

**Role of microglial activation and norepinephrine transporter abnormalities in pathogenesis  
of MS-related fatigue**

**NCT04144257**

**Study Protocol and Statistical Analysis Plan 8.19.21**

## DETAILED PROTOCOL

### **Study Title: Role of microglial activation and norepinephrine transporter abnormalities in pathogenesis of MS-related fatigue**

#### **I. Background and Significance**

##### **a. Historical background**

Fatigue is reported as the most disabling symptom in up to 60% of MS patients<sup>1</sup> and is estimated to have a lifetime prevalence of 80%.<sup>2</sup> The anatomical and physiological substrate, and mechanism of MS-related fatigue is not clear. Neurochemical dysfunction, alteration of circadian rhythms, and hypothalamic-pituitary axis abnormalities may play a role but there is a lack of a comprehensive model.<sup>1</sup> Inflammation is proposed as a potential mechanism but suffers from the lack of sufficient evidence.<sup>1</sup> Abnormalities in functional connectivity of the resting default mode network have been linked with fatigue in MS.<sup>3</sup> However, the mechanisms of these abnormalities are not known.

Abnormally activated immune cells, predominantly lymphocytes and macrophages are thought to mediate injury to the myelin sheath in multiple sclerosis (MS) as these cells enter the CNS from the periphery. However, the innate immune system of the CNS, particularly, the microglia may play a key role in development of MS pathology through antigen presentation and secretion of pro-inflammatory cytokines.<sup>4,5</sup> Microglial activation may be responsible for neuronal dysfunction associated with MS-related fatigue, but cannot be assessed using conventional MRI techniques.<sup>6</sup>

Experimental animal and post-mortem human studies have demonstrated damage to locus coeruleus in the pons, the primary brain nucleus that produces norepinephrine, and reduced norepinephrine levels in the brain.<sup>7</sup> Decreased serum norepinephrine levels and concomitant reduced sympathetic activity has been demonstrated in MS patients.<sup>8,9</sup> This reduction in sympathetic activity may contribute to higher incidence of depression and fatigue in MS.<sup>10</sup> Clinically used stimulants, such as modafinil act at least partially by interacting with norepinephrine transporter system<sup>11</sup> underscoring their potential relevance in pathogenesis of MS-related fatigue. However, a direct study of *in-vivo* changes in noradrenergic neurons in humans has been limited by the lack of appropriate technology so far, and has not been studied in assessment of fatigue in living MS patients.

Moreover, norepinephrine may have immunomodulatory effects, beyond its role as a neurotransmitter.<sup>12,13</sup> Stimulating  $\beta$ 2-adrenergic receptors on lymphocytes and antigen presenting cells in the periphery may lead to inhibition of inflammatory cytokine production and targeted cell killing.<sup>14</sup> In the central nervous system, *in vitro* studies have demonstrated that norepinephrine suppresses microglial transcription of proinflammatory genes and cytokine production (e.g., TNF-alpha)<sup>15</sup> but a detailed assessment of neuroanatomy of these changes and their interaction with microglial activation in MS patients has not been performed.

[F-18]PBR06 is a second generation PET radioligand targeting the 18kiloDalton translocator protein (TSPO), which is overexpressed on activated microglia/macrophages.<sup>16</sup> Our preliminary human data in MS suggests that this technique has the ability of demonstrating microglial activation in humans and that it correlates with disability and brain atrophy in MS patients.<sup>17,18</sup> On head-to head comparison with [C-11]PBR28 in MS patients, our data suggested a higher clinical relevance of white matter lesional changes measured by [F-18]PBR06.<sup>19</sup> On preliminary analysis, positive correlations of [F-18]PBR06 uptake with modified fatigue impact scale (MFIS) values were seen in pons and other brain regions.

