Widespread and Durable Knockdown of HTT in Non-Human Primate Brain by a Novel Oligonucleotide Modality

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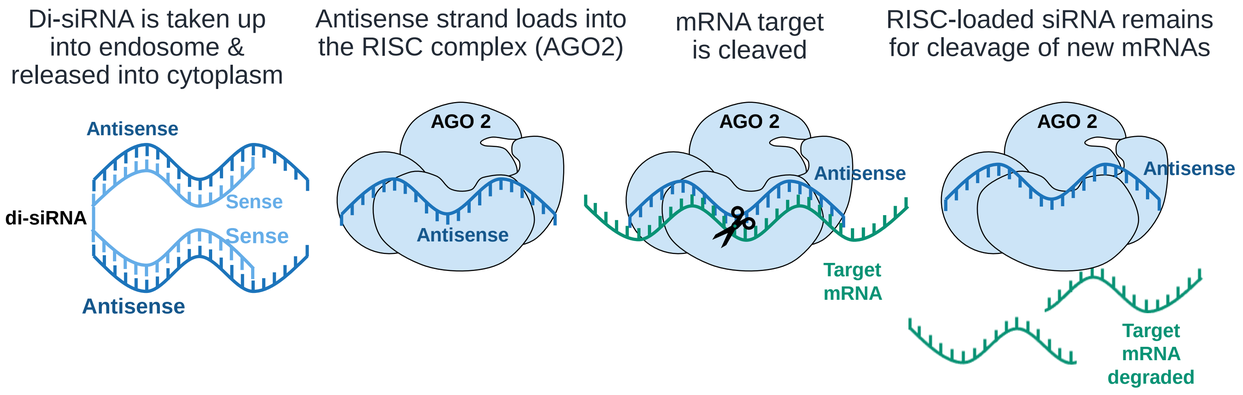
Abstract

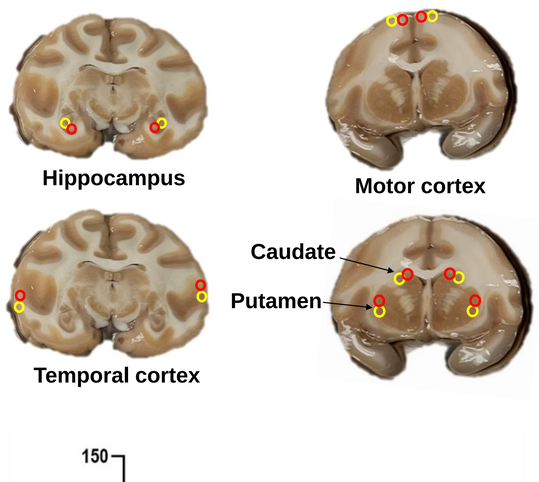
Oligonucleotide therapies offer the prospect of modifying Huntington's disease by knocking down transcripts of the *HTT* gene. Challenges include achieving significant and widespread reduction of HTT in the brain and possible adverse effects due to reduction of transcripts from the wildtype as well as the CAG-expanded pathological HTT allele. Here we present the application of divalent small interfering RNA (di-siRNA) technology to knockdown of HTT transcript and consequent reduction of the translated HTT protein. Like standard siRNAs, di-siRNA technology uses chemically modified oligonucleotides to leverage the RNA-induced silencing complex (RISC) and degrade a specific transcript sequence. Unlike other oligonucleotide modalities, di-siRNA distributes broadly throughout the nervous system following a single dose in saline directly into cerebrospinal fluid. In nonhuman primates, a single well-tolerated intrathecal dose of di-siRNA targeting HTT reduced HTT protein in all brain areas, including over 80% in cortex. Up to 75% of HTT protein reduction was observed in the caudate and putamen, areas critical to Huntington's that are difficult to reach with other oligonucleotide modalities. HTT silencing was sustained for at least six months following a single dose. Treatment-related side effects were not observed with clinical observations, clinical chemistry, or post-mortem histopathology, including in a cohort dosed three times over seven months with a saturating dose of di-siRNA. RNA-Seq experiments in mouse as well as NHP did not show major alterations in gene expression that would suggest that the HTT knockdown triggered inflammation or affected major signal transduction pathways. Mining of the UK Biobank showed that *HTT* genetic loss-of-function heterozygotes were not associated with phenotypes, suggesting no adverse effects from loss of a single HTT allele. Results support the application of the novel di-siRNA modality to Huntington's Disease by knockdown of HTT and suggest there may be a safety window for 50% or higher reduction of wildtype HTT in adults.

