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.

THIK-1 Inhibitors

Evidence Summary

THIK-1 regulates cell functions that are affected by the degree of cell excitability. It may be important in specific contexts, but its effects in animal models are minor. More human studies are needed.

Neuroprotective Benefit: THIK-1 helps regulate the functional state of microglia. It appears to promote a neuroprotective state under physiological conditions but may promote inflammation under pathological conditions.

Aging and related health concerns: THIK-1 may play minor roles in the susceptibility to inflammatory pain and atrial fibrillation, but more conclusive human data is needed.

Safety: Scant safety data is available. Due to redundancies, the effects of THIK-1 inhibition are expected to be minor and context-dependent. THIK-1 modulators may impact and/or be impacted by anesthesia.

What is it?

THIK-1 (Tandem pore-domain Halothane Inhibited K+ channel), also called K2P13.1 or KCNK13, is a two-pore domain containing potassium (K+) channel [1]. It produces background (leak) K+ conductance, meaning it has tonic activity, and is important for the regulation of the cell's resting membrane potential, which is important for the regulation of cell excitability. THIK-1 was found to be inhibited by the anesthetic halothane and activated by the inflammatory fatty acid arachidonic acid. Its expression is enriched in myeloid cells, including microglia in the brain, and by affecting membrane potential, THIK-1 is implicated in the regulation of the functional state of microglia [2]. It is currently unclear whether the modulation of THIK-1 function will have therapeutic value for any condition.

Neuroprotective Benefit: . THIK-1 helps regulate the functional state of microglia. It appears to promote a neuroprotective state under physiological conditions but may promote inflammation under pathological conditions.

Types of evidence:

- Several laboratory studies on THIK-1 function or expression

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:

A challenge in the study of THIK-1 is that it has been purported to be expressed ubiquitously in rodents, but its expression appears to be far more restricted in humans [1; 3]. Thus, some of the findings in preclinical rodent studies may not be translatable to humans. The most relevant studies are those in which the expression of THIK-1 has been confirmed in corresponding human tissue. Additionally, the expression of THIK-1 is severely reduced in response to the conditions typically used to prepare brain slices for electrophysiological recording and processing [4]. As a result, the expression and activity of THIK-1 could be influenced by experimental conditions in preclinical studies, which may compromise the

translatability of *in vitro* or *ex vivo* studies, and caution is warranted in the interpretation of results that have not been validated *in vivo*.

Microglial surveillance: The expression of THIK-1 is highly enriched in microglial cells and appears to play a role in regulating the functional state of microglia [2]. The tonic activity of THIK-1 influences the polarization of the cell membrane, and the polarization state can influence the degree of microglial cell ramification and surveillance [2]. Tonic THIK-1 activity promotes a ramified resting state of continual surveillance of the neuropil. There are several modes of surveillance, which include non-directed actin-dependent retraction and extension of fine filopodia, non-directed microtubule-mediated retraction and extension of larger processes, and the target-directed (cue-triggered) movement of large processes [2; 5; 6]. Filopodia-mediated surveillance is dependent on local increases in cyclic AMP (cAMP) [6], while cue-triggered movement is mediated by the purinergic receptor P2Y12 [2]. THIK-1 is not required for either of these surveillance programs. It is the intermediate state of resting non-directed large process surveillance that appears to be sensitive to the level of membrane polarization, and thus the activity of THIK-1. Resting state surveillance is thought to represent a neuroprotective state, thus the loss of this state in an otherwise healthy organism is generally considered detrimental [7]. The loss of THIK-1 during development results in reduced microglial phagocytosis and impaired synaptic pruning in rodents [8]. It is, however, unclear whether the neuroprotective function of THIK-1 is context-dependent, such that in a disease context it may promote a maladaptive microglial state.

