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

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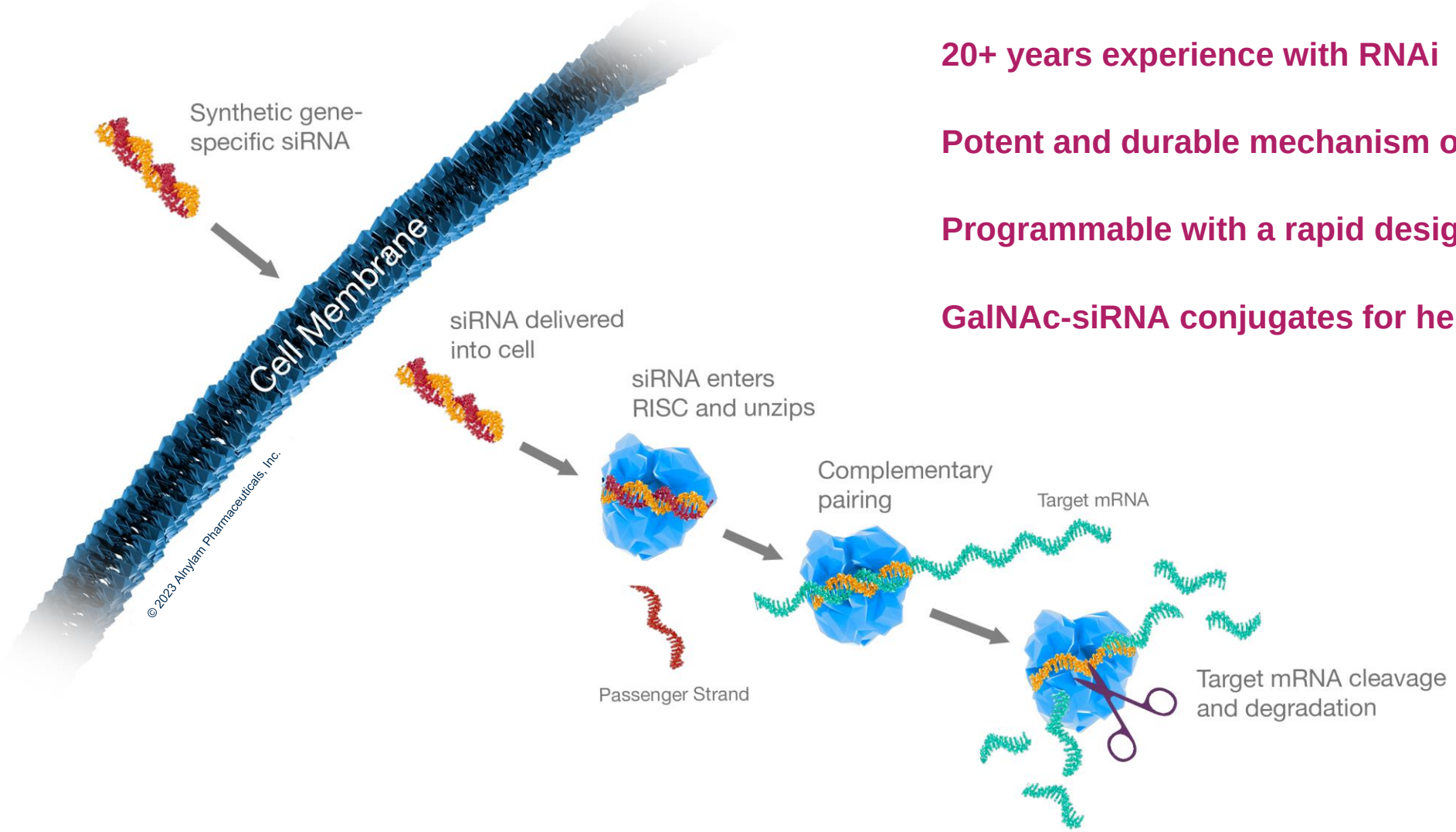
APRIL 26TH, 2023

This work is being conducted as a partnership between Alnylam 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

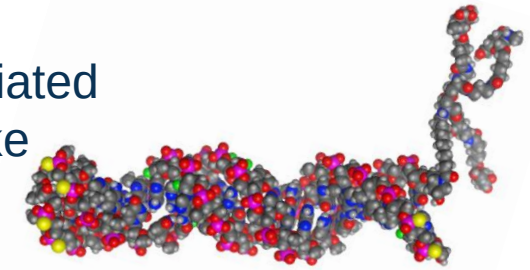
GalNAc-siRNA conjugates for hepatocyte delivery

RNAi, RNA interference siRNA, small-interfering RNA RISC, RNA Induced Silencing Complex GalNAc, N-Acetylgalactosamine ASGPR, asialoglycoprotein receptor

Adaptation of the siRNA Platform for CNS Delivery

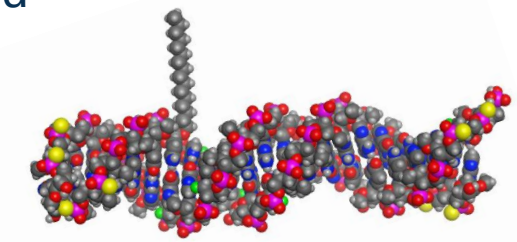
GalNAc

- ASGPR-mediated hepatic uptake



C16

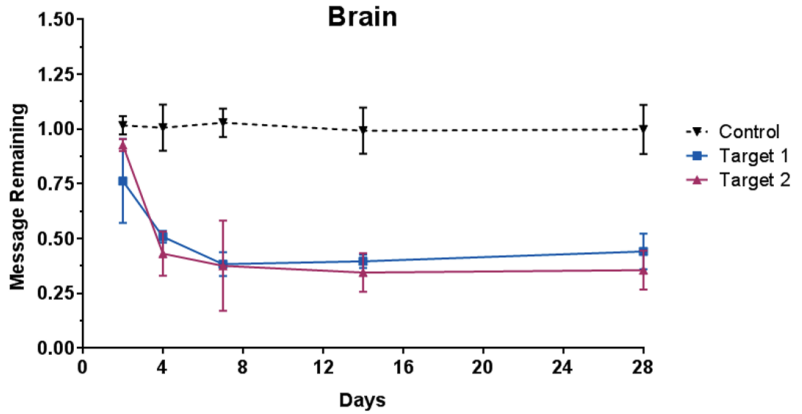
- Lipid-facilitated tissue distribution and/or cell uptake



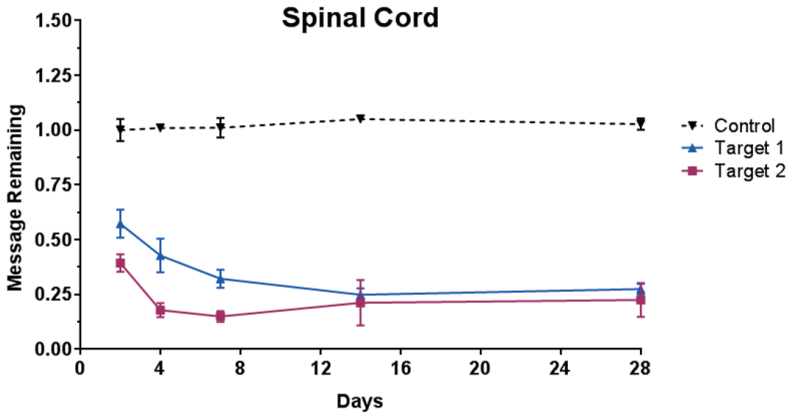
- VP-mediated RISC loading

Single Intrathecal Dose in Rat

- Sequence specific siRNAs designed against two unique gene targets



- Demonstration of target engagement across CNS (brain and spine after IT administration of siRNA

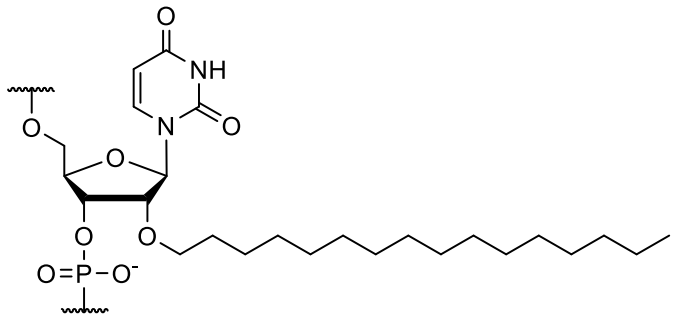


Modified from 2018 TIDES: Delivering on RNAi Therapeutics: Patisiran and Beyond.

