Journal of Controlled Release xxx (xxxx) 1–24

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Innovative drug delivery strategies to the CNS for the treatment of multiple sclerosis

Ariane Mwema [a, b], Giulio G. Muccioli [b, 1, **], Anne des Rieux [a, 1, *]

[a] Université catholique de Louvain, UCLouvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Avenue E. Mounier 73, 1200 Brussels, Belgium
[b] Université catholique de Louvain, UCLouvain, Louvain Drug Research Institute, Bioanalysis and Pharmacology of Bioactive Lipids, Avenue E. Mounier 73, 1200 Brussels, Belgium

ARTICLE INFO

Keywords:
Nanomedicine
Remyelination
Neuro-regeneration
Blood-brain-barrier
Central nervous system
Nose-to-brain

ABSTRACT

Disorders of the central nervous system (CNS), such as multiple sclerosis (MS) represent a great emotional, financial and social burden. Despite intense efforts, great unmet medical needs remain in that field. MS is an autoimmune, chronic inflammatory demyelinating disease with no curative treatment up to date. The current therapies mostly act in the periphery and seek to modulate aberrant immune responses as well as slow down the progression of the disease. Some of these therapies are associated with adverse effects related partly to their administration route and show some limitations due to their rapid clearance and inability to reach the CNS. The scientific community have recently focused their research on developing MS therapies targeting different processes within the CNS. However, delivery of therapeutics to the CNS is mainly limited by the presence of the blood-brain barrier (BBB). Therefore, there is a pressing need to develop new drug delivery strategies that ensure CNS availability to capitalize on identified therapeutic targets. Several approaches have been developed to overcome or bypass the BBB and increase delivery of therapeutics to the CNS. Among these strategies, the use of alternative routes of administration, such as the nose-to-brain (N2B) pathway, offers a promising non-invasive option in the scope of MS, as it would allow a direct transport of the drugs from the nasal cavity to the brain. Moreover, the combination of bioactive molecules within nanocarriers bring forth new opportunities for MS therapies, allowing and/or increasing their transport to the CNS. Here we will review and discuss these alternative administration routes as well as the nanocarrier approaches useful to deliver drugs for MS.

1. Introduction

Multiple sclerosis (MS) is a multifaceted neurodegenerative and autoimmune disease of the central nervous system (CNS), driven by a complex immune response towards mainly the myelin sheaths surrounding the axons of the CNS [1]. This phenomenon triggers an inflammatory environment which eventually results in demyelination and neuroaxonal damage [2,3]. MS is the leading cause of non-traumatic neurological disability in young adults, affecting approximately 2.8 million people worldwide, having a significant impact on their quality of life [4]. The origin of the disease remains incompletely understood, however, genetic and environmental factors including vitamin D deficiency, Epstein-Barr virus (EBV) infection, cigarette smoking, obesity and/or hormones may contribute to MS etiology [5].

MS exhibits different clinical phenotypes. In most cases, patients endure recurring clinical symptoms followed by partial or complete recovery (relapsing-remitting MS, RRMS). The first episode that occurs during the development of the disease is termed clinically isolated syndrome. The relapses coincide with CNS inflammation and demyelination [5,6]. Within 10 to 15 years, relapses decrease in frequency and the CNS gradually and continuously deteriorates, resulting in uninterrupted progression termed secondary progressive MS (SPMS) for 50% of RRMS patients. In this case, CNS atrophy and increased axonal loss accompany neurological decline [2]. Although the chronic-remitting form is the most frequent, between 15 to 20% of MS patients are diagnosed with primary progressive MS (PPMS) which is characterized by the accumulation of disabilities from the onset, with an absence of relapses [2,6,7]. Despite these differences, it is now recognized that all

* Corresponding author.
** Corresponding author at: Université catholique de Louvain, UCLouvain, Louvain Drug Research Institute, Bioanalysis and Pharmacology of Bioactive Lipids, Avenue E. Mounier 72, 1200 Brussels, Belgium.
E-mail addresses: giulio.muccioli@uclouvain.be (G.G. Muccioli), anne.desrieux@uclouvain.be (A. des Rieux).
[1] both authors contributed equally to the work.

https://doi.org/10.1016/j.jconrel.2023.10.052
Received 5 July 2023; Received in revised form 5 October 2023; Accepted 31 October 2023

clinical forms of MS present both inflammation and neurodegeneration. Early MS lesions are characterized by invading peripheral cells and leakage of the blood-brain-barrier (BBB). The infiltrating cells include activated autoreactive CD4+ T cells (T helper 1 (Th1) cells and Th17 cells) and cytotoxic CD8+ T cells, as well as B cells and activated myeloid cells. Together with activated CNS-resident cells, namely microglia and astrocytes, they secrete inflammatory cytokines and chemokines, as well as neurotoxic factors that will result in further recruitment of immune cells, oligodendrocytes injury, demyelination and neuro-axonal injury (Fig. 1). During the progressive phases of MS, which is characterized by a mostly intact BBB, chronic inflammation, acute and chronic oxidative stress induced by innate and adaptative immune cell activation, loss of myelin trophic support, pro-inflammatory environment and involvement of complement activation will contribute to the ongoing tissue injury, blockade of remyelination and neurodegeneration. For further details on MS physiopathology, refer to these excellent reviews [2,8–10].

The neurological symptoms associated with MS include visual, cognitive and motor deficits such as blurred vision, limb and facial weakness, bladder and bowel problems, stiffness, muscle spasms, vertigo and dizziness [7].

MS remains a global burden and its prevalence is on the rise [4]. Although more than 15 therapies that reduce disability and extend survival of MS patients are available, a cure has yet to be found [7]. The current therapies mostly act on the peripheral adaptive immune system and are largely insufficient to prevent accumulation of permanent disability, particularly during the progressive phase [11,12]. Moreover, these therapies are associated with several side effects, partly linked to their administration route, with some of them having poor solubility and rapid clearance in biological fluids [13]. As MS pathophysiology involves both peripheral and central immune cells, therapies should optimally also act at the level of the CNS [8]. However, most disease-modifying therapies (DMT) are restricted by the BBB, hindering their access to the CNS [14,15]. In this context, the use of alternative administration routes and/or approaches that avoid/disrupt the BBB could increase the efficacy of the drugs within the CNS [16]. Moreover, the use of nanotechnology offers several advantages that can be applied for the treatment of MS [17]. These nanovesicles (nanoparticles, natural and artificial vesicles) can overcome the aforementioned limitations and

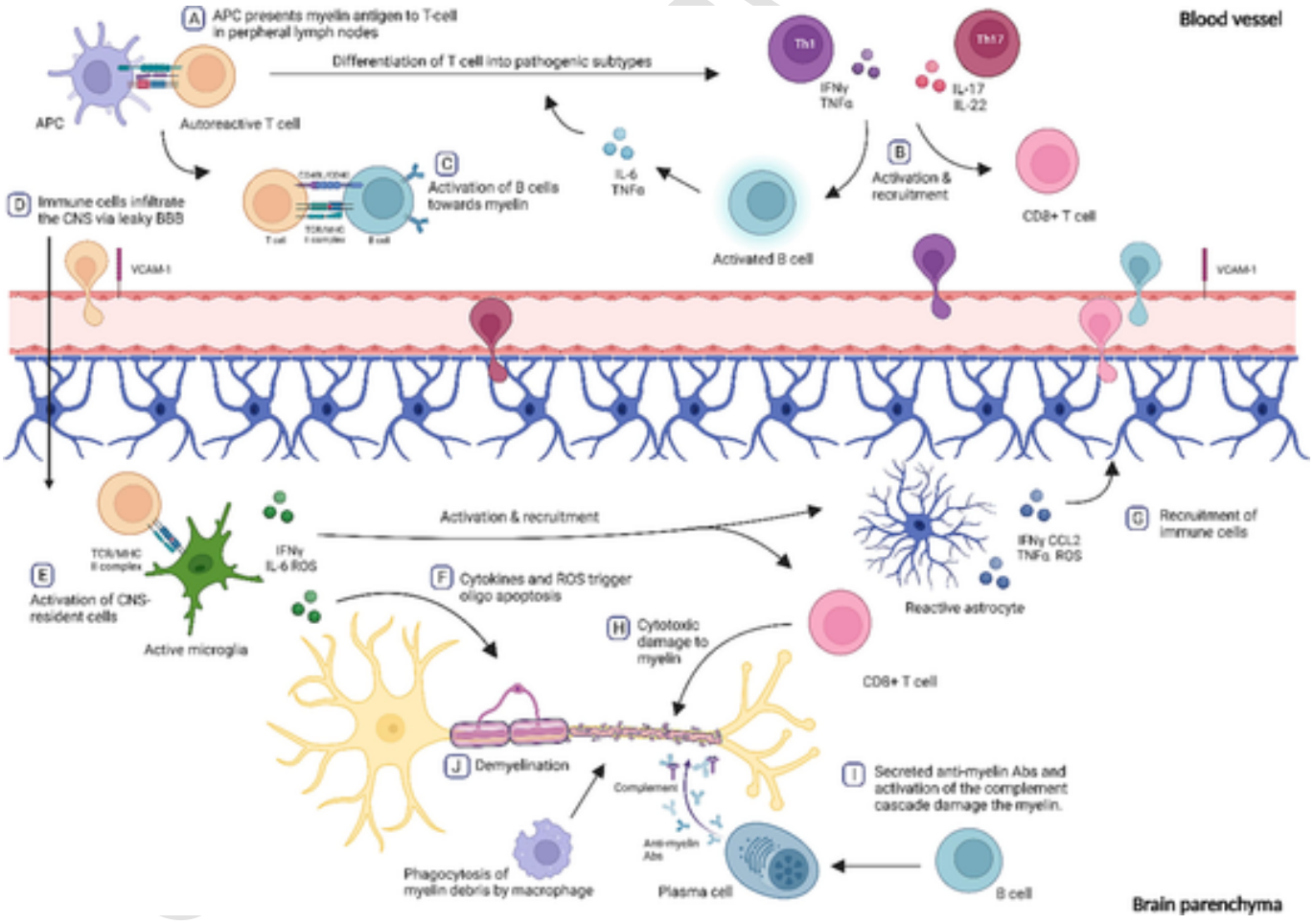

Fig. 1. Immune response in MS In the periphery, APC present myelin epitopes to autoreactive T cells leading to their activation and differentiation into pro-inflammatory Th cells (Th1, Th17). Activated T cells can recruit and activate CD8+ T cells and B cells towards myelin via secretion of pro-inflammatory cytokines and APC activity towards B cells. Pro-inflammatory cytokines secreted by activated B cells further induce aberrant Th1/Th17 response. Upregulation of integrins at the surface of the immune cells (i.e. integrin α4β1) enables their transport across the BBB via cell adhesion molecules (i.e. VCAM-1). Interaction of infiltrated cells with CNS-resident microglia & astrocytes initiates & sustains inflammation in the CNS by releasing pro-inflammatory factors. The latter induces oligodendrocytes apoptosis as well as enables recruitment of immune cells that contribute to cytotoxic damages to the myelin sheath. In the presence of antigen, B cells release anti-myelin Abs following differentiation into plasmocytes and further induce oligodendrocyte cell death via activation of the complement pathway. Together, this process contributes to sustained neuro-inflammation as well as demyelination. Abs: antibodies, Ag: antigen, APC: antigen-presenting cell, BBB: blood-brain-barrier, IFN: interferon, IL: interleukin, Oligo: oligodendrocyte, ROS: reactive oxygen species, Th: T helper, TNFα: tumor necrosis factor alpha. (created with BioRender.com).

barriers as most of them can cross the BBB. Moreover, they can serve as protective vehicles to further increase the delivery to the CNS, both for the already existing and the newly developed MS therapies, while using alternative routes of administration or strategies to by-pass the BBB [18,19].

In this review, we will briefly discuss the several delivery strategies and limitations of MS approved drugs and the current focus of research for the development of new MS treatments. Then we will highlight the potential of innovative delivery routes, as well as nanotechnology as tools for better delivery and efficiency of MS therapies in the CNS.

2. Current MS treatments and their administration route

The current therapeutic approach for MS aims at treating acute relapses, slowing the progression of the disease via disease-modifying-therapies (DMT) and decrease the symptoms. The latter involves all treatments used to improve complications and symptoms induced by the disease, such as tremors, itching, depression, bladder problems, fatigue, bowel dysfunction and pain [20,21]. Acute relapses, also known as acute attacks or exacerbations, are mainly treated using corticosteroids (i.e methylprednisolone) to decrease inflammation and to bring the relapse to an end more rapidly [21].

A better comprehension of the immune processes contributing to MS has led to the development and approval of several DMTs that are effective against the relapsing forms of MS (RMS), which include clinically isolated syndrome, RRMS and active SPMS [2,22]. Over 15 DMTs have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) that mainly target the inflammatory components of the disease. These DMTs reduce the rate of relapses, the accumulation of lesions and stabilize the delay between relapses ([6,7]. These drugs suppress circulating immune cells, inhibit their passage through the BBB and decrease the inflammatory responses [18]. A summary of the available DMTs, as well as their administration route and mechanism of action, are summarized in Table 1. These treatments are either injected (subcutaneously or intramuscularly), infused (intravenously) or administered via the oral route.

Additionally, autologous haematopoietic stem cell transplantation (aHSCT) is the most advanced experimental cell-based therapy against highly active RMS. It combines high-doses of immunosuppressive drugs to deplete immune cells, followed by autologous hematopoietic stem cell administration to boost bone marrow recovery and immune reconstitution in order to achieve long-term remission [23,24]. Interestingly, this treatment is gaining traction as a valuable option to treat RMS with promising outcomes for patients with SPMS.

Although great progresses have been made in the development of therapies against RMS, only modest breakthroughs have been made for the progressive forms of MS. Ocrelizumab was the first anti-CD20 monoclonal antibody approved for PPMS patients following the results obtained with the phase III ORATORIO trial [25]. Furthermore, the phase III EXPAND trial led to the approval of siponimod for the treatment of SPMS patients with active disease [26]. However, treatment of progressive MS remains an unmet need, mostly due to the fact that current therapies confer only partial protection against neurodegeneration associated with MS [7,22].

3. Challenges and limitations of current therapies

The development of effective therapies has allowed to reach near-complete control of relapses and focal brain inflammation [7]. However, most of the treatments are associated with adverse reactions, partly due to the administration route [13]. Moreover, no drug can hinder or counteract the progression of disabilities over time [14]. A major limitation to the development of new drugs and to their efficiency is the poor penetration across the BBB and the low accumulation into the CNS, particularly for the B-cell depleting monoclonal antibodies [14]. Also, it is currently considered that the resident cells of the CNS are an important driving force that mediate the progression of the disease [15], it is thus imperative to promote accumulation of drugs in the CNS, despite the presence of an intact BBB, to improve MS treatments.

3.1. Adverse effects of the DMTs

MS as a progressive and chronic CNS disease has yet no cure and most patients face neurological impairments after the onset of the disease [12]. Besides the preclinical and/or clinical efficacy of the aforementioned therapies, several adverse effects as well as the risk of severe infections have been reported in experimental studies and clinical trials [8,11] (Table 1). These adverse effects are linked to the properties of the drug, the administration route and mechanism of action. Their severity sometimes requires treatment discontinuation [55].

The most common side effects reported for DMTs administered by the oral route include gastro-intestinal disorders as well as hepatoxicity with elevated liver enzymes. The majority of patients using injectable DMTs show injection-site reactions including irritation, which is the more usual side effect following intramuscular (30%) or subcutaneous (90%) injections of glatiramer acetate and interferons (IFNs) for example [56]. With injectable therapeutics, the generation of neutralizing antibodies and poor drug compliance and tolerability are also reported [57]. Patients treated with MS therapies such as rituximab, the first anti-CD20 therapy commonly prescribed as an off-label treatment, ocrelizumab and alemtuzumab, display a higher risk of infections, usually attributable to “post-infusion reactions” [34,58].

Some side effects are attributed to the mechanism of action of the drug. For example, lymphopenia is a well-known side effect associated with the mechanism of action of cladribine [52]. As a synthetic deoxyadenosine analogue, cladribine resistance to adenosine deaminase-mediated deamination enable its accumulation in lymphocytes. Once inside, cladribine is phosphorylated to the cytotoxic triphosphorylated cladribine which causes adenosine deaminase deficiency and results in severe lymphopenia [59]. Fingolimod, a sphingosine-1-phosphate (S1P) agonist, is associated with cardiac side effects at treatment initiation [41]. The latter is most likely related to transient agonism to S1P receptors of cardiomyocytes, whose activation can induce bradycardia [60]. Concerning mitoxantrone, an antineoplastic agent with immunosuppressive effects, a high incidence of cardiotoxicity has been reported [40]. This is associated with the fact that mitoxantrone is able to delay Ca^{++} uptake and increase Ca^{++} release by sarcoplasmic reticulum [61]. Moreover, several MS patients treated with mitoxantrone present oncologic complications. The most common type of cancer developed following mitoxantrone treatment is acute myelocytic leukemia, which is caused by topoisomerase II inhibition and is associated with a higher risk of secondary leukemia [62].

3.2. DMTs and relevance of targeting the CNS

As MS progresses, neuro-inflammation is mostly mediated by phenomena occurring within the CNS and fewer peripheral cells are present in the brain lesions [2]. Despite the notion that CNS-intrinsic inflammation (i.e. driven by astrocytes and microglial cells) plays a critical role in CNS injury, it is poorly addressed by available therapies and needs further investigation [8]. A common feature of all MS therapies is that they primarily act on the peripheral adaptive immune system, thereby preventing damage within the CNS [3]. Indeed, most DMTs do not readily cross the BBB, meaning that their effects are not focused on the CNS compartment but act in the periphery [63]. It is thus assumed that the modified processes observed in the CNS following treatment is a result of the altered activity of the peripheral immune system [64]. Moreover, it is presumed that, immune cells localized in the CNS, such as lymphocytes, are relatively protected from therapeutics that cannot cross the BBB, and are thus not affected by these therapies.

Table 1
Overview of approved disease-modifying therapies (DMT) for MS. (MS drugs updates via [21,27,28]) Ab: antibody, APC: antigen-presenting cells, BBB: blood-brain-barrier, CNS: central nervous system, IFN: interferon, MHC: major histocompatibility complex, Nrf2: nuclear factor (erythroid-derived 2)- like 2, PPMS: primary progressive multiple sclerosis, p.o.: per os, RMS: relapsing forms of multiple sclerosis which include CIS (clinically isolated syndrome), RRMS (relapsing-remitting multiple sclerosis) and active SPMS (secondary progressive multiple sclerosis), S1P: sphingosine-1-phosphate, Treg: regulatory T cells

Drug	Proposed mechanism of action	MS phenotype	Dose	Most common adverse effects	Peripheral/central activity	References
Intramuscular						
IFN-β-1a (Avonex®)	Cytokine that reduces BBB disturbance and production of pro-inflammatory cytokines, increases anti-inflammatory factors and modulates T and B cells	RMS	30 μg once/ week	Flu-like symptoms (including fever, myalgia, asthenia and chills), injection site reactions (including necrosis)	Activity restricted to the periphery	[29,30]
Subcutaneous						
IFN-β-1b (Betaseron®, Extavia®)	Cytokine that activates JAK/STAT pathway, inhibits T cell activation & proliferation and reduces T cells migration across the BBB	RMS	0.25 mg/2 days	Injection-site reactions, lymphopenia, flu-like symptoms, myalgia, asthenia, headache	Activity restricted to the periphery	[31]
IFN-β-1a (Rebif®)	Cytokine that reduces BBB disturbance and production of pro-inflammatory cytokines, increases anti-inflammatory factors and modulates T and B cells	RMS	22 or 44 μg, 3 times/week	Injection-site disorders (including necrosis), flu-like symptoms, abdominal pain, depression, elevation of liver enzymes	Activity restricted to the periphery	[31,32]
PegIFN-β-1a (Plegridy®)	Cytokine that reduces BBB disturbance and production of pro-inflammatory cytokines, increases anti-inflammatory factors and modulates T and B cells	RMS	125 μg/ 2 weeks	Injection site reaction (including erythema, pain, pruritus), flu-like symptoms, pyrexia, headache	Activity restricted to the periphery	[31]
Glatiramer acetate, GA (Copaxone, Glatopa®)	Synthetic peptide that binds to MHC I & II molecules of APC to block myelin damaging T cells, increases brain-derived neurotrophic factors and anti-inflammatory cytokine production and decreases secretion of pro-inflammatory cytokines	RMS	20 mg /day or 40 mg 3 times a week	Injection site reactions (erythema, pain, edema, pruritus, mass), vasodilatation, rash, dyspnea and chest pain	Activity in the periphery	[33]
Ofatumumab, OFA (Kesimpta®)	Human recombinant IgG1 CD20 monoclonal Ab, selectively depletes B cells in the lymph nodes	RMS	20 mg/week for 3 weeks followed by once/ 4 weeks	Upper respiratory tract infections, injection-related reactions (systemic & local), headache	Activity in the periphery, no evidence of OFA capacity to reach the CNS	[34]
Intravenous						
Natalizumab, NAT (Tysabri®)	Anti-α4β1 integrin monoclonal Ab, inhibits lymphocyte access to the CNS by blocking adhesion and crossing of the BBB	RMS	300 mg/ 4 weeks	Headache, fatigue, arthralgia, urinary tract infection, depression, lower respiratory tract infection, pain in extremity	Activity in the periphery (molecular size hinders its entry via the BBB)	[35]
Alemtuzumab, ALE (Lemtrada®)	Humanized anti-CD52 monoclonal Ab, eliminates CD52 cells in the circulation, inducing Treg-dependent immune regulation	RRMS and active SPMS	96 mg over 2 years	Rash, headache, pyrexia, nasopharyngitis, nausea, urinary tract infection, fatigue	Activity in the periphery (molecular size hinders its entry via the BBB)	[36,37]
Ocrelizumab, OCR (Ocrevus®)	Humanized anti-CD20 monoclonal Ab, depletes circulating B cells	RMS & PPMS	300 mg/ 2weeks or 600 mg/ 6 months	Infusion-related reactions, upper respiratory tract infection, headache, skin infections	Activity in the periphery, no evidence of OCR capacity to reach the CNS	[34,38]
Mitoxantrone, MITO (Novantrone®)	Topoisomerase-II inhibitor, inhibits DNA synthesis affecting quickly dividing cells, immunosuppressor	RRMS & SPMS	12 mg/m^2 of body surface area/ 3 months	Cardiac toxicity, increased risk of ovarian failure, menstrual disorders, upper respiratory tract infection, amenorrhea	Activity in the periphery, MITO is hydrophilic and penetrates the CNS poorly when the BBB is intact	[39,40]
Oral						
Fingolimod, FINGO (Gilenya®)	Retains lymphocytes in the lymph nodes via S1P receptor inhibition	RMS	0.5 mg/day	Headache, gastro-intestinal disorders (nausea, diarrhea, abdominal pain), liver transaminase elevation, cough	Peripheral and central activity. The un-phosphorylated pro-drug form of FINGO can cross the BBB readily	[41,42]
Siponimod, SIPO (Mayzent®)	Retains lymphocytes in the lymph nodes via S1P receptor modulation + anti-inflammatory properties	RMS	0.25 or 2 mg/ day	Headache, hypertension, transaminase increases (in blood & liver), abnormal liver functions	Peripheral and central activity. SIPO can cross the BBB	[42,43]
Ozanimod, OZA (Zeposia®)	Retains lymphocytes in the lymph nodes via S1P receptor modulation	RMS	0.92 mg/day	Upper respiratory infection, hepatic transaminase elevation	Peripheral and central activity. OZA can reach the brain after following p.o. administration.	[42,44, 45]
Ponesimod, PONE (Ponvory™)	Retains lymphocytes in the lymph nodes via S1P receptor modulation	RMS	20 mg /day	Upper respiratory tract infection, hepatic transaminase elevation, hypertension.	Peripheral and central activity	[42,46]

(*continued on next page*)

Table 1 (*continued*)

Drug	Proposed mechanism of action	MS phenotype	Dose	Most common adverse effects	Peripheral/central activity	References
Teriflunomide, TFM (Aubagio®)	Pyrimidine synthesis inhibitor (dihydroorotate dehydrogenase inhibition), reduces T & B cell activation	RMS	7 or 14 mg/day	Hepatoxicity & nephrotoxicity (increase in alanine aminotransferase), headache, diarrhea	Activity in the periphery, no strong evidence that TFM enters the CNS	[47]
Dimethyl fumarate, DMF (Tecfidera®)	Induce anti-inflammatory immune response and neuroprotection via Nrf2-dependent & -independent pathways	RMS	240 mg twice/day	Flushing, gastro-intestinal disorders (abdominal pain, diarrhea, nausea)	Peripheral and central activity: its metabolite MMF can cross the BBB	[48,49]
Diroximel fumarate, DRF (Vumerity®)	Nrf2 pathway activator	RMS	462 mg (taken as two 231 mg capsules) twice/day	Flushing, gastro-intestinal disorders (abdominal pain, diarrhea, nausea)	Peripheral and central activity: its metabolite, MMF can cross the BBB	[50]
Monomethyl fumarate, MMF (Bafiertam™)	Active metabolite of DMF with anti-inflammatory and anti-oxidant effects via the Nrf2 pathway	RMS	190 mg (taken as two 95 mg capsules) twice/day	Flushing, gastro-intestinal disorders (abdominal pain, diarrhea, nausea)	Can cross the BBB	[49–51]
Cladribine, CLAD (Mavenclad®)	Adenosine analog prodrug that targets T and B cells without continuous immunosuppression	RRMS and active SPMS	3.5 mg/kg cumulative dose over 2 years	Upper respiratory tract infection, headaches and lymphopenia and nausea	Peripheral and central activity, able to cross the BBB	[52–54]

Up to date, the DMTs able to cross the BBB include cladribine [54,65], fingolimod [53,64], siponimod [42,66], ozanimod [45], ponesimod [67,68] and monomethyl fumarate [49]. These DMTs have the potential to directly influence the resident cells of the CNS. The high molecular size of several DMTs, including alemtuzumab (145 kDa) and nataliezumab (146 kDa), as well as the hydrophilicity of others, such as mitoxantrone, result in their inability to cross the BBB [14].