Neuroinflammation: The efflux of potassium has been identified as a critical common driver of NLRP3 inflammasome activation in response to a diverse array of stimuli [9]. There are numerous ways in which potassium efflux can be achieved, including the formation of pores in the membrane, increasing extracellular calcium levels, increasing intracellular sodium levels, and modulating the activity of potassium channels, among others [10]. It is this last mechanism which ties THIK-1 activity with NLRP3 activation. A large membrane depolarization, such as in response to nearby neuronal activity, can result in a large potassium efflux, in order to restore the membrane potential. This, then, provides one of the necessary steps for NLRP3 activation. Since NLRP3 activation is a multi-step process, tonic THIK-1 activity alone does not drive inflammasome activation, but it can facilitate activation the presence of the appropriate NLRP3-activating stimuli [2; 11]. Various potassium channels are able to drive this process, but it appears that there is some degree of specificity in which NLRP3-activiating stimuli couple to which potassium channels. THIK-1 appears to be coupled to ADP-mediated NLRP3 activation through its interaction with P2Y12 [5], and to ATP-mediated NLRP3 activation via P2X7 [11]. In response to ADP, P2Y12 can potentiate THIK-1 activity, resulting in a potassium efflux, and the activation of the NLRP3

inflammasome [2]. In the context of acute tissue damage resulting in very high levels of ADP (and ATP), damping down THIK-1 activity, or more specifically P2Y12-mediated THIK-1 activity, may help protect against excessive inflammation and neuronal loss. However, in a more chronic condition, there are likely to be multiple NLRP3-activiting stimuli present, which are coupled to different potassium channels. Therefore, the impact of inhibiting only one of these channels (i.e. THIK-1) is likely to be minor on the overall inflammatory environment. Due to the ability of THIK-1 to impact the functional state of microglia, it is possible that THIK-1 activity has additional influence on shaping the overall inflammatory state of microglia, but that remains to be determined.

Demyelination: THIK-1 expressing microglia have been shown to be localized to nodes of Ranvier as part of 'neuron-glial hubs' in postmortem hemispheric myelinated brain tissue from healthy donors [12]. A similar interaction was seen within the rodent brain [12]. In mice, the contact of the microglial processes with the nodes was neuronal activity dependent, with THIK-1 as the intermediatory coupling microglial and neuronal activity based on changes in potassium levels [12]. The change in axonal potassium following focal demyelination influences the microglial state. The decline in activity reduced microglia-neuronal contacts and fostered a more pro-inflammatory state associated with demyelination, while increased THIK-1 activity during the remyelination phase promoted increased contacts, and a pro-regenerative state. This suggest that THIK-1 may play a role in local myelin dynamics.
The contribution of THIK-1 to a pro-regenerative microglial state in the grey matter following a large-scale demyelinating injury is less clear. In mice, the degree of de-myelination and microglial activation state differs in different cortical layers [13]. In deep layers (V), there was extensive demyelination and microglial activation, while in upper layers (II/III), the microglia remained in a ramified surveillance state and there was less demyelination in response to cuprizone This process was not altered in THIK-1 knockout mice, suggesting that THIK-1 was not necessary for this protective microglial state, though compensation cannot be ruled out.

Alcohol dependence: THIK-1 has been implicated in alcohol dependence behavior in rodents [4; 14]. THIK-1 activity plays a role in the excitation of ventral tegmental area (VTA) neurons, the reward center of the brain, in response to ethanol consumption [4]. The excitation of VTA neurons in response to ethanol occurs in part due to the inhibition of THIK-1. In mice, decreasing THIK-1 levels promotes binge drinking behavior [14]. It has been hypothesized that genetic variants in the KCNK13 gene may affect susceptibility to alcoholism and binge drinking, though genetic variants in KCNK13 have not yet been identified, and the relevance to humans has not yet been established [15].

Anesthesia: THIK-1 is inhibited by a certain class of inhaled anesthetics, including halothane and isoflurane [1]. At least in rodents, the preservation of activity in the respiratory motor system, despite a general lack of systemic motor responses during anesthesia, may be related to the impact of these agents on THIK-1 [16]. Neurons in the retrotrapezoid nucleus of the rodent brainstem are important regulators of the respiratory motor system. The activity of these neurons increases in response to these inhaled anesthetics due to the inhibition of THIK-1 [16].
It has also been hypothesized that the inhibition of THIK-1 may play a role in anesthesia-related cognitive dysfunction and inflammation due to the impact of THIK-1 on microglial surveillance [7]. Microglia ramification and process complexity decreases in response to isoflurane, which is consistent with the effect of THIK-1 inhibition on microglia. Due to its coupling with inflammasome activation, a post-surgical/anesthesia rebound in THIK-1 activity may potentiate neuroinflammation. It has not yet been determined whether changes in THIK-1 activity are associated to long-term consequences of anesthetic exposure.

