

Exploring potential mechanisms of action of natalizumab in secondary progressive multiple sclerosis

Finn Sellebjerg, Diego Cadavid, Deborah Steiner, Luisa Maria Villar, Richard Reynolds and Daniel Mikol

Abstract: Multiple sclerosis (MS) is a common and chronic central nervous system (CNS) demyelinating disease and a leading cause of permanent disability. Patients most often present with a relapsing–remitting disease course, typically progressing over time to a phase of relentless advancement in secondary progressive MS (SPMS), for which approved disease-modifying therapies are limited. In this review, we summarize the pathophysiological mechanisms involved in the development of SPMS and the rationale and clinical potential for natalizumab, which is currently approved for the treatment of relapsing forms of MS, to exert beneficial effects in reducing disease progression unrelated to relapses in SPMS. In both forms of MS, active brain-tissue injury is associated with inflammation; but in SPMS, the inflammatory response occurs at least partly behind the blood–brain barrier and is followed by a cascade of events, including persistent microglial activation that may lead to chronic demyelination and neurodegeneration associated with irreversible disability. In patients with relapsing forms of MS, natalizumab therapy is known to significantly reduce intrathecal inflammatory responses which results in reductions in brain lesions and brain atrophy as well as beneficial effects on clinical measures, such as reduced frequency and severity of relapse and reduced accumulation of disability. Natalizumab treatment also reduces levels of cerebrospinal fluid chemokines and other biomarkers of intrathecal inflammation, axonal damage and demyelination, and has demonstrated the ability to reduce innate immune activation and intrathecal immunoglobulin synthesis in patients with MS. The efficacy of natalizumab therapy in SPMS is currently being investigated in a randomized, double-blind, placebo-controlled trial.

Keywords: natalizumab, pathophysiology, review, secondary progressive multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), affecting an estimated 2.0–2.5 million people worldwide [Milo and Kahana, 2010]. Relapsing–remitting MS (RRMS), the most common form of MS, in which disability accumulates in a step-wise fashion due to incomplete recovery from relapses, typically transitions over time towards a secondary progressive phase, in which relapses become less and less frequent and insidious disability progression begins [Weinshenker, 1998]. Thus, RRMS and secondary progressive MS (SPMS) are generally regarded as different phases

of the same disease, each with ‘active’ and ‘not active’ phenotypes that are differentiated by the presence of relapses with or without magnetic resonance imaging (MRI) activity [Lublin *et al.* 2014]. Despite being on a continuum, RRMS and SPMS appear to have somewhat distinct pathophysiological mechanisms. In RRMS, deterioration results from acute intermittent inflammation, demyelination and axonal injury following the transmigration of inflammatory cells into the CNS [Frischer *et al.* 2009; Kutzelnigg *et al.* 2005; Lassmann, 2007a]. Conversely, disability accumulation in SPMS results mostly from chronic persistent demyelination, continued widespread

Ther Adv Neurol Disord

2016, Vol. 9(1) 31–43

DOI: 10.1177/
1756285615615257

© The Author(s), 2015.

Reprints and permissions:
[http://www.sagepub.co.uk/
journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

Correspondence to:
Finn Sellebjerg, MD, PhD, DMSci
Danish Multiple Sclerosis
Center, Rigshospitalet,
University of Copenhagen,
Copenhagen, Denmark
sellebjerg@dadlnet.dk

Diego Cadavid, MD
Deborah Steiner, MD, MS
Daniel Mikol, MD, PhD
Biogen, Cambridge, MA,
USA

Luisa Maria Villar, PhD
Department of
Immunology, Ramón y
Cajal University Hospital,
Institute Ramón y Cajal
for Biomedical Research,
Madrid, Spain

**Richard Reynolds, BSc,
AKC, PhD**
Division of Brain Sciences,
Imperial College London,
Hammersmith Hospital
Campus, London, UK

axonal injury, and a compartmentalized inflammatory process behind the blood–brain barrier (BBB) distinguished by chronic meningeal inflammation, ectopic lymphoid tissues and widespread microglial activation that is most notable in the white matter [Frischer *et al.* 2009; Kutzelnigg *et al.* 2005]. During the transition from RRMS to SPMS, both peripherally driven and compartmentalized inflammatory processes may coexist [Compston and Coles, 2008].

Natalizumab is a humanized monoclonal antibody that selectively binds to the α 4-subunit of α 4 β 1- and α 4 β 7-integrins expressed on the surface of human leukocytes [Rice *et al.* 2005]. Natalizumab is approved in the United States for relapsing MS [Biogen, 2015a] and in Europe for the treatment of RRMS [Biogen, 2015b], based on the outcomes of two phase III studies [Polman *et al.* 2006; Rudick *et al.* 2006]. These studies in patients with relapsing forms of MS demonstrated that natalizumab significantly reduced the annualized relapse rate, the risk of confirmed disability deterioration over 2 years and the accumulation of new brain MRI lesions. In other placebo-controlled studies, natalizumab has been shown to suppress the evolution of new gadolinium-enhanced lesions into T1-hypointense lesions in patients with relapsing MS (70.5% RRMS; 29.5% SPMS) [Dalton *et al.* 2004]. There is also evidence that natalizumab efficacy in RRMS is accompanied by reductions in the rate of brain volume loss [Miller *et al.* 2007; Radue *et al.* 2010; Rinaldi *et al.* 2012] and enhanced tissue integrity on diffusor tensor imaging [Fox *et al.* 2011].

In light of these findings, the efficacy of natalizumab in slowing progression of disability independent of relapses is currently being investigated in a phase IIIb trial in patients with SPMS (ASCEND [ClinicalTrials.gov identifier: NCT01416181]). Here, we summarize the relevant pathophysiology of SPMS and present a biological rationale for the treatment of SPMS with natalizumab, including its potential to reduce chronic intrathecal inflammation and disability progression through several potential mechanisms, as illustrated both by pathophysiological data and *post hoc* analyses of natalizumab studies that included patients with SPMS.

Natalizumab mechanism of action in RRMS

The principal clinical effect of natalizumab in RRMS is thought to be secondary to blockade of

the molecular interaction between α 4 β 1-integrin (also known as very late antigen-4 [VLA-4], expressed by mononuclear inflammatory cells) and vascular cell adhesion molecule-1 (expressed by cerebral vascular endothelial cells). The net effect is reduced leukocyte migration into the CNS and consequent reduction of disease activity [Miller *et al.* 2003; Polman *et al.* 2006].

Effects on CNS cellular infiltration and intrathecal antibody production

Among untreated RRMS patients, high lymphocyte counts in the cerebrospinal fluid (CSF) and the presence of oligoclonal bands (OCBs) of immunoglobulin types G and M (IgG and IgM) are associated with markers of axonal degeneration and MS disease activity, including higher CSF levels of neurofilament light-chain protein, with higher MS Severity Score (MSSS) and MRI lesion number [Villar *et al.* 2015]. Similarly, IgG OCB-negative patients exhibit less global and regional brain atrophy than IgG OCB-positive patients [Ferreira *et al.* 2014].

