Progress in Extrahepatic Silencing with siRNA Conjugates

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Alnylam PHARMACEUTICALS

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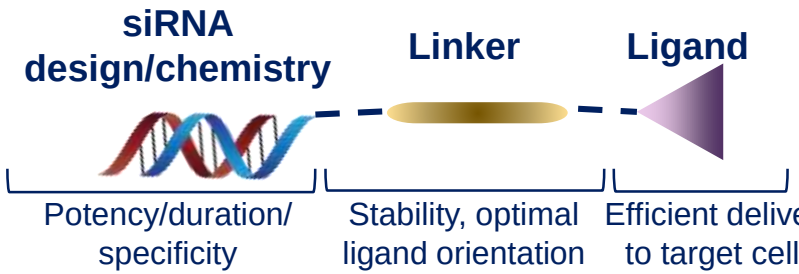
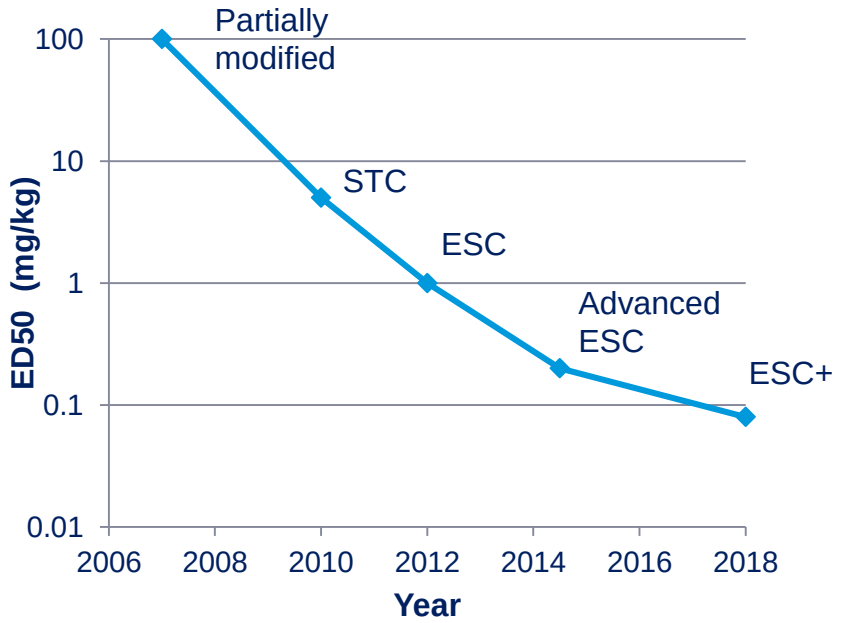
Transformative Advancements in Conjugate-Based Delivery

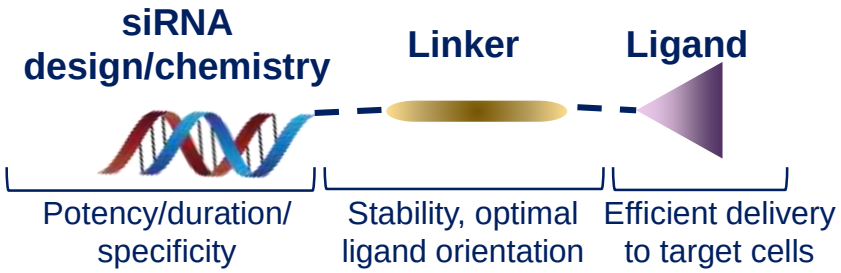
siRNA designs with enhanced potency and stability may extend to extrahepatic tissues



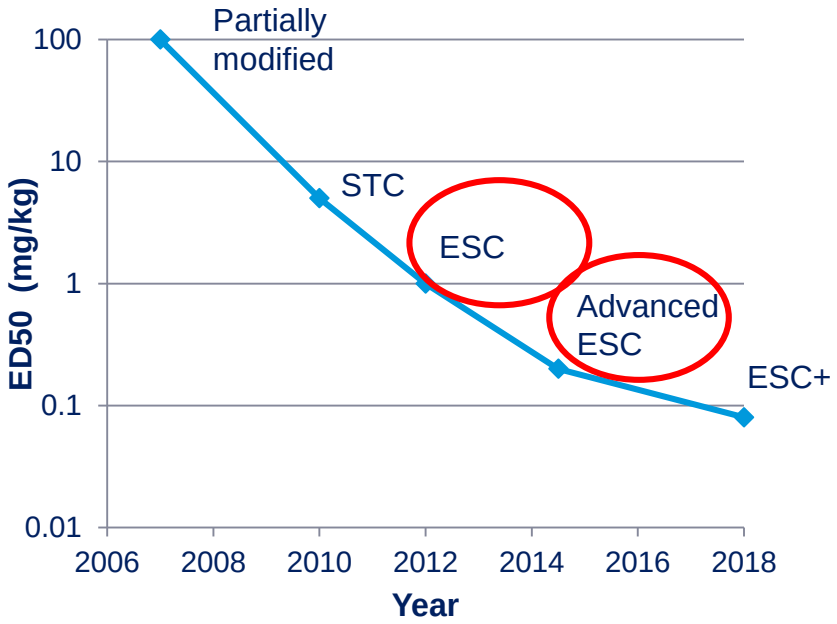
Evolution of conjugate potency (mouse, SD ED_{50})



Extensive Durability and Safety Demonstrated in Liver Programs



Evolution of conjugate potency (mouse, SD ED_{50})



Alnylam Clinical Development Pipeline

Focused in 4 Strategic Therapeutic Areas (STArs):

- Genetic Medicines
- Cardio-Metabolic Diseases
- Hepatic Infectious Diseases
- CNS & Ocular Diseases

		HUMAN POC[1]	BREAKTHROUGH DESIGNATION	EARLY STAGE (IND or CTA Filed-Phase 2)	LATE STAGE (Phase 2-Phase 4)	REGISTRATION/ COMMERCIAL[3]	COMMERCIAL RIGHTS
ONPATTRO® (patisiran)[2]	*Hereditary ATTR Amyloidosis*	✓	✓			●	Global
Givosiran	*Acute Hepatic Porphyria*	✓	✓			●	Global
Patisiran	*ATTR Amyloidosis Label Expansion*	✓			●		Global
Fitusiran	*Hemophilia and Rare Bleeding Disorders*	✓			●		15-30% royalties
Inclisiran	*Hypercholesterolemia*	✓			●		Milestones & up to 20% royalties
Lumasiran	*Primary Hyperoxaluria Type 1*	✓	✓		●		Global
Vutrisiran	*ATTR Amyloidosis*	✓			●		Global
Cemdisiran	*Complement-Mediated Diseases*	✓		●			Global
ALN-AAT02	*Alpha-1 Liver Disease*			●			Subject to partner option rights
ALN-HBV02 (VIR-2218)	*Hepatitis B Virus Infection*			●			50-50 option rights post-Phase 2
ALN-AGT	*Hypertension*			●			Global

[1]POC, proof of concept – defined as having demonstrated target gene knockdown and/or additional evidence of activity in clinical studies
[2]Approved in the U.S. for the polyneuropathy of hATTR amyloidosis in adults, and in the EU for the treatment of hATTR amyloidosis in adults with stage 1 or stage 2 polyneuropathy
[3]Includes marketing application submissions

As of March 2019

Investigational RNAi Therapeutics for CNS and Ocular Diseases

Expanding Alnylam opportunities beyond liver

Devastating diseases with enormous burden and unmet need

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

- AMD, dry
- AMD, wet
- Birdshot chorioretinopathy
- Dominant retinitis pigmentosa 4
- Fuch's dystrophy
- hATTR amyloidosis
- Hereditary and sporadic glaucoma
- Stargardt's disease

Number of genetically validated targets known but few disease modifying therapies for these devastating or life threatening disorders.

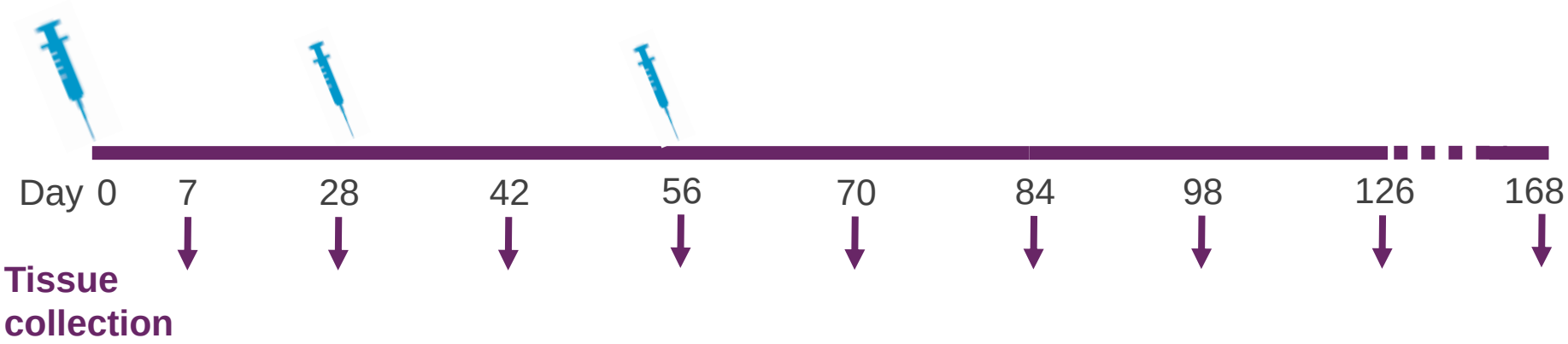
RNAi therapeutics directed to disease-causing, CNS- or ocular-expressed genes represent a potential opportunity to address diseases with some of the greatest unmet need.

