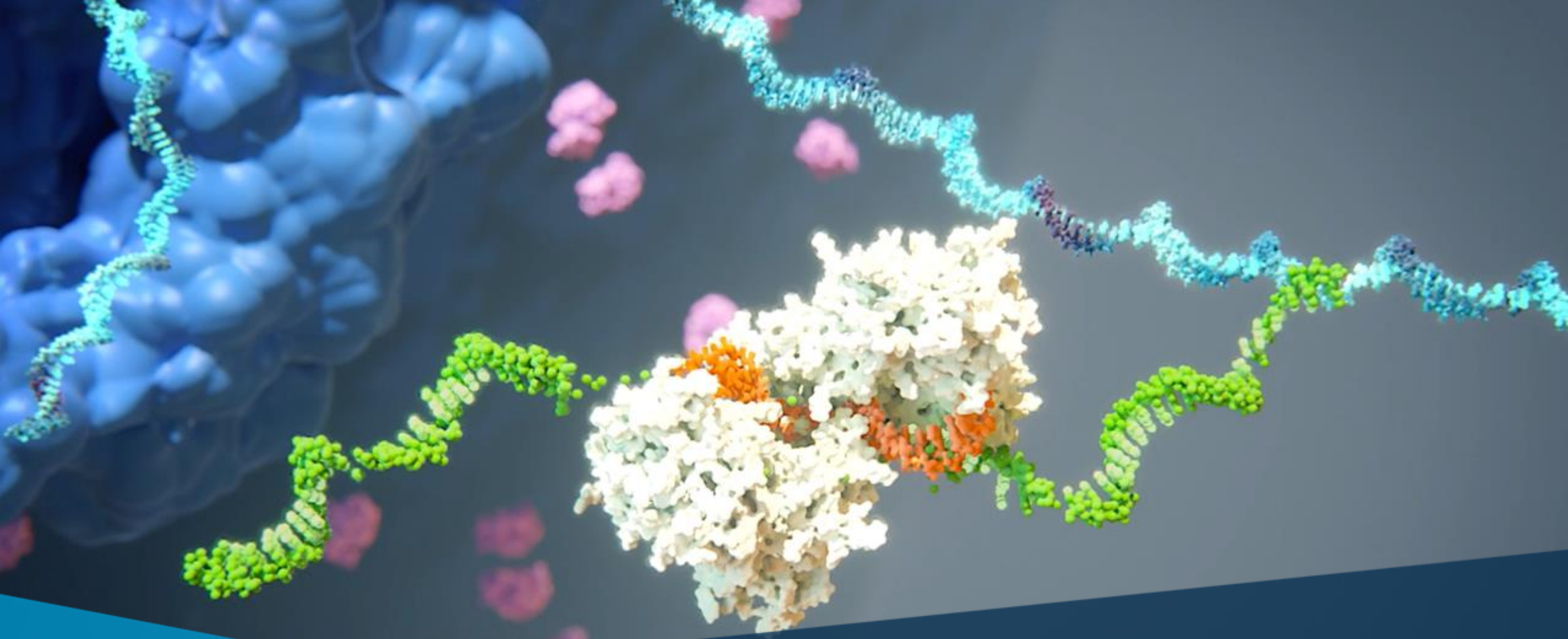


# Expanding the Reach of RNAi Therapeutics

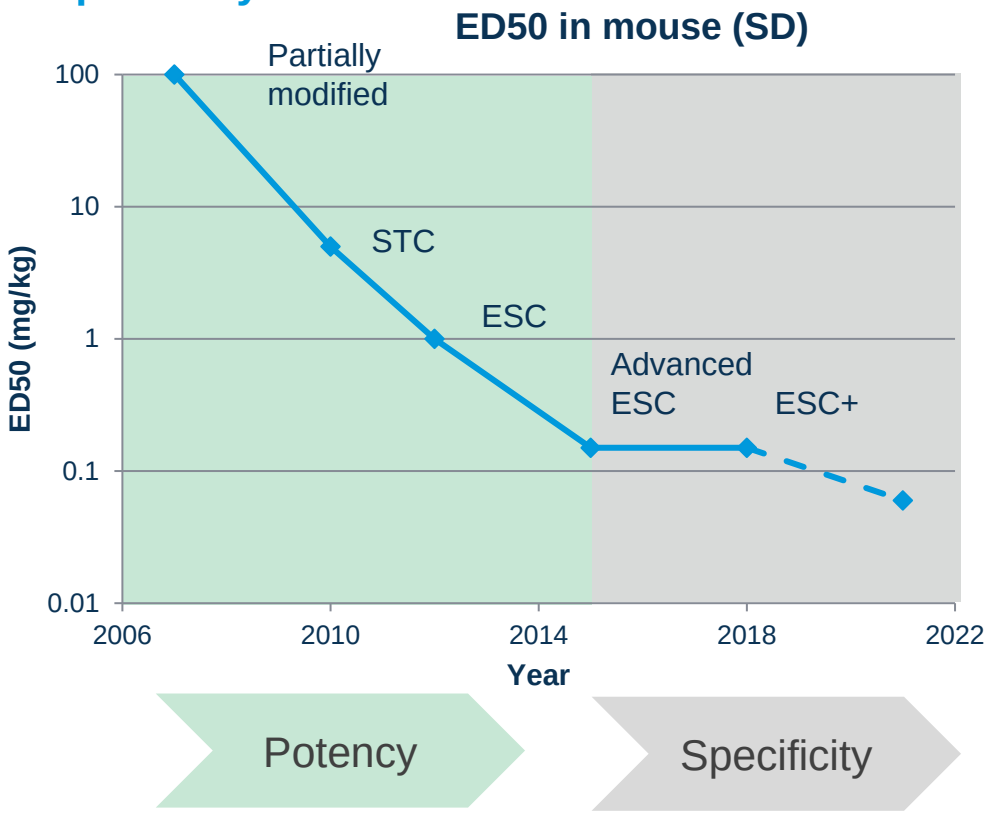
**Kirk M Brown**

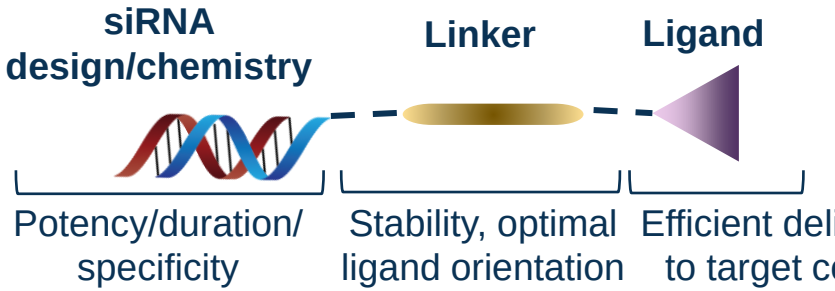
**OTS 2021**

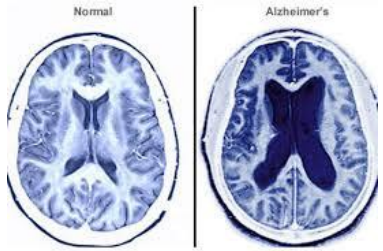
Alnylam PHARMACEUTICALS

# Advancements in Conjugate-Based Delivery Serve as a Blueprint for Extrahepatic Applications

## Evolution of conjugate design with improved potency and specificity



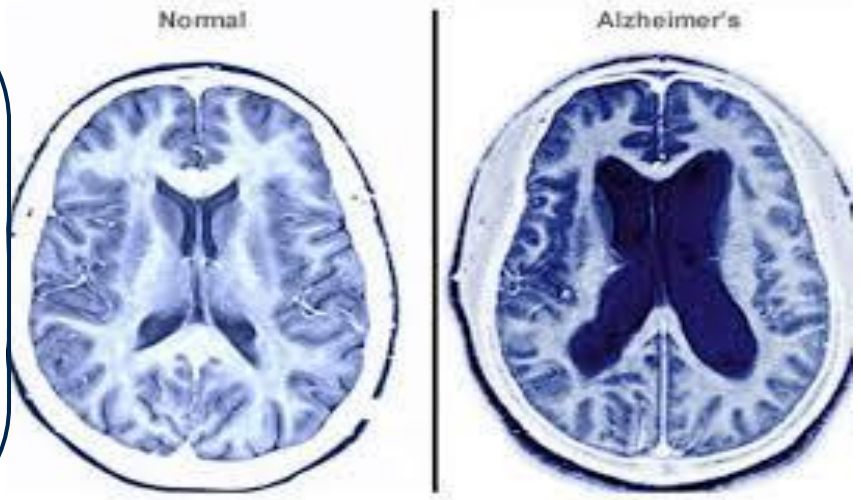

Investigational RNAi therapeutics for CNS

# Investigational RNAi Therapeutics for CNS Diseases

## 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

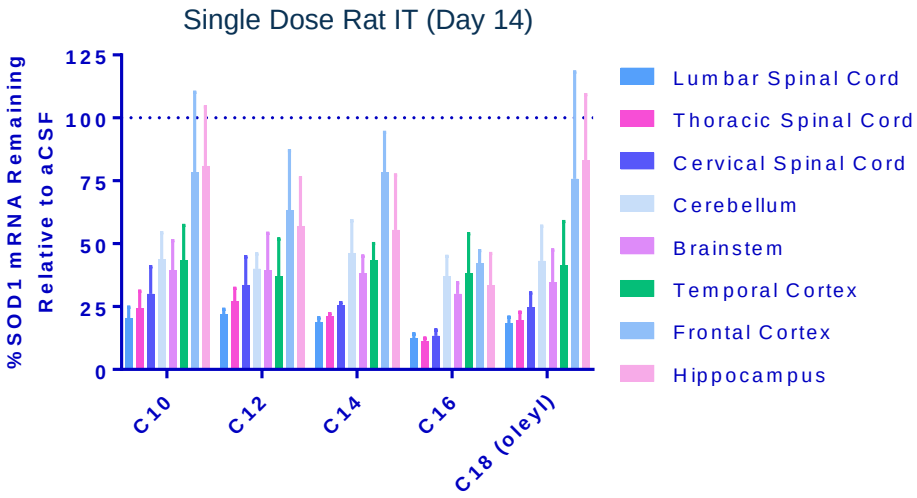



A large number of genetically validated targets are known but few disease modifying therapies for these devastating, life threatening disorders

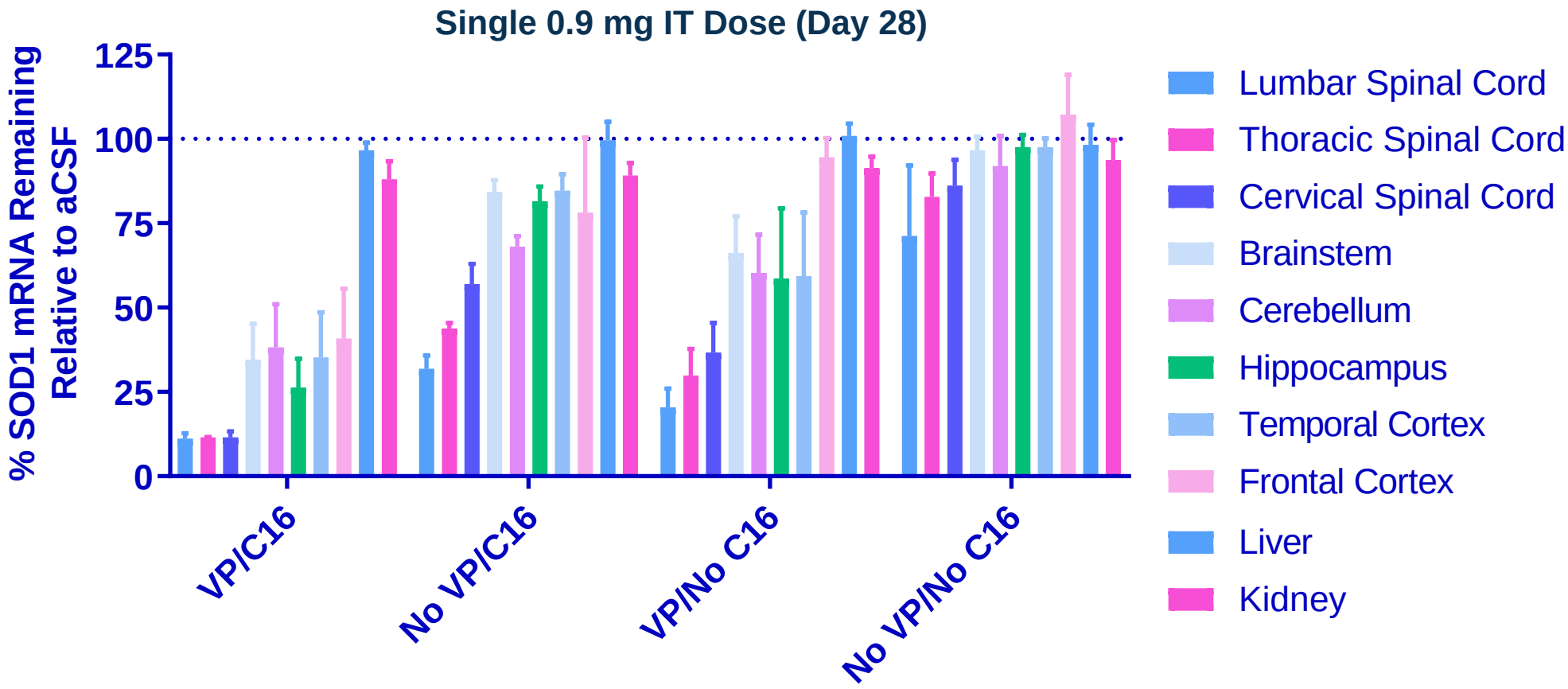
