



Review

Pharmacogenetics of siponimod: A systematic review

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ABSTRACT

Multiple sclerosis is a chronic inflammatory neurological disease, and siponimod (Mayzent) is the first oral treatment option for adult patients with secondary progressive multiple sclerosis. We performed a systematic review of the pharmacogenetics of Siponimod, and we found that (430 C>T; rs1799853) and CYP2C9 * 3 (1075 A>C; rs1057910), both translated no-function alleles, have been related to a lower metabolism of siponimod by CYP2C9 enzyme. The FDA-approved drug label and EMA risk management plan for siponimod require testing patients for CYP2C9 genotype before treatment starts. The FDA drug label states that siponimod is contraindicated in patients carrying a CYP2C9 * 3/* 3 genotype, and a daily maintenance dose of 1 mg in patients with CYP2C9 * 1/* 3 and * 2/* 3 genotypes. The EMA reported the potential long-term safety implications in CYP2C9 poor metabolizer patients treated with this drug. Based on this systematic review we concluded that CYP2C9 SNPs influence on siponimod response might be stated by assessing not only CYP2C9 * 2 and CYP2C9 * 3 but other genetic variants resulting in CYP2C9 IM/PM status. CYP2C9 IM phenotype translated from the CYP2C9 * 2 genotype should be revised since it is contradictory compared to other CYP2C9 no-function alleles, and CYP2C9 * 2 might be excluded from PGx testing recommendation before treatment starts with siponimod since it is not translated into a therapeutic recommendation.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory neurological disease in which focal demyelination of the central nervous system occurs [1]. Its etiology is not well known, although it is thought that there is an autoimmune basis, with the participation of cellular and humoral immunity, triggered by an unknown stimulus in a genetically predisposed subject.

There are 2.5 million cases worldwide, with a mean prevalence of 80–100 cases per 100,000 population/year, varying by country, and 1 per 1000 in the Caucasian population [2]. Four different types of MS had been described depending on the evolution of the disease: relapsing-remitting multiple sclerosis (RRMS), secondarily progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS), and progressive recurring (PRMS) [3], but nowadays it is used the phenotypic classification, differentiating into relapsing-remitting forms or progressive forms. The former includes isolated clinical

syndrome and RRMS, while the progressive ones are PPMS and SPMS. Each group can be classified with or without activity depending on whether there is clinical or radiological activity [4]. Most patients (85 %) show an RRMS phenotype, and approximately half of them will develop SPMS at 15–20 years of follow-up, characterized by increased disability unrelated to outbreaks [5]. There are no clear criteria that differentiate the transition from RRMS to SPMS. SPMS is retrospectively diagnosed by a history gradually worsening independent of relapses for ≥ 6 months, following an initial relapsing-remitting course after an initial setting of recurrent illness, and may occur with or without acute exacerbations during the follow-up [6]. The therapeutic management of MS includes both the search for control of its activity, its clinical outbreaks, and the modification of the progression of the disease, such as symptomatic treatment of complications/sequelae. The remaining 10–15 % start with PPMS, with the sustained progression of disability.

In Spain, among different available treatments, the “Document of the Consensus Group of the Spanish Society of Neurology on the use of drugs

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in multiple sclerosis” [7] recommends using the following options in the initial treatment of RRMS: β -Interferon, glatiramer acetate, teriflunomide, and dimethyl fumarate. In those cases of fast and aggressive evolution, natalizumab or fingolimod are considered treatment alternatives. For those patients who do not respond to treatment with immunomodulators, they continue to present flare-ups and lesions (evidenced with neuroimaging techniques), natalizumab, fingolimod, ocrelizumab, and cladribine are indicated, depending on factors depending on the patient, such as clinical severity, comorbidities or others or in those patients with very severe onset forms. Alemtuzumab is reserved for patients with very active disease despite having received a full course of treatment with other drugs, or for patients with the severe, rapidly evolving disease.

