Effects of ACTHAR on Advanced MRI Surrogate Markers of Disease Activity and on Comprehensive Immune Signature During MS Relapses

IRB16-1390

NCT3021317

Protocol Date 10/23/19

Clinical Protocol

Effects of ACTHAR on advanced MRI surrogate markers of disease activity and on comprehensive immune signature during MS relapses

This investigator-initiated trial (IST) is divided into two complementary parts:

1. Advanced serial MRI studies on patients during and after an acute MS relapse. MRI will be performed at baseline, 1 month after the 1st dose of ACTHAR, and months 3, 6, and 12. ACTHAR will be administered for 10 days. Patients will start ACTHAR within 48 hours of relapse assessment.
2. Serial immune assays on patients during and after an acute MS relapse. Serum and blood samples with be collected at baseline, last day of ACTHAR (day 10 of therapy), 1 month post 1st dose, and months 3 and 6.

ACTHAR will be administered by a self-injectable syringe under the skin fat tissue (subcutaneous injection). The first subcutaneous injection of ACTHAR will be done in the clinic. Subjects will undergo a teaching session with the PI (Dr. Javed) about how to give a subcutaneous injection of ACTHAR. All subsequent injections will be self-administered by the patient.
At each research visit, subjects will have a physical/neurological examination, blood pressure, height, weight, and temperature taken at the time of visits. Urine pregnancy test or urine analysis may be done if there is a clinical concern for pregnancy or urine infection. This will be based on the discretion of the investigators.

Part I. Investigational Plan-MRI

1.1 Overview

Multiple sclerosis (MS) is a demyelinating disease of the CNS. In a vast majority of patients, its clinical course is characterized by transient attacks of acute neurological compromise, followed by variable degree of recovery. Each relapse leaves a patient with some degree of residual disability. Higher number and longer duration of relapses are associated with greater loss of function. Hence, it is imperative that these relapses are optimally treated and curtailed in duration to allow for maximal recovery and repair.

ACTH (ACTHAR or IV formulation) has long been used for the treatment of MS relapses [1, 2]. ACTH has equivalent efficacy to high-dose IV methyprednisolone in curtailing the duration of MS relapses [3, 4]. ACTH has an advantage over steroids in that it has a short half-life and much less deleterious steroidogenic effect on bone and fat metabolism [5]. Importantly, ACTH has a unique mechanism of action on immune and brain cells through melanocortin receptors (MCRs), which promote production of regulatory and anti-inflammatory cytokines and support oligodendrocyte precursors and neuronal function, all of which could lead to better repair of MS lesions and favorable clinical outcome [6].

Several studies have shown beneficial effects of ACTHAR on clinical relapses [7]. However, there is little *if any* knowledge about the benefits of ACTHAR in suppressing inflammatory lesion activity and enhancing remyelination over time as assesed by MRI. Improvement in the inflammatory activity, and particularly repair, in MS lesions leads to

improved clinical outcome and less residual clinical deficit. Often during the MS course, the ratio of new MRI disease activity to clinical relapses can be 10:1. Hence, suppression of this MRI actvity is relavant to clinical outcome.

The studies proposed herein will provide a better understanding of the effects of ACTHAR in improving MRI lesion characteristics over time. The complementary immune and genetic studies will further provide evidence for the mechanism of action (MOA) of ACTHAR in improving immune dysfunction related to MS relapse. This is a one of a kind study, involving both advanced/state-of-the art MRI techniques and immune studies to assess the beneficial effects of ACTHAR in MS relapses in the same patients over time.

1.2 Hypothesis: ACTHAR during acute attacks will reduce inflammation and promote CNS repair as assessed by conventional and advanced MRI techniques and it will shift the inflammatory immune response associated with a MS relapses towards a more regulatory and nurturing immune response.

1.3 MRI Assessments

All images will be performed at the University of Chicago's Magnetic Resonance Imaging Research Center (MRIRC). The MRIRC is fully dedicated to MRI research and located in the Q300 suite in the Department of Radiology of the University of Chicago Medical Center. The MRIRC houses two state of the art MRI scanners and is staffed by 2 full-time MRI technologists, research nurses, and coordinators. Dr. Carroll has over 20 years' experience in neurological imaging and is the Director of Neuroimaging for the MRIRC and will oversee all aspects of imaging.

The primary outcome of all MRI techniques is to determine whether there is an improvement and subsequent stabilization/repair over time of tissue damage caused by inflammatory MS disease activity. Multiple conventional and nonconventional MR imaging modalities are examined here to determine which of these are the most sensitive and reliable in detecting microstructural damage and repair over time. The results of this study will also greatly impact the design of future MS trials by providing a guide for selecting the most appropriate MRI and immune methods to assess treatment efficacy in MS.