[C-11] Methylreboxetine (MRB) (also known as [C-11]MeNER) is a novel PET radiopharmaceutical targeting norepinephrine transporter in the central nervous system that has been validated as a quantitative biomarker of norepinephrine transporter binding in the human brain. Our previous work demonstrated high concentrations of MRB in locus ceruleus, raphe nuclei, thalamus, red nucleus, hypothalamus and several cortical regions.<sup>20</sup> [C-11]MRB has been already validated to demonstrate alterations in NET binding in conditions such as cocaine abuse, attention deficit hyperactivity disorder and obesity.<sup>21</sup>

#### b. Rationale and potential benefits

As described above, microglial activation likely plays a crucial role in neural injury in MS. *In vivo* neuroimaging biomarkers of microglial activation are crucially needed to further study the role of microglia in pathogenesis, to enable better prognostication, to monitor disease evolution, and to assess putative anti-inflammatory therapies.

A direct study of in-vivo changes in noradrenergic neurons in living humans has been limited by the lack of appropriate technology so far. [C-11] Methylreboxetine (MRB) (also known as [C-11]MeNER) is a novel Positron Emission Tomography (PET) radiopharmaceutical targeting norepinephrine transporter in the central nervous system that has been validated as a quantitative biomarker of norepinephrine transporter binding in the human brain, which demonstrated high concentrations in locus ceruleus, raphe nuclei, thalamus, red nucleus, hypothalamus and several cortical regions.<sup>16</sup> [C-11]MRB has been already validated to demonstrate alterations in NET binding in conditions such as cocaine abuse, attention deficit hyperactivity disorder and obesity.<sup>17</sup>

PET agents targeting TSPO that is overexpressed in activated microglia have the potential advantage of being direct and specific markers of chronic innate neuroinflammation.<sup>22</sup> Improved radiopharmaceuticals targeting TSPO are urgently needed. We propose to use a novel TSPO PET ligand, <sup>18</sup>F-PBR06 ((18)F-N-fluoroacetyl-N-(2,5-dimethoxybenzyl)-2-phenoxyaniline), to assess microglial activation in MS.<sup>23</sup> This study will provide a unique opportunity to assess the utility of a novel TSPO PET ligand that has a longer radioisotope half life (approximately, 110 minutes) and hence, has the potential for widespread use depending upon the results of this study.

These strategies are targeted to address an unmet need because currently available MRI techniques lack sensitivity and specificity for assessing such changes.<sup>18</sup> For example, in subjects with SPMS, conventional MRI may fail to distinguish between chronic active MS plaques and inactive plaques, and MRI findings may appear unchanged while subjects continue to deteriorate clinically. Moreover, therapies effective in RRMS are ineffective in SPMS and hence, new and improved treatments are urgently needed. One potential therapeutic avenue would be to target microglial activation that may contribute to worsening of RRMS and SPMS. An in vivo biomarker of microglia activation would play a crucial role in the study of biologic disease mechanisms and development of such new therapies.

Molecular changes are expected to precede changes detected on structural MRIs. There is data to suggest that microglial activation signal on Positron Emission Tomography (PET) precedes contrast enhancement on MRI.<sup>2</sup> High resolution 7-Tesla (7T) MRI has recently become available at Brigham and Women's Hospital. 7T MRI enables imaging with increased signal-to-noise and contrast-to-noise-ratio that allow higher spatial resolution and better detection of anatomical and pathological features in MS.

This study will provide a unique opportunity to assess the utility of novel PET ligands that have the potential for widespread use depending upon the results of this study. This would trigger further studies using [C-11]MRB-PET and [F-18]PBR06-PET for assessing prognostication, treatment response and drug development for MS subjects.

## **II. Specific Aims**

The overarching aim is to assess the role of microglial activation and norepinephrine transporter binding in pathogenesis of MS-related fatigue, using novel Positron Emission Tomography (PET) radiotracers. [F-18]PBR06 and [C-11]MRB.

The specific aims of the study are:

*Specific Aim 1:* To determine the relationship of cerebral microglial activation, as assessed by [F-18]PBR06 PET, with MS-related fatigue. The hypothesis is that there is a positive correlation between microglial activation with fatigue in MS. We further hypothesize that cognitive and physical aspects of fatigue are mediated by microglial activation in cortical and subcortical grey matter regions, respectively. We'll also perform an exploratory analysis on correlation of [F-18]PBR06 PET and fatigue with serum measurements of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, MCP-1 and MIF-1.