Several studies have demonstrated the effects of natalizumab therapy on immunological mechanisms involved in inflammatory MS disease activity in the CNS, as summarized in Table 1. In studies of CSF profiles in RRMS patients treated with natalizumab for at least 1 year, reductions were seen in CSF levels of CD4+ T cells, B cells (especially CD5+) and OCBs (IgM, and to a lesser extent, IgG) [Mancuso *et al.* 2014; Villar *et al.* 2012; Harrer *et al.* 2013]; whereas OCBs reappeared in the CSF of patients who discontinued natalizumab [Harrer *et al.* 2013; von Glehn *et al.* 2012]. Similarly, a longitudinal study revealed that the CSF CD4+/CD8+ ratio, IgG and IgM levels and IgG-CSF index declined in samples collected during natalizumab treatment compared with pretreatment [Warnke *et al.* 2015]. A decrease from pretreatment levels in the CD4+/CD8+ ratio in CSF was also observed in a separate longitudinal prospective study of natalizumab-treated patients [Stüve *et al.* 2006]. Moreover, the proportion of CD19+ B cells in CSF has been shown to be lower in natalizumab-treated MS patients ($n = 59$) than in those not treated with natalizumab ($n = 17$) [Warnke *et al.* 2015]; whereas increases in mature CD19+ B cells and CD19+, CD10+ pre-B cells in peripheral blood of natalizumab-treated patients were greater than increases in the total peripheral lymphocyte population [Krumbholz *et al.* 2008].

Table 1. Summary of the CNS effects of natalizumab in patients with RRMS and in patients with SPMS.

Type of effect	Reference(s) reporting effect in RRMS	Reference(s) reporting effect in SPMS
Lower CSF number or proportion of the following cells relative to untreated controls:		
• CD4+ T cells	Stüve <i>et al.</i> [2006]	NR
• CD8+ T cells	Stüve <i>et al.</i> [2006]	NR
• CD19+ B cells	Stüve <i>et al.</i> [2006], Warnke <i>et al.</i> [2014]	NR
• CD138+ plasma cells	Stüve <i>et al.</i> [2006]	NR
Lower CSF CD4/CD8 ratio relative to untreated controls	Warnke <i>et al.</i> [2014]	NR
Reductions in the following CSF parameters relative to pretreatment levels:		
• Total cell counts	Mellergård <i>et al.</i> [2010]	NR
• CD4+ T-cell counts	Mancuso <i>et al.</i> [2014], Villar <i>et al.</i> [2012]	NR
• B-cell counts (especially CD5+)	Mancuso <i>et al.</i> [2014], Villar <i>et al.</i> [2012]	NR
• Proportion of patients with OCBs	Mancuso <i>et al.</i> [2014], Villar <i>et al.</i> [2012]	NR
• IgM and to a lesser extent IgG OCB indices	Mancuso <i>et al.</i> [2014], Villar <i>et al.</i> [2012]	NR
• IgG concentration	Warnke <i>et al.</i> [2014]	NR
• IgG production, represented by IgG _{IF}	Harrer <i>et al.</i> [2013]	NR
• IgM concentration	Warnke <i>et al.</i> [2014]	NR
• IgG CSF index	Warnke <i>et al.</i> [2014]	NR
• Concentrations of pro-inflammatory cytokines (IL-1 β , IL-6, IL-8)	Mellergård <i>et al.</i> [2010]	NR
• Concentrations of chemokines associated with both Th1 (CXCL9, CXCL10, CXCL11) and Th2 immunity (CCL22)	Mellergård <i>et al.</i> [2010]	NR
• CXCL13 concentration	Sellebjerg <i>et al.</i> [2009]	Romme Christensen <i>et al.</i> [2014]
• Osteopontin concentration	Khademi <i>et al.</i> [2009]	Romme Christensen <i>et al.</i> [2014]
• Neurofilament light chain concentration	Gunnarsson <i>et al.</i> [2011]	Romme Christensen <i>et al.</i> [2014]
Decreased microglial activation relative to pretreatment levels	Maoz <i>et al.</i> [2014], Politis <i>et al.</i> [2013] ¹	Politis <i>et al.</i> [2013]
Increased total N-acetylaspartate, creatine, phosphocreatine and glutamate in lesional white matter relative to pretreatment levels	Wiebenga <i>et al.</i> [2015]	NR
Reduced accumulation of T2-weighted lesions observed by MRI relative to comparator	Polman <i>et al.</i> [2006], Rudick <i>et al.</i> [2006]	NR
Suppressed development of new T1 gadolinium-enhanced lesions relative to comparator	Miller <i>et al.</i> [2003], Polman <i>et al.</i> [2006], Rudick <i>et al.</i> [2006]	Miller <i>et al.</i> [2003]
Reduced evolution of new gadolinium-enhanced to T1-hypointense lesions	Dalton <i>et al.</i> [2004]	Dalton <i>et al.</i> [2004]
Reduction in the rate of volume loss relative to comparator	Miller <i>et al.</i> [2007], Radue <i>et al.</i> [2010], Rinaldi <i>et al.</i> [2012]	NR
Enhanced tissue integrity on MRI diffusor tensor imaging relative to pretreatment levels	Fox <i>et al.</i> [2011]	NR
¹ Politis <i>et al.</i> [2013] study population included eight RRMS and two SPMS patients. CNS, central nervous system; CSF, cerebrospinal fluid; IgG, immunoglobulin type G; IgG _{IF} , relative intrathecal IgG fraction; IgM, immunoglobulin type M; IL, interleukin; MRI, magnetic resonance imaging; NR, not reported; OCB, oligoclonal band; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.		

In addition to its effects on CSF cell counts and OCBs, natalizumab affects other markers of inflammation in the CSF and plasma. In a longitudinal study of RRMS, patients starting natalizumab therapy [Mellergård *et al.* 2010], had baseline levels of some pro-inflammatory cytokines and chemokines that were significantly higher than in patients with other noninflammatory neurological disorders (for CSF comparisons) or healthy blood donors (for plasma comparisons). Over 1 year, natalizumab therapy resulted in marked declines in total CSF cell counts and CSF levels of pro-inflammatory cytokines (interleukin [IL]-1 β , IL-6 and IL-8) and chemokines associated with both T helper (Th) type 1 (CXCL9, CXCL10 and CXCL11) and Th2 immunity (CCL22), as well as declines in plasma levels of some cytokines [Mellergård *et al.* 2010]. In a separate longitudinal study, RRMS patients treated with natalizumab for 1 year showed an increase in the percentage of activated CD4+ and CD8+ T cells producing pro-inflammatory cytokines in the peripheral blood that was thought to be due to sequestration of activated cells in the peripheral circulation [Kivisäkk *et al.* 2009]. Furthermore, most RRMS patients treated with natalizumab have undetectable, presumably normal CSF levels of the B-cell chemokine CXCL13 [Sellebjerg *et al.* 2009], which is expressed in demyelinating lesions [Krumbholz *et al.* 2006] and is associated with the formation of ectopic lymphoid tissue. Collectively, these data illustrate the wide-reaching effects of natalizumab on various CSF markers of inflammation known to be associated with disease activity in RRMS.

Other effects of natalizumab in RRMS

Monoclonal antibodies including natalizumab are thought to reach levels in the intrathecal compartment in the range of approximately 0.1–1.0% of their respective serum levels [Deng *et al.* 2012]. Consistent with this estimate, a recent study of 15 natalizumab-treated patients with MS (tested 1.0–7.4 weeks after the last natalizumab infusion) found that the median natalizumab concentration was 50.9 $\mu\text{g/ml}$ (range 4.3–109.0) in the serum and 0.11 $\mu\text{g/ml}$ (range 0.01–0.42) in the CSF [Harrer *et al.* 2015]. When present within the intrathecal compartment, natalizumab may have the potential to exert local direct effects on intrathecal CNS inflammation in addition to its known impact on peripheral leukocyte migration. A CSF substudy of ASCEND will provide further information

regarding the biological effects of natalizumab in the CNS [ClinicalTrials.gov identifier: NCT01416181].

