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Acting centrally or peripherally: A renewed interest in the central nervous system penetration of disease-modifying drugs in multiple sclerosis

Jorge Correale [a,*,#], Mario Javier Halfon [b,#], Dominic Jack [c], Adrián Rubstein [d], Andrés Villa [e,#]

[a] Department of Neurology, Fleni, Buenos Aires, Argentina
[b] Hospital Británico de Buenos Aires, Buenos Aires, Argentina
[c] Merck Serono Ltd, Feltham, United Kingdom (an affiliate of Merck KGaA)
[d] Merck S.A., Buenos Aires, Argentina (an affiliate of Merck KGaA)
[e] Hospital Ramos Mejía, Universidad de Buenos Aires, Argentina

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ABSTRACT

With the recent approval of cladribine tablets, siponimod and ozanimod, there has been a renewed interest into the extent to which these current generation disease-modifying therapies (DMTs) are able to cross into the central nervous system (CNS), and how this penetration of the blood-brain barrier (BBB) may influence their ability to treat multiple sclerosis (MS).

The integrity of the CNS is maintained by the BBB, blood-cerebrospinal fluid barrier, and the arachnoid barrier, which all play an important role in preserving the immunological environment and homeostasis within the CNS. The integrity of the BBB decreases during the course of MS, with a putative temporal relationship to disease worsening. Furthermore, it is currently considered that progression of the disease is mediated mainly by resident cells of the CNS.

The existing literature provides evidence to show that some of the current generation DMTs for MS are able to penetrate the CNS and potentially exert direct effects on CNS-resident cells, in particular the CNS-penetrating prodrugs cladribine and fingolimod, and other sphingosine-1 phosphate receptor modulators; siponimod and ozanimod. Other current generation DMTs appear to be restricted to the periphery due to their high molecular weight or physicochemical properties.

As more effective brain penetrant therapies are developed for the treatment of MS, there is a need to understand whether the potential for direct effects within the CNS are of significance, and whether this brings additional benefits over and above treatment effects mediated in the periphery. In turn, this will require an improved understanding of the structure and function of the BBB, the role it plays in MS and subsequent treatments.

This narrative review summarizes the data supporting the biological plausibility of a potential benefit from therapeutic molecules entering the CNS, and discusses the potential significance in the current and future treatment of MS.

1. Introduction

Although the etiology of multiple sclerosis (MS) remains unknown, current evidence suggests that MS is an immune-mediated disease in which both genetic and environmental factors contribute (Belbasis et al., 2015; Dendrou et al., 2015). Clinical phenotypes include clinically isolated syndrome (also referred to as a first clinical demyelinating event), relapsing-remitting MS, and progressive forms of MS (Lublin et al., 2014; Zettl et al., 2012).

The characteristic pathophysiological processes of MS include the breakdown of the blood-brain barrier (BBB), the presence of multifocal inflammatory lesions, reactive gliosis, oligodendrocyte loss, demyelination, axonal damage and neuronal loss as a result of diffuse neurodegeneration (Cohen and Rae-Grant, 2012; Trapp and Nave, 2008; Baecher-Allan et al., 2018; Duffy et al., 2014). The BBB plays a crucial role in homeostasis and regulating immune processes within the central

* Corresponding author. Department of Neurology, Fleni, Montañeses 2325, Buenos Aires 1428, Argentina.
E-mail address: jcorreale@fleni.org.ar (J. Correale).
Co-lead authors

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nervous system (CNS). However, during the evolution of MS, the integrity of the BBB is reduced, increasing its permeability. There is a putative temporal relationship between MS worsening and changes to BBB permeability, especially in cases of relapsing-remitting MS (Daneman and Prat, 2015; Ortiz et al., 2014).

It is thought that the progression of the disease is mediated, at least in part in the earlier stages of MS, by the ability of autoreactive lymphocytes and inflammatory monocytes to cross the BBB into the CNS (Du Pasquier et al., 2014). Inflammation within the CNS occurs when these autoreactive lymphocytes are re-activated mainly by microglia cells acting as antigen presenting cells (APCs) (Zeinstra et al., 2000), but also by dendritic cells that infiltrate into the CNS where they act as potent APCs (Chastain et al., 2011). As the disease progresses, chronic neuroinflammation and neurodegeneration are seemingly driven by resident cells within the CNS (i.e. astrocytes and microglia cells) and fewer peripheral immune cells are detected in brain lesions (Dendrou et al., 2015; Duffy et al., 2014; Bar-Or, 2008). This has led to the concept that immune processes relevant to MS may be operating in a body compartment protected by the BBB or other barriers within the CNS. These processes start early in the course of the disease and may become increasingly important and independent of immune processes occurring in the periphery as disease progresses.

The treatment of MS with disease-modifying therapies (DMTs) aims to diminish the effects of immune processes on the nervous system and address key pathological factors leading to disability progression. However, one of the striking features in the development of MS therapeutics is that most therapies cannot be transported across the BBB, thus leaving a knowledge gap about the ability of DMTs to exert a direct effect within the CNS. It is therefore presumed that these MS therapies affect cells of the immune system within the periphery, and this altered peripheral activity then translates into a modification of the processes occurring within the CNS (Antel and Miron, 2008).

A pathological feature of MS, particularly in the later stages of the disease, is the presence of tertiary lymphoid structures (TLS) in the meningeal space, i.e. on the CNS side of the blood-cerebrospinal fluid barrier (BCB) (Serafini et al., 2004). Immunohistochemical studies of these structures demonstrated that they contain B cells, T cells, plasma cells, and follicular dendritic cells expressing the lymphoid chemokine CXCL13 (Serafini et al., 2004; Pikor et al., 2015). The role of these structures has been debated, but it is thought that the meningeal lymphocytic aggregates are a source of soluble factors that degrade the glial limitans, and promote a gradient of demyelination and neuronal injury, particularly in the brain cortex of patients with MS (Magliozzi et al., 2007; Magliozzi et al., 2010). Indeed, the presence of meningeal TLS in secondary progressive MS (SPMS) correlates with the degree of microglial activation, gray matter cortical demyelination, accelerated disease progression, and age at death compared with SPMS cases without meningeal TLS (Howell et al., 2011). Pathogenic B cells likely act on both sides of the BBB by recirculating from within the brain to the secondary lymphoid tissue situated outside the brain and back again (von Büdingen et al., 2012; Stern et al., 2014). However, it is presumed that, while in the CNS, lymphocytes and other immune cells are relatively protected from agents that cannot access the CNS. The long lifespan of some lymphoid lineage cells potentially means that therapies could take a long time to act fully if they can only influence immune cells during the time that those cells are trafficking in the blood.

Animal modeling, in particular experimental autoimmune encephalomyelitis (EAE), has made an important contribution to the understanding of inflammation-induced neurodegenerative processes in MS pathogenesis (Lassmann and Bradl, 2017; Russi and Brown, 2015). Although EAE is characterized by loss of focal BBB integrity and involves CNS-infiltrating adaptive and innate immune cells, no experimental model covers the full spectrum of clinical, pathological, or immunological features of the MS. There are numerous models available to study different aspects of inflammation, demyelination, remyelination, and neurodegeneration in the CNS and results from these need to be interpreted carefully when extrapolating findings to human disease (Lassmann and Bradl, 2017).

