

A new approach to HTT-lowering using C16-siRNA conjugates

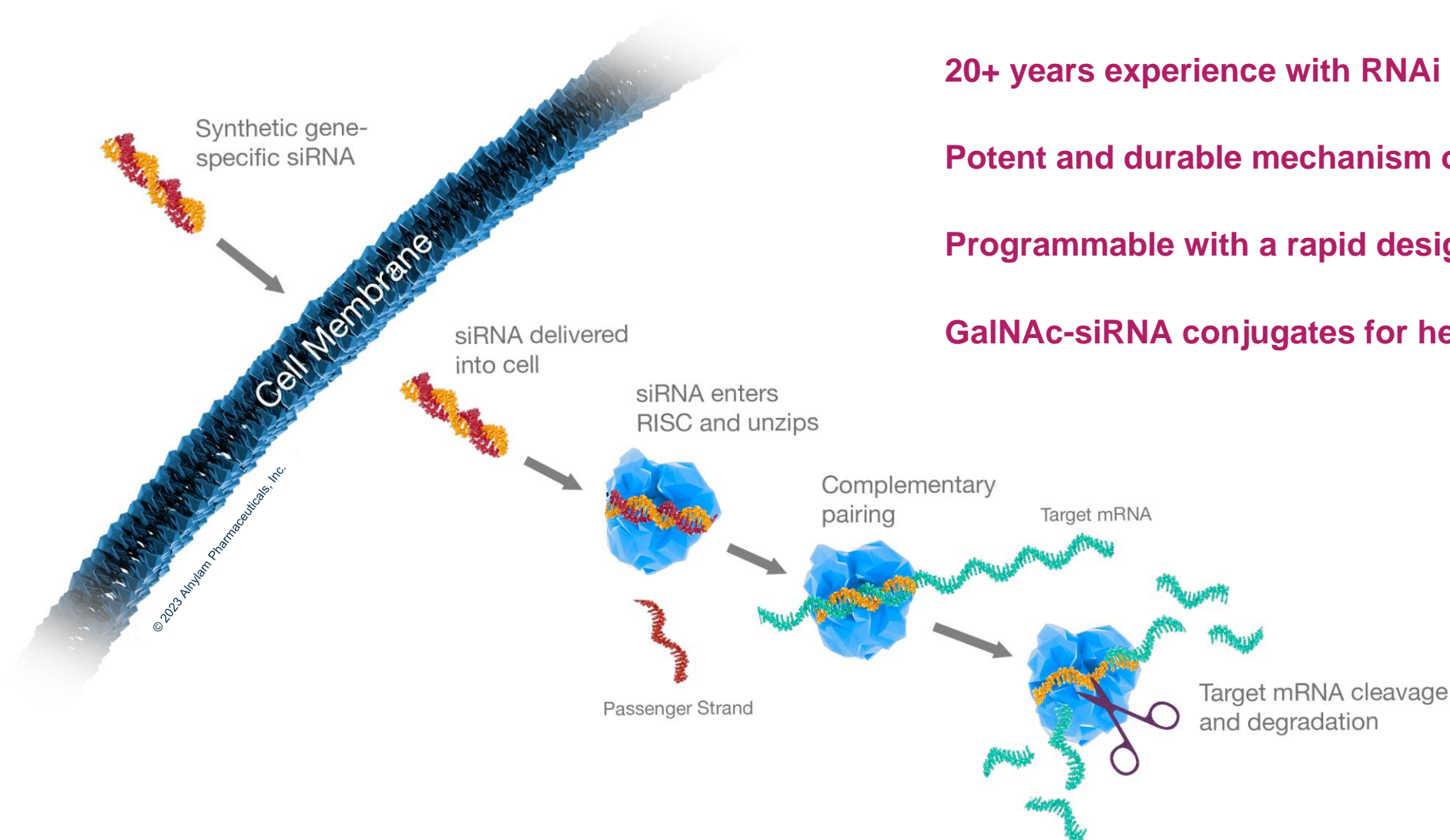
William Cantley, PhD; Anylam Pharmaceuticals

APRIL 26TH, 2023

This work is being conducted as a partnership between Anylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

RNAi Therapeutics

Clinically Established Platform in Liver



20+ years experience with RNAi

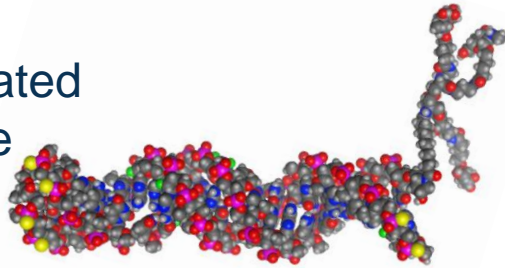
Potent and durable mechanism of action

Programmable with a rapid design/test cycle

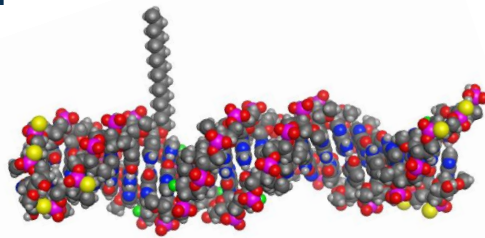
GalNAc-siRNA conjugates for hepatocyte delivery

Adaptation of the siRNA Platform for CNS Delivery

GalNAc



C16



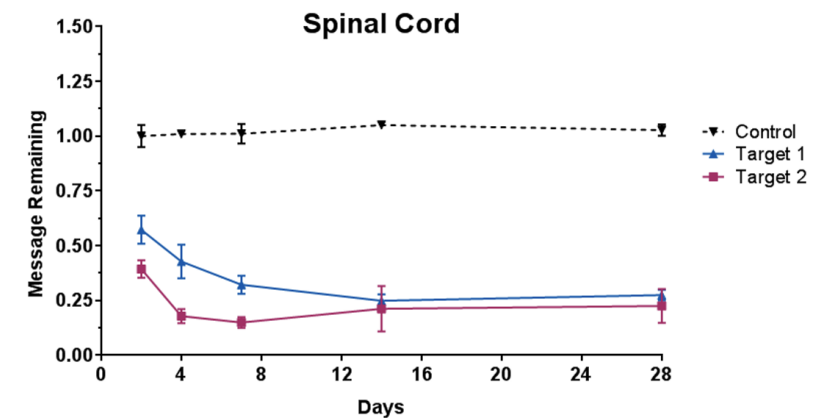
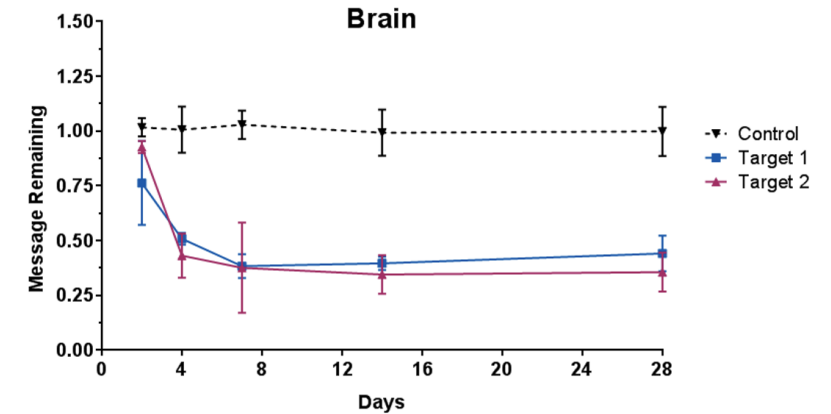
- ASGPR-mediated hepatic uptake

- Lipid-facilitated tissue distribution and/or cell uptake

- VP-mediated RISC loading

- Sequence specific siRNAs designed against two unique gene targets
- Demonstration of target engagement across CNS (brain and spine after IT administration of siRNA)

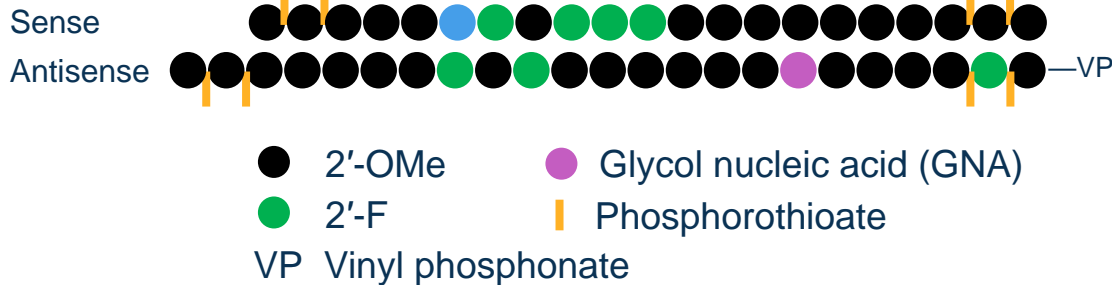
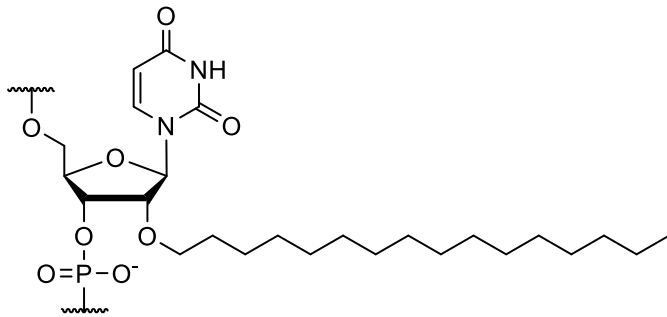
Single Intrathecal Dose in Rat



