Natalizumab and Chronic Inflammation

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Background

- *The Importance of the Problem to be Addressed*

Multiple sclerosis (MS) is a chronic inflammatory-neurodegenerative disease of the central nervous system (CNS), affecting about 900,000 patients in the United States [1]. The disease starts with a relapsing remitting (RR) course followed by a secondary progressive phase in about 70% of patients. About 10% of MS patients have a primary progressive course. RRMS is characterized by the periodic occurrence of neurological symptoms or clinical flares [2]. Disease flare ups are due to the presence of inflammatory lesions sustained by the breakdown of the blood brain barrier (BBB) [3] and associated with variable degrees of demyelination and axonal injury. Upon the resolution of the BBB, some of these lesions undergo repair, whilst many persist as focal areas of demyelination and axonal damage, potentially leaving permanent functional impairment [4].

As the disease advances (or by the time of its onset in primary progressive MS patients), the inflammatory events no longer occur but patients progress into a relentless neurological decline. This decline is thought to be sustained by chronic demyelination which in its turn fosters neurodegeneration. Axons cannot survive chronic demyelination which ultimately leads them to death. The mechanisms inducing and sustaining chronic demyelination are largely known. Studies from histopathology indicate that *there is always chronic inflammation where chronic demyelination occurs* [5]. This chronic inflammation hinders repair and is trapped within what appears to be a subtly impaired BBB. Subtle changes in BBB permeability have been identified both postmortem [6] and in vivo [7] and, when present, predict future disease activity and progression [8,9].

Magnetic resonance imaging (MRI) assists with the diagnosis and monitoring of MS patients. Active lesions are visible on Tl-weighted (Tl-w) post-gadolinium MRI as hyper-intense contrast-enhancing lesions (CELs). The identification of CELs has revolutionized the diagnosis and treatment of MS [10]. A multitude of clinical trials evaluating drugs' efficacy in reducing CELs are continuously conducted and have led to the armamentarium of disease modifying agents (DMAs) available to treat patients with RRMS today [11]. Conversely, although established MRI surrogates of subtle BBB breakdown and demyelination are available, these are not routinely used as measures of outcome both in clinical trials and practice.

Incorporating measures of BBB and myelin integrity in clinical studies will reduce uncertainty on the role of available medications as neuroprotective agents and disease control, beyond the resolution of overt inflammation.

- *Our Proposed Solution: Studying the Effect of Natalizumab on Chronic Inflammation and Demyelination*

The 21st century is facing the presence of a multitude of DMAs to treat patients with MS [11]. While each of these DMAs is sustained by different mechanisms of actions, all act in preventing overt BBB breakdown and inflammation. None of these DMAs acts upon the actual resolution of the BBB.

Thus, after the resolution of CEL, subtle leakages of the BBB may persist and continue to favor T-lymphocytes trafficking through the CNS. This trafficking may foster a state of chronic inflammation ultimately responsible of chronic demyelination.

Natalizumab (Tysabri®) is a humanized monoclonal antibody against the a4 chain of integrins and acts as a selective adhesion molecule antagonist [12]. Opposite to other DMAs, due to its mechanisms of action, natalizumab is the only medication directly acting upon the BBB. By binding VLA-4 and inhibiting the translocation of activated VLA-4-expressing leukocytes across the BBB into the CNS, Natalizumab is the only DMA that can completely abolish the lymphocytic permeation.

In the long term, this effect shall favor the resolution of chronic inflammation with the full restoration of the BBB integrity, a potential foster of remyelination and better quality of life (QoL), and certainly an obstacle to chronic demyelination. *Thus, studying the effect of Natalizumab on BBB permeability and demyelination has the potential to unveil neuroprotective properties of this medication, thus far uncovered.*

Rationale and Specific Aims

This is an open-label, prospective study. Natalizumab will be dosed according to the FDA approved label. We propose to apply dynamic contrast enhanced (DCE) and selective inversion recovery quantitative magnetization transfer imaging (SIR-qMT) in a cohort of patients with RRMS to assess the effect of Natalizumab on BBB and myelin integrity; and on QoL as measured using the electronic visual analog scales (EVAS). If the electronic version of the VAS is not available, paper copy will be provided to the participant.

