Progress of RNAi in Extrahepatic Tissues

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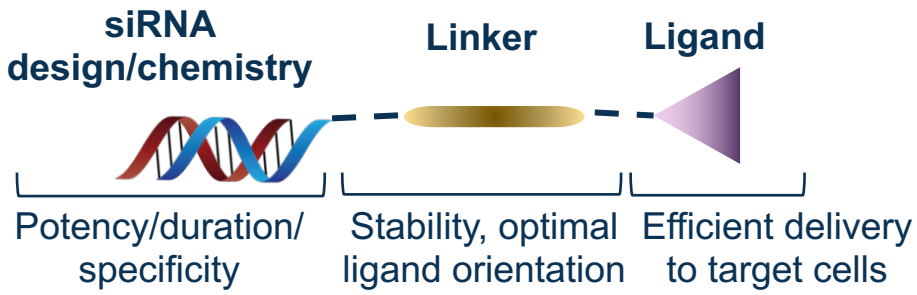
Alnylam Pharmaceuticals
TIDES, September, 2021

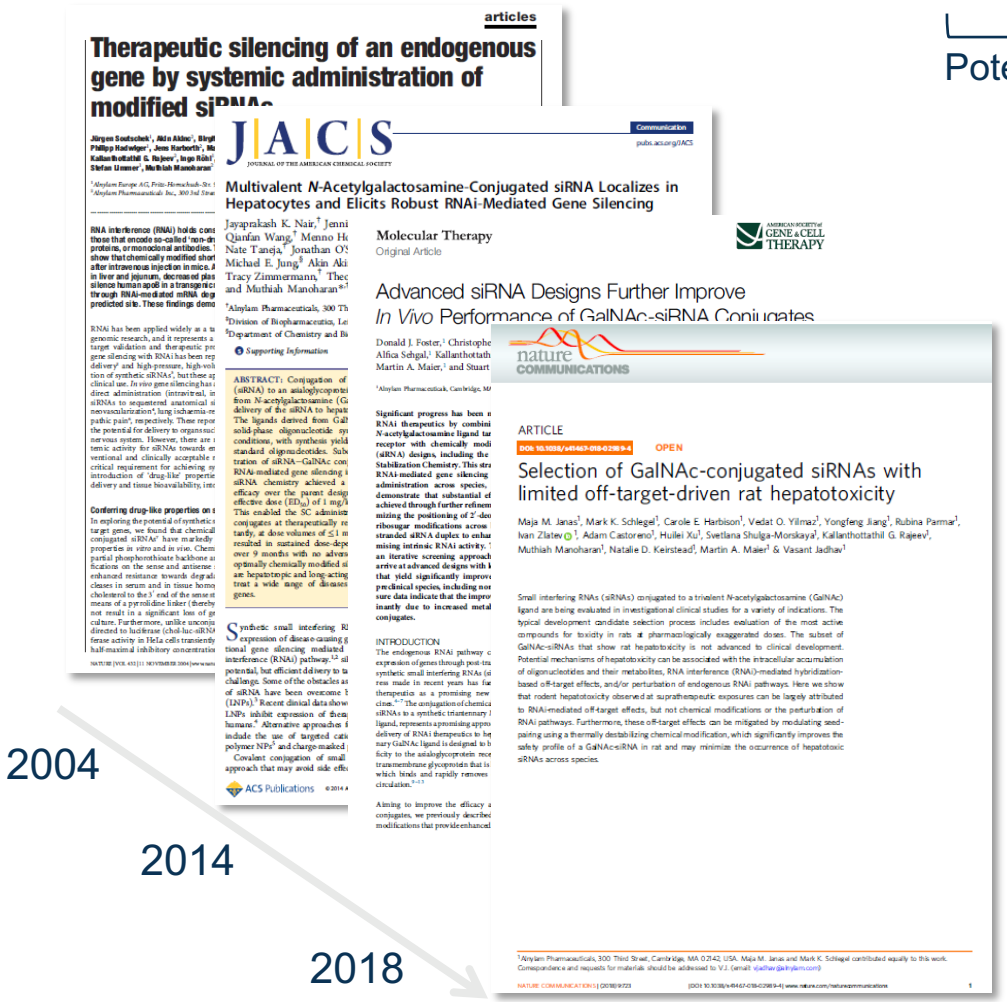
Alnylam PHARMACEUTICALS

Agenda

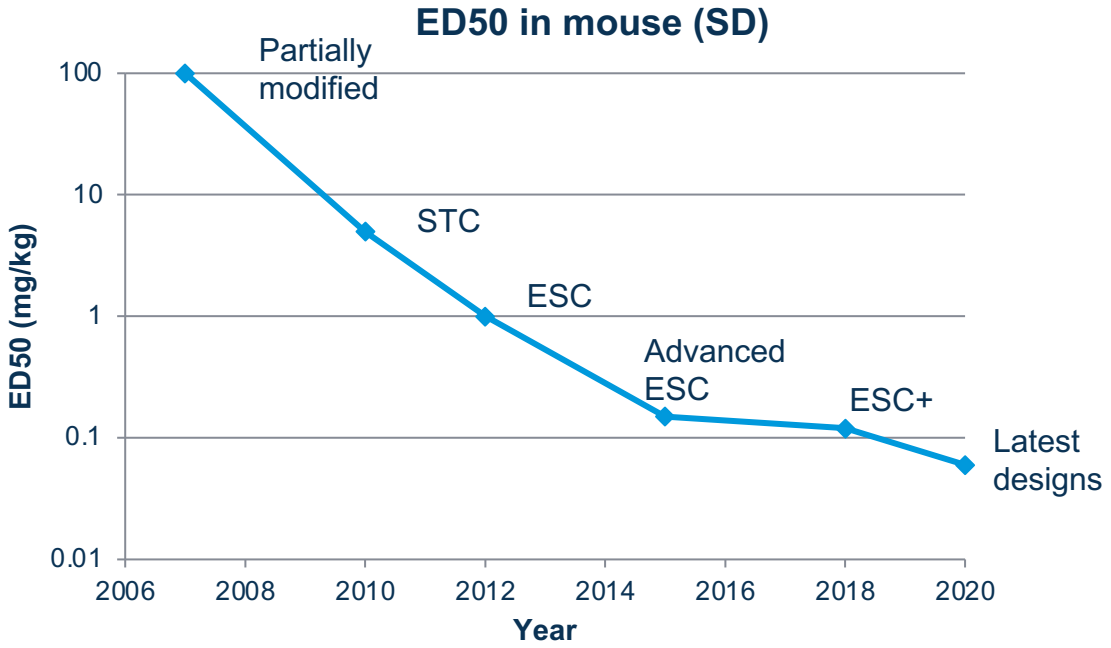
- Background of Alnylam RNAi platform
- Progress of RNAi in CNS
  - PK/PD profiles of RNAi in Mouse ICV
  - PK/PD profiles of RNAi in Rat IT
  - Translation to NHP
- Progress of RNAi in Ocular
- Summary

Transformative Advancements in Conjugate-Based Delivery



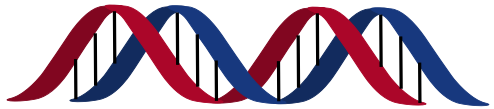


Evolution of conjugate design with improved potency and specificity

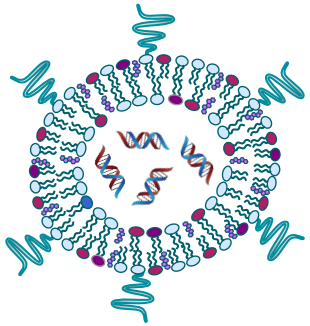


Addressing the Delivery Challenge

Mechanisms for siRNA Delivery to Liver



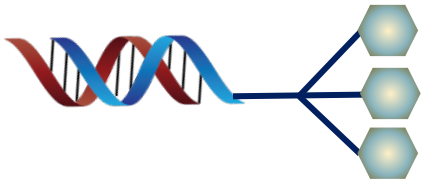
siRNA



Patisiran

Lipid Nanoparticles (LNPs)

- siRNA (limited modifications) in a multi-component lipid formulation (LNP; ~85 nm)
- Targeted delivery to liver
- Intravenous (IV) administration



Current Clinical siRNAs

GalNAc-siRNA Conjugates

- Single chemical entity
- Tri-GalNAc ligand conjugated to sense strand of extensively modified siRNA
- Targeted delivery to liver
- Subcutaneous (SC) administration

Complementary Approaches for Efficient siRNA Delivery to Liver

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

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.