Ataxia: There is currently no evidence linking altered THIK-1 activity to ataxia, however, there is growing appreciation for the contribution of microglia to cerebellar ataxia [17]. The microglia in the cerebellum have a unique transcriptome, which allows for the maintenance of a hyper alert state of continual surveillance. Due to the role of THIK-1 in surveillance, it may play a role. In the rodent cerebellum, THIK-1 is implicated in the activity of Purkinje cells [18]. However, the expression of THIK-1 in the cerebellum appears to be lower than in many other brain regions according to the human protein atlas [3], and more information is needed to determine whether or not THIK-1 contributes to cerebellar function in humans.

APOE4 interactions: Not established

Aging and related health concerns: THIK-1 may play minor roles in the susceptibility to inflammatory pain and atrial fibrillation, but more conclusive human data is needed.

Types of evidence:

- Several laboratory studies on THIK-1 function or expression

Inflammatory pain: THIK-1 ACTIVITY IS SENSITIVE TO PAIN STIMULI IN RODENTS (Preclinical)
Potassium channels have been shown to play a role in inflammatory pain via their contribution to setting the resting membrane potential of cells [19]. Changes to the membrane potential influences the threshold for sensory neurons to be activated by stimuli, thus, differences in the activity/expression of these potassium channels can increase or decrease the threshold for pain. In rats, THIK-1 is expressed in dorsal root ganglion, including neurons involved in pain processing [20; 21]. Lower levels of THIK-1 expression following a cutaneous inflammatory pain stimulus (Complete Freund's Adjuvant) were associated with an increase in behaviors indicative of spontaneous pain [21]. A reduction in the levels of THIK-1 increases the resting membrane potential, which lowers the threshold to activate neurons in response to pain-inducing stimuli. THIK-1 expression appears to be sensitive to the levels of pro-inflammatory mediators. However, a separate study has shown that THIK-1 is one of many potassium channels modulated by inflammatory pain-inducing stimuli, and THIK-1appears to be a minor contributor relative to some of the other channels [20]. The relevance of THIK-1 in the mediation of inflammatory pain in humans remains to be determined.

Atrial fibrillation: THIK-1 IS REDUCED IN ATRIAL FIBRILLATION (Preclinical)
Two pore potassium channels play important roles in cardiac function and have been proposed as anti-arrhythmic targets [22]. THIK-1 channels have been found to be expressed in the human heart, primarily in the atria [23]. Levels were found to be reduced in patients with atrial fibrillation, and heart failure [23; 24]. In a pig model (atrial burst pacing), atrial fibrillation was associated with a 66% reduction in cardiac levels of THIK-1 [22]. The activity of THIK-1 was also found to be sensitive to anti-arrhythmic drugs in cell culture [22]. This suggests that individuals with lower levels of THIK-1 activity may be at higher risk for atrial fibrillation.

Safety: Scant safety data is available. Due to redundancies, the effects of THIK-1 inhibition are expected to be minor and context-dependent. THIK-1 modulators may impact and/or be impacted by anesthesia.

Types of evidence:

- 0 studies for THIK-1 specific inhibitors
- Several laboratory studies using non-specific inhibitors and/or knockout animals

Published literature on the safety of THIK-1 specific inhibitors is lacking. Most studies use potassium channel inhibitors that are not specific for THIK-1 and/or THIK-1 knockout animals. THIK-1 knockout

mice are viable [8] suggesting that THIK-1 does not have an essential function and/or that its activity can be at least partially compensated for by other potassium channels. Overall, the phenotypes seen with the loss of THIK-1 activity, such as reduced microglial ramification, a decrease in ADP/ATP-induced neuroinflammation, an increased propensity for binge drinking, an increase in sensitivity to inflammatory pain, and an increased risk for atrial fibrillation are all quite minor in rodents/animal models [8; 11; 14; 21; 22]. Due to the differential expression profile for THIK-1 between humans and rodents, some of these rodent phenotypes may not translate to humans. Side effects for THIK-1 modulators would be expected to be subtle and context-dependent, such that they may not be apparent under physiological conditions in otherwise healthy animals/individuals.

Drug interactions: Interactions have not been established, however, based on preclinical models, THIK-1 inhibitors may have interactions with alcohol, anesthesia, and arrhythmia-related drugs.