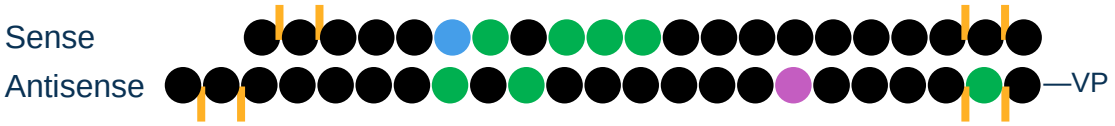
siRNA, small-interfering RNA GalNAc, N-Acetylgalactosamine ASGPR, asialoglycoprotein receptor VP, vinylphosphonate CNS, Central Nervous System IT, Intrathecal

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

2'-O-palmityl (C16) nucleotide

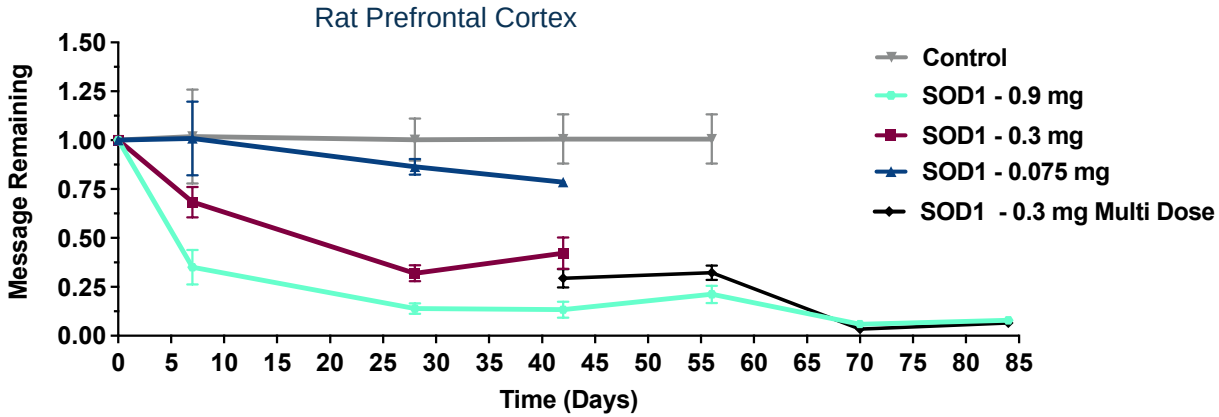


2′-C16

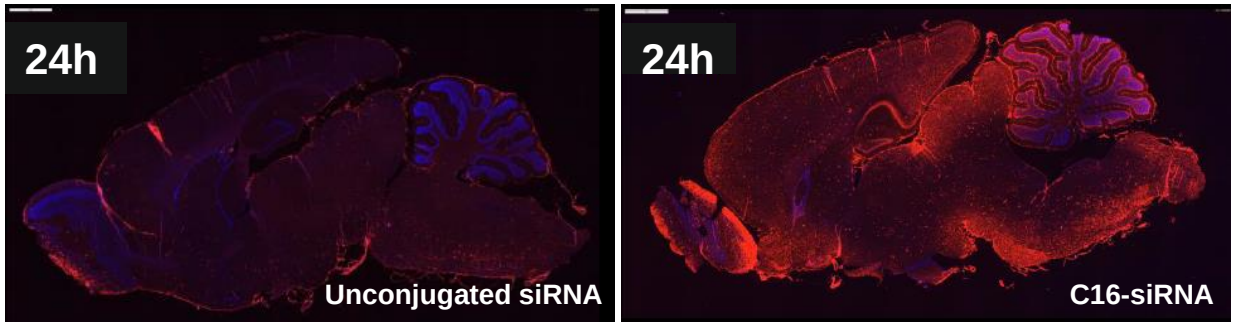


2′-OMe
2′-F
VP Vinyl phosphonate
Glycol nucleic acid (GNA)
Phosphorothioate

Example Rodent PD Data



Distribution

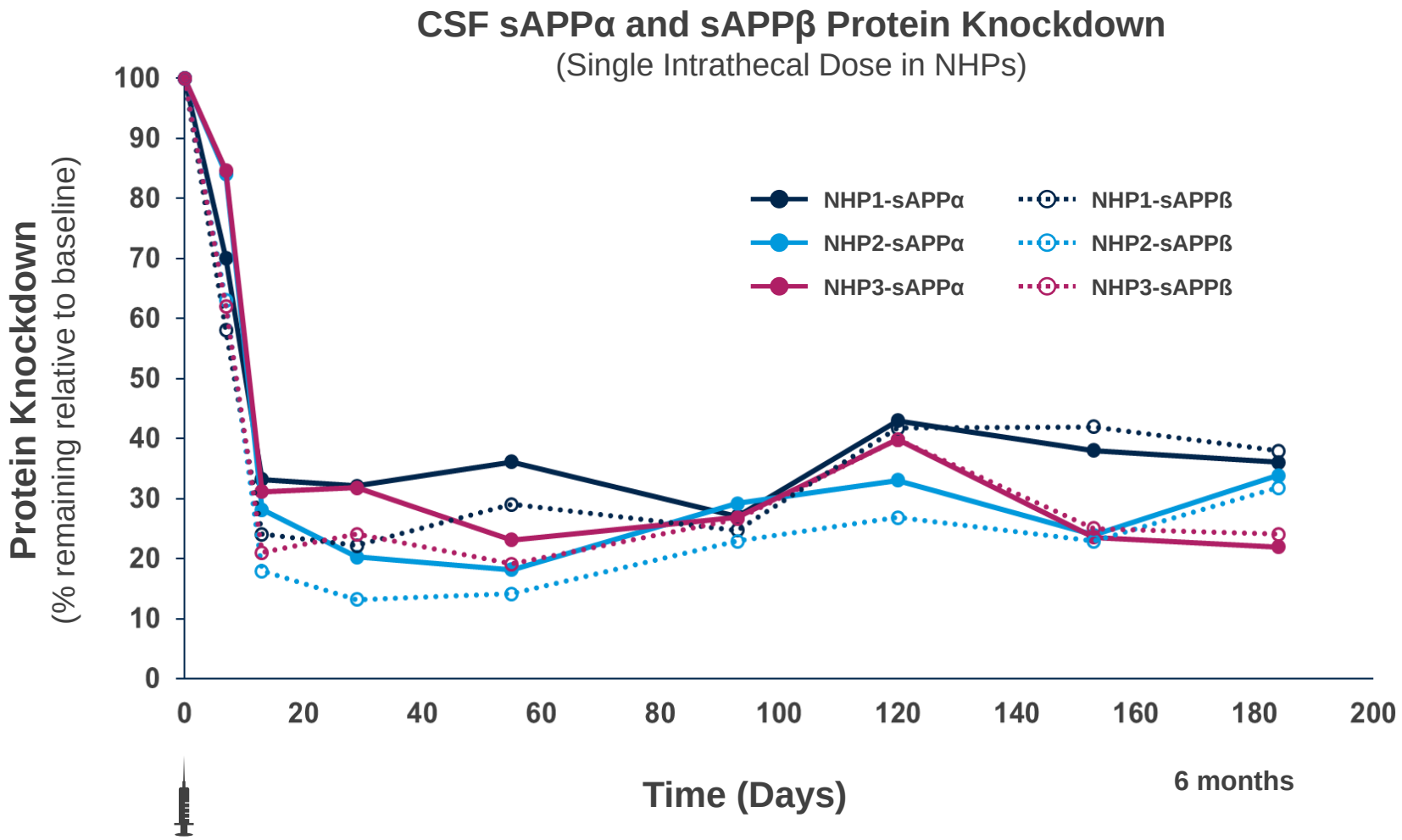


Unconjugated or C16-modified siRNA administered as single IT bolus injection to rats at 0.9 mg. siRNA biodistribution was assessed in whole brain at 24 h post-dose using IHC with anti-siRNA antibody

Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates
Brown et al., Nature Biotechnology, 2022

Potent, Durable Reduction of sAPPα and β in CNS of NHPs

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



Results

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

From 2023 ADPD, Kirk Brown

APP, amyloid precursor protein; CNS, central nervous system; CSF, cerebrospinal fluid; IT, intrathecal; mRNA, messenger RNA; NHP, non-human primate; RNA, ribonucleic acid; sAPP, soluble APP

Potential Applications for C16-siRNA Conjugates

Devastating diseases with enormous burden and unmet need

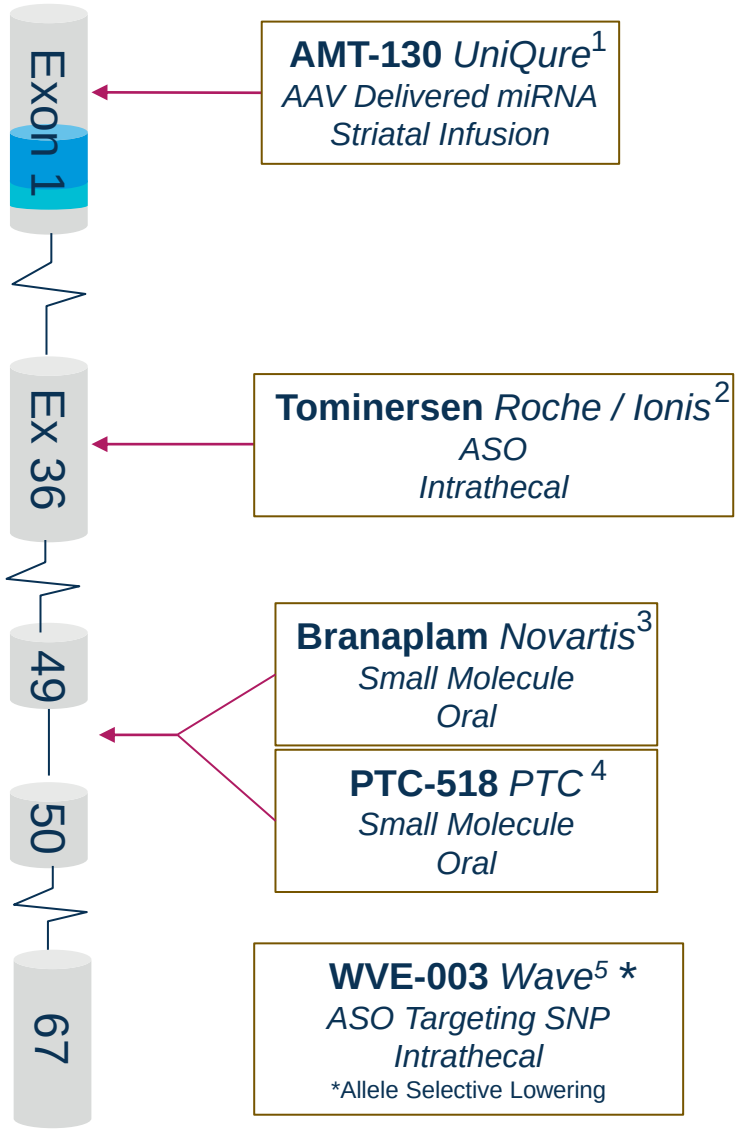
Many dominantly inherited neurodegenerative diseases:

- 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



AMT-130 *UniQure*[1]
AAV Delivered miRNA
Striatal Infusion

Tominersen *Roche / Ionis*[2]
ASO
Intrathecal

Branaplam *Novartis*[3]
Small Molecule
Oral

PTC-518 *PTC*[4]
Small Molecule
Oral

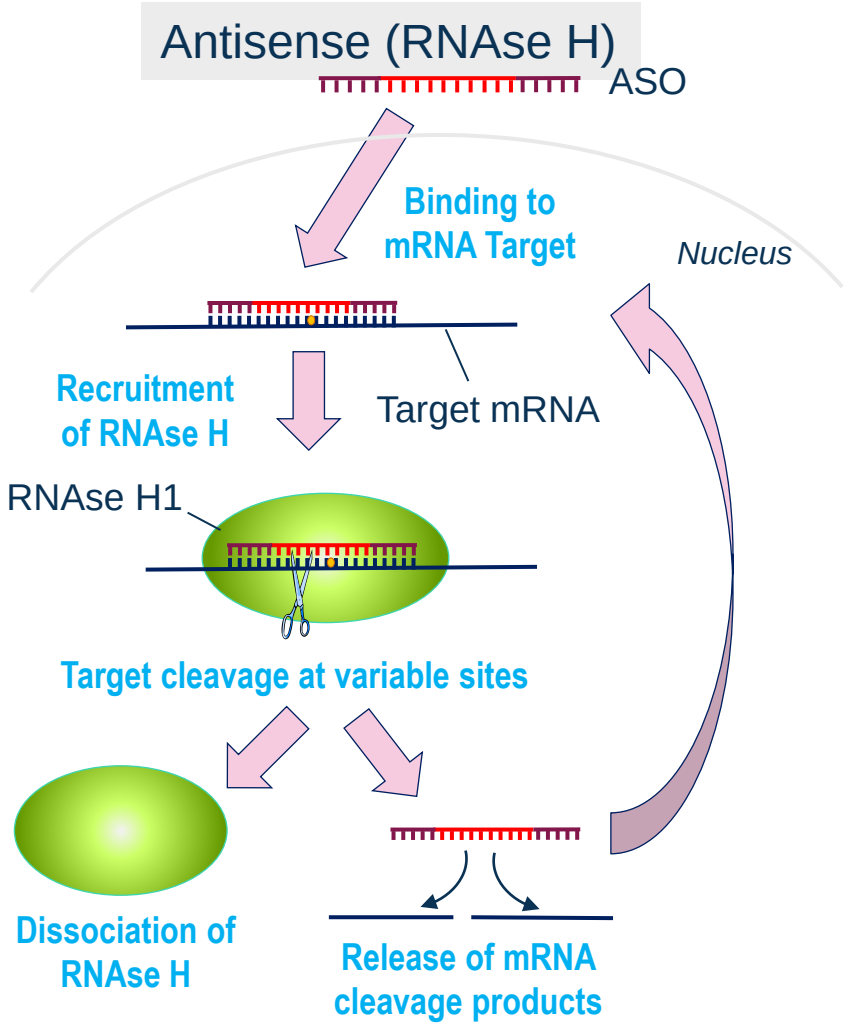
WVE-003 *Wave*[5] *
ASO Targeting SNP
Intrathecal
*Allele Selective Lowering


6
Neuron
Review
Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease
7
nature COMMUNICATIONS
ARTICLE
OPEN
Small molecule splicing modifiers with systemic HTT-lowering activity
8
JAMA Neurology | Clinical Implications of Basic Neuroscience Research
Huntingtin-Lowering Therapies for Huntington Disease
A Review of the Evidence of Potential Benefits and Risks