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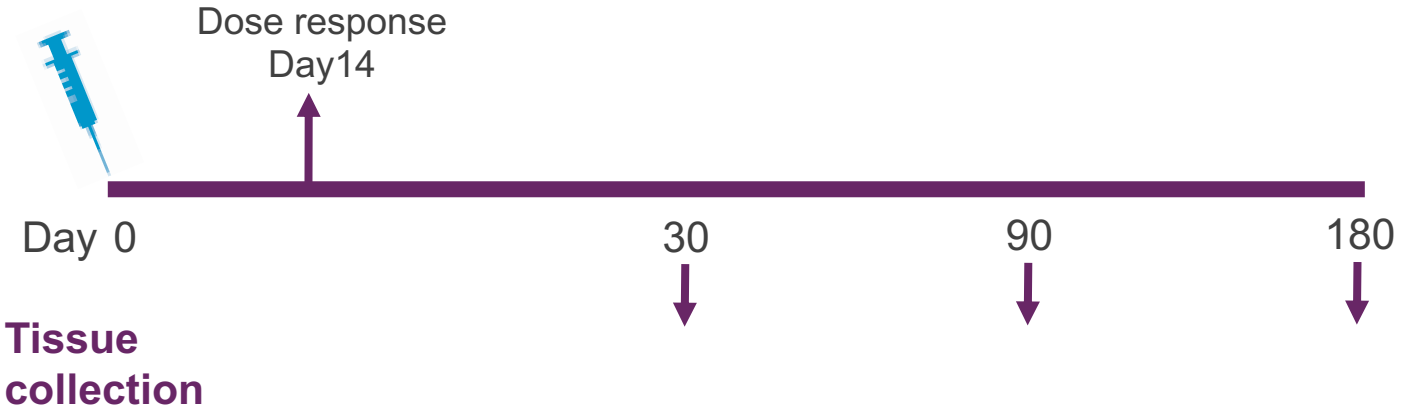
Intracerebroventricular (ICV) Delivery of CNS Optimized siRNA Conjugates

Dose response and duration in mouse ICV

siRNA targeting SOD1

- Dose response study: single siRNA conjugate doses of 25ug, 50ug, 100ug, 150ug, 200ug and 300ug
- Duration: time points through 6 months with 50ug, 150ug and 300ug



Tissues:

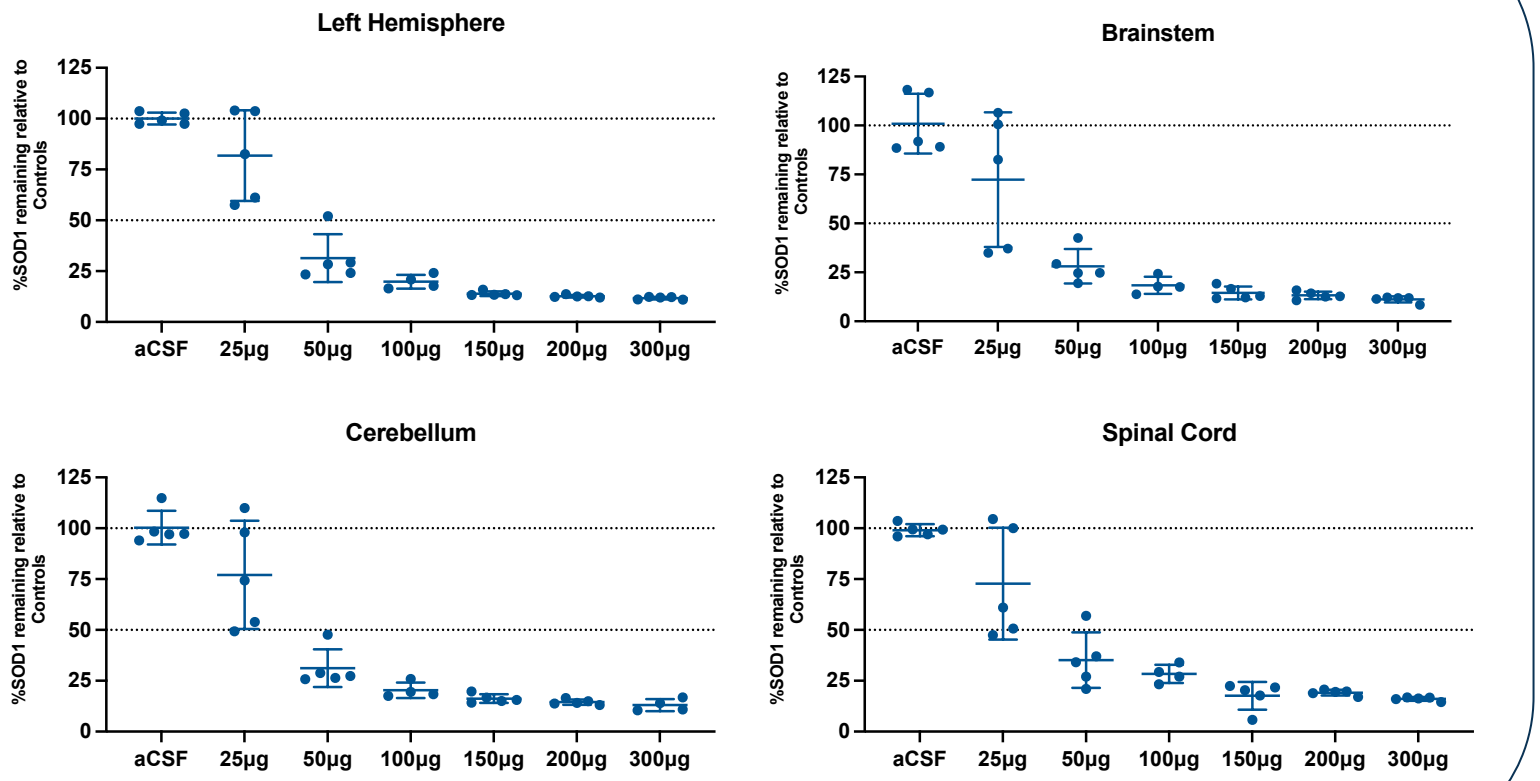
Spinal cord, left and right hemispheres, cerebellum and brain stem

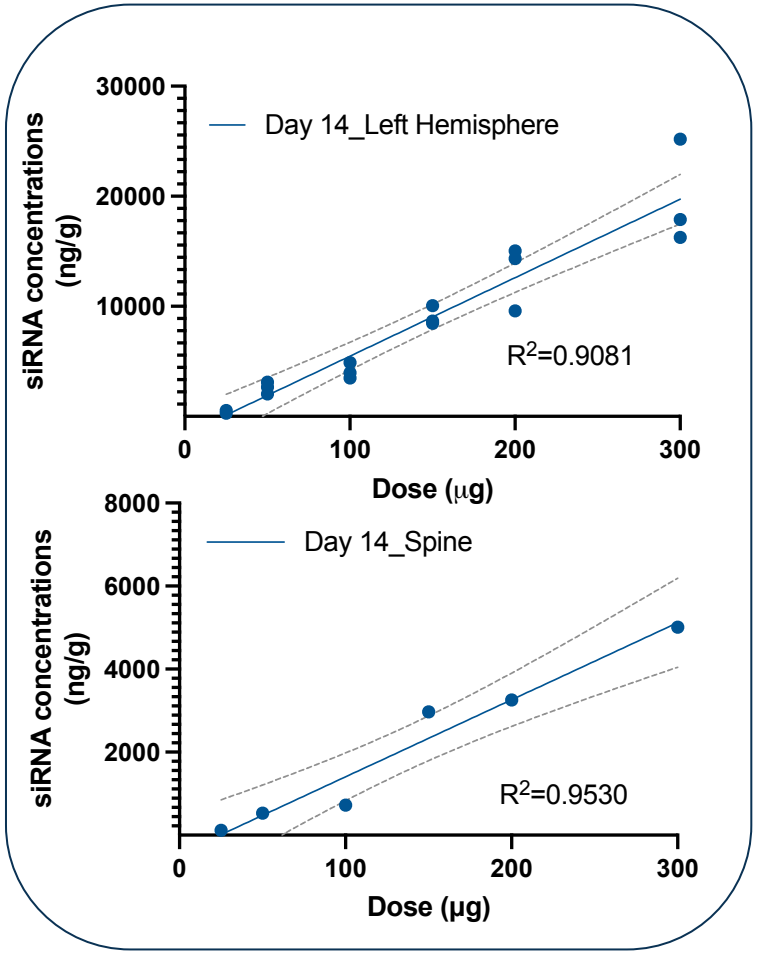
Assays:

mRNA by qPCR and tissue siRNA levels

Dose Dependent Silencing Throughout Mouse Brain

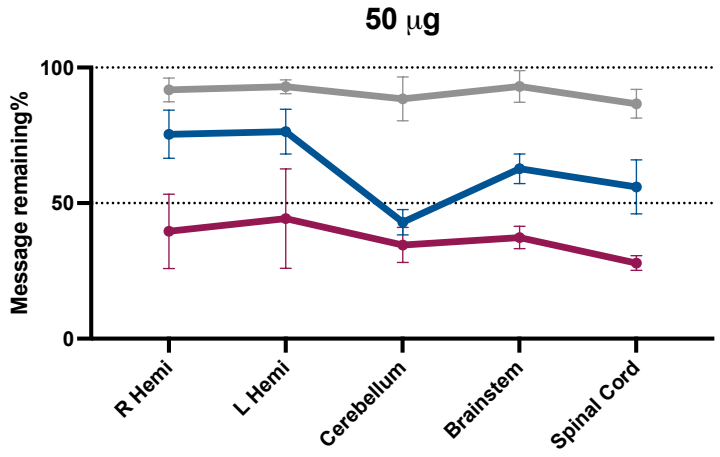
Single dose and dose response in mouse ICV