Some of the currently approved therapies used in the treatment of MS, including cladribine tablets (Hermann et al., 2019; Kearns et al., 1994; Liliemark, 1997) and the sphingosine-1 phosphate receptor modulators fingolimod (Miron et al., 2008; Chun and Hartung, 2010; Hunter et al., 2016; Foster et al., 2007), siponimod (Tavares et al., 2014; Aslanis et al., 2012), and ozanimod (Lamb, 2020; Scott et al., 2016), can enter the CNS and potentially exert direct effects on CNS-resident cells. Direct actions of such DMTs on cells within the CNS could potentially provide neuroprotective effects and/or promote endogenous repair mechanisms (Antel and Miron, 2008; Hunter et al., 2016). However, it is unclear whether the CNS penetration of current generation DMTs gives rise to any additional treatment benefit over and above the treatment effects observed in the periphery.

In this narrative review, we summarize the ability of current generation DMTs to cross into the CNS, and what evidence exists to suggest that this penetration of the BBB has an additional affect beyond that of DMTs which are restricted to the periphery and act to stabilize the BBB.

1.1. Factors affecting CNS penetration of therapeutic molecules

If therapeutic molecules are to pass from peripheral blood into the CNS, they need to overcome the main biological barriers: the BBB, the epithelial cells of the choroid plexus forming the BCB, and the epithelium of the arachnoid mater that covers the outer brain surface above the layer of the subarachnoid cerebrospinal fluid (CSF) and forms the arachnoid barrier (**Fig. 1**) (Engelhardt et al., 2017; Dominguéz et al., 2013; Deczkowska et al., 2016).

These interfaces between blood vessels and CNS tissue or non-neural tissue have different cellular or biochemical properties that form the anatomical and immunological basis for these barriers and, in turn, influence penetration of molecules into the CSF or CNS tissue (Monaco et al., 2020). The BBB is considered the primary interface of the brain, separating the brain, CSF, and extracellular fluid of the CNS from the peripheral blood system (Dominguéz et al., 2013). The BCB plays a critical role in both the secretion of CSF and the exchange of various molecules between the blood and CSF (Ortiz et al., 2014; Correale and Villa, 2009). The choroid plexus consists of a single layer of epithelial cells that surrounds a core of capillaries and connective tissues (Lun et al., 2015). The epithelium of the choroid plexus is considered to be the most important part of the BCB due to its presence in each of the ventricles of the brain, which produces the majority of CSF (Ransohoff and Engelhardt, 2012), and also as a result of the direction of the flow of the CSF (Ortiz et al., 2014). The arachnoid barrier, comprised of a cell layer with numerous tight junctions, surrounds the brain and spinal cord, forming part of the BCB and is the most structurally complex but the least studied barrier to the brain (Correale and Villa, 2009; Yasuda et al., 2013).

The BBB in particular regulates the movement of cells and molecules between the peripheral blood system and the CNS, and is thought to effectively block between 98%–100% of all small and large molecule drugs from entering the CNS (Pardridge, 2005). This barrier therefore represents a potential therapeutic obstacle that needs to be overcome if the intent is to target cells within the CNS and have a direct action on neurodegeneration, chronic inflammation, and myelin repair.

The integrity of the BBB is reduced in MS, and there is a temporal relationship between MS worsening and increases in BBB permeability (Daneman and Prat, 2015; Ortiz et al., 2014). Furthermore, pathophysiological changes underlying disability and neurodegeneration in progressive MS are thought to be related to immune responses which are compartmentalized in the brain parenchyma and CSF-filled regions of the CNS (Monaco et al., 2020). Dysfunction of the BBB is, in part, caused by alterations to various components that are responsible for the integrity of the barrier, including tight junction proteins, molecule transporters, and the expression of leukocyte adhesion molecules

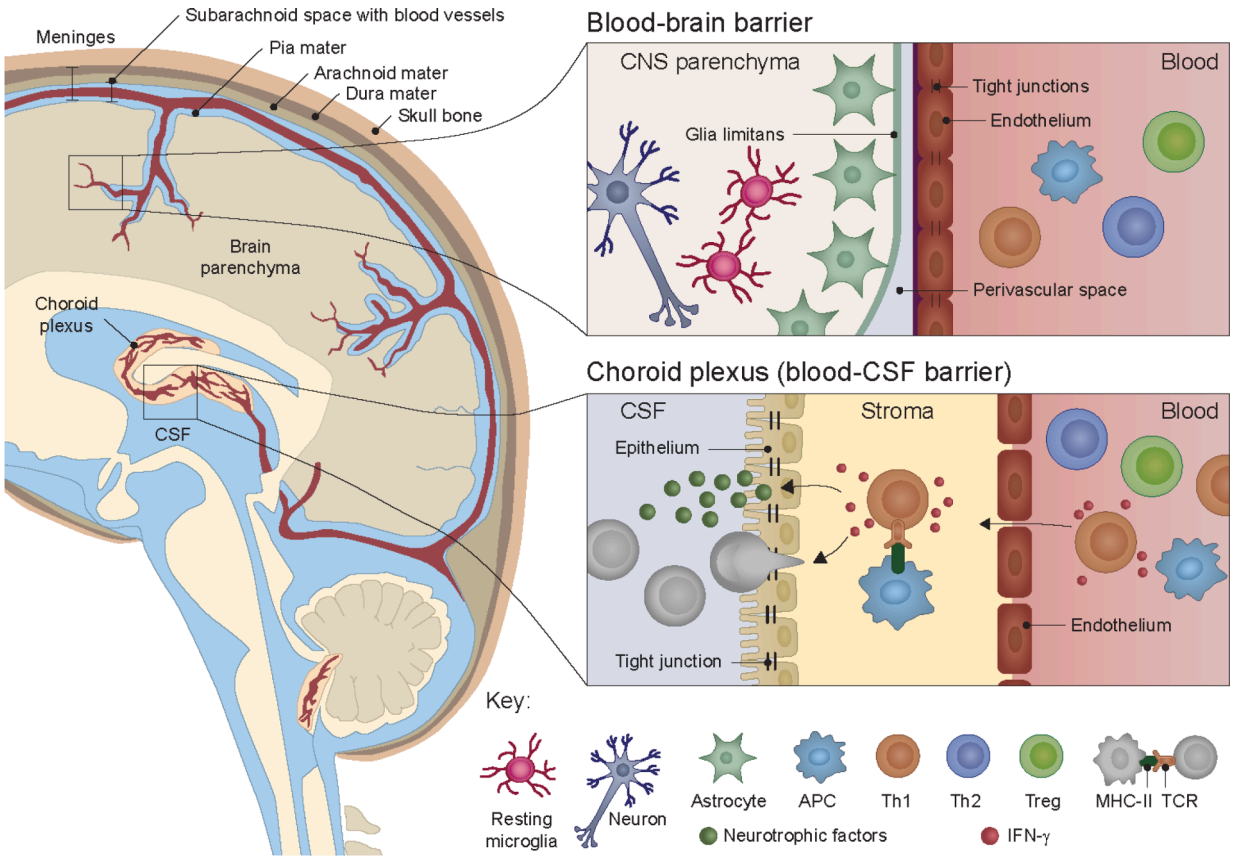

Fig. 1. The location of two main barriers that maintain separation of the periphery and the central nervous system (Deczkowska et al., 2016).

(Daneman and Prat, 2015). Disruption of the tight junction proteins, claudin and occludin, decrease the integrity of the BBB and allows for leukocytes to infiltrate the BBB via paracellular movement (Liebner et al., 2018).

