Development of RNA Therapeutics for Prion Disease and Other Dementias

Holly B. Kordasiewicz

Disclosures & Disclaimers

- I am an employee of Ionis and own Ionis stock
- This material contains scientific information about investigational medicines that are not approved by the U.S. Food and Drug Administration or any regulatory body
- All investigational medicines are being evaluated in pre-clinical and clinical trials
- Information contained within this presentation is not medical advice and decisions about care should be directed to and discussed with your doctors

Ionis has a large neurology pipeline with disease modifying medicines

Disease state	Medicine or investigational ASO	Gene/Target	Phase 1	Phase 2	Phase 3	Approved
Spinal muscular atrophy	Spinraza	SMN2				
Amyotrophic lateral sclerosis	Qalsody	SOD1				
Amyotrophic lateral sclerosis	ulefnersen	FUS				
Alexander disease	zilganersen	GFAP				
Alzheimer's disease	Ionis-MAPTRx	MAPT/Tau				
Angelman syndrome	ION582	UBE3A-ATS				
Parkingson's disease	ION859	LRRK2				
Multiple system atrophy	ION464	SNCA				
Huntington's disease	tominersen	HTT				
Prion disease	ION717	PRNP				
Pelizaeus-Merzbacher disease	ION356	PLP1				
Spinal muscular atrophy	ION306	SMN2				
AD in Down Syndrome	ION269	APP				

- CNS ASO delivery experience with ASOs, >15,000 patients doses IT with Ionis ASOs
- 3 Approved Medicines in Neurological Diseases

SPINRAZA® (nusinersen) injection 12 mg/5 mL

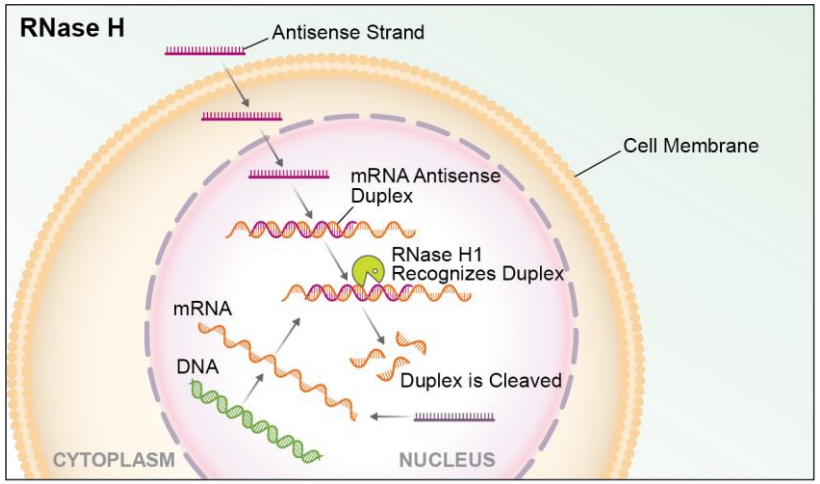
QALSODY® (tofersen) 100 mg/15 mL injection

WAINUA (eplontersen) 45 mg injection for subcutaneous use

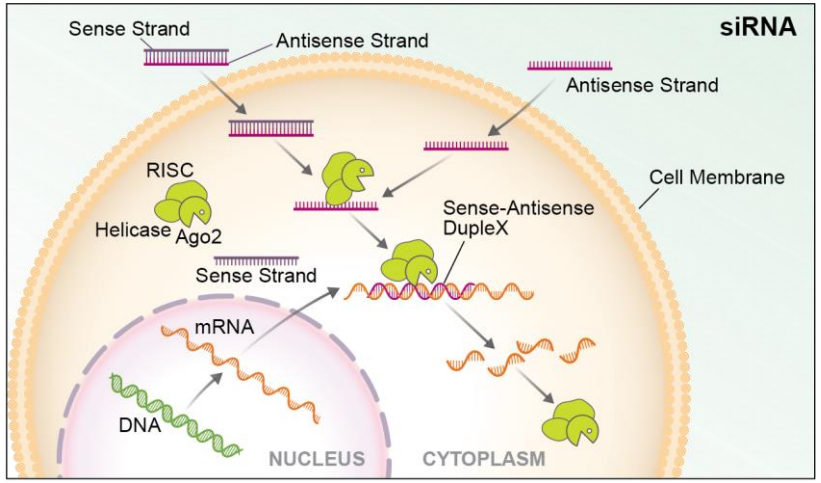
RNA therapeutics target RNA using multiple different mechanisms

Mechanisms that degrade RNA
(and reduce gene expression)

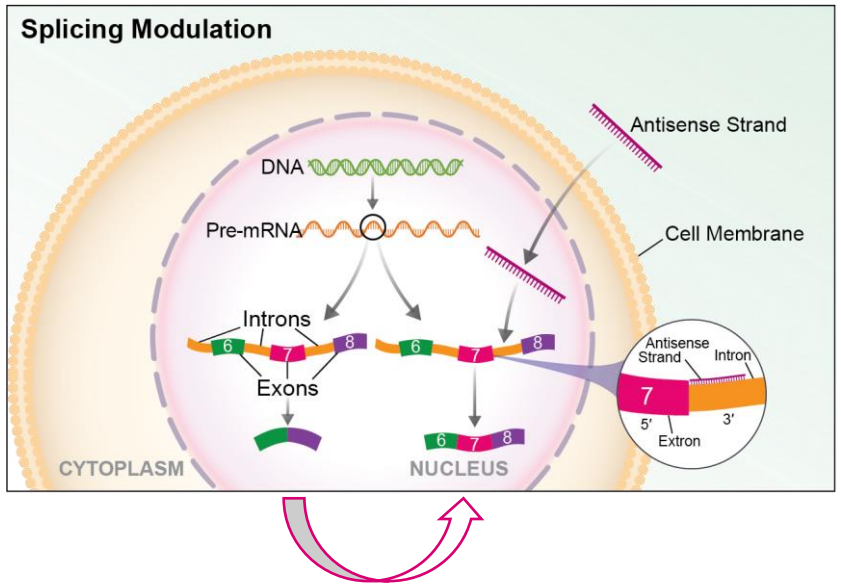
Gapmer ASOs like Qalsody lower protein via RNase H



siRNAs like vutrisiran lower protein via RNA interferance



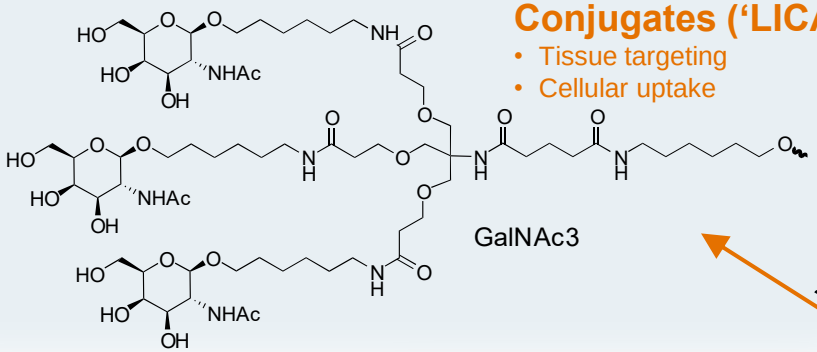
Mechanisms that modulate RNA
(and can alter / increase gene expression)