For the DMTs crossing the BBB, it remains to be clarified if direct actions on CNS-resident (immune) cells is required to promote endogenous repair mechanisms and/or provide neuroprotective effects [53,64]. Another open question is whether higher CNS penetration of the periphery-restricted DMTs would result in higher efficacy. Indeed, some DMTs including IFNs, glatiramer acetate and natalizumab have their molecular targets in the circulating compartment and thus require no BBB penetration to exert their therapeutic efficacy [69]. Therefore, a potential strategy for future therapies may be a combination of an early targeting of peripheral immune cells and of CNS-compartmentalized inflammation, along with the provision of neuroprotective and/or neuroregenerative drugs [2,14].

4. Focus of research for the development of new MS therapies

During the past two decades, the development of immunotherapies that act on the inflammatory component of the disease has allowed for a substantial reduction of CNS lesion formation and relapse rate [70]. However, as the accumulation of permanent disabilities, particularly during the progressive phase of MS, remains insufficiently prevented by current therapies, the research community has looked for new treatments. Research focuses on different strategies, including the reduction of neuro-inflammation, promotion of remyelination and neuroprotection [71–73]. On the other hand, new promising studies focusing on alternative avenues including targeting immune tolerance, chronic EBV infection, Bruton's tyrosine kinase, dysbiosis of the gut microbiome, specific metabolites linked with MS pathogenicity and the coagulation system have also recently emerged [74,75]. This review will focus on neuro-inflammation, remyelination and neuroprotection as the other strategies have been discussed elsewhere [74,75].

Due to the complexity of the disease, no pre-clinical model fully recapitulates the full spectrum of human MS. However, several models modeling different aspects of the pathology are available to study new drug and therapeutic strategies against MS [76–79]. A model is selected based on the specific question or the mode of action of the new therapeutics to be tested. For experimental drugs targeting neuro-inflammation as well as for therapies targeting different aspects of the pathology simultaneously (e.g. neuro-inflammation, neuroprotection, remyelination) the use of the experimental autoimmune encephalomyelitis (EAE) mouse model is recommended as it is an autoimmune-mediated CNS inflammatory and demyelinating model. Indeed, by using a specific mouse strain with a corresponding autoantigen, one could mimic different forms of MS [77,78]. For drugs specifically targeting the de-/remyelination processes, toxin-induced demyelination models would provide an adequate platform (i.e. cuprizone (CPZ), lysophosphatidycholines (LPC), bromide ethidium). However, as activation of CNS-resident cells also occurs in these models, the impact of anti-inflammatory compounds on remyelination can also be assessed, especially in the CPZ model.

4.1. Neuro-inflammation

Neuro-inflammation is one of the pathological hallmarks of MS, with microglia and astrocytes as key regulators within the CNS [80]. Given the importance of inflammation at the onset of MS, treatments are mainly based on anti-inflammatory drugs, including some DMTs [13]. Activated microglia and astrocytes secrete a series of pro-inflammatory and anti-inflammatory cytokines and chemokines during the course of the disease, that can be used as direct or indirect immune or inflammatory mediators [81]. Specific targeting of the microenvironment surrounding the glial cells to either enhance phagocytosis of myelin debris (that hinders effective remyelination) by both microglia and astrocytes, or to reduce the prolonged inflammatory process, could contribute to slow down MS evolution [82,83].

Using common models of MS (EAE and CPZ), several molecules have shown efficacy against neuro-inflammation, the most studied of which will be discussed hereunder and illustrated in Fig. 2.

An interesting compound expected to become a treatment for MS is arsenic trioxide, known to treat several autoimmune diseases [84]. Not only could it reduce several pro-inflammatory cytokines in EAE mice, but also reduce demyelination, inflammation and microglial activation in that experimental model. Guo et al. showed that fasudil-modified encephalitogenic mononuclear cells reduced the inflammatory processes in the spinal cords of EAE-induced mice as well increased the levels of neurotrophic factors. [85]. Investigations from other groups also showed that fasudil was able to inhibit neuro-inflammation in an EAE mouse model [86,87]. Other compounds such as cordycepin [88], ganoderic acid A [89], epimedium flavonoids [90], and icariin [91] were found to hinder the overactivation of microglia and/or astrocytes in the CNS, improving the symptoms of CNS damage in EAE and CPZ mice. Several drugs, including lenalidomide, ethyl pyruvate, minocycline, resveratrol and forskolin to name a few, have been investigated as potential MS therapies for their capacity to target macrophages, microglia and their polarization [92]. Indeed, lenalidomide, ethyl pyruvate and forskolin improved MS disease in rodent models by promoting mi-

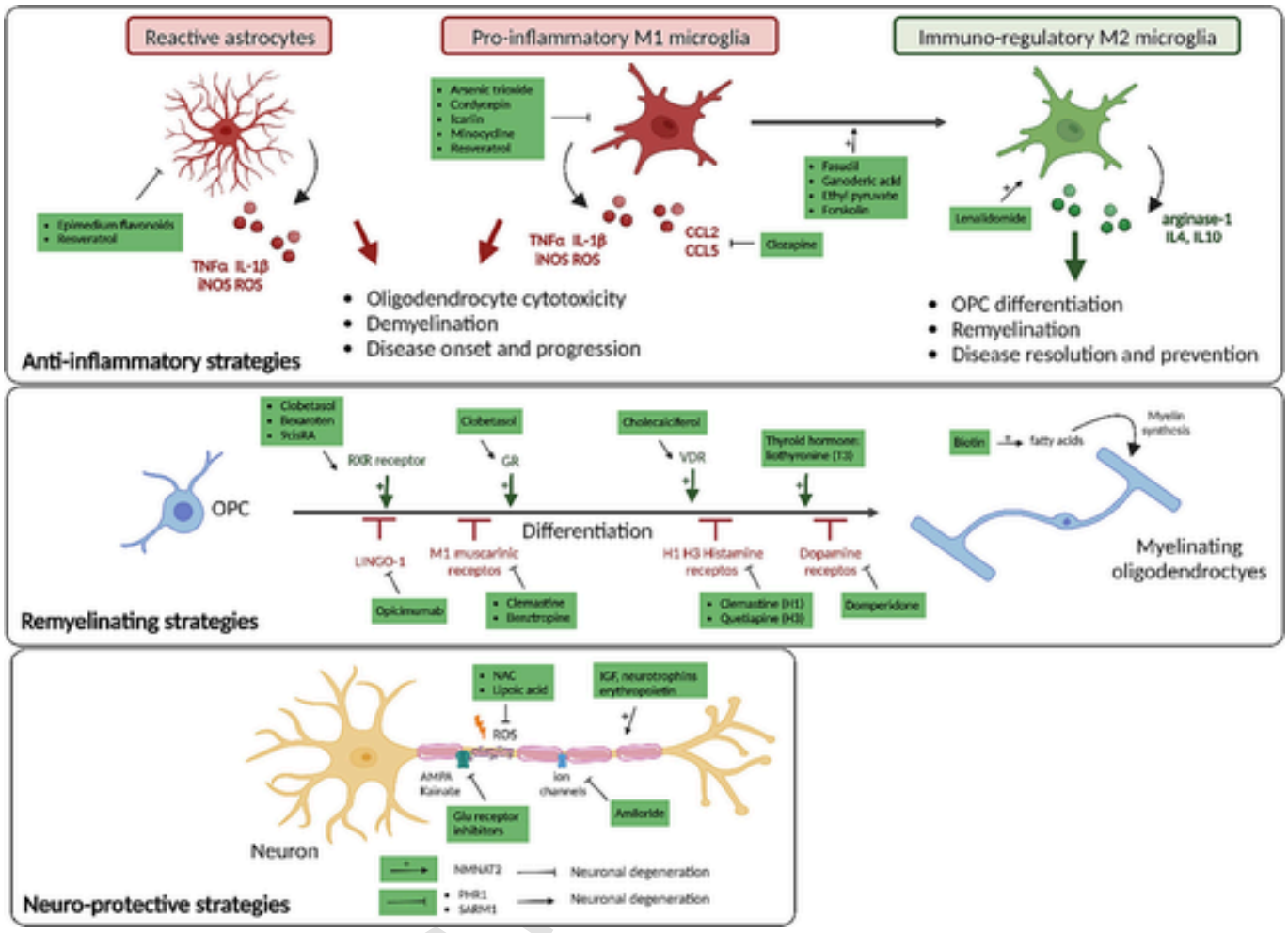

Fig. 2. Promising strategies to reduce neuro-inflammation, promote remyelination and neuroprotection for progressive MS. Strategies targeting neuro-inflammation (anti-inflammatory strategies) include the use of inhibitors of astrocytic (e.g. epimedium flavonoids, resveratrol) and microglial activity (e.g. arsenic trioxide, cordycepin, icariin, minocycline, resveratrol), the use of molecules reducing the activation of pro-inflammatory microglia (M1) while promoting the activation of anti-inflammatory and immune-regulatory microglia (M2) (e.g. fasudil, ganoderic acid, ethyl pyruvate, forskolin), the use of M2 microglia inducers (e.g. lenalidomide) and the repurposed antipsychotic clozapine, for its ability to reduce pro-inflammatory chemokine expression. Strategies promoting remyelination include stimulators of OPC differentiation (e.g. clobetasol, bexarotene, 9cisRA, cholecalciferol, liothyronine), molecules neutralizing differentiation inhibitory signals (opicimumab, clemastine, benztropine, quetiapine, domperidone) and promoting synthesis of myelin (e.g. biotin). Neuroprotective approaches include preventing oxidative stress (e.g. NAC, lipoic acid), targeting pathways used by neurotrophic factors (e.g. IGF2, neurotrophins, erythropoietin), targeting glutamate receptors to avoid excitotoxicity, targeting ion channels (e.g. amiloride) and promoting or reducing factors that hinder (e.g. NMNAT2) or promote (e.g. PHR1, SARM1) neuronal degeneration, respectively. AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, CCL: chemokine ligand, Glu: glutamate, GR: glucocorticoid receptor, IGF2: insulin growth factor, IL: interleukin, iNOS: inducible nitric oxide synthase, LINGO-1 (leucine-rich repeat and Ig domain-containing, nogo receptor-interacting protein 1), NAC: N-acetyl cysteine, NMNAT2: nicotinamide mononucleotide adenylyltransferase 2, Oligo: oligodendrocyte, OPC: oligodendrocyte progenitor cell, PHR1: phosphate starvation response 1, ROS: reactive oxygen species, RXR: retinoid X receptor, SARM1: sterile alpha and TIR motif containing 1, TGFβ: transforming growth factor beta, TNFα: tumor necrosis factor alpha, VDR: vitamin D receptor. (created with BioRender.com).

croglia anti-inflammatory and pro-regenerative phenotype (M2) [93–95]. Moreover, inhibition of microglia activation and the subsequent expression of pro-inflammatory cytokines via minocycline or resveratrol treatment, reduced the inflammatory response and the demyelination process in the CNS of diseased mice [96–99].

Another research avenue is the repurposing of drugs already in clinical use for other diseases. In this case, researchers identify new therapeutic targets for these drugs, decreasing thus the toxicological and preclinical tests necessary for their validation as a therapy [100]. Atypical antipsychotics have gained interest for their immunomodulatory properties and their capacity to curb neuro-inflammation [101]. Compounds such as clozapine have been investigated for their effects on immune-mediated neuro-inflammation that occurs in MS (See review: [63]).

4.2. Remyelination

Recently, a lot of efforts have been made to target remyelination in the scope of MS, as it has been suggested that, in some types of lesions, persistent demyelination may be the main cause of neurological disability in patients [72]. Targeting and/or promoting different stages involved in the remyelination process to subsequently increase remyelination could be an essential therapeutic method to prevent axonal degeneration and, consequently, accumulation of neurological disability. These research strategies have the ultimate goal to find new treatment for progressive MS [70].

The number of potential drug candidates to enhance remyelination has remarkably expanded over the years. Several experimental strategies have led to the identification of pro-remyelinating drugs, both repurposed and new, with only a few selected for further clinical trials

(see reviews: [70–72,78,102]. These molecules promote remyelination via three main strategies, namely (1) stimulation of oligodendrocyte progenitor cell (OPC) differentiation, (2) neutralization of OPC differentiation inhibitory signals and (3) provision of cofactors for myelin forming enzymes (Fig. 2). Due to the large amount of available data, a selection of a few are listed below as examples:

(1) Agonists of the following nuclear receptors; retinoid X receptor; RXR (***bexarotene, 9cis-retinoic acid***), vitamin D receptor; VDR (***cholecalciferol***), as well as hormone agonist (***liothyronine***). ***Clobetasol***, a synthetic glucocorticoid, has been found to induce OPC differentiation in several pre-clinical models through activation of multiple hormone receptors (i.e. RXR) or via glucocorticoid receptor signaling.
(2) Inhibitors of LINGO-1 (leucine-rich repeat and Ig domain-containing, nogo receptor-interacting protein 1), a receptor that negatively regulates OPC maturation (***opicinumab***), inhibitors of muscarinic receptors M1/M3, both associated with maintaining OPC in the proliferative state (***clemastine, benztropine***), inhibitors of histamine receptors H1 (***clemastine***) and H3 (***quetiapine***) as well as inhibitors of dopamine receptors (***domperidon***e).
(3) Acetyl-CoA carboxylase cofactor (***biotin***) promotes remyelination by increasing the supply of fatty acid precursors for subsequent myelin synthesis.

Interestingly, several oligodendrocyte maturation enhancers have demonstrated a similar mechanism. Muscarinic antagonists (***clemastine, benztropine***), the repurposed antifungal drug ***miconazole*** and selective estrogen modulators (i.e. ***tamoxifen***) increase oligodendrocyte maturation by enhancing the production of 8,9-unsaturated sterols, in addition to acting on their known targets [103]. Conversely, RXR modulators as well as thyroid hormones target transcriptions factors. Indeed, effective expression of transcription factors such as olig1, olig2 and NKx2.2 is required for the generation of mature oligodendrocytes [71,104].

Despite the major advances made in remyelination research, MS therapies targeting remyelination have yet to reach clinical use in MS. Ploughman et al., recently reported the failure of three clinical trials, testing high-dose biotin, bexarotene and opicinumab [105]. It was suggested that the failure was mainly due to the lack of consideration of the deleterious impact of the microenvironment surrounding lesions. Indeed, the non-permissive environment owing to the secretions of activated microglia and/or astrocyte might have hindered the efficacy of the treatment [105]. Moreover, inclusion of trial participants with no capacity of repair and ignoring the potential synergistic effect of neurorehabilitation and exercise with remyelinating compounds could also explain this failure. With ageing, oligodendrocytes undergo degeneration and the remyelination process becomes less efficient [106]. Therefore, recruiting younger participants whose CNS have higher capacity for remyelination and repair or combining treatment of older participants with an agent that reverses biological ageing in OPC (i.e. metformin) [107] could address these problems. Moreover, several experimental studies in animal models confirm that exercise and rehabilitation enhance neurogenesis, neuronal survival and differentiation [108]. In addition, Bierhansl et al. emphasized the importance of the timing of drug administration. Indeed, as remyelination immediately starts after the onset of inflammatory demyelination, drug administration should be carried out in the early remyelination phase, targeting thus the inflammatory oligodendrocyte/OPC phenotype [75]. Lastly, the poor CNS penetration of the remyelinating drugs could also hinder their capacity to induce robust remyelination in MS patients [15,109]. Increasing the delivery of these drugs into the CNS could contribute to an increased therapeutic effect. The discovery and development of new pro-remyelinating drugs remain thus a major unmet medical need.

4.3. Neuroprotection

Researchers and clinicians have also recognized the importance of developing strategies that aim at preventing neuronal and oligodendrocyte injury in MS, independent of inflammatory processes. Such approaches may be beneficial in progressive forms of MS during which neurodegeneration occurs without evident inflammatory injury [74,110]. From 2014 to 2020, 221 studies aiming to either increase trophic support for oligodendrocytes and axons, stimulate oligodendrogenesis, stimulate remyelination or promoting axonal regeneration and integrity have been reported [110].

The goal of neuroprotection is to preserve CNS architecture and function in potentially damaging situations (Fig. 2). At present, neuroprotection is being investigated by targeting the pathways used by neurotrophic factors, and by using ion channel modulators or compounds that trigger the antioxidant response [71]. Neurotrophic factors (e.g. insulin growth factor, neurotrophins or erythropoietin) have long been investigated as potential treatments for MS. However, the use of recombinant neurotrophic factors has been unsuccessful in different clinical trials owing to their poor penetration inside the CNS and poor clinical trial design [111,112].

Another strategy involves prevention and/or reduction of oxidative stress, a process involved in cell degeneration in all stages of MS. Several compounds with antioxidants properties have been explored as potential therapies [113], among which *N*-acetyl cysteine (NAC) and lipoic acid. NAC is a glutathione precursor previously studied in progressive MS [114] whereas lipoic acid is a commonly used antioxidant with potential neural protective properties [115,116]. Both molecules are each being tested in a phase 2 study of progressive MS with the objective to show a beneficial impact of NAC on CNS atrophy (NCT05122559) and to determine whether lipoic acid can protect the brain (NCT03161028) [74].

Ion channel modulators (i.e. amiloride) have also been investigated to induce neuroprotection, as they have demonstrated benefits in animal models and small clinical studies [71]. Moreover, molecules modulating calcium signaling have gained more interests due to the critical role of calcium in several signaling cascades in the neurons. Indeed, the overactivation of glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (also known as excitotoxicity) is a well-known neuronal damaging mechanism in MS leading to increased levels of intracellular calcium and consequently provoking cell death [117,118]. ROS formation and release of apoptotic proteins have also been associated with increased intracellular calcium. Compounds with the capacity to reduce glutamate-induced neuronal excitotoxicity and drugs that directly interfere with calcium homeostasis have thus been identified and thoroughly investigated for their neuroprotective capacities in MS [110,118].

Lastly, therapies preventing axon loss have been explored thanks to the understanding of the biology of axon damage. Following activation of the neuronal degenerative process subsequent to axon damage, key molecules act to protect axons from degeneration such as nicotinamide mononucleotide adenylyltransferase 2, or to promote degeneration (i.e. phosphate starvation response 1 and sterile alpha and TIR motif containing 1) [119]. Targeting these factors has been used as an approach to develop neuroprotective agents [71,120].

The following strategies allowed the identification of promising drugs, targeting different aspects of the disease mainly focused in the CNS compartment. While some of the listed compounds act on CNS processes via mechanisms in the periphery, others require entry to the CNS. In this context, several strategies have been developed to increase drug delivery to the CNS. Among these strategies, nanocariers have been used as vehicles to protect the drug of interest during its transport to the CNS or as a strategy to reach the CNS as some of them have been shown to cross the BBB. These approaches will further be discussed in the following sections.

5. Drug delivery strategies to the CNS

5.1. Barriers limiting access to the CNS

As mentioned above, access to the CNS is hindered by the presence of several biological barriers, namely the arachnoid layer, the epithelial cells of the choroid plexus forming the blood-cerebrospinal fluid barrier (BCSFB) and the BBB (Fig. 3). Thus, strategies that would increase/facilitate access and accumulation in the CNS could offer MS drugs additional opportunities to resolve the neuro-inflammatory and neurodegenerative processes involved in the progressive forms of MS.

The arachnoid layer is the external layer of the meninges that surrounds the CNS. It forms a relatively avascular membrane that has a surface area smaller than either the BCSFB and the BBB, thus does not promote nutrient and drug exchanges and in consequence, will not be considered as a drug exchanging interface [121].

The BCSFB is located at the interface between the blood and the CSF. The CSF is produced by the choroid plexus, and is contained within the two lateral ventricles and lines up the midline within the third and fourth ventricles. The ventricle main function is to produce the CSF [16]. The BCSFB is not as limiting as the BBB as certain drugs that do not cross the BBB do cross the BCSFB and enter the CSF at a rate inversely related to molecular weight, but do not enter the brain parenchyma [122]. Moreover, this barrier does not cover a surface area as wide as the BBB (3 folds less) [123]. However, it washes up a significant amount of drug, based mainly upon the permeability coefficient of the drug, via a constant flow of CSF [122].

The main biological barrier limiting drug delivery to the brain remains the BBB. This barrier is comprised of different cell types, including brain microvessel endothelial cells (BMECs), astrocytes, pericytes, and neuronal cells (Fig. 3). The BBB main functions are to protect the brain parenchyma from toxic agents circulating in the blood as well as providing a significant barrier to the entry of drugs and other exogenous compounds into the CNS [124,125]. It therefore ensures the homeostasis of the CNS by regulating transport of specific compounds and nutrients as well as barring the passage of potentially deleterious molecules [126]. Compared to peripheral endothelial cells, BMECs have several specific characteristics, including no fenestrations, a relatively low endocytic activity, which limits the transcellular flux, and high trans-endothelial electrical resistance, due to the continuous tight junctions between the cells that restrict paracellular transport [16,124]. This highly selective structural and biochemical barrier restricts and controls the entry of circulating molecules (amino acids, glucose, drugs, etc.) from the blood into the CNS and vice versa. Under physiological conditions, the BBB prevents entry of large molecules (> 1 kDa) as well as more than 98% of small molecules, proteins and peptides (500 – 1000 Da) [127].