RNAi therapeutics directed to disease-causing, CNS-expressed genes represent an opportunity to address diseases with some of the greatest unmet need

# Conjugation of 2’-O-palmityl (C16) to siRNAs Enables Robust and Durable Target Knockdown in the Rat CNS

## Optimization of siRNA lipophile, position and design chemistry for CNS delivery



# Greatest Potency Across Rat CNS with siRNA Combining Both 5'-VP and 2'-C16



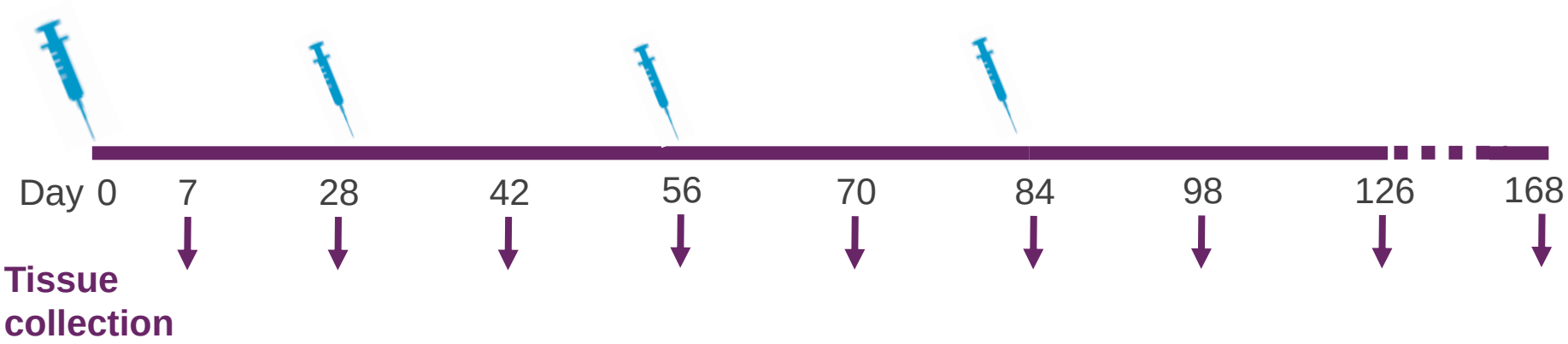
# IT Studies with CNS siRNA Conjugates in Rats

# Intrathecal 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**

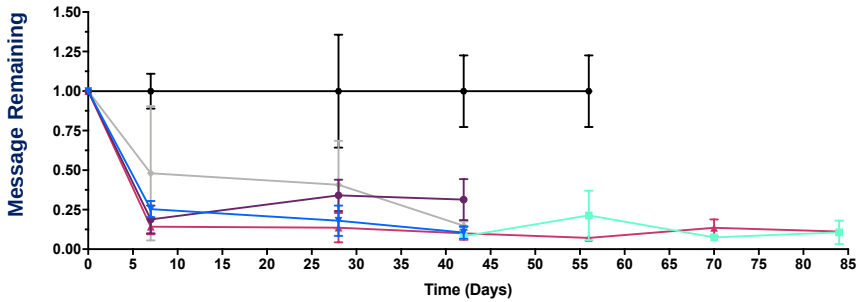
**Assays: mRNA, tissue siRNA levels, Histology**

| | Strand (5'-3') | Sequence |
|---|---|---|
| SOD1 | S | CAUUUUAAUCCUCACUCUAAA |
| | AS | UUUAGAGUGAGGAUUAAAAUGAG |

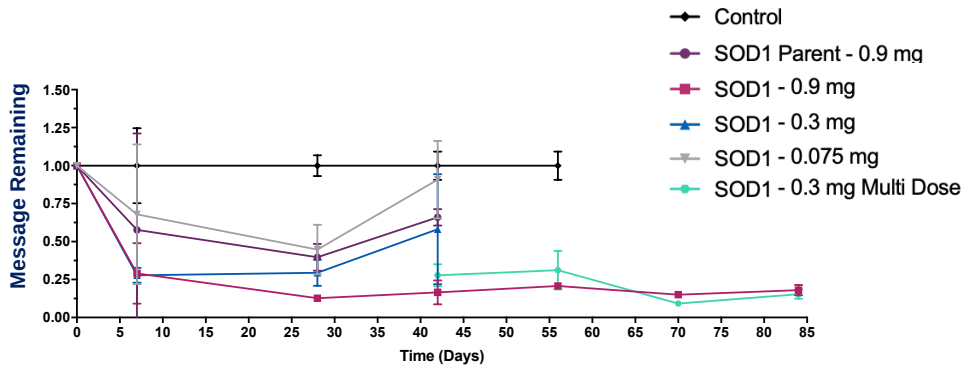
# Robust and Durable Silencing Demonstrated Following a Single IT Dose