Intrathecal Delivery of CNS Optimized siRNA Conjugates

Single dose and dose response in rat

siRNAs targeting β-catenin or SOD1 in single dose or dose response

- Single siRNA conjugate doses of 0.9 mg, 0.3 mg, 0.07 mg
- Multidose arm- 0.3 mg monthly x 5
- Time points through 1 month for β-catenin and 6 months for SOD1



Tissues: Spinal cord: Lumbar, thoracic and cervical

Brain: prefrontal cortex, cerebellum and remaining brain

Fluids: CSF and plasma

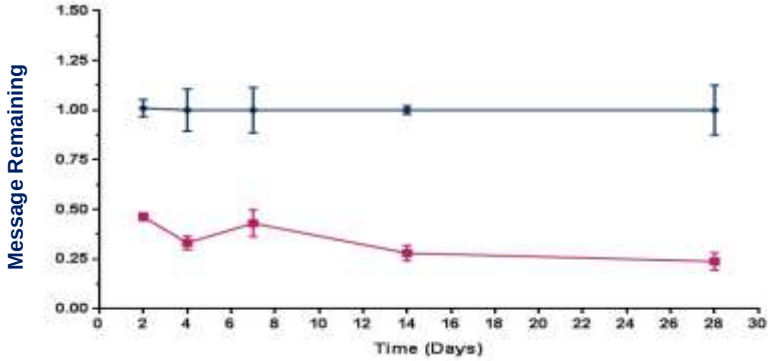
Assays: mRNA, tissue siRNA levels, Histology

	Strand (5'-3')	Sequence
β-catenin	S	UACUGUUGGAUUGAUUCGAAA
	AS	TUUCGAAUCAAUCCAACAGUAGC
SOD1	S	CAUUUUAAUCCUCACUCUAAA
	AS	UUUAGAGUGAGGAUUAAAAUGAG

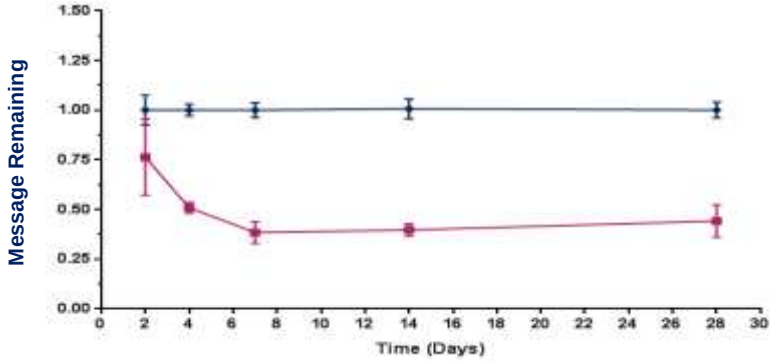
Robust and Durable Silencing Demonstrated Following a Single IT Dose

Silencing of β-catenin following a single IT dose

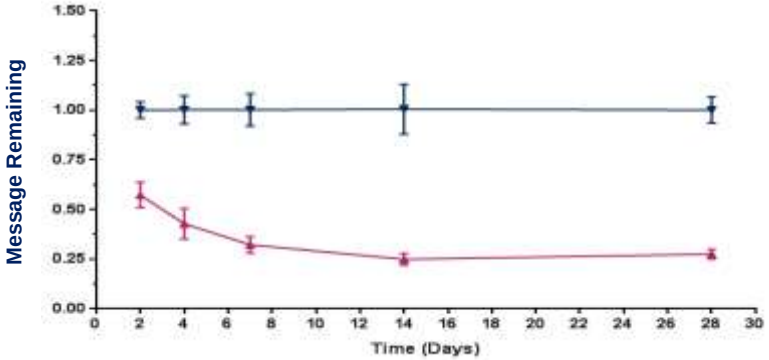
Lumbar



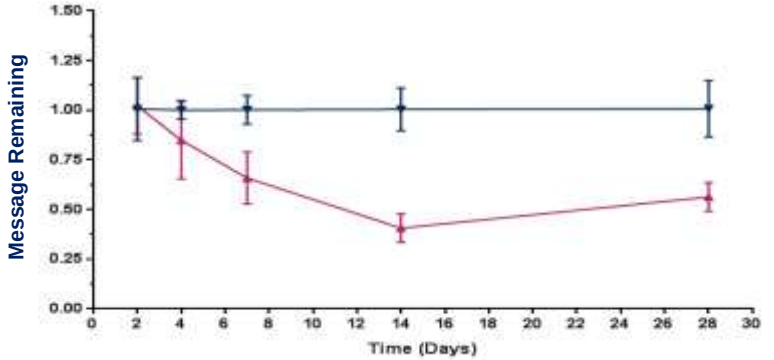
Cerebellum



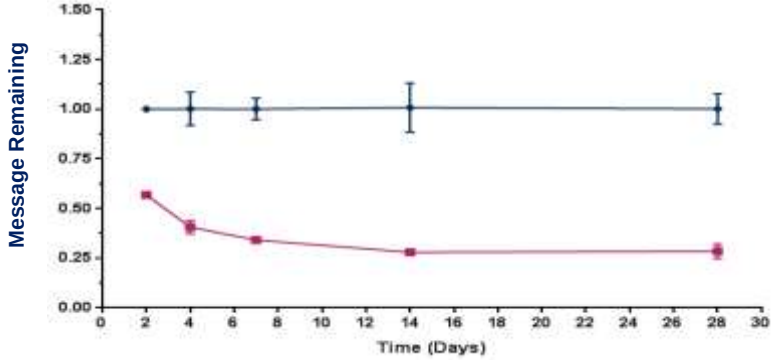
Thoracic



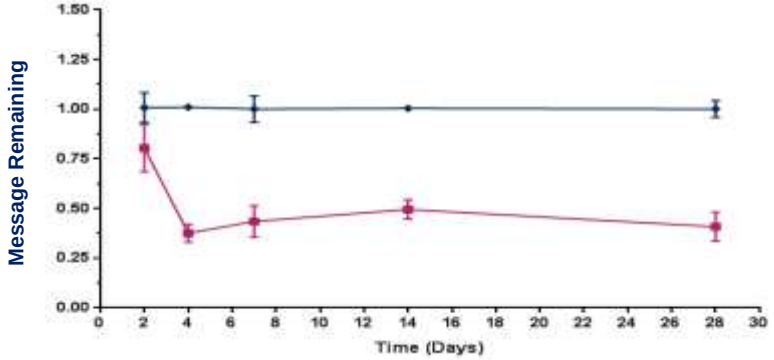
Frontal Cortex



Cervical



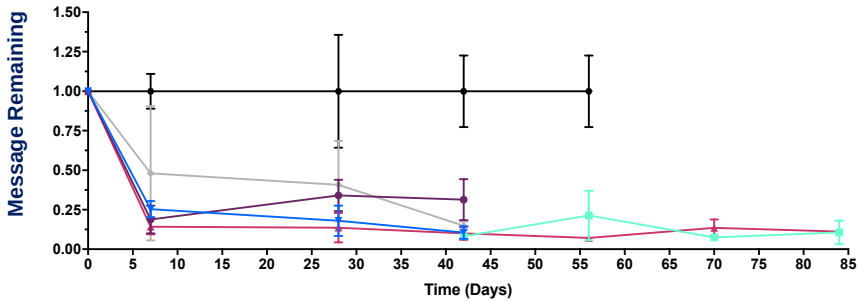
Remaining Brain



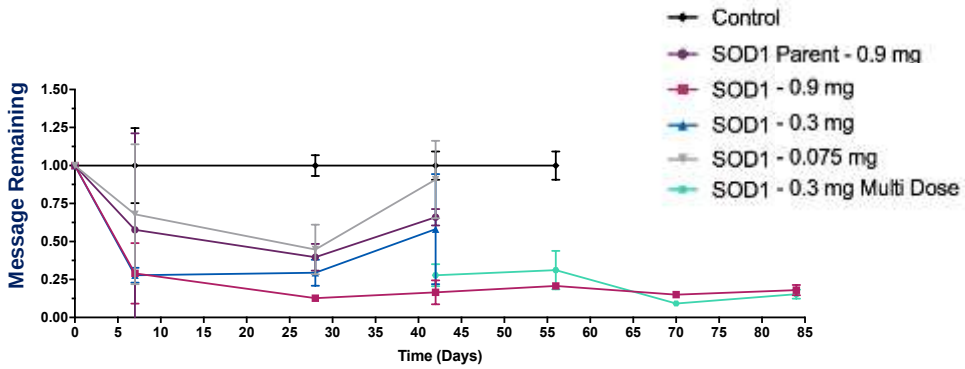
Robust and Durable Silencing Demonstrated Following a Single IT Dose