Specific Aim 2: To determine the relationship of norepinephrine transporter (NET) binding, as assessed by [C-11]MRB PET, with MS-related fatigue. The hypothesis is that there is an inverse correlation between NET binding with fatigue in MS. We'll also perform an exploratory analysis on correlation of [C-11]MRB PET and fatigue with measurements of serum norepinephrine.

Specific Aim 3: To determine the relationship of microglial activation and NET binding, with grey matter pathology (lesion load and brain atrophy) assessed using 7T MRI, and evaluate their independent contribution in development of MS-related fatigue. The hypothesis is that microglial activation and NET binding demonstrate positive and inverse correlations respectively, with grey matter pathology (lesion load and atrophy) and are independent predictors of MS-related fatigue.

### **III. Subject Selection**

#### **a. Recruitment methods:**

Subjects will be recruited during routine clinical appointments by their physician or one of the other co-investigators listed on the protocol at the Partners MS Center, 60 Fenwood Road, Boston, MA. Established subjects of the MS Center will be sent a letter describing the study and a copy of the consent form. Subjects will also be recruited through the Partners Rally website (<https://rally.partners.org>).

#### **b. Inclusion/Exclusion criteria:**

##### Subject Inclusion Criteria:

1. Subjects meeting the definition for RRMS or SPMS by International Panel (2017 McDonald) Criteria.<sup>24</sup>
2. We will recruit patients with RRMS diagnosed in the last 2 years and patients with SPMS.
3. Male and female subjects age 18 to 70 years.

##### Subject Exclusion Criteria:

1. Relapse/Corticosteroid treatment in the past 4 weeks to avoid transient effects on imaging

2. Individuals with a known alternate neurologic disorder, previous head injury, or substance abuse.
3. Individuals with bipolar disease and schizophrenia
4. Concurrent medical conditions that contraindicate study procedures.
5. Women who are pregnant or nursing. Also, any woman who is seeking to become pregnant or suspects she is pregnant will be excluded from enrollment.
6. Claustrophobia
7. Non-MRI compatible implanted devices
8. Low affinity binders
9. Subjects unable or unwilling to withhold taking symptomatic treatment for fatigue (specifically, modafinil, methylphenidate and amphetamine salts) on the day of [C-11]MRB scan

c. Source of subjects and recruitment methods

As described above, subjects will be recruited during routine clinical appointments by their physician or one of the other co-investigators listed on the protocol. Established subjects at the Partners MS clinic may be sent a letter describing the study and a copy of the consent document. Subjects will also be recruited through the Partners Rally website (<https://rally.partners.org>).

#### **IV. Subject Enrollment**

a. Methods of enrollment, including procedures for subject registration and/or randomization.

Physicians at the MS clinic may present the study to a subject during a regular scheduled clinic visit. If the subject is interested in the study, a copy of the consent form will be given. The subject will then be contacted by telephone prior to the screening visit appointment. At the time of the subject's screening visit, an investigator will go over the study in detail, answer any questions the subject may have regarding the study and a licensed physician investigator will obtain informed consent. In accordance with NIH guidelines, efforts will be made to attain a mix of study participants, in terms of gender and racial/ethnic representation.

Our goal sample size is 12 MS subjects (6 RRMS subjects and 6 SPMS subjects). In order to attain our goal sample size, we estimate that we may need to screen up to approximately 20 subjects.

b. Procedures for obtaining informed consent (including timing of consent process)

Informed consent will be obtained from the subjects by a licensed physician investigator on the study protocol. Existing Multiple Sclerosis clinic patients may be sent a letter describing the study and a copy of the consent document. Interested subjects are directed to contact research staff via a telephone number provided in the letter for participation in the study. They will have the

opportunity to discuss the study with research study staff prior to giving consent as outlined above and they may be screened over telephone for eligibility for the study. Subjects approached for participation in the study during a routine clinical visit will have the opportunity to participate in the study at that time or they may choose to return for participation at another time in the future. All subjects will be informed that they are free to withdraw consent from the study at any time without affecting the quality or type of care that they receive at Partners institutions.