## Silencing of SOD1 following a single or multiple IT doses

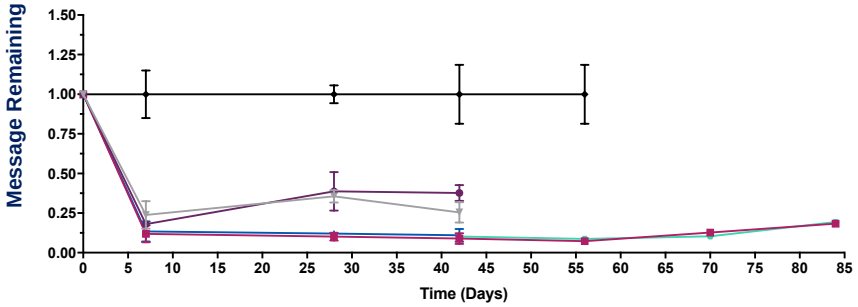
Lumbar



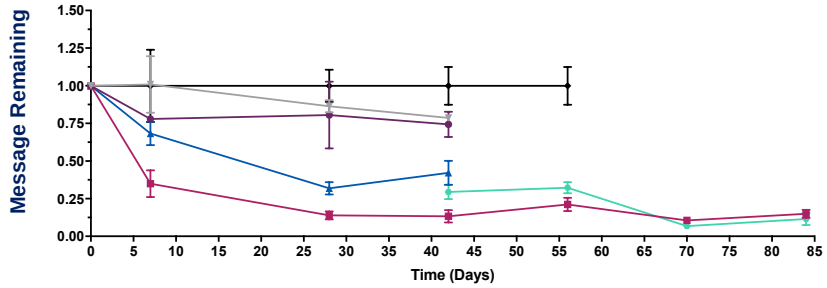

Cerebellum



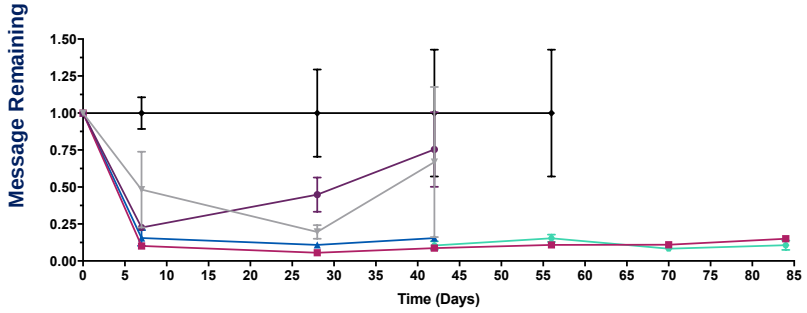

Thoracic



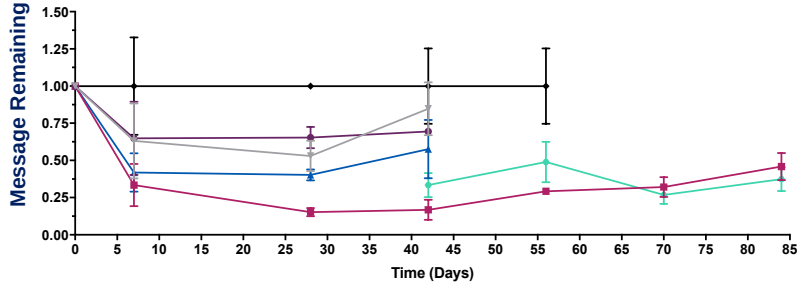

Frontal Cortex




Cervical

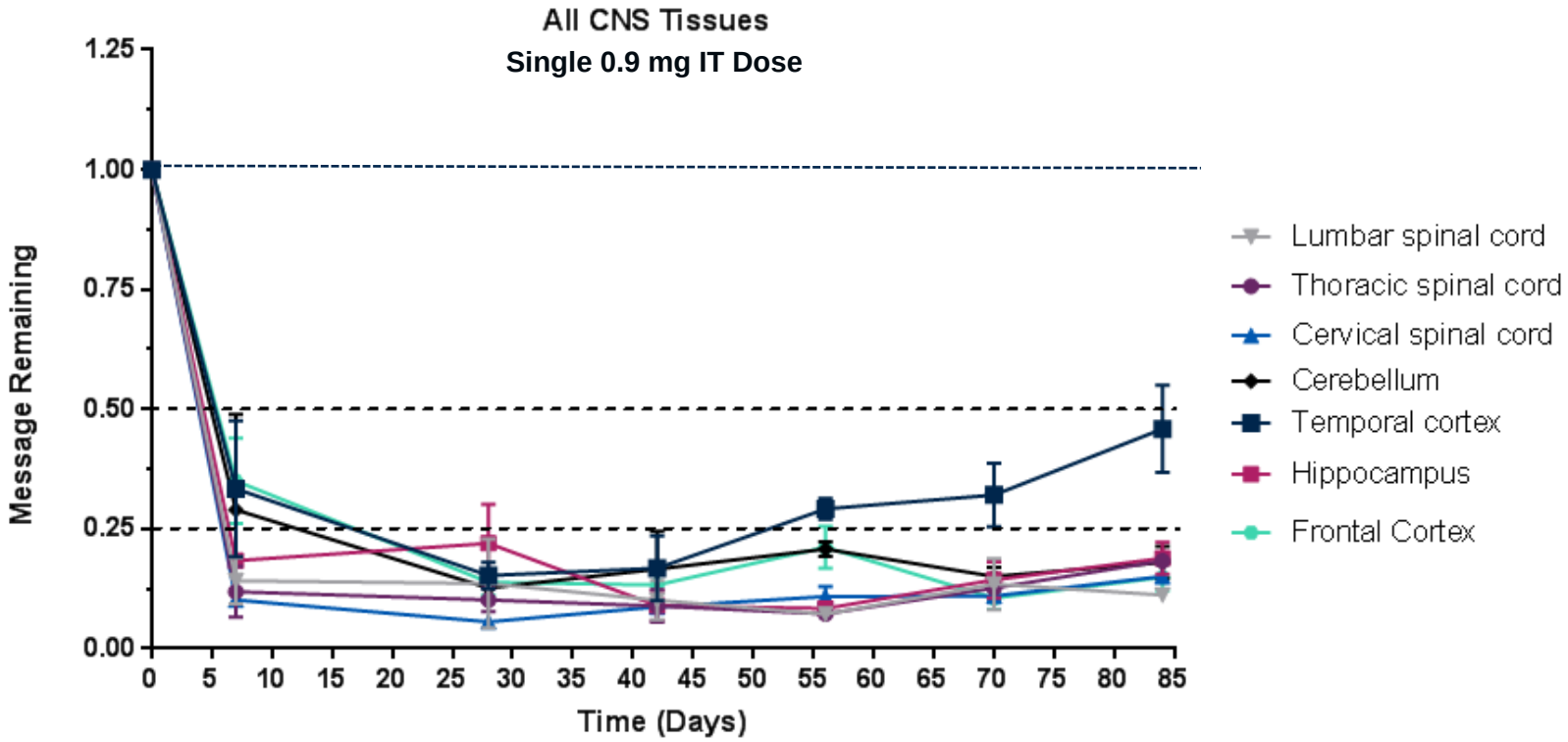



Temporal Cortex



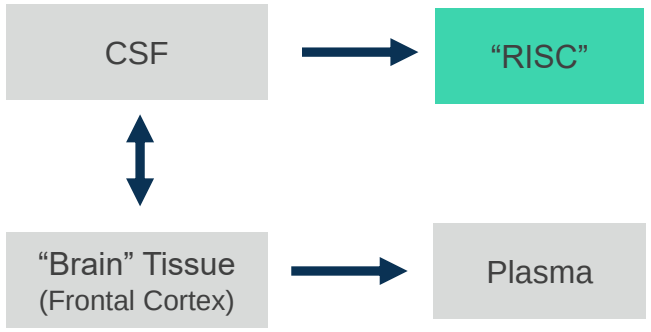
# Robust Silencing of *SOD1* Transcript Throughout the Brain

