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Siponimod Therapy and *CYP2C9* Genotype

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Introduction

Siponimod (brand name Mayzent) is a sphingosine-1-phosphate (S1P) receptor modulator used in the treatment and management of relapsing forms of multiple sclerosis (MS) in adults. It works by targeting lymphocytes to decrease the number of circulating cells that are associated with MS symptomatic attacks and disease progression and may also have a direct neuroprotective impact. Siponimod strongly binds to the S1P type 1 and type 5 receptors that are abundantly expressed on lymphocytes and multiple other cell types in the central nervous system (CNS). Off-target interactions and effects on cardiac cells may occur, also. The use of a dose titration schedule is recommended to decrease the risk of bradycardia (see Table 1, Table 2) (1, 2). This medication is approved for multiple forms of relapsing MS (RMS) in the United States (1) and for active, secondary progressive disease in Europe and Canada (2, 3).

Siponimod is metabolized by members of the cytochrome P450 family, specifically CYP2C9 and, to a lesser extent CYP3A4. The *CYP2C9* gene is polymorphic and activity scores are used to categorize diplotype into phenotype. Decreased CYP2C9 metabolic activity is associated with increased exposure to siponimod and increased risk of adverse effects. Therefore, individuals with the *CYP2C9**3/*3 diplotype (activity score = 0) are contraindicated from taking siponimod (1, 2). Individuals with one copy of the no-function **3* allele (diplotype with activity scores of 0.5 or 1.0) are advised to take half the standard maintenance dose (1, 2). Consideration of genotype and activity score is essential for CYP2C9-based siponimod dosing because labeled dose recommendations are not categorized by phenotype. In the US, there is a modified titration schedule for individuals with a *CYP2C9**3 allele (Table 1)(1); however, the European prescribing guidelines do not modify the titration schedule for individuals with a single copy of the *CYP2C9*3* allele (heterozygous for *CYP2C9*3*) (Table 2) (2). The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy similarly recommends a 50% reduced maintenance dosage for intermediate metabolizers (IM) (Table 3) (4). It should be noted that dose recommendations in the Siponimod package label are limited to diplotypes consisting of only *CYP2C9 *1*,**2*, and **3* alleles due to lack of clinical data on the impact of other decreased or no-function alleles(1), while other medication and testing guidelines also consider**5, *6, *8*, and **11* (5, 6).

Table 1: The FDA Recommended Titration Schedule and Dosage based on *CYP2C9* Genotype (2023)

Titration day	Standard dose[a]	Reduced dose[b]
Day 1	0.25 mg	0.25 mg

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Table 1 continued from previous page.

Titration day	Standard dose[a]	Reduced dose[b]
Day 2	0.25 mg	0.25 mg
Day 3	0.5 mg	0.5 mg
Day 4	0.75 mg	0.75 mg
Day 5	1.25 mg	1 mg
Day 6 (maintenance)	2 mg	1 mg

[a] The standard dose of 2 mg maintenance dose and titration schedule is indicated for those with the following *CYP2C9* genotype: **1/*1*, **1/*2*, **2/*2*
[b] The reduced dose of 1 mg maintenance dose and modified titration schedule is indicated for those with the following *CYP2C9* genotype: **1/*3*, **2/*3*
This table is adapted from (1).

Table 2: The EMA Recommended Titration Schedule and Dosage based on *CYP2C9* Genotype (2023)

Titration day	Standard dose[a]	Reduced dose[b]
Day 1	0.25 mg	0.25 mg
Day 2	0.25 mg	0.25 mg
Day 3	0.5 mg	0.5 mg
Day 4	0.75 mg	0.75 mg
Day 5	1.25 mg	1.25 mg[c]
Day 6 (maintenance)	2 mg	1 mg[c]

[a] The standard dose is recommended for those individuals who do not have a *CYP2C9*2* or **3* allele.
[b] The reduced maintenance dose of 1 mg is recommended for individuals with *CYP2C9*1/*3* or **2/*3* genotype.
[c] Note that the EMA recommended titration schedule is the same regardless of *CYP2C9* genotype. The additional exposure of 0.25 mg on day 5 for CYP2C9 IM individuals does not compromise safety. EMA – European Medicines Agency, IM – intermediate metabolizers
This table is adapted from (2).

