Abbreviated Title: PET in MSK Conditions
NIH IRB #: 19M0079
Version Date: 04/16/2024

Title: Evaluation of a Novel PET Radioligand as an Inflammatory Biomarker in Musculoskeletal Conditions

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Total requested accrual
(42) Patients with rheumatoid arthritis
(15) Patients with myositis
(15) Patients with axial spondyloarthritis
(39) Healthy Volunteers

Project Uses Ionizing Radiation: ☐ No ☒ Yes
☐Medically-indicated only
☒Research-related only
☐Both

IND/IDE ☐ No ☒Yes
Drug/Device/#_142872 for [^{11}C]MC1_____
Sponsor: __NIMH IRP_____

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☐No ☒ Yes
Institution#1___University of Maryland School of Medicine _FWA #___0007145_____
Date of IRB approval ___07/28/2022_____
Institution#2_____ FWA #_____
Date of IRB approval _____

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☒ No ☐ Yes

Samples are being stored ☒ No ☐ Yes

Covered Protocol Requiring DEC Clearance (per SOP 21) ☐ No ☒ Yes

Approved for Short Form Consent Process for Non-English Speakers ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form:

- Healthy volunteer for Phase 1 study: 8.5
- Healthy volunteer for Phase 2 study: 8.7
- RA patient for Phase 2 study: 8.7
- IIM patient for Phase 2 study: 8.7
- AxSpA patient for Phase 2 study: 8.7
- Healthy volunteer for Phase 3: 8.7
- RA patient for Phase 3: 8.7
- RA patient for Phase 4: 8.7

PRÉCIS:

I. Objective

Cyclooxygenase-2 (COX-2) is implicated in the pathophysiology of various inflammatory disorders, suggesting that both may serve as potential biomarkers of inflammation in brain as well as periphery. Our laboratory recently developed [^{11}C]MC1 to image COX-2. Using whole-body imaging, this study seeks to determine whether PET imaging using this new radioligand can differentiate three inflammatory conditions—rheumatoid arthritis (RA), axial spondyloarthritis (AxSpA) and idiopathic inflammatory myopathies (IIM)—from healthy conditions. To determine if [^{11}C]MC1 uptake is specific to COX-2, we will also conduct a blocking study with a selective COX-2 inhibitor (celecoxib). Using brain-dedicated imaging, this seeks to determine whether RA patients and healthy volunteers have specific binding in brain – i.e., uptake that can be blocked celecoxib.

II. Study population

Healthy volunteers (n = 17), patients with RA (n = 15), and patients with IIM (n = 15) will undergo whole-body PET/CT scans. Patients with AxSpA (n=15) will undergo two whole-body PET/MRI scans. In addition, healthy volunteers (n = 22) and patients with RA (n = 12) will have brain-dedicated imaging using [^{11}C]MC1 concurrent with arterial blood sampling. Finally, 15 patients with RA will be imaged during a period of moderate to severe symptoms and after clinically indicated treatment for two to four months. Thus, the entire population will be healthy volunteers (n = 39), patients with RA (n = 42), patients with AxSpA (n=15) and patients with IIM (n = 15).

III. Design

a) Phase 1: We will begin by injecting up to 10 mCi of [^{11}C]MC1 in one healthy male and one healthy female and then conducting a whole-body PET scan. Uptake will be measured in the ovaries and testes, and the dose of radioactivity will be calculated. We will proceed only if the dose to these organs with the higher injected activity proposed for Phase 2 will not exceed the limits specified by the Radioactive Drug Research Committee (RDRC).

b) Phase 2: 15 RA patients, 15 IIM patients and 15 age-, sex-, and genotype-matched healthy subjects will undergo two whole-body PET/CT scans using 15 mCi of [^{11}C]MC1. 15 AxSpA patients will undergo two whole-body PET/MRI scans using 15 mCi of [^{11}C]MC1. The first scan will serve as the baseline scan for comparison; the second scan will be a blocking study using celecoxib.

c) Phase 3: 12 RA patients and 22 age- and sex-matched healthy subjects will undergo two brain-dedicated PET/CT scans, each using 20 mCi [^{11}C]MC1, and concurrent with arterial blood sampling. The first scan will be a baseline scan, and the second will be after blockade by celecoxib.

d) Phase 4: 15 RA participants will be imaged twice with [^{11}C]MC1: while having moderate to severe symptoms and after clinically-indicated therapy for about two to four months. Participants will have whole-body scan after injection of 15 mCi of [^{11}C]MC1.

IV. Outcome measures

For whole body imaging, radioligand uptake in a selected region of interest will be quantified as a Standardized Uptake Value (SUV), which normalizes for injected activity and

body weight. Possible differences in actual blood radioligand level may be adjusted by venous blood data obtained during the PET scan. Regional uptake after blockade with celecoxib will be expressed as a percentage of the baseline value. The baseline uptake and the percentage blockade by celecoxib of each radioligand will be compared between patients and healthy subjects as well as between inflamed and non-inflamed regions of the body in RA, AxSpA, and IIM patients.

For brain-dedicated imaging, the density of COX-2 will be measured with pharmacokinetic modeling and expressed as distribution volume (V_T).

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LIST OF ABBREVIATIONS

TSPO	18-kDa translocator protein
PET	positron emission tomography
FDG	fluorodeoxyglucose
MDD	major depressive disorder
LAB	low-affinity binder
HAB	high-affinity binder
MAB	mixed-affinity binder
COX	cyclooxygenase
IC_{50}	half maximal inhibitory concentration
HPLC	high-performance liquid chromatography
NSAID	non-steroidal anti-inflammatory drug
LPS	lipopolysaccharide
MRI	magnetic resonance imaging
SUV	standardized uptake value
V_T	total (specific plus nondisplaceable) distribution volume
RA	rheumatoid arthritis
AxSpA	axial spondyloarthritis
AS	ankylosing spondylitis
IIM	idiopathic inflammatory myopathies
PM	polymyositis
DM	dermatomyositis
IMNM	immune-mediated necrotizing myopathy
IBM	inclusion body myositis

1. INTRODUCTION AND BACKGROUND

Inflammation plays a significant role in various disorders that involve neurodegeneration or autoimmune reaction as one of their mechanisms. Although positron emission tomography (PET) imaging is capable of visualizing, characterizing, and measuring inflammatory processes at the molecular level, most PET radioligands that target inflammatory mediators have not made the successful transition from preclinical to human study due to the low sensitivity or non-specific bindings of the radioligands themselves or to other issues such as species differences. Although it is not a specific biomarker of inflammation, [^{18}F]FDG remains the most widely used PET radioligand in inflammation studies in humans. With regard to inflammation-specific PET imaging biomarkers, 18-kDa translocator protein (TSPO) radioligands have been the most successful in human studies.

COX-2 as a new target for inflammatory biomarkers

Just as neuroinflammation may play a pathophysiological role in neuropsychiatric disorders, anti-inflammatory drugs may be therapeutically useful. For this reason, we have developed PET radioligands for cyclooxygenase (COX), a well-known target of non-steroidal anti-inflammatory drugs (NSAIDs). COX is the rate-limiting enzyme in the synthesis of prostaglandins from arachidonic acid and exists as two primary isoforms—COX-1 and COX-2 (Fig. 3). NSAIDs such as aspirin and ibuprofen inhibit the function of both COX-1 and COX-2

non-selectively or selectively. Inhibition of the COX system by NSAIDs attenuates both central and peripheral inflammation.

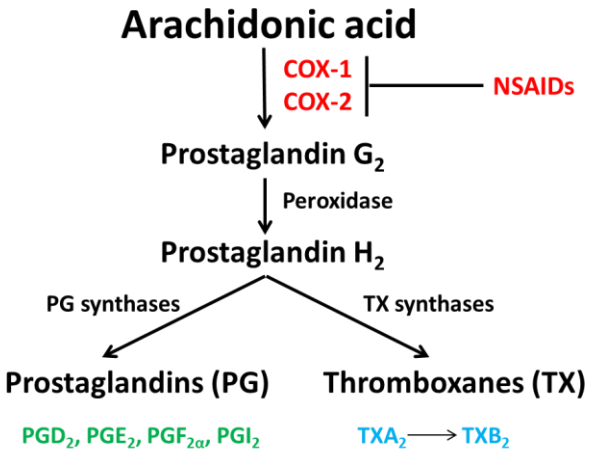

Figure 3. The two primary COX enzymes, COX-1 and COX-2, regulate the rate-limiting steps in the metabolic pathway of arachidonic acid and in the production of pro-inflammatory agents such as prostaglandins and thromboxanes.

The COX-1 radioligand, [^{11}C]PS13, is the first direct-acting radioligand and is now being tested in healthy subjects (Protocol 17-M-0179). While COX-1 is constitutively expressed, COX-2 is known to be inducible, contributing to increased production of pro-inflammatory agents in pathologic conditions. Accordingly, COX-2 has been an important target for developing anti-inflammatory treatment to prevent or decelerate the progression of neuropsychiatric and autoimmune disorders. For instance, celecoxib and naproxen have been used in a clinical prevention trial in Alzheimer's disease, but had no significant effect on clinical outcome (The Alzheimer's Disease Anti-inflammatory Prevention Trial Research, 2013). However, because no reliable COX-2 biomarker exists, interpreting negative results in this and other studies has been limited; specifically, it remains unclear whether the agents' ability to inhibit COX activity was insufficient or whether COX inhibition itself did not significantly affect disease progression. Thus, developing a COX-2 PET radioligand as an inflammatory biomarker is necessary to further evaluate and monitor the effects of anti-inflammatory treatments.

Development of a PET radioligand selective for COX-2

Substituted pyrimidines, such as 6-methoxy-2-(4-(methylsulfonyl)phenyl)-N-(thiophen-2-ylmethyl)pyrimidin-4-amine (MC1), display many desirable properties for development as PET radioligands. A set of five substituted pyrimidines, including MC1, was synthesized by modifying published procedures (Orjales *et al.*, 2008). When these ligands were tested for affinity to COX-2 and COX-1 in human blood, they showed high affinities for COX-2 in the nM range. Because MC1 had one of the highest affinities (IC_{50} = 3 nM) and selectivities (> 1000-fold) over binding to COX-2, it was selected for labeling with carbon-11 by treating the desmethyl-precursor 1 and base in DMF with [^{11}C]MeI. [^{11}C]MC1 was separated by HPLC and formulated for intravenous injection.