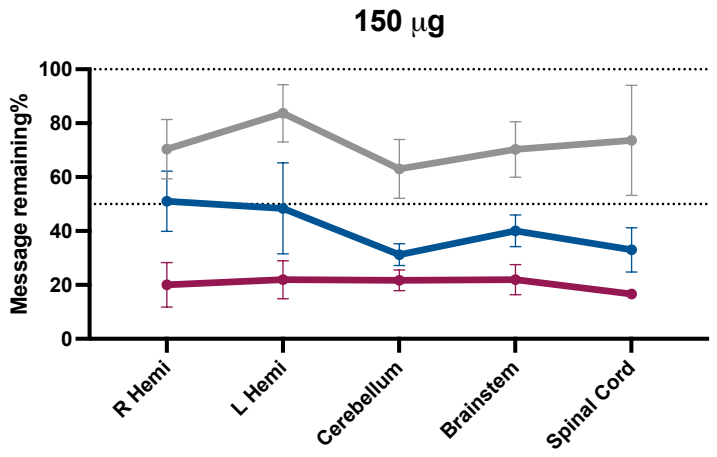


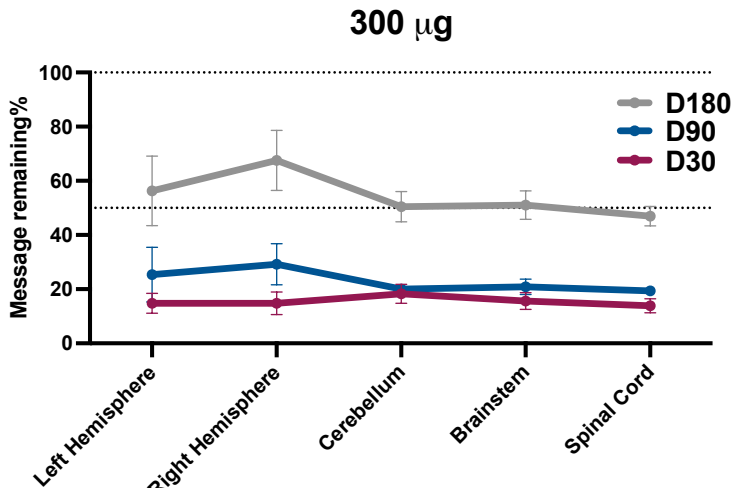


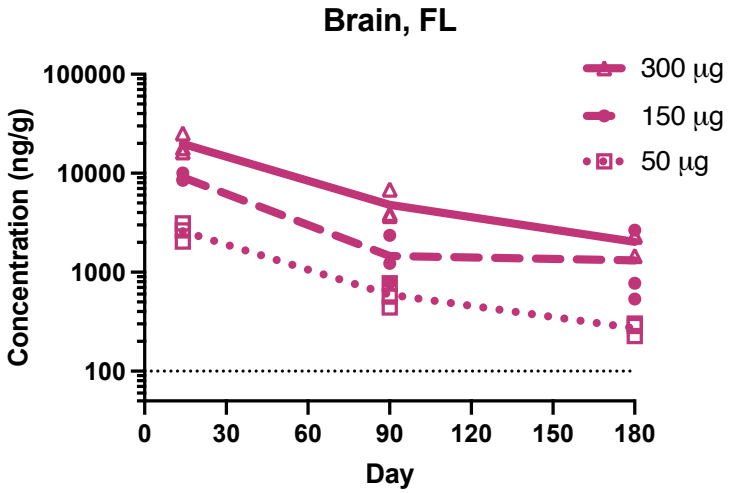
Robust and Sustained Activity of siRNA in the Mouse CNS

