

# Developing Therapeutics for the Treatment of Multiple Sclerosis

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**Summary:** Multiple sclerosis (MS) is both a complex and chronic neurological disease of the CNS. This poses unique challenges for drug discovery in terms of delineating specific targets related to disease mechanisms and developing safe and effective molecules for clinical application. Preclinical animal models of MS provide the necessary test bed for evaluating the effects of novel therapeutic strategies. Because the clinical manifestations and pathological consequences of disease vary dramatically from individual to individual, as well as treatment response to existing therapies, this creates a significant research endeavor in terms of translating preclinical methodologies to the clinical domain. Potentially exciting treatments have emerged in the form of natalizumab (Tysabri), an  $\alpha 4$  integrin antagonist, and more recently FTY720, a sphingosine-1 phosphate receptor modulator, providing a compelling proof-of-principle from bench to bedside. However, further research is

required to discharge safety concerns associated with these therapeutic avenues. Future prospects in the guise of disease-modifying therapies that target the inflammatory and neurodegenerative components of disease have come to the forefront of preclinical research with the sole aim of reducing the underlying irreversible progressive disability of MS. Significant progress with novel therapies will be made by implementing biomarker strategies that extrapolate robustly from animal models to the divergent patient populations of MS. The future therapeutic options for MS will depend on improvements in understanding the precise factors involved in disease onset and progression and subsequently the development of oral therapeutics that translate sustained benefit from the preclinical context into clinical reality. **Key Words:** Multiple sclerosis, inflammation, demyelination, regeneration, experimental autoimmune encephalomyelitis, therapeutics.

## INTRODUCTION

Multiple Sclerosis (MS) is the most common demyelinating disease of the CNS, affecting young adults in their formative years, where current treatments have limited effectiveness. MS is typified pathologically by multiple inflammatory foci, plaques of demyelination, gliosis, and axonal pathology within the brain and spinal cord, all of which contribute to the clinical manifestations of neurological disability. Although the causal events in precipitating the disease are not fully understood, most evidence implicates an autoimmune etiology together with environmental factors, as well as specific genetic predispositions. Functional impairment, disability, and handicap are expressed as paralysis, sensory and cognitive disturbances, spasticity, tremor, lack of coordination, and visual impairment. All these symptoms significantly impact on the quality of life of the individ-

ual. The clinical course of MS can vary from individual to individual, but invariably the disease can be categorized into three forms: relapsing-remitting, secondary progressive, and primary progressive. In approximately 85% of patients with MS, the disease starts with alternating episodes of neurological impairment characterized by relapses with subsequent complete or partial remission.<sup>1</sup> In the majority of patients over a variable period, this course is followed by a secondary progressive phase where recovery is absent. A minority of patients (~15%) display primary progressive characteristics where irreversible worsening of clinical signs manifest from disease onset.<sup>1</sup> The disease as a whole places a huge burden on economic and societal resources and highlights the importance of developing novel, safe, and effective therapies for MS in treating the underlying and progressive course of the disease.

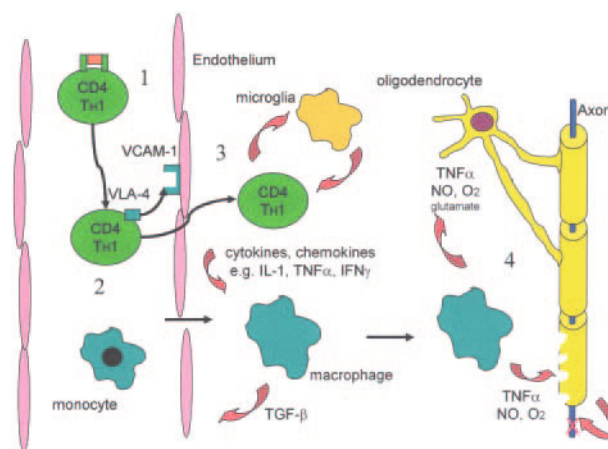
This article, will review key challenges for drug discovery in MS, based initially on the existing clinical outcome measurements, available preclinical models to simulate the disease process, and treatment response to current therapeutics. Specific emphasis will then be placed on novel therapeutic challenges for MS, drawing

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on the weight of evidence from natalizumab (Tysabri) and examples of novel anti-inflammatory, neuroprotective, and regenerative approaches. Improving the translational quality of candidate compounds from bench to bedside, involving the utility of biomarkers will also be highlighted to help guide the future development of robust treatment options for MS.

