FACULDADE DE MEDICINA
UNIVERSIDADE D
COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

DIOGO FERRARO JUNQUEIRA

Sphingosine-1-phosphate and remyelination in pediatric-onset multiple sclerosis

REVISÃO NARRATIVA

ÁREA CIENTÍFICA DE FARMACOLOGIA

Trabalho realizado sob orientação de:

FILIPE MANUEL FARTO PALAVRA
JOANA AFONSO RIBEIRO

MARÇO 2023

Sphingosine-1-phosphate and remyelination in pediatric-onset multiple sclerosis

ARTIGO DE REVISÃO NARRATIVA

ÁREA CIENTÍFICA DE FARMACOLOGIA

Diogo Ferraro Junqueira[1]
[1]Faculdade de Medicina da Universidade de Coimbra
diogoferraro@hotmail.com

Filipe Manuel Farto Palavra[1,2]
[1]Faculdade de Medicina da Universidade de Coimbra
[2]Centro de Desenvolvimento da Criança – Neuropediatria, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Joana Afonso Ribeiro[2]
[2]Centro de Desenvolvimento da Criança – Neuropediatria, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Table of Contents

List of abbreviations

ADEM: Acute Disseminated Encephalomyelitis
ADS: Acquired Demyelinating Syndrome
AOMS: Adult-Onset Multiple Sclerosis
AQP4: Aquaporin-4
ARR: Annualized Relapse Rate
BBB: Blood-Brain Barrier
CNS: Central Nervous System
DMTs: Disease-Modifying Therapies
EMA: European Medicines Agency
FDA: Food and Drug Administration
HDL: High-Density Lipoprotein
IFN-beta: Interferon beta
IPMSSG: International Pediatric Multiple Sclerosis Study Group
MOG: Myelin Oligodendrocyte Glycoprotein
MOGAD: Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease
MS: Multiple Sclerosis
NK cells: Natural Killer Cells
NMOSD: Neuromyelitis Optica Spectrum Disorder
POMS: Pediatric-Onset Multiple Sclerosis
PPMS: Primary Progressive Multiple Sclerosis
RCT: Randomized Clinical Trial
RRMS: Relapsing-Remitting Multiple Sclerosis
S1P: Sphingosine-1-Phosphate
S1Pr: Sphingosine-1-Phosphate Receptor
SphK: Sphingosine Kinase
SPMS: Secondary Progressive Multiple Sclerosis

Abstract

Background: Pediatric-Onset Multiple Sclerosis (POMS) is a rare disease that represents 3-10% of all MS cases. Despite the therapeutic developments that have taken place for the form of the disease that begins in adults, most approved disease-modifying therapies (DMTs) lack studies that demonstrate their efficacy and safety in the pediatric population. Sphingosine-1-Phosphate (S1P) receptor agonists, like fingolimod and siponimod, have shown interesting results in adult-onset MS and there is a rise in the research regarding their effectiveness in treating POMS. This review explores the potential use of S1P receptor agonists in controlling neuroinflammation and promoting remyelination in children and adolescents diagnosed with MS.

Methods: Scientific articles, systematic reviews, narrative reviews, clinical trials, and clinical case reports were analysed, accordingly to their relevance, using the platform *PubMed*. Considering the previously defined inclusion criteria, 45 references have been selected, according to their relevance, which formed the basis for the elaboration of this review.

Results: S1P is a pleiotropic lysophospholipid mediator involved in various cellular processes, particularly in the immune and vascular systems. Its main role is to regulate lymphocyte egress from the thymus and secondary immune organs, via interaction with a S1P gradient. Fingolimod is the only drug acting on this system that is formally approved for use in POMS, based on the results of the PARADIGMS clinical trial. Its agonism for all types of S1P receptors, except for $S1Pr_2$, made it assume an interesting role in the treatment of children and adolescents, but the attempt to mitigate some of its adverse effects, increasing the selectivity on some of the receptors is causing other drugs to be investigated and may even bring more clinical benefit, particularly by promoting remyelination. Siponimod, ozanimod, ponesimod, ceralifimod and amiselimod are also S1P modulators, but still not approved for POMS.

Discussion: The overall usage of S1P modulators in MS patients is linked to a significant improvement in clinical and imaging measures and the knowledge acquired with the adult population has also been confirmed in children and adolescents, at least in relation to fingolimod. However, cardiovascular adverse effects, opportunistic infections, and an increased signal in the report of basal cell carcinomas are also associated with the use of this family of drugs. The development of molecules with more selective affinity for receptors that obviate some of these adverse effects is obviously very important, but no less relevant is their potential promoting effect on remyelination and neural repair, which is only now beginning to be properly explored.

Conclusion: Early diagnosis and treatment initiation are crucial in reducing major and irreversible lesions in POMS patients. The treatment approach can be an escalation strategy or an aggressive

initial intervention, with fingolimod approval changing the therapeutic attitude to consider the drug earlier in treatment algorithms. Pediatric patients may be considered an optimal clinical model for studying and refining the use of S1P modulators as agents that promote remyelination and early repair of the disease.

Keywords: multiple sclerosis; children; adolescents; remyelination; sphingosine-1-phosphate; fingolimod; siponimod.

1 – Background

Multiple sclerosis (MS) is a neuroinflammatory and neurodegenerative disease normally diagnosed in young adults, particularly in the age group between 20 and 40 years old (1). Nevertheless, it can have its onset before the age of 18, being defined as Pediatric-Onset Multiple Sclerosis (POMS). This is estimated to be 3-10 % of all the patients diagnosed with MS, thus being considered a relatively rare disease in children and adolescents (2). There are also authors who refer to this disease as early-onset MS or juvenile MS (3).

From a clinical point of view, the first event of central nervous system (CNS) demyelination, before the age of 18, is referred to with the generic term of Acquired Demyelinating Syndrome (ADS). When dealing with children, around 20% of the cases are diagnosed as MS, right at the first event (4). The development of laboratory and imaging techniques in recent years has allowed important advances in differential diagnosis and other relevant ADS are now to be considered in the pediatric age: myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD), aquaporin-4 antibody-associated neuromyelitis optica spectrum disorder (AQP4-NMOSD) or even monophasic conditions, as with most acute disseminated encephalomyelitis (ADEM), which may be the first manifestation of any of the previously mentioned syndromes.