#### Planning for Potential Study Disruptions due to COVID-19

Under contingent circumstances related to Covid-19, informed consent may need to be obtained remotely. The consent discussion will occur by phone or video conferencing (preferably the latter unless chosen otherwise by the subject). The consent discussion will be conducted by the principal investigator or another licensed physician on the protocol. The consent form will be mailed or e-mailed to the participant. The participant will have the option of proving consent in the following manner:

- Electronic consent (Adobe E-Sign or COVID MyStudies App for FDA regulated trials or Partners REDCap e-consent)

OR

- A remote consent process where the participant will be asked to sign consent and return back by email, mail, or fax.

The study team shall obtain and document informed consent before the participant is enrolled and study procedures begin.

Subjects will be paid for taking part in this study as it follows:

- If they complete the screening visit, they will be paid \$50
- For each PET scan they complete, they will be paid \$200
- If they complete the MRI scan, they will be paid \$100

The total amount subjects may receive for taking part in all study visits is \$550 if they complete the PET scans, the MRI scan, and the screening visit. If the subject has to stop any visit early for any purpose, he/she will still receive \$25 for his/her time.

Meals and parking in the hospital will be provided to the subjects for the visits. Subjects may also be reimbursed for the cost of transportation on the day of the research visits.

c. Treatment assignment, and randomization (if applicable)

Not applicable

## V. Study Procedures

- a. Study visit and parameters to be measured (e.g., laboratory tests, x-rays, and other testing)

### Initial Visit:

During the first visit, subjects will be administered the screening questionnaire. If a subject is on methylphenidate, modafinil or amphetamine salts (medications to treat fatigue), they will be informed about the need to not take the medication on the day of the [C-11]MRB-PET scan. These medications are routinely withheld prior to other FDA-approved nuclear medicine procedures like [I-123]Ioflupane scans<sup>25</sup>, is well tolerated and considered safe. The ultimate decision to withhold these medications for 1 day in each individual case will be based on a discussion of the PI with the patient and the patient's referring physician. However, if a potential subject is unwilling to do so or after discussion with the patient's referring physician it is considered clinically unsafe for any reason to withhold taking symptomatic treatment for fatigue (specifically, modafinil, methylphenidate and amphetamine salts) on the day of [C-11]MRB scan, he/she will be excluded from further recruitment in the study. Subjects will review and eventually sign the consent form. They will be administered standardized questionnaires for cognitive testing and/or other comorbidities. In the event of suicidal ideation during the administration of the Beck Depression Inventory (if a subject answers 2 or 3 on Question 9 of the BDI or if there are spontaneous reports of suicidality), the subject will be evaluated promptly by Dr. Singhal or another licensed clinician member of the study staff for appropriate assessment and triage. In addition, a blood sample will be drawn for genotype testing to identify high affinity, medium affinity and low affinity binders. Subjects will also undergo cross-sectional estimations of blood markers of inflammation, and serum norepinephrine measurements.

### Genotype Testing

Blood sample drawn on the initial screening visit will be used to obtain genomic DNA for genotyping for polymorphism within the TSPO gene on chromosome 22q13.2, using a Taqman assay.<sup>26</sup> High affinity and medium affinity binders will be included while the low affinity binders will be excluded from the study.

### PET Visit:

During the PET scan visits, all women subjects of child bearing age will undergo a stat quantitative serum hCG pregnancy test and only women with a negative test will undergo the radiopharmaceutical injection.

### PET imaging procedures

PET scanning sessions will be at MGH Gordon PET Core scanning facility, 55 Fruit Street, Boston, MA and BWH PET scanning facility, 75 Francis Street, Boston, MA. Radiopharmaceutical will be injected as a bolus (up to 15mCi for [C-11]MRB and 4mCi for [F-18]PBR06) followed by a saline flush. Each PET session will last up to 120 minutes. At the time of imaging, the subjects will be positioned in the gantry of a high-resolution PET/CT camera. Head alignment will be made, relative to the canthomeatal line, using projected laser lines whose positions are known with respect to the slice positions of the scanner. A head support apparatus will be used to minimize head motion. For [C-11]MRB PET scans, brain PET data acquisition will begin at the moment of radiotracer injection. For [F-18]PBR06 PET scans, brain PET data acquisition in list mode, will begin 60 seconds prior to radiotracer injection. Dynamic data over up to 120 minutes for PET quantification will be acquired, according to previously described methods for both tracers.<sup>17,18,20,27-30</sup> Images will be acquired using a high-resolution PET/CT scanner.