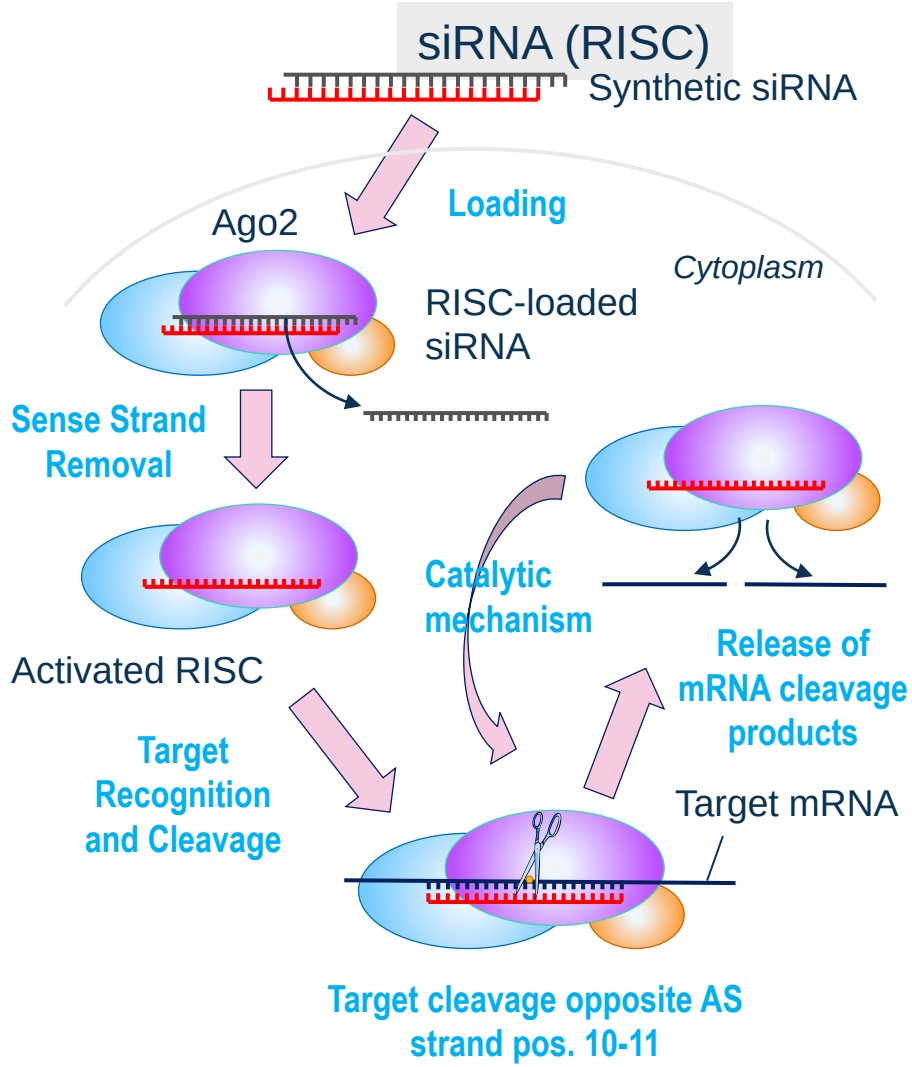
[1] UniQure, 2022
[2] Tabrizi et.al., NEJM, 2019
[3] Keller et.al., Nature Communications, 2022
[4] PTC Bio, 2021
[5] Carroll et.al. Molecular Therapy, 2011
[6] Tabrizi et.al., *Neuron,* 2019
[7] Bhattacharyya et.al, Nature Communications, 2021
[8] Leavitt et.al, JAMA Neurology, 2020

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

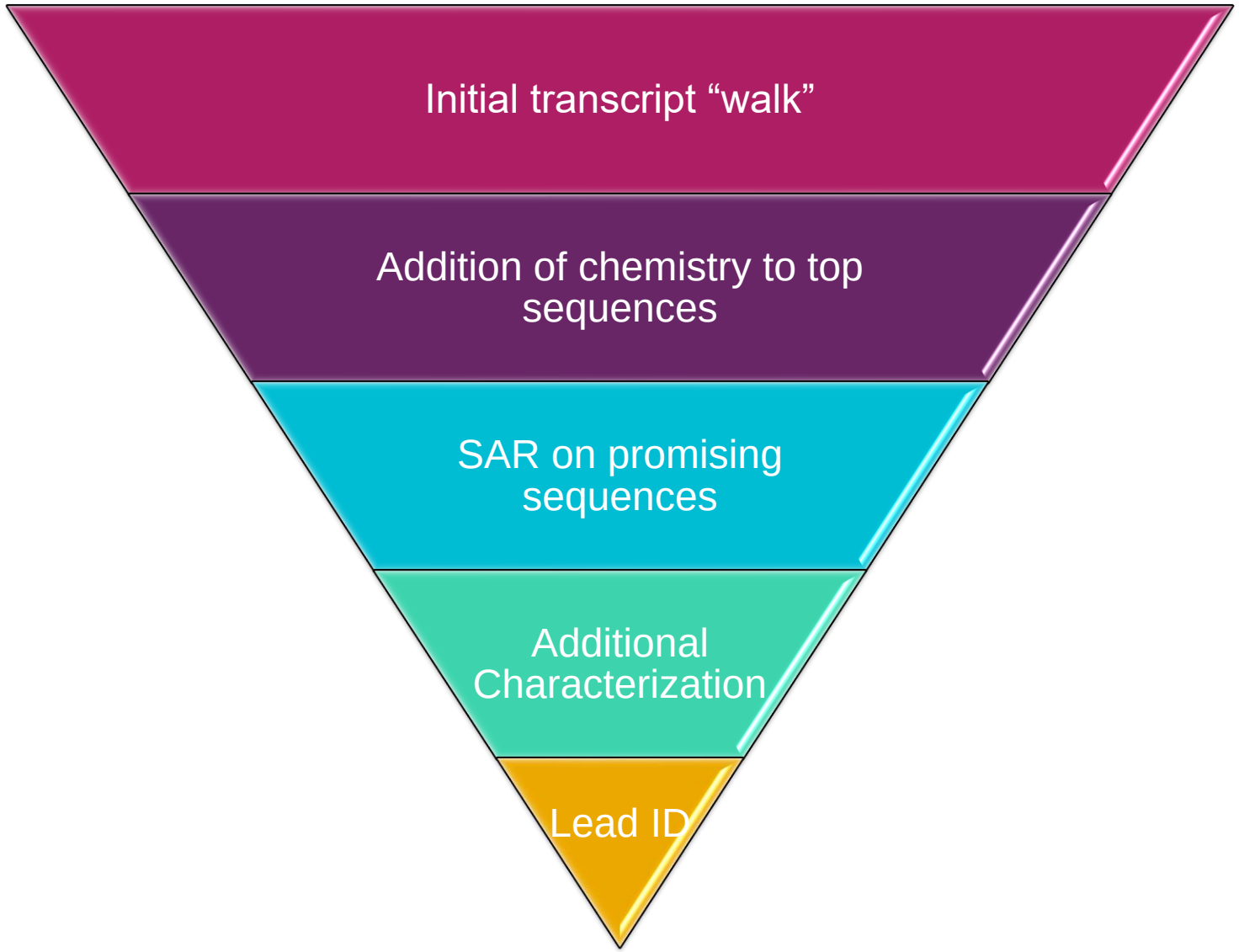
Impact of Targeting Location on HTT mRNA

👍 Desired
👎 Undesired

mRNA Targeted	HTT siRNA Targeting Strategy			
	HTT_{EX1} siRNA	HTT_{IN1} siRNA	HTT_{FL} siRNA	HTT_{SNP} siRNA
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"

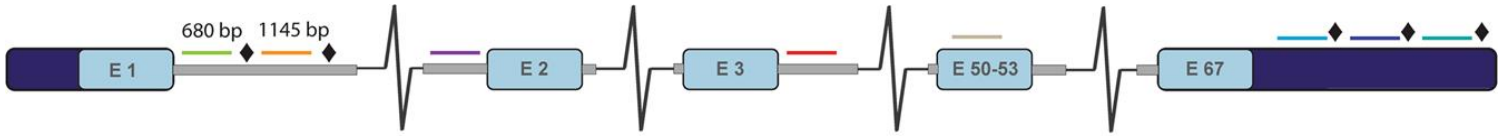


- Initial Screen for Absolute Potency
- Variations to improve sequence specificity
- Modifications to increase drug like properties
- In vivo characterization of top performers