Sources and dosing:

THIK-1 modulators are under development, but none are currently available for research or human use.

Research underway:

THIK-1 inhibitors are in clinical development; however, the details of this work have not been made public. Additionally, there are efforts underway to develop specific THIK-1 activators [25].

Search terms:

Pubmed, Google: THIK-1; KCNK13

- Alzheimer's disease, brain, neuroinflammation, aging, cardiovascular

References:

1. Rajan S, Wischmeyer E, Karschin C *et al.* (2001) THIK-1 and THIK-2, a Novel Subfamily of Tandem Pore Domain K+Channels * 210. *Journal of Biological Chemistry* **276**, 7302-7311https://doi.org/10.1074/jbc.M008985200.

2. Madry C, Kyrargyri V, Arancibia-Cárcamo IL *et al.* (2018) Microglial Ramification, Surveillance, and Interleukin-1b; Release Are Regulated by the Two-Pore Domain K+ Channel THIK-1. *Neuron* **97**, 299-312.e296https://doi.org/10.1016/j.neuron.2017.12.002.

3. KCNK13. *The Human Protein Atlas*https://www.proteinatlas.org/ENSG00000152315-KCNK13.

4. You C, Vandegrift BJ, Brodie MS (2021) KCNK13 potassium channels in the ventral tegmental area of rats are important for excitation of ventral tegmental area neurons by ethanol. *Alcoholism: Clinical and Experimental Research* **45**, 1348-1358https://onlinelibrary.wiley.com/doi/abs/10.1111/acer.14630.

5. Kyrargyri V, Madry C, Rifat A *et al.* (2020) P2Y13 receptors regulate microglial morphology, surveillance, and resting levels of interleukin 1β release. *Glia* **68**, 328-344https://onlinelibrary.wiley.com/doi/abs/10.1002/glia.23719.

6. Bernier L-P, Bohlen CJ, York EM *et al.* (2019) Nanoscale Surveillance of the Brain by Microglia via cAMP-Regulated Filopodia. *Cell Reports* **27**, 2895-2908.e2894https://doi.org/10.1016/j.celrep.2019.05.010.

7. Shih J, Dulla CG (2018) The THIK and Thin of Microglia Dynamics. *Neuron* **97**, 253-255https://doi.org/10.1016/j.neuron.2018.01.002.

8. Izquierdo P, Shiina H, Hirunpattarasilp C *et al.* (2021) Synapse development is regulated by microglial THIK-1 K+ channels. *Proceedings of the National Academy of Sciences* **118**, e2106294118https://www.pnas.org/doi/abs/10.1073/pnas.2106294118.

9. Muñoz-Planillo R, Kuffa P, Martínez-Colón G *et al.* (2013) K+ Efflux Is the Common Trigger of NLRP3 Inflammasome Activation by Bacterial Toxins and Particulate Matter. *Immunity* **38**, 1142-1153https://www.sciencedirect.com/science/article/pii/S1074761313002434.

10. Xu Z, Chen Z-m, Wu X *et al.* (2020) Distinct Molecular Mechanisms Underlying Potassium Efflux for NLRP3 Inflammasome Activation. *Frontiers in Immunology* **11**https://www.frontiersin.org/articles/10.3389/fimmu.2020.609441.

11. Drinkall S, Lawrence CB, Ossola B *et al.* (2022) The two pore potassium channel THIK-1 regulates NLRP3 inflammasome activation. *Glia* **70**, 1301-1316https://onlinelibrary.wiley.com/doi/abs/10.1002/glia.24174.

12. Ronzano R, Roux T, Thetiot M *et al.* (2021) Microglia-neuron interaction at nodes of Ranvier depends on neuronal activity through potassium release and contributes to remyelination. *Nature Communications* **12**, 5219https://doi.org/10.1038/s41467-021-25486-7.

13. Roufagalas I, Avloniti M, Fortosi A *et al.* (2021) Novel cell-based analysis reveals region-dependent changes in microglial dynamics in grey matter in a cuprizone model of demyelination. *Neurobiology of Disease* **157**, 105449https://www.sciencedirect.com/science/article/pii/S0969996121001984.

