Exploring the therapeutic potential of LINE-1 RT inhibitors in Parkinson's disease

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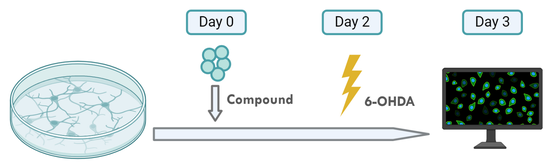
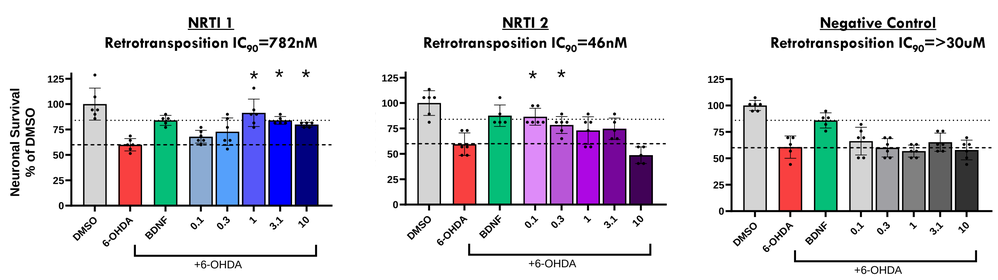
Abstract

Aging is a major risk factor for Parkinson's disease (PD), and increased LINE-1 expression is associated with aging, which may contribute to neuroinflammation and neurodegeneration. Furthermore, increases in LINE-1 insertion polymorphisms correlate with PD progression in the Parkinson's Progression Marker Initiative (PPMI) data set. Additionally, retrospective analysis has shown lower incidence of neurodegenerative disorders, such as PD, in HIV patients treated with nucleoside anti-retroviral therapies, which are known to be weak LINE-1 reverse transcriptase (RT) inhibitors. Using ROME's proprietary nucleoside compound library, we identified selective and potent LINE-1 RT inhibitors having excellent oral bioavailability, tolerability, and brain penetrance in rodents. We assessed the activity of LINE-1 RT inhibitors in mouse primary neurons injured with 6-hydroxydopamine (6-OHDA). We observed a protective effect of treatment on TH+ neuron survival in this model, as well as reduced α-synuclein aggregation, a hallmark of PD. Additionally, upon 6-OHDA stress, Orf1p expression was increased in TH+ neurons. Treatment with LINE-1 RT inhibitors reduced Orf1p upregulation, which was associated with the protective effect. Translation of these cellular results in an *in vivo* model of acute 6-OHDA injury will be informative for demonstrating CNS target engagement, potentially enabling long-term efficacy studies in an α-synuclein aggregation disease model. Preclinical validation of LINE-1 RT inhibition may indicate potential for LINE-1 RT inhibitors as a novel and differentiated therapy in PD.

Neuroprotective effects are observed with LINE-1 RT inhibitors, and correlate with potency against LINE-1 RT, in an acute injury (6-OHDA) *in vitro* model of Parkinson's disease

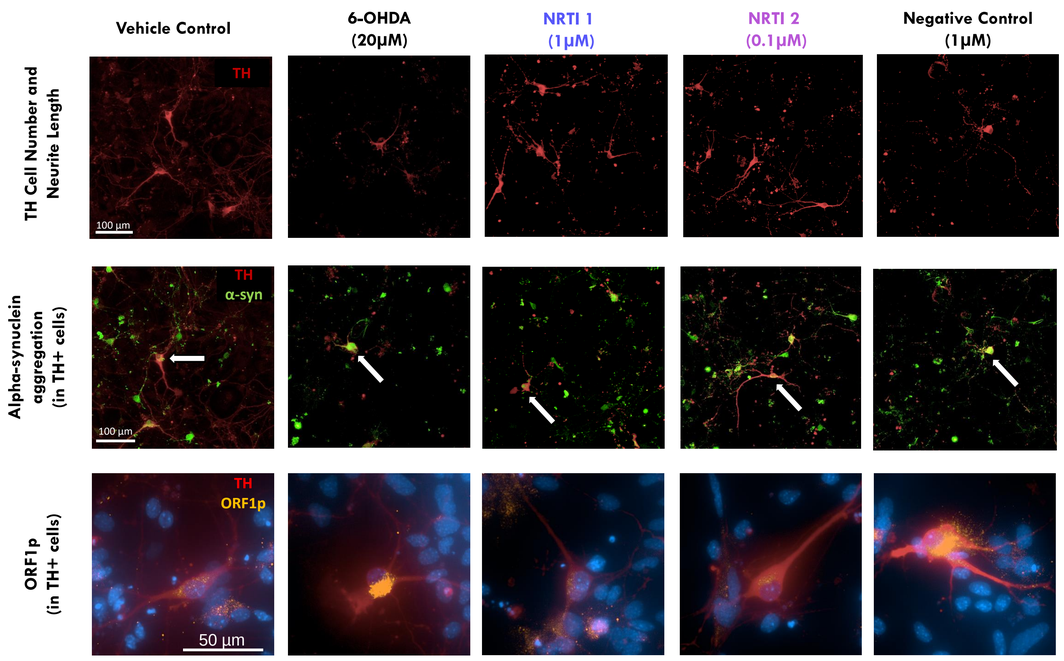




* p<0.05, one-way ANOVA, Dunnett's test

BDNF-Brain derived neurotrophic factor (positive control)

Reduction of alpha-synuclein aggregation and Orf1p upregulation in 6-OHDA injured neurons correlates with protective effects of LINE-1 RT inhibitors