Notable advances in the treatment of all forms of MS, especially for relapsing disease forms, have improved the long-term outlook for many patients. The emergence of higher-efficacy drugs requiring less frequent administration has made these preferred options in terms of tolerability and adherence. Many experts now recommend using these as first-line treatment for many patients with early disease, before permanent disability is evident [8] (Table 1). With highly effective therapies, relapses are further reduced or eliminated. However, it has been seen that despite the absence of attacks, relapses occur. For this reason, the use of highly effective therapies in the early stages of the disease has grown to control relapses as much as possible.

Treatment options for patients who enter a phase of secondary progression are rare and are restricted to patients in whom inflammatory activity persists. Siponimod is the first oral treatment option for adult patients with SPMS (Table 1), effective in delaying the progression of disability and impaired cognitive processing speed and reducing the number of flare-ups.

1.1. Siponimod in multiple sclerosis

Siponimod is an oral medicine recently licensed to treat adult patients with SPMS with active disease evidenced by relapses or imaging features of inflammatory activity. Siponimod is a sphingosine-1-phosphate (S1P) receptor modulator (Fig. 1).

It binds selectively two out of five G-protein-coupled receptors (GPCRs) for S1P, namely S1P1 and S1P5 [9]. By acting as a functional

Table 1

Approved disease-modifying therapies for multiple sclerosis. Classification based on drug efficacy according to Hauser et al. [8].

Drugs name	Illness*
Highly effective	
1. Ocrelizumab 600 mg IV	RMS and PPMS
2. Natalizumab 300 mg IV	RMS
3. Alemtuzumab IV 60 mg/1st year, 36 mg 2nd year	RMS
4. Ofatumumab 20 mg SC	RMS
Moderately effective	
1. Fingolimod 0,5 mg	RMS
2. Siponimod 0,5 mg, 1 mg	SPMS
3. Dimethyl fumarate 240 mg	RMS
4. Cladribine oral 3,5 mg/kg/2 years	RMS
5. Ozanimod 0.23 mg/0.46 mg/0.92 mg	RMS
Modestly effective	
1. Teriflunomide 14 mg	RMS
2. Glatiramer acetate	CIS, RMS
3. IFN- β 1a IM 30 mcg	CIS, RMS
4. PegIFN β -1a SC	RMS
5. IFN- β 1a SC 20/40 mcg	CIS, RMS
5. IFN β -1b	CIS, RMS, SPMS

CIS: clinical isolated syndrome; RMS: relapsing forms of multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; IFN- β : Interferon beta; PEG-IFN: Pegylated interferon; SC: Subcutaneous; IV: Intravenous

* According to the technical data sheet approved by the European Medicines Agency.

antagonist on S1P1 receptors on lymphocytes, siponimod prevents egress from lymph nodes. This reduces the recirculation of T cells into the central nervous system to limit central inflammation [10]. It also binds the S1P5 sub-receptor on specific central nervous system (CNS) cells, including astrocytes and oligodendrocytes. It has shown neuro-protective effects and favors pro-remyelination in preclinical models of MS [11].

Siponimod induces a dose-dependent reduction of the peripheral blood lymphocyte count within 6 h of the first dose due to the reversible sequestration of lymphocytes in lymphoid tissues. Most SPMS patients (90 %) show lymphocyte counts returning to the normal range within ten days of stopping therapy. Towards stopping siponimod treatment, residual lowering effects on peripheral lymphocyte count may persist for up to 3–4 weeks after the last dose.

The efficacy of siponimod has been investigated in a phase III study evaluating once-daily doses of 2 mg in patients with SPMS [12].

In the time to event analysis, the confirmed disability progression (CDP) was significantly delayed in siponimod-treated patients with active disease, with 21% risk reduction compared to placebo at 3 months (hazard ratio [HR] = 0.79; 95 % CI: 0.65–0.95; $p = 0.013$) and 26 % at 6 months (HR = 0.74; 95 % CI: 0.60–0.92; $p = 0.0058$). The annualized relapse rate (ARR; confirmed relapses) was lower with siponimod than with placebo (rate ratio = 0.45; 95 % CI= 0.34–0.59; risk reduction 55 %, $p < 0.0001$), as was time to confirmed first relapse (HR = 0.54; 95 % CI= 0.41–0.70; risk reduction 46 %; $p < 0.0001$) [12].

