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.

Caspase-2 Inhibitors

Evidence Summary

Optimized levels of caspase-2 are important for healthy aging. Inhibitors may protect against excessive stress-induced cell death, but a basal level of activity is necessary for tissue homeostasis.

Neuroprotective Benefit: Preventing excessive caspase-2 activity may protect against ischemic injury or proteotoxicity-induced neuronal cell death.

Aging and related health concerns: Complete loss of caspase-2 accelerates aging phenotypes in mice and may promote hematological malignancies, but partial inhibition may protect against metabolic stress and disease.

Safety: Caspase-2 specific inhibitors have not yet been tested in humans. The side effect profile will likely depend on the degree of inhibition. Use in acute conditions will likely have the best therapeutic profile.

Availability: For research use	**Dose**: N/A	**Z-VDVAD-FMK** **Chemical formula:** $C_{32}H_{46}N_5O_{11}F$ **MW**: 695.7 Da Z-Val-Asp(OMe)-Val-Ala-Asp(OMe)-CH_2F
Half-life: N/A	**BBB**: N/A	
Clinical trials: None	**Observational studies**: Elevated caspase-2 activity has been associated with cognitive dysfunction in neurodegenerative disease. Reduced caspase-2 activity is associated with poor prognosis in acute myeloid leukemia.	

What is it?

Caspase-2 is a cysteine aspartate protease, and is the most evolutionarily conserved caspase across species [1]. Most caspases are classified as either initiator or effector caspases, but caspase-2 is unique in showing features of both types. Caspases are the executioner enzymes of apoptotic cell death, however, caspase-2 has a broader scope of action relative to other caspases, with roles in autophagy, genomic stability, and responses to oxidative stress [2]. The activation of caspase-2 involves dimerization and recruitment to high molecular weight complexes, such as the PIDDosome [1]. Caspase-2 appears to play an important role in the regulation of age-related phenotypes, such that the loss of caspase-2 can accelerate aging processes due to the buildup of oxidative stress damage, while excessive caspase-2 can drive pathological cell loss. Therefore, caspase-2 activity needs to be carefully balanced to ensure a healthy aging process. Caspase-2 inhibitors are currently in preclinical development, with potential utility in neurodegenerative diseases, and fatty liver disease.

Neuroprotective Benefit: Preventing excessive caspase-2 activity may protect against ischemic injury or proteotoxicity-induced neuronal cell death.

Types of evidence:

- 5 studies of caspase-2 levels in postmortem brain tissue for neurodegenerative diseases
- Numerous 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:

Cell death: Studies of postmortem brain tissue indicate that the expression and localization of proteins involved in cell death are altered in a variety of neurodegenerative diseases, including Alzheimer's disease (AD). One study (n=20) found that the cell death-related proteins Bim/BOD and p21 were significantly increased in the frontal cortex of AD patients, but there were no significant differences in the levels of procaspase-2 or RIP-associated Ich-1/Ced-3 homologous protein with a death domain (RAIDD), which is involved in the activation of caspase-2 [3]. Other studies have found that caspase-2 is elevated in the particulate fraction, but not the cytosolic fraction of AD brain tissue [4].

Preclinical studies show that inhibition of caspase-2 can protect against Aβ-mediated cell death. In cell culture, the inhibition of caspase-2, via the upregulation of miR-34a, restores cell viability in SH-SY5Y cells treated with Aβ40. Levels of miR-34a, and thus caspase-2 activity, is regulated by inositol-requiring enzyme 1 (IRE1) [5]. Similarly, downregulation of caspase-2 using antisense oligonucleotides protects PC12 cells and murine hippocampal neurons from Aβ42-mediated cell death [6]. Notably, cultured neurons derived from caspase-2 deficient mice were resistant to Aβ42-mediated cell death [6]. These studies suggest that excess caspase-2 activity may drive neuronal cell death under conditions of stress, and that inhibiting caspase-2 could preserve neuron viability.