We performed [^{11}C]MC1 whole-body PET/CT in rhesus monkeys. As shown in Figure 4, there was no specific binding of [^{11}C]MC1 in any organs in healthy monkeys. Although a few organs have been shown to constitutively express COX-2 under normal conditions, COX-2 is

predominantly induced with inflammation. Thus, we developed an inflammation model in monkey with lipopolysaccharide (LPS) injection into the brain. LPS was injected into the right putamen of two monkeys to induce transient neuroinflammation, and brain PET scans were obtained on post-injection Days 1, 3, and 8. To measure specific uptake, blocked scans were also performed with non-radioactive MC1 (0.3 mg/kg). The specific uptake of [^{11}C]MC1 was not observed in brain under baseline conditions, and brain uptake was increased after LPS injection on Day 1; the percentage of increased uptake was ~60% (Fig. 5). This upregulation was confirmed postmortem with measurements of COX-2 protein and its cognate mRNA (Shrestha et al. J. Neuroimmunology, submitted).

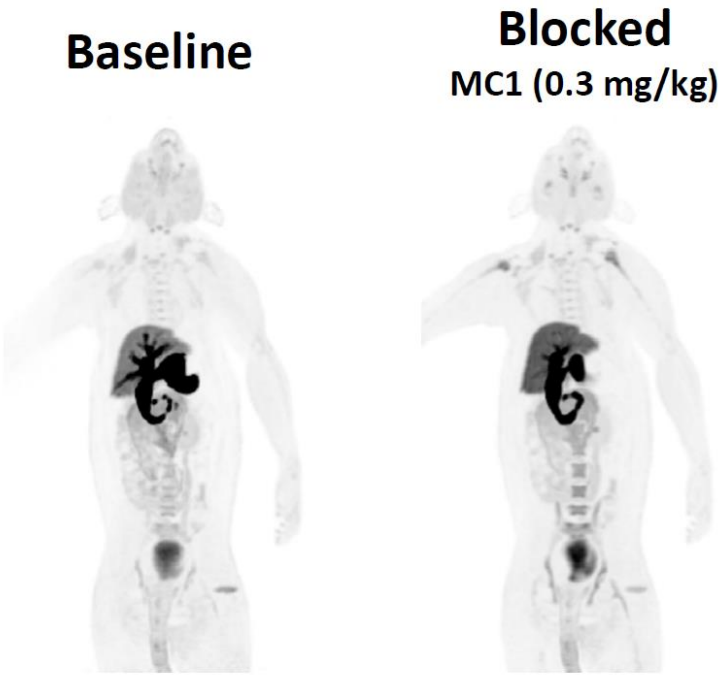

Figure 4. Uptake of radioactivity in a rhesus monkey after injection of [^{11}C]MC1 under baseline conditions (left) and 10 minutes after injection of nonradioactive MC1 (0.3 mg/kg i.v.) (right). High uptake was only observed in excretory organs such as the biliary system, the liver, and the urinary bladder at baseline. Uptake was not blocked in any of these areas by pharmacological dose of MC1.

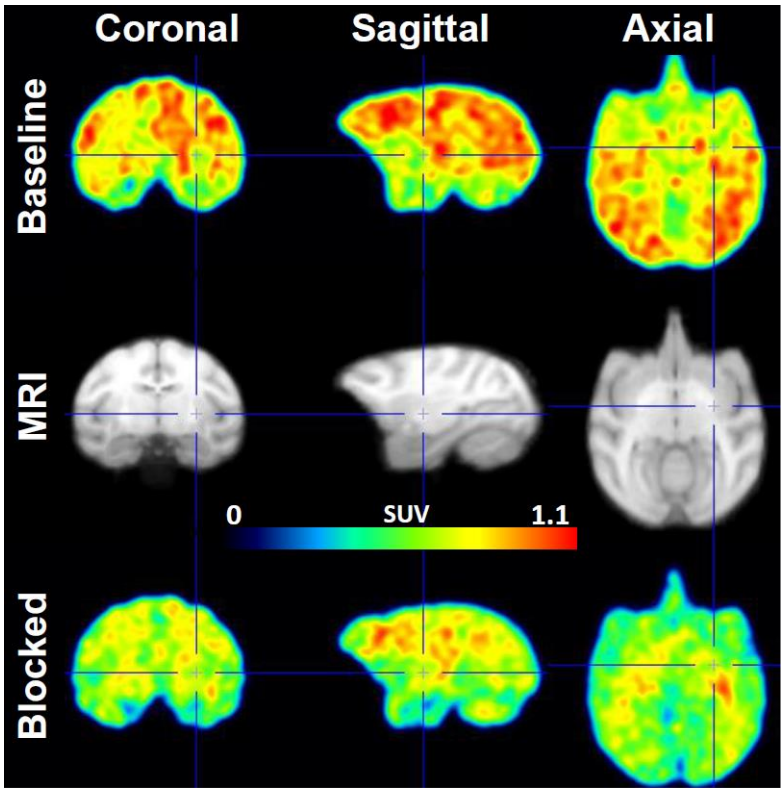

Figure 5. Brain uptake of radioactivity in a rhesus monkey one day after injection of lipopolysaccharide (LPS) into the right putamen. To obtain specific binding of [^{11}C]MC1 to COX-2, non-radioactive MC1 (0.3 mg/kg i.v.) was injected 10 minutes before the second scan. Non-radioactive MC1 blocked radioligand binding to COX-2 and decreased brain uptake compared to the baseline condition. The PET images are shown as standardized uptake value (SUV), which adjusts for injected activity and body weight. MRI images show the anatomical structures for the co-registered PET images

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To examine whether a second LPS injection would cause an even larger increase of COX-2, two animals were injected with LPS a second time (Shrestha et al., *J. Neuroinflammation*, submitted). The second injection caused a marked increase of COX-2 at the injection site, which could be blocked by nonradioactive MC1 (Fig. 6) – i.e., the uptake was specifically bound to COX-2.

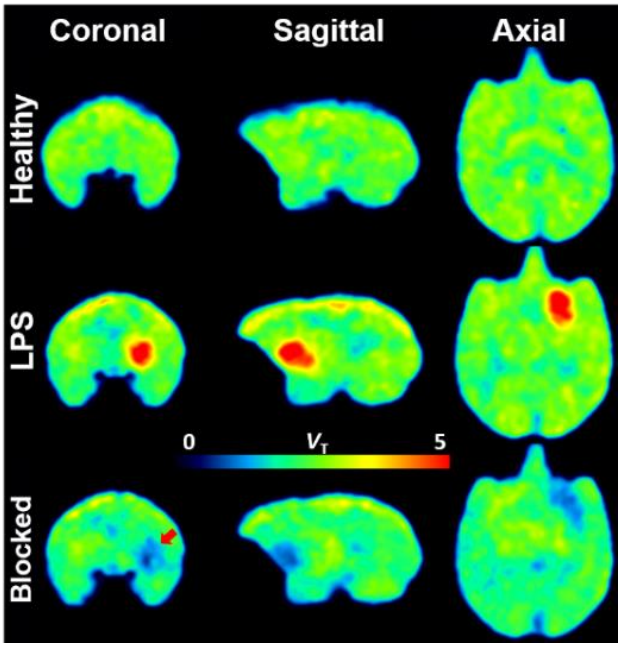

Figure 6. Parametric distribution volume (V_T) images of [^{11}C]MC1 uptake on Day 1 after the second lipopolysaccharide (LPS) injection in a monkey. *Upper row:* [^{11}C]MC1 uptake was markedly increased surrounding the injection area in the right putamen. *Bottom row:* MC1 blocked radioligand binding to COX-2 at the injection site (marked by red arrow) to a level lower than that in the remainder of the brain.

Our interpretation was that [^{11}C]MC1 lacked the sensitivity to measure the low basal concentrations of COX-2, but could detect the enzyme after upregulation, which can be a 10- to 20-fold effect. We wondered if postmortem measurements of the density of COX-2 in monkey and human brain could guide the feasibility of detecting COX-2 in humans, either healthy volunteers or patients with neuroinflammation. Unfortunately, such postmortem measurements are not possible because COX-2 is rapidly upregulated and metabolized, with a degradation half-life of only a few hours (Learn *et al.*, 2000; Kang *et al.*, 2007). We confirmed this finding by measuring COX-2 via ELISA in monkey brain that was rapidly harvested after death (i.e., within 15 min) and postmortem human brain (typical with a postmortem interval of one to two days). The concentration of COX-2 in human brain was 10-100 fold lower than that in rapidly harvested monkey brain. Thus, a study of COX-2 in human diseases cannot be performed with postmortem tissue. Instead, it will require PET to measure in vivo concentrations of this dynamic biomarker of inflammation.

To determine whether [^{11}C]MC1 can detect COX-2 in any human condition, we studied patients with rheumatoid arthritis (RA), as COX-2 is upregulated in the affected joints.

Preliminary Results in RA Patients

To date, we have studied three RA patients. The first two patients had moderately symptomatic joints in the hands, arms, and/or shoulders. Those symptomatic joints had increased uptake of [^{11}C]MC1, which was partially blocked by celecoxib, a COX-2 selective inhibitor (Fig. 7). The third RA patient had joints that were relatively mildly affected at the time of the PET scan and showed no increased uptake of [^{11}C]MC1.

