Cladribine tablets after treatment with natalizumab (CLADRINA) – rationale and design

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Abstract

Background: Individual disease modifying therapies approved for multiple sclerosis (MS) have limited effectiveness and potentially serious side effects, especially when administered over long periods. Sequential combination therapy is a plausible alternative approach. Natalizumab is a monoclonal therapeutic antibody that reduces leukocyte access to the central nervous system that is associated with an increased risk of progressive multifocal leukoencephalopathy and disease reactivation after its discontinuation. Cladribine tablets act as a synthetic adenosine analog, disrupting DNA synthesis and repair, thereby reducing the number of lymphocytes. The generation of prospective, rigorous safety, and efficacy data in transitioning from natalizumab to cladribine is an unmet clinical need.

Objectives: To test the feasibility of transitioning patients with relapsing forms of MS natalizumab to cladribine tablets.

Design: Cladribine tablets after treatment with natalizumab (CLADRINA) is an open-label, single-arm, multicenter, collaborative phase IV, research study that will generate hypothesis regarding the safety, efficacy, and immunological impact of transition from natalizumab to cladribine tablets in patients with relapsing forms of MS.

Methods and analysis: Participants will be recruited from three different sites. The primary endpoint is the absolute and percent change from baseline of lymphocytes and myeloid cell subsets, as well as blood neurofilament light levels. The secondary endpoint is the annualized relapse rate over the 12- and 24-month trial periods. Exploratory endpoints include the expanded disability status scale, and magnetic resonance imaging outcomes.

Discussion: The CLADRINA trial will generate data regarding the safety, efficacy, and immunological impact of the transition from natalizumab to cladribine. As the pace of immunological knowledge of MS continues, insight into disease modifying therapy transition strategies is needed.

Keywords: cladribine tablets, multiple sclerosis, natalizumab, neurofilament light chain, progressive multifocal leukoencephalopathy

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Background

Multiple sclerosis (MS) is an immune-mediated inflammatory, and ultimately neurodegenerative disorder of the central nervous system (CNS). MS is the most common non-traumatic cause of disability in young people.[1] The treatment options for early relapsing forms of MS have expanded substantially over the past three decades, with more than 20 different disease-modifying therapies (DMTs) having achieved FDA approval to

date.[2] All current FDA-approved therapies essentially have immunomodulatory properties through depletion of leukocyte subsets, impacting leukocyte activation or differentiation, or by their sequestration out of the brain, optic nerves, and spinal cord. Each therapy has its own balance of benefits and risks that are selected with the patient to optimally treat their disease.[3]

Natalizumab is a therapeutic recombinant monoclonal antibody (mAb) that binds to alpha (α)4-integrin and interferes with the adhesion and diapedesis of leukocytes into the CNS. Natalizumab is considered to be highly effective in diminishing MS disease activity, namely the occurrence of disease relapses and new signal changes on brain magnetic resonance imaging (MRI). Unfortunately, natalizumab is associated with an increased risk of progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the brain with the polyomavirus JC. Patients with MS at high risk for PML can be identified by the JC virus antibody serum status, the duration of natalizumab treatment, and the prior treatment with immunosuppressive agents.[4] Importantly, two of these risk factors change over time, and at least one, namely an increased risk with prolonged treatment duration will invariably occur. Not surprisingly, many MS patients and their neurologists aim to transition to another DMT once an increased level of risk is established. However, cessation of natalizumab has been associated with MS disease reactivation[5] during which patients are at risk of accumulating neurological disability.[6] Thus, both treatment with natalizumab, and discontinuation of this agent have been challenging for neurologists.

Given the immunomodulatory properties of natalizumab, and that of other approved DMTs, the concept of delaying the initiation of a follow-up agent after natalizumab, commonly referred to as a 'washout', was conceptually attractive as an attempt to avoid compounding immunosuppressive effects, and in the example of natalizumab, reduce the risk of PML. While well-intentioned, the washout from natalizumab yielded a second problem, namely the risk of MS disease reactivation.[6,7]

The cladribine sequential therapy following natalizumab (CLADRINA) trial is a phase IV clinical study that was designed to test whether sequential natalizumab-to-cladribine tablets therapy will prevent disease activation after cessation of natalizumab, and whether it will provide sustained disease remission in many patients. The goal of this trial is to establish a disease-free state over a 24-month period in patients who received the natalizumab-cladribine sequential therapy.