Table 3: The DPWG Recommended Dosage based on *CYP2C9* Genotype (2020)

CYP2C9 genotype	Recommended action	Rationale and risk summary
*CYP2C9*1/*3, CYP2C9*2/*3, or comparable genotype classified as IM*	Use 50% of the normal maintenance dose Reconsider the choice and potential benefit of siponimod if the individual is also using a moderate CYP3A4 inducer, such as modafinil.	Theoretically, the risk of adverse effects is increased, as the genetic variation results in higher plasma concentrations of siponimod. For the comparable genetic variation *1/*3, the moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model
*CYP2C9*3/*3* *or comparable genotype classified as PM*	Avoid siponimod	Siponimod is contraindicated in individuals with the comparable genetic variation *3/*3. Theoretically, the risk of adverse effects is greatly increased, as the genetic variation results in much higher plasma concentrations of siponimod
*CYP2C9*1/*2* *CYP2C9*2/*2*	No action is required for this drug-gene interaction	The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects

DPWG - The Dutch Pharmacogenetics Working Group, IM - Intermediate metabolizer, PM - Poor metabolizer
This table is adapted from (4).

Drug: Siponimod

Siponimod is a modulator of S1P receptors, specifically type 1 and type 5, used in the treatment of some forms of RMS. It is approved for use in the United States by the FDA, in the European Union by the European Medicines Agency (EMA), and in Canada by Health Canada. In the US, siponimod is authorized for all forms of RMS, including isolated syndrome, relapsing-remitting disease, and active secondary progressive MS (SPMS)(1). In contrast, the EMA and Health Canada have authorized it only for use in active SPMS (progressive MS with evidence of active disease based on relapses or imaging features of inflammatory activity) (2, 3). In all cases, the medication is approved for adults and is contraindicated in individuals with the *CYP2C9*3/*3* genotype. (1, 2, 7) Due to its nonselective binding and adverse effects in cardiac cells, siponimod is also contraindicated in individuals with a recent history (previous 6 months) of myocardial infarction, unstable angina, stroke, transient ischemic attack, heart failure requiring hospitalization, or class III/IV heart failure (1). Cardiac conduction defects, such as Mobitz type II second- or third-degree atrioventricular (AV) block, or sick sinus syndrome without a functioning pacemaker, are also contraindications for siponimod therapy (1).

Siponimod is extensively absorbed (≥70%) with high oral bioavailability (84%), reaching maximum plasma concentration approximately 4 hours following dose administration (1). Following the prescribed dose titration period, siponimod usually reaches steady-state levels in the circulation after 6 days of the maintenance dosage. Although siponimod can cross the blood brain barrier, it is estimated that 68% of the medication remains in plasma (1). Siponimod is primarily metabolized via oxidation by the cytochrome P450 family members 2C9 (CYP2C9, accounting for 79% of the metabolism) and 3A4 (CYP3A4, accounting for 18.5%) (1, 8). The elimination half-life is 30 hours, and no unchanged siponimod is detected in urine, indicating it is fully metabolized before excretion. The main metabolites, M3 and M17, are not thought to contribute to the efficacy or safety of siponimod. Following discontinuation of therapy, complete elimination from the body may take up to 10 days, and the residual effect of reduced peripheral lymphocyte count may persist for 3 to 4 weeks. (1, 8, 9)

