*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.*

# URMC-099

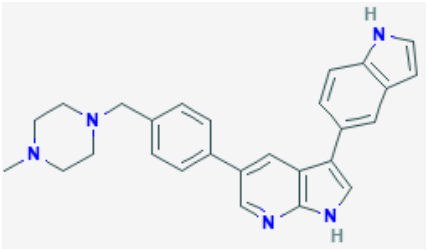
**Evidence Summary**

Has potential to mitigate neuropathology associated with microglia driven neuroinflammation, but is likely to have pleiotropic effects due to broad-spectrum activity, and safety testing in humans is needed.

**Neuroprotective Benefit:** May protect against neural pathology associated with innate immune system mediated neuroinflammation by modulating microglia. May also lead to activation of endogenous neuronal survival pathways.

**Aging and related health concerns:** May reduce inflammation mediated lipotoxicity in the liver, but is not protective against metabolic dysfunction.

**Safety:** Has not been tested in humans and long-term safety profile in unknown. May affect response to some viruses by dampening the immune system.

| | | |
|---|---|---|
| **Availability:** Research studies | **Dose:** Not established | **Chemical formula:** $C_{27}H_{27}N_5$<br>**MW:** 421.5 g/mol<br>  <br>Source: PubChem |
| **Half-life:** ~2 hours | **BBB:** Penetrant | |
| **Clinical trials:** None | **Observational studies:** None | |

**What is it?**

URMC-099 is a broad-spectrum mixed lineage kinase (MLK) inhibitor with preferential activity toward MLK3 ($IC_{50}$ = 14 nM) [1]. MLKs are serine/threonine kinases which **regulate upstream MAPK signaling including JNK and p38 MAPK.** MLK3 (MAP3K11) is the most widely expressed MLK, undergoes a wide array of interactions, and thus may serve as an integrator of multiple signaling pathways [2]. It is activated through interactions with cdc42/Rac1 in response to cell stressors. URMC-099 also exhibits >90% inhibition (at 1uM) toward the kinases: ABL1, CDK11, CDK4, CDKL2, CLK1, CLK2, CLK4, DYRK1B, FLT3, KIT, MELK, PDGFRB, SRPK2, ALK, ARK5, AXL, IKKα, IKKβ, ROCK1, TYK2, DLK, and LRRK2 [1]. URMC-099 is orally bioavailable (%F=41) and blood-brain barrier (BBB) penetrant. It was developed in the lab of Dr. Harris Gelbard at the University of Rochester Medical Center, and has primarily been tested in preclinical studies for its ability to mitigate pathogenic neuroinflammation driven by the innate immune system.

**Neuroprotective Benefit:** May protect against neural pathology associated with innate immune system mediated neuroinflammation by modulating microglia. May also lead to activation of endogenous neuronal survival pathways.

*Types of evidence:*

- 1 observational study (MAPK signaling in Superagers)
- 7 laboratory studies for URMC-099

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

URMC-099 has not yet been tested in humans, but there is evidence suggesting that people with reduced levels of a signaling pathway targeted by URMC-099 are less likely to experience cognitive decline during aging. Superagers are defined as people ≥ 80 years old with episodic memory scores of people in their 50s and 60s [3]. Superagers with superior memory are less susceptible to age-related cortical atrophy. Single nucleotide polymorphisms (SNPs) in the MAP2K3 gene, rs2363221, rs2230435 and rs736103, were found to be more common in superagers. **Individuals with these SNPs have a lifelong reduction in MAP2K3 activity, which is hypothesized to confer neuroprotection as they age.** MAP2K3, also known as MKK3, is one of the kinases activated by MLK3, the best characterized target of URMC-099. MAP2K3/MKK3 is enriched in microglia, and is part of an inflammatory signaling cascade that is upregulated in the brain of Alzheimer's disease (AD) patients. This suggests that inhibition of MAP2K3/MKK3 signaling via URMC-099 could potentially be protective against neuroinflammation-associated age-related cognitive decline.