1.2. Pharmacogenetics and Siponimod

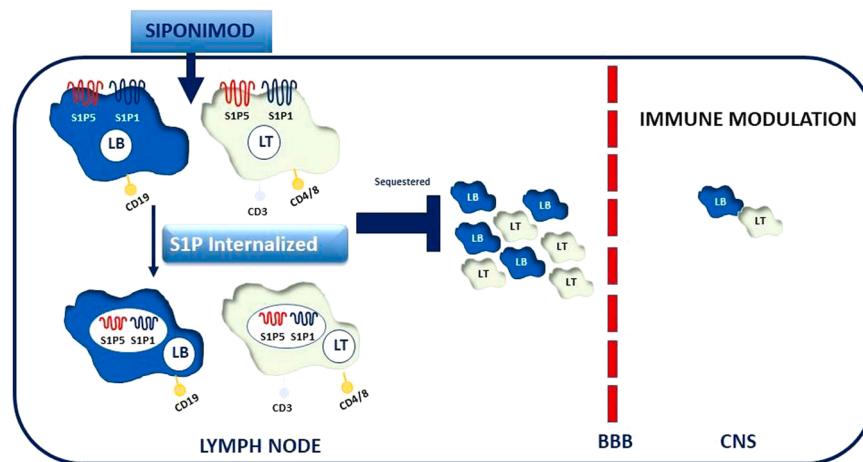
The main aim of pharmacogenetics (PGx) is to predict the response of patients to different drugs. We know that genetic polymorphisms or mutations in genes encoding the expression of biomolecules involved in the pathway of drugs, usually enzymes related to their metabolism, may lead to differences in their activity, thus interindividual differences in the response of patients to these drugs.

In this regard, many genetic polymorphisms have been related to interindividual differences in the response to drugs with the highest level of evidence. There are available dosing guidelines based on PGx information as those from the “Clinical Pharmacogenetics Implementation Consortium” (CPIC) [13], the “Dutch Pharmacogenomics Working Group” (DPWG) [14], the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) [15], and the French National Network of Pharmacogenetics (RNPgX) [16]; and sanitary and public authorities as the “Federal Drug Administration” (FDA), and “European Medicines Agency” (EMA) have warned about the need of genotyping many drug-gene interactions before treatment start.

Especially about Siponimod, the drug label requires studying the *CYP2C9* genotype, contraindicating its use in patients with the *CYP2C9* * 3/* 3 genotype. Also, in patients with *CYP2C9* * 1/* 3 and * 2/* 3 genotypes it recommends a daily maintenance dose of 1 mg starting on day 5 of treatment, but without dosing recommendation in patients with *CYP2C9* * 1/* 1, * 1/* 2, or * 2/* 2 genotypes.

Based on this, nowadays the *CYP2C9* genotyping is mandatory before treatment starts and many laboratories included this test in their portfolio, but there is a lack of knowledge about the basis of this practice, and providing structured information about the state of the art in this topic might help in the translation of genetic results into dosing recommendations.

This systematic review aims to resume and discuss all the information about the PGx of siponimod, including results of studies exploring the influence of genetic variants on the response to this drug, information provided by sanitary authorities (FDA and EMA), and pharmacogenetic dosing guidelines from the most relevant PGx institutions as the CPIC, DPWG, CPNDS, and RNPgX.



Siponimod is an oral compound that acts on the S1P receptor and selectively targets 2 (S1P1 and S1P5) of the 5 known S1P receptors. Siponimod has immunomodulatory properties required for therapeutic approaches in multiple sclerosis. T cells selectively require S1P1 activation for emigration from the thymus, and both T and B cells require this receptor for egress from peripheral lymphoid organs. Siponimod promotes a marked and long-lasting internalization and degradation of S1P1 receptors, thereby acting as a functional antagonist on S1P1. This makes lymphoid cells unresponsive to S1P signalling, thus depriving them of their capacity to egress from lymphoid organs and thereby preventing the recirculation of lymphocytes to blood and tissues including the CNS

Fig. 1. Siponimod action.

