Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

S1P4R Antagonists

Evidence Summary

S1P4R may contribute to the progression of cancer and chronic inflammation-associated conditions via immune regulation, but the extent of this contribution and impact of targeting is unclear.

Neuroprotective Benefit: S1P4R may play a role in influencing the neuroinflammatory profile and neurovascular dynamics, but the effect of modulating it is unclear, and may be disease-context dependent.

Aging and related health concerns: S1P4R may influence the anti-tumor immune response and be useful as a prognostic marker, but the utility of S1P4R antagonists as immunotherapy adjuncts may depend on the tumor microenvironment.

Safety: The safety profile of selective S1P4R modulators has not been established, but due to its limited tissue distribution, S1P4R does not appear to be a major contributor to side effects in clinically tested broad-spectrum S1PR modulators.

Availability: Research use	**Dose**: N/A	**Chemical formula:** N/A **MW**: N/A
Half-life: N/A	**BBB**: N/A	
Clinical trials: None	**Observational studies**: S1P4R expression is associated with prognosis in various cancers.	

What is it?

Sphingosine-1-phosphate receptor 4 (S1P4R) is one of the five G-protein coupled receptors (GPCRs) ($S1PR_{1-5}$) for sphingosine-1-phosphate (S1P) [1]. S1P is a bioactive sphingolipid involved in autocrine and paracrine signaling. S1P can activate a variety of downstream signaling cascades depending on which G-proteins are engaged. As such, S1P-mediated signaling is highly context dependent. S1P4R has been reported to couple to $G\alpha_{i/0}$ and $G\alpha_{12/13}$, and thus can lead to the phospholipase C-mediated release of Ca^{2+} from internal stores and activate downstream Ca^{2+}-associated signaling [2]. S1P4R is the least studied and characterized of the S1PRs. It is predominantly expressed on lymphoid and hematopoietic tissue, and its best characterized role is in the regulation of T cell cytokine secretion [3]. It also plays roles in the activation and migration of different immune cell subsets, including lymphocytes, dendritic cells, and neutrophils [4]. Selective S1P4R modulators with good drug-like properties are not yet available [5]. S1P4R is best characterized in the context of cancer as a prognostic biomarker.

Neuroprotective Benefit: S1P4R may play a role in influencing the neuroinflammatory profile and neurovascular dynamics, but the effect of modulating it is unclear, and may be disease-context dependent.

Types of evidence:

- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function: None

Human research to suggest benefits to patients with dementia:

There is currently no evidence specifically linking S1P4R to dementia, but dysregulation of sphingolipid and S1P signaling is found in a variety of neurodegenerative diseases [1]. The pattern of dysregulation in the Alzheimer's disease brain typically involves an increase in relative levels of ceramide and a corresponding decrease in sphingomyelin and S1P. Since S1P4R does not appear to be expressed on CNS resident cells, it is unlikely to contribute to altered S1P signaling within neural cells. It may, however, play an indirect role in influencing the immune profile of the CNS due to its roles in regulating peripheral immune cell activation and migration.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

There is limited information regarding the role of S1P4R signaling within the CNS [1]. It may be involved in influencing the neuroinflammatory profile. In a rat model of experimental autoimmune encephalitis (EAE), S1P4R expression was found to be upregulated six to eight-fold in the spinal cord, and normalized in response to treatment with the S1PR inhibitor, fingolimod [6]. It is unclear, however, the extent to which the increase in S1P4R per se influenced disease activity, as the increase in S1P4R levels may have been related to the migration of T cells into the spinal cord in EAE. It has not been established whether S1P4R-mediated signaling meaningfully contributes to inflammatory processes involved in the induction and/or progression of neurodegenerative diseases.

There is some evidence to suggest that S1P4R may play a role in regulating the vascular endothelium. S1P4R was found to be expressed on primary brain microvascular endothelial cells (BMECs) from mice, cows, and pigs, and was localized to the abluminal endothelial membrane [7]. Apical treatment of S1P led to a tightening of the endothelial barrier, whereas basolateral treatment of S1P led to a more permeable endothelial barrier. The effect was mediated by the balance between S1PR1/S1P4R and S1P2R-mediated signaling, with the former promoting barrier integrity, and the latter promoting permeability. Mice treated with a S1P4R antagonist also showed evidence of increased blood brain barrier (BBB) permeability, suggesting that S1P4R plays a role in endothelial homeostasis and barrier function. S1P4R was shown to be down regulated on brain microvessels in the transient middle cerebral artery occlusion (tMCAO) mouse model of stroke, a condition associated with increased BBB permeability. This suggests that, if these findings are applicable to humans, long-term treatment with S1P4R antagonists may impact BBB function and integrity.

In patients with aneurysmal subarachnoid hemorrhage (n=44), elevated mRNA expression of S1P4R in the blood was associated with increased risk for cerebral vasospasm [8], providing further support for S1P4R playing a role in the regulation of the cerebral vasculature. Since this was a blood biomarker, it is unclear whether S1P4R is causally involved, and if so, which cell type(s) S1P4R acts upon to mediate this effect. In rats, S1P4R was found to be expressed on the media of pulmonary arteries, and the activation of S1P4R by S1P resulted in pulmonary vasoconstriction [9].

