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.

MSUT2 Inhibitors

Evidence Summary

MSUT2 levels are altered with Alzheimer's disease progression, and may play a role in tau-mediated neurodegeneration. Specific inhibitors are not yet available. More research on this target is needed.

Neuroprotective Benefit: Inhibition of MSUT2 may reduce tau aggregation and tau-mediated neurodegeneration, but MSUT2 also plays a role in working memory and neuronal function.

Aging and related health concerns: MSUT2 expression may be a prognostic biomarker for hepatocellular carcinoma. It is not known whether MSUT2 modulation affects cancer risk or any other aging-related disease.

Safety: There is no safety data regarding MSUT2 inhibitors. There is a theoretical risk for cancer and a potential impact on cognition. Side effect profile would be influenced by the specificity of the inhibitor for MSUT2.

Availability: Tool compounds are available for research use	**Dose**: N/A	**Chemical formula:** N/A
Half-life: N/A	**BBB**: N/A	**MW**: N/A
Clinical trials: None	**Observational studies**: MSUT2 levels are depleted in brain regions with advanced tau pathology in AD postmortem tissue.	

What is it?

Mammalian suppressor of tau pathology (MSUT2), also called ZC3H14, is an RNA-binding protein involved in the post-transcriptional processing of mRNAs [1]. It is primarily localized to nuclear speckles, which are enriched in pre-mRNA splicing factors and other RNA processing proteins. The orthologs in yeast and flies, Nab2 and dNab2, respectively, are essential genes, while the mammalian gene is not essential [2]. There are multiple alternatively spliced isoforms of MSUT2 in mammals. While primarily understood for its role in RNA processing, especially the regulation of poly-adenylation, MSUT2 was identified in a screen to be a modifier of tau pathology [3]. There are efforts underway to develop MSUT2 inhibitors with the expectation that they may reduce tau-mediated neurodegeneration in the context of Alzheimer's disease [4].

Neuroprotective Benefit: Inhibition of MSUT2 may reduce tau aggregation and tau-mediated neurodegeneration, but MSUT2 also plays a role in working memory and neuronal function.

Types of evidence:

- 4 Postmortem brain tissue studies of MSUT2 expression in AD
- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function: None

Human research to suggest benefits to patients with dementia: None

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

RNA processing: MSUT2 IS A REGULATOR OF RNA PROCESSING

MSUT2 was originally identified as zinc finger CCCH-type containing 14 (ZC3H14), an RNA binding protein involved in RNA processing, which is an ortholog to the yeast protein Nab2 [5]. MSUT2 is localized to nuclear speckles, which are sites of active transcription and RNA processing [1]. MSUT2 binds to poly-adenylated RNA, and plays a role in the regulation of mRNA poly(A) tail length, an important contributor to mRNA stability. Loss of MSUT2 leads to an increase in poly(A) tail length, suggesting that MSUT2 shortens poly(A) tails [6; 7]. Although longer poly(A) tails generally confer greater stability, short poly(A) tails are common in highly expressed genes, which may be important for them to be translated efficiently [8]. Transcriptomic analysis following MSUT2 depletion indicates that MSUT2 is only critical for the regulation of a small subset of RNAs (~1%) [9]. Consequently, the phenotypes associated with loss of MSUT2 are related to that RNA subset. Although ubiquitously expressed, MSUT2 interacts with a variety of other RNA processing proteins, and the effects of MSUT2 activity vary in a tissue specific and context-dependent manner, depending on the overall array of RNA processing factors and expression of specific transcripts within a given cell [10]. The brain and testes are the tissues most dependent on precise spatial and temporal control of gene expression, and correspondingly are the tissues that show phenotypes when MSUT2 is knocked out in mice [2].

Due to its role in the precise spatio-temporal control of gene expression, levels of MSUT2 that are too high or too low can both lead to negative phenotypes, with most studies indicating that elevated levels are worse. Two MSUT2-regulated RNAs that are important for neuronal function are Atp5g1, which is involved in ATP synthesis, and Psd95, which is involved in synaptic plasticity [11]. In cell culture, knockdown of MSUT2 results in decreased cellular ATP levels and mitochondrial fragmentation [9]. It is unclear whether this has a significant impact on neuronal function *in vivo*, but this suggests that MSUT2 inhibitors may be most beneficial as partial inhibitors, to normalize levels, rather than to fully block its activity.

Brain development and cognition: MSUT2 IS INVOLVED IN BRAIN CIRCUIT DEVELOPMENT

MSUT2 plays a role in brain development, such that the loss of MSUT2 during this period results in impaired cognition. The role it plays in the maintenance of cognitive function throughout adulthood is unclear, such that it is not known whether loss or inhibition of MSUT2 only in adulthood would impact cognition.

A mutation in MSUT2, 25-base pair deletion that is 16 base pairs downstream of the 3'-end boundary of the annotated common exon 16 of MSUT2 (ZC3H14) located on chromosome 14q31.3, is associated with a non-syndromic form of intellectual disability, identified in a cohort of 200 Iranian families [6].