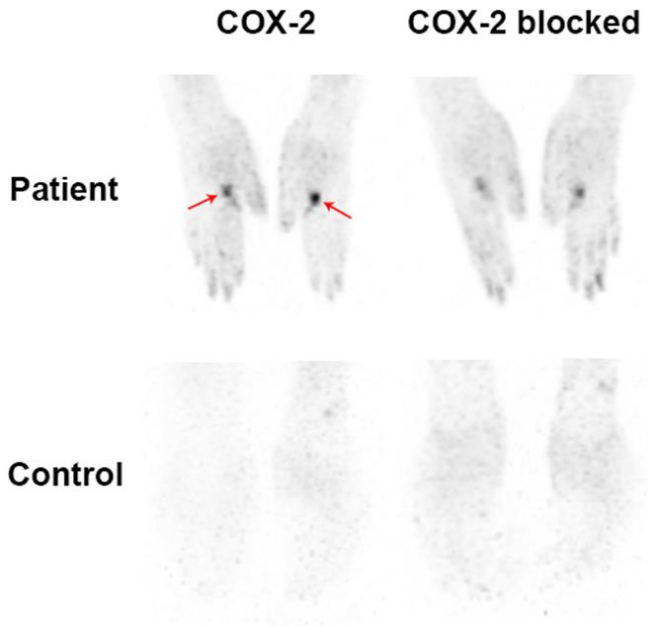

Figure 7. Human positron emission tomography (PET) images of cyclooxygenase-2 (COX-2) in Patient 1 with rheumatoid arthritis and a healthy control. Increased [^{11}C]MC1 uptake in the bilateral hand joints reflected increased COX-2 binding in Patient 1 compared to the healthy control. The increased [^{11}C]MC1 uptake in Patient 1 with rheumatoid arthritis was partially blocked by celecoxib (400 mg) – i.e., the uptake was not reduced to that in control subjects. We seek to determine whether a higher dose (600 mg) will cause a greater blockade.

As described above, we found no specific binding of [^{11}C]MC1 to COX-2 in the brain of healthy monkeys, where specific uptake means it can be blocked by celecoxib. For this reason, we intracerebrally injected lipopolysaccharide (LPS) to induce COX-2 expression to determine whether the radioligand works in vivo. When we started in human subjects, we also expected to find no specific binding in brain. To our surprise, the first two RA patients and two healthy

volunteers had brain uptake that was blocked by celecoxib: about 30% in RA and 20% in controls (Fig. 8). However, these results from only PET imaging (i.e., time-activity curves) can be misleading, as celecoxib could have had a peripheral rather than a central effect. For example, celecoxib might have increased the peripheral clearance of [^{11}C]MC1. To determine whether celecoxib's effect is central (i.e., blocking the enzyme) rather than peripheral (e.g., increased clearance), we need to measure the amount of radioligand delivered arterially to brain. That is, pharmacokinetic modeling with arterial blood measurements can separate central from peripheral effects.

We doubt celecoxib's effect was peripheral for two reasons. First, if it increased clearance, then all organs should have less uptake. However, most organs, including lung, liver, thyroid, kidney, and GI tract, were unaffected by celecoxib. Second, the effect on clearance would have had to be specific for [^{11}C]MC1, as celecoxib was unable to block uptake of [^{11}C]ER176 (which binds to TSPO) in affected joints of RA patients.

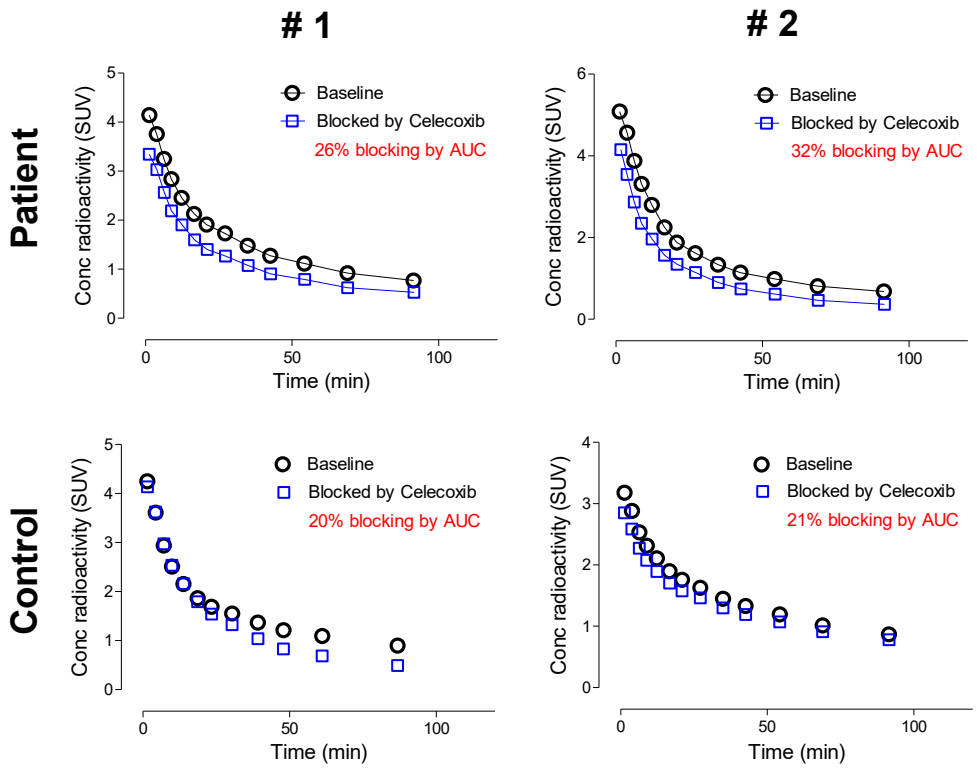

Figure 8. Brain uptake of [^{11}C]MC1 at baseline and after blockade by celecoxib (400 mg) in two patients with rheumatoid arthritis and two healthy controls. These time-activity curves suggest celecoxib is blocking radioligand binding to COX-2 in brain, by ~20% in controls and ~30% in patients. An alternative interpretation is that celecoxib acted peripherally to decrease the amount radioligand delivered to the brain. To address this question, we propose quantitative brain imaging with arterial blood sampling to measure and then to correct for any peripheral effect of celecoxib. A third RA patient was recently analysed and had only 9% blockade by area-under-the-curve (AUC).

Necessity of a human study in peripheral inflammatory condition

The COX-2 radioligand, [^{11}C]MC1, is the first successful radioligand for this target. COX-2 is more typically expressed in peripheral organs than in brain. Indeed, most selective COX-2 inhibitors were primarily developed to treat peripheral inflammatory disorders such as arthritis and chronic musculoskeletal pain, and limited data exist on their penetration to brain. As noted above, COX-2 is rarely detected in non-inflammatory conditions, suggesting that any new radioligand should be tested in inflammatory conditions with more robust expression of COX-2. For example, MDD would not be appropriate to evaluate this radioligand, as a negative result could reflect either that the radioligand does not work in vivo or that MDD does not have elevated COX-2. Although results from the LPS-injected monkey study (Fig. 5) suggested significant specific binding of [^{11}C]MC1 to COX-2, which was induced by neuroinflammation, low signal-to-noise ratio in the image was still a limitation; high non-specific binding in the background was due to the high lipophilicity of [^{11}C]MC1 and to the high lipid content of the entire brain. To resolve this, [^{11}C]MC1 would need to be tested in peripheral inflammation with lower background lipid content. We observed [^{11}C]MC1 uptake in the ovaries of a female monkey that was blocked by both nonradioactive MC1 and celecoxib (a selective COX-2 inhibitor). This is in line with the notion that the ovary highly expresses COX-2 even under normal conditions as long as it is actively involved in the ovulation cycle (McCann *et al.*, 2013). Even if the ovaries showed relatively higher specific uptake of [^{11}C]MC1 than other organs in monkey study, our dosimetry calculation confirmed that actual radiation exposure to the ovaries is not higher than those with other ^{11}C radioligands. The radiation exposure in the ovaries is compared in Table 1 assuming that 15 mCi of each radioligand is injected. Most of these ^{11}C radioligands have been widely used in human studies.

Table 1. Comparison of radiation exposure to ovaries with 15 mCi of ^{11}C radioligands

Radioligand	[^{11}C]MC1	[^{11}C]PK11195	[^{11}C]CUMI	[^{11}C]PIB	[^{11}C]DPA-713
Radiation exposure to ovaries (rem)	0.21	0.18	0.21	0.18	0.22

However, ovaries are not an appropriate organ for testing our new radioligand because of their small size and significant movement during the scans. Thus, from the perspective of radioligand development, this first-in-human study of [^{11}C]MC1 would be most useful if conducted in patients with representative peripheral inflammatory disorders: rheumatoid arthritis (RA) and idiopathic inflammatory myopathies (IIM).

If [^{11}C]MC1 effectively images COX-2 in RA, AxSpA, and in IIM, it will be used in two general ways relevant to neuropsychiatric disorders, to investigate pathophysiology and to guide therapeutic trials with NSAIDs. Having validated [^{11}C]MC1 in human disorders known to have elevated COX-2, it could be used to determine whether AD and MDD also show such an elevation. With regard to treatment, it is largely unknown the extent to which various NSAIDs enter brain and their relative in vivo selectivity for COX-1 versus COX-2. Validated radioligands for both COX-1 and COX-2 would help address these questions. In the brain, however, the relative roles of these two isozymes are controversial. Increasing evidence suggests that COX-1 is more pathologic than COX-2 (Choi *et al.*, 2009). Furthermore, the selective COX-2 inhibitor, rofecoxib, had detrimental effects on cognition in a relatively large study of AD patients (Aisen *et al.*, 2008). In summary, both COX-1 and COX-2 may have important pathophysiological roles

in neuropsychiatric disorders. Radioligands selected for each isoform would be useful both to explore pathophysiological and potentially to guide therapeutic trials of NSAIDs.