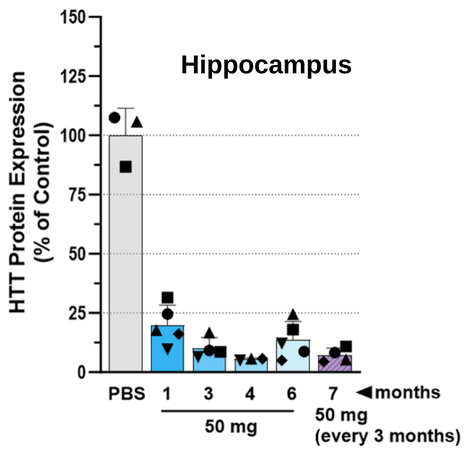
Background

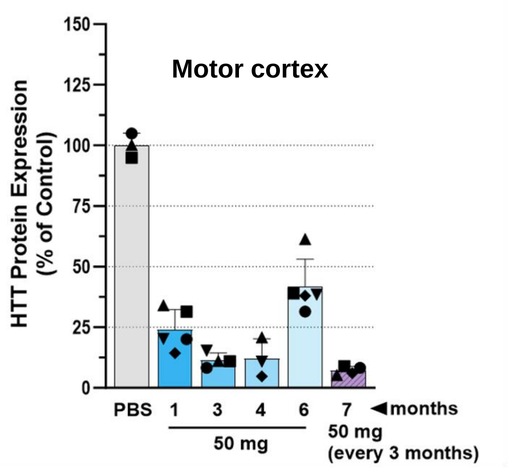


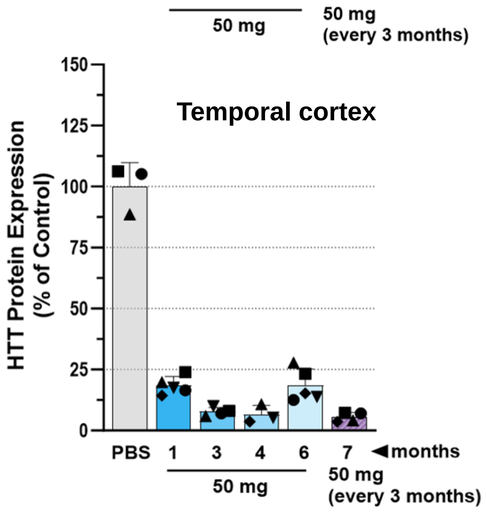
- siRNAs enable selective knockdown of individual transcripts via Argonaute-2 and the RNA-induced silencing complex (RISC).
- Divalent siRNAs (di-siRNAs) distribute broadly through the CNS following dosing into the cerebrospinal fluid

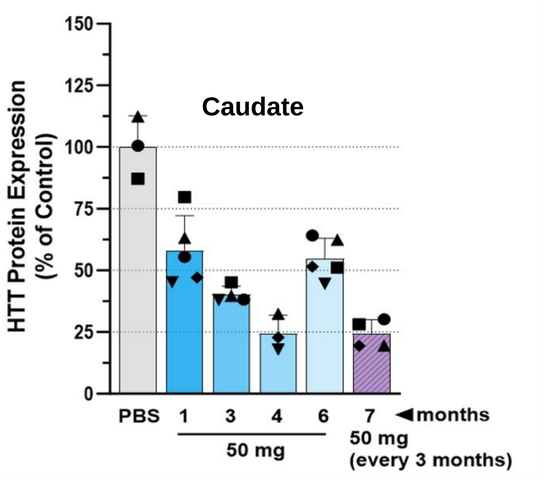
HTT Protein Knockdown

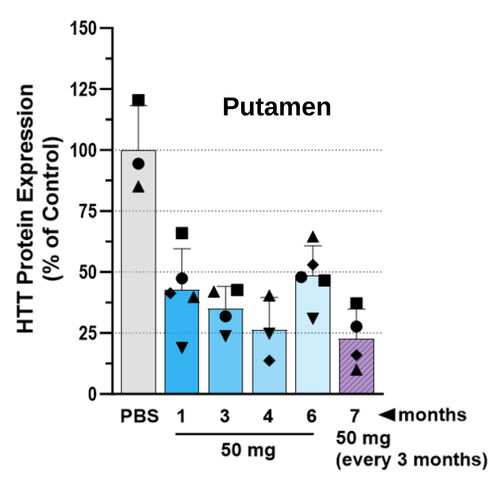












Study Goal: Test the extent and durability of knockdown of HTT protein by ATL-101, a di-siRNA targeting *HTT,* dosed IT-L in non-human primates

