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.

TRPML1 Agonists

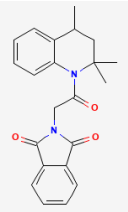
Evidence Summary

TRPML1 is an important regulator of autophagy and lysosomal function. Modulating it has the potential to protect against age-related diseases, but it is unclear whether that can be done safely or reliably.

Neuroprotective Benefit: TRPML1 activation may help restore lysosomal function and autophagy in neurons, but due to its numerous context-dependent effects, the impact of modulating it may vary by condition and individual.

Aging and related health concerns: TRPML1 regulates longevity-related pathways. Its modulation may benefit some cancers, but its sensitivity to specific environmental conditions may limit its therapeutic utility in other indications.

Safety: There is a dearth of evidence regarding the safety of TRPML1 agonists currently in preclinical development. Its ubiquitous expression and context-dependent activity raises the concern about the potential for side effects.

Availability: For research use, in preclinical drug development.	**Dose**: Not established	**ML-SA1** **Chemical formula:** $C_{22}H_{22}N_2O_3$ **MW**:362.4 g/mol  Source: PubChem
Half-life: Not established	**BBB**: Not established	
Clinical trials: None	**Observational studies**: None	

What is it?

Transient receptor potential channel mucolipin 1 (TRPML1) is an endolysosomal cation channel [1]. It is one of three TRPML channels expressed in humans. TRPML1 has ubiquitous expression, while TRPML2 and TRPML3 show more restrictive expression, primarily to immunological tissue. TRPML1 fluxes both monovalent (Na+≅ K+>Cs+) and divalent cations (Ba2+>Mn2+>Fe2+>Ca2+> Mg2+> Ni2+>Co2+> Cd2+>Zn2+>>Cu2+) (IUPHAR/BPS). It is involved in the maintenance of lysosomal and cellular Ca2+ homeostasis, as it interacts with other Ca2+ storage organelles, including the endoplasmic reticulum (ER) and mitochondria [2]. The biological consequences of its other ionic fluxes are less well understood, and similar to Ca2+, are likely context dependent [3]. TRPML1 plays numerous essential roles in the maintenance of lysosomal homeostasis. Most prominently, it is involved in the regulation of autophagy. Endogenously it is regulated by phosphoinositide, such that its activity is affected by lipid membrane composition. Its localization to the endolysosomal compartment is tied to its activation by the endolysosomal-specific phosphoinositide, PI(3,5)P2.

Due to increasing evidence regarding the role of lysosomal dysfunction in aging and age-related diseases, TRPML1 has emerged as a potential therapeutic target and several companies are working on developing TRPML1 modulators [2]. TRPML1 agonists have not yet been clinically tested, and most of the compounds used in research studies lack specificity for TRPML1 and/or have poor pharmacokinetic properties. There are several companies that appear to be developing TRPML1 agonists, primarily for neurodegenerative diseases, though they are in early stages. Due to the buzz around this target, there may be additional companies working on it which have not yet disclosed their target.

Neuroprotective Benefit: TRPML1 activation may help restore lysosomal function and autophagy in neurons, but due to its numerous context-dependent effects, the impact of modulating it may vary by condition and individual.

Types of evidence:

- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

GWAS studies implicate lysosomal genes in risk for dementia, and endolysosomal dysfunction has been identified as the earliest pathology in the context of Alzheimer's disease (AD) [4]. The accumulation of amyloid appears to stem from its inability to be properly degraded in deacidified lysosomes [5]. Deficits in lysosome exocytosis have also been implicated in the failure to clear tau and alpha-synuclein [6]. Together these suggest that the maintenance or restoration of lysosomal function is critical for cognitive health. To date, it has been technically challenging to determine whether alterations in TRPML1, specifically, contribute to endolysosomal dysfunction in AD.
The accumulation of perinuclear endolysosomes, identified through the marker LAMP1, along with the vacuolization of these intracellular vesicles, has been observed in neurons and astrocytes in postmortem hippocampal brain tissue from AD patients [7]. These LAMP1+ vesicles were found to be enriched around Aβ plaques and in cells with tau aggregates. Phosphoinositols are endogenous ligands of TRPML1, and levels of total PIP_3 and PIP_2 were also found to be elevated in the temporal cortical region in brain tissue from AD patients, relative to controls, suggesting that TRPML1 activity could be impacted. In cell culture, depletion of the endogenous phosphoinositol agonist of TRPML can induce a similar AD-like cellular phenotype of perinuclear accumulation and vacuolization of the endolysosomes, which can be rescued through the use of a synthetic TRPML1 agonist [7]. Due to variability and lack of specificity with available TRPML1 antibodies, it has been challenging to determine whether TRPML1 protein expression and/or localization is altered in the AD brain [7]. In human iPSC-derived cortical neurons from ApoE4 carriers, TRPML1 activity was found to be reduced, resulting in elevated Ca2+ levels in the endolysosomal compartment [7; 8].
Mutations in the endosomal/lysosomal ion channel, TRPML1, lead to the neurodevelopmental neurodegenerative disorder, Mucolipidosis type IV (MLIV) [9]. This lysosomal storage disorder results in mental retardation, motor deficits, and vision loss. The prominent neurological effects implicate TRPML1 as critical for the maintenance of lysosomal function in the brain. Most of the mechanistic studies

regarding TRPML1 have been conducted in rodents, and there are limited studies looking at its activity in human brain tissue. A proteomics study analyzing postmortem brain tissue from a patient with MLIV was conducted. In addition to changes in lysosome, autophagy, and inflammation-related proteins, there were prominent changes in apolipoproteins, such as an increase in ApoD, which is also elevated in other neurodegenerative conditions, such as AD [9]. Ultimately, more studies are needed to assess the contribution of TRPML1 to neurodegenerative disease in human tissue.

Human research to suggest benefits to patients with dementia:

TRPML1 agonists have not yet been tested in dementia patients.

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

Autophagy: The accumulation of misfolded proteins is a common feature of many neurodegenerative diseases, thus mechanisms to enhance protein clearance are considered as potentially beneficial therapeutic interventions. One of the major clearance mechanisms is autophagy. TRPML1 has been implicated in autophagy, however, its role is complex, and not completely understood. The impact of TRPML1 activation on autophagy and downstream cellular processes appears to be context-dependent, as it varies across studies depending on the cell type and experimental conditions.
The proposed mechanisms by which TRPML1 impacts autophagy pathways are both direct and indirect. TRPML1 is part of a positive feedback loop with TFEB and a negative feedback loop with mTORC1 [6; 12]. Calcium release from TRPML1 promotes the activation and nuclear translocation of TFEB, a transcription factor that serves as a master regulator of autophagy, which influences the expression of lysosome and autophagy-related genes. TRPML1 is one of its targets, thereby leading to a positive feedback loop. This process also increases lysosomal exocytosis, which facilitates the clearance of cellular debris and the recycling of essential cellular building blocks. TRPML1 can also promote autophagosome biogenesis in a TFEB-independent manner [13]. TRPML1 also contributes to the negative regulation of autophagy, which is necessary to prevent cell death [1]. TRPML1 can activate mTORC1, a negative regulator of autophagy, which in turn inhibits TRPML1 [14]. The cumulative effect of these feedback loops is to ensure that autophagy is finely tuned to the needs of the cell. The relative flux of ions through TRPML1 may play a role in determining which of these pathways dominate, as some studies indicate that the flux of calcium promotes autophagy, while the flux of zinc may inhibit it [10]. Due to the range of interacting players, the impact of activating TRPML1 highly depends on cellular environmental conditions, such as ion concentrations and the expression/activity level of other players in the autophagy network. Some

studies suggest that the enhancement of autophagy by TRPML1 is largely driven by the fusion of autolysosomes with lysosomes, which facilitates the breakdown of cellular waste/aggregates through their transfer to acidified compartments [11; 12]. If, for example, lysosome acidification was impaired, the increased transfer of cargo to lysosomes would not necessarily enhance clearance. Therefore, increasing some parts of the autophagy process may not drive productive protein clearance if there are additional impairments in other parts of the network. This suggests that the modulation of TRPML1 may be best suited as part of a network approach.