It has been suggested that natalizumab may inhibit the interaction of $\alpha 4\beta 1$ -expressing leukocytes with ligands in the extracellular matrix in the CNS, such as osteopontin and fibronectin, inhibiting further leukocyte recruitment and thereby contributing to the suppression of intrathecal inflammation [Yaldizli and Putzki, 2009]. Osteopontin is present at elevated levels in the plasma of MS patients compared with healthy control subjects [Kivisäkk *et al.* 2014], and at elevated levels in the CSF of MS patients compared with patients with other neurological diseases [Khademi *et al.* 2009]. Osteopontin is expressed in the lesions of progressive MS patients and has been found to be elevated in the blood [Hur *et al.* 2007] and CSF [Szalardy *et al.* 2013] of MS patients during relapse, decreasing significantly during recovery from relapse [Börnsen *et al.* 2011]. Natalizumab treatment has been shown to decrease osteopontin levels in the CSF [Khademi *et al.* 2009] and plasma [Kivisäkk *et al.* 2014] of RRMS patients. In a study of RRMS patients receiving natalizumab for 2 years, mean plasma osteopontin levels decreased by approximately 31% ($p < 0.005$), and this decrease correlated with significant ($p < 0.005$) improvements in mean global cognitive impairment index values [Iaffaldano *et al.* 2014].

Fibronectin is expressed in chronically demyelinated MS lesions [van Horssen *et al.* 2006], where it appears to accumulate [Stoffels *et al.* 2013]. Natalizumab treatment in patients with RRMS significantly reduced the adhesion of *ex vivo* peripheral blood mononuclear cells to activated transfected human brain microvascular endothelial cells (THBMECs) under flow conditions; fibronectin containing the CS1 region was identified as the ligand of $\alpha 4$ -integrin on the THBMECs [Man *et al.* 2009].

Pathophysiology of SPMS

Demyelination and loss of axons and neurons

SPMS is characterized by chronic white matter and gray matter neuro-axonal degeneration, cortical demyelination and acceleration of CNS tissue loss, resulting in prominent brain volume loss against a background of global innate inflammatory responses in the CNS parenchyma and adaptive immune responses in the meninges [Friese

et al. 2014; Mahad *et al.* 2015; Reynolds *et al.* 2011]. Thus, a cascade of mechanisms, including cellular and humoral inflammation, oxidative damage, iron accumulation and mitochondrial damage have been hypothesized to collaboratively induce the sustained CNS tissue damage responsible for the progressive accumulation of disability characteristic of SPMS [Friese *et al.* 2014; Mahad *et al.* 2015; Reynolds *et al.* 2011]. Patients with SPMS at autopsy demonstrate slow expansion of pre-existing demyelinating white matter plaques, the borders of which show active demyelination and axonal injury with infiltration by small numbers of diverse inflammatory cells, including T cells, B cells (predominantly in perivascular cuffs), plasma cells, monocytes and activated microglia [Frischer *et al.* 2009; Kutzelnigg *et al.* 2005; Reynolds *et al.* 2011].

Brain volume loss in SPMS affects cortical gray matter much more than white matter [Fisniku *et al.* 2008; Furby *et al.* 2010]. Subpial lesions are the most abundant type of cortical lesion in SPMS [Kutzelnigg *et al.* 2005; Magliozzi *et al.* 2007; Moll *et al.* 2008], and appear to be specific to MS, as they are absent in human non-MS inflammatory and noninflammatory brain diseases [Kutzelnigg *et al.* 2005; Mahad *et al.* 2015; Moll *et al.* 2008]. Diffuse injury of normal-appearing white matter (NAWM) is also a hallmark of SPMS [Kutzelnigg *et al.* 2005], with prominent widespread white matter-microglial activation and perivascular inflammation, accompanied by structural changes at the nodes of Ranvier [Howell *et al.* 2010] despite a paucity of gadolinium-enhanced and new T2 lesions on brain MRI [Cohen *et al.* 2002; Kutzelnigg *et al.* 2005]. The resultant neuro-axonal loss is reflected in increased sodium concentration within lesions, NAWM and cortical and deep gray matter, with higher concentrations seen in SPMS patients and in MS patients with greater levels of disability across the MS spectrum [Paling *et al.* 2013].

It has been speculated that disability progression in SPMS may result from neurodegeneration, independent of inflammation [Lassmann, 2007a]. Evidence for this includes data from serial MRI studies showing that gadolinium-enhanced lesions are less common in primary progressive MS (PPMS) patients and SPMS patients without superimposed relapses [Kidd *et al.* 1996]. Similarly, areas of cortical demyelination in patients with chronic forms of MS (SPMS and PPMS) do not demonstrate increased leukocyte

infiltration or altered distribution of lymphocyte subsets or microglial activation [Bø *et al.* 2003]. In contrast, cortical demyelinating lesions in patients with early MS are generally inflammatory, as demonstrated in a study of biopsy specimens, which also revealed that meningeal inflammation was topographically associated with cortical demyelination: in patients who had sufficient meningeal tissue for study [Lucchinetti *et al.* 2011]. The relatively noninflammatory nature of cortical demyelinating lesions in SPMS is consistent with the observation that despite reducing focal white matter inflammation measured by gadolinium enhancement, immunotherapies have had little effect on brain volume loss and disability progression in progressive forms of MS [Coles *et al.* 2006]. However, a detailed autopsy study using age-matched control subjects without neurological disease found that neurodegeneration in RRMS and SPMS does not occur above the levels observed in normal aging in the absence of CNS inflammation [Frischer *et al.* 2009]. This supports the contrary hypothesis that disease progression in SPMS results from chronic CNS-based inflammation trapped behind the BBB, which causes widespread, lower-grade, ongoing diffuse demyelination and axonal injury [Kutzelnigg *et al.* 2005; Lassmann, 2007b].

CXCL13 and CNS ectopic lymphoid tissue

B cells and plasma cells are found in the CSF and focal lesions of MS patients [Esiri, 1977; Lucchinetti *et al.* 2000], and are responsible for intrathecal production of OCBs [Colombo *et al.* 2000; Holmøy, 2009]. In addition, several studies in MS and animal models of autoimmune CNS demyelination, such as experimental autoimmune encephalitis (EAE), have documented prominent intrathecal inflammation [Aloisi *et al.* 2008; Columba-Cabezas *et al.* 2003, 2006; Magliozzi *et al.* 2004, 2007; Serafini *et al.* 2004, 2006]. A notable finding in SPMS is the presence of mature meningeal ectopic lymphoid follicle (ELF)-like structures in 41% of cases who had autopsy brain tissues available for examination from the UK MS tissue bank [Magliozzi *et al.* 2007]. Moreover, when the presence of either ELF-like structures or more diffuse lymphocyte infiltrates was assessed in a larger sample of SPMS autopsies, evidence of meningeal lymphocytic infiltration was found in the majority (87%) [Howell *et al.* 2011].