During MS pathology, the impairments of the BBB have a double effect. During the onset of the disease, immune system abnormalities induce alterations in the BBB tight junctions which facilitate migration of activated peripheral cells to the CNS. It Is unclear whether the disruption of the BBB is a result of the pathology or its cause but it is also reflected during the relapse phases of the disease. However, as the disease progresses, demyelination and neurodegeneration increase due to CNS self-sustained processes [128,129]. The absence of gadolinium positive lesions (used in magnetic resonance imaging as a consequence of BBB disruption) in the progressive forms of MS, suggests a compartmental-

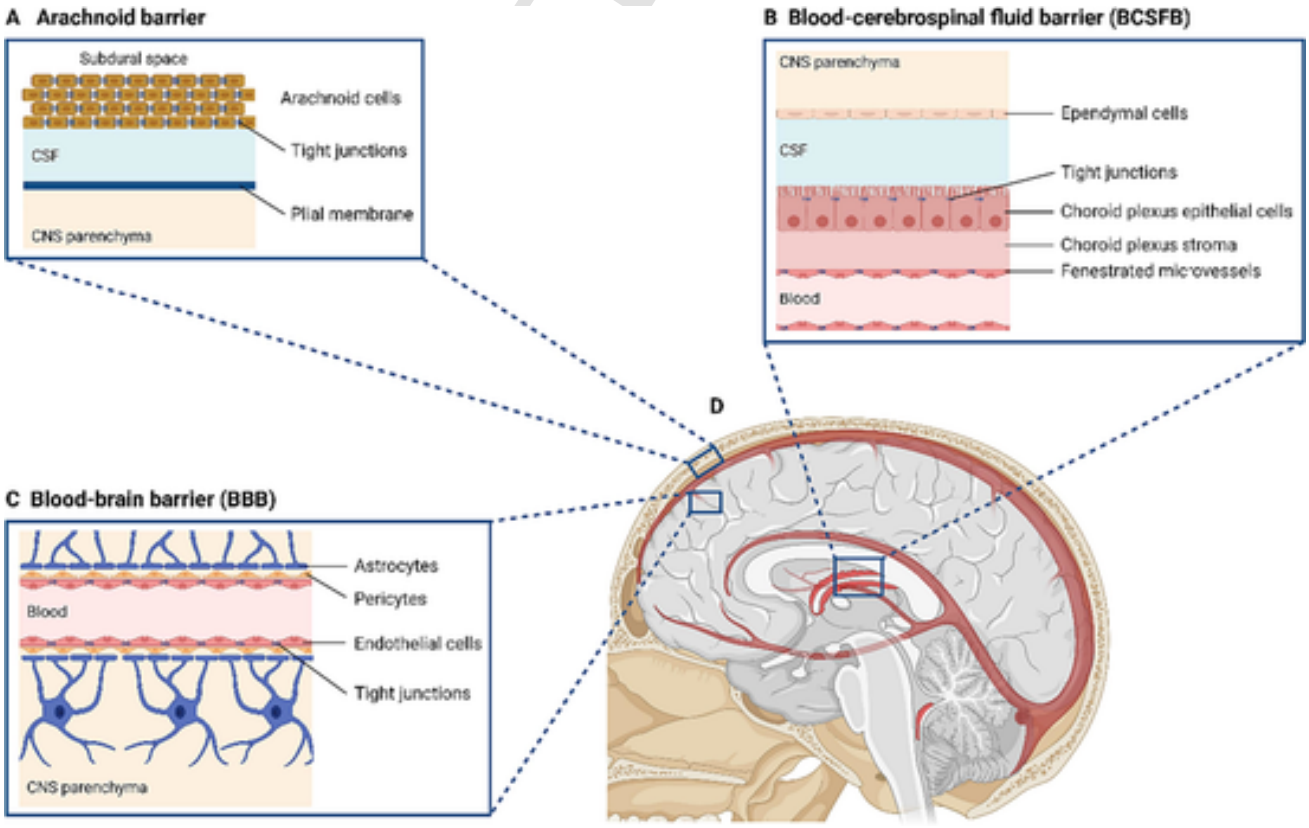

Fig. 3. Biological barriers of the CNS. The three main barriers include (A) the arachnoid barrier, (B) the blood cerebrospinal fluid barrier (BCSFB) and (C) the blood-brain barrier (BBB), identified in a (D) schematic sagittal brain/skull section. The arachnoid barrier is the external layer of the meninges that encases the CNS. The BBB is formed at the endothelial plasma membrane of the brain parenchyma capillary and is composed of blood microvessel endothelial cells (endothelial cells), astrocyte processes end-feet forming the "glia limitans" and pericytes. The BCSFB is formed by the epithelial plasma membrane of the choroid plexus and separates CSF from blood. Of the three barriers, the BBB is at the closest vicinity to the brain parenchyma and thus offers the most opportunities for CNS drug delivery. CNS: central nervous system, CSF: cerebrospinal fluid. (created with BioRender.com).

ized injury in the CNS that is secondary to the integrity of the BBB after an unknown period of MS evolution [129].

5.2. Strategies to overcome/bypass the BBB

In order to reach therapeutic levels of drugs in the CNS, several strategies have been developed to bypass and/or overcome the BBB. These strategies include invasive and non-invasive methods as depicted in Fig. 4, where in both cases, nanocarriers can serve as a protective shuttle during the transport of the therapeutic drugs to the CNS. Additionally, as several nanocarriers have the ability to cross the BBB, their use alone (with the encapsulated or grafted cargo) can serve as a CNS drug delivery strategy.

Some approaches take advantage of the main transport pathways used by molecules that can cross the BBB, including the passive transcellular diffusion, carrier-mediated transport and receptor-mediated transcytosis [125]. These strategies are considered non-invasive as they only require modifications of the drug of interest to allow their transport across the BBB (Fig. 4). Chemical modifications include increasing the lipophilicity of the drug by attaching a lipophilic moiety (able to cross the BBB) to form a prodrug that will undergo transformations to become biologically active when localised in the brain parenchyma [130,131]. Genetic modifications are carried out when drugs cannot tolerate chemical modifications. This include the use of a molecular Trojan Horse which consist of the genetic fusion of a vector that binds a specific endogenous receptor or transporter on the luminal side of the BBB, enabling its transport in the CNS [132]. Molecules such as the enzymes α-l-iduronidase and sulfamidase [133,134] as well as nanoparticles (e.g. liposomes) [135,136] have used this approach to increase their accumulation in the CNS. This strategy allowed the delivery of 1 to 3 % of the administered dose of therapeutics in the brain following intravenous administration [132]. The main limitation of these strategies are the properties of the drugs of interest and their compatibility with such modifications. For the progressive forms of MS, as the BBB is considered intact, its composition in terms of receptor distribution should not be affected, increasing thus the feasibility of these approaches.

Other strategies aim at transiently modifying the permeability of the BBB by opening the tight junctions using hyperosmotic solutions, chemical disruption or focused ultrasound (FUS) (Fig. 2). Hyperosmotic shock is achieved by intracarotid injection of a highly concentrated saccharide solution (e.g. mannitol or arabinose) [137,138]. Alternatively, the simultaneous intracarotid injection of the therapeutic drug with vasoactive agents, stabilizers or adjuvants (e.g. bradykinin [139], alkylglycerols [140], and histamine [141]) enables a temporary chemical disruption of the BBB. However, these methods do not allow any specific targeting nor any control of drug release and are associated with a potential risk of accumulation of neurotoxic compounds [124]. The use of focused ultrasound (FUS) on the other hand, represents a less invasive technique that can stimulate local and reversible opening of the BBB when used in conjunction with microbubble contrast agents (e.g. optison, perflutren lipid microsphere) [142–144]. The latter are intravenously administered before exposure to FUS and enable a controlled and consistent multi-target BBB disruption when infused [144]. In the context of MS, increasing the permeability of the BBB should be operated with caution to avoid worsening of the disease. FUS is advantageous over the other strategies as it can be targeted to a small focal volume. However, while opening of the BBB using FUS in Alzheimer disease rodent models resulted in the amelioration of the pathology [145], Schregel et al. used this technique to increase lesion occurrence at the sonicated location in EAE-induced mice [146].

Several approaches bypass the BBB altogether when delivering therapeutic compounds to the CNS (Fig. 4). These strategies include direct injections of the drug into the brain parenchyma (intracerebral or intraparenchymal injections) or direct delivery to the CSF either through the skull into the CSF-filled lateral ventricle (intracerebroventricular) or

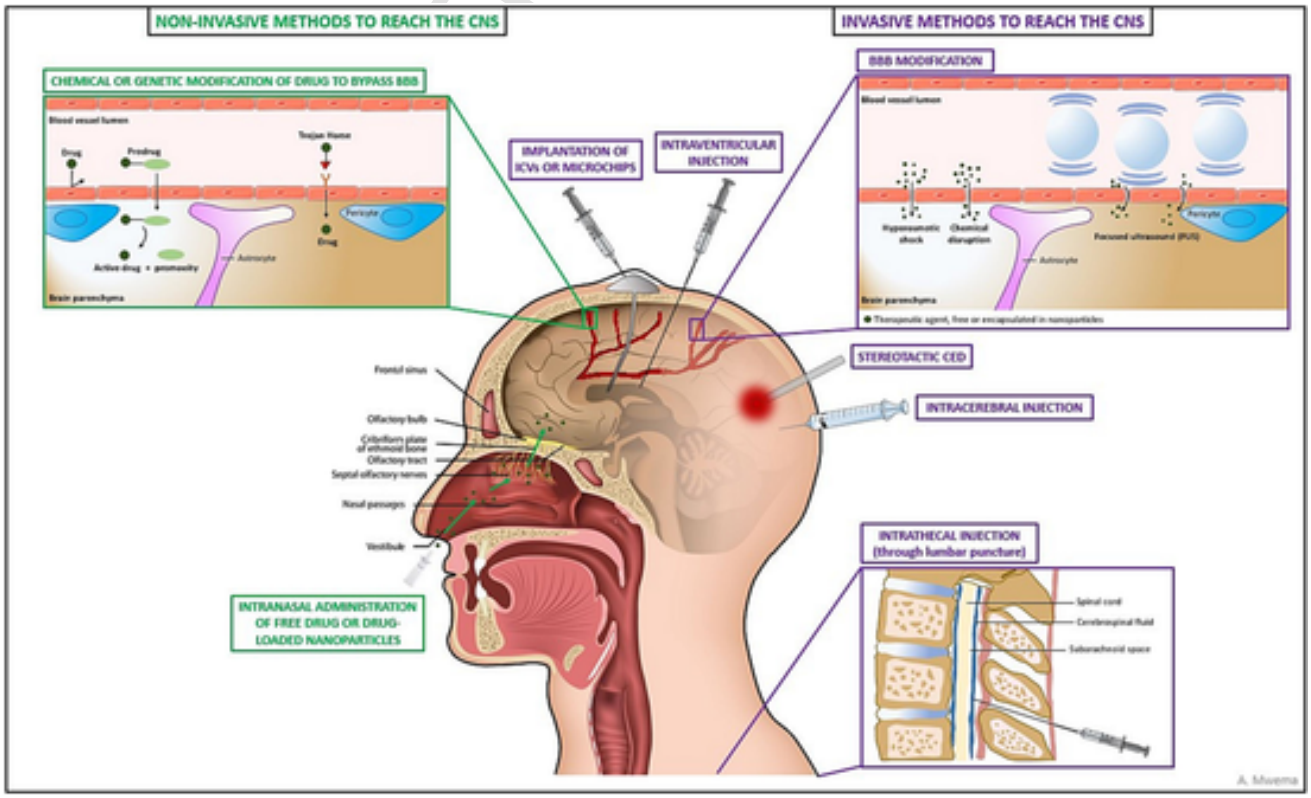

Fig. 4. Overview of the several strategies used to avoid, bypass or overcome the BBB to reach the CNS [19]. BBB: blood-brain barrier, ICVs: intracerebroventricular devices

via lumbar puncture into the spinal canal (intrathecal injections) [16,124]. To achieve a more continuous and controlled release of the bioactive molecules, intracerebroventricular devices, such as the Ommaya reservoir [147] or microchips [148,149] are implanted into the brain parenchyma and/or the ventricles. These approaches have several advantages including reduced systemic penetration and no metabolism in the blood stream. Nonetheless, the drugs infused via these methods have a slow diffusion coefficient which is dependent on the drug molecular weight, polarity, tissue affinity and localisation of administration [124]. Although a great percentage of the drug is locally delivered to the CNS, these methods are quite invasive and cannot be routinely carried out. Alternatively, a minimal invasive strategy involving the stereotactic placement of micro-pumps that maintain hydrostatic pressure, namely convection enhanced delivery (CED), have also been used [150,151]. This system allows to homogenously distribute a greater volume of drug to targeted regions of the CNS using a positive pressure gradient.

Although very interesting, these last strategies require surgical procedures and are thus less suitable for multiple dosing, the latter being essential for MS therapies. Moreover, as BBB disruption and lymphocyte trafficking are among the most important pathological processes of MS [129], using strategies that increase BBB permeability to increase CNS delivery might not only exacerbate autoreactive lymphocyte infiltration but also increase the potential accumulation of toxic agents. Modification of the drug of interest to exploit transport pathways of the BBB is thus the best strategy in the context of MS. Alternatively, an interesting non-invasive approach to deliver drugs to the CNS, avoiding the BBB altogether, is the use of the nose-to-brain (N2B) route, further discussed in the following sections.

5.3. Nose-to-brain delivery - general concepts

In the past decade, an increased interest in nasal delivery, mainly for the growing field of N2B delivery in the context of CNS diseases has been observed [152–155]. This administration route enables direct transport of the therapeutic drugs from the nasal cavity to the brain, avoiding thus the BBB. Furthermore, in addition to the many advantages provided by the intranasal administration route, including minimized systemic exposure, avoidance of hepatic first-pass metabolism, rapid onset of action, as well as increased patient comfort and compliance [156], the potential of self-medication as well as multiple dosing are particularly interesting for chronic diseases [157].

The transport mechanisms of the drug from the nasal cavity to the brain are not fully understood, however, four main pathways have been described [158–160] (Fig. 5). Once in the nasal cavity, the drug is either transported via the olfactory (in the olfactory region) and/or the trigeminal nerve (in the respiratory region of the nasal cavity) via intracellular and/or extracellular mechanisms. The intracellular mechanism begins with internalisation of the drug in the neuron, followed by trafficking of the endocytic vesicle within the neurone's axon and exocytosis of the cargo in the olfactory bulb and brainstem for the olfactory and trigeminal nerve, respectively [161]. The extracellular pathway entails the transport of the drugs in the perineural space alongside the nerve's axons via bulk flow processes [155]. The high vasculature of the respiratory epithelium exposes the drugs to the systemic circulation, allowing their accumulation in the brain depending on their capacity to cross the BBB [160,161]. Lastly, a transport through or alongside the supporting cells located at the olfactory epithelium can also occur [159].

Some limitations have been associated with this delivery route. Indeed, due to the small surface area of the nasal cavity, the efficacy of the N2B transport is restricted to small volumes administered (20-200 μl for rodents [154,162] and up to 500 μl for humans [163]). The administration of higher volumes leads to drainage of the excess into the oesophagus or increase leakage. Moreover, these volumes help minimize the potential for suffocation [154,162]. Studies using human nasal cast models and mathematical algorithms have shown that ideal particle size for olfactory region deposition and efficient N2B in humans is 1 nm to 10 μm, with a flow rate between 5-20 L/min [164,165]. Based on these studies, several devices have been developed to promote deposition to the olfactory region including ViaNase [166], an electronic atomizer developed by Kurve Technology®, Opt-Powder by Optinose®, a bi-directionnal delivery device [167], the Unit Dose system by Aptar Pharma, suitable for liquid and powdered drug delivery [168], the Precision Olfactory Delivery® by Impel Neuropharma that uses a gas propellant to deliver liquids and powders to the olfactory epithelium [169] and the Sipnose device by SipNose LTD, a pressurized delivery system that enables delivery of small particle aerosols without deposition in the lower airways [170]. Among these devices, the Precision Olfactory Delivery®, Optinose® and ViaNase have been specifically evaluated for delivery to the brain, making them thus more appropriate for a potential MS treatment. Indeed, the ViaNase™ device was tested in a clinical trial with patients with cognitive impairment related to MS (NCT02988401). However, it is important to note that cross-comparisons of such devices are difficult as these devices use different formulations (powders vs liquids) and measure different endpoints. The selection of the most appropriate device should thus be based on their advantages and drawbacks depending on each specific drug tested in the context of MS. The presence of enzymes in both the olfactory and respiratory mucosa lead to local enzymatic degradation and limited drug absorption [168]. Furthermore, drug residence time in the nasal cavity is further diminished by the nasal ciliary clearance as well the presence of efflux pumps (i.e. P-glycoprotein (P-gP) efflux pump) [152,159].

Successful CNS drug delivery through the nasal route is thus greatly influenced by the physico-chemal properties of the drug of interest. Some properties reduce the ability of the drug to reach the CNS following this administration route (Table 2). Several strategies have thus been proposed to increase their N2B transport (Table 2). Among these strategies, the use of nanocarriers to encapsulate the therapeutics drugs was proposed, enabling their protection against the actions of the nasal cavity enzymes. Moreover, via the functionalization or the composition of the surface of the nanocarriers, evasion of the P-gP efflux pump mechanisms can be mediated, increasing thus the bioavailability of the drug during the N2B transport [168].

5.4. Nanomedicine for CNS drug delivery in the scope of MS

5.4.1. Criteria for drug delivery in MS

In addition to increasing the delivery of MS drugs in the CNS while protecting them from potential enzymatic degradation, nanotechnology offers other advantages in the scope of neuro-degenerative diseases like MS. Although increased therapeutic options for MS patients are offered, there are still some limitations regarding the routes of administration available and the necessity for long-term and repeated dosing, that induces systemic disadvantageous consequences and patient non-compliance [18]. Less than half of patients with chronic diseases such as MS adheres to their prescribed treatments, which impedes the full benefit of the medication, worsens disease outcomes, and accelerates disease progression [182,183]. In MS, poor adherence to treatment negatively impacts the quality of life, disease progression and MS-related hospitalization [184]. Highly effective medications that require less frequent administration are favored in terms of tolerability and adherence [7]. In that regard, nanomedicine is a promising approach to not only allow prolonged release of the therapeutic compounds, but also to target specific cells or CNS regions when delivering drugs to the CNS. The latter would thus allow to increase local drug delivery and reduce systemic adverse effects.

5.4.2. Main nanocarriers for CNS drug delivery

A wide array of nanocarriers such as nanoparticles (NP), natural and artificial vesicles, has been widely investigated as drug delivery systems

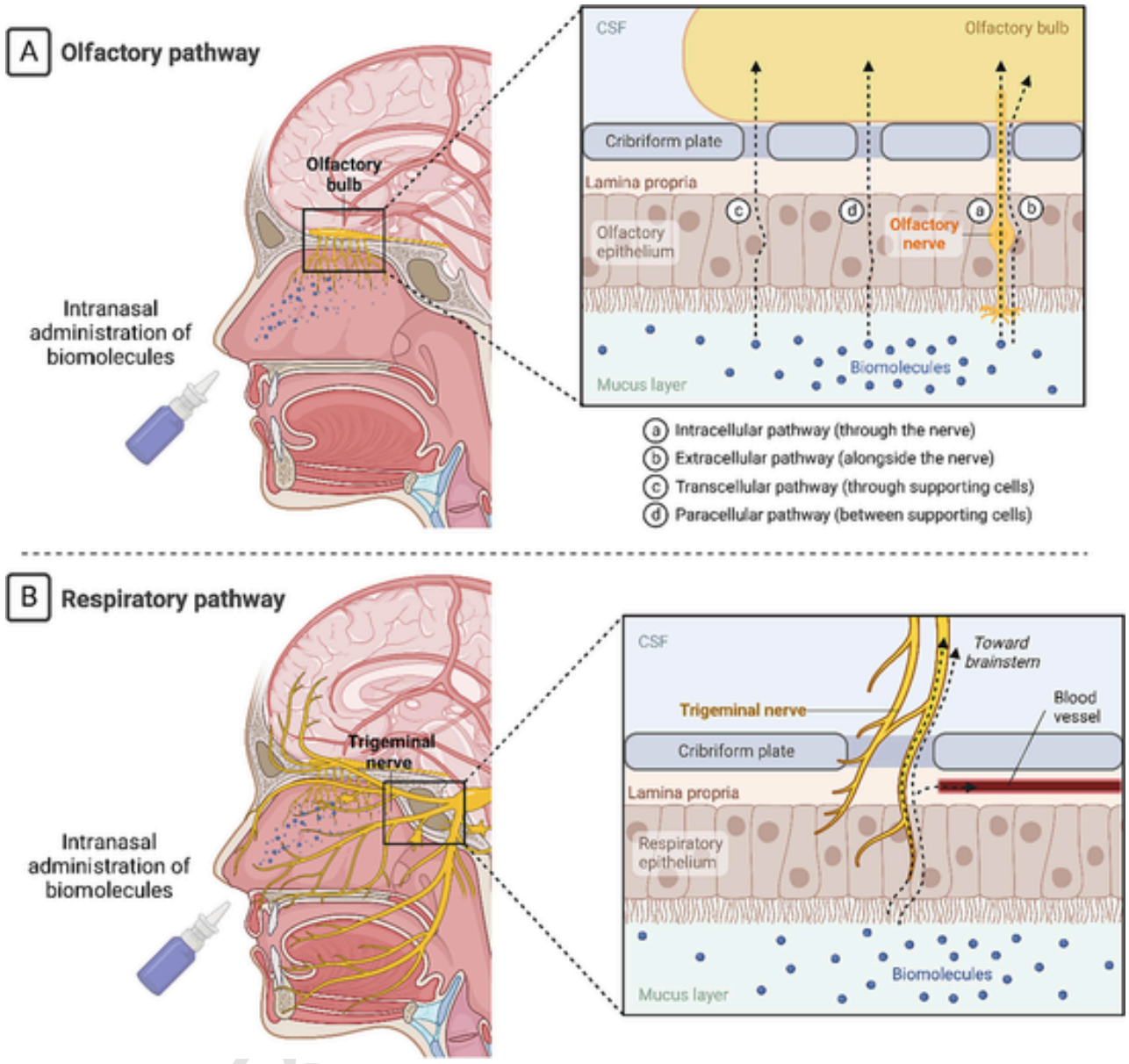

Fig. 5. Overview of the N2B transport mechanisms. Drugs administered to the nasal cavity reach the CNS via the olfactory (A) and/or the trigeminal (B) nerve pathways. The drugs can be a) internalised into the nerve cell and exocytosed at the nerve's projection site, b) transported alongside the nerve in the perineural space via bulk flow processes, c) through and/or d) between the supporting cells of the olfactory epithelium. Moreover, drugs can enter the circulation in the respiratory region of the nasal cavity and reach the CNS according to their ability to cross the BBB. CSF: cerebrospinal fluid. (created with Biorender.com).

for CNS application, over the past few years [18,185,186]. Among them, the main characteristics of the nanocarriers most frequently used in the context of MS will be discussed hereunder (Fig. 6).

Poly (lactic-co-glycolic acid) (PLGA) NP are well-studied polymeric NP that can encapsulate small hydrophobic drugs, proteins and nucleic acids. Although their formulation requires the use of organic solvents, PLGA NP are biocompatible, biodegradable and suitable for lyophilization [187]. Extracellular vesicles (EVs) or exosomes are small vesicles in the nanometer scale composed of a lipid bilayer surrounding an aqueous core (Fig. 6). They can be secreted by most cell types of the body and their lipid, protein and nucleic acid content depend on the parent cell [188]. Although their drug loading efficiency is low, their low immunogenicity and their natural affinity to target cells distinguish them from other nanocarriers [19,188]. Lipid-based nanocarriers including liposomes, solid lipid nanocarriers (SLN), nanostructured lipid carriers (NLC), lipid nanoparticles (LNP) and lipid nanocapsules (LNC) are ideal drug delivery systems (Fig. 6), mostly for lipophilic drugs, with the capacity to interact with different molecules/cell types via surface functionalization [185,189,190].

The main considerations when selecting a nanovesicle for CNS delivery are the physico-chemical properties of the drug of interest, as well as the drug release profile wanted and the localization of its potential targets within the CNS. For hydrophilic drugs, the use of liposomes, as well as polymeric NP, such as PLGA NP, or EVs are often used, although PLGA NP and EVs are known for low encapsulation efficiency of water-soluble drugs. The use of modified formulation processes, such as the use of microfluidics, can increase the loading efficiency in PLGA NP [191,192]. With microfluidics, the encapsulation of macromolecules such as protein and siRNA in PLGA NP is also possible. The brain uptake mechanisms of these NP include passive diffusion across the BBB for unmodified PLGA NP or active endocytosis mechanisms, such as adsorption-mediated transcytosis, receptor-mediated transcytosis and carrier-mediated transport [193]. These pathways require the modification of the NP surface with components favoring the BBB endocytic pathways

Table 2
Strategies to facilitate the N2B transport.

