Targeting Cerebral Amyloid Angiopathy at its Source: An Amyloid-beta Precursor Protein-Targeted siRNA Reduced Vascular Amyloid-Beta and Hemorrhage in Rodent Models

Kirk Brown[1], Lan T. H. Dang[1], Feng Xu[2], Xiaoyue Zhu[2], Louis-Philippe Croteau[1], Sarah LeBlanc[1], Mark Schlegel[1], Adam Castoreno[1], Kevin Fitzgerald[1], William Van Nostrand[2]

[1]Alnylam Pharmaceuticals, Inc., Cambridge, MA, USA; [2]George & Anne Ryan Institute for Neuroscience, Department of Biomedical and Pharmaceutical Sciences, The University of Rhode Island, Kingston, RI, USA

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Disclosures

Presenter: Kirk Brown, PhD

Conflict	Disclosure
Paid Employee and Stockholder of Alnylam Pharmaceuticals	Alnylam Pharmaceuticals

Mivelsiran:

Mivelsiran is an investigational drug being studied for the treatment of cerebral amyloid angiopathy and Alzheimer's disease. Mivelsiran is not approved by any health authority, and the safety and efficacy of mivelsiran have not been established.

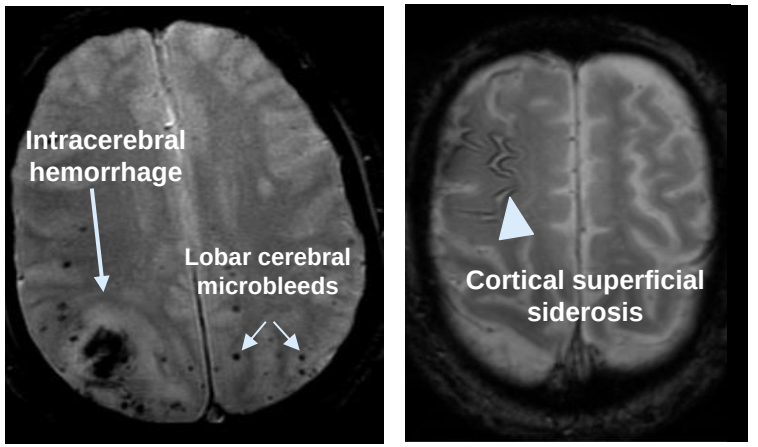
Funding:

The mivelsiran clinical program is funded by Alnylam Pharmaceuticals.

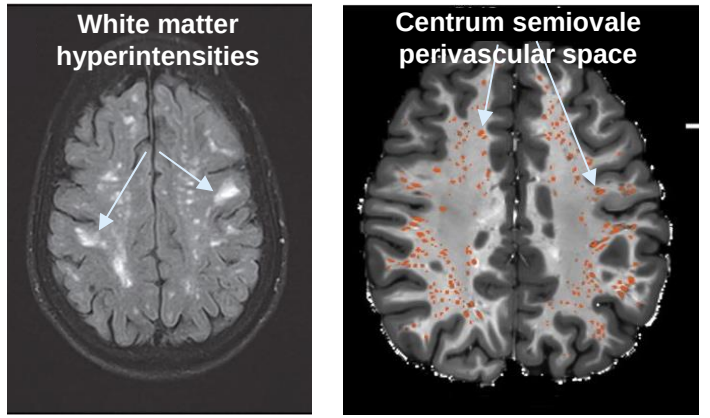
CAA is a Serious, Progressive Cerebrovascular Disease Characterized by Deposition of Aβ in Cerebral Blood Vessels

- Amyloid-beta (Aβ) accumulation leads to altered cerebrovascular physiology and nonhemorrhagic and hemorrhagic brain injury[1–5]
- Cerebral angiopathy (CAA) often co-occurs with Alzheimer's disease (AD) [2,3]
  - Greater CAA severity increases the likelihood of severe cognitive impairment in AD[2]
- CAA is often sporadic, but can occur due to amyloid-beta precursor protein (APP) variants and overexpression[1,3,6]
- There are no disease-modifying therapies for CAA[7]

Lobar Hemorrhagic Lesions[3,8,a]



White Matter Abnormalities[9,a]



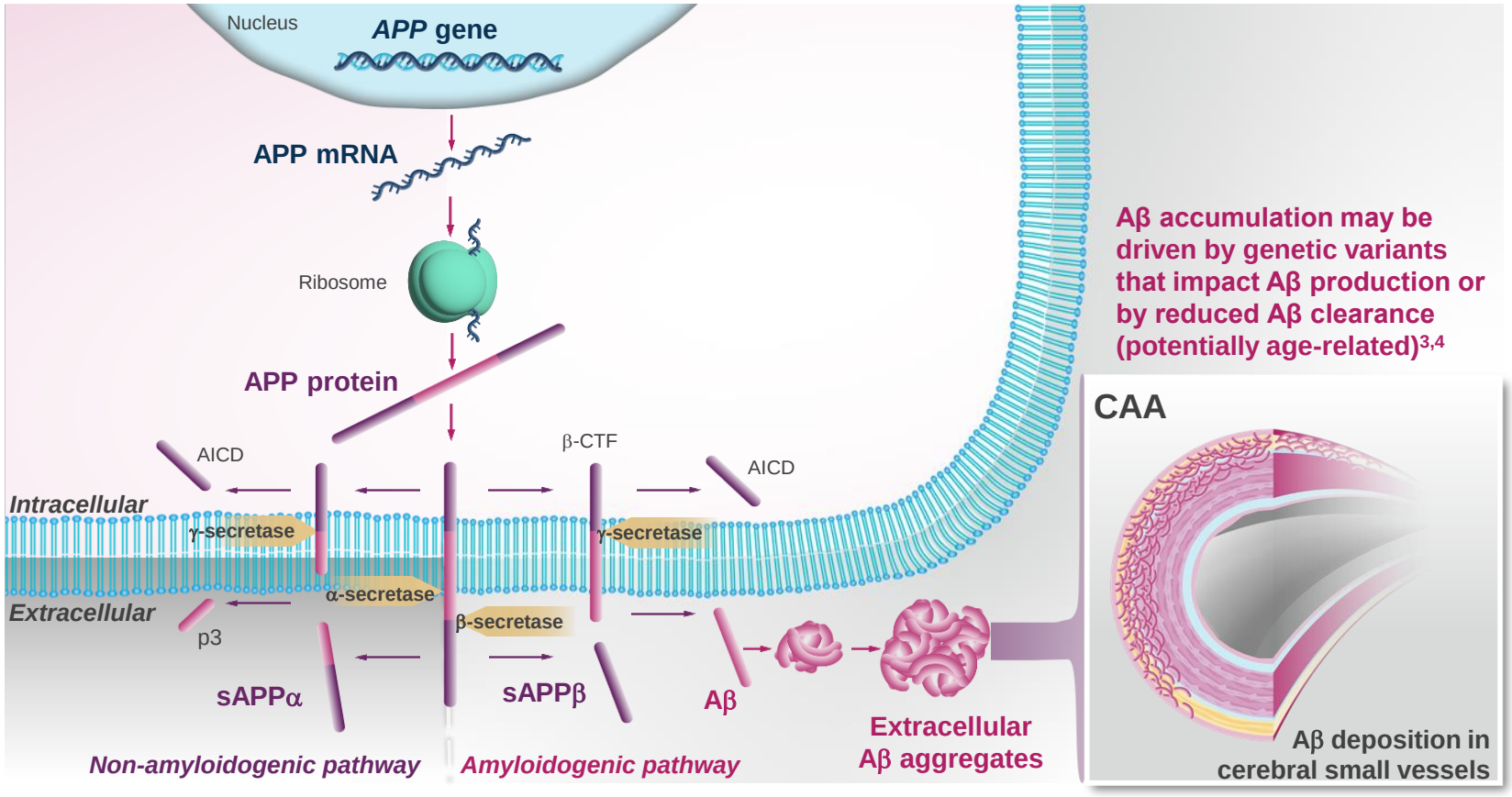
[a]Available through Creative Commons Attribution License, image labels added.