Conventional MRI

<u>Quantitative T1 Gd+, T2, T1 chronic, and BPF analysis</u>:

Commonly used MRI metrics in most studies include a post-contrast T1 weighted sequence and a T2 weighted sequence. These are useful for detecting new inflammatory lesions. The contrast-enhancing lesions are transient (less than 2 months on average) and are indicative of active lesions. The T2 lesions are more permanent and are thought to develop after enhancement ceases. Measurement of lesion load in terms of number and volume, both active and inactive (T1 Gd+, T2 chronic lesions, and T1 chronic lesions) will be examined over time.

Clinical Protocol

Specific Aim: determine the effects of ACTHAR in improving the characteristics of contrast enhancing lesions (T1 Gd+) observed at baseline and followed squentially over time to the development of T2 and chronic T1 signal intensities.

Quantitative nonconventional MRIs (see Supplement for protocol details**):**

Quantitative T1/T2: Standard anatomic MRI parameters include T1-weighted 3D and PD/T2 sequences. Only gross lesion characteristics such as number, size, and volume can be assessed using these sequences. However, the actual signal intensities that are correlated with water content/lesion pathology cannot be determined with these routine sequences. We have developed a unique approach to converting standard anatomic images acquired through routine scanning procedures to quantitative values by modeling of signal intensities and physics equations. Hence, standard anatomic images are converted to quantitative values (T1 in milliseconds). The greatest advantage of this technique is that we are able to quantify T1 characteristics of lesions from MRI scans done prior to the study start and compare these values to those MRI scans obtained on study. Hence, T1 lesion characteristics can be assessed months before ACTHAR treatment and thereafter.

Specific Aim: compare the effects of ACTHAR on T1 lesion characteristics over time to the T1 lesion features derived from previous MRI scans without ACTHAR treatment.

Quantitative Perfusion: in active MS lesions, perivascular inflammation and hyalinization of blood vessels is seen. These changes in the blood vessel walls affect tissues hemodynamics. Consistent with this concept, there is reduced cerebral blood flow (CBF) and increased mean transit time (MTT) in white matter lesions compared to normal appearing white matter [8].

Specific Aim: examine the effects of ACTHAR in improving CBF and MTT in active MS lesions.

Quantification of Tissue Permeability (Ktrans): This measure is used to evaluate the integrity of the blood brain barrier (BBB). Active MS lesions are characterized by intact BBB. In active MS lesions, the integrity of the BBB is compromised, leading to the extravasation of immune cells into brain parenchyma. This technique will specifically assess BBB permeability [9].

.

Specific Aim: examine the effects of ACTHAR in improving BBB permeability in active MS lesions.

Oxygen Extraction Fraction: MS relapses are often characterized by excessive fatigue and slow cognitive processing. These symptoms are thought to be due to increased inflammatory activity in the brain during MS relapses. There is accumulating evidence that cortical inflammation contributes to metabolic disturbances in MS and these are related to cognitive decline [10, 11]. PET studies have shown a reduction in cerebral oxygen metabolism in both gray and white matter [10].

Specific aim: Compare OEF in MS patients undergoing a relapse before and after ACTHAR treatment and observe any improvement in OEF indices post-relapse treatment.

Multi-component driven equilibrium single pulse obervation of T1/T2 (mcDESPOT): This is a fairly new technique to assess the water content of myelin. In an active MS lesion, the overall water content of the lesion increases, but the water associated with myelin decreases, implying loss of integrity of myelin. Hence, a decrease in MWF is a marker of myelin damage [12, 13]. MS lesions are associated with a low MWF initially which improves over time as the lesion repairs.

Specific aim: Determine whether ACTHAR treatment is associated with an improvement in MWF of active (Gd+) and inactive T2 lesions over time.

Magnetization transfer imaging (MTI): this imaging modality complements MWF in identifying changes in the microstructure of both white matter and gray matter. MTI improves over time as lesions heal/remyelinate [14, 15, 16]. This technique will be used to assess lesion resolution/repair over time.

Specific Aim: Determine whether ACTHAR treatment is associated with an improvement in MTR of active (Gd+) and inactive T2 lesions over time

Diffusion Tensor Imaging (DTI): This is a technique has an advantage over the MWF imaging and MTR in that it can interrogate both myelin ($\lambda\perp$) and axonal loss (λII) based on transverse and axial diffusivities. There is evidence that alteration in DTI parameters in acute MS lesions have predictive value in terms of long term T1 black hole evolution [17].

Specific aim: Determine whether ACTHAR treatment is associated with an improvement in DTI metrics of active (Gd+) and inactive T2 lesions over time.

Part II. Corresponding immune studies:

2.1 Overview and Rationale

Purpose and Rationale

MS is immune-mediated. The process involves deregulated immune response of T and B lymphocytes, and macrophages. Autoreactive T cells expand, creating a shift in the balance of pro- and anti-inflammatory cytokines that favors inflammation. The antigen triggering MS is unknown.