### MRI Visit:

All subjects will undergo a 7T Brain MRI during an MRI visit that may precede or follow the PET scan visit. Efforts will be made to perform the PET and MRI scans within 2 weeks of each other. However, due to limited PET scan availability, this time frame may be longer.

### Pregnancy screening prior to MRI:

All women will be queried about their pregnancy status, use of contraception and last menstrual period. If they are reliably using contraception, can state the timing of their last menstrual period, and do not believe they could be pregnant, we will proceed with the MRI scan without pregnancy testing. If they cannot do this, then they will undergo a urine pregnancy test. If a woman is seeking pregnancy and not using contraception, she will undergo a urine pregnancy test.

### MRI imaging procedures

MRI scanning session will be performed at BWH MRI facility at 60 Fenwood Road, Boston, MA. Using the 7T Siemens MAGNETOM Terra MRI unit at BWH, several pulse sequences will be performed to image the brain. There will be no intravenous contrast used for the MRIs. The MRI scan session will take approximately 1 hour.

The details of the parameters of the pulse sequences are as follows: 3D MRI will be obtained with 0.7 mm<sup>3</sup> isotropic voxels using pre-contrast T2\*, T2, MP2RAGE, FLAIR sequences.

The pulse sequences are standard research protocols, and may include any or all of the following: scout series, 3D FLAIR, 3D T2, 3D MP-RAGE/SPGR, 2D T1, 2D T2, 2D FLAIR, DTI, STIR, T2\*, PSIR.

Serum assays:

Serum measurements for inflammatory markers (IL-1 $\beta$ , TNF- $\alpha$ , IL-6, MCP-1 and MIF-1) and serum norepinephrine measurement will be performed according to established procedures.<sup>31,32</sup>

### **Planning for Potential Study Disruptions due to COVID-19**

Some of the study screening procedures may be performed remotely. During this visit, a physician with or without assistance from a study staff will contact subjects via zoom video call platform or telephone and conduct the following types of research activities:

- Clinical review of symptoms and past medical history
- Review of study procedures
- Clinical questionnaires for assessment of disability, depression, anxiety, cognition and fatigue including: Expanded Disability Status Scale (EDSS), Modified Fatigue Impact Scale (MFIS), Fatigue Severity Scale (FSS), Hospital Anxiety and Depression Scale (HADS), Beck's Depression Inventory (BDI), Pittsburgh Sleep Quality Index (PSQI), Center for Epidemiological Studies-Depression Scale (CES-D) and MSQOL-54 scale (MSQOL-54)

Timed 25-foot walk (T25W), MS Functional Composite (MSFC), and Symbol-Digit Modality test (SDMT) will be skipped.

Research staff will follow the MGB requirements for using the appropriate virtual tools including Zoom instance (Enterprise Zoom or HealthCare Secure Zoom) per [https://pulse.partners.org/hub/departments/emergency\\_preparedness/coronavirus/virtual\\_care](https://pulse.partners.org/hub/departments/emergency_preparedness/coronavirus/virtual_care)

b. Drugs to be used

No therapeutic drugs will be used for the study. Tracer amounts of [C-11]MRB and [F-18]PBR06 have no pharmacological activity.

c. Devices to be used

No special or extra devices will be used other than the PET scanners during the PET scans and MRI scanner during the MRI scans.

d. Procedures/surgical interventions, etc.

The radiotracer dose will be injected intravenously for the PET scan.

Production of radiopharmaceuticals

[F-18]PBR06 will be produced in the PET radiochemistry facility at BWH according to standardized procedures.<sup>16</sup> [C-11]MRB will be produced in the PET radiochemistry facility at MGH Gordon Center according to standardized procedures.<sup>33</sup> The products will be purified by HPLC and sterilized by membrane filtration using 0.22-micron membrane filter. The final product will be an isotonic solution that is sterile and pyrogen free, ready for intravenous administration. The volume injected will be adjusted so that the proper dose of radiotracer is administered. The radiochemical purity (RCP) of radiopharmaceuticals will be determined using SG-ITLC and/or high-pressure liquid chromatography (HPLC). The organic solvents will be determined using gas chromatography (GC). The expected RCP of radiolabeled tracers is >95%. The dose for the scans will be 4mCi (148MBq) of [F-18]PBR06 and 15mCi (555MBq) of [C-11]MRB.

e. Data to be collected and when the data is to be collected

Imaging Data

The following data will be collected on all subjects during scanning sessions:

During PET scanning, brain images will be acquired in 3-D mode, and dynamic acquisition scans will continue for up to 120 minutes. A non-diagnostic CT scan will be performed for attenuation correction of PET transmission data at the time of initiation of scanning.

