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.

Cambinol/Neutral Sphingomyelinase Inhibitors

Evidence Summary
nSMase2 inhibitors may restore the age-related imbalance of bioactive lipids and reduce inflammation by inhibiting extracellular vesicle formation, but may accelerate neurodegeneration if nSMase2 is not elevated.

**Neuroprotective Benefit:** May protect against ceramide induced cell-death, and the propagation of inflammation by glial cells by modulating lipid composition and extracellular vesicle formation, but only in the context of nSMase2 overactivity.

**Aging and related health concerns:** By regulating extracellular vesicle content, nSMase2 inhibitors could reduce inflammatory signaling and cancer metastasis.

**Safety:** Safety profile in humans has not been established, though well-tolerated in rodents. Has potential to cause adverse effects by disrupting lipid homeostasis.

*Assessments for Neutral Sphingomyelinase Inhibitors as a class of drugs*
What is it? Cambinol is a class III histone deacetylase (HDAC) inhibitor and can regulate sphingolipid metabolism by inhibiting neutral sphingomyelinase 2 (nSMase2). It is a cell permeable β-naphthol that inhibits the NAD dependent deacetylase activity of SIRT1 and SIRT2 with an IC$_{50}$ ≈ 50 uM in a substrate competitive, but not NAD competitive manner (also known as SIRT1/2 inhibitor IV) \[1\]. SIRT inhibition leads to cell cycle arrest, the inhibition of cell growth, and induction of apoptosis. Consequently, cambinol has been tested in preclinical models as an anti-cancer therapy.

Cambinol was subsequently found to be a 10X more potent inhibitor of nSMase2, which is a Mg$^{2+}$ dependent enzyme involved in the breakdown of sphingomyelin into phosphocholine and ceramide, and is important for ceramide-mediated exosome biogenesis and secretion \[2\]. Sphingolipids are fatty acids derived from sphingosine that are involved in cell signaling. Cambinol acts as a reversible, uncompetitive inhibitor, which binds to an allosteric site rather than the substrate binding site of nSMase2. The activity of the enzyme is modulated by the repositioning of critical residues (Asp430 and Lys435) at the active site \[3\]. Based on its ability to inhibit ceramide and cell stress associated exosome production, cambinol has been tested in preclinical models for its neuroprotective potential in Alzheimer’s disease (AD).

Cambinol is not the only nSMase inhibitor available for research use. The drug GW4869 is the nSMase inhibitor most widely used in research, but has poor solubility due to its highly hydrophobic nature, which limits its clinical potential. While still compromised by low solubility, cambinol was thought to be a more attractive candidate due to its moderate oral bioavailability, but was found to have a poor in vivo pharmacokinetic profile. Moreover, it is unclear whether its additional activity as a weak SIRT1/2
inhibitor would augment or antagonize its neuroprotective potential. Recently MS-882 was identified from a high throughput screen, and found to have a good pharmacokinetic profile [4]. Contingent on a good safety profile, this drug has the potential for future clinical testing.

**Neuroprotective Benefit:** May protect against ceramide induced cell-death, and the propagation of inflammation by glial cells by modulating lipid composition and extracellular vesicle formation, but only in the context of nSMase2 overactivity.

*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:* None

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

Sphingolipids are essential components of cell membranes and important mediators of signal transduction affecting a variety of cell functions. Alterations in sphingolipid metabolism occur during aging, and these changes may promote a proapoptotic environment conducive to neurodegenerative processes [5]. **Ceramide is one of the key sphingolipids affected by aging.** While cell membrane localized ceramide has important functions in regulating receptor compartmentation and signaling, its production in response to elevated oxidative stress and inflammation is also associated with neuronal pathology. During conditions of oxidative stress, there are high levels of reactive oxygen species (ROS) and low levels of the antioxidant glutathione. Ceramide production is favored under these conditions because glutathione inhibits the activation of neutral sphingomyelinases [6]. nSMase2 is the most abundant neutral sphingomyelinase, and is enriched in the brain. Upon activation, nSMase2 translocates from the Golgi to the plasma membrane where it can act to produce ceramide through the breakdown of sphingomyelin [7]. **In the aging brain, there is an increase in membrane nSMase activity and associated imbalance of sphingolipids favoring proapoptotic ceramide [8].**