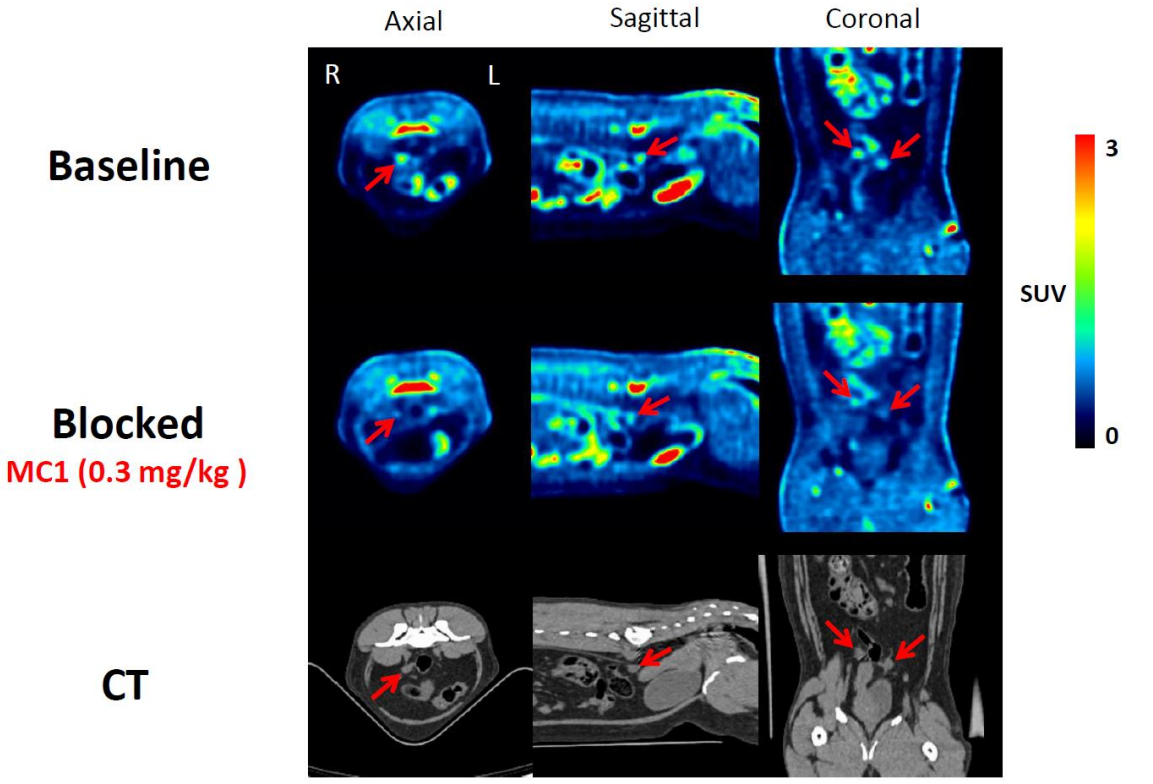

Figure 6. Uptake of radioactivity in ovaries of a female rhesus monkey after injection of [^{11}C]MC1 at baseline (top), 10 minutes after injection of nonradioactive MC1 (0.3 mg/kg i.v.) (middle), and CT images showing the corresponding anatomical structures (bottom). High uptake was observed in both ovaries and was blocked by a pharmacological dose of MC1, consistent with specific binding to COX-2. The PET images are shown as standardized uptake value (SUV), which adjusts for injected activity and body weight.

Rheumatoid arthritis (RA)

RA is estimated to affect between one and four percent of the United States population, causing significant losses in work and social functioning as well as increases in health-related costs and disability across the general population, including in the minority community. Early diagnosis and monitoring of disease activity is important for proper treatment and good prognosis, lending increased urgency to the importance of developing an inflammatory biomarker for RA.

RA is an inflammatory autoimmune disease of the joints and involves migration of macrophages and T helper lymphocytes into inflamed areas (Tak *et al.*, 1997; Steiner *et al.*, 1999). An [^{18}F]FDG PET imaging study found that inflamed joints could be differentiated from normal non-inflamed joints (Elzinga *et al.*, 2007). However, because glucose metabolism can be increased for a number of reasons, more specific markers of inflammation are needed to be clinically useful.

NSAIDs target COX-1 and 2 and are often recommended as first-line therapy in RA. To prevent the common gastrointestinal side effect of NSAIDs, which mostly result from COX-1 inhibition, selective COX-2 inhibitors have been developed to treat RA. One of these is celecoxib, which showed significant anti-inflammatory and analgesic activity in RA patients in

large clinical trials (Emery *et al.*, 1999; Tsuji *et al.*, 2012). Increased expression of COX-2, but not COX-1, has also been demonstrated in rheumatoid synovial tissues (Crofford *et al.*, 1994). Thus, COX-2 is considered to be a key mediator in the inflammatory processes that underlie the development of clinical symptoms in RA.

Idiopathic inflammatory myopathies (IIM)

The IIM are characterized by the presence of inflammation in skeletal muscle. The clinical course of IIMs can vary widely ranging from a slowly progressive weakness over years with no extraskeletal muscle involvement to rapidly disabling weakness with severe interstitial lung fibrosis within a matter of weeks to months. Approximately five to 10 new cases per million per year occur in the United States and the disorders are somewhat more common in females than males. Bohan and Peter classified IIM patients into two major categories: polymyositis (PM) and dermatomyositis (DM) (Bohan and Peter, 1975a, b). In PM and DM, lymphocytes, dendritic cells, and macrophages infiltrate the inflamed muscle. Among the IIMs, immune-mediated necrotizing myopathy (IMNM) is considered to be a predominantly macrophage-mediated immune response. Inclusion body myositis (IBM) is another type of IIM, which shows distinctive clinical presentation and pathologic findings. Any of these conditions could be associated with the high expression of COX-2.

PET studies in IIM have been almost exclusively limited to [^{18}F]FDG PET, and muscle biopsy remains the diagnostic gold standard despite the invasiveness of the procedure. COX-1 and COX-2 were also shown to be increased in muscle tissues from IIM patients, and immunosuppressive treatment led to significant downregulation of COX-2 (Studynkova *et al.*, 2004; Korotkova *et al.*, 2008).

Axial spondyloarthritis (AxSpA)

Spondyloarthritis encompasses several immune-mediated inflammatory diseases that exhibit overlapping features but differ from other types of inflammatory arthritis in genetic predisposition, pathogenesis, and outcome. Axial spondyloarthritis (AxSpA), the most common form of SpA includes ankylosing spondylitis (AS), which is the prototypic form of disease. AxSpA frequently begins with back pain and stiffness often leads to aberrant ossification and ankylosis (fusion) of the spine (AS). Enthesitis, or inflammation where tendons and ligaments connect to bones, is the primary pathologic lesion in AxSpA. Our ability to recognize AxSpA (early form of AS) involving the axial skeleton, and our understanding of the cause and progression of this disease, is limited.

PET studies in AS have been almost exclusively limited to the evaluation of bone metabolism with bone-seeking agents. However, both COX-2 and COX-1 (encoded by *PTGS2* and *PTGS1*, respectively) are implicated in AS. *PTGS1* variants are associated with progression of AS (PMID: 24651623) while *PTGS2* variants are associated with disease risk (PMID: 33565113). In addition, COX-2 may be overexpressed *in vivo* in patients with AS, and COX1/2 inhibitors are routinely used to treat patients with AS.

2. STUDY OBJECTIVES

a. Primary objectives

- To evaluate the PET radioligand [^{11}C]MC1 as a new inflammatory biomarker in representative disorders with active inflammation: RA, AxSpA and IIM, compared to healthy condition.

- To evaluate the specific binding of [^{11}C]MC1 to COX-2 at the inflammatory focus in patients versus the same body part in healthy subjects through blocking study using a selective COX-2 inhibitor.
- To measure the brain uptake of [^{11}C]MC1 in brain of RA patients, in comparison to healthy volunteers.
- To evaluate the change of [^{11}C]MC1 binding to COX-2 at the inflammatory focus in RA patients: while having moderate to severe symptoms and after two to four months of clinically indicated treatment.

b. Secondary objectives

- To collect the human biodistribution and dosimetry data of [^{11}C]MC1.
- To see if the uptake of [^{11}C]MC1 at the inflammatory focus is correlated with the patient's available clinical profiles: clinical severity, serum biomarkers, X-ray or MRI findings, synovial/muscle biopsy findings, etc.
- To see if the uptake of [^{11}C]MC1 at the inflammatory focus is correlated with the patient's clinical rating scales and laboratory tests.

3. SUBJECTS

a. Description of study populations

We propose to study four groups of participants:

1) 17 healthy subjects (2 subjects in Phase 1, and 15 subjects in Phase 2, who are age-, sex-, and genotype-matched with patient groups) as well as 22 healthy subjects who are age- and sex-matched with the RA patients in Phase 3.
2) 15 patients with RA (in Phase 2) and 12 patients in Phase 3.
3) 15 patients with IIM (in Phase 2)
4) 15 patients with AxSpA (in Phase 2)
5) 15 patients with RA (in Phase 4)

Note: As described below in Section 10 ("Statistical Analysis"), these requested numbers account for and include subjects who may drop out.

NIH employees are eligible to participate, but NIH employees who are subordinates/relatives/co-workers of investigators will be excluded. NIMH employee participation is guided by intramural institute policy.

b. Inclusion criteria

1) Healthy subjects
   - Aged18 years or older.
   - Willing and able to complete all study procedures.
   - Able to provide informed consent.
   - Healthy based on medical history, physical examination, and laboratory testing.
   - Enrolled in protocol #01M0254 "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" or #17M0181"Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies".
   - Be age-, and sex-matched with patient groups for the 15 subjects in the Phase 2.

2) RA patients
   - Aged 18 years or older.

- Willing and able to complete all study procedures.
- Able to provide informed consent.
- Have been given a diagnosis of RA based on the published criteria (Aletaha *et al.*, 2010).
- Have moderate to severe symptoms, as defined by a DAS28-ESR score >3.2, but RA patients may be in remission for the repeat scan in phase 4.

3) IIM patients
   - Aged 18 years or older.
   - Willing and able to complete all study procedures.
   - Able to provide informed consent.
   - Meets Bohan and Peter criteria (Bohan and Peter, 1975a, b) for probable or definite DM or PM, or
   - Meets criteria for IBM as defined by Lloyd et al. (Lloyd *et al.*, 2014): 1) finger flexor or quadriceps weakness, and 2) endomysial inflammation, and 3) either invasion of non-necrotic muscle fibers or rimmed vacuoles.

