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.

L3MBTL1 Inhibitors

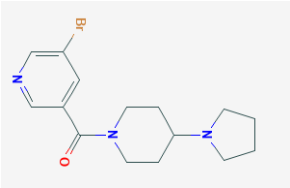
Evidence Summary

L3MBTL1 promotes transcriptional repression, and tumor suppression pathways. L3MBTL1 inhibition influences hematopoiesis, but effects are dependent on the epigenetic status of a given cell.

Neuroprotective Benefit: L3MBTL1 inhibition may promote proteasomal activation through modulation of p53, but could produce heterogenous effects in patients depending on disease-related changes to chromatin state.

Aging and related health concerns: L3MBTL1 may act as tumor suppressor; loss of expression is associated with myeloproliferative disorders and worse prognosis in breast cancer. May suppress p53 mediated cellular senescence.

Safety: L3MBTL1 inhibition may increase cancer risk, but the spectrum of potential side effects is not known since its targets have not been fully characterized.

Availability: Research use (tool compounds)	**Dose**: N/A	L3MBTL1 Antagonist UNC669 **Chemical formula:** $C_{15}H_{20}BrN_3O$
Half-life: N/A	**BBB**: N/A (UNC669 penetrant)	**MW**: 338.24 g/mol
Clinical trials: None	**Observational studies**: None for L3BMTL1 inhibitors. L3MBTL1 expression is increased following exercise and higher expression is associated with better survival in breast cancer.	 Source: PubChem

What is it?

Lethal(3)malignant brain tumor-like protein, L3MBTL1, belongs to the MBT domain containing family of methyl lysine readers, which is the least characterized class of chromatin code readers. It acts as a chromatin reader by binding mono and di-methylated lysines on histones, primarily histone H4 at lysine 20 (H4K20) and histone H1b at lysine 26 (H1bK26) [1]. In this role it acts as a chromatin compaction factor by compacting nucleosomal arrays through its MBT domains. This compaction makes the genes associated with that region of chromatin inaccessible for transcription machinery, and thus acts to repress gene expression. L3MBTL1 also binds to mono and di-methylated lysines on non-histone proteins, primarily transcription factors, and can also regulate gene expression in this manner [2]. While primarily a transcriptional repressor, its effects are context and cell type dependent, and associated with transcriptional activation under certain conditions [3]. In humans l3mbtl1 is an imprinted gene, where the paternal allele is active, and the maternal allele is epigenetically silenced.

Neuroprotective Benefit: L3MBTL1 inhibition may promote proteasomal activation through modulation of p53, but could produce heterogenous effects in patients depending on disease-related changes to chromatin state.

Types of evidence:

- 3 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:

Synaptic Plasticity: A study in primary neuronal cultures and organotypic hippocampal slices in mice found that L3MBTL1 is involved in homeostatic scaling induced by high levels of excitatory synaptic transmission [3]. It was found to be the most highly downregulated chromatin regulatory gene in response to excitatory neuronal activity, as it was decreased to 30% of its baseline level in response to the blockade of inhibitory GABAergic neurotransmission. These effects were attributed to the activity dependent occupation of L3MBTL1 at the promoters for beta-catenin (Ctnnb1) and the GABA-A2 receptor subunit (Gabra2). L3MBTL1 appears to play a role in regulating the basal expression of these genes, and in the context of elevated activity, L3MBTL1 dissociates from the chromatin, which provides access for other transcriptional complexes to bind and regulate activity-dependent gene expression. These studies suggest that the ability of L3MBTL1 to be dynamically regulated may be important for synaptic plasticity. Consequently**, epigenetic changes including histone methylation states could negatively impact synaptic function** by disrupting the transcriptional regulation by chromatin readers such as L3MBTL1.

Transcriptional regulation: L3MBTL1 has primarily been studied in the context of hematopoietic cells and heterologous cell lines, however, in humans, it is most highly expressed in reproductive tissue and brain (Human Protein Atlas). A study assessing the occupancy of L3MBTL1 at gene promoters in mouse neurons, found that unlike previous studies showing that L3MBTL1 is primarily bound to regions with inactive chromatin marks, the L3MBTL1 in neurons was primarily bound at sites containing open chromatin [3]**. Chromatin readers may preferentially bind to different genomic regions in different cell types based on differences in the chromatin environment, thus they can have distinct cell type specific functions.** Therefore, studies done in other cell types may not be translatable to neurons, and the effect of modulating L3MBTL1 needs to be directly assessed in CNS cells. A study in mice found that loss of L3MBTL1 altered anxiety and depression-associated phenotypes, suggesting it may regulate genes involved in mood regulation [4]. However, caution should be warranted in extrapolating the function of L3MBTL1 in humans based on mouse studies because the L3MBTL1 gene is imprinted in humans, but not in mice [5].