APOE4 interactions: Not established

Aging and related health concerns: S1P4R may influence the anti-tumor immune response and be useful as a prognostic marker, but the utility of S1P4R antagonists as immunotherapy adjuncts may depend on the tumor microenvironment

Types of evidence:

- 6 biomarker studies linking S1P4R expression and prognosis in cancer
- Several laboratory studies

Cancer: SIP4R EXPRESSION MAY BE A PROGNOSIS MARKER
There are numerous studies showing that S1P4R expression is a potential biomarker of prognosis for various types of cancer. These studies generally examine tissue from cancer databases, such as The Cancer Genome Atlas (TCGA), and look for associations between gene expression and survival. In the majority of these studies, high expression of S1PR4 was associated with better prognosis. However, high levels of S1P are also associated with poor prognosis [10], and some studies suggest that this may be mediated via S1P4R [5]. The association between S1P4R and prognosis may be confounded by the association between S1P4R and CD8 T cells in combination with the association between CD8 T cells and survival. Preclinical studies suggest that CD8 T cells with high S1P4R expression are immunosuppressive, and thus hinder the anti-tumor response [5; 11]. The potential utility of S1P4R antagonists in cancer remains to be determined, and will likely vary based on tumor type.

Ovarian cancer: Based on 1,129 ovarian cancer tissue samples from TCGA, S1P4R was identified as a prognostic classifier, along with four other genes, CXCL11, TNFRSF17, FPR1 and DHRS95, to differentiate high and low risk patients [12]. The risk groups could be characterized by the differential distribution of five immune cell subsets (monocytes, macrophages M1, macrophages M2, CD4 memory T cells, and CD8

T cells). High risk groups had higher proportions of monocyte cells and M2 macrophages, and decreased levels of CD8 T cells and M1 macrophages. These genes tracked with the different subpopulations of immune cells. Higher levels of S1P4R were associated with higher levels of CD8 T cells, and thus a lower risk score.

Cervical squamous cell carcinoma: Based on 309 tumor tissue samples from TCGA, S1P4R, along with ITGA5, and HHEX were identified as prognostic factors [13]. Low expression of ITGA5 and high expression of HHEX and S1P4R was associated with better survival. S1P4R was also an independent prognostic factor (Hazard Ratio [HR]: 0.787, 95% Confidence Interval [CI] 0.657 to 0.944, P=0.010).

Non-smoker, lung adenocarcinoma: Based on 1,927 NSCLC samples from TCGA, high expression of S1PR4 was associated with better overall survival (HR: 0.79, 95% CI 0.69 to 0.91, P = $7e^{-4}$) [14]. This was driven by an association between S1PR4 expression and the levels of tumor-infiltrating lymphocytes.

Breast cancer: Based on 622 breast cancer samples from TCGA, higher expression of S1P4R was associated with better survival (HR: 0.73, 95% CI 0.59 to 0.9) [15]. This study found an enrichment in immune response genes as predictors of prognosis, with a higher immune response score showing better prognosis. Similarly, a study focused on ***triple-negative breast cancer*** using the UALCAN cancer database found that higher expression of S1P4R was associated with better release-free survival (HR: 0.81, 95% CI 0.73 to 0.91, P=0.00023) [16]. This was driven by a correlation between S1P4R expression and levels of tumor infiltrating immune cells. However, a study examining 140 samples from patients with ***estrogen receptor-negative breast cancer*** found that high expression of S1PR4 was associated with worse disease-free survival and disease-specific survival [17]. This could be related to differential signaling downstream of S1P4R due to different tumor environment conditions. However, this study instead suggests that the association of S1P4R with prognosis can be confounded by the association between S1PR4 and CD8 T cells. While having more tumor infiltrating immune cells is generally beneficial, S1P4R is preferentially associated with immunosuppressive T cells, thus tumor infiltrating T cells with high S1P4R expression may be indicative of a weak anti-tumor immune response.

The association between S1P4R and an immunosuppressive tumor environment is supported by preclinical studies. S1P is generally considered to be pro-tumorigenic, and preclinical studies suggest that the this may be mediated, at least in part, through S1P4R. IL-33 promotes tumorigenesis by expanding T regulatory cells within the tumor microenvironment [11]. Under conditions of nutrient deprivation, which is common in the tumor microenvironment, CD8 T cells were found to show reduced

expression of CD8 and increased IL-33. These T cells showed markers associated with immunosuppressive T regulatory cells. In response to IL-33, S1P4R was found to be to upregulated on these immunosuppressive CD8 low T cells. In the AOM/DSS-induced model of colitis-associated cancer, mice lacking S1P4R had delayed tumor growth and improved response to therapy, due to increased survival and proliferation of anti-tumor CD8 T cells [5]. The mice lacking S1P4R also had less metastases. Similarly, in a mammary tumor cell spheroid coculture system, treatment with the S1P4R antagonist, CYM 50358, led to an increase in anti-tumor CD8 T cells. Altogether these studies suggest that S1P4R antagonists may help promote anti-tumor immune responses, especially when used in combination with immunotherapies, though the efficacy may depend on the immunogenicity of the tumor as well as the signaling landscape of the tumor microenvironment.