Rationale – natalizumab

Natalizumab is a recombinant humanized mAb that binds to the alpha α4 chain of the integrin very late activation antigen (VLA)-4.[6,8,9] Initially approved by the FDA in 2005, natalizumab has an extensive history as one of the more effective therapies in reducing clinical and paraclinical MS disease activity, in addition to a firmly established long-term safety profile. Despite its efficacy, there are three observations that have limited the use of natalizumab in patients with MS. First is the risk of PML, a polyomavirus infection of the CNS that is associated with a 30–50% mortality risk.[10] While this risk can be mitigated by patient selection, duration of exposure is one of the risk factors for PML infection, and therefore this risk is cited as the primary reason for discontinuation of the therapy.[11,12] The second observation is MS disease reactivation following cessation of natalizumab therapy. Disease activity returns 3–6 months after treatment discontinuation in a predictable manner in up to 36% patients.[5,12,13] In O'Connor *et al.*, who analyzed clinical relapses in 1866 patients, and gadolinium (Gd)-enhancing lesions in 341 patients from the AFFIRM, SENTINEL, and GLANCE studies of natalizumab, annualized relapse rate (ARR) and Gd-enhancing lesions both increased shortly after natalizumab interruption and peaked between 4 and 7 months following discontinuation.[5] A consistent return of disease activity was observed regardless of overall natalizumab exposure, or if they received an alternative FDA-approved MS therapy, particularly in patients with highly active MS disease. As stated above, the first two observations in themselves often create a dilemma for neurologists and their patients. Lastly, with natalizumab, continued therapy exposure is necessary for efficacy, with incomplete efficacy often ultimately occurring. Immune tolerance and prolonged disease remission may not be achieved with natalizumab, as evidenced by the occurrence of MS disease reactivation following discontinuation of natalizumab. Even still, while highly efficacious, 17% of patients with relapsing forms of MS experienced disability progression in the phase III clinical trial AFFIRM.[14] Whether the

incomplete efficacy of natalizumab can be attributed to failure of lymphocyte sequestration, or its incomplete effect on innate immunity, and more specifically myeloid cells, remains an unanswered question.

Once a patient with MS has initiated natalizumab, a clinician must consider how and when they should counsel the patient to discontinue the DMT to maximize benefits while minimizing risk, sometimes balancing PML risk with the potential for disease rebound. Much effort has been dedicated to understanding the immunology of natalizumab, which has generated a number of hypotheses regarding the mechanisms of disease rebound following its cessation. With natalizumab treatment, leukocytes are sequestered out of the CNS into the peripheral blood, where they assume a more inflammatory phenotype. Krumbholz *et al.* demonstrated that natalizumab therapy increased $CD19^+$ mature B-cells in peripheral blood two- to three-fold more than that of other lymphocytes and monocytes compared to pre-treatment levels.[15] The increase of immature $CD19^+CD10^+$ pre-B cells in peripheral blood was even greater at 7.4-fold. This pattern remained stable during treatment for up to 16 months, and Kivisakk *et al.* showed that the frequency of $CD4^+$ T-cells producing interferon gamma, tumor necrosis factor, and interleukin (IL)-17 upon anti-CD3 stimulation increased 6 months after initiation of natalizumab treatment and remained elevated throughout the 16 month follow-up.[16] In a cohort of 23 patients who experienced return of disease activity following cessation of natalizumab, there was correlated reconstitution of $CD4^+$ T-cells and $CD8^+$ T-cells in the cerebrospinal fluid (CSF), suggesting that these cells might have particular pathological relevance.[17]

Rationale – cladribine tablets

Our current understanding of the mechanisms of action for each MS DMT could inform on the most ideal therapeutic strategy for natalizumab discontinuation. Cladribine tablets are an oral treatment option for MS patients with relapsing forms of the disease, which were approved by the FDA in 2019.[19] The mechanism of action of cladribine is thought to be lymphocyte depletion by the accumulation of deoxyadenosine nucleotides within lymphocytes. Cladribine mimics the same process as a synthetic purine nucleoside analog, targeting lymphocytes preferentially as it requires intracellular phosphorylation by deoxycytidine kinase (DCK) for its activation and 5′-nucleotidase (5′-NTase) for its inactivation.[18,19] Once within the cell, cladribine undergoes initial phosphorylation by DCK to finally become the active 2-chlorodeoxyadenosinetriposphate.[20] To inactivate cladribine-triphosphate nucleotides and to prevent intracellular accumulation, 5′-NTase is required. Compared with other cell types, resting and activated lymphocytes have high levels of DCK but low levels of 5′-NTase. Thus, cladribine becomes its active form within lymphocytes making these cell types preferentially vulnerable to its effect.[21] The accumulation of cladribine nucleotides leads to breaks in DNA strands, interferes with DNA synthesis, and ultimately results in a sustained reduction of lymphocyte counts, with a recovery by 6 months in the majority of patients.[22] The main therapeutic effects of cladribine tablets in MS are thought to be mediated by immune cell reduction of both the proliferating and the quiescent lymphocytes.[23,24]