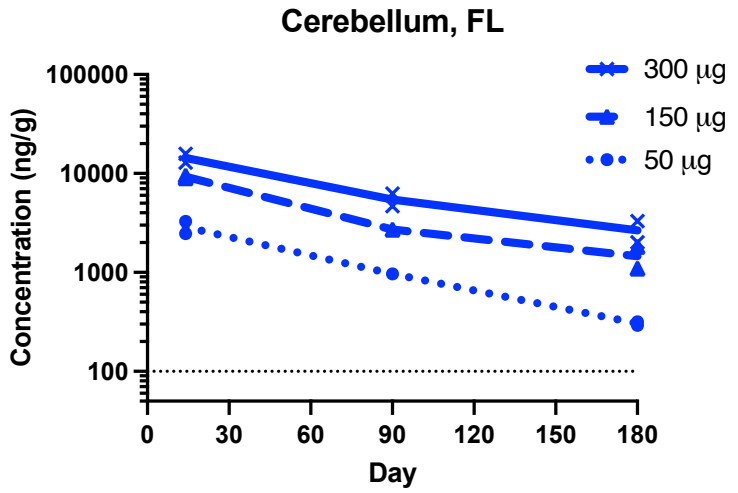
Single dose and duration in mouse ICV

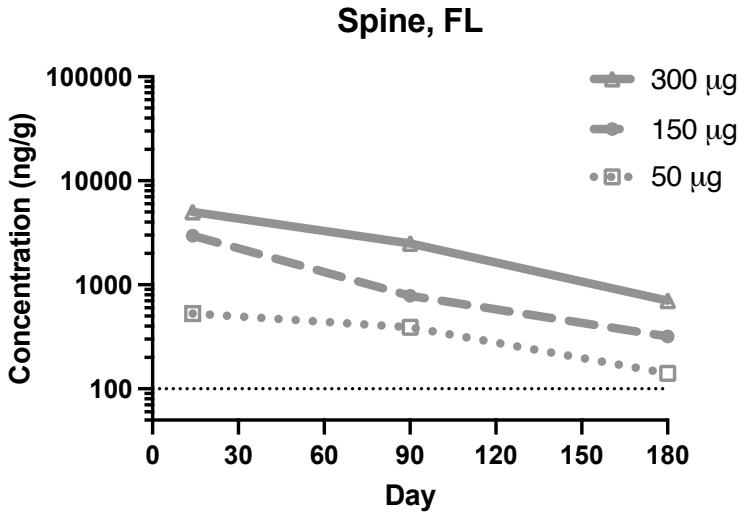




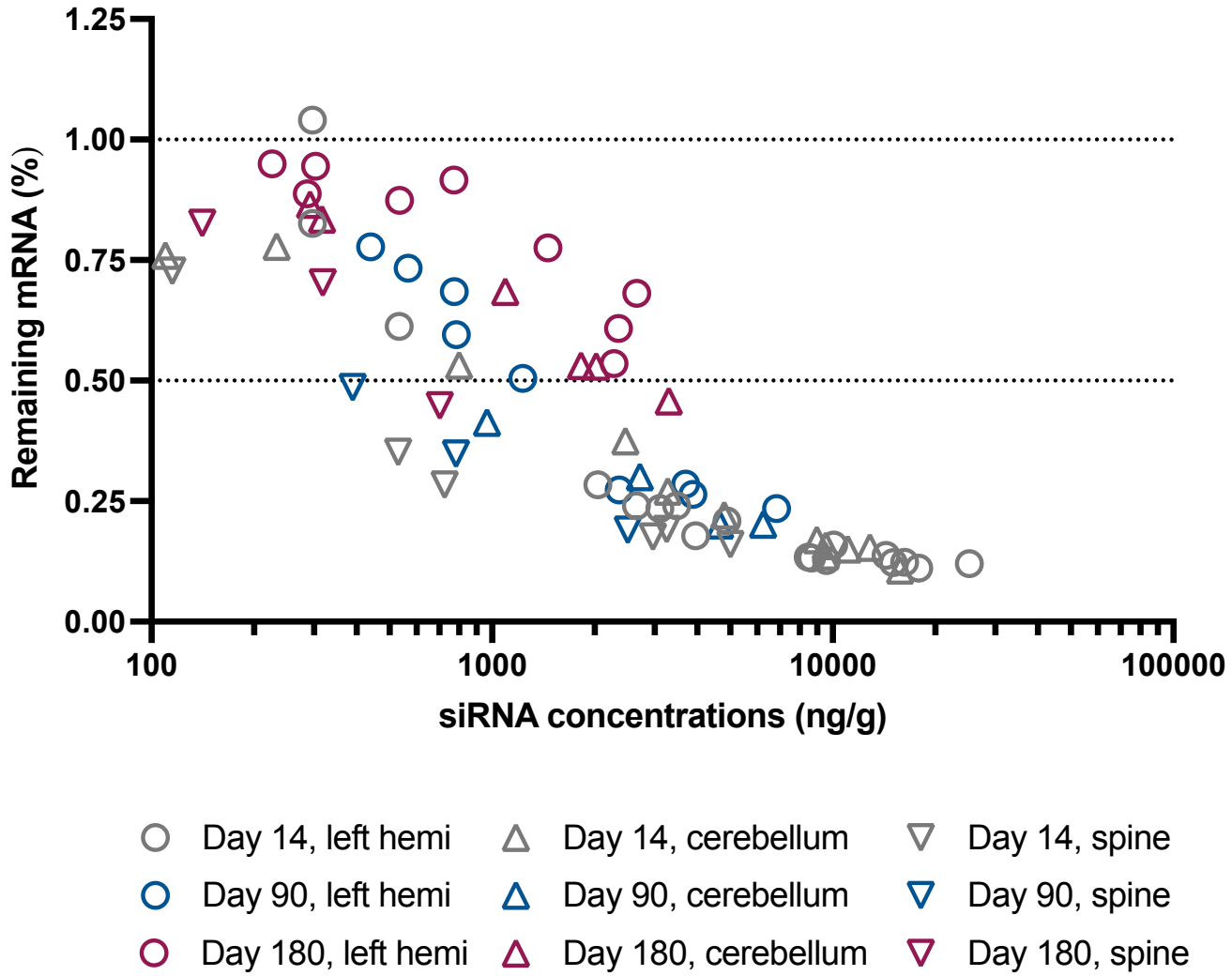








Strong PK/PD Relationship in Mouse Brain

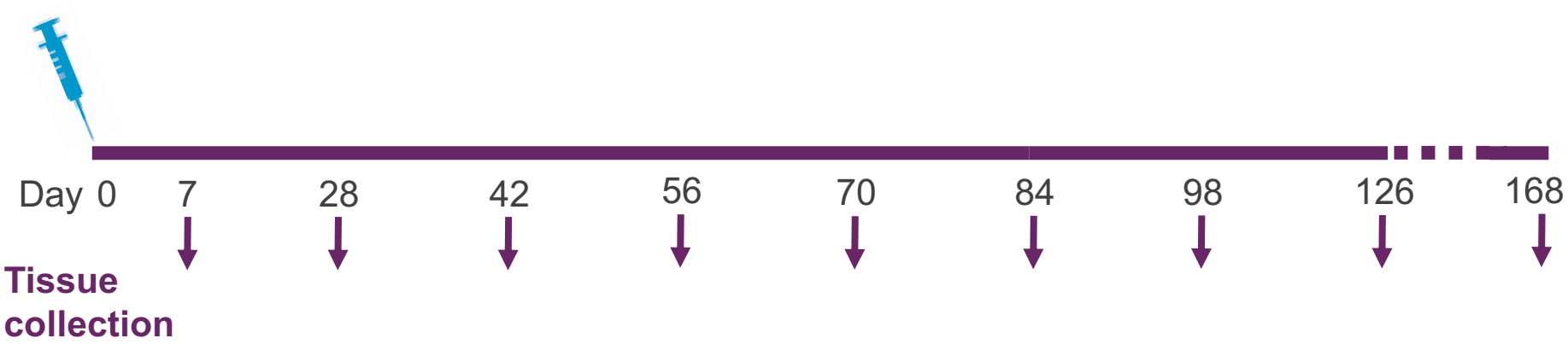


Intrathecal (IT) Delivery of CNS Optimized siRNA Conjugates

Single dose and dose response in rat

siRNAs targeting 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 6 months for SOD1



Tissues: Spinal cord: Lumbar, thoracic and cervical

Brain: prefrontal cortex, cerebellum and remaining brain

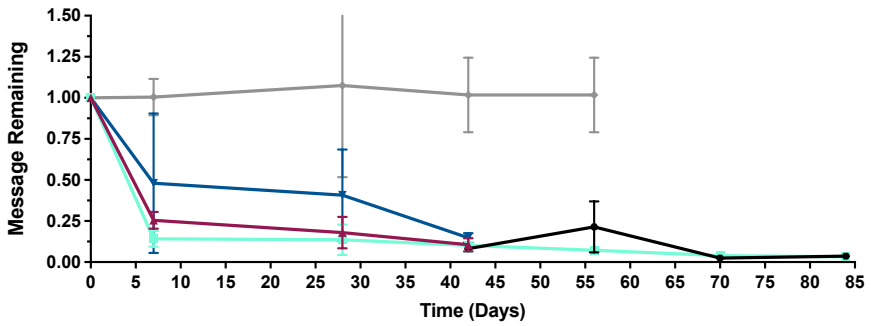
Fluids: CSF and plasma

Assays: mRNA, tissue siRNA levels, Histology

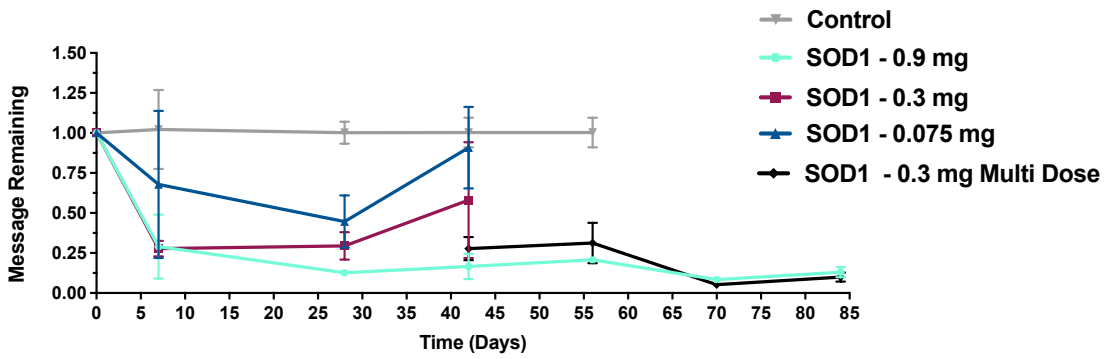
Robust and Durable Silencing Demonstrated Following a Single IT Dose