ATL-101 targets sequence identical among mouse, rat, cyno, and human orthologs. It is not selective for the pathological allele

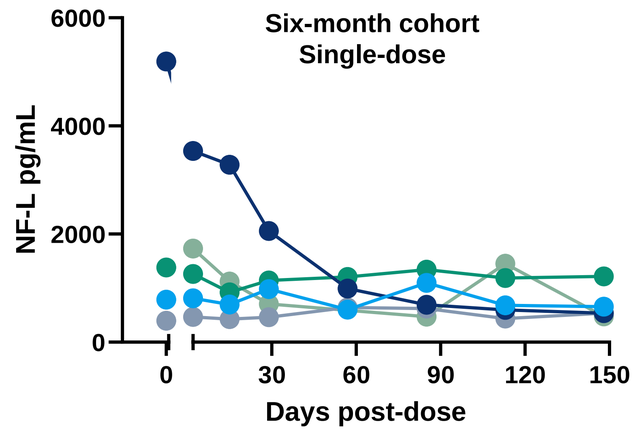
The 1, 3, 4, and 6-month groups received a single dose. The 7-month group was dosed at 0, 3, and 6 months. Dosing was in a volume of 3 mL in PBS over 2 to 3 minutes via a pre-implanted catheter.

Dose	Duration (months)	# NHPs
PBS	3	3
50 mg ATL-101	1	5
	3	5
	4	5
	6	5
3x 50 mg ATL-101	7	4

3 NHPs total were not dosed properly and were excluded from analysis.

CSF and 1.5 mm punches from frozen, sectioned tissue were analyzed with RT-PCR (transcript○), ELISA (protein○), and dual hybridization (siRNA○).

Neurofilament Light Chain



Summary Pathology and Clinical Chemistry

- No ATL-101-related clinical observations, changes in body weight, or clinical pathology parameters over the course of the study.
- Histopathology analyzed (Horus Scientific, Worcester MA) post-mortem from n=3 PBS, n=5 six-month, and n=4 seven-month (triple-dosed) animals. Tissues paraffin-embedded, sectioned at 5 microns, stained with H&E. Brain slides also prepared with GFAP and IBA1 IHC.
- "No microscopic evidence of treatment-related adverse effects (e.g., neuronal necrosis, inflammation), within the CNS tissues examined."
- "No evidence of adverse changes (e.g., thrombosis, inflammation) in the systemic organs examined" including liver and kidneys.