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

1. Kozberg MG *et al. Int J Stroke* 2021;16:356–69. 2. Boyle PA *et al. Neurology* 2015;85:1930–6. 3. Biffi A, Greenberg SM *J Clin Neurol* 2011;7:1–9. 4. Mintun MA *et al. N Engl J Med* 2021;384:Suppl. 5. Vilela P, Wiesmann M. Diseases of the Brain, Head and Neck, Spine 2020–23. Springer; 2020. 6. Chatterjee P *et al. J Alzheimers Dis* 2021;79:895–903. 7. Cozza M *et al. J Neurol Sci* 2023;454:120866. 8. Image obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Website: adni.loni.usc.edu. 9. Sepehrband F *et al. Sci Rep*. 2019;9:12351.

The Amyloidogenic Pathway Underlies CAA Pathophysiology

- APP mRNA encodes APP[1]
- APP is cleaved to produce Aβ[2]
- Aβ deposits in the vessel walls of small arteries, arterioles, and capillaries[2]



Altered cerebrovascular physiology leads to hemorrhagic and nonhemorrhagic brain injury[5–9]

Image created by Alnylam from data published in Hampel, *et al.* 2021[10] and Banerjee, *et al.* 2017.[11]

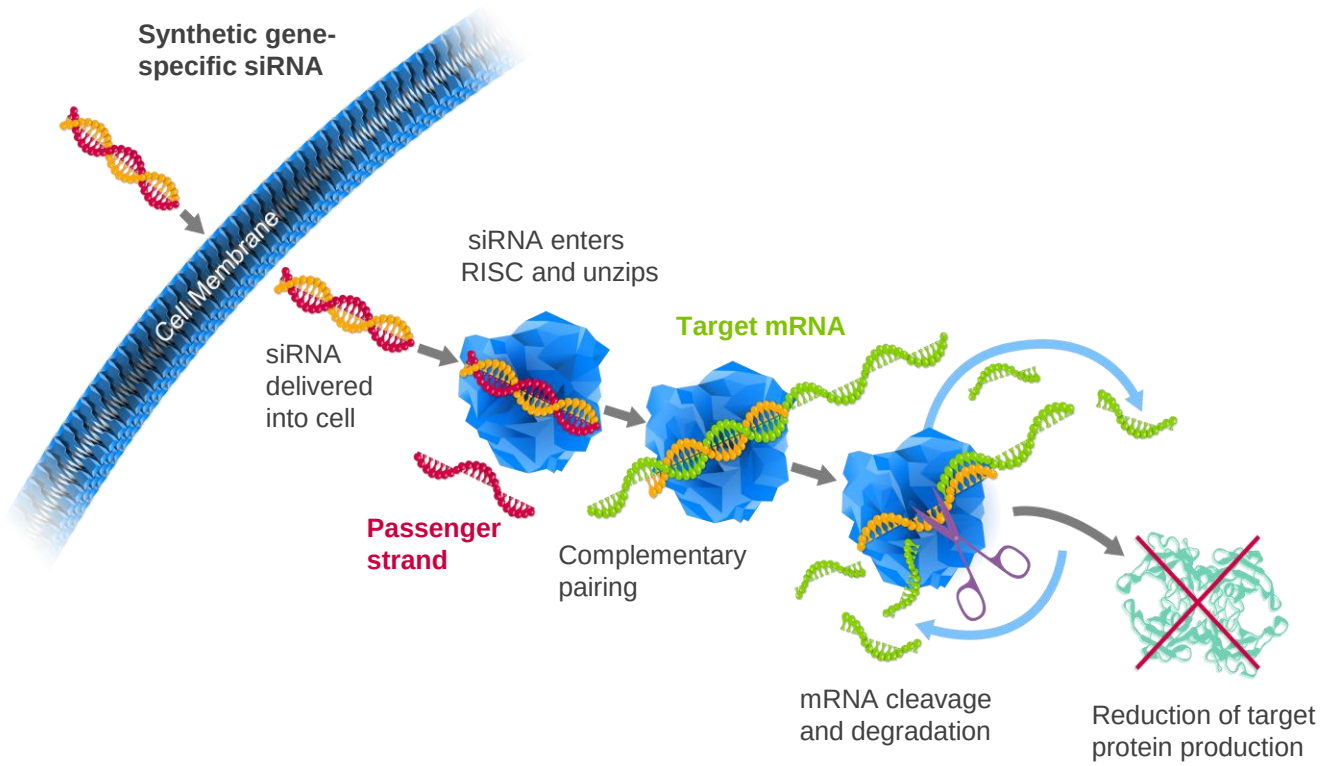
Aβ, amyloid-beta; AICD, activation-induced cell death; APP, amyloid-beta precursor protein; β-CTF, β-C-terminal fragment; CAA, cerebral amyloid angiopathy; sAPP, soluble amyloid precursor protein.

1. Zheng *et al. Mol Neurodegener* 2006;1. 2. DeSimone CV *et al. J Am Coll Cardiol* 2017;70(9):1173-82. 3. van Veluw *et al. Cell Mol Life Sci* 2024;81:239. 4. Hawkes *et al. Acta Neuropathol* 2011;121(4):431-43. 5. Kozberg MG *et al. Int J Stroke* 2021;16:356–69. 6. Boyle PA *et al. Neurology* 2015;85:1930–6. 7. Biffi A, Greenberg SM *J Clin Neurol* 2011;7:1–9. 8. Mintun MA *et al. N Engl J Med* 2021;384:Suppl. 9. Vilela P, Wiesmann M. Diseases of the Brain, Head and Neck, Spine 2020–23. Springer; 2020. 10. Hampel *et al*. *Mol Psychiatry* 2021:26:5481–503. 11. Banerjee *et al*. *J Neurol Neurosurg Psychiatry* 2017;88:982–94.