MRI data will be acquired as described above. T2/FLAIR and other MRI images will be coregistered with PET images.

Non Imaging Data/Clinical Data

The following non-imaging, clinical data will be obtained:

Modified Fatigue Impact Scale (MFIS)<sup>34</sup>

Fatigue Severity Scale (FSS)<sup>35</sup>

Expanded Disability Status Scale (EDSS)<sup>36</sup>  
Timed 25-Foot Walk (T25FW)<sup>37</sup>  
MS Functional Composite (MSFC)<sup>38</sup>  
Symbol Digit Modalities Test (SDMT)<sup>39</sup>  
MSQOL-54 scale (MSQOL-54)<sup>40</sup>  
Pittsburgh Sleep Quality Index (PSQI)<sup>41</sup>  
Beck's Depression Inventory (BDI)<sup>42</sup>  
Center for Epidemiological Studies-Depression Scale (CES-D)<sup>43</sup>  
Hospital Anxiety and Depression Scale (HADS)<sup>44</sup>

In addition, each subject's medical record will be reviewed at the time of study onset. Age, gender, disease duration, disease symptoms, and medications will be extracted. The medical records will also be reviewed before each PET scan to assess any interval changes.

## **VI. Biostatistical Analysis**

### **a. Study endpoints/variables**

Binding potential (BP<sub>nd</sub>) will be estimated for [C-11]MRB according to established protocols.<sup>20,45</sup> Tissue volume of distribution (V<sub>t</sub>), distribution volume ratios (DVR) and standardized uptake value ratios (SUVR) will be estimated for [F-18]PBR06, according to our prior experience and previously reported literature.<sup>17,27,28,46</sup> For regional analysis, we will focus on the thalamus and pons/locus ceruleus (both are norepinephrine transporter rich areas)<sup>20</sup>, in addition to other cortical and subcortical regions (segmented according to the AAL/Hammers templates). PET analyses will be performed on PET only images as well as those co-registered with clinically obtained intrasubject MRI (using mutual information algorithms).<sup>47</sup> Coregistered PET and MRI images will be transformed in the standardized MNI (Montreal Neurological Institute) space and segmentation of the brain will be performed using standardized atlases, including AAL (Automated Anatomical Labeling) template<sup>48</sup> and Hammers atlas.<sup>49</sup> Additionally, voxel-by-voxel statistical parametric mapping (SPM) analysis will also be performed to assess correlations of PET uptake with fatigue measures, after adjustment of age and gender.<sup>50</sup>

MRI data will be assessed for presence and extent of brain atrophy using the brain parenchymal fraction (BPF) and cerebral normalized grey matter fraction (GMF), along with grey matter lesional load, using standard methods.<sup>51,52</sup>

#### b. Statistical methods

Primary analyses: For Aim 1, the primary analysis will estimate the correlation between modified fatigue impact scale (MFIS) score and [F-18]PBR06 SUVR using Pearson's correlation coefficient and Spearman's correlation coefficient. Similarly, for Aim 2, the primary analysis will estimate the correlation between MFIS scores and [C-11]MRB BPnd. For Aim 3, we will estimate the correlations between PET measures ([F-18]PBR06 SUVR and [C-11]MRB BPnd) and 7T MRI grey matter lesional load and brain atrophy. We will also estimate the correlations between MFIS and 7T MRI grey matter lesional load and brain atrophy. Given the exploratory nature of the study, an uncorrected p-value <0.05 will be considered statistically significant.