The subsequent migration of B and T cells and macrophages across the BBB and BCB, as well as a secondary barrier, the glia limitans, is of central importance in the progression of CNS injury, demyelination and neuronal loss (Liebner et al., 2018; Noseworthy et al., 2000; Engelhardt and Ransohoff, 2012). Increased permeability of the BBB, or loss of BBB integrity, has been observed to precipitate periods of disease worsening in MS (Ortiz et al., 2014). These observations may suggest that changes to BBB integrity are important in MS worsening, perhaps due to the increased permeability to autoreactive immune cells which may, in turn, promote neuroinflammation and lesion formation. It is also true, however, that gray matter demyelination and neuroaxonal degeneration associated with the activation of microglia may be independent of BBB dysregulation (Herranz et al., 2016; Koudriavtseva and Mainero, 2016).

The mechanisms by which molecules are able to enter the CNS include passive diffusion, or involve carrier-mediated transport, receptor-mediated transport and active efflux transport at sites on the BBB and BCB (**Fig. 2**) (Dominguéz et al., 2013; Chen and Liu, 2012; Ghersi-Egea et al., 2018; Shawahna et al., 2011). Generally, it is only small molecules with a low molecular weight of <400–500 Da and/or high lipophilicity that can reach the CNS by passive or transcellular diffusion across the BBB (Dominguéz et al., 2013; Mikitsh and Chacko, 2014; Banks, 2009). Molecules with a high molecular weight and/or low lipid solubility require the presence of transporters to be able to cross the BBB. Efflux transporters move a variety of lipophilic substrates up the concentration gradient (Daneman and Prat, 2015), while influx transporters transport small hydrophilic molecules (Dominguéz et al., 2013; Deeken and Loscher, 2007; Sanchez-Covarrubias et al., 2014). A number of efflux and influx carrier-mediated transporters have been identified as barriers to drug delivery to the CNS, including ATP-binding cassette (ABC) transporters such as ABCG2 and P-glycoprotein (Sanchez-Covarrubias et al., 2014).

Initial attempts at improving drug delivery to the CNS focused on increasing lipid solubility (Correale and Villa, 2007). More recently, the development of therapeutic molecules targeting delivery within the CNS has concentrated on transportation across the BBB using receptors such as transferrin (Johnsen et al., 2019). Likewise, the BCB and the arachnoid barrier also express high numbers of transport proteins that facilitate drug penetration into the CNS (Yasuda et al., 2013). In MS research, CNS penetrant libraries are now used to investigate the abilities of small molecules to cross the BBB. However, it has previously been reported that CNS penetration for small molecule therapeutics does not increase despite the reported disruption to the BBB observed in MS (Cheng et al., 2010).

After crossing the BBB, a drug is able to distribute within the interstitial space by diffusion and convection, and, where possible, may also distribute into brain cells (Loryan et al., 2020). The diffusion of molecules within the CNS is governed by the features of the extracellular space, as well as properties of the molecule itself, and in turn determines the potential for transport across the cellular membrane (Wolak and Thorne, 2013). The role of convection, however, is more critical for the distribution of large molecules; yet, the exact mechanisms are still debated (Abbott et al., 2018).

In recent years, the MS treatment landscape has changed considerably with new biologic and small molecule drugs becoming available, and other compounds still in development. Many currently available DMTs are not able to enter the CNS and so their primary effects are exerted on the peripheral immune system, or on the function/integrity of the BBB. Such DMTs can change the biological function of immune cells or cytokines that may be able access the CNS, and so these drugs may be able to produce what have been referred to as 'indirect' effects

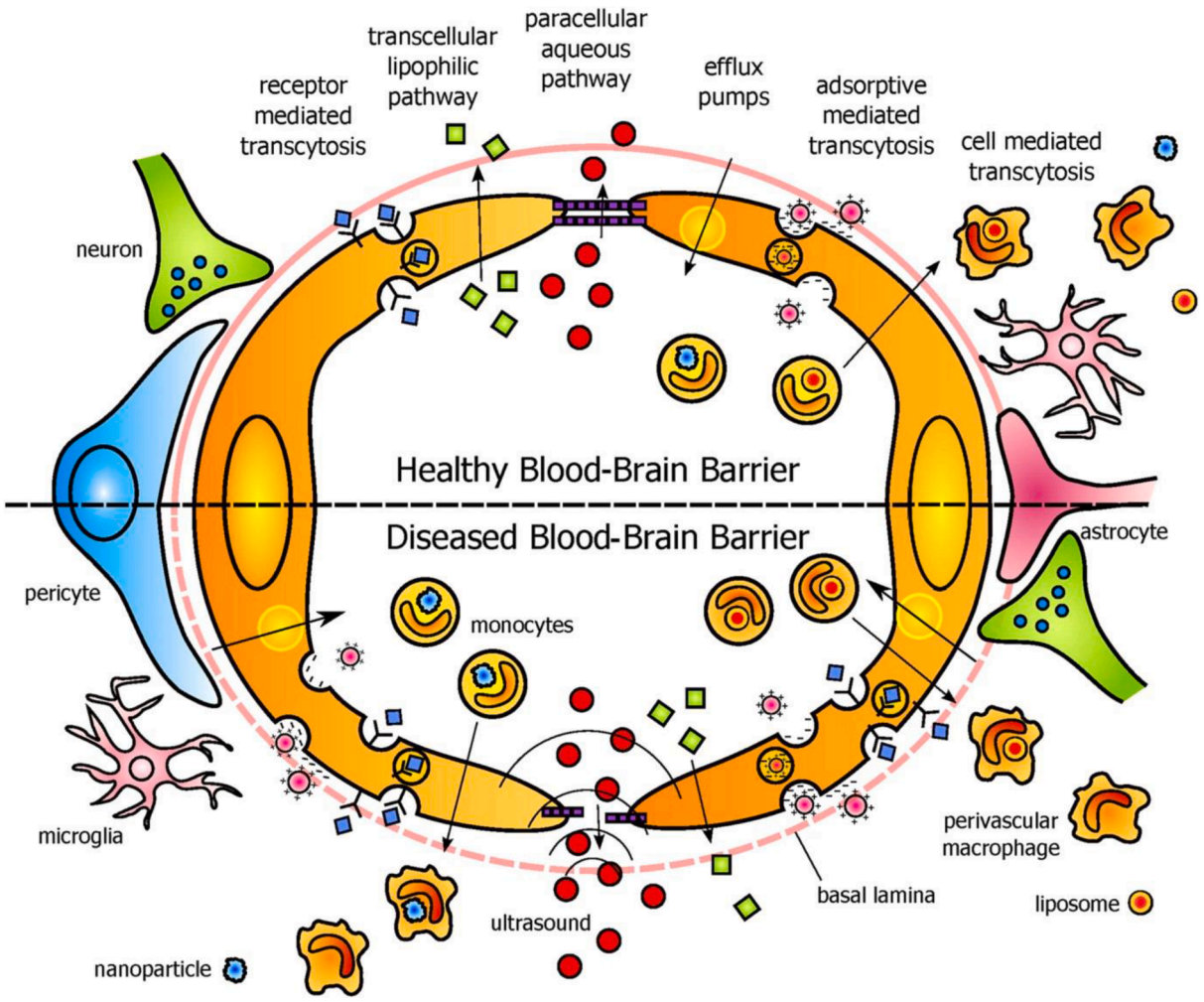

Fig. 2. Schematic representation of the blood-brain barrier (BBB) in healthy and disrupted states (Chen and Liu, 2012).