RNA Interference Harnesses an Endogenous Process that Regulates Gene Expression

- Synthetic small interfering RNAs (siRNAs) target entry into this cellular mechanism[1]
- siRNAs direct the targeted reduction of mRNA to reduce the disease-associated protein[2,3]
- C16 modification enhances delivery of siRNA to brain cells[4]

RNAi Mechanism of Action[3,a]



RNAi works catalytically to reduce target protein expression while leaving DNA intact[2]

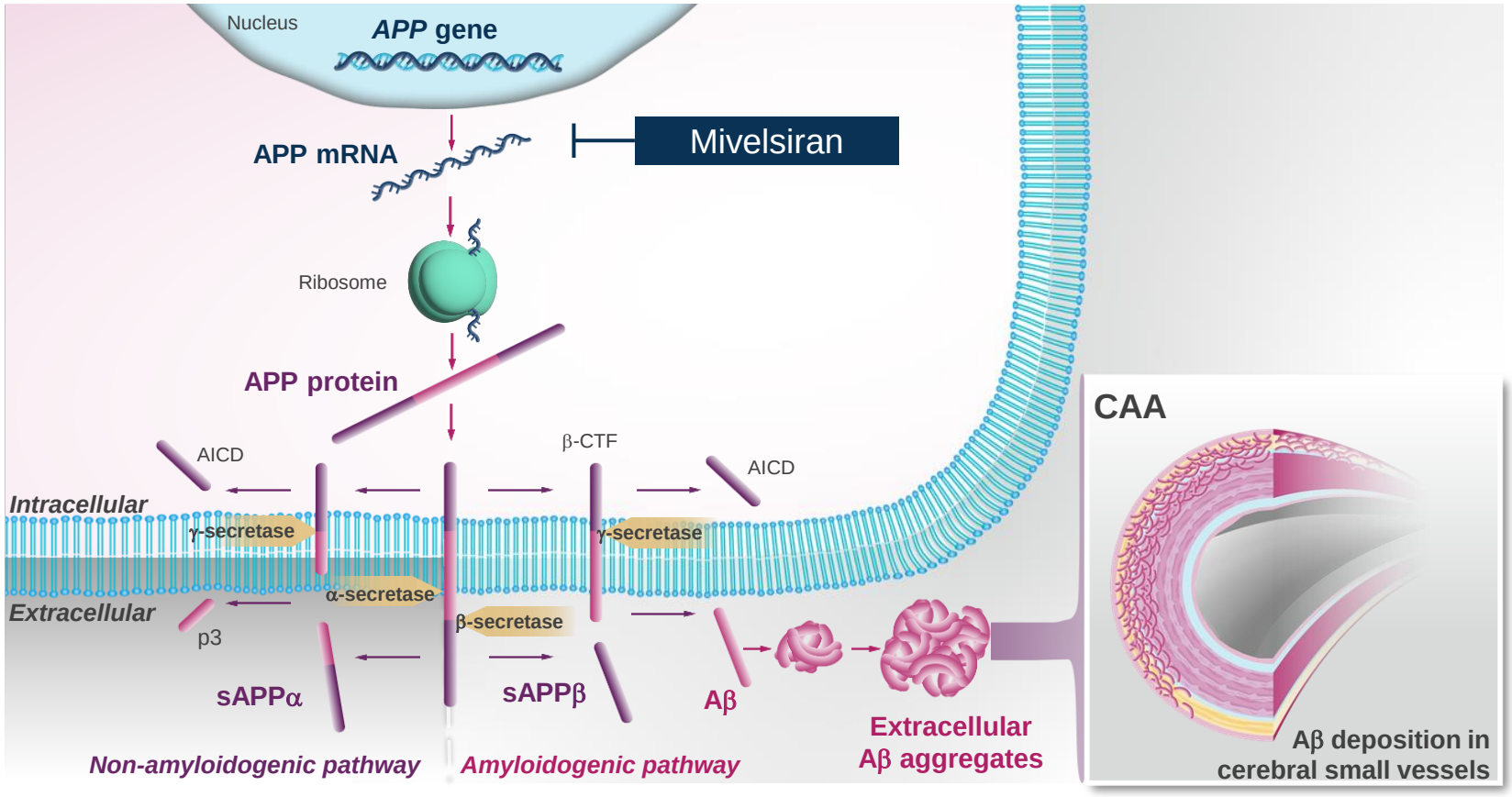
[a]Image created by Alnylam Pharmaceuticals from data published in Jadhav *et al.* 2024.[3]

C16, 2′-*O*-hexadecyl; mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNAi, RNA interference; siRNA, small interfering RNA.

1. Niemietz C *et al. Molecules* 2015;20:17944–75. 2. Ranasinghe R *et al. Br J Pharmacol* 2023;180:2697–720. 3. Jadhav V *et al*. *Nature Biotechnol* 2024;42:394–405. 4. Brown *et al. Nat Biotechnol* 2022;40:1500–8.

Mivelsiran is Designed to Reduce APP Production

- Mivelsiran is an investigational therapeutic currently in development for treatment of CAA and AD
- Targets APP production in the CNS
- Administered intrathecally



Lowering APP reduces substrate for amyloid accumulation and may enable natural clearance of Aβ

Image created by Alnylam from data published in Hampel, *et al.* 2021[1] and Banerjee, *et al.* 2017.[2]

Aβ, amyloid-beta; AD, Alzheimer's disease; AICD, activation-induced cell death; APP, amyloid-beta precursor protein; β-CTF, β-C-terminal fragment; CAA, cerebral amyloid angiopathy; CNS, central nervous system; sAPP, soluble amyloid precursor protein.

1. Hampel, *et al. Mol Psychiatry*. 2021:26(10):5481-503. 2. Banerjee, *et al*. *J Neurol Neurosurg Psychiatry*. 2017:88(11):982-94.