Considering POMS, the symptoms that may arise in children are the same as those associated with the adult-onset form of the disease. They include frequent involvement of long tracts, brainstem symptoms, optic neuritis, and myelitis, as more suggestive of the diagnosis (5). Some patients may have polyregional symptoms, which corroborates a relatively aggressive aspect of this disease at an early stage of life (6). However, it is unlikely that progressive forms of the disease, either primary (PPMS) or secondary (SPMS) will be seen at a pediatric age, with most cases corresponding to relapsing-remitting forms (RRMS) (7). Finally, it is more common for POMS patients to show a higher relapse rate when compared to adult-onset MS.

As for the diagnosis, the McDonald criteria, defined for adults and revised in 2017, are also used in children, apart from having to comply simultaneously with the recommendations of the International Pediatric MS Study Group (IPMSSG) (6). The criteria are fundamentally based on the affirmation of the existence of dissemination in time and space, after excluding any other explanation for the clinical manifestations presented by the patient (6). With the evolution of this diagnostic process, new therapies and regimens have been studied, each

of them directly affecting some specific mechanism in the complex pathophysiology of MS. After making the diagnosis, it is possible to decide on a more incisive therapeutic plan, to be started as soon as possible, or in a more conservative approach. Some disease-modifying therapies (DMTs) used in adult-onset MS (AOMS) have also been tested on children, but most of the molecules that are used in the treatment of adults do not yet have studies that support their use in the pediatric population.

Agonists of the Sphingosine-1-Phosphate (S1P) receptors are an example of DMTs for MS and have been used in AOMS therapy since 2010, having fingolimod first in class. S1P is a lipid complex, which binds G protein-coupled receptors and can influence immune cells, so, these S1P receptors (S1PRs) can be considered targets in the treatment of many inflammatory and immune-mediated conditions (8). Fingolimod is the only molecule that acts by agonism of S1PRs which has already been tested in POMS. This drug was compared with interferon beta-1a in the PARADIGMS trial, demonstrating interesting results, both from a clinical point of view, with a significant reduction in the annualized relapse rates, and from an imaging perspective (9). Another S1P agonist, siponimod, has been approved for the treatment of AOMS in both the RRMS and SPMS forms. Additionally, a clinical trial is currently underway that aims to compare the use of this molecule with fingolimod and with ofatumumab (a fully human anti-CD20 monoclonal antibody) in children aged between 10 and 17 years. Fingolimod-phosphate, the active form of the molecule, binds to four of the five subtypes of S1PRs (it binds to $S1Pr_1$, $S1Pr_3$, $S1Pr_4$ and $S1Pr_5$), whereas siponimod binds only to receptors $S1Pr_1$ and $S1Pr_5$. This allows siponimod to alleviate the cardiac adverse effects that are recognized with fingolimod (and which result from $S1Pr_3$ receptor agonism) and allows exploring the potential promoter of remyelination that has been associated with the $S1Pr_5$ receptor (10).

The purpose of this narrative review is to look up the scientific evidence regarding the S1P functioning and its receptors' agonists, in view of the control of neuroinflammation and the potential effect promoting remyelination in the pediatric population.

2 – Material and methods

To elaborate this narrative review, scientific articles, reviews, and clinical case reports were analysed, having been extracted from the *Pubmed* platform. The searching equation was initially: ((fingolimod)) AND (siponimod)) AND (multiple sclerosis); 79 results have been found. After that, another search was done, to include scientific evidence related to pediatric MS, using the equation: (pediatric multiple sclerosis)) AND (fingolimod); 77 results have been found, many of them already present in the previous search. In addition, both searches were restrained to articles written in english, portuguese or french, as well as published between 2017 and 2022. After reading the abstracts of all the articles, 45 references have been selected, according to their relevance, which formed the basis for the elaboration of this narrative review.

3 – Results

3.1 – Sphingosine-1-Phosphate

S1P is a lysophospholipid, resulting from the phosphorylation of sphingosine, by sphingosine kinase 1 or 2 (SphK1, SphK2) (10). It is a pleiotropic lipid mediator involved in different cellular processes, such as proliferation, migration, adhesion, and inflammation, especially in the immune and vascular systems. Among all sphingolipids, S1P is the most well-characterized intracellular signalling molecule (11). In normal conditions, the major reserves of S1P are erythrocytes and endothelial cells, however, during inflammation, platelets and mast cells may also lead to its overproduction (10).

This molecule can influence cellular processes, such as growth, survival, motility, angiogenesis and vascular integrity, neural development, and immune cell trafficking (11,12). The main one is the regulation of lymphocyte egress from the thymus and secondary immune organs, and it is in this step that most of the S1P modulators, described in this review, have their mechanism of action, by interacting with the S1P gradient. Normally, S1P has low concentrations in intracellular and interstitial fluids. On the other hand, in the blood and lymph, there is a higher concentration, which leads to an S1P gradient. Lymphocytes use this gradient to egress from the circulation to the lymphoid organs. When in the higher concentration fluids, S1P of lymphocytes is internalized, whereas in the lymphoid organs these cells gradually recover the surface expression of S1P, because of the low concentration, which gives them the ability to migrate out of the lymphoid organs. This gradient-dependent process is not unique to lymphocytes, it is also utilized by other immune cells, such as dendritic cells, natural killer cells (NK cells), and splenic B-cells (11).

Regarding vascular integrity, some authors theorize about a probable vascular-intrinsic stabilization capacity of S1P, leading to the better response of vessels to inflammatory signals. This molecule stabilizes the endothelial cells' cytoskeleton and strengthens the adherence junctions; consequently, the endothelial barrier becomes less permeable, one of the inflammatory processes' basal features. The blood-brain barrier (BBB) is also affected by S1P, as it acts the same way on the brain endothelial cells (13).

S1P activity may be controlled in multiple steps, being its metabolism the first one: sphingolipids’ synthesis starts with the condensation of serine and palmitoyl-CoA into 3-keto-dihydrosphingosine. After multiple enzymatic reactions, ceramide is obtained, being hydrolysed, by ceramidase, to sphingosine and, ultimately, leading to the production of S1P

(14). As for S1P, S1P phosphatase and S1P lyase are the two enzymes that keep the intracellular balance and affect its concentration. The second step refers to its transport: most of the S1P, in the plasma, is attached to High-Density Lipoprotein (HDL) (around 70%), and the rest is transported by albumin (11,12). It is also known that apolipoprotein M has a specific chaperone function, which was proven to ameliorate the exportation of S1P from erythrocytes to HDL, in comparison to albumin (15). Finally, S1P has its effects projected via specific receptors, which have a high affinity to G-proteins. Till now, there are 5 subtypes described: $S1Pr_{1-5}$, widely spread in the body (11) and summarized in Table 1.

