# Discovery and Nonclinical Development of ALN-APP, an Investigational RNAi Therapeutic

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The ALN-APP clinical program is being conducted as a partnership between Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

# Disclosures for Kirk Brown


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| | |
|---|---|
| | No, nothing to disclose |
| X | Yes, please specify: |

| *Company Name* | *Honoraria/ Expenses* | *Consulting/ Advisory Board* | *Funded Research* | *Royalties/ Patent* | *Stock Options* | *Ownership/ Equity Position* | *Employee* | *Other (please specify)* |
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| Alnylam Pharmaceuticals | | | | | X | | X | |
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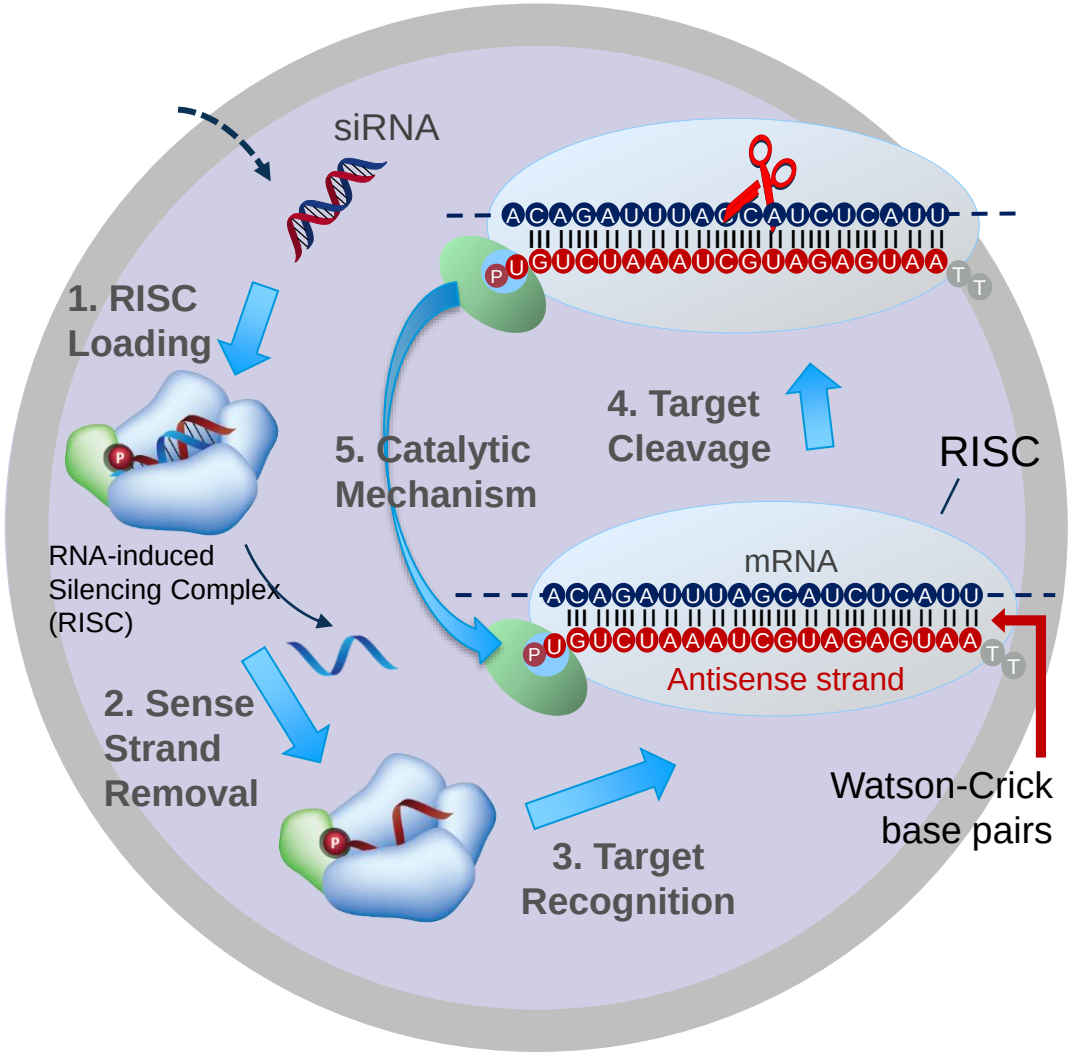
# RNAi Therapeutics for the CNS

## Significant Unmet Need for New Therapeutic Approach for Diseases of the CNS




### CNS siRNA Design Objectives for Intrathecal Lumbar Puncture Administration

- Potent siRNA for robust target reduction
- Ligand selection for wide biodistribution in CNS
- Chemically modified for specificity and long duration of action
- Safe and tolerated

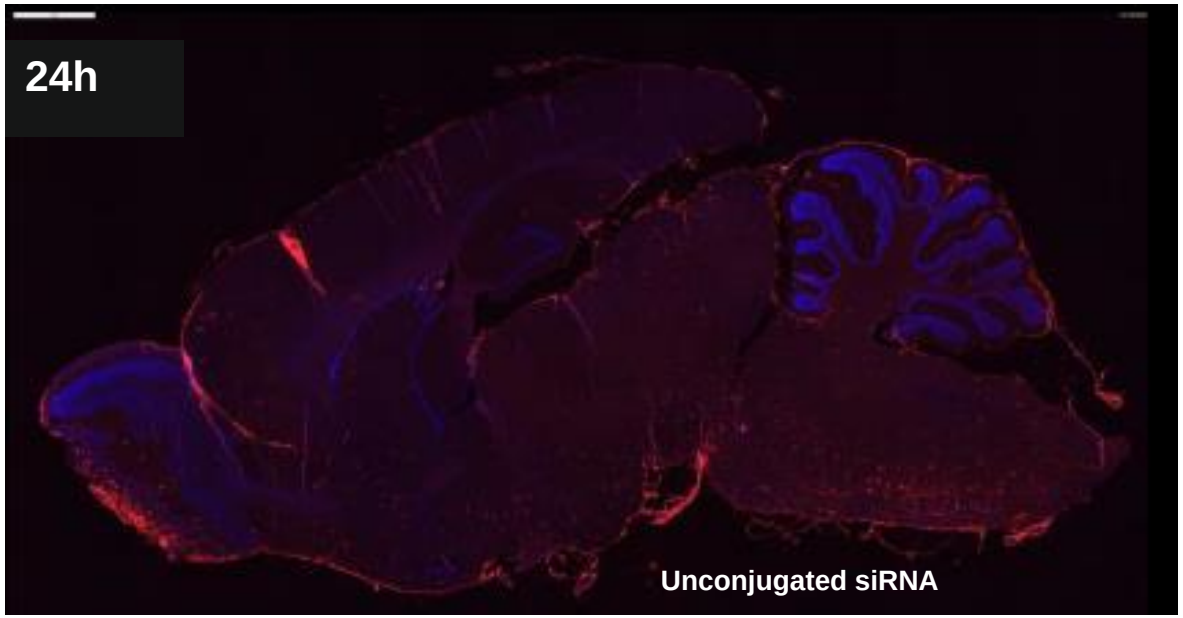


CNS, central nervous system; mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNA, ribonucleic acid; siRNA, small interfering RNA

# RNAi Therapeutics Can Be Delivered to the CNS

## Robust Distribution Throughout Rat CNS Achieved with C16 Conjugates

### C16 conjugate achieves distribution across brain regions





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

### OTS Paper of the Year 2022

*Nature Biotechnology*
**Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates**