Synaptic function: Caspase-2 can cleave tau at Asp314, generating a truncation product, Δtau314 [7]. The C-terminal Δtau314 fragment can be generated from all six tau splice isoforms. The production of this cleaved fragment promotes the mis-sorting of tau to dendritic spines, leading to synaptic dysfunction.

Alzheimer's disease*:* A study involving postmortem brain tissue from 85 elderly individuals found that those with cognitive impairments had approximately three-fold higher brain levels of Δtau314 [7]. In a separate study (n=90), levels of H1485- and 4F3- reactive Δtau314 proteins normalized to total tau were 1.8- and 1.6- fold higher in individuals with cognitive impairment, AD or mild cognitive impairment (MCI), relative to those with normal cognition [8]. The level of Δtau314, as measured by ELISA, had moderate predictive value for dementia, with an AUC of 0.68. Levels of caspase-2 were also 1.3-fold higher with cognitive impairment.

Huntington's disease*:* Elevations of Δtau314 and caspase-2 have also been shown in the postmortem tissue from individuals with Huntington's disease (HD) (n=27) [9]. The ratio of Δtau314 to total tau, as assessed by Western blot, was 3.8-fold higher in the prefrontal cortex and 21.9-fold higher in the

caudate in those with HD. The normalized level of caspase-2 in the prefrontal cortex was 1.7 to 2.5-fold higher in HD patients, and there was good correlation between the normalized levels of Δtau314 and caspase-2.

***Lewy body dementia*:** A similar elevation in both Δtau314 and caspase-2 has also been seen in postmortem brain tissue from the superior temporal gyrus from individuals with Lewy body dementia (LBD) [10]. When compared to brain tissue from individuals with Parkinson's disease (PD) without dementia (n=12), those with LBD (n=21) had levels of Δtau314 normalized to total tau that were approximately four-fold higher. The Δtau314: tau ratio showed predictive value for LBD with an AUC of 0.869. Caspase-2 levels were 59% higher in the superior temporal gyrus from those with LBD relative to PD, which was associated with a 50% decrease in neuronal markers, suggesting there was extensive neuronal loss in this region, in those with LBD.

In the rTg4510 AD transgenic mouse model expressing a human form of P301L mutant tau, caspase-2 levels are elevated by 25% [7]. Reducing caspase-2 levels with morpholino oligonucleotides restored long-term memory in these mice. Mutating the Asp314 residue in tau to an amino acid that cannot be cleaved by caspase-2 prevents the localization of tau to dendritic spines, suggesting that caspase-2 plays a critical role in the tau-mediated synaptic dysfunction. The mislocalized tau disrupts synaptic function by interfering with the trafficking of glutamatergic AMPA receptors, which disrupts neurotransmission. Strain differences in caspase-2 may underlie strain-related differences in disease phenotypes amongst AD mouse models. In this study, rTg4510 mice on a 129/FVB F1 background show elevated Δtau314 levels and cognitive impairment, while the addition of a C57bl/6 background confers resistance to transgene phenotypes. Notably, the C57bl/6 background mice also do not show an increase in Δtau314. In the J20 AD mouse model, which overexpresses a human form of mutant amyloid precursor protein (APP), caspase-2 deficiency protected against Aβ-related declines in synaptic density and spatial memory [11]. The synaptic effects were related to the recruitment of RhoA and ROCK-11 to dendritic spines, leading to their collapse. These studies suggest that caspase-2 mediates synaptic dysfunction associated with both Aβ and tau, and that the normalization of caspase-2 levels could preserve synaptic integrity in the context of AD.

Developmentally, capase-2 activity is important for the establishment of normal cognition, as mutations in genes required for caspase-2 activation are associated with intellectual disability [12; 13]. Due to its role in reducing dendritic spine density, mice lacking caspase-2 have deficits in dendritic spine pruning and synaptic plasticity [14]. Synaptic pruning is important developmentally as a mechanism of structural synaptic plasticity. These mice have decreased cognitive flexibility. In the context of disease, excessive caspase-2 activity leads to changes in spine density that hinder rather than promote cognition. However,

its role in synaptic plasticity suggests that the normalization, rather than complete inhibition of caspase-2 activity, would be the most therapeutically beneficial strategy.