(Antel and Miron, 2008). There is currently limited evidence for the ability of newer-generation DMTs to cross the BBB and exert a direct effect in the CNS.

1.2. Strategies for delivering drugs through the BBB

As previously discussed, the BBB is impermeable to almost all therapeutic molecules (Pardridge, 2005). The development of new DMTs targeting delivery within the CNS tend to focus on transportation across the BBB, yet the BCB and the arachnoid barrier also present opportunities to target the CNS (Yasuda et al., 2013). Recent attempts to improve drug delivery across the BBB have investigated the use of colloidal carriers such as liposomes and nanoparticles. Systems such as these allow relatively large amounts of drug to be incorporated into the delivery vectors, offering the possibility for significant concentrations of drug to be delivered within the CNS (Dong, 2018). The surfaces of these colloidal delivery systems can also be modified to target specific BBB transport mechanisms (Dominguéz et al., 2013). One example is the use of monoclonal antibodies attached to liposome-drug complexes, which can be recognized as ligands by receptors in the BBB; thus, allowing them to be transported into the CNS (Deeken and Loscher, 2007). Alternative methods have been investigated including the inhibition of efflux transport mechanisms. Efflux transporters are transmembrane protein pumps that actively transport molecules out of the cell. Inhibition of these transporters should prevent the removal of drug molecules from the CNS, effectively enhancing the net uptake of molecules across the BBB. However, it is important to note that the inhibition of efflux transporters for prolonged periods may result in the accumulation of potential neurotoxins within the CNS, and therefore, the long-term use of such inhibitors would not be advisable (Correale and Villa, 2007).

Further research has looked at the potential of recombinant adeno-associated viruses (AAV) to cross the BBB. Such AAV capsids have been shown to infiltrate the CNS after intravenous administration in animal models, thus demonstrating their potential as a drug delivery system (Deverman et al., 2016). However, one concern with the use of AAV capsids in humans is the presence of anti-AAV antibodies, which may prevent efficient brain transduction (Bourdenx et al., 2014).

2. Potential relevance of the CNS penetration of DMTs in multiple sclerosis

The progression of MS is thought to be mediated by the ability of autoreactive lymphocytes and inflammatory monocytes to cross the BBB, penetrating the CNS and thus causing localized inflammation, leading to demyelination, glial scarring, axonal damage, and neuronal loss (Du Pasquier et al., 2014). However, it has been observed that, as the disease progresses, neuroinflammation is seemingly largely driven by local, but poorly understood mechanisms within the CNS and that fewer peripheral cells are detected in brain lesions (Dendrou et al., 2015). Thus, during the progressive phase of the disease, resident cells within the CNS (i.e. astrocytes and microglial cells) play a critical role in the pathogenesis of MS, and additional age-related factors (e.g. iron accumulation) and vascular comorbidities may also play a role in neurodegeneration (Lassmann, 2017). The BBB represents an important

barrier that needs to be overcome to target the sites of inflammation, demyelination, and neuroaxonal damage within the CNS in order to have an action on neurodegeneration and myelin repair.

Evidence from the existing literature concerning the ability of newer-generation DMTs to enter the CNS is summarized in Table 1 and described in more detail below.

2.1. Interferons

Interferon beta (IFNβ) compounds have been a mainstay in the treatment of MS since the 1990s. These agents are part of the cytokine family of signaling proteins, which have a broad range of biological effects, and have an important role in preventing the migration of leukocytes across the BBB (Zettl et al., 2018). Treatment with IFNβ has been shown to increase serum concentrations of soluble vascular cell adhesion molecule-1 (VCAM-1) which may in turn reduce the ability of T cells to bind to and cross the BBB and this increase was correlated with decreased MRI lesion load (Graber and Dhib-Jalbut, 2014; Graber et al., 2005). Matrix metalloproteinases (MMPs) have also been implicated in the disruption of the BBB and immune cell trafficking in MS. These endopeptidases are secreted by activated T cells and macrophages, and may facilitate their migration into the CNS (Waubant et al., 1999). Treatment with IFNβ has been seen to reduce the number of leukocytes secreting MMPs, whilst increasing the expression of tissue inhibitor matrix metalloproteinase 1 (TIMP-1) that regulates the activity of MMPs (Özenci et al., 2000; Karabudak et al., 2004).

2.2. Glatiramer acetate

Glatiramer acetate (GA) is a random polymer of glutamic acid, lysine, alanine, and tyrosine, and was designed as an analog of myelin basic protein. The hydrophilic nature of GA might prevent it from crossing the BBB, thus suggesting that the therapeutic effect would preferentially occur in the periphery. Furthermore, data from animal models using radiolabeled-GA show very low levels in the CNS (Carter and Keating, 2010). However, it has been shown in animal models that GA-reactive Th2 cells migrate to the CNS where they are re-activated, producing anti-inflammatory cytokines and growth factors (Blanchette and Neuhaus, 2008; Aharoni et al., 2003; Lalive et al., 2011). More recent evidence indicates that APCs are the initial target important to the mode of action of GA and it is the modulation of the APC compartment to an anti-inflammatory phenotype that is responsible for the expansion of Th2 cells, CD8+ *T* cells, and Treg cells (Prod'homme and Zamvil, 2019).

2.3. Dimethyl fumarate and monomethyl fumarate

Dimethyl fumarate is a prodrug that is hydrolyzed to monomethyl fumarate and fumarate within cells (di Nuzzo et al., 2014). Neither the effects of dimethyl fumarate on the distribution of lymphocyte subsets within the CNS of patients with MS, nor its effects on resident cells within the CNS have been studied and the impact of the drug directly within the CNS of patients with MS remains largely unknown (Mills et al., 2018). Efforts to improve the delivery of dimethyl fumarate to brain tissue using nanolipidic carriers have reported some success in pre-clinical studies (Kumar et al., 2017). Dimethyl fumarate and monomethyl fumarate each have a hypothetical role affecting the brain endothelial cell layer, thus stabilizing the BBB (Kunze et al., 2015; Lim et al., 2016). However, there is limited *in vitro* evidence to suggest that monomethyl fumarate is able to exert a neuroprotective effect within the CNS, as it has been shown to reduce the severity of neuronal excitotoxicity mediated by glutamate (Luchtman et al., 2016). *In vitro* exposure to monomethyl fumarate has been shown to reduce VCAM-1 expression on human brain-derived microvascular endothelial cells; an effect that was also observed when the agent was added 24 h after the onset of TNFα-mediated inflammation (Breuer et al., 2017). This downregulation of VCAM-1 subsequently led to a reduced adhesion of T cells to the endothelium, and therefore reducing transmigration across the BBB (Breuer et al., 2017). Both dimethyl fumarate and monomethyl fumarate have been shown to reduce the number of T cells and suppress macrophage infiltration in the spinal cord of EAE mouse models (Mills et al., 2018; Schilling et al., 2006).

2.4. Teriflunomide

Teriflunomide in an immunomodulatory agent that selectively, and reversibly, inhibits enzymes involved in the synthesis of pyrimidine (Sanofi-Aventis Groupe, 2020) and reduces the proliferation of B and T lymphocytes in the periphery (Miller, 2017). A search of the literature did not identify any published studies into the effects of teriflunomide on the BBB in MS, but a study of experimental traumatic brain injury in rodents suggested that teriflunomide could restore BBB integrity and reduce brain permeability (Prabhakara et al., 2018). There is also limited evidence to suggest that teriflunomide enters the CNS, or has a direct effect on neurons or other cells of the CNS (Palmer, 2013). In EAE rat models, teriflunomide has been observed to reach CNS concentrations of 4.1 μM, or approximately 2–4% of the blood concentration (Kaplan et al., 2015), and also inhibit demyelination and prevent axonal loss (Merrill et al., 2009).