Silencing of SOD1 following a single or multiple IT doses

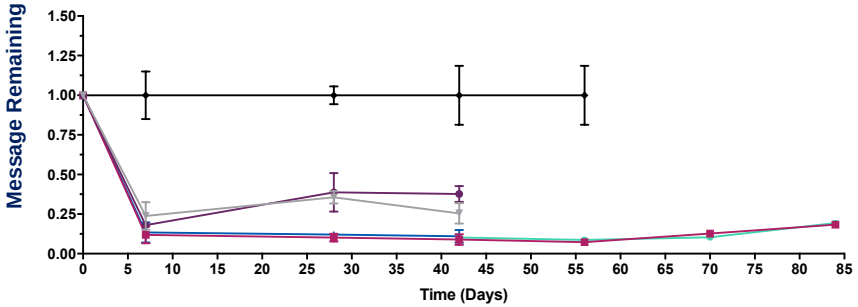
Lumbar



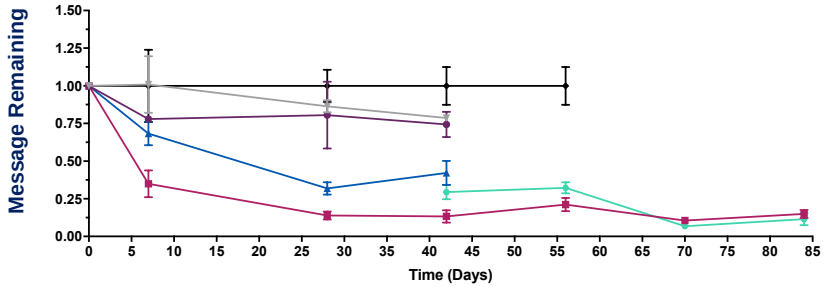
Cerebellum



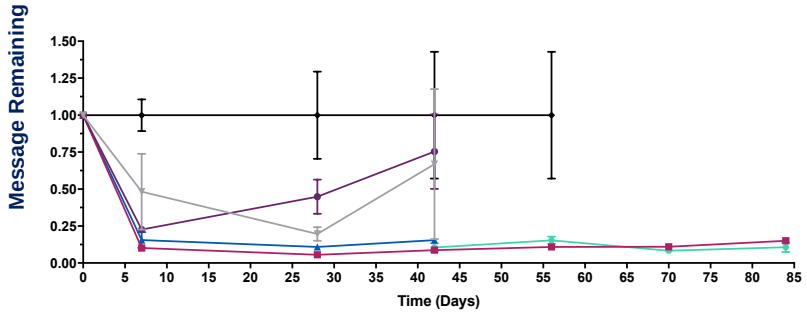
Thoracic



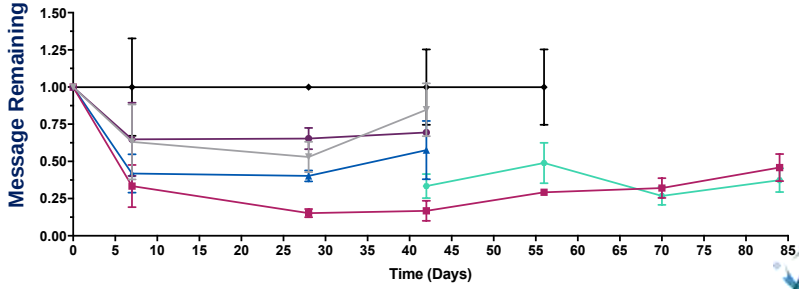
Frontal Cortex



Cervical

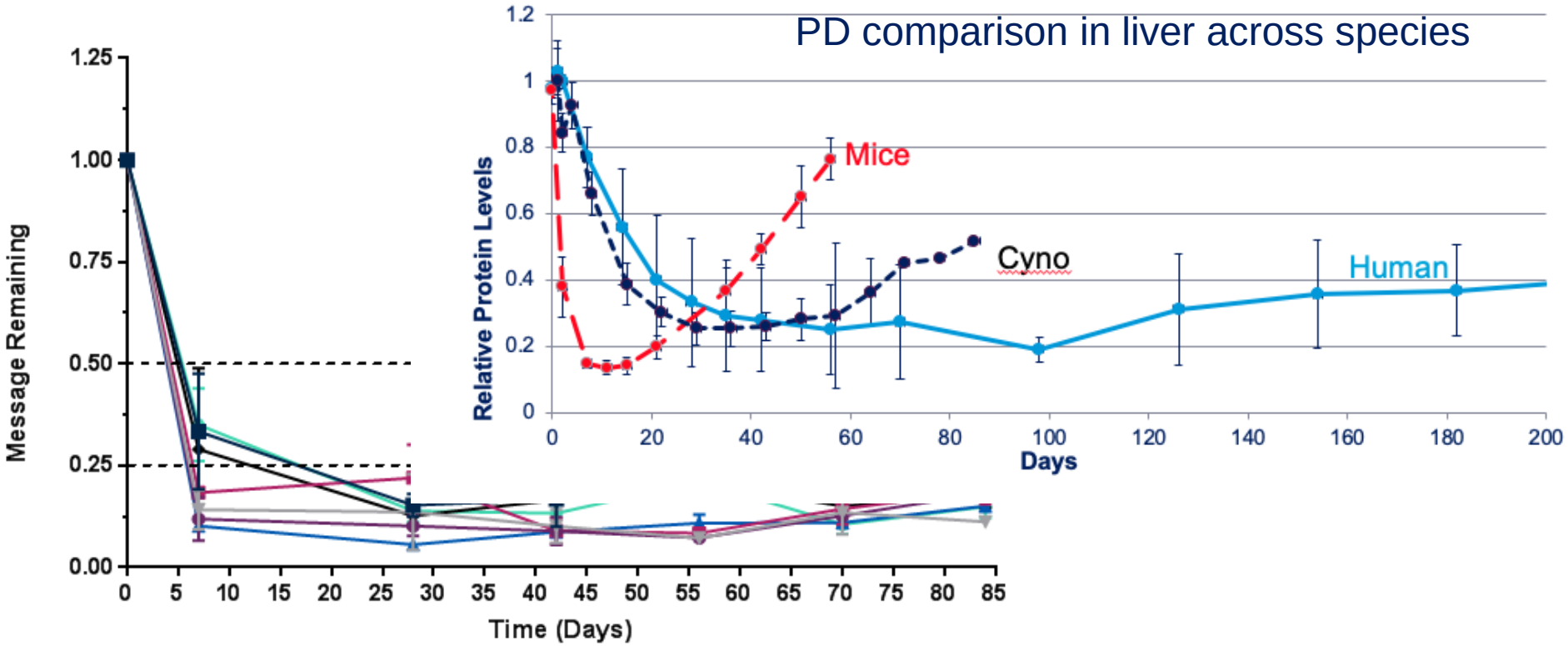


Temporal Cortex



Robust Silencing Throughout the Brain

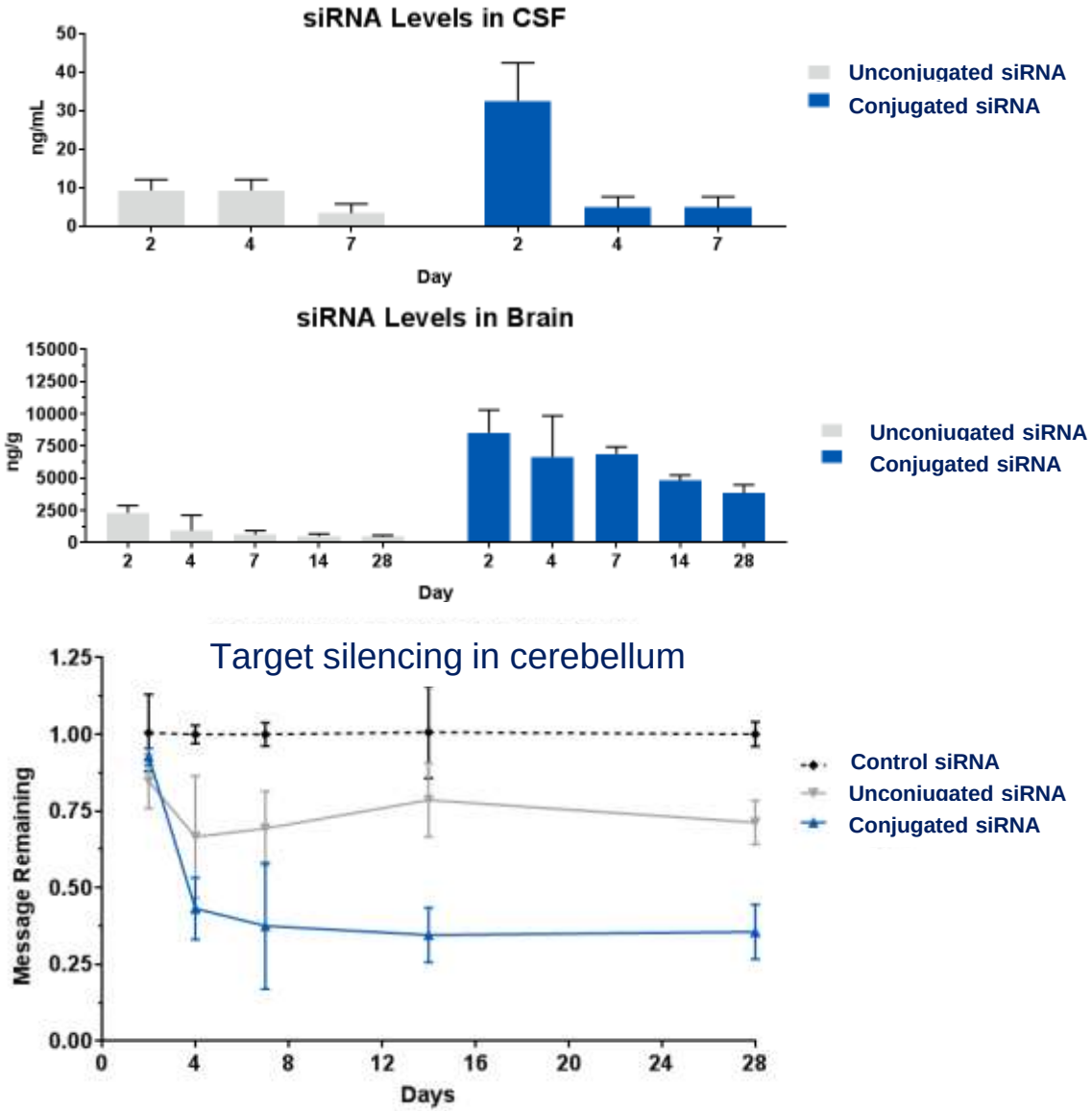
Intrathecal delivery of siRNA provides durable knockdown throughout CNS



- Consistent lowering across animals in most regions of the brain
- PD comparison in liver across species together with extended duration seen in rodents expected to support infrequent dosing in human

siRNA Conjugates Show Enhanced Uptake and Activity

Higher drug levels and robust silencing observed in brain with SOD1 siRNA conjugate

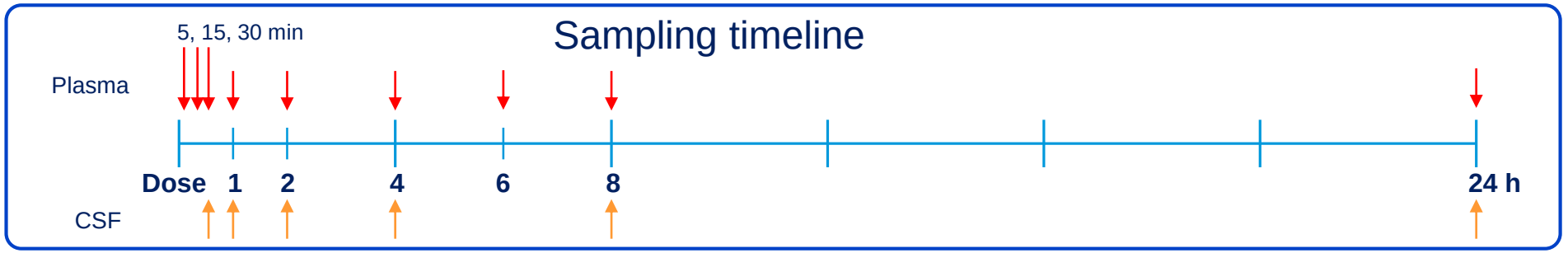


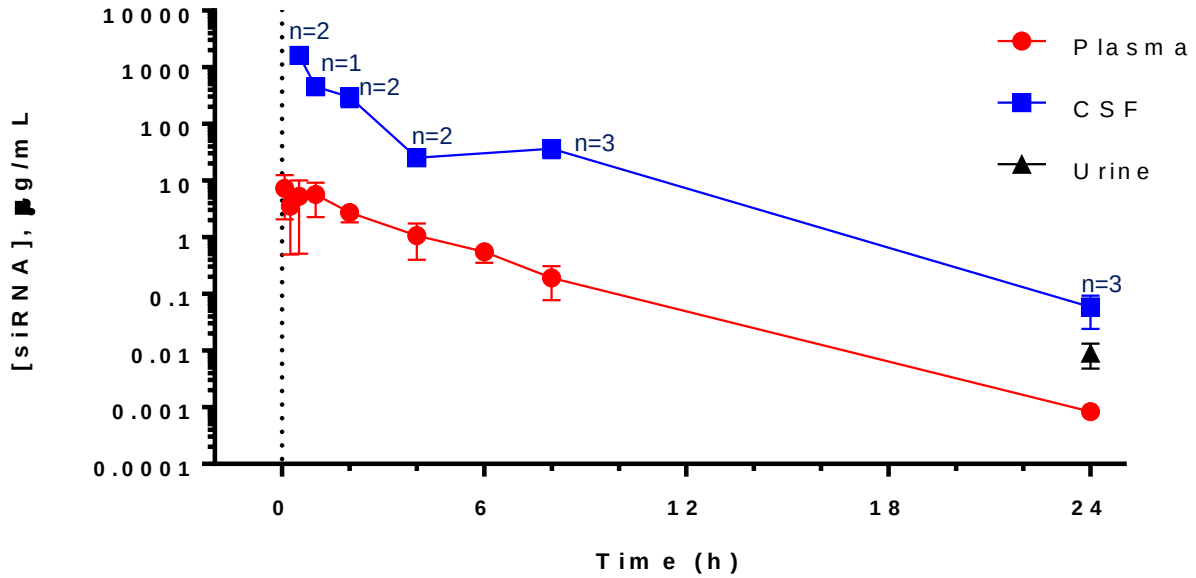
- Rapid siRNA clearance from CSF
- Conjugate reveals superior uptake and stability in brain over unconjugated siRNA
- Increased uptake in brain results in substantial improvement in mRNA knockdown