Drug characteristics hindering N2B transport	Impact on N2B transport	Strategies to increase N2B transport	References
Size > 1000 Da	Lower adsorption through the olfactory and nasal epithelium	Permeation enhancers to increase membrane fluidity and/or tight junction permeability and/or generate hydrophilic pores (e.g. cyclodextrins, surfactants, saponins)	[170–172]
		Cell-penetrating peptides to facilitate transport across membranes (e.g. penetratin, TAT)	[168,173,174]
		Mucoadhesive agents (e.g. chitosan) to enhance permeation by opening tight junctions	[159,172]
Not mucoadhesive	Low nasal residence time and brain uptake	Mucoadhesive agents (e.g. carbopol) to increase the nasal mucosa permeation by opening tight junctions while also increasing adhesion into nasal cavity	[159,172]
Substrates of the P-gP efflux pump	Active efflux transport as the multidrug resistance pump is extensively distributed and expressed across the nasal mucosa	P-gP inhibitors (e.g. verapamil, cyclosporine)	[175,176]
		Broad category of nanocarriers to by-pass this barrier and enhance absorption and delivery of bioactive drugs to brain	[160,177–179]
Substrates of enzymes present in the nasal cavity (mainly proteins and peptides)	Enzymatic degradation or metabolism in the nasal cavity	Enzyme inhibitors (e.g. bestatin, amastatin, phospholipids)	[180,181]
		Broad category of nanocarriers to protect the encapsulated cargo against drug metabolism in the nasal cavity	[160,177–179]

(e.g. positive charges, ligands, etc.) and increases the low brain uptake observed with the unmodified NP [193,194]. On the other hand, EV transport mechanisms across the BBB have not yet been fully elucidated. However, evidence suggests that transcytosis is their main route of transport [195]. The encapsulation efficiency of small hydrophilic drugs in liposomes is highly influenced by their size (the larger the size, the greater the loading) [196]. Moreover, these NPs have been shown to cross the BBB and deliver a sufficient amount of both hydrophilic and lipophilic anti-cancer drugs to the CNS [197].

For lipophilic drugs, several options are available. However, the use of lipid-based NP is preferred as the bioavailability of such drugs is when encapsulated in lipid-based NP in comparison with polymeric NP, due to the physiological stability of the lipids [197]. Between the different lipid-based nanocarriers, the choice can be made depending on the specificity and practicality of the nanocarrier itself. For long stability and straightforward production, LNC are of particular interest as their preparation process is organic solvent-free with the possibility to scale-up their formulation [198]. Moreover, due to the presence of the pegylated non-ionic surfactant (Kholliphor HS 15) in their formulation, a transient inhibition of the P-gP efflux pump (present at the surface of the BBB) as well as an evasion of the mononuclear phagocyte system clearance increase their ability to cross the BBB [199–201]. Although less stable than LNC, liposomes have their advantages, especially in the translational aspect, as several medications using these NP have reached the market (e.g. Doxil®, Onivyde®, Vyxeos®, etc.) [202]. Alternatively, SLN and NLC can cross the BBB via endocytosis influenced by the developing concentration gradient [203,204]. The presence of surfactant in the formulation contributes to the increased membrane fluidity as well as better attachment to lipoprotein receptors that support brain delivery [204]. However, even though higher drug loading and stability can be achieved with SLN and even more with NLC compared to liposomes, the clinical application of these NP is limited due to challenges with scale up aspects [205].

Altogether, each nanocarrier has its own limitations and advantages. The selection of the most appropriate NP will depend on the specific requirements of the cargo as well as its application. In the context of MS, and more specifically for the progressive forms of MS, the nanocarrier must efficiently deliver therapeutic amounts of the drug of interest in the CNS, its formulation process must be reproducible and scalable for a potential clinical application, as well as depict a prolonged sustained release for reduced administration frequency and increased patient compliance. However, the latter is not as essential if the administration route is non-invasive and patient friendly (i.e. using the nasal route, as discussed in section 5.3), suitable for multiple administrations.

5.4.3. Nanomedicine-based approaches in the scope of MS

Nanotechnology presents promising approaches to improve autoimmune disease treatment, such as MS, with the ability to overcome many of the limitations discussed above associated with the current biological and immunosuppressive therapies. Nanotechnology has recently been employed for the diagnosis of MS with an improved efficacy [206]. Development of nanomedicine-derived sensors to detect biomarkers in the CSF, and nanomedicine-based contrast agents to scan MS lesions using MRI have shown the potential of using nanotechnology as a diagnostic tool for MS (see review [207]). In this review, we will focus on the use of nanomedicine as drug delivery carriers and/or therapeutic agents.

Several nanocarriers, as depicted in Fig. 6, have been used for their intrinsic properties and/or as nanocarriers to deliver specific drugs in different preclinical models of MS. The main nanovesicles used include polymeric-based NP (PLGA), lipid-based NP (SLN, NLC and liposomes) and EVs.

Polymeric NP, with a focus on PLGA NP, have been used in MS research due to their numerous advantages as mentioned previously. PLGA NP have successfully delivered several interesting drugs to the CNS. Leukemia inhibitory factor (LIF), a pro-remyelinating cytokine easily degraded *in vivo*, was encapsulated in PLGA NP to promote OPC differentiation in animal models [208]. An anti-NG2 antibody was grafted at the surface of the NP to further target OPCs. Intralesional delivery of the NG2-targeted LIF-NP significantly increased OPC differentiation and remyelination in a mouse model of lysolecithin (LPC)-induced demyelination [208]. Moreover, LIF-PLGA NP, targeted to CD4+ T cells by conjugating an anti-CD4 antibody at the NP surface, were able to cross the BBB and accumulate in the brain following intravenous injections [209]. These NP demonstrated neuro-protective and anti-inflammatory properties in two EAE mouse models, following intraperitoneal and intra-cranial injections [209]. Additionally, the use of PLGA NP was also explored to deliver IFN β-1a, an FDA-approved MS drug, as its efficiency is limited by its low plasmatic half-life following intravenous injections. Fodor-Kardos et al. produced IFN β-1a-loaded PEG-PLGA NP via nanoprecipitation and showed that following subcutaneous injections, mild toxicities were observed in the kidneys of the rats due to the nanocarrier [210]. Further investigations are thus needed to produce IFN β-1a-loaded PEG-PLGA NP with low or no toxicities *in vivo,* to further assess the neuroprotective effect of the system.

Among the existing lipid nanocarriers, liposomes, SLN and NLC are considered as great tools to protect and deliver drugs to specific CNS targets (via functionalization of their surface) in the scope of MS (Table 3).

Liposomes have been often used to deliver glucocorticoids such as prednisolone in experimental animal models of MS. To increase plasma circulation time, PEG-coated liposomes were produced. Besides im-

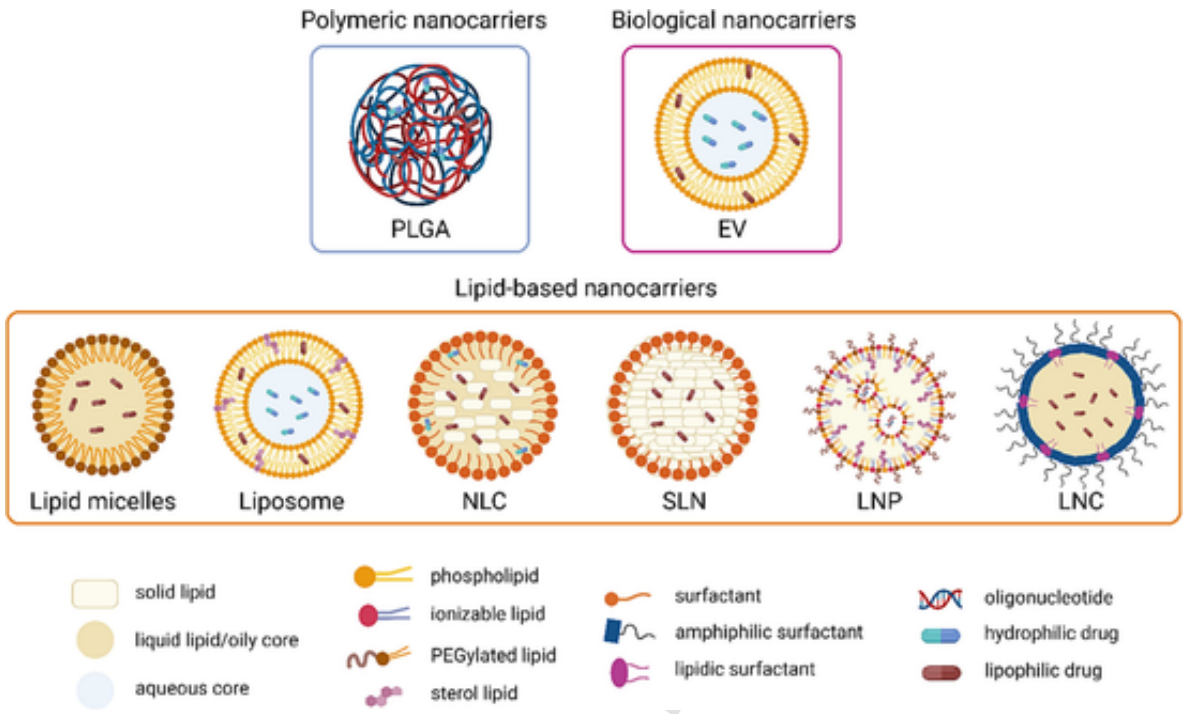

Fig. 6. Overview of the main nanocarriers used as drug delivery vehicles. Hypothetic representation of the main nanocarriers and their potential cargo. EV: extracellular vesicle, LNC: lipid nanocapsules, LNP: lipid nanoparticles, NLC: nanostructured lipid carriers, PLGA: Poly (lactic-co-glycolic acid), SLN: solid lipid nanoparticles. (created with BioRender.com).

proved clinical scores observed in EAE rats, PEG-coated liposomes loaded with prednisolone showed better accumulation in inflamed organs (brain and spinal cord) compared to free drug [211,212]. Nevertheless, conjugation of a BBB targeting ligand (i.e. glutathione, as its transporter has been shown to facilitate drug delivery to the CNS) significantly improved PEG-liposome accumulation in EAE mice spinal cord and further improved EAE clinical score and pathology in rats [211] and mice [213]. Similarly, sterically stabilized PEG liposome, loaded with methylprednisolone pro-drug (methylprednisolone hemisuccinate) or tempamine, an antioxidant and anti-inflammatory agent, ameliorated clinical signs of EAE in mice [214]. However, active targeting of these liposomes via covalent attachment of short peptide fragments of ApoE or β-amyloid was not more efficient than the unmodified liposomes [214]. Although Turjeman et al. do not discuss their targeting choices, functionalization with ApoE-derived peptides has been shown to increase the interaction of nanoliposomes binding amyloid-beta peptide with brain capillary endothelial cells [215] and to improve the transport of NP across an *in vitro* model of the BBB [216,217].

SLN are used in MS research to enable oral delivery of promising compounds as well as promote delivery to the brain. Pegylated methylprednisolone-loaded SLN were produced for efficient delivery to the brain [218]. SLN surface was modified via conjugation of an antibody (anti-contactin 2 or anti-neurfascin) to target antigens expressed at the node of Ranvier of myelinated axons. Contrary to *in vitro* results, the unmodified SLN showed better penetration ability of the BBB compared to the antibody-functionalized and PEGylated SLN after injection in CPZ mice [218]. Ohja et al. study depicted the development of dimethylfumarate (DMF)-loaded SLN. The latter showed a longer half-life compared to the free drug in rats following oral administration, a promising system for better management of MS [219]. Lastly, SLN were used to orally deliver methylthioadenosine, a natural nucleoside with interesting MS-related properties [220]. Oral administration of the formulation increased locomotor activity, motor coordination and myelination of CPZ-exposed mice.

NLC are mainly used to improve the bioavailability of MS-approved drugs in the brain and plasma as well as to reduce their toxicity. Ghasemian et al. produced baclofen-loaded NLC, a hydrophilic drug used in MS to treat spasticity [221]. Intraperitoneal injection of baclofen-NLC increased baclofen half-life in plasma (10 fold) and brain (1.5 fold) of rats and provided a prolonged effect compared to the aqueous solution of the drug. NLC were also utilized to encapsulate DMF, an MS drug in need of increased brain permeability and gastric tolerance [222,223]. Oral and brain bioavailability of DMF was significantly increased in rats following oral administration of DMF-loaded NLC compared to free drug [222]. Furthermore, a single oral administration of DMF-NLC increased motor coordination, locomotive activity and myelin sheath recovery in CPZ-exposed mice compared to free DMF [223]. Lastly, NLC loaded with teriflunomide induced rapid remyelination of damaged axons in CPZ-exposed rats compared to control, with no toxicity observed in the liver and kidneys [224]. However, no comparison was made with the free drug.

EVs have been used in MS research owing to their interesting intrinsic properties (i.e. their lipid, protein, nucleotide content), dependent on their parent cell. The immunomodulatory and anti-inflammatory properties of mesenchymal stem cell (MSC)-derived EVs have supported their evaluation in several models of MS [225–228]. EVs derived from activated dendritic cells have shown greater efficacy in EAE mice compared to EVs derived from inactivated cells [229]. This was hypothesized to be due to the presence of anti-inflammatory microRNAs (miRNA) as well as miRNA involved in oligodendrocyte differentiation in EVs-derived from activated cells [226,229,230]. Promising results in EAE and CPZ mice were also obtained with EVs derived from other parent cells, including neural stem cells (NSC), rhesus monkey MSC and oligodendrocytes [231–233]. In addition to this, EVs are used as nanocarriers for their targeting properties, their capacity to solubilize and stabilize lipophilic drugs as well as their ability to cross the BBB. They have been used to administer several types of molecules (e.g. growth factors, miRNAs, anti-inflammatory and/or neuroprotective compounds) in different animal models of MS [234–238]. Zheng et al.

Table 3
Non-exhaustive list of nanomedicine-based approaches in the scope of MS. The papers were selected to illustrate the different roles these nanocarriers were used for in the scope of MS, namely to induce immune tolerance, to deliver different types of drugs or to take advantage of their intrinsic properties. AhR: aryl hydrocarbon receptor, EAE: experimental autoimmune encephalomyelitis, i.c.: injection into cisterna magna, CPZ: cuprizone, i.n.: intranasal, i.p.: intraperitoneal, i.v.: intravenous, LIF: Leukaemia inhibitory factor, MBP: myelin basic protein, MOG: myelin oligodendrocyte glycoprotein, MSC: mesenchymal stem cells, NP: nanoparticle, NSC: neural stem cells, NV: nanovesicle, OPCs: oligodendrocyte progenitor cells, PGDFα: platelet-derived growth factor alpha, PLP: proteolipid protein, s.c.: subcutaneous TMEV: Theiler's murine encephalomyelitis virus

Type of NV	Function of the NVs	Characteristic of the NV and administration route	Main effects in preclinical models	References
PLGA	Used to induce immune tolerance	PLGA loaded with antigens (i.e. MOG_{35-55}) and IL10 (s.c.)	Amelioration of EAE clinical scores, decreased CNS lesions and inflammation	[245]
		PLGA loaded with $PLP_{139-151}$ together with rapamycin used as adjuvant (s.c. and i.v.)	Inhibition of autoreactive T cells and stimulation of regulatory immune cells, prevention of EAE and relapses in EAE mice	[246]
		Small PLGA loaded with vitamin D and MOG_{35-55} + large PLGA loaded with GM-CSF and TGF-β1 (s.c.)	Reduced activation of macrophage and microglia, decreased infiltration of CD4+ T cells and recruitment of dendritic cells towards tolerogenic phenotype in EAE mice	[247]
		PLGA loaded with several regulatory molecules (i.e. TGF-β and myelin peptides) with MHC I/II peptides at the surface (i.v)	Ameliorated EAE with reduced neuro-inflammation and demyelination in EAE mice	[248]
		PLGA loaded with $PLP_{139-151}$	No assessment of the nanomedicine efficacy *in vivo*	[249]
		MOG_{35-55}-conjugated PLGA (i.v.)	Amelioration of EAE clinical scores and reduced infiltration of immune cells in the brain of EAE mice	[250]
		PEG-PLGA loaded with MOG_{35-55} (s.c.)	Amelioration of EAE symptoms associated with lower complement activation, neutrophil recruitment thus creating a tolerogenic microenvironment in EAE mice	[251]
	Used as nanocarrier	PLGA loaded with LIF with antibody against NG2 on their surface to target OPC (intralesional)	Increased differentiation of OPC and remyelination with increased number of myelinated axons and increased thickness in mouse model of LPC-induced demyelination	[208]
		PLGA loaded with LIF with antibody against CD4 on their surface to target CD4+ T cells (i.p., i.v. and intra-cranial)	Reversal of EAE-induced paralysis and reduced inflammation in EAE mice	[209]
		PLGA loaded with IFN β-1a (s.c.)	Mild toxicity observed in rats thus no assessment of the nanomedicine therapeutic efficacy	[210]
Liposome	Used to deliver glucocorticoids	PEG-liposomes loaded with prednisolone (i.v.)	Successful accumulation in inflamed organs and improved EAE compared to free drug in rats	[212]
		PEG-liposomes conjugated with glutathione (GSH) (for brain targeting) loaded with methylprednisolone (i.v.)	Decreased EAE clinical signs more than PEG liposome and better accumulation in CNS of EAE rats in the presence of GSH	[211]
		GSH-PEG liposomes loaded with methylprednisolone (i.v.)	Decreased EAE severity, infiltration of immune cells, activation of astrocytes, axonal loss and demyelination in EAE mice	[213]
		Sterically stabilized PEG liposomes loaded with tempamine or methylprednisolone prodrug, the latter with or without active targeting (covalent link to ApoE or β-amyloid) (i.v.)	Active targeting similar to passive, both nanomedicines improved clinical signs and pathology of EAE in mice	[214]
	Used to induce immune tolerance	Mannosylated liposomes loaded with MBP_{46-62}, MBP_{124-39} or $MBP_{147-170}$ (s.c.)	Mannosylation and the liposome-mediated synergistic effect of the mixture of 3 MBP peptides significantly suppressed the progression of EAE, prevented circulation of autoantibodies and reduced production of Th1 pro-inflammatory cytokines in EAE rats	[252]
		Liposomes loaded with MOG_{35-55} and AhR agonist (s.c.)	Suppressed development of EAE and infiltration of immune cells and induced tolerogenic dendritic cells in EAE mice	[253]
		Phosphatidylserine-rich liposomes loaded with MOG_{40-55} (i.p.)	Reduced incidence and severity of EAE and delayed onset of the disease in EAE mice	[254]
		Doxorubicin-loaded liposomes coated with MBP or PLP (i.v.)	Reduced EAE symptoms, infiltration of immune cells in the spinal cord and demyelination in EAE mice.	[255]
	Used as nanocarrier	Honokiol-loaded liposomes (i.v.)	Ameliorated EAE severity and decreased demyelination, inflammation and infiltration of immune cells in the spinal cord of EAE mice	[256]
SLN	Used as nanocarrier	SLN loaded with methylprednisolone either pegylated or conjugated with contactin 2 or neurofascin antibodies	Better penetration ability of the BBB of blank SLN compared to PEG and antibody-functionalized SLN in CPZ mice. No *in* vivo efficacy study of methylprednisolone-SLN was done	[218]
		SLN loaded with dimethylfumarate (DMF) (oral)	Prolonged half-life in rats but no *in vivo* efficacy study of DMF-SLN was done	[219]
		SLN loaded with methylthioadenosine (oral)	Increased locomotor activity, motor coordination and myelination compared to free drug in CPZ mice	[220]
NLC	Used to deliver MS approved drugs	NLC loaded with baclofen (i.p.)	Prolonged release compared to baclofen solution and increased half-life of drug in plasma and brain of rats	[221]
		NLC loaded with dimethylfumarate (oral)	Oral bioavailability in rats was enhanced and brain availability was substantially improved compared to plain drug	[222]
		NLC loaded with dimethylfumarate (oral)	Increased locomotive activity, motor coordination and reduced demyelination in *corpus callosum* of CPZ mice	[223]
		NLC loaded with teriflunomide (i.n.)	Induced rapid remyelination in CPZ-exposed rats without toxicity in liver and kidneys	[224]
Extracellular vesicles (EVs)	Used as therapeutic agents	EV derived from human adipose tissue MSC (i.v.)	Improved motor deficit, reduced brain atrophy and production of pro-inflammatory mediators and modulated microglia activation state in TMEV model	[225]

(*continued on next page*)

Table 3 (*continued*)

Type of NV	Function of the NVs	Characteristic of the NV and administration route	Main effects in preclinical models	References
		EVs derived from IFNγ-stimulated dendritic cells (i.n.)	Increased myelination of CNS of rats	[229]
		EVs derived from IFNγ-stimulated MSC (i.v.)	Decreased EAE development, spinal cord demyelination and neuro-inflammation in EAE mice	[226, 230]
		EVs derived from placental MSC (i.v.)	Increased motor functions and myelination in spinal cord of EAE mice	[227]
		EVs derived from human adipose tissue MSC (i.v.)	Attenuated EAE, reduced mean clinical score, leukocyte infiltration, and demyelination in EAE mice	[228]
		EVs derived from bone-marrow MSC (i.v.)	Improved clinical symptoms and decreased demyelination, M1 pro-inflammatory cytokines in EAE rats	[257]
		EVs derived from rhesus monkey MSC (i.v.)	Improved clinical signs of EAE mice and enhanced myelin levels and decreased demyelination in EAE and CPZ mice	[233]
	Used as nanocarriers	EVs loaded with BDNF and engineered for brain targeting (i.n.)	Increased remyelination and coordination abilities of CPZ-induced mice	[234]
		Macrophage-derived EVs loaded with resveratrol to target microglia (i.n.)	Successful co-localization with microglia, inhibited CNS inflammatory responses and improved clinical evolution of MS in EAE mice.	[235]
		Montekulast-loaded EVs with PGDFα expressed at their surface to target OPC (i.n.)	Successful targeting of OPC, reduced demyelination and promotion of OPC differentiation and remyelination in CPZ mice	[236]
		Bryostain-1 loaded EVs derived from NSC, with PGDFα expressed at their surface to target OPC (i.v.)	Ameliorated EAE, reduced infiltration of pro-inflammatory cells, reduced myelin loss and astrogliosis in EAE mice	[237]
		miR-219a-5p-loaded EVs derived from a human embryonic kidney cell line (i.n.)	Improved EAE clinical symptoms and promotion of remyelination in EAE mice	[238]
	Used as targeting tool	EVs derived from NSC with PGDFα at their surface to target OPC (i.v.) + triiodothyronine (T3)	Co-administration of EVs +T3 improved EAE development, decreased myelin damage, increased oligodendrocyte survival and myelin regeneration of lesions in EAE mice	[231]
		EVs derived from microglial cell line BV2 engineered to target phagocyte and containing IL-4 (i.c.)	Protection against neuro-inflammation and activation of anti-inflammatory phenotype of microglia in EAE mice	[239]
	Used to induce immune tolerance	EVs derived from oligodendrocytes (naturally contain multiple myelin antigens) (i.v.)	Immune tolerance was restored by promoting immunosuppressive monocytes and apoptosis of autoreactive CD4+ T cells in EAE mice	[232]

loaded an anti-inflammatory compound in EVs derived from macrophages, to exploit their microglia-targeting properties [235]. The formulation reduced inflammation as well as improved clinical symptoms of EAE mice. Moreover, on top of their natural targeting properties, EVs can be engineered to express different ligands and/or peptides to target different cell types and/or brain regions (i.e. PGDFα to target OPCs) and to subsequently promote accumulation at lesion sites [231,236,237,239].

Other NP including chitosan alginate NP (DMF [240]), inorganic-organic hybrid NP (glucocorticoids [241]) and heparin nanocomplex (IFN β-1a [242]), were investigated for the delivery of FDA-approved MS drugs with promising results *in vitro* and *in vivo*.

Of special note, PLGA NP, as well as liposomes and EVs to some extent, have been also used in the context of MS to induce durable immune tolerance as listed in Table 3. For more details on this strategy refer to these reviews [75,243,244].