The **short-term goal** of our study is to demonstrate the ability of Natalizumab to decrease lesional and non lesional DCE-derived *ktrans* and preserve stable SIR-qMT derived macromolecular to free pool size ratio (*PSR*) values over a time period of 12 months.

The **long-term goal** of our line of inquiry is to discover hidden mechanisms of actions and neuroprotective properties of currently available DMA.

The **central hypothesis** of our study is that Natalizumab fosters the restoration of the BBB integrity as early as three months after the first infusion and that this restoration ultimately 1) preserves myelin integrity and 2) favors improvement of QoL. We plan to prove our hypothesis through the following complementary aims.

- *Aim 1: To establish that DCE metric ktrans decreases with treatment.*

We hypothesize that *ktrans* differs between baseline (pre- treatment) and both 3 and 12 months' post-treatment as a result of better BBB restoration operated by natalizumab.

- *Aim 2: To establish that qMTI metric PSR increases with treatment.*

We hypothesize that upon a significant early (3 months post treatment) increase in PSR due to decrease in BBB permeability and subtle leakages operated by natalizumab, PSR will maintain stable levels over time secondary to myelin integrity preservation.

- *Aim 3: To explore changes in quality of life (QoL) operated by natalizumab***.**

We hypothesize that natalizumab improves QoL as measured using the electronic visual analog scales (EVAS) or VAS, via the restoration of the BBB and that such an effect

correlates with drug serum level.

- *Additional Secondary Explorative Endpoints:*

1. Associations between QoL measures and *ktrans* values.
2. Associations between *ktrans* and *PSR* values

Animal Studies and Previous Human Studies

In recent years, the use of DCE MRJ has emerged as a tool for probing BBB integrity in vivo [7-9]. DCE estimates hemodynamic parameters (e.g., cerebral blood flow, cerebral blood volume, and mean transit time) during the passage of the paramagnetic contrast agent gadolinium. In addition, it assesses vascular integrity by acquiring high spatial resolution Tl-w images before and after gadolinium injection. For intact BBB, gadolinium is too large to move into the tissue. However, when the BBB is compromised, the contrast agent will move into the tissue, shorten the surrounding tissue water Tl and increase the MRJ signal on a Tl-w scan. In addition to providing a qualitative description of the spatial location of BBB breakdown, it is possible to quantify kinetic parameters, such as *Ktrans* (ml/l00g/min). *Ktrans* provides a measure of the efflux rate of the gadolinium contrast from blood plasma into tissue and is an of subtle changes in the BBB [8,9].

Our work and that of others [14-18] proved that indices derived from qMT are solid predictors of myelin contents in brains of MS patients. By employing a two-pool model of magnetization transfer exchange between bound macromolecules (like myelin) and unbound pools (free water), qMT quantifies characteristics of single pools. This feature distinguishes it from the commonly used magnetization transfer ratio in that qMT is more specific to myelin content and less sensitive to non-biological scanner-related factors, which are not possible to be separated from edema using the standard magnetization transfer ratio measurements. We optimized a SIR-qMT protocol at 16 inversion times both a 3.0 Tesla (3T) [17,18] and 7T [14,15].

SIR-qMT is based on a biexponential model of inversion recovery data and its derived metrics are not influenced by the assumption of the macromolecular pool line shape such as in other quantitative magnetization transfer methods. The derived measure relevant to the studies I propose include *PSR*, reflective of the (%) quantity of pool of free molecules not bound to macromolecules. Upon validation of the sequence with bovine serum albumin phantoms [19] and animals with the experiential model of MS [20], we proved that SIR-qMT generates reproducible results between scans and that regional differences in PSR and RI fare also in accordance with the well-known biological differences in myelin content between white matter and grev matter tissues in both healthy subjects and MS patients as well as between lesional and non-lesional tissue in patients [17, 18].