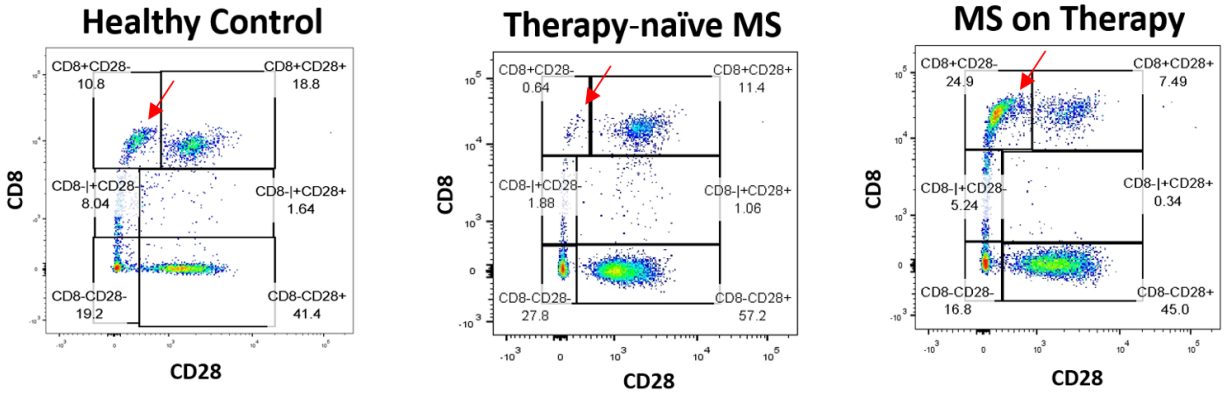
ACTH therapy produces rapid and robust recoveries during exacerbations [18-20]. ACTH induces anti-inflammatory and immunomodulatory functions involving T and B lymphocytes and macrophages; it activates the sympathetic nervous system to reduce inflammation; it reduces pro-inflammatory cytokines through inhibition of NF-κB signaling, and inhibits expression of nitric oxide and adhesion molecules; and it enhances production of anti-inflammatory IL-10 [6, 21].

Clinical Protocol

In MS, there are defects In CD4 and CD8 regulatory cell function. Tregs normally reduce and control the intensity of an immune response [22]. Accumulating evidence indicates an immunosuppressive role for CD4+CD25+Tregs in immune-mediated diseases. Venken and colleagues suggested that the suppressive capacity of these CD4+CD25+Tregs appears to be more affected in the early phases of MS [23], but Antel et al. found that CD8 Tregs were more affected in the progressive phase [24].

There are differences in function that correlate with expression of FOXP3, a master regulator in the development and function of regulatory CD4+CD25+T cells), which are adequate in patients with SPMS but reduced in those with RRMS [23]. More recently, Correale and Villa investigated the role of CD8+CD25+FOXP3 cells in RRMS patients, as the role of CD8+ has received less recent attention than CD4+ cells [22]. CD8+CD25+FOXP3 cells are suppressed to a greater degree in the peripheral blood during MS exacerbations than during remission or in healthy controls. Likewise, in the cerebrospinal fluid (CSF) of MS patients during exacerbations, lower levels of CD8+ CD25+ FOXP3 T cells are detected, providing evidence for a previously unknown CNS mechanism involved in immune regulation in MS [22].

We and others find the regulatory deficit is largely constrained to the CD8 population [22, 25, 26]. CD8 regulatory/suppressor cell function is slightly reduced in stable MS, very low during attacks and progression, and rebounds during recovery [25]. The defect is localized to the CD8+CD28- population [22, 27, 28], a better marker than FOXP3 in this subpopulation [26]. We have found that CD8+CD28-Treg cells are very low in therapy-naive MS compared to healthy controls. Some MS immune modulators can elevate numbers of this subpopulation, and thus enhance anti-inflammatory functions and reduce inflammatory Th1/Th17 functions (Figure 1 below) [29].