Differences in CYP2C9 activity, either due to genetic variation or inhibition from the dual CYP2C9/3A4 inhibitor fluconazole, are associated with reduced metabolism of siponimod (9) and increased overall exposure to the medication (1, 10). The most common CYP2C9 loss-of-function alleles are the *CYP2C9*3* no-function allele and the **2* reduced reduced-function allele. Individuals with 2 copies of the *CYP2C9*3* no-function allele are contraindicated for siponimod treatment, and those with reduced CYP2C9 enzyme activity due to a single **3* allele, specifically, the *CYP2C9*1/*3* or **2/*3* diplotype, are advised to take a lower daily maintenance dose (1 mg) compared to individuals without these genotypes (2 mg daily dose) (1). Although the FDA-approved label mentions other *CYP2C9* alleles with reduced or no functional activity that might have a similar effect on metabolism, there is inadequate evidence to justify a recommendation for altered dosing based on the presence of those alleles. In contrast, the DPWG guideline for siponimod recommends a similar dose adjustment for those with a genotype comparable to *CYP2C9*3* heterozygous individuals, and avoidance of siponimod for those with a genotype comparable to homozygous *CYP2C9*3*. (4)

Multiple sclerosis is an immune-mediated disorder of the CNS, characterized by neuroinflammation, focal demyelination, and axonal damage (11). It affects more than 2 million people worldwide, with approximately 85% of these individuals having the relapsing-remitting form of MS, which is characterized by periodic attacks followed by partial or complete remission, at disease onset (12). The relapsing-remitting phenotype is usually followed by a secondary progressive phase (SPMS) consisting of gradual disability accrual, with or without overlapping relapses (13). Typically, MS affects individuals between the ages of 20 to 40 and can lead to significant disability (14). The specific symptoms and severity of the disease vary depending on the site and severity of lesions in the CNS. Common symptoms include fatigue, spasticity, weakness, tremor, visual impairment, pain, motor paralysis, cognitive impairment, and bladder and bowel problems (12). The clinical presentation of MS forms a spectrum, ranging from less severe forms of radiologically or clinically isolated syndrome to more aggressive primary progressive MS in which there is little to no relapse from symptoms, with

persistent disease progression (15). Both genetics and environmental exposures are believed to play a role in determining the risk of developing MS, similar to other autoimmune disorders (11).

Receptors for S1P are expressed by a variety of cell types including astrocytes, oligodendrocytes, erythrocytes, myocytes, lymphocytes, and cells of the eyes and spleen (16). Prolonged activation of S1P1 receptors on lymphocytes leads to their internalization and prevents cellular exit from lymph nodes. This reduces migration of these immune cells from the peripheral bloodstream into the CNS, preventing inflammatory relapses (17) . Siponimod can also readily cross the blood brain barrier. Preclinical and clinical data suggest that binding of siponimod to S1P1 and S1P5 receptors in the CNS may also provide a direct protective and regenerative effect on neuronal cells, and this may be achieved independent of effects on peripheral lymphocyte counts (18, 19, 20).

Unlike a previous generation nonselective S1P modulator, fingolimod, siponimod targets only the type 1 and 5 receptors and does not require phosphorylation. This increased specificity reduces, but does not eliminate, the off-target adverse effects of siponimod on cardiac tissue (21). Specifically, siponimod can cause a transient and dose-dependent decrease in heart rate, which can be largely improved by following a specific dose titration schedule at the initiation of therapy (or following daily treatment interruption for more than 4 days) (22). Additionally, for individuals with preexisting cardiac conditions such as sinus bradycardia and first- or second-degree AV block, the drug labeling recommends a first dose monitoring period of 6 hours to address potential symptomatic bradycardia. (1, 2)