### EVALUATION OF CLINICAL OUTCOME IN MS

Due to the fluctuating nature and breadth of symptoms, robust measurement of the clinical manifestations of MS is problematic. For appropriate assessment of efficacy of drug treatment within clinical trials, the measurement tool(s) should be sensitive and reproducible enough to detect a significant treatment effect. The Expanded Disability Status Scale (EDSS) is considered the most widely used instrument to evaluate therapeutic strategies in MS, despite drawbacks of reproducibility and inadequate representation of upper limb function and cognitive decline.<sup>2</sup> More recent developments by the National MS Society's clinical outcomes task force in MS have provided a more quantitative and sensitive tool in the MS Functional Composite (MSFC).<sup>3</sup> The MSFC provides more objective measures of leg function, arm and hand function, and cognitive function. This instrument comprises of three specific tests that probe walking speed (timed 25 foot walk), fine upper limb dexterity (9 hole peg test), and cognitive processing (paced auditory serial additional test) on a continuous scale. The MSFC has been shown to be more sensitive to change than EDSS, and during and after treatment with the corticosteroid, methylprednisolone, clinical improvements from acute relapses were more consistently measured.<sup>4</sup> Furthermore, MSFC scores have been found to correlate with EDSS, magnetic resonance imaging (MRI) lesion load, and self-reported quality of life. In terms of application to clinical trials, the MSFC has been reported to be strongly predictive of clinical and MRI status in relapsing-remitting patients and may offer improved sensitivity to assessing progression in the course of disease and ultimately, the effects of novel disease-modifying therapies for MS. Recent concurrent validation of the MSFC with MRI has been established to determine biological sensitivity to disease severity.<sup>5</sup> In this study, the EDSS was directly compared with the MSFC in relation to MRI measurements of lesion load. The EDSS was not shown to correlate with MRI measures of disease, whereas the MSFC was shown to correlate with both T1 and T2 lesion load, especially in both relapsing-remitting and secondary progressive MS patients. Although MRI provides valuable primary end-points in phase II clinical trials and supportive outcome measures to phase III clinical trials, as a putative surrogate marker of disease ac-



**FIG. 1.** Schematic view of the putative pathogenic steps in MS. 1: Activation of autoreactive T cells by antigen presenting cells in the periphery. 2: Migration of T cells and monocytes through the blood brain barrier. 3: Amplification of local inflammation and activation of resident microglia. 4: Release of toxic mediators damages myelin and oligodendrocytes with the culmination of axonal loss.

tivity, further developments on assessing additional MRI parameters are required to improve the association with clinical disability. The evolving development of more sensitive, predictive, and practical measures of impairment and disability aligned with more comprehensive and quantitative assessments of MRI tissue signatures in the brain and spinal cord will help evaluate novel therapeutic strategies for MS.

### ANIMAL MODELS OF MS

A major thrust of preclinical research is to identify and validate novel targets within appropriate disease-relevant models that mimic the clinical situation as closely as possible. Animal models form an essential part of the drug development process to assess the validity of the target for therapeutic intervention and provide proof-of-concept for clinical progression. Although there is no gold standard model of MS, experimental autoimmune/allergic encephalomyelitis (EAE) models simulate the clinical and pathological hallmarks of MS in various guises and can provide the necessary predictive index for clinical therapeutic application.<sup>6</sup> EAE is primarily induced by generating T-cell-mediated immunity to CNS antigens and is commonly modeled in rodents (mice, rats, and guinea pigs). The range of autoantigen preparations used to induce EAE range from whole CNS homogenate (spinal cord) to purified protein and peptides. Myelin basic protein (MBP), proteolipid protein, myelin oligodendrocyte glycoprotein (MOG), S100 $\beta$ , and glial fibrillary acidic protein as well as specific peptides from respective parent proteins are encephalitogenic in the appropriate host, as the major histocompatibility complex (MHC) is one of the major determinants of immune