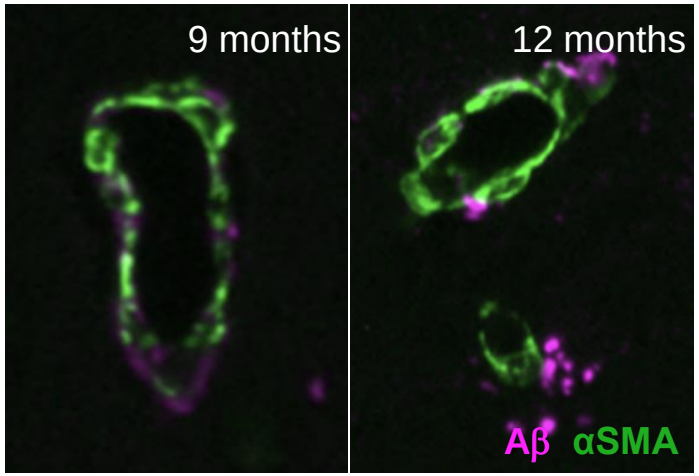
APP-Lowering siRNA in Rodent Models

Rodent Models of Aβ-related Pathology Used to Assess Impact of APP-Lowering siRNA

CVN mouse model[1,2]

- Transgenic model of AD and CAA, which develop over time
- APP Swedish, Dutch, and Iowa variants expressed
- Levels of Aβ40 over double that of Aβ42
- Amyloid accumulates in both brain and vessels
- CMBs do not typically occur in model

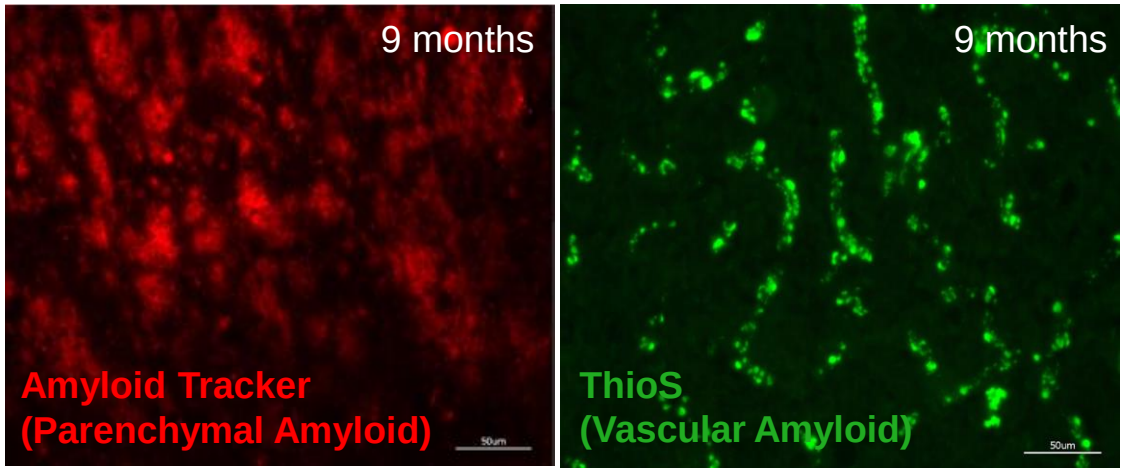
Blood Vessel Cross-Section in Hippocampus[4]



rTg-DI rat model[3]

- Transgenic model of CAA
- APP Dutch and Iowa APP variants expressed
- Amyloid accumulates in both brain and vessels
- CMBs observed in this model, mainly in the thalamus

Amyloid Staining in Hippocampus[4]

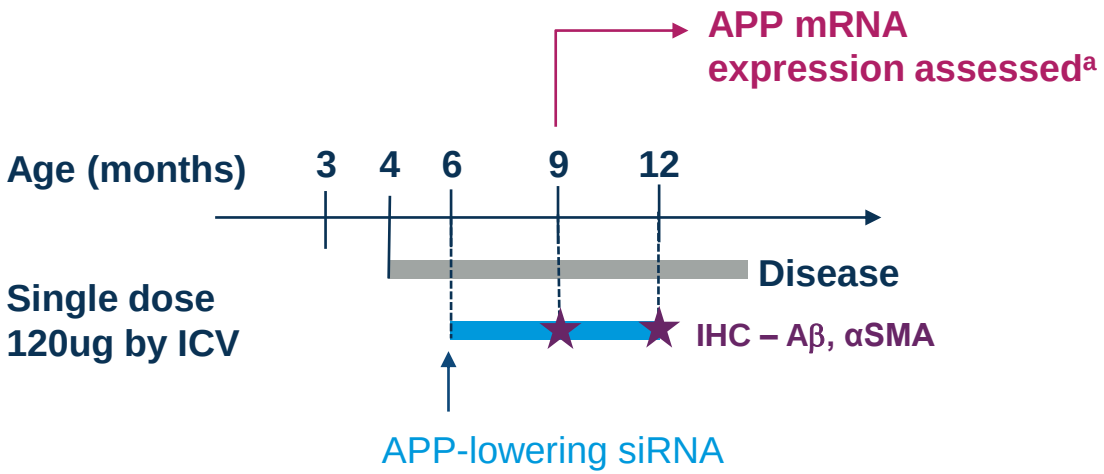


αSMA, alpha-smooth muscle actin; Aβ, amyloid-beta; AD, Alzheimer's disease; APP, amyloid-beta precursor protein; CAA, cerebral amyloid angiopathy; CMB, cerebral microbleed; siRNA, small interfering RNA; ThioS, Thioflavin S.

1. Coulton *et al. J Neuropathol Exp Neurol* 2014;73:752–69. 2. Wilcock *et al. J Neurosci* 2009;29:7957–65. 3. Davis *et al. Am J Pathol* 2018;188:2877–89. 4. Alnylam data on file.

Timeline of Aβ Accumulation and Occurrence of CMBs Assessed in Interventional Rodent Studies

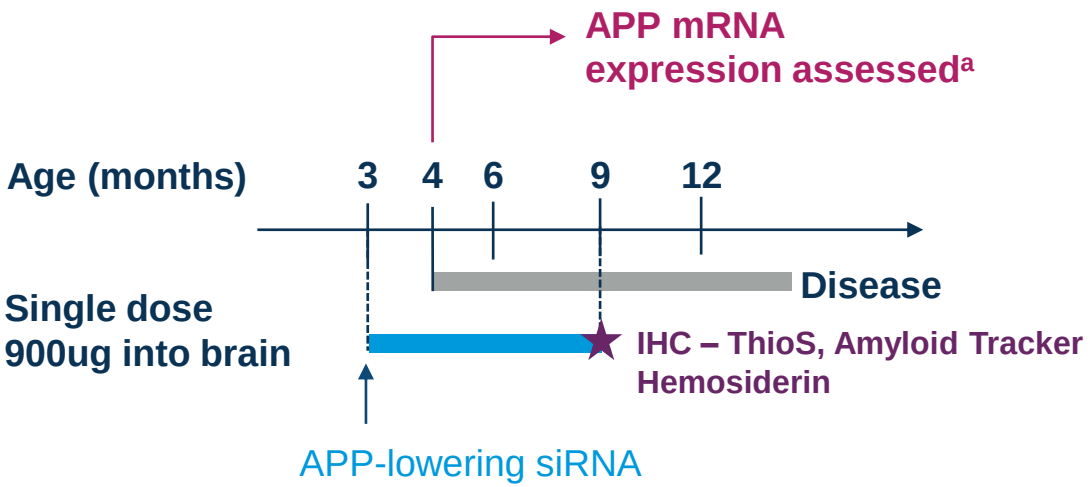
CVN mouse study



Assessments

- **Accumulation of Aβ:** Immunohistochemical staining of brain tissue 3 and 6 months post-dose

rTg-DI rat study



Assessments

- **Accumulation of Aβ:** Immunohistochemical staining of unilateral brain tissue 6 months post-dose
- **Occurrence of cerebral microbleeds:** Hemosiderin staining 6 months post-dose