Compared to placebo, siponimod improves imaging-based metrics for disease progression (for example, lesion volume by MRI) and reduces the risk of confirmed disability progression, relapses, and the annual relapse rate (23). The benefits of siponimod are more substantial in individuals with active MS, as observed in the EXPAND extension study (24). Secondary analysis of the EXPAND trial reported a clinically meaningful improvement in cognitive function for individuals in the siponimod arm versus placebo (25). A systemic review of the BOLD (phase II) and core EXPAND (phase III) trials found low-certainty evidence supporting the benefit of 2 mg daily siponimod for the primary and secondary measures reported in those studies. However, the review called for additional trials to examine longer treatment duration for potential adverse effects and inclusion of other active controls(12). In contrast, a review of multiple disease-modifying therapies for RMS and active SPMS concluded that the EXPAND trial showed a significant reduction in disability progression and relapse rates (26). The neuroprotective effect of siponimod has led some groups to begin preclinical studies to assess its efficacy in autoimmune neuritis and optic nerve damage due to glaucoma (27, 28). Additional, ongoing clinical studies of siponimod can be found in the online database ClinicalTrials.gov, including a study for pediatric individuals (ages 10-17) with MS (NCT04926818) (29).

As siponimod specifically targets the immune system, it is essential to assess individuals before treatment for signs of active infection and to test for immunity to varicella zoster virus (either history of chicken pox or vaccination). Effective therapy with siponimod can lead to a 20–30% decrease in peripheral lymphocyte counts, increasing the individual's risk for infection. A recent (within prior 6 months) complete blood count should be taken before initiating treatment (1). Similarly, individuals without immunity to varicella zoster infection should complete a vaccination course 4 weeks before starting siponimod (1). Studies have found that individuals on a S1P receptor modulator therapy (including siponimod and fingolimod) also have impaired responses to vaccination to SARS-CoV-2 and may be at an increased risk of break-through infection following vaccination (30, 31, 32). Siponimod may have a limited effect on the immune response following other vaccinations such as influenza or pneumococcal polysaccharide (33).

Other side effects including macular edema, elevated liver enzymes, and hypertension were reported in a higher proportion of individuals on siponimod compared to placebo (23). Thus, the FDA recommends an ophthalmic evaluation, as well as measurement of transaminase and bilirubin levels before starting treatment (1). Treatment with S1P receptor modulating therapies is also associated with a higher risk of cutaneous malignancies, such as basal cell carcinoma. Therefore, individuals should be monitored for suspicious skin lesions before starting

treatment and periodically during treatment with siponimod (1). The extension of the EXPAND trial observed an increase in basal cell carcinoma incidence with increased time on siponimod, specifically for 3–5 years, with an incidence rate of 0.9 per "100 patient-years" compared to 0.7 for the placebo arm (24).

The use of siponimod in pediatric individuals, nursing mothers, pregnant women, or individuals aged 65 and over has not been sufficiently studied to decide if this medication is safe or if modified administration would be needed for these specific groups. Safety and efficacy in a pediatric population have not been established. Animal studies have suggested that siponimod may be able to cause fetal harm when taken by a pregnant woman. There is a pregnancy exposure registry available for enrollment: MotherToBaby (www.mothertobaby.org/join-study). Rat studies suggest siponimod, its metabolites, or both are detectable in milk (1). However, there are no data on the presence of siponimod in human breastmilk, its effects on breastfed infants, or the impact on milk production in lactating females. While it is unlikely that siponimod would be present in breast milk in significant amounts, it is potentially toxic to a breastfed infant. Based on the related drug fingolimod, experts recommend avoiding its use during breastfeeding (34). Individuals aged 65 or older should be managed with care due to the greater frequency of renal, cardiac, or hepatic dysfunction in this population. However, there is no specific recommendation in the drug labeling to suggest altered dosing for these individuals. (1)

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic, and genetic variants can lead to reduced, absent, or increased enzyme activity.

The CYP2C9 enzyme metabolizes approximately 15–20% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the *CYP2C9* gene can play a significant role in adverse drug reactions (35, 36). Among CYP2C isoforms expressed in the liver, CYP2C9 is the most abundant (37, 38).