5.5. Nose-to-brain (N2B) route to deliver drugs and nanomedicines in MS

Several studies have investigated the N2B route as a non-invasive alternative to deliver FDA-approved MS therapies to the CNS (Table 4). Ross et al. showed that the anti-inflammatory cytokine IFN-β1b, one of the first line therapies usually given systematically to MS patients, reached the brain intact and induced tyrosine phosphorylation of IFN receptor in the CNS following nasal administration. Moreover, at similar blood levels, intravenous IFN-β1b had lower brain concentrations compared to intranasal administration [258]. More recently, intranasal administration of IFN-β significantly improved EAE mouse clinical symptoms but only when loaded into chitosan polymeric NP [259]. A similar dose of intranasal or systemic free IFN-β had no effect, highlighting thus the additional protective role of nanoencapsulation against enzymatic degradation and/or other microenvironmental factors. Nasal administration of encapsulated IFN-β also reduced demyelination in the spinal cord of EAE mice and the activation of microglia and astrocytes [259]. In another study, Gadhave et al., developed the formulation of NLC loaded with teriflunomide, an MS drug associated with hepatotoxicity and nephrotoxicity when administered orally [224]. They showed that intranasal delivery of teriflunomide-loaded NLC significantly promoted remyelination in a CPZ mouse model compared to oral administration of the formulation, without any signs of toxicity in the liver. Similar conclusions were made when comparing the efficacy of intranasal and oral administrations of similar doses of free fingolimod, an MS drugs known to cross the BBB, in a rat model of LPC-induced demyelination [260]. Significant reduction of astrocyte activation and demyelination, as well as higher concentrations of fingolimod in the brain, were observed following nasal administration of fingolimod compared to oral fingolimod. Lastly, Rassy et al. investigated the efficacy of methylprednisolone in EAE mice using the N2B route for local delivery to the CNS. Their study was motivated by the fact that intravenous methylprednisolone, the standard treatment of relapses, requires hospitalization and 3 to 5 days of invasive administrations to patients. The data showed that intranasal delivery of methylprednisolone is as efficient as the intravenous route to treat neuroinflammation in EAE mice, highlighting thus the nasal route as a potential non-invasive alternative [261].

Other studies have used the intranasal route with experimental drugs, using different strategies to develop new therapies against MS (Table 4). A few strategies will be discussed hereunder.

Xiao et al., were the first to show that intranasal but not peripheral (subcutaneous) administration of free IL-10 suppressed neurologic symptoms in a rat model of MS [262]. This was associated with decreased myelin antigen-specific T-cell proliferative responses and IFNγ secretion as well as inhibition of MHC class II expression in microglia. This indicates that IL-10 administration by the nasal route prevented the activation of microglia. In our laboratory, we demonstrated the capacity of a bioactive lipid, prostaglandin D_2 glycerol ester (PGD_2-G) [263], encapsulated in LNC, to reduce the expression of several pro-inflammatory chemokines in the brain and spinal cord of EAE-induced mice following intranasal administration [264]. The nanocarrier itself also reduced the mRNA expression of pro-inflammatory chemokines in EAE mouse brains and spinal cord. Other groups focused on inducing immune tolerance (silence and/or remove autoreactive T cells that recognize CNS via MHC class II proteins [244]) by nasal administration of myelin peptides (i.e. MBP, MOG) in pre-clinical models of MS. Indeed, intranasal delivery of MBP prevented EAE development in rats [265,266]. However, intranasal delivery of MOG_{36-44} using filamentous

Table 4
Overview of experimental strategies using the N2B route in the scope of MS. The papers were selected to illustrate the different types of therapies delivered using the nasal route in the scope of MS. CNS: central nervous system, CPZ: cuprizone, EAE: experimental autoimmune encephalomyelitis, EV: extracellular vesicles, IFN: interferon, LNC: lipid nanocapsules, LPC, lysophosphatidylcholine, MBP: myelin basic protein, MOG: myelin oligodendrocyte glycoprotein, MS: multiple sclerosis, MSC: mesenchymal stem cells, NLC: nanostructured lipid carriers, NP: nanoparticles, OPC: oligodendrocyte progenitor cells, PGD_2-G: prostaglandin D2 glycerol ester, SDF-1α: stromal cell-derived factor 1 alpha, Treg: regulatory T cells

Treatment delivered	Type of treatment	Animal model	Main effects	References
MS FDA-approved drugs	Free IFN-β1b	Healthy rats	Increased accumulation in the brain after nasal administration compared to the intravenous route	[258]
	IFN-β-loaded chitosan polymeric NP	EAE mice	Reduction of EAE clinical score, demyelination, microgliosis and astrogliosis when loaded in NP	[259]
	Teriflunomide-loaded NLC	CPZ mice	Promotion of remyelination with no toxicity in the liver	[224]
	Free fingolimod	LPC-induced demyelination in rats	Increased accumulation in the brain compared to oral administration and reduction of demyelination and astrogliosis	[260]
	Free methylprednisolone	EAE mice	Reduction of neuro-inflammation and CNS demyelination	[261]
Myelin peptides	Bovine MBP	EAE rats	Prevention and reduction of EAE progression	[265]
	Guinea-pig MBP	EAE rats	Prevention of EAE progression via nasal tolerance induction	[266]
	MOG_{36-44} fused to the main coat protein of a filamentous phage	EAE mice	Improved EAE clinical score and reduction of inflammation	[267]
Cells	Engineered Treg cells	EAE mice	Suppression of ongoing inflammation and reduction of EAE clinical progression	[275]
	Fasudil-modified mononuclear cells	EAE mice	Reduction of EAE clinical severity, demyelination and inflammation in the spinal cord	[85]
	Human oligodendroglioma cells	Healthy mice	Successful delivery to mice CNS and preferential migration of cells to OPC niche in the brain	[273]
	SDF-1α pre-conditioned MSC derived from bone marrow	CPZ mice	Improvement of remyelination and reduction of microglia and astrocyte activation	[272]
	MSC derived from bone marrow	CPZ mice	Promotion of myelin recovery in *corpus callosum* of mice and reduced astrogliosis and microgliosis	[274]
	MSC derived from abdominal adipose tissue	EAE mice	Reduction of EAE clinical severity and increased immunomodulatory response	[276]
EVs or exosomes	Small EVs derived from adipose tissue-derived MSC	EAE mice	Increased reduction of EAE clinical severity compared to intranasal MSC and increased immunomodulatory response	[276]
	EVs derived from IFNγ-stimulated dendritic cells	Healthy rats	Increased CNS myelination	[229]
	Resveratrol-loaded macrophage exosomes	EAE mice	Reduction of EAE clinical severity and inhibition of inflammatory responses in the CNS	[235]
	BDNF-loaded exosomes	CPZ mice	Reduction of demyelination and increase of remyelination and motor coordination	[234]
	Montelukast-loaded EV	CPZ mice	Promotion of oligodendrocyte differentiation and remyelination in mice brain	[236]
Others	IL-10	EAE rats	Reduction of EAE clinical severity and of microglia activation	[262]
	PGD_2-G-loaded LNC	EAE mice	Reduction of pro-inflammatory chemokines in EAE mice brain and spinal cord	[264]

phage improved clinical score of EAE mice as well as reduced inflammation in the CNS but only when the intranasal administration of the system started at day 1 post-immunization and not when clinical signs had already developed (at day 13 post immunization) [267].

The N2B route has also been used to deliver cells to promote remyelination in the scope of MS (Table 4). The extracellular pathway (Fig. 5) has been described as the main pathway used by large molecules and cells to reach the CNS following intranasal administration [268,269]. However, other studies suggest that cells can reach the cranial cavity by entering the olfactory nerve and the olfactory tract [270,271]. Their N2B transport can further be enhanced by using cell permeation enhancers such as hyaluronidase, to increase the permeability of the olfactory epithelium, promoting thus the passage across the nasal mucosal barrier [270–274]. Intranasal administration of human oligodendroglioma (HOG) cells, which behave similarly to OPCs *in vitro*, allowed the cells to reach the brain. Moreover, a preferential migration of these cells to OPC niches in the brain of experimental animals was observed, which supports the hypothesis that intranasal delivered HOG may help restore the pool of OPCs to induce remyelination [273]. Other immune cells, including engineered Treg cells or modified mononuclear cells, have been delivered to the CNS through the nasal route, and showed promising results in EAE-induced mice [85,275]. Other studies have exploited the immunomodulatory and anti-

inflammatory properties of MSC to develop new therapies. Intranasal delivery of MSC improved remyelination and reduced activation of astrocytes and microglia in the CPZ mouse model [272,274] as well as improved clinical signs and increased Treg population in EAE-induced mice [276]. However, intranasal administration of small EVs derived from MSC were more effective than the administration of MSC to reduce clinical score and histological lesions of the CNS of EAE mice [276]. Pusic et al. showed that intranasal administration of EVs derived from IFNγ-stimulated dendritic cells in rats increased myelin levels in the brain [229]. In addition to their intrinsic properties, EVs or exosomes, have also been used as vehicles to deliver specific drugs to the CNS of MS experimental animals, via the nasal route, to target microglia [235], promote remyelination [234] or induce OPC differentiation [236].

6. Limitations and regulations of nanomedicine-based approaches via the N2B route

As stated in previous sections, nanotechnology has a promising future in the treatment of CNS disorders such as MS owing to the ability of NP to cross the BBB, capacity to be engineered according to the need and application, and to enable targeted delivery. While their use in drug delivery appears to be straightforward, there are several challenges that should be addressed during their development and clinical translation. Challenges include understanding of their interactions with the BBB, assessing their long-term safety and scaling up their production process [277]. In most cases, the more elaborate and complex the process is (e.g. adding targeting agents), the more difficult it is to scale up the formulation. Moreover, the use of potentially toxic agents (e.g. organic solvents) during synthesis can lead to safety issues. Efficient encapsulation of the drug of interest (requires adequate quantification and separation methods to evaluate the amount of unencapsulated drug), efficiency in reaching the targeted sites (the latter usually being distant from the region of administration), delivery of therapeutic doses (limited by the drug loading of the nanocarrier and its interaction with the environment) and controlled delivery within a limited and predictable timescale represent major challenges in the drug delivery field [202,277]. Therefore, the road from laboratory to market in nanoscience has a high level of risk, owing to the instability, fragility and reactivity of nanoscale materials, in addition to financial, ethical and regulatory challenges. Indeed, most universities are not able to leverage the required funds to support the transition of NP research to clinical trials. Furthermore, the ethical issues surrounding the assessment of nano-based products in clinical trials are based on the benefit/harm ratio on the adverse effects caused by the nano-formulations. Ethical committees exert thus increased caution owing to the inability to provide sufficient precise information about the risks, benefits, and likely outcome of the trials. Furthermore, the established regulations laid down by drug authorities (e.g. FDA), represent an additional challenge on the road to new marketed nanomedicines [202]. In 2016, the FDA published a document addressing the general regulations for all nanomaterial based-products related to food ingredients and animal feedstuff [202,278,279]. However, owing to the fact that nanomaterials behave differently *in vivo* than small molecules (e.g. biodistribution, toxicity, etc.) and that current testing methods are unable to clarify these aspects, no definite guidance has yet been issued [202]. Regulation for the nasal delivery of nanoparticles brings an additional challenge. As nasal drug products are generally a reprocessing of pre-approved products, the safety aspect is justified by previously approved products with a requirement of additional data from new studies [172,280]. In this case, nano-formulations are being delivered via the nasal route, thus their previous approval is required to justify the safety aspect of the delivery route.

During the early stages of the formulation development process, the nasal delivery method and/or device is an essential factor to consider. Indeed, it is rare that a given medical device on the market can specifically target only one delivery pathway [281]. While intranasal delivery has been tested in CNS diseases such as Alzheimer's disease (i.e. insulin [282]), the efficiency of the N2B pathway may be influenced by MS pathology. Indeed Sahin et al. studies demonstrated that RRMS patients showed a longer mucociliary transport time compared to healthy people [283]. This could significantly influence the nasal residence time and subsequent brain uptake of the intranasally administered drugs. To our knowledge, there are no nanomedicine-based medication, delivered conventionally or via the N2B route, that have received approval for clinical use in the prevention and treatment of CNS disease, including MS [172,284]. In most cases, nanotechnology have been assessed clinically as a diagnostic tool in the context of MS (e.g. gold NP-based strategies (NCT01206023, NCT01465087), iron NP (NCT02511028)). However, some clinical trials have evaluated the nasal route or nanotechnology as therapeutic options for MS. Among them, two phase I clinical trials assessing the safety and efficacy of intranasal Dexamethasone or Foralumab for RRMS (NCT00674141) and the progressive forms of MS (NCT00674141), respectively, were designed but withdrawn due to the ministry of health's disapproval and business reasons. On the other hand, there is an ongoing phase II trial assessing the effects of gold nanocrystals (CNM-Au8) on the impaired neuronal redox state in RMS patients (NCT03993171) and a completed phase I/II trial assessing the safety and impact of intranasal insulin on MS cognitive function (NCT02988401). However, no results have been reported yet.

7. Future directions

As a complex multifaceted disease whose exact physiopathology remains not fully understood, targeting different pathological processes involved in MS pathophysiology at the same time could improve the efficacy of MS therapies. Focusing on the CNS compartment would also bring forth new therapeutic options for the progressive forms of MS. Data from the several preclinical studies suggest that the promotion of remyelination as well as neuroprotection are strategies that prevent neurodegeneration. However, failure of pro-remyelinating drugs in MS clinical trials have notably been partly attributed to the lack of consideration of the deleterious inflammatory micro-environment surrounding the demyelinated lesions as well as the limited access of the drugs to the CNS. To circumvent the former, targeting neuro-inflammation in addition to promoting myelin repair and/or neuronal protection is an interesting strategy. However, as the BBB is altered during the pathology, one could contend its capacity to limit the entry of potential therapeutics to the CNS and thus the relevance of the discussed strategies. However, based on current evidences, it is known that BBB disruption is transient and heterogenous, but only occurs during the onset and the aggressiveness of the disease [129,285]. The neurodegenerative processes in the progressive forms of MS are thought to arise following the integrity of the BBB after a variable period of MS evolution [129]. Thus, the optimal goal would be to increase BBB impenetrability against activated immune cells but also to promote transport of neuroregenerative and neuroprotective therapeutics into the CNS to reach their target. The use of nanotechnology, despite its challenges, to further increase the availability, delivery and thus potentially the efficacy of the therapeutic drugs in the CNS, give rise to new opportunities for MS therapies. The selection of the most appropriate nanocarrier, as discussed above, is of the outmost importance and will depend on the properties, mechanism of action and the specific needs of the drug of interest, as well as their desired application in the context of MS.

The several strategies discussed in this review, as well as the N2B pathway, offer new prospects to increase drug delivery to the CNS. However, these strategies remain interesting as long as the mechanism of action of the drug of interest requires entry to the CNS. Thus, a proof of concept study assessing the efficacy of the drug after direct injection into the CNS is highly recommended. Moreover, a study in an adequate

model to assess the successful delivery of the drug of interest and its accumulation in the CNS following nasal administration should be carried out. As there is a lack of studies correlating an increased drug accumulation in the brain with increased efficacy in the literature, a question remains on whether the observed effect of the drugs following their delivery to the CNS, are due to their increased accumulation in the brain. Moreover, although systemic side effects are reduced with the strategies described in previous sections, an enhanced risk of CNS toxicity is also associated with these strategies, even if no studies, that we found off, in the literature have reported such toxicities. Thus, targeting specific CNS cells or CNS regions (i.e. using nanotechnology) could also reduce this potential risk.

8. Conclusion

The field of nanomedicine together with alternative routes of administration such as the N2B pathway offer MS drugs new tools and routes to accumulate in the CNS and target CNS-specific aspects of the disease. The use of several nanocarriers have successfully improved the delivery and efficacy of several experimental drugs in several preclinical models of MS. Additionally, the design of CNS-targeting therapies using the non-invasive intranasal route, could also increase the safety (reduced systemic adverse effects) and efficacy (increased bioavailability) of those therapies. However, further work is needed to overcome the key challenges to ensure successful clinical translation of both nanomedicine-based therapy and the N2B delivery of such treatments in the context of MS. Given the technological advances of nanotechnology nowadays, it can however be anticipated that nanotechnology-based delivery systems could revolutionize current traditional delivery for CNS diseases such as MS.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI-assisted technologies.

CRediT authorship contribution statement

Ariane Mwema : Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization. **Giulio G. Muccioli :** Writing – review & editing, Validation, Supervision, Conceptualization. **Anne Des Rieux :** Writing – review & editing, Visualization, Validation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by the Fondation Charcot Stitching and the Walloon Region (ARC EViMS), Ariane Mwema was supported by a FRIA doctoral grant funded by FNRS (Belgium) and the UCLouvain ("COVID mandate" and "Patrimoine"). Anne des Rieux is an Honorary FNRS Senior Research Associate.

References

[1] J. van Langelaar, L. Rijvers, J. Smolders, M.M. van Luijn, B and T cells driving multiple sclerosis: identity, mechanisms and potential triggers, Front. Immunol. 11 (2020) 760.
[2] C.A. Dendrou, L. Fugger, M.A. Friese, Immunopathology of multiple sclerosis, Nat. Rev. Immunol. 15 (9) (2015) 545–558.
[3] H. Wiendl, R. Gold, T. Berger, T. Derfuss, R. Linker, M. Maurer, O. Aktas, K. Baum, M. Berghoff, S. Bittner, A. Chan, A. Czaplinski, F. Deisenhammer, F. Di Pauli, R. Du Pasquier, C. Enzinger, E. Fertl, A. Gass, K. Gehring, C. Gobbi, N. Goebels, M. Guger, A. Haghikia, H.P. Hartung, F. Heidenreich, O. Hoffmann, B. Kallmann, C. Kleinschnitz, L. Klotz, V.I. Leussink, F. Leutmezer, V. Limmroth, J.D. Lunemann, A. Lutterotti, S.G. Meuth, U. Meyding-Lamade, M. Platten, P. Rieckmann, S. Schmidt, H. Tumani, F. Weber, M.S. Weber, U.K. Zettl, T. Ziemssen, F. Zipp, G. Multiple Sclerosis Therapy Consensus, Multiple Sclerosis Therapy Consensus Group (MSTCG): position statement on disease-modifying therapies for multiple sclerosis (white paper), Ther. Adv. Neurol. Disord. 14 (2021) 17562864211039648.
[4] C. Walton, R. King, L. Rechtman, W. Kaye, E. Leray, R.A. Marrie, N. Robertson, N. La Rocca, B. Uitdehaag, I. van der Mei, M. Wallin, A. Helme, C. Angood Napier, N. Rijke, P. Baneke, Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition, Mult. Scler. 26 (14) (2020) 1816–1821.
[5] A.J. Thompson, S.E. Baranzini, J. Geurts, B. Hemmer, O. Ciccarelli, Multiple sclerosis, Lancet 391 (10130) (2018) 1622–1636.
[6] W. Wei, D. Ma, L. Li, L. Zhang, Progress in the application of drugs for the treatment of multiple sclerosis, Front. Pharmacol. 12 (2021) 724718.
[7] S.L. Hauser, B.A.C. Cree, Treatment of multiple sclerosis: a review, Am. J. Med. 133 (12) (2020) 1380–1390 e2.
[8] M. Filippi, A. Bar-Or, F. Piehl, P. Preziosa, A. Solari, S. Vukusic, M.A. Rocca, Multiple sclerosis, Nat Rev Dis Primers 4 (1) (2018) 43.
[9] S. Faissner, J.R. Plemel, R. Gold, V.W. Yong, Progressive multiple sclerosis: from pathophysiology to therapeutic strategies, Nat. Rev. Drug Discov. 18 (12) (2019) 905–922.
[10] B.A.T. Hart, A. Luchicchi, G.J. Schenk, P.K. Stys, J.J.G. Geurts, Mechanistic underpinning of an inside-out concept for autoimmunity in multiple sclerosis, Ann Clin Transl Neurol 8 (8) (2021) 1709–1719.
[11] A. Rafiee Zadeh, M. Askari, N.N. Azadani, A. Ataei, K. Ghadimi, N. Tavoosi, M. Falahatian, Mechanism and adverse effects of multiple sclerosis drugs: a review article. Part 1, Int J Physiol Pathophysiol Pharmacol 11 (4) (2019) 95–104.
[12] A. Rafiee Zadeh, K. Ghadimi, A. Ataei, M. Askari, N. Sheikhinia, N. Tavoosi, M. Falahatian, Mechanism and adverse effects of multiple sclerosis drugs: a review article. Part 2, Int J Physiol Pathophysiol Pharmacol 11 (4) (2019) 105–114.
[13] M.A. Barbieri, E.E. Sorbara, A. Battaglia, G. Cicala, V. Rizzo, E. Spina, P.M. Cutroneo, Adverse drug reactions with drugs used in multiple sclerosis: an analysis from the italian pharmacovigilance database, Front. Pharmacol. 13 (2022) 808370.
[14] J. Avasarala, It's time for combination therapies: in multiple sclerosis, Innov Clin Neurosci 14 (5–6) (2017) 28–30.
[15] J. Correale, M.J. Halfon, D. Jack, A. Rubstein, A. Villa, Acting centrally or peripherally: a renewed interest in the central nervous system penetration of disease-modifying drugs in multiple sclerosis, Mult. Scler. Relat. Disord. 56 (2021) 103264.
[16] D. Furtado, M. Bjornmalm, S. Ayton, A.I. Bush, K. Kempe, F. Caruso, Overcoming the blood-brain barrier: the role of nanomaterials in treating neurological diseases, Adv. Mater. 30 (46) (2018) e1801362.
[17] G. Ghalamfarsa, M. Hojjat-Farsangi, M. Mohammadnia-Afrouzi, E. Anvari, S. Farhadi, M. Yousefi, F. Jadidi-Niaragh, Application of nanomedicine for crossing the blood-brain barrier: Theranostic opportunities in multiple sclerosis, J. Immunotoxicol. 13 (5) (2016) 603–619.
[18] S. Dolati, Z. Babaloo, F. Jadidi-Niaragh, H. Ayromlou, S. Sadreddini, M. Yousefi, Multiple sclerosis: therapeutic applications of advancing drug delivery systems, Biomed. Pharmacother. 86 (2017) 343–353.
[19] V. Gratpain, A. Mwema, Y. Labrak, G.G. Muccioli, V. van Pesch, A. Des Rieux, Extracellular vesicles for the treatment of central nervous system diseases, Adv. Drug Deliv. Rev. 174 (2021) 535–552.
[20] I. Callegari, T. Derfuss, E. Galli, Update on treatment in multiple sclerosis, Presse Med. 50 (2) (2021) 104068.
[21] N.M.S. Society, Treating MS, Medications. https://www.nationalmssociety.org/Treating-MS/Medications, 2022, Accessed 7 July 2022.
[22] M. Bross, M. Hackett, E. Bernitsas, Approved and emerging disease modifying therapies on neurodegeneration in multiple sclerosis, Int. J. Mol. Sci. 21 (12) (2020).
[23] P.A. Muraro, R. Martin, G.L. Mancardi, R. Nicholas, M.P. Sormani, R. Saccardi, Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis, Nat. Rev. Neurol. 13 (7) (2017) 391–405.
[24] A.G. Willison, T. Ruck, G. Lenz, H.P. Hartung, S.G. Meuth, The current standing of autologous haematopoietic stem cell transplantation for the treatment of multiple sclerosis, J. Neurol. 269 (7) (2022) 3937–3958.
[25] X. Montalban, S.L. Hauser, L. Kappos, D.L. Arnold, A. Bar-Or, G. Comi, J. de Seze, G. Giovannoni, H.P. Hartung, B. Hemmer, F. Lublin, K.W. Rammohan, K. Selmaj, A. Traboulsee, A. Sauter, D. Masterman, P. Fontoura, S. Belachew, H. Garren, N. Mairon, P. Chin, J.S. Wolinsky, O.C. Investigators, Ocrelizumab versus placebo in primary progressive multiple sclerosis, N. Engl. J. Med. 376 (3) (2017) 209–220.
[26] L. Kappos, A. Bar-Or, B.A.C. Cree, R.J. Fox, G. Giovannoni, R. Gold, P.

