A Narrative Review on Axonal Neuroprotection in Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) resulting in demyelination and neurodegeneration. The therapeutic strategy is now largely based on reducing inflammation with immunosuppressive drugs. Unfortunately, when disease progression is observed, no drug offers neuroprotection apart from its anti-inflammatory effect. In this review, we explore current knowledge on the assessment of neurodegeneration in MS and look at putative targets that might prove useful in protecting the axon from degeneration. Among them, Bruton’s tyrosine kinase inhibitors, anti-apoptotic and antioxidant agents, sex hormones, statins, channel blockers, growth factors, and molecules preventing glutamate excitotoxicity have already been studied. Some of them have reached phase III clinical trials and carry a great message of hope for our patients with MS.

Keywords: Neuroprotection; Multiple sclerosis; Progressive form; Statins; BTKi; Antioxidant
The therapeutic strategy in multiple sclerosis (MS) is largely based on reducing inflammation with immunosuppressive drugs. Unfortunately, when disease progression is observed, no drug offers neuroprotection apart from its anti-inflammatory effect.

Assessment of MS progression relies clinically on both no evidence of progression and progression independent of relapse activity to reveal the axonal degenerative process that occurs independently of inflammation but also gives pride of place to non-conventional MRI or serological biomarkers such as serum glial fibrillary acidic protein (sGFAP).

Bruton’s tyrosine kinase inhibitors, anti-apoptotic and antioxidant agents, sex hormones, statins, channel blockers, growth factors, and molecules preventing glutamate excitotoxicity share neuroprotective properties that have been studied in MS. Some of them have reached phase III clinical trials.

INTRODUCTION

Axonal Lesions and the Progressive Phase of Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) resulting in demyelination and neurodegeneration. Autoimmunity plays a major role in the disease pathogenesis. However, it is now recognized that MS is not only an inflammatory disease but also a neurodegenerative condition [1].

The different courses of MS were described in the revised criteria of MS, published in 2017 [2]. The relapsing–remitting form of MS (RRMS) occurs in about 80% of patients. In RRMS, the inflammatory process is predominant and relapses are explained by the massive infiltration of the CNS by activated lymphocytes through the blood–brain barrier (BBB). The progressive form (20% of patients) can occur primarily or be secondary to the relapsing form. This progression is characterized clinically by a slow neurological deterioration over a period of many years and it is referred to as a neurodegenerative process [3, 4]. Even though the etiology of MS remains elusive, there are now strong arguments to suggest that inflammation could drive the neurodegenerative process [5, 6]. Both courses of the disease are characterized, albeit to varying degrees, by an inflammatory environment in the CNS, demyelination, and axonal loss. Consequently, the therapeutic strategy is now largely based on reducing inflammation with immunosuppressive drugs. In RRMS, intensive drugs, such as natalizumab and ocrelizumab, allow a complete remission to be achieved in around 37–48% of patients and in 72% of patients with MS when rebaseline was performed for ocrelizumab at week 24 [7, 8]. Unfortunately, when progression is observed, no drugs offer neuroprotection other than their anti-inflammatory effect and none specifically induce remyelination. Our limited understanding of the neurodegeneration process in MS has not fully unraveled the complex interactions between glia, immune cells, and neurons.

Recent data indicate that progression may be linked to several possibly interrelated mechanisms. Formation of ectopic lymphoid follicles has been observed in meninges of around 40% of secondary progressive MS tissues [9]. These structures exhibit an architecture similar to that found in germinal centers of secondary lymphoid organs: B and T cells in compartmentalized areas with follicular dendritic cells to support differentiation and activation of B cells. Although the contribution of these follicles has not yet been deciphered, a positive correlation exists between their presence and MS progression [10]. The substantial cortical degeneration associated with lymphoid tissue formation may be the consequence of cytotoxic factors diffusing from these follicles [11]. A second
mechanism contributing to MS progression involves slowly expanding lesions (SELS). These lesions exhibit a slow radial expansion within the CNS. They are characterized by a hypomyelinated core surrounded by a thin rim of iron-rich cells mostly composed of microglial cells [12]. Brain magnetic resonance imaging (MRI) scans have shown that SELs are associated with disability progression in people with secondary progressive MS (SPMS) [13]. Quantification of SEL burden associated with other MRI sequences has been proposed as a predictive biomarker to identify people with a higher risk of SPMS conversion [14]. A third mechanism involves oxidative stress as a major factor in demyelination and neurodegeneration in patients with MS. It is suggested that oxidative damage leads to mitochondrial damage, which in turn leads to functional disturbances or cellular degeneration due to lack of energy. A breakdown of energy in neurons and axons will lead to an ion imbalance leading to Ca^{2+} overload and cellular degeneration [15]. Mitochondrial damage can also amplify oxidative stress by releasing oxygen radicals as a result of impaired respiratory chain function [16].

As outlined in this section, inflammation seems to drive a pathogenic cascade in patients with MS, leading to oxidative damage and mitochondrial injury. As a consequence, progression is likely to occur when axonal loss exceeds CNS compensatory capacity, resulting in irreversible neurological disability [17].

**Therapeutic Targets for Neuroprotection**

Acquired immune effectors are represented by clonal expansion of B cells, ectopic formation of follicular lymphoid structures, and CD8\(^+\) cytotoxic T lymphocytes [18]. The effectors of neurodegeneration are less well understood but could relate to:

1. Dysfunctions that occur directly in the axon, such as mitochondrial injury, glutamate excitotoxicity, anomalous distribution of ion channels, excess of intra-axonal Ca\(^{2+}\), and deficit in axonal transport
2. Glial cell dysregulation, such as astrocyte or microglia activation
3. Loss of BBB integrity secondary to infiltration of immune cells (macrophages and lymphocytes) or astrocyte activation

Finally, neurodegeneration may be a consequence of an indirect process related to loss of myelin-protective functions, such as the loss of myelin-derived trophic support, release of Fe\(^{3+}\) from damaged oligodendrocytes, regulation of other glial cells, including microglia, or repair of the BBB [19–21].

The main targets now identified to combat axonal degeneration are illustrated in Fig. 1.

In this review, we explore current knowledge on neuroprotection in MS and look at putative targets that could help to protect the axon from degeneration. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

**The Problem of Drug Access to the CNS**

The CNS needs a precisely balanced microenvironment to function properly. The BBB enables cerebral homeostasis by regulating the highly selective passage of molecules. The endothelial cells of the capillary wall have typical characteristics such as the expression of specific transporters causing a selective transcellular passage, a low proportion of transport vesicles thus limiting the passage of molecules with high molecular weight, and the presence of tight junctions restricting the penetration of water-soluble compounds [22]. As a consequence, brain penetration of drugs is basically limited to small (<0.5 kDa) lipophilic molecules and the large size of interferon and IgG antibody therapies strongly restricts their ability to cross the non-disrupted BBB [23, 24].

Astrocyte–endothelial cell interactions are thought to be crucial in regulating the BBB phenotype [25]. This regulation includes control of angiogenesis, transporter and tight junction protein expression, and morphology [26]. Intracerebral inflammation is frequently associated with reactive oxygen species (ROS) production, which contributes to BBB dysfunction through oxidative damage to proteins, lipids, and DNA, through tight junction protein...
modulation, cytoskeletal rearrangements and upregulation of inflammatory mediators, and matrix metalloproteinase (MMP) activation [27]. The increased permeability of the BBB under these inflammatory conditions may also be mediated by increased endothelial transcellular passage. The influence of pathological changes on BBB dynamics and ultimately on brain drug delivery is frequently overlooked. Currently, a correlation between BBB disruption revealed by gadolinium-based contrast agents and brain delivery of disease-modifying treatments (DMTs) such as siponimod and ocrelizumab has not been established. Thus, it is important to the clinical success of brain-targeting drugs to account for pathological changes, to fully understand the impact of the BBB on drug delivery, and how these issues will be addressed.

Most DMTs marketed for MS influence the peripheral immune compartment, suppressing the immune attack of proinflammatory leukocytes oriented towards the BBB. Some DMTs act directly on the dysregulated BBB. These molecules modulate the adhesion molecule expression, such as VCAM-1 (dimethyl fumarate, corticosteroids), ICAM-1 (dimethyl fumarate, laquinimod, cladribine), or inactivate some matrix metalloproteases, including types 2 or 9 (laquinimod, cladribine, corticosteroids). In addition, fingolimod reduces the microvascular luminal expression of S1P1 and S1P3 receptors,

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Fig. 1 Main targets to achieve neuroprotection in multiple sclerosis. Drugs under development to achieve a neuroprotective effect have different mechanisms of actions including the promotion of (1) axonal functioning, (2) glial regulation, (3) the integrity of the blood–brain–barrier myelin integrity, and (4) recovery of myelin-protective functions.
decreasing lymphocyte transmigration. In an S1P-receptor-independent manner, fingolimod reduces vascular endothelial growth factor (VEGF) expression. Consequently, immune cell passage across the BBB is reduced [28–34]. One of the main pitfalls in MS is the need for DMTs to be able to cross the dysregulated BBB to address the progression of the disease. After reaching the CNS, neuroinflammation increases the neurodegenerative patterns and DMTs therefore have to cross the dysregulated BBB in order to act directly on the CNS inflammatory cells [35, 36]. Several active substances used in MS are presumed to act directly on the CNS. This is the case with monomethyl fumarate (dimethyl fumarate metabolite), S1P receptor modulators (e.g., fingolimod and siponimod), laquinimod, and cladribine [28, 37–40]. In pharmacokinetic experiments in rodents and cynomolgus monkeys, monomethyl fumarate exhibited brain penetration [41]. In experimental autoimmune encephalomyelitis (EAE) rodent models, fingolimod and siponimod were distributed in the brain [38, 42]. On the other hand, clinical pharmacokinetics of cladribine has been described in cerebrospinal fluid (CSF). The concentration of cladribine in the CSF was approximately 25% of the plasma concentration [43]. However, no data are available on the penetration of cladribine into the human cerebral parenchyma.

MODALITY OF PROGRESSION ASSESSMENT IN THE CLINICAL SETTING

Clinical Measures

Assessment of axonal integrity is a crucial challenge to define some biomarkers for neuroprotection. In clinical settings, the relevant criteria for identifying neurodegeneration are linked to the progression of the disability noted on the Expanded Disability Status Scale (EDSS). New concepts have emerged in trials using possible neuroprotective drugs, such as no evidence of progression (NEP), no evidence of progression or active disease (NEPAD), and progression independent of relapse activity (PIRA) [44, 45]. Both NEP and PIRA tend to reveal the axonal degenerative process that occurs independently to the direct axonal destruction due to inflammation but their definition comes from different forms of MS. NEPAD includes the inflammatory process in the assessment related to protocol-defined relapses and brain MRI measure of disease activity. NEP is a secondary endpoint in patients with a progressive form, used in the ORATORIO study to measure the absence of progression of disability beyond EDSS limits, including functional assessment with upper limb function and walking speed, which are non-overlapping areas of the burden of disability associated with MS [46]. NEP status is defined as the absence of 12-week confirmed disability accumulation (CDA), as measured by no 12-week clinical disability progression (CDP) on EDSS (increase of at least 1.0 point if baseline EDSS was at most 5.5 points or at least a 0.5-point increase if baseline EDSS was more than 5.5 points), no 12-week confirmed 20% or greater progression on hand/arm function as measured by the nine-hole peg test (9HPT), and no 12-week confirmed 20% or greater progression on ambulation as measured by the timed 25-foot walk (T25FW) test. The limitations for isolating the neurodegenerative process with this clinical assessment are that NEP is defined although clinical and MRI measurements of acute disease activity are not captured. Thus, because the direct consequences of inflammation are not extracted from this framework, PIRA has been defined in relapsing forms of MS. The composite PIRA corresponds to CDA confirmed at 12 or 24 weeks, with the following modifications to verify the independence of the event from the relapse activity: the baseline assessment (EDSS, T25FW, or 9HPT values) has been rebaselined 30 days or more after the onset of each relapse; No protocol-defined relapse should occur between baseline reference assessment and within 30 days after the initial increase of disability (IID) and 30 days prior to and after the IID confirmation.

In the pooled OPERA I and II population, comprising 1656 of the 2096 eligible
participants with relapsing MS, most disability accumulations were not associated with relapses [44]. These data indicate an underlying progression in this typical relapsing MS population, a finding that could pave the way for a new therapeutic strategy using neuroprotective drugs in addition to anti-inflammatory agents.

**Fluids Biomarkers**

The neurofilament light chain (NfL) is a protein in the cytoskeleton of neurons. There are numerous lines of evidence that serum NfL (sNfL) concentrations reflect ongoing inflammatory neuroaxonal damage (e.g., relapses or disease activity on MRI) and that sNfL levels predict disease activity over the next few years [47, 48]. A number of studies have confirmed that high levels of sNfL have predictive value for future brain atrophy based on MRI over the next 2–5 years, and two studies have found predictive value for atrophy in the longer term at 10 and 12 years [49–51]. On the other hand, data regarding the longer-term predictive value of sNfL for disability progression are less convincing. Precisely defined progression states in large cohorts of patients may clarify the exact value of sNfL reflecting gradual degenerative processes, since even patients classified as RRMS may experience increased disability in phases without relapse of the disease (as shown with PIRA). Overall, sNfL is more likely a predictor of brain atrophy than a predictor of conversion to SPMS.

Glial fibrillary acidic protein (GFAP) is the main cytoskeletal protein in astrocytes and is released during changes in cell integrity. GFAP is gaining increasing research interest as the second major blood biomarker that can be reliably measured in serum samples and is moderately correlated with sNfL. Early studies in patients with MS suggested that GFAP is not elevated in association with acute relapses and focal inflammatory infiltrates, and therefore could be used to elucidate neurodegenerative pathology induced by glia [52]. The combination of sNfL with sGFAP highlighted that a combination of the two markers could be useful in differentiating patients with progressive MS [53, 54]. This notion is supported by a recent explorative study which evaluated GFAP and chitinase-3-like protein 1 (CHI3L1) in serum and CSF as markers of astrocyte and microglial activation. A simplified “glia score” (GFAPxCHI3L1/NfL) was significantly higher in a group of patients with primary progressive MS (PPMS) or SPMS than in patients with RRMS. In serum, GFAP (but not NfL), correlated with the disease severity only in patients with PPMS [54]. These studies are of interest because they are consistent with the concept that glia activation is closely related to axonal damage and disability progression in patients with SPMS. Another study suggests that neither sNfL nor sGFAP was associated with clinical severity or progression in patients with PPMS [55].