Proteostasis: L3MBTL1 was identified as a negative regulator of proteasome mediated degradation of mutant aggregation prone SOD1 ($SOD1^{G85R}$) in a *C. elegans* screen [6]. Knockdown of L3MBTL1 promoted the clearance of mutant SOD1 and/or C9orf72 in Drosophila and mouse models, and in a human heterologous cell line. The enhancement of proteasomal activity associated with the loss of L3MBTL1 was attributed to the activation of p53 and the lysine methylation activity of the methyltransferase SET8. However, this association runs counter to previous work showing that lysine methylation of p53 (at K382) by SET8 represses p53 through the recruitment of L3MBTL1 [7]. Yet, the results of this study are consistent with a prior Drosophila study showing that the retention of lysine methylation on p53 (at K370) could promote the activation of both p53 and protein clearance pathways [8]. The discrepancy may be resolved by looking further into the multiple methylation marks that influence the binding of chromatin readers/transcriptional regulators on p53, as well as examining other proteostasis associated genes that are regulated by SET8 mediated methylation.

The underlying mechanism of enhanced proteasomal activity appears to stem from the activation of the DNA damage response pathway. RNA misprocessing and aggregated protein formation, such as those associated with neurodegenerative diseases, can induce cellular stress and lead to the activation of damage related pathways, including DNA double strand break (DSB) repair [9]. The ubiquitin E3 ligase, 53BP1, is important for DSB repair and the activation of p53 dependent transcription, and thus may be a critical intermediary [10]. The study in Drosophila found that the activation of p53 and protein clearance in response to changes in the lysine methylation pattern were related to increased binding of 53BP1 to p53 [8]. The competition of L3MBTL1 and 53BP1 for SET8 generated histone H4K20me sites on chromatin is important for the proper regulation of DSB repair mechanisms, and may provide the link to the regulation of proteostasis. L3MBTL1 binds to H4K30me under basal conditions, but in the context of DNA damage, it is removed by the ubiquitin-selective chaperone valosin-containing protein (VCP/p97), which then allows 53BP1 to bind and facilitate DSB repair [11]. VCP is known to be a critical mediator of protein homeostasis, and its inhibition leads to the accumulation of misfolded ubiquitinated proteins [12]. VCP also plays a role in the regulation of p53. Mutations in VCP lead to multisystem proteinopathies, including Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) [13]. Therefore, the presence or absence of L3MBTL1 may influence proteostasis by placing a brake on these repair pathways.

Frontotemporal dementia/Amyotrophic lateral sclerosis: Potential benefit (preclinical)

The L3MBTL1 inhibitor, UNC669, was found to be neuroprotective against proteotoxicity in the SOD^{G93A} ALS mouse model, and in mammalian neurons transfected with mutant $SOD1^{G85R}$ or C9orf72 [6]. However, UNC669 is not completely specific for L3MBTL1, as it also inhibits the related family member

L3MBTL3 [14]. L3MBTL1 levels were found to be increased in the spinal cords of ALS model mice and a subset of ALS/FTD patients [6]. Since **one of the functions of L3MBTL1 is to inhibit DNA damage response programs in the absence of damage**, levels of (chromatin bound) L3MBTL1 would be expected to decrease in response to the high levels of cellular stress experienced by ALS/FTD patients. Therefore, it is unclear whether the increased levels seen in patients represent a compensatory mechanism to reduce chronic overactivation of damage repair pathways, or if it stems from pathogenic changes to the chromatin environment. As a chromatin reader, the downstream effects of L3MBTL1 binding depend on the epigenetic state and availability of other chromatin regulators in a given cell. **Due to these context dependent effects, the modulation of L3MBTL1 would likely produce heterogenous outcomes in patients.**

APOE4 interactions: Unknown

Aging and related health concerns: L3MBTL1 may act as tumor suppressor; loss of expression is associated with myeloproliferative disorders and worse prognosis in breast cancer. May suppress p53 mediated cellular senescence.

Types of evidence:

- 1 meta-analysis (L3MBTL1 methylation following exercise)
- 1 clinical trial (L3MBTL1 expression in breast cancer)
- 2 observational studies (L3MBTL1 expression in 20q deletion myelodysplastic disorders)
- Several laboratory studies

Cancer: L3MBTL1 may act as a tumor suppressor

L3MBTL1 (l(3)mbt) was originally identified as a potential tumor suppressor in Drosophila, because its loss in flies led to the formation of brain tumors [15]. In this model system, the loss of l(3)mbt mediated transcriptional repression led to the ectopic reactivation of a broad range of germline associated genes [16]. The ectopic activation of germline genes is also common in human cancers, and associated with the ability of cells to acquire neoplastic characteristics [17]. This suggests the chromatin depression due to the **loss of L3MBTL1 may promote the malignant transformation of cells**.