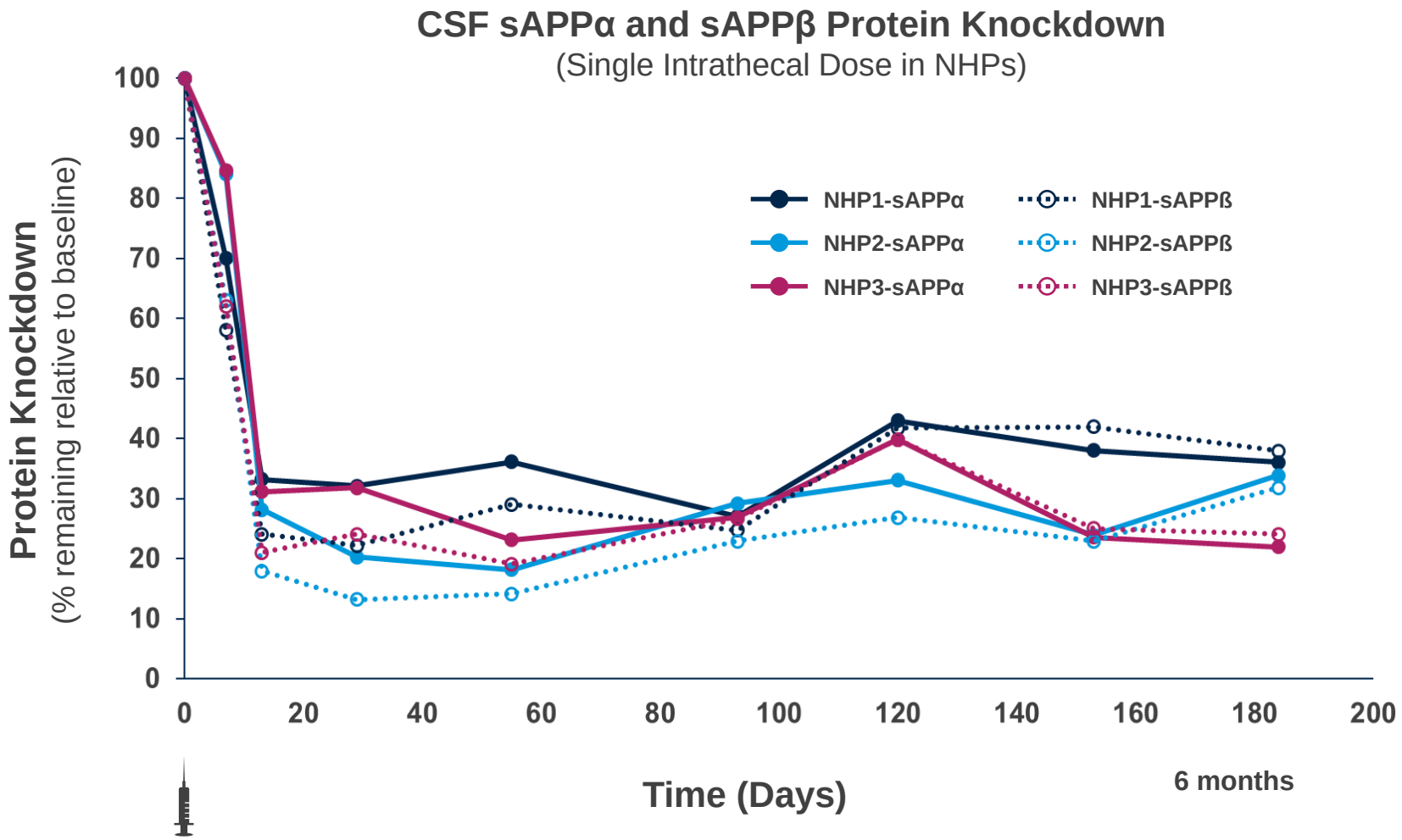
*Brown, et al. Nature Biotechnology 2022*

CNS, central nervous system; h, hours; IHC, immunohistochemistry; OTS, Oligonucleotide Therapeutics Society; RNAi, RNA interference; siRNA, small interfering RNA

Brown K et al. *Nature Biotech* 2022;40:1500–1508.

# 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

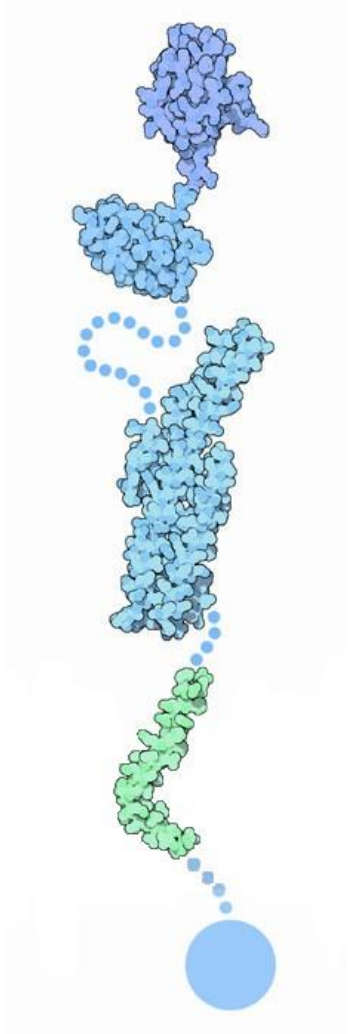


APP, amyloid precursor protein; CNS, central nervous system; CSF, cerebrospinal fluid; IT, intrathecal; mRNA, messenger RNA; NHP, non-human primate; RNA, ribonucleic acid; sAPP, soluble APP

# ALN-APP Target Identification and Lead Discovery

# Amyloid Precursor Protein (APP)

## Genetically Validated Target for Alzheimer's Disease and Cerebral Amyloid Angiopathy



### One target, two distinct pathological processes[1,2]

- APP is an 87 kDA membrane-associated protein produced in many tissues, but with the highest expression in the nervous system
- APP is processed via serial cleavage by various enzymes (α-, β-, and γ-secretase) to produce a variety of peptides, including Aβ
- APP is a genetically validated target for both Alzheimer's Disease and Cerebral Amyloid Angiopathy

**Alzheimer's Disease (AD)**

- APP mutations and duplications cause Early Onset AD
- Amyloid deposits in brain tissue, preceding tau tangle formation and neurodegeneration

**Cerebral Amyloid Angiopathy (CAA)**

- APP mutations cause hereditary CAA
- Amyloid deposits in the walls of the arteries in the brain and causes cerebral hemorrhages and dementia

Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; kDA, kilodalton.