CXCL13 plays a key role in the accumulation and maintenance of ectopic lymphoid tissues [Aloisi

et al. 2008; Gunn *et al.* 1998; Legler *et al.* 1998; Magliozzi *et al.* 2004; Serafini *et al.* 2004] and is present at elevated levels in the CSF [Krumbholz *et al.* 2006; Sellebjerg *et al.* 2009; Edwards *et al.* 2013] and serum [Festa *et al.* 2009] of patients with active MS. Although CSF levels of CXCL13 and osteopontin in SPMS patients are similar to or lower than those in stable RRMS patients, they correlate with CSF markers of neuro-axonal damage and demyelination [Edwards *et al.* 2013; Khademi *et al.* 2011; Romme Christensen *et al.* 2013]. Importantly, CSF levels of CXCL13 are greater in OCB-positive than OCB-negative MS patients [Khademi *et al.* 2011] and are positively correlated with CSF B-cell counts as well as the IgG-CSF index [Khademi *et al.* 2011; Krumbholz *et al.* 2006; Ragheb *et al.* 2011; Sellebjerg *et al.* 2009].

Microglial activation

Although macrophages recruited into the CNS and resident brain macrophages (microglia) react to any CNS injury, playing a well-established role in clearing myelin debris [Vogel *et al.* 2013], it is increasingly recognized that these cells may also be pathogenic effector cells in MS [Hickey, 1999]. Positron emission tomography (PET) studies have shown that activated microglia/macrophages are increased in patients with RRMS and SPMS compared with healthy controls [Politis *et al.* 2012] and that their increased presence was correlated with disability assessed by the Expanded Disability Status Scale (EDSS) [Politis *et al.* 2012]. This most likely reflects changes occurring in the gray matter of the SPMS patients, since another study found no increase in activated microglia in patients with RRMS relative to healthy controls [Takano *et al.* 2013]. In a more recent PET study, the presence of activated microglia/macrophages was increased diffusely in NAWM but not in the cortical gray matter of patients with SPMS relative to healthy controls [Rissanen *et al.* 2014]. The authors hypothesized that the difference between their findings and those of Politis and colleagues [Politis *et al.* 2012] might have resulted from different data processing and modeling approaches; although they cannot rule out neuropathological differences between the two relatively small study groups [Rissanen *et al.* 2014].

Regardless of their location within the CNS, the pro-inflammatory and demyelinating activity of activated resident microglia and CNS-infiltrating

blood-derived macrophages may be crucial for MS lesion formation [Koning *et al.* 2007; Trapp and Nave, 2008; Weiner, 2008]. In progressive forms of MS, active demyelination and axonal injury in white matter are associated with microglial activation and oxidative burst in microglia. This in turn leads to gene deletions in neuronal mitochondrial DNA and clonal expansion of defective mitochondria, which may lead to hypoxia and apoptosis in the final pathways of tissue injury in MS lesions [Mahad *et al.* 2015]. The importance of activated microglia/macrophages in the pathogenesis of SPMS is highlighted by an autopsy study of patients who died after therapeutic chemotherapy followed by bone marrow transplantation, which revealed an absence of meningeal B-cell follicles, B cells and plasma cells in the brain, while there was ongoing axonal injury associated with activated macrophages/microglia [Metz *et al.* 2007].

Potential impact of natalizumab on pathophysiological mechanisms in SPMS

As mentioned previously, data exist showing that natalizumab reduces CSF levels of chemokines relevant to the formation and maintenance of meningeal lymphocyte aggregates, which may be involved in the pathophysiology of SPMS. In addition to the effects observed with natalizumab treatment in patients with RRMS [Sellebjerg *et al.* 2009], reductions of CXCL13, osteopontin and neurofilament in the CSF have also been demonstrated in patients with progressive MS (SPMS or PPMS) treated with natalizumab compared with pretreatment levels [Romme Christensen *et al.* 2014] (Table 1). In contrast, no change was seen in these CSF biomarkers of inflammation, axonal damage or demyelination after 1 year of treatment with placebo or MBP₈₂₋₉₈ peptide [Romme Christensen *et al.* 2013], a synthetic peptide with amino acid sequence corresponding to amino acids 82–98 of myelin basic protein, in patients with SPMS [Freedman *et al.* 2011]. The fact that natalizumab has effects in SPMS patients on biomarkers that are not affected by other compounds such as MBP₈₂₋₉₈ suggests a potential role for natalizumab in this population. However, further exploration of specific biomarkers is needed to better understand their relevance in SPMS. For example, while osteopontin is known to be expressed in MS lesions [Chabas *et al.* 2001], its expression within the meningeal space has not been demonstrated. Likewise, the relevance of fibronectin expression in chronic MS lesions [van

Table 2. Key eligibility criteria in ASCEND [ClinicalTrials.gov identifier: NCT01416181].

<p>Key inclusion criteria</p> <ul style="list-style-type: none"> • SPMS defined as relapsing–remitting disease followed by progression of disability independent of or not explained by MS relapses for at least two years • Confirmed evidence of disease progression independent of clinical relapses during the prior year • MS severity score of 4 or higher • Age 18–58 years • EDSS score of 3.0–6.5, inclusive <p>Key exclusion criteria</p> <ul style="list-style-type: none"> • RRMS or primary progressive MS as defined by the revised McDonald Committee criteria • Clinical relapse (within three months) prior to randomization • T25FW test of >30 seconds during the screening period <p>EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; T25FW, Timed 25-Foot Walk.</p>
--

Horssen *et al.* 2006] is unclear, but it is possible that natalizumab's effect on the binding of peripheral blood cells to fibronectin [Man *et al.* 2009] could contribute to reductions in intrathecal inflammation.

Since natalizumab's target, the VLA-4 integrin, plays a major role in B-cell trafficking across the BBB [Stüve *et al.* 2009], there are fewer B cells in the CSF of natalizumab-treated patients with MS than in MS patients who are not treated with natalizumab [Stüve, 2008; Stüve *et al.* 2009]. It is possible that natalizumab's inhibition of B-cells ingressing through a compromised BBB may have a positive impact in SPMS, given the presence of B cells in lymphoid-like follicles of SPMS. However, once the immune response becomes largely confined to the CNS behind a relatively intact BBB, local expansion of B-cell clones within the lymphoid structures may primarily account for increases in intrathecal B cells [Reynolds *et al.* 2011].

Natalizumab also appears to modulate microglial function by inhibiting the transmigration of VLA-4-expressing macrophages and dendritic cells into the CNS [de Andres *et al.* 2012; Khademi *et al.* 2009; Mindur *et al.* 2014]. This has been demonstrated in studies using markers of microglial activation with PET imaging. Natalizumab treatment of RRMS patients resulted in decreased cerebral microglial activation [Maoz *et al.* 2014], and reductions in cortical microglial activation following a year of natalizumab therapy were associated with stabilization of physical disability [Politis *et al.* 2013]. Finally, inhibition of VLA-4 on dendritic cells may decrease the capacity of these and other cells to efficiently present antigen and

activate T cells [Börnsen *et al.* 2012; de Andres *et al.* 2012; del Pilar Martin *et al.* 2008]. These effects on CNS macrophage infiltration, microglial and/or blood-derived macrophage activation and dendritic-cell function may be directly, and more likely indirectly, beneficial in SPMS by decreasing the number of inflammatory cells in the perivascular and subarachnoid spaces.

Clinical experience with natalizumab in SPMS

The efficacy of natalizumab in SPMS patients is currently being evaluated in ASCEND, a multi-center, randomized, double-blind, placebo-controlled phase IIIb study of the efficacy of natalizumab in reducing disability progression independently of relapses [ClinicalTrials.gov identifier: NCT01416181]. Criteria for enrollment in ASCEND are shown in Table 2.