CSF and Plasma PK in Rat Following IT dosing

0.9 mg SOD1, lumbar puncture

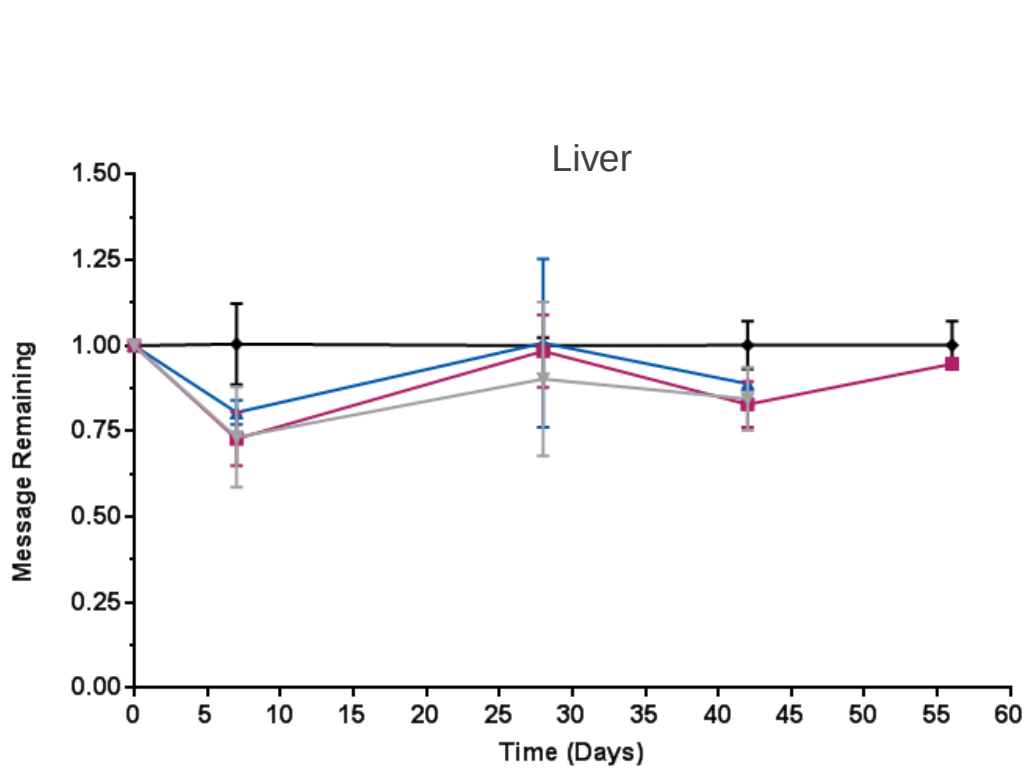
Intrathecal (IT) dosing, 0.9 mg

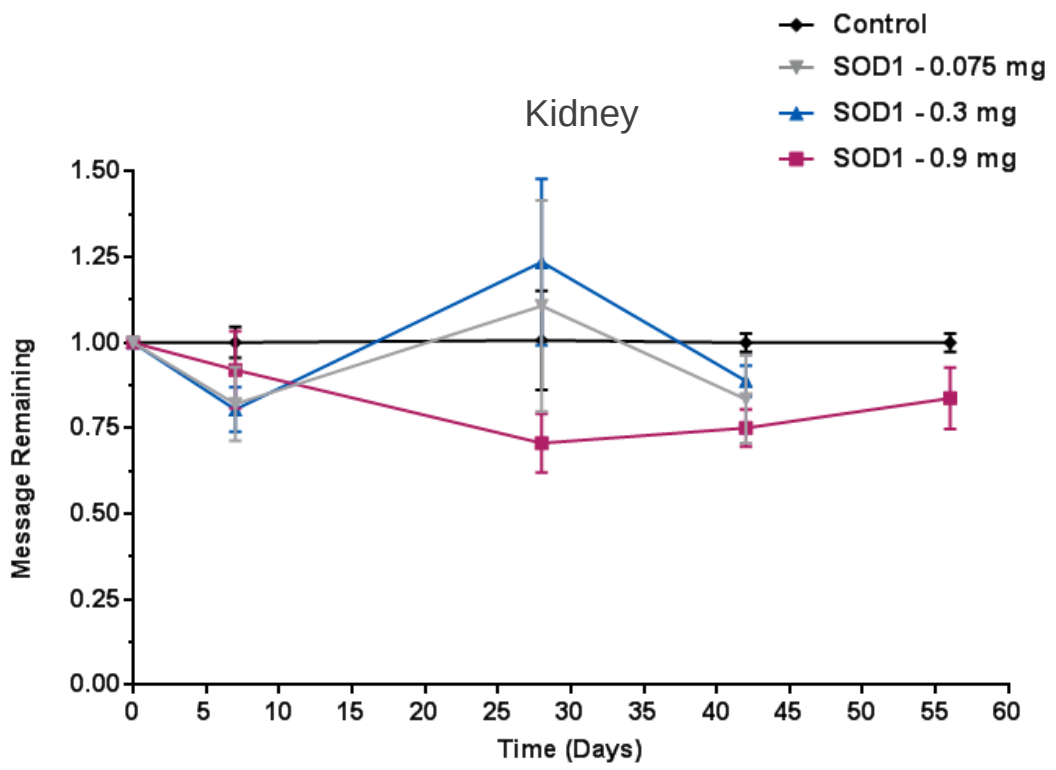




- Rapid disappearance from CSF
- Rapid appearance in plasma, t_{max} 5 min – 1 h
- Plasma concentrations parallel CSF, but ~2 logs lower

SOD1 IT Dose Response Reveals Minimal Silencing in the Liver and Kidney

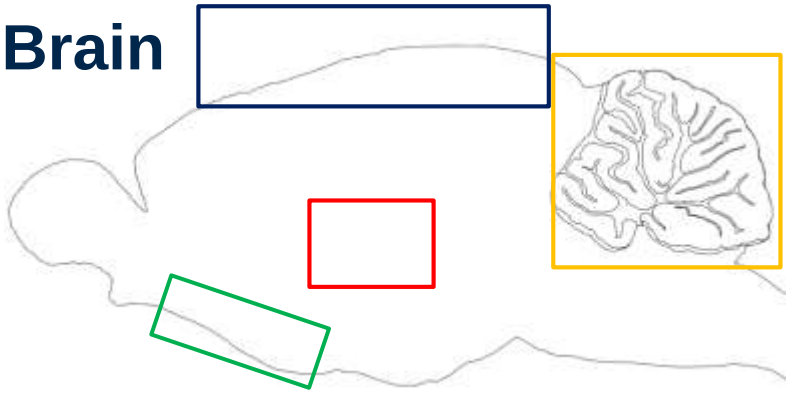


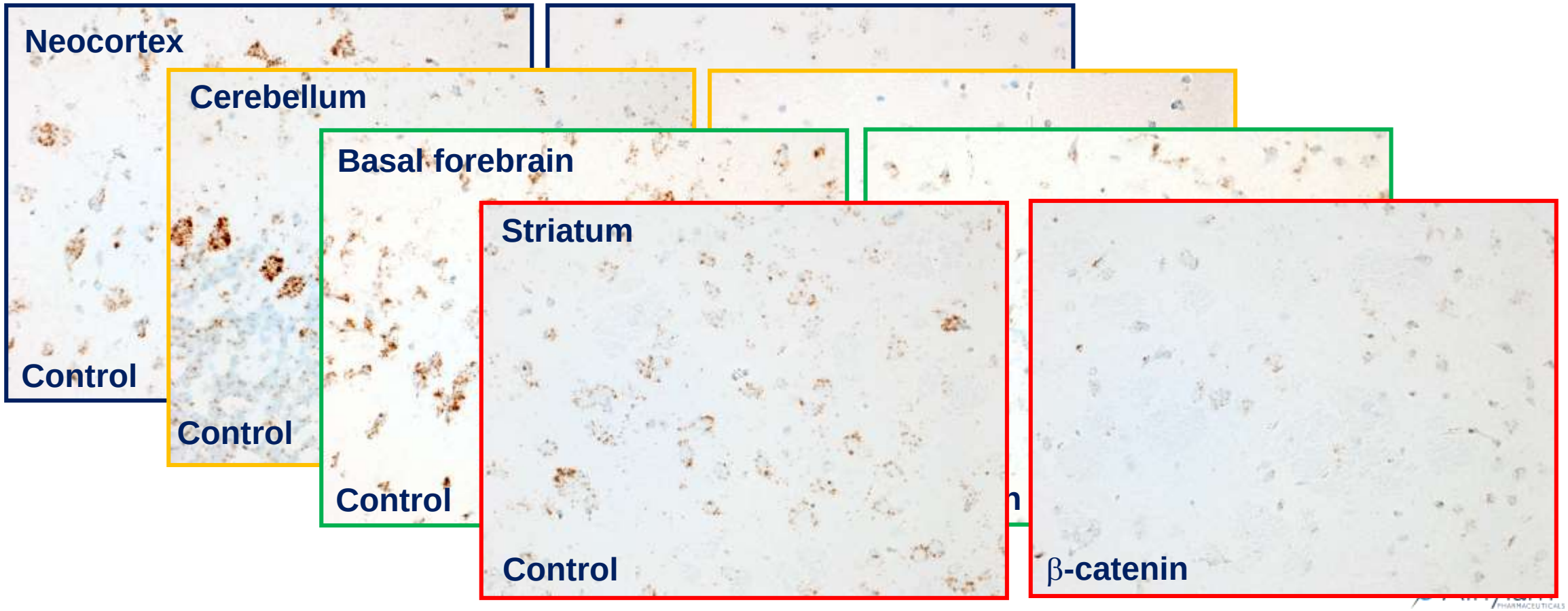


Though rapidly cleared from the CSF to the systemic circulation, conjugated siRNAs do not show robust silencing in the liver or kidney

Silencing Is Seen In All Regions of the Brain

Rat IHC probed for β-catenin





Evaluating Translation of CNS siRNA Conjugate Delivery to NHP

Single dose NHP study design

- siRNA conjugate dosed at 72 mg – IT bolus
- Evaluated a single target – β-catenin

	Strand (5'-3')	Sequence
β-catenin	S	UACUGUUGGAUUGAUUCGAAA
	AS	TUUCGAAUCAAUCCAACAGUAGC

Tissue collection

Tissues: Spinal cord: Lumbar, thoracic and cervical

Brain: Prefrontal cortex, temporal cortex, cerebellum, brain stem hippocampus

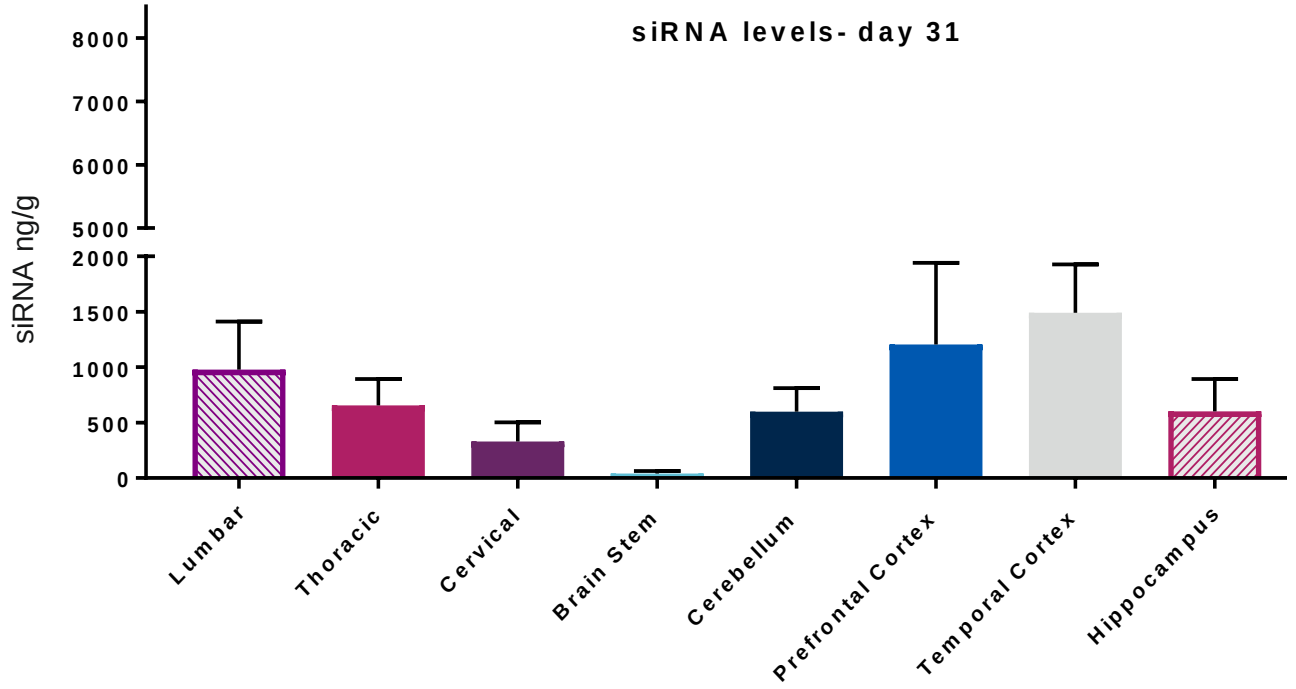
Fluids: CSF and plasma

Assays: mRNA, siRNA levels

Histology

IT Dosed β-catenin siRNA is Detected Throughout the Spinal Cord and Brain Regions of NHP