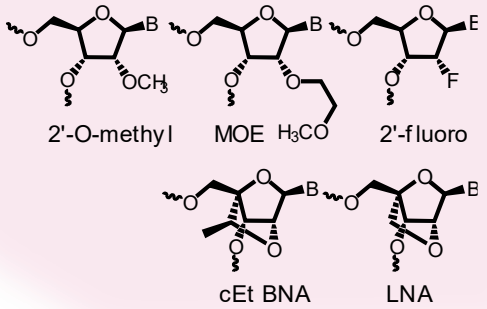
Spinraza alters splicing to make a functional mRNA that makes SMN protein

Advances in medicinal chemistry have driven the evolution of Ionis antisense drugs

Conjugates ('LICA')
- Tissue targeting
- Cellular uptake

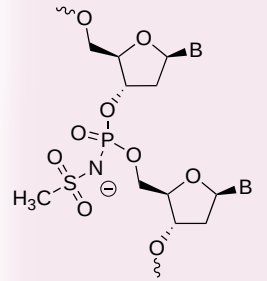


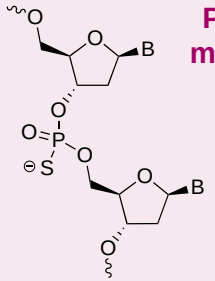
Sugar Modifications
- Increase binding affinity to RNA
- Stability against nucleases
- Decrease pro-inflammatory properties



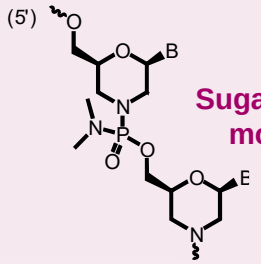
Backbone Modifications
- Stability against nucleases
- Modulate protein binding

A potential breakthrough that improves stability, reduces nonspecific protein binding while maintaining RNase H1 activity

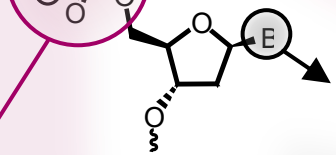

Mesyl phosphoramidate (MsPA)


Phosphorothioate

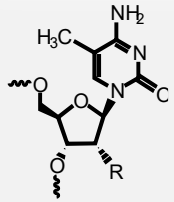
Phosphate modification


Morpholino

Sugar-phosphate modification


DNA phosphodiester

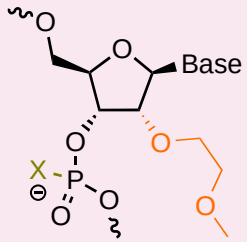
Base Modifications
- Decrease pro-inflammatory properties
- Slight increase in binding affinity


5-methylcytosine

Chimeric (Gapmer) RNase H1 Oligo Design

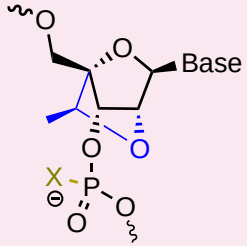
MOE/cEt	DNA	MOE/cEt
↑ affinity ↑ stability ↑ tolerability	RNase H1 Substrate	↑ affinity ↑ stability ↑ tolerability

2'-O-methoxyethyl (MOE)



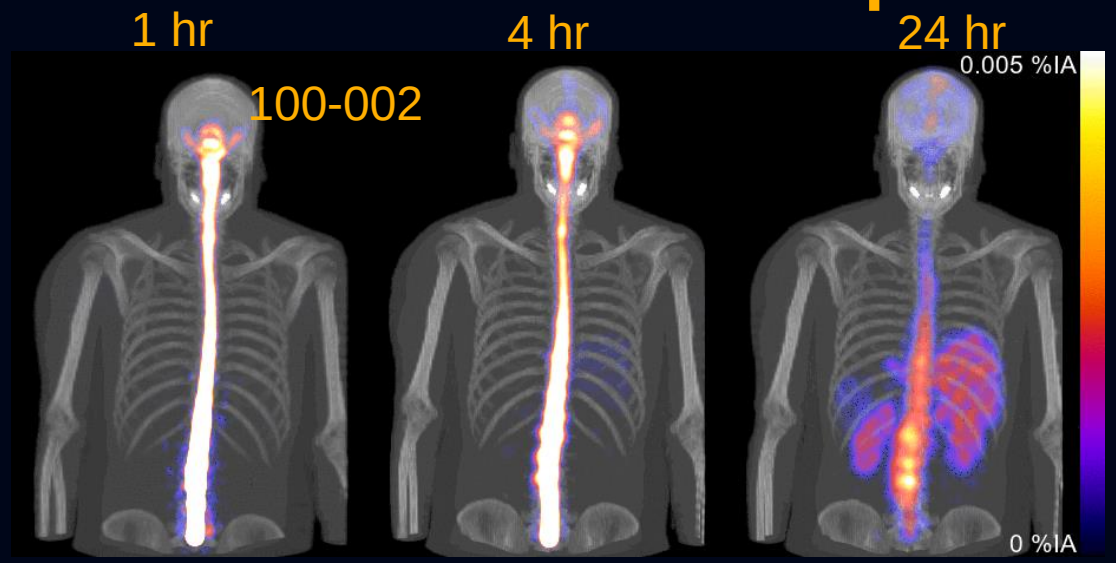
'Generation 2.0'