*Human research to suggest benefits to patients with dementia*: None

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

### Neuroinflammation

MLK3 acts as part of an intracellular signaling system that links external inflammatory signals with changes in gene expression [2]. In this capacity, **MLK3 appears to play a critical role in regulating innate immune responses associated with neuroinflammation.** MLK3 is activated in response to mediators of inflammation and cell stress, and MLK3 inhibitors have been shown to inhibit LPS-induced release of pro-inflammatory mediators (TNFα) in microglia and macrophages [1]. MLK3 inhibitors can also inhibit the release of pro-inflammatory mediators (NO, IL-6) from astrocytes through inhibition of JNK and p38 MAPK signaling [4]. **URMC-099 has been shown to modulate the activation state of microglia toward a less pathogenic state** following exposure to various cell stressors [1; 5; 6; 7; 8; 9], and this anti-inflammatory activity is generally attributed to the targeting of MLK3.

### Neuronal survival

The JNK and p38 MAPK signaling pathways are drivers of neuronal cell death in response to toxic stimuli, and upstream drivers of these pathways may vary in different neuronal cell types, therefore, the **neuroprotection exhibited by URMC-099 is attributed to its ability to target multiple kinases**

[10]. URMC-099 targets numerous kinases associated with neurodegeneration, including MAPKKKs, ABL/DLK, LRRK2, and IGF1R [1]. However, the constellation of kinases targeted by a given broad-spectrum kinase inhibitor and affinities toward those most relevant toward cell survival in a given cell type likely account for differences in neuroprotective efficacy across multiple neurodegenerative diseases. One study found that MAP4Ks were most relevant for ER-stress mediated neurodegeneration, particularly in motor neurons [10]. While URMC-099 can inhibit MAP4Ks, it has higher affinity for MAP3Ks, and it was found to offer less neuroprotection in motor neurons with an amyotrophic lateral sclerosis (ALS) mutation ($SOD^{A4V}$), relative to compounds with higher affinity for MAP4Ks.

MLK3 can also influence neuronal survival by linking glutamatergic signaling through the NMDA receptor to JNK activation. Low levels of NMDA receptor activation are protective, as they lead to the activation of Akt, and inhibition of MLK3 [11; 12]. In contrast, high levels of NMDA receptor activation, such as those associated with excitotoxicity, promote the activation of MLK3 and downstream JNK signaling, leading to the induction of neuronal apoptotic processes [13].

### Autophagy

The ability of URMC-099 to promote mechanisms of proteostasis in neural cells has not yet been established, however, there is evidence from rodent and cell culture studies that URMC-099 can promote autophagy by regulating the nuclear localization of the autophagy promoting transcription factor TFEB in an mTORC1 dependent manner [14]. URMC-099 appears to promote the fusion of endosomes with autophagosomes [14; 15; 16]. In the context of HIV-1 infected cells, URMC-099 was able to enhance cellular metabolic activity and mitochondrial health [14].

### Alzheimer's disease: Potential benefit (Preclinical)

MLK3 mediated JNK and p38 MAPK signaling has been shown to contribute to oligomeric Aβ induced neurotoxicity. In cultured cortical neurons, a non-specific inhibitor which blocked Aβ induced activation of MLK3 and JNK, was neuroprotective [17; 18]. In rats, protection against Aβ oligomer mediated neuronal loss by brief ischemic postconditioning was found to stem from the inhibition of Aβ induced MLK3-MKK3-p38 MAPK activation [12]. **URMC-099 was shown to exert similar protection against Aβ mediated neurotoxicity in preclinical models.** The neuroprotection was primarily related to modulation of the inflammatory phenotype in microglia. In Aβ42 treated microglia, URMC-099 treatment reduced expression of pro-inflammatory mediators (TNFα, IL-1β), induced expression of anti-inflammatory cytokines (IL-4, IL-13), and promoted the phagocytic uptake of Aβ. APP/PS1 AD model

mice were found to have increased levels of activated (phosphorylated) MKK3, MKK4, and JNK in the cortex and hippocampus. URMC-099 treatment (10 mg/kg i.p. daily for 3 weeks) reduced levels of JNK pathway activation, and shifted the polarization state of microglia toward the protective M2 (Arginase+) subtype. This modulation of microglial state was associated with reduced synapse loss and increased Aβ clearance, suggesting that URMC-099 can protect against Aβ mediated neurotoxicity *in vivo*.