While no previous trial has assessed the efficacy of natalizumab in an exclusively SPMS population, retrospective analyses [Cadavid *et al.* 2013] have been conducted with data from natalizumab-treated SPMS patients who participated in the following two studies: (1) a 6-month placebo-controlled phase IIb study (MS231) of RRMS and relapsing SPMS patients, who were monitored for an additional 6 months after the randomized treatment period [Miller *et al.* 2003] and (2) an 8-month open-label phase Ib study (MS102) of natalizumab in RRMS patients, or SPMS patients with a history of disability progression without superimposed relapses [Woodworth *et al.* 2013]. A total of 66 patients with SPMS from MS231 and 52 patients with SPMS from MS102 were included in the

analyses. Given the relatively short durations of the MS231 and MS102 studies and the observation that EDSS in patients with a baseline score > 5.0 is less responsive to change than T25FW [Cadavid *et al.* 2010], a *post hoc* analysis of efficacy on ambulation was conducted using the T25FW. ‘Responders’ were defined as patients who walked faster in two of three post-baseline T25FW assessments than in their best pre-dose walk. In MS231, the percentage of T25FW responders was higher in the natalizumab group (24% [10 of 41]) than in the placebo group (12% [3 of 25]), and in MS102, which lacked a placebo group, a similar proportion of T25FW responders was seen with natalizumab (22% [10 of 45]) [Cadavid *et al.* 2013]. The potential therapeutic efficacy of natalizumab in SPMS is further supported by the results of a larger *post hoc* T25FW responder analysis of 548 SPMS patients and a more disabled subset of 358 RRMS patients (baseline EDSS score ≥ 3.5 and T25FW time > 5 seconds) enrolled in previous clinical studies [Cadavid *et al.* 2013]. The proportion of responders, using various definitions of improvement in T25FW (e.g. faster walking time over a shorter [6–9 month] or longer [24–30 month] treatment period relative to the best predose walking time), was greater in patients treated for up to 30 months with natalizumab alone, or in combination with intramuscular interferon beta-1a (IFN β -1a) than among patients given placebo or IFN β -1a alone. Finally, in a phase IIa study in patients with PPMS and SPMS, EDSS scores improved and T25FW times were stable after 60 weeks of natalizumab therapy [Romme Christensen *et al.* 2014].

In summary, while the above studies were not designed to assess whether natalizumab is effective in reducing disability progression unrelated to relapses in an SPMS population, the exploratory findings formidably support the prospective evaluation of natalizumab in the ongoing ASCEND SPMS study.

Discussion

As RRMS and SPMS represent a continuum of the same disease [Lublin *et al.* 2014], mechanisms that predominate in RRMS or SPMS are likely to overlap in individual patients, particularly during the gradual transition from RRMS to SPMS. In practice, diagnosis of SPMS can be challenging given that (1) superimposed relapses during the transition period of RRMS to SPMS may make it difficult for the clinician to identify and

differentiate insidious disability progression from stepwise accumulation of disability due to relapses [Lublin *et al.* 2014] and (2) the manifestations of progression may be more subtle at onset [Rudick *et al.* 2014], particularly in patients with less severe disease as reflected by MSSS [Roxburgh *et al.* 2005]. These factors are accounted for in the eligibility criteria of ASCEND (see Table 2, first three bullets of inclusion criteria).

The overlap between RRMS and SPMS suggests treatments that are efficacious in RRMS patients might also benefit SPMS patients. Some treatments approved for RRMS have been studied in SPMS [Ontaneda *et al.* 2015]. However, anti-inflammatory treatments investigated for treating SPMS should have a plausible biological mechanism to address the CNS-compartmentalized inflammatory process that predominates in SPMS. As natalizumab prevents the transmigration of leukocytes across the BBB [Yednock *et al.* 1992], clinical benefit might be facilitated not only in RRMS but also in SPMS. The net effect of natalizumab treatment in SPMS may be a reduction in chronic intrathecal (meningeal and CNS parenchymal) inflammation and production of pro-inflammatory cytokines, which in turn may decrease the ongoing CNS demyelination, axonal injury and neuronal loss that are key drivers of progression in SPMS.

In addition to the clinical benefits of natalizumab therapy seen in phase III studies of patients with relapsing forms of MS [Polman *et al.* 2006; Rudick *et al.* 2006], other studies have revealed evidence of enhanced tissue integrity and improved axonal metabolism in some natalizumab-treated RRMS patients, suggesting that reducing inflammation may enable endogenous repair mechanisms to take place during natalizumab therapy [Fox *et al.* 2011; Wiebenga *et al.* 2015]. While it is unclear whether or to what extent this may be pertinent in SPMS, it is encouraging that natalizumab treatment improved magnetization transfer ratios in the cerebral cortex and normal-appearing white matter of SPMS/PPMS patients [Romme Christensen *et al.* 2014].

In conclusion, clinical findings from short-term studies with natalizumab in patients with SPMS, combined with evidence indicating its potential to exert beneficial effects on multiple aspects of the pathophysiology associated with the development of SPMS, suggest that natalizumab represents a promising candidate for the treatment of SPMS,

a condition for which available therapeutic options are limited. The efficacy of natalizumab for treatment of secondary progression, independent of relapses, is being evaluated in the large, ongoing phase IIIb ASCEND study. There is a high unmet need for effective treatments for SPMS, and data from ASCEND should demonstrate whether preliminary findings with natalizumab in short-term studies and its multiple potentially beneficial mechanisms of action of VLA-4 blockade translate into clinically meaningful efficacy in this serious disease.

Acknowledgment

Mary Goodsell, on behalf of Infusion Communications, wrote the first draft of the paper based on input from authors, and Joshua Safran of Infusion Communications copyedited and styled the manuscript per journal requirements. Biogen reviewed and provided feedback on the manuscript to the authors. The authors had full editorial control of the manuscript, made extensive contributions to follow-up drafts, and provided final approval of all content.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Biogen provided funding for editorial support in the development of this manuscript.

Conflict of Interest Statement

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: F.S. has served on scientific advisory boards, been on the steering committees of clinical trials, served as a consultant, received support for congress participation, received speaker honoraria, or received research support for his laboratory from Bayer Schering, Biogen, Genzyme, Lundbeck, Merck Serono, Novo Nordisk, Novartis, Sanofi, Schering Plough and Teva. D.C. and D.S. are full-time employees of Biogen and own stock/stock options in Biogen. D.M. was a full-time employee of Biogen and owned stock/stock options in Biogen when this manuscript was developed. L.M.V. has served as a consultant, received support for congress participation, received speaker honoraria, or received research support for her laboratory from Bayer Schering, Binding Site, Biogen, Genzyme, Merck Serono, Novartis and Teva. R.R. has received speaker honoraria from Biogen and Novartis and research support from EMD Serono.