The *CYP2C9* gene is polymorphic, with more than 80 known alleles (39). The wild-type allele is designated *CYP2C9*1.* In a test of multiple CYP2C9 variants, when none of the tested variants are detected, the genotype is assigned as *CYP2C9*1,* and the gene is predicted to produce a fully functional enzyme with normal enzyme activity (40). Individuals who have 2 normal-function alleles, for example, *CYP2C9*1/*1*, are classified as "normal metabolizers" (Table 4). Each allele is assigned an activity score of 0 (no-function), 0.5 (reduced-function), or 1 (normal-function), and the combined score for both alleles is used to determine the phenotype. This approach was initially described for standardization of phenotype for the gene *CYP2D6* and is used for multiple CYP loci by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (37, 41). A combination of alleles resulting in an activity score of 1.5 or 1 is classified as an IM phenotype, while a combined activity score of 0 or 0.5 is classified as a poor metabolizer (PM) phenotype (Table 4) (40). However, the effect of the reduced-function allele yielding a higher activity score than the no-function allele may result in different recommendations for IMs with activity scores of 1.5 and 1. For example, the CPIC guideline for the CYP2C9-phenytoin interaction recommends no adjustments are needed from typical dosing strategies for IMs with an activity score of 1.5, but recommends subsequent doses are reduced by 25% for IMs with an activity score of 1 (40).

Allelic variants associated with reduced, or no enzyme activity include *CYP2C9*2* (p.Arg144Cys), *CYP2C9*3* (p.Ile359Leu), *CYP2C9*5* (p.Asp360Glu), *CYP2C9*8* (p.Arg150His) and *CYP2C9*11* (p.Arg335Trp). The **2* reduced-function allele is more common in European (13%), Near Eastern (13%), and Central/South Asian (11%) populations than in Latino (8%), Oceanian (3%), East Asian (0.2%), or African (1–2%) descent populations. The **3* no-function allele is less common (<10% in most populations, except for Central/South Asian, at approximately 11%), and it is extremely rare in African populations. In African descent populations, the *CYP2C9*5* no-function (~1%), and **8* (6–8%), and **11* (1–3%) reduced-function alleles are also common. (42, 43, 44, 45)

Table 4. The CPIC Assignment of likely *CYP2C9* Phenotype based on Genotype

CYP2C9 phenotype[a,b]	Genotype	Activity score	Examples of diplotype
Normal metabolizer	An individual with 2 normal-function alleles	2	*1/*1, *1/*9
Intermediate metabolizer	An individual with one normal-function allele plus one decreased-function allele OR one normal-function allele plus one no-function allele OR 2 decreased-function alleles	1.5	*1/*2, *1/*8
		1	*1/*3, *2/*2, *8/*11
Poor metabolizer	An individual with one no-function allele plus one decreased-function allele; OR 2 no-function alleles	0.5	*2/*3, *2/*6
		0	*3/*3, *3/*6, *3/*5

[a] Assignment of allele function and associated citations can be found at the CPIC website, also see (46)
[b] See the *CYP2C9* Frequency Table (45) for population-specific allele and phenotype frequencies.
Note: There are no known cases of CYP2C9 ultrarapid metabolizers. CPIC - Clinical Pharmacogenetics Implementation Consortium
This CPIC table has been adapted from (5)

Phenoconversion

Medications can alter CYP2C9 enzymatic activity, resulting in conversion to a different metabolizer phenotype than would be predicted from an individuals' genotype. This phenomenon is referred to as phenoconversion. Any medication that is a substrate for CYP2C9 may compete with other concomitant CYP2C9 substrate medications, leading to a reduced effective enzyme activity level for all CYP2C9-metabolized medications. Other non-substrate medications that inhibit the CYP2C9 enzyme can alter the pharmacokinetic parameters of any drug that is a substrate of CYP2C9 through mechanisms other than competition for metabolism. An individual who is predicted from their genotype to be an IM and is taking more than one CYP2C9 substrate or inhibitor, such as amiodarone, fluconazole, metronidazole, sulfaphenazole, or voriconazole (37, 38), may present phenotypically like a PM. Conversely, CYP2C9 can be induced by other medications, leading to increased enzyme activity compared to the activity predicted by the genotype. Administration of rifampin and carbamazepine can induce CYP2C9 and the related enzyme CYP3A4 (1). Additional CYP2C9 inducers include nifedipine, hyperforin (found in St. John's wort herbal supplement), phenobarbital, phenytoin, dicloxacillin, flucloxacillin, and tamoxifen (37). If an individual who is predicted to be an IM is taking a CYP2C9 substrate and an inducer, the individual may present phenotypically more like a normal metabolizer because the increased activity produced by the inducer may counteract some of the decreased activity produced by the reduced-function allele.