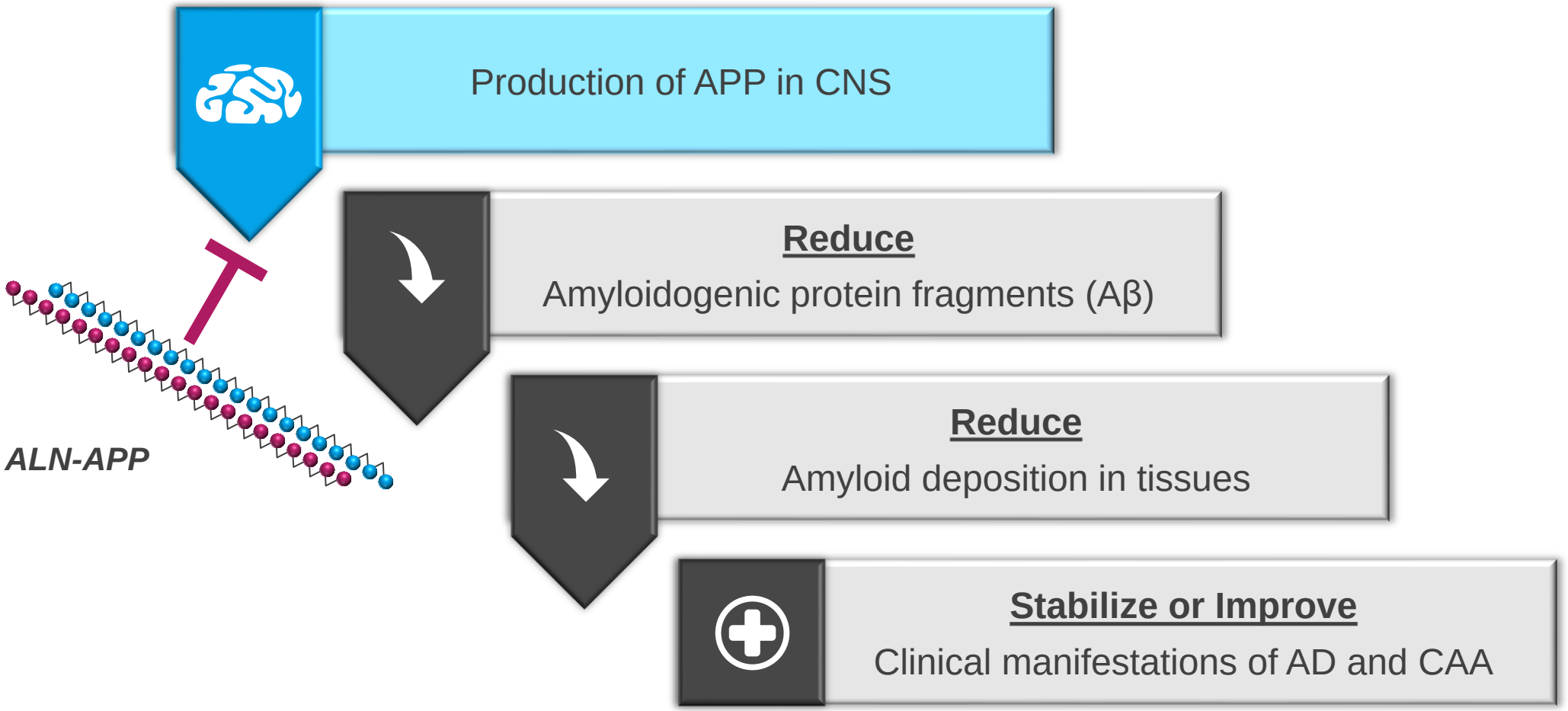
APP Protein Structure courtesy of David S. Goodsell and the RCSB Protein Data Bank

1. Biffi, A. and Greenberg, S.M., *J Clin Neurol* 2011;7(1):1–9. 2. Selkoe, D. J. and Hardy, J. *EMBO Mol Med* 2016;8(6):595-608.

# ALN-APP Therapeutic Hypothesis

## Reduce APP Protein Production Upstream of Amyloidogenic Process by Targeting *APP* mRNA

*Therapeutic hypothesis*



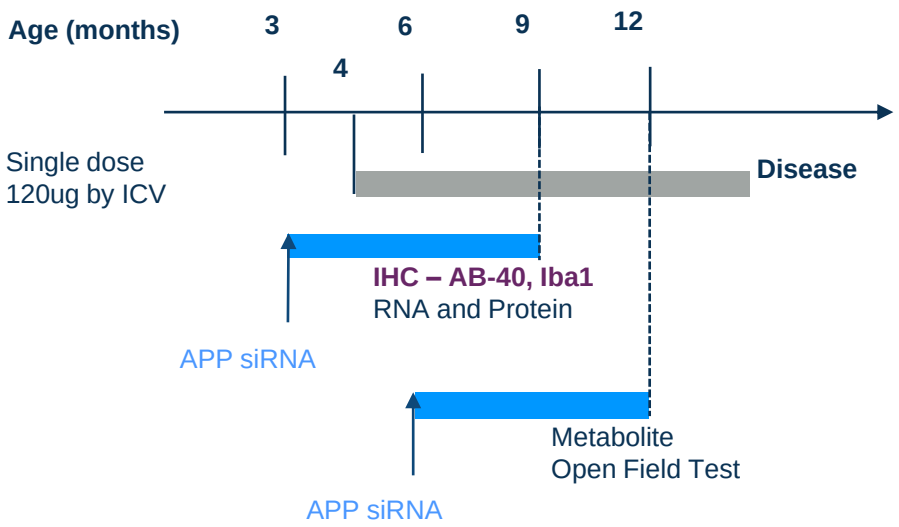



Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; CNS, central nervous system; mRNA, messenger RNA; RNA, ribonucleic acid

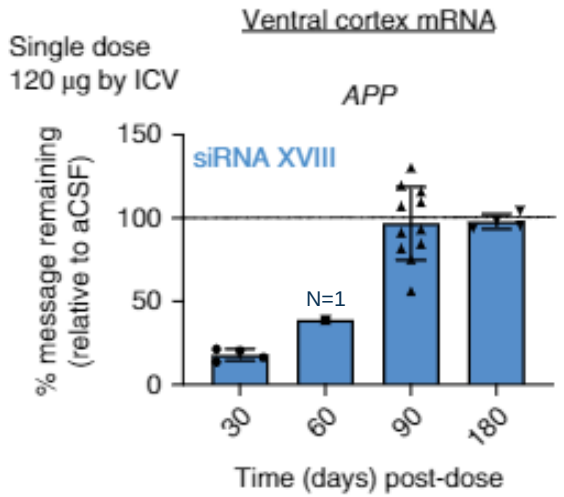
# APP-Lowering Reduced Amyloid in AD Mouse Model

## Single Dose of APP siRNA Reduced APP Production and Brain Amyloid Deposition

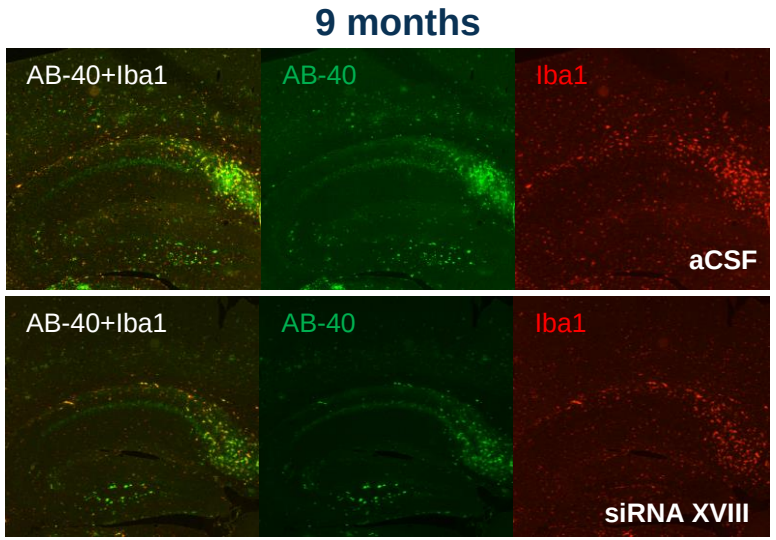
### Study Design[1]




### APP mRNA Reduction[1]




### IHC 6 Months Post ICV Dose[1]




- APP targeting siRNA was administered by ICV in the CVN transgenic mouse expressing human *APP*[2], harboring the Swedish K670N/M671L, Dutch E693Q and Iowa D694N variants, which accumulates Aβ in the brain parenchyma and vasculature