Retinoblastoma protein (RB) is important for cell cycle control and is mutated or inactivated in a wide spectrum of cancers [18]. RB inhibits cell cycle progression from G1 to S phase by binding to and

repressing E2F family transcription factors at the promoters of genes involved in S phase progression. RB also plays a role in maintaining genomic stability, as it is recruited to double-strand breaks, and localizes to repetitive elements, such as LINES and SINES [19]. The activity of RB is controlled by various post-translational modifications, including methylation. L3MBTL1 can bind to RB that has been mono methylated at lysine 860 [20]. The **recruitment of L3MBTL1 has been shown to promote repression on at least a subset of RB/E2F bound promoters** [1; 20]. L3MBTL1 may also contribute to the tumor suppressor functions of RB in other ways, as the spectrum of RB containing complexes where L3MBTL1 can be recruited to enhance RB's repressive activity has not yet been fully characterized.

c-Myc is an oncogene that promotes cell cycle progression and cell proliferation. Its dysregulation toward a constitutively active state is a common feature in many cancers [21]. **c-Myc has been identified as one of the genes repressed by L3MBTL1** through histone methylation mediated chromatin compaction [1]. Loss of L3MBTL1 is associated with an increase in protein levels of c-Myc [1; 22], and it is hypothesized that L3MBTL1 may be decreased or absent from the c-Myc promoter in malignant cells due to changes in chromatin structure and/or histone methylation patterns.

L3MBTL1 plays a role in the maintenance of genomic stability by acting as a negative regulator of the DNA damage response. **The loss of L3MBTL1 induces replicative stress, DNA breaks, and activation of the DNA damage response in human cells *in vitro*** [23]**.** p53 is one of the key targets repressed by L3MBTL1 in the absence of DNA damage. L3MBTL1 binds to p53 that is mono methylated at lysine 382 by the methyltransferase SET8, and prevents it from activating its target genes in the absence of DNA damage [7]. Although p53 is typically understood to protect genomic integrity by inducing growth arrest and DNA repair programs in the context of cellular stress, there is evidence that its activity can also contribute to genomic instability if left unchecked, due to the disruption of positive regulatory feedback loops [24]. Therefore, the inhibition of L3MBTL1 may be beneficial in the context of p53 inactivation driven cancers, if the regulation of SET8 mediated p53 methylation is still intact, as well as in cancers overexpressing SET8. Due to the numerous mechanisms by which p53 is regulated *in vivo*, it is unclear whether inhibition of L3MBTL1 alone would significantly increase the risk for p53 overactivation mediated pathology.

Breast Cancer: Higher L3MBTL1 associated with better prognosis

The methylation of L3MBTL1 was found to be high in breast cancer tumor samples, and associated with lower levels of L3MBTL1 gene expression [25]. Relative to tumors with low expression**, high mRNA expression of L3MBTL1 was associated with a lower risk for mortality** (Hazard ratio: 0.37, 95% Confidence Interval 0.17 to 0.80, p=0.012). In a 6-month RCT testing the effect of moderate intensity

aerobic exercise (150 min/week) on breast cancer patient outcomes, a sub study found that exercise induced changes in the methylation pattern of a subset of genes in peripheral blood leukocytes, including L3MBTL1, were associated with patient survival [25]. The methylation of L3MBTL1 decreased in patients in response to exercise (-1.48% in exercise vs +2.15% control, difference 3.63%, $p=2.9 X10^{-5}$), leading to higher L3MBTL1 expression. This suggests that L3MBTL1 may be a relevant biomarker for breast cancer, with higher expression associated with better prognosis.

Exercise: L3MBTL1 activated by exercise in older adults

A bioinformatics meta-analysis assessing the regulation of imprinted genes in response to exercise found that in skeletal muscle, **the methylation of L3MBTL1 decreased in response to exercise in older adults** [26]**.** This suggests that exercise may alter the 'epigenetic age' of cells. Notably, the changes in gene methylation in muscle due to exercise differed depending on age. In young adults (<40), genes involved in muscle stem cell activity were activated, while in older adults, exercise primarily led to the de-repression of tumor suppressor genes regulated by the miR-519b network, such as L3MBTL1, that restrict cell growth. Therefore, the ability to promote muscle growth with age is compromised by mechanisms designed to prevent tumor formation.