responsiveness and disease susceptibility to these self-antigens. The pathogenic autoimmune steps that are thought to initiate and amplify tissue damage in EAE and MS are described in Figure 1. The key steps are: 1) activation of autoreactive CD4<sup>+</sup> T-cells in the periphery to an antigen; 2) transmigration of proinflammatory T-cells and monocytes through the blood brain barrier (BBB); 3) amplification of local inflammation and activation of resident antigen-presenting cells (APCs), such as microglia; and 4) destruction of oligodendrocytes, myelin sheath, and axons culminating in demyelination and axonal pathology. Neurological deficits in rodent EAE models are typically manifested in an ascending manner, beginning with loss of tail tone and progressing to hind limb paralysis, hind and forelimb paralysis, and death. However, the clinical course of EAE is greatly dependent on the type of CNS antigen used, immunization protocols, species and strain of animal used to induce disease. For a valid model of EAE to adequately mimic the clinical condition of acute or chronic progressive MS, enduring pathological signatures such as inflammation, gliosis, oligodendrocyte degeneration, demyelination, and axonal loss should be readily observed within the brain and spinal cord. A number of EAE models possess some but not all these characteristic features, each of which can provide valuable insight into target identification and validation for drug discovery in MS. Depending on the hypothesis being tested for a specific target of interest, the choice of model in the appropriate species allows assessment of the target in the pathological process and the putative mode of action of a therapeutic acting at that specific target. Therefore, a number of rodent EAE models can recapitulate different phases of the disease process such as a rapidly progressing acute monophasic disease, a relapsing-remitting clinical course or a chronic progressive outcome with varying degrees of inflammation, gliosis, oligodendrocyte degeneration, demyelination, gliosis, and axonal pathology in the CNS.

The identification of a target antigen that significantly contributes to clinical severity, lesion topography and the extent of demyelination in animal models of EAE has been attributed to MOG. MOG is a quantitatively minor myelin protein (less than 0.05% of total myelin proteins), with an Ig-like extracellular domain that is expressed in abundance on the outer most layer of myelin sheaths, which may render it accessible to antibody attack. Autoantibodies against MOG have been shown to enhance demyelination in several EAE models and localized to disintegrating myelin around axons in lesions of acute MS patients on pathological inspection. Furthermore, anti-MOG antibodies have been demonstrated within the peripheral blood and CSF of MS patients, further associating MOG in the pathogenesis of the disease. On the weight of this evidence, MOG (35-55 peptide) induced

EAE in the C57BL/6 mouse is a robust model of EAE with a chronic clinical course of disease with accompanying pathological hallmarks of inflammation, gliosis, and demyelination.<sup>7</sup> The consistency of disease incidence and severity is usually maintained with the addition of *Bordetella pertussis* toxin, which is thought to open BBB and facilitate the entry of autoreactive T-cells primed by MOG.<sup>8,9</sup> The clinical and pathological signs of MOG-induced EAE are thought to mimic the chronic sustained and progressive phase of MS, particularly relevant to secondary progressive and primary progressive clinical courses of MS.