3.2 – S1P receptors

3.2.1. $S1Pr_1$

This receptor has its role in neurogenesis, angiogenesis, endothelial barrier function, as well as in regulating vascular tone. $S1Pr_1$ is the one with a stronger influence on the S1P gradient described before, with lymphocyte expression, being also expressed in the endothelium where it affects vascular permeability (16). Studies in mouse models of MS, namely with experimental autoimmune encephalomyelitis, have shown that blocking $S1Pr_1$ leads to survival of the neurons and inhibition of demyelination (17), this being a critical aspect to consider this receptor as an important therapeutic target in MS.

3.2.2. $S1Pr_2$

This receptor appears in relation to mast cell degranulation, histamine secretion, contraction of bronchial smooth muscle, and hair cell survival in the inner ear. $S1Pr_2$ deletion was proven to reduce the secretion of reactive oxygen species, leading to the protection of the neural cells (18). It is also related to the development of the heart and the auditory and vestibular systems (19).

In a 2021 study, Jonnalagadda D *et al* described the link between glutamate uptake by astrocytes and this specific subtype of S1Pr. S1P was identified as an inhibitor of glutamate uptake, in a dose-dependent manner, as well as an increasing factor of mitochondrial oxygen consumption, especially because of the activation of $S1Pr_2$. Usually, the excess of glutamate is controlled by the astrocytic uptake, followed by the conversion into non-toxic glutamine. The investigation in receptor knock-out mice found that $S1Pr_2$ was the receptor responsible for this inhibition in contrast to the other S1P receptors, which were not associated with this pathway. With the discovery of this interaction, the authors point to

the search for $S1Pr_2$ antagonists, to prevent glutamate neurotoxicity, present in neurological diseases, such as MS, and presenting with high concentrations of S1P (20).

3.2.3. $S1Pr_3$

This receptor has an important influence on heart rate, as well as $S1Pr_1$ (16). There are also authors who affirm $S1Pr_3$ regulates vascular tone, by vasodilation (19). Others refer to its vasoconstrictive effect, by developing specific $S1Pr_3$ antagonists and showing that its inhibition leads to a decrease in the concentration of calcium and protein Rho activation, involved in the contraction of smooth muscle cells (21). This receptor might have a dual effect on the immune system, as inducing pro and anti-inflammatory responses, but more research is needed, regarding this issue.

Most of the evidence linking MS to $S1Pr_3$ is related with its effect on the heart's conductive system, since fingolimod (the first in class acting on these receptors), specifically by inhibiting $S1Pr_3$ function, generates a negative chronotropic response, which will be further explored.

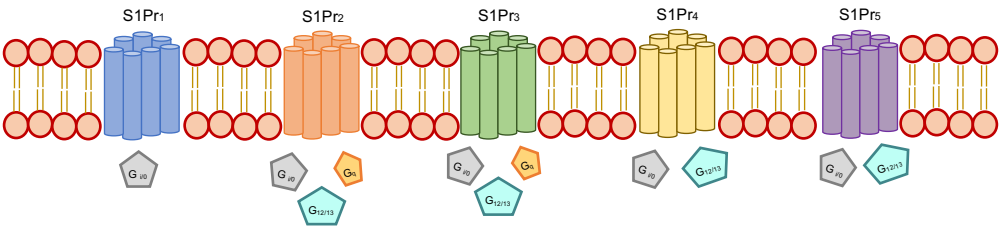
3.2.4. $S1Pr_4$

This receptor is expressed especially in immune cells, but it is also present in airway smooth muscle cells (16). The function of this subtype is maybe the less well studied, but is associated with cytokine production by T cells, being $S1Pr_4$ a negative regulator of its response (19).

3.2.5. $S1Pr_5$

Mainly expressed in oligodendrocytes and brain endothelial cells, $S1Pr_5$ can alter BBB integrity, having an important role in its maintenance. Its activation leads to a decrease in inflammatory cell adhesion on the surface and improves the protection of endothelial cells against penetration by monocytes (22). In addition, it regulates NK cell trafficking and seems to be involved in regulating distinct oligodendrocyte precursor cells and oligodendrocyte related processes (and these could be, in theory, important for remyelination). However, it is still unclear if the S1P modulators' interaction with this specific subtype of receptor leads to clinically valuable results in MS (16).

Table 1. S1Pr subtypes; function, cell and tissue expression and the respective antagonists available.



CELL EXPRESSION	Lymphocytes Neuronal cells Endothelial cells Cardiac conductive system Smooth muscle	CNS cells Endothelial cells Smooth muscle cells	Neuronal cells Cardiac conductive system Endothelial cells Smooth muscle cells	Lymphocytes	CNS cells NK cells
FUNCTION	Egress from lymph nodes Neuron migration and function Heart rate slowing Barrier permeability	Hearing and balance Barrier permeability Vascular tone	Neuron migration and function Barrier permeability	Lymphoid tissue expression Dendritic and Th17 cell modulation	Oligodendrocyte function NK cell migration
ORGAN EXPRESSION	Brain, heart, spleen, liver, lung, thymus, kidney, skeletal muscle, lymphoid organs	Brain, heart, spleen, liver, lung, thymus, kidney, skeletal muscle	Brain, heart, spleen, liver, lung, thymus, kidney, skeletal muscle, testis	Lymphoid organs, lung	Brain, skin, spleen
DRUG	Fingolimod Siponimod Ozanimod Ponesimod Ceralifimod Amiselimod		Fingolimod	Fingolimod Amiselimod	Fingolimod Siponimod Ozanimod Ponesimod Ceralifimod Amiselimod

3.3 – S1P modulators approved for POMS

During the past years, there has been a vast increase in the investigation of POMS, regarding its epidemiology, pathophysiology, diagnosis, and treatment. This led to a change in the number and types of drugs used in the therapeutic plan, as well as an earlier diagnosis and initiation of DMTs. However, in this special population, the evidence of DMT's effectiveness is not as strong as in adults, since most of it comes from observational studies and, that is why, to date, there is only approval of one S1P modulator, fingolimod, to treat this disease in the pediatric age (3).

Currently, the first line treatment considered for POMS is still interferon beta (IFN-beta) or glatiramer acetate (>12 years of age), since observational studies have shown these drugs to be safe and effective, with no serious adverse effects noted in this population (3,5).