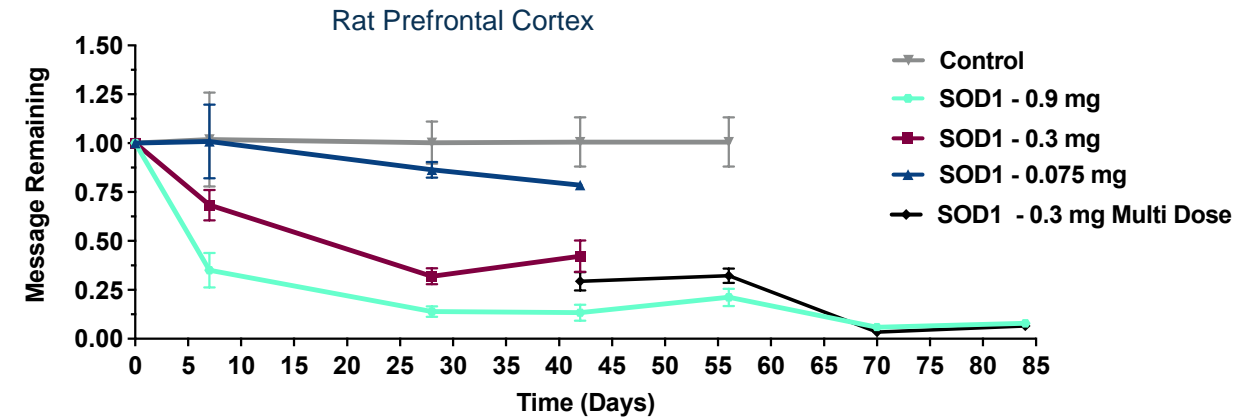
Modified from 2018 TIDES: Delivering on RNAi Therapeutics: Patisiran and Beyond.

C16- siRNA Conjugates Enable Robust and Durable Target Knockdown and Distribution in Rat CNS Post Intrathecal (IT) Injection

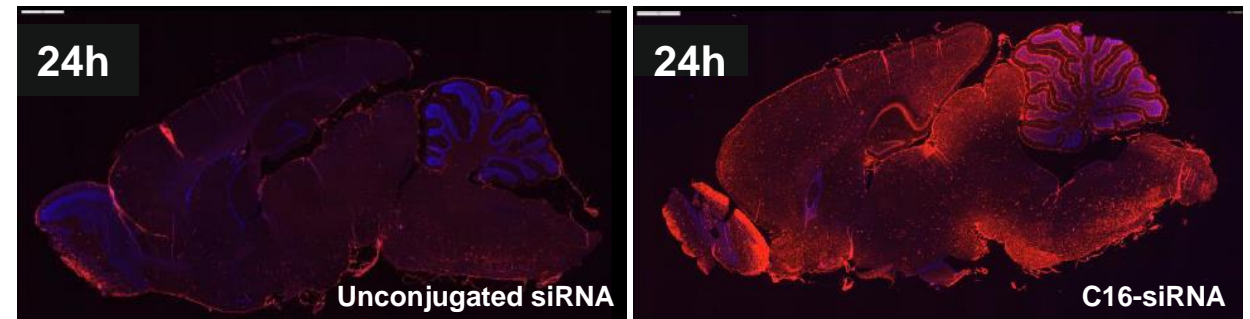
2'-O-palmitoyl (C16) nucleotide



Example Rodent PD Data



Distribution

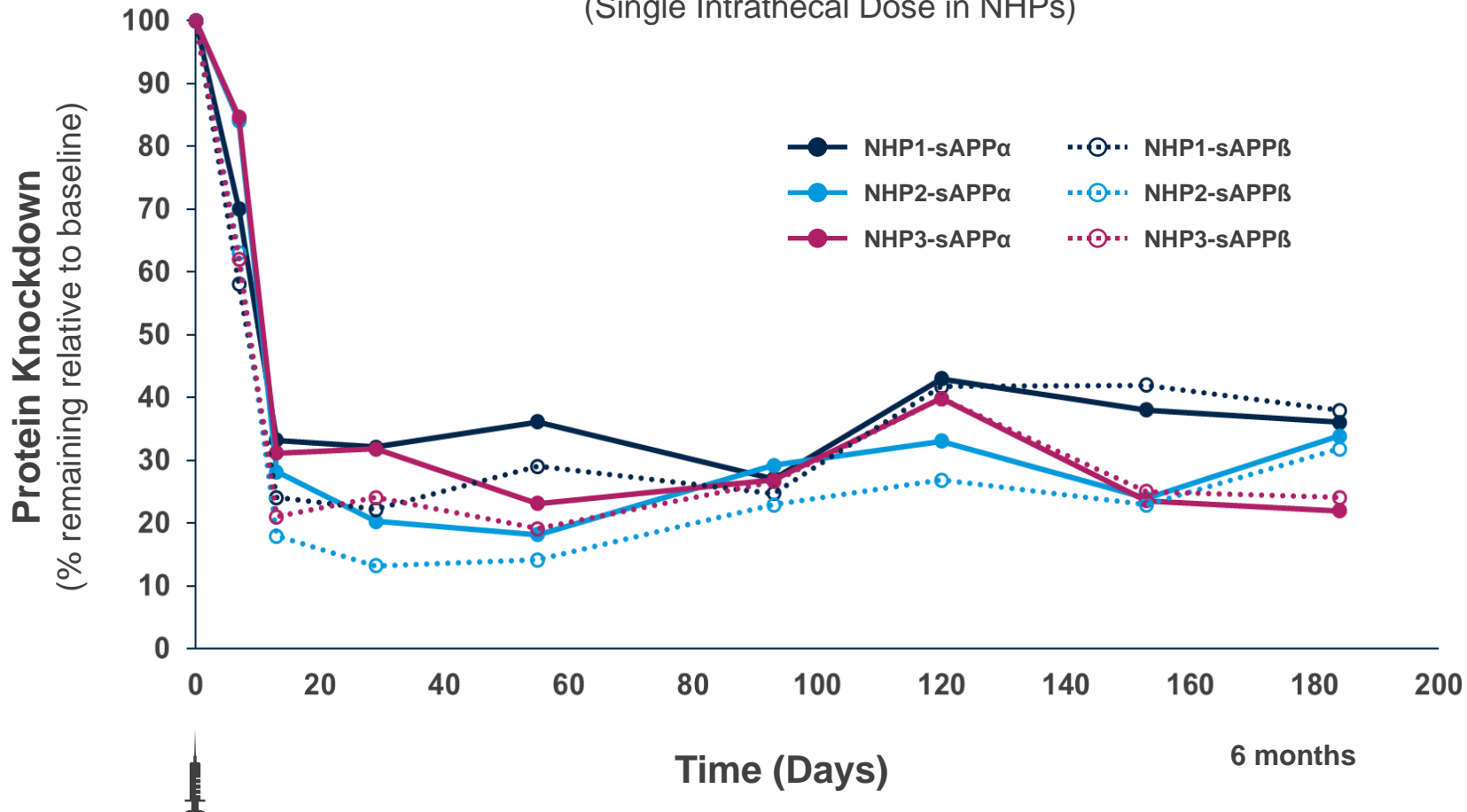


Unconjugated or C16-modified siRNA administered as single IT bolus injection to rats at 0.9 mg. siRNA biodistribution was assessed in whole brain at 24 h post-dose using IHC with anti-siRNA antibody

Potent, Durable Reduction of sAPP α and β in CNS of NHPs

sAPP Knockdown with Single Intrathecal 60 mg Dose of siRNA targeting APP mRNA

CSF sAPP α and sAPP β Protein Knockdown
(Single Intrathecal Dose in NHPs)



Results

- >50% APP reduction
- Well-tolerated out to 6 months
- Durability of effect consistent with infrequent IT dosing