## Intrathecal delivery of siRNA provides durable knockdown throughout CNS



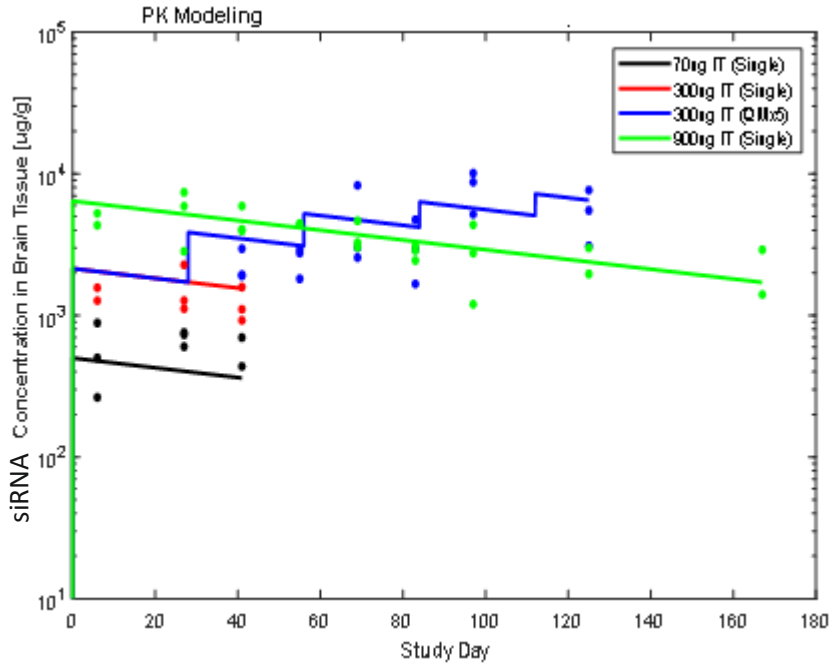

- Target lowering across animals throughout the spinal cord and the brain

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



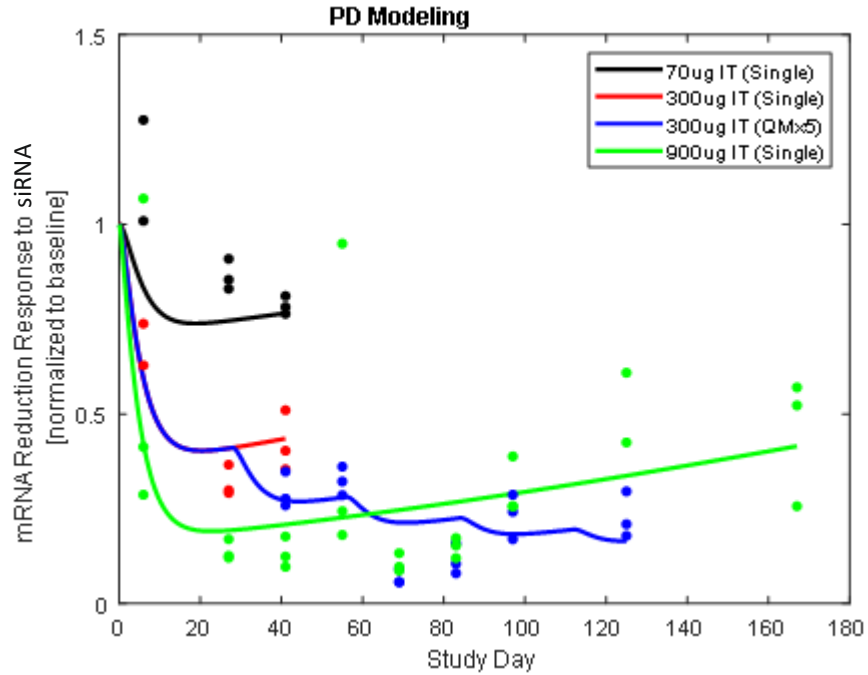

**PK in Frontal Cortex**

Half-life ~ 4 months




**PD**

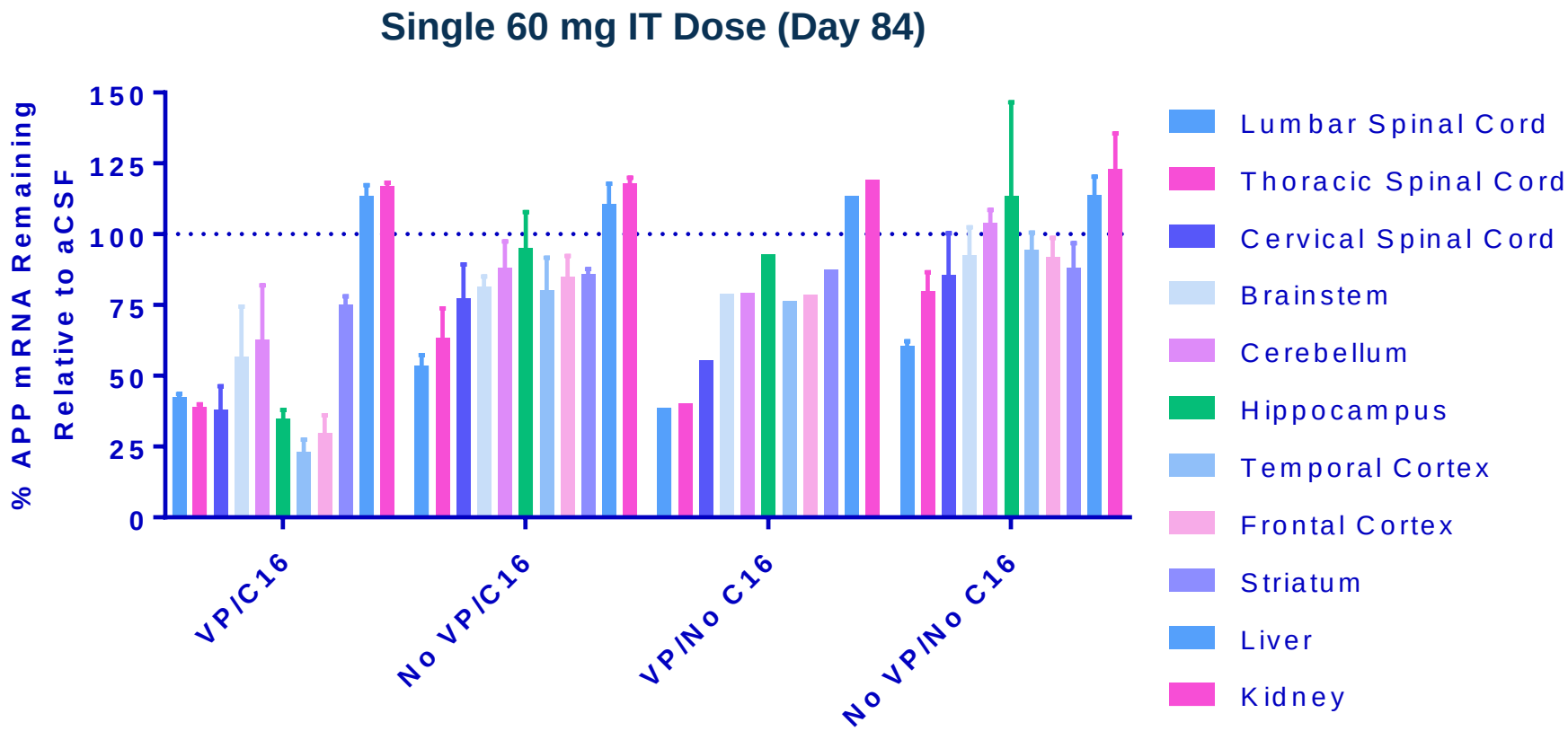
$ED_{50}$ ~ 0.3 mg




- 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

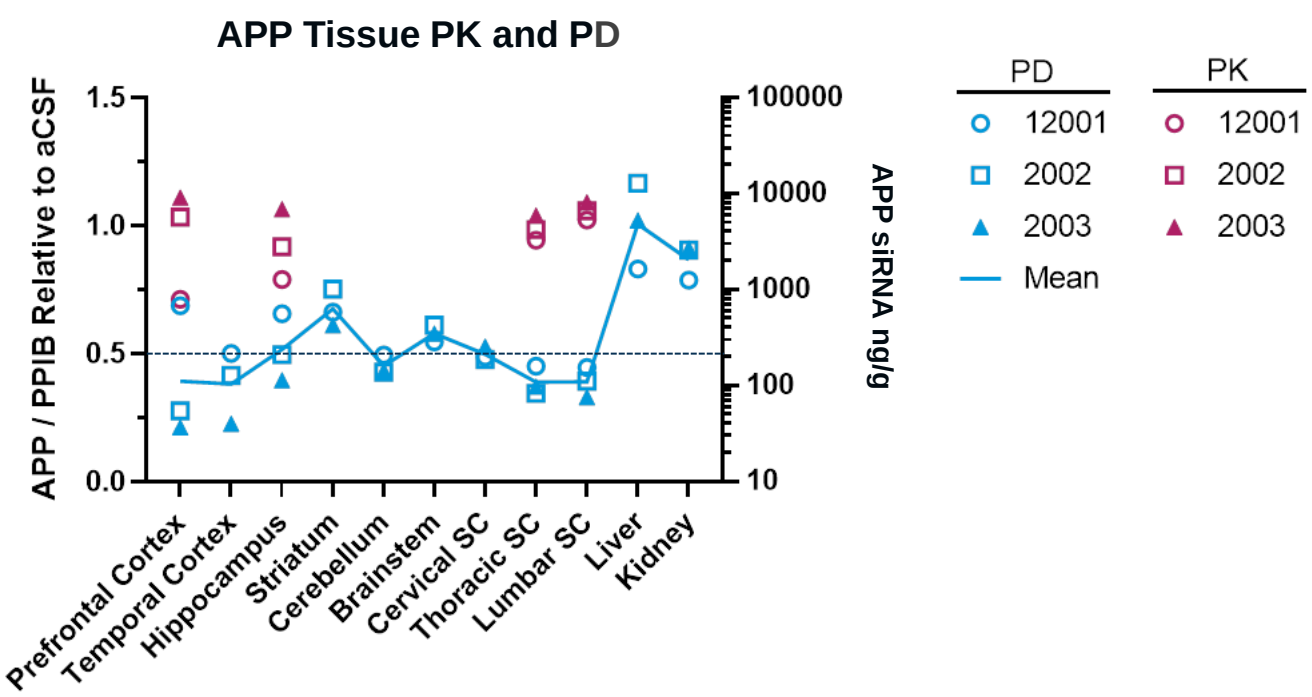
# Intrathecal Studies in NHP with Toolkit CNS siRNA Conjugate Targeting APP

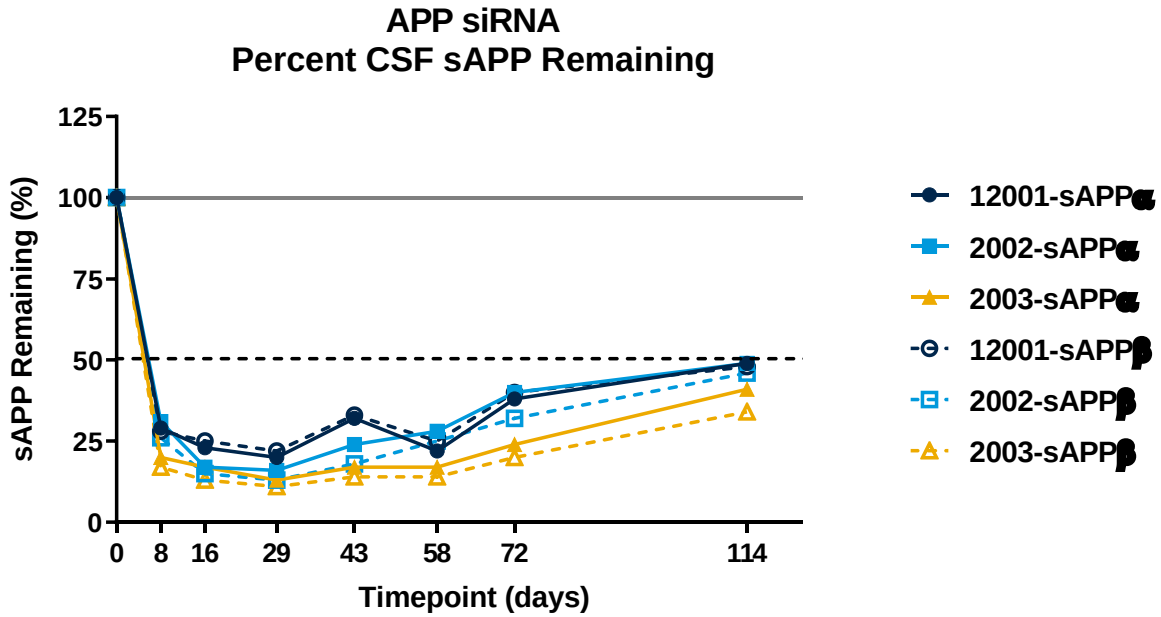
# Greatest Potency Across NHP CNS with siRNA Combining Both 5'-VP and 2'-C16



# Durable Silencing and Good PK/PD Relationship in CNS Tissue after Single IT Injection in NHP

## Sustained silencing across CNS regions at 18 weeks following a single IT administration (45 mg)



# Toxicology Summary for C16 Conjugates

## No test article-related changes in platform studies or CTA-enabling studies for ALN-APP

### 6-month Platform PD Study in Rat

- Single IT LP dose (0.07, 0.3, 0.9 mg) and Multi-IT LP dose (5 x 0.3 mg)