SAR: Structure-Activity Relationship

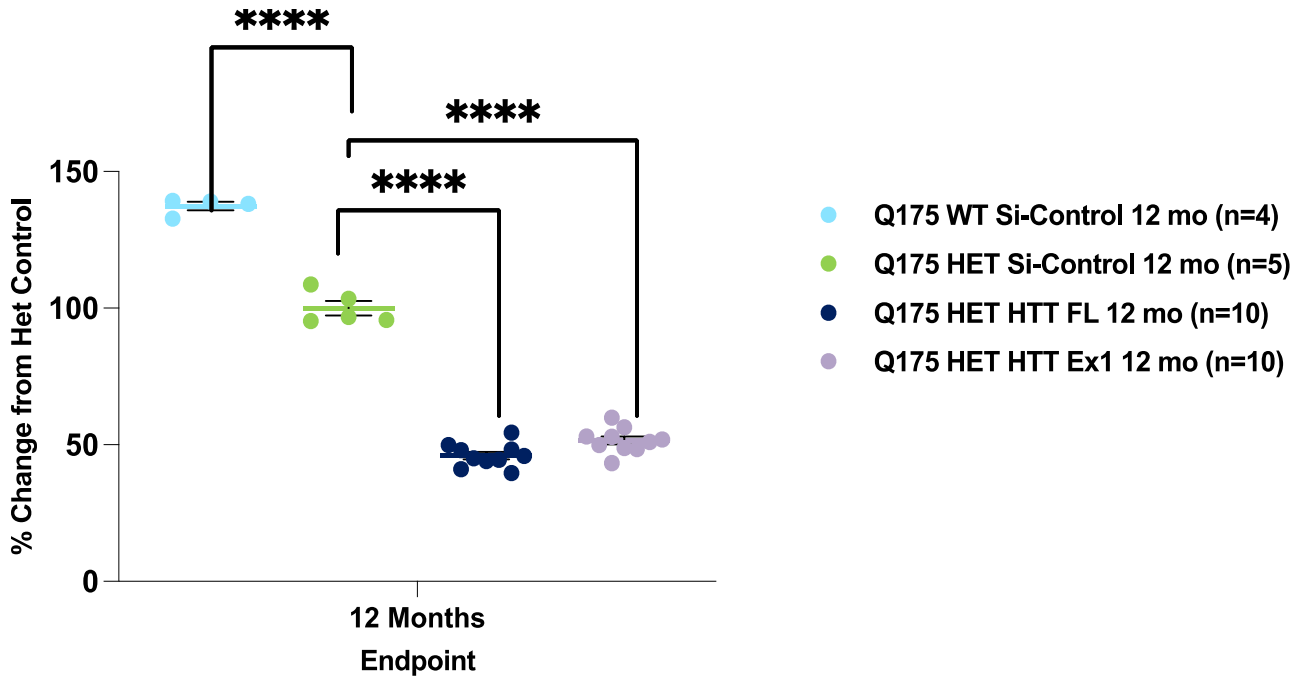
HTT Transcript Engagement Impacted by Targeting Location

Q175 mouse Striatal Tissue; 3-Month Post-Dose

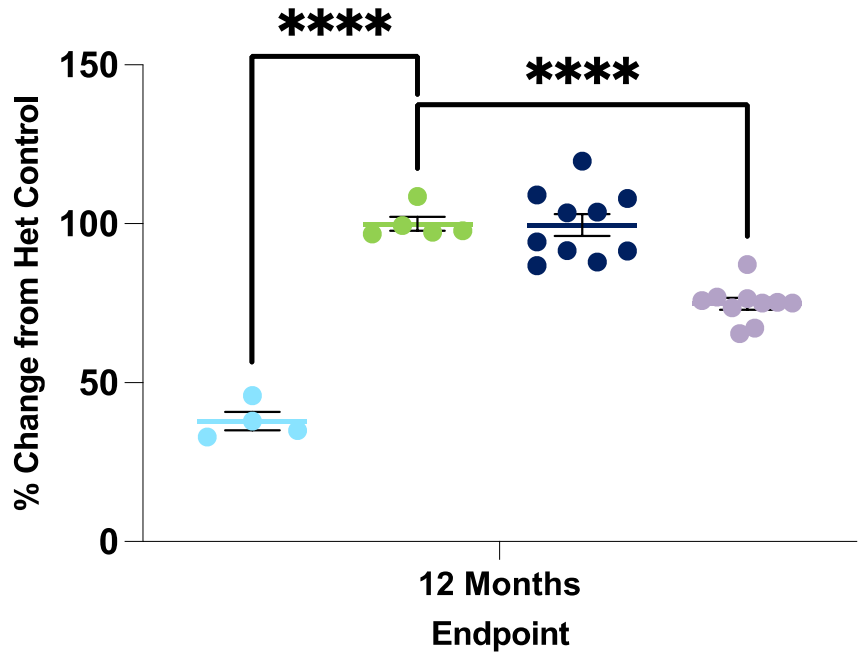


Papadopoulou et al., Sci. Rep. 2019

Full-Length HTT Transcript



HTT1a Fragment Transcript



Both FL and Ex1 Targeting siRNA reduce HTT FL transcript

Only Ex1 Targeting siRNA reduces HTT1a transcript

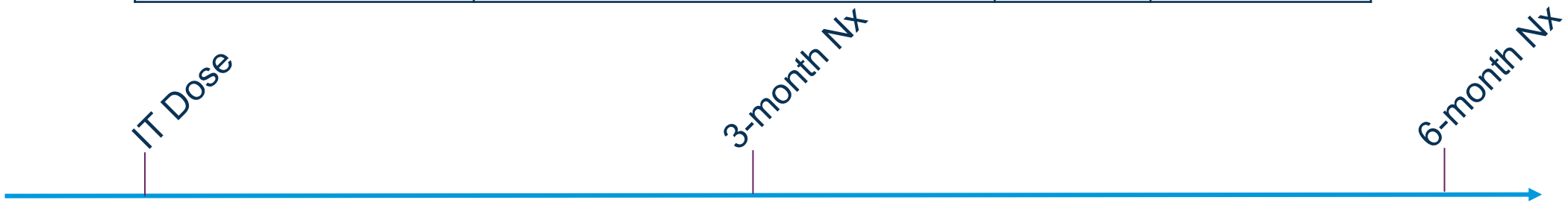
The QuantiGene Plex Assay. Data are presented as mean ± SEM, n = 3-10 per group. Statistical significances **** $p < 0.0001$, Q175 WT Si-Control 12 mo & Q175 HET HTT Ex1 12 mo vs. Q175 HET Si-Control 12 mo (Unpaired t test with Welch correction/Welch's ANOVA test, Dunnett's T3 multiple comparisons test).