Fingolimod, as a DMT, is considered more effective than IFN-beta and glatiramer acetate. Its usage comes after its approval by the American Food and Drug Administration (FDA) and European Medicines Agency (EMA), after the findings of the PARADIMGS clinical trial, published in 2018 (3,5,9). In a 2021 study, Sandesjö *et al* proceeded with a survey which questioned clinicians regarding their therapeutic approach to POMS and weather the COVID-19 pandemic had affected their way of act, namely considering the possible reduction in the prescription of more effective drugs, such as fingolimod. The interesting finding was the increasing use of highly effective DMTs, in an earlier stage of the disease, being this slightly affected by COVID-19 (23).

3.3.1. Fingolimod (FTY720)

The mechanism of action of this S1P modulator is based on the inhibition of the gradient described before. By having a high affinity for all the S1P receptors, except $S1Pr_2$, this drug acts as an antagonist of the receptors, inhibiting the normal egress of lymphocytes from the lymphoid organs. That turns to a reduction in the circulating lymphocytes, which leads to a limited inflammatory cell migration into the CNS, resulting, in consequence, in a smaller activity of the disease (16). Since it is an S1P analogue, fingolimod is phosphorylated by SphK and then fingolimod-P will bind to S1P receptors, which are coupled to different G proteins. When this bond happens, the G protein is activated, leading to the internalization of the receptor which would let lymphocytes egress to the lymph (24).

As already stated, the expression of S1P is not exclusive of immune cells, being present also in endothelial cells and astrocytes, main components of the BBB. Fingolimod can preserve BBB properties, by reducing its permeability by other cells. The increased barrier permeability is one of MS features, leading to the passage of aggressive cells which induce demyelination and axonal loss. Fingolimod lipophilic nature was proven to allow it to penetrate the CNS and, by modulating S1P functions, diminish the endothelial leakage previously present (25,26).

As mentioned, fingolimod was approved to be used in children and adolescents in face of the results arising from the PARADIGMS study (9). This trial enrolled 10- to 17-year-old patients with the diagnosis of MS. For 24 months, the comparison between the efficacy and safety of oral fingolimod (0.5 mg daily or 0.25 mg, for weights less than 40 kg) and intramuscular interferon beta-1a (30 µg weekly) was made. The results showed clearly superior efficacy of fingolimod, with an 82% relative reduction in the annualized relapse rate, compared to interferon. It should be noted that the annualized relapse rate in the interferon beta-1a group was almost twice what was observed in adult patients receiving the same treatment, in the TRANSFORMS trial (that underpinned the approval of fingolimod for adults), and so, the authors conclude that interferon might be considered weak in the pediatric population, when compared with fingolimod. Nevertheless, this drug is still used as first line treatment for POMS (9).

Concerning safety and tolerability, the PARADIGMS study demonstrated that fingolimod is well tolerated in POMS. No serious adverse events were reported, and the trial described some cases of seizures and leukopenia (what would be expected, given the mechanism of action of the drug). Mild adverse effects were reported, such as lymphopenia and mild infectious complications. The association of fingolimod and skin carcinomas was not found in the pediatric population studied, in contrast with the increased risk described in adult trials (9). Nevertheless, there were some cases of bradyarrhythmia and/or macular edema reported, as well as Herpes virus infections, thus being recommended to screen for these conditions prior to the administration of fingolimod in children and adolescents, as it already happens with adults. As for the long-term effects of fingolimod on neurodevelopment, there is not enough evidence available to conclude anything, since there is a lack of longitudinal data (27). Fingolimod has a long circulatory half-life, which leads to a longer reduction of lymphocyte count, even after the administration, and this aspect can be related to more adverse reactions (28). This topic will be explained further in this review.

3.4 – S1P modulators under study for POMS

3.4.1. Siponimod (BAF312)

This drug was the second to be tested in clinical trials in AOMS, having been approved by FDA in 2018 for the treatment of SPMS. Siponimod was developed having its precursor fingolimod in mind, with the aim of finding a more selective $S1Pr_1$ modulator: siponimod has high affinity with $S1Pr_1$ and $S1Pr_5$ receptors (only) and, unlike fingolimod, it does not require phosphorylation to be activated and does not have as many adverse effects on the heart, as it does not bind to $S1Pr_3$ receptors. Still, the mechanism of action is similar and so siponimod also makes use of the S1P gradient, preventing the egress of lymphocytes (10), and, with this, a decrease the disease's autoimmune activity.

Some preclinical studies have demonstrated a protective effect of siponimod within CNS, through direct influence on astrocytes and oligodendrocytes (10,25). Other authors point out that siponimod reduces oligodendrocytes' kinetics and axonal loss, during both acute and chronic phases of demyelination (29). Spampinato *et al*, in a study published in 2021, using an in-vitro BBB model of endothelial-astrocytes co-culture exposed to an inflammatory insult, demonstrated that siponimod reduced the migration of peripheral blood mononuclear cells through the endothelial layer, only in the presence of astrocytes, concluding about this drug's effect on the stability and reduced permeability of the astrocyte/endothelial barrier (26). In respect to remyelination, even though some authors did not find significative evidence in siponimod's potential (29), there are others who stated, with preclinical observations, that the drug has promyelinating and neuroprotective properties, because of other effects of $S1Pr_1$ and essentially of $S1Pr_5$, still not completely understood. Montarolo *et al* investigated the expression of NR4A2, a transcription factor of the steroid nuclear hormone receptor family 4, group A (NR4A), in a sample treated with siponimod (30). This hormone family is related to an anti-inflammatory and protective function of the nervous system, by reducing the transcription of pro-inflammatory factors, such as NF-kB, in blood and CNS. In this study, authors found that siponimod increased the expression of NR4A1 and NR4A2, in the N9 microglial cell line, as well as increased expression of TREM-2, a protein expressed in microglia, which facilitates recovery from brain injury, proposing its beneficial effects in CNS cells. However, no effect was found on oligodendrocytes and peripheral blood mononuclear cells, in this study (30). In contrast, a clinical experience with *Xenopus tadpoles*, in 2018, pointed to the particular interest of $S1Pr_5$ receptor activity in the

myelin sheath repair; by comparing the effect on myelination of multiple compounds, the study stated siponimod was the most efficient, with its effect primarily based on $S1Pr_5$ receptor subtype activity, since with the S1Pr5-knocked-out model, the drug was no longer active (31). The protective capacity of siponimod was also mentioned in relation with astrocytes; a 2020 experiment generated human fibroblast-derived astrocytes, to identify the pharmacological effects of S1P modulators; the direct impact of siponimod on NFkB activation and glutamate transporters was shown, supporting the anti-inflammatory function of this drug (32).