### Parkinson's disease: Potential benefit based on mechanism of action

MLK3 activates MAP2Ks, including MKK4 and MKK7, which activate JNK and its associated signaling cascade. MLK3 can be activated by neuroinflammatory mediators including TNFα and ceramide [19]. The activation of JNK can promote cell death, and MLK inhibitors have been shown to prevent dopaminergic neuron loss in rodent models of Parkinson's disease (PD) [20]. This suggests that **MLK3 may activate the cascade that leads to cell death in the context of neuroinflammation.** The MLK3 inhibitor CEP1347 failed to show protection in a Phase 2 clinical trial in patients with early onset PD (NCT00040404), however, it is unknown whether this stems from insufficient brain levels of the drug, or if MLK3 is a suboptimal target [21]. Many studies have found that broad spectrum kinase inhibitors tend to be more effective and have higher neuroprotective potential than more specific kinase inhibitors [10]. URMC-099 is expected to overcome both of the pitfalls of CEP1347 in that it has higher BBB penetrance and targets a broader range of kinases, including LRRK2, which is mutated in some familial forms of PD and elevated in idiopathic PD [22]. URMC-099 is being tested in a preclinical PD rodent model in a study, and results are expected to inform whether URMC-099 is a viable clinical candidate for PD.

### Stroke: Theoretical benefit based on role of MLK3 in cerebral ischemia

MLK3 is implicated in mechanisms of neuronal loss associated with cerebral ischemic-reperfusion events in preclinical models. The Akt and JNK pathways regulate neuronal cell death during cerebral ischemia [23]. MLK3 activation promotes the MKK7-JNK pathway and neuronal apoptosis [17]. Meanwhile, activation of Akt leads to the inhibition of MLK3 and promotes pro-survival signaling. MLK3 has been implicated as a sensor of oxidative stress, and regulator of downstream cellular responses. In cells exposed to low levels of reactive oxygen species (ROS), JNK signaling is low and mechanisms of cellular proliferation dominate, but following exposure to high levels of ROS, MLK3 is activated, leading to the activation of JNK and pro-apoptotic signaling [24]. The accumulation of free radicals leads to the activation of MLK3. One study found that MLK3 is S-nitrosylated in the presence of nitric oxide (NO),

and that this post-translational modification was associated with its activation and ability to promote neurotoxicity [13].

The damage minimizing effects of ischemic preconditioning, in which short bouts of ischemia are induced in a tissue prior to a major ischemic injury, is partially attributed to the modulation of MLK3 and associated downstream JNK signaling. In rats, preconditioning increased levels of activated Akt, which then prevented the enhancement of MLK3 activity which typically occurs in response to an ischemic-reperfusion injury [11]. As a result, preconditioning shifts the balance toward pro-survival Akt signaling and away from pro-apoptotic JNK signaling.

These studies suggest that URMC-099 may be protective against neuronal loss in the context of cerebral ischemic injury, however, URMC-099 has not yet been specifically tested for this indication, and as a multi-kinase inhibitor, it may have different effects relative to a more specific MLK3 inhibitor.

### HIV-associated neurocognitive disorders (HAND): Potential benefit (preclinical)

The viral infection of innate immune cells can promote pathogenic neuroinflammation and trigger cognitive dysfunction. HAND is characterized by the dysregulation of inflammatory cytokines, microglial dysfunction, and synaptodendritic damage. MLK3 has been found to play a role in mediating neurotoxicity in response to HIV-1 through the activation of JNK (via phosphorylation of p46 and p54 JNK) and activation of pro-inflammatory microglia [9]. In HIV-1 Tat infected mice, URMC-099 treatment was neuroprotective. URMC-099 treatment normalized levels of pro-inflammatory cytokines (TNFα, IL-6), and reduced dendritic spine loss by phagocytic microglia. This suggests that **URMC-099 exerts its neuroprotective effects by modulating the activation state and inflammatory profile of the microglia**. URMC-099 is a particularly attractive candidate for HAND because separate studies have found that URMC-099 increases the efficacy of anti-retroviral therapy [14; 15; 16].