  - No TA-related findings in spinal cord, brain, liver, kidney, lung, diaphragm, pituitary, sciatic nerve, skeletal muscle, spleen or thymus
  - No TA-related findings on clin signs, body weight or body weight gain

### Platform non-GLP Tox Studies in Rat

- 15-day systemic tox
  - No changes observed in serum chemistry, hematology or histopath
- 15-day IT LP tox
  - No test article findings across all parameters examined: clinical observations, body weight, functional observational battery, clinical pathology parameters, macroscopic findings, and microscopic examinations including expanded neurological assessment with Fluoro-Jade

### Platform non-GLP Tox Study in NHP

- 15-day IT LP tox
  - No test article related findings across all parameters examined: clinical observations, body weight, neurological exams, macroscopic findings, and microscopic examinations including neurological assessment with Fluoro-Jade

### GLP Tox Studies in rat and NHP

- Completed for ALN-APP
  - No test article related findings

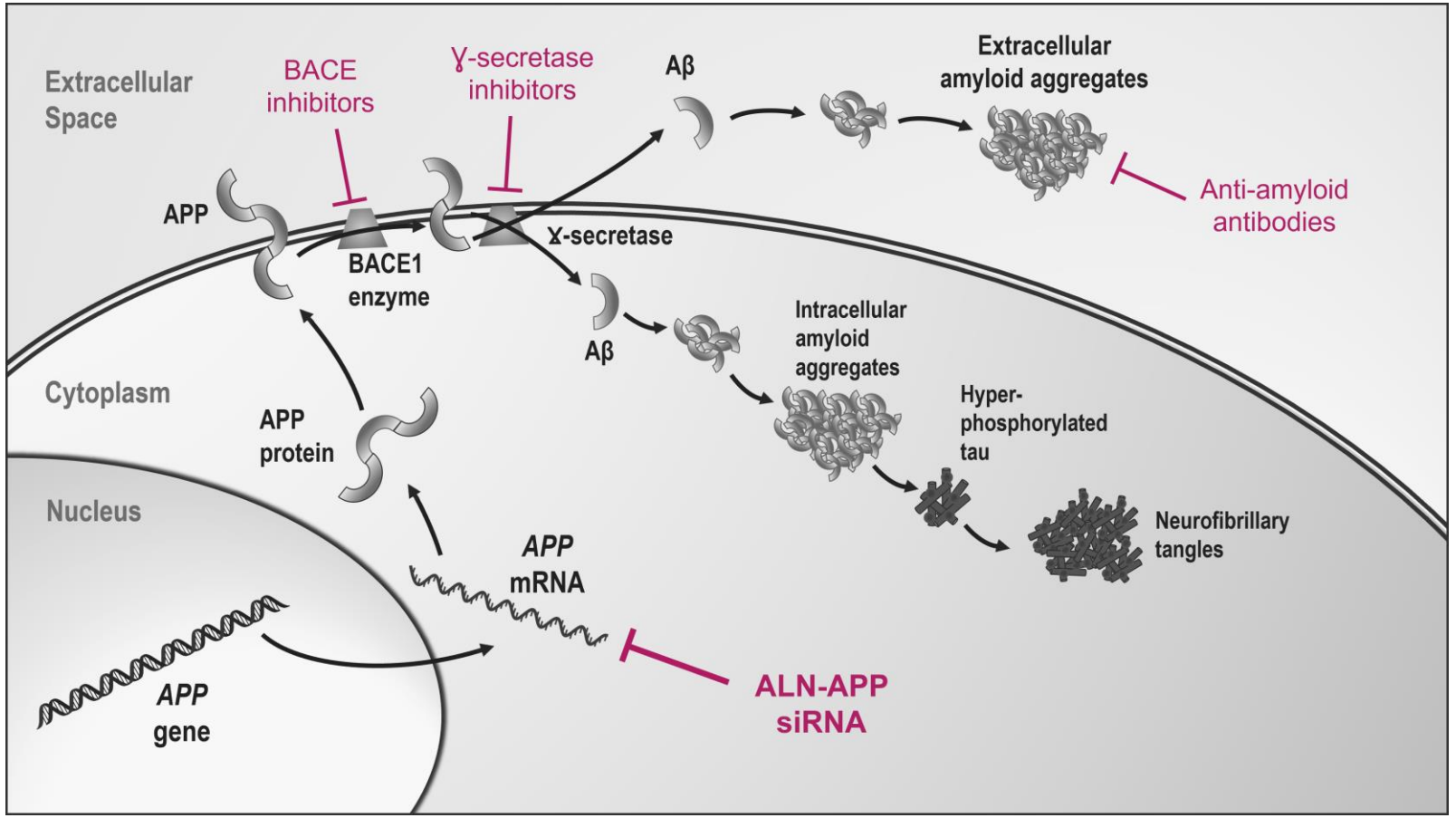
# Targeting Amyloid Precursor Protein for Early Onset Alzheimer's Disease