Due to the majority of MS patients presenting relapsing-remitting symptoms before progressing onto a chronic phase, a number of animal models of EAE have been designed to simulate the more dynamic clinical and pathological features of relapsing-remitting MS. One such model using the Biozzi AB/H mouse,<sup>10</sup> involves the inoculation of homologous spinal cord homogenate (or more specifically MOG peptide)<sup>11,12</sup> in adjuvant without the additional use of *Bordetella pertussis* toxin, and reproducibly induces a chronic relapsing-remitting demyelinating disease. The dynamic chronicity of symptoms is expressed as an acute induction of disease (loss of tail tone and hindlimb paralysis), followed by reduced severity (remission) and then a relapse disease episode. The development of clinical signs in this model are preceded by a loss in weight, whereas remission periods are associated with an increase in body weight, implicating changes in weight as surrogate markers of disease status. Reductions in the degree of inflammation and evidence for remyelination are thought to reflect the remission period in this EAE model, whereas relapses are thought to be indicative of an amplified inflammatory response, gliosis, and demyelination within the CNS.

A key challenge for investigators using rodent EAE models in preclinical drug development for MS is the assessment of neurological deficits in a more sensitive, objective, and quantifiable manner as opposed to the more traditional, qualitative clinical-grading scales. More specific functional measures assessed in rodent EAE models over time, such as hindlimb sensorimotor behavior<sup>13</sup> and fine motor coordination, may provide a more powerful and sensitive means in extrapolating more closely to the clinical situation (such as the MSFC outcome measure) and provide a more comprehensive assessment of novel therapeutics targeted for MS.

The utility of nonhuman primate EAE models has provided an improved insight into CNS autoimmunity and ensuing pathology due to their close evolutionary relationship with humans.<sup>14</sup> Nonhuman primate models of EAE have advantages over rodent models in that they simulate more closely the relapsing-remitting and progressive course of disease and have a more sophisticated neuroanatomy with a greater ratio of white to gray mat-

ter, similar to humans. Furthermore, monkeys are outbred in nature, unlike rodents which are inbred, making the individual response to EAE more variable, similar to human MS. The use of the common marmoset (*Callithrix jacchus*), a small new-world monkey, allows a practical and more sophisticated functional and pathological analysis of EAE disease progression, as well as providing essential middle-ground for the development of novel putative therapeutic agents from rodent models to human clinical trials. The incidence of EAE in marmosets immunized with whole myelin, myelin proteins (MOG), recombinant human MOG 1-125 or specifically MOG 14-36 peptide in adjuvant is 100%, with clinical signs following a relapsing-remitting or chronic progressive course.<sup>14</sup> The pathological hallmarks relating to large foci of demyelination surrounding perivascular infiltrates (inflammation, gliosis, and remyelination) can be readily visualized by serial *in vivo* MRI in this animal species, providing valuable pathological correlates to human MS.<sup>15</sup> Clinical signs are usually preceded by weight loss, and include motor weakness, visual defects and paralysis usually scored on a qualitative grading scale. However, a thorough objective characterization of quantitative functional deficits, particularly locomotor activity, fine-motor movement, visuo-spatial neglect and cognitive function, has yet to be interrogated in the marmoset. The EAE model in the marmoset may bridge the gap for novel therapeutic strategies being progressed for clinical trials, such as humanized antibody approaches (e.g., CD40),<sup>16</sup> and provide definitive MRI surrogate markers of disease activity and treatment response to help guide phase II proof-of-principle clinical trials.

### CURRENT THERAPIES FOR MS

The treatment of MS is still in its infancy with limited therapeutic options, where the main-stay therapies involve the utility of corticosteroid and immunosuppressive interventions. There are currently only five Food and Drug Administration (FDA)-approved treatments for relapsing-remitting MS: two interferon (IFN)- $\beta$ 1a agents (Avonex and Rebif), one IFN- $\beta$ 1b (Betaseron), glatiramer acetate (GA) (Copaxone) and Mitoxanthrone (Novantrone). For patients with secondary progressive MS, cyclophosphamide (Cytoxan) and mitoxanthrone<sup>17</sup> are prescribed, although provide only modest benefit with significant toxicity. There are currently no available treatment options for primary progressive MS. The main therapeutic options for patients with MS will now be discussed (i.e., corticosteroids, IFN $\beta$ , and GA).