### Post-operative cognitive dysfunction (POCD): Potential benefit (preclinical)

Neuroinflammation stemming from surgery and/or anesthesia is hypothesized to be a key driver of POCD. URMC-099 pre-treatment (10 mg/kg i.p. 3x prior to surgery) reduced microgliosis and leakage through the BBB in mice where the tibia was fractured under anesthesia [5]. This **reduction in neuroinflammation** was associated with increased performance on object place and discrimination memory tests following surgery relative to the vehicle treated counterparts, suggesting that cognitive function was preserved. Notably, URMC-099 had no significant effects on peripheral inflammation or bone healing processes.

*APOE4 interactions*: Unknown

**Aging and related health concerns:** May reduce inflammation mediated lipotoxicity in the liver, but is not protective against metabolic dysfunction.

*Types of evidence:*

- 3 laboratory studies for URMC-099

**Non-alcoholic steatohepatitis (NASH): Potential benefit (Preclinical)**

MLK3 mediated JNK signaling was found to promote high-fat diet induced insulin resistance, obesity, and hepatic steatosis in mice [25]. Mice lacking MLK2 and MLK3 had improved insulin sensitivity and glucose tolerance, while retaining smaller adipocytes and lower body weight in response to a high fat diet [26]. The metabolic benefits were attributed, in part, to increased energy expenditure and physical activity levels. However, due to redundancy in MLK family members, and the possible contribution of kinase-independent activity, URMC-099 treatment was not protective against metabolic syndrome or weight gain in mice fed a high fat/high carbohydrate diet [27]. Instead, URMC-099 is selectively protective against lipotoxic stress associated inflammation and cell death. MLK3 is important for fat metabolism in the liver, and appears to be a key driver of hepatocyte lipoapoptosis and lipotoxicity. Saturated free fatty acids act as stressors to activate MLKs and stimulate JNK signaling [25]. MLK3 appears to play a role in driving hepatic inflammation in response to lipotoxic stress, in part, by releasing extracellular vesicles enriched with macrophage chemotaxis molecules (CXCL10) [28]. In a NASH mouse model, **URMC-099 treatment reduced hepatic inflammatory infiltrates** by preventing macrophage activation and migration in response to elevated lipid load [27]. The reduction in inflammation protected against hepatocyte cell death and fibrosis. This suggests that URMC-099 may be beneficial for addressing the inflammatory, but not the metabolic pathology associated with NASH.

**Cancer: Unclear benefit**

MLK3 has been shown to play roles in cell motility and migration *in vitro* [2]. Based on these studies, MLK3 is implicated in the migration and metastasis of cancer cells. Colorectal cancers are characterized by high levels of oxidative stress, which can serve as a trigger to activate MLK3 and drive colon cancer cell invasion [29]. However, it is **unclear how well these findings will translate *in vivo***. URMC-099 was found to be capable of inhibiting the migration of triple negative breast cancer cells *in vitro*, but was ineffective at inhibiting metastasis in a mouse xenograft model [30]. This could indicate that due to redundancy in MLK family members, that MLK3 is not an effective target, or that the pleiotropic effects

of broad-spectrum kinase inhibition with URMC-099 mitigates any potential therapeutic benefit. Whether the efficacy of URMC-099 is enhanced when used in combination with other anti-cancer therapies remains to be determined.

**Cardiovascular: Potential harm/unclear (Preclinical)**

The role of MLK3 and JNK activation in cardiovascular function is unclear, as different studies show conflicting results, suggesting that the outcome may depend on the downstream cellular targeting of JNK in a particular context [31]. Most studies find that inhibiting JNK signaling is cardioprotective, however, a study examining cardiac hypertrophy found that activation of JNK signaling may be protective in this context. MLK3 expression was found to be increased by over six-fold in patients with nonischemic or hypertrophic cardiomyopathy, and mice deficient in MLK3 were more susceptible to cardiac dysfunction worsening in response to pressure overload [32]. URMC-099 treatment also promoted cardiomyocyte hypertrophy, in cell culture. Although the impact of URMC-099 on cardiac function remains to be confirmed *in vivo*, this study suggest that it could potentially promote adverse cardiac remodeling in a subset of patients, which should be assessed during clinical safety testing.

**Safety:** Has not been tested in humans and long-term safety profile in unknown. May affect response to some viruses by dampening the immune system.