From 2023 ADPD, Kirk Brown

Potential Applications for C16-siRNA Conjugates

Devastating diseases with enormous burden and unmet need



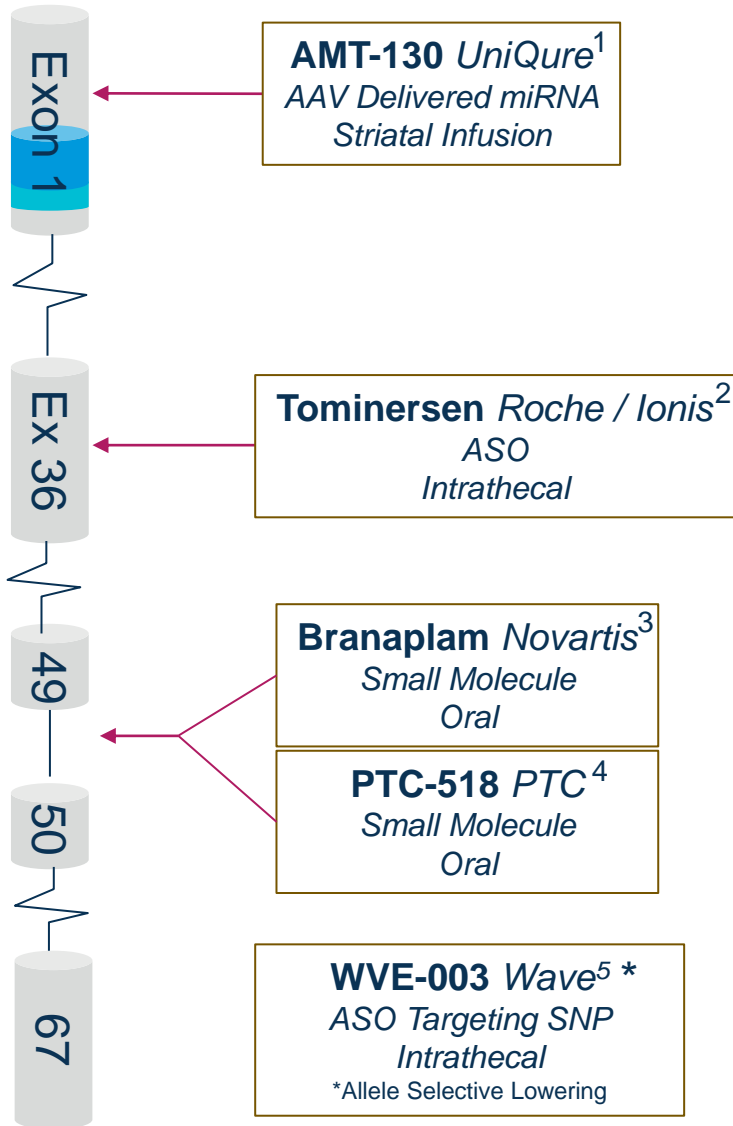
Many dominantly inherited neurodegenerative diseases:

- Alzheimer's disease
- Amyotrophic lateral sclerosis (ALS)
- Cerebral amyloid angiopathy
- Frontotemporal dementia
- **Huntington's disease**
- Multi-system atrophy
- Parkinson's disease
- Spinocerebellar ataxia

Large number of genetically validated targets, but few disease-modifying therapies exist for these devastating, life threatening disorders

RNAi therapeutics could potentially offer a new approach

HTT Lowering is a Leading Therapeutic Approach for HD



¹ UniQure, 2022
² Tabrizi et al., NEJM, 2019

³ Keller et al., Nature Communications, 2022
⁴ PTC Bio, 2021

⁵ Carroll et al., Molecular Therapy, 2011
⁶ Tabrizi et al., Neuron, 2019

⁷ Bhattacharyya et al., Nature Communications, 2021
⁸ Leavitt et al., JAMA Neurology, 2020

Neuron Review

Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease

Sarah J. Tabrizi,^{1,2} Ria Ghosh,¹ and Blair R. Leavitt¹

Huntington's disease is caused by an abnormal gene, which confers a predominant toxic gain of function. There are currently no disease-modifying therapies in disease pathogenesis hold great promise. These finger proteins, transcription activator-like effector huntingtin-lowering approaches such as RNAi, antisense modulators, and novel methods to clear the mHTT. Improvements in the delivery and distribution of biomarkers of disease and of HTT lowering clinical therapies to the forefront of Huntington's disease under way.

Huntington's disease (HD) is an inherited autosomal-dominant neurodegenerative disorder characterized by a triad of motor, cognitive, and psychiatric features. HD typically displays onset in mid-life, with irreversible progression of symptoms over 10-15 years (Filla and Tabrizi, 2015). All cases of HD are caused by an abnormally expanded CAG repeat near the N-terminus of the huntingtin gene (HTT), which leads to the production of mutant huntingtin protein (mHTT) on translation. It has now been 25 years since the identification of the genetic mutation in 1993, and intensive research efforts have described many cellular pathological mechanisms underlying disease development. Nearly all are driven by the presence of the mHTT protein, which is ubiquitously expressed and is thought to cause disease by a predominant toxic gain-of-function mechanism (Bliss et al., 2019).

Currently, a major focus is development and testing of therapies that target proximal to HD pathogenesis, namely, by targeting HTT DNA, RNA, and protein. These approaches ultimately aim to reduce mHTT levels and, therefore, ameliorate all of its downstream pathogenic effects, which are multiple and varied. In animal models of HD, reduction of mHTT improves disease phenotypes and reverses neuropathology (Tomer et al., 2000; Wang et al., 2014), confirming the importance of mHTT lowering as a therapeutic aim.

This review will cover the methods we think are most important now in therapy development for HD; namely, HTT lowering therapies that will mitigate all downstream pathogenic effects of mHTT protein (Figure 1). An overview of the treatments that have been or are being taken forward into clinical development is shown in Table 1.

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ARTICLE

Small molecule splicing modifiers with systemic HTT-lowering activity

Anuradha Bhattacharyya¹, Christopher R. Trotta², Kerstin A. Effenberger¹, Matthew G. Woll¹, Minakshi Nadiya Sydorenko¹, Young-Choon Moon¹, Gary M. Nikolai A. Naryshkin¹, Jason D. Graci¹, Thomas Tripathi¹, Joseph M. Colacino¹ & Stuart W. Peltz¹

Huntingtin's disease (HD) is a hereditary neurodegenerative of cytosine-adenine-guanine (CAG) trinucleotide repeats. Consequently, the mutant protein is ubiquitously expressed through a toxic gain-of-function mechanism. Animal model reducing huntingtin (HTT) protein levels alleviates motor and cognitive deficits. Investigational drugs aim to reduce HTT levels by RNA interference or translation. These drugs require invasive procedures to the central nervous system (CNS) and do not achieve broad CNS distribution. Here, orally bioavailable small molecules with broad distribution reduce HTT expression consistently throughout the CNS and periphery of pre-messenger RNA splicing. These compounds act through a pseudonucleotide containing a premature termination codon (PTC) that causes mRNA degradation and reduction of HTT levels.

JAMA Neurology | Clinical Implications of Basic Neuroscience Research
Huntingtin-Lowering Therapies for Huntington Disease: A Review of the Evidence of Potential Benefits and Risks

Blair R. Leavitt, MD, PhD, Holly B. Kordasiewicz, PhD, Scott S. Schohl, MD

Huntingtin disease (HD) is caused by a cytosine-adenine-guanine trinucleotide repeat expansion in the huntingtin gene, HTT, that results in expression of mutant huntingtin protein (HTT). Therapeutic strategies that reduce HTT levels are currently being pursued to slow or stop disease progression in people with HD. These approaches are supported by robust preclinical data indicating that reducing mutant huntingtin protein is associated with decreased HD pathology. However, the risk-benefit profile of reducing either variant HTT or both variant and wild-type HTT is currently an open question that is being addressed in ongoing clinical trials. This review aims to examine the current data available regarding lowered HTT in humans, normal animals, and animal models of HD. Studies indexed in PubMed were searched using the MeSH term Huntington disease or the text words huntingtin or huntingtin from August 31, 1999, to August 31, 2019, with no language restrictions. Additional studies were included from the reference lists of relevant studies and the authors' personal files. Articles describing at least 1 aspect of HTT reduction were included, prioritizing those published within the last 10 years. In vivo studies were also prioritized, with a focus on studies that examined the consequences of wild-type HTT reduction in adults. In a recently completed phase 1/2a study of RG6042 in 46 adults with early manifest HD, antisense oligonucleotide-mediated partial reduction of HTT was reported to be generally safe and well tolerated over the course of 4-monthly RG6042 doses. In case studies of people with rare genetic variations in huntingtin alleles, the loss of 1 wild-type allele was not associated with HD. People with homozygous cytosine-adenine-guanine expansions developed normally until the onset of HD, although they may have experienced a more aggressive disease course. In mouse models of HD, partial reduction of HTT was beneficial, with improvements in motor, cognitive, and behavioral phenotypes. The partial reduction of wild-type HTT in normal adult rodents and nonhuman primates was generally safe and well tolerated. The body of evidence reviewed in this article indicates a positive risk-benefit profile for the partial reduction of either variant HTT alone or both variant and wild-type HTT. These strategies target the underlying cause of HD and are currently being tested in several investigational clinical trials.

