


# The sequential natalizumab – alemtuzumab therapy in patients with relapsing forms of multiple sclerosis (SUPPRESS) trial – Part I: Rationale and objectives

Journal of Central Nervous System Disease  
Volume 14: 1–6  
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DOI: 10.1177/11795735221123911



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## ABSTRACT

**BACKGROUND:** Natalizumab is a recombinant humanized monoclonal antibody (mAb) against  $\alpha 4$ -integrin that is approved for relapsing forms of multiple sclerosis (MS). Natalizumab is associated with an increased risk of developing progressive multifocal leukoencephalopathy (PML), and with disease reactivation after cessation of treatment that is likely mediated by an accumulation of pro-inflammatory lymphocytes in the blood during therapy. Alemtuzumab is a mAb against CD52 that reduces the number of peripheral lymphocytes.

**RATIONALE:** To determine if treatment with alemtuzumab after natalizumab reduces disease activity in patients with relapsing forms of MS. This review article will outline the rationale and objectives of the sequential natalizumab – alemtuzumab therapy in patients with relapsing forms of multiple sclerosis (SUPPRESS; [ClinicalTrials.gov](https://clinicaltrials.gov) ID: NCT03135249) trial in greater detail than would be feasible in a manuscript that summarizes the study results.

**METHODS:** The SUPPRESS trial is single arm, open-label, multicenter, efficacy pilot study that aims to establish a disease-free state over a 24-months period in patients who received the natalizumab- alemtuzumab sequential therapy. Participants will be recruited from four different sites. The primary endpoint is the annualized relapse rate (ARR) from the time of cessation of natalizumab treatment. Key secondary endpoint is freedom of relapse at 12-months, the number of new/enlarging T2 lesions on magnetic resonance imaging (MRI), and the number of gadolinium (Gd)-enhancing lesions on MRI. An exploratory endpoint is the Expanded Disability Status Scale (EDSS), retinal nerve fiber layer (RNFL) thickness assessment by optic coherence tomography (OCT) and assessment of quality of life (QoL) measures by a pre-defined, self-administered testing battery. To evaluate immunological effects, blood leukocytes will be collected and immunophenotyped by multi-parameter flow cytometry.

**CONCLUSION:** The SUPPRESS trial will provide clinical, imaging, and biological data to determine whether sequential natalizumab to alemtuzumab combination therapy establish a disease-free state in patients with relapsing forms of MS.

**TYPE:** Study Protocol

**DECLARATION OF CONFLICTING INTERESTS** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**FUNDING** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Dr. Stuve is funded by Merit Review grants (federal

award document number (FAIN) I01BX001674 and BX005664-01 from the United States (U.S.) Department of Veterans Affairs, Biomedical Laboratory Research and Development.

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## Background

Multiple sclerosis is an inflammatory disorder of the central nervous system (CNS).<sup>1</sup> A pathological hallmark of this disorder is the infiltration of immune-competent leukocytes into the brain and spinal cord. Natalizumab is a humanized recombinant monoclonal antibody that binds to the alpha ( $\alpha$ )<sub>4</sub> chain of the integrin very late activation antigen (VLA)-4. Natalizumab is currently considered the most effective approved therapy in reducing clinical and paraclinical MS disease activity. Our group has made several novel observations with regard to the pharmacodynamic properties of natalizumab: (1) Compared to controls, natalizumab-treated MS patients had

significantly fewer white blood cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, CD19<sup>+</sup> B cells, and CD138<sup>+</sup> plasma cells in cerebrospinal fluid (CSF); (2) CD4<sup>+</sup>:CD8<sup>+</sup> ratios in the CSF of MS patients treated with natalizumab were reversed, and not statistically different from those in HIV-infected controls; (3) elevated serum anti-Human Herpesvirus (HHV)-6 IgG, and HHV-6A DNA were detected in the CSF of a subset of patients on natalizumab therapy. In summary, these data indicate that natalizumab therapy substantially alters the composition of immune-competent cells in the CSF of patients with MS.

Since its first approval for patients with MS in November 2004, more than several hundred patients treated with



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natalizumab have been diagnosed with progressive multifocal leukoencephalopathy (PML), a CNS infection with the polyomavirus JC. PML is typically observed in the setting of prolonged and severe immunosuppression, most commonly in patients infected with HIV. Natalizumab is currently administered monthly as monotherapy to approximately 100,000 patients with MS, and treatment is recommended to continue indefinitely without treatment interruptions.