Vermersch, D.L. Arnold, S. Arnould, T. Scherz, C. Wolf, E. Wallstrom, F. Dahlke, E.C. Investigators, Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study, Lancet 391 (10127) (2018) 1263–1273.
[27] M. Clinic, MS, Diagnosis - Treatment. https://www.mayoclinic.org/diseases-conditions/multiple-sclerosis/diagnosis-treatment/drc-20350274, 2022, Accessed 7 July 2022.
[28] J.H. Yang, T. Rempe, N. Whitmire, A. Dunn-Pirio, J.S. Graves, Therapeutic advances in multiple sclerosis, Front. Neurol. 13 (2022) 824926.
[29] S.K. Shahi, S.N. Jensen, A.C. Murra, N. Tang, H. Guo, K.N. Gibson-Corley, J. Zhang, N.J. Karandikar, J.A. Murray, A.K. Mangalam, Human commensal prevotella histicola ameliorates disease as effectively as interferon-beta in the experimental autoimmune encephalomyelitis, Front. Immunol. 11 (2020) 578648.
[30] L. Yu, E. Croze, K.D. Yamaguchi, T. Tran, A.T. Reder, V. Litvak, M.R. Volkert, Induction of a unique isoform of the NCOA7 oxidation resistance gene by interferon beta-1b, J. Interf. Cytokine Res. 35 (3) (2015) 186–199.
[31] S. Dhib-Jalbut, S. Marks, Interferon-beta mechanisms of action in multiple sclerosis, Neurology 74 (Suppl. 1) (2010) S17–S24.
[32] E.M. Agency, Merck Europe B.V. REBIF. EMA Summary of Product Characteristics. https://www.ema.europa.eu/en/documents/product-information/rebif-epar-product-information_en.pdf, 2020, Accessed 04 July 2022.
[33] A. Kasindi, D.T. Fuchs, Y. Koronyo, A. Rentsendorj, K.L. Black, M. Koronyo-Hamaoui, Glatiramer acetate immunomodulation: evidence of neuroprotection and cognitive preservation, Cells 11 (9) (2022).
[34] A. Ovchinnikov, O. Findling, An overview of pivotal trials and real-world evidence for CD20-depleting therapy in multiple sclerosis : immunotherapy with rituximab, ocrelizumab, and ofatumumab, Wien. Med. Wochenschr. (2022) 172 (15-16):359-364.
[35] A. Babaesfahani, N.R. Khanna, B. Kuns, Natalizumab, StatPearls, Treasure Island (FL), 2022.
[36] N. Kiapour, B. Wu, Y. Wang, M. Seyedsadr, S. Kapoor, X. Zhang, M. Elzoheiry, E. Kasimoglu, Y. Wan, S. Markovic-Plese, Therapeutic effect of anti-CD52 monoclonal antibody in multiple sclerosis and its animal models is mediated via t regulatory cells, J. Immunol. 209 (1) (2022) 49–56.
[37] C. Hilger, C. Riedhammer, E. Orso, R. Weissert, Effects of alemtuzumab on (auto)antigen-specific immune responses, Front. Immunol. 11 (2020) 563645.
[38] D. Florou, M. Katsara, J. Feehan, E. Dardiotis, V. Apostolopoulos, Anti-CD20 agents for multiple sclerosis: spotlight on ocrelizumab and ofatumumab, Brain Sci 10 (10) (2020).
[39] M.J. Fabis-Pedrini, W.M. Carroll, A.G. Kermode, Efficacy and safety of mitoxantrone use in aggressive multiple sclerosis (P3.414), Neurology 90 (15 Supplement) (2018) P3.414.
[40] E.M. Agency, Accord Healthcare Ltd, Mitoxantrone, in: EMA Summary of Product Characteristics, 2016. https://www.ema.europa.eu/en/documents/referral/novantrone-article-30-referral-annex-iii_en.pdf. (Accessed 7 July 2022).
[41] L. Kappos, J. Cohen, W. Collins, A. de Vera, L. Zhang-Auberson, S. Ritter, P. von Rosenstiel, G. Francis, Fingolimod in relapsing multiple sclerosis: an integrated analysis of safety findings, Mult. Scler. Relat. Disord. 3 (4) (2014) 494–504.
[42] S.L. Cohan, R.H.B. Benedict, B.A.C. Cree, J. DeLuca, L.H. Hua, J. Chun, The two sides of siponimod: evidence for brain and immune mechanisms in multiple sclerosis, CNS Drugs 36 (7) (2022) 703–719.
[43] N. Behrangi, F. Fischbach, M. Kipp, Mechanism of siponimod: anti-inflammatory and neuroprotective mode of action, Cells 8 (1) (2019).
[44] A.M. Kuczynski, J. Oh, Ozanimod for the treatment of relapsing forms of multiple sclerosis, Neurodegener Dis Manag 11 (3) (2021) 207–220.
[45] F.L. Scott, B. Clemons, J. Brooks, E. Brahmachary, R. Powell, H. Dedman, H.G. Desale, G.A. Timony, E. Martinborough, H. Rosen, E. Roberts, M.F. Boehm, R.J. Peach, Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity, Br. J. Pharmacol. 173 (11) (2016) 1778–1792.
[46] S. Ruggieri, M.E. Quartuccio, L. Prosperini, Ponesimod in the treatment of relapsing forms of multiple sclerosis: an update on the emerging clinical data, Degener. Neurol. Neuromuscul. Dis. 12 (2022) 61–73.
[47] G. Tilly, M. Cadoux, A. Garcia, J. Morille, S. Wiertlewski, C. Pecqueur, S. Brouard, D. Laplaud, N. Degauque, Teriflunomide Treatment of multiple sclerosis selectively modulates CD8 memory T cells, Front. Immunol. 12 (2021) 730342.
[48] S.K. Yadav, D. Soin, K. Ito, S. Dhib-Jalbut, Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis, J Mol Med (Berl) 97 (4) (2019) 463–472.
[49] E.A. Mills, M.A. Ogrodnik, A. Plave, Y. Mao-Draayer, Emerging understanding of the mechanism of action for dimethyl fumarate in the treatment of multiple sclerosis, Front. Neurol. 9 (2018) 5.
[50] E. Jonasson, T. Sejbaek, Diroximel fumarate in the treatment of multiple sclerosis, Neurodegener Dis Manag 10 (5) (2020) 267–276.
[51] A.A. Berger, E.R. Sottosanti, A. Winnick, J. Izygon, K. Berardino, E.M. Cornett, A.D. Kaye, G. Varrassi, O. Viswanath, I. Urits, Monomethyl fumarate (MMF, bafiertam) for the treatment of relapsing forms of multiple sclerosis (MS), Neurol. Int. 13 (2) (2021) 207–223.
[52] N. Fissolo, L. Calvo-Barreiro, H. Eixarch, U. Boschert, C. Espejo, X. Montalban, M. Comabella, Immunomodulatory effects associated with cladribine treatment, Cells 10 (12) (2021).
[53] S.F. Hunter, J.D. Bowen, A.T. Reder, The direct effects of fingolimod in the central nervous system: implications for relapsing multiple sclerosis, CNS Drugs 30 (2) (2016) 135–147.
[54] J. Liliemark, The clinical pharmacokinetics of cladribine, Clin. Pharmacokinet. 32 (2) (1997) 120–131.
[55] S.R. Mehr, M.P. Zimmerman, Reviewing the unmet needs of patients with multiple sclerosis, Am Health Drug Benef. 8 (8) (2015) 426–431.
[56] D.M. Balak, G.J. Hengstman, A. Cakmak, H.B. Thio, Cutaneous adverse events associated with disease-modifying treatment in multiple sclerosis: a systematic review, Mult. Scler. 18 (12) (2012) 1705–1717.
[57] A.M. Palmer, Pharmacotherapeuetic options for the treatment of multiple sclerosis, clinical medicine insights, Therapeutics 4 (2012) CMT.S8661.
[58] S. Brancati, L. Gozzo, L. Longo, D.C. Vitale, F. Drago, Rituximab in multiple sclerosis: are we ready for regulatory approval? Front. Immunol. 12 (2021) 661882.
[59] D. Baker, G. Pryce, S.S. Herrod, K. Schmierer, Potential mechanisms of action related to the efficacy and safety of cladribine, Mult. Scler. Relat. Disord. 30 (2019) 176–186.
[60] E. Jozefczuk, T.J. Guzik, M. Siedlinski, Significance of sphingosine-1-phosphate in cardiovascular physiology and pathology, Pharmacol. Res. 156 (2020) 104793.
[61] K. Seiter, Toxicity of the topoisomerase II inhibitors, Expert Opin. Drug Saf. 4 (2) (2005) 219–234.
[62] F. Ravandi, Therapy-related acute promyelocytic leukemia, Haematologica 96 (4) (2011) 493–495.
[63] E. Stamoula, A. Ainatzoglou, V.P. Stamatellos, I. Dardalas, S. Siafis, A. Matsas, K. Stamoulas, G. Papazisis, Atypical antipsychotics in multiple sclerosis: a review of their in vivo immunomodulatory effects, Mult. Scler. Relat. Disord. 58 (2022) 103522.
[64] V.E. Miron, A. Schubart, J.P. Antel, Central nervous system-directed effects of FTY720 (fingolimod), J. Neurol. Sci. 274 (1–2) (2008) 13–17.
[65] R. Hermann, M.O. Karlsson, A.M. Novakovic, N. Terranova, M. Fluck, A. Munafo, The clinical pharmacology of cladribine tablets for the treatment of relapsing multiple sclerosis, Clin. Pharmacokinet. 58 (3) (2019) 283–297.
[66] A. Tavares, O. Barret, D. Alagille, T. Morley, C. Papin, R. Maguire, E. Briard, Y. Auberson, G. Tamagnan, Brain distribution of MS565, an imaging analogue of siponimod (BAF312), in non-human Primates (P1.168), Neurology 82 (10 Supplement) (2014) P1.168.
[67] S. Cohan, E. Lucassen, K. Smoot, J. Brink, C. Chen, Sphingosine-1-phosphate: its pharmacological regulation and the treatment of multiple sclerosis: a review article, Biomedicines 8 (7) (2020).
[68] S. Faissner, R. Gold, Efficacy and safety of multiple sclerosis drugs approved since 2018 and future developments, CNS Drugs 36 (8) (2022) 803–817.
[69] A.M. Palmer, Multiple sclerosis and the blood-central nervous system barrier, Cardiovasc. Psychiatry Neurol. 2013 (2013) 530356.
[70] C. Lubetzki, B. Zalc, A. Williams, C. Stadelmann, B. Stankoff, Remyelination in multiple sclerosis: from basic science to clinical translation, Lancet Neurol. 19 (8) (2020) 678–688.
[71] P. Villoslada, L. Steinman, New targets and therapeutics for neuroprotection, remyelination and repair in multiple sclerosis, Expert Opin. Investig. Drugs 29 (5) (2020) 443–459.
[72] N. Gacem, B. Nait-Oumesmar, Oligodendrocyte development and regenerative therapeutics in multiple sclerosis, Life (Basel) 11 (4) (2021).
[73] A. Manousi, P. Gottle, L. Reiche, Q.L. Cui, L.M. Healy, R. Akkermann, J. Gruchot, J. Schira-Heinen, J.P. Antel, H.P. Hartung, P. Kury, Identification of novel myelin repair drugs by modulation of oligodendroglial differentiation competence, EBioMedicine 65 (2021) 103276.
[74] B.A.C. Cree, H.P. Hartung, M. Barnett, New drugs for multiple sclerosis: new treatment algorithms, Curr. Opin. Neurol. 35 (3) (2022) 262–270.
[75] L. Bierhansl, H.P. Hartung, O. Aktas, T. Ruck, M. Roden, S.G. Meuth, Thinking outside the box: non-canonical targets in multiple sclerosis, Nat. Rev. Drug Discov. 21 (8) (2022) 578–600.
[76] C. Procaccini, V. De Rosa, V. Pucino, L. Formisano, G. Matarese, Animal models of multiple sclerosis, Eur. J. Pharmacol. 759 (2015) 182–191.
[77] I. Bjelobaba, V. Begovic-Kupresanin, S. Pekovic, I. Lavrnja, Animal models of multiple sclerosis: focus on experimental autoimmune encephalomyelitis, J. Neurosci. Res. 96 (6) (2018) 1021–1042.
[78] G.S. Melchor, T. Khan, J.F. Reger, J.K. Huang, Remyelination pharmacotherapy investigations highlight diverse mechanisms underlying multiple sclerosis progression, ACS Pharmacol. Transl. Sci. 2 (6) (2019) 372–386.
[79] S. Glatigny, E. Bettelli, Experimental Autoimmune Encephalomyelitis (EAE) as Animal Models of Multiple Sclerosis (MS), Cold Spring Harb Perspect Med 8 (11) (2018).
[80] H.S. Kwon, S.H. Koh, Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes, Transl Neurodegener 9 (1) (2020) 42.
[81] W. Yi, D. Schluter, X. Wang, Astrocytes in multiple sclerosis and experimental autoimmune encephalomyelitis: Star-shaped cells illuminating the darkness of CNS autoimmunity, Brain Behav. Immun. 80 (2019) 10–24.
[82] S. Zia, K.S. Rawji, N.J. Michaels, M. Burr, B.J. Kerr, L.M. Healy, J.R. Plemel, Microglia diversity in health and multiple sclerosis, Front. Immunol. 11 (2020) 588021.
[83] L. Starost, M. Lindner, M. Herold, Y.K.T. Xu, H.C.A. Drexler, K. Hess, M. Ehrlich, L. Ottoboni, F. Ruffini, M. Stehling, A. Ropke, C. Thomas, H.R. Scholer, J. Antel, J. Winkler, G. Martino, L. Klotz, T. Kuhlmann, Extrinsic immune cell-derived, but not intrinsic oligodendroglial factors contribute to oligodendroglial differentiation block in multiple sclerosis, Acta Neuropathol. 140 (5) (2020) 715–736.
[84] K. An, M.J. Xue, J.Y. Zhong, S.N. Yu, T.S. Lan, Z.Q. Qi, J.J. Xia, Arsenic trioxide ameliorates experimental autoimmune encephalomyelitis in C57BL/6 mice by inducing CD4(+) T cell apoptosis, J. Neuroinflammation 17 (1) (2020) 147.

[85] S.D. Guo, C.Y. Liu, J.W. Yu, Z. Chai, Q. Wang, X.T. Mi, G.B. Song, Y.H. Li, P.W. Yang, L. Feng, B.G. Xiao, C.G. Ma, Nasal delivery of Fasudil-modified immune cells exhibits therapeutic potential in experimental autoimmune encephalomyelitis, CNS Neurosci Ther 25 (6) (2019) 783–795.
[86] S.W. Hou, C.Y. Liu, Y.H. Li, J.Z. Yu, L. Feng, Y.T. Liu, M.F. Guo, Y. Xie, J. Meng, H.F. Zhang, B.G. Xiao, C.G. Ma, Fasudil ameliorates disease progression in experimental autoimmune encephalomyelitis, acting possibly through antiinflammatory effect, CNS Neurosci Ther 18 (11) (2012) 909–917.
[87] C. Liu, Y. Li, J. Yu, L. Feng, S. Hou, Y. Liu, M. Guo, Y. Xie, J. Meng, H. Zhang, B. Xiao, C. Ma, Targeting the shift from M1 to M2 macrophages in experimental autoimmune encephalomyelitis mice treated with fasudil, PLoS One 8 (2) (2013) e54841.
[88] Y. Jia, H. Li, H. Bao, D. Zhang, L. Feng, Y. Xiao, K. Zhu, Y. Hou, S. Luo, Y. Zhang, L. Xiao, X. Chen, J. Zhou, C. Wang, G. Wang, H. Yu, C. Xiao, J. Du, Cordycepin (3'-deoxyadenosine) promotes remyelination via suppression of neuroinflammation in a cuprizone-induced mouse model of demyelination, Int. Immunopharmacol. 75 (2019) 105777.
[89] Y. Jia, D. Zhang, H. Li, S. Luo, Y. Xiao, L. Han, F. Zhou, C. Wang, L. Feng, G. Wang, P. Wu, C. Xiao, H. Yu, J. Du, H. Bao, Activation of FXR by ganoderic acid A promotes remyelination in multiple sclerosis via anti-inflammation and regeneration mechanism, Biochem. Pharmacol. 185 (2021) 114422.
[90] L.L. Yin, L.L. Lin, L. Zhang, L. Li, Epimedium flavonoids ameliorate experimental autoimmune encephalomyelitis in rats by modulating neuroinflammatory and neurotrophic responses, Neuropharmacology 63 (5) (2012) 851–862.
[91] H. Cong, M. Zhang, H. Chang, L. Du, X. Zhang, L. Yin, Icariin ameliorates the progression of experimental autoimmune encephalomyelitis by down-regulating the major inflammatory signal pathways in a mouse relapse-remission model of multiple sclerosis, Eur. J. Pharmacol. 885 (2020) 173523.
[92] T. Kuntzel, D. Bagnard, Manipulating Macrophage/Microglia Polarization to Treat Glioblastoma or Multiple Sclerosis, Pharmaceutics 14 (2) (2022).
[93] Y. He, J. An, J.J. Yin, R.X. Sui, Q. Miao, Z.B. Ding, Q.X. Han, Q. Wang, C.G. Ma, B.G. Xiao, Ethyl pyruvate enhances spontaneous remyelination by targeting microglia phagocytosis, Int. Immunopharmacol. 77 (2019) 105929.
[94] Q. Weng, J. Wang, J. Wang, J. Wang, F. Sattar, Z. Zhang, J. Zheng, Z. Xu, M. Zhao, X. Liu, L. Yang, G. Hao, L. Fang, Q.R. Lu, B. Yang, Q. He, Correction: Lenalidomide regulates CNS autoimmunity by promoting M2 macrophages polarization, Cell Death Dis. 11 (2) (2020) 108.
[95] T. Veremeyko, A.W.Y. Yung, M. Dukhinova, I.S. Kuznetsova, I. Pomytkin, A. Lyundup, T. Strekalova, N.S. Barteneva, E.D. Ponomarev, Cyclic AMP Pathway Suppress Autoimmune Neuroinflammation by Inhibiting Functions of Encephalitogenic CD4 T Cells and Enhancing M2 Macrophage Polarization at the Site of Inflammation, Front. Immunol. 9 (2018) 50.
[96] K. Kobayashi, S. Imagama, T. Ohgomori, K. Hirano, K. Uchimura, K. Sakamoto, A. Hirakawa, H. Takeuchi, A. Suzumura, N. Ishiguro, K. Kadomatsu, Minocycline selectively inhibits M1 polarization of microglia, Cell Death Dis. 4 (3) (2013) e525.
[97] W.W. Li, A. Setzu, C. Zhao, R.J. Franklin, Minocycline-mediated inhibition of microglia activation impairs oligodendrocyte progenitor cell responses and remyelination in a non-immune model of demyelination, J. Neuroimmunol. 158 (1–2) (2005) 58–66.
[98] X. Lu, L. Ma, L. Ruan, Y. Kong, H. Mou, Z. Zhang, Z. Wang, J.M. Wang, Y. Le, Resveratrol differentially modulates inflammatory responses of microglia and astrocytes, J. Neuroinflammation 7 (2010) 46.
[99] H.R. Ghaiad, M.M. Nooh, M.M. El-Sawalhi, A.A. Shaheen, Resveratrol promotes remyelination in cuprizone model of multiple sclerosis: biochemical and histological study, Mol. Neurobiol. 54 (5) (2017) 3219–3229.
[100] V. Parvathaneni, N.S. Kulkarni, A. Muth, V. Gupta, Drug repurposing: a promising tool to accelerate the drug discovery process, Drug Discov. Today 24 (10) (2019) 2076–2085.
[101] M.M. Al-Amin, M.M. Nasir Uddin, H. Mahmud Reza, Effects of antipsychotics on the inflammatory response system of patients with schizophrenia in peripheral blood mononuclear cell cultures, Clin Psychopharmacol Neurosci 11 (3) (2013) 144–151.
[102] S. Balestri, A. Del Giovane, C. Sposato, M. Ferrarelli, A. Ragnini-Wilson, The current challenges for drug discovery in CNS Remyelination, Int. J. Mol. Sci. 22 (6) (2021).
[103] Z. Hubler, D. Allimuthu, I. Bederman, M.S. Elitt, M. Madhavan, K.C. Allan, H.E. Shick, E. Garrison, D.C. Factor, Z.S. Nevin, J.L. Sax, M.A. Thompson, Y. Fedorov, J. Jin, W.K. Wilson, M. Giera, F. Bracher, R.H. Miller, P.J. Tesar, D.J. Adams, Accumulation of 8,9-unsaturated sterols drives oligodendrocyte formation and remyelination, Nature 560 (7718) (2018) 372–376.
[104] V.E. Miron, T. Kuhlmann, J.P. Antel, Cells of the oligodendroglial lineage, myelination, and remyelination, Biochim. Biophys. Acta 1812 (2) (2011) 184–193.
[105] M. Ploughman, V.W. Yong, B. Spermon, S. Goelz, G. Giovannoni, Remyelination trial failures: repercussions of ignoring neurorehabilitation and exercise in repair, Mult. Scler. Relat. Disord. 58 (2022) 103539.
[106] R.A. Hill, A.M. Li, J. Grutzendler, Lifelong cortical myelin plasticity and age-related degeneration in the live mammalian brain, Nat. Neurosci. 21 (5) (2018) 683–695.
[107] B. Neumann, R. Baror, C. Zhao, M. Segel, S. Dietmann, K.S. Rawji, S. Foerster, C.R. McClain, K. Chalut, P. van Wijngaarden, R.J.M. Franklin, Metformin restores CNS remyelination capacity by rejuvenating aged stem cells, Cell Stem Cell 25 (4) (2019) 473–485 e8.
[108] M.W. Voss, C. Vivar, A.F. Kramer, H. van Praag, Bridging animal and human models of exercise-induced brain plasticity, Trends Cogn. Sci. 17 (10) (2013) 525–544.
[109] P.E.M. van Schaik, I.S. Zuhorn, W. Baron, Targeting fibronectin to overcome remyelination failure in multiple sclerosis: the need for brain- and lesion-targeted drug delivery, Int. J. Mol. Sci. 23 (15) (2022).
[110] J.R. Allanach, J.W. Farrell 3rd, M. Mesidor, S. Karimi-Abdolrezaee, Current status of neuroprotective and neuroregenerative strategies in multiple sclerosis: A systematic review, Mult. Scler. 28 (1) (2022) 29–48.
[111] J.A. Frank, N. Richert, B. Lewis, C. Bash, T. Howard, R. Civil, R. Stone, J. Eaton, H. McFarland, T. Leist, A pilot study of recombinant insulin-like growth factor-1 in seven multiple sclerosis patients, Mult. Scler. 8 (1) (2002) 24–29.
[112] Y. Huang, C.F. Dreyfus, The role of growth factors as a therapeutic approach to demyelinating disease, Exp. Neurol. 283 (Pt B) (2016) 531–540.
[113] E.D. Miller, A. Dziedzic, J. Saluk-Bijak, M. Bijak, A review of various antioxidant compounds and their potential utility as complementary therapy in multiple sclerosis, Nutrients 11 (7) (2019).
[114] S. Shahrampour, J. Heholt, A. Wang, F. Vedaei, F.B. Mohamed, M. Alizadeh, Z. Wang, G. Zabrecky, N. Wintering, A.J. Bazzan, T.P. Leist, D.A. Monti, A.B. Newberg, N-acetyl cysteine administration affects cerebral blood flow as measured by arterial spin labeling MRI in patients with multiple sclerosis, Heliyon 7 (7) (2021) e07615.
[115] G.P. Biewenga, G.R. Haenen, A. Bast, The pharmacology of the antioxidant lipoic acid, Gen. Pharmacol. 29 (3) (1997) 315–331.
[116] C. Waslo, D. Bourdette, N. Gray, K. Wright, R. Spain, Lipoic acid and other antioxidants as therapies for multiple sclerosis, Curr. Treat. Options Neurol. 21 (6) (2019) 26.
[117] D. Pitt, P. Werner, C.S. Raine, Glutamate excitotoxicity in a model of multiple sclerosis, Nat. Med. 6 (1) (2000) 67–70.
[118] M. Enders, T. Heider, A. Ludwig, S. Kuerten, Strategies for neuroprotection in multiple sclerosis and the role of calcium, Int. J. Mol. Sci. 21 (5) (2020).
[119] C. Ding, M. Hammarlund, Mechanisms of injury-induced axon degeneration, Curr. Opin. Neurobiol. 57 (2019) 171–178.
[120] R.O. Hughes, T. Bosanac, X. Mao, T.M. Engber, A. DiAntonio, J. Milbrandt, R. Devraj, R. Krauss, Small molecule SARM1 inhibitors recapitulate the SARM1(-/-) phenotype and allow recovery of a metastable pool of axons fated to degenerate, Cell Rep. 34 (1) (2021) 108588.
[121] A. Misra, S. Ganesh, A. Shahiwala, S.P. Shah, Drug delivery to the central nervous system: a review, J. Pharm Pharm. Sci. 6 (2) (2003) 252–273.
[122] W.M. Pardridge, CSF, blood-brain barrier, and brain drug delivery, Expert Opin Drug Deliv 13 (7) (2016) 963–975.
[123] E.C. de Lange, Potential role of ABC transporters as a detoxification system at the blood-CSF barrier, Adv. Drug Deliv. Rev. 56 (12) (2004) 1793–1809.
[124] M. Tajes, E. Ramos-Fernandez, X. Weng-Jiang, M. Bosch-Morato, B. Guivernau, A. Eraso-Pichot, B. Salvador, X. Fernandez-Busquets, J. Roquer, F.J. Munoz, The blood-brain barrier: structure, function and therapeutic approaches to cross it, Mol. Membr. Biol. 31 (5) (2014) 152–167.
[125] H. Kadry, B. Noorani, L. Cucullo, A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity, Fluids Barr. CNS 17 (1) (2020) 69.
[126] C.M. Bellettato, M. Scarpa, Possible strategies to cross the blood-brain barrier, Ital. J. Pediatr. 44 (Suppl. 2) (2018) 131.
[127] B. Srinivasan, A.R. Kolli, M.B. Esch, H.E. Abaci, M.L. Shuler, J.J. Hickman, TEER measurement techniques for in vitro barrier model systems, J. Lab Autom. 20 (2) (2015) 107–126.
[128] M. Xiao, Z.J. Xiao, B. Yang, Z. Lan, F. Fang, Blood-brain barrier: more contributor to disruption of central nervous system homeostasis than victim in neurological disorders, Front. Neurosci. 14 (2020) 764.
[129] R. Balasa, L. Barcutean, O. Mosora, D. Manu, Reviewing the significance of blood-brain barrier disruption in multiple sclerosis pathology and treatment, Int. J. Mol. Sci. 22 (16) (2021).
[130] J. Rautio, K. Laine, M. Gynther, J. Savolainen, Prodrug approaches for CNS delivery, AAPS J. 10 (1) (2008) 92–102.
[131] E. Puris, G. Fricker, M. Gynther, Targeting transporters for drug delivery to the brain: can we do better? Pharm. Res. 39 (7) (2022) 1415–1455.
[132] W.M. Pardridge, Delivery of biologics across the blood-brain barrier with molecular trojan horse technology, BioDrugs 31 (6) (2017) 503–519.
[133] R.J. Boado, W.M. Pardridge, Brain and organ uptake in the rhesus monkey in vivo of recombinant iduronidase compared to an insulin receptor antibody-iduronidase fusion protein, Mol. Pharm. 14 (4) (2017) 1271–1277.
[134] R.J. Boado, J.Z. Lu, E.K. Hui, W.M. Pardridge, Reduction in brain heparan sulfate with systemic administration of an igg trojan horse-sulfamidase fusion protein in the mucopolysaccharidosis Type IIIA Mouse, Mol. Pharm. 15 (2) (2018) 602–608.
[135] W.M. Pardridge, Brain delivery of nanomedicines: trojan horse liposomes for plasmid DNA gene therapy of the brain, Front. Med. Technol. 2 (2020) 602236.
[136] D. Jiang, H. Lee, W.M. Pardridge, Plasmid DNA gene therapy of the Niemann-Pick C1 mouse with transferrin receptor-targeted Trojan horse liposomes, Sci. Rep. 10 (1) (2020) 13334.
[137] S.I. Rapoport, Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications, Cell. Mol. Neurobiol. 20 (2) (2000) 217–230.
[138] D.H. Dobrogowska, A.W. Vorbrodt, Immunogold localization of tight junctional proteins in normal and osmotically-affected rat blood-brain barrier, J. Mol. Histol. 35 (5) (2004) 529–539.
[139] T. Nomura, T. Inamura, K.L. Black, Intracarotid infusion of bradykinin selectively increases blood-tumor permeability in 9L and C6 brain tumors, Brain