Cladribine tablets 3.5 mg/kg (cumulative over 2 years) have had significant positive clinical/imaging effects in patients with clinically isolated syndrome (CIS) and patients with relapsing remitting MS (RRMS).[25–27] A recent analysis compared the effect of cladribine tablets on the dynamics of immune cell reduction and reconstitution in ORACLE-MS, CLARITY, and CLARITY Extension during the first year of treatment (in which patients received the first course of cladribine tablets) in patients randomized to cladribine tablets over 2 years.[28] Changes in cell counts and relative proportions of lymphocytes were evaluated at Weeks 5, 13, 24, and 48. In each study, consistent and comparable depletion of individual immune cell populations occurred following the first treatment year with cladribine tablets. A rapid reduction in $CD16^+/CD56^+$ cells (Week 5 nadir), a more marked reduction in $CD19^+$ B-cells (Week 13 nadir), and a less pronounced effect on $CD4^+$ (Week 13 nadir) and $CD8^+$ T-cells (Week 24 nadir) was shown. Lymphocyte recovery begins following treatment with cladribine tablets. When relative proportions of naïve and memory T-cell subtypes were studied in ORACLE-MS, the proportion of naïve-like naturally occurring T regulatory cells (nTregs) decreased, and the proportion of memory-like nTregs increased.[29] It was concluded that, in patients with CIS or RRMS, cladribine tablets' effects on the immune system are

comparable. The pronounced reduction and recovery dynamics of $CD19^+$ B-cells and relative changes in the proportion of some immune cell subtypes was hypothesized to underlie the clinical effects of cladribine tablets. This hypothesis was supported by data from the MAGNIFY-MS study, a phase IV open-label, single-arm study demonstrating that 1 year after treatment with cladribine tablets, memory B-cells remained low while regulatory B and naïve B-cells recovered.[30]

Cladribine may have mechanisms of efficacy beyond that of lymphocyte reduction. Some recent reports indicate the therapy may also impede the influx of T-cells into the CNS by affecting levels of soluble adhesion molecular levels such as Soluble intercellular adhesion molecule-1 (sICAM-1) or Soluble E-Selectin (sE-Selectin).[31] Cladribine may affect the expression of proinflammatory cytokine profiles; mean values of IL-2 and soluble IL-2 receptor levels measured 12 months after cladribine treatment for chronic progressive MS were lowered.[32] Similarly, IL-8 levels were decreased in CSF of cladribine-treated RRMS patients, whereas chemokine ligand 5 (CCL-5) levels were decreased both in CSF and serum.[33] Based on these observations, cladribine not only has a leukocyte reducing effect, but may also exert a direct effect on the remaining effector T-cells' function.

Regarding its pharmacological properties, cladribine is rapidly absorbed, with its oral bioavailability between 37% and 51%. The half-life varies from 5.7 to 19.7 h, meaning its biological half-life as measured in the reduction of leukocyte subsets is far greater than expected and cannot be explained with direct effects of cladribine on cellular metabolism or survival. In CSF, the concentration has been reported to be approximately 25% of that in plasma in patients without CNS disease, indicating the ability of cladribine to cross the blood–brain barrier.[22] Cladribine's CNS penetrance, while limited, may present an advantage over therapeutic mAbs that are currently being utilized or developed in MS, which are large molecules with presumably very little ability to cross the blood–brain barrier.

Rationale – sequential natalizumab to cladribine tablets combination therapy

Studies examining the transition from natalizumab to cladribine tablets have largely either been limited to expert opinion or retrospective chart reviews. In Hersh *et al.*, there was increased risk of disease activity within the first 6 months of natalizumab withdrawal that was sustained at 24 months, even with switching to high efficacy therapy including CD20 depleting therapies.[34] In a retrospective study from Mustonen *et al.*, DMTs subsequent to natalizumab failed to prevent reactivation of MS, although longer washout times were associated with higher reactivation risk at 6 months and the authors suggest washout times should not exceed 3 months.[35] None of these patients received cladribine tablets as a subsequent therapy. Similarly, a multicenter, retrospective study by Zanghì *et al.*, incorporating an extended washout when switching from natalizumab to cladribine tablets revealed an increased risk of a higher ARR when controlled for covariates.[36] Pfeuffer *et al.* simultaneously prospectively examined the MS patients prescribed cladribine tablets in a multicenter study, 23 of which transitioned to cladribine tablets from natalizumab. Seemingly unique to this transition, they published an increased risk of disease activity; however, median washout period was 66 days with an interquartile range of 49–81 days.[37]