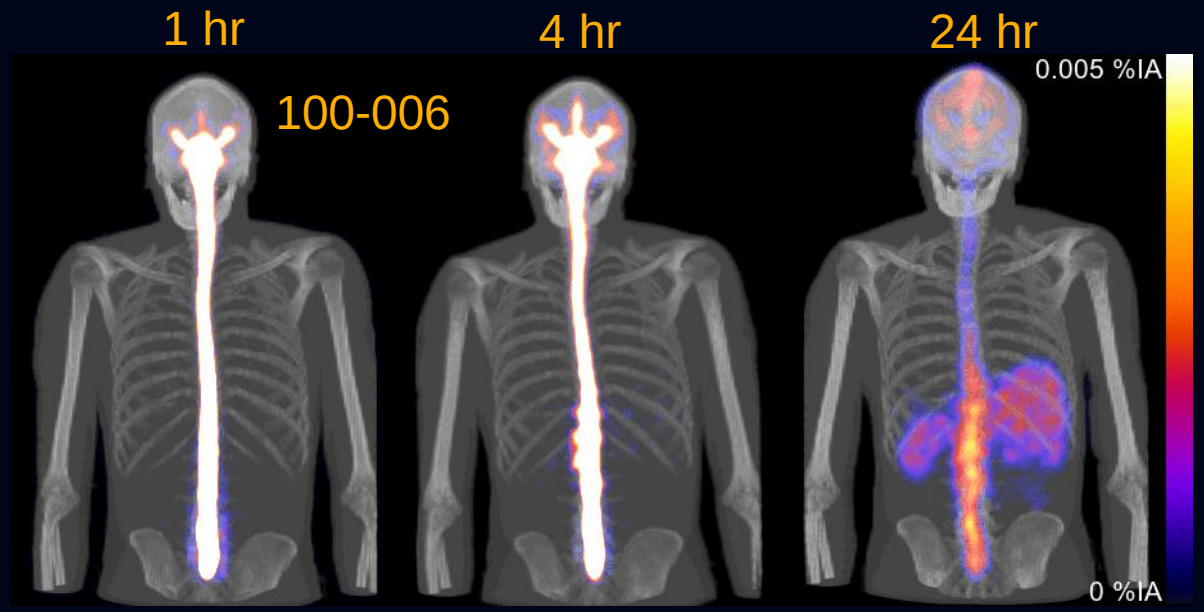
Constrained ethyl (cEt)

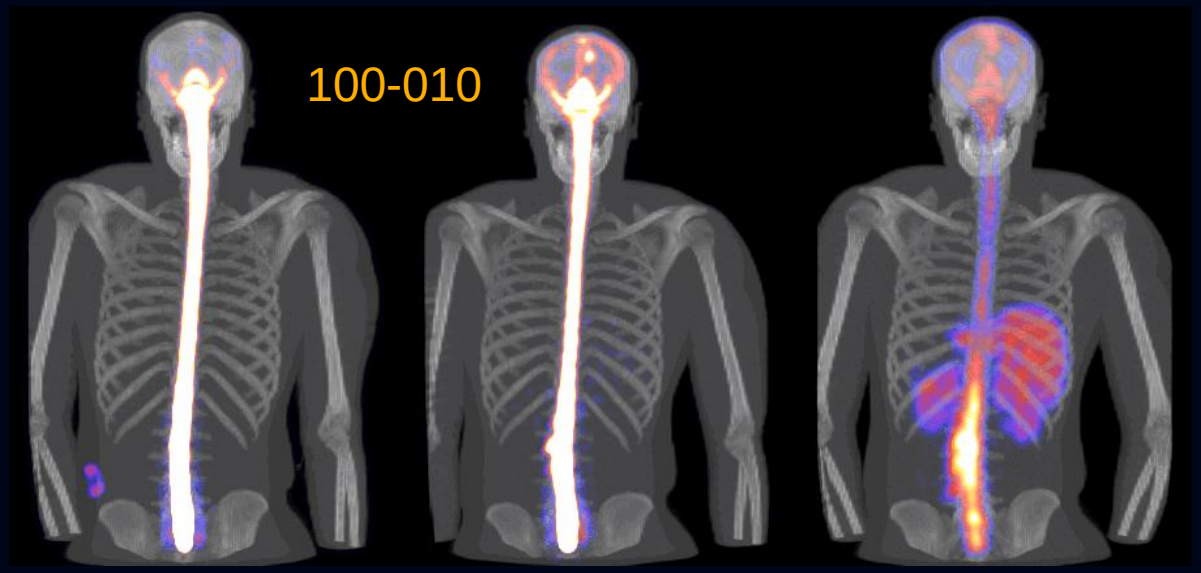


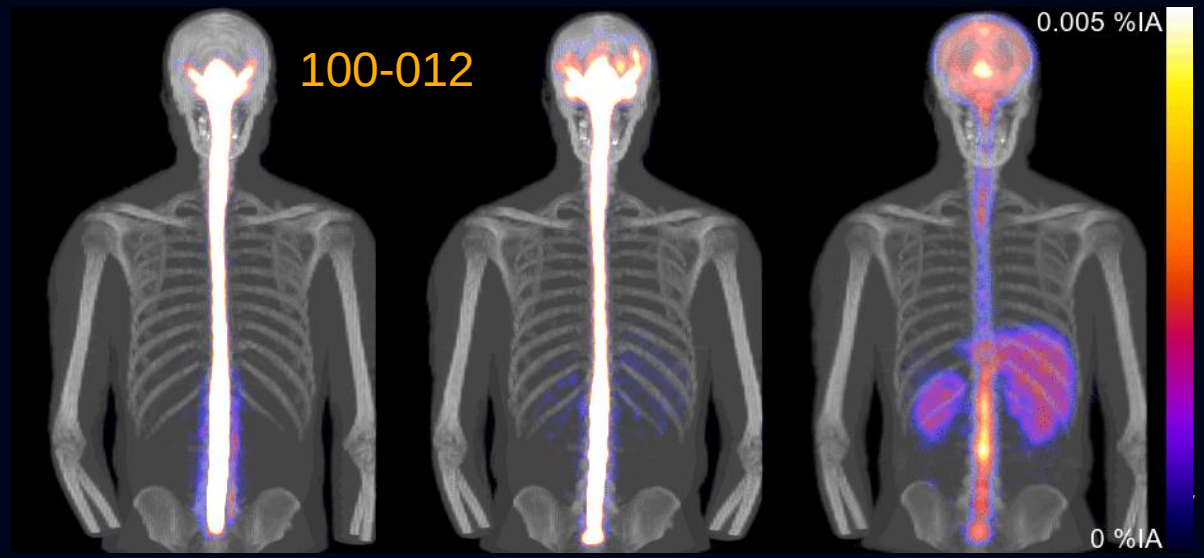
'Generation 2.5'

^{99m}Tc-MAG3-SOD1 ASO SPECT imaging in humans shows robust ASO distribution up the neuraxis