# Therapeutic Hypothesis: Alzheimer's Disease

## A New Approach: Targeting the *APP* mRNA

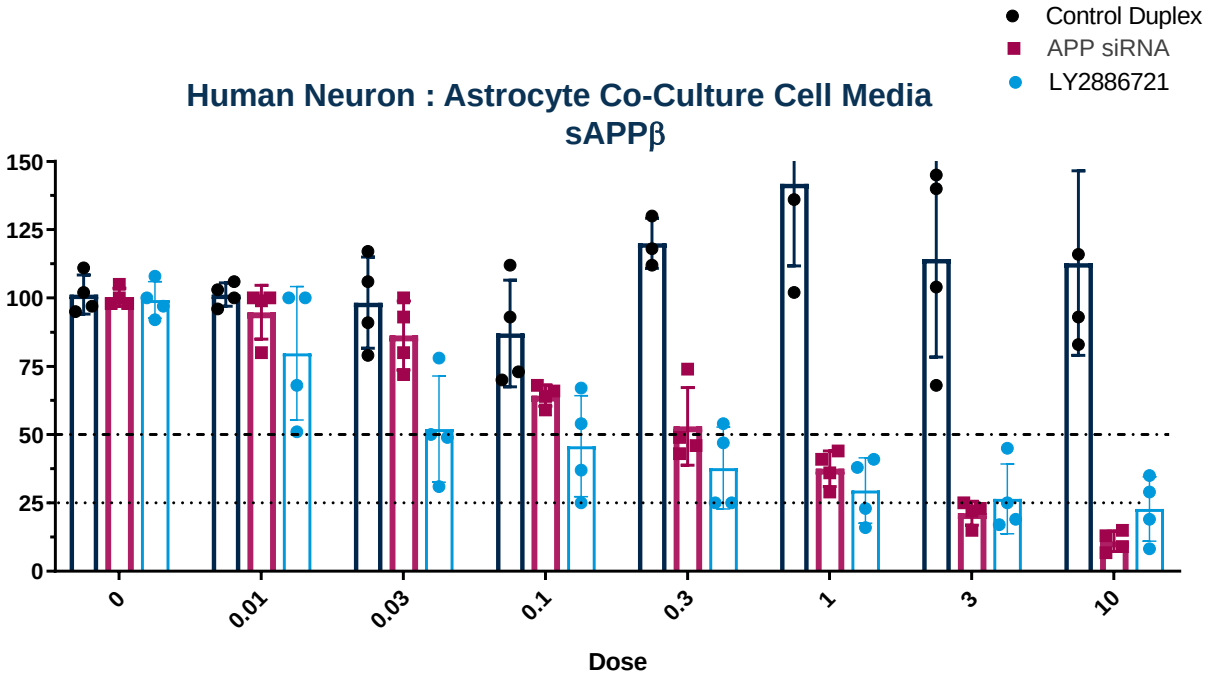
**Lower APP production *at its source*, upstream of the pathogenic process**

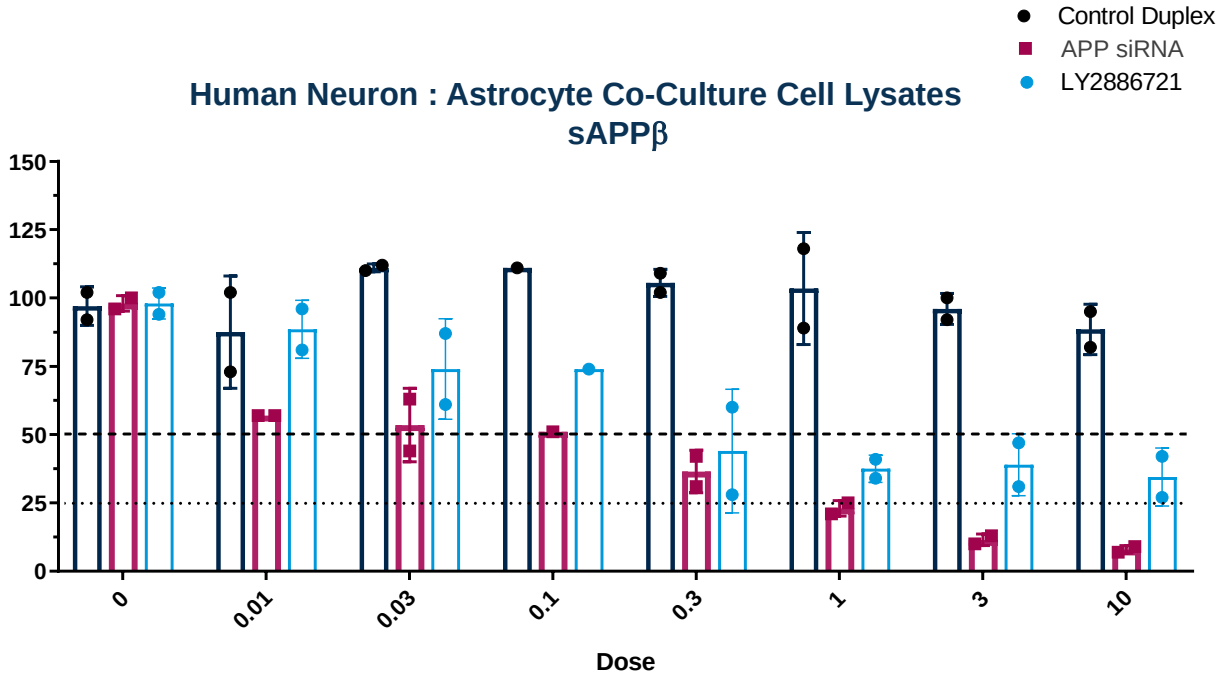
- Reduce ***both intracellular and extracellular drivers of disease pathology***
- ***Reduce all APP cleavage products*** including all species of Aβ and other non-Aβ drivers of disease
- Removing substrate for amyloid deposit formation and ***allow for natural clearance***



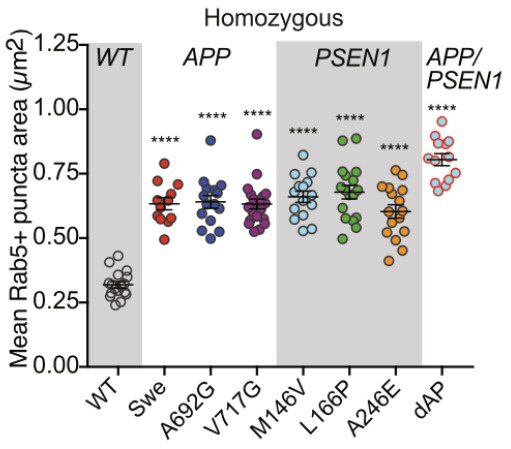
# siRNA Targeting APP Shows Greater Intracellular Reductions in sAPPβ Compared to BACE Inhibition