Therefore, there has been an interest in finding inhibitors of nSMase2 which could help restore the balance of sphingolipids and promote neuronal survival.
Alzheimer’s disease: Potential benefit based on preclinical models

In Alzheimer’s disease (AD) patients, there is a shift in the balance of brain sphingolipids toward more long chain ceramides and higher levels of oxidative stress markers [9]. In response to elevations in ceramide, neural cells release extracellular vesicles, in a nSMase2 dependent manner. These microvesicles/exosomes, particularly those derived from astrocytes and microglia, have been implicated in the promotion of inflammation, the sequestering of Aβ oligomers, and tau propagation [10; 11; 12; 13]. In preclinical AD models, inhibiting the formation and release of nSMase derived extracellular vesicles was found to be moderately beneficial in reducing AD pathology and associated cognitive impairment [13; 14]. However, the beneficial effects were sex dependent, and nSMase2 generated ceramide has been shown to play a role in synaptic function and memory performance [15; 16]. This suggests that nSMase2 modulation should be used within the framework of personalized medicine, in order to modify nSMase2 activity in a manner that restores the balance of brain sphingolipids.

Aβ pathology: In the 5XFAD model, which has prominent amyloid pathology, inhibition of nSMase2 activity through genetic deletion (fro/fro mutant mice) reduced brain ceramide levels by 60-70%, decreased Aβ levels in 10-month-old male mice by 30%, and improved performance on a contextual fear conditioning task in male mice [13]. Similarly, nSMase inhibition using GW4896 reduced levels of brain extracellular vesicles, ceramide, and Aβ plaque load by 40% in male 5XFAD mice [14]. Treatment with MS-882 (10 mg/kg i.p. daily) from 12-35 weeks of age rescued performance to wild-type levels on a fear conditioning task in 5XFAD mice (P<0.001) [17]. Sex differences were not noted, but these results are still preliminary (not peer-review published). It is hypothesized that the extracellular vesicles in the presence of Aβ may contribute to the seeding of neuritic plaques. In cell culture, Aβ has been shown to promote the secretion of ceramide (C18) rich extracellular vesicles from astrocytes through activation of nSMase2 [18]. The elevated ceramide also induces mitochondrial fragmentation in astrocytes [19]. When taken up by neighboring cells, these extracellular vesicles could promote cell death. The extracellular vesicles contained PAR-4, which sensitized the cells to the proapoptotic effects of ceramide [18]. The extracellular vesicles also promoted glial reactivity. Furthermore, the extracellular vesicles promoted the aggregation of Aβ, and interfered with its ability to be cleared by astrocytes and microglia [13; 14]. MS-882 has also been shown to dose-dependently inhibit stimulated exosome release from glial cells in culture and following IL-1β stimulation in rats in vivo [4].

Neuroinflammation: Inhibition of nSMase2 by cambinol has also been shown to protect against inflammation associated toxicity in rat cortical neurons [2]. The proinflammatory cytokines TNFα and IL-1β promote the activation of nSMase2 and the generation of apoptosis-inducing ceramide. nSMase2 activity may also relay the transmission of inflammatory signals between the brain and periphery. IL-1β
induced nSMase2 activation in astrocytes was shown to induce blood brain barrier (BBB) penetrant extracellular vesicles which could regulate the expression of inflammatory cytokines in peripheral tissues and facilitate leukocyte recruitment to the CNS [20]. These processes could be blocked with GW4869.

**Tau pathology:** Exosomes have been implicated in the spreading of tau pathology in the brain. Treatment with the nSMase inhibitor, GW4869, reduced the secretion of hTau containing exosomes in cultured microglia, and reduced levels of tau oligomers in the hippocampus by 44% in an AAV-tau injection mouse model of tau propagation [11]. Cambinol treatment also suppressed extracellular vesicle formation and reduced tau seeding propagation in HEK 293T Tau RD P301S biosensor cells [10]. It also inhibited the spread of AD patient synaptosome-derived tau oligomers in cell culture.