4) AxSpA patients
   - Aged 18 years or older.
   - Willing and able to complete all study procedures.
   - Able to provide informed consent.
   - Have been given a diagnosis of AxSpA based on Assessment of Spondylo-Arthritis International Society (ASAS) criteria AxSpA (Appendix 1).

c. Exclusion criteria

1) Common for all participants
   - Because non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2, subjects should not have taken NSAIDs or willow bark tea for two weeks prior to the PET scan.
   - For Phase 2, 3 and 4, *contraindications to taking COX-2 inhibitors that, in the opinion of the investigators, have the potential to affect the results or the safety of the participant.

     These may include:
     - History of hypersensitivity reaction to COX inhibitors or History of aspirin- or NSAID-induced asthma;
     - History of upper or lower gastrointestinal bleeding, gastritis, peptic ulcer disease;
     - History of uncontrolled gastroesophageal reflux disease (GERD), but not medically controlled GERD;
     - Coagulation disorder;
     - Thrombocytopenia;
     - G6PD deficiency;
     - History of gout;
     - History of hepatic or renal impairment;
     - History of cardiovascular disease or presence of cardiovascular risk factors such as uncontrolled or poorly controlled hypertension;

  - Current use of probenecid;
  - Patients clinically in remission or who have low disease activity.
- HIV Infection.
- Any other history of severe medical illness or injury with the potential to affect study data interpretation or to be any medical contraindication to the procedures performed in the study, including active infection and untreated malignancy.
- Unable to travel to NIH.
- 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.
- Inability to lie flat on camera bed for at least two hours, including claustrophobia and overweight greater than the maximum for the scanner (500 lb).
- Pregnancy or breastfeeding.
- Participants must not have substance use disorder or alcohol use disorder. However, alcohol or cannabis use by themselves are not exclusion criteria, unless that use affects the function of daily life.
- NIMH employees or an NIH employee who is a subordinate/relative/co-worker of the investigators.

*These criteria will not be applied to the two healthy volunteers participating in the Phase 1 of this study.

2) Healthy subjects
   - Clinically significant laboratory abnormalities based on tests performed under screening protocol 01M0254 or 17M0181 and specified in Section 4.c "Screening."

3) AxSpA and IIM patients
   - Unable to have an MRI scan (e.g., pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pumps, or shrapnel fragments, metal fragments in the eye).

4. STUDY DESIGN AND METHODS

a. Study overview

Although most of our prior targets were primarily, if not exclusively, expressed in brain, COX-1 and COX-2 are present in the entire body and play important roles in the periphery: physiology, pathophysiology, and drug treatment. In fact, our prior studies in monkeys demonstrated that COX-1 is expressed at much higher densities in some peripheral organs (e.g., spleen, GI tract, and kidney) than in brain. For this reason, the phases of evaluating this COX-2 radioligand differ from those for brain-dedicated radioligands. That is, we normally do dedicated brain kinetic imaging before whole body imaging. For this target, by whole-body imaging in patients with RA and IIM, we wish to determine whether the radioligand actually labels COX-2 (based on blocking studies using a selective COX-2 inhibitor), and whether the most robust signal for COX-2 is in the peripheral inflammatory focus by whole-body imaging.

This is a multi-site center study in three groups of participants: patients with RA, patients with IIM, patients with AxSpA, and healthy volunteers as a control group. As noted above, participants must meet inclusion and exclusion criteria and sign an informed consent document. This study consists of four phases: 1) initial [^{11}C]MC1 whole-body imaging of two single healthy volunteers with a low injection activity; 2) [^{11}C]MC1 whole-body imaging in baseline and pre-blocked condition with a COX-2 selective antagonist (celecoxib). 3) Dedicated brain imaging using [^{11}C]MC1, concurrent arterial blood sampling, in RA patients compared to healthy volunteers. 4) [^{11}C]MC1 whole-body imaging of RA patients at baseline and after clinically indicated therapy.

To estimate the radiation exposure from the new radioligand [^{11}C]MC1, we will follow the prior suggestions from our laboratory (Zanotti-Fregonara and Innis, 2012), which have been approved by the NIH Radiation Safety Committee. Specifically, we found that the average exposure of all published human studies with ^{11}C-radioligands more accurately reflected the exposure to humans than estimation based on imaging in monkeys with that particular radioligand.

Phase 1: We will do whole-body scanning in one male and one female healthy volunteer with [^{11}C]MC1 using up to 10 mCi. We will measure uptake in the ovaries and testes and calculate the dose of radioactivity. Phase 2 will be proceeded only if the dose to these organs from the higher injected activity proposed in Phase 2 will not exceed the limits specified by the Radioactive Drug Research Committee (RDRC). If the dose of radioactivity already exceeds the RDRC limitation in the first participant of Phase 1, IRB will be consulted before the second participant of Phase 2 undergoes [^{11}C]MC1 PET scan.

Phase 2: Fifteen patients with RA, fifteen patients with IIM, and fifteen healthy volunteers will have two whole-body PET/CT scans using 15 mCi of [^{11}C]MC1. Fifteen patients with AxSpA will undergo two whole-body PET/MRI scans using 15 mCi of [^{11}C]MC1. The first scan will serve as the baseline for comparison to pre-blocked scan and will provide dosimetry information, and the second scan will be a pre-blocking study using the COX-2 selective antagonist celecoxib. Two scans are necessary because the specific binding can only be estimated by comparing a scan at baseline and a scan after blockade.

Phase 3: Twelve RA patients and twenty-two healthy volunteers will have dedicated brain imaging with 20 mCi of [^{11}C]MC1 at baseline and after blockade by celecoxib.

Phase 4: Fifteen RA patients will have a whole-body scan with 15 mCi of [^{11}C]MC1 at baseline and after two to four months of clinically indicated therapy. The repeat scan will be initiated if the RA participant has substantial improvement of symptoms or by four months, whichever occurs first.

NOTE: A subject may participate in only one phase of the study.

We will proceed in the order of Phase 1 and 2. In Phase 2, all three groups of participants will be recruited and scanned randomly without specific order. Each PET scan will take up to two hours. Two PET scans on a day will be separated by at least 2.5 hours to allow for decay of the first injection ($T_{1/2}$ = 20 min). In IIM patients, for the purpose of localizing inflammatory

focus in muscle, a whole-body MRI will be obtained before or after the PET scan and can be performed on the same day depending on the schedule of the radiology and PET departments. The completion of MRI will take approximately one hour. For AxSpA patients, a whole body PET/MRI will be obtained meaning that MRI imaging will be done simultaneously with the PET scan. MRI is not obtained in RA patients since the inflamed joints are generally well-identified by other clinical parameters.

Number of visits and time commitment of participants

Phase 1: whole-body imaging with 10 mCi [^{11}C]MC1 injection
1st outpatient visit: pregnancy test; whole body PET (4 hours)

Phase 2: whole-body imaging with 15 mCi [^{11}C]MC1 at baseline and after celecoxib
1st outpatient visit (only for patients with RA, AxSpA or IIM): clinical evaluations and screening procedures;
2nd outpatient visit (all participants): pregnancy test; baseline & blocked whole body PET/CT imaging (8 hours). For AxSpA patients, this will be PET/MRI (8 hours) instead of PET/CT.
3rd or 4th outpatient visit (only for patients with IIM): pregnancy test; whole-body MRI (2 hours)

Phase 3: brain-dedicated imaging with 20 mCi [^{11}C]MC1 at baseline and after celecoxib
1st outpatient visit: clinical evaluations and screening procedures
2nd or 3rd outpatient visit: pregnancy test; baseline & blocked [^{11}C]MC1 PET imaging (8 hours)
2nd or 3rd outpatient visit: pregnancy test; brain MRI (2 hours)

Phase 4: whole-body imaging with 15 mCi [^{11}C]MC1 at baseline and after two to four months of therapy
1st outpatient visit (only for patients with RA): clinical evaluations and screening procedures
2nd outpatient visit: pregnancy test; baseline whole body PET imaging (4 hours)
3rd outpatient visit: pregnancy test; post-therapy whole body PET imaging (4 hours)

All procedures and their associated exposure to radiation are for research purposes only. Each subject will not participate in more than one phase of this study.

b. Recruitment

Patients with RA, AxSpA or IIM will be recruited by advertisements *[IRB-approval for which will be obtained based on the requirements in OHSRP Policy 302 (“*Subject Recruitment and Compensation*”)* placed in medical journals, on websites/email list-servers of medical professionals, in patient support group or registry mailings, and/or in a variety of patient support group literature and websites, including new patient communication sites, such as Facebook, MySpace, Twitter, and Google Adwords for distribution as allowed by NIH policies as needed to reach the target enrollment numbers. AxSpA patients may also be referred from NIAMS protocol 11AR0223 (Studies on the Natural History and Pathogenesis of Spondylarthritis).

Healthy volunteers will be recruited under protocol 01M0254 “The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers” or 17M0181,

"Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies".

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

Recruitment for Healthy volunteers will also include use of Research Match, Listservs, Craig's List, advocacy websites, resource listings or other website postings and Study-specific page on the NIMH-IRP: Join A Study Website with URL (placeholder). Short text descriptions of this study will also appear on Facebook. Facebook posts will be made only via official NIMH accounts. Associate Investigators and members of the NIMH Marketing & Community Relations Unit will distribute recruitment materials.

All recruitment materials, including listserv notices, social media, letters to physicians, recruitment website content, and other venues will be approved by the IRB prior to use.