#### *Biological and medical rationale for the SUPPRESS trial – Natalizumab*

Natalizumab is a humanized recombinant monoclonal antibody against alpha( $\alpha$ )4-integrin that was first approved in November 2004 for patients with relapsing forms of multiple sclerosis (MS) based on the results of two phase III trials.<sup>2,3</sup> Natalizumab blocks the egress of leukocytes from the peripheral blood into the CNS.<sup>4,5</sup> In the short term, the therapeutic benefits are likely due to its effect on lymphocytes as shown by our laboratory and other investigators.<sup>6-9</sup> Long-term, the number of myeloid cells that serve as antigen presenting cells (APC) in perivascular spaces is likely also substantially reduced.<sup>10</sup> Despite its tremendous efficacy, there are two observations that have limited the use of natalizumab in patients with MS, namely the risks of PML and MS disease reactivation after cessation of natalizumab therapy.

Approximately 1:250 patients with MS under natalizumab will develop PML, an infection caused by the human polyomavirus JC.<sup>11</sup> This potential side effect has substantially limited the use of an effective therapy. An algorithm was recently developed to estimate PML incidence in MS patients considering or receiving natalizumab based on duration of natalizumab treatment (1-24 or 25-48 months), prior immunosuppressant use (yes or no), and anti-JCV antibody status (positive or negative).<sup>12</sup> Based upon the two established risk factors for PML, PML risk was lowest in patients treated with natalizumab for 1-24 months without prior immunosuppressant use, (.19 cases per 1000 patients), and greatest in those with both risk factors, natalizumab treatment for 25-48 months and prior immunosuppressant use (4.3 cases per 1000). When anti-JCV antibody status was included as a third risk factor, PML risk was lowest in patients who were anti-JCV antibody negative (.11 per 1000), and highest in patients with all three factors, (11 per 1000). The most recent data indicate that MS patients on natalizumab with all three risk factors appear to have a risk of 1:44 to develop PML.<sup>11,13</sup>

As stated above, the second risk associated with natalizumab is that of disease-reactivation after cessation of therapy. This is a complex issue for many patients and their neurologists, given that there is an increased risk of PML with cumulative dosing. While natalizumab is a tremendously effective therapy, disease activity returns 3 to 6 months after treatment discontinuation in a predictable manner. The best data in this regard were generated by 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, and their respective safety extension studies.<sup>14</sup> Annualized relapse rates and Gd-enhancing lesions both increased shortly after natalizumab interruption and peaked between 4 and 7 months. A consistent return of disease activity was observed regardless of overall natalizumab exposure, whether or not patients received the FDA-approved alternative MS therapies, particularly in patients with highly active MS disease.

The return of disease activity may be explained by natalizumab's biological activities. Leukocytes are sequestered out of the CNS in the peripheral blood, where they assume a more inflammatory phenotype. Krumbholz et al. demonstrated that natalizumab therapy increased CD19<sup>+</sup> mature B cells in peripheral blood 2-3-fold more than that of other lymphocytes and monocytes compared to pre-treatment levels.<sup>15</sup> The increase of immature CD19<sup>+</sup>CD10<sup>+</sup> pre-B cells in peripheral blood was 7.4-fold. This pattern remained stable during treatment for up to 16 months. Kivisakk et al showed that the frequency of CD4<sup>+</sup> T cells producing interferon gamma (IFN $\gamma$ ), tumor necrosis factor, and interleukin (IL)-17 upon anti-CD3 stimulation increased 6 months after initiation of natalizumab treatment and remained elevated throughout the follow-up.<sup>16</sup> The frequency of CD4<sup>+</sup> T cells expressing CD25, HLA-DR, and CCR6 ex vivo was increased at one or more time points during treatment. Our lab showed in a cohort of 23 patients that return of clinical disease activity in patients who stopped taking natalizumab correlated with the re-constitution of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells in the cerebrospinal fluid.<sup>8</sup>