**MRI Measures**

Neuronal degeneration and axonal loss are reported on postmortem samples histologically and with imaging techniques in the early stages of the disease, even in patients who have not developed clinically obvious neurological disabilities. Neurodegeneration seen in MS includes loss of neurons within the gray matter (GM) and loss of axons within the white matter (WM) lesions and normal appearing white matter (NAWM) [56, 57].

Brain atrophy, also referred to as progression of brain volume change, can be observed in different regions using 3D T1 sequences. Indeed, persons with MS see their brain volume diminish by approximately 0.5–1.3% per year, whereas the physiologic rate is 0.1–0.3% per year [58, 59]. Brain atrophy correlates with cognitive function and has a prognostic role in disability progression at 10 years [60, 61]. Atrophy can be specifically measured according to brain regions, giving additional information in terms of pathophysiological processes:

- GM atrophy is classically used to test the neuroprotective effect of DMT [62]. This value has been associated with disability progression in all MS forms and correlated with clinical disability and cognitive deterioration [63–65]. This cortical atrophy...
worsens according to the stages of the disease, with atrophy rates lowest in isolated clinical syndromes and highest in progressive forms.

- WM atrophy progresses faster in patients with progressive MS in comparison to patients with RRMS but its modification is not specifically related to progression [66].
- Spinal cord atrophy is correlated with disability in cross-sectional and longitudinal studies [65, 67] and it is also correlated with the risk of conversion to a progressive form [68].
- Finally, thalamic atrophy can also be evaluated since it is correlated with increased risk of disability progression and cognitive impairment [69, 70].

Other characteristic features of a degenerative process in MS can therefore be monitored with MRI:

- Ectopic lymphoid follicles can be observed with the 3D-T2-FLAIR sequence [71].
- Iron depletion in basal ganglia can be detected with T2-FLAIR and susceptibility-weighted imaging (SWI) sequences [72].
- SELs can be detected with T2-FLAIR, SWI sequences but also through 3D-T1 sequences [73, 74].

Conventional MRI provides useful tools to diagnose and predict the course of MS, but still has some limitations. Indeed, its prognostic value remains insufficient, mainly because of the lack of specificity of the visible lesions. In this regard, advanced MRI sequences have been developed. Techniques such as diffusion tensor imaging (DTI), magnetization transfer ratio (MTR), and myelin water fraction (MWF) are sufficiently sensitive techniques to allow the detection and quantification of myelin content and brain microstructural tissue integrity, and are able to predict cognitive impairment [75], disability progression [76], and decreased intervention response in patients with MS related to progression [77].

CLINICAL DATA WITH USUAL DRUGS, INDEPENDENT OF THEIR ACTION ON INFLAMMATION

RRMS

In general, studies with interferon (IFN) have not shown any beneficial effects on the rate of brain atrophy. Intramuscular IFNβ1a produced lower rates of brain volume loss (BVL) compared to placebo during the second year of treatment in patients with RRMS (−0.23% vs. −0.51%; \( p = 0.03 \)) [78]. However, subcutaneous IFNβ1a has produced inconsistent results in patients with clinically isolated syndrome (CIS) and RRMS [79–81]. A possible delayed effect in reducing cerebral atrophy has been reported for glatiramer acetate (GA) [82–84]. In the PreCiSe clinical trial, GA did not show an immediate effect on brain volume compared to placebo (−0.38% vs. 0.33%), but the subsequent open-label phase of the trial showed a net benefit on percentage of brain volume change (PBVC) for the early treatment group, compared to patients with delayed onset of treatment (40% reduction, \( p = 0.0209 \)) [85, 86].

Brain volume outcomes have been reported for teriflunomide with CIS and RRMS in the TOPIC and TEMSO clinical trials, respectively; doses of 7 mg or 14 mg both failed to show a clear effect in terms of slowing the course of brain atrophy when compared to placebo [87, 88]. However, when tissue-specific volume changes were examined a significant reduction in the rate of WM loss was detected for the 14-mg teriflunomide treatment arm versus placebo [89]. Similar results were recently reported in retrospective analyses of the TOWER and TEMSO trials when alternative methods of BVL evaluation, such as SIENA, was implemented [90, 91].

Dimethyl Fumarate

Dimethyl fumarate (DMF) showed a 21% reduction in the course of brain atrophy compared to placebo in the DEFINE study (the 240 mg twice daily regimen only) and produced only marginal but beneficial effects in BVL reduction in the CONFIRM study [92, 93].
pilot study of 20 patients with RRMS showed a protective effect of DMF treatment in whole brain atrophy (PBVC \(-0.37 \pm 0.49\%\) vs. \(-1.04 \pm 0.67\%, p = 0.005\)) and putamen atrophy (\(-0.06 \pm 0.22\) vs. \(-0.32 \pm 0.28\) ml, \(p = 0.02\)), but no effect on other subcortical volumes or total GM atrophy [94].

**Fingolimod**

For fingolimod, data concerning the evolution of cerebral atrophy (measured by SIENA) extracted from phase III FREEDOMS 1 and 2 and TRANSFORMS trials found an annual decrease in brain volume of between 0.3% and 0.5%, present from the first year of treatment and maintained for the following year [95]. The progression of this atrophy was more marked as the patients presented MRI characteristics showing disease activity at the time of inclusion. The evolution of thalamic atrophy was 0.82% the first year of treatment and 0.51% the second year [96]. Prospective studies comparing the evolution of atrophy with fingolimod and natalizumab found comparable data in patients without any inflammatory activity on brain MRI [97].

**Natalizumab**

The study of neuroprotection under natalizumab shows that the evolution of WM atrophy and to a lesser extent GM atrophy is quite low and presents the risk of the effects of atrophied structures being masked by the effects of those which are not. On the other hand, the analysis of the literature shows that the evaluation of thalamic atrophy seems to be the best marker of the neuroprotective effect in patients with MS [98–101]. These data can be extracted from the standard sequences. The increase of this atrophy in patients taking natalizumab was around 1.5% after 48 weeks of treatment and then seemed to be relatively stable and around 0.5% per year after this period. Note that this picture is close to the natural course of cerebral atrophy, the annual rate of which is 0.4% in healthy subjects.

**Ocrelizumab**

For ocrelizumab, the data from the pivotal studies OPERA I and II and its extension phase made it possible to identify a change in cerebral atrophy of 0.6% over 18 months from week 24. Thalamic atrophy was 2% in the first year, then around 0.4% each year for 5 years of follow-up [102]. Lastly, ocrelizumab (vs. IFNβ1a) was associated with a reduced risk of composite clinical disease activity (hazard ratio [HR] 0.67) and confirmed PIRA (HR 0.78) and relapse-associated worsening (HR 0.47) events [44].

These data show that we should observe the same neuroprotective effect with fingolimod, ocrelizumab, and natalizumab in stable patients without recent inflammatory activity on MRI.

**Progressive MS**

There is a clear gap between the numerous drugs that are widely available and effective in RRMS and the comparatively few drugs available for progressive forms of MS. Until a few years ago there were no on-label drugs available in this field. Recently, following the positive results of two studies, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved two drugs with only a mild intensity of effect (reduction of around 20–25% of the disability progression compared with placebo). The first one is ocrelizumab in PPMS (ORATORIO study) and the second one is with siponimod, an S1P1 agonist, in SPMS (EXPAND study) [46, 103]. An important question addressed following the results of these trials (especially for SPMS) is the role of both forms of inflammation, mainly represented by relapses/new T2 lesions, and neurodegeneration, represented by disability/atrophy. The primary progressive form of MS is the best demonstration of the occurrence of progression without any relapses or imaging evolution, but the real mechanisms of this progression remain uncertain.

**Ocrelizumab**

Ocrelizumab has been demonstrated as a highly efficacious drug in RRMS, with a significant reduction of both relapse rate and progression
of the disease compared with IFNβ1a subcutaneously three times a week, in two parallel studies (OPERA I and II) [104]. However, the effect was essentially attributed to the anti-inflammatory mechanism of ocrelizumab rather than a mixed effect on both the inflammatory process, expressed by relapse, and progression. A third study, focusing on PPMS, showed a significant effect on EDSS progression against placebo [46]. This study was the first time we found a drug working on PPMS, even if the impact value (reduction of 24% of the disability progression) was not very impressive. The results were also positive for MRI markers such as new T2 lesions and brain atrophy that are usually attributed to neurodegeneration. In addition, ocrelizumab treatment increased the proportion of patients with PPMS maintaining NEP from baseline to week 120 by 47% compared to placebo. Very recently, an extrapolation study evaluated from the ORATORIO study the mean time to requiring a wheelchair, which may be considered as a good indirect marker of the neurodegenerative process [105]. The study found a mean time to requiring a wheelchair of 12 years on placebo against 19 years on ocrelizumab.

**Siponimod**

Siponimod is an S1P drug derived from the same class of drugs as fingolimod but with a small difference of S1P receptor affinity. The latter binds with nanomolar affinity as an agonist at four of the five S1P receptors, namely, S1P1, S1P3, S1P4, and S1P5. Siponimod may have a direct neurobiological effect in the CNS, independent from its effects on peripheral lymphocytes, through selective modulation of S1P1 on astrocytes and S1P3 on oligodendrocytes [106]. Fingolimod was not able to demonstrate any effect on PPMS in a phase III, placebo-controlled study even with stratification of some subgroups [107]. A specific study, EXPAND, was designed for SPMS with siponimod; the results were positive, with a reduction of 21% of the confirmed disability progression, but this effect was mainly driven by the subgroup of patients with active SPMS [103]. The FDA and EMA approved this new drug for active SPMS but not for non-active SPMS, which remains a clear unmet need in this field.

### NEUROPROTECTIVE EFFECT OF DRUGS UNDER DEVELOPMENT

Studies using neuroprotective agents and reported in this review are summarized in Fig. 2.

#### Agents Acting on Microglia

**Rationale for the Use of Bruton’s Tyrosine Kinase (BTK) Inhibitors in MS**

BTK was initially identified in human X-linked agammaglobulinemia, an inherited disorder characterized by a very low level of immunoglobulins. A defect in the BTK gene impairs normal B cell development and maturation [108]. Indeed, BTK is a cytoplasmic non-receptor tyrosine kinase modulating several intracellular signaling cascades, such as phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), phospholipase C (PLC), and nuclear factor-κB (NF-κB). These key pathways regulate activation, proliferation, survival, and differentiation, dysregulation of which participates in the development of B cell malignancies and autoimmune diseases, including MS [109, 110]. Thus, the X-linked immunodeficient (XID) mouse line lacking functional BTK exhibited milder clinical signs after induction of EAE [111]. Further analysis demonstrated that myeloid cells also undergo abnormalities during development in XID mice. Indeed, in addition to B cells, BTK is also expressed in other immune cells, including monocytes, macrophages, dendritic cells, granulocytes, mast cells, and platelets [112]. In physiological conditions, BTK-positive cells are barely detectable in the CNS. After lysophosphatidylcholine (LPC)-induced demyelination of murine organotypic cerebellar slices, BTK was detected in microglia, i.e., a resident myeloid immune cell of the CNS, and to a lesser extent in astrocytes [113]. High expression of BTK was also found in microglia associated with MS lesions in postmortem patient brain samples [114]. Both B cells and microglia are important...
actors of MS pathogenesis as mentioned in Sect. “Axonal Lesions and the Progressive Phase of Multiple Sclerosis”. Consequently, BTK appears as a valuable therapeutic target in the treatment of this disease. Twenty-four BTK inhibitors (BTKi) are currently under clinical evaluation [115]. Preclinical studies indicated that treatment of EAE mice with the BTKi tyrphostin AG126 improved the clinical course of the disease, with a reduced immune cell infiltration, milder myelin damage, and attenuation of microglia activation. Cell culture experiments showed that AG126 affects microglial functions directly [116]. Recent data have highlighted the implication of microglia during neurodegenerative processes [117]. Notably, genetic data from patients with MS have revealed an enrichment of susceptibility variants in microglial cells [118]. Microglia, the resident macrophages of the CNS, can adopt a diversity of polarization states after activation, but the precise protective or detrimental role of microglia in neurodegenerative diseases is far from being elucidated [119]. Specific microglial phenotypes have been identified in cortical lesions during the progressive phases of MS [120]. Studies have highlighted the essential role of microglia in remyelination by promoting oligodendrocyte differentiation [121], a process dependent on phagocytosis [122]. Microglia–axon interaction at the level of the nodes of Ranvier has been shown to contribute to remyelination [123]. Thus, modulation of microglial activation by BTKi appears to be a potential treatment for MS as the support given in remyelination would promote neuroprotection. Ibrutinib, the first approved BTKi, suppressed the proinflammatory polarization in microglia and decreased microglial phagocytic activity [124, 125].

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Fig. 2 Stages of development of drugs (phases II and III) used for neuroprotection in multiple sclerosis. CIS clinically isolated syndrome, MS multiple sclerosis, RRMS relapsing–remitting MS, PPMS primary progressive MS, SPMS secondary progressive MS
specificity of ibrutinib precluded its evaluation in autoimmune diseases and led to the development of new BTKi, such as evobrutinib, a highly selective covalent inhibitor [126].