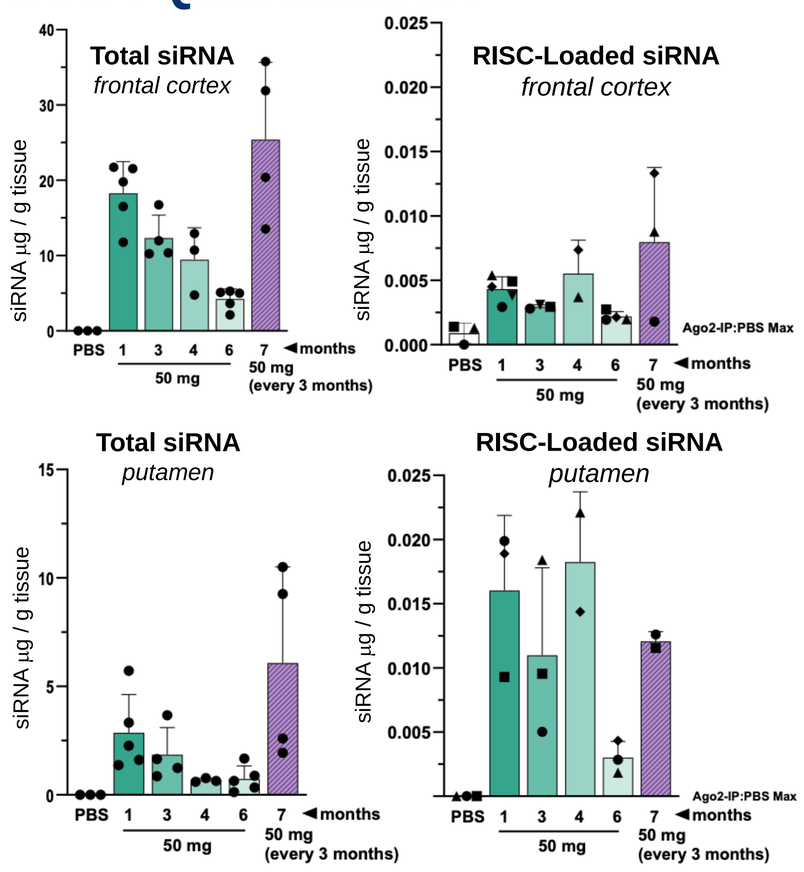
siRNA Quantitation

siRNA measured in total tissue and within RISC with antibody pull-down of AGO2

siRNA in total tissue decreases whereas siRNA within RISC stays fairly constant

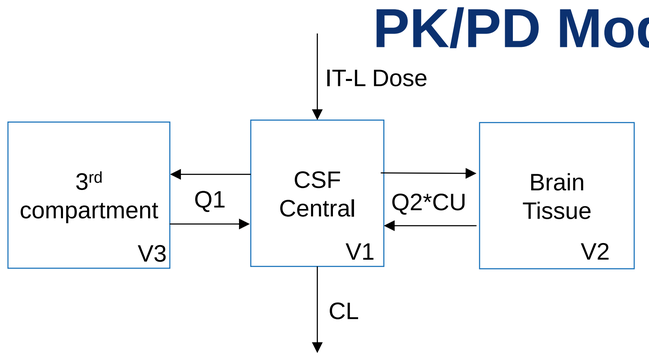
Durability of RISC-loaded siRNA is comparable to the durability of HTT protein knockdown

Higher bulk tissue siRNA in cortex than putamen but comparable or slightly less amounts loaded into RISC



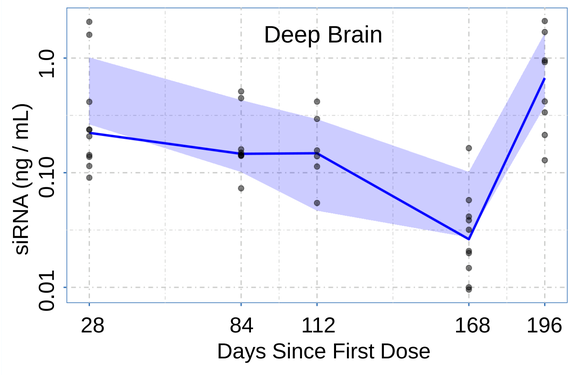
PK/PD Modeling

A2-AI

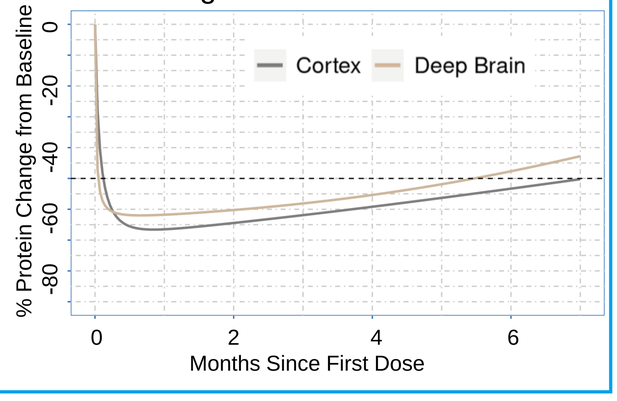


Three-compartment model used to predict knockdown in cortex and in deep brain as a function of bulk tissue siRNA levels. V2 fixed to physiological values for cortex and deep brain.

Model and data points for knockdown in striatum (cortex similar)



Predicted HTT knockdown in humans by a single 50 mg dose, based on scaling to human CSF volume



Summary

- A single 50 mg dose of ATL-101 di-siRNA delivered intrathecally in NHPs produced >80% HTT knockdown in cortex and 75% in striatum
- Knockdown maintained ≥50% at 6 months in all brain regions
- Durability of knockdown was driven by RISC-loaded siRNA
- No safety signals observed, and NfL did not increase with HTT knockdown
- Modeled clinical dose of 50 mg for >50% six-month knockdown in striatum