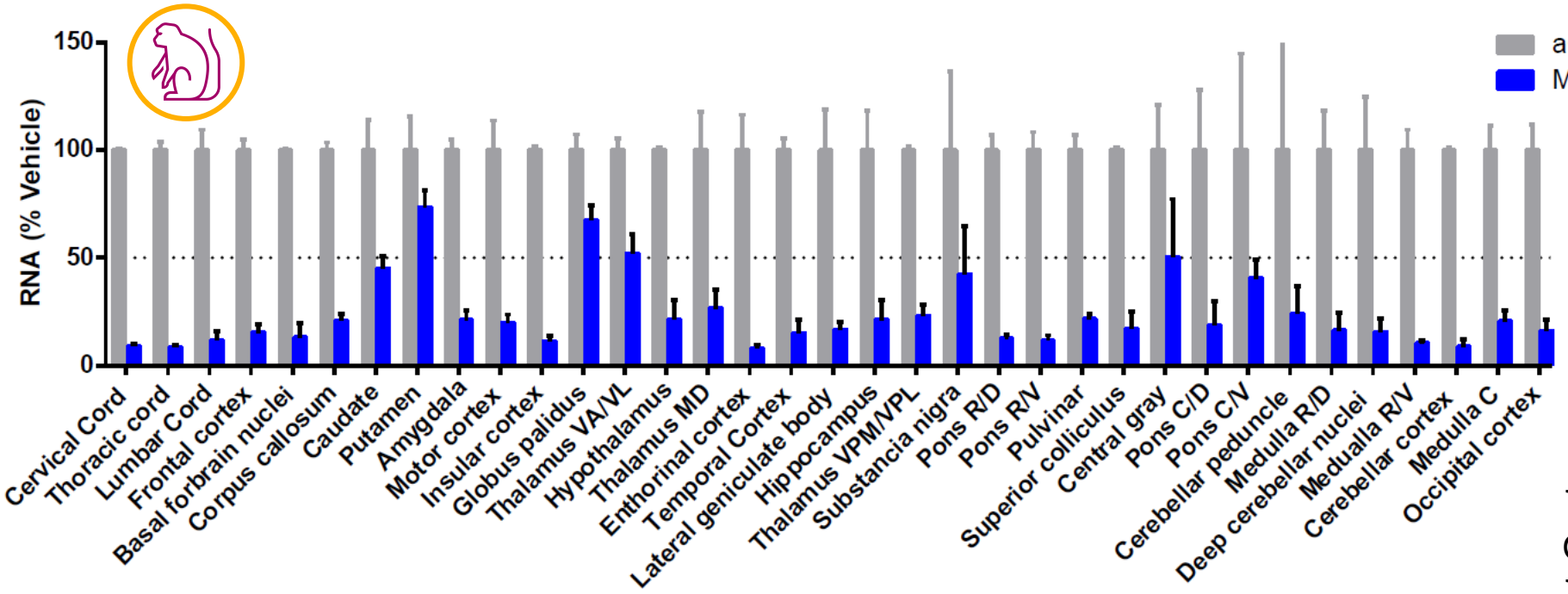




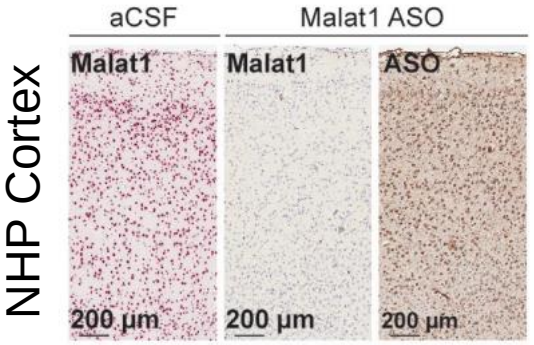
Jenna Sullivan et al, OTS 2021

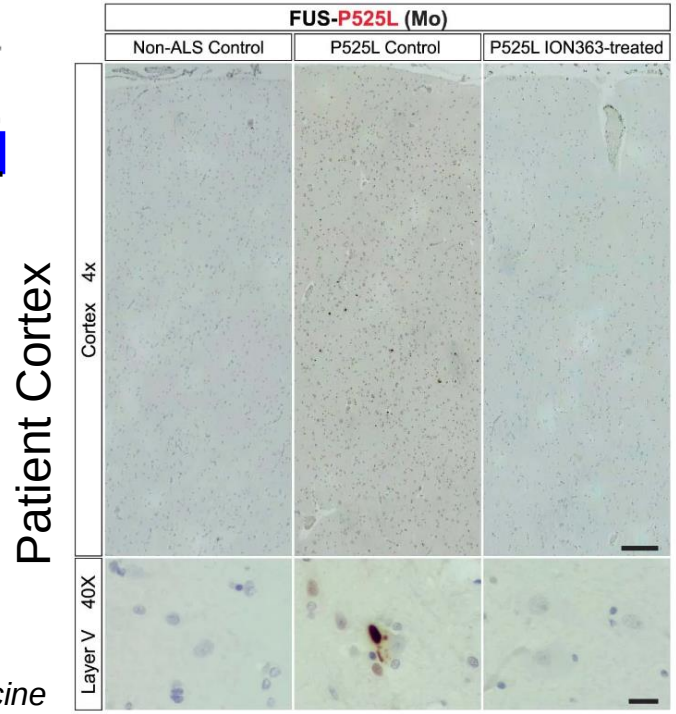
ASOs distribute throughout NHP CNS following intrathecal dosing

IT delivery in NHP of an ASO targeting the ubiquitously expressed long-non-coding RNA MALAT1 (MALAT1) or vehicle (aCSF)



Jafar-nejad et al. 2021 *Nuclei Acids Res.*

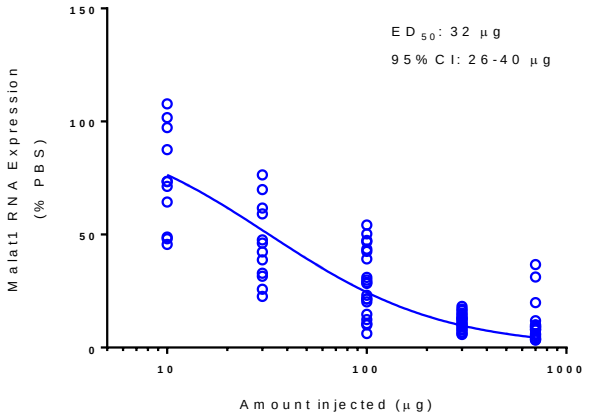




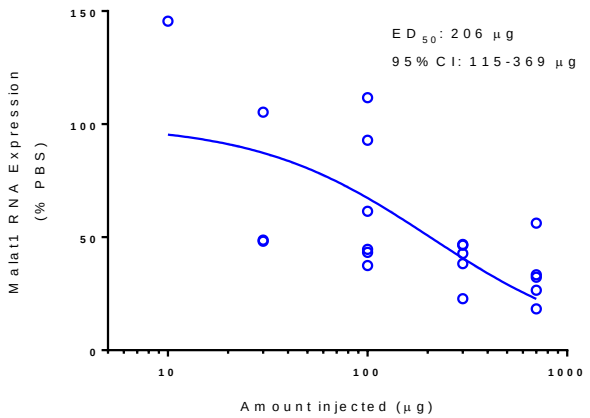
Korobeynikov et al. 2022 *Nature Medicine*