**Clinical Trials with BTKi in MS**

Evobrutinib has been successfully tested in several EAE models [112, 127] (Table 1). A phase II trial (NCT02975349) concluded on its safety and efficacy to reduce the total cumulative number of T1 gadolinium-enhancing lesions in patients with relapsing MS [128]. In comparison to other trials realized on MS, participants included in this study were older, with a longer disease duration, and experienced fewer relapses within the 2 years before baseline, which might have indicated the inclusion of patients with SPMS. Thus, longer and larger trials were required to investigate the effect and risks of this molecule in patients with MS. Evobrutinib has now entered two phase III trials on relapsing MS (EVOLUTION RMS 1 and 2) expected to be completed by September 2023.

The data obtained with evobrutinib represented the proof of concept that inhibiting BTK in patients with MS can improve clinical and MRI outcomes. To further enhance efficacy during MS, BTKi drugs have to better penetrate the CNS. Tolebrutinib (SAR442168) has been shown to cross the BBB efficiently and reach pharmacologically relevant concentrations in the CNS [129]. This phase IIb study reported good drug tolerability and a dose-dependent reduction in the number of new gadolinium-enhancing lesions after 12 weeks of treatment. The reduction of inflammation monitored by MRI in this study was comparable to that reported in other trials related to DMTs that were subsequently shown to be highly effective in clinical practice. The most efficacious and well-tolerated dose of tolebrutinib, i.e., 60 mg daily, was selected for further investigations. Phase III trials are currently recruiting patients to test fenebrutinib in MS. Two identical trials will investigate the effect of this molecule on the relapsing form (FENhance 1 and FENhance 2) and a third on the primary progressive form (FENtrepid). Most current DMTs work outside the CNS and aim to prevent peripheral immune cells from attacking the myelin. During the progressive forms of MS, resistance to the effective DMTs could be partly due to compartmentalized inflammation. Using drugs able to cross the BBB, such as the newly developed BTKi, should show higher efficacy as it would help to fight the endogenous immune attack against myelin directly in the CNS.
### Table 1  Summary of studies on neuroprotection with Bruton’s tyrosine kinase inhibitors (BTKi) in multiple sclerosis

| Dose and Duration | Study type | Sample size/patients (number) | Results (outcomes) | References |
|-------------------|------------|------------------------------|--------------------|------------|
| **Evobrutinib**   |            |                              |                    |            |
| 48 weeks          | Phase II,  | 267 patients (18–65 years)   | The dose 75 mg/day  | [128]      |
| (covalent,        | randomized, | with relapsing MS (including  | of evobrutinib  | NCT02975349|
| irreversible)     | parallel   | RRMS and SPMS with relapses) | significantly reduced |            |
| 25 mg/day or      | assignment  |                              | T1 gadolinium-     |            |
| 75 mg/day or      | double-blind, |                              | enhancing lesions |            |
| 150 mg/day, or    | placebo-controlled |                              | during weeks 12    |            |
| 240 mg/day        |             |                              | through 24. There   |            |
| dimethyl fumarate |             |                              | was no significant  |            |
|                   |             |                              | difference in the   |            |
|                   |             |                              | annualized relapse  |            |
|                   |             |                              | rate or disability  |            |
|                   |             |                              | progression at any  |            |
|                   |             |                              | dose               |            |
| **150 mg/day**    | Phase III,  | 930 patients (18–55 years)   | Estimated primary   | NCT04338061|
| versus            | randomized, | with relapsing MS (including  | completion date,   |            |
| teriflunomide     | double-blind, | RRMS and SPMS with relapses) | September 2023      |            |
|                   | parallel assignment |                              | Intended primary   |            |
|                   | (EVOLUTION  |                              | endpoint: ARR at    |            |
|                   | RMS 2 study) |                              | week 96            |            |
|                   |             |                              | Intended secondary  |            |
|                   |             |                              | endpoints: time to   |            |
|                   |             |                              | first occurrence of  |            |
|                   |             |                              | 12- and 24-week     |            |
|                   |             |                              | confirmed EDSS      |            |
|                   |             |                              | progression, total   |            |
|                   |             |                              | number of Gd -       |            |
|                   |             |                              | enhancing T1 lesions |            |
|                   |             |                              | and new or enlarging |            |
|                   |             |                              | T2 lesions assessed  |            |
|                   |             |                              | by MRI              |            |
| Dose                          | Duration of treatment | Study type                                                                 | Sample size/patients (number) | Results (outcomes)                                                                                                                                                                                                 | References   |
|-------------------------------|----------------------|-----------------------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 150 mg/day versus teriflunomide | Up to 108 weeks     | Phase III, multicenter, randomized, double-blind, parallel assignment (EVOLUTION RMS 1 study) | 930 patients (18–55 years) with relapsing MS (including RRMS and SPMS with relapses) | Estimated primary completion date, September 2023 Intended primary endpoint: ARR at week 96 Intended secondary endpoints: time to first occurrence of 12- and 24-week confirmed EDSS progression, total number of Gd-enhancing T1 lesions and new or enlarging T2 lesions assessed by MRI | NCT04338022  |
| Tolebrutinib (covalent, irreversible) | 16 weeks             | Phase IIb, randomized, double-blind, crossover assignment, placebo-controlled | 130 patients (18–55 years) with RMS | Dose-dependent reduction in the number of new Gd-enhancing lesions by MRI. Good drug tolerability                                                                                                           | [129]        |
| 5, 15, 30, or 60 mg/day       |                      |                                                                             |                               |                                                                                                                                                                                                                |              |
| 60 mg/day in addition to intravenous anti-CD20 antibody treatment | 96 weeks             | Phase II, non-randomized, open-label                                       | 20 patients (above 18 years) with chronically inflamed white matter lesions in MS | Estimated study completion date, December 2022 Intended primary endpoint: effect on the paramagnetic rim of chronically inflamed white matter lesions by MRI                                                                 | NCT04742400  |
| Dose | Duration of treatment | Study type | Sample size/patients (number) | Results (outcomes) | References |
|------|-----------------------|------------|------------------------------|--------------------|------------|
| 60 mg/day tolebrutinib versus 14 mg/day teriflunomide | 36 months | Phase III, randomized, double-blind, parallel assignment (GEMINI 1 study) | 900 patients (18–55 years) with RRMS | Estimated study completion date, August 2023 | NCT04410978 |
| 60 mg/day tolebrutinib versus 14 mg/day teriflunomide | 36 months | Phase III, randomized, double-blind, parallel assignment (GEMINI 2 study) | 900 patients (18–55 years) with RRMS | Estimated study completion date, August 2023 | NCT04410991 |
| 60 mg/day tolebrutinib | 48 months | Phase III, randomized, double-blind, parallel assignment, placebo-controlled (HERCULES study) | 1290 patients (18–60 years) with SPMS | Estimated study completion date, August 2024 | NCT04411641 |
| **Dose** | **Duration of treatment** | **Study type** | **Sample size/patients (number)** | **Results (outcomes)** | **References** |
|----------|--------------------------|----------------|----------------------------------|------------------------|---------------|
| 60 mg/day tolebrutinib | 48 months | Phase III, randomized, double-blind, parallel assignment, placebo-controlled (PERSEUS study) | 990 patients (18–55 years) with PPMS | Estimated study completion date, August 2024 | NCT04458051 |
| **Orelabrutinib** (covalent, irreversible) | Up to 120 weeks | Phase II, randomized, double-blind, placebo-controlled, parallel assignment | 160 patients (18–55 years) with RRMS | Estimated primary completion date, March 2024 | NCT04711148 |
| Low, medium, high dose or placebo | | | | Intended primary endpoint: cumulative number of new Gd-enhancing T1 brain lesions by MRI | |
| **Fenebrutinib** (non-covalent, reversible) versus teriflunomide | At least 96 weeks | Phase III, randomized, double-blind, parallel assignment (FENhance 1 study) | 736 patients (18–55 years) with RMS | Estimated primary completion date, October 2025 | NCT04586023 |
| | | | | Intended primary endpoint: ARR | |
| | | | | Intended secondary endpoint: number of Gd-enhancing T1 lesions, new and/or enlarging T2-weighted lesions by MRI | |
Anti-apoptotic Agents

Rationale for the Use of Ibudilast in MS
Ibudilast is a non-selective phosphodiesterase (PDE) inhibitor (PDE3, 4, 10, and 11), preventing the hydrolysis of cGMP or cAMP and in some cases both [136]. It is approved in Asia for the treatment of asthma and chronic obstructive pulmonary disease, and is used in ischemic stroke. Its peripheral anti-inflammatory effect results from the decrease in leukotriene release and tumor necrosis factor alpha (TNFα) or IFNγ production from peripheral white blood cells. At the central level, its anti-inflammatory effect is based on the attenuation of kainate-induced oligodendrocyte cell toxicity and astrocyte apoptosis in vitro, and on the dose-dependent decrease of microglial activation [137]. In vivo, ibudilast attenuates rat EAE, by reducing spinal cord inflammation and modulating the severity of clinical signs in a dose-dependent manner, after prophylactic administration [138]. In a genetic model of Krabbe’s disease, ibudilast (10 mg/kg/day, with a daily intraperitoneal injection) decreases the number of TNFα-labeled cells, reduces the number of oligodendrocytes undergoing apoptosis, and attenuates demyelination [139]. Among the

Table 1 continued

| Dose     | Duration of treatment | Study type                        | Sample size/patients (number) | Results (outcomes)                                                                 | References     |
|----------|-----------------------|-----------------------------------|-------------------------------|-----------------------------------------------------------------------------------|----------------|
| Versus teriflunomide | At least 96 weeks | Phase III, randomized, double-blind, parallel assignment (FENhance 2 study) | 736 patients (18–55 years) with RMS | Estimated primary completion date, October 2025                                    | NCT04586010   |
| Versus ocrelizumab   | At least 120 weeks  | Phase III, randomized, double-blind, parallel group (FENtrepid study)  | 946 patients (18–65 years) with PPMS | Estimated primary completion date, October 2025                                    | NCT04544449   |

ARR annualized relapse rate, RRMS relapsing-remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis, PPMS primary progressive multiple sclerosis

△ Adis
neuroprotective mechanisms mentioned, PDE inhibition reduces inflammatory activities of several non-neuronal cell types: it reduces the production of ROS, suppresses TNFα release from the astrocytes and microglia cells [140], and other neurotoxic mediators (interleukin-6 [IL-6], nitric oxide [NO]) that can damage both neurons and oligodendrocytes [141]. Indeed, roflupram, a PDE4 inhibitor, suppresses inflammasome activation through autophagy in microglial cells [142], and it attenuates lipopolysaccharide (LPS)-induced neuroinflammatory responses in microglial cells in both the hippocampus and cortex [143]. Roflupram also prevents the increase of IL-1β and suppresses microglial reactivity in the hippocampus of mice subjected to the chronic unpredictable mild stress mouse model of depression [144]. The neuroprotective effects of PDE inhibitors have been demonstrated in PC12 cell lines, where concomitant treatment with epidermal growth factor (EGF) and cAMP is required to induce morphological differentiation and cell survival [145].

The passage of ibudilast across the BBB is difficult to demonstrate in clinical practice. The presence of ibudilast in the spinal cord and brain of rats 7 h after an intraperitoneal injection of ibudilast 5 mg/kg suggests its passage through the BBB, although concentrations in the CSF appeared to be low, probably equivalent to the unbound fraction in plasma [146].

**Clinical Trials with Ibudilast in MS**
The first proof-of-concept study suggesting a neuroprotective effect of ibudilast is based on a phase II study, comparing two doses of ibudilast (30 and 60 mg/day, for 12 months), in 297 patients with RRMS and significant disease activity. Although ibudilast treatment showed no beneficial effect on the primary endpoint (brain MRI lesion development, the volume of enhancing lesions) and relapses, post analysis highlighted the reduction in the brain atrophy rate. Furthermore, over 2 years, the percentage of patients with EDSS score progression was lower in those on active treatment (10.4%) versus placebo (21%, \( p = 0.026 \)) [147] (Table 2). These data suggest that ibudilast has a neuroprotective effect rather than an anti-inflammatory action but, because for the second year of evaluation neither the patients nor the investigators were blinded, this needs to be confirmed in another large study, with a better primary endpoint. In the SPRINT-MS, phase II trial, ibudilast reduced the rate of brain atrophy by 48% compared to placebo, i.e., approximately 2.5 mL less brain tissue loss over 96 weeks, but at the expense of some adverse events, such as gastrointestinal side effects and depression [148]. A second analysis of the SPRINT-MS trial suggests that ibudilast has more effect on neurodegenerative processes in patients with PMS than on the inflammatory ones, by reducing the gray matter atrophy (\( p = 0.038 \)) and slowing progression of whole brain atrophy by SIENA (\( p = 0.08 \)), but without effect on new or enlarging T2 lesions, or new T1 lesions, and without a change in either serum or CSF NfL [149, 150].

However, in the SPRINT-MS trial, the absence of a positive effect of ibudilast on all the evaluation criteria still raises questions about either the mechanism of action or the extrapolation of brain atrophy or diffusivity results in post hoc longitudinal analyses performed with different hardware [151]. Despite very promising preclinical results, the repositioning of ibudilast in progressive MS remains in its infancy (only two phase II studies have been conducted, with a limited sample size, mixing different diagnoses of MS and with different ongoing treatments) and needs more evaluation of predictive clinical markers of neurodegeneration.