Inclusion/Exclusion Criteria

- *Inclusion Criteria:*

1. Adult (age 18+) RRMS per McDonald Criteria (Attachment-1)
2. Eligible to receive Natalizumab treatment
3. Creatinine levels within normal range within 42 days (6 weeks) of MRI

- *Exclusion Criteria:*

1. Pregnancy or breastfeeding
2. Known sensitivity/allergy to MRI contrast
3. Steroid treatment (oral or intravenous) within past 30 days

Enrollment/Randomization

Enrollment will occur at the Vanderbilt Comprehensive MS Center; no specific ethnic groups will be targeted or excluded from the study. Thirty-six (36) patients will be identified and enrolled by the study team. This studywill not require randomization. This study will receive review and approval from the Vanderbilt Human Research Protection Program (HRPP) prior to enrollment of any participants.

There will be recruitment advertisements (Attachment-2A,2B) posted or provided in the VUMC MS clinic, where potential recruitment can happen. Patients visiting the VUMC hospital or MS clinic will be informed of the study by their hospital or clinic personnel, inclusive of nurses and physicians, or can inquire based on the presence of the flyer. If interested, patients will be notified by the research team for screening and subsequent informed consent. By nature, the subject demographic will mimic that of the pathologies examined found in the MidSouth region.

Additionally, potential research subjects will be identified based on data available in the VUMC clinical system (e.g. Epic/eStar). Based on the list of upcoming appointments in the relevant clinics, patients' Epic record will be reviewed for the specified inclusion/exclusion criteria. If the patient is deemed a candidate for the study, the study personnel will notify the patient's care provider who will then ask the patient if they would be interested in communicating with study personnel about the research study.

Subjects interested in participating in clinical research will be screened using the MRI screening form (Attachment-3). Informed consent will be obtained in a private setting (e.g., an otherwise empty conference room or waiting area) by one of the study Investigators.

Each subject will also be able to complete the MRI screening form online, from her/his own home using the REDCAP software at the subjects' computer. All data transmitted within REDCAP is secure with password-protected access. Prior to the study start, any of the associate investigators will take care of explaining the details of the study to the subject. The subject will receive a copy of the signed informed consent document. The study PI will be available to meet and/or talk in person with any of the subject shall any questions arise.

The patient will be made aware that if they choose not to participate in the study, their care will not be altered in any way. It is possible that the person obtaining consent will have an existing relationship with the participant (e.g., he or she may be a colleague or coworker). Only peers or persons who do not work directly with a potential participant will obtain consent. The treating neurologist may also obtain consent. The potential participant will be told that agreement or refusal to participate will in no way affect his/her job or academic standing.

Study Procedures

Procedures	*Baseline (M0)*	*Month 3 (M3)*	*Month 12 (M12)*
Obtain Informed Consent	x		
Review of McDonald Criteria for RRMS	x		
Concomitant Medication Review	x	x	x
Inclusion/Exclusion Review	x		
Adverse Event/SAE Review specific to MRI	x	x	x
QoL measurement with EVAS/Visual Analog Scale	x	x	x
Pre-infusion Blood draw for future analyses	x	x	x
Creatinine level present on chart prior to MRI	x	x	x
Urine pregnancy test prior to MRI[1]	x	x	x
MRI with/without contrast[2]	x	x	x
Standard clinical assessments	x	x	x

1. For females of child-bearing potential
2. MRI at M0 should occur immediately prior to start of 1st dose of Natalizumab. M3 and M12 MRIs should occur on same date ± 5 days as Natalizumab dose

- *Baseline:*

The baseline visit will last approximately 4 hours. This will include the following tests/procedures:

1. Review, sign Informed Consent
2. Inclusion/Exclusion Review
3. Medical History, including MS history
4. Concomitant medication review, including history of steroids within past 30 days
5. Urine pregnancy test for females of child-bearing potential
6. Creatinine level results on chart within past 42 days and within normal limits
7. MRI with and without contrast (to occur prior to first dose of Natalizumab)
8. Standard of care clinical assessments
9. Blood sample obtained when IV line is placed for contrast MRI

- *Month 3 (M3) and Month 12 (M12):*

M3 and M12 visits will last approximately 4 hours each. The visit will include the following tests/procedures:

1. Concomitant medication review
2. Urine pregnancy test for females of child-bearing potential
3. Confirm creatinine level results on chart within past 42 days
4. MRI with and without contrast immediately prior to, or following, infusion
5. Standard of care clinical assessments
6. Adverse event review for MRI only
7. QoL measurement utilizing the EVAS or VAS
8. Blood sample obtained when IV line is placed for contrast MRI

- *MRI*

MRI will be obtained at the Vanderbilt University Institute of Imaging Science. Participants will have IV inserted at the Vanderbilt MS Clinic prior to MRI. The participant will be accompanied to MRI facility by MS Research staff member.