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



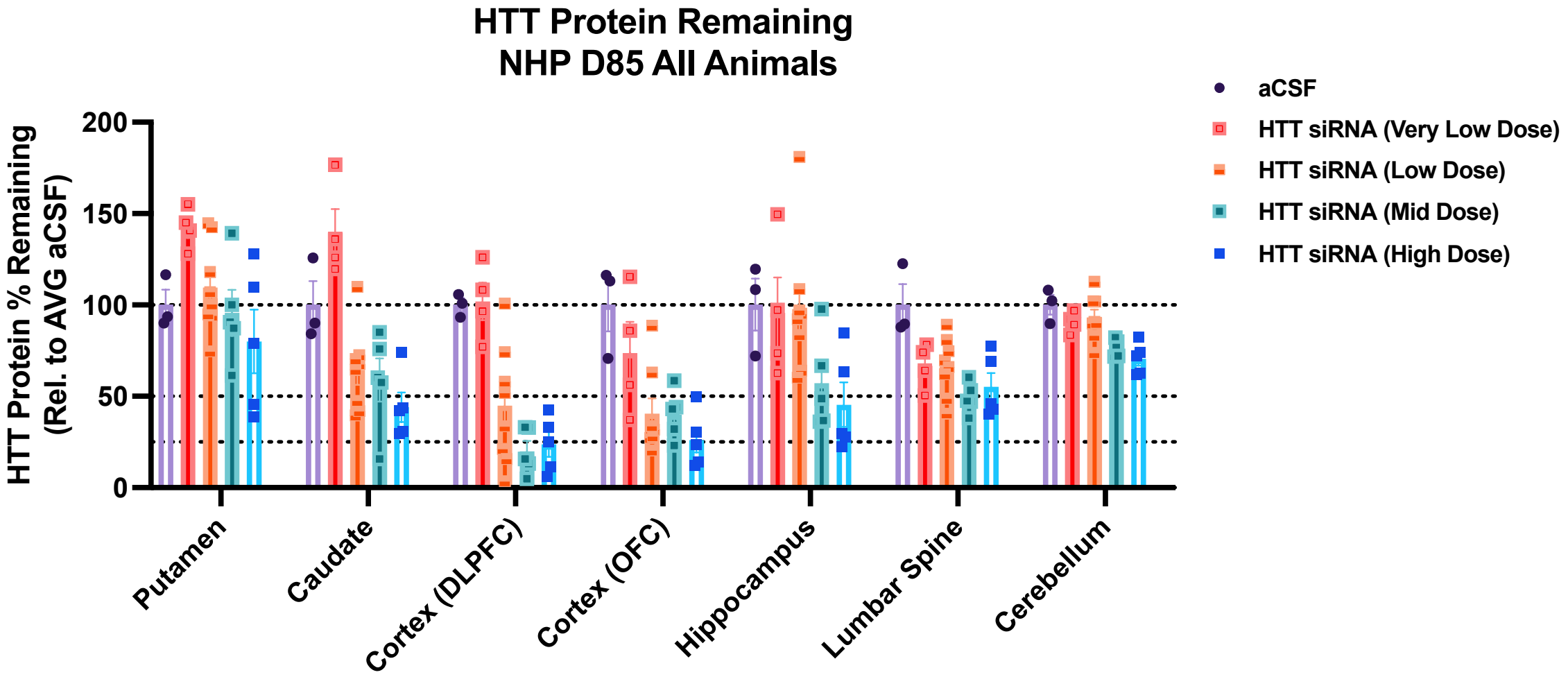
In Life
- Weekly Clinical Obs.
- Monthly Neuro Evals.
- Serial CSF Collections

Terminal
- Tissue KD
- Histopathological Evaluation

NHP – Non-Human Primate
IT – Intrathecal
KD - Knockdown
Nx - Necropsy

Characterizing HTT Target Engagement in NHP with a C16-siRNA

HTT Protein Remaining 3-Months After Single IT Dose



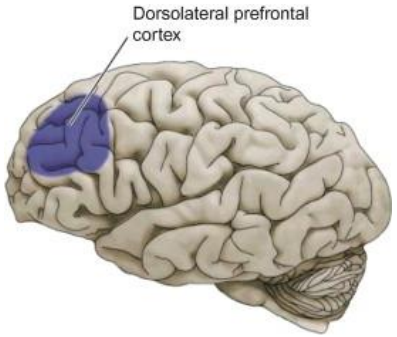
DLPFC- Dorsolateral Prefrontal Cortex

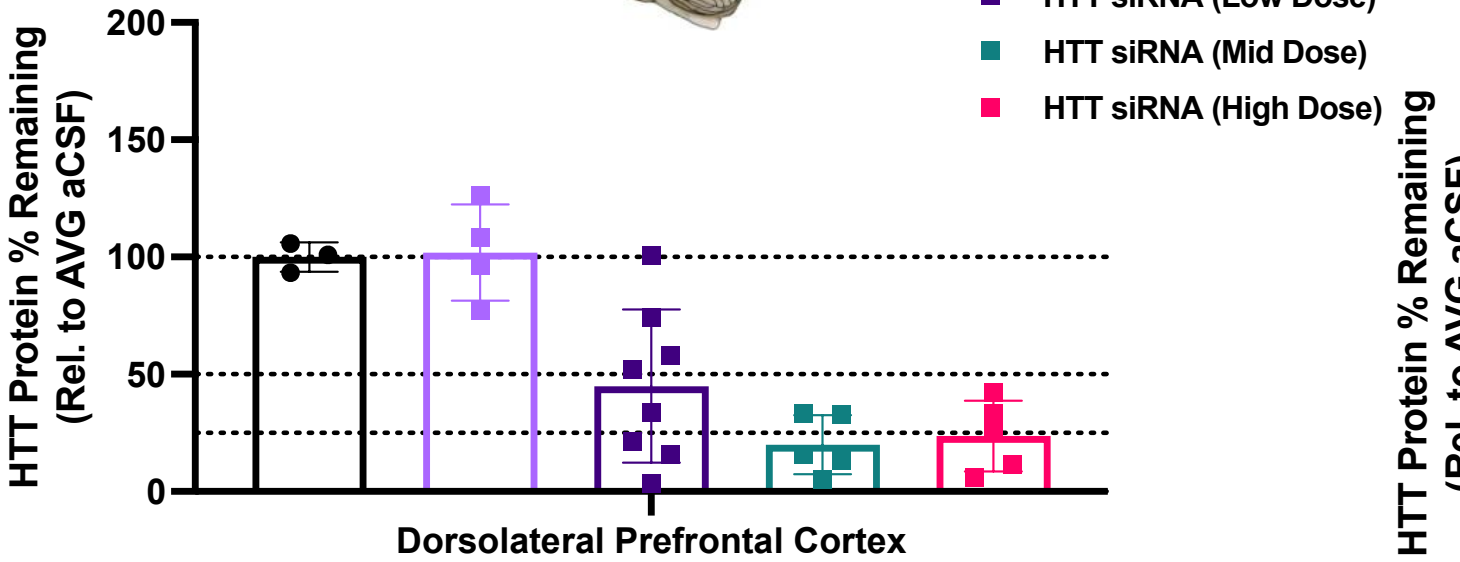
OFC – Orbitofrontal Cortex

Characterizing HTT Target Engagement in NHP with a C16-siRNA

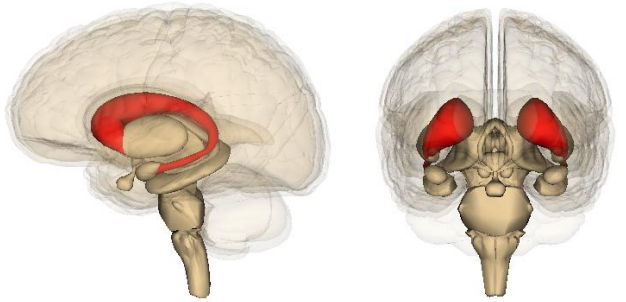
HTT Protein Reduction in Cortex and Caudate 3 months after single IT dose

