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.

CaMKK2 Inhibitors

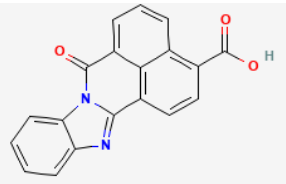
Evidence Summary

CaMKK2 activity promotes age-related disorders, including cancer, osteoporosis, and metabolic diseases, in preclinical models. The safety of inhibitors may be impacted by its pleiotropic activity.

Neuroprotective Benefit: CaMKK2 may act as a mediator of synaptic loss downstream of amyloid, but lower expression is associated with working memory and executive function impairments based on gene association studies.

Aging and related health concerns: CaMKK2 inhibition may protect against cancer, age-related bone loss, and metabolic disease.

Safety: Due to the pleiotropic, context-dependent nature of CaMKK2 activity, the therapeutic profile will depend on the selectivity and mechanism of the inhibitor. More *in vivo* and human studies are needed.

Availability: Research use	**Dose**: N/A	STO-609 **Chemical formula:** $C_{21}H_{14}N_2O_5$ **MW**: 374.3 g/mol  Source: PubChem
Half-life: Varied STO-609: 3.74 hours	**BBB**: N/A	
Clinical trials: None	**Observational studies**: Elevated CaMKK2 expression is associated with poor prognosis in cancer, and low levels are associated with schizophrenia.	

What is it?

Calcium/Calmodulin-dependent Kinase Kinase 2 (CaMKK2) is a serine/threonine kinase and an important regulator of metabolic homeostasis [1]. It is activated by calcium/calmodulin (CaM), but it also has activity in the absence of calcium/CaM. Its major targets are the calcium/CaM kinases, CaMK1 and CaMK4, as well as the metabolic regulator AMP-activated kinase (AMPKα). CaMKK2 activity plays diverse roles in different tissues depending on whether its activity is calcium-mediated as well as the composition of the downstream effectors. It is highly expressed in the brain. CaMKK2 expression is associated with poor prognosis in a variety of cancers, and CaMKK2 inhibitors may be clinically useful in this context, particularly in metastatic prostate cancer [2].

Neuroprotective Benefit: CaMKK2 may act as a mediator of synaptic loss downstream of amyloid, but lower expression is associated with working memory and executive function impairments based on gene association studies.

Types of evidence:

- 2 gene association studies for CaMKK2 and cognition
- Numerous laboratory studies

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

There are data from gene association studies implicating CaMKK2 in cognition. CaMKK2 is abundantly expressed in the brain, particularly in the cerebellum and frontal cortex [3]. The single nucleotide

polymorphism (SNP) rs1063843 (T allele), is associated with reduced expression of CaMKK2. The rs1063843 variant was found to be weakly associated with working memory in an Irish cohort of 285 schizophrenic cases and 85 controls [3]. In a cohort of 342 healthy Chinese college students (age 18-23), rs1063843 showed an association with executive function, based on performance on the Wisconsin card sorting task. Similarly, rs1140886, which shows linkage with rs1063843 and an association with expression levels, was associated with working memory [3]. The variant rs2686346 showed weak associations with IQ and reversal learning. In a study with 84 healthy volunteers, carriers of the rs1063843 (T allele) variant showed increased activation of the dorsolateral prefrontal cortex (DLPFC) on measures of attention, executive control, and working memory [4]. They also exhibited increased activity in the cerebellum and caudate. The increased activation is indicative of inefficiencies, such that more brain cells need to be recruited to complete a given task. It is unclear whether these effects are developmental in origin, leading to altered brain structure and function.