Silencing of SOD1 following a single or multiple IT doses

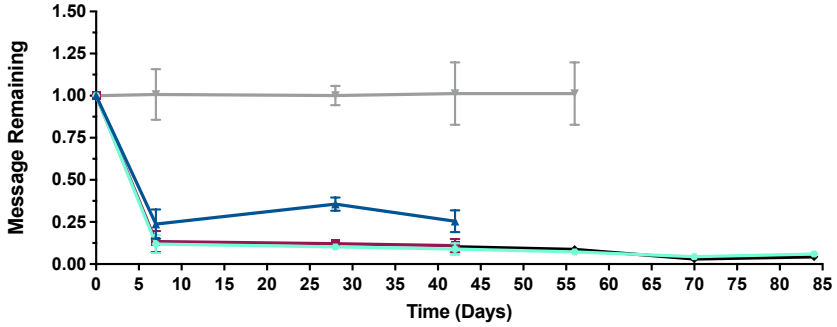
Lumbar



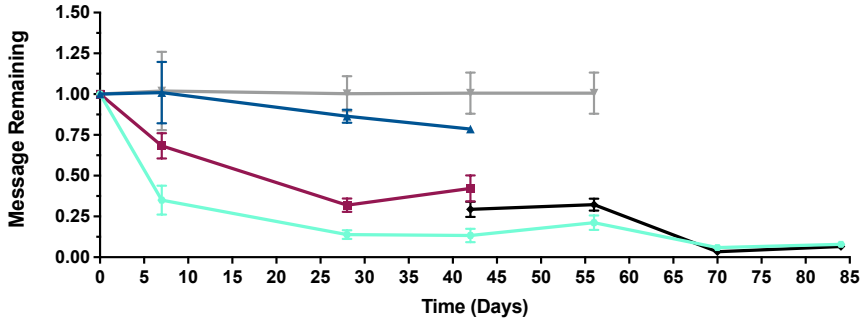
Cerebellum



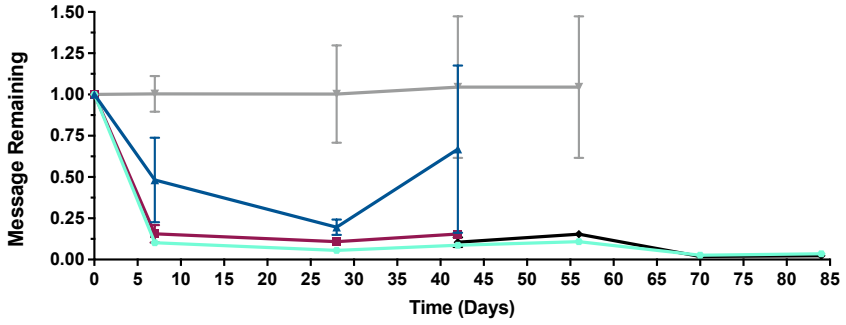
Thoracic



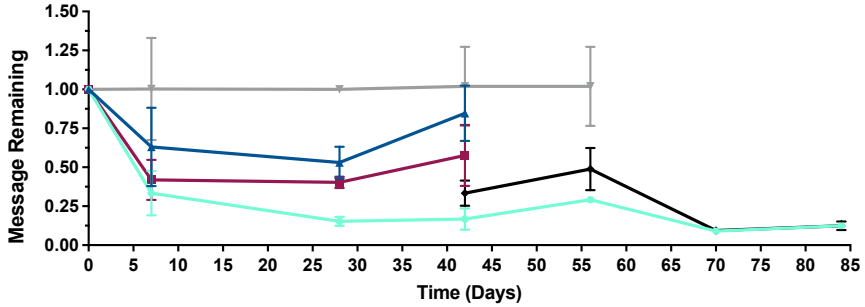
Frontal Cortex



Cervical

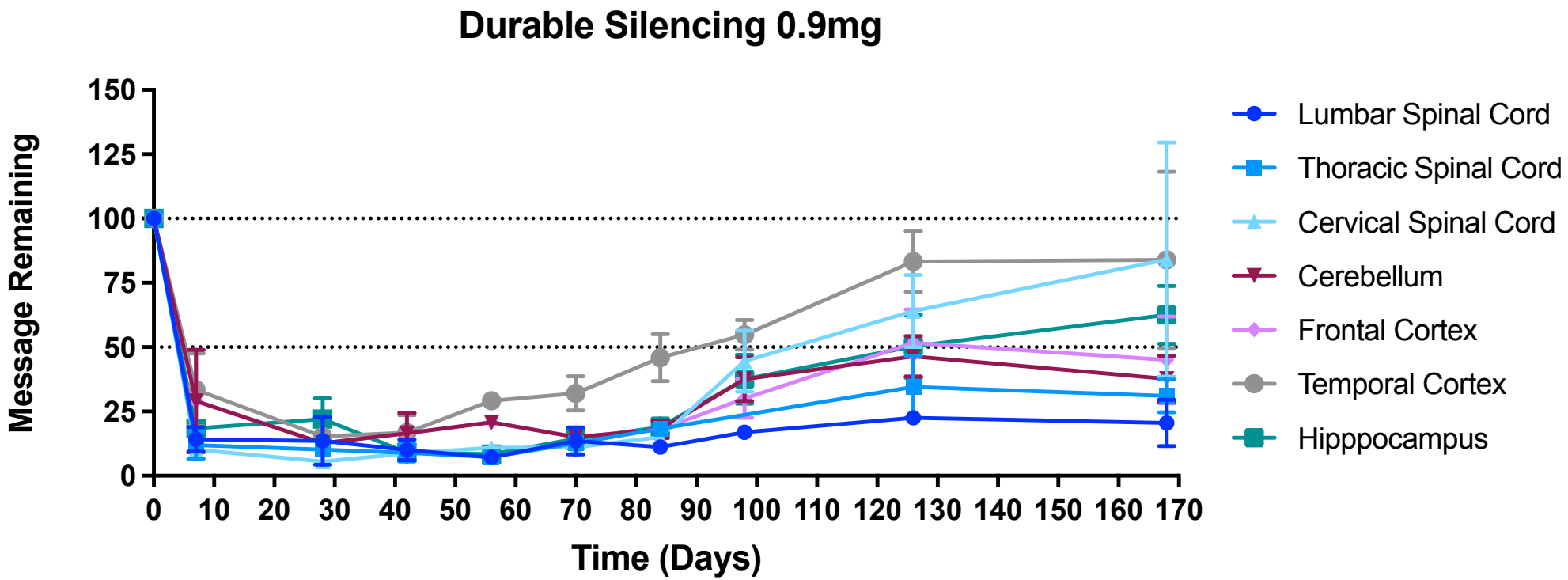


Temporal Cortex



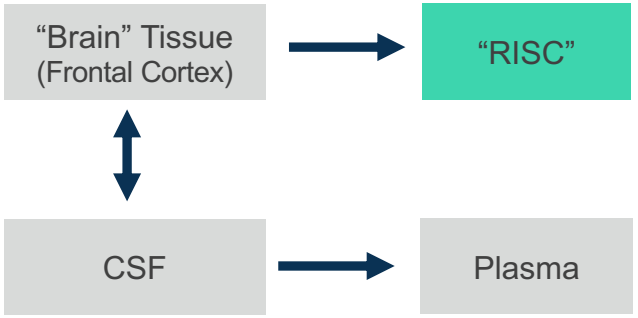
Robust Silencing Throughout the Rat Brain

Intrathecal delivery of siRNA provides durable knockdown throughout CNS in rat

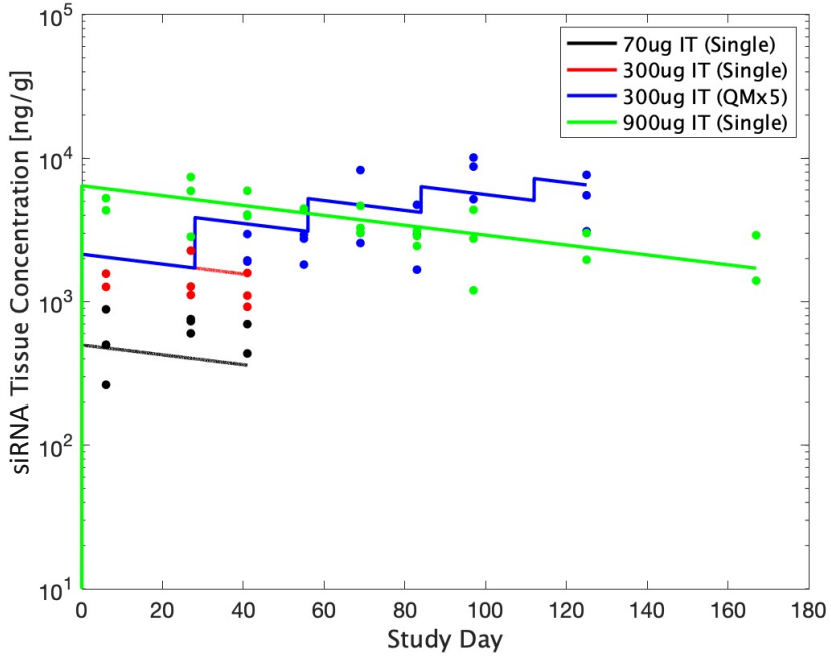


Consistent lowering across animals in most regions of the brain

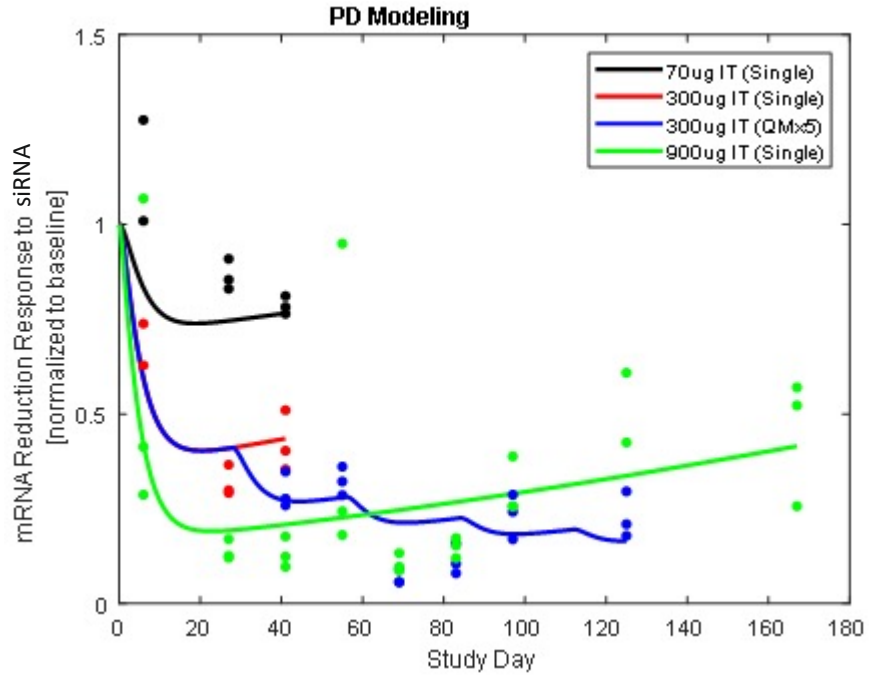
PKPD Model Can Describe Exposure-Pharmacology Relationship For Single And Repeat Doses in Rats