#### Corticosteroids

Corticosteroid treatment is extensively used in MS for promoting a hastened recovery following a period of an acute attack.<sup>18</sup> High dose methylprednisolone, via the

intravenous route, is now more popular than oral prednisone, as it provides a stable therapy for MS patients at the onset of an acute relapse. Although short-term therapy has shown benefit to varying degrees, long-term administration is more useful in the treatment and management of relapsing-remitting MS patients. Dramatic improvement in the clinical course of secondary progressive MS has not been shown with corticosteroid treatment. Although the mechanism of action of corticosteroids in MS is not completely understood, evidence from preclinical research has highlighted a number of putative mechanisms: reduction in BBB disruption, an inhibition of the Th1 immune response, a dampening of T-cell migration and the response to antigens, suppression in the expression of adhesion molecules, and protection of oligodendrocytes from cytokine-induced cell death. EAE models have confirmed suppressive actions of corticosteroid treatment on the clinical course of disease and the use of the anti-glucocorticoid, RU 38486 (mifepristone), has been shown to intensify and reverse steroid-induced inhibition of disease.<sup>19,20</sup> However, the side effects of corticosteroid treatment should not be underestimated.<sup>18</sup> Short-term treatment can induce transient changes in mood, headache, gastrointestinal pain, and myalgias. Chronic treatment may decrease bone density, leading to osteoporosis with risk of fractures, and infections making the suspension of treatment more appropriate for management of the patient.

#### IFN- $\beta$

The IFN- $\beta$ -based therapies have been established after 25 years of clinical development. The original rationale for exploring the effects of IFNs in MS was based on the premise that MS was thought to be a virally mediated disease. However, this antiviral hypothesis was untenable based on a clinical trial assessing IFN $\gamma$  where clinical symptoms worsened, suggesting that IFN $\gamma$  played a role in the pathological process of MS. IFN $\beta$ , like other IFNs, is a species-specific glycoprotein that has numerous biological properties. Although its mechanism of action is still poorly understood, immunomodulatory as opposed to antiviral and antiproliferative effects seem to predominate. IFN $\beta$ -1a is identical to the natural IFN- $\beta$ , whereas IFN $\beta$ -1b differs by two amino acids and is not glycosylated. Irrespective of these subtle structural differences IFN $\beta$ -1b shows similar biological activity to IFN $\beta$ -1a. The putative effects of IFN $\beta$  on MS progression primarily relate to antiinflammatory effects: dampening the stimulatory effects of IFN $\gamma$ , tumor necrosis factor (TNF) $\alpha$ , interleukin (IL)-12, and lymphotoxin secretion; inhibiting monocyte activation; preventing the disruption of the BBB and thereby reducing the entry of lymphocytes into the CNS; reducing antigen presentation to T-cells; and up-regulation of anti-inflammatory cytokines such as TGF $\beta$  and IL-10. EAE models have dem-



onstrated that IFN $\beta$  reduces the progression of disease, delays the exacerbation onset and rate,<sup>21</sup> and may modulate the IL10/IL-12 circuit reducing the effect of epitope spreading and disease severity.<sup>22</sup>

In the clinical trials that have been conducted with both IFN $\beta$  formulations, the key efficacy findings relate to: one third reduction in relapse rate at higher doses; rapid onset of effect, within 1 year for relapse rate and within a few weeks for MRI disease activity; disproportionately large effects on inflammation as measured by MRI activity; slowing of the accumulation of MRI burden of disease; and a tendency for a reduction in the number of patients with observed progression of disability.<sup>23–25</sup> However, adverse effects are associated with IFN $\beta$  therapy, such as flu-like symptoms and injection site reactions. Discontinuation of IFN $\beta$  therapy is warranted where patients show no improvement over a 6-month period, and where disability progresses or more relapses occur with three or more courses of corticosteroids over a 1-year period. Additional concerns over severe depression or suicidal ideation, drug toxicity, and noncompliance highlight the need for alternative classes of drug with a better therapeutic index. Issues concerning the effects of neutralizing antibodies on IFN $\beta$  efficacy also need to be elucidated in relation to the potential long-term complications for MS patients on IFN $\beta$  treatment.