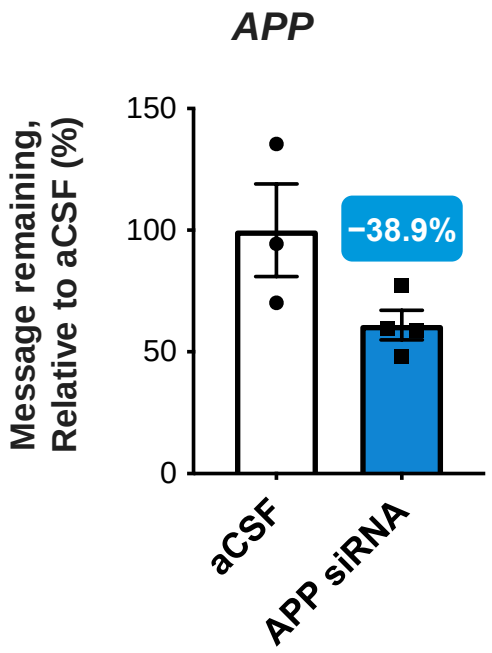
[a]APP mRNA expression was assessed postmortem in a separate study; CVN mouse: 120ug ICV dosed, evaluated 3 months post-dose; rTg-DI rat: 900ug intrathecally dosed, evaluated 1 month post-dose.

αSMA, alpha-smooth muscle actin; Aβ, amyloid-beta; APP, amyloid-beta precursor protein; CMB, cerebral microbleed; IHC, immunohistochemical; ICV, intracerebroventricular; siRNA, small interfering RNA; ThioS, Thioflavin S.

APP mRNA Expression in Models After Single Dose of siRNA

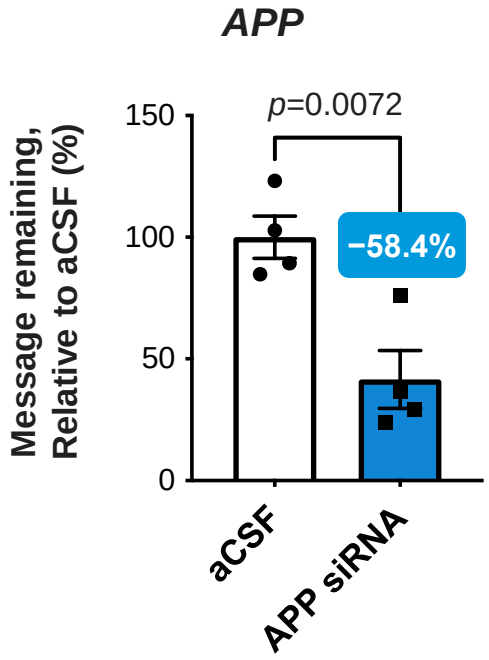
CVN mouse – Hippocampus[a,b]

3 months post-dose



rTg-DI rat – Hemibrain[a,c]

1 month post-dose

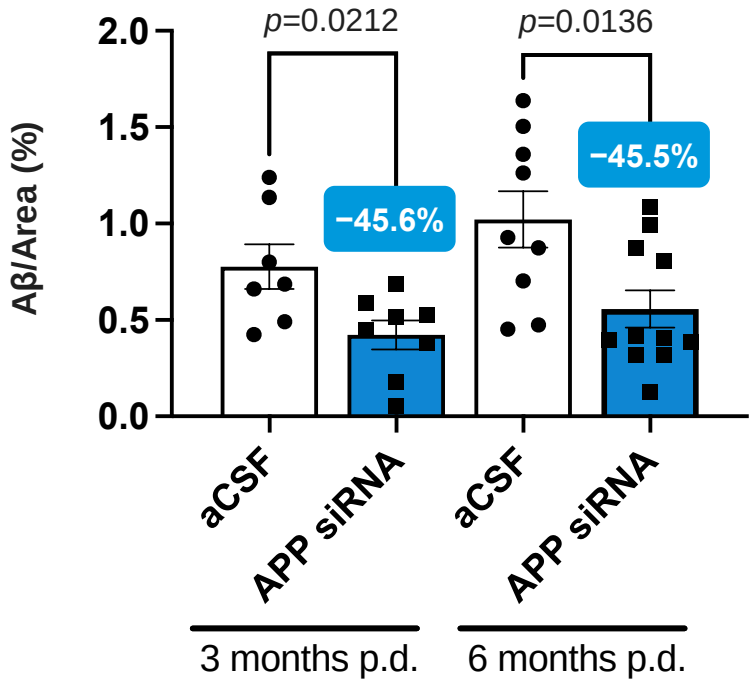


[a]Normalized to Xpnpep1. [b]CVN mouse brain assessed in hippocampus. [c]rTg-DI rat brain assessed in hemibrain without cerebellum.
aCSF, artificial cerebrospinal fluid; APP, amyloid-beta precursor protein; mRNA, messenger RNA; siRNA, small interfering RNA.

