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.

LINE1 inhibition (with nucleoside reverse transcriptase inhibitors)

Evidence Summary
L1 activation may be associated with age-related diseases, and nucleoside reverse transcriptase inhibitors (NRTIs) suppress L1 activation and may be beneficial in age-related diseases.

**Neuroprotective Benefit:** Increased retrotransposition of transposable elements is implicated in neurodegenerative diseases, so NRTIs could theoretically reverse these actions, but only preclinical evidence exists to date.

**Aging and related health concerns:** Preclinical studies suggest that NRTIs may be an effective anti-aging therapy, and clinical studies suggest they may be effective for cancer.

**Safety:** NRTIs are associated with some mild side effects and rare severe, possibly life-threatening, side effects.
**What is it?**

Retrotransposons are stretches of DNA that can replicate and reinsert into other parts of the genome. Their presence in the DNA comes from retroviral infection of germline cells throughout evolutionary history. Although their presence is often detrimental, sometimes retrotransposons can benefit the host by modifying gene-regulatory networks and creating phenotypic heterogeneity (Kassiotis and Stoye, 2016). For instance, somatic retrotransposition is enriched in the human hippocampus, and may be related to neural plasticity or diversity (Baillie et al, 2011). Normally, cells epigenetically suppress retrotransposons, especially in germline cells and in the embryo. However, these epigenetic mechanisms become less efficient with age, and retrotransposon reactivation is thought to contribute to neurodegenerative diseases and cancer.

There are three active families of retrotransposons, L1, Alu, and SVA. Long interspersed nuclear element 1 (LINE1 or L1 – 6kb) is a retrotransposon ubiquitous in mammalian genomes and comprises about 20% of the genomic DNA in humans. Most L1s are unable to retrotransposition due to a 5’ truncation. However, there are an estimated 80-100 retrotransposition-competent L1s with about 10% classified as highly active. L1 is not only able to replicate itself but can replicate other retroelements that use L1-encoded proteins necessary for retrotransposition (Simon et al, 2019; Muotri et al, 2005). L1 is a non-long-term repeat (non-LTR) retrotransposon that produces two proteins, ORF1p, an RNA binding protein, and ORF2p, an endonuclease with reverse transcriptase activity. Short interspersed nuclear elements (SINEs) are another group of non-LTR retrotransposons that lack the machinery for retrotransposition and require the L1 retrotransposition machinery (Kassiotis and Stoye, 2016).

Increased retrotransposition of transposable elements (TEs) have been implicated in several neurodegenerative diseases including Alzheimer’s, ALS, Rett’s syndrome, frontotemporal dementia (FTD), prion disease, age-related macular degeneration, and fragile X (Li et al, 2012). Nucleoside reverse transcriptase inhibitors (NRTIs), antiretroviral HIV drugs, have been used to suppress retrotransposition in preclinical studies. They function by incorporating nucleoside analogs into the transcription machinery.
and blocking transcription. One potential concern with NRTIs is genotoxicity if they are incorporated into the DNA, and this is a dose-limited toxicity (Olivero, 2007). However, HIV patients have been on them chronically for many years. HIV patients do have a greater risk for cognitive decline, but this is thought to be due to HIV infection of the brain. It is unrelated to Alzheimer’s.

**Neuroprotective benefit:** Increased retrotransposition of transposable elements is implicated in neurodegenerative diseases, so NRTIs could theoretically reverse these actions, but only preclinical evidence exists to date.

**Types of evidence:**
- Five postmortem studies
- Five preclinical 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:**
In post-mortem tissue from patients with Alzheimer’s disease or progressive supranuclear palsy (PSP), Sun et al (2018) reported an upregulation of endogenous retroviruses and a down regulation of non-LTR retrotransposons. Transposable element expression profiles were more similar between patients with Alzheimer’s disease and PSP than between diseased patients and controls.

Tau pathology is associated with global chromatin reorganization and dysregulated gene expression, and Guo et al (2018) hypothesized that chromatin relaxation due to tau pathology may de-repress silenced transposable elements. In postmortem tissue from 636 individuals, they reported increased transcriptional activation from several L1s, SINEs, and endogenous retroviruses (ERVs). These were correlated with neurofibrillary tangle pathology but less so (or non-significantly) with amyloid pathology.