The cognitive deficits seen in patients are similar to what is seen in MSUT2 mutant animal models. In mice, MSUT2 is expressed in hippocampal neurons, and the hippocampi of transgenic mice lacking MSUT2 (Zc3h14Δex13/Δex13) have altered steady-state levels of several proteins involved in proper synaptic function, including CaMK2α, and show a specific impairment in working memory [2]. These mice show impaired performance on the Y-maze, as well as impaired performance on the water radial arm maze, a measure of spatio-temporal working memory. Notably, these mice perform similar to wild type mice in terms of motor function, and other measures of cognition, such as the novel cage, light-dark box, and fear conditioning paradigms.

The Drosophila ortholog of MSUT2, dNab2, is important for the proper brain development in flies. Specifically, dNab2 is involved in proper axon projection and branching patterns in the Mushroom body, the region of the fly brain important for associative olfactory learning and memory [12]. Loss of dNab2 impairs short-term memory. Proteomic analysis revealed that the effect on axon growth was mediated by dNab2's regulation of a small pool of brain proteins involved in brain morphogenesis, neuroblast proliferation, circadian cycles, and synaptic development [10]. Some of the key proteins include the microtubule-binding protein Futsch, the neuronal Ig-family transmembrane protein turtle, the glial:neuron adhesion protein contactin, the Rac GTPase-activating protein tumbleweed, and the planar cell polarity factor Van Gogh.

Tau pathology: MSUT2 PROMOTES TAU PATHOLOGY

SUT2 was identified in a *C. elegans* screen for modifiers of tau pathology [3]. Overexpression of SUT2 promoted the accumulation of insoluble tau aggregates and degeneration of GABAergic neurons in tau transgenic worms (T337 Tg), while loss of SUT2 suppresses tau aggregation and tau-mediated neurodegeneration [13]. The effect is specific for tau, as SUT2 does not affect neurotoxicity associated with other aggregation prone proteins, such as poly-glutamine [3]. The protective effects are conserved in the mammalian form of SUT2, MSUT2, such that it shows protection from tau-mediated neurotoxicity in transgenic mice and human cells. In the PS19 tau overexpression model, deletion of MSUT2 was associated with better learning and memory performance on the Barnes maze, reduced neurodegeneration, and an 85% reduction in tau neurofibrillary tangles in the hippocampus [7]. Similarly, overexpression of MSUT2 in the Tau4Rtg2652 tau transgenic model increases tau pathology and a neuroinflammatory response to tau. It is currently unclear whether this neuroinflammatory

response is detrimental or adaptive. The overexpression of tau led to a two-fold increase in MSUT2 expression in HEK cells [13]. Knockdown of MSUT2 in cultured human cells overexpressing tau also reduces levels of insoluble tau and tau aggregation. It is hypothesized that high levels of MSUT2 make neurons more vulnerable to tau.

The mechanism is not clear, but may involve the modulation of microtubule dynamics [14]. MSUT2 interacts with HOOK2, which links subcellular structures to the microtubule cytoskeleton [3]. Together they play a role in the formation of aggresomes, which form when the ubiquitin proteasome and chaperone systems are overwhelmed or disrupted. Aggresomes contain misfolded proteins and related chaperones, which are transported via microtubules to cellular sites where they can be degraded by autophagy. Due to its role in the stabilization of microtubules, mutant tau may impair axonal transport needed for aggresome degradation [1]. While the sequestration of misfolded proteins could be a protective mechanism, it becomes deleterious in the specific case of tau. As levels of free non-microtubule-associated tau increase, MSUT2 promotes its aggregation, however, due to the impairment of microtubule dynamics, this aggregated tau cannot be cleared, and may instead seed further tau accumulation. In addition to promoting tau aggregation, altered expression of MSUT2 may promote neuronal degeneration through a disruption in RNA processing.

Alzheimer's disease: MSUT2 IS DEPLETED IN TAU-AFFECTED REGIONS AT LATE-STAGES

Postmortem brain tissue studies have found that MSUT2 is depleted in areas of the brain with high tau pathology in AD patients [13]. It has been proposed that as seen in cell culture, tau may promote the expression of MSUT2, and then high MSUT2 increases vulnerability to tau-related neurodegeneration. Therefore, the decrease in MSUT2 levels in affected areas is due to extensive neuronal loss, rather than a decline in MSUT2 at the cellular level. Depletion of MSUT2 correlated with loss of a related, interacting RNA-binding protein involved in the regulation of poly(A) length, PABPN1, suggestive of an overall dysregulation of RNA processing in regions with high levels of neurodegeneration [7]. Those with lower MSUT2 had an earlier age of onset and more advanced neuronal loss at the time of analysis. Similar to what was seen in rodent models, individuals with higher levels of MSUT2 also had higher levels of neuroinflammation. A separate study found that the nuclear speckle localized protein SRRM2, which is involved in mRNA splicing, is mis-localized from the nucleus to the cytoplasm with the progression of tauopathy in AD [15]. In the frontal cortex, nuclear SRRM2 was seen in cases with intact MSUT2 levels and less tauopathy, while it was cytoplasmic in more severe cases where MSUT2 was depleted. This suggests that there is an overall disruption to RNA processing activities with increasing tau accumulation, and this loss of proper gene regulation may be a key driver of neurodegeneration. Thus

far, there is evidence to show that tau leads to a dysregulation of MSUT2, but there is a lack of direct human data supporting a role for MSUT2 as a driver of tau pathology.