Human research to suggest benefits to patients with dementia: None

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

Alzheimer's disease: CAMKK2 PLAYS MIXED ROLES (Preclinical)

Preclinical studies suggest that CaMKK2 may play diverse roles in the pathophysiology of Alzheimer's disease (AD). The loss of CaMKK2 leads to a mild impairment of working memory in mice, similar to what is seen with genetic variants in humans [3; 5]. The loss of CaMKK2 also impairs iron homeostasis in the mouse brain, in a manner similar to iron dyshomeostatic phenotypes in the AD brain [6]. However, the loss or inhibition of CaMKK2 protects against Aβ42-mediated synaptic toxicity in mouse and cell culture models [7]. While a study examining postmortem frontal cortex tissue (n=6) did not find a difference in the percentage of CaMKK2 positive neurons in AD [8], there may be a disease-related change in kinase activity. Since AD-related phenotypes are consistent with both elevated and reduced CaMKK2, there may be an altered activity pattern, such that certain downstream activation patterns (i.e. AMPKα phosphorylation) are increased while other patterns (i.e. CamK4 activation) are decreased. This could be due to differences in the ability of CaMKK2 to be activated by calcium, as part of a global calcium dyshomeostasis, and/or due to differences in the expression or coupling of the downstream effectors. Due to the pleiotropic effects of CaMKK2, a better understanding of how CaMKK2 is altered in AD may be needed to design an appropriate therapeutic for this target.

Aβ-related toxicity: Aβ42 can trigger increases in intracellular calcium concentrations, leading to the activation of CaMKK2 [7]. This calcium-triggered activation of CaMKK2 then promotes the

phosphorylation and activation of AMP-activated kinase (AMPK). AMPK then phosphorylates tau at S262, which leads to a reduction in synaptic spine density. This synaptotoxic effect of Aβ can be prevented in neurons from AD mice (3-month-old J20) through overexpression of a kinase-dead version of CaMKK2, or treatment with the CaMKK2 inhibitor, STO-609 [4]. The synapse-enriched microRNA, miR-9, which is decreased in AD, was shown to target CaMKK2, such that overexpression of miR-9 inhibited the Aβ42-mediated activation of the CaMKK2-AMPK-ptau S262 pathway, and prevented synaptic spine loss in neurons [9; 10]. However, there may be trade-offs to inhibiting CaMKK2-mediated activation of AMPK. The drug, J147, which inhibits ATP synthase (ATP5A) activity by approximately 20% was found to promote CaMKK2-depedent activation of AMPK and mTOR, which prevent age-related drifts in the hippocampal transcriptome in SAMP8 mice [11].

Learning and memory: CaMKK2 plays a role in nervous system development and synaptic plasticity associated with learning and memory. CaMKK2 knockout mice show impaired migration of cerebellar granule cells stemming from reduced levels of the neurotrophic factor BDNF [12]. As adults, male CaMKK2 knockout mice show deficits in spatial memory formation due to impaired activation of the transcription factor CREB [5]. Notably, CREB activation in spatial learning does not appear to be dependent on CaMKK2 activity in female mice, and accordingly, females do not show spatial memory impairments. The genetic associations between CaMKK2 variants that reduce expression or activity with lower performance on some cognitive measures, such as working memory and executive function, suggests that CaMKK2 may play a similar role in synaptic plasticity underlying mechanisms of learning and memory in humans [3]. However, since CaMKK2 function is reduced or absent throughout the duration of the lifespan in these cases, it is unclear how much developmental processes contribute to these effects, and whether inhibition during adulthood would impact cognitive function, and/or whether the effect would be sex dependent.

Iron homeostasis: CaMKK2 has been implicated in iron homeostasis through the regulation of the trafficking and turnover of the iron transporter, transferrin. The loss of CaMKK2 in neurons reduces the level of phosphorylated transferrin, and a specific loss in the levels of transferrin localized to acidic (PH 3-4) compartments [6]. In the 3xTg-AD mouse model, altered levels of CaMKK2 in the negatively charged brain fraction was associated with reduced levels of phosphorylated transferrin in the cortex, and serum levels of phosphorylated transferrin were found to be decreased in CaMKK2 knockout mice. Levels of (acidic fraction) phosphorylated transferrin were also found to be decreased in the cerebrospinal fluid (CSF) and serum from patients with early-onset or late-onset AD, suggesting this is a clinically relevant mechanism, although the relevance of altered CaMKK2 to the effect on transferrin was not assessed in the human study.