As natalizumab treatment sequesters lymphocytes into the periphery where they assume a more inflammatory cellular phenotype, and cladribine depletes peripheral lymphocytes, a rapid transition from natalizumab to cladribine tablets might (1) reduce the risk of disease rebound from natalizumab, and (2) lead to sustained disease remission by depletion of encephalitogenic lymphocytes (Figure 1). In addition, as cladribine tablets have no association with PML, this particular transition might normalize their PML risk. Thus, based on our understanding of the immunology of cladribine tablets and natalizumab, we designed CLADRINA to evaluate the safety, efficacy, and immunological impact of this transition.

Accordingly, CLADRINA is an open-label, single-arm, multicenter, collaborative phase IV, research study in the United States to generate hypotheses regarding the safety, efficacy, and immunological impact of transition from natalizumab to cladribine tablets in patients with relapsing forms of MS. The primary endpoints are the absolute and percent change from baseline for $CD3^+$ lymphocytes, $CD19^+$ B lymphocytes, $CD11c^+$ dendritic cell subsets, and serum

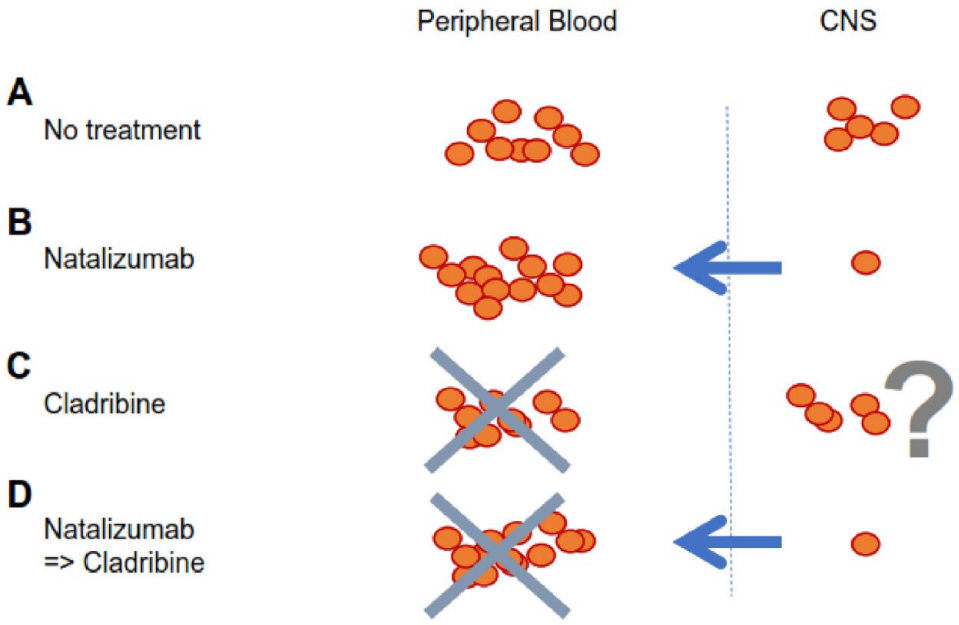

Figure 1. Study rationale for CLADRINA. (a) Natalizumab treatment sequesters encephalitogenic leukocytes out of the central nervous system (CNS) into the peripheral blood. (b) Long-term natalizumab therapy reduces the ability of the innate and adaptive immune system to replace CNS leukocytes. (c) Cladribine tablets reduces the number of encephalitogenic lymphocytes in the peripheral blood. Cladribine has incomplete CNS bioavailability its reducing effects on encephalitogenic leukocytes in the brain and spinal cord are currently unknown. (d) There is good biological and pharmacological plausibility that immediate initiation of cladribine tablets after treatment with natalizumab will deplete the absolute number of encephalitogenic cells more completely than initiation of cladribine tablets after other therapies, as many encephalitogenic leukocytes will be sequestered out of the CNS into compartments (peripheral blood and secondary lymphoid organs) where they can be targeted by cladribine.