#### *Biological and medical rationale for the SUPPRESS trial – Alemtuzumab*

Alemtuzumab is a humanized monoclonal therapeutic antibody that rapidly depletes CD52<sup>+</sup> cells. Alemtuzumab is effective in ameliorating MS disease activity. In the CARE-MS I phase III trial, a 55% relapse rate reduction with alemtuzumab (12 mg/d) over interferon-beta (IFN $\beta$ ) was observed for alemtuzumab treated patients after 24 months.<sup>17</sup> Significantly more (78%) alemtuzumab treated patients remained relapse-free at month 24 compared with 59% of IFN $\beta$ 1a-treated patients, which equates to a 55% risk reduction. In the CARE-MS II phase III trial a 49% reduction in relapse rate was observed in patients treated with alemtuzumab (12 mg/d) compared with those treated with IFN $\beta$ -1a over the 2 years.<sup>18</sup> Significantly more alemtuzumab treated patients remained relapse-free at month 24 compared with IFN $\beta$ -1a treated patients. While these data provide a rationale for the use of alemtuzumab in patients with MS, many experts were surprised that alemtuzumab did not display even greater efficacy based on the hypothesis that the peripheral immune system perpetuates disease activity in MS. Again, its biological effects may explain this incomplete treatment effect of alemtuzumab. Mainly, there is currently no

evidence that alemtuzumab has any biological effect in the CNS. Thus, the number and function of autoimmune-prone lymphocytes and pre-inflammatory myeloid cells that reside in the brain and spinal cord is not reduced.

MS is considered an autoimmune disorder of the CNS. However, an autoantigen has not been identified. Thus, the creation of peripheral tolerance will best be inferred by the disease status. Recent data from an Immune Tolerance Network (ITN) trial that tested autologous hematopoietic stem cell transplant (HSCT) in patients with very aggressive MS (HALT trial) showed that the reconstitution of the T cell receptor repertoire predicts treatment responses:

- Patients who failed treatment had a significantly less diverse TCR (CD4+ and CD8+) at 2 months post-transplant than responders
- Treatment effectively reduced dominant baseline CD4+ TCR clones, did not reduce dominant CD8 TCR clones
- Patients who recovered CD8+ T cells at year 1 had a less diverse CD8+ TCR repertoire
- The reconstituted CD8+ T cell repertoire was dominated by large clonal expansions
- There was up to a 100% “renewal” (ablated→new) of the CD4+ T cell repertoire; there was less renewal within the CD8+ T cell repertoire
- Treatment resulted in a “new” Top 100 CD4+ TCR repertoire at 2 months post-transplant

Immune-reconstitution after alemtuzumab therapy is very likely similar to that after autologous HSCT. With both treatment regimens, there is an extensive re-constitution of the lymphocyte compartment that is driven by mobilization of autologous CD34+ bone marrow cells.<sup>19-22</sup> As stated above, alemtuzumab targets CD52, which is a 12-amino-acid glycosylated GPI-bound membrane protein.<sup>23,24</sup> CD52 is expressed on most cells derived from the lympho-monocytic cell lineage, including T and B cells, natural killer (NK) cells, dendritic cells and most monocytes and macrophages. By contrast, neutrophils and precursors cells of the hematopoietic lineage do not express CD52.<sup>25-27</sup> The exact biological function of CD52 is not fully understood; CD52 binding may induce T cell activation, and CD52 may be a stimulatory co-factor required for regulatory T cells (Treg).<sup>25,26</sup> Alemtuzumab depletes CD52+ cells through antibody-dependent cellular cytotoxicity (ADCC) and likely also through activation of the complement cascade.<sup>27-30</sup> Cellular depletion is initiated rapidly resulting in almost complete disappearance of CD52+ cells from the circulation shortly after alemtuzumab administration. Experimentally, complement-mediated cell lysis of leukemic B cells occurs within 1-4 hours after addition of alemtuzumab *in vitro*.<sup>31</sup> Lympho-monocytic cells in the periphery are eventually repopulated from

pools of stem cells and certain progenitor cells that do not constitutively express CD52. Monocytes and B cells reach pre-alemtuzumab levels in the peripheral blood approximately three to six months after treatment, with B cell levels exceeding baseline levels by 124-165%.<sup>28,29,32,33</sup> T cells repopulate considerably slower; CD8+ T cells reach baseline levels only after a median of 30 months, and CD4+ T cells after a median of 61 months.<sup>28</sup> The lower limits of normal for CD4+ and CD8+ T cells are reached earlier with medians of 12 and 11 months, respectively.<sup>34</sup> It is particularly noteworthy that Tregs repopulate distinctly before CD4+ and CD8+ T cells, resulting in their specific enrichment in the peripheral blood.<sup>28,33</sup>