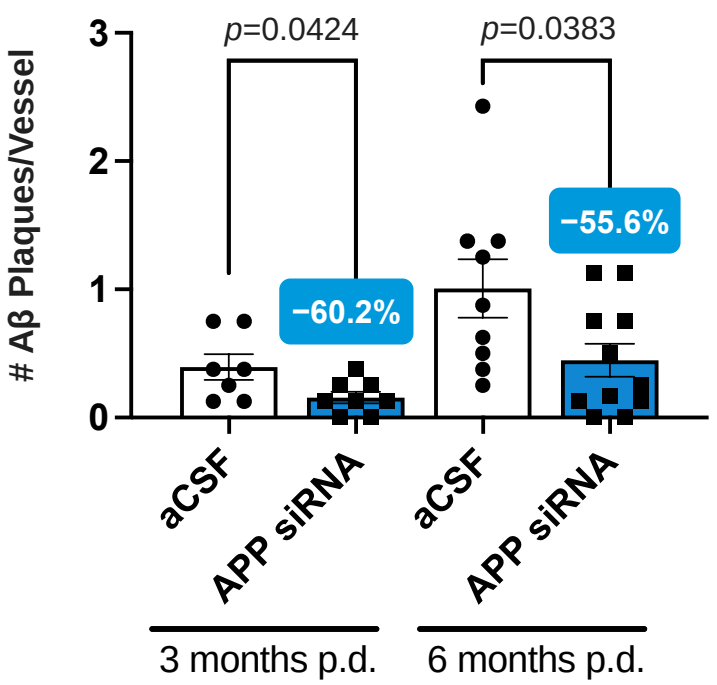
CVN Mouse Model Data

Plaque Deposition in Mouse Hippocampus

Parenchymal Aβ



Vascular Aβ



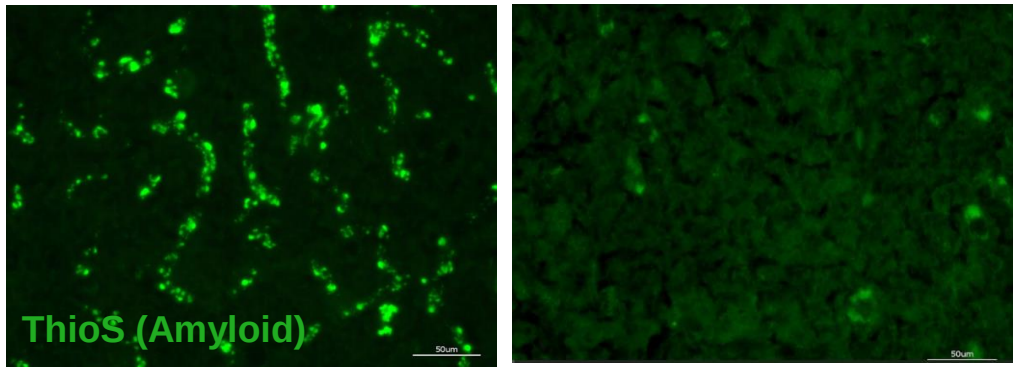
Significant reductions in Aβ parenchymal and vascular accumulation in the hippocampus
compared with controls 3 and 6 months post-dose

Number of Aβ40 staining along SMA+ vessels were determined by counting the number of distinct Aβ40 positive areas that are colocalized to the SMA+ vessel area.
Aβ, amyloid-beta; aCSF, artificial cerebrospinal fluid; APP, amyloid-beta precursor protein; p.d., post-dose; siRNA, small interfering RNA; SMA, smooth muscle actin.

rTg-DI Transgenic Rat Model Data

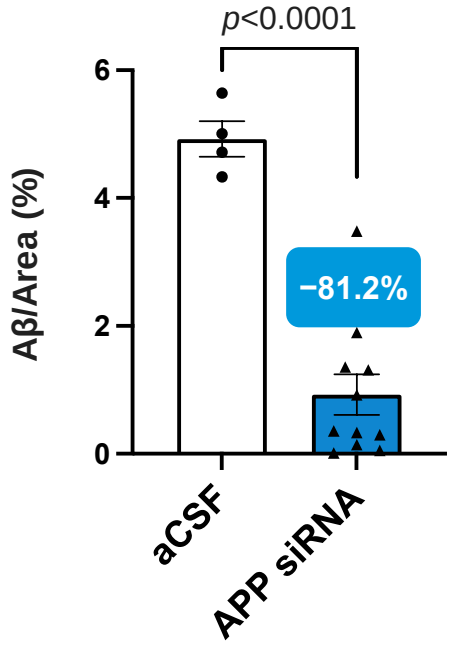
Amyloid Deposition in Rat Hippocampus

Amyloid deposits in vessels
6 months post-dose



aCSF Control | APP-lowering siRNA

Vascular area occupied by amyloid
6 months post-dose



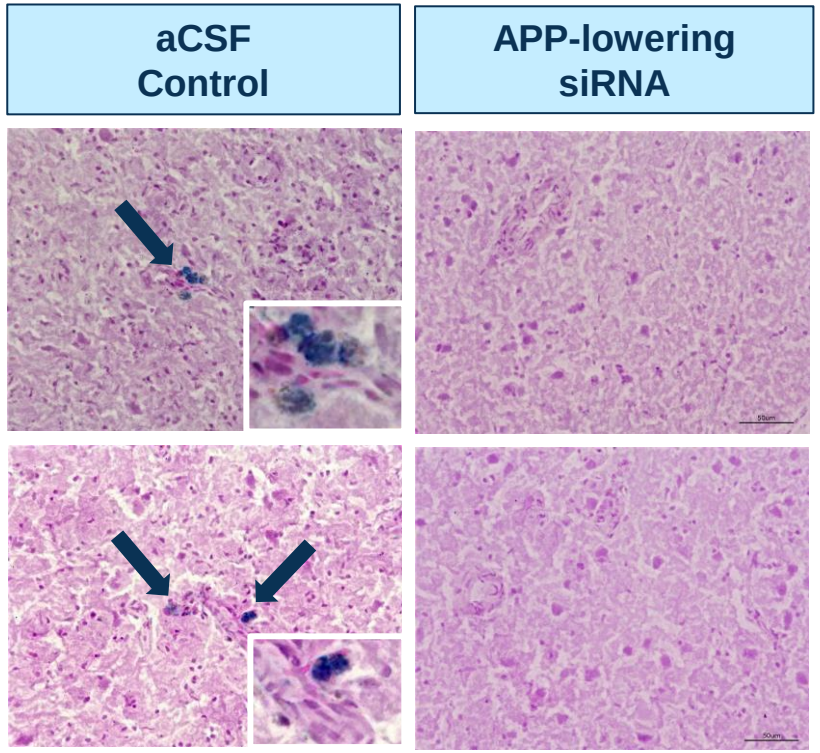
rTg-DI transgenic rats had a significant **reduction in Aβ accumulation in the hippocampus vasculature** compared with controls

[a]Staining greater than background was quantified.

Aβ, amyloid-beta; aCSF, artificial cerebrospinal fluid; APP, amyloid-beta precursor protein; siRNA, small interfering RNA; ThioS, Thioflavin S.