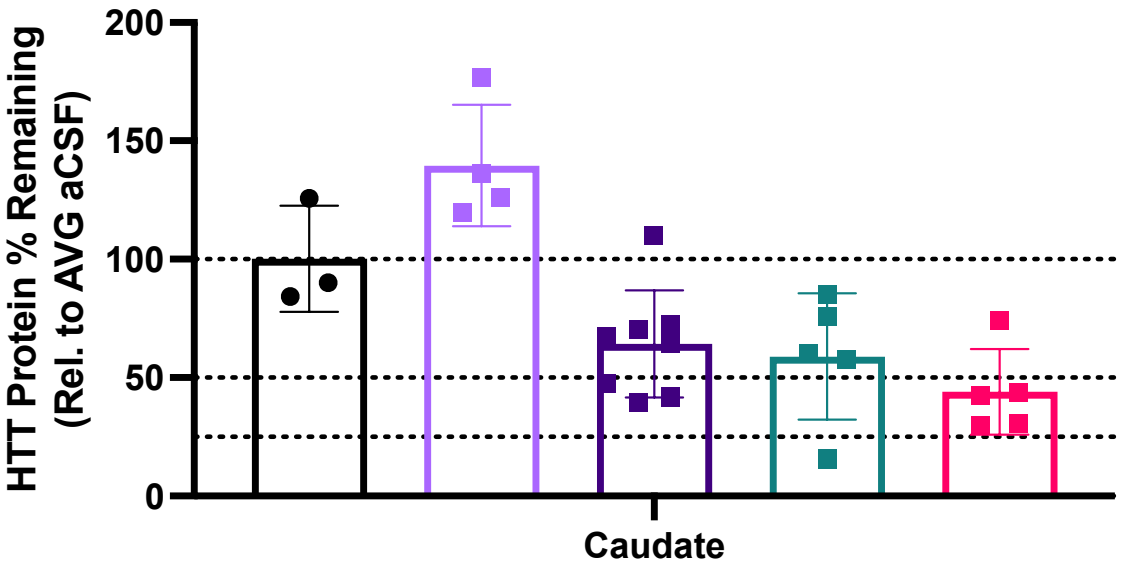
HTT Protein Remaining DLPFC





HTT Protein Remaining Caudate

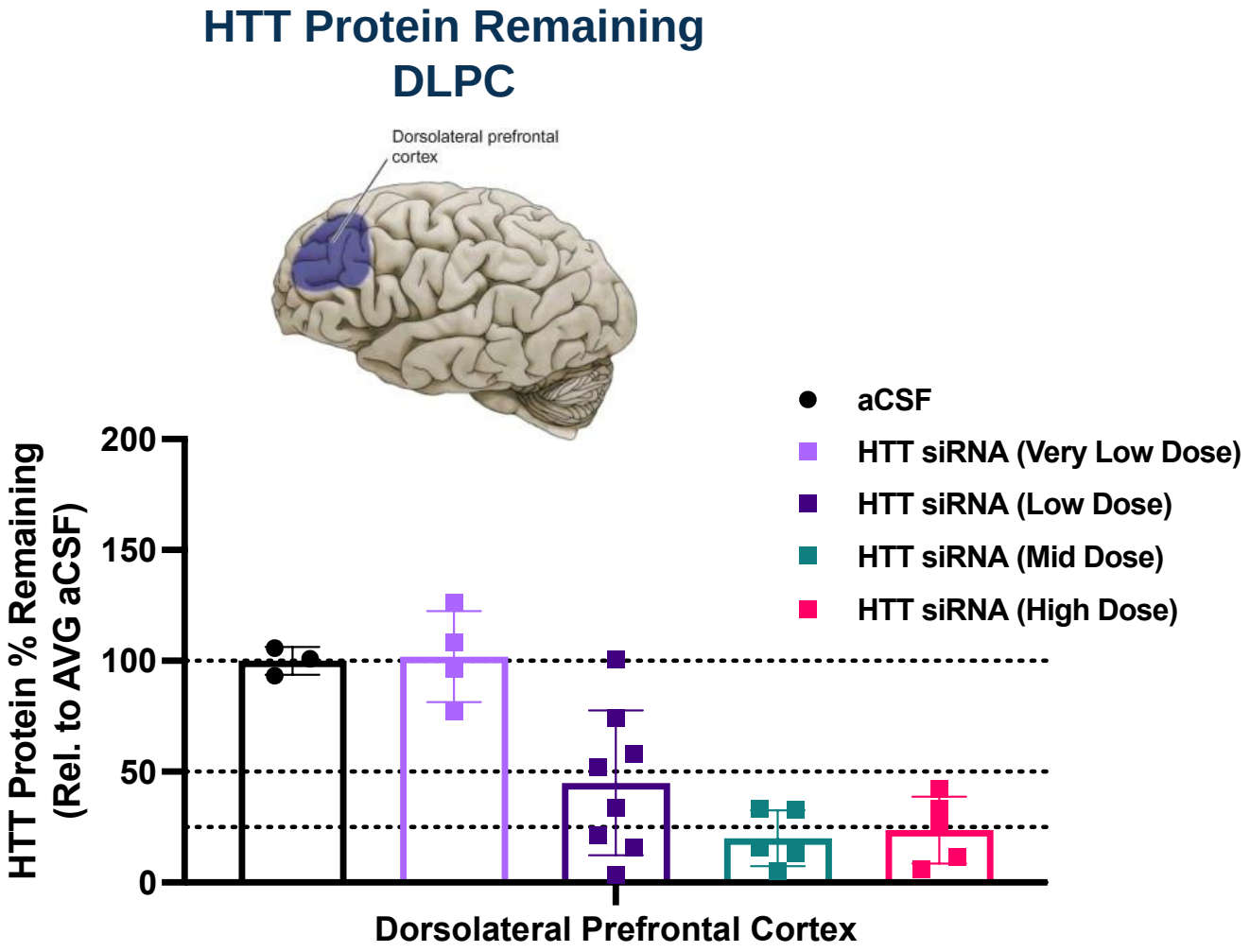


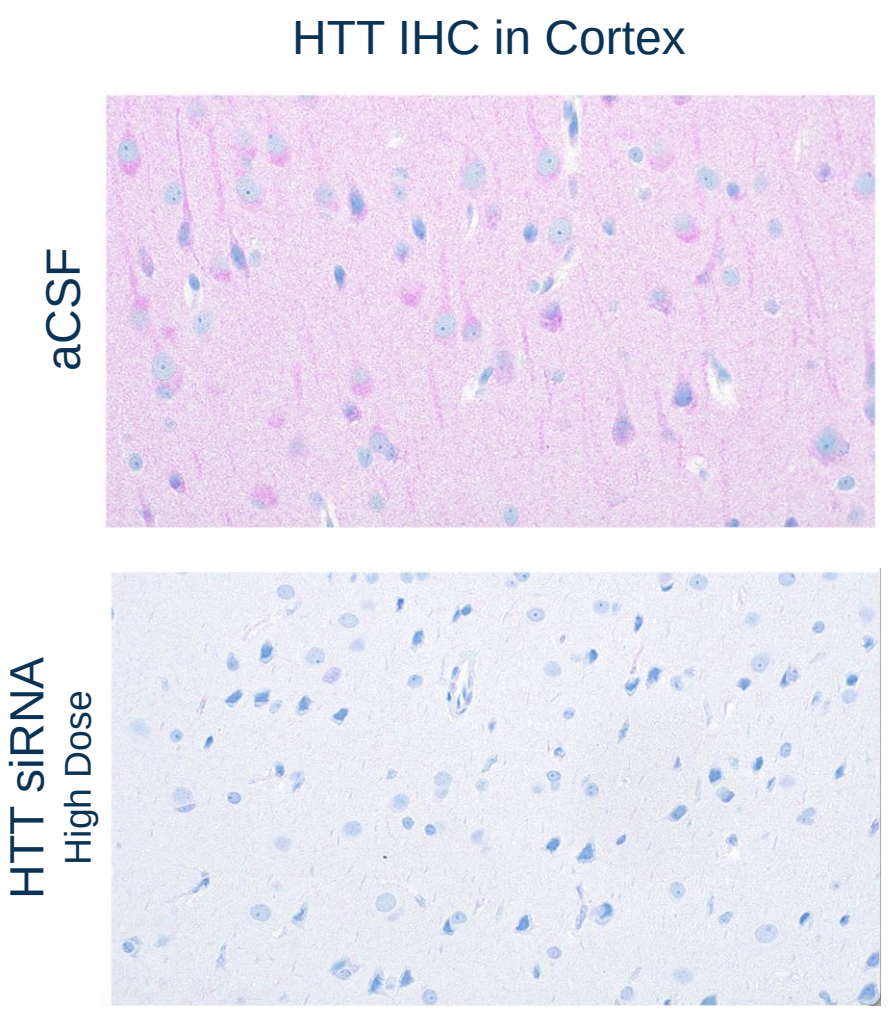


DLPFC: Dorsolateral Prefrontal Cortex

Characterizing HTT Target Engagement in NHP with a C16-siRNA

HTT Protein Reduction in Cortex 3 months after single IT dose





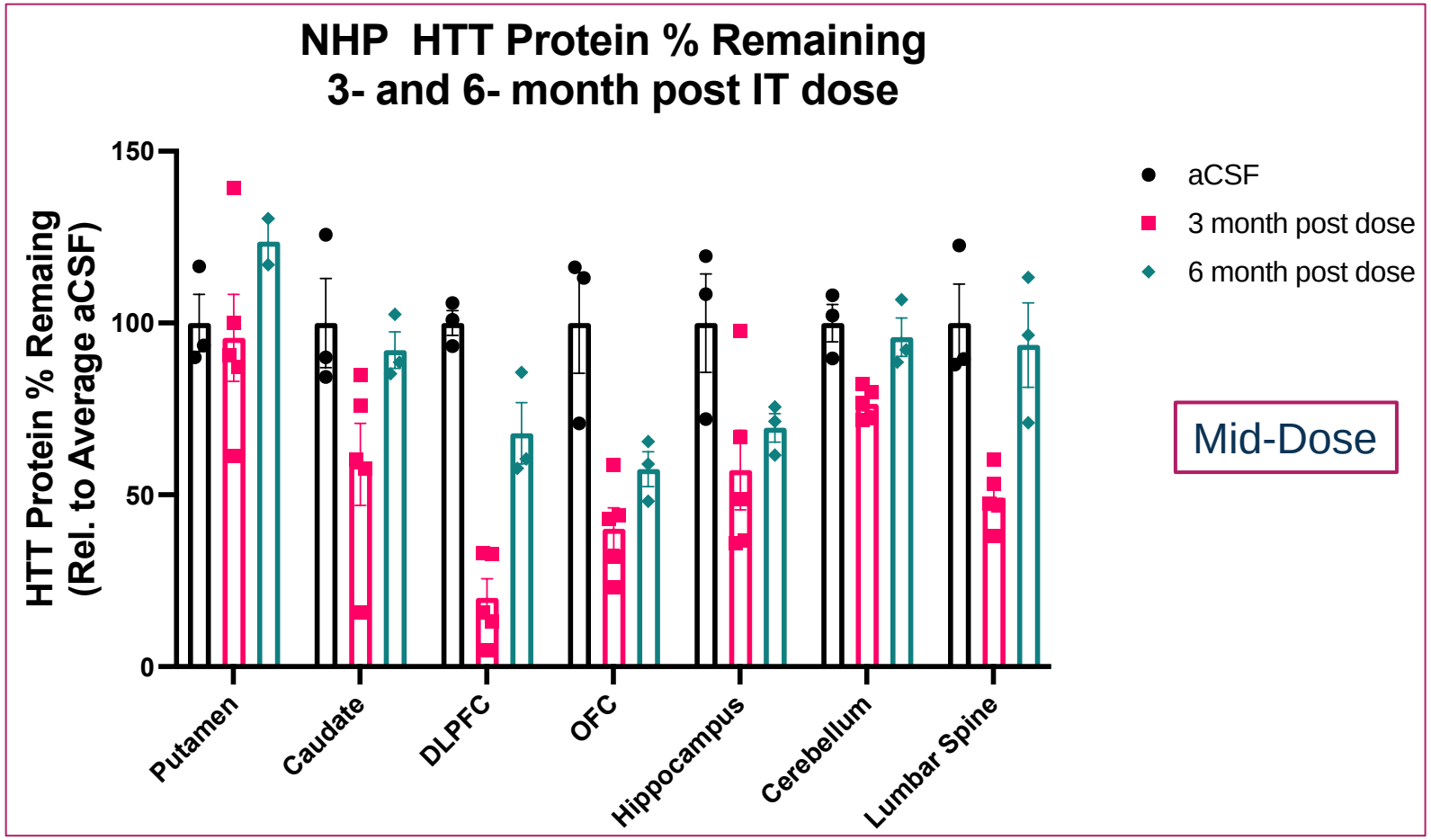
DLPC: Dorsolateral Prefrontal Cortex