Stroke: CASPASE-2 PROMOTES NEURONAL DEATH AFTER INJURY (Preclinical)

Caspase-2 is a mediator of neuronal cell death in the context of ischemic injury. In male rats, transient global cerebral ischemia led to the induction of caspase-2 [15]. Pre-treatment with the caspase-2 like inhibitor, VDVAD-FMK, improved neuronal survival. Similarly, this inhibitor prevented apoptosis in N2a cells exposed to oxygen-glucose deprivation/reoxygenation conditions [16]. The miRNA miR-1247-3p also inhibits caspase-2. In male mice, overexpression of miR-1247-3p reduced the size of the cerebral infarction by 22% and improved neurological function following an ischemia/reperfusion injury [16]. Caspase-2 knockdown also protected against neuronal loss in the context of neonatal hypoxia-ischemia in mice [17]. These studies suggest that acute treatment with caspase-2 inhibitors could protect against delayed neuronal death in the context of cerebral ischemic injury, though the optimal therapeutic window for treatment needs to be determined.

APOE4 interactions: In a study assessing levels of caspase-2 and Δtau314 in postmortem brain tissue from individuals with LBD or PD, the levels of Δtau314 were approximately four-fold higher in ApoE4 carriers, suggesting that the presence of ApoE4 may lead to higher levels of caspase-2 activity [10].

Aging and related health concerns: Complete loss of caspase-2 accelerates aging phenotypes in mice and may promote hematological malignancies, but partial inhibition may protect against metabolic stress and disease.

Types of evidence:

- 5 studies assessing relationships between caspase-2 and prognosis in cancer
- Numerous laboratory studies

Lifespan: LOSS OF CASPASE-2 ACCELERATES AGING PHENOTYPES (Preclinical)

Caspase-2 plays a role in the regulation of cellular redox status and metabolism, which leads to a premature aging phenotype in caspase-2 deficient mice [18]. The lack of caspase-2 leads to an impaired endogenous antioxidant response, due to reduced expression of stress response-related transcription factors FOXO1 and FOXO3 [19]. Caspase-2 acts as a negative regulator of autophagy, such that loss of caspase-2 leads to an upregulation of autophagy in various cell types [20]. The loss of caspase-2 impairs

the expression of FOXO family transcription factors, leading to increased levels of reactive oxygen species (ROS), such as superoxide. The elevation in ROS leads to the induction of the autophagic pathways. Through these mechanisms, caspase-2 plays a role in protecting against oxidative stress.

Caspase-2 is induced in response to oxidative stress and is important for mitochondrial oxidative stress-induced apoptosis, and induction of endogenous antioxidants [19]. Caspase-2 deficient mice have higher levels of oxidized proteins, which is tied to a decreased capacity to remove oxidatively damaged cells. These mice have 10% shorter maximum lifespans (1137 versus 1254 days), and show a range of aging-related phenotypes including, reduced hair growth, increased bone loss, and reduced body fat [18]. The reduction in bone mineral density was due to a reduction in oxidative stress-induced apoptosis of bone absorbing osteoclasts [21]. The activity of caspase-2 increases with age in hepatocytes, due to higher levels of oxidative stress [22]. The loss of capase-2 activity accelerates age-related changes in mitochondrial metabolism in these cells. Caspase-2 may play a role in the remodeling of the proteome and metabolome during aging [23]. The metabolic profile of young caspase-2 deficient mice is similar to that of old wildtype mice, such as decreased amino acid and carbohydrate metabolites and altered energy and lipid metabolism. However, male caspase-2 deficient mice are resistant to age-related glucose intolerance. Female caspase-2 deficient mice do not show this protective effect, which appears to be related to sex differences in fasting responses and lipid metabolism [24]. Lack of caspase-2 also increases the risk for tumors, due to the increased numbers of aneuploid cells, as caspase-2 is involved in the removal of mitotically aberrant cells [25]. The role of caspase-2 in preventing aneuploidy appears to be most prominent in the hematopoietic compartment [26].