*Types of evidence:*

- 16 laboratory studies

**URMC-099 has not yet been tested in humans.** Cell culture studies show that it has minimal interference with human CYP450 or hERG channels [1]. Treatment in mice at doses up to 10 mg/kg (i.p. 2x/day) is well tolerated, and not associated with changes in body weight or obvious toxicities [14; 27]. However, the studies to date have all been short in duration, generally under a month, so **long-term safety remains to be determined.** Preclinical pharmacokinetic studies suggest that URMC-099 has reasonable drug properties for neurological diseases in that it satisfies Lipinski's rule of five with an oral bioavailability (%F) of 41, and has a level of BBB penetrance (brain: plasma ratio 0.81) that allows biologically active levels of the drug to reach the brain with systemic dosing [1].

As a broad-spectrum kinase inhibitor, the side effect profile of URMC-099 may vary in different patient populations, depending on which kinases are preferentially affected.

Since MLK3 is involved in immune system regulation, its chronic inhibition could potentially affect the ability of the body to clear certain pathogens. One study found that MLK3 acts as a host restriction factor against Zika virus, and that URMC-099 facilitated Zika viral replication in the neonatal mouse brain [33].

**Sources and dosing:**

URMC-099 is available for research use from commercial suppliers, and has not yet been tested in humans. Rodent studies have used a dose of 10 mg/kg, which translates to a human equivalent dose (HED) of 0.8 mg/kg. It is under development for neuroinflammatory conditions by the University of Rochester Medical Center, which holds the relevant patents.

**Research underway:**

URMC-099 continues to be studied in preclinical studies, and there are currently no active clinical trials, but the developers of URMC-099 are reported to be working on filing an investigational new drug (IND) application in preparation for future clinical trials.

**Search terms:**

Pubmed, Google: URMC-099, MLK3

- Alzheimer's disease, Parkinson's disease, neurodegeneration, ischemia, cardiovascular, aging, inflammation, cancer, metabolism, autophagy, safety

Websites visited for URMC-099:

- PubChem

**References:**

1. Goodfellow VS, Loweth CJ, Ravula SB *et al.* (2013) Discovery, synthesis, and characterization of an orally bioavailable, brain penetrant inhibitor of mixed lineage kinase 3. *J Med Chem* 56, 8032-8048.https://www.ncbi.nlm.nih.gov/pubmed/24044867 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4032177/

2. Polesskaya O, Wong C, Lebron L *et al.* (2014) MLK3 regulates fMLP-stimulated neutrophil motility. *Mol Immunol* 58, 214-222.https://www.ncbi.nlm.nih.gov/pubmed/24389043 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946811/

3. Huentelman MJ, Piras IS, Siniard AL *et al.* (2018) Associations of MAP2K3 Gene Variants With Superior Memory in SuperAgers. *Front Aging Neurosci* 10, 155-155.https://www.ncbi.nlm.nih.gov/pubmed/29896098

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987172/

4. Falsig J, Pörzgen P, Lotharius J *et al.* (2004) Specific Modulation of Astrocyte Inflammation by Inhibition of Mixed Lineage Kinases with CEP-1347. *The Journal of Immunology* 173, 2762-2770.https://www.jimmunol.org/content/jimmunol/173/4/2762.full.pdf

5. Miller-Rhodes P, Kong C, Baht GS *et al.* (2019) The broad spectrum mixed-lineage kinase 3 inhibitor URMC-099 prevents acute microgliosis and cognitive decline in a mouse model of perioperative neurocognitive disorders. *J Neuroinflammation* 16, 193-193.https://www.ncbi.nlm.nih.gov/pubmed/31660984 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6816182/

6. Bellizzi MJ, Hammond JW, Li H *et al.* (2018) The Mixed-Lineage Kinase Inhibitor URMC-099 Protects Hippocampal Synapses in Experimental Autoimmune Encephalomyelitis. *eNeuro* 5, ENEURO.0245-0218.2018.https://www.ncbi.nlm.nih.gov/pubmed/30627663 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6325567/

7. Kiyota T, Machhi J, Lu Y *et al.* (2018) URMC-099 facilitates amyloid-β clearance in a murine model of Alzheimer's disease. *J Neuroinflammation* 15, 137-137.https://www.ncbi.nlm.nih.gov/pubmed/29729668 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5935963/