Linking *CYP2C9* Genetic Variation with Treatment Response

The manufacturer provided data for EMA and FDA authorization indicates that individuals with *CYP2C9*3/*3* genotype (activity score 0) should not take siponimod due to substantially elevated plasma levels of the medication, increased by 285% compared to *CYP2C9*1/*1* exposure (1, 2). The DPWG also recommends avoiding siponimod in individuals with *CYP2C9*3/*3* genotype (4). However, this contraindication is not extended to other genotypes with a PM phenotype, as the total exposure of *CYP2C9*2/*3* (activity score 0.5) was only 91% higher (1). Citing a lack of experimental or clinical data on the impact of other no-function alleles (including alleles like *CYP2C9*6*), the FDA-approved label states it is likely that other no-function alleles "will have similar effects on siponimod pharmacokinetics" though clinical guidance is not provided(1).

Individuals with the reduced-function genotype of *CYP2C9*1/*3* (activity score of 1) or *CYP2C9*2/*3* (activity score of 0.5) are recommended to take half of the standard maintenance dose, as stated by the FDA, EMA drug labels, and the DPWG (1, 2, 4). Again, other alleles that confer an IM phenotype are not specifically addressed by the FDA drug labeling, and the reduced dosage recommendation is specific to these genotypes. It should be noted that the versions of the drug label approved in the US (1) and Canada specifically recommend that individuals with *CYP2C9*2/*2* genotype be given the standard dose (2 mg daily maintenance dose).The

European approved version of the drug label does not explicitly address dosing for individuals with *CYP2C9*2/*2* genotype (2).

Currently, the dose recommended for *CYP2C9*3* heterozygotes cannot be extrapolated to all IM-associated genotypes. Much of the available literature for siponimod pharmacokinetics was published before the use of activity scoring and phenotype assignments were standardized (5). *In vitro* metabolism studies with human liver microsomes reported a 2.7-fold decrease in siponimod metabolism in samples with *CYP2C9*2/*2* genotype and simulations of plasma concentration of siponimod were elevated in both the **2/*2* and **3/*3* genotype (9). However, *in vivo* studies described in the drug label show a 25% increase in plasma concentration (measured as area under the curve) with the *CYP2C9*2/*2* genotype compared to **1/*1* (1).The clearance of siponimod reported for *CYP2C9*2/*2* was higher than *CYP2C9*1/*3*, and the *CYP2C9*2/*3* clearance was well above *CYP2C9*3/*3*(47). The difference in *in vitro* and *in vivo* metabolism of siponimod raises a question as to whether the *CYP2C9*2* variant may not confer as significant of a decrease in enzyme activity for siponimod as compared to other CYP2C9 substrates. Contrary to other medications, siponimod pharmacogenetic dosing recommendations are not made based on metabolizer phenotype but rather specific allele identification based on known effects of those alleles.

This model for pharmacogenetic recommendations highlights the need for more studies to assess the impact of CYP2C9 alleles found in populations with non-European genetic ancestry. Some authors have observed that the incidence of MS is higher in blacks (racial assignment extracted from health and military records, most likely reflecting self-reported identification (48, 49); incidence rates averaged to 10.2 per 100,000 as compared to 6.9 in whites, 2.9 in Hispanics and 1.4 in Asians) and the EXPAND and BOLD clinical trials for siponimod did not report race or ethnicity in their studies (50). The FDA review documentation for siponimod approval indicated that approximately 95% of the participants in EXPAND (also referred to as CBAF312A2304) were white (51). Additional decreased or no-function *CYP2C9* alleles (for example, **5, *6, *8* and **11)* are highly relevant across a broad range of racial and ethnic backgrounds, indicating the need for additional analysis and consideration for dosing adjustment (50). Based on altered pharmacokinetics of phenytoin and warfarin in individuals with a *CYP2C9*11* allele, analysis of this allele is highly recommended before prescribing medications like siponimod, especially due to their narrow therapeutic window and CYP2C9 substrate status (52).