PK in Frontal Cortex
Half-life ~ 4 months



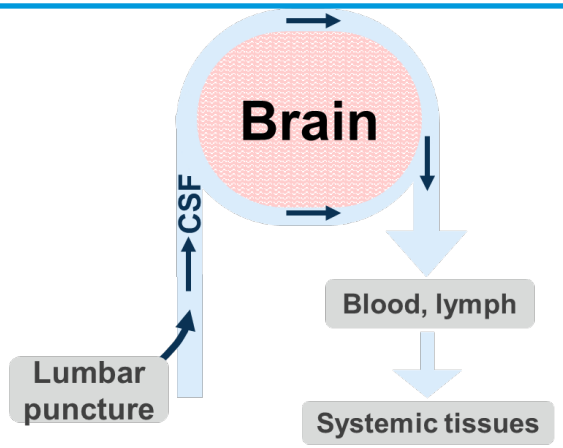
PD
ED_{50} ~ 0.3 mg



- Drug is absorbed very rapidly from CSF (majority with-in first 24 hours) and then cleared very slowly from tissue with siRNA half-life ~ 4 months (frontal cortex)
- Dose dependent exposure; multidose exposure on monthly schedule is additive
- Similar behavior in other tissue groups
- Integrating an indirect PD model via a stand-in "RISC" PK compartment allows the PK model to describe mRNA reduction observed in same study

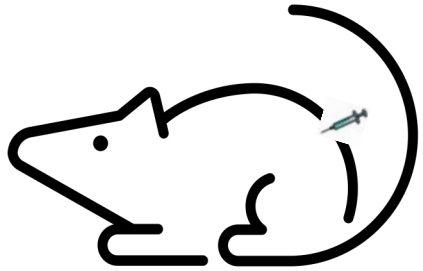
Rat CNS distribution of radiolabeled siRNAs

IT drug delivery



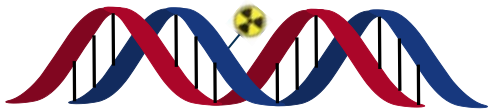
Study Groups

Three groups:
Lumbar IT catheter
N = 2 animals/siRNA
3 toolkit siRNAs

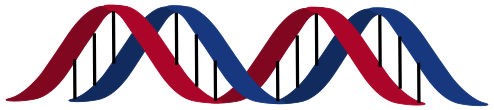


Dose Formulation

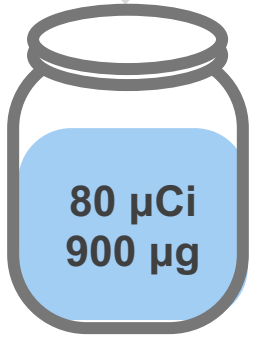
[^{111}Indium]-DTPA-conjugated siRNA



Unmodified siRNA



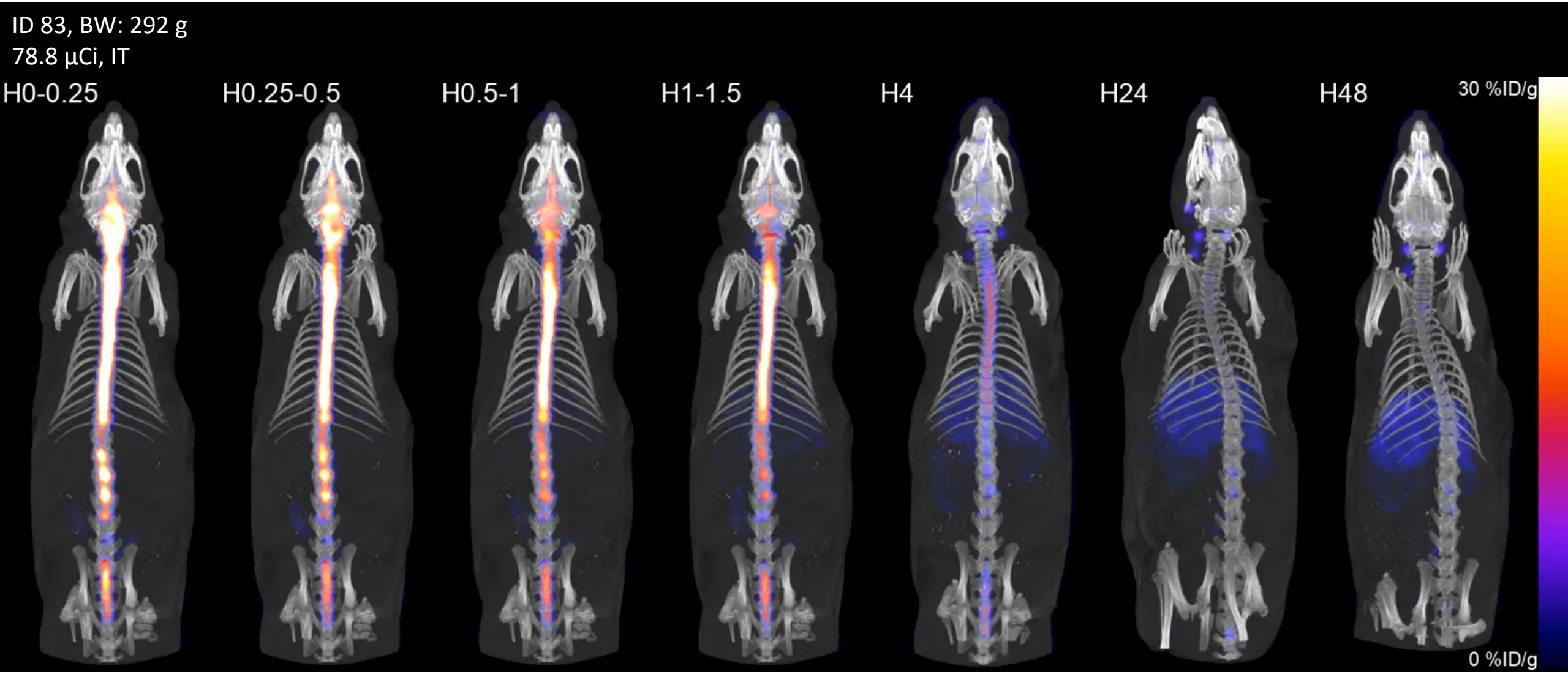
Dosing (per animal)



SPECT/CT Imaging	Sampling	Radiological quantification
0-1.5 (dynamic), 4, 24 and 48 h	Whole-organ and blood gamma counting, and immunohistochemistry for siRNA	^{111}In whole-body uptake per timepoint, and uptake by 13 brain regions

Pharmacokinetics of IT-dosed ^{111}In-siRNA in rodents

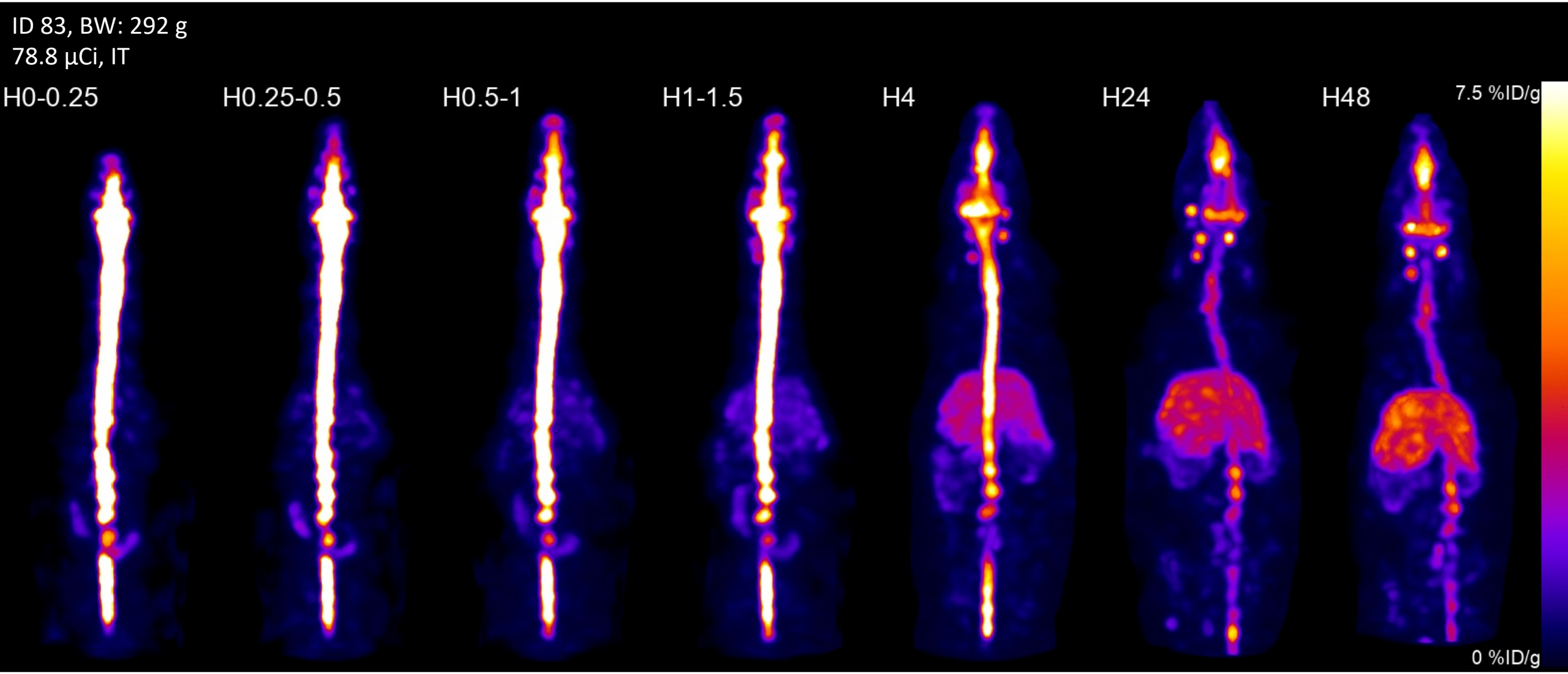
Co-registered SPECT/CT images facilitate anatomical orientation