8. Dong W, Embury CM, Lu Y *et al.* (2016) The mixed-lineage kinase 3 inhibitor URMC-099 facilitates microglial amyloid-β degradation. *J Neuroinflammation* 13, 184-184.https://www.ncbi.nlm.nih.gov/pubmed/27401058 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4940949/

9. Marker DF, Tremblay M-È, Puccini JM *et al.* (2013) The new small-molecule mixed-lineage kinase 3 inhibitor URMC-099 is neuroprotective and anti-inflammatory in models of human immunodeficiency virus-associated neurocognitive disorders. *J Neurosci* 33, 9998-10010.https://www.ncbi.nlm.nih.gov/pubmed/23761895 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682381/

10. Bos PH, Lowry ER, Costa J *et al.* (2019) Development of MAP4 Kinase Inhibitors as Motor Neuron-Protecting Agents. *Cell Chemical Biology* 26, 1703-1715.e1737.http://www.sciencedirect.com/science/article/pii/S2451945619303502

11. Yin X-H, Zhang Q-G, Miao B *et al.* (2005) Neuroprotective effects of preconditioning ischaemia on ischaemic brain injury through inhibition of mixed-lineage kinase 3 via NMDA receptor-mediated Akt1 activation. *Journal of Neurochemistry* 93, 1021-1029.https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1471-4159.2005.03096.x

12. Li H (2019) A Brief Ischemic Postconditioning Protects Against Amyloid-β Peptide Neurotoxicity by Downregulating MLK3-MKK3/6-P38MAPK Signal in Rat Hippocampus. *Journal of Alzheimer's disease* 71, 671-684

13. Hu S-Q, Ye J-S, Zong Y-Y *et al.* (2012) S-nitrosylation of mixed lineage kinase 3 contributes to its activation after cerebral ischemia. *J Biol Chem* 287, 2364-2377.https://www.ncbi.nlm.nih.gov/pubmed/22123824 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3268398/

14. Gnanadhas DP, Dash PK, Sillman B *et al.* (2017) Autophagy facilitates macrophage depots of sustained-release nanoformulated antiretroviral drugs. *J Clin Invest* 127, 857-873.https://www.ncbi.nlm.nih.gov/pubmed/28134625 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5330738/

15. Thomas MB, Gnanadhas DP, Dash PK *et al.* (2018) Modulating cellular autophagy for controlled antiretroviral drug release. *Nanomedicine (Lond)* 13, 2139-2154.https://www.ncbi.nlm.nih.gov/pubmed/30129397 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6219451/

16. Zhang G, Guo D, Dash PK *et al.* (2016) The mixed lineage kinase-3 inhibitor URMC-099 improves therapeutic outcomes for long-acting antiretroviral therapy. *Nanomedicine* 12, 109-122.https://www.ncbi.nlm.nih.gov/pubmed/26472049 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4728028/

17. Xu Y, Hou X-Y, Liu Y *et al.* (2009) Different protection of K252a and N-acetyl-L-cysteine against amyloid-β peptide–induced cortical neuron apoptosis involving inhibition of MLK3–MKK7–JNK3 signal cascades. *Journal of Neuroscience Research* 87, 918-927.https://onlinelibrary.wiley.com/doi/abs/10.1002/jnr.21909

18. Zhou F, Xu Y, Hou X-Y (2014) MLK3-MKK3/6-P38MAPK cascades following N-methyl-D-aspartate receptor activation contributes to amyloid-β peptide-induced apoptosis in SH-SY5Y cells. *Journal of Neuroscience Research* 92, 808-817.https://onlinelibrary.wiley.com/doi/abs/10.1002/jnr.23354

19. Sathyanarayana P, Barthwal MK, Kundu CN *et al.* (2002) Activation of the Drosophila MLK by Ceramide Reveals TNF and Ceramide as Agonists of Mammalian MLK3. *Molecular Cell* 10, 1527-1533.https://doi.org/10.1016/S1097-2765(02)00734-7