HIV may be associated with cognitive impairment. Cooley et al (2018) reported that tau PET imaging was not elevated in HIV-negative patients without Alzheimer’s, HIV patients without cognitive impairment, or HIV patients with cognitive impairment. However, tau PET imaging was elevated in HIV-negative patients with Alzheimer’s disease, suggesting that cognitive impairment in HIV patients is mechanistically different.
Hernandez et al (2014) reported no difference in L1 methylation in blood samples from patients with Alzheimer’s disease. Likewise, Protasova et al (2017) reported no increase in active L1 repeats in brain samples from patients with Alzheimer’s disease.

TAR DNA-binding protein 43 (TDP-43)
TDP-43 binds to DNA and RNA and is involved in transcriptional repression. Aggregates of TDP-43 are present in patients with frontotemporal dementia (FTD), ALS, and some with Alzheimer’s. Li et al (2012) reported a significant enrichment of TDP-43 binding to LINE’s and SINE’s. In patients with FTD, they found the association of TDP-43 to its transposable elements is reduced, suggesting that TDP-43 pathology may be associated with a loss of transposable element regulation.

Mechanisms of action for neuroprotection identified from laboratory and clinical research
In a Drosophila tau transgenic model, Guo et al (2018) reported increased transcriptional expression of several transposable elements that also increased with age. Sun et al (2018) reported aberrant expression of transposable elements (50 upregulated, 60 downregulated) in a Drosophila tau transgenic model at day 10. The most common transposable elements that were differentially expressed were class 1 long terminal repeat (LTR) retrotransposons. In another transgenic Drosophila model that increases transposable element mobilization (flamenco), there was an increase in neuronal death in tau transgenic Drosophila. There was also a reduction of piRNAs (a group of regulatory RNAs that inhibit transposon activity) in tau Drosophila and a decondensation of heterochromatin. Overexpression of piwi (a regulatory protein of piRNA biogenesis) also reduced neuronal death. Finally, treatment with lamivudine (a nucleoside reverse transcriptase inhibitor - NRTI) reduced transposable element mobilization and neuronal death. In vitro, stavudine, another NRTI, was reported to reduce NLRP3 assembly but had no effect on the release of IL-1β (La Rosa et al, 2019). These studies suggest that increased retrotransposon activity may increase neurodegeneration.

Amyotrophic lateral sclerosis (ALS)
The expression of human endogenous retrovirus was higher in postmortem tissue from patients with ALS than those with chronic systemic illness, Parkinson’s disease, or those that died in accidents. The highest expression was found in the prefrontal and sensory cortices and was especially prevalent in neurons (Douville et al, 2011). In an open-label study, 40 patients with ALS were treated with Triumeq (abacavir, lamivudine, and dolutegravir) for 24-weeks after a 10-week lead-in period. Patients declined 32% less on the ALSFRS-R (ALS-functional rating score) than would be expected from the lead-in period. There was less decline on other functional outcome measures than would be expected. There was also a
reduction in transcription levels of human endogenous retroviruses. There were no changes in neurofilament light (Gold et al, 2019).

**APOE4 Interactions:**

Bollati et al (2013) reported no association between ApoE status and DNA methylation at L1 or Alu sites.

**Aging and related health concerns:** Preclinical studies suggest that NRTIs may be an effective anti-aging therapy, and clinical studies suggest they may be effective for cancer.

**Types of evidence:**

- Three systematic reviews and meta-analyses on cancer
- One clinical study on the effects of exercise
- Three studies of L1 hypomethylation in the blood
- Five preclinical lifespan studies
- One preclinical study on the effects of exercise
- One preclinical study on inflammation

**Lifespan**

There are mixed results regarding L1 hypomethylation in blood samples from aged individuals which could be due to methodological differences (Bollati et al, 2009; Protasova et al, 2017). Erichsen et al (2018) reported evidence of L1 hypomethylation in cell free DNA, but not cellular DNA, from aged patients.