Res. 659 (1–2) (1994) 62–66.
[140] B. Erdlenbruch, M. Alipour, G. Fricker, D.S. Miller, W. Kugler, H. Eibl, M. Lakomek, Alkylglycerol opening of the blood-brain barrier to small and large fluorescence markers in normal and C6 glioma-bearing rats and isolated rat brain capillaries, Br. J. Pharmacol. 140 (7) (2003) 1201–1210.
[141] E. Dux, F. Joo, Effects of histamine on brain capillaries. Fine structural and immunohistochemical studies after intracarotid infusion, Exp. Brain Res. 47 (2) (1982) 252–258.
[142] N. Sheikov, N. McDannold, N. Vykhodtseva, F. Jolesz, K. Hynynen, Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles, Ultrasound Med. Biol. 30 (7) (2004) 979–989.
[143] N. Sheikov, N. McDannold, S. Sharma, K. Hynynen, Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium, Ultrasound Med. Biol. 34 (7) (2008) 1093–1104.
[144] N.A. Lapin, K. Gill, B.R. Shah, R. Chopra, Consistent opening of the blood brain barrier using focused ultrasound with constant intravenous infusion of microbubble agent, Sci. Rep. 10 (1) (2020) 16546.
[145] J.F. Jordao, E. Thevenot, K. Markham-Coultes, T. Scarcelli, Y.Q. Weng, K. Xhima, M. O'Reilly, Y. Huang, J. McLaurin, K. Hynynen, I. Aubert, Amyloid-beta plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound, Exp. Neurol. 248 (2013) 16–29.
[146] K. Schregel, C. Baufeld, M. Palotai, R. Meroni, P. Fiorina, J. Wuerfel, R. Sinkus, Y.Z. Zhang, N. McDannold, P.J. White, C.R.G. Guttmann, Targeted blood brain barrier opening with focused ultrasound induces focal macrophage/microglial activation in experimental autoimmune encephalomyelitis, Front. Neurosci. 15 (2021) 665722.
[147] J. Lane, B.E. Zacharia, Endoscopic-assisted ommaya reservoir placement: technical note, Cureus 9 (7) (2017) e1490.
[148] G.Y. Kim, B.M. Tyler, M.M. Tupper, J.M. Karp, R.S. Langer, H. Brem, M.J. Cima, Resorbable polymer microchips releasing BCNU inhibit tumor growth in the rat 9L flank model, J. Control. Release 123 (2) (2007) 172–178.
[149] B.C. Masi, B.M. Tyler, H. Bow, R.T. Wicks, Y. Xue, H. Brem, R. Langer, M.J. Cima, Intracranial MEMS based temozolomide delivery in a 9L rat gliosarcoma model, Biomaterials 33 (23) (2012) 5768–5775.
[150] R.R. Lonser, M. Sarntinoranont, P.F. Morrison, E.H. Oldfield, Convection-enhanced delivery to the central nervous system, J. Neurosurg. 122 (3) (2015) 697–706.
[151] Y.E. Seo, T. Bu, W.M. Saltzman, Nanomaterials for convection-enhanced delivery of agents to treat brain tumors, Curr Opin Biomed Eng 4 (2017) 1–12.
[152] Z. Wang, G. Xiong, W.C. Tsang, A.G. Schatzlein, I.F. Uchegbu, Nose-to-brain delivery, J. Pharmacol. Exp. Ther. 370 (3) (2019) 593–601.
[153] S. Md, S.K. Bhattmisra, F. Zeeshan, N. Shahzad, M.A. Mujtaba, V. Srikanth Meka, A. Radhakrishnan, P. Kesharwani, S. Baboota, J. Ali, Nano-carrier enabled drug delivery systems for nose to brain targeting for the treatment of neurodegenerative disorders, J. Drug Deliv. Sci. Technol. 43 (2018) 295–310.
[154] Y. Fan, M. Chen, J. Zhang, P. Maincent, X. Xia, W. Wu, Updated progress of nanocarrier-based intranasal drug delivery systems for treatment of brain diseases, Crit. Rev. Ther. Drug Carrier Syst. 35 (5) (2018) 433–467.
[155] T.P. Crowe, W.H. Hsu, Evaluation of recent intranasal drug delivery systems to the central nervous system, Pharmaceutics 14 (3) (2022).
[156] V. Bourganis, O. Kammona, A. Alexopoulos, C. Kiparissides, Recent advances in carrier mediated nose-to-brain delivery of pharmaceutics, Eur. J. Pharm. Biopharm. 128 (2018) 337–362.
[157] M.J. Stenslik, A. Evans, F. Pomerleau, R. Weeks, P. Huettl, E. Foreman, J. Turchan-Cholewo, A. Andersen, W.A. Cass, Z. Zhang, R.C. Grondin, D.M. Gash, G.A. Gerhardt, L.H. Bradley, Methodology and effects of repeated intranasal delivery of DNSP-11 in awake Rhesus macaques, J. Neurosci. Methods 303 (2018) 30–40.
[158] S.V. Dhuria, L.R. Hanson, W.H. Frey 2nd, Intranasal delivery to the central nervous system: mechanisms and experimental considerations, J. Pharm. Sci. 99 (4) (2010) 1654–1673.
[159] S. Ganger, K. Schindowski, Tailoring formulations for intranasal nose-to-brain delivery: a review on architecture, physico-chemical characteristics and mucociliary clearance of the nasal olfactory mucosa, Pharmaceutics 10 (3) (2018).
[160] P.C. Pires, A.O. Santos, Nanosystems in nose-to-brain drug delivery: a review of non-clinical brain targeting studies, J. Control. Release 270 (2018) 89–100.
[161] T.P. Crowe, M.H.W. Greenlee, A.G. Kanthasamy, W.H. Hsu, Mechanism of intranasal drug delivery directly to the brain, Life Sci. 195 (2018) 44–52.
[162] P.V. Turner, T. Brabb, C. Pekow, M.A. Vasbinder, Administration of substances to laboratory animals: routes of administration and factors to consider, J. Am. Assoc. Lab. Anim. Sci. 50 (5) (2011) 600–613.
[163] O. Santos-Morales, A. Diaz-Machado, D. Jimenez-Rodriguez, Y. Pomares-Iturralde, T. Festary-Casanovas, C.A. Gonzalez-Delgado, S. Perez-Rodriguez, E. Alfonso-Munoz, C. Viada-Gonzalez, P. Piedra-Sierra, I. Garcia-Garcia, D. Amaro-Gonzalez, E.P.O.S.G. Neuro, J.C. Garcia-Rodriguez, I. Sosa-Teste, A. Lagarto-Parra, L. Barrero-Viera, M. David-Baldo, M. Tamayo-Rodriguez, I. Rivero-Vazquez, G. Gonzalez-Gamiz, A. Martin-Trujillo, Y. Rodriguez-Fernandez, A.A. Ledo-de la Luz, M. Alvarez-Delgado, I. Howland-Alvarez, Y. Cruz-Gomez, Nasal administration of the neuroprotective candidate NeuroEPO to healthy volunteers: a randomized, parallel, open-label safety study, BMC Neurol. 17 (1) (2017) 129.
[164] J. Dong, Y. Shang, K. Inthavong, H.K. Chan, J. Tu, Numerical comparison of nasal aerosol administration systems for efficient nose-to-brain drug delivery, Pharm. Res. 35 (1) (2017) 5.
[165] M. Stützle, S. Carle, L. Engelhardt, U. Simon, A. Schafmeister, C. Mavoungou, K. Schindowski, Protein aerosol for intranasal nose to brain (N2B) delivery, BMC Proc. 9 (S9) (2015).
[166] S. Craft, L.D. Baker, T.J. Montine, S. Minoshima, G.S. Watson, A. Claxton, M. Arbuckle, M. Callaghan, E. Tsai, S.R. Plymate, P.S. Green, J. Leverenz, D. Cross, B. Gerton, Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial, Arch. Neurol. 69 (1) (2012) 29–38.
[167] P.G. Djupesland, A. Skretting, Nasal deposition and clearance in man: comparison of a bidirectional powder device and a traditional liquid spray pump, J Aerosol Med Pulm Drug Deliv 25 (5) (2012) 280–289.
[168] J.T. Trevino, R.C. Quispe, F. Khan, V. Novak, Non-invasive strategies for nose-to-brain drug delivery, J. Clin. Trials 10 (7) (2020).
[169] H.D. Hoekman, A. Brunelle, Relethford Ho RJY, Nasal drug Delivery Device, 2017.
[170] Z.N. Warnken, H.D.C. Smyth, A.B. Watts, S. Weitman, J.G. Kuhn, R.O. Williams, Formulation and device design to increase nose to brain drug delivery, J. Drug Deliv. Sci. Technol. 35 (2016) 213–222.
[171] Y. Ozsoy, S. Gungor, E. Cevher, Nasal delivery of high molecular weight drugs, Molecules 14 (9) (2009) 3754–3779.
[172] H. Jain, B. Prabhakar, P. Shende, Modulation of olfactory area for effective transportation of actives in CNS disorders, J. Drug Deliv. Sci. Technol. 68 (2022).
[173] M. Rizzuti, M. Nizzardo, C. Zanetta, A. Ramirez, S. Corti, Therapeutic applications of the cell-penetrating HIV-1 Tat peptide, Drug Discov. Today 20 (1) (2015) 76–85.
[174] N. Kamei, N. Okada, T. Ikeda, H. Choi, Y. Fujiwara, H. Okumura, M. Takeda-Morishita, Effective nose-to-brain delivery of exendin-4 via coadministration with cell-penetrating peptides for improving progressive cognitive dysfunction, Sci. Rep. 8 (1) (2018) 17641.
[175] E. Jouan, M. Le Vee, A. Mayati, C. Denizot, Y. Parmentier, O. Fardel, Evaluation of P-glycoprotein inhibitory potential using a rhodamine 123 accumulation assay, Pharmaceutics 8 (2) (2016).
[176] P.P. Martins, H.D.C. Smyth, Z. Cui, Strategies to facilitate or block nose-to-brain drug delivery, Int. J. Pharm. 570 (2019) 118635.
[177] F. Sonvico, A. Clementino, F. Buttini, G. Colombo, S. Pescina, S. Staniscuaski Guterres, A. Raffin Pohlmann, S. Nicoli, Surface-modified nanocarriers for nose-to-brain delivery: from bioadhesion to targeting, Pharmaceutics 10 (1) (2018).
[178] B. Chatterjee, B. Gorain, K. Mohananaidu, P. Sengupta, U.K. Mandal, H. Choudhury, Targeted drug delivery to the brain via intranasal nanoemulsion: available proof of concept and existing challenges, Int. J. Pharm. 565 (2019) 258–268.
[179] S.U. Islam, A. Shehzad, M.B. Ahmed, Y.S. Lee, Intranasal Delivery of Nanoformulations: a potential way of treatment for neurological disorders, Molecules 25 (8) (2020).
[180] S.B. Bhise, A.V. Yadav, A.M. Avachat, R. Malayandi, Bioavailability of intranasal drug delivery system, Asian J. Pharmaceut. 2 (2008) 201.
[181] V. Dhamankar, M.D. Donovan, Modulating nasal mucosal permeation using metabolic saturation and enzyme inhibition techniques, J. Pharm. Pharmacol. 69 (9) (2017) 1075–1083.
[182] V. Devonshire, Y. Lapierre, R. Macdonell, C. Ramo-Tello, F. Patti, P. Fontoura, L. Suchet, R. Hyde, I. Balla, E.M. Frohman, B.C. Kieseier, G.A.P.S. Group, The Global Adherence Project (GAP): a multicenter observational study on adherence to disease-modifying therapies in patients with relapsing-remitting multiple sclerosis, Eur. J. Neurol. 18 (1) (2011) 69–77.
[183] F. Kleinsinger, The unmet challenge of medication nonadherence, Perm J 22 (2018) 18–033.
[184] N. Giedraitiene, V. Taluntiene, G. Kaubrys, Relationship between cognition and treatment adherence to disease-modifying therapy in multiple sclerosis: a prospective, cross-sectional study, Sci. Rep. 12 (1) (2022) 12447.
[185] M. Chountoulesi, C. Demetzos, Promising nanotechnology approaches in treatment of autoimmune diseases of central nervous system, Brain Sci 10 (6) (2020).
[186] M. Sidoryk-Wegrzynowicz, B. Dabrowska-Bouta, G. Sulkowski, L. Struzynska, Nanosystems and exosomes as future approaches in treating multiple sclerosis, Eur. J. Neurosci. 54 (9) (2021) 7377–7404.
[187] D.M. Teleanu, C. Chircov, A.M. Grumezescu, R.I. Teleanu, Neuronanomedicine: an up-to-date overview, Pharmaceutics 11 (3) (2019).
[188] M. Zhang, X. Zang, M. Wang, Z. Li, M. Qiao, H. Hu, D. Chen, Exosome-based nanocarriers as bio-inspired and versatile vehicles for drug delivery: recent advances and challenges, J. Mater. Chem. B 7 (15) (2019) 2421–2433.
[189] R.P. Moura, C. Pacheco, A.P. Pego, A. Des Rieux, B. Sarmento, Lipid nanocapsules to enhance drug bioavailability to the central nervous system, J. Control. Release 322 (2020) 390–400.
[190] H.N. Jung, S.Y. Lee, S. Lee, H. Youn, H.J. Im, Lipid nanoparticles for delivery of RNA therapeutics: current status and the role of in vivo imaging, Theranostics 12 (17) (2022) 7509–7531.
[191] S. Rezvantalab, M. Keshavarz Moraveji, Microfluidic assisted synthesis of PLGA drug delivery systems, RSC Adv. 9 (4) (2019) 2055–2072.
[192] Y. Guo, X. Li, R.B. Macgregor Jr, H. Yan, R.X. Zhang, Microfluidics-based PLGA nanoparticles of ratiometric multidrug: from encapsulation and release rates to cytotoxicity in human lens epithelial cells, Heliyon 9 (7) (2023) e18318.
[193] K. Zhi, B. Raji, A.R. Nookala, M.M. Khan, X.H. Nguyen, S. Sakshi, T. Pourmotabbed, M.M. Yallapu, H. Kochat, E. Tadrous, S. Pernell, S. Kumar, PLGA nanoparticle-based formulations to cross the blood-brain barrier for drug delivery: from R&D to cGMP, Pharmaceutics 13 (4) (2021).
[194] K.M. El-Say, H.S. El-Sawy, Polymeric nanoparticles: promising platform for drug delivery, Int. J. Pharm. 528 (1–2) (2017) 675–691.
[195] M. Abdelsalam, M. Ahmed, Z. Osaid, R. Hamoudi, R. Harati, Insights into