Non-alcoholic steatohepatitis: POTENTIAL BENEFIT AT VERY EARLY STAGES (Preclinical)
The expression of S1P4R was found to be six-fold higher in the livers of patients with non-alcoholic steatohepatitis (NASH) (n=9) relative to controls (n=10) [18]. S1P4R expression was similarly increased in the liver in several mouse models of NASH. In the high-fat, high-cholesterol-diet model of NASH, mice lacking one copy of S1P4R ($S1pr4^{+/-}$) showed evidence of steatosis without corresponding hepatic fibrosis. The protective effect was mediated via the hepatic macrophages, by preventing the S1P-induced activation of the NLRP3 inflammasome, and associated pro-inflammatory (IL-1β) signaling in the liver. This involved the release of calcium from internal stores down stream of S1P4R activation. NASH-model mice treated with the selective S1P4R functional antagonist, SLB736 (1 mg/kg orally for 4 weeks), showed a similar phenotype as the $S1pr4^{+/-}$ mice in that they still showed evidence of steatosis, but liver inflammation and fibrosis were strongly attenuated. These studies suggest that preventing the induction of S1P4R in the context of steatosis may prevent inflammation-related liver injury and fibrosis, but it is unclear if S1P4R antagonists would offer any benefit once the mechanisms of liver inflammation and fibrosis have been induced.

Muscle atrophy/repair: POTENTIAL BENEFIT (Preclinical)
In myoblast (C2C12) cell culture, the activation of S1P4R was shown to promote TGFβ1-mediated muscle precursor cell apoptosis. TGFβ1 is an established negative regulator of skeletal muscle cell repair, and S1P4R may play a role in this process. Treatment with the S1P4R antagonist, CYM 50358, attenuated TGFβ1-mediated proapoptotic signaling in myoblasts. If this pathway is relevant to muscle tissue in humans, then S1P4R antagonists may play a beneficial role in the context of age-related muscle atrophy.

Safety: The safety profile of selective S1P4R modulators has not been established, but due to its limited tissue distribution, S1P4R does not appear to be a major contributor to side effects in clinically tested broad-spectrum S1PR modulators.

Types of evidence:

- Reviews of broad-spectrum S1PR modulators
- Several laboratory studies

Although S1PR modulators, which impact several S1PRs, have been in clinical use for autoimmune conditions, primarily multiple sclerosis, S1P4R is not the predominant target [19]. In general, the serious adverse effects of broad S1PR modulators, particularly with respect to the cardiovascular system, are attributed to S1P3R, while lymphopenia is primarily attributed to S1P1R, which plays a major role in lymphocyte trafficking [20; 21]. The side effect profiles of S1PR modulators which also show activity toward only S1P1R, S1P4R, and S1P5R tend to be more favorable than those which also show activity toward S1P2R and S1P3R. The effects appear to be primarily attributable to S1P1R, suggesting that that modulation of S1P4R and S1P5R does not greatly contribute to systemic side effects. This could be due to the relatively localized expression of these receptors and/or that the drugs tested thus far show preferential activity toward S1P1R.

The S1P4R antagonists currently available for research use have poor pharmacokinetic profiles, thus the safety profile of this class of drugs has not been well characterized [5].

Since S1P4R is primarily expressed on lymphoid tissue, its modulation would be expected to primarily impact the activation and migration of different immune cell subsets [1]. Due to S1P4R's roles in immunity, its modulation may impact the risk and response to infections [22]. Unlike S1P1R, it is not a major player in lymphocyte trafficking, thus its inhibition would not be expected to result in clinically relevant lymphopenia. Due to the pleiotropic, context-dependent nature of S1PR signaling, the therapeutic profile of S1P4R modulators is likely to vary with different disease indications depending on the overall S1P associated landscape [1]. Biased S1P4R modulators, which affect its coupling to only one or a subset of its associated G proteins, and thus influence only a subset of potential downstream signaling pathways, may offer the best therapeutic profiles.

Drug interactions: Not established. Due to its potential immunomodulatory properties, S1P4R modulators may have interactions with immunosuppressants.

Sources and dosing:

Selective S1P4R modulators are not available for clinical use, though S1P4R can be affected by the broad-spectrum S1PR functional antagonist, fingolimod, as well as other S1PR modulators in clinical development, such as etrasimod. Selective S1P4R antagonists which have been tested in preclinical studies include CYM 50358 and SLB736.

Research underway:

There are preclinical efforts underway to better characterize S1P4R and develop novel modulators.

Search terms:

Pubmed, Google: S1P4R

Antagonist, Alzheimer's disease, brain, aging, cardiovascular, cancer, safety

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