In cells, a reduction in CaMKK2 leads to increased transferrin uptake and transcytosis, along with decreased trafficking to subcellular organelles [13]. This shift leads to reduced intracellular iron content, and defective bioenergetics. The effect of CaMKK2 on transferrin involves calcium signaling and appears to be mediated through the activation of CaMK4 by CaMKK2. Thus, the dysregulation of iron trafficking is downstream of altered calcium homeostasis and signaling. The distribution of iron is altered in the AD brain, leading to abnormal tissue accumulation. These studies suggest that altered expression or activity of CaMKK2 may play a role in AD-associated iron dyshomeostasis.

Schizophrenia: REDUCED CAMKK2 SHOWS GENETIC ASSOCIATION WITH SCHIZOPHRENIA

CaMKK2 has been shown to be downregulated in individuals with schizophrenia, and the T variant of the SNP, rs1063843, which is associated with reduced levels of CaMKK2, was found to be significantly associated with schizophrenia ($P=5.17 \times 10^{-5}$) in multiple independent cohorts totaling 130, 623 subjects [3]. Due to the associations with executive function in other populations, the reduction in CaMKK2 may play a causal role in some of the executive function deficits commonly associated with schizophrenia.

Cerebral ischemic injury: CAMKK2 ACTIVATION MAY BE PROTECTIVE (Preclinical)

The downregulation of CaMKK2 by the microRNA, miR-378a-5p, was shown to reduce cell viability in the context of oxygen/glucose deprivation/reoxygenation (OGDR) in primary rat hippocampal neurons [14]. The protective effect of CaMKK2 was related to the phosphorylation and activation of AMPK. The induction of CaMKK2-AMPK was also found to be protective in a model of germinal matrix hemorrhage in neonatal rats [15].

APOE4 interactions: Not established

Aging and related health concerns: CaMKK2 inhibition may protect against cancer, age-related bone loss, and metabolic disease.

Types of evidence:

- 4 observational studies for CaMKK2 expression and cancer outcomes
- Numerous laboratory studies

Cancer: ELEVATED CAMKK2 IS ASSOCIATED WITH POOR PROGNOSIS

Prostate cancer: The impact of CaMKK2 on cancer is best characterized in the context of prostate cancer. CaMKK2 is a target of the microRNA, miR-224, and in prostate cancer patient tissue, there is an inverse correlation between expression levels of miR-224 and CaMKK2 (Spearman's correlation: r = −0.66, P = 0.004) [16]. Furthermore, the combination of low miR-244 and high CaMKK2 was correlated with advanced clinical stage, metastasis, and shorter survival. CaMKK2 is thought to promote anabolic pathways downstream of androgen receptor activation in prostate cancer. In human prostate cancer cell lines, CaMKK2 promotes *de novo* lipogenesis in an AMPK-independent manner, which facilitates cell growth. Treatment of prostate cancer model mice ($Pten^{fl/fl}$) with the CaMKK2 inhibitor STO-609, reduced prostate weight by 30% [17]. CaMKK2 inhibitors may be particularly beneficial in the context of prostate cancer because the primary site of metastasis is the bone, and treatment with androgen receptor inhibitors can weaken the bone [2]. Due to its role in the regulation of bone remodeling, inhibition of CaMKK2 may both inhibit tumor cell growth and strengthen the damaged bone.

Glioma: An expression analysis (n=866) found that hypomethylation of the promoter resulted in elevated expression of CaMKK2 in high-grade glioma [18]. Higher CaMKK2 expression was associated with worse prognosis, such that those with higher CaMKK2 promoter methylation had better overall survival. Cell culture work in the U87 glioma cell line found that CaMKK2 promoted cell proliferation and migration.

Lung cancer: The loss of LKB1 is associated with increased metastasis and poor prognosis [19]. AMPK can be phosphorylated by LKB1 or CaMKK2, and in LKB1-deficient lung cancer, phosphorylation of AMPK occurs primarily by CaMKK2. In this context, CaMKK2 is activated by glutamate dehydrogenase (GDH1).