**Normal brain function:** nSMase2 generated ceramide plays a role in the clustering of proteins within lipid rafts in cell membranes. This includes receptors that are important for neuronal signaling and mediating synaptic function. Consequently, altering the lipid composition of membranes also alters receptor composition. In rodents, the striatum has the highest level of nSMase2, and inhibition of striatal nSMase2 with GW4896 induces minor motor deficits [16]. In the hippocampus, nSMase2 inhibition impairs spatial reference memory and episodic-like working memory, which may be related to a shift in the composition of glutamate receptor subunits in postsynaptic densities [15]. In these experiments, inhibiting nSMase2 shifts the balance of brain lipids away from a healthy state. Whereas, in the context of AD, the lipid content is already dysregulated, thus inhibiting nSMase2 could help restore it back to a healthy state. These studies highlight the importance of maintaining the proper balance of membrane sphingolipids.

**Stroke: Potential benefit based on preclinical models**

In two models of cerebral ischemia, ceramide accumulation was found to contribute to neuronal damage. Following ischemic injury, ceramide production was highly induced in astrocytes in response to inflammation (TNFα, IL-1β) induced activation of nSMase2 by p38 MAPK. nSMase2 inhibition via GW4869 prevented neuronal damage by reducing the production of ceramide and pro-inflammatory mediators by astrocytes [12].

**Parkinson’s disease: Potential harm based on preclinical models**

Studies using the nSMase inhibitor GW4869 indicate that nSMase2 activity may regulate dopamine levels and protect against dopaminergic toxicity.

Expression of nSMase2 was found to be decreased in the substantia nigra of Parkinson’s disease (PD) patients relative to healthy donors [21]. However, it is unclear whether this decrease contributes to
pathology or is a compensatory response. In PC12 cells, dopamine stimulated the formation of ceramide by increasing the activity of nSMase2. This nSMase2 activation was shown to be important for recycling of the dopamine transporter, and inhibition of nSMase2 activity using GW4869 decreased dopamine uptake [22]. The reduction in dopamine transporters is being developed as a potential diagnostic marker in disease progression [23], suggesting that inhibiting nSMase2 may accelerate PD. Additionally, nSMase2 generated ceramide was found to promote autophagy and protect against dopaminergic toxicity in PC12 cells [21].

*These studies indicate that context and cell type matter with respect to the potential benefits of nSMase2 modulation. In the context of elevated nSMase2 activity and ceramide production, as may occur during AD, partial inhibition of nSmase2 may be beneficial in restoring sphingolipid homeostasis. However, if nSMase2 is not elevated, inhibiting it could be detrimental. Additionally, activation of nSMase2 within glial cells is associated with inflammation and pathology, but within neurons it may be protective.

**APOE4 interactions:**

In the white matter of ApoE4 AD patients there are increased levels of ceramide and oxidative stress markers, suggestive of elevated nSMase2 activity [9]. Based on postmortem tissue analysis, the level of brain microvesicles/exosomes is reduced in ApoE4 carriers [24]. In ApoE4 mice, brain-derived extracellular vesicle levels are also reduced, and lipid content is altered. These extracellular vesicles are 10x enriched in cholesterol and 50x enriched in ceramide and gangliosides relative to total brain levels. This suggests that due to a deficit in the endosomal-lysosomal pathway, the production of classical ESCRT-machinery associated exosomes is greatly reduced, and there may be a compensatory increase in the production of plasma membrane derived extracellular vesicles by nSMase2. Since membrane bound cholesterol is released in association with nSMase2 activity, the corresponding elevation in free cholesterol may further exacerbate ApoE4 mediated pathology.

**Aging and related health concerns:** By regulating extracellular vesicle content, nSMase2 inhibitors could reduce inflammatory signaling and cancer metastasis.

Types of evidence:

- Several laboratory studies
Cancer: Potential benefit based on preclinical models

SIRT1 overexpression is associated with poor long-term survival in cancer patients [25]. Cambinol was initially identified as a SIRT1/2 inhibitor, and tested for its ability to inhibit cell viability in a variety of cancer cell lines and models [26; 27; 28; 29; 30; 31; 32; 33]. Benefits were found to be modest and variable with different tumor types. Cambinol analogs are unlikely to have much utility as a monotherapy, but may be beneficial in patients with high SIRT expression, and for sensitizing tumor cells to other chemotherapeutic agents [26; 32]. Medicinal chemistry efforts have been underway to increase the potency and selectivity toward SIRT1 or SIRT2 in order to enhance efficacy [34; 35; 36].