**Rationale for the Use of Minocycline in MS**
As part of a repositioning strategy in MS for old molecules administered orally and having proven their safety, minocycline, a second-generation tetracycline antibiotic used to treat acne and suppress inflammation, was tested as a drug candidate. In vitro, minocycline reduces matrix metalloproteinase 9 (MMP-9) production and attenuates T cell transmigration across a fibronectin barrier [152]. The anti-apoptotic effect of minocycline was studied in mixed spinal cord (glial and microglial cells) cultures treated with glutamate or kainate for 24 h. Minocycline inhibited excitotoxin-induced neuronal death.
and proliferation of microglial cells by inhibiting the release of nitric oxide (NO) metabolites and IL-1β [153]. Moreover, minocycline inhibited microglial activation, in brain cell cultures treated with IFNγ and LPS, and promoted remyelination by enhancing the oligodendrocyte precursor cells and immature oligodendrocytes [154]. In vivo, minocycline pretreatment delays the course of the disease in EAE mice, with minimal signs of inflammation and demyelination in the CNS, leading to improvements in motor coordination [152]. The neuroprotective effect of minocycline was confirmed in a rat model of EAE induced by myelin oligodendrocyte glycoprotein (MOG), where functional and histopathological data of retinal ganglion cells and optic nerves revealed neuronal and axonal protection, when administration was started on the day of immunization and also when it started on the day of disease onset [155]. Minocycline-induced neuroprotection is the consequence of the induction of anti-apoptotic intracellular signaling pathways (upregulation of Bcl2 and decrease of Bax) and of the decrease in glutamate excitotoxicity. Recently, in an EAE mouse model, the injection of minocycline into the dentate gyrus prevented microglial activation, dentate gyrus neurodegeneration, and memory impairment [156]. Minocycline, by inhibiting microglial activation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, reversed

| Dose                  | Duration of treatment | Study type                                      | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|-----------------------|-----------------------|------------------------------------------------|------------------------------|--------------------------------------------------------------------------------|------------|
| 30 mg/day or 60 mg/day or placebo | 12 months             | Phase II, multicenter, double-blind, placebo-controlled, parallel group | 297 patients (18–55 years) with RRMS (and Gd-enhancing lesions) | No reduction between the mean number of active lesions and relapse rate | [147]     |
| < 100 mg/day or placebo | 96 weeks              | Phase II, multicenter, randomized, double-blind, placebo-controlled, parallel group (SPRINT-MS/NeuroNEXT) | 255 patients (21–65 years) with PPMS or SPMS (ibudilast n = 129, 53% with PPMS, placebo n = 126, 52% with PPMS) | Slower progression of brain atrophy on ibudilast treatment vs. placebo (measured by the brain parenchymal fraction) Reduction in gray matter atrophy (p = 0.038), 20% slower progression of whole brain atrophy with SIENA in the ibudilast vs. placebo group (p = 0.08) No difference between groups in neurofilament light chain in either serum or CSF | [148–150] |

RRMS relapsing–remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis, PPMS primary progressive multiple sclerosis
synaptic and learning deficits in a chronic relapsing EAE mouse model, without preventing the development of disease [157]. Finally, Chen et al. demonstrated that minocycline could upregulate the expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), in both the cerebral cortex and the lumbar spinal cord of EAE mice [158]. Prior to clinical testing, minocycline was combined with other immunomodulatory therapies for MS. The experimental treatment, combining IFNβ-secreting mesenchymal stem cells with minocycline in a mouse model of EAE, reduced the clinical severity by maintaining the microvascular integrity of the BBB, and by inhibiting the production of MMP-2 and MMP-9 in the spinal cord, leading to a beneficial effect on neuroinflammation [159]. Another combination therapy involving minocycline and corticoids alleviated clinical scores and improved MRI outcome in the EAE mouse model. In the same model, the association with prednisolone reduced phenotype severity, inflammation, and demyelination, by preventing the reduction of BDNF and NGF mRNA expression in cerebral cortex [160]. Also, association with methylprednisolone improved severe clinical deficit and suppressed histopathological events in C57Bl/6 EAE mice by reducing the levels of IFNγ and increasing IL-4 expression/production in the splenocyte culture supernatants and brains of EAE mice [161]. Finally, the combination of minocycline and glatiramer acetate significantly reduced the severity of the disease by attenuating inflammation, axonal loss, and demyelination, through alleviation of neuronal T cell toxicity [162].

Regarding its pharmacokinetic properties, minocycline is a highly lipophilic molecule that crosses the BBB, with a good oral bioavailability of 95–100% and seems to be well tolerated in humans at 200 mg/day, which was the only dose tested in phase II trials [163].

**Clinical Trials with Minocycline in MS**

Three open-label consecutive studies were conducted to evaluate the potential of minocycline in the same 10 patients with RRMS [164–166] (Table 3). In the first study, minocycline treatment (100 mg twice daily for 6 months) reduced the number of gadolinium-enhancing lesions, compared to baseline [164]. In the second study, minocycline treatment at the same dosage for 24 months confirmed the reduction in the mean of gadolinium-enhancing lesions, and no relapses occurred after 18 months of treatment, despite a moderately high pretreatment annualized relapse rate [165]. These clinical and MRI outcomes are supported by systemic immunological changes: a decrease in MMP-9 activity and an increase in levels of the p40 subunit of IL-12, which might antagonize the proinflammatory IL-12 receptor. The third study, evaluating minocycline treatment over 36 months, highlighted a reduction of the ARR, and the proportion of active scans was lower during the first 6 months of treatment (5.6%, $p < 0.001$) and during the extension phase (8.7% $p = 0.002$) than during the 3-month run-in period (47.5%) [166]. Moreover, with minocycline administration for over 3 years, T2 lesion volume tended to remain stable and an attenuation of the brain volume impairment was observed, suggesting a possible neuroprotective role of minocycline in patients with RRMS. In a randomized, double-blind, placebo-controlled clinical trial, minocycline treatment, as an add-on therapy to glatiramer acetate, tended to reduce the total number of T1 gadolinium-enhanced lesions (mean 1.47 versus 2.95, $p = 0.08$), the total number of new and enlarging lesions (mean 1.84 versus 5.14, $p = 0.06$), and the total T2 disease burden ($p = 0.10$) [167]. The authors also reported a lower risk of relapse in the combination arm. The treatment was safe and well tolerated, but the lack of statistical significance makes it impossible to conclude on the efficacy of this association (minocycline 100 mg twice daily with glatiramer acetate) and could be attributed to an underpowered clinical trial. Another, larger, randomized, double-blind, multicentric, placebo-controlled clinical trial evaluated the effectiveness and safety of minocycline (100 mg twice daily, for 96 weeks), added to IFNβ1a therapy [168]. No differences were observed for primary (time to first relapse) and secondary (annualized relapse rate, number of new/enlarging T2 lesions, change in brain volume)
| Dose                     | Duration of treatment | Study type                          | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|-------------------------|-----------------------|-------------------------------------|------------------------------|-----------------------------------------------------------------------------------|------------|
| 100 mg twice daily      | 6 months              | Open label                          | 10 patients (18–50 years) with RRMS | Significant reduction in the mean total of GELs                                    | [164]      |
| 100 mg twice daily      | 24 months             | Open label                          | 10 patients (18–50 years) with RRMS | Reduction in the mean total of GELs, no relapses under treatment                  | [165]      |
| 100 mg twice daily      | 36 months             | Open label                          | 10 patients (18–50 years) with RRMS | Reduction of the ARR and lower active scans under treatment                       | [166]      |
| 100 mg twice daily      | 9 months              | Phase II, multicenter, randomized, double-blind, placebo-controlled | 44 RRMS (18–50 years) patients (GA + minocycline $n = 21$; GA + placebo $n = 23$) | Tendency for a reduction of the total number of T1 lesions ($p = 0.08$), total number of new and enlarging T2 lesions ($p = 0.06$), and the total T2 disease burden ($p = 0.10$), under treatment. Lower risk of relapse in the combination arm ($p = 0.08$) | [167]      |
| 100 mg twice daily as add-on therapy to glatiramer acetate (GA) 20 mg daily | 96 weeks              | Phase II, multicentric, double-blind, placebo-controlled, randomized, parallel group (RECYCLINE) | 149 RRMS (18–55 years) patients (IFNβ + minocycline $n = 149$; IFNβ + placebo $n = 155$) | No statistical difference between the two groups on primary and secondary endpoints | [168]      |
outcomes between the two groups. Finally, in a Canadian multicentric trial, minocycline significantly reduced the risk of conversion from a clinically isolated syndrome to MS, compared to placebo, after 6 months of treatment, but not over 24 months [169]. This study is seeking to evaluate an early role for minocycline treatment; however, some potential biases limit this interpretation (small sample size, short study duration, lack of MRI scans for more than one-third of patients after 3 months, and higher risk of conversion in the placebo group patients since they had a greater number of baseline MS lesions). In addition, the preclinical results of minocycline suggest a protective effect in patients with progressive multiple sclerosis (SPMS and PPMS), a population not yet evaluated in well-conducted clinical trials with sufficient power.

### Pleiotropic Effects Including Modulation of Excitotoxicity

**Rationale for the Use of Statin in MS**

Statins are powerful inhibitors of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, the rate-limiting enzyme of hepatic cholesterol synthesis [170]. This cholesterol-lowering treatment is indicated for the prevention of cardiovascular-related morbidity and mortality in individuals with coronary artery disease, with good tolerance [171]. In the mid-1990s, the lower incidence of rejection episodes and mortality in heart transplant patients treated with pravastatin led to the investigation of a potential immunomodulatory effect of statins [172]. In fact, experimental and clinical studies have shown that statins can modulate immune responses through mevalonate pathway-dependent and -independent mechanisms. The pleiotropic effect of statins could be related to the inhibition of post-translational protein modifications, known as isoprenylation, by blocking the synthesis of isoprenoid compounds, such as farnesylpyrophosphate and geranylpyrophosphate, thus preventing the activation of small G proteins, such as Ras (farnesylated proteins), Rho and Rab (geranylated proteins). Proof of concept has been demonstrated in the EAE mouse model, where lovastatin treatment inhibited leukocyte migration into the CNS and significantly attenuated the development of both acute and relapsing clinical disease by inhibiting the Rho-mediated transendothelial T cell migration [173]. The immune effects of statins were analyzed in vitro on the pathogenic cascade of immune peripheral cells obtained from patients with MS. The inhibition of mononuclear cell transmigration/penetration across the BBB, the inhibition of dendritic cell maturation, the decreased T cell activation, inhibition of glutamate-mediated

| Dose              | Duration of treatment | Study type                                                                 | Sample size/patients (number)                                                                 | Results (outcomes)                                                                 | References |
|-------------------|-----------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------|
| 100 mg twice daily| 24 months             | Multicentric, double-blind, randomized, placebo-controlled, parallel group | 142 patients (18–60 years) with first demyelinating symptoms within the previous 180 days (minocycline n = 72; placebo n = 70) | Less conversion to multiple sclerosis within 6 months of randomization to minocycline versus placebo (33.4% vs. 61% respectively, p = 0.001). No significant difference at 24 months | [169]      |

ARR annualized relapse rate, RRMS relapsing–remitting multiple sclerosis, GELs Gd-enhancing lesions
excitotoxicity, and alteration of the NO production by endothelial cells were observed [174]. In murine models, statins inhibit MHC class II-restricted antigen presentation, downregulate T cell activation and proliferation, and induce a shift from a proinflammatory Th1 to a Th2 phenotype. Statins also block adhesion molecule expression and inhibit leukocyte migration through the BBB, supporting their therapeutic use in early MS and their inconsistent effect depending on the inflammatory stage [175]. Among the described central neuroprotective effects induced by statins, atorvastatin, in primary cortical neurons, significantly protected from glutamate-induced excitotoxicity, independently of HMG-CoA reductase inhibition [176]. Moreover, after traumatic brain injury, simvastatin has been shown to induce the expression of BDNF. Some authors suggest that the neuroprotective effects may derive from statin-induced immunomodulatory effects, protecting oligodendrocytes from Th1 (TNFα) and Th17 (IL-17) phenotype cytokine toxicity in vitro, by inhibiting small Rho GTPases, via a peroxisome proliferator-activated receptor alpha (PPARα)-dependent mechanism, which in turn increases ROS detoxifying defense [177]. Statins induce central neuroprotection by inhibition of NO and glutamate synthesis in the CNS. Indeed, lovastatin inhibits the expression of inducible NO synthase and cytokines (IL-1β, TNFα, IL-6) in rat astrocytes, microglia, and macrophages [178]. In astrocytes, NO secretion appears to be dependent on the tropomyosin receptor kinase B (TrkB), a neurotrophin receptor whose expression is induced in white matter lesions of people with MS. Stimulation of astrocytes with BDNF, an agonist of TrkB, leads to neuronal death [179], as NO impairs the energy metabolism of these cells [180]. A similar mechanism connects microglia to the neuro-axonal degeneration that leads to irreversible MS progression [181].

Finally, a vascular effect could also contribute to the neuroprotective effects of statins by modulating endothelial cell eNOS activity, thereby improving cerebral vasomotor reactivity and protecting against long-term hypoxic damage [170]. Since vascular comorbidity is a risk factor for disability in MS, the benefit could also come from a reduction in total cholesterol levels.