## GA

GA is non-IFN, nonsteroidal therapy that constitutes a mixture of synthetic random base copolymers of four amino acids (alanine, glutamic acid, lysine, and tyrosine), in a highly specific molar ratio. Original research investigated the potential encephalitogenic role of GA in animal models of EAE, but unexpectedly GA suppressed the acute and chronic clinical and pathological hallmarks of EAE in a number of animal species.<sup>26</sup> These effects translated into clinical benefit, in that an initial phase II trial demonstrated GA to reduce relapse rates by 76% in relapsing-remitting MS patients.<sup>27</sup> Further clinical development confirmed reductions in relapse rates by a third and a higher preponderance of patients relapse-free.<sup>28</sup> These effects were confirmed on follow-ups for more than 5 years on treatment and demonstrated sustained efficacy for GA in slowing the progression of disability. Lesion burden assessed by MRI has shown a beneficial profile for GA in relapsing-remitting patients, in that treatment reduced the frequency of new enhancing lesions and lesion load compared to baseline pretreatment measures.<sup>29</sup> However, no significant improvement in the course of the disease with GA has been demonstrated for secondary progressive MS patients.<sup>26</sup> A number of mechanisms have been proposed related to its biological activity in relapsing-remitting MS: induction of antigen-specific suppressor T cells and competitive inhibition of

MBP and related-peptides from antigen-presenting cells. Although GA is well tolerated in MS patients, administration by the subcutaneous route induces localized injection site reactions in the majority. Generally, it is viewed that GA has the most favorable adverse effect profile in that there is a reduced propensity to develop depression, menstrual disorders, neutralizing antibodies compared with the other therapeutic options available for MS. However, there is clear need to develop more improved treatment options for MS patients, which offer sustained relief with greater efficacy without associated risks. This poses a huge challenge for the pharmaceutical and biotechnology industry. A number of alternative disease-modifying strategies will now be presented each of which exert different modes of action and target different phases of the disease process.

## EXAMPLES OF NOVEL THERAPEUTIC CHALLENGES FOR MS

### Blockade of lymphocyte migration

**Very late antigen-4: natalizumab (Tysabri) and small molecule antagonists.** A key step in the early phase of EAE and MS is the binding of leukocytes to the vascular endothelium of the BBB, before their penetration through it by diapedesis to enter the brain parenchyma (FIG. 1). A substantial body of evidence has now been accumulated that implicates very late antigen-4 (VLA-4,  $\alpha 4$ - $\beta 1$  integrin) in this process, via its interaction with receptors such as vascular cell adhesion molecule 1 (VCAM-1) and the CS1 domain of fibronectin.<sup>30,31</sup> For example, surface expression of VLA-4 has been shown to be essential for the entry of T-cell clones into the brain,<sup>32</sup> and in a number of different EAE models treatment with anti-VLA-4 monoclonal antibodies has been effective in suppressing the clinical signs of disease and T cell infiltration into the CNS.<sup>32–35</sup> Peptide blockers of VLA-4 have likewise been shown to be effective in EAE,<sup>36</sup> preventing the development of clinical signs and cellular infiltration.<sup>37</sup> Direct *in vivo* evidence has suggested that VLA-4 may be important not only in the capture and adhesion of T cells to microvascular endothelium through interaction with VCAM-1,<sup>38</sup> but also in facilitation of T-cell entry into the brain parenchyma (by the induction of metalloproteinase-2) and in maintenance of the residency of T cells within the CNS.<sup>39</sup> However, caution should be exercised based on the preclinical EAE relapsing-remitting model data generated with the PS/2 VLA-4 antibody, demonstrating that, although prophylactic administration suppressed onset and severity of EAE, therapeutic administration at the peak of acute disease or during remission exacerbated disease relapses and increased the accumulation of CD4<sup>+</sup> T cells and VCAM-1 expression in the CNS.<sup>35</sup> The concerns highlighted by the authors<sup>35</sup> were that the

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