NIH Employees/staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

c. Screening

Patients with RA, AxSpA or IIM will be screened under this protocol. The screening process for AxSpA and IIM patients will additionally ensure that there are no contraindications to MRI. Basic screening may include physical exam, medical history, questionnaires, and laboratory tests: hematology (CBC with differential); chemistry (electrolytes, LFT's, glucose, BUN, creatinine, Ca, Mg, phosphorus, uric acid, transaminases, total protein, albumin, CK, LDH, alkaline phosphatase); urinalysis; urine pregnancy test (females); acute phase reactants (ESR, CRP); serology (RF, ANA, immunoglobulins, anti-cardiolipin (ACA), anti-dsDNA, ANCA); anti-CCP antibodies and, if clinically indicated, spot urine protein to creatinine ratio. In addition, myositis associated or myositis specific antibodies will be obtained, if clinically indicated. HIV screening test; hepatitis panel (A, B, C); Tb test; others as required. X-ray, CT or MRI of clinically involved area will be obtained if clinically indicated. Additional studies on selected subjects, when indicated for diagnosis, will include: muscle biopsy or synovial biopsies with a separate informed consent; electromyography (EMG); swallowing tests; and others. Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures. No subjects will be withdrawn from NSAIDs including aspirin or corticosteroids exclusively for the purpose of participation in this protocol.

If the RA, AxSpA or IIM subject has had prior NIH laboratory and clinical examinations as part protocol #'s 01AR0227 Natural History of Rheumatic Disease in Minority Communities, 00AR0222 Natural History of Arthritis and Related Conditions, and 11AR0223 Studies on the Natural History and Pathogenesis of Spondylarthritis, those results may be used to better understand the subject's current condition. Finally, results within the past year from these three natural history protocols can be used as part of screening, rather than repeat these tests under this protocol.

For Phase 4 patients, in addition to a basic rheumatology exam, we will do a Health Assessment Questionnaire Disability Index (HAQ-DI for rheumatoid arthritis) and the Disease Activity Score in 28 joints (DASB28). The DAS28 is a disease activity score (Prevoo *et al.*, 1995). These assessments will be done before both scans of Phase 4.

Healthy volunteers will be screened under protocol 01M0254 or 17M0181. Basic screening may include physical exam, medical history, and laboratory tests: CBC; acute care

panel; urine pregnancy test (females); HIV screening test; and EKG. Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures.

Consent will be obtained before any study procedures, including screening procedures done under this protocol.

d. Study procedures

1) PET Procedures

i. Radioligands

[^{11}C]MC1 will be prepared according to the IND and administered via an indwelling intravenous catheter over approximately one to three minutes.

ii. Insertion of the intravenous and/or arterial line

Two intravenous lines may be placed in the arms. They are used for the injection of radioligand and for drawing venous blood to measure the plasma radioactivity level in each time point during the scan. The lines are removed at the end of the day. For Phase 3, an arterial catheter will be inserted in the radial artery.

iii. PET scan

For whole-body scans (Phase 2 and 4), PET imaging will be performed using PET/CT scanner for about two hours for RA, IIM patients and healthy volunteers. AxSpA patients will undergo imaging using PET/MRI scanner for about two hours. Participants will be placed on the scanner bed, and to minimize extraneous motion, they may wear a head-holding mask and have their arms and abdomen wrapped with body-restraining sheets. Almost all subjects find this restraint comforting (i.e., they are not afraid of falling off the table), and none have found it intolerable. Pre-injection CT transmission scan (for PET) will be performed to measure and correct for attenuation. After an intravenous bolus of up to 15 mCi, several venous blood samples may be drawn from the venous catheter at discrete time points during the PET scan. The total amount of sampling volume will be up to 50 mL in each scan. PET image will be acquired in three-dimensional mode with increased length of frame for a total of approximately two hours. After the scan, the venous line will be removed, and the subject will be instructed to void frequently to minimize radiation exposure.

For brain-dedicated scans (Phase 3), the PET procedures will be the same as that for whole-body, with two exceptions. 1) Only the head will be scanned and 2) The subject's head may be secured with a thermoplastic mask to prevent excessive motion, but it can be removed if the subject finds it uncomfortable.

Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

iv. Administration of celecoxib

In these whole-body scans, specific binding of [^{11}C]MC1 to COX-2 in the inflamed focuses will be verified by pre-blocking study using celecoxib. We will also

obtain plasma samples just before and after the PET scan to measure the concentration of celecoxib. The dose of celecoxib will not be higher than 600 mg, which is the highest single dose for clinical therapeutic purpose. For comparison, the typical adult dose of celecoxib is 200 – 400 mg per day. The dose may be adjusted to achieve 40 - 80% enzyme occupancy in order to avoid possible side effects, but not to exceed the highest dose of 400 mg.

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 to three hours (Paulson *et al.*, 2001). Thus, we will administer celecoxib about two to three hours before the second PET scan. Participants will be monitored by study clinicians after drug administration to assess drug associated adverse events.

2) Whole-body MRI

A whole-body MRI will be obtained for localization of the inflamed muscle in patients with IIM and AxSpA will be performed on a scanner located at the NIH Clinical Center (Bethesda, Maryland) and will take about one hour. Subjects will undergo safety screening prior to the MRI to rule out contraindications such as cardiac pacemaker.

Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

3) Brain MRI

Subjects in Phase 3 will receive a T-1 weighted MRI of the head within six months of the PET scan. A previously acquired MRI scan can be substituted for one on this protocol, if acquired within six months of the PET scan.

e. End of participation

Results of clinical testing from the screening process, diagnostic impression, and MRI findings may be shared with patients or referring physicians through the CRIS portal.

Such practice is typical for participants undergoing PET studies at the NIMH, since these scans are performed for research only, and not routine medical care. Eligible subjects may be offered enrollment in other protocols at the NIH. No treatment will be offered.

Subjects will return to the referring health care provider for treatment and long-term management. Subjects who do not have an appropriate health care provider will be referred to one in the community.

5. MANAGEMENT OF DATA AND SAMPLES

a. Storage

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

All data will be stored on the NIMH server under password-protected accounts accessible only to the principal investigator and directly involved study personnel to preserve subject

privacy. All data are regularly backed up, either by the NIMH system administrator or by NIMH CIT personnel.

b. Data and sample sharing plan

No samples will be saved or shared. However, data may be shared with collaborating laboratories at the NIH or outside of the NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data from this protocol may be open-access or restricted access.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data are shared, the key to the code will not be provided to collaborators but will remain at the NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submission to NIH-sponsored or supported databases and repositories will be reported at the time of the Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

c. Collaborative Agreement

This is a multi-site study with NIH as the lead site. The other site is the University of Maryland School of Medicine.

This protocol is a collaboration with investigators at the University of Maryland School of Medicine, and it will be performed under a Reliance Agreement in which NIH is the responsible IRB. Investigators at the University of Maryland School of Medicine will recruit and screen volunteers with rheumatoid arthritis and myositis and give NIMH's contact information for potential PET study. They may also ask the volunteers if their contact information may be shared with NIMH investigators to contact them directly. If the volunteer is interested in participating, we'll ask him/her to sign the University of Maryland School of Medicine'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 the University of Maryland School of Medicine will bilaterally share clinical and research data that may include PII.

6. ADDITIONAL CONSIDERATIONS

a. Research with investigational drugs or devices

[^{11}C]MC1 will be prepared under IND 142872. The PET radioligand will be synthesized in recently re-established NIMH cGMP facility.

b. Gene therapy

Not applicable.

7. RISKS AND DISCOMFORTS

Risks include those associated with: a) medical examinations and screening procedures, b) MRI, c) placement of a venous line, d) blood sampling, e) radiation exposure from [^{11}C]MC1, and the transmission scan, f) PET scanning, and g) celecoxib administration.

a. Medical examination and screening procedures

Medical examination and screening procedures are associated with minimal risks that are mostly related with phlebotomy for laboratory tests in all participants, and with invasive procedures in selected patients such as EMG, muscle biopsy, and synovial biopsy. Phlebotomy can be associated with discomfort or bruising. Invasive procedures in selected patients will be performed only when clinically indicated under screening protocols with separate informed consents. In the present protocol, all subjects are expected to undergo recruitment and assessment procedures without any difficulties.

b. MRI

MRI scans will be obtained from IIM patients in Phase 2 and in all participants in Phase 3 as well as for AxSpA patients undergoing PET/MRI scans. People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if they have any, they will not receive an MRI scan. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. Subjects will be asked to complete an MRI screening form for each MRI scan they have. There are no known long-term risks associated with MRI scans.

c. PET/MRI Scans

PET/MRI scans involve the risks associated with MRI as stated above. Specifically, the risks of MRI include exposure to strong magnetic fields, which may be unsafe for individuals with certain implants or devices, and the potential for claustrophobia or discomfort due to the enclosed space of the scanner.

The PET/MRI combines MRI with PET imaging, which involves the use of a radioactive tracer. However, the radiation exposure from a PET/MRI is lower compared to a PET/CT scan, as it does not involve a CT scan. The risks associated with the PET scan include exposure to a small amount of radiation, which is generally considered to be within safe limits for diagnostic procedures.

d. Placement of venous and/or arterial lines

Venous catheter insertion can be associated with discomfort, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks. In case of tracer extravasation, we will stop the study, remove the venous line from the arm, and apply cold to the site.

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

e. Blood sampling

Participants may have venous blood sampling during PET scans. The total amount of blood drawn under this protocol, or the screening protocols will not exceed 400 mL in each subject. Blood sampling may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. We will ask participants not to donate blood within 8 weeks prior to the study or for 8 weeks following the study.

f. Radiation exposure risks

Radiation exposure in this protocol will be from [^{11}C]MC1 and the associated transmission scans.

A whole-body acquisition has been performed in a male and a female volunteer to estimate dosimetry. The estimates show an effective dose of 4.6 μSv/MBq (0.017 rem/mCi) and that the liver is the target organ, with an equivalent dose of 18 μSv/MBq (0.067 rem/mCi). These values are well below the regulatory limits and similar to those of other ^{11}C tracers. Thus, the radiation exposure from a single injection of 15 mCi yields an effective dose of 0.26 rem, not including the transmission scan (described below).

With regard to exposure from the transmission scan, the PET Department recently implemented Dr. Innis's suggestion to decrease the current (amperage) and, thereby, the radiation from the CT. We do not need a high resolution (high current) image for attenuation correction; a low resolution, like that 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 1/3 of the previous value.