Enhancement of nSMase inhibitory activity by cambinol may also result in inhibition of tumor-derived exosomes and reduction of the cancer’s capacity to metastasize. nSMase2 plays an important role in the sorting of cargo into extracellular vesicles, especially miRNAs [37]. Extracellular vesicle-derived miRNAs are involved in the ability of cancer cells to modulate the microenvironment. miRNA-210 enhances the activity of vascular endothelial cells to promote angiogenesis and support metastasis [38]. Inhibiting nSMase2 prevents loading and release of miRNA-210 containing tumor extracellular vesicles. Cambinol can also disrupt organelle trafficking, particularly lysosomes, mediated by cytoskeletal dynamics which is necessary for tumor invasion [27].

Cardiovascular function: Potential mixed in preclinical models

Inhibiting nSMase2 may be protective against vascular calcification and atherosclerosis. nSMase2 generated extracellular vesicles in macrophages have been found to play a role in pathological biomineralization [39]. Inhibition of nSMase with GW4869 decreased vascular macrophage infiltration and the inflammatory response of endothelial cells to oxidized LDL in a mouse model of atherosclerosis [40]. However, these protective effects may be mitigated with cambinol due to its additional SIRT1/2 inhibitory activity. SIRT1 activation has atheroprotective properties [41], and the SIRT2 inhibitory activity of cambinol has been shown to reduce functional responses of platelets, including aggregation and granule release [42].

While blocking extracellular vesicle-derived miRNA-210 may help protect against tumor metastasis, it could potentially be harmful to endogenous vascular function and repair. Inhibiting nSMase2 could also block the benefits of mesenchymal stem cell (MSC) therapy. Paracrine signaling by MSCs and cardiosphere-derived cells (CDCs) promotes cardiac functional recovery [43; 44]. Extracellular vesicle release contributes to the angiogenic and promigratory effects of hCDCs on vascular endothelial cells [44]. These beneficial effects appear to be related to miRNA-210. MSCs cultured under hypoxic conditions led to improved cardiac recovery, less cardiomyocyte apoptosis (18.3 ± 4.8% vs 25.2 ± 5.5%),
less fibrosis, and increased progenitor cell recruitment in an infarct heart model than MSCs cultured under normal conditions [43]. The benefits were associated with the increased production of miR-210 containing extracellular vesicles via nSMase2 in order to promote an angiogenesis response to hypoxia. The same pathway likely underlies the induction of miR-210 containing exosomes in tumor cells.

**Inflammation: Potential benefit based on preclinical models**

nSMase2 activity is driven in response to inflammation and oxidative stress. Extracellular vesicles are important mediators of immune cell responses, and inhibiting nSMase2 induced extracellular vesicle formation can **block the propagation of pro-inflammatory mediators**. nSMase2 inhibitors may be beneficial in the mitigation of pathological inflammation. However, due to its role in T cell activation and polarization, partial inhibition may be necessary in order to prevent immunosuppression.

In cell culture, cambinol inhibits the expression of cytokines (TNFα, IL-1β, IL-6, IL-12p40, and IFN-γ) by macrophages, dendritic cells, splenocytes and whole blood stimulated by a range of inflammatory stimuli [45]. These anti-inflammatory effects were not found with other SIRT inhibitors, and thus thought to stem from its nSMase inhibitory activity. The anti-inflammatory activity also occurs **in vivo**, as cambinol (10 mg/kg i.p.) pre-treatment was found to **protect against inflammation associated toxicity** in mouse models. TNFα levels were reduced by 1.5-fold (P<0.05) and survival was improved from 8 to 46% (P<0.001) in an endotoxemia model and from 13 to 60% (P=0.013) in a sepsis model.

nSMase2 activity also plays an important role in **T cell receptor signaling, polarization, migration, and extracellular vesicle release** [46; 47]. Cytoskeletal rearrangement drives the polarized redistribution of organelles and receptors during T cell activation, and involves the reorganization of lipid membrane domains. nSMase activity controls this process by altering the composition of lipids in the membrane, and thus regulates protein/receptor distribution [47]. It is also necessary for extracellular vesicle formation and cargo loading. Extracellular vesicles are important mediators of the T cell response by transferring biologically active miRNAs to antigen presenting cells [46]. nSMase2 is not required for T cell receptor signal initiation, but is important for signal amplification, particularly when the antigen is present at low doses [47]. While nSMase is involved in the directional migration of T cells in the periphery, it does not appear to affect immunosurveillance of the CNS [46], since the transmigration of T cells into the brain is regulated by acidic sphingomyelinase. Therefore, partial nSMase2 inhibition may protect against pathological overactivation of the immune system without compromising normal immunity, but more **in vivo** testing is needed.
Liver protection: Potential benefit based on preclinical models

nSMase2 inhibition may help prevent age-related inflammation-associated liver dysfunction. Aged hepatocytes show hyper-responsiveness to IL-1β, which is driven by changes in levels of different ceramide species through increased expression of nSMase2 due to decreases in glutathione levels [6; 48].