### *Biological and medical rationale for sequential natalizumab – alemtuzumab therapy*

Natalizumab treatment sequesters leukocytes out of the CNS into the peripheral blood. Immediate sequential alemtuzumab therapy will deplete these cells more completely than alemtuzumab monotherapy and prevent reactivation of disease activity previously treated with natalizumab. Thus, we hypothesized that sequential natalizumab – alemtuzumab therapy will prevent disease activation after cessation of natalizumab and will provide sustained disease remission in many patients. The goal of this trial is to establish a disease-free state over a 24-months period in patients who received the natalizumab-alemtuzumab sequential therapy.

### *Study purpose of SUPPRESS*

The purpose was to provide preliminary evidence that the sequential treatment approach would provide feasibility and estimates of effectiveness on which to plan a larger study. The biological rationale was that the proposed sequential combination therapy of natalizumab and alemtuzumab induces peripheral tolerance and reduces the AAR in patients with RRMS.

### **Study objectives of SUPPRESS**

The primary objective of the SUPPRESS trial is to determine if treatment with alemtuzumab after natalizumab maintains or reduces the ARR in patients with relapsing forms of MS. The goal of this trial is to establish a disease-free state over a 24-months period in patients who received the natalizumab-alemtuzumab sequential therapy.

Secondary objectives of SUPPRESS are to evaluate the T cell, B cell, and autoreactivity characteristics of immune cells in RRMS patients before and after alemtuzumab treatment.

### **Study design of SUPPRESS**

The SUPPRESS trial is a single arm, open-label, multicenter, efficacy pilot study of sequential natalizumab-alemtuzumab treatment in patients with relapsing forms of MS. Study participants will use commercial drug alemtuzumab treatment.

Alemtuzumab (Lemtrada®) will be administered at a dose of 12 mg/d by intravenous (i.v.) infusion every day for five consecutive days within 14 days of the last dose of natalizumab. After 12 months, patients will be treated with a second course of alemtuzumab and they will be followed open-label for another 12 months per standard of care. Outside the scope of this study, the intention is to follow all study participants in participating centers long-term as well as in a Risk Evaluation and Mitigation Strategy (REMS) program mandated by the Food and Drug Administration (FDA) outline below, and to record disease activity and treatment response.

### *Study approval of SUPPRESS*

Institutional approval was obtained at all study sub-sites by their respective Institutional Review Boards (IRB) for all procedures proposed in the study protocol.

### **Study endpoints of SUPPRESS**

The primary endpoint is the annualized relapse rate (ARR) from the time of cessation of natalizumab treatment.

Key secondary endpoints are freedom from relapse at 12 months and the number of new/enlarging T2 lesions on MRI.

Other secondary endpoints are: Number of gadolinium (Gd)-enhancing lesions on MRI. Exploratory endpoints are the assessment of neurological disability by the EDSS scale, assessment of the RNFL thickness by OCT, and the evaluation of QoL measures by a pre-defined, self-administered testing battery.

### **Study population**

The target population for this study are RRMS patients nearing the end of their natalizumab treatment regimen as determined by recipients and their treating clinical providers. Participants are recruited from four different sites. Recruitment occurs on a competitive basis. Participants will be eligible for the trial if they meet the following criteria: (1) Age 18-60 years, (2) Diagnosis of relapsing forms of MS using revised McDonald Criteria<sup>35</sup> (3) EDSS 0-6.5, (4) Has had a minimum of 12 monthly doses of continuous natalizumab therapy (300 mg/d), either regular or extended interval dosing (5) Understands and gives informed consent. Patients will be excluded from the study if (1) Natalizumab failure based on clinician's discretion (2) Has progressive MS (3) A diagnosis of PML (4) Known hypersensitivity to alemtuzumab (5) Any prior exposure to alemtuzumab (6) Initiation of new immunosuppressant treatment after the subject becomes protocol-eligible (except for corticosteroids) or enrollment in a concurrent trial with immuno-active pharmacotherapies (7) Uncontrolled diabetes mellitus defined as HbA1c >8% and/or requiring intensive management (8) History of cytopenia consistent with the diagnosis of myelodysplastic syndrome (9) Clinically significant

autoimmune disease other than MS that may affect the CNS, including neuromyelitis optica (NMO), systemic lupus erythematosus (SLE, or Behcet disease (10) Active hepatitis B or C infection or evidence of cirrhosis (11) Human immunodeficiency virus (HIV) positivity (12) Uncontrolled viral, fungal, or bacterial infection (13) Positive pregnancy test or inability or unwillingness to use effective means of birth control (14) Presence of metallic objects implanted in the body that would preclude the ability of the subject to safely have MRI exams (15) Psychiatric illness, mental deficiency, or cognitive dysfunction making compliance with treatment or informed consent impossible.