Concerning clinical trials, the most important study of the development of siponimod was the EXPAND study, a double-blind phase 3 multicentric trial, where the effect of siponimod was assessed on the progression of SPMS, by comparing its administration with a placebo. The S1P modulator decreased the risk of disease progression and has shown a safety profile similar to the previously tested S1P modulator, fingolimod (33). In 2020, data of EXPAND core and its extension up to >5 years were, also, analysed, corroborating the long-term clinical efficacy and consistent safety profile presented before. In the extension phase, patients receiving the placebo before were switched to siponimod; when comparing this sample with the group treated with the active drug since the beginning, both clinical and MRI features supported the fact thar earlier intervention with siponimod leads to better overall disease behaviour (34).

Even considering all the potential of siponimod described in the adult population, so far fingolimod is the only S1P modulator approved worldwide for POMS (9). Nevertheless, there is an on-going clinical trial, the phase 3 NEOS study, which pretends to assess the efficacy and safety of siponimod and ofatumumab, in patients with POMS, from 10 to 17 years, in comparison with fingolimod. NEOS includes a 2-year double blind core phase and an open-label extension part, and its primary outcome is the reduction of the annualized relapse rate in treated population, to conclude by the non-inferiority of siponimod and ofatumumab in comparison to fingolimod. Variables related to safety and imaging data will also be collected and analysed (35).

3.4.2. Ozanimod (RPC1063)

This drug has specific affinity for $S1Pr_1$ and $S1Pr_5$, inducing $S1Pr_1$ internalization and reduction of circulating lymphocytes. Preclinical studies presented this drug as effective as fingolimod, in the EAE model, reducing the inflammatory activity of the disease (28). Plus,

some authors found ozanimod's safety profile better, when comparing it with fingolimod (36). Ozanimod does not need to be phosphorylated to be activated, but it presents delayed absorption. This was revealed to be beneficial since the lower peak in blood concentration provokes fewer first-dose effects on heart rate, which, in themselves, are not very frequent, considering the weak affinity for the $S1Pr_3$ receptor (10).

Even though the drug will enter clinical trials recruiting children and adolescents soon, till now, there is no evidence of its efficacy in this special population.

3.4.3. Ponesimod (ACT-128800)

Ponesimod is an orally active S1P modulator with selective activity mostly on $S1Pr_1$ receptor (it has much less activity on the $S1Pr_5$ one, compared to the previously mentioned) (37). It leads to the same lymphopenia as the others, manipulating the S1P gradient, by internalization and further degradation of the receptors (29). Some preclinical studies revealed that ponesimod reduced the amount of both B and T cells in the blood and spleen, as well as general inflammatory activity: pro-inflammatory cytokines, development of edema, and cell infiltration. Another important pharmacological aspect is its half-life; contrary to fingolimod's long half-life and slow elimination, ponesimod is excreted after 1 week of discontinuation, which is an advantage when speaking of after-therapy adverse effects (37).

As ozanimod, there are clinical trials expected to be conducted with ponesimod, studying children and adolescents with MS. However, so far there is no evidence supporting the prescription of ponesimod to the pediatric population.

3.4.4. Ceralifimod (ONO-4641)

This is another selective $S1Pr_1$ modulator, with some activity on the $S1Pr_5$ receptor as well. A phase II clinical trial was completed in adults, showing a reduction of gadolinium-enhanced lesions in patients receiving this drug (38). Ceralifimod was not studied in further trials, since the manufacturers had referred to changes in the overall MS therapeutic paradigm and did not proceed with the investigation (37).

3.4.5. Amiselimod (MT-1303)

Some authors also mention this selective $S1Pr_1$ modulator, stating that its clinical studies have revealed a good outcome of treated patients, with a low risk for bradycardia (39). Once again, the developing company decided not to proceed the investigation with a phase III study.

Table 2 summarizes some of the most significant aspects related to the pharmacokinetics of these products.

Table 2. Pharmacokinetics of the different S1P modulators tested till the present moment.

S1P Modulator	***Targets***	***Time to maximum plasma concentration***	***Half-life***	***Effect on lymphocyte count***	***Lymphocyte recovery***	***Approval for MS treatment***
Fingolimod	$S1P_1$, $S1P_3$, $S1P_4$, $S1P_5$	12-16 hours	6-9 days	~73% reduction	4-8 weeks	AOMS and POMS
Siponimod	$S1P_1$, $S1P_5$	3-8 hours	22-38 hours	~70% reduction	7-10 days or 3-4 weeks	AOMS (RR and SP forms)
Ozanimod	$S1P_1$, $S1P_5$	6-8 hours	19-22 hours	~68% reduction	30 days-3 months	AOMS (relapsing MS)
Amiselimod	$S1P_1$, $S1P_5$	12-16 hours	32 hours	60-66% reduction	7 days	Discontinued
Ceralifimod	$S1P_1$, $S1P_5$	4-6 hours	~ 3 days	40-65% reduction	14 days	Discontinued
Ponesimod	$S1P_1$, $S1P_5$	2-4 hours	15-17 days	~70% reduction	~7 weeks	AOMS (relapsing MS)

4 – Discussion

The approval of the use of fingolimod for the treatment of POMS opened a new page in the therapeutic approach of this clinical situation, in children and adolescents. SP1 modulators are being increasingly used as an alternative to parenteral formulations of IFN-beta and glatiramer acetate, which is why they have effectively allowed for a true change in the paradigm of disease treatment, prioritizing the use of oral drugs.

Since the first line of prolonged treatment in POMS had been consisting in injectable drugs, most of the S1P modulators have been tested in comparison to IFN-beta. A recent meta-analysis of published randomized controlled trials regarding the safety and efficacy of S1P modulators in the treatment of RRMS patients revealed interesting conclusions. First, the overall usage of S1P modulators was linked to a significant decrease in annualized relapse rates, as well as in the number of new or enlarging T2 lesions on MRI scans. This aspect consolidates the effective anti-inflammatory role of these drugs, which is crucial, given the frankly inflammatory nature of the pediatric disease. Also, S1P modulators' use was associated with better scores in the MSQOL-54 (Multiple Sclerosis Quality of Life-54), a physical health self-report, reinforcing their benefit in quality of life (36).