Clinical Trials with Statins in MS

The first clinical evidence of both immunomodulatory and neuroprotective effects of statins comes from three open-label studies in patients with RRMS, demonstrating positive effects on both the number and the size of MRI gadolinium-enhancing lesions, compared to baseline, after 12, 6, and 9 months of treatment, respectively, with a high-dose of statins [182–184] (Table 4). Despite the beneficial effects, the analysis design is questionable and is exposed to the risk of probabilistic phenomena, such as regression to the mean, i.e., an expected decrease in disease activity in a population of patients with high initial activity [184]. In subsequent randomized, placebo-controlled trials, atorvastatin or simvastatin was consistently combined with IFNβ in patients with RRMS, at different doses. However, in view of the inconsistency of the results, some showing a worsening of the disease, an antagonistic effect of this association (statin and IFNβ) has been suggested [185]. The interaction between statin and interferon on blocking the signal transducer and activator of transcription 1 (STAT-1) phosphorylation and so the induction of IFNβ-stimulated genes was evaluated in the SIMCOMBIN trial, to remove any doubt about a possible antagonistic effect of the combination [186]. The evaluation of in vivo IFNβ bioactivity was assessed by measuring mRNA expression of the biomarkers IL-10, TNFSF10, MX1, and IRF7. All patients treated with IFNβ1a 30 μg/week and simvastatin had a full in vivo response, thereby providing reassurance on the concomitant use of the combination. The ACTIVE, SWABIMS, SIMCOMBIN, and ARIANNA [186–188] studies investigated the long-term treatment effect of statins (atorvastatin or simvastatin), between 12 and 24 months, in combination with IFNβ in RRMS. They were all negative on their primary endpoint [186, 188, 189]. A post hoc analysis of the SENTINEL trial, and thus a retrospective study, found no significant changes on clinical (ARR, disability progression) or MRI (number of Gd-enhancing lesions [GELs], number or new enhancing T2-hypertensive lesions) endpoints between the 40 patients treated with statins, at doses used to treat hypercholesterolemia, and IFNβ1a, compared to the 542 patients treated.
Table 4 Summary of studies on neuroprotection with statins in multiple sclerosis

| Dose       | Duration of treatment | Study type                                      | Sample size/patients (number) | Results (outcomes)                                                                                                                                                                                                 | References |
|------------|-----------------------|------------------------------------------------|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| **Lovastatin** |                       |                                                |                               |                                                                                                                                                                                                               |            |
| 20 mg/day  | 12 months             | Add-on therapy with IFNβ One-center, open-label, non-placebo-controlled | 7 patients (24–45 years) with RRMS | EDSS unchanged, decreased mean annual relapse rate for 3 patients, decreased average number of GELs for 4 patients                                                                                     | [183]      |
| 40 mg/day  | for 1 month, then     |                                                |                               |                                                                                                                                                                                                               |            |
|            | if no AE              |                                                |                               |                                                                                                                                                                                                               |            |
| **Simvastatin** |                       |                                                |                               |                                                                                                                                                                                                               |            |
| 80 mg/day  | 6 months              | No previous treatment with IFN or glatiramer 3 months before | 30 patients (18–55 years) with RRMS | 44% and 41% reduction in mean number of GELs ($p < 0.0001$) and mean volume ($p = 0.0018$), respectively, compared to 3-month baseline period. No effect on T2 lesion volume, brain parenchymal fraction, EDSS, and yearly relapse rate | [182]      |
|            |                       | Multicenter, open-label, single-arm           |                               |                                                                                                                                                                                                               |            |
| 80 mg/day  | 24 months             | Multicenter, phase II, double-blind, placebo-controlled trial (MS-STAT study) | 140 patients (18–65 years) with SPMS (statin $n = 70$, placebo $n = 70$) | Reduction of the annualized rate of whole-brain atrophy and disability ($−0.254\%$ per year; $p = 0.003$)                                                                                     | [192]      |
| 80 mg/day  | 24 months             | Multicenter, phase II, double-blind, placebo-controlled (MS-STAT study) | 140 patients (18–65 years) with SPMS (statin $n = 70$, placebo $n = 70$) | Improvement in the frontal lobe function (T score decline of 5.7 points on tests of verbal memory, $p < 0.0001$ and 6.8 points on tests of non-verbal memory, $p < 0.0001$) and physical quality of life (increase of 2.5 points on mean physical component score of the SF-36; $p = 0.028$) | [193]      |
| Dose       | Duration of treatment | Study type                                                                 | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|------------|-----------------------|-----------------------------------------------------------------------------|-------------------------------|----------------------------------------------------------------------------------|------------|
| 80 mg/day  | 12 months             | Add-on therapy with IFNβ1a (30 µg/week, IM)                                 | 307 treatment-naïve patients with RRMS (18–55 years) | No significant improvement in ARR and atrophy progression                         | [186]     |
|            |                       | Phase IV, multicenter, placebo-controlled, double-blind, randomized trial, parallel group (SIMCOMBIN study) | IFNβ + statin n = 151, IFNβ + placebo n = 156 |                                                                                  |            |
| Atorvastatin | 9 months             | Phase II, open-label, baseline to treatment trial                           | 41 patients (18–55 years) with RRMS (IFNβ + statin, n = 16, statin n = 25) | Trend towards reduction of GELs (with IFNβ + atorvastatin, p = 0.060), increase in IL-10 production, no suppression in T cell response | [184]     |
| 80 mg/day  |                       | Alone or with IFNβ (IFNβ1a 22 µg × 3/week or IFNβ1b every other day)       |                                                                             |                                                                                  |            |
| 40 mg/day  | 6 months              | Phase II, double-blind, placebo-controlled, randomized trial               | 26 patients with RRMS (IFNβ + placebo n = 9, IFNβ + statin n = 17)          | Increased MRI and clinical disease activity with atorvastatin (p = 0.019)         | [185]     |
| (n = 7) or|                       |                                                                             |                                                                             |                                                                                  |            |
| 80 mg/day  |                       | Add-on therapy with high-dose of IFNβ1a (44 µg × 3/week, SC)               |                                                                             |                                                                                  |            |
| (n = 10)   |                       |                                                                             |                                                                             |                                                                                  |            |
| 20 mg/day  | 24 months             | Add-on therapy with high-dose IFNβ1a (44 µg × 3/week, SC)                  | 45 patients (18–50 years) with RRMS and poorly responder to IFNβ1a (IFNβ + statin n = 21, IFNβ n = 24) | Reduced GELs vs. baseline (p = 0.007) and relapses (p < 0.001) vs. the two pre-randomization years with atorvastatin | [187]     |
|            |                       | Open-label, randomized, controlled, longitudinal (24 months follow-up) trial (ACTIVE study) |                                                                             |                                                                                  |            |
| Dose       | Duration of treatment | Study type                                                                 | Sample size/patients (number)                  | Results (outcomes)                                                                 | References |
|------------|-----------------------|----------------------------------------------------------------------------|-----------------------------------------------|----------------------------------------------------------------------------------|-----------|
| 40 mg/day  | 15 months             | Add-on therapy with IFNβ1b (3 months)                                       | 76 patients (18–55 years) with RRMS (IFNβ + statin n = 38, IFNβ n = 38) | No significant difference in number of patients with new T2 lesions and in secondary endpoints, after 12 months of treatment | [189]     |
|            |                       | Multicenter, randomized, control trial, parallel group, rater-blinded study (SWABIMS study) |                                               |                                                                                  |           |
| Atorvastatin (n = 26) | Different dosages, different durations: median duration of concomitant IFNβ1a and statin administration = 657 days | Post hoc analysis (SENTINEL, prospective trial of 1171 patients with RRMS treated with natalizumab (n = 589) vs. placebo (n = 582)) | 582 patients (18–55 years) with RRMS (IFNβ + statin n = 40, IFNβ n = 542) | No significant changes in terms of clinical (AAR, disability progression) and MRI (number of GELs, number of new or enhanced T2-hyperintense lesions) efficacy at 1 and 2 years. More musculoskeletal pain and hepatic enzyme abnormalities in the statin group | [190]     |
| Simvastatin (n = 13) |                       | Add-on therapy with IFNβ1a (30 µg/week IM)                                 |                                               |                                                                                  |           |
| Atorvastatin 40 mg/day | 24 months             | Phase II, multicenter, randomized, double-blind, placebo-controlled (ARIANNA study) | 154 patients (18–50 years) with early RRMS (diagnostic MS within 5 years (statin n = 75, placebo n = 79) | No difference in rate of brain atrophy between the two groups after 1 and 2 years, compared to baseline | [188]     |
|            |                       | Add-on therapy with IFNβ1b (250 µg, every other day, SC)                   |                                               |                                                                                  |           |

AE adverse events, AAR annualized relapse rate, GELs Gd-enhancing lesions, RRMS relapsing–remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis
with IFNβ1a alone, at 1 and 2 years [190]. Despite some biases (retrospective study, non-identical dose, two statins used, different statin treatment durations), the observation of musculoskeletal pain, as well as hepatic enzyme abnormalities, in the statin-treated group suggests that the dose received was sufficient to induce an effect on MS [190]. Even if the comparison of the results between these different studies is made difficult by the different endpoints and by the selection of patients being specific to each trial (years since onset of MS symptoms, EDSS score, number of relapses within previous year), there is no strong evidence of adapted doses of atorvastatin (low or high) or simvastatin, nor of effective treatment duration in add-on therapy with IFNβ, in patients with RRMS. The variable response to statins in MS trials may be explained by an inhomogeneous enrolled population with different inflammatory and progression stages.

In view of these contradictory results, a meta-analysis including eight clinical trials, five on RRMS and one on SPMS, showed no significant effect of statins when added to IFNβ therapy in RRMS. However, statins may be beneficial in SPMS in reducing brain atrophy and disability progression, but they have no effect on relapse rate [191]. This hypothesis was confirmed in the MS-STAT study, where a high dose of simvastatin (80 mg/day, for 2 years) attenuated brain atrophy and disability in patients with SPMS, supporting a real effect on disease progression. The lack of effect of simvastatin on five immune markers (IFNγ, IL-4, IL-10, IL-17, and CD4 Fox P3 levels) did not confirm a neuroprotective effect despite the clinical evidence [192]. Another post hoc analysis of the same study (MS-STAT) highlighted the positive effect of simvastatin on frontal lobe function and physical quality of life [193].

Sodium, Calcium, and Potassium Channels Blockers

**Rational for the Use of Sodium, Calcium, and Potassium Channel Blockers in MS**

Blocking cation channels, such as sodium and calcium channels, has long been used as a neuroprotective approach in clinical situations such as stroke. This approach makes sense if we consider a potential mechanism of neurodegeneration involving these channels. Thus, it appears that NO, a mediator of inflammation, could strongly slow down or even stop the propagation of action potential in demyelinated fibers [194, 195]. Shrager et al. showed that this NO effect depends on the axonal environment, in their study a sciatic nerve [194]. Surprisingly, Chen and Schofield showed that NO could activate calcium channels in cervical ganglion neurons through a cGMP-dependent mechanism [196, 197]. Thus, NO would block nerve conduction, not by blocking depolarizing channels but by causing a depolarizing block of conduction [194].

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from neurons and express voltage-dependent channels such as sodium and potassium channels that seem to play a key role in the demyelination process [203]. Interestingly, glatiramer acetate, used as an immunomodulator by acting on B lymphocytes, affects the expression of K⁺ and Cl⁻ channels and the entry of Ca²⁺ into the cells [204]. The effect of drugs affecting ion channels must therefore be considered in a broad way by taking into account the effects on neurons and glial and immune cells. This multicellular impact greatly complicates interpretations and the transition from in vitro studies to in vivo results, especially in clinical trials.

In oligodendroglial cells, numerous Na⁺ (Nav), Ca²⁺, and K⁺ channels are expressed [203]. Voltage-dependent Na⁺ channels have been proposed as links between demyelinated axonal sections and oligodendrocyte progenitors [205]. In demyelinating diseases, Na⁺ channels appear to play opposing pathophysiological roles. Thus, Nav1.2 appears to support conduction by means of rapidly activating and inactivating currents. In contrast, Nav1.6 produces sustained depolarizations that can cause Ca²⁺ overload and all the associated intracellular deleterious effects [206, 207]. This means that targeting of Na⁺ channels in MS must be selective to reduce the risk of antagonistic actions. This led to the testing of PF-01247324, a selective blocker of Nav1.8, in mice with EAE. This compound was able to improve locomotor coordination disorders and the cerebellar syndrome [208]. Regarding voltage-dependent Ca²⁺ channels (Cav), all families are expressed in oligodendrocytes (L, T, N, and P/Q) [209]. Their roles are fundamental in the proliferation, migration, and maturation processes of oligodendrocyte progenitor cells (OPCs). Moreover, invalidation of Cav1.2 in these OPCs reduces their maturation and myelination in the mouse brain and disrupts remyelination in a non-inflammatory demyelination model induced by cuprizone [210, 211]. Thus, Ca²⁺ channel blockade appears to be deleterious. Nevertheless, here again, there would appear to be a balance between the different cell types since genetic invalidation of Cav1.2 in astrocytes decreases the activation and proliferation of astrocytes and microglia, as well as the inflammatory response measured by the production of TNFα, IL-1β, and TGFβ1. These effects are reproduced by nimodipine, a brain-tropic calcium antagonist [212]. Concerning this particular calcium channel antagonist, the origin of its neuroprotective effect appears elusive. Schampel et al. tested nimodipine in experimental autoimmune encephalomyelitis and demonstrated that this drug attenuates clinical signs and spinal cord degeneration and promotes remyelination [213]. This protection is due to the induction of microglia apoptosis. This effect is reproduced with high nimodipine concentrations (5 and 10 μM) in two microglia cell lines (N9 and BV-2) but not by nifedipine, another dihydropyridine calcium channel antagonist. The mechanism by which nimodipine induces such a neuroprotection is still unknown but seems drug-specific and not related to calcium channels. With respect to K⁺ channels, there is also a balance among various cell types. The main K⁺ channels opening at the resting membrane potential are the inward-rectifier Kir4.1 and the two-pore K⁺ channels [209]. Genetic knockout of Kir4.1 strongly disrupts oligodendrocyte maturation and myelination during development, an effect originating in mature oligodendrocytes since selective invalidation in these cells reproduces the phenotype [214, 215]. Interestingly, antibodies against Kir4.1 have been identified in patients with MS, suggesting a pathophysiological role associated with the blockade of this channel [216]. Thus, K⁺ channel blockade seems to be a poor therapeutic option to slow down demyelination or stimulate remyelination. However, at the neuronal level, demyelination leads to alterations in the expression and distribution of K⁺ channels [217]. This delocalization could disrupt neuronal function. Overall, the contribution of cationic channels to the pathophysiology of demyelination and remyelination is far from clear, nor is its contribution to the maintenance of neuronal functionality in MS. Nevertheless, several molecules have been evaluated in clinical trials.
**Clinical Trials**

**Sodium Channel Blockers** The sodium channel blocker phenytoin has been tested in a phase II randomized, double-blind, placebo-controlled trial in 86 patients aged 18–60 years with acute optic neuritis [218] (Table 5). The primary outcome was retinal nerve fiber layer (RNFL) thickness in the affected eye at 6 months. This criterion was analyzed in a modified intention-to-treat population. Patients of the phenytoin group received 4 (n = 29) or 6 mg/kg/day (n = 13) depending on the time of randomization. In the 81 patients finally analyzed at 6 months, a 30% reduction in RNFL thickness deterioration was observed in the affected eye compared to placebo, with treatments being well tolerated. A similar reduction was observed concerning macula volume. Regarding the secondary criteria, no difference was observed in terms of visual function, visual evoked potentials (VEP) latency, or VEP amplitude. Importantly, no impairment of vision was observed after discontinuation of the phenytoin treatment. These results argue in favor of protection of the ganglion cells and their axons in the RNFL and the optic nerve and validate the hypothesis of protection given by sodium channel blockers in an inflammatory demyelination. Unfortunately, the design of the study and its small power did not offer the possibility to observe a clinical improvement.