Hepatic cancer: CaMKK2 is upregulated in hepatocellular carcinoma (HCC) with expression approximately 2.5 times higher than adjacent liver tissue, and is inversely associated with patient survival [20]. In a mouse model, inhibition of CaMKK2 with STO-609 (30 µg/kg/bw i.p. 2x/week for 4 weeks) resulted in a 21% reduction in tumor burden. CaMMK2 promotes tumor cell growth by increasing protein translation.

Gastric cancer: Gene expression data for gastric adenocarcinoma samples from the Cancer Genome Atlas (TCGA) found overlap in the gene signature between the tumor samples and the effect of modulating CaMKK2 activity in gastric cancer cell lines [21]. Inhibition of CaMKK2 in these cell lines with STO-609 reduced cell proliferation, and inhibited the epithelial-mesenchymal transition.

Osteoporosis: CAMKK2 INHIBITON PROMOTES BONE FORMATION (Preclinical)

CaMKK2 is expressed in bone forming osteoblasts and bone absorbing osteoclasts [22]. Activation promotes differentiation of osteoclasts over osteoblasts, while inhibition promotes the building of bone. Therefore, CaMKK2 inhibitors may help prevent or reverse age-related bone loss. Aged (32-month-old) male CaMKK2 knockout mice had 2.4-fold higher trabecular bone volume and 1.7-fold higher breaking force threshold [23]. Treatment of wildtype 32-month-old male mice with the CaMKK2 inhibitor STO-609 (10 uM tri-weekly) for six weeks produced similar phenotypes including a 40% increase in the bone formation rate, a 1.7-fold increase in trabecular and cortical bone strength, and a restoration of trabecular bone volume fraction toward levels seen in 12-month-old (middle-aged) mice. The inhibition of CaMKK2 has also been shown to accelerate bone healing and improve bone strength following a fracture-inducing injury [22].

Metabolic Disease: CAMKK2 INHIBITON MAY PROTECT AGAINST METABOLIC SYNDROME (Preclinical)

Obesity: CaMKK2 plays a role in the regulation of appetite [1]. In the hypothalamus, it is involved in the induction of the orexigenic hormone NPY, and is important for ghrelin-related stimulation of appetite. In response to a ghrelin challenge, male mice treated with the CaMKK2 inhibitor STO-609 do not increase food intake [24]. A similar effect was seen in rats treated with a separate CaMKK2 inhibitor, 4t [25]. CaMKK2 knockout mice are protected from high-fat diet-related obesity, in part through decreased food consumption.

Diabetes: In addition to being protected against high-fat diet-related weight gain, CaMKK2 knockout mice are also protected from glucose intolerance and insulin resistance [26]. These effects occur independent of the effect of CaMKK2 on food consumption. The latter effects appear to be driven by the effects of CaMKK2 on pancreatic islet cells and liver hepatocytes. In the pancreatic islet cells, loss of CaMKK2 results in higher production and secretion of insulin in response to a glucose challenge [27]. It is hypothesized that CaMKK2 acts as a molecular rheostat for insulin secretion, such that under conditions of metabolic stress, CaMKK2 promotes the overproduction of insulin to glucose, which can lead to insulin resistance. In the absence of CaMKK2, insulin is produced at a higher baseline level, but production does not increase under high glucose conditions, which preserves insulin sensitivity. In the hepatocytes, CaMKK2 is involved in the regulation between glucose and lipid metabolism [26]. In the absence of CaMKK2, there is a shift to less glucose production and increased lipogenesis, which is coupled with increased lipid utilization, based on rates of beta-oxidation. This shift results in improved glucose homeostasis, particularly in the context of metabolic stress, such as high glucose conditions.

However, due to the pleiotropic and context-dependent nature of CaMKK2 signaling, inhibition of CaMKK2 may exacerbate some diabetes-related complications. With respect to diabetic cardiomyopathy and vascular damage, activators of CaMKK2-AMPKα signaling have been shown to be protective in rodent models. Tetrahydrobiopterin (BH4) was identified as an endogenous activator of CaMKK2, and alleviates mitochondrial dysfunction in rodent models of diabetic cardiomyopathy [28]. The mitigation of vascular oxidative stress by FGF21 was also shown to be dependent on CaMKK2 in diabetic mouse models [29].

