are cleared by the IC Clinical Director. In such situations, use of an MTA is not necessary for these transfers.

This is a multisite study with NIH as the lead site. The other site is University of Maryland, Baltimore.

This protocol is a collaboration with investigators at UMB, and it will be performed under a Reliance Agreement in which NIH is the responsible IRB. Investigators at UMB will recruit and screen volunteers with MS and give NIMH's contact information for potential PET study. They may also ask the volunteers if their contact information with NIMH investigators to contact them directly. If the volunteer is interested to participate, we'll ask him/her to sign UMB's release of medical information for both clinical and research data to be sent to NIMH. If the volunteer is eligible based on this information, the volunteer will be asked to visit NIMH to review and sign the consent form. That is, only NIMH investigators will obtain consent. After the consent is obtained, we will order any additional data for inclusion and exclusion criteria as specified in this protocol. Investigators at NIMH and UMB will bilaterally share clinical and research data that may include PII.

10.12 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 managing 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
COX	Cyclooxygenase
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
f_P	Free fraction in plasma
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LPS	Lipopolysaccharide
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NIMH	National Institutes of Mental Health
NSAID	Non-steroidal anti-inflammatory drug
OHRP	Office for Human Research Protections
PBR	Peripheral benzodiazepine receptor
PET	Positron emission tomography
PI	Principal Investigator
SAE	Serious Adverse Event

SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
V_T	Total (specific plus nondisplaceable) distribution volume

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