Clinical Protocol

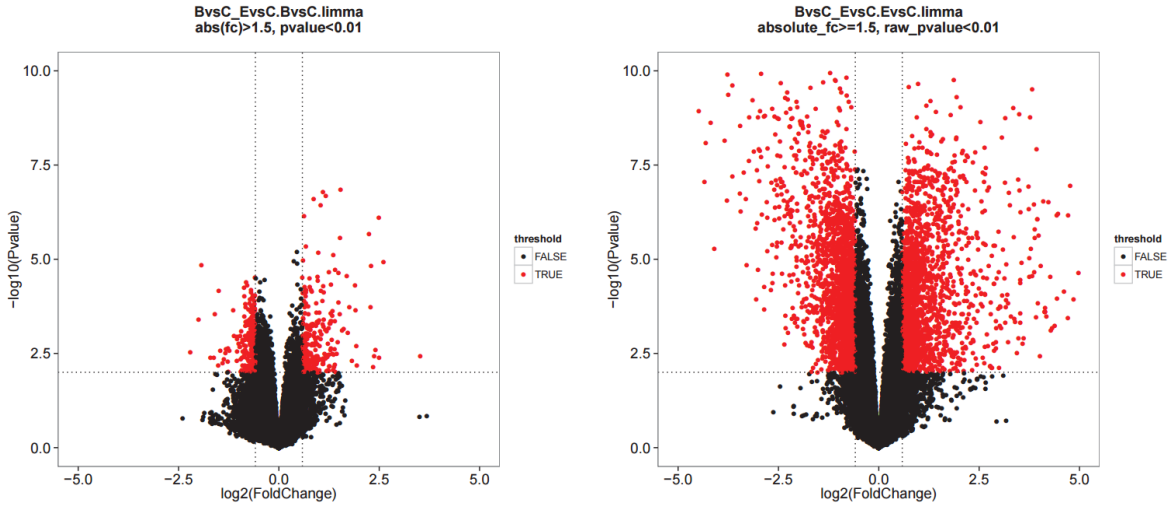
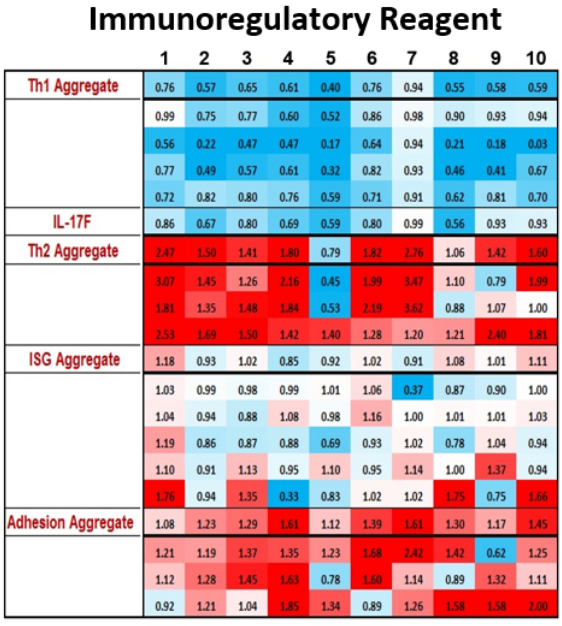


Figure 1. CD8 regulatory cell expression (CD8+CD28- T cells, arrows) is subnormal in MS (second panel) compared to healthy controls (first panel), however this low expression can be up-regulated with immune modulator (third panel).

We also illustrate our capabilities for measuring gene expression (Figure 2) and serum proteins with multiplex assays (Figure 3).



1: HC (n=18)
2: RRMS-s Ø Rx (n=17)
3: RRMS-a Ø Rx (n=15)
4: SPMS Ø Rx (n=13)
5: PPMS Ø Rx (n=15)
6: RRMS-s Drug 1 (n=18)
7: RRMS-a Drug 1 (n=14)
8: RRMS-s Drug 2 (n=12)
9: SPMS Drug 2 (n=10)
10: SPMS Drug 3 (n=8)

Figure 2. Volcano plots of RNA expression in MNC during exacerbations, comparing patients on IFN-b therapy (L) vs. no therapy (R). Patients in left panel had washed out IFN-b therapy for 72 hours, yet still had markedly suppressed RNA expression for 5,000 differentially-expressed genes compared to therapy-naïve exacerbations in right panel. x-axis = fold down (to L) or up (to R); y-axis = significance

Figure 3. Multiplex analysis of proteins expressed by activated MNC. Cells were exposed to media only or to an agent that affects MS exacerbations and progression, then activated for 48 hours. Supernatants were analyzed for Th1, Th17, Th2, IFN-stimulated genes, and adhesion molecules (shown here), in addition to monokines, B cell factors, and neuroprotective proteins (dns). Columns are different patient and control groups, row are heat maps of average expression. Red = increased expression vs. media control; Blue = decreased expression vs. control; hue = statistical significance, with more saturation having lower p values (more zeros).

2.2 The hypothesis is that ACTHAR treatment in MS relapses generates a tolerogenic immune environment that suppresses inflammation and facilitates brain repair. We've found that CD8+CD28- suppressor cells are low in MS and are up-regulated by several classes of immune modulators. The tolerogenic immune environment will be assessed in 18 ACTHAR-treated MS patients.

2.3 Overall objective is to determine Treg expression and neuroprotection signature, as well as identify gene and immune protein signatures in ACTH-treated MS:

2.4 Specific aims and experiment design with serum, MNC, and RNA samples from baseline, last day of ACTHAR (day 10 of therapy), 1 month post 1st dose, and months 3 and 6:

1. **Aim1**. Determine immune subset expression in CD4+FOXP3 Tregs and CD8+CD28- T suppressor cells by flow cytometry at each visit above.
2. **Aim 2**. Determine global gene expression profiling in MNC with 913,000 probes at each visit above. Bioinformatics will include pathway analysis and ACTHAR-induced RNA signature.
3. **Aim 3**. Determine serum protein profiling for immune-regulated cytokines (Th1, Th2, Th17, monokines…) and neuroprotective proteins (NGF, BDNF, ACTH, HGF, CNTF, IL-10…) at each visit above.