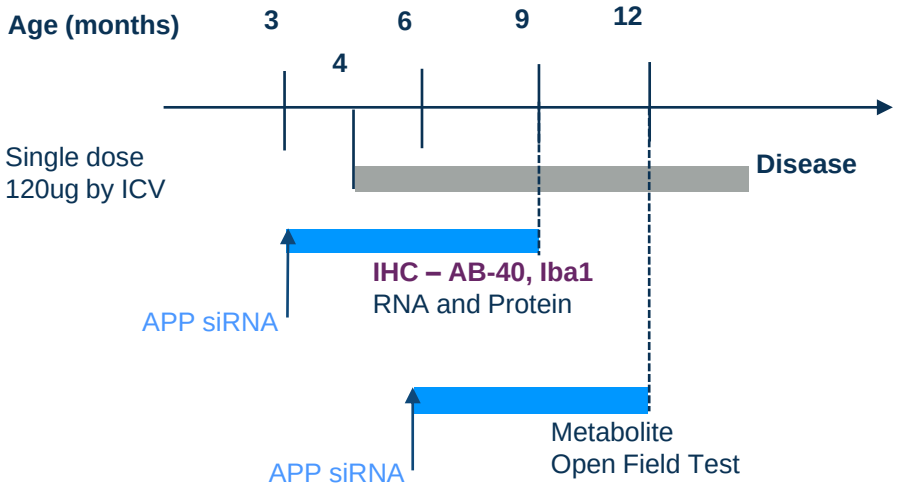
Aβ, amyloid beta; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; APP, amyloid precursor protein; CVN, Tg-hAPP$^{SwDI}$/mNos2$^{-/-}$; ICV, intracerebroventricular injection; IHC, immunohistochemistry; mRNA, messenger RNA; RNA, ribonucleic acid; siRNA, small interfering RNA

1. Brown K et al. *Nature Biotech* 2022;40:1500–1508. 2. Colton et. Al., *J Neuropath & Exp Neurology* 2014;73(8):752–769

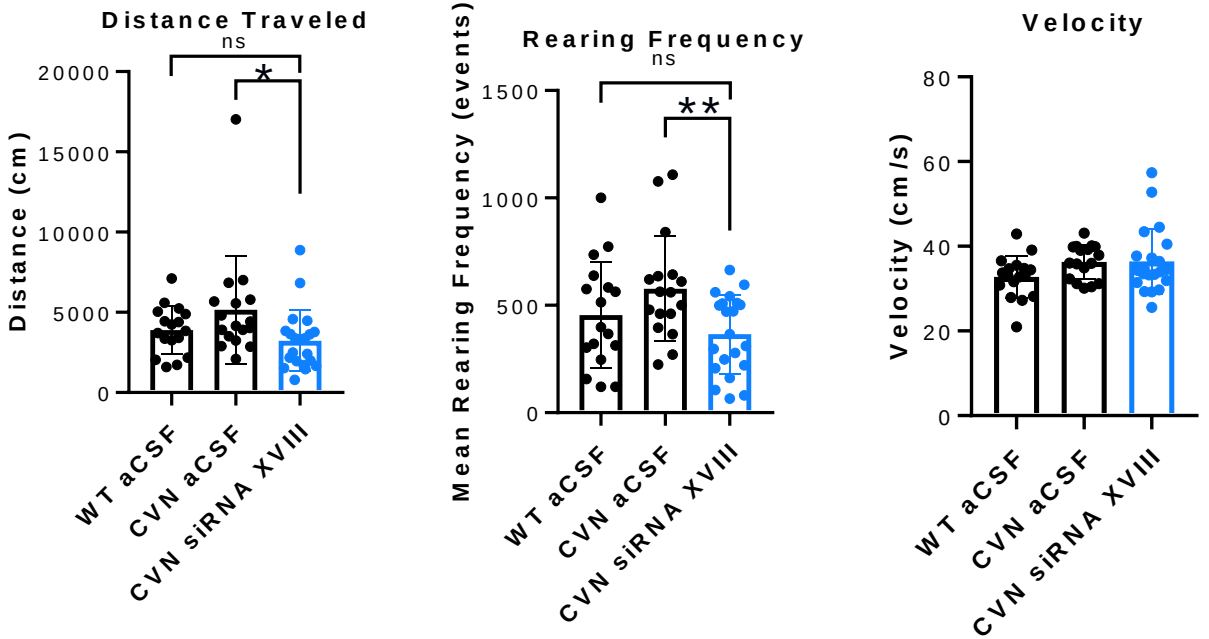
# APP-Lowering Normalized Behavior in AD Mouse Model

## Single Dose of APP siRNA Resulted in Normalization of Behavior in the Open Field Test in CVN mice[1,2]

### Study Design




### Total Distance Traveled and Rearing Frequency




**Treatment Groups**

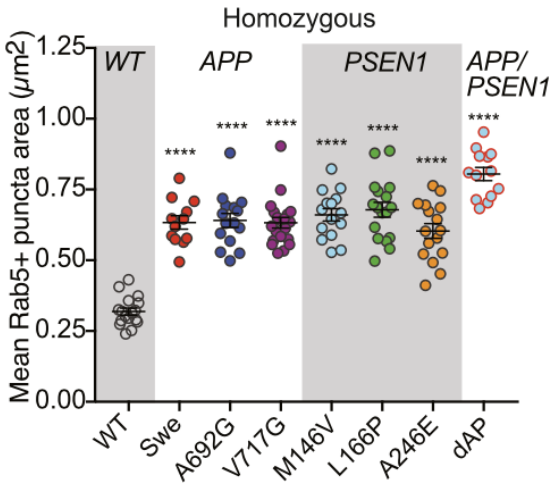
WT aCSF – Wild Type mice treated with aCSF
CVN aCSF – CVN mice treated with aCSF
CVN siRNA XVIII – CVN mice treated with *APP* targeting siRNA

aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; APP, amyloid precursor protein; CVN, Tg-hAPP$^{SwDI}$/mNos2$^{-/-}$; NS, not significant; RNA, ribonucleic acid; siRNA, small interfering RNA; WT, wild type