The effective dose for each whole-body transmission scan from a PET/CT is 0.56 rem. We routinely include the dose from additional transmission scan in the event that it must be repeated in a subject, so the effective dose for three transmission scans during each day with two PET scans (3 * 0.56) is ~1.68 rem. Thus, the total effective dose in two [^{11}C]MC1 PET scans (1.68 + 2 * 0.26 rem) is ~2.2 rem. Therefore, the total effective dose in this study is ~2.2 rem, which is less than the NIH limit of 5 rem.

In Phase 3 study, the effective dose for each brain transmission scan from a PET/CT is 0.02 rem. The effective dose for four transmission scans in the event that it must be repeated in a subject during each day with two PET scans (4 * 0.02) is ~0.08 rem. We plan to inject 20 mCi of [^{11}C]MC1 in each brain scan which yields an effective dose of 0.34. Thus, the total effective dose in two [^{11}C]MC1 scans on each day (0.08 + 2 * 0.34 rem) is ~0.76 rem, which is far less than the NIH limit of 5 rem. This dose is described in the consent form as "about 1 rem".

In Phase 4 study, the total effective dose in two [^{11}C]MC1 PET scans (1.68 + 2 * 0.26 rem) is 2.7 rem, which is described in the consent form as "about two rem."

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

g. PET scan

PET scans, which detect injected radioactivity within the body, are not associated with any known physical hazards to the subject lying on the table. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom participants will have ready access should they experience any problems. Participants can communicate with the trained health professionals while in the scanner and can be removed from the scanner and withdraw from the study at any time if they wish to do so. Participants can also request that the operator stop the scan.

h. Celecoxib administration

Participants in Phases 2 and 3 will receive a single dose of celecoxib up to 600 mg on either day one (Phase 3) or day two (Phase 2). This dose is beyond the normal therapeutic range (100- 200 mg BID), but we feel it is safe based on prior experience and celecoxib will be administered only short-term – i.e., one or two doses.

Prior Experience.

a) Although the typical dose of celecoxib is 100-200 mg BID, the Food and Drug Administration (FDA)-approved package insert (page 1) allows 400 mg BID for familial adenomatous polyposis. 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 patients did not result in serious toxicity (FDA-approved package insert, page 9).
c) Large clinical trials have used celecoxib at doses of 400 mg twice per day for 6 months. For example, Silverstein and colleagues (Silverstein *et al.*, 2000) studied 8,059 RA patients taking either celecoxib (400 mg twice per day) or ibuprofen or diclofenac, had three arms, with the celecoxib arm patients taking 400 mg twice per day for 6 months (Silverstein *et al.*, 2000). This study found a decrease in GI toxicity among patients using celecoxib as compared to NSAIDs but they did not note any increase in cardiovascular events among these patients (Silverstein *et al.*, 2000).
d) Our collaborator, James Katz, MD, has used up to 600 mg as a single dose in RA patients and had no untoward effects.

Short-Term Use. A few studies have examined whether increased cardiac risk is associated with short-term use. In two randomized, double-blind studies patients were administered 200 mg of celecoxib daily for 6 weeks without significant increase in cardiovascular adverse events (McKenna *et al.*, 2001; Geba *et al.*, 2002). In fact, one study demonstrated a superior safety and tolerability profile for celecoxib as compared to diclofenac (McKenna *et al.*, 2001). Furthermore, a trial of 7 day treatment of 200 mg celecoxib twice daily actually improved outcomes in myocardial injury in elective percutaneous intervention for stable angina (Pelliccia *et al.*, 2006). To more thoroughly investigate this, a retrospective analysis was conducted of the Veterans Integrated Service Network 17 Veterans Affairs database. This identified that patient with long-term

celecoxib use was associated with increased cardiovascular risk, but short term use did not carry this risk (Motsko *et al.*, 2006).

Common reactions to celecoxib administration include: headache, dyspepsia, urinary tract infection, diarrhea, abdominal pain, nausea, vomiting, rash, flatulence, peripheral edema, dizziness, AST/ALT elevation, elevated blood urea nitrogen, and photosensitivity. Celecoxib carries a Black Box warning for cardiovascular and gastrointestinal risks and that the drug might rarely lead to death. Rare but serious reactions associated with celecoxib 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; and blood dyscrasias (https://online.epocrates.com/rxmain accessed 3/10/2016). We feel that the risks of serious reactions are low as we are giving a single dose of celecoxib within the recommended dose range. Subjects will be monitored for adverse reactions. Rarely, a subject will have an allergic reaction to their very first dose. Because there is possible teratogenic or nonteratogenic effect of celecoxib on pregnancy, participants with 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.

8. SUBJECT SAFETY MONITORING

The subjects will be evaluated upon entry into the protocol. If there are any medical contraindications to performing a procedure, that procedure will not be performed. No safety labs or EKG will be performed on the day of the PET scan. If the patient has metal in their body preventing the safe administration of the MRI, that test will not be performed.

a. Toxicity criteria

Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

b. Criteria for individual subject withdrawal

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

9. OUTCOME MEASURES

a. Primary outcome measures

- Phase 2: Difference of [^{11}C]MC1 binding between patients and healthy subjects, and between inflamed and uninflamed body parts in RA patients.
- Phase 2: The specificity of [^{11}C]MC1 binding to COX-2 evaluated by celecoxib-blocking study.
- Phase 3: Quantitative measure of enzyme density (V_T) at baseline and after celecoxib blockade in patients compared to healthy controls.
- Phase 4: The change of [^{11}C]MC1 binding to COX-2 after two to four months of clinically-indicated therapy.

b. Secondary outcome measures

- Biodistribution and dosimetry of [^{11}C]MC1.
- Clinical correlation of [^{11}C]MC1 inRA, AxSpA and IIM patients based on their clinical or laboratory parameters.

10. STATISTICAL ANALYSIS

a. Analysis of data/ study outcomes

Phase 2 and 4: SUV will be calculated in region-of-interests based on involved body parts in patients (e. g. knee joints in RA, thigh muscles in IIM), spine in AxSpA) using PMOD software (PMOD Technologies Ltd., Zurich, Switzerland). Whole-body CT and/or MRI will provide guidance for anatomical location of the radioligand uptake and/or significant inflammation. In each region-of-interest, time-activity curve will be obtained from the dynamic PET image. Region uptake after blockade with celecoxib or after treatment will be expressed as a percentage of the baseline value. Possible difference of actual blood radioligand level may be adjusted by venous blood data obtained during the PET scans.

Phase 3: The Quantitative measure of enzyme density (V_T) in whole brain will be compared between patients and controls both at baseline and after celecoxib blockade.

b. Power analysis

Phases 1 and 2: This study is exploratory and we do not know how much would [^{11}C]MC1 uptake be different between patients and healthy subjects. Thus, a formal power calculation is impossible. We wish to scan at least 10 subjects in each group of Phase 2 and request a maximum of 15 in each group to account for drop-outs. In total, we request permission to scan two subjects for Phase 1 and 60 subjects for Phase 2, for a total of 62 subjects.

Phase 3 is also exploratory, and we request 12 RA patients and 22 healthy volunteers.

Phase 4 is also exploratory, and we request 15 RA patients.

11. HUMAN SUBJECTS PROTECTION

a. Subject selection

Participants will be selected based on the study's eligibility criteria. Participants will be admitted to the protocol regardless of gender, race, or ethnicity. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

b. Justification for 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.

c. Justification for exclusion of other vulnerable subjects

Pregnant women will be excluded because this protocol involves exposure to ionizing radiation. Lactating women will be excluded because radioisotopes may be excreted in milk. Persons with HIV infection are excluded to ensure that the results we see are related only to COX-2 binding and not to another abnormality such as a viral infection.

d. Justification for sensitive procedures

Not applicable

e. Safeguards for vulnerable populations and sensitive procedures

Pregnancy testing will be performed before PET and MRI scanning for any participants of child-bearing potential. Protections for NIH employees and staff participating in this study include: 1) ensuring that participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research" prior to obtaining consent, and 3) ensuring that there will be no direct solicitation of employees or staff.

This study collects sensitive information. For example, we may collect information on drug and alcohol use, medical history and diagnoses. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

12. BENEFIT/RISK

a. Benefit

This study offers no direct benefit to individual subjects but will lead to generalizable knowledge about RA, AxSpA, and myositis, and development of novel inflammatory biomarkers.

13. CONSENT DOCUMENTS AND PROCESS

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".

All participants will receive a verbal explanation in person and in the Clinical Center in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. To minimize undue coercion, participants may take as long as they wish to ask questions of anyone prior to signing. Consent may be obtained in-person or using NIH-approved telehealth platforms.

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

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent

document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

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

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

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

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

14. DATA AND SAFETY MONITORING

a. Data and safety monitor

Data and safety will be monitored by an independent safety monitor (ISM) for this study: Dr. Peter Grayson. Dr. Grayson is a board-certified rheumatologist with full clinical privileges at the NIH Clinical Center. He runs the NIAMS Vasculitis Translational Research Program and relies heavily on advanced imaging for his work. He is also associated with the NIAMS Systemic Autoimmunity Branch (Kaplan Lab). His primary responsibility will be to provide independent safety monitoring in a timely fashion.

b. Data and safety monitoring plan

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.

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

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

15. QUALITY ASSURANCE (QA)

a. Quality assurance monitor

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequatelymonitored. 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, andapplicable 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 inspectionby 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 studystaff. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

b. Quality assurance plan

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.

16. REPORTING OF UNANTICIPATED PROBLEMS, ADVERSE EVENTS AND PROTOCOL DEVIATIONS

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

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

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

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

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

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

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

The PI will immediately report all deaths and SAEs to the Sponsor by disclosing all event-related information in a completed MedWatch Form 3500A. This form should include the IND number, protocol number, PI name, and an assessment on the reasonable possibility of a relationship between the event and the study drug or intervention. **MRSs 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.

a. For Research Radioligand Studied under IND

The PI will report SAEs according to the requirements of 21 CFR 312.64(b). The PI will record nonserious AEs to the IRB at the time of Continuing Review, and to the Sponsor at regular intervals per request.