2. Methodology

2.1. Search strategy and inclusion/exclusion criteria

We performed a systematic review of the PGx of siponimod. We tried to find all the relevant information in this regard, including studies exploring genetic variants affecting the response to siponimod, review articles on this topic, and data reported by the EMA, FDA, or relevant institutions in PGx as the DPWG, CPNDS, and the RNPGx. First, two independent researchers searched in Pubmed on 15th May 2022 using the following argument: (MAYZENT OR SIPONIMOD) AND (PHARMACOGENETICS OR PHARMACOGENOMICS OR SNP OR GENETIC VARIANT OR POLYMORPHISM). Then both two researchers also checked the webs and clinical guidelines from the CPIC, DPWG, CPNDS, and RNPGx. Finally, these two researchers checked PharmGKB to find any other publication related to the studied topic.

The publications found in the initial search were included for review according to the following inclusion/exclusion criteria:

1. Publications not written in English were excluded.
2. Publications assessing the association of genetic variants with the illness, thus without studying the association of genetic variants with response to Siponimod, were excluded.
3. Comments, short reports, and editorials were excluded.
4. Review articles were excluded.
5. Publications reporting results about the influence of genetic variants on siponimod response, PGx dosing guidelines for siponimod, and warnings or reports from the EMA or FDA, were included.

First, among publications found in the initial search in Pubmed, all abstract titles were checked looking for publications not written in English. Then, both researchers extensively read complete manuscripts to find those publications meeting the inclusion/exclusion criteria and checked out the review articles found in the initial search before being excluded to find other publications not found in the initial search meeting the inclusion/exclusion criteria. Finally, they manually checked the provided literature on PharmGKB about genetic variants affecting siponimod response to verify that we had not excluded any relevant publication related to the topic of this systematic review.

2.2. Data extraction and quality assessment

As commented above, two different researchers carried out the search strategy. In case of discrepancies about publications published studies exploring the influence of genetic variants on siponimod response meeting the inclusion/exclusion criteria, another researcher blinded to the decision of these two researchers performed an evaluation and took the final inclusion/exclusion decision.

Regarding the studies considered for inclusion of genetic variants affecting siponimod response, we performed a quality assessment using the Newcastle–Ottawa quality assessment Scale (NOS) [17]. We judged each study on three categories (selection, comparability, and exposure) and eight items, with up to nine “stars/points” as the top score, and research articles with NOS scores higher than five points were included.

All the information provided by FDA, EMA, CPIC, DPWG, CPNDS, and RNPGx about PGx of siponimod was included in the results and discussed below.

3. Results

In the initial search in Pubmed, we found eight publications, including three reviews [18–20], and one study protocol [21], thus excluded from the results (n = 4). Finally, we found four original research articles [22–25] (Table 2), all of them meeting the quality criteria based on the NOS. Those three excluded reviews did not include original research articles not found in our search. We also found information from the FDA [26] and EMA [27] about the PGx of Siponimod, based on those original researches partly, and dosing guidelines from the DPWG.

3.1. Pharmacogenetics of siponimod

Jin et al. [22] conducted in vitro metabolism studies using human liver microsomes (HLM) to investigate the enzyme responsible for siponimod metabolism and the impact of *CYP2C9* genetic polymorphisms on its response. They found the *CYP2C9* enzyme as the main responsible in humans for siponimod metabolism and differences about its effect after comparing the *CYP2C9* wildtype genotype (*CYP2C9* *1/*1) versus *CYP2C9* *2/*2 and *3/*3. Based on HLM incubations patients carrying these *2/*2 and *3/*3 genotypes showed

Table 2

Original research articles exploring the influence of genetic variants on the response to siponimod.