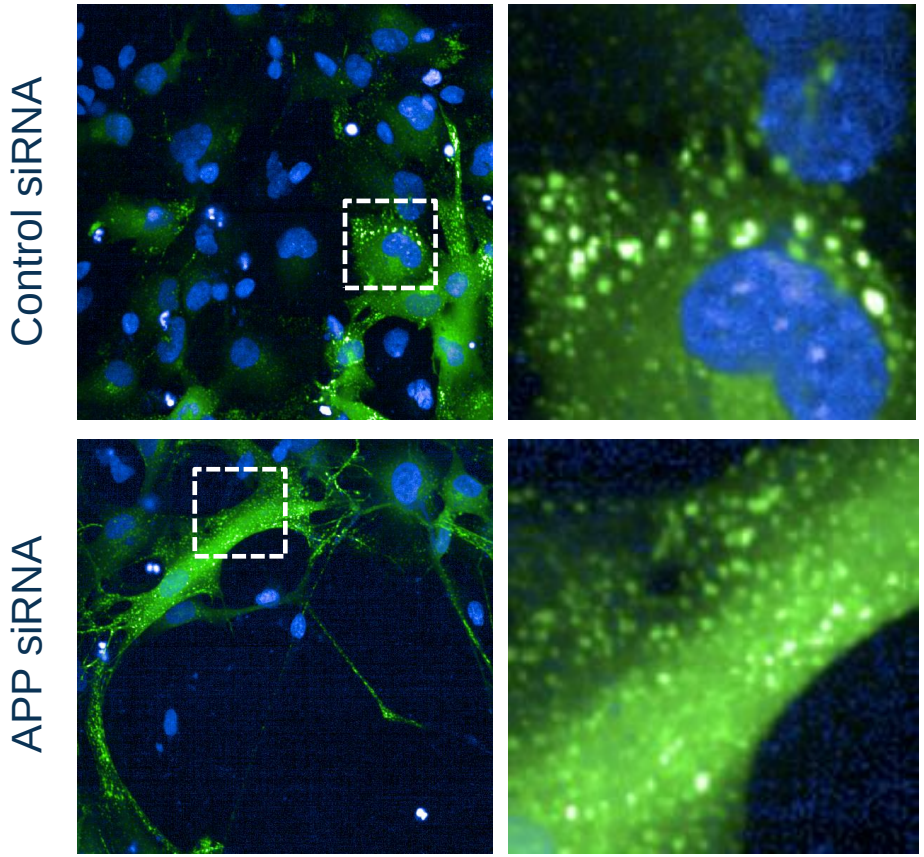
1. Colton et. Al., *J Neuropath & Exp Neurology* 2014;73(8):752–769; 2. Brown K et al. *Nature Biotech* 2022;40:1500–1508.

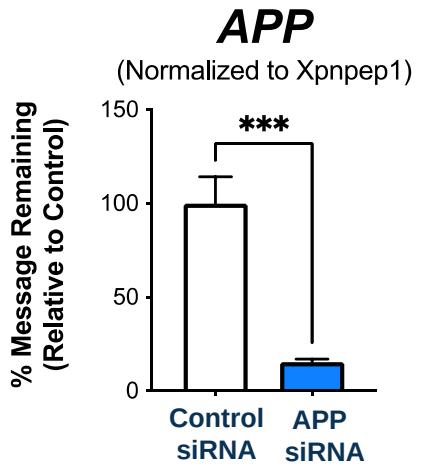
# APP-Lowering in Human ADAD iPSC-derived Neurons

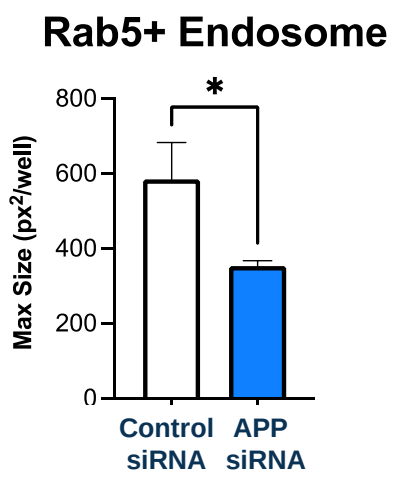
## PSEN1 A246E Patient iPSC-derived Neurons Treated with APP siRNA Showed a Reduction in Rab5+ Early Endosome Size[1]




Mutations in APP and PSEN1 cause enlargement of Rab5+ early endosomes in human iPSC derived neurons[2]




- Phenix high content imaging, 63X, analysis on harmony
- Rab5+ endosome Alexa 488 for live imaging

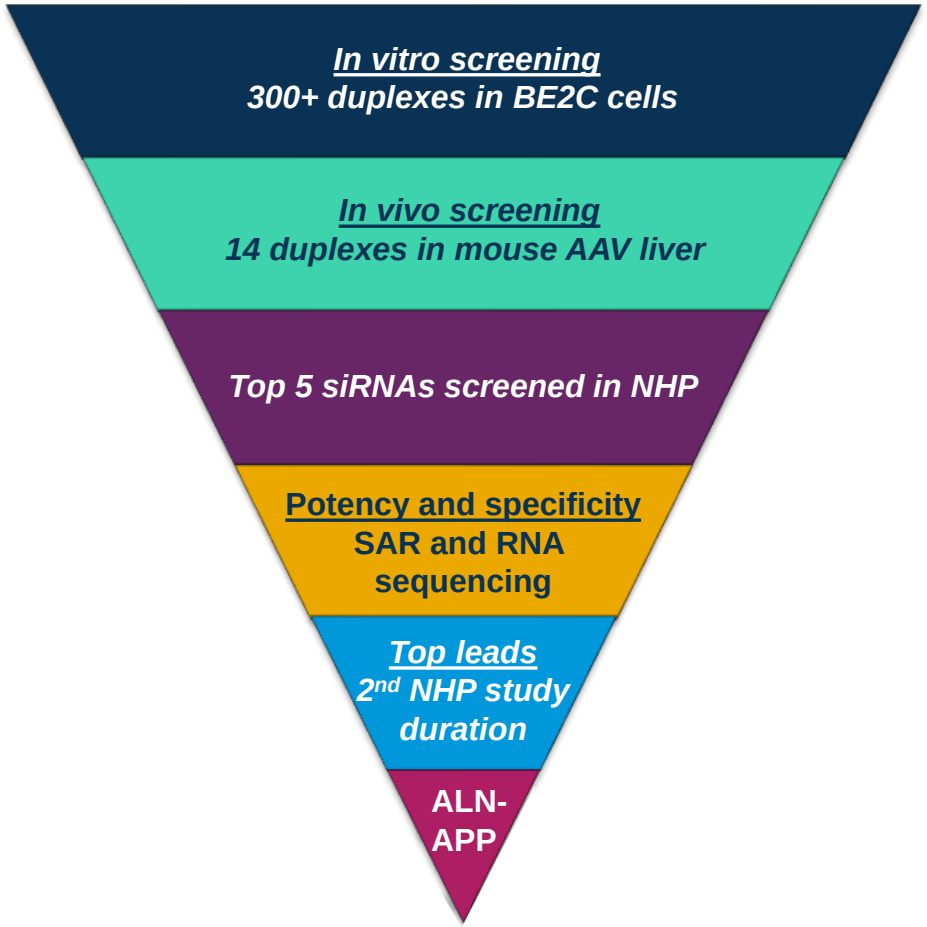
aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; ADAD, autosomal dominant AD; APP, amyloid precursor protein; iPSC, induced pluripotent stem cells; PSEN, presenilin; RNA, ribonucleic acid; siRNA, small interfering RNA; WT, wild type