- *Visual Analog Scale*

Assessment of quality of life will be completed at each time pint. The scale will be administered electronically (I-Pad) in the clinic by the research staff.

- *Routine Laboratory Assessments*

Urine pregnancy test will be performed on all females of child-bearing potential. This test will be paid for by research.
Serum Creatinine results will be on file within 42 days of MRI. Serum creatinine will be obtained, and results reviewed prior to MRI.
Blood Draw will occur at each time point when IV line is placed for contrast MRI. The research team will process and freeze specimens. Specimens will not be sent to the sponsor at this time.

- *Adverse Events*

Adverse events related to MRI procedure will be collected and reported. The Investigator will review all MRI-associated adverse events.

Risks

Potential risks related to MRI include:

1. No major risks are associated with MRI
2. Certain metal objects like watches, credit cards, hairpins, writing pens, etc. may be damaged by the machine or may be pulled away from the body during the scan.
3. Hammering and clicking noise will occur throughout the scan
4. Claustrophobia may occur
5. Allergic reaction to the IV dye may occur
6. Placing needle in arm for dye may cause pain and bruising

Reporting of Adverse Events or Unanticipated Problems Involving Risk to Participants or Others

Adverse events related to MRI will be collected on an ongoing basis. Adverse events will be reported promptly to the investigator for review and causality. Serious adverse events will be reported to Vanderbilt Human Research Protections Program within 24 hours of notification to site. Participants safety will be reviewed on an ongoing basis.
Non-serious adverse events will be reported to HRPP during annual review.

Study Withdrawal/Discontinuation

Participants may withdraw from the study at any time.
Participants may be withdrawn from the study for the following reasons:

1. Study discontinued by PI
2. Pregnancy
3. Discontinuation of Natalizumab
4. Lost to follow-up

Statistical Considerations

- *Power Analysis*

The sample size is enumerated on the basis of aim 1. Cramer and co-authors observed that the minimally detectable difference in DCE-derived metric k-trans was between NAWM of patients and that of healthy controls [7].
On the basis of their study number, and assuming that differences in normal appearing white matter BBB permeability between baseline and M12 will be of the same magnitude of that observed between patients and healthy controls in Cramer's study, a cohort of 30 patients will be sufficient to detect such a difference (i.e. 0.05) with a power of 80% and a Type I Error alpha of 5%. A total of 36 subjects will be screened/enrolled for an anticipated dropout rate of 15%.

- *Statistical Plan*

Baseline clinical, demographical and MRI patients' characteristics will be reported as mean±standard deviation and median (range) for continuous variables, and as frequency and percentage for categorical variables.
Multivariable analyses will be used to compare group differences in absolute values and over-time changes of *Ktrans* and *PSR*.

Ktrans values collected during the follow-up (i.e. M0, M3 and M12) will be analyzed using a longitudinal linear model, adjusted for potential confounders, using a spatial power covariance matrix to account for unequally spaced time visits. Within this model, using appropriate contrasts, we will assess:

1. The mean change over the whole follow-up time of *Ktrans*
2. The mean change between M0 and M3
3. The mean change between M0 and M12

The same statistical framework will be used to assed temporal change of *PSR* and QoL measurements.

Privacy/Confidentiality Issues

Participants will be enrolled and assigned a unique ID number, along with initials. Blood samples will be stored in -20 freezer at the Vanderbilt Comprehensive MS Center for future analyses of immune correlates and Natalizumab serum levels. The specimens will be de-identified prior to storage. Only the research team will have the codes to identify

participants.

Follow-up and Record Retention

Records and documents pertaining to the conduct of this study will be retained by the Principal Investigator for 5 years after completion of study.

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List of Attachments

McDonald criteria_2017	Attachment-1
Study Advertisement Flyer	Attachment-2A-B
MRI Screening Form	Attachment-3