Author	refSNP (rs)	Translated allele	Study subjects	CYP2C9 Genotype distribution	Endpoint	Main results
Gardin A et al. [23]	rs1799853 rs1057910	CYP2C9 * 2 CYP2C9 * 3	Healthy (Caucasians)	N = 24 * 1/* 1: n = 12 * 2/* 3: n = 6 * 3/* 3: n = 6	AUC and Cmax (siponimod)	Coadministration with fluconazole led to siponimod AUC approximately twofold and fourfold greater in the CYP2C9 * 2/* 3 and CYP2C9 * 3/* 3 genotypes, respectively
Jin Y et al. [22]	rs1799853 rs1057910	CYP2C9 * 2 CYP2C9 * 3	human liver microsomes	NA	AUC and Cmax (siponimod)	The predicted mean AUC is 2.7-, 3.0- and 4.5-fold higher in the CYP2C9 * 2/* 2, CYP2C9 * 2/* 3, and CYP2C9 * 3/* 3 genotypes, respectively, compared with the CYP2C9 * 1/* 1 genotype.
Huth F et al. [24]	rs1799853 rs1057910	CYP2C9 * 2 CYP2C9 * 3	NA	NA	DDI behavior of siponimod	CYP2C9 genotype has a relevant influence on the DDI behavior of siponimod in the presence of different CYP3A4/CYP2C9 inducers and inhibitors. With decreased CYP2C9 metabolic activity, CYP3A4 becomes the dominant elimination pathway for the PMs (CYP2C9 * 3/* 3).
Wanounou M et al. [25]	rs28371685	CYP2C9 * 11	Healthy (Jewish)	N = 143 * 1/* 11 = 13 * 11/* 11 = 3	(S)-warfarin oral clearance and PMR 24/12 and PMR 24/24 derived from the ratio of 5-(4-hydroxyphenyl)-5-phenylhydantoin	CYP2C9 * 11 may be at increased risk to experience siponimod-associated adverse effects (i.e., bradycardia, risk of infection, and elevated liver enzymes) if treated with the standard dose.

N: Total number of patients; AUC: Area under the Curve; refSNP: reference Single Nucleotide Polymorphism; DDI: Drug-Drug Interaction; PMs: Poor Metabolizers; PMR 24/12: Phenytoin Metabolic Ratio in 24-hour urine collection 12 h post dosing; PMR 24/24: Phenytoin Metabolic Ratio in 24-hour urine collection 24 h post dosing.

close to 3 and 10 decreases in siponimod metabolism respectively. They also found that the predicted mean area under the curve, in patients treated with siponimod only, is between 2.7 and 4.5-fold higher when carrying the CYP2C9 * 2 (430 C>T; rs1799853) or CYP2C9 * 3 (1075 A>C; rs1057910) alleles compared to CYP2C9 wildtype genotype.

Another study by Gardin A. et al. [23] studied in healthy subjects the pharmacokinetics (PK) of siponimod depending on CYP2C9 * 2 (430 C>T; rs1799853) and * 3 (1075 A>C; rs1057910) genotypes. In subjects with CYP2C9 * 2/* 3 and * 3/* 3 their results showed 2–4-fold greater in the area under the curve (AUC) of siponimod with a minor increase of Cmax than the CYP2C9 * 1/* 1 genotype. Their results confirmed the impact of CYP2C9 activity on siponimod metabolism in humans.

Both two studies also assess the influence of fluconazole on siponimod PK. Jin Y. et al. studied the inhibitory effects of fluconazole on siponimod treatment. For CYP2C9 * 1/* 1 carriers treated with fluconazole 200 mg, they found that the predicted exposure increase of siponimod was 2.0–2.4-fold. Gardin A. et al. found in healthy subjects treated with siponimod and fluconazole (CYP2C9 and CYP3A4 inhibitor) a twofold increase in AUC versus siponimod alone (AUC: 1110–2160 h*ng/mL), an increase in maximum plasma concentration (Cmax: 31.2–34.0 ng/mL) and an increase in elimination half-life (T_{1/2}: 40.6–61.6 h). These results confirmed the impact of CYP2C9 activity on siponimod metabolism in humans.