1. Alnylam Data on File. 2. Kwart et al., *Neuron* 2019; 04(2):256-270.

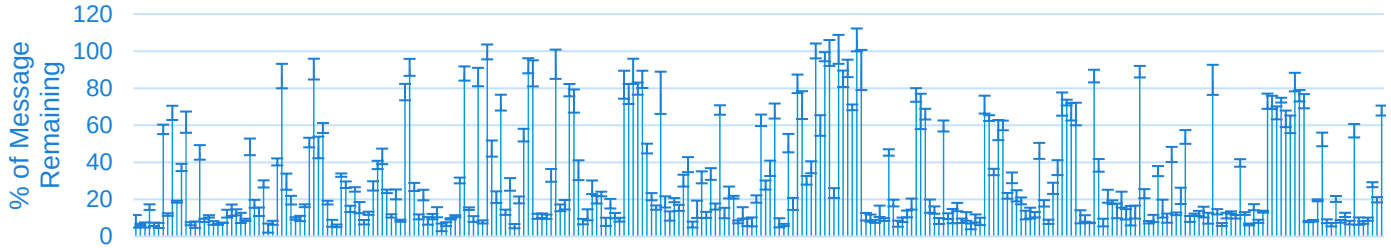
# ALN-APP Preclinical Development

# Lead Identification Strategy for ALN-APP

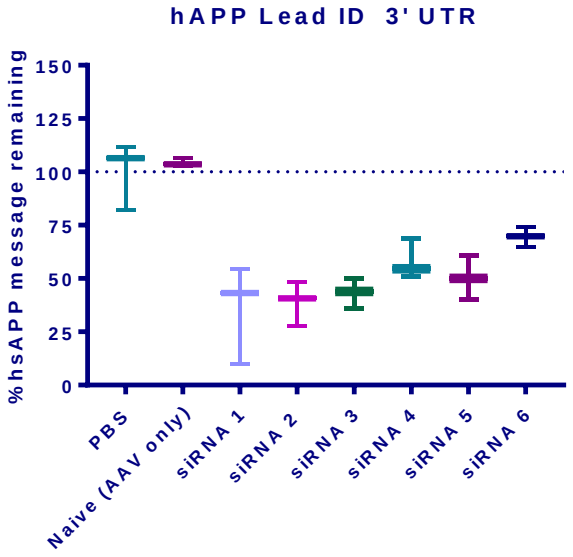
## Process to Identify and Optimize First CNS Development Candidate

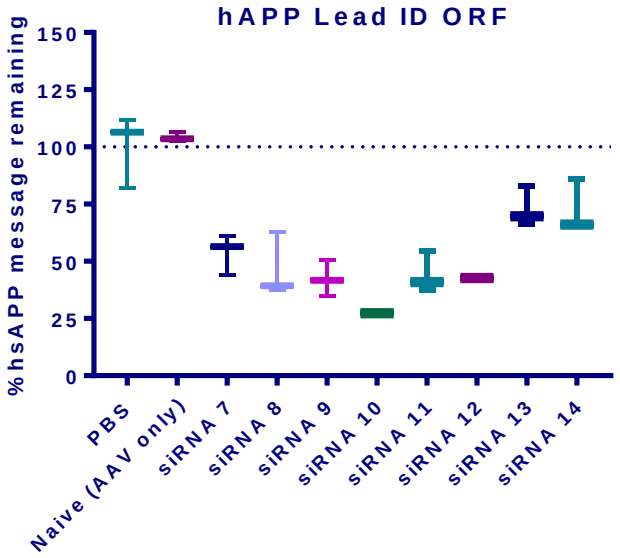



### BE2C in vitro screen identified potent sequences




### Mouse in vivo screen confirmed in vivo activity



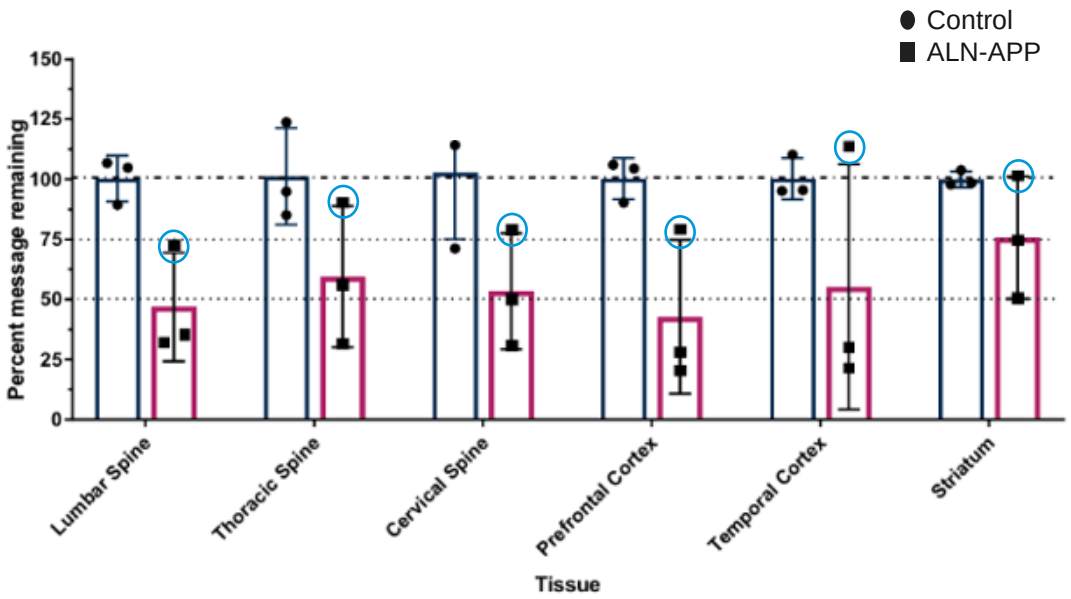




AAV, adeno-associated virus; hAPP, human amyloid precursor protein; hsAPP, human soluble amyloid precursor protein; NHP, non-human primate; ORF, open reading frame; PBS, phosphate buffered saline; RNA, ribonucleic acid; SAR, structure activity relationship; siRNA, small interfering RNA; UTR, untranslated region

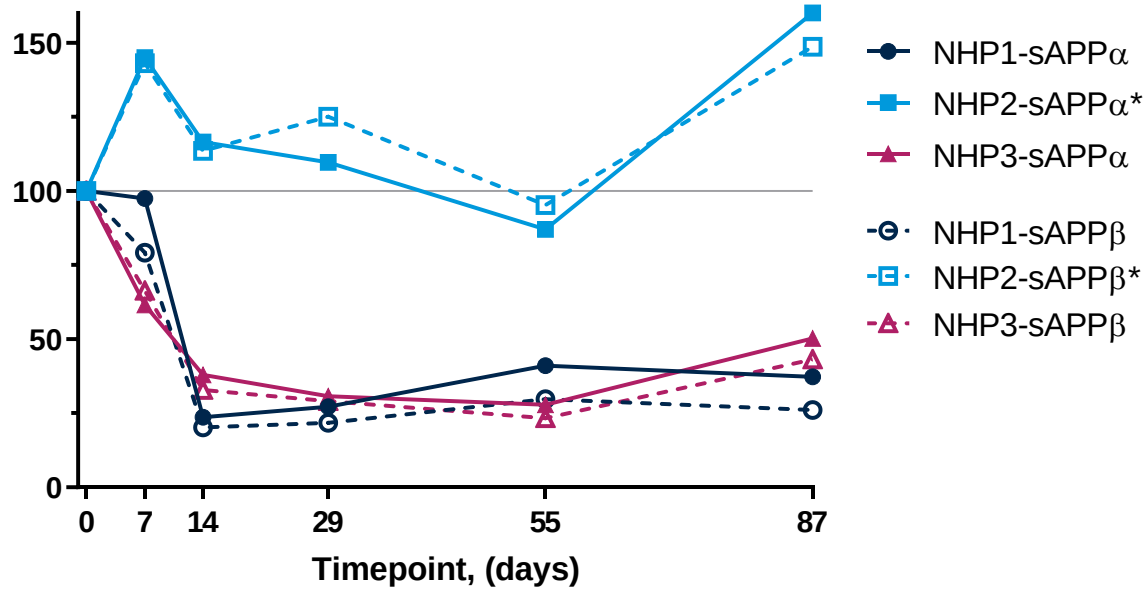
# Non-Clinical Development of ALN-APP

## PD Profile in NHPs for Single 60 mg IT Injection of ALN-APP

### *APP* mRNA in CNS tissue




### CSF PD biomarker in NHP




- ALN-APP displayed pharmacologic activity in monkeys, measured by reduction of *APP* mRNA in CNS tissue and CSF sAPP
- ALN-APP characterized by persistent target protein reduction in NHP