MIP: Maximum intensity projections derived from projecting the voxel with the highest value on every view throughout the volume onto a 2D image.

Pharmacokinetics of IT-dosed ^{111}In-siRNA in rodents

SPECT reveals rapid movement through CSF to brain (<1h) followed by drainage to systemic circulation



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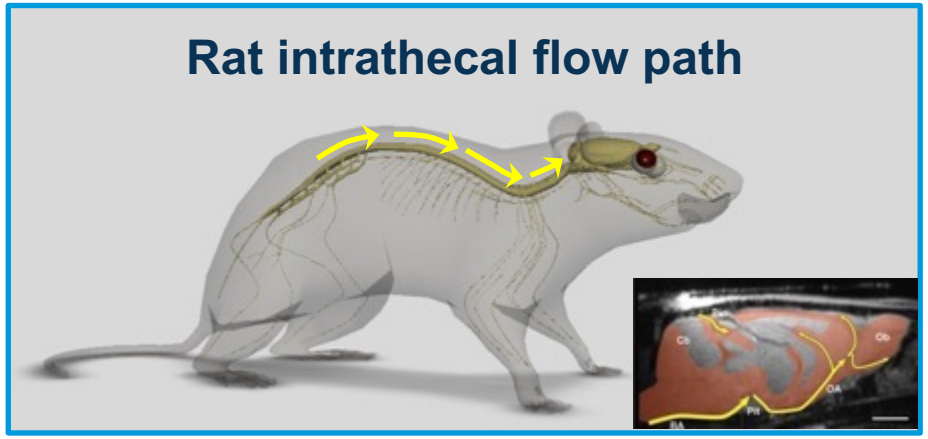
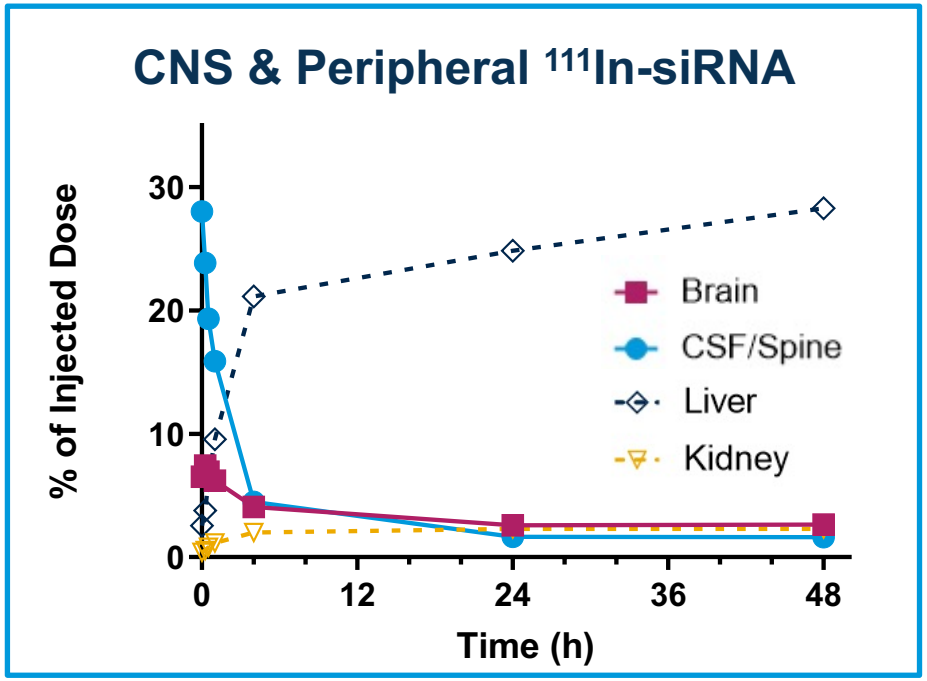
Rat distribution of intrathecally administered siRNA

- Summary of SPECT/CT with ^{111}In-siRNA:
  - Follows primary CSF flow routes up the spine and around the brain.
  - Clears CSF fast due to systemic drainage. CSF turnover (9×/day in rat) is primary clearance route.
  - Small fraction distributed to brain: 2-3% of injected dose at 48 hours.
  - Rapid and substantial peripheral tissue distribution (highest concentration in liver)
  - Consistency with “cold” studies suggests radiolabel is stable, accurately reflecting distribution of duplex.



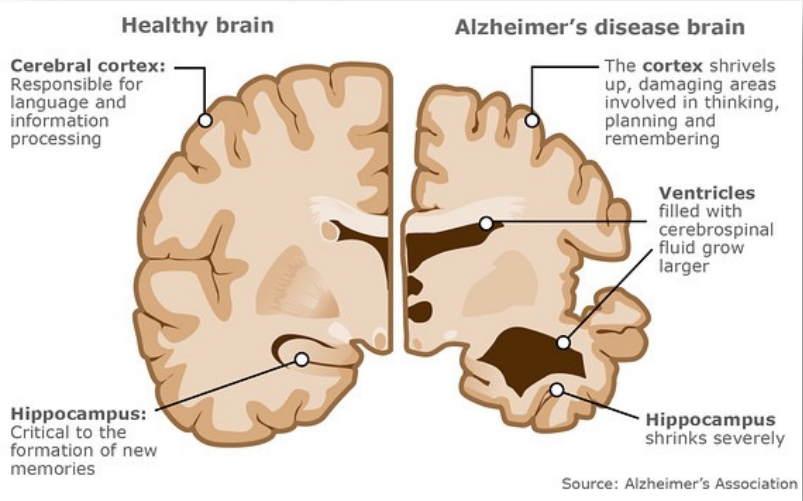


Iliff et al 2013 J Clin Inv; 3D Rat Anatomy, Biosphera

APP Targeting for Autosomal Dominant Alzheimer's Disease (ADAD)

ADAD

Patients develop rapidly progressive Alzheimer's-type dementia



BURDEN

- ~50,000 affected globally
- Mean age of onset 44 years with rapid progression over 6-8 years

TARGET IDENTIFICATION

- All ADAD causative genes identified to date (*APP, PSEN1, PSEN2*) regulate APP protein metabolism by increasing production of amyloid products, including Aß42
- Autosomal dominant, nearly 100% penetrant genetic syndrome