2.5. Mitoxantrone

Mitoxantrone is a synthetic anthracenedione derivative with established cytotoxic and antineoplastic properties, which is licensed in some countries for patients with MS when other DMTs are not effective or available (Accord Healthcare Ltd, 2016). The molecule is water soluble and penetrates the CNS poorly when the BBB is intact (Accord Healthcare Ltd, 2016; Reif et al., 2007). It has been reported that the efflux transporter ABCG2 plays a minor role in the active efflux transport of mitoxantrone from the CNS to the periphery, and other efflux transporters distinct from ABCG2 or P-glycoprotein may be involved in the brain efflux of mitoxantrone (Lee et al., 2005). Although it has been reported that mitoxantrone can cross a disrupted BBB, there is limited evidence of an immunosuppressive or regulatory effect on mouse microglial cells *in vitro* (Li et al., 2012). However, a review of studies in patients with brain tumors showed that mitoxantrone does penetrate the CNS in these circumstances, with brain/tissue concentration ratios over 30 (Pitz et al., 2011).

2.6. Monoclonal antibodies

In general, the BBB prevents entry of large molecules such as monoclonal antibodies into the CNS tissue; however, in diseases characterized by BBB disruption, the situation is more complex (Lampson, 2011).

The molecular weights of antibodies approved for use in the treatment of MS are largely thought to prevent these DMTs from crossing the BBB. Alemtuzumab is a CD52 monoclonal antibody that binds to circulating B and T lymphocytes within the periphery and depletes their number through antibody-dependent cell cytotoxicity, complement-dependent cytotoxicity, and apoptosis (Sanofi Belgium, 2020; Hu et al., 2009). The number of studies on the effects on alemtuzumab on the CNS are limited, but there is some evidence to suggest that it may have neuroregenerative properties (Ruck et al., 2015). There is an ongoing study into the effects of alemtuzumab on the BBB (ClinicalTrials.gov Identifier: NCT03193086). However, there are currently no publications on the findings of this study. In a small scale study by Möhn et al., alemtuzumab was found to significantly decrease the quantitative fraction of intrathecal IgG synthesis within the CSF at 12 and 24 months post-administration, thus suggesting the inhibition of immune processes within the CNS. This was also reflected in a decrease of oligoclonal bands (OCBs) within the CSF, and furthermore, for 2

Table 1
A summary of the peripheral and central activity of current generation DMTs that are licensed in the treatment of MS.

Drug	Entity	Dosing	MOA	Biodistribution	Peripheral/central activity	Comments
DMTs restricted to the periphery						
Interferons (β−1a and β−1b)	Cytokines	Various doses administered by intramuscular or subcutaneous injection	The MOA of interferons is not fully understood, but it has been proposed that interferons inhibit T cell activation and proliferation, and induce apoptosis of autoreactive T cells (Biogen Netherlands B. V. AVONEX 2020; Bayer, 2019; Novartis Europharm Ltd 2019; Biogen Netherlands B. V. PLEGRIDY 2020; Merck Europe B.V. REBIF 2020)	The bioavailability for some interferons has been reported as 40–50% (Bayer, 2019; Novartis Europharm Ltd 2019; Biogen Netherlands B.V. PLEGRIDY 2020)	Restricted to the periphery	Interferons change the response of the immune system and may reduce the ability of T cells to bind to and cross the BBB (Graber and Dhib-Jalbut, 2014) Interferons have been shown to reduce the secretion of MMPs, while increasing the expression of TIMP-1 (Ozenci et al., 2000; Karabudak et al., 2004)
Glatiramer acetate	Random polymer of glutamic acid, lysine, alanine, and tyrosine	Subcutaneous injection; 20 mg once daily or 40 mg 3 times a week	Modulation of immune processes		Restricted to the periphery	Glatiramer acetate alters inflammatory processes, and may provide neuroprotective and neuroregenerative effects (Lalive et al., 2011). More recent evidence indicates that antigen presenting cells are the initial target important to the mode of action of glatiramer acetate (Prod'homme and Zamvil, 2019)
Dimethyl fumarate and monomethyl fumarate	Derivatives of fumaric acid	Dimethyl fumatate: oral; 240 mg twice daily Monomethyl fumarate: oral; 95 mg twice daily	Primarily mediated through activation of the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway (Biogen Netherlands B.V. TECFIDERA 2020)	C_{max} of dimethyl fumarate: 1.72 mg/L with volume of distribution of 60–90 L (Biogen Netherlands B. V. TECFIDERA 2020) C_{max} of monomethyl fumarate is bioequivalent to dimethyl fumarate (Banner Life Sciences LLC 2020)	Dimethyl fumarate is rapidly hydrolyzed to monomethyl fumarate, which is able to cross the BBB (Mills et al., 2018)	Dimethyl fumarate acts to stabilize and increase BBB integrity through various established mechanisms (Kunze et al., 2015; Dubey et al., 2015)
Teriflunomide	Immunomodulatory agent with anti-inflammatory properties	Oral; 14 mg once daily	Inhibitor of dihydroorotate dehydrogenase in the *de novo* synthesis of pyrimidines (di Nuzzo et al., 2014)	High oral bioavailability (approximately 100%) Extensively bound to plasma protein (>99%) and mainly distributed in plasma (Sanofi-Aventis Groupe 2020)	There is no strong evidence indicating that teriflunomide enters the CNS	There is no strong evidence that teriflunomide has a direct effect on resident cells of the CNS (di Nuzzo et al., 2014; Palmer, 2013)
Mitoxantrone	Synthetic anthracenedione derivative	IV; 12 mg/m^2 of body surface area	Inhibits B cell, T cell, and macrophage proliferation (Accord Healthcare Ltd 2016)	Volume of distribution exceeds 1000 L/m^2 with plasma concentrations decreasing rapidly (Accord Healthcare Ltd 2016)	The molecule is water soluble and penetrates the CNS poorly when the BBB is intact (Accord Healthcare Ltd 2016; Reif et al., 2007). In patients with brain tumors, brain to tissue concentration ratios were over 30 (Pitz et al., 2011)	Limited evidence of an immunosuppressive or regulatory effect of mitoxantrone on mouse microglial cells *in vitro* (Li et al., 2012)
Alemtuzumab	Monoclonal antibody	IV infusion; 96 mg over two years	Binds to circulating B and T lymphocytes, depleting their number through apoptosis (Sanofi Belgium 2020)	C_{max}: 3014 ng/mL on Day 5 of initial treatment course C_{max}: 2276 ng/mL on Day 3 of the second treatment course (Sanofi Belgium 2020)	Restricted to the peripheral component due to its molecular size	Recent data have shown that alemtuzumab may play a role in restoring the integrity of the BBB (Ruck et al., 2015)
Natalizumab	Monoclonal antibody	IV; 300 mg once every four weeks	Binds to α4-integrin on lymphocyte surfaces thus blocking T cells from entering the CNS (	Maximum serum concentration 110 μg/mL following repeat IV administration of 300	Molecular size prevents it from crossing the BBB restricting it to the	Natalizumab is a monoclonal IgG4 antibody that binds to α4-integrin, thus interfering with

(*continued on next page*)

Table 1 (*continued*)