ASOs are active in all major cell types in the CNS

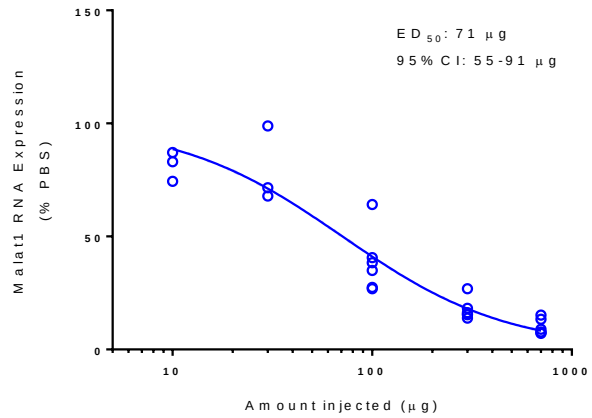
Total cortex



Neurons

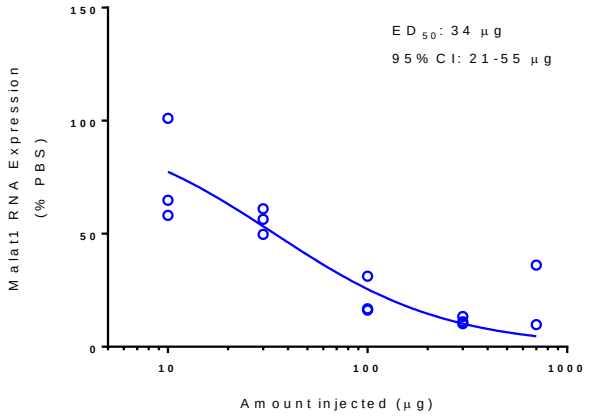


Microglia

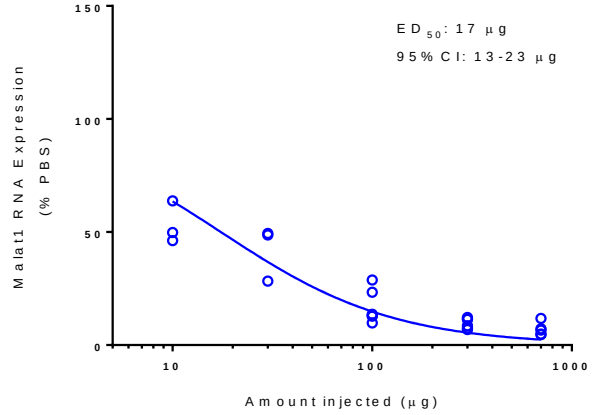


Magnetic-Activated Cell Sorting of key cell populations in the CNS following ASO dose response in mice

Oligodendrocytes



Astrocytes



Jafar-nejad et al. 2021 *Nuclei Acids Res.*

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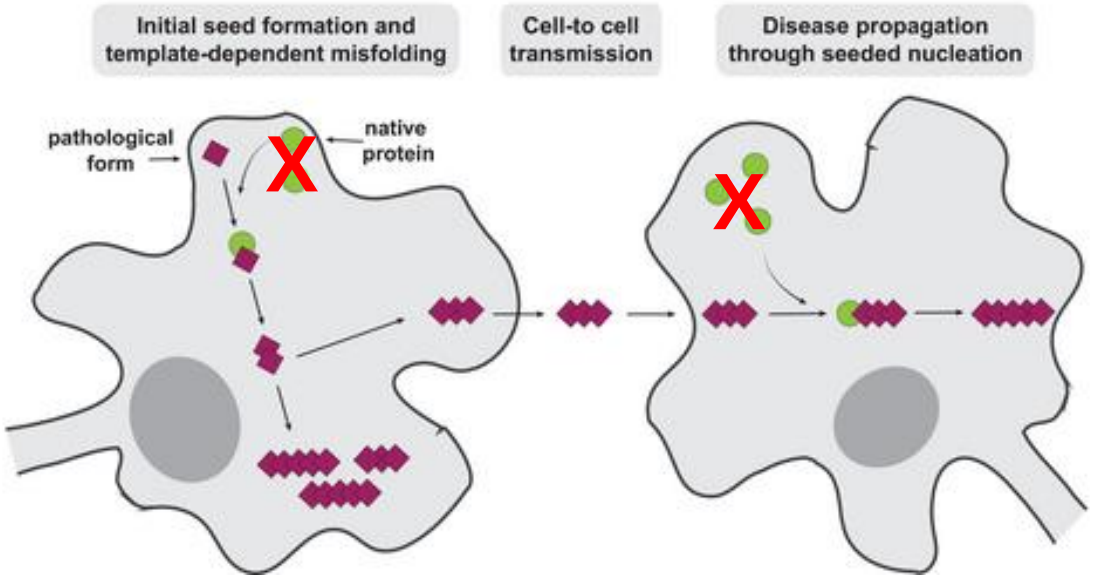
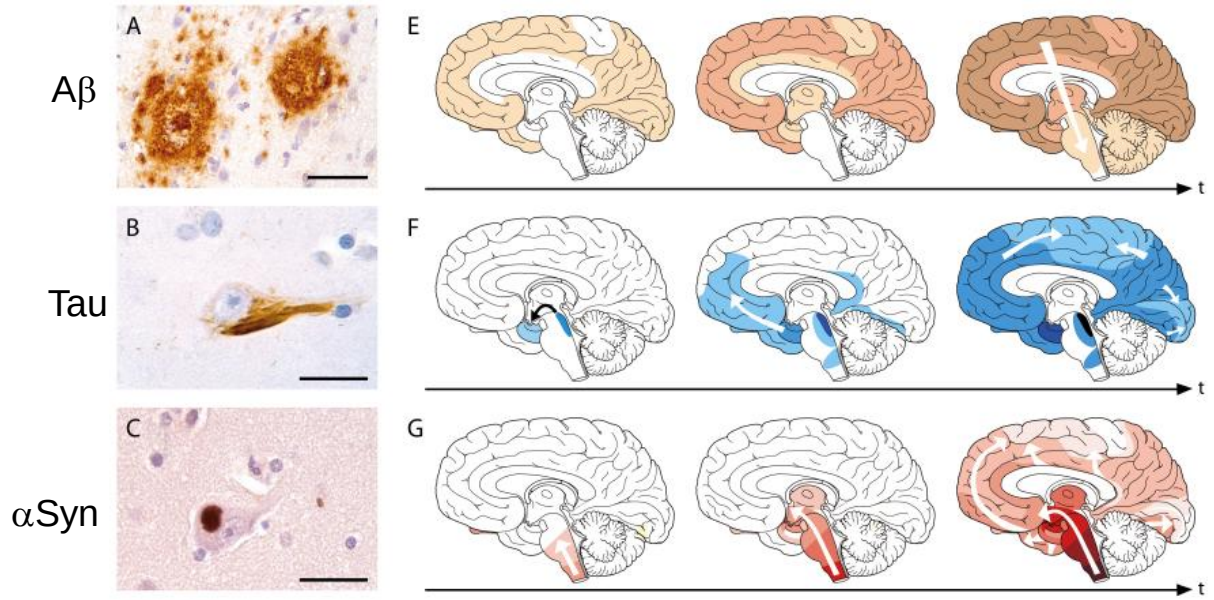
Transmission hypothesis of neurodegenerative diseases