### **Safety assessments**

To minimize the risk of transitioning MS patients from natalizumab to alemtuzumab who already have PML, all enrolled patients will undergo a brain MRI within 14 days prior to receiving alemtuzumab.<sup>36</sup> Any untoward effects of pharmacotherapy will be monitored, and the study will be terminated should a case of PML arise to allow for a thorough safety assessment that will include JCV antibody titers and other biomarkers. To minimize the risk of herpetic infections while on alemtuzumab, anti-viral prophylaxis for herpetic viral infections should be administered per USPI on the first day of each treatment course and continued for a minimum of 2 months following treatment, or until the CD4+ lymphocyte count is >200 cells/microliter, whichever occurs later.

There may be an exaggerated cytokine response in some patients. Thus, patients will be monitored and managed based on the treating physician's best judgement from the final dose of natalizumab to the end of the first course of alemtuzumab treatment.

Each relapse will be treated with pulse corticosteroids as per best clinical judgment. Two or more confirmed clinical relapses during the trial would allow rescue therapy as per best clinical judgment. Five or more Gd<sup>+</sup> lesions at the 6-month MRI assessment over the baseline assessment will result in a follow-up scan after 60 days and will count as one relapse.<sup>37</sup> Should a confirmed clinical relapse occur within the 60-day period, it will still count as a single relapse. This conclusion is based on our previous observation that the number of lesions on a brain MRI scan is the best predictor of a relapse within a defined period.<sup>37</sup>

Sensitivity analyses will be conducted using intent-to-treat (ITT) principles for efficacy to ensure that safety considerations and withdrawals have not altered results. All patients who receive alemtuzumab will automatically be enrolled into a Risk Evaluation and Mitigation Strategy (REMS) program mandated by the Food and Drug Administration (FDA). A REMS program is a strategy to manage known or potential serious risks associated with a drug product. The purpose of the alemtuzumab REMS program is to inform prescribers, pharmacies, healthcare facilities, and patients about the potential risks of this agent. Specifically, the duration of the alemtuzumab REMS

program is 4 years, and thus exceeds the duration of this study by 2 years. Patients who withdraw from study treatment will be observed as mandated by REMS.

The relationship or attribution between an adverse event and an investigational product is determined by the site investigator and recorded on the appropriate case report form and/or SAE reporting form. The Common Terminology Criteria for Adverse Events (CTCAE) provides the following descriptors and definitions (one category classified as unrelated [Code 1] and 4 categories classified as related [Codes 2-5]) for assigning an attribution to each adverse event (for most recent update of terminology see: [http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE\\_4.03\\_2010-06-14\\_QuickReference\\_5x7.pdf](http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)). Toxicity grades are assigned by the study site to indicate the severity of adverse events occurring in study participants. The principal investigator (PI) has adopted the use of the National Cancer Institute's manual Common Terminology Criteria for Adverse Events v3.0 (CTCAE; published June 10, 2003) for application in adverse event reporting. The purpose of using the CTCAE system is to provide a standard language to describe toxicities, to facilitate tabulation and analysis of the data, and to facilitate the assessment of the clinical significance of all adverse events. The CTCAE provides the following grades and descriptions in the CTCAE manual (v3.0). Adverse events will be recorded and graded 1 to 5 according to the CTCAE grades: Grade 1 = Mild adverse event, Grade 2 = Moderate adverse event, Grade 3 = Severe and undesirable adverse event, Grade 4 = Life-threatening or disabling adverse event ND Grade 5 = Death. In contrast to the CTCAE guidelines provided the National Cancer Institute's Common Terminology Criteria for Adverse Events v3.0 (published June 10, 2003), all adverse events are to be reported and graded whether or not they are related to disease progression or treatment.