20. Kanthasamy A, Jin H, Mehrotra S *et al.* (2010) Novel cell death signaling pathways in neurotoxicity models of dopaminergic degeneration: relevance to oxidative stress and neuroinflammation in Parkinson's disease. *Neurotoxicology* 31, 555-561.https://www.ncbi.nlm.nih.gov/pubmed/20005250 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2888638/

21. The Parkinson Study Group PRECEPT Investigators (2007) Mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson disease. *Neurology* 69, 1480-1490.https://n.neurology.org/content/neurology/69/15/1480.full.pdf

22. Di Maio R, Hoffman EK, Rocha EM *et al.* (2018) LRRK2 activation in idiopathic Parkinson's disease. *Science Translational Medicine* 10, eaar5429.https://stm.sciencemag.org/content/scitransmed/10/451/eaar5429.full.pdf

23. Shvedova M, Anfinogenova Y, Atochina-Vasserman EN *et al.* (2018) c-Jun N-Terminal Kinases (JNKs) in Myocardial and Cerebral Ischemia/Reperfusion Injury. *Frontiers in Pharmacology* 9.https://www.frontiersin.org/article/10.3389/fphar.2018.00715

24. Lee H-S, Hwang CY, Shin S-Y *et al.* (2014) MLK3 Is Part of a Feedback Mechanism That Regulates Different Cellular Responses to Reactive Oxygen Species. *Science Signaling* 7, ra52-ra52.https://stke.sciencemag.org/content/sigtrans/7/328/ra52.full.pdf

25. Craige SM, Reif MM, Kant S (2016) Mixed – Lineage Protein kinases (MLKs) in inflammation, metabolism, and other disease states. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1862, 1581-1586.http://www.sciencedirect.com/science/article/pii/S0925443916301399

26. Kant S, Barrett T, Vertii A *et al.* (2013) Role of the mixed-lineage protein kinase pathway in the metabolic stress response to obesity. *Cell Rep* 4, 681-688.https://www.ncbi.nlm.nih.gov/pubmed/23954791 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3769115/

27. Tomita K, Kohli R, MacLaurin BL *et al.* (2017) Mixed-lineage kinase 3 pharmacological inhibition attenuates murine nonalcoholic steatohepatitis. *JCI Insight* 2, e94488.https://www.ncbi.nlm.nih.gov/pubmed/28768902 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5543922/

28. Ibrahim SH, Hirsova P, Tomita K *et al.* (2016) Mixed lineage kinase 3 mediates release of C-X-C motif ligand 10-bearing chemotactic extracellular vesicles from lipotoxic hepatocytes. *Hepatology* 63, 731-744.https://www.ncbi.nlm.nih.gov/pubmed/26406121 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4764421/

29. Schroyer AL, Stimes NW, Abi Saab WF *et al.* (2018) MLK3 phosphorylation by ERK1/2 is required for oxidative stress-induced invasion of colorectal cancer cells. *Oncogene* 37, 1031-1040.https://www.ncbi.nlm.nih.gov/pubmed/29084209 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5823719/

30. Rhoo KH, Granger M, Sur J *et al.* (2014) Pharmacologic inhibition of MLK3 kinase activity blocks the in vitro migratory capacity of breast cancer cells but has no effect on breast cancer brain metastasis in a mouse xenograft model. *PLoS One* 9, e108487-e108487.https://www.ncbi.nlm.nih.gov/pubmed/25264786 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4180451/

31. Rose BA, Force T, Wang Y (2010) Mitogen-activated protein kinase signaling in the heart: angels versus demons in a heart-breaking tale. *Physiol Rev* 90, 1507-1546.https://www.ncbi.nlm.nih.gov/pubmed/20959622 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3808831/

32. Calamaras TD, Baumgartner RA, Aronovitz MJ *et al.* (2019) Mixed lineage kinase-3 prevents cardiac dysfunction and structural remodeling with pressure overload. *Am J Physiol Heart Circ Physiol* 316, H145-H159.https://www.ncbi.nlm.nih.gov/pubmed/30362822 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383356/

33. Xu H, Cheng M, Chi X *et al.* (2019) High-Throughput Screening Identifies Mixed-Lineage Kinase 3 as a Key Host Regulatory Factor in Zika Virus Infection. *Journal of Virology* 93, e00758-00719.https://jvi.asm.org/content/jvi/93/18/e00758-19.full.pdf