Mitochondrial function: Mitochondrial dysfunction is observed in a variety of neurodegenerative conditions resulting in impaired cellular bioenergetics and signaling. The interplay between lysosomes and mitochondria has recently been understood to play an important role in regulating mitochondrial dynamics, through calcium signaling between the two organelles [13]. TRPML1 has been shown to play an important role in facilitating contacts and rapid calcium transfer between lysosomes and mitochondria [14]. Cell culture studies suggest that, at least in certain cell types, the loss of this TRPML1-mediated calcium signaling negatively impacts mitochondrial structure and function, resulting in fragmentation and impaired bioenergetics [14]. TRPML1 can also influence mitochondrial activity through the promotion of mitophagy, or the clearance of damaged mitochondria. Consequently, this inter-organelle calcium signaling may impact the production of reactive oxygen species (ROS) and oxidative tissue damage.

Alzheimer's disease: POTENTIAL BENEFIT, MORE HUMAN AND *IN VIVO* STUDIES ARE NEEDED
(Preclinical)
TRPML1 plays numerous roles in the maintenance of lysosomal homeostasis [6]. Due to the evidence for prominent endolysosomal dysfunction in AD, TRPML1 has emerged as a potential therapeutic target. The preclinical data regarding the effect of boosting TRPML1 function has been mixed, which is likely a reflection of the context-dependent and multifaceted nature of this ion channel. Additionally, a major challenge in interpreting the role of TRPML1 in disease processes and its therapeutic potential stems from the lack of high-quality reagents suitable for *in vivo* and *ex vivo* use [8; 15]. Due to challenges with antibody specificity and drug solubility/bioavailability, the majority of studies assessing the impact of TRPML1 modulation have been conducted in cell culture. This has limited the ability to assess the translatability of these findings. It is possible that the companies working on developing TRPML1 agonists have developed better tools, which have not yet been publicly disclosed.
Lysosome deacidification is seen in the context of AD [5]. Mutations in presenilin (PS1) can lead to lysosomal deacidification through deficits in the function of vATPase, the proton pump which acidifies

the lysosome [16]. In presenilin knockout cells, lysosomal Ca2+ efflux is elevated due to TRPML1 hyperactivity. The increased efflux of lysosomal Ca2+ leads to numerous downstream effects, leading to increases in cytosolic Ca2+ and the initiation of Ca2+-sensitive signaling cascades, such as autophagy induction, and altering levels of Ca2+ within coupled organelles, including mitochondria and the ER. In contrast to studies using other models where the enhancement of TRPML1 activity is therapeutic, the amelioration of deficits in this model system involves the reduction in TRPML1 activity. Notably, targeting TRPML1 alone was not sufficient to restore lysosomal function, as this is a secondary effect. This required targeting the primary deficit, the acidification of the lysosome. This study attributed the hyperactivity of TRPML1 to elevated pH within the lysosome, though it is unclear whether this is a byproduct of this model system, as the vast majority of studies indicate that the maximum activity for TRPML1 is at pH 4.6, the average pH of the lysosomal lumen, and that it decreases at elevated pH [3]. In PS1 mutant cells, the TRPML1 hyperactivity led to the stalling of late endosomes/lysosomes, resulting in a decrease in retrograde transport [17].

In contrast, TRPML1-mediated lysosomal Ca2+ release was found to be reduced in human iPSC-derived neurons expressing ApoE4, resulting in elevated levels of Ca2+ within lysosomes [7]. The lysosomes were properly acidified, thus this defect was not a result of altered pH. Treatment of the neurons with the synthetic TRPML1 agonist ML-SA1 enhanced TRPML1-mediated Ca2+ release.