neurofilament light levels. The secondary endpoints are the ARR over the 12- and 24-month periods following initiation of cladribine tablets. Exploratory endpoints include the expanded disability status scale (EDSS), and MRI outcomes, including new/enlarging T2 lesions, as well as the number of new Gd-enhancing lesions. Study participants will receive cladribine tablets 3.5 mg/kg up to 100 kg as per the United States Package Insert (USPI).[38] A total of 40 study participants with relapsing forms of MS, who meet the criteria for treatment with cladribine tablets as per the approved USPI are planned to be enrolled at three centers in the United States. All study participants will receive treatment with cladribine 10 mg tablets during year 1 and year 2 according to the approved USPI. Given what is known about the lymphocyte depletion dynamics following cladribine tablets, a rapid transition from natalizumab to cladribine tablets will likely temporally coincide the potential disease rebound associated with natalizumab withdrawal with the lymphocyte nadir. Therefore, cladribine 10 mg tablets will be provided in an unblinded fashion, with treatment initiated approximately 14 days after the last infusion of natalizumab (e.g. a 14-day washout), with a maximum permissible washout period of no more than 4 weeks.

Patients with at least 12 months treatment with natalizumab therapy, standard dosing, extended interval dosing, or a combination thereof, will be transitioned to cladribine tablets, with an MRI completed and reviewed at the baseline visit as a PML screening tool (Figure 2). Participants will return at month 3 for neurological and EDSS examinations, in addition to serological testing including a CBC as per the USPI, with prophylactic antiviral therapy indicated should the absolute lymphocyte count (ALC) $\leqslant$200 K/μL. Participants will return at month 6 for examinations, including a repeat MRI to review for

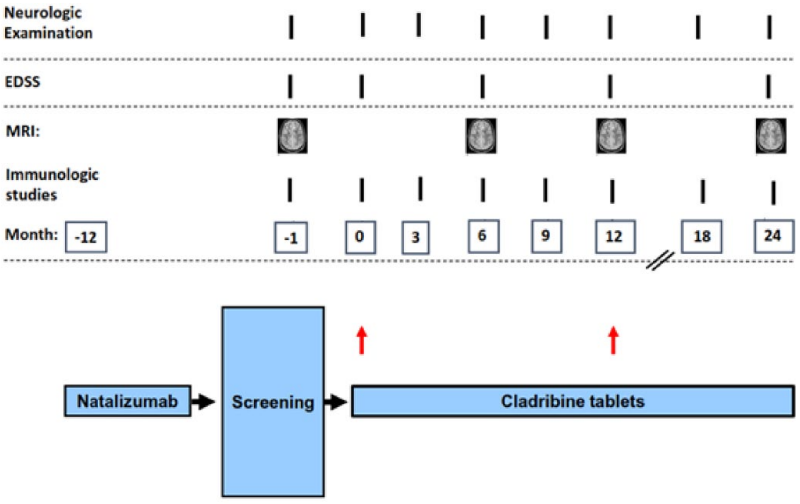

Figure 2. CLADRINA study design. CLADRINA will be an open-label, single-arm, multicenter, collaborative phase IV research study, designed to generate hypotheses regarding the transition to cladribine tablets after treatment with natalizumab in patients with relapsing forms of MS, to include RRMS and active SPMS. The total duration of this interventional study will be 2 years. A total of 40 study participants with relapsing forms of MS, to include RRMS and active SPMS, who meet the criteria for treatment with cladribine tablets as per the approved USPI are planned to be enrolled in three centers in the United States. All study participants will receive treatment with cladribine 10 mg tablets during year 1 and year 2 according to the approved USPI (EMD Serono, 2019). Treatment with cladribine tablets is intended to be initiated approximately 14 days after the last infusion of natalizumab (e.g. a 14-day 'washout'), with a maximum permissible washout period of no more than 4 weeks.
MS, multiple sclerosis; RRMS, relapsing remitting MS; SPMS, secondary progressive MS; USPI, United States Package Insert.

potential disease activity, and similarly return at month 9 for similar assessments. Year 2 of cladribine tablets will be initiated at month 12, with another screening MRI for baseline disease activity. In conclusion, examinations will be conducted at month 18, and then at the end of the study at month 24, including another MRI.