Due to the more rapid metabolism of siponimod, co-medication with drugs that strongly induce CYP2C9 and CYP3A4 is not recommended in all individuals. Specifically, co-medication with moderate or strong CYP3A4 inducers such as modafinil or efavirenz, is not recommended in individuals with *CYP2C9*1/*3* or **2/*3* IM genotype. Conversely, co-medication with drugs that inhibit CYP2C9 and CYP3A4, such as fluconazole is also not recommended for all individuals due to the risk of increased exposure and potential adverse effects. (1)

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests available for siponimod drug response and the *CYP2C9* gene.

For *CYP2C9*, the Association for Molecular Pathology has recommended alleles that should be included in clinical genotyping assays (6). Testing results are typically reported as a diplotype, such as *CYP2C9*1/*2,* and may include an interpretation of the individual's predicted metabolizer phenotype (normal, intermediate, or poor) and an activity score (Table 5). Testing laboratories will report an allele assignment of *CYP2C9*1/*1* if no tested variant is detected in the sample. However, if an allele is not covered by the test, the *1/*1 diplotype does not mean that the individual does not have that variant; it only indicates the person does not have any of the variants included in the test. Therefore, reviewing the testing methods and alleles covered by the specific test used is crucial to understanding what a **1/*1* report truly means for the individual tested.

The *CYP2C9* Gene Interactions with Medications Used for Additional Indications

The CYP2C9 enzyme is involved in the metabolism of a wide range of medications, making genotyping results informative for many drugs. Medications that may be affected by *CYP2C9* genetic variation include:

- NSAIDs like celecoxib, flurbiprofen, piroxicam, all of which are metabolized by CYP2C9; thus individuals with reduced enzyme activity will experience increased exposure and have a higher risk of side effects.
- Cannabinoids like dronabinol, a synthetic form of delta-9-tetrahydrocannabinol, are metabolized by CYP2C9 and decreased enzyme activity increases an individual's exposure to the active compound
- Anti-convulsant medications like phenytoin, which are metabolized by CYP2C9, and a loss of function in this enzyme increases exposure to the medication and the risk of adverse effects.
- Uric acid lowering medications like lesinurad; individuals who lack CYP2C9 activity have an increased exposure to lesinurad, and an increased risk of side effects.
- Anticoagulants like warfarin, individuals with reduced or no function *CYP2C9* alleles require lower doses of this medication.

Additional information on gene-drug interactions for *CYP2C9* are available from PharmGKB, CPIC and the FDA (search for "CYP2C9").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

CYP2C9 Genotype Determination

Test patients for CYP2C9 variants to determine CYP2C9 genotype.

[…]

Recommended Dosage in Patients With CYP2C9 Genotypes *1/*1, *1/*2, or *2/*2

Maintenance Dosage

After treatment titration (see Treatment Initiation), the recommended maintenance dosage of [siponimod] is 2 mg taken orally once daily starting on Day 6.

[…]

Recommended Dosage in Patients With CYP2C9 Genotypes *1/*3 or *2/*3

Maintenance Dosage

In patients with a CYP2C9*1/*3 or *2/*3 genotype, after treatment titration (*see Treatment Initiation*), the recommended maintenance dosage of [siponimod] is 1 mg taken

orally once daily starting on Day 5.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Administer tablets whole; do not split, crush, or chew [siponimod] tablets.