In other AD models, such as the APP/PS1 mouse, TRPML1 has been shown to be downregulated [18]. In this context, the activation of TRPML1 is protective, stemming from its role in the regulation of autophagy. TRPML1 interacts with autophagy pathways via multiple routes. When functional, this system is neuroprotective. TRPML1-TFEB mediated lysosomal exocytosis prevents tau accumulation by promoting its exocytosis from the cell [6]. TRPML1 can also promote the clearance of amyloid, though this seems to be dependent on TRPML1-mediated acidification of the lysosome. In cell culture, LDL led to lysosome de-acidification and increased levels of Aβ42 [19]. Treatment of the cells with the TRPML1 agonist, ML-SA1, led to lysosome acidification and reduced Aβ accumulation. A similar effect has been shown with this TRPML1 agonist in the context of HIV antiretroviral-mediated Aβ accumulation in de-acidified endolysosomes [20]. Promotion of lysosome acidification is expected to be a beneficial therapeutic strategy in AD. TRPML1 does not directly control lysosome acidification, but as a cation channel, it can influence the level of charge buildup across the membrane, and thus impact the activity of the proton pump [21]. However, the studies in different cell systems indicate that the overall impact of TRPML1 on pH and the other parameters it regulates depends on the cellular environment. TRPML1 has extensive crosstalk with other Ca2+ storing organelles, and has the capacity to flux numerous cations, including several metals, such as iron, nickel, zinc, and copper [3]. This suggests that in a pathophysiological setting, where there is dysregulation in several of these interacting pathways, the net

effect of activating TRPML1 may be difficult to predict. Empirical evidence is needed regarding the function of TRPML1 in the AD brain in humans, including whether it is uniformly affected across cell types, and the interindividual variability.

Parkinson's disease: POTENTIAL BENEFIT (Preclinical)
The accumulation of alpha-synuclein aggregates and iron dyshomeostasis are observed in the PD brain [22]. TRPML1 activity has been shown to facilitate the clearance of alpha-synuclein aggregates and impact iron-related cytotoxicity in cell culture models, however, the direction of TRPML1 modulation needed to positively impact these disease-related processes is not necessarily consistent. Regional increases in iron and decreases in copper levels have been observed in postmortem brain tissue from PD patients with sporadic and genetic (G2019S-LRRK2) forms compared to healthy age-matched controls [11]. In cell culture, the activation of TRPML1 promoted endolysosomal release of Fe2+ resulting in increased cytosolic Fe2+ and cytotoxicity under iron overload conditions [11]. Meanwhile, the induction of TFEB through TRPML1-mediated Ca2+ release could prevent cytotoxicity by promoting the exocytosis of iron-laden lysosomes. In the brain, iron is typically bound with ferritin, which stores iron in a non-toxic, bioavailable form [23]. TRPML1 was shown to promote lysosomal ferritin secretion in primary rat astrocytes under conditions of elevated iron, suggesting that this mechanism may help protect against free iron oxidation and associated cell damage [23]. Thus, the net impact of TRPML1 depends on its relative flux of Fe2+ to Ca2+, as well as the functionality of machinery involved in lysosomal biogenesis and exocytosis.
In contrast, treatment with TRPML1 agonists generally enhances the clearance of alpha-synuclein aggregates in cell culture systems. Treatment with ML-SA1 reduced levels of alpha-synuclein aggregates in HEK293T cells overexpressing mutant A53Tα-synuclein by shifting the balance of alpha-synuclein into acidified vesicles and facilitating autophagosome-lysosome fusion [24]. Human-induced pluripotent stem cells (hiPSC)-derived neurons transduced with tagged alpha-synuclein also showed a trend to reduced alpha-synuclein aggregates in the presence of ML-SA1 and increased aggregates with the TRPML1 inhibitor ML-SI3 [24]. A screen for compounds that promote lysosomal clustering, and autophagic flux identified several compounds that potentiated TRPML1 activity [12]. In iPSC-derived dopaminergic neurons from patients with PARK9 mutations, lysosomal calcium homeostasis is disrupted [19]. Loss of PARK9 leads to a reduction in lysosomal calcium levels. This, in turn, impairs lysosomal exocytosis, leading to the accumulation of alpha-synuclein. The activation of TRPML was able to restore lysosomal exocytosis in this model system.
Caraway Therapeutics developed TRPML1 activators for use in GBA-PD, the most common genetic form of PD. In this condition, PD occurs downstream of mutations in GBA, which disrupts the function of the