In a double-blind, parallel-group phase II trial, lamotrigine (400 mg/day) was tested against placebo for 2 years in patients with SPMS [219]. The primary outcome was the rate of change of central cerebral volume. Of the 108 patients analyzed for the primary outcome, 52 received lamotrigine and 52 were in the placebo group. No significant difference was observed. However, lamotrigine reduced the deterioration of the T25FW without changing any other clinical endpoint. Of note, an exploratory modelling analysis seemed to show a greater partial (central) cerebral volume loss in the first year of the lamotrigine treatment that reversed after treatment discontinuation, arguing in favor of a “treatment effect”. Nevertheless, the effects were not spectacular and the results were limited by the small power of the study.

Carbamazepine, another sodium channel blocker, is reported to be neuroprotective in patients with MS. Nevertheless, there are only case reports and as yet no randomized clinical trial. Iorio et al. reported the case of a patient with paroxysmal ataxia and dysarthria that developed while he was recovering from an MS relapse [220]. A treatment with carbamazepine fully reversed the symptoms. Similarly, Li et al. reported that carbamazepine alleviated paroxysmal dysarthria in two patients with MS [221]. The first patient had dysarthria and a decrease of bilateral vision. Brain MRI showed lesions in the bilateral periventricular white matter. He received carbamazepine (400 mg/day) in combination with methylprednisolone (1 g/day, 3 days) and improved progressively until 6 weeks of treatment. The second patient presented with paroxysmal dysarthria-ataxia syndrome and paresthesia in the tongue, numbness of the right face, and incoordination of the left limbs. MRI revealed multiple lesions. He was treated with carbamazepine for 4 weeks and showed a reduction in the number of episodes followed by a complete suppression. The three cases described in these two publications argue in favor of building a randomized clinical trial testing carbamazepine in symptomatic MS.

**Calcium Channel Blockers** Despite encouraging preclinical data on the anti-inflammatory properties of calcium channel blockers in MS models [203], there are no data reporting their clinical effects so far. Outside the field of degenerative diseases, the calcium blocker pregabalin, already used to manage pain, was shown to reduce spatial working memory when used postoperatively in humans [222]. No classical calcium channel blocker, such as nimodipine, verapamil, or bepridil, has been tested in MS and it is debatable if this could be done in view of the safety data reported by Myhre et al. [222].

**Potassium Channel Blockers** Among potassium channel blockers, fampridine has been marketed in some countries for the treatment of walking disability in adult patients with MS. Dalfampridine is an extended-release form that is only marketed in the USA. Its mechanism of
Table 5: Summary of studies on neuroprotection with ion channel blockers in multiple sclerosis

| Dose | Duration of treatment | Study type | Sample size/patients (number) | Results (outcomes) | References |
|------|-----------------------|------------|------------------------------|--------------------|------------|
| Na⁺  | 6 months              | Phase II, randomized, double-blind, placebo-controlled | 86 patients (18–60 years), acute optic neuritis | 30% reduction of retinal nerve fiber layer thickness in the affected eye compared to placebo | [218] |
| Phenytoin 4 or 6 mg/kg/day |                        |            |                              |                    |            |
| Na⁺  | 2 years               | Phase II, randomized, double-blind, parallel group | 108 patients, SPMS | Primary outcome: central cerebral volume. No difference. Improved performance on the timed 25-foot walk | [219] |
| Lamotrigine 400 mg/day |                        |            |                              |                    |            |
| Na⁺  | 6 or 4 weeks          | Case reports | 3 patients                   | Reversal of paroxysmal ataxia and dysarthria | [220, 220] |
| Carbamazepine 400 mg/day |                      |            |                              |                    |            |
| K⁺   | 24 weeks              | Phase III, ENHANCE trial | Relapsing or progressive MS | Improvement of the MSWS-12 in 43.2% vs. 33.6% in placebo | [223] |
| Fampridine  Dalfampridine 10 mg twice daily |                      |            |                              |                    |            |
| K⁺   | 24 weeks              | Phase II, double-blind, placebo-controlled | 262 patients | Endpoint: total motor score of American Spinal Injury Association manual motor test. No effect | NCT00093275 |
| Nerispidine 100–400 mg once daily |                      |            |                              |                    |            |
| K⁺   | 1 day                 | Phase II, double-blind, placebo-controlled, randomized, crossover | 31 patients | Endpoint: VEP P100 latency. No effect | Sanofi-Aventis website NCT00772525 |
| Nerispidine 50 and 400 mg |                      |            |                              |                    |            |
| K⁺   | 14 weeks              | Phase II, randomized, double-blind, placebo-controlled, parallel group | RRMS, SPMS, PPMS | Primary outcome: improved performance on the timed 25-foot walk. No effect compared to placebo | Sanofi-Aventis website NCT00811902 |

RRMS relapsing–remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis, PPMS primary progressive multiple sclerosis
action involves the blockade of voltage-dependent potassium channels, thereby improving conduction in demyelinated nerve fibers [223, 224]. Nerispirdine has been developed by Sanofi-Aventis as an acetylcholine release enhancer, but it is also a blocker of potassium and sodium channels [225]. These molecules have been tested in phase II trials as neuron protectors in MS and chronic spinal cord injury but did not show any neuroprotective effect. For nerispirdine, no phase III trial was performed and the drug was withdrawn from clinical development.

Glutamate Antagonist

**Rationale for the Use of Glutamate Antagonists in MS**

Gray matter damage occurring early in the disease course contributes to clinical disabilities [226, 227]. Neuronal loss occurring in normal appearing gray matter emphasizes the need to explore neuronal endangering mechanisms independent of demyelination, such as synaptic dysfunctions [228, 229]. In inflammatory bursts of MS, experimental models have identified actors detrimental for synapses such as components of complement C1q and C3 [230], TNFα [231], IL-1β and oxidative stress [232]. Besides, proinflammatory cytokines released in the lesion disturb neurotransmission by increasing glutamate-mediated transmission and reducing GABA transmission. Glutamate accumulates in the synaptic cleft and induces excitotoxic damage [233]. Moreover, the maintenance of this excess of excitatory input observed during remission phases of EAE could alter the establishment of neuronal connections [234]. Indeed, when long-term plasticity is impaired in EAE models, disability recovery is also strongly reduced [157].

Because of its potent reversibility, pharmacological treatment of synaptopathy remains attractive. Targeting of the glutamatergic pathway has therefore been tried with many drugs because of its role in the pathophysiology of MS. Increased glutamate concentration in the CSF correlates with relapsing phases of MS or disabling secondary progressive forms [235]. Therapeutic strategies have been tested through the targeting of glutamate ionotropic receptors (N-methyl-D-aspartate receptor [NMDAR], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor [AMPA], kainate receptor) as well as metabotropic receptors. Already known for its toxic effect on neurons, glutamate also affects oligodendrocytes, astrocytes, endothelial cells, and immune cells. In inflammatory lesions, the immune cells involved in extracellular glutamate release are monocytes, macrophages, microglia, and dendritic cells via the cysteine/glutamate antiporter Xc⁻ [236–238]. Altered glutamate transport likely contributes to this dysregulation, as seen for the glutamate transporter GLT-1, whose expression in oligodendrocytes decreases around active MS lesions [239].

AMPA receptors can lead to motor neuron damage through an excessive increase of calcium in cytosol and mitochondria resulting in oxidative stress [240]. Therefore, in the Theiler’s murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) chronic viral model of MS, the NBQX (2,3-dioxo-6-nitro-7-sulamoylbenzo[f]quinoxaline) AMPA/kainate receptor antagonist reduced the clinical score and axonal damage visualized by lower dephosphorylation of the heavy chain of neurofilament H [241]. An alternative targeting of AMPA receptors was performed with a TAT-fusion peptide disrupting the interaction between the AMPA receptor subunit GluR2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [242]. Zhai et al. observed increased GluR2/GAPDH complexes in plaques of MS. Already shown to prevent neuronal damage, this peptide successfully reduced the clinical score and axonal damage [243].

Extrasynaptic NMDARs are mediators of neuronal damage whereas synaptic NMDARs are mediators of plasticity, thus complicating neuroprotective approaches [244, 245]. However, preclinical studies using NMDAR antagonists were promising. When inhibited by NMDAR antagonists memantine or amantadine, rats subjected to an EAE protocol developed lower clinical scores [244, 246]. Conversely, antagonists of group I metabotropic glutamate receptors, mGluRs (LY 367385 and MPEP) failed to demonstrate a beneficial effect [246].
**Clinical Trials with Glutamate Antagonists in MS**

To date, clinical trials have failed to demonstrate any significant effect of memantine on patients with MS whatever the parameter investigated: fatigue, cognitive performance, or spasticity [247–251] (Table 6). Worse still, adverse events have been reported in several studies. Trials regarding treatment of fatigue by amantadine similarly have failed to reach a consensus [252, 253]. The first studies found a reduction of fatigue [254], but this effect was not found in more recent studies [255]. Ketamine, another NMDAR antagonist, emerged recently as a potential modulator of mood disorders with a rapid and sustained antidepressant effect when infused at low doses in patients suffering from treatment-resistant depression. A clinical trial conducted on participants with bipolar disorders showed also a strong and prolonged reduction in fatigue scores, indicating that ketamine may be a valuable approach in fatigue management in multiple diseases [256]. Thus, in patients with MS, ketamine infusions, which were shown to be safe and well tolerated, led to a decrease in fatigue severity over a long period [257]. Riluzole constitutes another approach via inhibition of glutamate release. It reduced inflammation, demyelination, axonal damage, and clinical score in a MOG-induced murine EAE model [258]. Despite encouraging data in a pilot study [259], phase II trials turned out to be disappointing since riluzole failed to reduce brain atrophy progression in early MS or in SPMS [260–262]. However, drug efflux transporters present in the BBB like P-glycoprotein (P-gp) and breast cancer-resistant protein (BCRP) could limit riluzole’s effect. One possible solution has been successfully experimented in a mouse model where P-gp/BCRP inhibitor rescued riluzole’s effect [263].

**Sex Hormones**

**Rationale for the Use of Sex Hormones in MS**

Steroids synthesized by peripheral glands act on several tissues in the body, including the peripheral and central nervous systems, since free steroids are capable of crossing the BBB. Indeed, steroid hormones (progesterone, estrogens, androgens, and corticosteroids) that are lipophilic molecules may control several processes in the CNS by crossing the BBB (by simple diffusion or via influx transporters) or by targeting BBB cells, which in turn affect the brain parenchyma by modulating inflammatory and oxidative mechanisms [264, 265]. Sex steroids exert protective effects on the BBB or restore its integrity and permeability in experimental models of stroke or LPS-induced inflammation in rodents [266, 267]. Decreased levels of circulating estrogens during aging have been correlated with increased BBB permeability while testosterone depletion is associated with glial cell (microglia and astrocytes) activation and increased inflammation and BBB permeability [268]. These parameters are improved by testosterone treatments [269]. Moreover, progesterone administration exhibited positive effects on the BBB physiology after stroke and traumatic brain injury [270, 271]. Altogether, these data strongly support the rationale for the use of steroidal hormones to tackle the central disorders observed in MS. Furthermore and more importantly, it is also demonstrated that in MS, female to male prevalence is approximately 3:1, primarily in RRMS forms [272]. Various hormone-related physiological conditions in women significantly impact both the frequency and the course of disease. Numerous studies have pointed to the fact that steroid hormones exert a large array of biological effects, including the modulation of diverse biological processes such as neuroprotection and myelination [273–279]. In terms of immune response, decreased concentrations of sex steroids are associated with higher serum levels of proinflammatory cytokines such as TNFα and INFγ [280, 281]. The influence of sex hormones on the immune system has also been observed in the EAE mouse model. Sex hormones (estrogens, progesterone, and androgens) likely play a role in the complex mechanism of the course of the disease. Indeed, the relapsing forms of MS are more frequent in young women; moreover, the relapse rate decreases during late pregnancy as hormonal estrogen secretions increase. These effects could be related to the anti-inflammatory properties.
| Dose       | Duration of treatment | Study type                                      | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|------------|-----------------------|------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------|------------|
| **Memantine** |                       |                                                |                               |                                                                                  |            |
| 1st week: 10 mg daily, then 20 mg daily until end of study | 3 months | Phase I, randomized, parallel assignment, double-blind, placebo-controlled | 64 patients (20–55 years) with RRMS | No efficacy for the management of MS-related fatigue. AEs led to dropouts in this study | [251]      |
| 10 mg twice a day: 4 weeks titration followed by 12 weeks on the highest tolerated dose | 16 weeks | Phase II–III, randomized, parallel assignment, double-blind, placebo-controlled | 82 patients (18–65 years), with RRMS, SPMS, PPMS | No improvement in cognitive performance | [250]      |
| 1st week: 5 mg, 2nd week: 10 mg, 3rd week: 15 mg, from 4th week until end of study: 20 mg | 52 weeks | Phase III, randomized, parallel assignment, double-blind, placebo-controlled (EMERITE study) | 93 patients (18–60 years), with RRMS and cognitive impairment | No differences in the Paced Auditory Serial Addition Test (PASAT) scores, short-term memory; attention scores, EDSS, and relapse rate. Tolerability was significantly worse than expected | [248]      |
| **Amantadine** |                       |                                                |                               |                                                                                  |            |
| 10 mg twice a day | 12 weeks | Phase IV, randomized, parallel assignment, double-blind, placebo-controlled | 21 patients (18–65 years), with MS and spasticity | No efficacy in treatment of spasticity although well tolerated | [249]      |
| Week 1: 137 mg/day, week 2–4: 274 mg/day | 4 weeks | Phase II, randomized, parallel assignment, double-blind, placebo-controlled | 60 patients (18–70 years) with MS and walking impairment | Treatment was generally safe and tolerated in MS patients. Improvement of walking speed. Greater proportion of treated patients experiencing a ≥ 20% improvement | [253]      |
Table 6 continued