In any case, the adverse effects reported in children and adolescents are not negligible. Several cardiovascular adverse effects (AEs) of fingolimod, such as atrioventricular block, sinus bradycardia, orthostatic hypotension, and hypertension, have been reported, even throughout the pharmacological development program, having been corroborated in the PARADIGMS clinical trial (9,10). These effects are explained by the expression and localization of the S1Pr subtypes and by the drug activity profile on them. As already mentioned, $S1Pr_1$ is also expressed in atrial myocytes and endothelial cells and $S1Pr_3$ is found in both smooth muscle and endothelial cells. The activation of the G protein-coupled receptors, particularly $S1Pr_1$ and $S1Pr_3$, leads to myocyte hyperpolarization and a transitory reduction of their excitability state, elucidating why patients may present bradyarrhythmias and initial hypotension, after initiating these drugs. The continuous dosing and consequent effect on S1P gradient were found to shift the receptor profile, leading to an opposite finding in blood pressure, and hypertension (40). Since the cardiac events were believed to be triggered by the activation of $S1Pr_1$ and $S1Pr_3$ in cardiomyocytes, one of the purposes of the development of more specific agents, after fingolimod's trials, was to understand if a non-interaction with $S1Pr_3$ could lead to less frequent cardiac AEs. In 2022, Al-Yafeai *et al* reviewed the cardiac AEs of all S1P modulators approved and concluded that

even though siponimod's activity is restricted to $S1Pr_1$ and $S1Pr_5$ modulation, cardiac AEs were still present (although to a much lesser extent). However, ponesimod and ozanimod did not show that association, suggesting these last two might be better therapeutic options, to avoid cardiac interference, particularly if that comorbidity is present (41). Considering this potential cardiovascular impact, it is recommended that all patients receive the first dose of these S1P modulators in a hospital environment, with monitoring of blood pressure, electrocardiogram, and heart rate.

Opportunistic infections are also an important aspect that deserves some discussion, regarding the use of these drugs. It makes sense that a drug that incites a decrease in lymphocyte count can be associated with some infections, as it happens with any immunocompromised patient. Sharma *et al* reviewed the existing data on the most common infections associated with S1P modulators, being them caused by Varicella Zoster Virus, Herpes Simplex Virus and *Cryptococcus neoformans* (42). Since immunocompromised patients can sometimes present clinically atypical manifestations, it is very important to take into account the possibility of these infections in all patients starting treatment with S1P modulators, accessing all the possible symptoms of an initial infectious process, before they might become serious or even fatal conditions.

Schoedel *et al* also reviewed the available evidence around S1P modulators' use to search for correlation with abuse of the prescription. Nevertheless, these drugs do not appear to be associated with abuse or any dependence potential (43).

Some authors point to the association of treatment with S1P modulators and skin cancers, particularly, basal cell carcinomas. By analysing FDA's databases of adverse effects, populations treated with fingolimod and siponimod were found to have an increased signal in the report of basal cell carcinomas. The pathophysiological hypothesis described was focused on the mechanism of action of S1P modulators, as lymphopenia could result in a more difficult identification and elimination of malignant cells (44). Because of this correlation, it is imperative to screen for pre-cancerous skin lesions, as well as to have regular skin examinations once treatment is initiated.

The PARADIGMS study revealed a higher frequency of seizures in the pediatric population treated with fingolimod than what was known in the adult (9). This naturally deserves some concern, but it does not appear that this adverse effect can be directly attributed to the use of the drug. The disease, being much more inflammatory in the pediatric age, naturally courses with more significant lesion loads, including the appearance of cortical

lesions, which can be potentially epileptogenic. When approached in this way, this AE does not require, in clinical practice, any other type of evaluation or monitoring.

One of the most interesting chapters in the history of the use of these drugs, in POMS, may still be written and that concerns their potential to promote remyelination. The biology of $S1Pr_5$ receptors is still not perfectly characterized and it is likely that it is through them that S1P modulators can promote remyelination and neural repair, acting on oligodendrocytes and respective precursor cells. In patients who participated in the EXPAND study, it was possible to identify siponimod in the cerebrospinal fluid, proving that the drug crosses the BBB (33). However, the concentration it reaches in this same location may be critical for the occurrence of remyelination and this aspect is still unclear. If, in fact, this repair takes place and effectively, children may be the ideal clinical model to better study it and to optimize the positioning of S1P modulators in the POMS treatment algorithm.

5 – Conclusion

Most investigators claim that the most important aspect when treating patients with POMS is the earlier diagnosis and initiation of any treatment since it is the most efficient way of reducing the appearance of major and/or irreversible lesions in patients' CNS. There is an increasing debate regarding the therapeutic attitude, with some clinicians defending an escalation strategy, used most of the time, versus an aggressive initial intervention (the so-called induction therapy). Since the approval of the use of fingolimod in the treatment of POMS, the therapeutic attitude has changed, in the sense of considering this drug earlier in the treatment of the disease, which also allows to obviate some of the adverse effects that are attributed to the classic injectable drugs (45).

There are several ongoing clinical trials that try to expand the range of therapeutic options for POMS, and this is a clinically desirable situation. More than that, a set of potential remyelination-promoting strategies is also being developed, and S1P modulators may have a word to say in this regard. The future will certainly be interesting in this field.

Acknowledgments

I express my gratitude to Dr. Filipe Palavra for his invaluable guidance and support throughout this process. His expertise in the field of neuropediatrics has been an inspiration, and his passion for the subject powered my enthusiasm while writing.

I am also grateful to my family, especially to my mother, who has been a constant source of encouragement and patience throughout my medical course. Their unwavering support and wise advice have been instrumental in helping me navigate the challenges of this journey.

Finally, I acknowledge my dear friends, for their constant support and companionship throughout these years. Their motivation and humour have been a source of inspiration and joy in my life.