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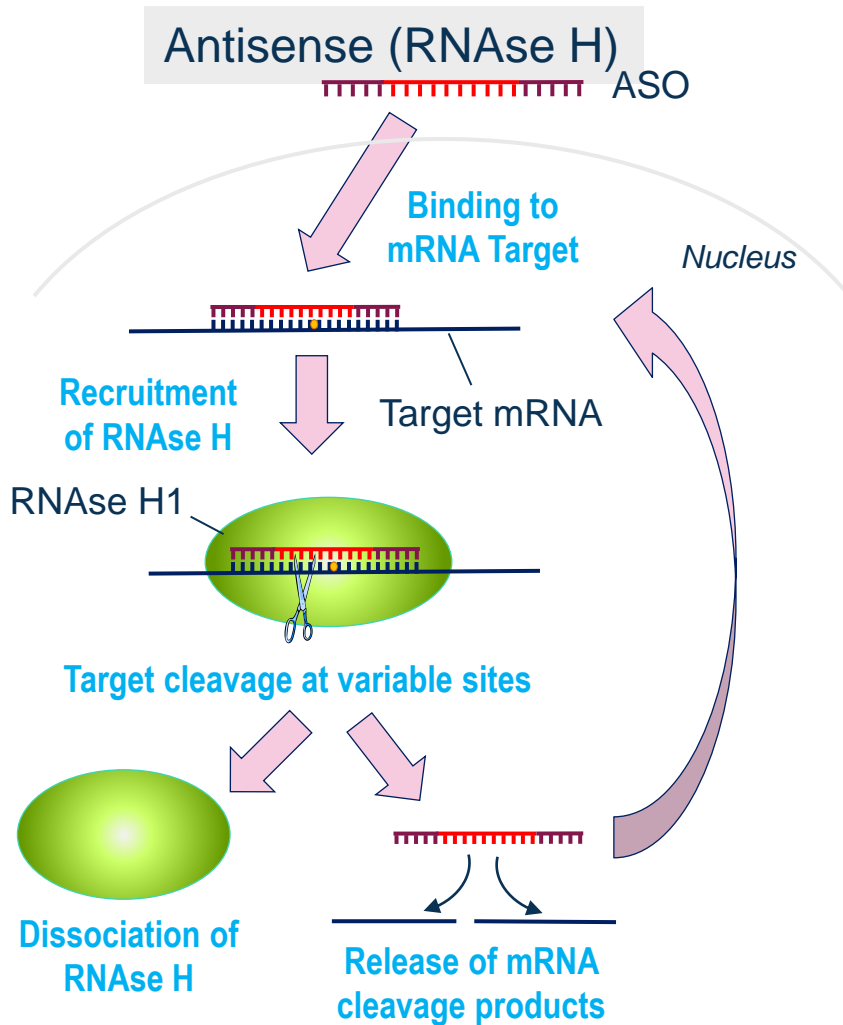
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Huntingtin disease (HD, OMIM 613004) is a rare genetic neurodegenerative disease characterized by a triad of cognitive, behavioral, and motor symptoms.^{1,2} Disease onset typically occurs in the prime of life, between ages 30 and 50 years, and is associated with increasing disability, worsening of function, and loss of independence, leading to death within approximately 15 years, on average, after the onset of motor signs and symptoms.^{1,4} A cytosine-adenine-guanine (CAG) repeat expansion variant (mutation) in only 1 of the 2 alleles of the huntingtin gene, HTT (OMIM 613004), is sufficient to be associated with the onset of HD with an autosomal-dominant pattern of inheritance.¹ The expansion variant on the affected allele is encoded for an abnormally long polyglutamine tract within the huntingtin protein (HTT), resulting in the formation of variant HTT.^{1,4,5} The expression of variant HTT throughout the brain is associated with progressive age-dependent neurodegeneration, primarily owing to toxic gain-of-function mechanisms.^{1,4,5} Strategies to decrease or suppress the production of variant HTT are in clinical development, with the ultimate goal of stopping or slowing clinical progression of the affected cognitive, behavioral, and motor domains.^{1,7} These strategies include antisense oligonucleotide (ASO)-mediated HTT-lowering approaches, which target the RNA product of either the variant HTT allele or both HTT alleles (the variant HTT and the unaffected and unexpanded or wild-type HTT allele) as well as adeno-associated viral (AAV) vector-delivered short interfering RNA (siRNA) or microRNA (miRNA) HTT-lowering approaches, which target the products of both HTT alleles.

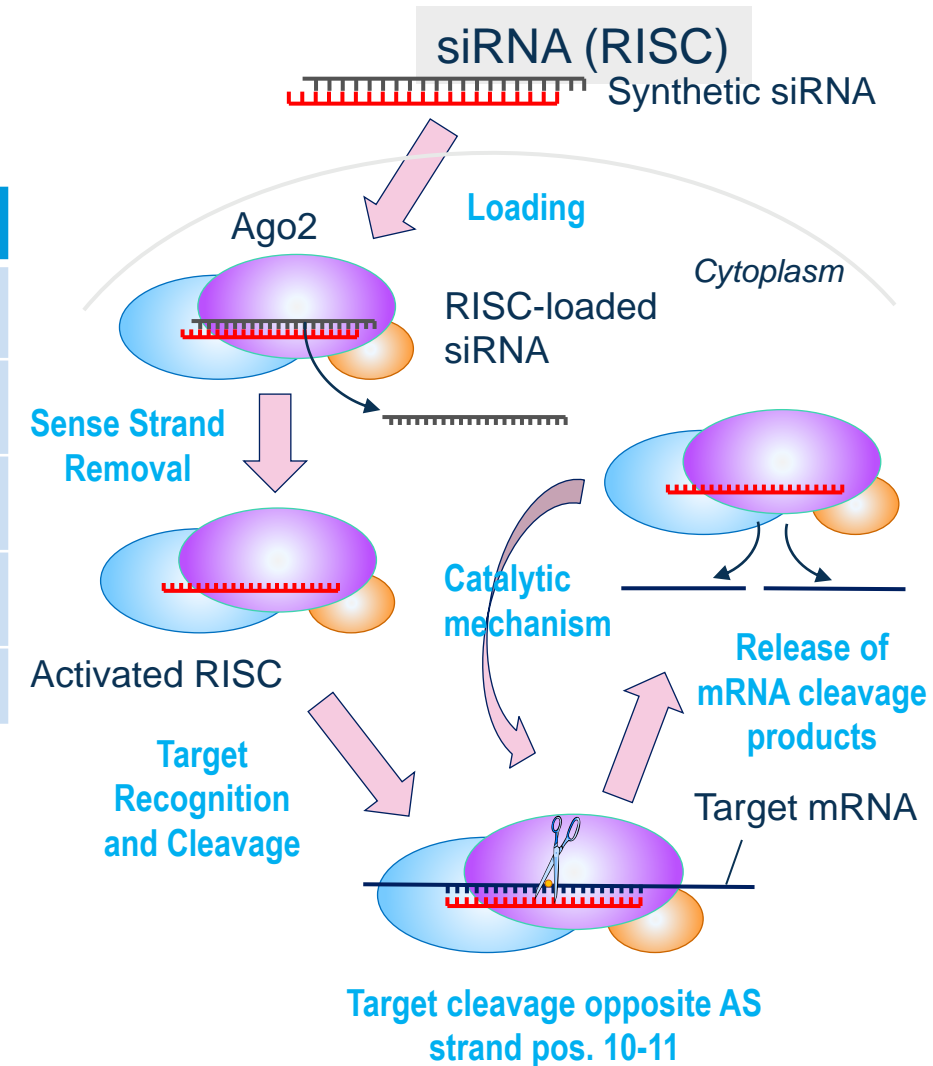
This review examines all available evidence from the relevant published human and animal literature to assess the potential benefits and risks of HTT-lowering therapeutic strategies in clinical development for the treatment of HD (Figure 1).¹⁰⁻¹⁹ The huntingtin protein is large and ubiquitously expressed (3144 amino acids), with

Biology of Antisense and RNAi Pathways

Distinct mechanisms for Single-Stranded “DNA” vs Double-Stranded “RNA”

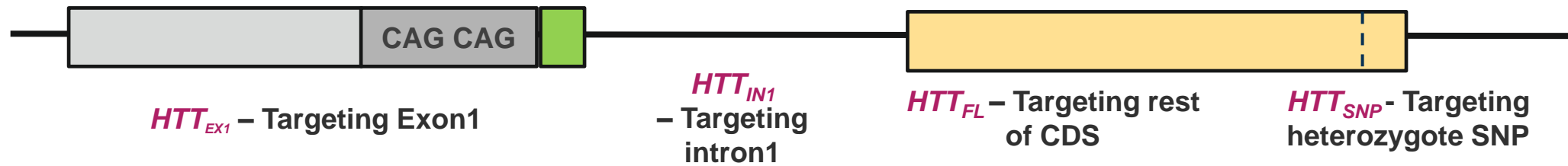


ASO		siRNA
RNase H	Mechanism of Action	RISC
mRNA or pre-mRNA	Target	mRNA
Nucleus	Location of Engagement	Cytoplasm
1:1	Nature of Interaction	Catalytic
Intrathecal	RoA	Intrathecal