## APP siRNA lowers sAPPβ in both media and lysates in co-culture system of human neurons and astrocytes.



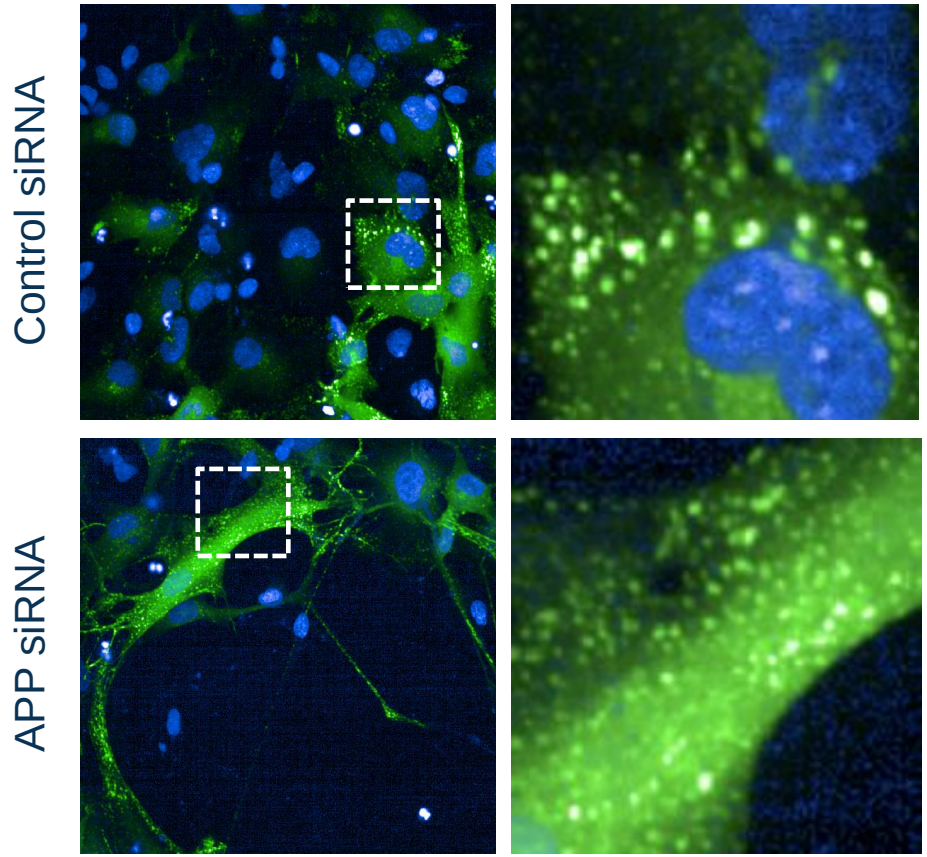



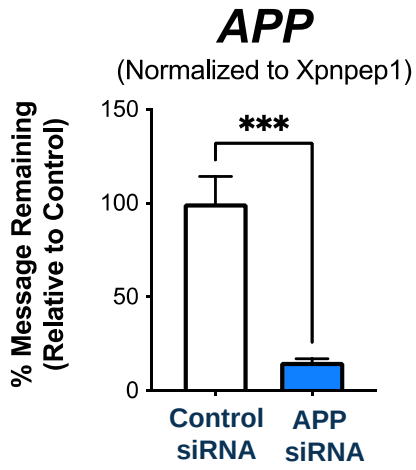
# *PSEN1* $^{A246E}$ patient iPSC-derived Neurons treated with APP siRNA show a reduction in Rab5+ Early Endosome size

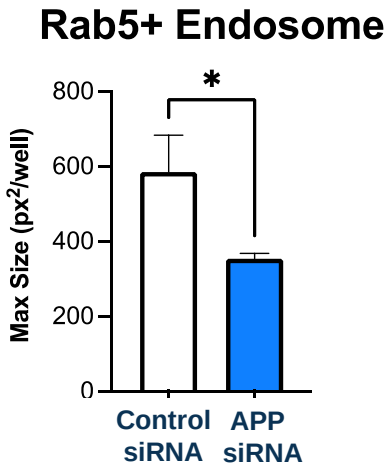



Mutations in APP and PSEN1 cause enlargement of Rab5+ Early Endosomes in human iPSC derived Neurons

Kwart et al., Neuron 2019



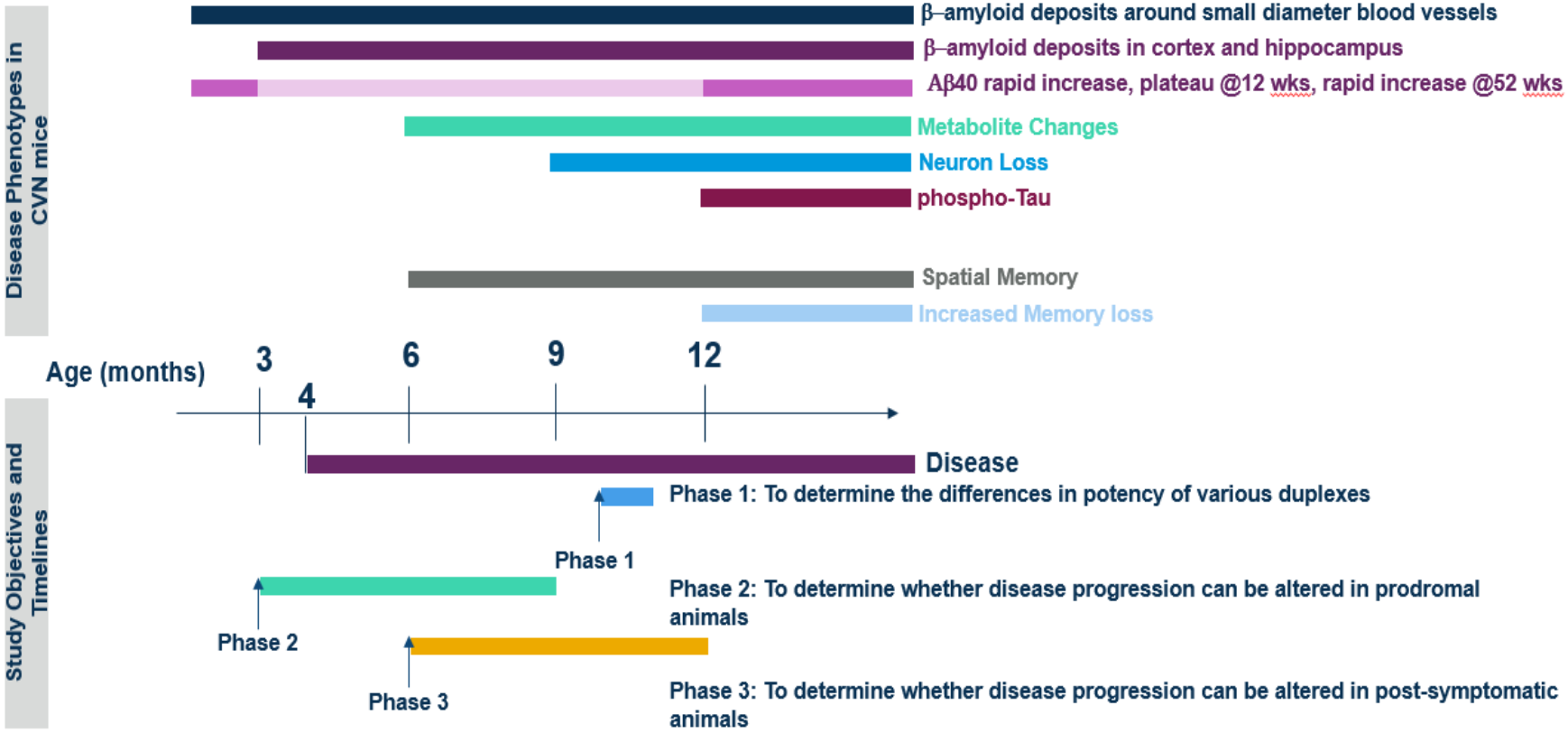



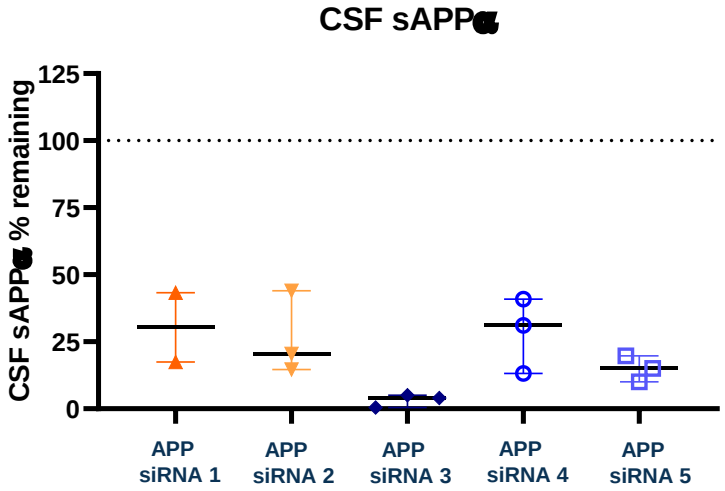




- Phenix High Content Imaging, 63X, analysis on Harmony
- Rab5+ endosome Alexa 488 for live imaging

# Silencing of Human CSF sAPP in Transgenic AD Mice Following ICV Treatment with siRNAs Targeting APP

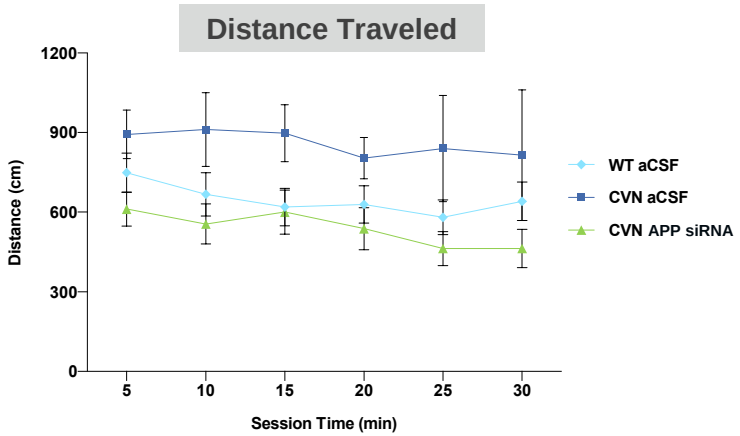
## Comparison of APP siRNAs with single 60 μg ICV dose in CVN mice ($APP^{SwDI}/Nos2^{-/-}$) at D30