References

- Aloisi, F., Columba-Cabezas, S., Franciotta, D., Rosicarelli, B., Magliozzi, R., Reynolds, R. *et al.* (2008) Lymphoid chemokines in chronic neuroinflammation. *J Neuroimmunol* 198: 106–112.
- Biogen (2015a) TYSABRI® (natalizumab) injection. Full prescribing information. <http://www.tysabri.com/prescribingInfo> (accessed 29 July 2015).
- Biogen (2015b) TYSABRI summary of product characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000603/WC500044686.pdf (accessed 30 June 2015).
- Bø, L., Vedeler, C., Nyland, H., Trapp, B. and Mørk, S. (2003) Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Mult Scler* 9: 323–331.
- Börnsen, L., Christensen, J., Rätzer, R., Oturai, A., Sørensen, P., Søndergaard, H. *et al.* (2012) Effect of natalizumab on circulating CD4+ T-cells in multiple sclerosis. *PLoS One* 7: e47578.
- Börnsen, L., Khademi, M., Olsson, T., Sørensen, P. and Sellebjerg, F. (2011) Osteopontin concentrations are increased in cerebrospinal fluid during attacks of multiple sclerosis. *Mult Scler* 17: 32–42.
- Cadavid, D., Jurgensen, S. and Lee, S. (2013) Impact of natalizumab on ambulatory improvement in secondary progressive and disabled relapsing–remitting multiple sclerosis. *PLoS One* 8: e53297.
- Cadavid, D., Tang, Y. and O’Neill, G. (2010) Responsiveness of the Expanded Disability Status Scale (EDSS) to disease progression and therapeutic intervention in progressive forms of multiple sclerosis. *Rev Neurol* 51: 321–329.
- Chabas, D., Baranzini, S., Mitchell, D., Bernard, C., Rittling, S., Denhardt, D. *et al.* (2001) The influence of the pro-inflammatory cytokine, osteopontin, on autoimmune demyelinating disease. *Science* 294: 1731–1735.
- Cohen, J., Cutter, G., Fischer, J., Goodman, A., Heidenreich, F., Kooijmans, M. *et al.* for the IMPACT Investigators (2002) Benefit of interferon beta-1a on MSFC progression in secondary progressive MS. *Neurology* 59: 679–687.
- Coles, A., Cox, A., Le Page, E., Jones, J., Trip, S., Deans, J. *et al.* (2006) The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol* 253: 98–108.
- Colombo, M., Dono, M., Gazzola, P., Roncella, S., Valetto, A., Chiorazzi, N. *et al.* (2000) Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. *J Immunol* 164: 2782–2789.

- Columba-Cabezas, S., Griguoli, M., Rosicarelli, B., Magliozzi, R., Ria, F., Serafini, B. *et al.* (2006) Suppression of established experimental autoimmune encephalomyelitis and formation of meningeal lymphoid follicles by lymphotoxin beta receptor-Ig fusion protein. *J Neuroimmunol* 179: 76–86.
- Columba-Cabezas, S., Serafini, B., Ambrosini, E. and Aloisi, F. (2003) Lymphoid chemokines CCL19 and CCL21 are expressed in the central nervous system during experimental autoimmune encephalomyelitis: implications for the maintenance of chronic neuroinflammation. *Brain Pathol* 13: 38–51.
- Compston, A. and Coles, A. (2008) Multiple sclerosis. *Lancet* 372: 1502–1517.
- Dalton, C., Miszkiel, K., Barker, G., MacManus, D., Pepple, T., Panzara, M. *et al.* (2004) Effect of natalizumab on conversion of gadolinium-enhancing lesions to T1 hypointense lesions in relapsing multiple sclerosis. *J Neurol* 251: 407–413.
- de Andrés, C., Teijeiro, R., Alonso, B., Sánchez-Madrid, F., Martínez, M., Guzmán de Villoria, V. *et al.* (2012) Long-term decrease in VLA-4 expression and functional impairment of dendritic cells during natalizumab therapy in patients with multiple sclerosis. *PLoS One* 7: e34103.
- del Pilar Martin, M., Cravens, P., Winger, R., Frohman, E., Racke, M., Eagar, T. *et al.* (2008) Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. *Arch Neurol* 65: 1596–1603.
- Deng, R., Jin, F., Prabhu, S. and Iyer, S. (2012) Monoclonal antibodies: what are the pharmacokinetic and pharmacodynamic considerations for drug development? *Expert Opin Drug Metab Toxicol* 8: 141–160.
- Edwards, K., Goyal, J., Plavina, T., Czerkowicz, J., Goelz, S., Ranger, A. *et al.* (2013) Feasibility of the use of combinatorial chemokine arrays to study blood and CSF in multiple sclerosis. *PLoS One* 8: e81007.
- Esiri, M. (1977) Immunoglobulin-containing cells in multiple-sclerosis plaques. *Lancet* 2: 478.
- Ferreira, D., Voevodskaya, O., Imrell, K., Stawiarz, L., Spulber, G., Wahlund, L. *et al.* (2014) Multiple sclerosis patients lacking oligoclonal bands in the cerebrospinal fluid have less global and regional brain atrophy. *J Neuroimmunol* 274: 149–154.
- Festa, E., Hankiewicz, K., Kim, S., Skurnick, J., Wolansky, L., Cook, S. *et al.* (2009) Serum levels of CXCL13 are elevated in active multiple sclerosis. *Mult Scler* 15: 1271–1279.
- Fisniku, L., Chard, D., Jackson, J., Anderson, V., Altmann, D., Miszkiel, K. *et al.* (2008) Gray matter atrophy is related to long-term disability in multiple sclerosis. *Ann Neurol* 64: 247–254.
- Fox, R., Cronin, T., Lin, J., Wang, X., Sakaie, K., Ontaneda, D. *et al.* (2011) Measuring myelin repair and axonal loss with diffusion tensor imaging. *AJNR Am J Neuroradiol* 32: 85–91.
- Freedman, M., Bar-Or, A., Oger, J., Traboulsee, A., Patry, D., Young, C. *et al.* (2011) A phase III study evaluating the efficacy and safety of MBP8298 in secondary progressive MS. *Neurology* 77: 1551–1560.
- Friese, M., Schattling, B. and Fugger, L. (2014) Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat Rev Neurol* 10: 225–238.
- Frischer, J., Bramow, S., Dal-Bianco, A., Lucchinetti, C., Rauschka, H., Schmidbauer, M. *et al.* (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 132: 1175–1189.
- Furby, J., Hayton, T., Altmann, D., Brenner, R., Chataway, J., Smith, K. *et al.* (2010) A longitudinal study of MRI-detected atrophy in secondary progressive multiple sclerosis. *J Neurol* 257: 1508–1516.
- Gunn, M., Ngo, V., Ansel, K., Ekland, E., Cyster, J. and Williams, L. (1998) A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 391: 799–803.
- Gunnarsson, M., Malmeström, C., Axelsson, M., Sundström, P., Dahle, C., Vrethem, M. *et al.* (2011) Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. *Ann Neurol* 69: 83–89.
- Harrer, A., Pilz, G., Wipfler, P., Oppermann, K., Sellner, J., Hitzl, W. *et al.* (2015) High interindividual variability in the CD4/CD8 T-cell ratio and natalizumab concentration levels in the cerebrospinal fluid of patients with multiple sclerosis. *Clin Exp Immunol* 180: 383–392.
- Harrer, A., Tumani, H., Niendorf, S., Lauda, F., Geis, C., Weishaupt, A. *et al.* (2013) Cerebrospinal fluid parameters of B cell-related activity in patients with active disease during natalizumab therapy. *Mult Scler* 19: 1209–1212.
- Hickey, W. (1999) The pathology of multiple sclerosis: a historical perspective. *J Neuroimmunol* 98: 37–44.
- Holmøy, T. (2009) The discovery of oligoclonal bands: a 50-year anniversary. *Eur Neurol* 62: 311–315.
- Howell, O., Reeves, C., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S. *et al.* (2011) Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain* 134: 2755–2771.
- Howell, O., Rundle, J., Garg, A., Komada, M., Brophy, P. and Reynolds, R. (2010) Activated microglia mediate axoglial disruption that contributes