exosome transport through the blood-brain barrier and the potential therapeutical applications in Brain diseases, Pharmaceuticals (Basel) 16 (4) (2023).
[196] Q. Li, X. Li, C. Zhao, Strategies to obtain encapsulation and controlled release of small hydrophilic molecules, Front Bioeng Biotechnol 8 (2020) 437.
[197] K.R. Mittal, N. Pharasi, B. Sarna, M. Singh, S. Rachana, S.K. Haider, K. Singh, S.K. Dua, A. Jha, S. Dey, S. Ojha, N.K. Jha Mani, Nanotechnology-based drug delivery for the treatment of CNS disorders, Transl. Neurosci. 13 (1) (2022) 527–546.
[198] D. Urimi, R. Widenbring, R.O. Perez Garcia, L. Gedda, K. Edwards, T. Loftsson, N. Schipper, Formulation development and upscaling of lipid nanocapsules as a drug delivery system for a novel cyclic GMP analogue intended for retinal drug delivery, Int. J. Pharm. 602 (2021) 120640.
[199] J.S. Coon, W. Knudson, K. Clodfelter, B. Lu, R.S. Weinstein, Solutol HS 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance, Cancer Res. 51 (3) (1991) 897–902.
[200] A. Lamprecht, J.P. Benoit, Etoposide nanocarriers suppress glioma cell growth by intracellular drug delivery and simultaneous P-glycoprotein inhibition, J. Control. Release 112 (2) (2006) 208–213.
[201] E. Roger, F. Lagarce, E. Garcion, J.P. Benoit, Reciprocal competition between lipid nanocapsules and P-gp for paclitaxel transport across Caco-2 cells, Eur. J. Pharm. Sci. 40 (5) (2010) 422–429.
[202] F. Farjadian, A. Ghasemi, O. Gohari, A. Roointan, M. Karimi, M.R. Hamblin, Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities, Nanomedicine (London) 14 (1) (2019) 93–126.
[203] G. Graverini, V. Piazzini, E. Landucci, D. Pantano, P. Nardiello, F. Casamenti, D.E. Pellegrini-Giampietro, A.R. Bilia, M.C. Bergonzi, Solid lipid nanoparticles for delivery of andrographolide across the blood-brain barrier: in vitro and in vivo evaluation, Colloids Surf. B: Biointerfaces 161 (2018) 302–313.
[204] E.E. N, M.O. M, A.M. H, A.A.-T. H, Nanostructured lipid carriers to mediate brain delivery of temazepam: design and in vivo study, Pharmaceutics 12 (5) (2020).
[205] M. Agrawal, S. Saraf, S. Saraf, S.K. Dubey, A. Puri, R.J. Patel, V. Ajazuddin, U.S. Ravichandiran, A. Alexander Murty, Recent strategies and advances in the fabrication of nano lipid carriers and their application towards brain targeting, J. Control. Release 321 (2020) 372–415.
[206] S. Ojha, B. Kumar, A review on nanotechnology based innovations in diagnosis and treatment of multiple sclerosis, J. Cell. Immunother. 4 (2) (2018) 56–64.
[207] Q. Zhang, X. Dai, H. Zhang, Y. Zeng, K. Luo, W. Li, Recent advances in development of nanomedicines for multiple sclerosis diagnosis, Biomed. Mater. 16 (2) (2021) 024101.
[208] S. Rittchen, A. Boyd, A. Burns, J. Park, T.M. Fahmy, S. Metcalfe, A. Williams, Myelin repair in vivo is increased by targeting oligodendrocyte precursor cells with nanoparticles encapsulating leukaemia inhibitory factor (LIF), Biomaterials 56 (2015) 78–85.
[209] R. de la Flor, J. Robertson, R.V. Shevchenko, M. Alavijeh, S. Bickerton, T. Fahmy, S.M. Metcalfe, Multiple Sclerosis: LIFNano-CD4 for trojan horse delivery of the neuro-protective biologic "lif" into the brain: preclinical proof of concept, Front. Med. Technol. 3 (2021) 640569.
[210] A. Fodor-Kardos, A.F. Kiss, K. Monostory, T. Feczko, Sustained in vitro interferon-beta release and in vivo toxicity of PLGA and PEG-PLGA nanoparticles, RSC Adv. 10 (27) (2020) 15893–15900.
[211] P.J. Gaillard, C.C. Appeldoorn, J. Rip, R. Dorland, S.M. van der Pol, G. Kooij, H.E. de Vries, A. Reijerkerk, Enhanced brain delivery of liposomal methylprednisolone improved therapeutic efficacy in a model of neuroinflammation, J. Control. Release 164 (3) (2012) 364–369.
[212] J. Schmidt, J.M. Metselaar, M.H. Wauben, K.V. Toyka, G. Storm, R. Gold, Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis, Brain 126 (Pt 8) (2003) 1895–1904.
[213] D.H. Lee, C. Rotger, C.C. Appeldoorn, A. Reijerkerk, W. Gladdines, P.J. Gaillard, R.A. Linker, Glutathione PEGylated liposomal methylprednisolone (2B3-201) attenuates CNS inflammation and degeneration in murine myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis, J. Neuroimmunol. 274 (1–2) (2014) 96–101.
[214] K. Turjeman, Y. Bavli, P. Kizelsztein, Y. Schilt, N. Allon, T.B. Katzir, E. Sasson, U. Raviv, H. Ovadia, Y. Barenholz, Nano-drugs based on nano sterically stabilized liposomes for the treatment of inflammatory neurodegenerative diseases, PLoS One 10 (7) (2015) e0130442.
[215] F. Re, I. Cambianica, S. Sesana, E. Salvati, A. Cagnotto, M. Salmona, P.O. Couraud, S.M. Moghimi, M. Masserini, G. Sancini, Functionalization with ApoE-derived peptides enhances the interaction with brain capillary endothelial cells of nanoliposomes binding amyloid-beta peptide, J. Biotechnol. 156 (4) (2011) 341–346.
[216] F. Re, I. Cambianica, C. Zona, S. Sesana, M. Gregori, R. Rigolio, B. La Ferla, F. Nicotra, G. Forloni, A. Cagnotto, M. Salmona, M. Masserini, G. Sancini, Functionalization of liposomes with ApoE-derived peptides at different density affects cellular uptake and drug transport across a blood-brain barrier model, Nanomedicine 7 (5) (2011) 551–559.
[217] G.R. Topal, M. Meszaros, G. Porkolab, A. Szecsko, T.F. Polgar, L. Siklos, M.A. Deli, S. Veszelka, A. Bozkir, ApoE-targeting increases the transfer of solid lipid nanoparticles with donepezil cargo across a culture model of the blood-brain barrier, Pharmaceutics 13 (1) (2020).
[218] N. Gandomi, R. Varshochian, F. Atyabi, M.H. Ghahremani, M. Sharifzadeh, M. Amini, R. Dinarvand, Solid lipid nanoparticles surface modified with anti-Contactin-2 or anti-Neurofascin for brain-targeted delivery of medicines, Pharm. Dev. Technol. 22 (3) (2017) 426–435.
[219] S. Ojha, B. Kumar, Preparation and statistical modeling of solid lipid nanoparticles of dimethyl fumarate for better management of multiple sclerosis, Adv Pharm Bull 8 (2) (2018) 225–233.
[220] P. Kumar, G. Sharma, V. Gupta, R. Kaur, K. Thakur, R. Malik, A. Kumar, N. Kaushal, O.P. Katare, K. Raza, oral delivery of methylthioadenosine to the brain employing solid lipid nanoparticles: pharmacokinetic, behavioral, and histopathological evidences, AAPS PharmSciTech 20 (2) (2019) 74.
[221] E. Ghasemian, A. Vatanara, N. Navidi, M.R. Rouini, Brain delivery of baclofen as a hydrophilic drug by nanolipid carriers: Characteristics and pharmacokinetics evaluation, J. Drug Deliv. Sci. Technol. 37 (2017) 67–73.
[222] P. Kumar, G. Sharma, R. Kumar, R. Malik, B. Singh, O.P. Katare, K. Raza, Enhanced brain delivery of dimethyl fumarate employing tocopherol-acetate-based nanolipidic carriers: evidence from pharmacokinetic, biodistribution, and cellular uptake studies, ACS Chem. Neurosci. 8 (4) (2017) 860–865.
[223] P. Kumar, G. Sharma, V. Gupta, R. Kaur, K. Thakur, R. Malik, A. Kumar, N. Kaushal, K. Raza, Preclinical explorative assessment of dimethyl fumarate-based biocompatible nanolipoidal carriers for the management of multiple sclerosis, ACS Chem. Neurosci. 9 (5) (2018) 1152–1158.
[224] D.G. Gadhave, C.R. Kokare, Nanostructured lipid carriers engineered for intranasal delivery of teriflunomide in multiple sclerosis: optimization and in vivo studies, Drug Dev. Ind. Pharm. 45 (5) (2019) 839–851.
[225] F. Laso-Garcia, J. Ramos-Cejudo, F.J. Carrillo-Salinas, L. Otero-Ortega, A. Feliu, M. Gomez-de Frutos, M. Mecha, E. Diez-Tejedor, C. Guaza, M. Gutierrez-Fernandez, Therapeutic potential of extracellular vesicles derived from human mesenchymal stem cells in a model of progressive multiple sclerosis, PLoS One 13 (9) (2018) e0202590.
[226] M. Riazifar, M.R. Mohammadi, E.J. Pone, A. Yeri, C. Lasser, A.I. Segaliny, L.L. McIntyre, G.V. Shelke, E. Hutchins, A. Hamamoto, E.N. Calle, R. Crescitelli, W. Liao, V. Pham, Y. Yin, J. Jayaraman, J.R.T. Lakey, C.M. Walsh, K. Van Keuren-Jensen, J. Lotvall, W. Zhao, Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders, ACS Nano 13 (6) (2019) 6670–6688.
[227] K. Clark, S. Zhang, S. Barthe, P. Kumar, C. Pivetti, N. Kreutzberg, C. Reed, Y. Wang, Z. Paxton, D. Farmer, F. Guo, A. Wang, Placental mesenchymal stem cell-derived extracellular vesicles promote myelin regeneration in an animal model of multiple sclerosis, Cells 8 (12) (2019).
[228] M. Jafarinia, F. Alsahebfosoul, H. Salehi, N. Eskandari, M. Azimzadeh, M. Mahmoodi, S. Asgary, M. Ganjalikhani Hakemi, Therapeutic effects of extracellular vesicles from human adipose-derived mesenchymal stem cells on chronic experimental autoimmune encephalomyelitis, J. Cell. Physiol. 235 (11) (2020) 8779–8790.
[229] A.D. Pusic, K.M. Pusic, B.L. Clayton, R.P. Kraig, IFNgamma-stimulated dendritic cell exosomes as a potential therapeutic for remyelination, J. Neuroimmunol. 266 (1–2) (2014) 12–23.
[230] D. Giunti, C. Marini, B. Parodi, C. Usai, M. Milanese, G. Bonanno, N. Kerlero de Rosbo, A. Uccelli, Role of miRNAs shuttled by mesenchymal stem cell-derived small extracellular vesicles in modulating neuroinflammation, Sci. Rep. 11 (1) (2021) 1740.
[231] Y. Xiao, J. Tian, W.C. Wu, Y.H. Gao, Y.X. Guo, S.J. Song, R. Gao, L.B. Wang, X.Y. Wu, Y. Zhang, X. Li, Targeting central nervous system extracellular vesicles enhanced triiodothyronine remyelination effect on experimental autoimmune encephalomyelitis, Bioact. Mater. 9 (2022) 373–384.
[232] G. Casella, J. Rasouli, A. Boehm, W. Zhang, D. Xiao, L.L.W. Ishikawa, R. Thome, X. Li, D. Hwang, P. Porazzi, S. Molugu, H.Y. Tang, G.X. Zhang, B. Ciric, A. Rostami, Oligodendrocyte-derived extracellular vesicles as antigen-specific therapy for autoimmune neuroinflammation in mice, Sci. Transl. Med. 12 (568) (2020).
[233] J. Zhang, B.A. Buller, Z.G. Zhang, Y. Zhang, M. Lu, D.L. Rosene, M. Medalla, T.L. Moore, M. Chopp, Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system, Exp. Neurol. 347 (2022) 113895.
[234] Y. Zhai, Q. Wang, Z. Zhu, Y. Hao, F. Han, J. Hong, W. Zheng, S. Ma, L. Yang, G. Cheng, High-efficiency brain-targeted intranasal delivery of BDNF mediated by engineered exosomes to promote remyelination, Biomater Sci 10 (19) (2022) 5707–5718.
[235] X. Zheng, K. Sun, Y. Liu, X. Yin, H. Zhu, F. Yu, W. Zhao, Resveratrol-loaded macrophage exosomes alleviate multiple sclerosis through targeting microglia, J. Control. Release 353 (2022) 675–684.
[236] Y. Xiao, Y. Zhang, Y.H. Gao, Z.H. Zhao, J. He, R. Gao, Y.X. Guo, L.B. Wang, X. Li, A targeted extracellular vesicles loaded with montelukast in the treatment of demyelinating diseases, Biochem. Biophys. Res. Commun. 594 (2022) 31–37.
[237] W.C. Wu, J. Tian, D. Xiao, Y.X. Guo, Y. Xiao, X.Y. Wu, G. Casella, J. Rasouli, Y.P. Yan, A. Rostami, L.B. Wang, Y. Zhang, X. Li, Engineered extracellular vesicles encapsulated Bryostatin-1 as therapy for neuroinflammation, Nanoscale 14 (6) (2022) 2393–2410.
[238] I. Osorio-Querejeta, S. Carregal-Romero, A. Ayerdi-Izquierdo, I. Mager, A. Egimendia Wood, M. Betanzos, A. Alberro, L. Iparraguirre, L. Moles, I. Llarena, M. Moller, F. Goni-de-Cerio, G. Bijelic, P. Ramos-Cabrer, M. Munoz-Culla, D. Otaegui, MiR-219a-5p enriched extracellular vesicles induce OPC differentiation and EAE improvement more efficiently than liposomes and polymeric nanoparticles, Pharmaceutics 12 (2) (2020).
[239] G. Casella, F. Colombo, A. Finardi, H. Descamps, G. Ill-Raga, A. Spinelli, P. Podini, M. Bastoni, G. Martino, L. Muzio, R. Furlan, Extracellular vesicles containing IL-4 modulate neuroinflammation in a mouse model of multiple Sclerosis, Mol. Ther. 26 (9) (2018) 2107–2118.
[240] S. Sinha, V. Garg, R.P. Sonali, R. Dutt Singh, Chitosan-alginate core-shell-corona shaped nanoparticles of dimethyl fumarate in orodispersible film to

improve bioavailability in treatment of multiple sclerosis: preparation, characterization and biodistribution in rats, J. Drug Deliv. Sci. Technol. 64 (2021).
[241] E. Montes-Cobos, S. Ring, H.J. Fischer, J. Heck, J. Strauss, M. Schwaninger, S.D. Reichardt, C. Feldmann, F. Luhder, H.M. Reichardt, Targeted delivery of glucocorticoids to macrophages in a mouse model of multiple sclerosis using inorganic-organic hybrid nanoparticles, J. Control. Release 245 (2017) 157–169.
[242] S. Beyer, L. Xie, M. Schmidt, N. de Bruin, M. Ashtikar, S. Ruschenbaum, C.M. Lange, V. Vogel, W. Mantele, M.J. Parnham, M.G. Wacker, Optimizing novel implant formulations for the prolonged release of biopharmaceuticals using in vitro and in vivo imaging techniques, J. Control. Release 235 (2016) 352–364.
[243] G. Cappellano, C. Comi, A. Chiocchetti, U. Dianzani, Exploiting PLGA-based biocompatible nanoparticles for next-generation tolerogenic vaccines against autoimmune disease, Int. J. Mol. Sci. 20 (1) (2019).
[244] A. Lutterotti, H. Hayward-Koennecke, M. Sospedra, R. Martin, Antigen-specific immune tolerance in multiple sclerosis-promising approaches and how to bring them to patients, Front. Immunol. 12 (2021) 640935.
[245] G. Cappellano, A.D. Woldetsadik, E. Orilieri, Y. Shivakumar, M. Rizzi, F. Carniato, C.L. Gigliotti, E. Boggio, N. Clemente, C. Comi, C. Dianzani, R. Boldorini, A. Chiocchetti, F. Reno, U. Dianzani, Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis, Vaccine 32 (43) (2014) 5681–5689.
[246] R.A. Maldonado, R.A. LaMothe, J.D. Ferrari, A.H. Zhang, R.J. Rossi, P.N. Kolte, A.P. Griset, C. O'Neil, D.H. Altreuter, E. Browning, L. Johnston, O.C. Farokhzad, R. Langer, D.W. Scott, U.H. von Andrian, T.K. Kishimoto, Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance, Proc. Natl. Acad. Sci. U. S. A. 112 (2) (2015) E156–E165.
[247] J.J. Cho, J.M. Stewart, T.T. Drashansky, M.A. Brusko, A.N. Zuniga, K.J. Lorentsen, B.G. Keselowsky, D. Avram, An antigen-specific semi-therapeutic treatment with local delivery of tolerogenic factors through a dual-sized microparticle system blocks experimental autoimmune encephalomyelitis, Biomaterials 143 (2017) 79–92.
[248] W. Pei, X. Wan, K.A. Shahzad, L. Zhang, S. Song, X. Jin, L. Wang, C. Zhao, C. Shen, Direct modulation of myelin-autoreactive CD4(+) and CD8(+) T cells in EAE mice by a tolerogenic nanoparticle co-carrying myelin peptide-loaded major histocompatibility complexes, CD47 and multiple regulatory molecules, Int. J. Nanomedicine 13 (2018) 3731–3750.
[249] A.F. Lima, I.R. Amado, L.R. Pires, Poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles loaded with proteolipid protein (PLP)-exploring a new administration route, Polymers (Basel) 12 (12) (2020).
[250] M. Gholamzad, H. Baharlooi, M. Shafiee Ardestani, Z. Seyedkhan, M. Azimi, Prophylactic and therapeutic effects of MOG-conjugated PLGA nanoparticles in C57Bl/6 mouse model of multiple sclerosis, Adv Pharm Bull 11 (3) (2021) 505–513.
[251] P.Y. Li, F. Bearoff, P. Zhu, Z. Fan, Y. Zhu, M. Fan, L. Cort, T. Kambayashi, E.P. Blankenhorn, H. Cheng, PEGylation enables subcutaneously administered nanoparticles to induce antigen-specific immune tolerance, J. Control. Release 331 (2021) 164–175.
[252] A.A. Belogurov Jr, A.V. Stepanov, I.V. Smirnov, D. Melamed, A. Bacon, A.E. Mamedov, V.M. Boitsov, L.P. Sashchenko, N.A. Ponomarenko, S.N. Sharanova, A.N. Boyko, M.V. Dubina, A. Friboulet, D.D. Genkin, A.G. Gabibov, Liposome-encapsulated peptides protect against experimental allergic encephalitis, FASEB J. 27 (1) (2013) 222–231.
[253] J.E. Kenison, A. Jhaveri, Z. Li, N. Khadse, E. Tjon, S. Tezza, D. Nowakowska, A. Plasencia, V.P. Stanton Jr, D.H. Sherr, F.J. Quintana, Tolerogenic nanoparticles suppress central nervous system inflammation, Proc. Natl. Acad. Sci. U. S. A. 117 (50) (2020) 32017–32028.
[254] I. Pujol-Autonell, M.J. Mansilla, S. Rodriguez-Fernandez, M. Cano-Sarabia, J. Navarro-Barriuso, R.M. Ampudia, A. Rius, S. Garcia-Jimeno, D. Perna-Barrull, E. Martinez-Caceres, D. Maspoch, M. Vives-Pi, Liposome-based immunotherapy against autoimmune diseases: therapeutic effect on multiple sclerosis, Nanomedicine (London) 12 (11) (2017) 1231–1242.
[255] K. Shimizu, K. Agata, S. Takasugi, S. Goto, Y. Narita, T. Asai, Y. Magata, N. Oku, New strategy for MS treatment with autoantigen-modified liposomes and their therapeutic effect, J. Control. Release 335 (2021) 389–397.
[256] Y.P. Hsiao, H.T. Chen, Y.C. Liang, T.E. Wang, K.H. Huang, C.C. Hsu, H.J. Liang, C.H. Huang, T.R. Jan, Development of nanosome-encapsulated honokiol for intravenous therapy against experimental autoimmune encephalomyelitis, Int. J. Nanomedicine 15 (2020) 17–29.
[257] Z. Li, F. Liu, X. He, X. Yang, F. Shan, J. Feng, Exosomes derived from mesenchymal stem cells attenuate inflammation and demyelination of the central nervous system in EAE rats by regulating the polarization of microglia, Int. Immunopharmacol. 67 (2019) 268–280.
[258] T.M. Ross, P.M. Martinez, J.C. Renner, R.G. Thorne, L.R. Hanson, W.H. Frey 2nd, Intranasal administration of interferon beta bypasses the blood-brain barrier to target the central nervous system and cervical lymph nodes: a non-invasive treatment strategy for multiple sclerosis, J. Neuroimmunol. 151 (1–2) (2004) 66–77.
[259] L.F. Gonzalez, E. Acuna, G. Arellano, P. Morales, P. Sotomayor, F. Oyarzun-Ampuero, R. Naves, Intranasal delivery of interferon-beta-loaded nanoparticles induces control of neuroinflammation in a preclinical model of multiple sclerosis: A promising simple, effective, non-invasive, and low-cost therapy, J. Control. Release 331 (2021) 443–459.
[260] M. Ghasemi-Kasman, N. Nosratiyan, M. Hashemian, S.R. Ahmadian, H. Parsian, S. Rostami-Mansoor, Intranasal administration of fingolimod (FTY720) attenuates demyelination area in lysolecithin-induced demyelination model of rat optic chiasm, Mult. Scler. Relat. Disord. 59 (2022) 103518.
[261] D. Rassy, B. Barcena, I.N. Perez-Osorio, A. Espinosa, A.N. Peon, L.I. Terrazas, G. Meneses, H.O. Besedovsky, G. Fragoso, E. Sciutto, Intranasal methylprednisolone effectively reduces neuroinflammation in mice with experimental autoimmune encephalitis, J. Neuropathol. Exp. Neurol. 79 (2) (2020) 226–237.
[262] B.G. Xiao, X.F. Bai, G.X. Zhang, H. Link, Suppression of acute and protracted-relapsing experimental allergic encephalomyelitis by nasal administration of low-dose IL-10 in rats, J. Neuroimmunol. 84 (2) (1998) 230–237.
[263] M. Alhouayek, J. Masquelier, P.D. Cani, D.M. Lambert, G.G. Muccioli, Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6, Proc. Natl. Acad. Sci. U. S. A. 110 (43) (2013) 17558–17563.
[264] A. Mwema, P. Bottemanne, A. Paquot, B. Ucakar, K. Vanvarenberg, M. Alhouayek, G.G. Muccioli, A. Des Rieux, Lipid nanocapsules for the nose-to-brain delivery of the anti-inflammatory bioactive lipid PGD(2)-G, Nanomedicine (2022) 102633.
[265] X.F. Bai, F.D. Shi, B.G. Xiao, H.L. Li, P.H. van der Meide, H. Link, Nasal administration of myelin basic protein prevents relapsing experimental autoimmune encephalomyelitis in DA rats by activating regulatory cells expressing IL-4 and TGF-beta mRNA, J. Neuroimmunol. 80 (1–2) (1997) 65–75.
[266] H.L. Li, J.Q. Liu, X.F. Bai, P.H. Van Der Meide, H. Link, Dose-dependent mechanisms relate to nasal tolerance induction and protection against experimental autoimmune encephalomyelitis in Lewis rats, Immunology 94 (3) (1998) 431–437.
[267] I.S. Rakover, N. Zabavnik, R. Kopel, M. Paz-Rozner, B. Solomon, Antigen-specific therapy of EAE via intranasal delivery of filamentous phage displaying a myelin immunodominant epitope, J. Neuroimmunol. 225 (1–2) (2010) 68–76.
[268] Y. Jiang, J. Zhu, G. Xu, X. Liu, Intranasal delivery of stem cells to the brain, Expert Opin Drug Deliv 8 (5) (2011) 623–632.
[269] J. Matias-Guiu, J.A. Matias-Guiu, P. Montero-Escribano, J.A. Barcia, A.A. Canales-Aguirre, J.C. Mateos-Diaz, U. Gomez-Pinedo, Particles containing cells as a strategy to promote remyelination in patients with multiple sclerosis, Front. Neurol. 11 (2020) 638.
[270] L. Danielyan, S. Beer-Hammer, A. Stolzing, R. Schafer, G. Siegel, C. Fabian, P. Kahle, T. Biedermann, A. Lourhmati, M. Buadze, A. Novakovic, B. Proksch, C.H. Gleiter, W.H. Frey, M. Schwab, Intranasal delivery of bone marrow-derived mesenchymal stem cells, macrophages, and microglia to the brain in mouse models of Alzheimer's and Parkinson's disease, Cell Transplant. 23 (Suppl. 1) (2014) S123–S139.
[271] J. Sun, Z.Z. Wei, X. Gu, J.Y. Zhang, Y. Zhang, J. Li, L. Wei, Intranasal delivery of hypoxia-preconditioned bone marrow-derived mesenchymal stem cells enhanced regenerative effects after intracerebral hemorrhagic stroke in mice, Exp. Neurol. 272 (2015) 78–87.
[272] F. Beigi Boroujeni, P. Pasbakhsh, K. Mortezaee, V. Pirhajati, R. Alizadeh, R. Aryanpour, S. Madadi, I. Ragerdi Kashani, Intranasal delivery of SDF-1alpha-preconditioned bone marrow mesenchymal cells improves remyelination in the cuprizone-induced mouse model of multiple sclerosis, Cell Biol. Int. 44 (2) (2020) 499–511.
[273] U. Gomez-Pinedo, J.A. Matias-Guiu, M.S. Benito-Martin, L. Moreno-Jimenez, I. Sanclemente-Alaman, B. Selma-Calvo, S. Perez-Suarez, F. Sancho-Bielsa, A. Canales-Aguirre, J.C. Mateos-Diaz, M.A. Hernandez-Sapiens, E.E. Reza-Zaldivar, D.D. Ojeda-Hernandez, L. Vidorreta-Ballesteros, P. Montero-Escribano, J. Matias-Guiu, Intranasal administration of undifferentiated oligodendrocyte lineage cells as a potential approach to deliver oligodendrocyte precursor cells into brain, Int. J. Mol. Sci. 22 (19) (2021).
[274] D. Zarini, P. Pasbakhsh, M. Shabani, S. Mojaverrostami, M. Hashemi, S. Amirizadeh, J. Majidpoor, A. Omidi, K. Mortezaee, I.R. Kashani, Glial response to intranasal mesenchymal stem cells in intermittent cuprizone model of demyelination, Neurotox. Res. 40 (5) (2022) 1415–1426.
[275] M. Fransson, E. Piras, J. Burman, B. Nilsson, M. Essand, B. Lu, R.A. Harris, P.U. Magnusson, E. Brittebo, A.S. Loskog, CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery, J. Neuroinflammation 9 (2012) 112.
[276] A. Fathollahi, S.M. Hashemi, M. Haji Molla Hoseini, S. Tavakoli, E. Farahani, F. Yeganeh, Intranasal administration of small extracellular vesicles derived from mesenchymal stem cells ameliorated the experimental autoimmune encephalomyelitis, Int. Immunopharmacol. 90 (2021) 107207.
[277] C. Ekhator, M.Q. Qureshi, A.W. Zuberi, M. Hussain, N. Sangroula, S. Yerra, M. Devi, M.A. Naseem, S.B. Bellegarde, P.R. Pendyala, Advances and opportunities in nanoparticle drug delivery for central nervous system disorders: a review of current advances, Cureus 15 (8) (2023) e44302.
[278] M.A. Hamburg, Science and regulation. FDA's approach to regulation of products of nanotechnology, Science 336 (6079) (2012) 299–300.
[279] D. Bagchi, M. Bagchi, H. Moriyama, F. Shahidi (Eds.), B. R., FDA and Nanotech: Baby Steps Lead to Regulatory Uncertainty, John Wiley & Sons, Ltd, Chichester, UK, 2013.
[280] H. Goel, V. Kalra, S.K. Verma, S.K. Dubey, A.K. Tiwary, Convolutions in the rendition of nose to brain therapeutics from bench to bedside: Feats & fallacies, J. Control. Release 341 (2022) 782–811.
[281] W. Alabsi, B.B. Eedara, D. Encinas-Basurto, R. Polt, H.M. Mansour, Nose-to-brain delivery of therapeutic peptides as nasal aerosols, Pharmaceutics 14 (9) (2022).
[282] M. Hallschmid, Intranasal Insulin for Alzheimer's Disease, CNS Drugs 35 (1) (2021) 21–37.
[283] E. Sahin, M. Hamamci, Y. Kantekin, Measurement of mucociliary clearance in

the patients with multiple sclerosis, Eur. Arch. Otorhinolaryngol. 277 (2) (2020) 469–473.
[284] R.G.R. Pinheiro, A.J. Coutinho, M. Pinheiro, A.R. Neves, Nanoparticles for targeted brain drug delivery: what do we know? Int. J. Mol. Sci. 22 (21) (2021).
[285] N. Huntemann, L. Rolfes, M. Pawlitzki, T. Ruck, S. Pfeuffer, H. Wiendl, S.G. Meuth, Failed, interrupted, or inconclusive trials on neuroprotective and neuroregenerative treatment strategies in multiple sclerosis: update 2015-2020, Drugs 81 (9) (2021) 1031–1063.