Roberson et al (2019) reported no difference in L1 or ORF1 DNA in muscle biopsies between older or younger individuals. ORF1 mRNA was higher in older individuals. One hour of cycling (at 70% of max heart rate) reduced the levels of ORF1 mRNA (but not L1 mRNA) 2 hours post-exercise in both groups. Accelerometry data, measured two weeks prior to the study, suggested that there was an inverse correlation between activity levels and Orf1 mRNA. Preclinical studies also suggested that five months of wheel exercise in rats reduced L1 mRNA in skeletal tissue (Romero et al, 2019).

SIRT6 knock out (SIRT6-KO) mice have a shortened lifespan and growth retardation. *In vitro*, cells from SIRT6-KO animals have high levels of L1 retrotransposition events, increased L1 DNA copy numbers, and high levels of DNA damage compared to wild type cells. These phenotypes are repressed by treatment with NRTIs (lamivudine and stavudine), drugs which can inhibit L1 retrotransposition. Similarly, in SIRT6-
KO mice NRTI treatment reduces the level of genomic L1 DNA and cytoplasmic L1 DNA in multiple organs (though not significantly in the brain). Old, non-transgenic mice also have elevated cytoplasmic L1 at levels similar to SIRT6-KO mice in multiple organs (though in the brain levels are only slightly significantly elevated compared to young mice, and not nearly as elevated as SIRT6-KO mice). SIRT6-KO mice also have elevated IFN-α and IFN-β. This elevation is repressed with NRTI treatment. Finally, NRTI treatment extended lifespan in SIRT6-KO mice and reverses several age-related phenotypes (body weight, mobility, behavior, bone density, muscle mass, muscle fiber thickness, apoptosis in the thymus and spleen, and a reduction of sterile inflammation and SASP proteins). However, NRTI treatment did not completely reverse aging, as treated mice still died by 60 days of age (Simon et al, 2019).

Furthermore, in vitro studies suggested that SIRT6 binds to the 5’-UTR of the L1 locus. It interacts with KAP1 and facilitates KAP1 interaction with the heterochromatin factor, HP1α, packaging L1 elements into heterochromatin and suppressing transcription. In vitro, SIRT6 is depleted from the L1 loci in old or senescent cells, thereby derepressing L1 elements (Van Meter et al, 2014). Another study suggested that during cellular senescence L1 is depressed and accumulates in the cytoplasm, triggering a type-1 interferon (IFN-1) response. IFN-1 contributes to the maintenance of the senescence-associated secretory phenotype (SASP), and treatment with lamivudine repressed the L1 response (but not expression) and downregulated inflammaging in vivo. In aged human dermal fibroblasts, senescent cells were increased and 10.3% of them had evidence of L1 activation. Notably, none of the non-senescent cells showed evidence of L1 activation (De Cecco et al, 2019).

Li et al (2012) reported increased transcription of several transposable elements with age in Drosophila including R2 and R1, LINE-like elements, and gypsy, an LTR element. In Drosophila, Ago2 is involved in the suppression of transposable elements. Drosophila Ago2 mutants had elevated expression of transposable elements, impaired memory, and shortened lifespan. Dietary restriction increased Drosophila Ago2 lifespan and reduced the age-related increase in transposable elements. Drosophila Dicer-2 mutants show an increase in double-stranded breaks. Treatment with lamivudine also increased lifespan (Wood et al, 2016).

Inflammation
Fowler et al (2014) reported that Alus can activate P2X7 and the NLRP3 inflammasome. In vitro administration or in vivo treatment with NRTIs in mouse models of P2X7 driven diseases inhibited P2X7-mediated NLRP3 inflammasome activation and reduced levels of several inflammatory cytokines.
Cancer