### *Sample size and power calculations*

The sample size of 40 patients was chosen to obtain a result that would be seen as meaningful by neurologists. Disease activity is expected to be extremely low on natalizumab and subsequently on alemtuzumab. Thus, it will be impossible to power a superiority or non-inferiority study. It is expected that the induction treatment proposed will effectively stop all MS disease activity as defined as No Evidence of Disease Activity (NEDA)-3, namely a stable EDSS score, no new signal changes on brain MRI, and no evidence of clinical relapses. To be conservative, we set the probability of activity at 5%. Based on this, if we observe 7 or more participants out of the 40 participants with activity, we will say this approach is futile based on the probability that if the event rate is 5% or less, the probability of observing 7 or more is .042 and thus indicative of a higher rate of activity (a lower NEDA rate) that we feel is not worth the risks as noted by the reviewers.

All outcome measures will be assessed in the 12 months prior to natalizumab, on natalizumab, and on alemtuzumab. We will

control secondary endpoints for multiple comparisons by testing sequentially the proportion of relapse-free patients, EDSS change, and T2-hyperintense lesion volume change.

The analysis of the acquired standardized imaging data will include a qualitative assessment of structural features suggestive of progressive multi-focal leukoencephalopathy (PML), T2-weighted lesion volumes, T1-weighted lesion volumes, a determination of new or enlarging T2 foci, the presence of acute blood brain barrier compromise and number of identified contrast enhanced lesions, and an assessment of brain volumetric changes by SIENA between the baseline and year 2 MRI study.

We will analyze the proportion of patients who are relapse-free with a proportional hazards model. We will analyze changes from baseline in EDSS at the pre-defined time points with a mixed model for repeated measures. We will make treatment comparisons of all available 3-month assessments with a non-parametric test for repeated measures. We will analyze changes in T2-hyperintense lesion volume, and RNFL thickness with a ranked ANCOVA model. We will also analyze proportions of patients with new or enlarging T2-hyperintense lesions or Gd+ lesions, and those who were free from disease activity, with logistic regression.

## Conclusion

The SUPPRESS trial will provide comprehensive clinical, imaging, and biological data to determine whether sequential natalizumab to alemtuzumab combination therapy may establish a disease-free state in patients with relapsing forms of MS. If a beneficial effect on MS disease activity can be demonstrated, SUPPRESS would provide a strong rationale for alemtuzumab therapy for patients on natalizumab at high risk for PML and MS disease reactivation after cessation of natalizumab.

## Acknowledgments

The authors would like to thank their patients for participating in this research initiative.

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## REFERENCES

1. Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. *N Engl J Med*. 2018; 378(2):169-180.
2. Rudick RA, Stuart WH, Calabresi PA, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med*. 2006;354(9): 911-923.
3. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*. 2006;354(9): 899-910.
4. Shirani A, Stuve O. Natalizumab for Multiple Sclerosis: A Case in Point for the Impact of Translational Neuroimmunology. *J Immunol*. 2017;198(4): 1381-1386.
5. Shirani A, Stuve O. Natalizumab: Perspectives from the Bench to Bedside. *Cold Spring Harb Perspect Med*. 2018;8:a029066.