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

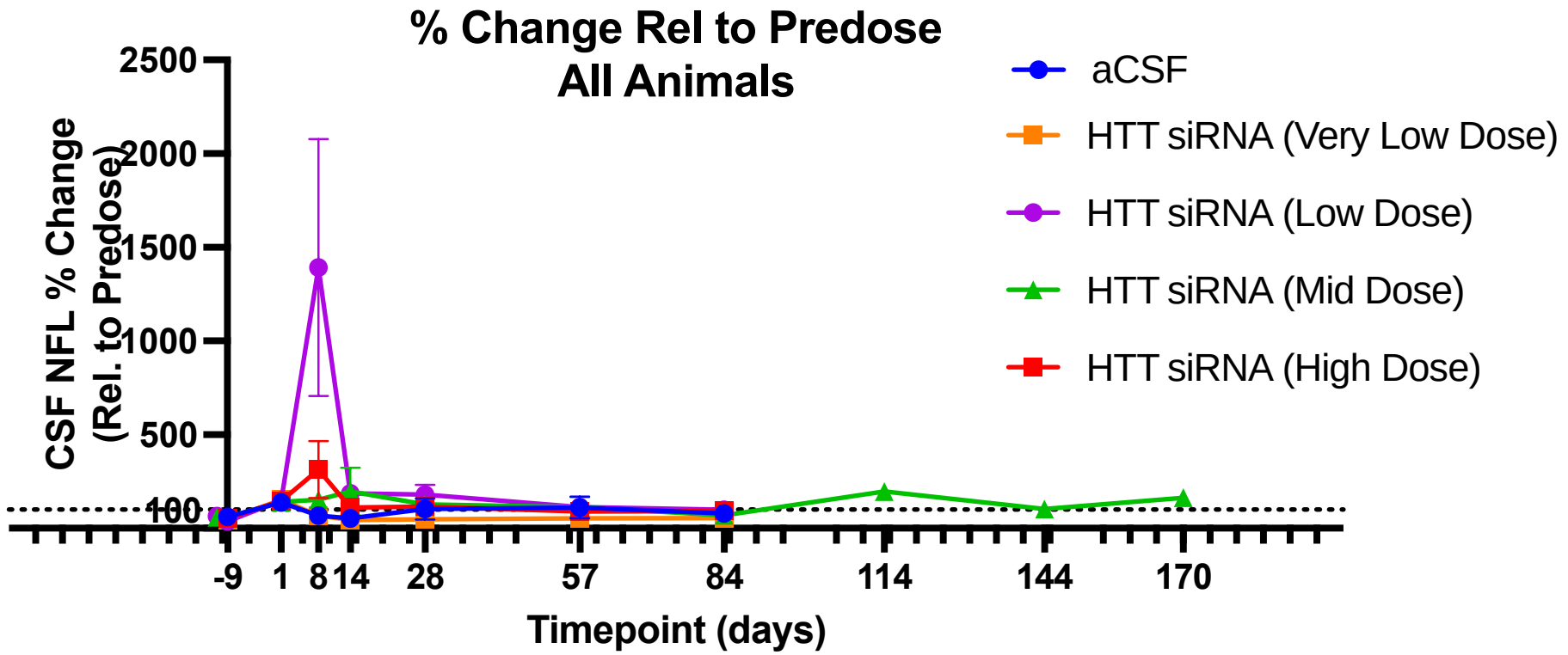
NfL from Longitudinal CSF Collections, Histopath Evaluation from Terminal Tissue

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



IT – Intrathecal NHP – non-human primate H&E - hematoxylin and eosin CSF – cerebrospinal fluid NFL – Neurofilament Light

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?