lysosomal lipid processing enzyme, glucocerebrosidase (GCase). According to Caraway Therapeutics, their TRPML1 activators enhanced lysosomal function and GCase activity in preclinical studies, which had positive effects on lipid and alpha-synuclein clearance (Press release).
In the MPTP model, treatment of male mice with the traditional Chinese herb, *Artemisia argyi Lev. et Vant*, also known as Chinese mugwort, (100 mg/kg) for two weeks, reduced the loss of dopaminergic neurons and associated motor deficits [25]. This was related to a reduction in the accumulation of reactive oxygen species, and enhanced autophagic clearance of alpha synuclein, due to the upregulation of TRPML1 activity.

Amyotrophic lateral sclerosis: POTENTIAL BENEFIT (Preclinical)
The accumulation of protein aggregates, such as SOD1, TDP-43, FUS, and C9orf72, is observed in both familial and sporadic cases of ALS, suggesting that impaired protein clearance may play a role in the disease [26]. Modulation of TRPML1 may be a therapeutic mechanism to enhance protein clearance through autophagy. Western blot analysis of postmortem brainstem tissue indicated that protein levels of TRPML1 were reduced in the medulla oblongata of patients with sporadic or familial ALS relative to controls, while levels of the lysosomal marker LAMP1 were unchanged [26]. A similar reduction in TRPML1 was observed in the brainstem and spinal cord of symptomatic $SOD1^{G93A}$ ALS model mice [26]. Notably, TRPML1 expression was not significantly impacted in these mice at the presymptomatic stage, suggesting that the change in TRPML1 occurs as a consequence of ongoing disease activity. Activation of TRPML1 through the administration of PEGylated self-assembling lipid nanoparticles (SANPs) administered once per week via intracerebroventricular injection for six weeks starting at 1.5 months of age delayed the onset of symptoms in $SOD1^{G93A}$ mice by about two weeks, attenuated body weight loss, and extended survival by about 10 days (from ~128 to ~138 days) [26]. Histologically, the mice had greater preservation of motor neurons and reduced gliosis in the spinal cord. This was accompanied by a normalization of autophagy related proteins, p62 and LC3-II.
It remains to be determined whether there is a critical window of intervention for TRMPL1-based therapeutic approaches, as efficacy could depend on disease stage and the functionality of downstream players in the autophagy process.

HIV-related cognitive impairment: POTENTIAL BENEFIT (Preclinical)
AD-like neuropathology, such as the accumulation of Aβ, is common in the context of HIV/AIDs. In a mouse model of HIV-related neuropathology (gp120/APP/PS1), there is an overproduction of sphingomyelins, which accumulated in the lysosome [27]. The sphingomyelin inhibits TRP channels, leading to the accumulation of calcium and Aβ within lysosomes. Treatment of the cells with a TRPML1

agonist reacidified the lysosomes and restored lysosomal calcium efflux, leading to a reduction in Aβ neuropathology. The antiretroviral drugs used to treat HIV themselves can also promote the accumulation of Aβ and lead to cognitive impairment. Several of these antiretrovirals, including, efavirenz, nevirapine, ritonavir, nelfinavir, darunavir, and dolutegravir, were found to de-acidify lysosomes, which promoted the secretion and accumulation of Aβ [20]. Additional treatment of the cells with the TRPML1 agonist, ML-SA1, reacidified the lysosomes and blocked the accumulation of Aβ. Not all antiretrovirals had this effect. Indeed, zidovudine and abacavir, acidified lysosomes, and cells treated with these drugs had lower levels of Aβ. Though more work is needed to verify the relevance *in vivo*, TRPML1 agonists may potentially be useful in reducing neuropathology in HIV-positive individuals treated with certain antiretrovirals.