COPD: Potential benefit based on preclinical models

Inhibiting nSMase2 has been proposed as a method to protect against COPD. The lungs of COPD patients and smokers show higher levels of nSMase2 compared to healthy controls, which is indicative of increased oxidative stress [49; 50]. In rodents, nSMase2 activity is responsible for ceramide production and lung cell apoptosis in response to cigarette smoke [49]. Therefore, inhibiting nSMase2 may help prevent the inflammation and cell toxicity stemming from oxidative stress.

Safety: Safety profile in humans has not been established, though well-tolerated in rodents. Has potential to cause adverse effects by disrupting lipid homeostasis.

Types of evidence:

- Several laboratory studies

Due to the poor solubility of GW4869 and cambinol, most testing has been performed in vitro. Based on the limited number of studies performed in vivo in rodents, nSMase2 inhibition does not exert any overt toxicity. Due to solubility constraints, cambinol can be administered orally at a maximum dose of 100 mg/kg [32]. At this dosage, the drug is well-tolerated, and no acute toxicity has been observed, while liver enzyme levels and regenerative potential are not adversely affected [30; 32; 33]. Cambinol could be detected in the brain 4 hours after oral administration, at 1.6% of the level in the plasma [10]. Despite this low penetrance, it could inhibit brain nSMase2 by 26.8%. Inhibition of nSMase2 in the brain by 64%, following GW4869 treatment (i.p.) did not alter CNS trafficking of blood derived monocytes, the activation state of microglia, or the ceramide or sphingolipid content of liver, heart or skeletal muscle [15]. The benefits and low level of side effects may stem from these drugs working as partial nSMase2 inhibitors in vivo. The novel nSMase2 inhibitor MS-882 currently in development has higher brain penetrance (brain to plasma ratio of 0.5-0.6) which may make it more potent [4; 17]. Further safety studies are needed to determine whether higher potency increases the risk for side effects. Long-term safety studies are also needed.
It is likely that nSMase2 inhibitors will only be safe and beneficial in a particular subgroup of individuals who have elevated levels of nSMase2 and corresponding alterations in lipid composition and dysregulated inflammatory processes. Biomarkers, perhaps related to extracellular vesicle content, will be needed to stratify patients. nSMase2 inhibition in people with healthy sphingolipid levels could instead induce lipid dysregulation and be pathogenic.

Sources and dosing:

Cambinol and GW4869 are available for research use from commercial suppliers, but there is nothing approved for human use.

Research underway:

There are currently no planned or ongoing trials for cambinol. Due to poor solubility, medicinal chemistry efforts are underway to develop cambinol-like drugs. Johns Hopkins and UCLA are the main academic institutions currently working in this area.

MS-882, which originated from a high throughput screen out of the Johns Hopkins Drug Discovery center, has a pharmacodynamic profile suggesting it may be a candidate for future clinical testing. Based on information presented at the Society for Neuroscience meetings in 2017 and 2018, this drug shows similar abilities to inhibit glial extracellular vesicle release and improve cognitive function in the 5XFAD mouse model as has been demonstrated with the other nSMase2 inhibitors [4; 17]. In contrast to these other drugs, MS-882 shows good pharmacokinetic properties, including high potency (IC<sub>50</sub> = 300 nM), brain permeability (50-60%), and excellent oral bioavailability (87% in rodents, and 100% in dogs).

Search terms:

Pubmed, Google: Cambinol (or neutral sphingomyelinase or nSMase2) +

Alzheimer’s disease, neurodegeneration, neuroprotection, apoE, aging, lifespan, SIRT, cancer, cardiovascular, cardiovascular, exosomes, ceramide, inflammation, safety

Websites visited for Cambinol:

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