- to axonal injury in multiple sclerosis. *J Neuropathol Exp Neurol* 69: 1017–1033.
- Hur, E., Youssef, S., Haws, M., Zhang, S., Sobel, R. and Steinman, L. (2007) Osteopontin-induced relapse and progression of autoimmune brain disease through enhanced survival of activated T cells. *Nat Immunol* 8: 74–83.
- Iaffaldano, P., Ruggieri, M., Viterbo, R., Mastrapasqua, M. and Trojano, M. (2014) The improvement of cognitive functions is associated with a decrease of plasma osteopontin levels in natalizumab-treated relapsing multiple sclerosis. *Brain Behav Immun* 35: 176–181.
- Khademi, M., Bornsen, L., Rafatnia, F., Andersson, M., Brundin, L., Piehl, F. *et al.* (2009) The effects of natalizumab on inflammatory mediators in multiple sclerosis: prospects for treatment-sensitive biomarkers. *Eur J Neurol* 16: 528–536.
- Khademi, M., Kockum, I., Andersson, M., Iacobaeus, E., Brundin, L., Sellebjerg, F. *et al.* (2011) Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. *Mult Scler* 17: 335–343.
- Kidd, D., Thorpe, J., Kendall, B., Barker, G., Miller, D., McDonald, W. *et al.* (1996) MRI dynamics of brain and spinal cord in progressive multiple sclerosis. *J Neurol Neurosurg Psychiatry* 60: 15–19.
- Kivisäkk, P., Healy, B., Francois, K., Gandhi, R., Gholipour, T., Egorova, S. *et al.* (2014) Evaluation of circulating osteopontin levels in an unselected cohort of patients with multiple sclerosis: relevance for biomarker development. *Mult Scler* 20: 438–444.
- Kivisäkk, P., Healy, B., Viglietta, V., Quintana, F., Hootstein, M., Weiner, H. *et al.* (2009) Natalizumab treatment is associated with peripheral sequestration of pro-inflammatory T cells. *Neurology* 72: 1922–1930.
- Koning, N., Bö, L., Hoek, R. and Huitinga, I. (2007) Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. *Ann Neurol* 62: 504–514.
- Krumbholz, M., Meinel, I., Kümpfel, T., Hohlfeld, R. and Meinel, E. (2008) Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. *Neurology* 71: 1350–1354.
- Krumbholz, M., Theil, D., Cepok, S., Hemmer, B., Kivisäkk, P., Ransohoff, R. *et al.* (2006) Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. *Brain* 129: 200–211.
- Kutzelnigg, A., Lucchinetti, C., Stadelmann, C., Brück, W., Rauschka, H., Bergmann, M. *et al.* (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128: 2705–2712.
- Lassmann, H. (2007a) Multiple sclerosis: is there neurodegeneration independent from inflammation? *J Neurol Sci* 259: 3–6.
- Lassmann, H. (2007b) New concepts on progressive multiple sclerosis. *Curr Neurol Neurosci Rep* 7: 239–244.
- Legler, D., Loetscher, M., Roos, R., Clark-Lewis, I., Baggiolini, M. and Moser, B. (1998) B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* 187: 655–660.
- Lublin, F., Reingold, S., Cohen, J., Cutter, G., Sørensen, P., Thompson, A. *et al.* (2014) Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 83: 278–286.
- Lucchinetti, C., Brück, W., Parisi, J., Scheithauer, B., Rodriguez, M. and Lassmann, H. (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 47: 707–717.
- Lucchinetti, C., Popescu, B., Bunyan, R., Moll, N., Roemer, S., Lassmann, H. *et al.* (2011) Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med* 365: 2188–2197.
- Magliozzi, R., Columba-Cabezas, S., Serafini, B. and Aloisi, F. (2004) Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol* 148: 11–23.
- Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M. *et al.* (2007) Meningeal B-cell follicles in secondary progressive multiple sclerosis associated with early onset of disease and severe cortical pathology. *Brain* 130: 1089–1104.
- Mahad, D., Trapp, B. and Lassmann, H. (2015) Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol* 14: 183–193.
- Man, S., Tucky, B., Bagheri, N., Li, X., Kochar, R. and Ransohoff, R. (2009) Alpha4-Integrin/FN-CS1-mediated leukocyte adhesion to brain microvascular endothelial cells under flow conditions. *J Neuroimmunol* 210: 92–99.
- Mancuso, R., Franciotta, D., Rovaris, M., Caputo, D., Sala, A., Hernis, A. *et al.* (2014) Effects of natalizumab on oligoclonal bands in the cerebrospinal fluid of multiple sclerosis patients: a longitudinal study. *Mult Scler* 20: 1900–1903.
- Maoz, A., Monohan, E., Kothari, P., Solnes, L., Nealon, N., Perumal, J. *et al.* (2014) Reduction in microglia activation measured by [¹¹C]PK11195-PET in patients treated with natalizumab [abstract P965]. *Mult Scler* 20: 488–489.