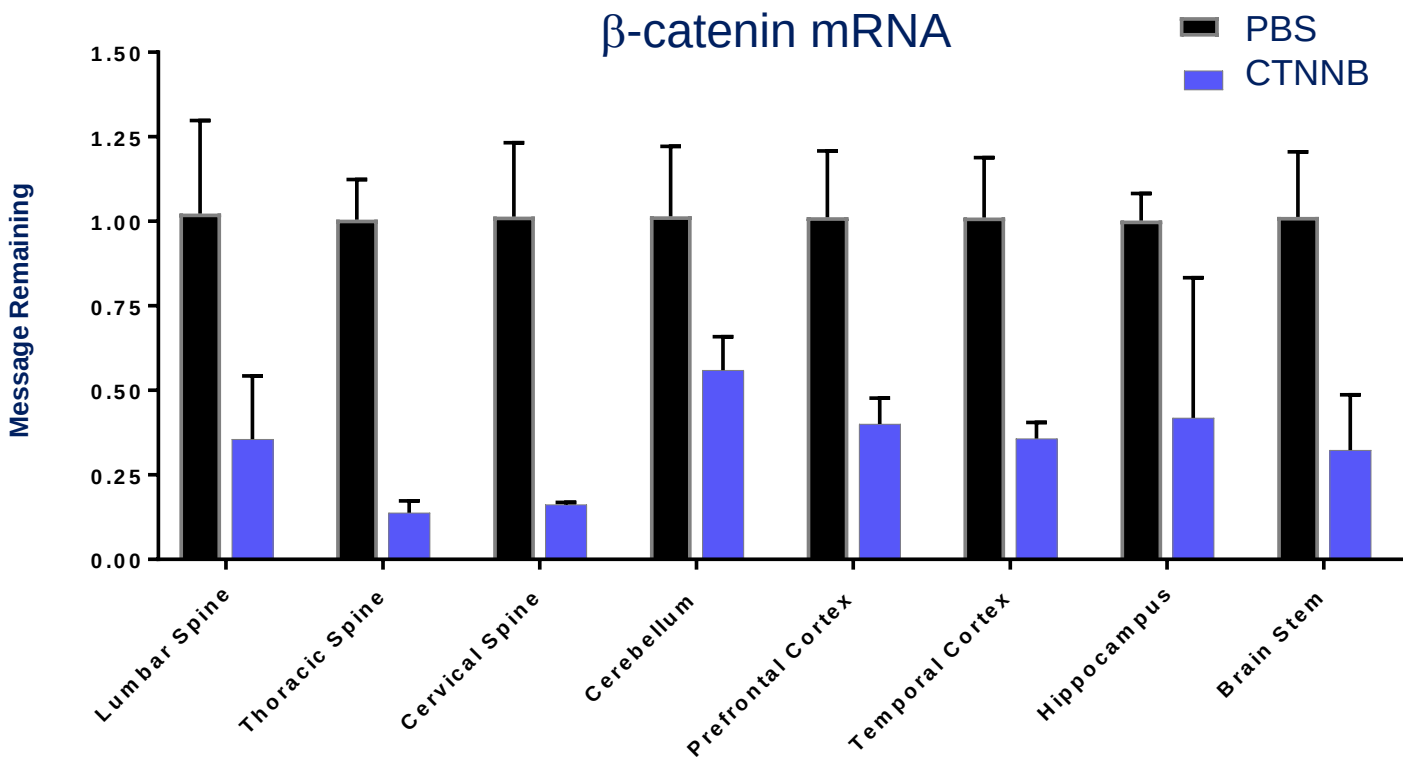
siRNA uptake varies between CNS regions



The day 31 time point shows the presence of significant siRNA levels across all tissues tested

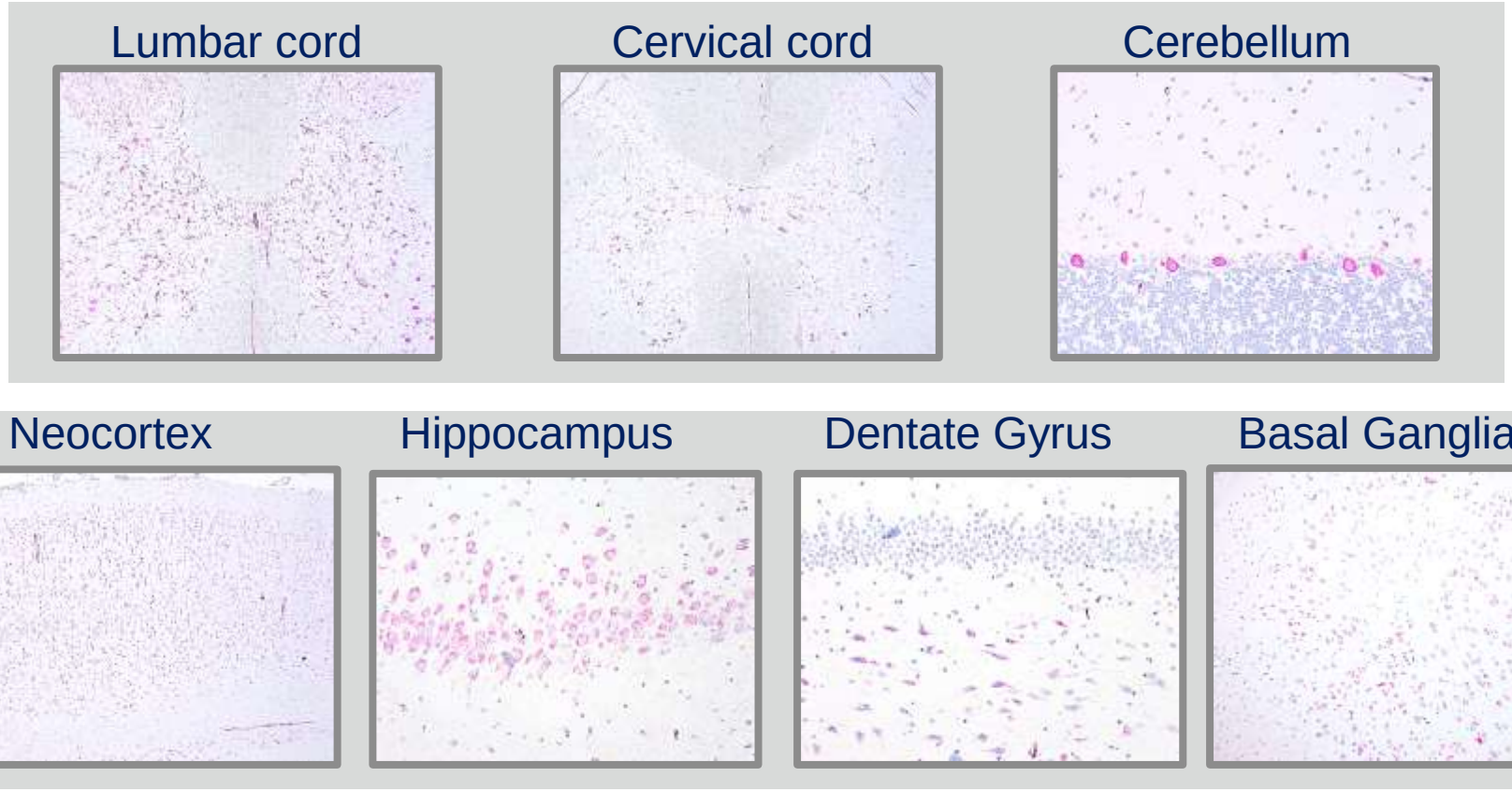
Robust Silencing Across CNS Demonstrating Successful Translation NHP

β-catenin mRNA knockdown by tissue, Day 31



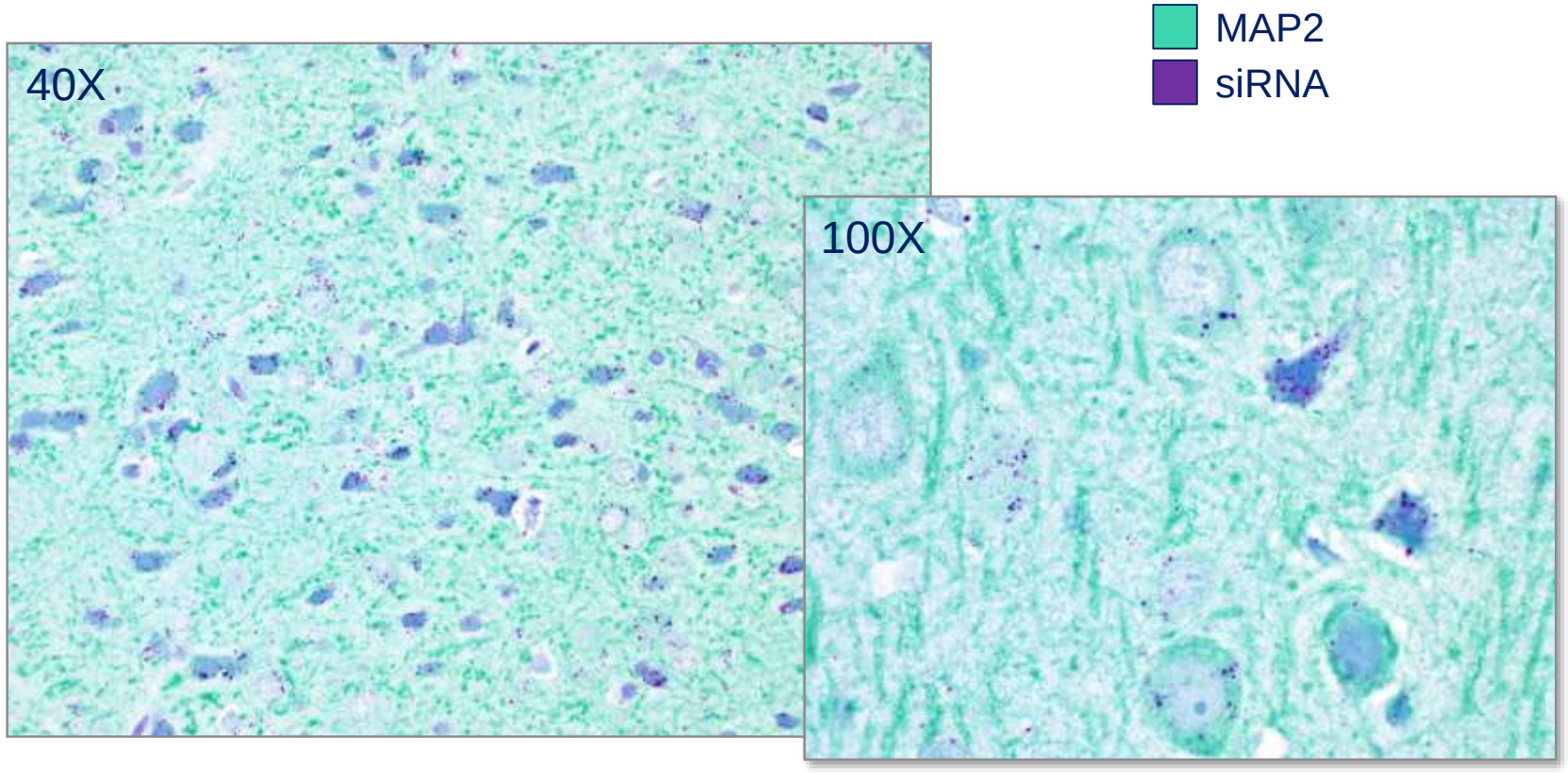
- The conjugate targeting β-catenin produces robust knockdown throughout the spinal cord and brain at the 31 day time point.