Drug	Entity	Dosing	MOA	Biodistribution	Peripheral/central activity	Comments
			Biogen Netherlands B. V. TYSABRI 2020)	mg (Biogen Netherlands B.V. TYSABRI 2020)	peripheral immune compartment	lymphocyte migration across the BBB (Stuve et al., 2006)
Ocrelizumab	Monoclonal antibody	IV infusion	Binds to CD20 and depletes circulating B lymphocytes (Roche Registration GmbH. OCREVUS 2020)	Volume of distribution 2.78 L (Roche Registration GmbH. OCREVUS 2020)	There is no direct evidence that ocrelizumab crosses the BBB	It has been suggested that anti-CD20 monoclonal antibodies may be able to cross the BBB (Sorensen and Blinkenberg, 2016). There is also evidence showing that intrathecal administration has a short half-life within the CNS (Lehmann-Horn et al., 2014; Weber, 2015)
Ofatumumab	Monoclonal antibody	Subcutaneous injection; 20 mg weekly for 3 weeks followed by once every 4 weeks	Binds to CD20 and induces B cell lysis and depletion (Bar-Or et al., 2018; Florou et al., 2020)	Subcutaneous dosing of 20 mg every 4 weeks provides a mean C_{max} of 1.43 μg/mL (Novartis Pharmaceuticals Corporation 2020)	Currently there is no evidence that ofatumumab penetrates the CNS	It has been shown to suppress new MRI lesions with dose-dependent B cell depletion (Bar-Or et al., 2018)
DMTs targeting the CNS						
Cladribine	Adenosine analog prodrug	Oral; 3.5 mg/kg cumulative dose over 2 years	Binds to circulating B and T lymphocytes depleting their number through apoptosis (Merck Europe B.V. MAVENCLAD 2021)	Volume of distribution: 480–490 L (Merck Europe B.V. MAVENCLAD 2021)	Able to cross the BBB, reaching concentrations in the CSF of approximately 25% of plasma concentrations (Liliemark, 1997)	Cladribine can potentially reduce the number of lymphocytes that have been recruited into the CNS as well as circulating lymphocytes (Baker et al., 2019). Cladribine may also affect adhesion molecule secretion by immune cells, inhibiting the recruitment of inflammatory cells into the CNS (Mitosek-Szewczyk et al., 2010; Leist and Weissert, 2011), and also inhibits microglial cell functions (Singh et al., 2012; Aybar et al., 2019)
Fingolimod	S1P receptor modulator	Oral; 0.5 mg once daily	Metabolized by sphingosine kinase to the active metabolite fingolimod phosphate (Novartis Europharm Ltd 2020)	Absolute oral bioavailability: 93% Volume of distribution: 1200 L (Novartis Europharm Ltd 2020)	Lipophilic fingolimod crosses the BBB accumulating in myelin (Hunter et al., 2016)	S1P receptors are present on most CNS cells, most notably glia and neurons (Chun and Hartung, 2010; Brinkmann, 2007). Fingolimod activates S1P and subsequently induces down-regulation, thereby reducing lymphocyte infiltration into the CNS (Chun and Hartung, 2010). Animal models have shown that fingolimod has some activity within the CNS, promoting myelin integrity and protecting against demyelination (Hunter et al., 2016)
Siponimod	Selective S1P receptor modulator	Oral; once daily	Selectively binds to S1P1 and S1P5 receptors (Novartis, 2019)	Absolute oral bioavailability: 84% Volume of distribution: 124 L (Novartis, 2019)	Siponimod is able to enter the CNS and bind directly to S1P5 and S1P1 sub-receptors on oligodendrocytes and astrocytes in animal models (Tavares et al., 2014; Bigaud et al., 2019; Novartis 2019), and can reach concentrations of ~10 times those in the blood (Bigaud et al., 2019)	Animal models have shown that siponimod distributes into the CNS exerting an effect on oligodendrocytes and astrocytes (Tavares et al., 2014; Aslanis et al., 2012)
Ozanimod	Selective S1P receptor modulator	Oral; once daily	Selectively binds to S1P1 and S1P5 receptors	Apparent volume of distribution: 5590 L (Bristol Myers Squibb Pharma EEIG 2020)	Ozanimod has been shown to reach brain to blood ratios of 10:1–16:1 in animal models (Scott et al., 2016)	Ozanimod reduces B and T lymphocytes (Scott et al., 2016)

BBB, blood-brain barrier; C_{max}, maximum concentration; CNS, central nervous system; CSF, cerebrospinal fluid; DMTs, disease-modifying therapies; IV, intravenous; MMPs, matrix metalloproteinases; MOA, mechanism of action; MRI, magnetic resonance imaging; MS, multiple sclerosis; S1P, sphingosine-1-phosphate; TIMP-1, tissue inhibitor matrix metalloproteinase 1.

patients OCBs were no longer detectable at 24 months (Möhn et al., 2020).

Natalizumab is a monoclonal IgG4 antibody that binds to α4-integrin, thus interfering with lymphocyte migration across the BBB. Natalizumab treatment dramatically reduces the number of CD4+ and CD8+ *T* cells, CD19+ *B* cells, and CD138+ plasma cells in the CSF of patients with MS (Stuve et al., 2006). Interestingly, natalizumab has a far greater effect on CD4+ *T* cells and B cells compared with other lymphocyte subsets (Stuve et al., 2006). Although restricted to the periphery, natalizumab has been observed to reduce OCBs in the CSF to undetectable levels (von Glehn et al., 2012; Mancuso et al., 2014).

Among other agents, ocrelizumab (a second-generation anti-CD20 monoclonal antibody) has no current evidence to suggest that it can penetrate the CNS despite possessing a humanized IgG1 tail that binds to a distinct but overlapping epitope to rituximab, another anti-CD20 monoclonal antibody (see below) (Oh and Calabresi, 2013; Sorensen and Blinkenberg, 2016). Ofatumumab, a recent FDA-approved fully human anti-CD20 monoclonal antibody, works by binding to the CD20 molecule on the B cell surface and inducing potent B cell lysis and depletion in the periphery (Bar-Or et al., 2018; Florou et al., 2020). However, there is also no current evidence to suggest that it can penetrate the CNS.

Although rituximab is not licensed for use in MS, it is currently used off-label in different countries.. It has been reported from a small case series in patients with MS that rituximab treatment results in significant and sustained reduction of circulating B cells and in a transient drop of CSF B cells (Cross et al., 2006; Martin Mdel et al., 2009; Stuve et al., 2005), but this did not translate to a change in the number or appearance of leptomeningeal contrast-enhancement on imaging or sCD21 used as surrogate marker for intrathecal B cells (Bhargava et al., 2019).

Rituximab has been reported to be detectable in the CSF of patients with MS, albeit at concentrations which are 1000-fold lower that serum concentrations (Petereit and Rubbert-Roth, 2009). However, a positron emission tomography study assessing the CNS penetration of radiolabeled rituximab in three patients with MS showed no strong evidence of cerebral penetration (Hagens et al., 2018). There is no compelling biological explanation presented in the literature providing a rationale as to why rituximab may be able to cross the BBB whereas other monoclonal antibodies are seemingly restricted to the periphery, and it may be that detection of low levels of rituximab in the CNS is confounded by the methods used in the studies reported to date (Petereit and Rubbert-Roth, 2009; Hagens et al., 2018). Moreover, recent clinical studies of intrathecally administered rituximab in patients with progressive MS have reported that treatment does not halt disease progression (Bergman et al., 2021, 2018; Bonnan et al., 2021).