- Mellergård, J., Edström, M., Vrethem, M., Ernerudh, J. and Dahle, C. (2010) Natalizumab treatment in multiple sclerosis: marked decline of chemokines and cytokines in cerebrospinal fluid. *Mult Scler* 16: 208–217.
- Metz, I., Lucchinetti, C., Openshaw, H., Garcia-Merino, A., Lassmann, H., Freedman, M. *et al.* (2007) Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. *Brain* 130: 1254–1262.
- Miller, D., Khan, O., Sheremata, W., Blumhardt, L., Rice, G., Libonati, M. *et al.*; International Natalizumab Multiple Sclerosis Trial Group. (2003) A controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 348: 15–23.
- Miller, D., Soon, D., Fernando, K., MacManus, D., Barker, G., Yousry, T. *et al.* AFFIRM Investigators. (2007) MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. *Neurology* 68: 1390–1401.
- Milo, R. and Kahana, E. (2010) Multiple sclerosis: geoeidemiology, genetics and the environment. *Autoimmun Rev* 9: A387–A394.
- Mindur, J., Ito, N., Dhib-Jalbut, S. and Ito, K. (2014) Early treatment with anti-VLA-4 mAb can prevent the infiltration and/or development of pathogenic CD11b+CD4+ T cells in the CNS during progressive EAE. *PLoS One* 9: e99068.
- Moll, N., Rietsch, A., Ransohoff, A., Cossoy, M., Huang, D., Eichler, F. *et al.* (2008) Cortical demyelination in PML and MS: similarities and differences. *Neurology* 70: 336–343.
- Ontaneda, D., Fox, R. and Chataway, J. (2015) Clinical trials in progressive multiple sclerosis: lessons learned and future perspectives. *Lancet Neurol* 14: 208–223.
- Paling, D., Solanky, B., Riemer, F., Tozer, D., Wheeler-Kingshott, C., Kapoor, R. *et al.* (2013) Sodium accumulation is associated with disability and a progressive course in multiple sclerosis. *Brain* 136: 2305–2317.
- Politis, M., Giannetti, P., Niccolini, F., Su, P., Turkheimer, F., Waldman, A. *et al.* (2013) Decreased microglial activation precedes stabilization of disability in multiple sclerosis patients treated with natalizumab [abstract]. *Neurology* 80 (Suppl 21): 004.
- Politis, M., Giannetti, P., Su, P., Turkheimer, F., Keihaninejad, S., Wu, K. *et al.* (2012) Increased PK11195 PET binding in the cortex of patients with MS correlates with disability. *Neurology* 79: 523–530.
- Polman, C., O'Connor, P., Havrdova, Hutchinson, M., Kappos, L., Miller, D. *et al.* AFFIRM Investigators. (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 354: 899–910.
- Radue, E., Stuart, W., Calabresi, P., Confavreux, C., Galetta, S., Rudick, R. *et al.* for the SENTINEL Investigators (2010) Natalizumab plus interferon beta-1a reduces lesion formation in relapsing multiple sclerosis. *J Neurol Sci* 292: 28–35.
- Ragheb, S., Li, Y., Simon, K., VanHaerents, S., Galimberti, D., De Riz, M. *et al.* (2011) Multiple sclerosis: BAFF and CXCL13 in cerebrospinal fluid. *Mult Scler* 17: 819–829.
- Reynolds, R., Roncaroli, F., Nicholas, R., Radotra, B., Gveric, D. and Howell, O. (2011) The neuropathological basis of clinical progression in multiple sclerosis. *Acta Neuropathol* 122: 155–170.
- Rice, G., Hartung, H. and Calabresi, P. (2005) Anti-alpha4-integrin therapy for multiple sclerosis: mechanisms and rationale. *Neurology* 64: 1336–1342.
- Rinaldi, F., Calabrese, M., Seppi, D., Puthenparampil, M., Perini, P. and Gallo, P. (2012) Natalizumab strongly suppresses cortical pathology in relapsing–remitting multiple sclerosis. *Mult Scler* 18: 1760–1767.
- Rissanen, E., Tuisku, J., Rokka, J., Paavilainen, T., Parkkola, R., Rinne, J. *et al.* (2014) *In vivo* detection of diffuse inflammation in secondary progressive multiple sclerosis using PET imaging and the radioligand ¹¹C-PK11195. *J Nucl Med* 55: 939–944.
- Romme Christensen, J., Börnsen, L., Khademi, M., Olsson, T., Jensen, P., Sørensen, P. *et al.* (2013) CSF inflammation and axonal damage are increased and correlate in progressive multiple sclerosis. *Mult Scler* 19: 877–884.
- Romme Christensen, J., Ratzer, R., Börnsen, L., Lyksborg, M., Garde, E., Dyrby, T. *et al.* (2014) Natalizumab in progressive MS: results of an open-label, phase IIA, proof-of-concept trial. *Neurology* 82: 1499–1507.
- Roxburgh, R., Seaman, S., Masterman, T., Hensiek, A., Sawcer, S., Vukusic, S. *et al.* (2005) Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. *Neurology* 64: 1144–1151.
- Rudick, R., Larocca, N. and Hudson, L. for MSOAC (2014) Multiple Sclerosis Outcome Assessments Consortium: genesis and initial project plan. *Mult Scler* 20: 12–17.
- Rudick, R., Stuart, W., Calabresi, P., Confavreux, C., Galetta, S., Radue, E. *et al.* for the SENTINEL Investigators (2006) Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med* 354: 911–923.
- Sellebjerg, F., Börnsen, L., Khademi, M., Krakauer, M., Olsson, T., Frederiksen, J. *et al.* (2009) Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. *Neurology* 73: 2003–2010.

- Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. and Aloisi, F. (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol* 14: 164–174.
- Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., Capello, E., Mancardi, G. *et al.* (2006) Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. *J Neuropathol Exp Neurol* 65: 124–141.
- Stoffels, J., de Jonge, J., Stancic, M., Nomden, A., van Strien, M., Ma, D. *et al.* (2013) Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain* 136: 116–131.
- Stüve, O. (2008) The effects of natalizumab on the innate and adaptive immune system in the central nervous system. *J Neurol Sci* 274: 39–41.
- Stüve, O., Cravens, P., Frohman, E., Phillips, J., Remington, G., von Geldern, G. *et al.* (2009) Immunologic, clinical, and radiologic status 14 months after cessation of natalizumab therapy. *Neurology* 72: 396–401.
- Stüve, O., Marra, C., Bar-Or, A., Niino, M., Cravens, P., Cepok, S. *et al.* (2006) Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *Arch Neurol* 63: 1383–1387.
- Szalaryd, L., Zadori, D., Simu, M., Bencsik, K., Vecsei, L. and Klivenyi, P. (2013) Evaluating biomarkers of neuronal degeneration and neuroinflammation in CSF of patients with multiple sclerosis—osteopontin as a potential marker of clinical severity. *J Neurol Sci* 331: 38–42.
- Takano, A., Piehl, F., Hillert, J., Varrone, A., Nag, S., Gulyás, B. *et al.* (2013) *In vivo* TSPO imaging in patients with multiple sclerosis: a brain PET study with [18F]FEDAA1106. *EJNMMI Res* 3: 30.
- Trapp, B. and Nave, K. (2008) Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci* 31: 247–269.
- van Horsen, J., Bö, L., Dijkstra, C. and de Vries, H. (2006) Extensive extracellular matrix depositions in active multiple sclerosis lesions. *Neurobiol Dis* 24: 484–491.
- Villar, L., Garcia-Sánchez, M., Costa-Frossard, L., Espiño, M., Roldán, E., Páramo, D. *et al.* (2012) Immunological markers of optimal response to natalizumab in multiple sclerosis. *Arch Neurol* 69: 191–197.
- Villar, L., Picón, C., Costa-Frossard, L., Alenda, R., García-Caldentey, J., Espiño, M. *et al.* (2015) Cerebrospinal fluid immunological biomarkers associated with axonal damage in multiple sclerosis. *Eur J Neurol* 22: 1169–1175.
- Vogel, D., Vereyken, E., Glim, J., Heijnen, P., Moeton, M., van der Valk, P. *et al.* (2013) Macrophages in inflammatory multiple sclerosis lesions have an intermediate activation status. *J Neuroinflammation* 10: 35.
- von Glehn, F., Farias, A., de Oliveira, A., Damasceno, A., Longhini, A., Oliveira, E. *et al.* (2012) Disappearance of cerebrospinal fluid oligoclonal bands after natalizumab treatment of multiple sclerosis patients. *Mult Scler* 18: 1038–1041.
- Warnke, C., Stettner, M., Lehmensiek, V., Dehmel, T., Mausberg, A., von Geldern, G. *et al.* (2015) Natalizumab exerts a suppressive effect on surrogates of B-cell function in blood and CSF. *Mult Scler* 21: 1036–1044.
- Weiner, H. (2008) A shift from adaptive to innate immunity: a potential mechanism of disease progression in multiple sclerosis. *J Neurol* 255(Suppl. 1): 3–11.
- Weinshenker, B. (1998) The natural history of multiple sclerosis: update 1998. *Semin Neurol* 18: 301–307.
- Wiebenga, O., Klauser, A., Schoonheim, M., Nagtegaal, G., Steenwijk, M., van Rossum, J. *et al.* (2015) Enhanced axonal metabolism during early natalizumab treatment in relapsing–remitting multiple sclerosis. *AJNR Am J Neuroradiol* 36:1116–1123.
- Woodworth, J., Duda, P., Fox, E., Lucas, N. and Plavina, T. (2013) Comparative pharmacokinetics and pharmacodynamics of subcutaneous and intravenous administration of natalizumab [abstract P529]. *Mult Scler* 19: 216–217.
- Yaldizli, O. and Putzki, N. (2009) Natalizumab in the treatment of multiple sclerosis. *Ther Adv Neurol Disord* 2: 115–128.
- Yednock, T., Cannon, C., Fritz, L., Sanchez-Madrid, F., Steinman, L. and Karin, N. (1992) Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha-4 beta-1 integrin. *Nature* 356: 63–66.