Muscular dystrophy: POTENTIAL BENEFIT (Preclinical)
In a mouse model of muscular dystrophy (mdx), muscle specific overexpression of TRPML1 reduced pathology and improved muscle function [28]. Similarly, treatment with the TRPML1 agonist, ML-SA5 (2 to 5 mg/kg i.p.) staring at P14, reduced muscle cell death and improved treadmill exercise function in one month old mice. Treatment was associated with the activation of TFEB and the promotion of muscle repair mechanisms. It is unclear whether there is a critical window of intervention, and whether chronic use would be safe and lead to the maintenance of benefits.

APOE4 interactions: TRPML1 activity was shown to be reduced in ApoE4 expressing neurons, relative to other ApoE genotypes, but it has not been established whether ApoE4 carriers are more likely to have reduced TRPML1 activity, or whether it is a relevant target *in vivo* [7; 8].

Aging and related health concerns: TRPML1 regulates longevity-related pathways. Its modulation may benefit some cancers, but its sensitivity to specific environmental conditions may limit its therapeutic utility in other indications.

Types of evidence:

- Several laboratory studies

Longevity: TRPML1 REGULATES LONGEVITY-ASSOCIATED MECHANISM
Rapamycin has been shown to increase lifespan in animal models. It has pleiotropic effects, but one of the major presumed mechanisms by which it promotes longevity is through the modulation of

autophagy via the inhibition of the mTOR complex. It was shown that TRPML1 can be activated by rapamycin, in an mTOR-independent manner [29]. It has been hypothesized that the induction of autophagy via TRPML-TFEB may be a major contributor to the anti-aging effects of rapamycin, though this requires further testing.

Cancer: POTENTIAL MIXED (Preclinical)
Different cancers may preferentially benefit from different types of TRPML1 modulators. Both TRPML1 activators and inhibitors have shown utility in preclinical cancer models [1]. TRPML1 interacts with nutrient sensing pathways (mTOR) and plays an important role in the adaptive lysosomal response to nutrient starvation. This is particularly relevant for the growth and survival of cancer cells. Cancer appears to be the condition best suited to a TRPML1-targeted therapy, due to the high sensitivity of these cells to TRPML1-mTORC1 signaling. TRPML1 has been shown to be overexpressed in some cancers, where its high levels are associated with better cancer cell growth and survival. These include triple negative breast cancer, melanomas, non-small-cell lung carcinomas (NSCLCs), pancreatic adenocarcinomas (PDACs) and p53-deficient bladder cancer [30]. Tumor cells driven by HRas proto-oncogene GTPase (HRAS) also appear to be particularly sensitive to TRPML1 inhibition [31]. But in other cases, the activation of TRPML1 can impair autophagy and lead to cell cycle arrest. For example, in glioblastoma, high expression of TRPML1 is associated with better prognosis and longer overall survival [30]. TRPML1 modulators may have utility in overcoming chemoresistance. In a xenograft model using a human leukemia cell line (OCI-AML3), knockdown of TRPML1 enhanced the anti-tumor efficacy of the chemotherapeutic, daunorubicin [32]. Notably, the knockdown of TRPML1 alone had no effect on tumor growth in this model. Due to the nutrient starved conditions of the tumor microenvironment, cancer cells may be especially sensitive to changes in the activity level of TRPML1, such that levels that are either too high or too low can impede their growth and survival, by impacting autophagy and mitochondrial function [33]. It may also depend on the stage, as the induction of autophagy can slow growth early on, and then accelerate it at later stages [1]. Studies in patient tissue highlight the variability in TRPML1 expression across individual patients [32], such that the prospective clinical benefits of TRPML1-targeted therapy are likely to vary based on the contribution of TRPML1 in a given patient, and may be best suited as part of targeted personalized medicine.