References

1. Jakimovski D, Awan S, Eckert SP, Farooq O, Weinstock-Guttman B. Multiple Sclerosis in Children: Differential Diagnosis, Prognosis, and Disease-Modifying Treatment. *CNS Drugs*. 2022;36(1):45–59. DOI: 10.1007/s40263-021-00887-w.
2. Deiva K. Pediatric onset multiple sclerosis. *Revue Neurologique* (Paris). 2020 Jan 1;176(1–2):30–6. DOI: 10.1016/j.neurol.2019.02.002
3. Brola W, Steinborn B. Paediatric multiple sclerosis-current diagnosis and treatment. *Neurol Neurochir Pol*. 2020 Dec 31;54(6):508–17. DOI: 10.5603/PJNNS.A2020.0069
4. Fadda G, Armangue T, Hacohen Y, Chitnis T, Banwell B. Paediatric multiple sclerosis and antibody-associated demyelination: clinical, imaging, and biological considerations for diagnosis and care. *Lancet Neurol.* 2021 Feb 1;20(2):136–49. DOI: 10.1016/S1474-4422(20)30432-4
5. de Chalus A, Taveira M, Deiva K. Pediatric onset multiple sclerosis: Future challenge for early diagnosis and treatment. *Presse Med.* 2021 Jun 1;50(2). DOI: 10.1016/j.lpm.2021.104069
6. Duignan S, Brownlee W, Wassmer E, Hemingway C, Lim M, Ciccarelli O, et al. Paediatric multiple sclerosis: a new era in diagnosis and treatment. *Dev Med Child Neurol.* 2019 Sep 1;61(9):1039–49. DOI: 10.1111/dmcn.14212
7. Alroughani R, Boyko A. Pediatric multiple sclerosis: A review. *BMC Neurol. 2018* Mar 9;18(1). DOI: 10.1186/s12883-018-1026-3
8. Rothhammer V, Kenison JE, Tjon E, Takenaka MC, de Lima KA, Borucki DM, et al. Sphingosine 1-phosphate receptor modulation suppresses pathogenic astrocyte activation and chronic progressive CNS inflammation. *Proc Natl Acad Sci U S A*. 2017 Feb 21; 114(8):2012–7. DOI: 10.1073/pnas.1615413114
9. Chitnis T, Arnold DL, Banwell B, Brück W, Ghezzi A, Giovannoni G, et al. Trial of Fingolimod versus Interferon Beta-1a in Pediatric Multiple Sclerosis. *N Engl J Med.* 2018 Sep 13; 379(11):1017–27. DOI: 10.1056/NEJMoa1800149
10. Roy R, Alotaibi AA, Freedman MS. Sphingosine 1-Phosphate Receptor Modulators for Multiple Sclerosis. *CNS Drugs*. 2021 Apr 1; 35(4):385–402. DOI: 10.1007/S40263-021-00798-W
11. Obinata H, Hla T. Sphingosine 1-phosphate and inflammation. *International immunology*. 2019 Sep 1; 31(9):617–25. DOI: 10.1093/INTIMM/DXZ037
12. Chun J, Giovannoni G, Hunter SF. Sphingosine 1-phosphate Receptor Modulator Therapy for Multiple Sclerosis: Differential Downstream Receptor Signalling and

Clinical Profile Effects. *Drugs*. 2021 Feb 1;81(2):207–31. DOI: 10.1007/s40265-020-01431-8

13. Yanagida K, Liu CH, Faraco G, Galvani S, Smith HK, Burg N, et al. Size-selective opening of the blood-brain barrier by targeting endothelial sphingosine 1-phosphate receptor 1. *Proc Natl Acad Sci U S A*. 2017 Apr 25; 114(17):4531–6. DOI: 10.1073/PNAS.1618659114/-/DCSUPPLEMENTAL
14. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol.* 2010; 688:1–23. DOI: 10.1007/978-1-4419-6741-1_1
15. Christensen PM, Bosteen MH, Hajny S, Nielsen LB, Christoffersen C. Apolipoprotein M mediates sphingosine-1-phosphate efflux from erythrocytes. *Sci Rep*. 2017 Dec 1;7(1). DOI: 10.1038/S41598-017-15043-Y
16. McGinley MP, Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis and other conditions. *Lancet.* 2021 Sep 25; 398(10306):1184–94. DOI: 10.1016/S0140-6736(21)00244-0
17. Gonzalez-Cabrera PJ, Cahalan SM, Nguyen N, Sarkisyan G, Leaf NB, Cameron MD, et al. S1P(1) receptor modulation with cyclical recovery from lymphopenia ameliorates mouse model of multiple sclerosis. *Mol Pharmacol.* 2012 Feb; 81(2):166–74. DOI: 10.1124/MOL.111.076109
18. Herr DR, Reolo MJY, Peh YX, Wang W, Lee CW, Rivera R, et al. Sphingosine 1-phosphate receptor 2 (S1P2) attenuates reactive oxygen species formation and inhibits cell death: implications for otoprotective therapy. *Sci Rep*. 2016 Apr 15; 6. DOI: 10.1038/SREP24541
19. Bravo GÁ, Cedeño RR, Casadevall MP, Ramió-Torrentà L. Sphingosine-1-Phosphate (S1P) and S1P Signaling Pathway Modulators, from Current Insights to Future Perspectives. *Cells.* 2022 Jul 1;11(13). DOI: 10.3390/cells11132058
20. Jonnalagadda D, Kihara Y, Rivera R, Chun J. S1p2-ga12 signaling controls astrocytic glutamate uptake and mitochondrial oxygen consumption. *eNeuro.* 2021 Jul 1;8(4). DOI: 10.1523/ENEURO.0040-21.2021
21. Murakami A, Takasugi H, Ohnuma S, Koide Y, Sakurai A, Takeda S, et al. Sphingosine 1-phosphate (S1P) regulates vascular contraction via S1P3 receptor: investigation based on a new S1P3 receptor antagonist. *Mol Pharmacol.* 2010 Apr; 77(4):704–13. DOI: 10.1124/MOL.109.061481