The target population comprises patients with relapsing forms of MS, RRMS, and active secondary progressive MS (SPMS), either by the 2005, 2010, and 2017 McDonald criteria, ages 18–60, regardless of JC virus serumstatus.[39–41] Patients must have exposure to natalizumab therapy for a minimum duration of 12 months, with no relapses in the 28 days prior to enrollment. Each subject's EDSS must be less than 5.5.[42] Prior to therapy, the ALC of participants must be within the normal range ($\geqslant$1000 cells/μL; $\leqslant$4800 cells/μL as per the CLL Society).[43] Participants cannot test positive for HIV, or hepatitis B or C, and must not have an active or history of malignancy at screening to be enrolled. Patients may be enrolled in this optional study regardless of JC virus serostatus as long as they were otherwise eligible for treatment with cladribine tablets as per the USPI.

Adverse events and serious adverse events will be recorded as defined in NCI CTCAE v5. Recording of adverse events will begin when the participant signs the consent form and will continue at least until the end of the study, between 24 and 30 months, depending on whether or not year 2 is delayed for recovery of the ALC to permit year 2 treatment. Given that MS disease metrics will be captured as outcomes, signs and symptoms of relapse or worsening MS will not be considered adverse events unless the participant's general condition is more severe than expected, and/or the outcome is fatality. Relapses, should they occur, will be treated as per their clinician's judgment. Participants are encouraged to continue with their healthcare team, will have access to their MRI data, and could voluntarily withdraw from the study at any time. If they opt to permanently discontinue cladribine tablets following consultation with their healthcare team, they will not remain in the study, but will be followed up for up to 30 days after intake for safety

monitoring. Safety laboratory monitoring will take place as per the USPI of cladribine tablets, with anti-herpes prophylaxis clinically indicated should the ALC be $\leqslant$200 cells/mm^3. Radiographic assessment for PML will occur at study baseline as well as throughout the study duration, specifically prior to initiation of the first and second year of treatment with cladribine tablets. The study is approved by the Institutional Review Board of the University of Texas Southwestern and is registered with clinicaltrials.gov (NCT 04178005).

Discussion

Given the multitude of FDA-approved therapies for MS, the number of possible switches between therapies is compounded, and additional data regarding the safety, efficacy, and immunological impact of these transitions are desperately needed. The transition from natalizumab to an alternative DMT carries particular import to the MS patient and their neurologist. To this end, the CLADRINA study will provide clinical data supporting the safety, efficacy, and immunological impact following the transition from natalizumab to cladribine tablets.

As an autoimmune disease, proven treatment response in MS is likely through the various immunomodulatory effects of DMT. A plethora of independent studies on animal models and clinical observations, converge on T-cells as a primary cellular mediator driving MS pathogenesis.[44–46] Natalizumab's mechanism of action emphasizes the pathogenic role of bone marrow-derived leukocyte infiltration into the CNS. The role of lymphocytes in the perpetuation of MS disease activity is further suggested by the observation of MS disease reactivation following cessation of treatment. Longitudinal monitoring of lymphocytes and myeloid cells by immunophenotyping and multi-parameter flow cytometry in peripheral blood following the initiation of oral cladribine therapy among patients transitioning from natalizumab may inform on pertinent cellular subsets that medicate disease remission, or, in patients who fail to achieve disease-free state, cellular subsets that are associated with disease activity.

Importantly, bone marrow-derived myeloid cells are of particular interest, given recent data showing that they may be critical mediators of disease activity and CNS tissue damage in experimental models and patients with MS.[47–49] Questions remain unanswered regarding the role of bone marrow-derived myeloid cells in the compartmentalization of inflammation as patients transition from RRMS to SPMS. As components of the innate immune system, bone marrow-derived myeloid cells were not intended as the original biological targets for most current MS DMT. Clinical and immunological data from this study provide a unique insight into the efficacy of cladribine on these cells; furthermore, the longitudinal design in this study will permit charting of the compartment-specific reconstitution of myeloid cell subsets as enrolled patients are transitioned off of natalizumab, with significant temporal resolution.

At the time of this manuscript submission, CLADRINA is fully enrolled. We believe that with completion of the CLADRINA study, there will be for the first time, high level evidence for disease prevention following transitioning from natalizumab.

Declarations

Ethics approval and consent to participate

CLADRINA will be undertaken in compliance with the Declaration of Helsinki and standards of Good Clinical Practice according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. The study protocol has been approved by UTSW Institutional Review Board (approval code STU 2019-1618). This trial is registered with clinicialtrials.gov (NCT04178005).

Consent for publication

All patients gave written informed consent before screening. The consent included permission to publish anonymized data.

Author contributions

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Olaf Stuve: Conceptualization; Data curation; Formal analysis; Funding acquisition; Writing – original draft; Writing – review & editing.

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Competing interests

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Availability of data and materials

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