Treatment Initiation

Initiate [siponimod] with a 4-day titration, as shown in Table 2... A 7-tablet starter pack should be used for patients who will be titrated to the 1-mg maintenance dosage.

[…]

[Siponimod] is contraindicated in patients who have:

A CYP2C9*3/*3 genotype

Please review the complete therapeutic recommendations that are located here: (1).

2023 Statement from the European Medicines Agency (EMA):

Before initiation of treatment, patients must be genotyped for CYP2C9 to determine their CYP2C9 metaboliser status …

In patients with a CYP2C9*3*3 genotype, siponimod should not be used …

Treatment initiation

Treatment has to be started with a titration pack that lasts for 5 days. Treatment starts with 0.25 mg once daily on days 1 and 2, followed by once-daily doses of 0.5 mg on day 3, 0.75 mg on day 4, and 1.25 mg on day 5, to reach the patient's prescribed maintenance dose of siponimod starting on day 6 (see Table 1).

[…]

Treatment maintenance

In patients with a CYP2C9*2*3 or *1*3 genotype, the recommended maintenance dose is 1 mg …

The recommended maintenance dose of siponimod in all other CYP2C9 genotype patients is 2 mg.

Please review the complete therapeutic recommendations that are located here: (2).

2023 Statement from Health Canada:

Contraindications:

Siponimod is contraindicated in: […]

• Patients with a CYP2C9*3*3 genotype

[…]

Dosing Considerations:

Prior to initiating treatment with MAYZENT the following assessments should be done to guide patient selection and treatment:

CYP2C9 genotype

The CYP2C9 genotype has a significant impact on siponimod metabolism.

• Determine the CYP2C9 genotype of the patient to establish CYP2C9 metabolizer status. CYP2C9 genotyping prior to initiating treatment with siponimod will be offered by the manufacturer through its Patient Support Program.

• MAYZENT is contraindicated in patients with a CYP2C9*3*3 genotype […]

• Dose adjustments are recommended for patients with CYP2C9*1*3 or a CYP2C9*2*3 genotype

[…]

Recommended Dose and Dosage Adjustment

Treatment has to be initiated in all patients with a starter pack that lasts for 5 days […] The dose titration starts with 0.25 mg once daily on day 1 and day 2, followed by once daily doses of 0.5 mg on day 3 (two tablets of 0.25 mg), 0.75 mg on day 4 (three tablets of 0.25 mg), and 1.25 mg on day 5 (five tablets of 0.25 mg), to reach the maintenance dose of 2 mg [siponimod] starting on day 6.

CYP2C9 Genotypes

In patients with a CYP2C9*2*3 or *1*3 genotype, the same starter pack should be used and treatment should be initiated as described above (see Table 1). On Day 6 the maintenance dose should be adjusted to 1 mg

Please review the complete therapeutic recommendations that are located here: (7)

2020 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

CYP2C9 IM ANDERS[other]: siponimod

Theoretically, the risk of adverse effects in increased, as the genetic variation results in higher plasma concentrations of siponimod.

- 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 the comparable genetic variation *1/*3, the moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model.

CYP2C9 PM ANDERS[other]: siponimod

Siponimod is contraindicated in patients with the comparable genetic variation *3/*3. Theoretically, the risk of adverse effects is greatly increased, as the genetic variation results in much higher plasma concentrations of siponimod.

- avoid siponimod

CYP2C9*1/*2: siponimod

NO action is required for this gene-drug interaction.

The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects.

CYP2C9*2/*2: siponimod

NO action is required for this gene-drug interaction.

The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects.

CYP2C9*2/*3: siponimod

Theoretically, the risk of adverse effects in increased, as the genetic variation results in higher plasma concentrations of siponimod.

- 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.

Please review the complete therapeutic recommendations that are located here: (4)

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.4:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.4:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.4:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.4:c.818del	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.4:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*9*	752A>G His251Arg	NM_000771.4:c.752A>G	NP_000762.2:p.His251Arg	rs2256871
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.4:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (53).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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