siRNAs Distribute Throughout the CNS in NHP Following IT Dosing



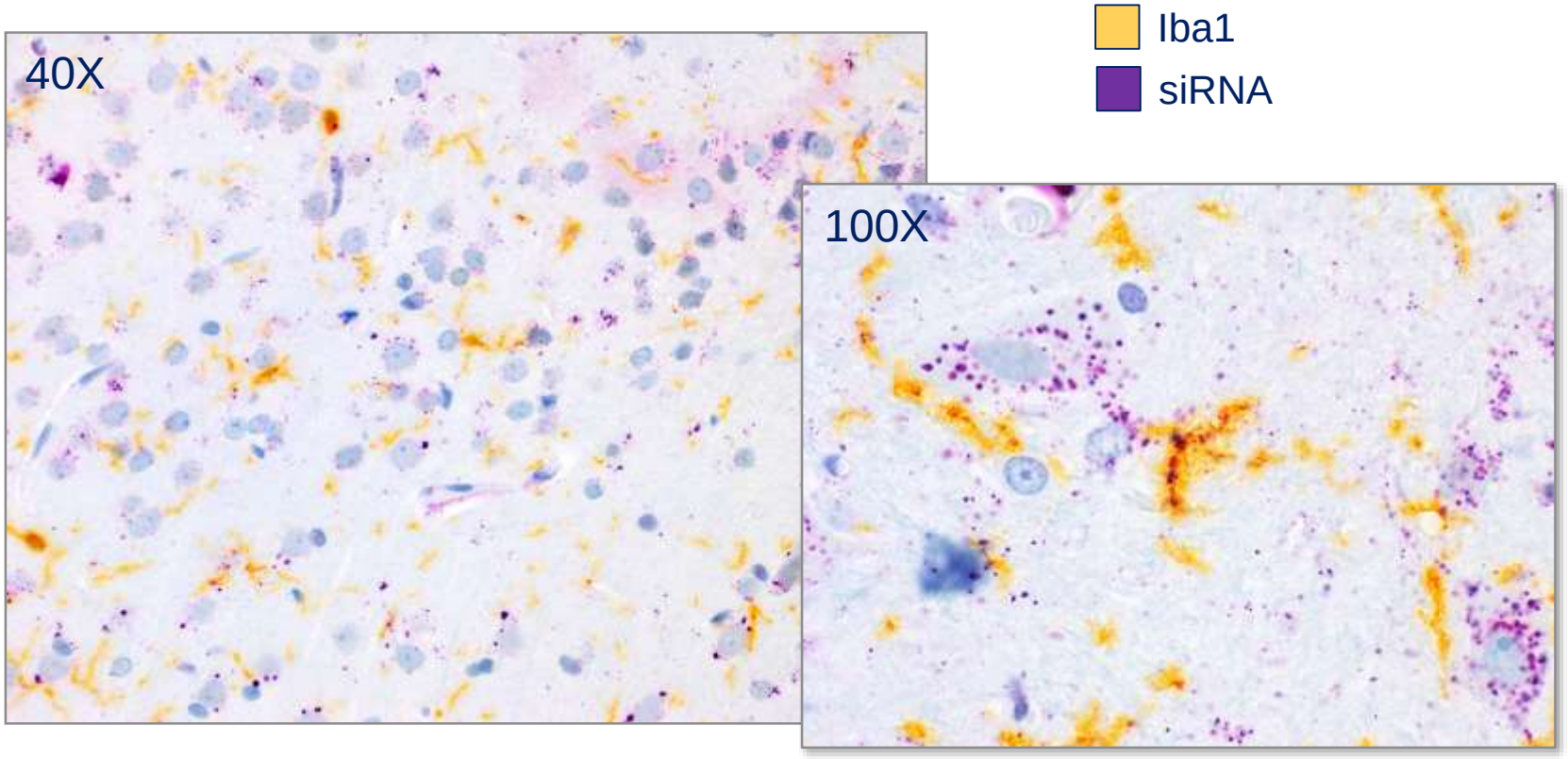
siRNA Conjugates are Localized to Neurons Following IT Dosing

Most neurons show siRNA uptake



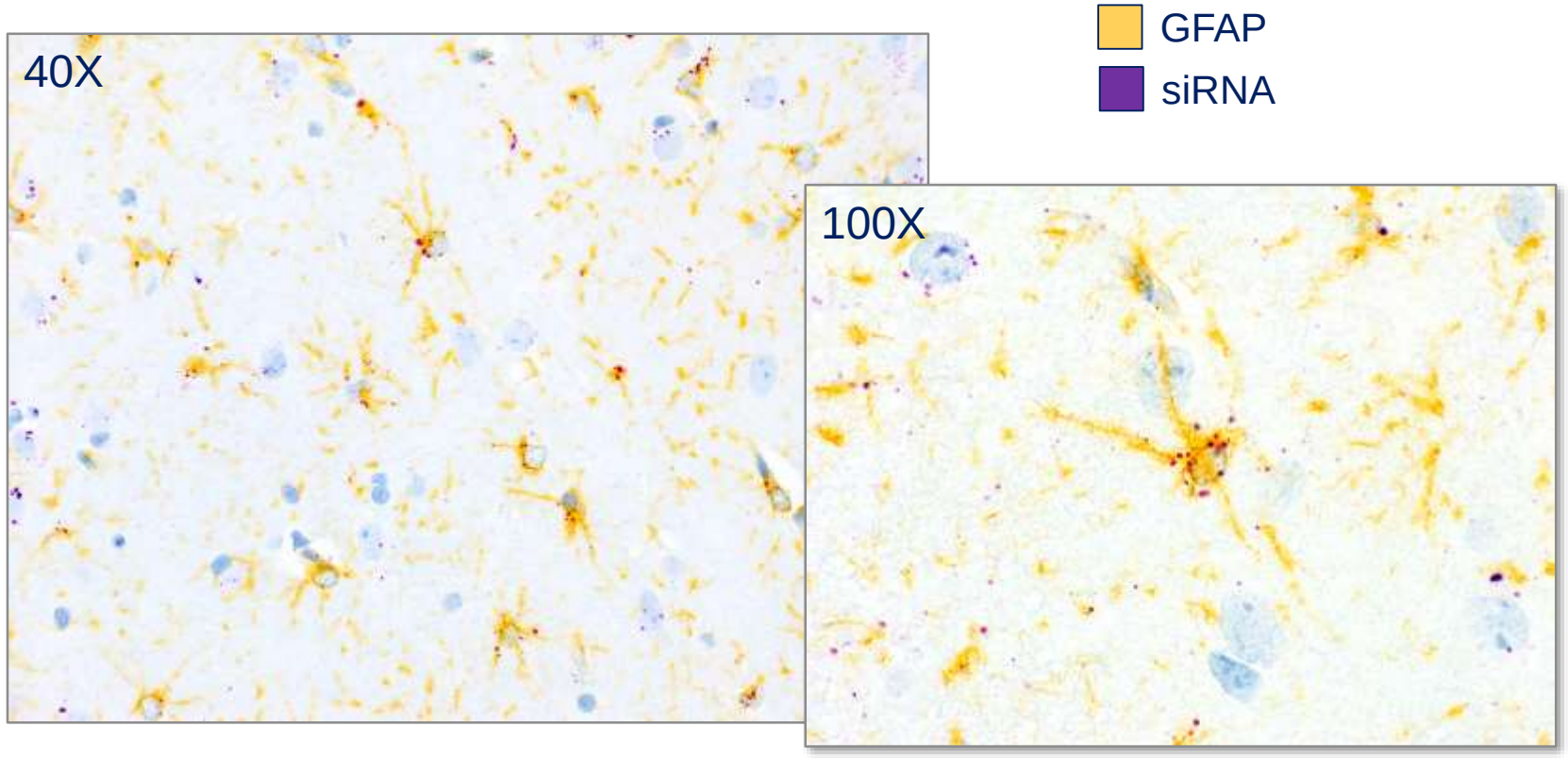
MAP2 is a neuronal marker
CTNNB siRNA probed with siRNA antibody

siRNA Conjugates are Localized to Microglia Following IT Dosing



Iba1 is a microglia marker
β-catenin siRNA probed with siRNA antibody

siRNAs Conjugates are Localized to Astrocytes Following IT Dosing



GFAP is an Astrocyte marker
β-catenin siRNA probed with siRNA antibody

siRNA vs ASO in hSOD1 (SOD1G93A) Rats

Day 7 collection after single IT dose

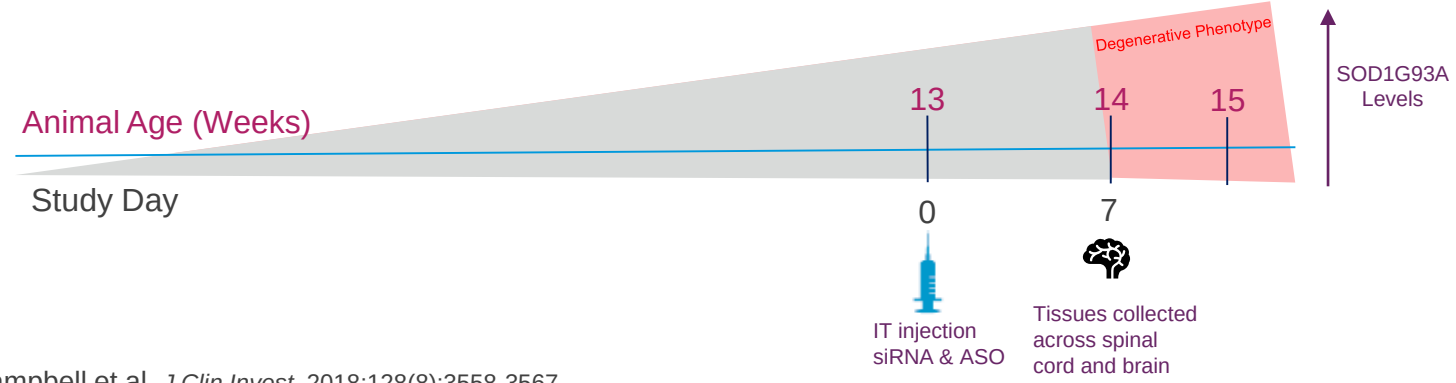
Study Purpose

- Head-to-head comparison of siRNA and ASO
  - 3 siRNAs selected from mouse AAV-hsSOD1 screen
  - ASO 1, based on McCampbell et al. (2018)
    - Demonstrated ~75% maximum silencing at 2 weeks in the same rat model