Multiple Opportunities for HTT Targeting with RNAi

Impact of Targeting Location on HTT mRNA

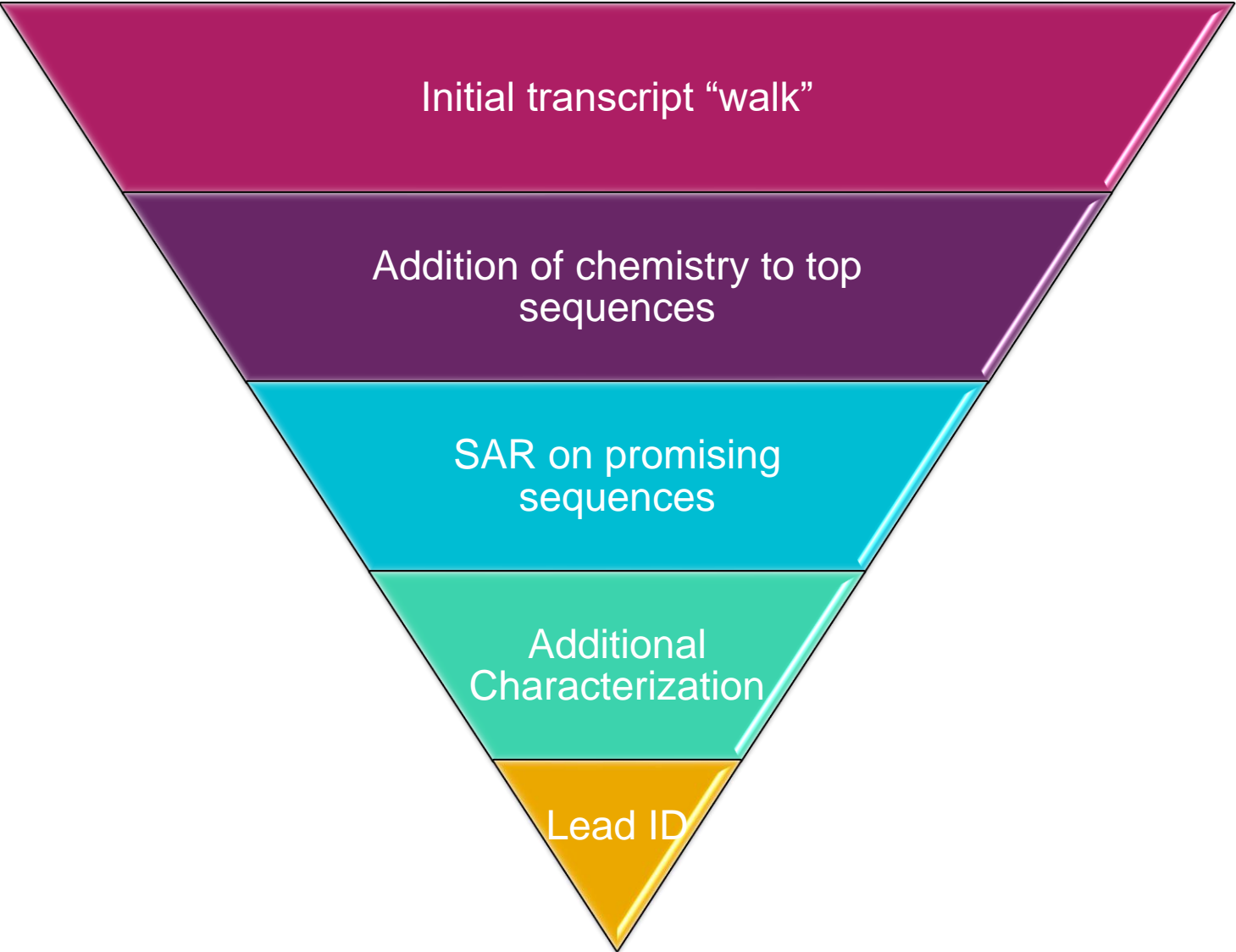


👍 **Desired**
👎 **Undesired**

		HTT siRNA Targeting Strategy			
		HTT _{EX1} siRNA	HTT _{IN1} siRNA	HTT _{FL} siRNA	HTT _{SNP} siRNA
mRNA Targeted	mHTT - Full Length	HITS 👍	MISSES 👎	HITS 👍	HITS 👍
	mHTT – HTT1a	HITS 👍	HITS 👍	MISSES 👎	MISSES 👎
	wtHTT – Full Length	HITS 👎	MISSES 👍	HITS 👎	MISSES 👍

Identifying a Potent and Specific Duplex

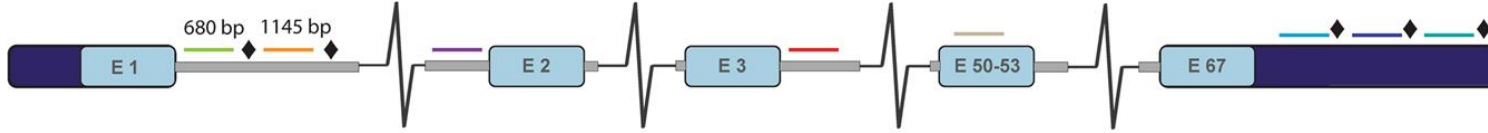
“Screening Funnel”



- Initial Screen for Absolute Potency
- Variations to improve sequence specificity
- Modifications to increase drug like properties
- In vivo characterization of top performers

HTT Transcript Engagement Impacted by Targeting Location

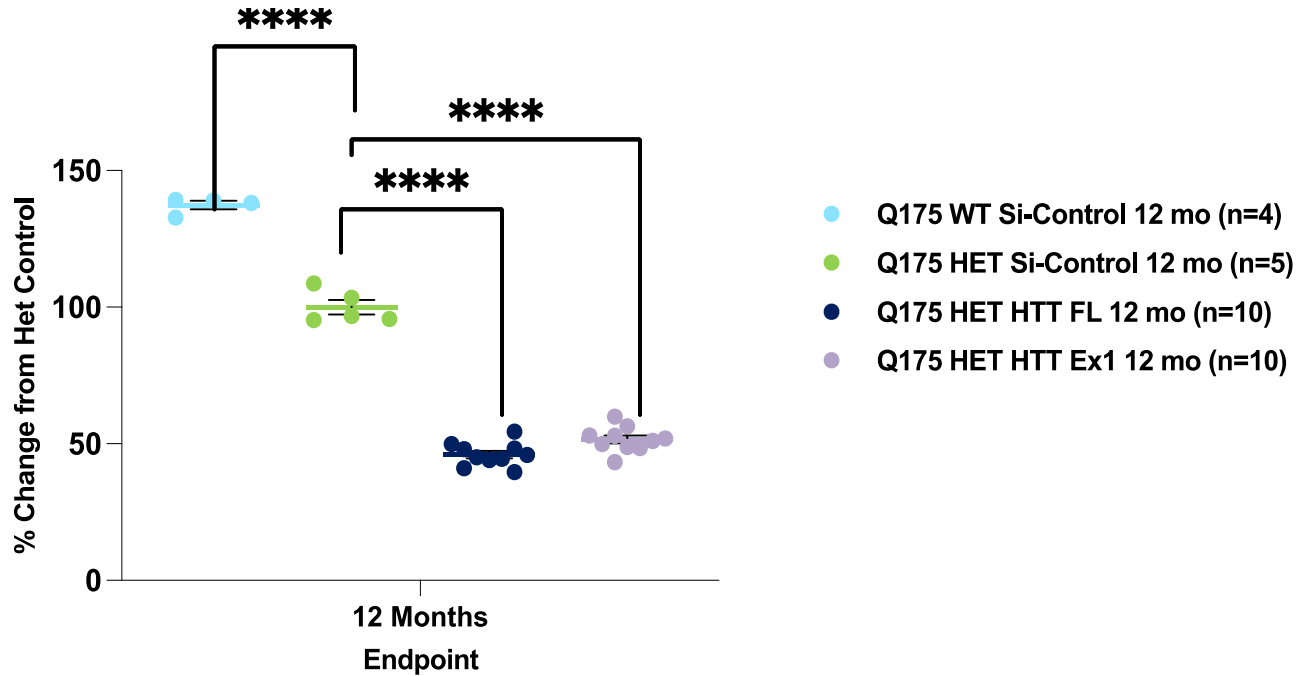
Q175 mouse Striatal Tissue; 3-Month Post-Dose