2.7. Cladribine

Cladribine is a deoxyadenosine analog prodrug that is sequentially phosphorylated by deoxycytidine kinase (DCK) and deoxyguanosine kinase to its biologically active form, 2-chlorodeoxyadenosine triphosphate (Cd-ATP). The dephosphorylation and deactivation of Cd-ATP is catalyzed by 5′-NT-ase (Liliemark, 1997; Merck Europe B.V. MAVENCLAD, 2021). The high DCK/5′-NT-ase ratio in B and T cells make them particularly sensitive to cladribine, which is able to accumulate within the lymphocytes and causing apoptosis by inhibition of DNA polymerase (Giovannoni, 2017). This effect on B and T cells interrupts the cascade of immune events that are central to the progression of MS (Merck Europe B.V. MAVENCLAD, 2021). In contrast, neutrophils express less DCK compared with 5′-NT-ase and this explains why these cells are affected by cladribine to a much lesser extent (Ceronie et al., 2018).

Studies have shown that cladribine has the potential to penetrate the CNS and achieve a CSF concentration of up to 25% of the concentration in plasma in patients both with and without MS (Hermann et al., 2019; Kearns et al., 1994; Liliemark, 1997). The fact that cladribine has been shown to be present in the CSF raises the possibility that this agent may act to reduce lymphocyte numbers within the CNS as well as those circulating in the periphery (Baker et al., 2019). It is also suggested that cladribine may affect adhesion molecule secretion by immune cells, thus inhibiting further recruitment of inflammatory cells into the CNS (Mitosek-Szewczyk et al., 2010; Leist and Weissert, 2011).

B cells appear to prominently drive the immune responses within the CNS, and this has led to an interest in studying the potential of cladribine to provide benefit in MS beyond what is achievable in respect of CNS depletion of B cells (Baker et al., 2019b, 2019a; Baker et al., 2018). In addition, cladribine has been shown to inhibit microglial cell proliferation, induce apoptosis, and suppress IL-1, IL-6, and TNF-α secretion; no effects were observed in the case of astrocytes (Singh et al., 2012; Aybar et al., 2019).

Parenteral cladribine, given off-label as a subcutaneous injection with a cumulative dose of 1.8 mg/kg (divided over 6 courses), has been shown to significantly decrease the number of OCBs in the CSF ($p < .0001$) (Rejdak et al., 2019). In this study by Rejdak et al. (2019), it was observed that 55% of patients tested negative for OCBs following treatment with cladribine. This reduction in OCBs was associated with a reduced disability progression after 10 years of follow up (Rejdak et al., 2019).

There is some evidence to show that cladribine possesses neuroprotective properties in EAE models when administered by intracerebroventricular minipump, independent of any peripheral immunosuppressant action (Musella et al., 2013). This study suggests that the neuroprotective effects of cladribine may be a result of interfering with IL-1β effects and thus blocking EAE synaptic alterations, rather than through an effect on astroglial or microglial activation (Musella et al., 2013).

2.8. Sphingosine 1-phosphate receptor modulators

Sphingosine-1-phosphate (S1P) receptors are expressed on the surface of lymphocytes, and have a key role in the regulation of many cellular processes, including the modulation of T cell migration into the CNS (Subei and Cohen, 2015; Bryan and Del Poeta, 2018). There are five subtypes of S1P receptors, however, of interest in MS are the S1P1, S1P3, and S1P4 receptors expressed by B and T lymphocytes, and the S1P5 receptors expressed by oligodendrocytes (Subei and Cohen, 2015).

2.8.1. Fingolimod

The prodrug fingolimod is an antagonist of S1P-1, 3, 4, and 5 receptors (Novartis Europharm Ltd, 2020). The binding of fingolimod to the S1P1 and S1P3 receptors on astrocytes is seen to induce astroglial activation (Lee et al., 2017). It has also been shown that fingolimod may have direct effects on brain microvascular endothelial cells and the blood-nerve barrier, which may restore their function through an action on S1P receptors (Nishihara et al., 2018; Nishihara et al., 2015; Prager et al., 2015). The lipophilic nature of fingolimod allows it to readily penetrate the CNS and exert direct effects (Miron et al., 2008; Chun and Hartung, 2010; Hunter et al., 2016; Foster et al., 2007), thereby modulating sphingosine-1 phosphate (S1P) receptors that are present on astrocytes, oligodendrocytes, microglia, and neurons (Lee et al., 2017; Healy and Antel, 2016).

A study into the effects of fingolimod on central and peripheral immune cells found that although numbers of B cells within the CNS remain unchanged, there is a significant decrease in the presence of leukocytes, and also that the numbers of CD4+ cells decrease and CD8+

cells increase leading to an inverted CD4+/CD8+ ratio (Kowarik et al., 2011). In animal models, fingolimod has been shown to act centrally promoting myelin integrity and protecting against demyelination, axonal and dendritic loss, and can also act to enhance the proliferation and survival of neuronal cells (Hunter et al., 2016). However, in patients with primary progressive MS, the anti-inflammatory effects of fingolimod have not been found to decrease the risk of disability progression (Lublin et al., 2016), nor has fingolimod been seen to affect OCBs in patients with MS (Kowarik et al., 2011).

2.8.2. Siponimod

Siponimod was developed to retain the efficacy of fingolimod in the treatment of MS, but to have selectivity for S1P1 and S1P5 receptors and faster elimination kinetics (Briard et al., 2015). This DMT is currently the only S1P1 agonist used in SPMS, possibly due to its favorable effects on the CNS. A study to investigate the relative concentrations of siponimod in the blood and brain in an EAE model revealed brain penetration with concentrations in the brain ~10 times those in the blood (Bigaud et al., 2019). As with fingolimod, animal models (rats and rhesus macaques) show that siponimod distributes into the CNS, potentially with direct effects on oligodendrocytes and astrocytes (Tavares et al., 2014; Aslanis et al., 2012); however, the clinical significance of this is not clear at present. A search of the literature did not identify any published studies into the effects of siponimod on OCBs in patients with MS.

2.8.3. Ozanimod

Ozanimod is a newly approved DMT with a high affinity to S1P1, and a lesser affinity to S1P5 (Cohan et al., 2020). In animal models, ozanimod has demonstrated a brain to blood ratio of 10:1 and 16:1 in mice and rats, respectively (Scott et al., 2016). Additionally, this study showed ozanimod to induce a rapid, but reversible, reduction in B and T lymphocytes *in vivo*. A search of the literature did not identify any published studies into the effects of ozanimod on OCBs in patients with MS.

2.8.4. Ponesimod

Ponesimod is a highly selective modulator of S1P1 receptors that induces a rapid, dose-dependent, and reversible reduction of peripheral blood lymphocytes (Baldin and Lugaresi, 2020). In EAE models, ponesimod was found to be effective in both preventive and therapeutic settings in which the overall severity of MS was reduced; this effect was also observed through improved histological outcomes (Pouzol et al., 2019). Recently, a Phase III, active-comparator, randomized trial demonstrated that ponesimod was superior to teriflunomide on annualized relapse rate reduction (the primary study outcome), fatigue symptoms, and MRI activity (Kappos et al., 2021). However, a search of the literature did not identify any published studies on the effects of ponesimod on the integrity of the BBB, or the CNS penetration of the drug in patients with MS.