Number and Size of CMBs in Rat

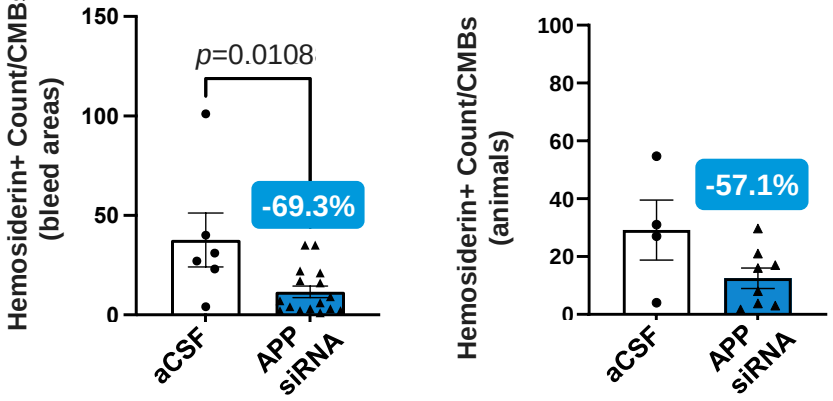
Thalamus
6 months post-dose



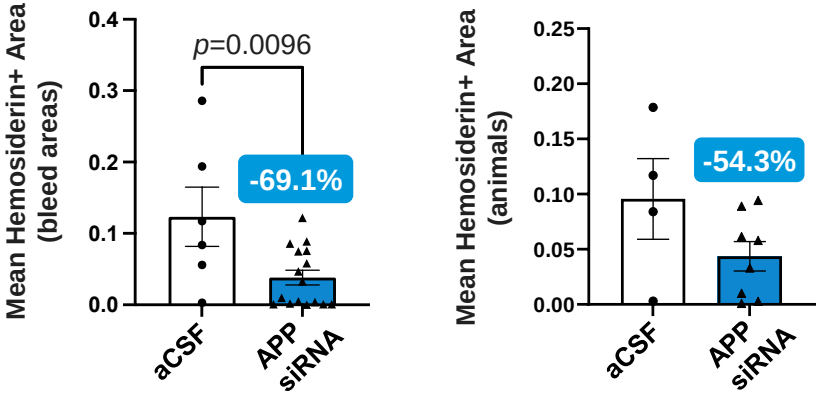
Microhemorrhages (blue) shown with arrows

6 months post-dose

Cerebral microbleed occurrence[a]



Cerebral microbleed size[a]

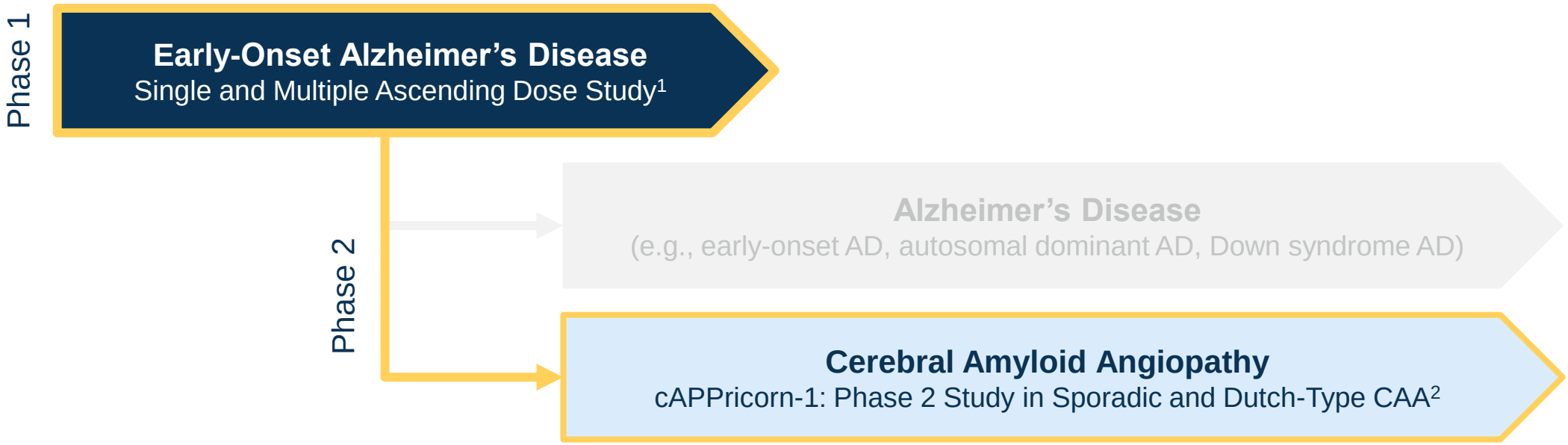


rTg-DI transgenic rats had a **reduction in number of CMBs[b] and mean CMB size**
6 months post-dose compared with controls

[a]Left panel: each dot plotted represents an individual bleed area. Right panel: each dot plotted represents a single animal. [b]Number of CMBs per cluster
aCSF, artificial cerebrospinal fluid; APP, amyloid-beta precursor protein; CMB, cerebral microbleed; siRNA, small interfering RNA.

Mivelsiran Clinical Trials

Phase 1 Early-Onset AD Data Support Development in CAA

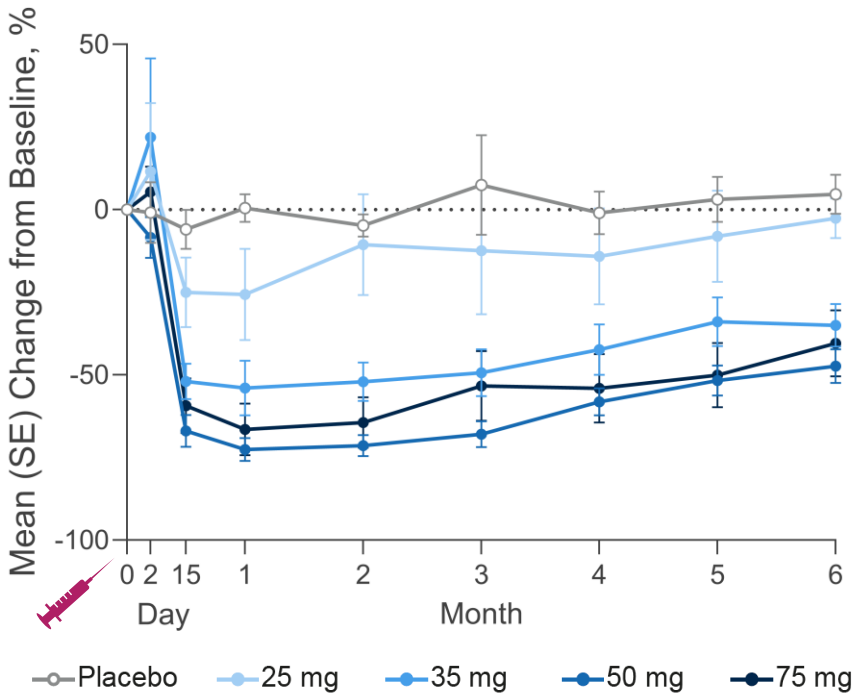


AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy.