17. ALTERNATIVES TO PARTICIPATION

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

18. PRIVACY

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

19. CONFIDENTIALITY

a. For research data and investigator medical records

Data will be kept in password-protected computers. Only study investigators will have access to the data. This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples

Blood samples will be discarded at the end of the PET scan. Data will be stored using codes that we assign.

c. Special precautions

Every necessary step will be taken to prevent identification of study participants and other violations of subject confidentiality. Information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.) will be used that could allow direct linking of database information to individual subjects. Where temporary linking of information with identifiers is needed, such identifiers will be temporarily attached to the data, and will be removed after information has been encoded. Secured e-mail will be used for all electronic communications of subject information between investigators.

Demographic and clinical data will be archived in EXCEL on a password protected PC server and the Clinical Trial Database (CTDB). Clinical Safety Monitoring data will be archived together with other data. Laboratory test results will be stored on the CRIS. Only study investigators and internal/external monitors will have access to the samples and data. De-identified results from this clinical trial will be posted on http://www.clinicaltrials.gov.

20. CONFLICT OF INTEREST

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts-of-interest to report.

21. RESEARCH AND TRAVEL COMPENSATION

Study participants will be compensated for time and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. Participants will be paid for each portion of the study they have completed whether or not they opt for early withdrawal from participation. Without any delay of study procedures or unanticipated inconvenience, the total possible compensation is $930. If the investigators need to delay study procedures or if additional time is needed for completion, subjects may receive additional compensation in accordance with NIH guidelines. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Healthy volunteers undergoing dosimetry whole-body scan (Phase 1)

Visit 1 to NIH	
PET scanning	$150
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$200

Healthy volunteers undergoing whole-body scans with blocking studies (Phase 2)

Visit 1 to NIH	
PET scanning x 2	$500
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Total	$610

RA or patients undergoing whole-body scans with blocking studies (Phase 2)

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90
Escort fee	$20
Visit 2 to NIH	
PET scanning x 2	$500
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Escort fee	$20
Total	$740

AxSpA patients undergoing whole-body scans with blocking studies (Phase 2)

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90
Escort fee	$20
Visit 2 to NIH	
PET/MRI x2	$700
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Escort fee	$20
Total	$940

IIM patients undergoing whole-body scans with blocking studies (Phase 2)

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90

Escort fee	$20
Visit 2 to NIH	
PET scanning x 2	$500
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Escort fee	$20
Visit to 3 to NIH	
MRI	$100
Pregnancy test	$10
Escort fee	$20
Total	$ 870

Healthy volunteers undergoing brain scans with blocking studies (Phase 3)

Visit 1 to NIH	
PET scanning x 2	$500
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Visit 2 to NIH	
MRI	$100
Pregnancy test	$10
Total	$780

RA patients undergoing brain scans with blocking studies (Phase 3)

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90
Escort fee	$20
Visit 2 to NIH	
PET scanning x 2	$500
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Celecoxib administration	$50
Escort fee	$20
Visit 3 to NIH	
MRI	$100
Pregnancy test	$10

Escort fee	$20
Total	$ 930

RA patients undergoing whole-body scans after treatment (phase 4)

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90
Escort fee	$20
Visit 2 to NIH	
PET scanning	$250
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Escort fee	$20
Visit 3 to NIH	
PET scanning	$250
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$20
Escort fee	$20
Total	**$770**

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

22. REFERENCES

Aisen PS, Thal LJ, Ferris SH, Assaid C, Nessly ML, Giuliani MJ*, et al.* Rofecoxib in patients with mild cognitive impairment: further analyses of data from a randomized, double-blind, trial. Current Alzheimer research 2008; 5(1): 73-82.

Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd*, et al.* 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis and rheumatism 2010; 62(9): 2569-81.

Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). The New England journal of medicine 1975a; 292(7): 344-7.

Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). The New England journal of medicine 1975b; 292(8): 403-7.

Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. Trends in pharmacological sciences 2009; 30(4): 174-81.

Crofford LJ, Wilder RL, Ristimaki AP, Sano H, Remmers EF, Epps HR*, et al.* Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1 beta, phorbol ester, and corticosteroids. The Journal of clinical investigation 1994; 93(3): 1095-101.

Elzinga EH, van der Laken CJ, Comans EF, Lammertsma AA, Dijkmans BA, Voskuyl AE. 2-Deoxy-2-[F-18]fluoro-D-glucose joint uptake on positron emission tomography images: rheumatoid arthritis versus osteoarthritis. Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging 2007; 9(6): 357-60.

Emery P, Zeidler H, Kvien TK, Guslandi M, Naudin R, Stead H*, et al.* Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: randomised double-blind comparison. Lancet (London, England) 1999; 354(9196): 2106-11.

Geba GP, Weaver AL, Polis AB, Dixon ME, Schnitzer TJ. Efficacy of rofecoxib, celecoxib, and acetaminophen in osteoarthritis of the knee: a randomized trial. Jama 2002; 287(1): 64-71.

Kang YJ, Mbonye UR, DeLong CJ, Wada M, Smith WL. Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. Prog Lipid Res 2007; 46: 108-25.

Korotkova M, Helmers SB, Loell I, Alexanderson H, Grundtman C, Dorph C*, et al.* Effects of immunosuppressive treatment on microsomal prostaglandin E synthase 1 and cyclooxygenases expression in muscle tissue of patients with polymyositis or dermatomyositis. Annals of the rheumatic diseases 2008; 67(11): 1596-602.

Kreisl WC, Jenko KJ, Hines CS, Lyoo CH, Corona W, Morse CL*, et al.* A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation. Journal of cerebral blood flow and

metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2013a; 33(1): 53-8.

Learn CA, Mizel SB, McCall CE. mRNA and protein stability regulate the differential expression of pro- and anti-inflammatory genes in endotoxin-tolerant THP-1 cells. J Biol Chem 2000; 275: 12185-93.

Lloyd TE, Mammen AL, Amato AA, Weiss MD, Needham M, Greenberg SA. Evaluation and construction of diagnostic criteria for inclusion body myositis. Neurology 2014; 83(5): 426-33.

Lockwood AH. Invasiveness in studies of brain function by positron emission tomography (PET). J Cereb Blood Flow Metab 1985; 5: 487-9.

McCann NC, Lynch TJ, Kim SO, Duffy DM. The COX-2 inhibitor meloxicam prevents pregnancy when administered as an emergency contraceptive to nonhuman primates. Contraception 2013; 88(6): 744-8.

McKenna F, Borenstein D, Wendt H, Wallemark C, Lefkowith JB, Geis GS. Celecoxib versus diclofenac in the management of osteoarthritis of the knee: a placebo-controlled, randomised, double-blind comparison. Scand J Rheumatol 2001; 30(1): 11-8.

Motsko SP, Rascati KL, Busti AJ, Wilson JP, Barner JC, Lawson KA*, et al.* Temporal Relationship between Use of NSAIDs, Including Selective COX-2 Inhibitors, and Cardiovascular Risk. Drug Safety 2006; 29(7): 621-32.

Orjales A, Mosquera R, López B, Olivera R, Labeaga L, Núñez MT. Novel 2-(4-methylsulfonylphenyl)pyrimidine derivatives as highly potent and specific COX-2 inhibitors. Bioorganic & Medicinal Chemistry 2008; 16(5): 2183-99.

Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B*, et al.* Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. The Journal of pharmacology and experimental therapeutics 2001; 297(2): 638-45.

Pelliccia F, Pasceri V, Granatelli A, Pristipino C, Speciale G, Roncella A*, et al.* Safety and efficacy of short-term celecoxib before elective percutaneous coronary intervention for stable angina pectoris. The American journal of cardiology 2006; 98(11): 1461-3.

Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38(1): 44-8.

Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A*, et al.* Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. Jama 2000; 284(10): 1247-55.

Steiner G, Tohidast-Akrad M, Witzmann G, Vesely M, Studnicka-Benke A, Gal A*, et al.* Cytokine production by synovial T cells in rheumatoid arthritis. Rheumatology (Oxford, England) 1999; 38(3): 202-13.

Studynkova JT, Kuchen S, Jeisy E, Schedel J, Charvat F, Jarosova K*, et al.* The expression of cyclooxygenase-1, cyclooxygenase-2 and 5-lipoxygenase in inflammatory muscle tissue of patients with polymyositis and dermatomyositis. Clinical and experimental rheumatology 2004; 22(4): 395-402.

Tak PP, Smeets TJ, Daha MR, Kluin PM, Meijers KA, Brand R*, et al.* Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis and rheumatism 1997; 40(2): 217-25.

The Alzheimer's Disease Anti-inflammatory Prevention Trial Research G. Results of a follow-up study to the randomized Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT). Alzheimer's & Dementia 2013; 9(6): 714-23.

Tsuji S, Miyoshi H, Tomita T, Nakase T, Hamada M, Oomae T*, et al.* Celecoxib, a cyclooxygenase-2 inhibitor, improved upper gastrointestinal lesions in rheumatoid arthritis patients as assessed by endoscopic evaluation. Modern rheumatology 2012; 22(3): 353-62.

Zanotti-Fregonara P, Innis RB. Suggested pathway to assess radiation safety of 11C-labeled PET tracers for first-in-human studies. Eur J Nucl Med Mol Biol 2012; 39: 396-8.

Appendix 1. ASAS Criteria for Axial Spondyloarthritis.

Patients with back pain for ≥3 months duration, and who are <45 years of age, are considered to have Axial Spondyloarthritis (Axial SpA) if they have either (1) sacroiliitis on imaging and one or more features of SpA, or (2) HLA-B27 and 2 or more features of SpA. Sacroiliitis on imaging is defined as active inflammation on MRI highly suggestive of sacroiliitis associated with SpA, or definite radiographic sacroiliitis according to the modified NY criteria. Features of SpA include (a) IBP, (b) arthritis, (c) enthesitis in the heel, (d) uveitis, (e) dactylitis, (f) psoriasis, (g) IBD, (h) a good response to an NSAID, (i) positive family history of SpA, (j) HLA-B27, and (k) elevated CRP.