6. Stuve O, Marra CM, Bar-Or A, et al. Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *Arch Neurol.* 2006;63(10):1383-1387.
7. Stuve O, Marra CM, Jerome KR, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann Neurol.* 2006;59(5):743-747.
8. Stuve O, Cravens PD, Frohman EM, et al. Immunologic, clinical, and radiologic status 14 months after cessation of natalizumab therapy. *Neurology.* 2009;72(5):396-401.
9. Kowarik MC, Pellkofer HL, Cepok S, et al. Differential effects of fingolimod (FTY720) on immune cells in the CSF and blood of patients with MS. *Neurology.* 2011;76(14):1214-1221.
10. del Pilar MM. Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. *Arch Neurol.* 2008;65(12):1596-1603.
11. Berger JR, Fox RJ. Reassessing the risk of natalizumab-associated PML. *J Neurovirol.* 2016;22:533-535.
12. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med.* 2012;366(20):1870-1880.
13. Berger JR, Fox RJ. Erratum to: Reassessing the risk of natalizumab-associated PML. *J Neurovirol.* 2016;22:536-537.
14. O'Connor PW, Goodman A, Kappos L, et al. Disease activity return during natalizumab treatment interruption in patients with multiple sclerosis. *Neurology.* 2011;76(22):1858-1865.
15. Krumbholz M, Meinl I, Kumpfel T, Hohlfeld R, Meinl E. Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. *Neurology.* 2008;71(17):1350-1354.
16. Kivisakk P, Healy BC, Viglietta V, et al. Natalizumab treatment is associated with peripheral sequestration of proinflammatory T cells. *Neurology.* 2009;72(22):1922-1930.
17. Cohen JA, Coles AJ, Arnold DL, et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet.* 2012;380(9856):1819-1828.
18. Coles AJ, Twyman CL, Arnold DL, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet.* 2012;380(9856):1829-1839.
19. Cox AL, Thompson S, Jones J, et al. Lymphocyte homeostasis following therapeutic lymphocyte depletion in multiple sclerosis. *Eur J Immunol.* 2005;35(11):3332-3342.
20. Baker D, Herrod SS, Alvarez-Gonzalez C, Giovannoni G, Schmierer K. Interpreting Lymphocyte Reconstitution Data From the Pivotal Phase 3 Trials of Alemtuzumab. *JAMA Neurol.* 2017;74(8):961-969.
21. Nash RA, Hutton GJ, Racke MK, et al. High-dose immunosuppressive therapy and autologous HCT for relapsing-remitting MS. *Neurology.* 2017;88(9):842-852.
22. Burt RK, Balabanov R, Burman J, et al. Effect of Nonmyeloablative Hematopoietic Stem Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in Patients With Relapsing-Remitting Multiple Sclerosis: A Randomized Clinical Trial. *JAMA.* 2019;321(2):165-174.
23. Hale G, Rye PD, Warford A, Lauder I, Brito-Babapulle A. The glycosylphosphatidylinositol-anchored lymphocyte antigen CDw52 is associated with the epididymal maturation of human spermatozoa. *J Reprod Immunol.* 1993;23(2):189-205.
24. Xia MQ, Tone M, Packman L, Hale G, Waldmann H. Characterization of the CAMPATH-1 (CDw52) antigen: biochemical analysis and cDNA cloning reveal an unusually small peptide backbone. *Eur J Immunol.* 1991;21(7):1677-1684.
25. Rowan WC, Hale G, Tite JP, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) triggers activation of normal human T lymphocytes. *International immunology.* 1995;7(1):69-77.
26. Hederer RA, Guntermann C, Miller N, et al. The CD45 tyrosine phosphatase regulates Campath-1H (CD52)-induced TCR-dependent signal transduction in human T cells. *International immunology.* 2000;12(4):505-516.
27. Stauch D, Dernier A, Sarmiento Marchese E, et al. Targeting of natural killer cells by rabbit antithymocyte globulin and campath-1H: similar effects independent of specificity. *PLoS One.* 2009;4(3):e4709.
28. Coles AJ, Cox A, Page E, et al. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol.* 2006;253(1):98-108.
29. Coles AJ, Compston DAS, Selmaj KW, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med.* 2008;359(17):1786-1801.
30. Hu Y, Turner MJ, Shields J, et al. Investigation of the mechanism of action of alemtuzumab in a human CD52 transgenic mouse model. *Immunology.* 2009;128(2):260-270.
31. Bologna L, Gotti E, Manganini M, et al. Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab. *J Immunol.* 2011;186(6):3762-3769.
32. Hill-Cawthorne GA, Button T, Tuohy O, et al. Long term lymphocyte reconstitution after alemtuzumab treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatr.* 2012;83(3):298-304.
33. Cossburn MD, Harding K, Ingram G, et al. Clinical relevance of differential lymphocyte recovery after alemtuzumab therapy for multiple sclerosis. *Neurology.* 2013;80(1):55-61.
34. Coles AJ, Fox E, Vladoic A, et al. Alemtuzumab more effective than interferon beta-1a at 5-year follow-up of CAMMS223 clinical trial. *Neurology.* 2012;78(14):1069-1078.
35. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* 2011;69(2):292-302.
36. Wijburg MT, Witte BI, Vennegoor A, et al. MRI criteria differentiating asymptomatic PML from new MS lesions during natalizumab pharmacovigilance. *J Neurol Neurosurg Psychiatr.* 2016;87(10):1138-1145.
37. Morgan CJ, Ranjan A, Aban IB, Cutter GR. The magnetic resonance imaging 'rule of five': predicting the occurrence of relapse. *Mult Scler.* 2013;19(13):1760-1764.