14. You C, Savarese A, Vandegrift BJ *et al.* (2019) Ethanol acts on KCNK13 potassium channels in the ventral tegmental area to increase firing rate and modulate binge–like drinking. *Neuropharmacology* **144**, 29-36https://www.sciencedirect.com/science/article/pii/S0028390818304076.

15. Blum K, Brodie MS, Pandey SC *et al.* (2022) Researching Mitigation of Alcohol Binge Drinking in Polydrug Abuse: KCNK13 and RASGRF2 Gene(s) Risk Polymorphisms Coupled with Genetic Addiction Risk Severity (GARS) Guiding Precision Pro-Dopamine Regulation. *Journal of Personalized Medicine* **12**, 1009https://www.mdpi.com/2075-4426/12/6/1009.

16. Lazarenko RM, Fortuna MG, Shi Y *et al.* (2010) Anesthetic Activation of Central Respiratory Chemoreceptor Neurons Involves Inhibition of a THIK-1-Like Background K+ Current. *The Journal of Neuroscience* **30**, 9324-9334https://www.jneurosci.org/content/jneuro/30/27/9324.full.pdf.

17. Ferro A, Sheeler C, Rosa J-G *et al.* (2019) Role of Microglia in Ataxias. *Journal of Molecular Biology* **431**, 1792-1804https://www.sciencedirect.com/science/article/pii/S0022283619300257.

18. Ishii H, Nakajo K, Yanagawa Y *et al.* (2010) Identification and characterization of Cs+-permeable K+ channel current in mouse cerebellar Purkinje cells in lobules 9 and 10 evoked by molecular layer stimulation. *European Journal of Neuroscience* **32**, 736-748https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1460-9568.2010.07336.x.

19. Du X, Gamper N (2013) Potassium channels in peripheral pain pathways: expression, function and therapeutic potential. *Current neuropharmacology* **11**, 621-640https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3849788/.

20. Marsh B, Acosta C, Djouhri L *et al.* (2012) Leak K+ channel mRNAs in dorsal root ganglia: Relation to inflammation and spontaneous pain behaviour. *Molecular and Cellular Neuroscience* **49**, 375-386https://www.sciencedirect.com/science/article/pii/S1044743112000036.

21. Haskins W, Benitez S, Mercado JM *et al.* (2017) Cutaneous inflammation regulates THIK1 expression in small C-like nociceptor dorsal root ganglion neurons. *Molecular and Cellular Neuroscience* **83**, 13-26https://www.sciencedirect.com/science/article/pii/S1044743117300325.

22. Staudacher I, Seehausen S, Illg C *et al.* (2019) Cardiac K2P13.1 (THIK-1) two-pore-domain K+ channels: Pharmacological regulation and remodeling in atrial fibrillation. *Progress in Biophysics and Molecular Biology* **144**, 128-138https://www.sciencedirect.com/science/article/pii/S0079610718300671.

23. Schmidt C, Wiedmann F, Voigt N *et al.* (2015) Upregulation of K2P3.1 K+ Current Causes Action Potential Shortening in Patients With Chronic Atrial Fibrillation. *Circulation* **132**, 82-92https://www.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.114.012657.

24. Schmidt C, Wiedmann F, Zhou X-B *et al.* (2017) Inverse remodelling of K2P3.1 K+ channel expression and action potential duration in left ventricular dysfunction and atrial fibrillation: implications for patient-specific antiarrhythmic drug therapy. *European Heart Journal* **38**, 1764-1774https://doi.org/10.1093/eurheartj/ehw559.

25. McCoull D, Ococks E, Large JM *et al.* (2021) A "Target Class" Screen to Identify Activators of Two-Pore Domain Potassium (K2P) Channels. *SLAS DISCOVERY: Advancing the Science of Drug Discovery* **26**, 428-438https://journals.sagepub.com/doi/abs/10.1177/2472555220976126.

***Disclaimer**: Cognitive Vitality Reports® do not provide, and should not be used for, medical advice, diagnosis, or treatment. You should consult with your healthcare providers when making decisions regarding your health. Your use of these reports constitutes your agreement to the **Terms & Conditions**.*

If you have suggestions for drugs, drugs-in-development, supplements, nutraceuticals, or food/drink with neuroprotective properties that warrant in-depth reviews by ADDF's Aging and Alzheimer's Prevention Program, please contact INFO@alzdiscovery.org. To view our official ratings, visit Cognitive Vitality's Rating page.