While excess caspase-2 activity can drive pathological cell loss in some age-related diseases, such as neurodegenerative disease and liver disease, the studies in caspase-2 deficient mice suggest caspase-2 is a critical component of the cellular response to oxidative stress. Therefore, a basal level of caspase-2 activity is necessary to maintain cellular function over the course of the lifespan. Potent caspase-2 inhibitors may be best suited for acute conditions, while moderate caspase-2 inhibitors, which preserve a basal level of activity, would be better suited for chronic conditions.

Cancer: LOSS OF CASPASE-2 MAY PROMOTE HEMATOLOGICAL MALIGANCIES

As a mediator of cell death, the loss of caspase-2 activity is associated with tumor cell survival in a variety of cancers [1]. However, there is a context-dependency, such that not all tumors are sensitive to the loss of caspase-2. Due to its role in the maintenance of myeloid progenitor cell populations, caspase-2 seems to be most relevant for hematological malignancies [26]. Caspase-2 is the target of several

miRNAs, and the differential expression of miRNAs in different tumor types may influence the expression levels and functional impact of caspase-2 in a given tumor.

Acute myelogenous leukemia (AML): Caspase-2 is highly expressed in myeloid lineage cells, and loss of caspase-2 leads to the expansion of myeloid progenitors during aging in mice [26]. Analyses of the NCI-Cancer Genome Atlas and BloodSpot databases indicate caspase-2 is expressed at lower levels in AML stem cells than in normal hematopoietic stem cells, and that low expression of caspase-2 in these cells is associated with poor outcomes [26]. A study examining caspase-2 and caspase-3 levels in peripheral blood of patients with AML (n=185) found that there were significantly increased levels of non-activated caspase-2 and caspase-3 [27]. High levels of both non-activated (uncleaved) caspase-2 and caspase-3 were a prognostic factor for poor survival (Relative risk [RR] 2.49, 95% confidence interval [CI] 1.59 to 3.90). The association was primarily driven by caspase-3, such that high levels of activated (cleaved) caspase-3 was associated with better survival.

***Acute lymphoblastic leukemia (ALL)*:** Levels of caspase-2 and caspase-3 were associated with cytogenic abnormalities in peripheral blood samples from patients with ALL (n=45) [28]. High levels of caspase-3 were associated with complete remission, but levels of caspase-2 did not show an association. The effect on survival was unclear due to the small sample size.

Glioma: Caspase-2 levels were found to be increased with tumor grade in a study of 82 clinical samples, such that grade 1 tumors were negative for caspase-2, while 21.3% of grade 2, 33.3% of grade 3, and 66.7% of grade 4 glioma tumors were positive for caspase-2 [29]. Caspase-2 is a functional target of miR-149, and in tumor samples, the expression of miR-149 is inversely associated with caspase-2. miR-149 promotes cell proliferation by suppressing p53. Abnormalities in p53 are common in glioma, and the functional effects of miR-149 may depend on the status of p53. The relationship with p53 may modify the effect of caspase-2 in the tumor tissue.

***Non-small cell lung cancer (NSCLC)*:** Caspase-2 is projected to be a target of miR-182-5p. Consistent with this, inhibition of miR-182-5p promotes tumor cell apoptosis [30]. miR-182-5p was increased, while caspase-2 was decreased in both tumor tissue and peripheral blood from patients with NSCLC relative to controls (n=59).

Breast cancer: In cohorts of 64 patients after neoadjuvant chemotherapy and 100 pretreatment patients with breast carcinomas, levels of the caspase-2S isoform, caspase-2L isoform, nor the ratio of S/L was associated with progression-free survival, or modified the response to chemotherapy [31]. The ability of caspase-2 to serve as a biomarker may be dependent on treatment status.