1. ClinicalTrials.gov. NCT05231785. Last updated January 24, 2025. Accessed January 27, 2024. https://clinicaltrials.gov/study/NCT05231785. 2. ClinicalTrials.gov. NCT06393712. Last updated December 6, 2024. Accessed January 27, 2024. https://clinicaltrials.gov/study/NCT06393712.

Mivelsiran Phase 1 Study in Patients with Early-Onset AD

Aβ40[a]



Adverse Events

Patients with events	Mivelsiran 25 mg or placebo (N=6, PY=6.9)	Mivelsiran 35 mg or placebo (N=8, PY=4.8)	Mivelsiran 50 mg or placebo (N=8, PY=7.6)	Mivelsiran 75 mg or placebo (N=14, PY=13.5)
Time from randomization, months, mean (SD)	13.89 (1.46)	7.27 (0.79)	11.45 (3.66)	11.60 (2.86)
At least one AE, n (%)	6 (100.0)	8 (100.0)	7 (87.5)	14 (100.0)
Related to study drug	0	0	1 (12.5)	2 (14.3)
Related to LP	4 (66.7)	7 (87.5)	6 (75.0)	7 (50.0)

- AEs deemed related to study drug were reported in three patients; all events resolved[b]
- One serious and severe AE of acute pancreatitis deemed unrelated to the study drug was fatal

Single doses of mivelsiran produced robust and durable **reductions of CSF amyloidogenic proteins** and were **generally well tolerated**, with **most AEs mild or moderate in severity and nonserious**[1]

NCT05231785. Safety data shown as of April 18, 2024, Aβ40 data shown as of August 2, 2024. [a]Similar reductions were observed for CSF Aβ42 levels. [b]Post-LP headache (n=3), post-LP nausea (n=1), post-LP vomiting (n=1), post-LP neck pain (n=1), vomiting due to LP (n=1), and lymphocytopenia (n=1).

AD, Alzheimer's disease; AE, adverse event; Aβ, amyloid-beta; Aβ40, amyloid-beta peptide length 40 amino acids; Aβ42, amyloid-beta peptide length 42 amino acids; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; LP, lumbar puncture; PY, patient-years; SD, standard deviation; SE, standard error.

1. Deering R *et al*. Oral Presentation at the International Cerebral Amyloid Angiopathy (ICAA) Conference, October 15–17, 2024, Munich, Germany.

cAPPricorn-1 Study Design

For US HCPs Only

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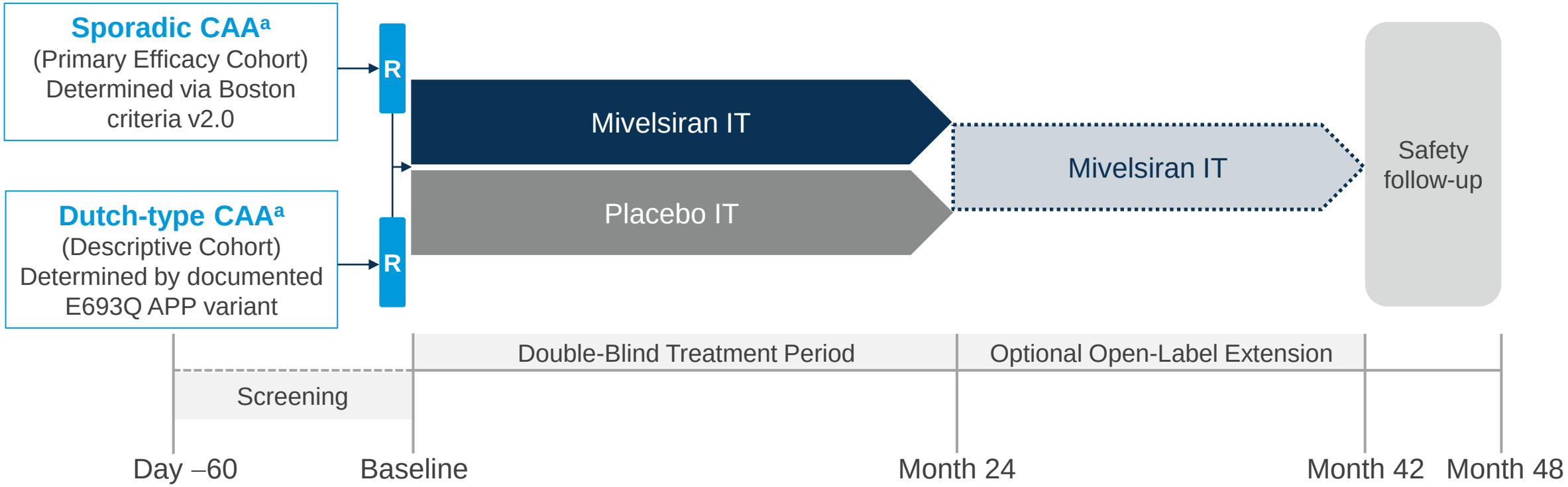
Phase 2 Multiple-Dose Study Evaluating Efficacy, Safety, and Pharmacodynamics



- Endpoints will span hemorrhagic and nonhemorrhagic manifestations of CAA

NCT06393712. [a]Sporadic and Dutch-type CAA cohorts will be analyzed separately.
APP, amyloid-beta precursor protein; CAA, cerebral amyloid angiopathy; IT, intrathecal; R, randomization.
1. ClinicalTrials.gov. NCT06393712. Last updated December 6, 2024. Accessed January 27, 2024. https://clinicaltrials.gov/study/NCT06393712.

Summary

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- Mivelsiran is an investigational RNAi therapeutic designed to reduce APP production, thereby reducing downstream Aβ accumulation and potentially slowing CAA progression
- When compared with controls, a single dose of an APP-lowering siRNA in rodent models of Aβ overproduction significantly:
  - Reduced production of APP
  - Reduced brain and vascular amyloid burden
- In the rat model, cerebral microbleed occurrence and size was reduced following a single dose of an APP-lowering siRNA
- First preclinical evidence that lowering APP by RNAi could slow or halt the progression of CAA
- cAPPricorn-1 is a proof-of-concept Phase 2 study (NCT06393712) to assess efficacy, safety, and pharmacodynamics of mivelsiran in patients with CAA

Investigators and referring physicians interested in cAPPricorn-1 may contact clinicaltrials@alnylam.com or visit cappricorn1.com

If you are seeking additional scientific information related to Alnylam therapeutics, US HCPs may visit the Alnylam US Medical Affairs website at RNAiScience.com. Non-US HCPs should contact medinfo@alnylam.com.
Aβ, amyloid-beta; AD, Alzheimer's disease; APP, amyloid-beta precursor protein; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; HCP, healthcare professional; ICH, intracerebral hemorrhage; RNAi, RNA interference.