Ischemia/Stroke: MIXED/UNCLEAR (Preclinical)
TRPML1 may play a role in regulating vascular dynamics through the modulation of calcium signaling in vascular endothelial cells. TRPML1 was found to promote lysosomal calcium mobilization in the human brain microvascular endothelial cell line, hCMEC/D3, which, in turn, induced the Ca2+-dependent

release of nitric oxide [34]. Thus, changes in TRPML1 activity may have the ability to influence a variety of vascular processes.

TRPML1 has been shown to be activated under conditions where reactive oxygen species (ROS) are elevated, such as during ischemia/reperfusion, but the net outcome of TRPML1 activation is highly variable across model systems. TRPML1 appears to be differentially responsive to different sources of ROS, which in some cases were shown to have no effect or even inhibit TRPML1 [3; 26]. While TRPML1 activation is associated with the induction of autophagy in many studies, it is associated with the inhibition of autophagic flux in other settings, including some ischemic/reperfusion models. The different effects may stem from different tissue/cell types, experimental paradigms, timing of therapeutic administration, or additional factors.

One of these factors may be the relative release of zinc (Zn^{2+}). In murine cardiomyocytes, TRPML1 activation in response to elevated ROS led to the release of lysosomal zinc and a disruption to autophagosome-lysosome fusion, resulting in an impairment of autophagic flux [35]. The expression of TRPML1 decreased during the ischemic period and then increased during the reperfusion period. A similar pattern was seen in rat neurons, where the ROS-mediated hyperactivity of TRPML1 was detrimental [36]. Preconditioning induced a protective adaptation involving an interplay between TRPML1 and an ER Ca^{2+} channel, STIM1, such that the expression of TRPML1 stayed low during the reperfusion period [36]. Direct infusion of the TRPML1 agonist, ML-SA1 into the ventricles of mouse prior to a transient global cerebral ischemic injury was protective in reducing infarct volume, neurological deficits, and mortality [37]. Similarly, pretreatment with ML-SA1 administered intracerebroventricularly starting three days prior to cerebrovascular ischemic injury (middle cerebral artery occlusion) attenuated neuronal loss and neurological deficits in rats, while administration of the TRPML1 inhibitor ML-SI3 exacerbated neuronal injury in this model [38]. It is unclear whether the TRPML1 agonist would also be protective if treatment had been started after the ischemic event, during the reperfusion period. In a model of myocardial ischemia/reperfusion, inhibition of TRPML1 via shRNA, administered one day after the injury, was protective in reducing infarct size [35]. It is unclear if the timing of administration, the tissue type, or other factors contributed to the difference in outcomes across these studies. The dynamics of TRPML1 during the vascular injury may affect the type of modulation that is needed to provide a therapeutic benefit. In contrast to ischemic stroke conditions, in which TRPML1 expression is reduced in early stages, TRPML1 expression was found to be increased early on following intracerebral hemorrhage in a rat model [39]. In this model, pretreatment with ML-SA1 exacerbated neurological injury, while downregulation of TRPML1 with shRNA attenuated cognitive impairments.

Since the tissue conditions are likely to determine the activity level of TRPML1, it may be too variable to reliably target for this indication.

Safety: There is a dearth of evidence regarding the safety of TRPML1 agonists currently in preclinical development. Its ubiquitous expression and context-dependent activity raises the concern about the potential for side effects.