HIV drugs have long been examined for their potential use as anti-cancer therapies after it was shown that highly active retroviral therapies (HAART) could reduce the risk of HIV-related Kaposi’s sarcoma (Chow et al, 2009). L1 insertions may be a cause of cancer as they have been reported to interrupt tumor suppressor genes, and cancer cells could have increased L1 activity. For instance, a meta-analysis of six studies of patients with chronic hepatitis C found that patients taking lamivudine had a reduced risk of developing hepatocellular carcinoma (HCC) versus those taking no therapy (OR = 0.48; 95%CI 0.38 – 0.61) (a caveat, Hepatitis C affects the liver) (Singal et al, 2013). A meta-analysis of 254 patients with acute T-cell leukemia/lymphoma (ATL) showed that those who received a first-line therapy of zidovudine and interferon-alpha had an improved 5-year survival (46%) than those who received first-line chemotherapy (20%) or those who received first-line chemotherapy followed by zidovudine and interferon-alpha as a maintenance dose (12%). These benefits were seen for those with acute, chronic, and smoldering ATL but not for patients with ATL lymphoma (where chemotherapy was better) (Bazarbachi et al, 2010).

Safety: NRTIs are associated with some mild side effects and rare severe, possibly life-threatening, side effects.

Types of evidence:
- 7 reviews
- 2 package inserts

There are several side effects associated with NRTIs. However, it should be noted that these drugs are usually taken in combination with other drugs.

The most common side effects associated with stavudine include nausea, diarrhea, headache, rash, vomiting, and neuropathy. In one trial, some patients developed pancreatitis (0.7%). In post-marketing analyses, cases of severe side effects included lactic acidosis, hepatic toxicity, neurologic symptoms, pancreatitis, immune reconstitution syndrome, and lipodystrophy (package insert).

The most common side effects associated with lamivudine include headache, nausea, malaise, fatigue, nasal symptoms, diarrhea, and cough. In clinical trials, some patients developed pancreatitis (0.3%). Similar to stavudine, cases of lactic acidosis, hepatic toxicity, pancreatitis, immune reconstitution syndrome, and fat redistribution have been reported (package insert).
NRTIs have also been associated with cases of myopathy and neuropathy. For instance, in clinical trials lamivudine was associated with elevated creatine kinase in 9% of patients (vs 5% in placebo) (Fleischer and Lok, 2009). In clinical trials of stavudine, peripheral neuropathy was a dose-limiting toxicity (Hill et al, 2007). Some NRTIs, such as first-generation NRTIs stavudine and zidovudine, also show an increased risk of insulin resistance, diabetes, dyslipidemia, and cardiovascular complications while others, such as tenofovir and later generation NRTIs, do not (Das, 2011; Bergersen, 2006; Lagathu et al, 2019). It is thought that NRTIs increase the nuclear localization of SREBP-1, which is involved in the expression of genes that regulate lipid synthesis (Pedro et al, 2018).

The safety profile of chronic treatment with an NRTI will be based on the specific dose. See the following for package inserts: zidovudine, didanosine, zalcitabine, abacavir, emtricitabine, entecavir.

**Drug interactions:** Stavudine is unlikely to interact with other drugs metabolized by cytochrome P450 enzymes. Potential drug interactions include zidovudine, doxorubicin, and ribavirin. It should not be used in pregnant women (package insert).

Other drug interactions can be found in the corresponding package inserts above.

**Sources and dosing:** NRTIs are available with a prescription (as generics). Dosing depends on the NRTI.

**Research underway:** There are 45 ongoing trials with NRTIs (link). Almost all studies are in HIV patients. There is one ongoing study in patients with ALS who have high levels of HERV-K in the blood (NCT02437110).

**Search terms:**
- antiretroviral therapy + Alzheimer
- retrotransposon + cardiovascular, aging
- nucleoside reverse transcriptase inhibitor [review, meta-analysis] + cancer, neuropathy, cardiovascular, diabetes, Alzheimer, ApoE
- HIV + Alzheimer

**Websites visited:**
- Clinicaltrials.gov
- Pubmed
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If you have suggestions for drugs, drugs-in-development, supplements, nutraceuticals, or food/drink with neuroprotective properties that warrant in-depth reviews by ADDeF’s Aging and Alzheimer’s Prevention Program, please contact [INFO@alzdiscovery.org](mailto:INFO@alzdiscovery.org). To view our official ratings, visit [Cognitive Vitality’s Rating page](#).