THERAPEUTIC HYPOTHESIS

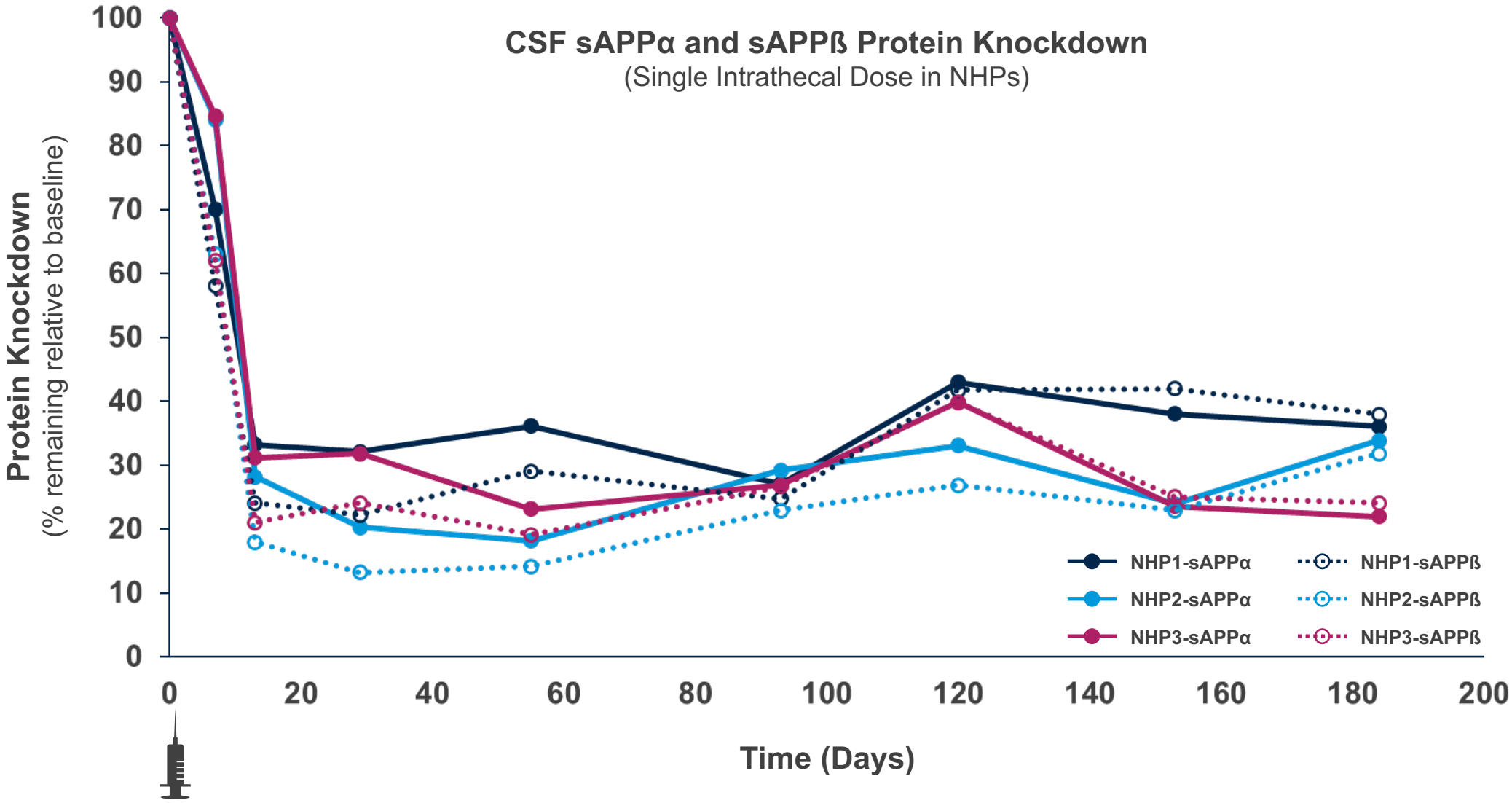
- Aß42 is made in neurons and aggregates in the intracellular and extracellular brain parenchyma
- RNAi-mediated knockdown of *APP* transcript in neurons will lower production of Aß42, halting aggregation and plaque formation

OPPORTUNITY

- Application of Alnylam's CNS platform to reduce parenchymal *APP*-derived amyloid in ADAD with no existing disease-modifying treatment
- Potential for expansion into sporadic Alzheimer's disease

Highly Durable Amyloid Precursor Protein (APP) Knockdown in NHP

Single Intrathecal Dose of ALN-APP Supports Bi-Annual or Less Frequent Regimen



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Investigational RNAi Therapeutics for Ocular Diseases

Expanding Alnylam opportunities beyond liver

Devastating diseases with enormous burden and unmet need

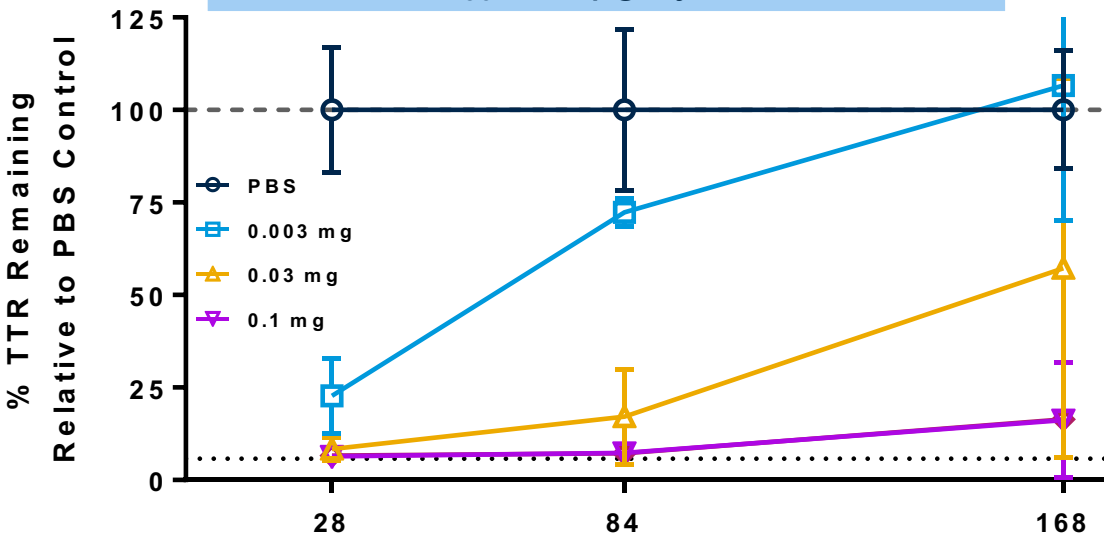
- AMD, dry
- AMD, wet
- Birdshot chorioretinopathy
- Dominant retinitis pigmentosa 4
- Fuch's dystrophy
- hATTR amyloidosis
- Hereditary and sporadic glaucoma

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

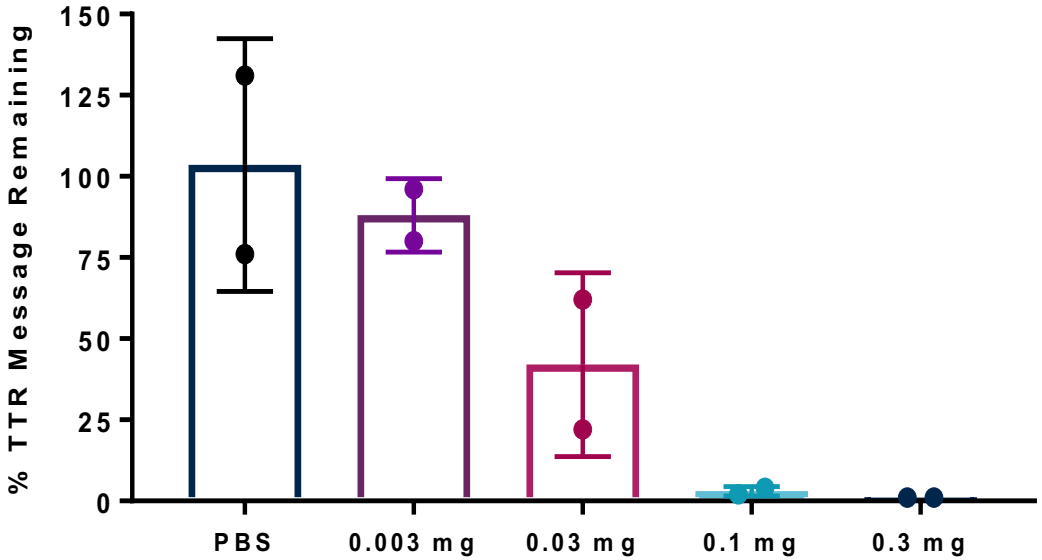
RNAi therapeutics directed to disease-causing, Ocular-expressed genes represent a potential opportunity to address diseases with some of the greatest unmet need.

Robust and Durable Activity in 6-month NHP Studies

TTR protein analysis in Aqueous Humor
ED_{90} < 10µg/eye D28



TTR mRNA analysis, Day 168



Knock Down of TTR Protein at 3 Month Demonstrated at 30µg

Sustained Protein and Message Knockdown Achieved at 100µg

Consistent Extra-hepatic 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 mouse, rat and NHP.
- siRNA uptake observed in all CNS tissues examined with drug levels in the ng/g to μg/g range
- In mouse, rat and NHP studies, central administration of the novel siRNA conjugates was well tolerated.
- Properties of the siRNAs in preclinical models suggest a major advancement of RNAi in CNS space

- TTR silencing demonstrated in rodents and NHP
- Target silencing is specific and found to be generally well tolerated
- Target knockdown in NHP seen for 168 days following a single IVT dose of siRNA
- Equivalent silencing demonstrated for mRNA and protein across the eye

Alnylam PHARMACEUTICALS