| Dose                        | Duration of treatment | Study type                                                                 | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|-----------------------------|-----------------------|-----------------------------------------------------------------------------|--------------------------------|----------------------------------------------------------------------------------|------------|
| 137 mg or 274 mg/day        | 16 weeks              | Phase III, 3-arm, randomized, parallel assignment, double-blind, placebo-controlled (INROADS study) | 594 patients (18–70 years) with MS and walking impairment | A higher proportion of participants achieved a clinically meaningful improvement in walking speed for 274 mg ADS-5102 compared with placebo | [252]      |
| Week 1: 137 mg/day, week 2: 205.5 mg/day, and 274 mg/day for the remainder of the study | 52 weeks              | Phase III, open-label extension of NCT03436199, single group assignment | 424 patients (18–70 years) with MS and walking impairment | Study completion date, April 2021 NCT03567057 |            |
| Amantadine: 100–200 mg/day, modafinil: 100–200 mg/day, methylphenidate: 5–20 mg/day or placebo, each given for up to 6 weeks | 30 weeks              | Phase III, randomized, double-blind, placebo-controlled, crossover assignment, 4-sequence, 4-period (TRIUMPHANT-MS study) | 141 patients (18 years old) with MS and reported fatigue | The tested drugs were not superior to placebo in improving MS-related fatigue and caused more frequent AE | [255]      |
| Ketamine                    | 28 days               | Phase I–II, randomized, parallel assignment, double-blind, placebo-controlled | 18 patients (18–65 years), with MS and reported fatigue | Ketamine infusions were safe and well tolerated. They led to a reduction of longer-term fatigue severity in MS patients | [257]      |
of estrogens [282]. The influence of this hormone on the immune system has also been observed in the EAE mouse model. When administered to mice prior to inoculation of EAE, exogenous estriol reduces disease activity [283]. In EAE, both females and males had a decreased proinflammatory cytokine profile with estriol treatment [281].

Progesterone is believed to have neuroprotective, promyelinating, and anti-inflammatory effects in the nervous system [284]. In EAE mice, progesterone attenuates the clinical severity, decreases demyelination, neuronal dysfunction, and the inflammatory response [281, 285]. More importantly, it has been well demonstrated that the progesterone derivative 3α,5α-tetrahydroprogesterone, also called neurosteroid allopregnanolone, critically modulates neuroinflammatory processes and MS symptoms. Gas chromatography–mass spectrometry assessment evidenced reduced levels of allopregnanolone in the brain of patients with MS [255]. Interestingly, allopregnanolone treatment after EAE induction decreased the disease development in mice [286–288]. Recently, we also combined various cellular and biochemical methods to show a direct effect of allopregnanolone on microglial morphology and phagocytic function, suggesting that allopregnanolone-based treatment may be of interest for the development of effective neuroprotective strategies against neurological disorders, including MS, that are evoked by microglia-related abnormalities [289].

We have previously reviewed extensively the key role played by testosterone in the modulation of immunodulatory, neuroprotective, and promyelinating mechanisms—a role that makes this sex steroid a potentially important candidate for the development of a hormone-based

| Dose | Duration of treatment | Study type | Sample size/patients (number) | Results (outcomes) | References |
|------|----------------------|------------|------------------------------|--------------------|------------|
| Riluzole | 24 months | Phase II, double-blind, randomized, parallel group design, placebo-controlled | 43 patients (18–55 years) with early MS or clinically isolated syndrome (CIS) in the previous 12 months | Riluzole treatment failed to modify brain atrophy measures and serum neurofilament levels | [260] |
| Week 1–4: 50 mg/day, and 100 mg/day for the remainder of the study | 96 weeks | Phase IIb, multi-arm, double-blind, randomized, parallel assignment, placebo-controlled (MS-SMART study) | 445 patients (25–65 years) with SPMS | No effectiveness in reducing disease progression for SPMS | [262] |

*AE adverse events, RRMS relapsing–remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis, PPMS primary progressive multiple sclerosis*
therapeutic strategy against MS [290]. Indeed, low testosterone has been reported in 40% of men with MS, correlating with physical and cognitive disability [291] and worse clinical outcomes [292]. Furthermore, in EAE models, there also seems to be a protective role of testosterone. EAE is less severe in female mice pretreated with dihydrotestosterone. In castrated mice the symptoms are more severe and can be ameliorated with testosterone replacement [283]. Administration of testosterone to men with MS could prevent the progression of the disease because of its potential neuroprotective and promyelinating effects [290].

**Clinical Trials with Sex Hormones in MS**

On the basis of their neuroprotective, promyelinating, and/or anti-inflammatory effects, estrogen and testosterone have been considered as good candidates for MS treatment [290]. Therefore, clinical studies were conducted to investigate their therapeutic potential in MS (Table 7). The first clinical trial using estrogen was conducted as a pilot assay in 10 women with MS in order to mimic the protective effects observed during pregnancy. The resulting study showed a reduced ARR in the patients treated with estriol compared to the placebo group [293]. A phase III study entitled POPART'MUS (Prevention of postpartum relapsing with progestin and estradiol in MS) was also conducted in pregnant women with MS with the objective of preventing postpartum MS attacks. Unfortunately, no beneficial effect was found on either the relapse rates or on the MRI data [294]. Very recently, the group POPART'MUS designed a new clinical trial in postpartum women treated with nomeostrol acetate (NOMAc) and 17-beta-estradiol. After 12 weeks, no treatment efficacy was observed compared to the placebo, likely as a result of the slow rate of inclusions [295]. Very recently, a phase II trial protocol was initiated with the aim of evaluating the beneficial effects of testosterone supplementation in testosterone-deficient men with RRMS [301].

**Growth Factors**

**Rational for Use of Growth Factors in MS**

Brain injuries induce the expression of growth factors within lesions, some of which can be measured in CSF and serum of patients. Long known for their trophic support of neurons, growth factors display their effect through pleiotropic target cells, including oligodendrocytes and immune cells.

**Basic Fibroblast Growth Factor (bFGF, FGF2)**

bFGF deposits are observed in active lesions and around chronic lesions [302]. bFGF is also reported to be elevated in CSF and serum of patients with MS [303]. bFGF was already known to increase survival of neurons in vitro [304]. Addition of bFGF in lysolecithin-induced demyelination models improves restoration of axonal conduction and myelin basic protein (MBP) expression and decreases immune response in EAE models [305, 306]. However, the roles of bFGF remain complex. Indeed, bFGF KO mice exhibit severe EAE [307], whereas depletion of its receptor FGFR1 in myelin proteolipid protein (PLP)-positive cells enhances myelination and axon integrity recovery after cuprizone treatment [308]. The opposite result is observed when FGFR1 and FGFR2 are knocked out in 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP)-positive oligodendrocyte lineage cells, which impair remyelination in the same model [309]. This is likely due to different effects of the cytokine, since bFGF promotes
Table 7  Summary of studies on neuroprotection with sex hormones in multiple sclerosis

| Dose                      | Duration of treatment | Study type                          | Sample size/patients (number) | Results (outcomes)                                                                 | References |
|---------------------------|-----------------------|-------------------------------------|-------------------------------|-----------------------------------------------------------------------------------|------------|
| *Estrogen/progestin*      |                       |                                     |                               |                                                                                   |            |
| Oral Estriol 8 mg + 100 mg progesterone daily | 18 months as crossover design. Treatment: 6 months on 6 months off and 4 months on | Crossover design             | 10 patients: 6 with RRMS and 4 with SPMS                                   | Decrease in number and volume of lesions IFNγ levels and TNFα production reduced Anti-inflammatory IL-5 and IL-10 upregulated | [293]      |
| Oral progestin and estradiol or placebo | 3 months | Double-blind, phase III POPART’MUS | 107 pregnant women          | No beneficial effect                                                             | [294]      |
| Ethinyl-estradiol (20 µg or 40 µg) plus INFβ (44 µg) or INFβ alone | 24 months | Phase II POPART’MUS                  | 148 women with RRMS          | Lesions decreased Anti-inflammatory effect of high-dose of estrogens (40 µg)     | [294]      |
| Oral estriol (8 mg) and injectable glatiramer acetate (20 mg) | 24 months | Double-bind phase II                  | 164 women with RRMS          | Relapse rate reduced                                                             | [296]      |
| Oral NOMAc 10 mg/day and transdermal 17-beta-estradiol (75 µg/week) or placebo | 12 weeks | POPART’MUS study, proof-of-concept trial | Postpartum women | No effect after 12 weeks                                                          | [295]      |
proliferation and migration of OPCs but inhibits oligodendrocyte differentiation [310]. The restrictive permeability of the BBB being a major limit for administration of large proteins such as growth factors, new approaches to facilitate their crossing, e.g., through the use of nanoparticle transport, have been developed to solve this problem. Thus, bFGF was loaded in chitosan nanoparticles conjugated with antibodies directed against transferrin receptor 1 to induce receptor-mediated transcytosis across the BBB and demonstrated a neuroprotective effect in a model of cerebral ischemia [311].

**Brain-Derived Neurotrophic Factor (BDNF)** BDNF is found in lesions and in perivascular infiltrates [312] and is generally reported to be elevated in patients with MS, as seen in the production of peripheral blood mononuclear cells [313]. Interestingly, CSF of patients with SPMS exhibits less BDNF than in CSF of patients with RRMS, arguing in favor of a link between BDNF deficit and disease progression [235]. In line with this, myelin deficits induced by cuprizone feeding are stronger in BDNF ± mice compared to wild-type (WT) animals [314]. Delivery of BDNF in an EAE model by transplantation of BDNF-engineered bone marrow stem cells decreased inflammation, apoptosis, and demyelination and reduced overall clinical severity [315]. To allow BBB crossing by BDNF, different strategies have been tried, such as fusion of BDNF with the cell-penetrating peptide TAT [316] or smaller BDNF mimetics mimicking a region binding its receptor [317]. Thus, several strategies have been developed to allow brain access of growth factors injected at the periphery.

**Insulin-Like Growth Factors (IGFs)** IGF receptors are enhanced in chronic demyelinating lesions but the level of IGF is not increased in serum and CSF of patients with MS [318–320]. In demyelinating lesions of animal models, IGF is increased in astrocytes and microglia, whereas IGF receptors are observed in

| Table 7 continued |
|-------------------|
| **Dose**          | **Duration of treatment** | **Study type**     | **Sample size/patients (number)** | **Results (outcomes)** | **References** |
| **Testosterone**  | 12 months                | Phase II pilot study | 10 men with RRMS                 | Cognitive performance improved |
| 10 g/day Androge containing 100 mg testosterone |                     |                          |                                   | Increased production of BDNF and PDGF-BB |
| Intramuscular injection 66 weeks, parallel-group | TOTEM protocol phase II randomized, double-blind | 40 testosterone deficient men with RRMS in 2 groups (treated and placebo) | Evolution evaluated by MRI parameters and clinical outcomes |
| 1000 mg/4 mL solution testosterone undecanoate or placebo |

*RRMS* relapsing–remitting multiple sclerosis, *SPMS* secondary progressive multiple sclerosis
Furthermore, the ablation of IGF1-R in a cuprizone model impairs remyelination [323]. Corroborating this result in EAE models, IGF injection reduces lesions and the clinical score [324, 325]. Despite these encouraging results, a pilot study of subcutaneous administration of recombinant human IGF-1 (rhIGF-1) in seven patients with MS displayed no significant effect [326] (Table 8).

Clinical Trials with Erythropoietin (EPO) in MS

In the development of new therapeutic strategies to treat MS, EPO is the most advanced growth factor. Essential for red blood cell production, EPO has multifunctional protective effects, due to its anti-inflammatory properties [327], blocking of ROS production and related apoptosis, neuroprotective effects, and stimulation of neurogenesis [328, 329]. EPO stimulates proliferation of OPCs and maturation of oligodendrocytes in vitro [330, 331]. In a rat model of EAE, recombinant human EPO (rhEPO) reduced inflammatory cytokines and infiltration of immune cells within lesions [332]. In a mouse model of EAE, rhEPO treatment led to a reduction of inflammatory infiltrates as well as a better functional recovery associated with an increase of OPC proliferation and BDNF-positive cells [333]. In a MOG-induced mouse model with optic neuritis, EPO combined with a high dose of methylprednisolone protected the optic nerve by reducing axonal damage and demyelination [334]. A double-blind, placebo-controlled phase II study evaluated the therapeutic potential of EPO on optical neuritis as add-on therapy (NCT00355095; VISION PROTECT). After 16 weeks of EPO treatment, retinal nerve fiber layer thinning was less apparent and visual evoked potentials were significantly shorter [335] (Table 8). A phase III study was then conducted and results have just been published [336]. However, the study did not confirm previous results and displayed neither functional nor structural neuroprotection in the visual pathways after optic neuritis. Further developments in EPO engineering deserve attention, such as EPO-derived small peptide with neuroprotective activity without hematologic effects, which in EAE models showed a protective effect, with lower clinical scores and decreased astrogliosis [337] or engineering with chimeric antibody targeting the transferrin receptor to improve EPO BBB penetrability [338].

Antioxidant Agents

Coenzyme Q10

Coenzyme Q10 is the main endogenous ubiquinone and acts as an essential cofactor of the electron transport chain. It is synthesized in the inner mitochondrial membrane and plays an essential role in the mitochondrial respiratory chain. Coenzyme Q10 is one of the most potent lipophilic antioxidants, acting as a catalytic antioxidant when chemically reduced from the ubiquinone to the ubiquinol form [339]. In progressive MS, oxidative stress and mitochondrial injury occur as a consequence of chronic inflammation and have a harmful impact on neurons and axons [340, 341].