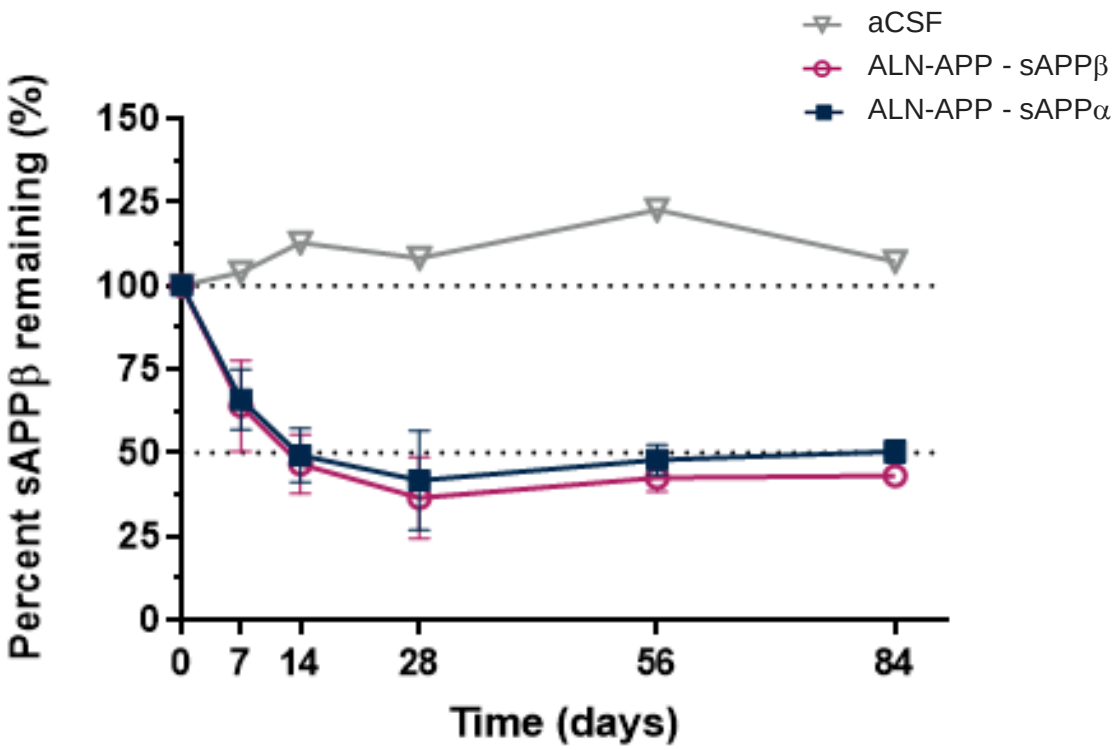
*NHP2 was a probable IT mis-dose.

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

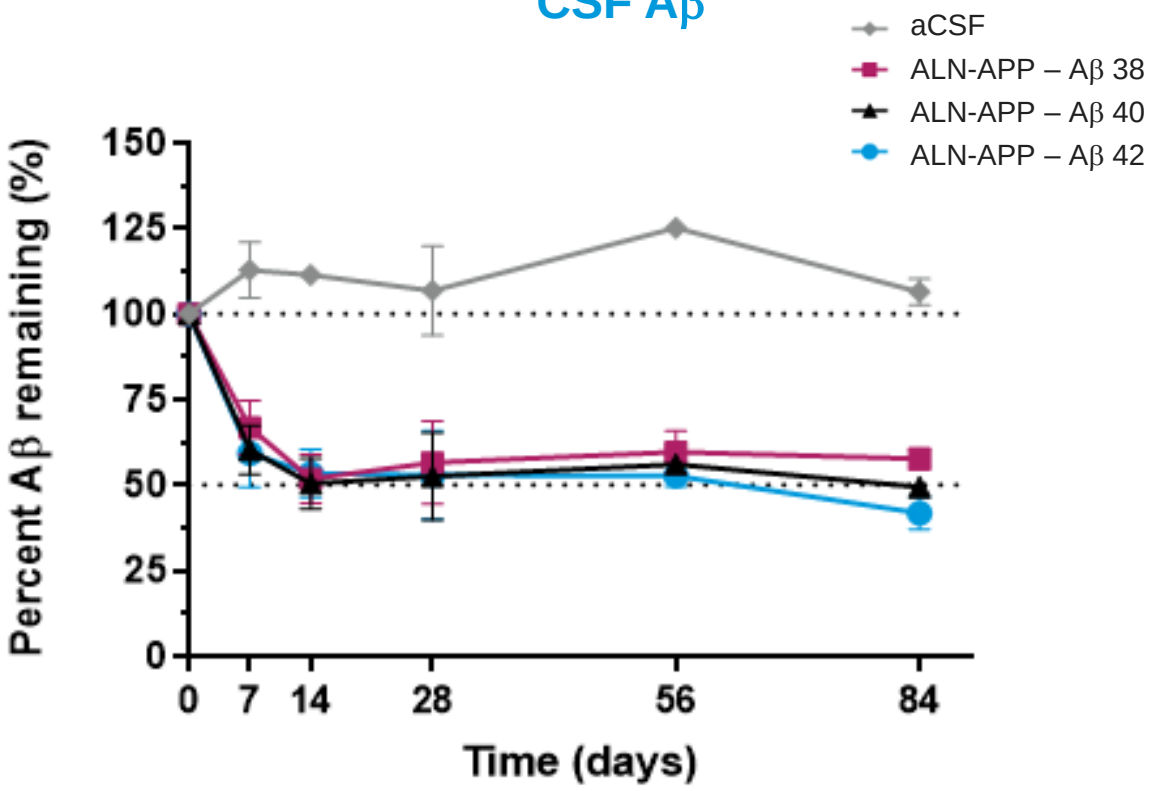
# Durable CSF sAPP and Amyloid Fragment Knockdown in NHP

## Single 10 mg IT administration of ALN-APP in NHP (N=3)

### CSF sAPP




### CSF Aβ





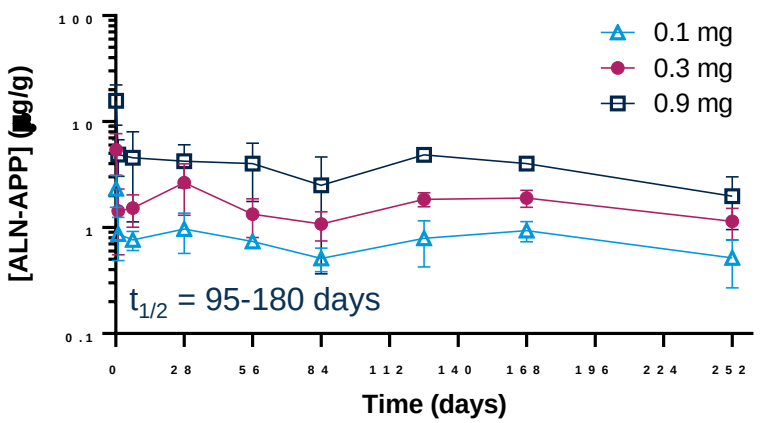

Aβ, amyloid beta; aCSF, artificial CSF; APP, amyloid precursor protein; CSF, cerebrospinal fluid; IT, intrathecal; NHP, non-human primate; sAPP, soluble APP