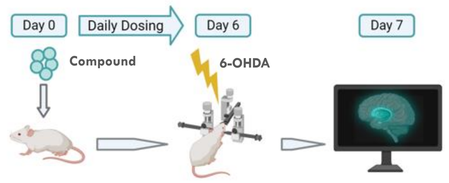


Select LINE-1 RT inhibitors are CNS penetrant and demonstrate good tolerability over 14 days

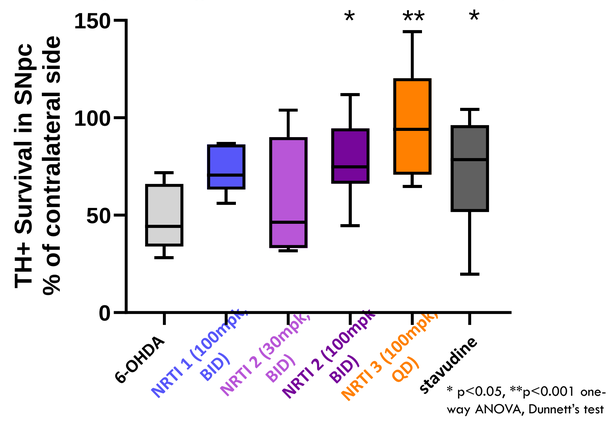
Compound	LINE-1 retrotransposition assay (IC_{90}, μM)	Dose (mg/kg)	Mean Plasma Exposure (μM) @ 4.5 h post last dose	Mean CSF Exposure (μM) @ 4.5 h post last dose	Mean Brain Exposure (μM) @ 4.5 h post last dose
NRTI 1*	0.782	100	1.9	0.653	0.575
NRTI 2*	0.046	100	6.2	0.329	0.551
NRTI 3	0.073	100	20.6	5.3	6.9
NRTI 4	0.016	100	2.64	0.326	0.520
NRTI 5	0.064	100	4.4	0.590	0.679

* Single day dosing

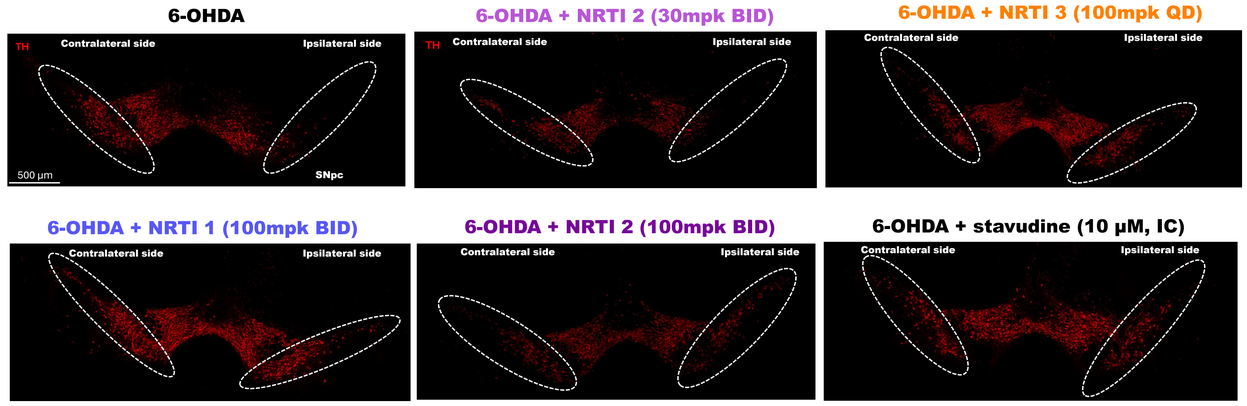
Orally bioavailable LINE-1 RT inhibitors can prevent loss of dopaminergic neurons from 6-OHDA injury *in vivo*



- 6-OHDA is injected on the ipsilateral side near the substantia nigra to induce injury, and brain samples are collected 24hr later for immunofluorescent analysis.
- TH cell survival is calculated as % surviving compared to contralateral (non-injected) side.

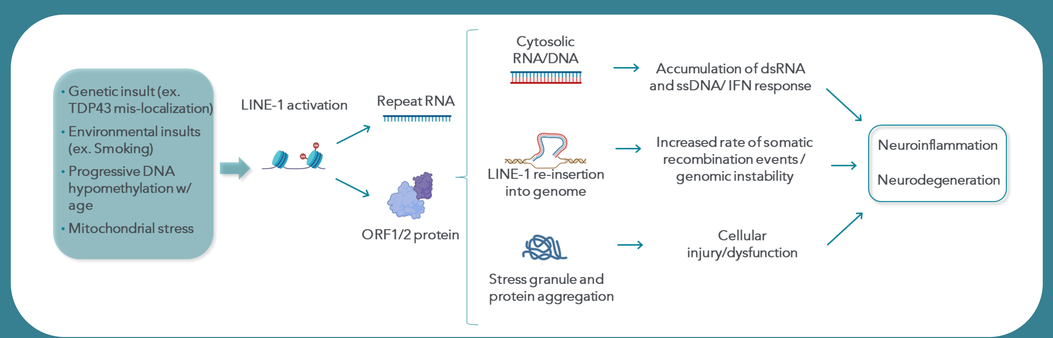


* p<0.05, **p<0.001 one-way ANOVA, Dunnett's test



Conclusions

- ROME has identified potent and brain penetrant LINE-1 RT inhibitors that are well-tolerated
- LINE-1 RT inhibition is neuro-protective in an *in vitro* acute model of Parkinson's disease
- Orally bioavailable LINE-1 RT inhibitors can prevent loss of dopaminergic neurons from 6-OHDA injury *in vivo*
- Follow on experiments include testing LINE-1 RT inhibitors in α-synuclein aggregation efficacy models and translating results into human cell-based models for target engagement
- These results support the working model of LINE-1 involvement in multiple pathogenic drivers of neurodegenerative disease



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