4. **Aim 4.** All data from protein and gene expression, as well as immune subset expression will be compared to our database generated from therapy-naïve stable and exacerbating MS. ACTHAR signature will analyzed based on these comparsions.

2.5 Methods

Blood is drawn from 18 MS patients at baseline, last day of ACTHAR (day 10 of therapy), 1 month post 1st dose, and months 3 and 6. Mononuclear cells (MNCs) from four 8cc heparin green-top tubes (40-60 million cells) are separated on Ficoll-Paque gradients. 30M cells are needed for flow cytometry. 20M cells are needed for total RNA preparation.

Specific Aim 1. Determination of ACTHAR effects on immune cell subsets in relapsing remitting MS (RRMS), emphasizing expression on CD8 cells including the CD8+CD28- regulatory subset. Flow cytometry will be conducted to determine the effect of ACTHAR on in vivo expression of surface markers on T cells (CD4, CD8, CD28, FOXP3). Reagents are from e-Biosciences and B-D. (MAbs = CD4, CD8, CD28, CD27, CD25, FOXP3).

Specific Aim 2. Global gene expression profiling in MNC will be measured with 913,000 probes at each sample. Bioinformatics will include pathway analysis and ACTHAR-induced RNA signature. Total RNA will be isolated from 20M cells, as the template for synthesizing cDNA for gene expression array analysis with Affy HTA kit. RT-PCR will be performed for validation of specific gene signatures.

Specific Aim 3. Serum from each sample will be applied on Procarta multiplex assay plates according to manufacturer's instruction. Immune, neuroprotection, and other proteins will be measured with 40 highly-selected targeted antibodies for Th1, Th2, Th17, monocyte, neuroprotection, cell proliferation and death, and adhesion molecule panels.

Specific Aim 4. Advanced bioinformatics with Gene Set Variation Analysis:
1. Identify differential gene expression (DEG) between groups;
2. Analyze pathways by Ingenuity Pathway Analysis (IPA);
3. Discover transcriptional signature of genes and gene pathways;
4. Build a predictive model using machine learning algorithms including Random Forests (RFs) and Support Vector Machines (SVMs).

2.6 Outcomes

This data may lead to better understanding of the mechanism of action of ACTHAR and identifying signatures/biomarkers in ACTHAR-treated MS. In addition, MRI, CD8+CD28- cell numbers, serum proteins, and RNA may be biomarkers for efficacy.

Part III. Patient criteria and publications

3.1a Inclusion Criteria

This study will be restricted to adult patients (>18 years) undergoing a clinical relapse who also must have a contrast-enhancing CNS lesion, either clinically or non-clinically relevant. Sample size under consideration is 18 patients. The population on which analyses will be conducted consists of all patients who have received at least 1 dose of study medication and for whom at least two time-points (baseline and at least one additional time-point) for MRI data are available (Intent-to-treat analysis).

3.1b Exclusion Criteria

1-Patients will be excluded if they only have a clinical relapse which is not associated with MRI findings of a new or enlarging MS lesion.

2-Patient will be excluded if they cannot give written consent

3-Patients will be excluded if they have an active infection

4-Patients will be excluded if they already received steroids (IV or oral) within last 30 days of receiving ACTHAR

5-Pregnant or lactating patients will be excluded from this study.

3.2 Treatment failures and/or inadequate response

Patients who have not had an adequate response to the ACTH treatment after 14 days may either be given a choice of getting another round of ACTH for additional 10 days or switching to IV Methylprednisolone 1000 mg for 3 days. The scheduling of blood draws and MRIs will not be affected and continue as planed.

3.3 Concomitant disease modifying therapies (DMTs)

Patients undergoing relapses will continue their current DMTs during the relapse. One month after the relapse (after the 1 month MRI time point and blood draw), physician and patient will decide whether to continue their current DMT over time or switch to another DMT. All DMTs and other symptomatic treatments will be recorded during the course of the study.

3.4 Statistical Analysis

All data collected will be documented using summary tables, figures, and patient data listings. For longitudinal data, mixed effects regression model will be used to summarize patterns over the one-year period. All hypothesis testing will be at the 0.05 level of significance. Correlation analyses between specific measures will be accomplished using Pearson's correlation co-efficient.

All summary statistics will be displayed as a function of study visit or assessment time. Summary statistics for continuous variables will include n, mean, standard deviation, minimum, median, and maximum. For categorical variables, frequencies and percentages will be presented. Graphical displays will be provided as appropriate.

3.5 Criteria for study withdrawal

Patients may withdraw from the study at any time for any reason. A patient's withdrawal from the main trial does not, by itself, constitute withdrawal of the data already collected. All data will be analyzed as intent-to-treat. No replacement for patients who withdraw from the study after drug treatment is planned.