Molecular hypothesis: Proteinaceous aggregates can induce pathology in healthy cells and propagate from cell to cell, underlying disease progression (endogenous protein is required for propagation and toxicity)



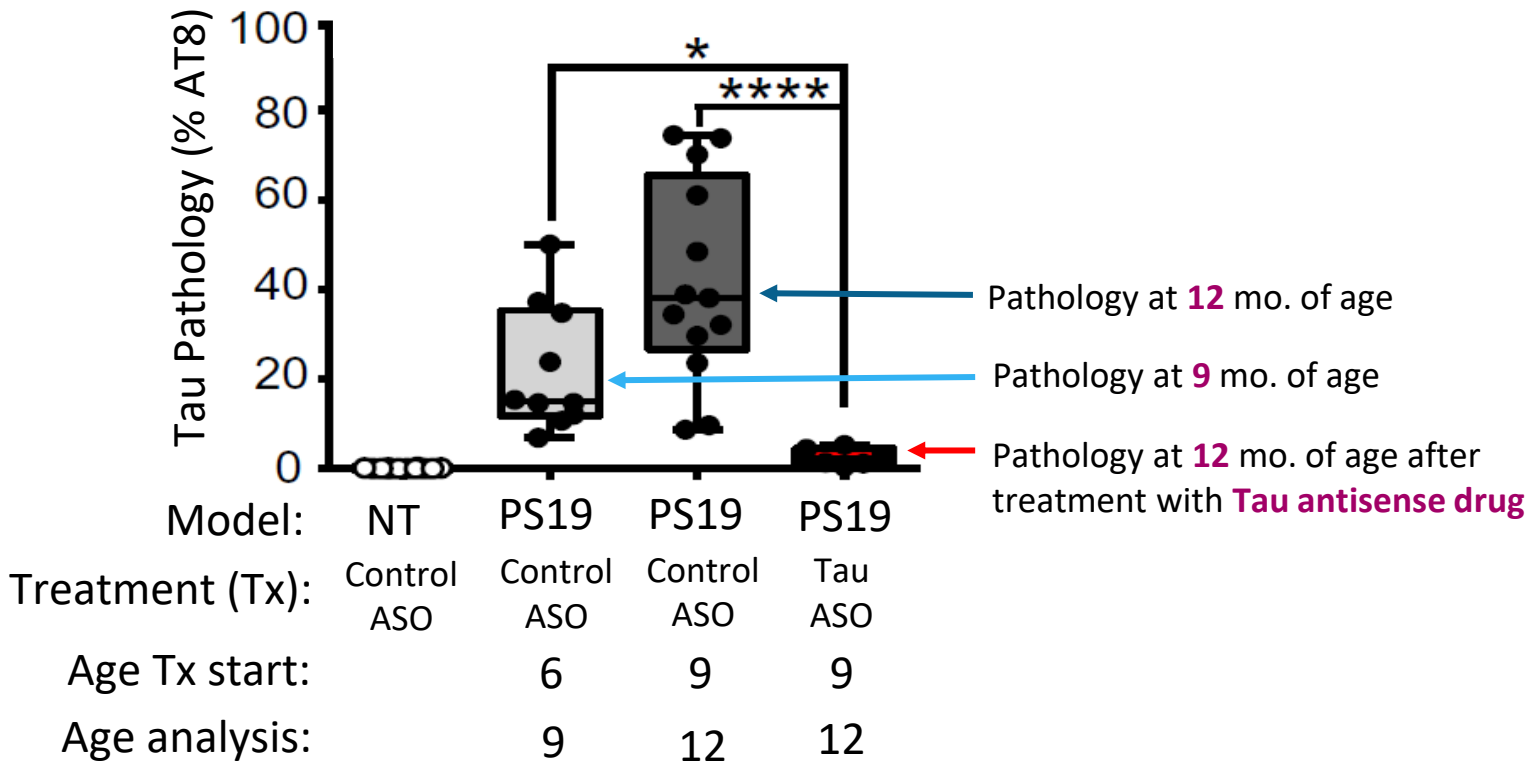
Hock and Polymenidou (2016)



Jucker and Walker. Nature (2013)

Therapeutic hypothesis: ASO-mediated substrate reduction should reduce pathological spread of protein aggregates and benefit diseases

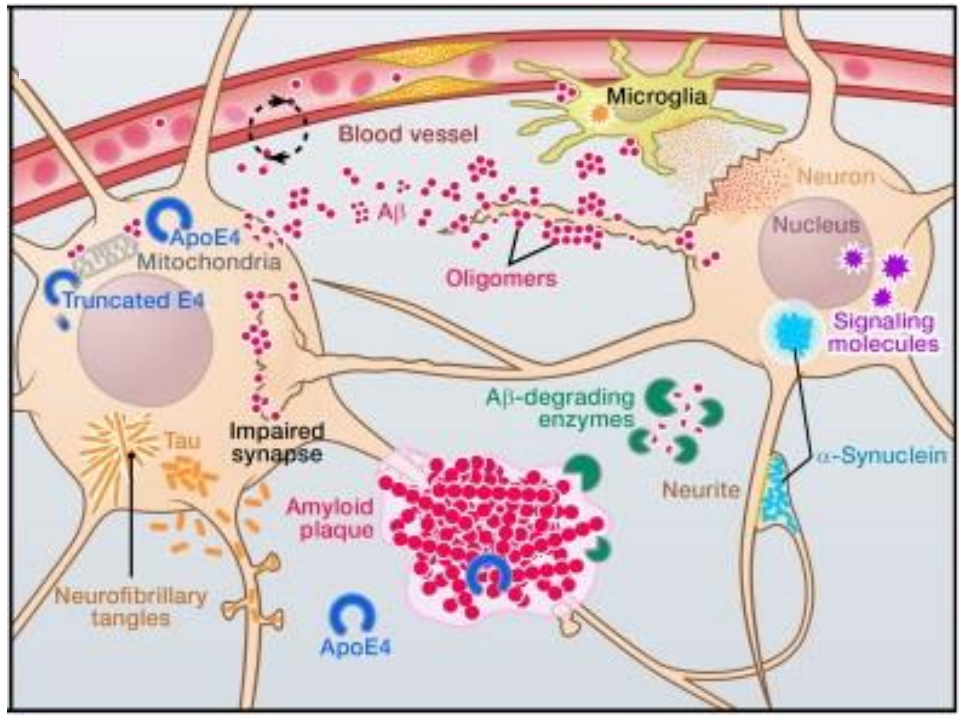
ASO-mediated Tau suppression can *reverse* Tau pathology