Also, Huth et al. [24] studied the influence of CYP2C9 genotype on drug-drug interaction (DDI) potential of siponimod in presence of CYP2C9/CYP3A4 inhibitors/inducers by physiologically based PK modeling. They predicted an increased DDI risk for siponimod and CYP2C9 * 3/* 3 genotype in presence of strong and moderate CYP3A4 inhibitors, and strong CYP3A4/moderate CYP2C9 inducers, compared to other genotypes, thus concluding the relevant influence of CYP2C9 * 3 on the DDI behavior of siponimod.

Finally, Wanounou et al. [25] assessed the in vivo activity of CYP2C9 depending on the CYP2C9 * 11 genotype. They studied n = 150 healthy Ethiopian Jewish participants who were non-smokers treated with 300-mg phenytoin and 20-mg warfarin, considering as markers of CYP2C9 activity the (S)-warfarin oral clearance and phenytoin metabolic ratio (PMR) derived from the ratio of 5-(4-hydroxyphenyl)-

5-phenylhydantoin in 24-hour urine collection to plasma phenytoin 12 h (PMR 24/12) or 24 h (PMR 24/24) post-dosing. They found a decrease of 50 % and 62.2 % in PMR 24/12 and PMR 24/24, respectively, among carriers of CYP2C9 * 1/* 11 compared to CYP2C9 * 1/* 1, and concluded that CYP2C9 * 11 genotyping should be deemed before prescribing many drugs such as phenytoin, warfarin, nonsteroidal anti-inflammatory drugs, or siponimod.

3.2. Drug label, FDA/EMA considerations, and PGx dosing guidelines for siponimod

Based on the results above, the FDA-approved drug label for siponimod requires testing patients for CYP2C9 genetic variants to determine the CYP2C9 genotype. This drug label also states that siponimod is contraindicated in patients with the CYP2C9 * 3/* 3 genotype. In patients with CYP2C9*1/*3 and *2/*3 genotypes, it is recommended a daily maintenance dose of 1 mg starting on day 5 of treatment. On the other hand, in patients with CYP2C9 * 1/* 1, * 1/* 2, or * 2/* 2 genotypes, there is no recommendation based on PGx information.

The EMA included in the risk management plan (RMP) for Mayzent [26] the potential long-term safety implications in CYP2C9 poor metabolizer (PM) patients treated with this drug based on studies by Jin et al. [22] and Gardin A. et al. [23]. This RMP includes as a must the genotyping of CYP2C9 to determine the metabolizer status of patients before the initiation of treatment with siponimod. Moreover, it recommends not using siponimod in patients with CYP2C9 * 3/* 3 genotype (PM phenotype) and a maintenance dose of 1 mg in patients with CYP2C9 * 1/* 3 and * 2/* 3 genotypes.

The DPWG guidelines included, in the 2020 update [28], the same therapeutic dose recommendations as those provided in the FDA-approved drug label for siponimod (Table 3).

In summarizing, siponimod is now considered the first-line treatment in SPMS patients, it is metabolized by the CYP2C9 enzyme, and two genetic polymorphisms in the gene encoding the expression of this enzyme, the CYP2C9 * 2 (430 C>T; rs1799853) and CYP2C9 * 3 (1075 A>C; rs1057910), both characterizing translated no-function alleles, have been significantly related to a lower metabolism by this enzyme, thus, the FDA and EMA bind genotyping these two single nucleotide polymorphisms (SNP) before the initiation of treatment and

Table 3

CYP2C9 genetic polymorphisms affecting siponimod response, genotype-translated phenotypes, and dosing recommendations based on DPWG guideline.