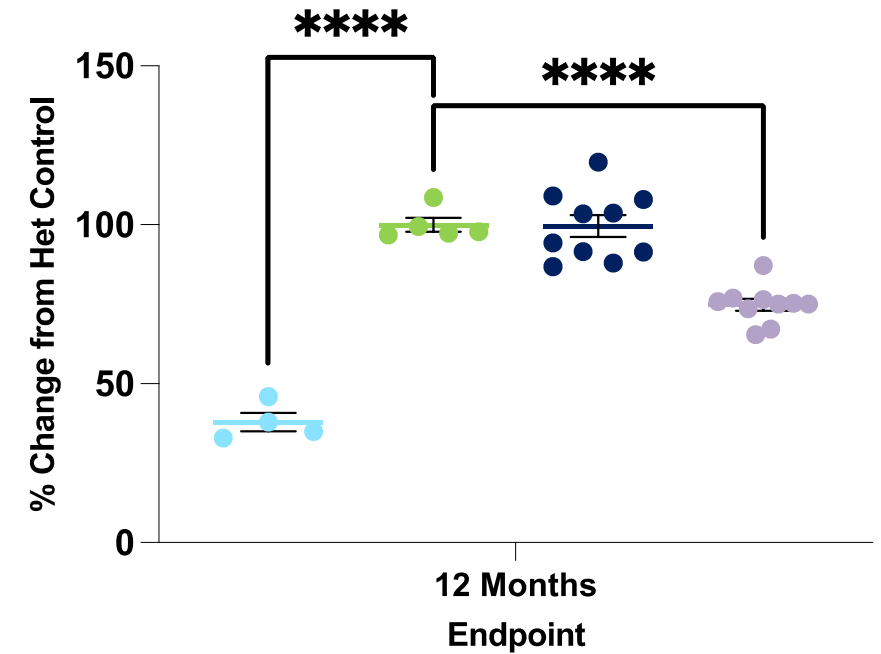
Papadopoulou et al., Sci. Rep. 2019



Full-Length HTT Transcript



HTT1a Fragment Transcript



Both FL and Ex1 Targeting siRNA reduce HTT FL transcript

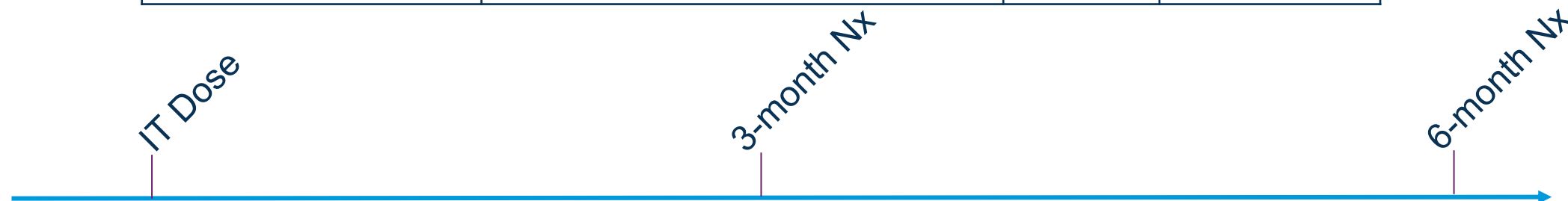
Only Ex1 Targeting siRNA reduces HTT1a transcript

Characterizing HTT Target Engagement in NHP with a C16-siRNA

3-Month Dose Range Exploratory Study of a HTT Targeting siRNA

Objective: Characterize potency & tolerability of a HTT targeting siRNA across range of dose levels

Group No.	Treatment Condition	NHP	
		N	Nx Day
1	aCSF	3	85
2	HTT siRNA: High Dose	5	85
3	HTT siRNA: Mid Dose	8	85, 171
4	HTT siRNA: Low Dose	8	85
5	HTT siRNA: Very-Low Dose	5	85



In Life

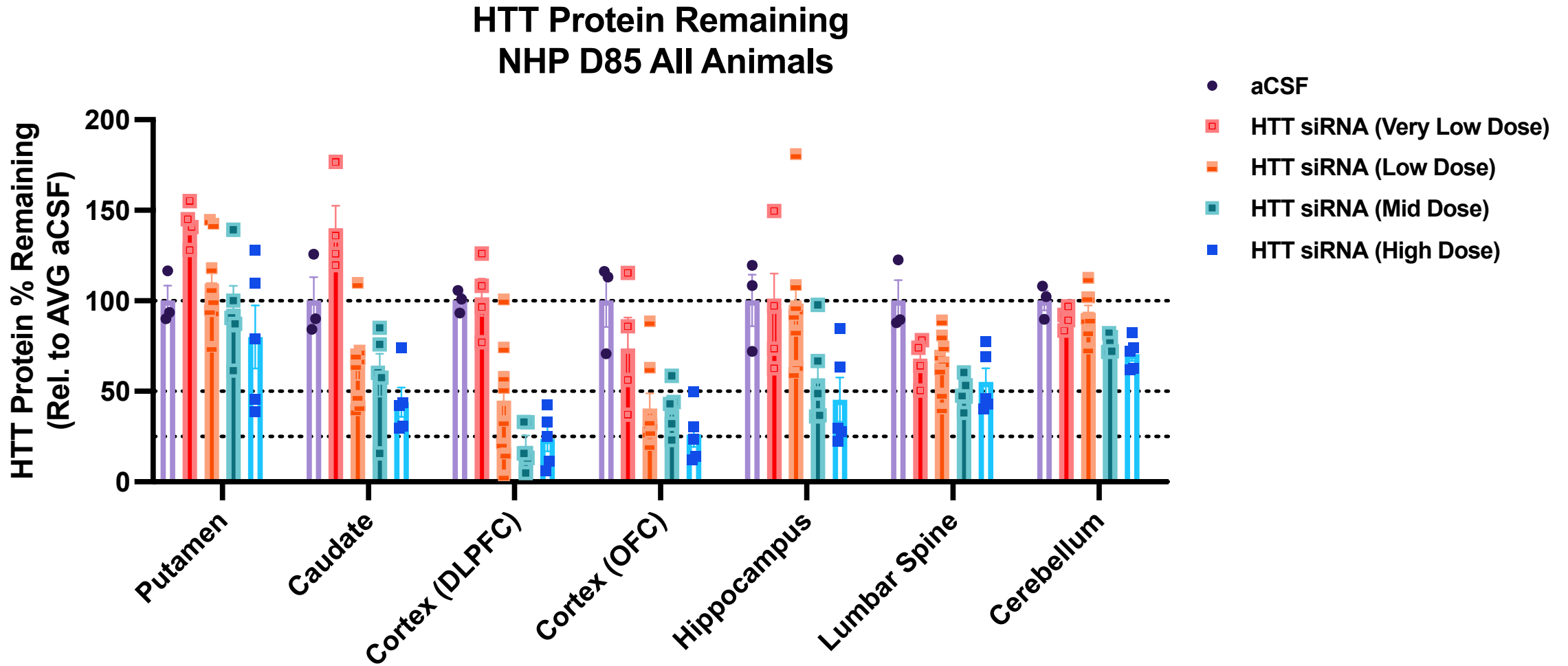
Weekly Clinical Obs.
Monthly Neuro Evals.
Serial CSF Collections

Terminal

Tissue KD
Histopathological Evaluation

Characterizing HTT Target Engagement in NHP with a C16-siRNA

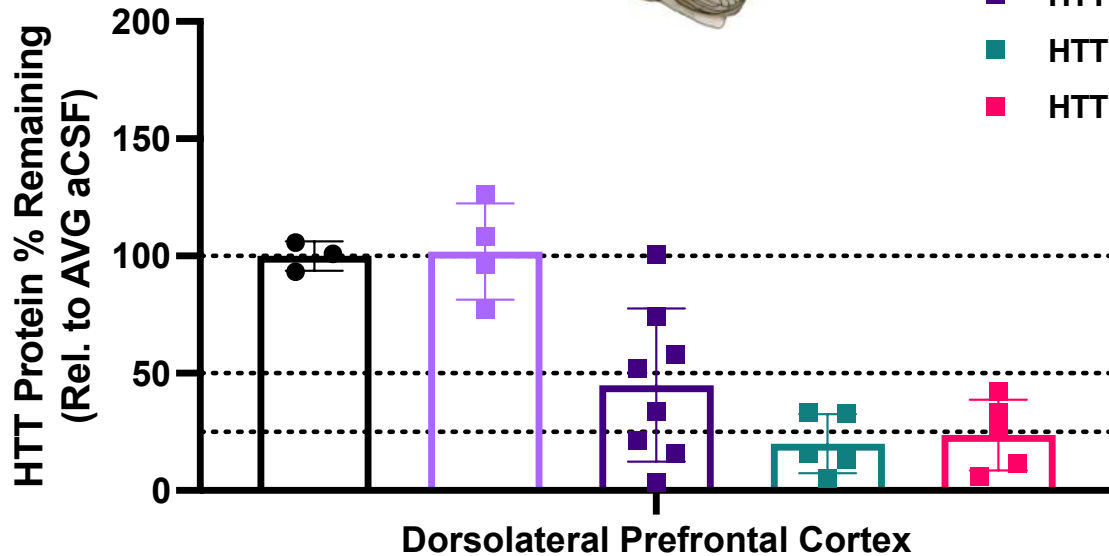
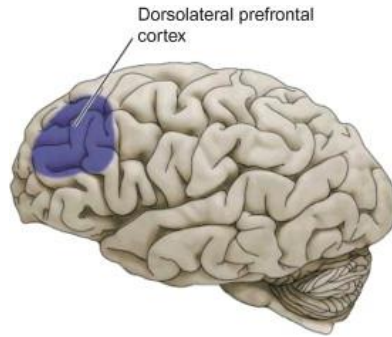
HTT Protein Remaining 3-Months After Single IT Dose