22. Cohan S, Lucassen E, Smoot K, Brink J, Chen C. Sphingosine-1-Phosphate: Its Pharmacological Regulation and the Treatment of Multiple Sclerosis: A Review Article. *Biomedicines*. 2020 Jul 18; 8(7). DOI: 10.3390/biomedicines8070227
23. Sandesjö F, Wassmer E, Deiva K, Amato MP, Chitnis T, Hemingway C, et al. Current international trends in the treatment of multiple sclerosis in children—Impact of the COVID-19 pandemic. *Mult Scler Relat Disord .*2021 Nov 1; 56:103277. DOI: 10.1016/J.MSARD.2021.103277
24. Zarzuelo Romero MJ, Pérez Ramírez C, Carrasco Campos MI, Sánchez Martín A, Calleja Hernández MÁ, Ramírez Tortosa MC, et al. Therapeutic value of single nucleotide polymorphisms on the efficacy of new therapies in patients with multiple sclerosis. *J Pers Med*. 2021;11(5). DOI: 10.3390/jpm11050335
25. Correale J, Halfon MJ, Jack D, Rubstein A, Villa A. Acting centrally or peripherally: A renewed interest in the central nervous system penetration of disease-modifying drugs in multiple sclerosis. *Mult Scler Relat Disord.* 2021 Nov 1;56. DOI: 10.1016/j.msard.2021.103264
26. Spampinato SF, Merlo S, Costantino G, Sano Y, Kanda T, Sortino MA. Decreased Astrocytic CCL2 Accounts for BAF-312 Effect on PBMCs Transendothelial Migration Through a Blood Brain Barrier in Vitro Model. *J Neuroimmune Pharmacol*. 2021 Oct 2; DOI: 10.1007/s11481-021-10016-5
27. Feng J, Rensel M. Review Of The Safety, Efficacy And Tolerability Of Fingolimod In The Treatment Of Pediatric Patients With Relapsing-Remitting Forms Of Multiple Sclerosis (RRMS). *Pediatric Health Med Ther.* 2019 Nov; 10:141–6. DOI: 10.2147/PHMT.S220817
28. Scott FL, Clemons B, Brooks J, Brahmachary E, Powell R, Dedman H, et al. Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br J Pharmacol.* 2016. DOI: 10.1111/BPH.13476
29. Tong J, Zou Q, Chen Y, Liao X, Chen R, Ma L, et al. Efficacy and acceptability of the S1P receptor in the treatment of multiple sclerosis: a meta-analysis. *Neurol Sci* 2021; DOI: 10.1007/s10072-021-05049-w/Published
30. Montarolo F, Martire S, Marnetto F, Valentino P, Valverde S, Capobianco MA, et al. The Selective Agonist for Sphingosine-1-Phosphate Receptors Siponimod Increases the Expression Level of NR4A Genes in Microglia Cell Line. *Curr Issues Mol Biol*. 2022 Mar 1;44(3):1247–56. DOI: 10.3390/cimb44030083

31. Mannioui A, Vauzanges Q, Fini JB, Henriet E, Sekizar S, Azoyan L, et al. The Xenopus tadpole: An in vivo model to screen drugs favoring remyelination. *Mult Scler.* 2018 Oct 1;24(11):1421–32. DOI: 10.1177/1352458517721355
32. Colombo E, Bassani C, de Angelis A, Ruffini F, Ottoboni L, Comi G, et al. Siponimod (BAF312) Activates Nrf2 While Hampering NFκB in Human Astrocytes, and Protects From Astrocyte-Induced Neurodegeneration. *Front Immunol.* 2020 Apr 8;11. DOI: 10.3389/fimmu.2020.00635
33. Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. *Lancet*. 2018 Mar 31; 391(10127):1263–73. DOI: 10.1016/S0140-6736(18)30475-6
34. Cree BAC, Arnold DL, Fox RJ, Gold R, Vermersch P, Benedict RHB, et al. Long-term efficacy and safety of siponimod in patients with secondary progressive multiple sclerosis: Analysis of EXPAND core and extension data up to >5 years. *Multiple Sclerosis Journal.* 2022 Sep 1;28(10):1591–605. DOI: 10.1177/13524585221083194
35. Study Record | Beta ClinicalTrials.gov [Internet]. [cited 2023 Jan 10]. Available from: https://beta.clinicaltrials.gov/study/NCT04926818
36. Yang S, Li X, Wang J, Wang T, Xu Z, Gao H, et al. Sphingosine-1-phosphate receptor modulators versus interferon beta for the treatment of relapsing–remitting multiple sclerosis: findings from randomized controlled trials. *Neuroll Sci*. 2022 Jun 1;43(6):3565–81. DOI: 10.1007/s10072-022-05988-y
37. Chaudhry BZ, Cohen JA, Conway DS. Sphingosine 1-Phosphate Receptor Modulators for the Treatment of Multiple Sclerosis. *Neurotherapeutics*. 2017 Oct 1;14(4):859–73. DOI: 10.1007/s13311-017-0565-4
38. A Study of the Safety and Efficacy of ONO-4641 in Patients With Relapsing-Remitting Multiple Sclerosis - Full Text View - ClinicalTrials.gov [Internet]. [cited 2023 Jan 11]. Available from: https://clinicaltrials.gov/ct2/show/study/NCT01081782
39. Kappos L, Arnold DL, Bar-Or A, Camm J, Derfuss T, Kieseier BC, et al. Safety and efficacy of amiselimod in relapsing multiple sclerosis (MOMENTUM): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* [Internet]. 2016 Oct 1 [cited 2023 Jan 11];15(11):1148–59. DOI: 10.1016/S1474-4422(16)30192-2
40. Al-Yafeai Z, Carvajal-González A, Abduljabar H, Arvas M, Patel S, Patel N. Risk for Cardiovascular Adverse Events Associated With Sphingosine-1-Phosphate Receptor Modulators in Patients With Multiple Sclerosis: Insights From a Pooled Analysis of 15

Randomised Controlled Trials. *Front Immunol*. 2021 Dec 7;12. DOI: 10.3389/fimmu.2021.795574

41. Al-Yafeai Z, Carvajal-González A, Abduljabar H, Arvas M, Patel S, Patel N. Novel multiple sclerosis agents-associated cardiotoxicity: A real-world pharmacovigilance study. *Int J Cardiol*. 2022 Sep 1;362:153–7. DOI: 10.1016/j.ijcard.2022.05.052
42. Sharma K, Chaudhary D, Beard K, Srivastava S, Khalid SH, Sriwastava S. A comprehensive review of varicella-zoster virus, herpes simplex virus and cryptococcal infections associated with sphingosine-1-phosphate receptor modulators in multiple sclerosis patients. *Mult Scler Relat Disord*. 2022 Mar 1;59. DOI: 10.1016/j.msard.2022.103675
43. Schoedel KA, Kolly C, Gardin A, Neelakantham S, Shakeri-Nejad K. Abuse and dependence potential of sphingosine-1-phosphate (S1P) receptor modulators used in the treatment of multiple sclerosis: a review of literature and public data. *Psychopharmacology* (Berl). 2022 Jan 1;239(1). DOI: 10.1007/s00213-021-06011-6
44. Stamatellos VP, Rigas A, Stamoula E, Lallas A, Papadopoulou A, Papazisis G. S1P receptor modulators in Multiple Sclerosis: Detecting a potential skin cancer safety signal. *Mult Scler Relat Disord.* 2022 Mar 1;59. DOI: 10.1016/j.msard.2022.103681
45. McGinley M, Rossman IT. Bringing the HEET: The Argument for High-Efficacy Early Treatment for Pediatric-Onset Multiple Sclerosis. *Neurotherapeutics*. 2017 Oct 1; 14(4):985–98. DOI: 10.1007/S13311-017-0568-1