Reference SNP (rs)	MNV	Allele *	MAF	Genotypes	Phen.	Dosing recommendation (DPWG guidelines)
rs1799853	430C>T	*2	4.79%	CYP2C9*1/ *1	EM	NO action is required for this gene-drug interaction.
				CYP2C9*1/ *2	IM	NO action is required for this gene-drug interaction.
				CYP2C9*2/ *2	PM	NO action is required for this gene-drug interaction.
				CYP2C9*1/ *3	IM	Use 50% of the normal maintenance dose. Reconsider the choice and the potential benefit of siponimod if the patient is also using a moderate CYP3A4 inducer, such as modafinil. For this genetic variation, a moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model.
rs1057910	1075A>C	*3	4.85%	CYP2C9*2/ *3	PM	Avoid siponimod.
				CYP2C9*3/ *3	PM	Avoid siponimod.

SNP: Single Nucleotide polymorphism; MNV: Major nucleotide variation; MAF: Minor Allele Frequency from the 1000 Genomes Project; Phen: Phenotype; DPWG: Dutch Pharmacogenetics Working Group; EM: Extensive Metabolizer; IM: Intermediate Metabolizer; PM: Poor Metabolizer

* Star allele

recommend avoid using siponimod in patients with CYP2C9 * 3/* 3, and a maintenance dose of 1 mg in patients with CYP2C9 * 1/* 3 and * 2/* 3 genotypes.

4. Discussion

Siponimod is the first oral treatment option for adult patients with SPMS, effective in delaying the progression of disability and impaired cognitive processing speed and reducing the number of flare-ups. Siponimod is also one of the low numbers of drugs with a required PGx test included on the drug label.

Based on results by Jin et al. [22] and Gardin et al. [23] the FDA-approved drug label for siponimod requires testing patients for CYP2C9 genetic variants to determine CYP2C9 metabolizer status, and the EMA included in the risk management plan (RMP) for Mayzent [27] the potential long-term safety implications in CYP2C9 PM patients treated with this drug.

The main limitation of this systematic review is the low number of original research articles found in the initial search (n = 4). Apart from that, this is the main topic for discussion in this manuscript. Despite the limited PGx information, the FDA-approved drug label for siponimod requires CYP2C9 genotyping before treatment start.

Based on this systematic review we found some circumstances and inconsistencies among dosing guidelines, EMA/FDA warnings, and information included in the drug label of siponimod.

The FDA and EMA bind genotyping CYP2C9 * 2 (430 C>T; rs1799853) and * 3 (1075 A>C; rs1057910) before the treatment starts with siponimod, recommend avoiding using siponimod in patients with CYP2C9 * 3/* 3 genotype, and a maintenance dose of 1 mg in patients with CYP2C9 * 1/* 3 and * 2/* 3. On the other hand, there is no recommendation in patients with CYP2C9 * 1/* 1, * 1/* 2, or * 2/* 2 genotypes, this is also stated by the FDA-approved drug label for Siponimod. Even, if we look at DPWG guidelines from the Royal Dutch Pharmacists Association, this information is also included.

Both the FDA and EMA warnings about siponimod/CYP2C9 drug-gene interaction, and siponimod drug label, refer to metabolizer status when talking about how genetic polymorphisms in CYP2C9 gene affect the response of patients to the treatment. In this regard, carrying a single copy of the CYP2C9 * 2 or * 3 alleles (CYP2C9 * 1/* 2 or CYP2C9 * 1/* 3 genotypes) translates into IM status, and two CYP2C9 * 2.* 3 combined alleles is translated into PM phenotype.

If we look at provided therapeutic recommendations based on these phenotypes, we can see that PM patients with CYP2C9 * 2/* 3 genotype are recommended to use 50% of normal maintenance dose, as intermediate metabolizer (IM) patients carrying CYP2C9 * 1/* 3; and, IM with CYP2C9 * 1/* 2 genotype, and PM with CYP2C9 * 2/* 2 are recommended to take no action on patient's treatment, as a normal

metabolizer with wildtype genotype.