Characterizing HTT Target Engagement in NHP with a C16-siRNA

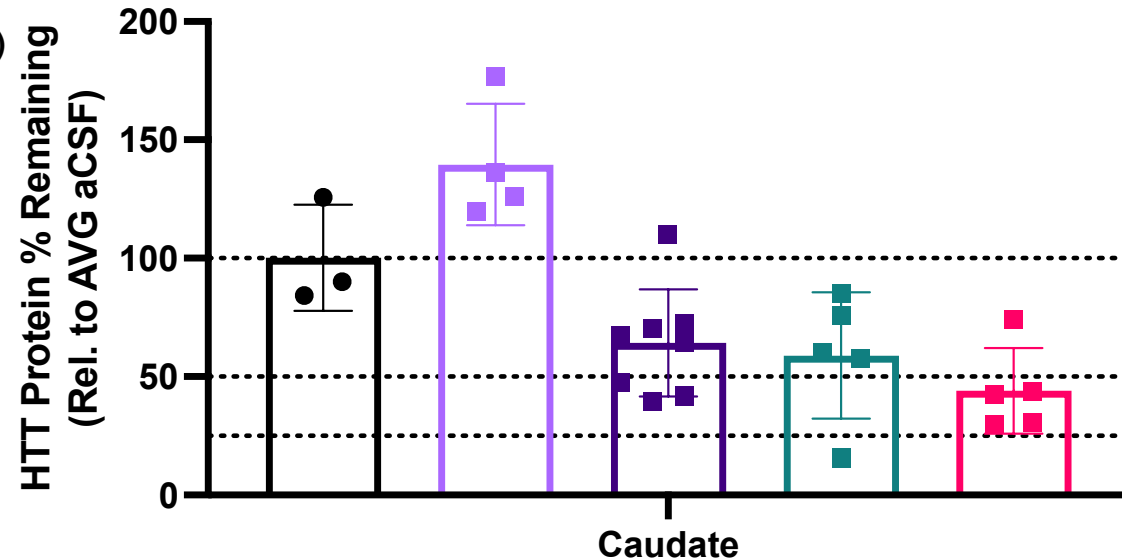
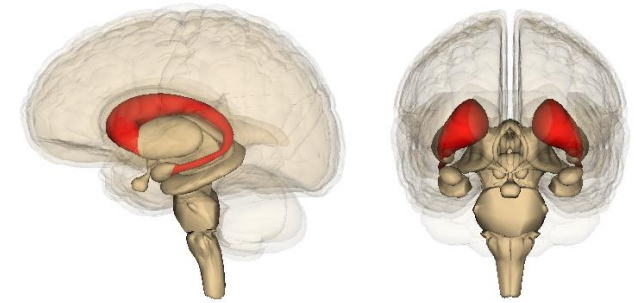
HTT Protein Reduction in Cortex and Caudate 3 months after single IT dose

HTT Protein Remaining DLPFC



- aCSF
- HTT siRNA (Very Low Dose)
- HTT siRNA (Low Dose)
- HTT siRNA (Mid Dose)
- HTT siRNA (High Dose)

HTT Protein Remaining Caudate

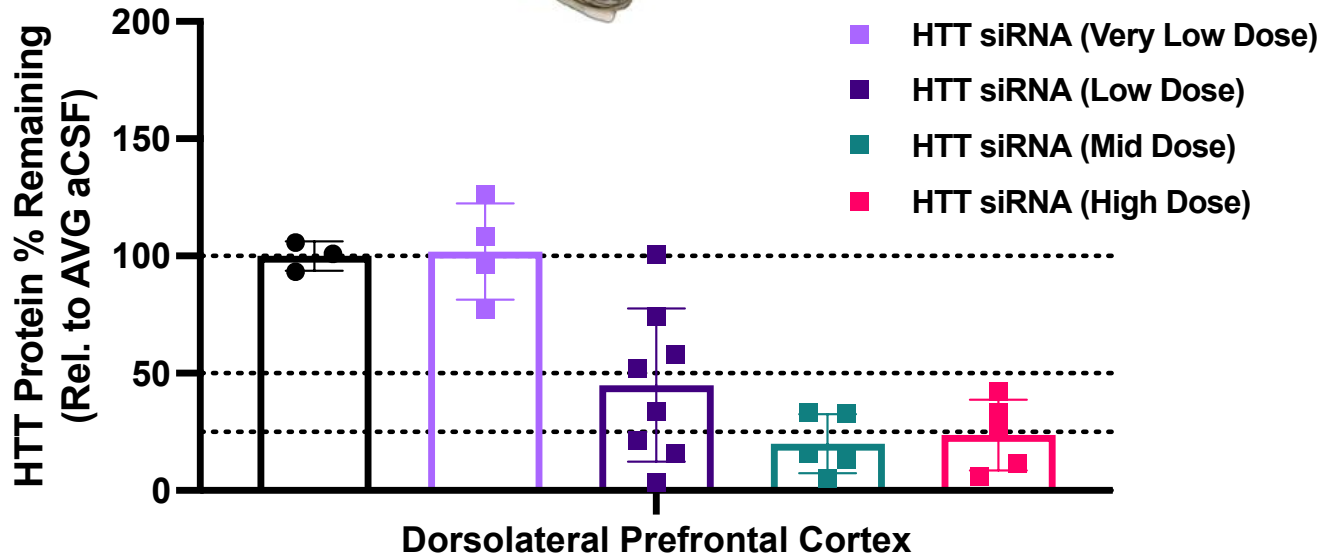
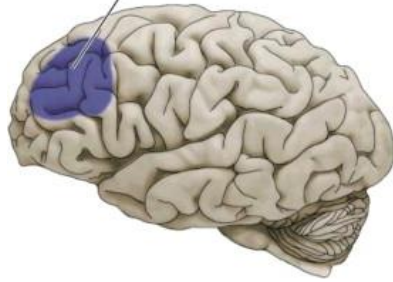