# Non-Clinical Development of ALN-APP

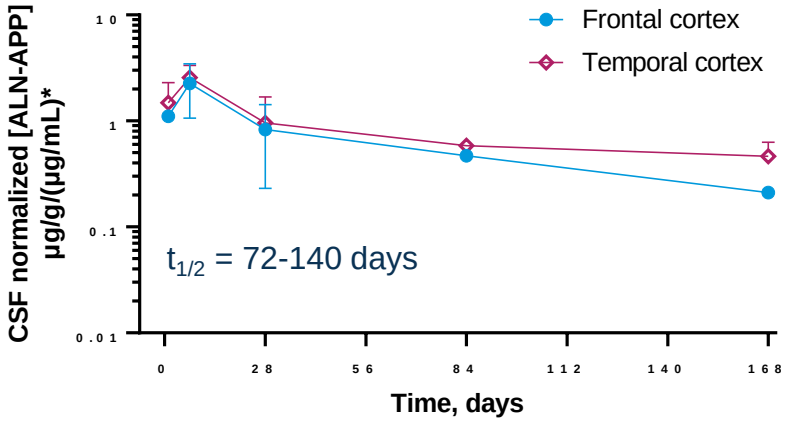
## PK and PD Profiles in Rats and NHPs for Single IT Injection

### Long brain $t_{1/2}$ in rat and NHP

**Rat cerebral cortex**

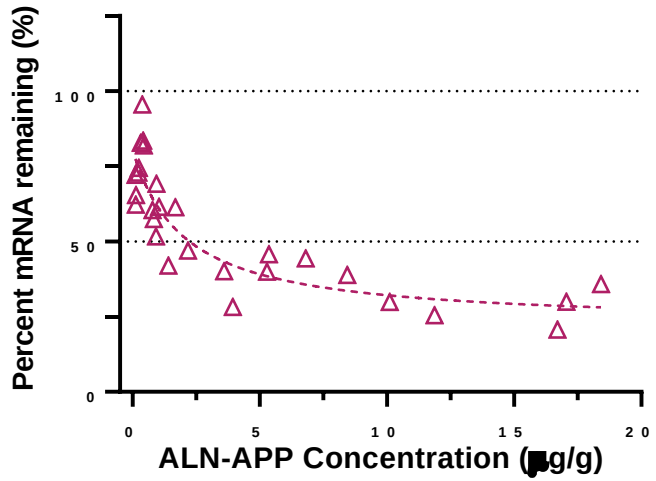



**NHP cerebral cortex (60 mg dose)**




### Strong PK/PD relationship

**Prefrontal cortex mRNA vs. [ALN-APP]**




- The pharmacodynamic effects were durable, consistent with drug levels that persisted for months in CNS tissues following single administration
- Strong PK/PD relationship in NHP brain

*NHP brain concentrations normalized to CSF exposure as surrogate for administered dose due to dosing variability. Mis-dosing is a phenomenon in NHP that has been previously observed for ASOs[1]

APP, amyloid precursor protein; CSF, cerebrospinal fluid; IT, intrathecal; mRNA, messenger RNA; NHP, non-human primate; PD, pharmacodynamics; PK, pharmacokinetics; RNA, ribonucleic acid

1. Sullivan et al, *J Transl Med* 2020;18(1):309

# Non-Clinical Safety and ADME

## CTA/IND-Enabling Studies for the Start of Phase I Clinical Trial

| Category | Brief Title | Route |
|---|---|---|
| Exploratory Studies | Evaluation of ALN-APP cytokine induction in human whole blood | In vitro |
| GLP-Compliant Safety Studies | Genetic toxicology (Ames, chromosomal aberrations in hPBL) | In vitro |
| | Genetic toxicology (rat bone marrow micronucleus) | SC |
| | Cardiovascular and respiratory safety evaluation in telemeterized conscious NHP | IT |
| | 4-week single or repeat dose toxicity in rat | IT or IV |
| | 4-week single or repeat dose toxicity in NHP with 12-week recovery | IT or IV |
| Absorption, Distribution, Metabolism, Excretion (ADME) Studies | Single dose PK/ADME in rat | IT |
| | Single dose PK/ADME and PD in NHP | IT |
| | TK for all GLP toxicology studies (plasma, CSF) | Study-dependent |
| | Metabolic stability and metabolite profiling (rat, NHP, and human brain homogenate, serum, primary hepatocytes) | In vitro |
| | Plasma and CSF protein binding (rat, NHP, human) | In vitro |
| | Cytochrome P450 and transporter (P-gp, BCRP) interactions | In vitro |

ADME, absorption, distribution, metabolism and excretion; APP, amyloid precursor protein; BCRP, breast cancer resistance protein; CSF, cerebrospinal fluid; CTA, clinical trial application; GLP, good laboratory practices; hPBL, human peripheral blood lymphocytes; IND, investigational new drug; IT, intrathecal; IV, intravenous; NHP, non-human primate; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetics; SC, subcutaneous; TK, toxicokinetics

# GLP IT/IV 4-week Toxicology Studies in Rat and NHP

## Results: 4-Week Rat Study and 4-week NHP Study With 12-Week Recovery

- ALN-APP was well-tolerated with no in-life findings
- No ALN-APP related changes in neurobehavioral function
- No ALN-APP related changes in clinical pathology
- No ALN-APP related adverse anatomic pathology findings
- NOAEL concluded to be 120 mg in monkeys and 1.8 mg in rats, IT or IV



APP, amyloid precursor protein; GLP, good laboratory practices; IT, intrathecal; IV, intravenous; NHP, non-human primate; NOAEL, no-observed-adverse-effect level

# ALN-APP Phase 1

# ALN-APP Phase 1 Overview

## Randomized, Double-Blind Study in Patients with Early-Onset Alzheimer's Disease (EOAD)

**Part A: Single Ascending Dose**
***(Ongoing)***

**Part B**: Multiple Dose
*(expected to begin 2023)*

- Population: Patients with Early Onset Alzheimer's Disease
- Primary Objective: Safety and tolerability of ALN-APP
- Secondary Objective: Pharmacology of ALN-APP
- Exploratory Objective: Impact of ALN-APP on disease
  - Fluid biomarkers for amyloid, tau, and neurodegeneration
  - Measures of synaptic health
  - Neuroimaging
  - Exploratory cognitive and functional clinical measures

**Progress Update**

Part A dosing ongoing

Preliminary data expected in 2023

# Summary

- RNAi therapeutics use a natural cellular process to suppress translation of specific target mRNA
- C16 conjugate delivery of siRNAs into cells in the CNS via intrathecal injection has shown robust protein lowering across different regions of the CNS with a long duration of effect in nonclinical experiments
- APP is a genetically validated target for Alzheimer's Disease and Cerebral Amyloid Angiopathy
  - In vitro and in vivo models suggest that lowering APP protein production via RNAi can reduce amyloid deposition and improve disease phenotypes in models of Alzheimer's disease
- ALN-APP targets *APP* mRNA;
  - IT administration of ALN-APP in rats and monkeys robustly lowered *APP* mRNA and CSF biomarkers of APP protein production with a long duration of action
  - Non-clinical safety studies have been conducted and support progression to human studies
- A phase 1 trial studying single ascending doses of ALN-APP in patients with early-onset Alzheimer's disease is ongoing with initial data expected in 2023



APP, amyloid precursor protein; CNS, central nervous system; CSF, cerebrospinal fluid; IT, intrathecal ; mRNA, messenger RNA; RNAi, RNA interference; siRNA, small interfering RNA