Non-alcoholic fatty liver disease: The metabolic shift in hepatocytes stemming from the loss of CaMKK2 has also been shown to be protective in the context of NAFLD. In male mice, treatment with the CaMKK2 inhibitor STO-609 (30 µM/kg i.p. for 4 weeks) reduced histological evidence of hepatic steatosis and improved glycemia in high-fat diet-based models of NAFLD [30]. Notably, the downstream effect of the inhibitor on CaMKK2 target genes varied in a model-dependent manner, highlighting the context-dependent nature of CaMKK2 signaling.

Safety: Due to the pleiotropic, context-dependent nature of CaMKK2 activity, the therapeutic profile will depend on the selectivity and mechanism of the inhibitor. More *in vivo* and human studies are needed.

Types of evidence:

- Numerous laboratory studies on CaMMK2 function, but few specifically for CaMKK2 inhibitors

CaMKK2 inhibitors are still in preclinical development, and have not yet been tested in humans. Most of the inhibitors identified thus far have only undergone *in vitro* testing. STO-609 is the best characterized CaMKK2 inhibitor, and has been used in numerous preclinical research studies. Although the liver is the primary target of STO-609, it does not affect liver enzyme levels [30]. It can also be detected in the kidney, but does not affect kidney function based on blood urea nitrogen, bilirubin or creatinine levels. It did lead to an initial reduction in body temperature in mice at all tested doses. Despite relatively good tissue bioavailability, STO-609 has poor solubility, which limits its utility. STO-609 is also not perfectly selective for CaMKK2, as it also affects CaMKK1, as well as at least seven other kinases, including ERK8, MNK1, AMPK, CK2, DYRK2, DYRK3, and HIPK2. CaMKK1 and CaMKK2 have high sequence similarity and partially overlapping roles, thus many inhibitors are best classified as dual CaMKK1/2 inhibitors [31]. There are, however, critical non-conserved residues, particularly outside of the ATP-binding pocket that can be exploited to make a selective inhibitor [31].

CaMKK2 knockout mice are viable, and show protection against some age-related phenotypes, such as metabolic dysfunction and bone loss [23; 26]. However, if being considered for neurological disorders, the effect of reduced CaMKK2 activity on cognition needs to be considered [3; 5], specifically whether this phenotype has a developmental origin.

The primary concern for CaMKK2 inhibitors stems from CaMKK2's context-dependent signaling, such that it can have different downstream effects under different conditions and in different cell types. Thus, the nature of the inhibitor would be expected to influence its therapeutic profile, namely whether it inhibits all CaMKK2 kinase activity, only affects calcium-mediated CaMKK2 activation and signaling, or only inhibits the activation of particular downstream targets, such as AMPKα. Certain diseases may preferentially benefit from a more selective targeting strategy than global CaMKK2 inhibition. Several gene variants have been identified that show altered CaMKK2 function and may serve as functional guides for therapeutic design [32].

Sex-effect: CaMKK2 knockout mouse studies indicate that the loss of CaMKK2 differentially affects brain function in males and females, due to different reliance on CaMKK2 for the activation of specific learning and memory-related signaling pathways [5]. Additionally, the studies assessing metabolic phenotypes from CaMKK2 loss or inhibition were primarily conducted in male animals. These studies suggest that the inhibition of CaMKK2 may show stronger effects in males, however, more studies are needed, and it has not yet been established whether a similar sex-effect occurs in humans.

Drug interactions: Not established

Sources and dosing:

CaMKK2 inhibitors are not available for human use, but some, such as STO-609, are available for research use from commercial suppliers.

Research underway:

There are preclinical efforts underway to develop and optimize novel CaMKK2 inhibitors.

Search terms:

Pubmed, Google: CaMKK2

- Alzheimer's disease, neurodegeneration, cognition, aging, metabolism, cancer, cardiovascular

Websites visited for CaMKK2 Inhibitors:

- PubChem

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