2.9. Other novel drugs

The treatment landscape of MS is evolving, and there are further agents in development that may also be able to cross the BBB and act centrally as well as in the periphery (Gregson et al., 2019; Kolahdouzan et al., 2019).

Ibudilast a cyclic nucleotide phosphodiesterase (PDE) inhibitor that reduces the inhibition of cyclic adenosine monophosphate, thus allowing for the activation of anti-inflammatory cascades which may prove beneficial in MS (Kolahdouzan et al., 2019). Ibudilast has also been shown to readily cross the BBB reaching high concentrations in the plasma, spinal cord, and brain 1 h after dosing (Ledeboer et al., 2006).

Another groups of developmental DMTs seek to inhibit Bruton's tyrosine kinase (BTK), which is expressed in many hematopoietic cells including B cells and myeloid cells, but not T cells (Hendriks, 2011). BTK inhibitors, such as evobrutinib and tolebrutinib, may therefore have an impact on multiple immune cell signaling pathways. Evobrutinib is an irreversible and highly selective BTK inhibitor, which may be suitable for the treatment of autoimmune diseases, through the inhibition of B cell receptor- and Fc receptor γ-chain-mediated signaling (Carnero Contentti and Correale, 2020). Early results suggest that evobrutinib is able to cross the BBB in EAE mouse models, and may have a broader therapeutic benefit in MS than solely B cell depletion (Boschert et al., 2017). There is preliminary evidence showing that tolebrutinib (also known as SAR442168, PRN2246, or BTK inhibitor '168) may cross the BBB in preclinical EAE mouse models (Francesco et al., 2017), and has been observed to reach a CSF to plasma ratio of 2.25 in first-in-human trials, with a geometric mean CSF concentration of 1.87 ng/ml, 2 h after a single dose of 120 mg (Smith et al., 2019). Two additional BBB-penetrating BTK inhibitors, fenebrutinib (NCT04586023) and orelabrutinib (NCT04711148), have started Phase III and Phase II trials, respectively. The first results from these trials are expected in 2024.

2.10. Monitoring intra-CNS impact of DMTs

Biomarkers are considered essential for the monitoring response to therapies that act within the CNS. Many biomarkers, such as cytokines and chemokines, are present in the blood or CSF as a consequence of disease pathology (Kothur et al., 2016), loss of BBB integrity (Xiao et al., 2020), or are indicators of neuronal damage such as increased levels of neurofilament (El Ayoubi and Khoury, 2017), or decreased N-acetyl-aspartate levels on magnetic resonance spectroscopy (MRS) (Narayana, 2005). In MS, the use of different *in vivo* imaging techniques during the course of the disease have been proposed to monitor therapeutic responses within the CNS. In this regard, the impact of different DMTs on microglia activation can be studied using positron emission tomography (PET) to examine the translocator protein 18-kDa (TSPO) as an indicator of neuroinflammation (Airas et al., 2017). Through the development of radiopharmaceuticals targeting TSPO, researchers have been able to better characterize the spatial-temporal evolution of MS. Therefore, it could be possible to use TSPO PET as a non-invasive biomarker to evaluate and monitor the efficacy of immunosuppressive therapies on MS disease activity (Ghadery et al., 2019). Similarly, increasing levels of myo-inositol in MRS may reflect astrocytic hypertrophy (Llufriu et al., 2014). In addition, changes to the permeability of the BBB, regarded as the hallmark of neuroinflammation, can be evaluated through the leakage of gadolinium using MRI (Shinohara et al., 2012; Miller et al., 1998). Furthermore, indicators of CNS infiltration by immune cells include markers of oxidative stress, such as myeloperoxidase bound to gadolinium, have been used in EAE models (Chen et al., 2008). Likewise, radiolabeled antibodies or radiolabeled cytokines imaged using PET, have been used to track CD4+ *T* cells, as well as IL-1 and IL-12 (Costa et al., 2001). These approaches have the potential to improve therapeutic monitoring for compounds that may have an intra-CNS impact. However, at this time they have only examined in pre-clinical models, with fewer available for clinical investigation.

3. Summary

The ever-changing treatment landscape in MS mirrors the progress made in the understanding of the BBB and CNS penetration of different DMTs. It is thought that the MS therapies able to exert an effect directly within the CNS may influence local disease processes, such as neuronal loss and demyelination, and allow for the inflammatory responses associated with MS to be treated in the CNS as well as the periphery. However, further studies are needed to determine if the lymphocytes involved in MS and resident in the CNS, or cells primarily resident in the CNS (e.g. microglial cells and astrocytes), are susceptible to these DMTs and, therein, the importance of these effects in the management of MS and the safety concerns this may raise.

Small molecule therapeutics for MS, in particular cladribine and the

S1P receptor modulators fingolimod, siponimod, and ozanimod, have been shown to cross the BBB. Cladribine can reach CSF concentrations of up to 25% of the concentration in plasma as determined in patients with and without MS (Hermann et al., 2019; Kearns et al., 1994; Liliemark, 1997). In EAE models siponimod is able to reach concentrations in the brain approximately 10 times those in the blood (Bigaud et al., 2019), and ozanimod has demonstrated brain to blood ratios of 10–16:1 (Scott et al., 2016), suggesting that lymphocytes recruited into the CNS can potentially be depleted, as well as circulating lymphocytes within the periphery. It is worth noting that cladribine has been shown to accumulate within B and T cells leading to a gradual depletion of these lymphocytes within the periphery, and possibly within the CNS, which extends beyond the dosing period (Giovannoni, 2017). The gradual reconstitution of B and T cells allows for the oral formulation of cladribine to be administered as two short courses over two annual treatment cycles. This mechanism of action is different to that of the other current generation DMTs targeting the CNS, which act on the S1P receptors, which need to be inhibited continuously in order to have an effect.

Future treatments for MS and other neurological conditions may work via direct action within the CNS. However, key to this is the translation of drug discovery in the laboratory to CNS-penetrating treatments in the clinic, which will, almost certainly, require a better understanding of the biology and function of the BBB as well as of the transport across the BBB. In time, translational studies may lead to the development of biomarkers suitable for use in the clinical management of MS. Studies are on-going to determine the full array of endothelial transporters and their substrates in order to identify target to aid drugs delivery across the BBB (Daneman and Prat, 2015).

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CRediT authorship contribution statement

Jorge Correale: Conceptualization, Writing – original draft, Writing – review & editing. **Mario Javier Halfon:** Conceptualization, Writing – original draft, Writing – review & editing. **Dominic Jack:** Conceptualization, Writing – original draft, Writing – review & editing. **Adrián Rubstein:** Conceptualization, Writing – original draft, Writing – review & editing. **Andrés Villa:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

JC is an advisory board member of Biogen, Genzyme, Merck, Novartis, and Roche; has received reimbursement for developing educational presentations for Biogen, Genzyme, Merck, Novartis, Roche, and Teva, as well as professional travel/accommodations stipends.

MJH has received reimbursement for developing educational presentations for Biogen Argentina, Genzyme Argentina, Merck S.A. Argentina (an affiliate of Merck KGaA), and Novartis Argentina, as well as professional travel/accommodations stipends.

DJ is an employee of Merck Serono Ltd, Feltham, UK (an affiliate of Merck KGaA).

AR is an employee of Merck S.A., Argentina (an affiliate of Merck KGaA).

AV has received reimbursement for educational presentations for Biogen Argentina, Genzyme Argentina, Merck S.A. Argentina (an affiliate of Merck KGaA), Novartis Argentina, and Roche.

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