Types of evidence:

- Few laboratory studies

TRPML1 agonists are still in preclinical development and have not yet been tested for any clinical condition. The preclinical studies conducted thus far do not provide good insight into the potential safety of these compounds, as the vast majority of them are *in vitro*, due to the poor pharmacokinetic properties of the commonly research used TRPML1 agonists [6]. Studies that have looked *in vivo*, have tended to be very short, lacking safety evaluations. Chronic dosing studies are needed.
There are several reasons why targeting TRPML1 could potentially pose a safety concern. TRPML1 is ubiquitously expressed, so with a systemically administered drug, attempts to correct a deficit in TRPML1 in one cell type could lead to unintended consequences in other cell types. Due to its role in coordinating Ca2+ homeostasis across organelles [40], altering TRPML1 function could result in non-lysosomal effects, such as by affecting mitochondrial function and integrity [41]. More studies are needed regarding the effects of TRPML1 agonists in healthy cells of different tissue types. The other members of the TRPML family, TRPML2 and TRPML3 have more restricted expression, but they have important roles in immune cell function and pathogen defense [42]. There is evidence to suggest that these receptors can form heteromers, and that changes in TRPML1 can influence the activity of the other TRPMLs [43]. TRPML1 has multiple context-dependent functions, thus it will be critical to understand how TRPML1 contributes to a particular disease state, and whether it is a primary or secondary contributor to pathology. With the exception of MLIV, a definitive role for TRPML1 as a driver of disease pathology, has not been established, and more studies in human tissue are needed.

Drug interactions: Not established

Sources and dosing:

TRPML1 agonists, such as ML-SA1, are available from commercial suppliers for research use. They have not yet been clinically tested for any indication.

Research underway:

A major hindrance to our understanding of TRPML1's contribution to physiological and pathophysiological processes *in vivo* stems from the lack of reliable tool compounds. There are efforts underway to develop TRPML1 modulators for research use with better drug-like properties. For example, one group found that the (S) enantiomer of ML-SA1 is a more reliable agonist of TRPML1 relative to the racemic mixture, and shows good cell permeability, but still lacks the properties required for systemic use [15]. Another group has identified the residues critical for allosteric modulation of TRPML and developed mutant channels targeting these residues which may be useful for identifying novel TRPML1 modulators [44].

There are currently no clinical trials underway for TRPML1 agonists. But, there are several companies working on the development of TRPML1 agonists for clinical use. These prospective therapeutic agents are currently in the preclinical development stage.

Arkuda Therapeutics collaborated with BioAscent to screen for new TRPML1 agonists [45]. In January 2025, Arkuda announced that Johnson & Johnson exercised its right to acquire Arkuda's portfolio of lysosomal function enhancers (Press release).

Caraway Therapeutics developed TRPML1 agonists, initially for a genetic form of Parkinson's disease, GBA-Parkinson's disease. While still in preclinical phases of development, Caraway Therapeutics and its assets were acquired by Merck, through a subsidiary, in late 2023 (Press release).

Casma Therapeutics is developing therapeutics to reprogram autophagy. One of their programs of interest targets lysosomal function through TRPML1. According to their pipeline, their work on the development of TRPML1 agonists for CNS indications is currently in the preclinical stage.

Libra Therapeutics is developing therapeutics to enhance lysosomal function and autophagy. TRPML1 is one of their targets of interest, and they have a TRPML1 agonist in preclinical development as part of their pipeline.

Lysoway Therapeutics is developing modulators for lysosomal ion channels, including TRPML1 to promote autophagy flux. According to their pipeline, they currently have two TRPML1 agonists in preclinical development, in the IND-enabling stage. These include a brain-penetrant TRPML1 agonist intended for neurodegenerative diseases, such as Parkinson's disease, ALS, and Frontotemporal dementia, as well as a non-brain penetrant TRPML1 agonist for rare non-CNS diseases.

Merck acquired *Calporta* in 2019, which was developing a TRPML1 agonist for lysosomal storage disorders and neurodegenerative diseases (Press release). The current status of development on this preclinical program is unclear.

Search terms:

Pubmed, Google: TRPML1

- agonists, Alzheimer's disease, Parkinson's disease, neurodegeneration, aging, cancer, cardiovascular

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