Secondary analyses: For Aim 1, secondary analyses will include estimating the correlations between [F-18]PBR06 SUVR and cognitive and physical fatigue subscales of MFIS; and between [F-18]PBR06 SUVR and serum measurements of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, MCP-1 and MIF-1. For Aim 2, secondary analyses will include correlation of [C-11]MRB PET BPnd and MFIS with measurements of serum norepinephrine. For Aim 3, secondary analysis will include estimation of correlation between [F-18]PBR06 SUVR and [C-11]MRB BPnd. We'll explore, using linear regression, the independent association between the PET measures and fatigue outcomes although the small sample size would limit our ability to make definitive statements.

## VII. Risks and Discomforts

a. Complications of surgical and non-surgical procedures, etc.

### PET scanning

The radiotracer dose will be injected intravenously prior for the PET scan. Inserting an IV catheter can cause pain, bleeding, or bruising at the place where the needle enters the vein. There is a small risk of an infection or a bruise at the site of IV catheter insertion. In addition, subjects will need to lie still in the PET camera for a period of time up to 120 min, and subjects may find it uncomfortable to remain still over this time. A standard head-support device will be used to ensure

that subjects do not move their heads during the imaging procedure. If subjects find the duration of scanning too uncomfortable, they are free to withdraw from the study at any time.

Staff at the Gordon Center will monitor PET scanning at MGH and Dr Singhal will monitor PET scanning at BWH. Dr. Singhal is certified in the Partners Health Care System Human Subjects Protection Education Requirements and is familiar with the procedures and steps necessary to report adverse events to the IRB committee. Subjects will also receive a follow up phone call 24-72 hours after the PET session, and again 2 weeks after the PET session.

### MRI scanning

Subjects may find the MRI scan uncomfortable. The 7T MRI magnet is approved for human experimentation at BWH. The scans take place in a confined space that makes some people claustrophobic. Also during the scan the subject will hear loud banging noises and will therefore wear ear plugs to reduce this noise.

The 7 Tesla MRI may cause dizziness, ringing in the ears, and less commonly nausea, especially as entering the magnet. However, these symptoms are temporary and disappear completely after scanning.

The switching of the magnetic field gradients can, under some circumstances, cause peripheral nerve stimulation that may be experienced as a mild twitching reaction in certain muscles. Such effects are rare and the switching rates are kept well below the levels at which such effects are known to occur frequently. The MR examination can and will be stopped at any time upon request by the subject, should he or she experience any of the above explained discomforts.

Subject monitoring during MRI and PET scans will be performed using a 2-way intercom system between the scanner operator and subject and by visual monitoring of the subject through the window into the scan room (the subject is visible to the operator at all times).

If investigators see something that looks like a medical problem on the PET/CT scans or MRI scan, we will ask a radiologist to review the results. If the radiologist thinks there might be a problem, we will assist the subject with receiving follow-up care. If the radiologist thinks the subject might have a medical problem, but it turns out that they do not, we may have caused the subject to worry needlessly about their health.

#### b. Drug side effects and toxicities.

No side effects from the radiopharmaceuticals are expected nor have they been reported. As this study is conducted under FDA/IND oversight, the administered doses of [C-11]MRB and [F-18]PBR06 are well below the known level to cause any pharmacologic effect. Subjects will also be exposed to a small amount of radiation.

A temporary withdrawal of fatigue medication may cause some subjects to experience some increase in perceived fatigue. The subjects will be advised to return to their normal (prescribed) dosing regimen of their symptomatic medication after the MRB PET scan.

c. Device complications/malfunctions.

No special and extra devices will be used except the PET and MRI scanners. Failure of PET scanner poses no risk to the subjects and would only cause delay or early termination of the study session. If the scan has to be abandoned after administration of the radiotracer, this could mean unnecessary radiation exposure as the subject will have to get another dose later if they choose to participate in a repeat of the scan.

d. Psychosocial (non-medical risks)

Cognitive and psychiatric assessments can be boring, fatiguing, anxiety provoking or distressing to some subjects and they have the option to take a break or not answer questions they feel uncomfortable answering.

e. Radiation Risks

For the PET portion, as these drugs are radioactive, the subject will be exposed to a small amount of radiation as a result of participation in this study. The per unit radiation dose for [C-11]MRB is 0.214mSv/mCi. The total amount of radiation is approximately 3.21mSv for [C-11]MRB scan based on an injected activity of 15mCi (555MBq). Additional dose from low dose CT on the GE Discovery MI PET/CT performed for attenuation correction of [C-11]MRB PET images is estimated to be 1mSv per CT attenuation scan. The total amount of radiation a subject will be exposed to for a C-11 MRB PET/CT imaging session will be not more than 4.21mSv, which is approximately 8.42% of the yearly amount of radiation allowed for persons who work with radiation or 1.40 years of natural background radiation exposure from the earth and sky.