An analysis of MSUT2 levels over the course of the disease is needed to understand the appropriate window to intervene. If, as predicted by the preclinical data thus far, that tau promotes the overexpression of MSUT2, then a MSUT2 inhibitor may be beneficial once tau can be detected. But it is unclear whether an inhibitor would be useful once significant MSUT2 depletion has already occurred. Since MSUT2 appears to play an important role in the maintenance of synaptic function, it would likely be disadvantageous to inhibit it when levels are normal, and when tau was not present. Specific MSUT2 inhibitors are not yet available. Some drugs that inhibit MSUT2 have been identified in a repurposing screen, including duloxetine, saquinavir, and clofazimine, but none have therapeutic potential as MSUT2 inhibitors [16]. Similarly, 4,4'-diisothiocyanostilbene-2,2'-sulfonic acid (DIDS) was identified in a high-throughput screen, and may be a useful tool compound, however, its low specificity for MSUT2 relative to other RNA binding proteins limits its utility [4].

Neuroprotection: MSUT2 MAY PLAY A ROLE IN CELL SURVIAL MECHANISMS

MSUT2 may play a role in non-cell autonomous astrocyte-mediated neuroprotection [17]. Astrocyte-conditioned media has neuroprotective properties in cell culture. PI3K signaling and platelet-derived growth factor (PDGF) are critical mediators of this neuroprotection. It was found that PDGF-induced neuroprotection requires the recruitment of MSUT2 within the neurons. The THO complex was also implicated in this processing. Since the interaction between MSUT2 and THO is important for proper RNA processing, it suggests that MSUT2 regulates transcripts important for neuronal cell survival programs.

APOE4 interactions: Not established

Aging and related health concerns: MSUT2 expression may be a prognostic biomarker for hepatocellular carcinoma. It is not known whether MSUT2 modulation affects cancer risk or any other aging-related disease.

Types of evidence:

- 1 laboratory study

Hepatocellular carcinoma: LOSS OF MSUT2 ASSOCIATED WITH WORSE PROGNOSIS

MSUT2 (Z3CH14) was identified as the cancer-associated gene in the genomic copy number deletion at chromosome14q31.1–32.13, which is commonly found in hepatocellular carcinoma [18]. MSUT2 is downregulated in primary carcinoma tissue, and this downregulation was found to be associated with worse outcomes (n=274). Having low MSUT2 was associated with lower overall survival (Hazard Ratio [HR] 1.82, P = 0.041) and disease-free survival (HR = 1.77, P = 0.048). A similar association was seen in an independent cohort. The downregulation of MSUT2 suppressed cell growth and metastasis in preclinical models, which was due to inhibition of integrin signaling. In the context of hepatocellular carcinoma, MSUT2 appears to function as a tumor suppressor.

Safety: There is no safety data regarding MSUT2 inhibitors. There is a theoretical risk for cancer and a potential impact on cognition. Side effect profile would be influenced by the specificity of the inhibitor for MSUT2.

Types of evidence:

- Several laboratory studies on the function of MSUT2 in animal models and human tissue

The development of MSUT2 inhibitors is only in the discovery phase [4]. The phenotypes in mouse knockouts of MSUT2 are mild and appear to be developmental in origin [2]. It is unclear whether loss of MSUT2 later in life would have a similar impact on cognitive function. Due to its context-dependent actions, inhibition of MSUT2 in individuals with tau pathology may be beneficial and well-tolerated, while those without tau pathology may experience adverse effects.

The side effect profile of any MSUT inhibitor is likely to be a function of its specificity for MSUT2. While targeting MSUT2 alone may be relatively safe, simultaneous inhibition of other related RNA binding proteins is likely to increase the risk for adverse effects.

Based on a possible role as a tumor suppressor in hepatocellular carcinoma, MSUT2 inhibition could theoretically increase the risk for cancer [18].

Drug interactions: Unknown

Sources and dosing:

MSUT2 inhibitors are not available, though 4,4'-diisothiocyanostilbene-2,2'-sulfonic acid (DIDS) has been identified as a tool compound that can be used for basic science research [4]. Although nontoxic, its use is limited by its low specificity for MSUT2 (IC_{50} = 5.346 µM) relative to the related RNA binding protein PABPN1 (IC_{50} = 11.27 µM).

Research underway:

There are high-throughput screens and optimization studies underway to develop specific MSUT2 inhibitors with appropriate drug-like properties, though they are very early stage. Identifying inhibitors that are specific for MSUT2 relative to other related RNA binding proteins may be a challenge [4].

Search terms:

Pubmed, Google: MSUT2; ZC3H14, Nab2

- Alzheimer's disease, tau, brain, inhibitor, aging, cancer

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