3.6 Safety instructions and emergency medical contacts

All study related adverse events (AEs) and serious adverse events (SAEs) will be collected. The medical treatment for relapses is given as part of standard medical care. Hence, all AE and SAEs will be handled per standard medical practices. The emergency medical contacts will be provided to all patients.

Risks associated with the study:

The potential risks of drawing blood include a bruise at the site of the puncture. This happens rarely and inflammation of the vein and infection have been reported. Care will be taken to avoid these complications.

The research-related MRI scans are not being done for diagnostic purposes. If any abnormalities are noted during the research scans, the treating physician will be informed.

Potential risks for ACTH are listed in the package insert. Patients will receive advice on the side effects as it is done in clinical practice. The most common side effects are water gain, injection site reaction, mild insomnia, and flushing. These will be managed as per standard medical care.

The risks associated with MRI are typically claustrophobia and risks associated with contrast agents. Patient's may be given some mild sedative before the MRI. Patient's who have had prior allergic reaction to contrast agents will be excluded. Also, pre-contrast renal function will be assessed prior to the contrast MRI. Hydration will be recommended after the MRI.

3.7 Data collection and management

MRI data for this study will be recorded via MRI machine and stored and disseminated to PIs or Sub-PIs by the Human Imaging Research Office (HIRO) at the University of Chicago. Data will be backed up and stored on-site. Accurate and reliable data collection will be assured by verification and cross–check of the MRI database against the Investigator's records. Immune data will be stored on password protected computers, with access to only the research team. These data will also be backed up routinely.

3.8 Ethical considerations

The Investigators will ensure that this study is conducted in full conformance with the principles of the “Declaration of Helsinki” (last amendment October 2000, with additional footnotes added 2002 and 2004).

All patients will sign the informed consent form for the study. The Consent Forms will be signed and dated by the patient. The clinical records for each patient will document the informed consent process and that written informed consent was obtained prior to participation in the study. The Consent Forms will be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. Patients will be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB policy) during their participation in the study. For any updated or revised Consent Forms, the clinical records for each patient will document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study. A copy of each signed Consent Form will be provided to the patient. All signed and dated Consent Forms will remain in each patient’s study file and be available for verification by study monitors at any time.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.

3.9 Confidentiality

The Investigator and personnel will ensure that patients’ anonymity will be maintained and that their identities are protected from unauthorized parties. The Investigator and personnel should keep a patient enrollment log showing codes, names and addresses. The data are kept in password protected computers. For transmission of data, only patient codes are transmitted, not any PHI type of information. The computers and database are all kept under locked and secure rooms. See below record keeping section.

3.10 Record keeping

All data stored electronically will be coded. Data will be stored within a firewall and password protected computer system within a secured building. Minimal, non-identifiable, subject/patient information (ie, subject/patient code, sex, age at time of scan, clinical condition) will be stored, with password protection, with the data.

3.11 Publication

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to Mallinckrodt prior to submission. This allows the Sponsor to protect proprietary information and to

provide comments based on information from other studies that may not yet be available to the Investigator.

Supplement: MRI Protocols

Quantitative T1/T2: We have developed a unique approach to converting standard anatomic images to quantitative values through the use of modeling of signal intensities and physics equations that define signal intensities from specific pulse sequences. We are able to standardize the T1 (in milliseconds), and then standardize the standard anatomic images that are normally used for diagnosis. In other words, we do not compromise scan time, image resolution, or patient throughput.

Standard anatomic MRI parameters include T1-weighted 3D (repetition time/echo time (TR/TE): 9.5 ms/2.3 ms, resolution: 0.63 × 0.63 × 1.2mm^3) and PD/T2 (TR/TE: 2500ms/10.7ms, resolution: 0.63×0.63×1.2mm^3), and will eaqui5ee before and 15 minutes post contrast. Image will be converted to T1 by calculating an MRI response function, by constraining unknowns using internal reference T1/T2 values (Normal Appearing White matter, Gray matter CSF).

Quantitative Perfusion: We have developed an MRI pulse sequence that calibrates dynamic susceptibility images to provide images of tissue perfusion in ml/100gg/min. Our pulse sequence is patented and Image parameters (FOV/matrix = 220 mm/128, 3.0 mm. 15 thick 2D slices TR/TE=1200 ms/50 ms. Fifty measurements (including calibration data) will be acquired in 3 minutes. Test-retest reliability of cerebral perfusion has been measured to be better than 14%.

Quantification of Tissue Permeability (Ktrans): A saturation recovery gradient recalled sequence will be used both for dynamic imaging of contrast agent leakage. Each slice will be acquired after application of a nonselective saturation prepulse with a saturation time delay (TD). The following acquisition parameters will be used: flip angle of 30°, TR/TE =3.82 ms/1.9 ms, centric phase ordering, scan matrix size 96 × 61 (interpolated to 256 × 256), SENSE factor 2, field of view 240 × 182 mm^2, and four slices of 8-mm thickness, resulting in a spatial resolution of 2.5 × 3.1 × 8 mm^3. The T_1 measurement was performed by varying the TD value (120 ms, 150 ms, 300 ms, 600 ms, 1 sec, 2 sec, 4 sec, 8 sec, 9 sec, 10 sec). Perfusion CBF can estimated by model-free deconvolution both for ROIs and CBF maps, blood volume Vb and the BBB permeability $Ktrans$ will estimated both by Patlak's method and the two-compartment model.