Characterizing HTT Target Engagement in NHP with a C16-siRNA

HTT Protein Reduction in Cortex 3 months after single IT dose

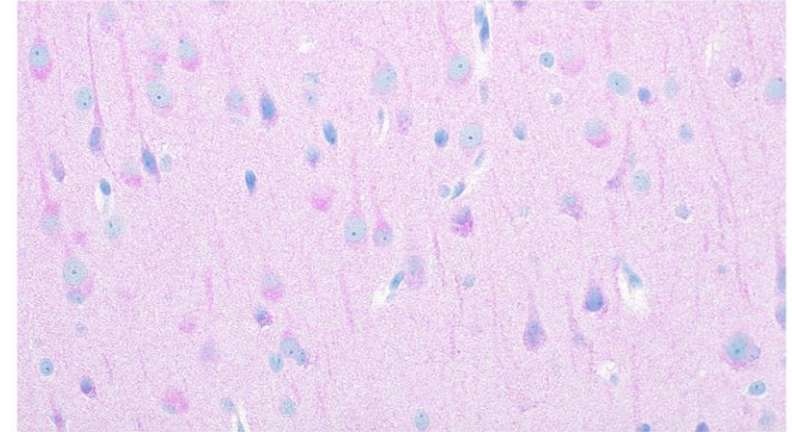
HTT Protein Remaining DLPC

Dorsolateral prefrontal cortex

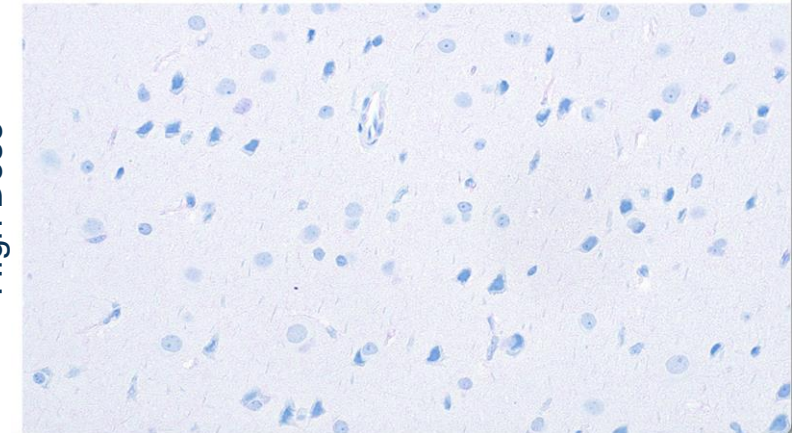


HTT IHC in Cortex

aCSF



HTT siRNA
High Dose



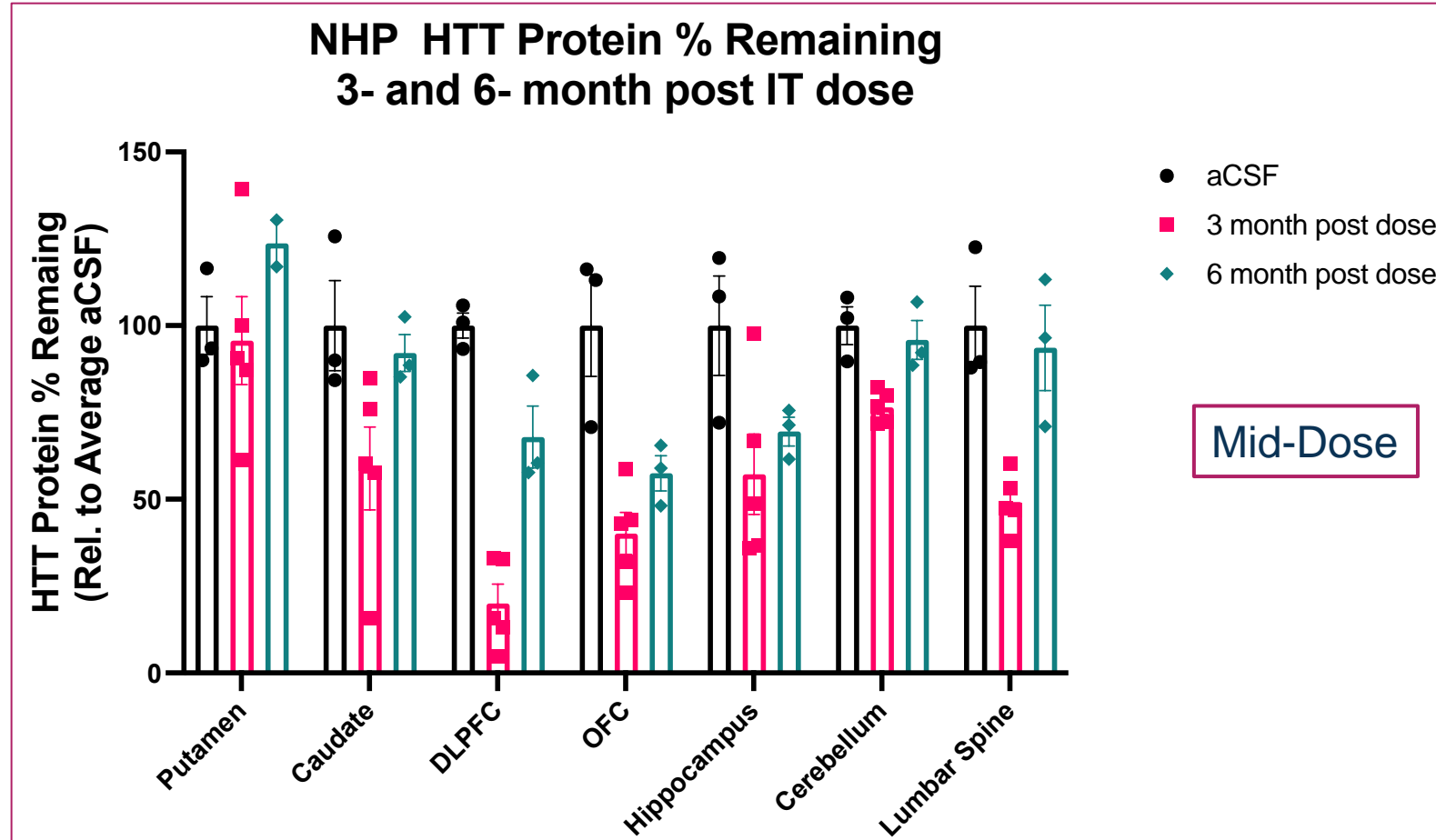
Characterizing HTT Target Engagement in NHP with a C16-siRNA

HTT Protein Reduction 3- and 6-months after single IT dose

Terminal Tissue

n=5 @ 3-month

n=3 @ 6-month



Tolerability Profile w/ 3-6 Month Sustained HTT Lowering in NHP

NfL from Longitudinal CSF Collections, Histopath Evaluation from Terminal Tissue

In-Life Evaluation:

Weekly Clinical Observations

Monthly Neuro Evaluations

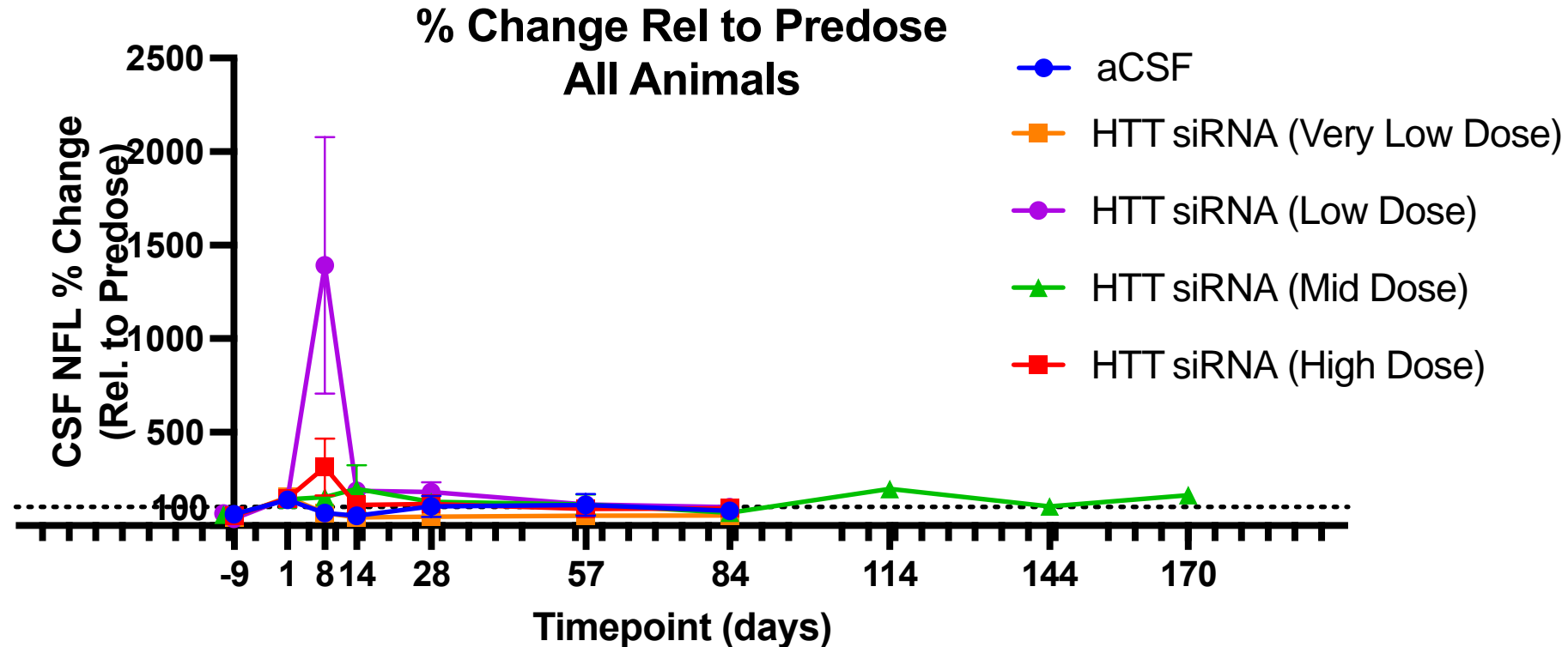
No treatment-associated adverse findings in NHPs treated with HTT siRNA at any dose level

Histopathological Evaluations:

15 sections per brain per animal

Analyzed via H&E

No treatment-associated adverse findings in NHPs treated with HTT siRNA at any dose level



Summary and Looking Forward

- Introduced C16-siRNA as a new approach for HTT lowering in the CNS
- Established differential engagement of HTT transcripts based on siRNA targeting location
- Demonstrated robust, tolerated HTT-lowering at 3- and 6-months after a single IT dose, as determined by terminal tissue protein levels, CSF NfL and histopathological evaluation
- Continue to explore HTT-targeting approaches & feasibility; work toward identifying potential development candidates
- Assess learnings from past & present clinical stage HTT-lowering programs
- Engage with the HD community to explore how our C16-siRNA platform might add value to the HD therapeutics landscape as a potential new way to pursue HTT-lowering

Thank You!

Questions?