The per unit radiation dose for [F-18]PBR06 is 18.5  $\mu$ Sv/MBq.<sup>23</sup> The total amount of radiation is approximately 2.72 mSv for each [F-18]PBR06 scan based on an injected activity of 4mCi (148MBq). Additional dose from low dose CT performed for attenuation correction of [F-18]PBR06 PET images is estimated to be 0.28mSv per CT scan. In case a subject decides to take the 15 minute break after 45 minutes of scanning, the subject has to undergo a repeat attenuation correction, low-dose head CT. In that case, the total exposure from CT is 0.56mSv (two times 0.28mSV). The total amount of radiation a subject will be exposed to for a [F-18]PBR06 PET/CT imaging session will be not more than 3.28 mSv, which is approximately 6.56% of the yearly amount of radiation allowed for persons who work with radiation or 1.09 years of natural background radiation exposure from the earth and sky.

For subjects participating in the study, the total radiation dose from the PET scans will be 5.93 mSv (3.21+2.72 mSv). If the subjects in the study decide to take a break during the [F-18]PBR06 scans, their total exposure from attenuation correction head CTs will be 1.56 mSv (1 + 0.56 mSv). The total radiation exposure for these subjects who choose to participate in both scans and take the available break will be 7.49 mSv (5.93mSv from PET scans plus 1.56 mSv from attenuation correction head CTs) or less. This is approximately 14.98% of the yearly amount of radiation allowed for persons who work with radiation or 2.50 years of natural background radiation exposure from the earth and sky.

Due to the exposure to radiation, pregnant or breast feeding women may not participate in the PET scan.

Subjects will be screened for prior research radiation exposure and will be asked to document any prior research radiation exposure within the past year on the consent form. If the subject has participated in research involving radiation exposure, study staff will review to ensure the subject's annual radiation research exposure with participation in this study results in less than 50mSv of radiation exposure, the current MGH RSC radiation exposure limit.

### **VIII. Potential Benefits**

#### **a. Potential benefits to participating individuals**

The individual subject should expect no direct benefits from the research procedures and results.

#### **b. Potential benefits to the society**

Although the individual subject should expect no direct benefits from the research procedures and results, the study will lead to a better understanding of the role of microglia and the noradrenergic system in the disease process of Multiple Sclerosis. The results of the study will also help to understand the underlying biological as well as structural changes associated with MS by assessing the relationship between clinical manifestations and imaging parameters, providing fundamental frameworks for better diagnosis and monitoring of treatment outcome.

### **IX. Monitoring and Quality Assurance**

#### **a. Independent Monitoring of Source Data**

Because no clinical diagnosis will be made as part of this study, no interventions will be used, and procedures have been shown to be safe, an independent data safety monitoring board (DSMB) will not be established. However, [F-18]PBR06 and [C-11]MRB are non-FDA approved radioligands so the study will be overseen by the FDA/IND and Radiation Safety Committee.

#### b. Safety Monitoring

The PI, co-investigators, and study coordinators will continuously provide oversight to ensure that participants are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to highest scientific and ethical standards.

#### c. Outcomes Monitoring

Clinical data are entered into an electronic database, checked against original instruments for inconsistencies, and corrected. PET scans are analyzed regularly to assure proper data acquisition and data quality. All data will be analyzed and assessed by the PI, co-investigators, and associated staff to assure quality and accordance with the IRB-approved protocol.

#### d. Adverse Event Reporting Guidelines

All serious and/or unexpected adverse events will be reported to PHRC in accordance with PHRC guidelines for AE reporting. All other adverse events will be reported in an annual continuing review report. This is not an interventional study, so the clinical care of these subjects will not be altered in any way.

## X. References

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