ASO 1	CAoGGoATACATTTCTACoAGoCT

Study Design

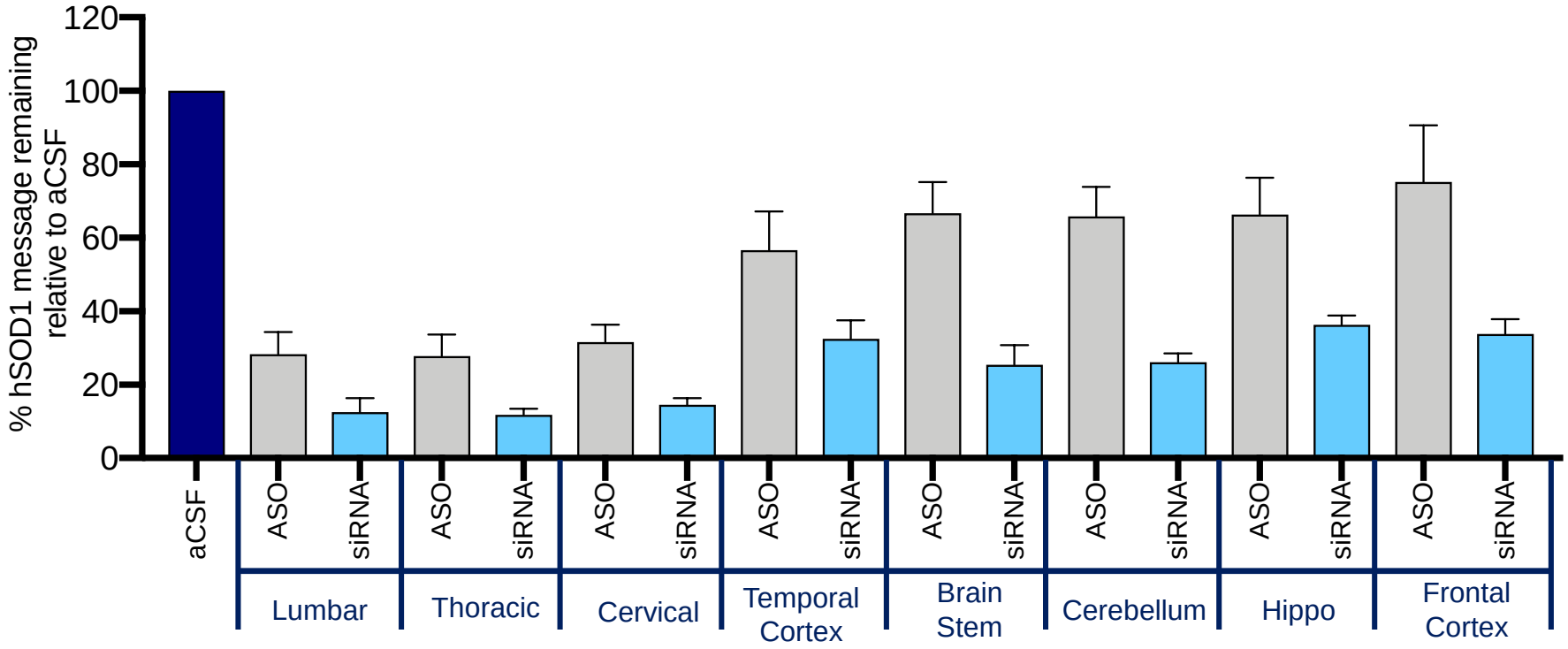
- Dose: single IT injection of 0.9 mg in 30µl (Study Day 0)
  - Same dose used for siRNA and ASO
- Dosed 13 week old hSOD1 rats (SOD1G93A)[1]
  - Single Day 7 timepoint
    - Single early time point was chosen to capture the highest expression of SOD1G93A before animals developed degenerative phenotype



McCampbell et al, *J Clin Invest.* 2018;128(8):3558-3567

siRNA vs ASO in hSOD1 (SOD1G93A) Rats

Improved hSOD1 mRNA Reduction with siRNA compared to ASO in CNS



An siRNA targeting hSOD1 showed superior silencing in all regions of the brain and spinal cord

Investigational RNAi Therapeutics for CNS and Ocular Diseases

Expanding Alnylam opportunities beyond liver

Devastating diseases with enormous burden and unmet need

- Alzheimer's disease
- Amyotrophic lateral sclerosis (ALS)
- Cerebral amyloid angiopathy
- Frontotemporal dementia
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Number of genetically validated targets known but few disease modifying therapies for these devastating or life threatening disorders.

RNAi therapeutics directed to disease-causing, CNS- or ocular-expressed genes represent a potential opportunity to address diseases with some of the greatest unmet need.

TTR is Produced in the Eye as Well as the Liver

Ocular manifestations of hATTR amyloidosis in patients

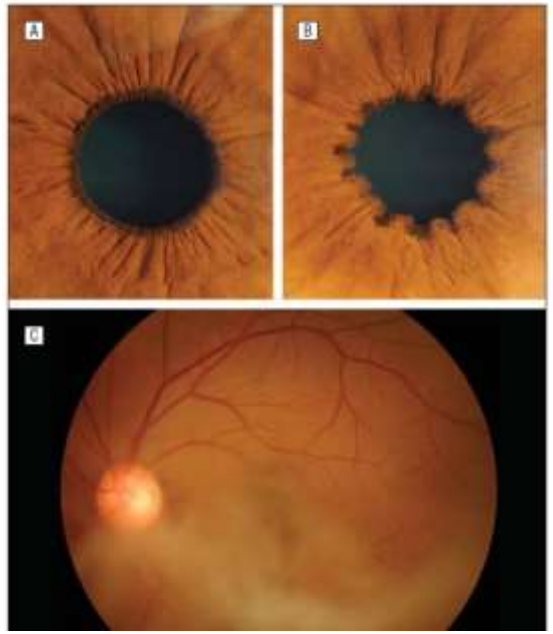
- Glaucoma (~12%-23%)
- Vitreous opacities (Wide range)
- Retinal abnormalities (~4% - 15%)
  - retinal amyloid deposit
- Iris abnormalities (~14% - 38%)
  - iris amyloid deposit
- Amyloid deposits on lens (~33%)

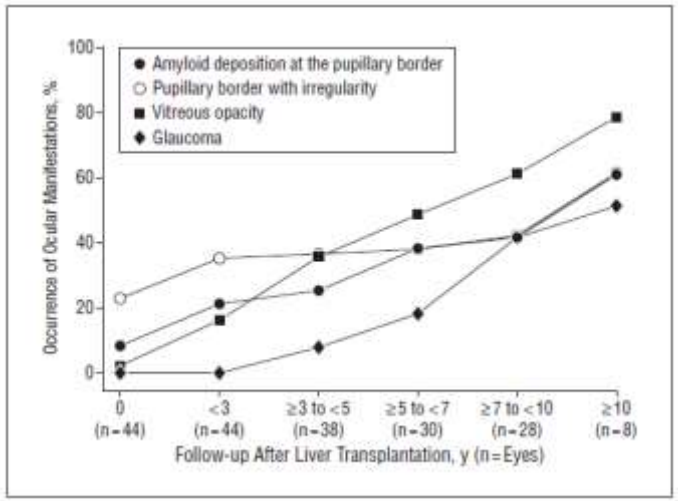
Pupillary deposits and abnormality predict onset of glaucoma

- V30M and T114C study
  - 62% eyes had high IOP

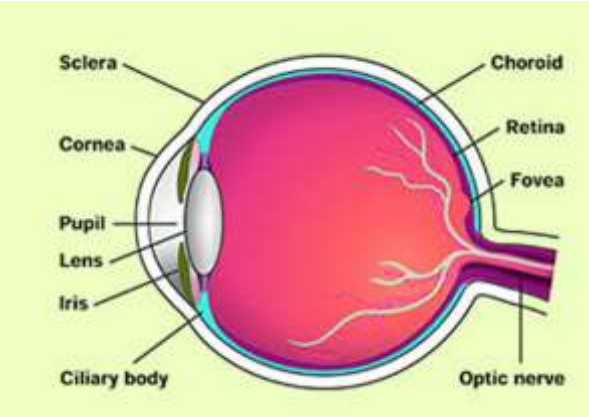
Liver transplant patients

- Study of Japanese liver transplant patients with V30M continue to have ocular symptoms

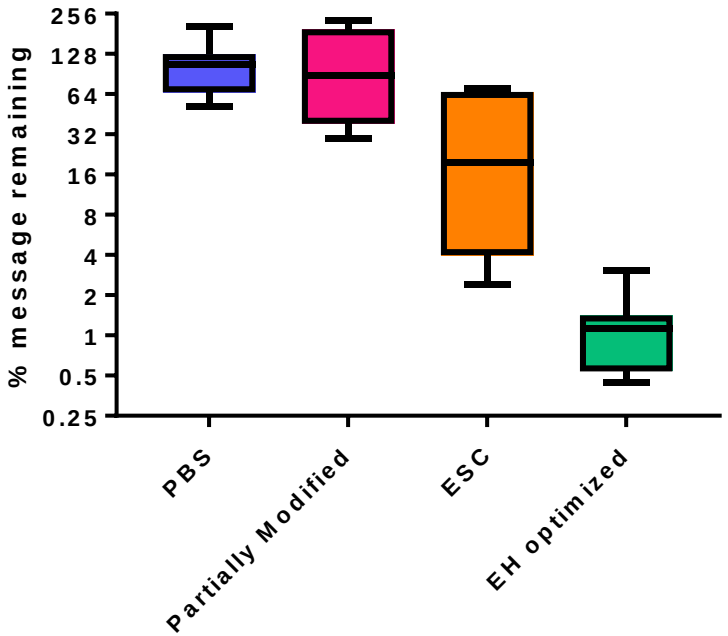




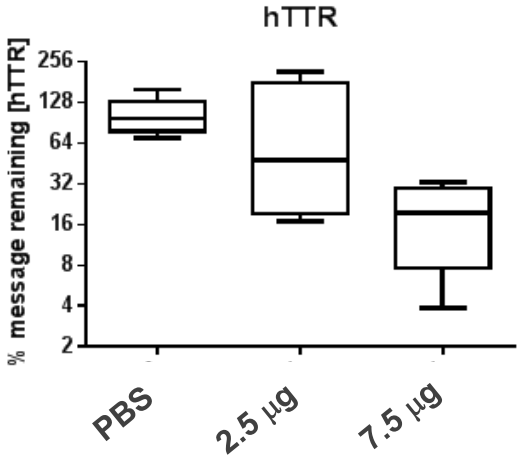
Ocular TTR Silencing by Differentially Modified siRNA Conjugates in Rat After Single Intravitreal Injection