Non-alcoholic steatohepatitis: CASPASE-2 MAY DRIVE DISEASE PROGRESSION (Preclinical)

Male caspase-2 deficient mice are resistant to high-fat diet-induced obesity, fatty liver, and insulin resistance [32]. Caspase-2 acts as an initiator of lipoapoptosis, or lipid-induced cytotoxicity [33]. Metabolic changes linked to impaired mitochondrial respiration lead to the accumulation of long-chain fatty acid metabolites, which triggers caspase-2 activation. Progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) involves hepatocyte apoptosis, thus caspase-2 may be a driver of this process [34]. Caspase-2 is upregulated in hepatocytes from patients with NASH [34]. In mouse models of NASH, caspase-2 deficiency did not limit inflammation, but it did reduce the level of apoptotic hepatocytes [34]. The death of hepatocytes triggers an injury response, including a regenerative fibrotic response driven by sonic hedgehog signaling. By limiting the amount of hepatocyte cell death, fibrosis is also mitigated in the livers of NASH model mice deficient in caspase-2. These studies suggest that caspsase-2 inhibitors may help slow the progression of NAFLD and ameliorate some of the associated metabolic phenotypes. However, more studies are needed to determine whether this protective effect is influenced by sex.

Safety: Caspase-2 specific inhibitors have not yet been tested in humans. The side effect profile will likely depend on the degree of inhibition. Use in acute conditions will likely have the best therapeutic profile.

Types of evidence:

- Numerous laboratory studies

Caspase-2 inhibitors have not been clinically tested, and limited preclinical work has been published. The most widely used caspase-2 inhibitor in preclinical research is the substrate peptide VDVAD [35]. These studies generally involve acute treatment and do not assess safety or toxicology. The majority of the work regarding caspase-2 involves the use of caspase-2 deficient mice or caspase-2 knockdown with siRNAs. These studies have revealed that caspase-2 deficient animals are viable [18]. These animals are generally normal under basal conditions; however, phenotypes emerge under challenge conditions, which reflects the role of caspase-2 in stress responses. This suggests that caspase-2 inhibition is unlikely to be associated with major toxicity, however, chronic inhibition could compromise oxidative stress responses, and lead to an acceleration of aging-related conditions. Therefore, caspase-2 inhibitors are likely to be best suited for acute conditions, such as ischemic injuries, or they need to be weak/partial inhibitors for chronic use.

Studies in mice suggest that caspase-2 inhibition is unlikely to significantly increase cancer risk, but it could increase the risk for some hematological malignancies, especially those of myeloid cell origin, in susceptible populations [26]. Thus, monitoring for hematological abnormalities may be necessary with the clinical use of caspase-2 inhibitors. Monitoring for changes to metabolic parameters would also be recommended.

Sex effect: Studies in caspase-2 deficient mice highlight sex-dependent effects with respect to metabolism [24]. The majority of the rodent studies have been conducted in male animals, so it is unclear whether the potential therapeutic benefit of caspase-2 inhibitors differs between males and females.

Drug interactions: Interactions have not been established, but due to its role in regulating cell death, caspase-2 inhibitors may interact with chemotherapeutic agents.

Sources and dosing:

Caspase-2 inhibitors have not yet been developed for clinical use. The peptide inhibitor, Z-VDVAD-FMK, is available for research use from commercial suppliers.

Research underway:

There are preclinical efforts underway to develop new peptide-based caspase-2 inhibitors and small molecule-based caspase-2 inhibitors. These include novel peptide-based caspase-2 inhibitors to be used for neurodegenerative diseases with intranasal delivery [36], and small molecule caspase-2 inhibitors, including the published champion inhibitor (NH-23-C2), which was shown to selectively block caspase-2 activation in reversine-treated HCT-116 colon cancer cells [37].

Search terms:

Pubmed, Google: Caspase-2

- Alzheimer's disease, neurodegeneration, stroke, aging, lifespan, cancer, cardiovascular, inhibitors

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