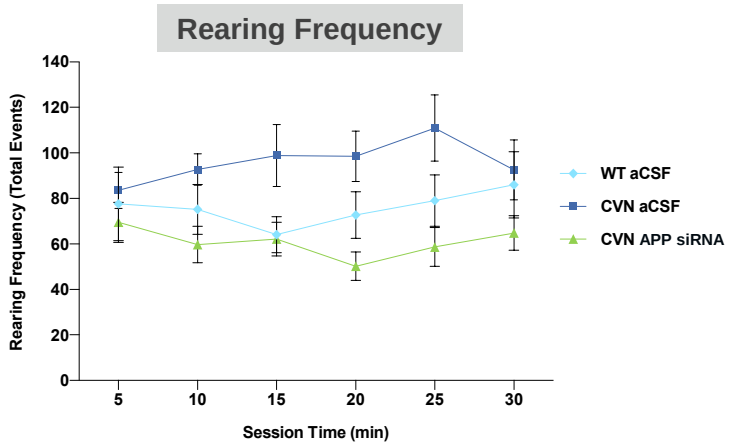


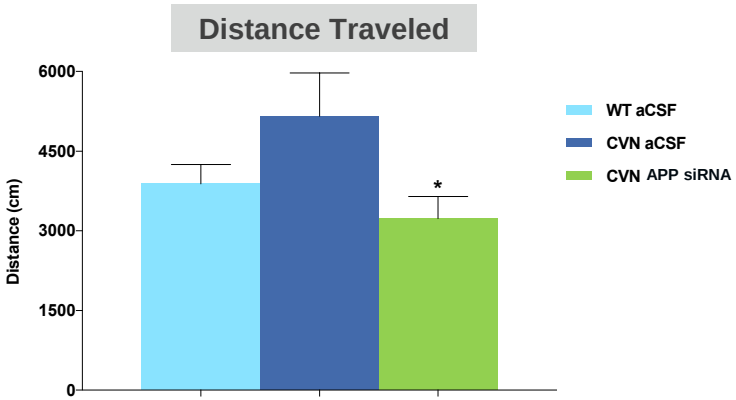



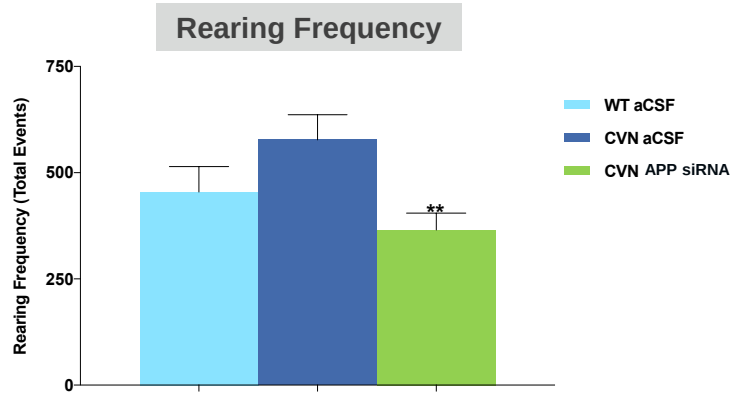
# Silencing of Human APP Showed Phenotypic Improvements in Open Field Test

## Phenotypic analysis of APP siRNA treated CVN mice following disease onset



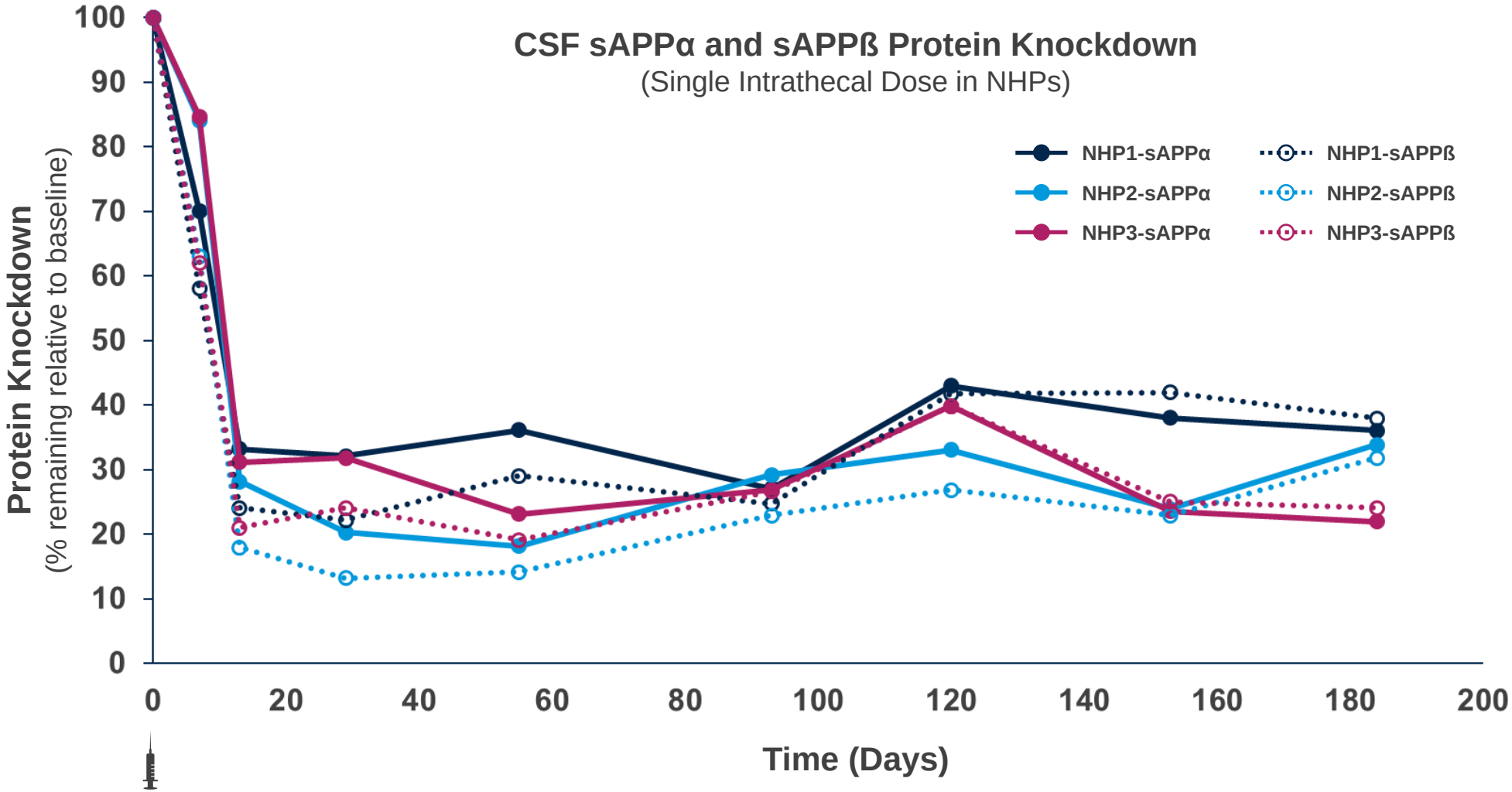




Phase 3

- Disease onset occurs at 4 months of age.
- Single ICV treatment at 6 months 120 μg.
- Phenotypic observations were taken at 9 months of age.
- Statistically significant change in behavior observed in treated animals in open field test.
- Amyloid burden and biomarker analysis pending.

# Highly Durable Amyloid Precursor Protein (APP) Knockdown in NHP

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



# Summary

- Advancements in siRNA chemistry together with improvements in mechanistic understanding have been the predominant drivers behind the evolution of the conjugate platform technology.

- Conjugation of 2'-O-palmityl (C16) to siRNAs enables safe, robust and durable target knockdown in the CNS of rats and NHP.

- Alnylam has developed an understanding of siRNA delivery, distribution and activity throughout the CNS across preclinical species.
  - siRNA conjugates are active across CNS regions
  - CNS conjugate designs show a good PK/PD relationship in the CNS
  - siRNA conjugates have long half-life in the CNS, resulting in durable activity (~ 6 months in NHP)
  - No test article-related safety findings to date in CNS Platform or ALN-APP rodent or monkey nonclinical studies

- C16 conjugates have been developed for multiple targets in the CNS and have shown promising results in pre-clinical disease models. First program (ALN-APP) expected to move to the clinic in early 2022.