Clinical Protocol

Oxygen Extraction Fraction: As an exploratory study, we will track changes in tissues oxygenation using a novel single shot image acquisition based on a rosette k-space sampling trajectory [30]. A Stack of 2D slices (3 mm thick, FOV 220/matrix 65) will be acquired after a single rf pulse. A maximum-likelihood recursion will yield image for R2*(ms), Mo and local frequency shift in tissue. dw(Hz). Frequency shifts can be converted directly to Oxygen Extraction Fraction through the simple algebraic expression:

$$\delta\omega = \gamma \times \frac{4}{3} \times \pi \times \Delta\chi_0 \times \mathrm{Hct} \times \mathrm{OEF} \times B_0$$

Multi-component driven equilibrium single pulse obervation of T1/T2 (mcDESPOT): This is a fairly new technique to assess the water content of myelin. In an active MS lesion, the overall water content of the lesion increases but the water associated with myelin decreases. The water pool associated with myelin has a very short T2 relaxation time compared to the other pool of intra- and extra-cellular water. Spoiled gradient echo (SPGR) and balanced steady-state free precession (bSSFP) scans are collected over a range of flip angles (FA) at constant repetition times to allow resolution of the two-pools of water, as has been previously reported [12,13]. The fast relaxing pool of water has been called the myelin fraction water (MWF), which has been shown to correlate with tissue myelin fraction [31].
Whole brain isotropic voxel size mcDESPOT imaging will be acquired using the following imaging parameters: FOV = 22 cm, matrix = 128 × 128, slice thickness = 2 mm; SPGR parameters: echo time/repetition time (TE/TR) = 2.1/6.7 ms, flip angle (α)=[3,4, 5, 6, 7, 8, 11, 13, 18]°; bSSFP parameters: TE/TR = 1.8/3.6 ms, α= [11, 14, 20, 24, 28, 34, 41, 51, 67]°, two phase cycles acquired per bSSFP flip angle. The total mcDESPOT acquisition time is ~13 min [13].

Magnetization transfer imaging (MTI): this imaging technique interrogates the transfer of magnetization (MT) from protons bound to macromolecules to more mobile water associated protons. This MT effect is quantified by acquiring two images, one with and the other without a resonance pulse. MTI will be performed using 2D gradient echo (GE) sequence (TR=640, TE=12, FA 20°) with and without an off resonance radiofrequency (RF) saturation pulse (off frequency=1.5 kHz, Gaussian envelope duration 7.68ms, FA=500°).

$MTR=(M_{off} - M_{on})/M_{off}$.

MTR of the active and inactive lesions as well as normal appearing white and gray matter will be determined. MTR of the lesions will be determined by the region of interest (ROI) analysis.

Diffusion Tensor Imaging (DTI): images will be acquired using single shot spin-echo planar pulse sequence TR= 7658 ms, TE= 70 ms, matrix size = 128 x 128, FOV = 224 x 224 with diffusion gradients (b-value =800 s/mm^2) applied in 32 noncollinear directions. Fractional Anisotropy, mean diffusivity, radial ($\lambda\perp$) and axial (λII) diffusivities of active and chronic lesions will be determined by ROI analysis.

References:

1. Miller H, Newell DJ, Ridley A. Multiple sclerosis. Treatment of acute exacerbations with corticotrophin (A.C.T.H.). Lancet 1961;2:1120-1122.
2. Rose AS, Kuzma JW, Kurtzke JF, Sibley WA, Tourtellotte WW. Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH vs placebo in acute exacerbation. Transactions of the American Neurological Association 1969;94:126-133.
3. Abbruzzese G, Gandolfo C, Loeb C. "Bolus" methylprednisolone versus ACTH in the treatment of multiple sclerosis. Italian journal of neurological sciences 1983;4:169-172.
4. Thompson AJ, Kennard C, Swash M, et al. Relative efficacy of intravenous methylprednisolone and ACTH in the treatment of acute relapse in MS. Neurology 1989;39:969-971.
5. Zaidi M, Sun L, Robinson LJ, et al. ACTH protects against glucocorticoid-induced osteonecrosis of bone. Proceedings of the National Academy of Sciences of the United States of America 2010;107:8782-8787.
6. Arnason BG, Berkovich R, Catania A, Lisak RP, Zaidi M. Mechanisms of action of adrenocorticotropic hormone and other melanocortins relevant to the clinical management of patients with multiple sclerosis. Multiple sclerosis 2013;19:130-136.
7. Berkovich R. Treatment of acute relapses in multiple sclerosis. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 2013;10:97-105.
8. Sowa P, Bjornerud A, Nygaard GO, et al. Reduced perfusion in white matter lesions in multiple sclerosis. European journal of radiology 2015;84:2605-2612.
9. Gaitan MI, Shea CD, Evangelou IE, et al. Evolution of the blood-brain barrier in newly forming multiple sclerosis lesions. Annals of neurology 2011;70:22-29.
10. Brooks DJ, Leenders KL, Head G, Marshall J, Legg NJ, Jones T. Studies on regional cerebral oxygen utilisation and cognitive function in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry 1984;47:1182-1191.
11. Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T. Cortical lesions in multiple sclerosis. Brain : a journal of neurology 1999;122 (Pt 1):17-26.
12. Deoni SC, Rutt BK, Arun T, Pierpaoli C, Jones DK. Gleaning multicomponent T1 and T2 information from steady-state imaging data. Magnetic resonance in medicine 2008;60:1372-1387.
13. Kitzler HH, Su J, Zeineh M, et al. Deficient MWF mapping in multiple sclerosis using 3D whole-brain multi-component relaxation MRI. NeuroImage 2012;59:2670-2677.
14. van Waesberghe JH, van Walderveen MA, Castelijns JA, et al. Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization transfer MR. AJNR American journal of neuroradiology 1998;19:675-683.
15. Filippi M, Rocca MA, Horsfield MA, Comi G. A one year study of new lesions in multiple sclerosis using monthly gadolinium enhanced MRI: correlations with changes of T2 and magnetization transfer lesion loads. J Neurol Sci 1998;158:203-208.
16. Filippi M, Rocca MA, Rizzo G, et al. Magnetization transfer ratios in multiple sclerosis lesions enhancing after different doses of gadolinium. Neurology 1998;50:1289-1293.
17. Naismith RT, Xu J, Tutlam NT, et al. Increased diffusivity in acute multiple sclerosis lesions predicts risk of black hole. Neurology 2010;74:1694-1701.

18. Rose AS, Kuzma JW, Kurtzke JF, et al. Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH vs. placebo: final report. Neurology 1970;20:1-70.
19. Thompson CB, Lindsten T, Ledbetter JA, et al. CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. Proceedings of the National Academy of Sciences USA 1989;86:1333-1337.
20. Sellebjerg F, Jensen J, Ryder LP. Costimulatory CD80 (B7-1) and CD86 (B7-2) on cerebrospinal fluid cells in multiple sclerosis. Journal of neuroimmunology 1998;84:179-187.
21. Feng X, Yau D, Holbrook C, Reder AT. Type I interferons inhibit Interleukin-10 production in activated human monocytes and stimulate IL-10 in T cells: Implications for Th1-mediated diseases. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 2002;22:311-319.
22. Correale J, Villa A. Role of CD8+ CD25+ Foxp3+ regulatory T cells in multiple sclerosis. Annals of neurology 2010;67:625-638.
23. Venken K, Hellings N, Broekmans T, Hensen K, Rummens J-L, Stinissen P. Natural naive $CD4^{+}CD25^{+}CD127^{low}$ regulatory T cell (Treg) development and function are disturbed in multiple sclerosis patients: Recovery of memory Treg homeostasis during disease progression. J Immunol 2008;180:6411-6420.
24. Antel JP, Bania MB, Reder AT, Cashman N. Activated suppressor cell dysfunction in chronic progressive multiple sclerosis. Journal of Immunology 1986;137:137-141.
25. Antel JP, Arnason BGW, Medof ME. Suppressor cell function in multiple sclerosis: correlation with clinical disease activity. Annals of neurology 1979;5:338-342.
26. Karandikar NJ, Crawford MP, Yan X, et al. Glatiramer acetate (Copaxone) therapy induces $CD8^{+}$ T cell responses in patients with multiple sclerosis. The Journal of clinical investigation 2002;109:641-649.
27. Karaszewski JW, Reder AT, Anlar B, Kim WC, Arnason BGW. Increased lymphocyte beta-adrenergic receptor density in progressive multiple sclerosis is specific for the CD8+, CD28- suppressor cell. Annals of neurology 1991;30:42-47.
28. Crucian B, Dunne P, Friedman H, Ragsdale R, Pross S, Widen R. Alterations in levels of CD28-/CD8+ suppressor cell precursor and CD45RO+/CD4+ memory T lymphocytes in the peripheral blood of multiple sclerosis patients. Clin Diagn Lab Immunol 1995;2:249-252.
29. AT R, Wang z, Causevic S, et al. Vitamin D enhances interferon-beta-1b response in multiple sclerosis. 31st ECTRIMS/ACTRIMS. Barcelona, Spain2015: P533.
30. Menon RG, Walsh EG, Twieg DB, et al. Snapshot MR technique to measure OEF using rapid frequency mapping. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2014;34:1111-1116.
31. Laule C, Leung E, Lis DK, et al. Myelin water imaging in multiple sclerosis: quantitative correlations with histopathology. Multiple sclerosis 2006;12:747-753.