Based on this, genotyping the CYP2C9 * 2 (430 C>T; rs1799853) might have no sense because, if it is not combined with CYP2C9 * 3 (1075 A>C; rs1057910) this is never translated into a therapeutic recommendation and even combined with CYP2C9 * 3 it does not modify translated therapeutic recommendations. This happens despite the CYP2C9 * 2 (430 C>T; rs1799853) allele being related to lower activity of the enzyme (IM or poor metabolizer, PM, phenotype) and higher plasma concentrations of siponimod.

On the other hand, many other genetic variants in the CYP2C9 gene have been related to decreased function of the enzyme, higher plasma concentrations, and IM or PM phenotype as those characterizing the CYP2C9 * 4, * 5, * 6, * 8, * 11, * 12, and * 13 alleles. DPWG guidelines recommend 50 % of the standard dose if carrying a single copy of these alleles (IM) and avoid siponimod if carrying two of them combined (PM phenotype/status).

These CYP2C9 no-function alleles different from CYP2C9 * 2 and * 3 have been related to many drugs response with the highest level of evidence. This may be easily looked at by PharmGKB [29].

Usually, the genotype-to-therapeutic recommendation translation process works as follows: We know a genotype as a result of genotyping one or many genetic mutations/polymorphisms, this is translated into a phenotype and this phenotype (e.g. metabolizer status regarding genes encoding an enzyme) is translated, or not, into a therapeutic recommendation depending on the drug. It does not usually happen that the same translated phenotype is related to a therapeutic recommendation depending on the genotype.

Curiously, the CYP2C9 * 2 (430 C>T; rs1799853) is the only genetic variant in the CYP2C9 gene related to a decreased function of the enzyme and higher plasma concentrations of siponimod, resulting in IM phenotype, but not related to a therapeutic recommendation for siponimod. Even more, it is included in the drug label and EMA/FDA warnings as one of the only two variants to be tested before treatment initiation.

If we deepen in this regard, the clinical trial supporting the approval of Mayzent [12] excludes CYP2C9 * 3/* 3 carriers based on results by Jin et al. [22] and Gardin et al. [23] commented above. On the other hand, CYP2C9 * 1/* 2, * 2/* 2, and * 1/* 3 carriers were included but this study does not provide results by genetic subgroups so we cannot know the real impact of these SNPs on siponimod response.

Furthermore, the RMP for Mayzent, drug label, FDA, and EMA warnings are based on data by Gardin A. et al., and results provided by these authors state that CYP2C9 * 2/* 3 and * 3/* 3 carriers showed 2–4-fold greater in the AUC of siponimod with a minor increase of C_{max} than CYP2C9 * 1/* 1 genotype; thus, without remarking differences among these genotypes and translated into the same phenotype (PM); instead, the considerations by these authorities about these genotypes

and genotype-resulting therapeutic recommendations are different.

The influence of *CYP2C9* no-function alleles on drug response, as it happens with other drug-gene interactions, depends on minor allele frequencies, drug pathways, and other related enzymes, or the influence of enzyme inducers/inhibitors, among many different parameters.

Based on this systematic review, we conclude that, first, *CYP2C9* SNPs influence on siponimod response might be stated considered not only *CYP2C9* * 2 (430 C>T; rs1799853) and *CYP2C9* * 3 (1075 A>C; rs1057910) but other genetic variants associated with *CYP2C9* IM or PM status. Second, *CYP2C9* IM/PM status translated from *CYP2C9* * 2 (430 C>T; rs1799853) genotype should be reconsidered since it is contradictory compared to other *CYP2C9* no function alleles. Finally, *CYP2C9* * 2 (430 C>T; rs1799853) might be excluded from PGx testing recommendation before treatment starts with siponimod since it is never translated into a therapeutic recommendation based on EMA, FDA data, and available PGx dosing guidelines.

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CRediT authorship contribution statement

Conceptualization, methodology, visualization, formal analysis and writing—original draft preparation, R.M.-R., and X.D.-V.; validation and writing—review and editing, R.P.-M. and F.J.B.-H.; software, investigation, resources and data curation, A.A.-R.; supervision and project administration, J.C.-B.; All authors have read and agreed to the published version of the manuscript.

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

Data will be made available on request.

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