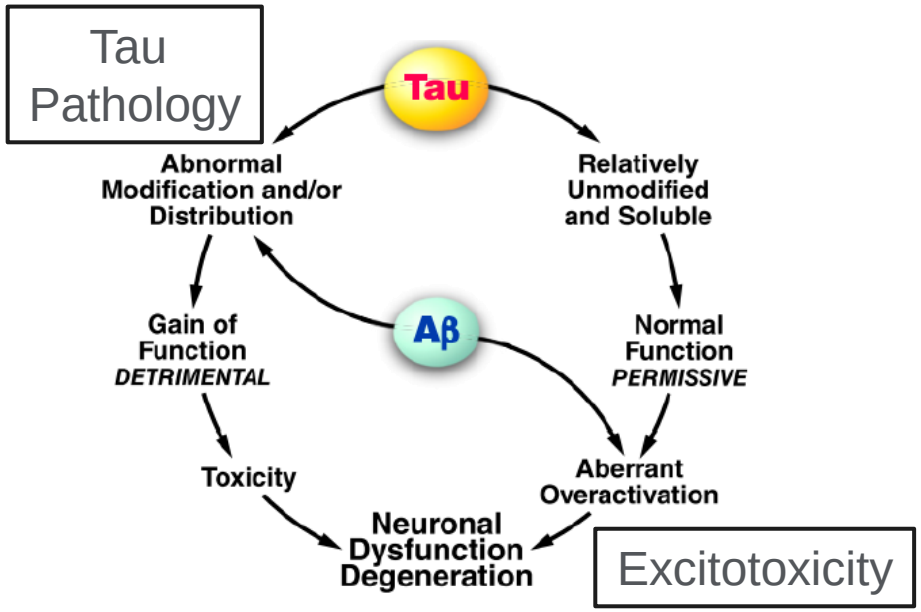
PS19 mice treated at 9 months with *MAPT*-targeting ASO had less severe pathology at 12 months of age (red) than control PS19 mice at 9 months of age (light gray)

DeVos, 2020

Both Tau pathology and Tau dependent excitotoxicity contribute to Alzheimer's disease (AD)



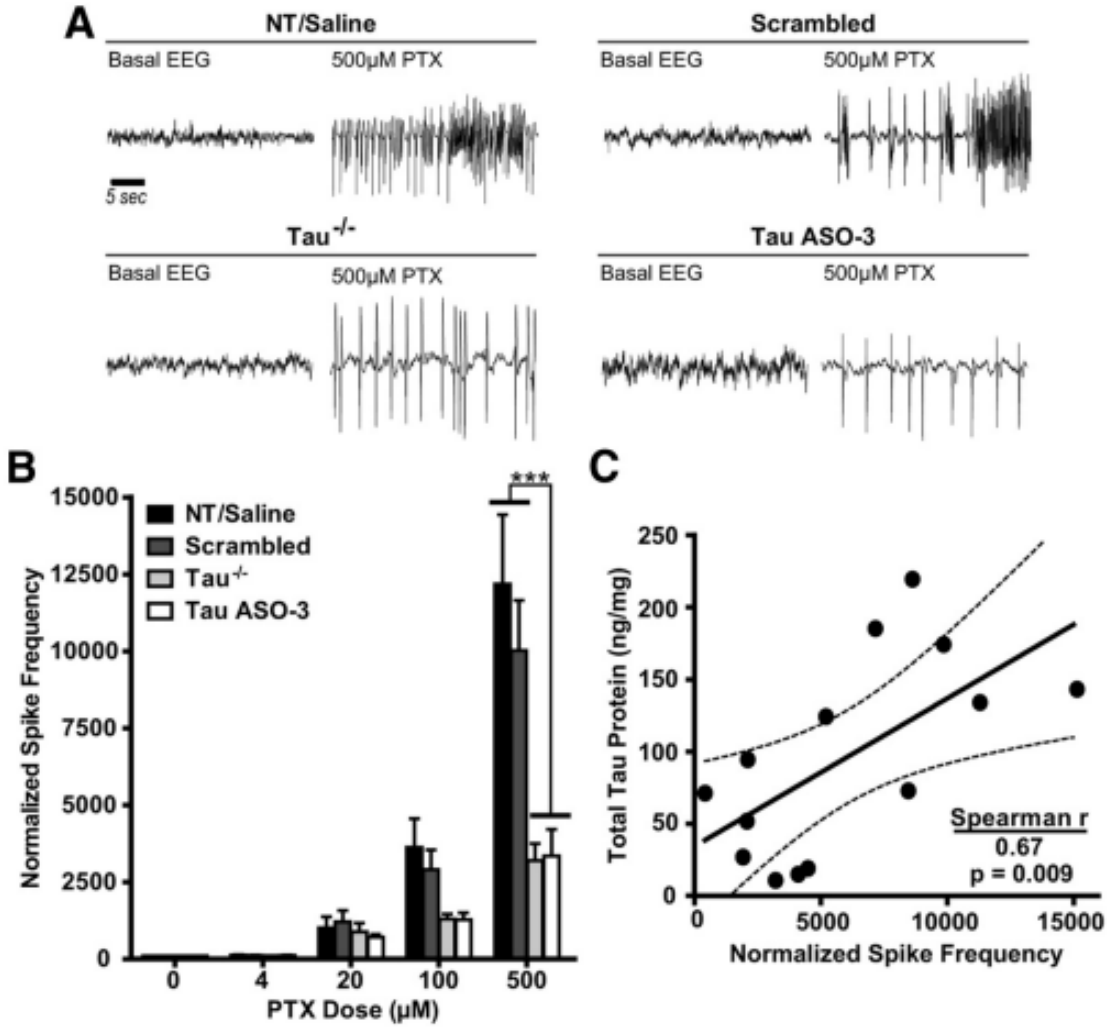
Huang and Mucke, *Cell* 2012



Morris et al., *Neuron* 2011

- The major pathological hallmarks of AD are Tau neurofibrillary tangles and Aβ plaques
- Aβ toxicity is dependent on Tau
- Lowering Tau reverses Tau pathology and prevents spread
- Lowering endogenous Tau protects against excitotoxicity