Myelodysplastic disorders: Loss of L3MBTL1 may promote pathology

The l3mbtl1 gene is located on the long arm of chromosome 20 (20q12), in the region commonly deleted in patients with myelodysplastic disorders [27]. Whether or not patients with the 20q deletion will lose expression of L3MBTL1 depends on whether the paternal or maternal allele is lost. Expression is expected to be lost with loss of the active paternal allele, but maintained if the silenced maternal allele is lost. One study found that loss of the paternal allele abrogated expression of L3MBTL1 [28], while an earlier study found evidence of possible epigenetic reactivation (demethylation) of the remaining maternal allele in some patients [27].

L3MBTL1 is expressed in CD34+ hematopoietic progenitor cells, and **plays a role in the differentiation of erythroid cells.** Cell culture experiments have indicated that the loss of L3MBTL1 promotes the production of erythroid cells, and that the downregulation of L3MBTL1 is a necessary step in the process of erythrocyte differentiation [8; 28]. The 20q deletion is the second most common primary chromosomal abnormality in hematological malignancies, and is most commonly found in patients with polycythemia vera, which involves the excess production of erythrocytes (red blood cells) [27; 28]. This evidence supports the hypothesis that the loss of expression of L3MBTL1 plays an active role in driving hematological malignancies. However, it has been difficult to confirm the importance of L3MBTL1

outside of the 20q deletion, since L3MBTL1 is associated with at least 15 different putative transcripts and normal transcript levels can vary as much as 6-fold between healthy individuals [27]. There is also evidence that the effects of losing L3MBTL1 expression are cellular context dependent, such that promotion of certain hematopoietic cell fates can be influence by the presence or absence of other genes found in the 20q region, such as SGK2 [28].

Therefore, promoting the (re)activation of L3MBTL1 could potentially be therapeutically beneficial in restoring hematological balance in a subset of patients with myelodysplastic disorders, but benefits may also require the simultaneous modulation of other affected genes/pathways.

Senescence: L3MBTL1 may inhibit p53 driven cellular aging

L3MBTL1 may play a role in inhibiting cellular senescence through its repression of SET8 methylated targets, including p53, p21, and p16 [29; 30]. SET8 is the methyltransferase responsible for monomethylating histone H4 at lysine 20 (H4K20 me), and the p21 and p16 genes are enriched for this methylation mark [29]. p53 is directly methylated by SET8 [7]. Therefore, the methylation activity of SET8 acts to maintain an epigenetic state unconducive to the induction of senescence programs [30]. Since L3MBTL1 is involved in the repression of SET8 methylated p53, and promotes chromatin compaction in regions containing H4K20me, it may play a role in promoting the repression of these senescence associated programs under basal conditions. However, the potential link between L3MBTL1 and senescence has not yet been experimentally validated, so it is unknown whether it significantly contributes to this process *in vivo*.

Safety: L3MBTL1 inhibition may increase cancer risk, but the spectrum of potential side effects is not known since its targets have not been fully characterized.

Types of evidence:

- Several laboratory studies

L3MBTL1 inhibitors are primarily used as research tool compounds for assessing the function of chromatin readers containing MBT domains, and the information about their potential safety is extremely limited [31]. No safety concerns were noted in a study using the L3MBTL1/L3MBT3 inhibitor, UNC669, in a mouse model of ALS, however, this study only treated mice for up to 7 weeks, and this study was not designed to assess safety [6]. **The long-term effects of L3MBTL1 inhibition are unknown,**

and will likely vary in different organ systems due to differences in the epigenetic state of the cells in different tissues.

The primary safety concern for L3MBTL1 inhibition is increased risk for malignancies, based on its proposed tumor suppressor activity. Additionally, the loss of L3MBTL1 is associated with myelodysplastic disorders [28] and worse prognosis in breast cancer [25]. The inhibition of L3MBTL1 could also potentially alter the differentiation of hematopoietic cells [8]. Since the targets of L3MBTL1 have not been fully characterized, there may be many additionally cell-specific side effects to L3MBTL1 modulation.

Sources and dosing:

L3MBTL1 tool compound inhibitors are available for research use from commercial suppliers, but there are no L3MBTL1 modulators available for clinical use.

Research underway:

L3MBTL1 inhibitors are primarily used for research projects aimed at better understanding the targets and function of the MBT domain-containing class of methyl lysine chromatin readers. It is not known whether L3MBTL1 modulators will be developed for any therapeutic indications.

Search terms:

Pubmed, Google: L3MBTL1

- dementia, cognitive decline, proteostatsis, aging, cancer, epigenetics, DNA repair, hematopoeisis, imprinting, safety

Websites visited for L3MBTL1:

- PubChem UNC669
- Human Protein Atlas

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