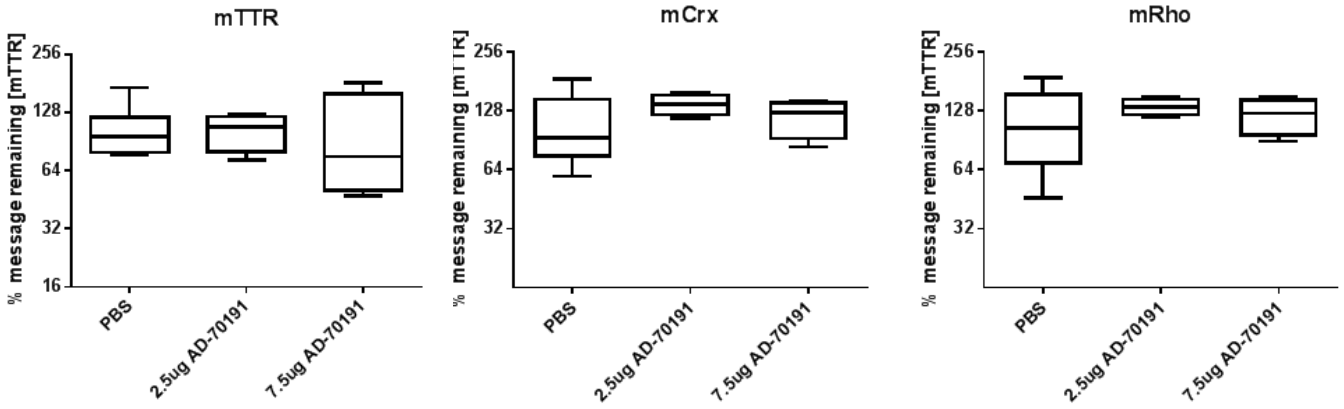
Mouse TTR mRNA
Day 14, 50 μg siRNA conjugate



Ocular silencing of hTTR in transgenic mice



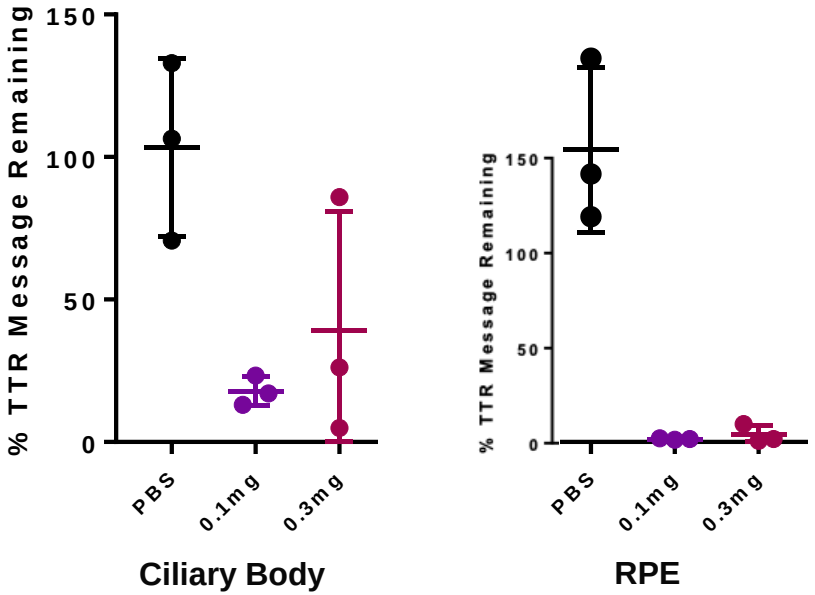
Specificity: No impact on expression of mTTR, Crx or Rhodopsin



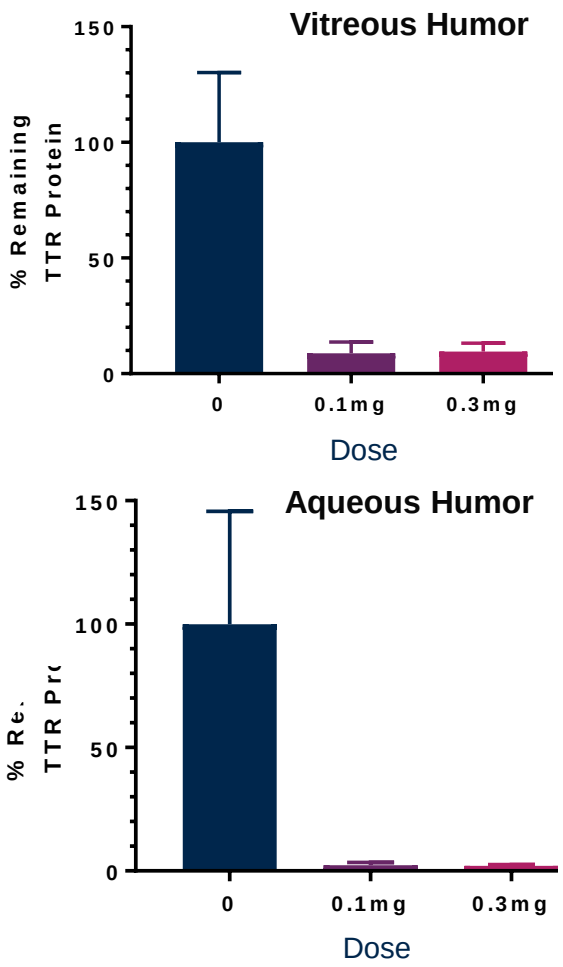
Robust Silencing of Ocular TTR by siRNA Conjugates in NHP

Near complete reduction of TTR mRNA and protein 28 days following a single IVT dose

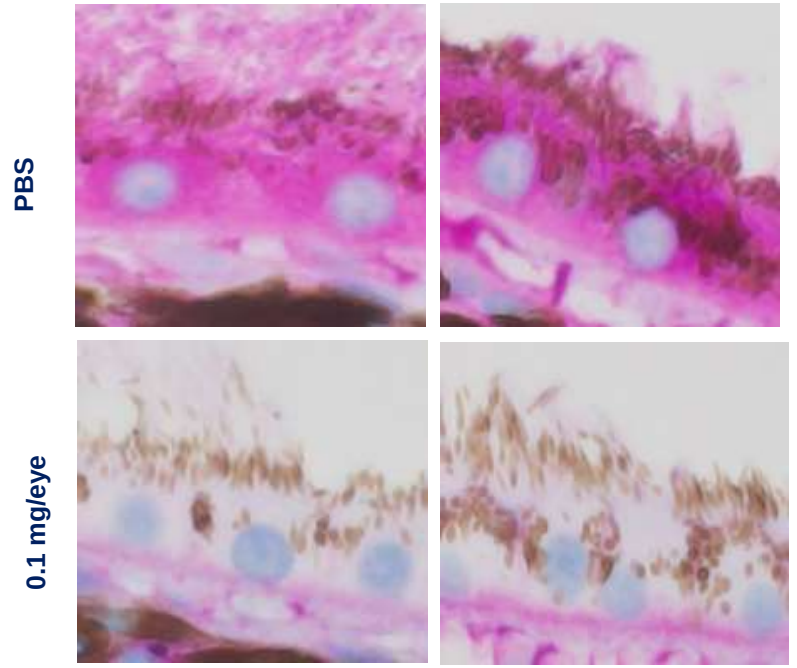
TTR mRNA analysis by qPCR



TTR protein analysis by ELISA

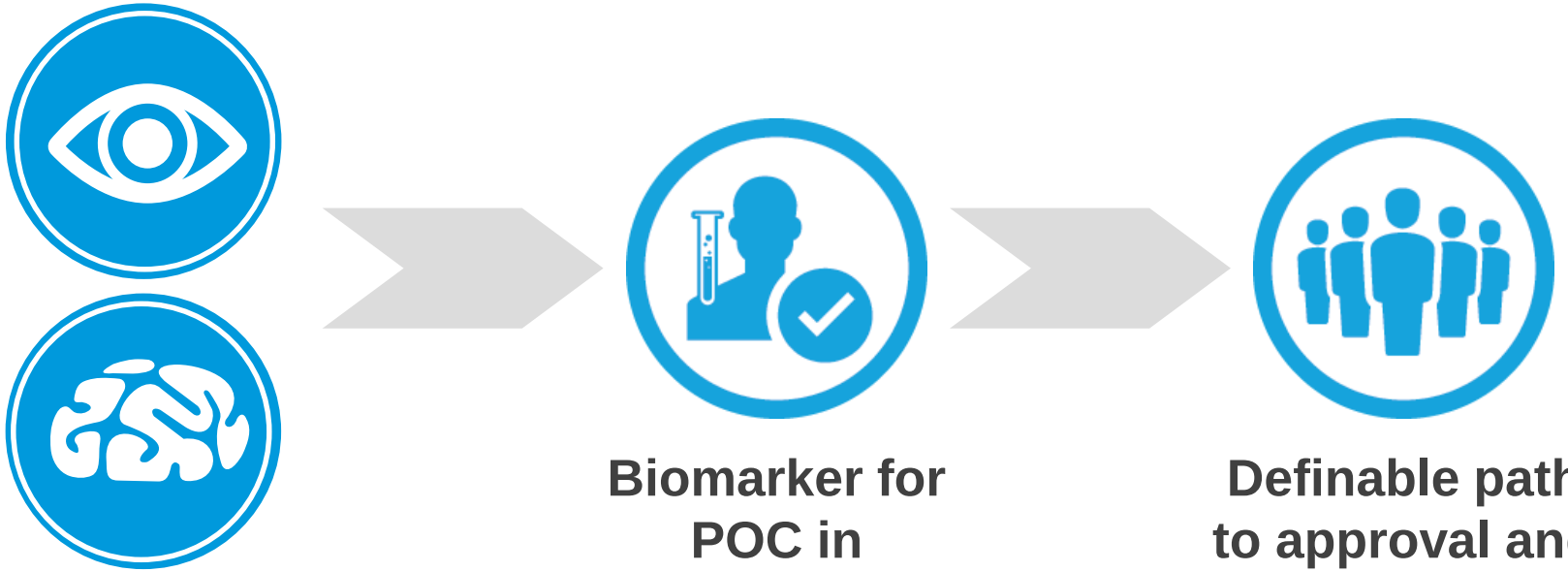


TTR protein analysis by IHC



Alnylam CNS and Ocular Pipeline Strategy

Expanding a pipeline of potentially transformative medicines



Consistent Silencing Observed Across Pre-clinical Species

Successful delivery of siRNA conjugate to the CNS and eye

- Durable silencing of target mRNA observed across the CNS of rat and NHP following IT administration
- Tissue uptake observed in all CNS tissues examined with drug levels in the ng/g to μg/g range
- In both rat and NHP studies, intrathecal administration of the novel siRNA conjugates was found to be generally well tolerated
- Robust silencing throughout the brain observed for an siRNA targeting SOD1

- TTR silencing demonstrated in rodents and NHP
- Target silencing seen in the CE and RPE
- Target silencing is specific
- Target knockdown demonstrated in NHP following a single IVT dose of siRNA
- Equivalent silencing demonstrated for mRNA and protein across the eye