Sanoobar et al. evaluated the effect of coenzyme Q10 supplementation on serum level of antioxidant factors in patients with RRMS (IRCT201102052602N5) (Table 9). Coenzyme Q10-treated patients had a significant increase in superoxide dismutase (SOD) activity and a decrease in malondialdehyde (MDA) levels compared with controls [342]. Coenzyme Q10 supplementation did not affect glutathione peroxidase activity. Moreover, the authors reported that coenzyme Q10 appears to decrease the inflammatory markers (TNFα, IL-6, and MMP-9) and improve fatigue and depression quantified by means of the fatigue severity scale and the Beck depression inventory [343, 344].

Moccia et al. reported that coenzyme Q10 in patients with RRMS improved various markers of scavenging activity (uric acid, bilirubin), oxidative damage (intracellular ROS, oxidative DNA damage), and induced a shift towards a more anti-inflammatory milieu in the peripheral blood [345].

Coenzyme Q10 exhibits limited oral bioavailability. For this reason, several synthetic
analogues of coenzyme Q10 such as idebenone and mitoquinone have been developed and proposed in numerous diseases with mitochondrial injury. They are currently being investigated as therapeutic options in MS.

**Idebenone**

Idebenone, (10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone), is a synthetic analogue of coenzyme Q10 described as a potent antioxidant. However, discrepancies between achievable tissue levels in brain and the doses required to show the proposed effects call into question the current proposed mechanism of action (MoA) [346]. Recent findings reviewed by Gueven et al. provide new insight into the MoA of idebenone. The authors hypothesize that to explain its pleiotropic effects, idebenone may modulate distinct signaling pathways, including inhibition of pS2Shc, increased Lin28A expression, activation of Akt, and the subsequent transcriptional changes [346]. In an EAE mouse model of MS, idebenone failed to affect disease incidence or onset when applied preventively, or to reduce disease severity when applied therapeutically [347]. In the clinical trial conducted by Kosa et al. (NCT00950248), idebenone was well tolerated but the change the area under the curve of the Combinatorial Weight-Adjusted Disability Score (CombiWISE) between idebenone and placebo did not reach statistical significance.

| Table 8 Summary of studies on neuroprotection with growth factors in multiple sclerosis |
|-------------------------------------------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Dose** | **Duration of treatment** | **Study type** | **Sample size/patients (number)** | **Results (outcomes)** | **References** |
| __________ | ______________________ | ______________ | ______________________ | ______________________ | ______________________ |
| **rhIGF-1** | 24 weeks | Pilot study | 7 patients (6 SPMS, 1 RRMS) | No difference between baseline and treatment periods for any MRI or clinical measures of disease activity | [326] |
| (CEP-151, Cephalon) | 0.05 mg/kg twice a day | | | | |
| **Erythropoietin** | 3 days of treatments and measures at week 16 | Phase II, double-blind, placebo-controlled | 40 patients with acute unilateral optic neuritis with or without prior diagnosis of MS | Lower thinning of retinal nerve fiber layer on EPO treatment vs. placebo ($p = 0.0357$) | [335] |
| 33,000 IU recombinant human erythropoietin or placebo as add-on therapy to methylprednisolone | | | | Smaller decrease in retrobulbar diameter of the optic nerve on EPO treatment ($p = 0.0112$) | |
| | | | | Shorter VEP latencies in EPO group ($p = 0.0011$) | |
| 33,000 IU recombinant human erythropoietin or placebo as add-on therapy to methylprednisolone | 96 weeks | Phase III, double-blind, placebo-controlled | 103 patients within 10 days after onset of unilateral optic neuritis without a previous diagnosis of MS | EPO as an adjunct conveyed neither functional nor structural neuroprotection in the visual pathways | [336] |
| | | | | AE in 81% of patients | |

**AE** adverse events, **RRMS** relapsing–remitting multiple sclerosis, **SPMS** secondary progressive multiple sclerosis
attesting that idebenone does not inhibit disability progression in PPMS [348].

**Mitoquinone**
Mitoquinone (MitoQ) is a quinone moiety linked to a triphenylphosphonium moiety by a 10-carbon alkyl chain [349]. The positive results in an EAE mouse model indicated that MitoQ could be a candidate for investigation in human MS [350]. The authors reported that MitoQ can exert protective effects on neurons and reduce axonal inflammation and oxidative stress. MitoQ is currently under investigation in the “MitoQ for fatigue in MS” phase I/II placebo-controlled trial (NCT04267926). In this trial, the investigators hypothesize that mitochondrial dysfunction and resultant neuronal energy depletion may be an important contributor to fatigue in MS. The study is still recruiting patients with MS with an EDSS score of 2–8, persistent fatigue (at least 2 months), and a Modified Fatigue Impact Scale (MFIS) score of 38 or greater. The primary outcome is change in fatigue impact as measured by the MFIS at 12 weeks.

**Pioglitazone**
Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors activated by small lipophilic molecules. Once activated by their ligand, these receptors bind to DNA sequences and regulate gene expression by transcriptional co-activation [351, 352]. More specifically, activated PPARs form a complex in the

### Table 9 Summary of studies on neuroprotection with antioxidant agents in multiple sclerosis

| Dose                        | Duration of treatment | Study type                | Sample size/patients (number) | Results (outcomes)                                                                 | References        |
|-----------------------------|-----------------------|---------------------------|-------------------------------|-----------------------------------------------------------------------------------|-------------------|
| Coenzyme Q10 500 mg/day for 12 weeks | 12 weeks              | Randomized, blinded, placebo-controlled phase III trial | 48 patients with RRMS          | Significant increase in SOD activity and decrease in MDA levels compared with controls | [342, 343] IRCT201102052602N5 |
| Coenzyme Q10 as an add-on therapy to IFNβ1a 200 mg/day | 3 months              | Open-label crossover design study | 60 patients with RRMS          | Increase of markers of scavenging activity (uric acid, bilirubin)                  | [345]             |
| Idebenone 2250 mg/day       | 2 years               | Randomized placebo-controlled phase I/II clinical trial | 77 patients with PPMS          | Idebenone was well tolerated but the CombiWISE score between idebenone and placebo did not reach statistical significance | [348] NCT00950248 |

RRMS relapsing–remitting multiple sclerosis, PPMS primary progressive multiple sclerosis
The complex finally targets the peroxisome proliferator response element in the promoter gene [354]. This is what causes the regulatory cascades. Pioglitazone is a member of the family of thiazolidinediones (TZDs). These drugs act as agonists of the PPARγ. Owing to their insulin sensitizing effect, two TZDs were originally marketed for the treatment of type 2 diabetes: pioglitazone and rosiglitazone. TZDs promote the differentiation of mesenchymal stem cells into adipocytes and lipogenesis in peripheral adipocytes, and they decrease hepatic and peripheral triglycerides and decrease visceral adipocyte activity [355]. These main effects of TZDs significantly improve insulin resistance and metabolic syndrome and decrease insulin requirements. However, TZD use has been limited because of concerns about safety issues and side effects. In particular, as a result of an increased risk of bladder cancer, pioglitazone was withdrawn by the French and German health authorities in 2011. However, a subsequent follow-up study in the USA involving over 193,000 patients aged 40 years and older found no correlation between bladder cancer and pioglitazone use. Bladder cancer may no longer be a significant issue [356]. Other side effects, such as edema, congestive heart failure, and bone fractures, require careful selection of patients who could benefit from these treatments [355].

Other effects of PPARγ agonists include reduction of TNFα, IL-1β, and inducible nitric oxide synthase (iNOS). PPARγ agonists also exert antiproliferative action, block cytokine production, and induce apoptosis in T cells [357–366]. Because of the anti-inflammatory properties of PPARγ agonists, several authors have hypothesized that the repositioning of pioglitazone in EAE could reduce clinical and histological manifestations. In a mouse model of EAE, pioglitazone reduced the incidence and severity of chronic single-phase disease [367]. The suppression of clinical signs was accompanied by a reduction in lymphocyte infiltration, a decrease in demyelination, a reduction in chemokine and cytokine expression, and an increase in inhibitor of kappa B expression in the brain. Pioglitazone also reduced antigen-dependent IFNγ production from EAE-derived T cells.

As a result of the neuroprotective and anti-inflammatory effects of pioglitazone and the encouraging results obtained from preclinical experiments in EAE models, a clinical trial dedicated to pioglitazone evaluation in RRMS was initiated. In this randomized, placebo-controlled phase I trial (NCT00242177), Stefoski et al. reported that pioglitazone was well tolerated, with a similar incidence of non-serious adverse events in the placebo and treatment groups (10 and 11 patients, respectively). After 1 year, there were no significant differences in clinical symptoms as assessed by EDSS. The authors reported that MRI showed a significant reduction in gray matter atrophy and that pioglitazone could reduce conversion of normal appearing white matter to lesions [368, 369].

Using a different approach, the TRAP-MS study (NCT03109288), a phase I/II open-label trial, is currently recruiting patients with progressive MS to evaluate whether signs of inflammation in CSF help predict a person’s response to different drugs, one of which is pioglitazone. The primary outcome is the change in CombiWISE progression rate at the end of the monotherapy plus combination therapy period in comparison to projected baseline disability progression. Clinical data on the use of pioglitazone in MS remain particularly limited to date. Pioglitazone concentrations reaching the brain could be an issue. Some BBB transporters restrict pioglitazone delivery to the brain and higher amounts than the usual diabetic dose could be required [370]. In the current state of knowledge, the therapeutic positioning of pioglitazone in MS is particularly unpredictable. Further studies are required to assess the interest of this molecule in MS.

DISCUSSION: TOWARDS A SCENARIO TO ASSESS THE EFFECT OF NEUROPROTECTIVE DRUGS

Many drugs have failed in their clinical trials in the treatment of PMS, and its treatment...
continues to be a challenge today. The causes of these failures are diverse.

The most obvious cause, as it is described in many sections in this paper, is that the drugs used in clinical trials have been identified in post hoc analysis of phase II trials in patients with RRMS. In PMS, inflammation is different from that observed in the remitting phase (compartmentalized with ectopic pseudofollicles, innate immunity with activated microglia), and the intact BBB prevents the migration of drugs into the CNS, thus limiting their effect.

Another reason for the failure of clinical trials could be an inappropriate sample size, population, or clinical trial design. For examples, trials with minocycline, phenytoin, or lamotrigine were underpowered and showed interesting results on substitution criteria but no significant effect in a clinical setting. Studies with ibudilast or statins included inhomogeneous population of patients with MS with different stages of inflammation and levels of neurodegeneration. Lastly, minocycline and statins were used as add-on therapy and this design limits the interpretation of the data, adding a possible antagonist effect between drugs. The duration of the trials is also a concern because phase II trials are in most cases limited to 6–12 months, which is too short to firstly stop the degenerative process and then to observe a clinical effect.

Finally, one of the most important challenges is evaluating the neuroprotective effect of these drugs. Despite the interesting putative role of conventional MRI in monitoring neurodegeneration, the fact that several approved MS drugs impact progression of MRI atrophy but not clinical worsening, and vice versa, underscores the lack of a strong surrogate for neuroprotection studies. New techniques such as ultrahigh-field MRI are currently being researched as they may provide benefits in terms of spatial resolution and accuracy [371]. PET imaging will provide improved anatomic localization, lead to a better understanding of MS pathophysiology, and enhance the monitoring of disease progression [56]. In particular, this technique has been applied to microglia activation, using PET-TSPO and communications are growing in this field of research. It therefore seems appropriate to conclude this discussion by outlining a putative ideal scenario in which patients with progressive MS will be selected on the basis of a recent progression of the disease without active inflammatory process, treated with a well-defined posology arising from strong evidence from preclinical and phase I studies. Time-to-event should be at least 12 months in phase II studies if a robust surrogate, such as PET-TSPO for microglia and chronic inflammation imaging or sNFL/sGFAP ratio for axonal damage, is used. Ambitious phase III trials should last long enough to identify a clinical effect that could be found outside of the EDSS score on a combined primary endpoint using EDSS and functional assessment. The “EDSS plus” includes the EDSS and two performance tests related to the 9HPT to assess upper extremity function and the T25FW to determine gait [372]. In addition to this clinical assessments, cognitive assessment with symbol digit modalities test or paced auditory serial audition could also be incorporated into the combined primary endpoint, as deterioration in cognition has been correlated with disease progression.

CONCLUSIONS AND PERSPECTIVES

Axonal transection and degeneration are a feature of MS and are predominant in the progressive form. Protecting the axon from metabolic changes induced by inflammation, demyelination, or direct metabolic alteration is the main challenge for years to come in the field of MS. To date, many candidates are well on their way to meeting these “unmet needs”. Among them, targeting microglia seems to be one of the most advanced approaches. To achieve this goal, molecules such as BTKi have entered phase III studies. On the other hand, in vivo assessment of microglia is being performed with MRI (SELS, iron rim) or PET-TSPO. Protecting the axon from oxidative stress or excitotoxicity could also be of interest and a therapeutic strategy could be proposed to use these treatments in addition to conventional DMTs. Much of the discovery of drugs for neuroprotection has been based on studies in
animal models such as EAE. Unfortunately, this model does not perfectly mimic the progressive form of MS in which the neuroprotection strategy may be most effective. Many studies have been successful in EAE models, but have failed to prove a neuroprotective effect in humans with MS. One reason could be the lack of sensitive and reliable outcome measures in MS to study neuroprotection. Another reason could lie in the BBB, which severely restricts the delivery of drugs to the CNS. Targeting the brain via the olfactory pathways or with a drug conjugate approach may need to be considered to bypass the BBB [373, 374]. Finally, major collaborative efforts are needed to improve animal models and outcome measures for the detection of neuroprotection and to develop ambitious clinical trials.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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