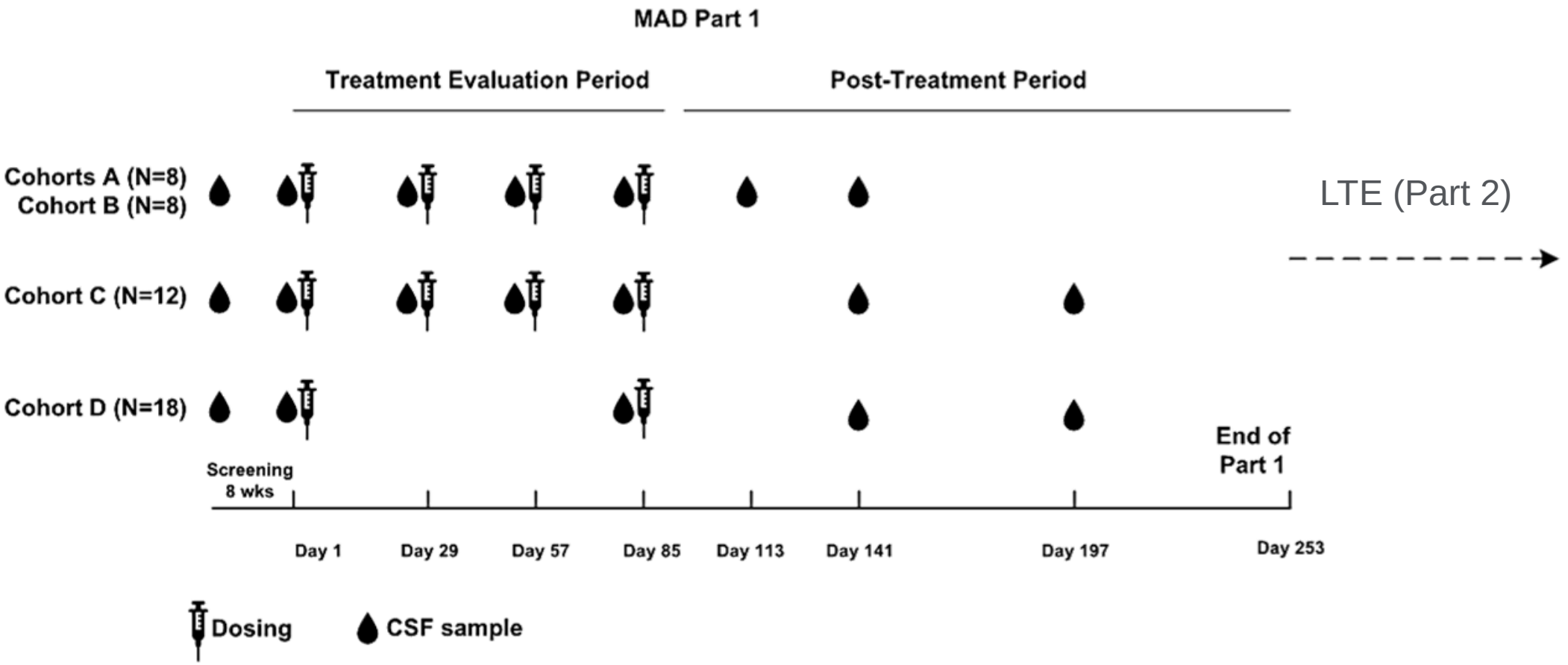
ASO-mediated Tau suppression prevents against overactivation



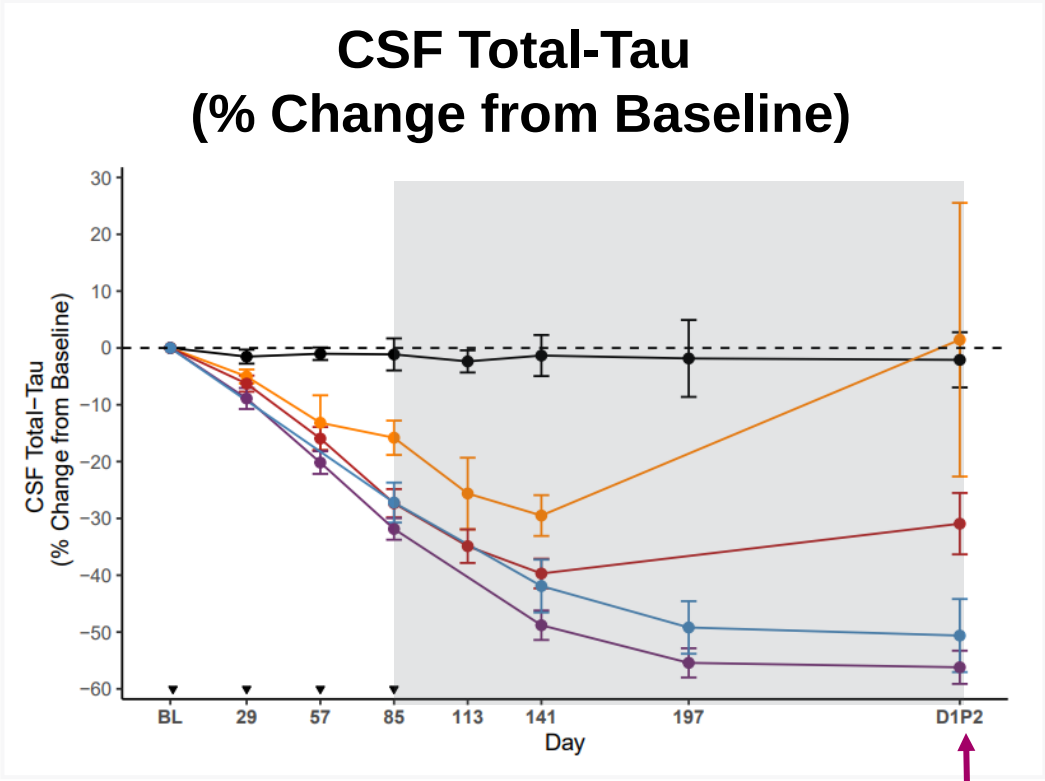
Suppression of endogenous Tau via genetic KO or ASO prevents chemically induced epileptic activity

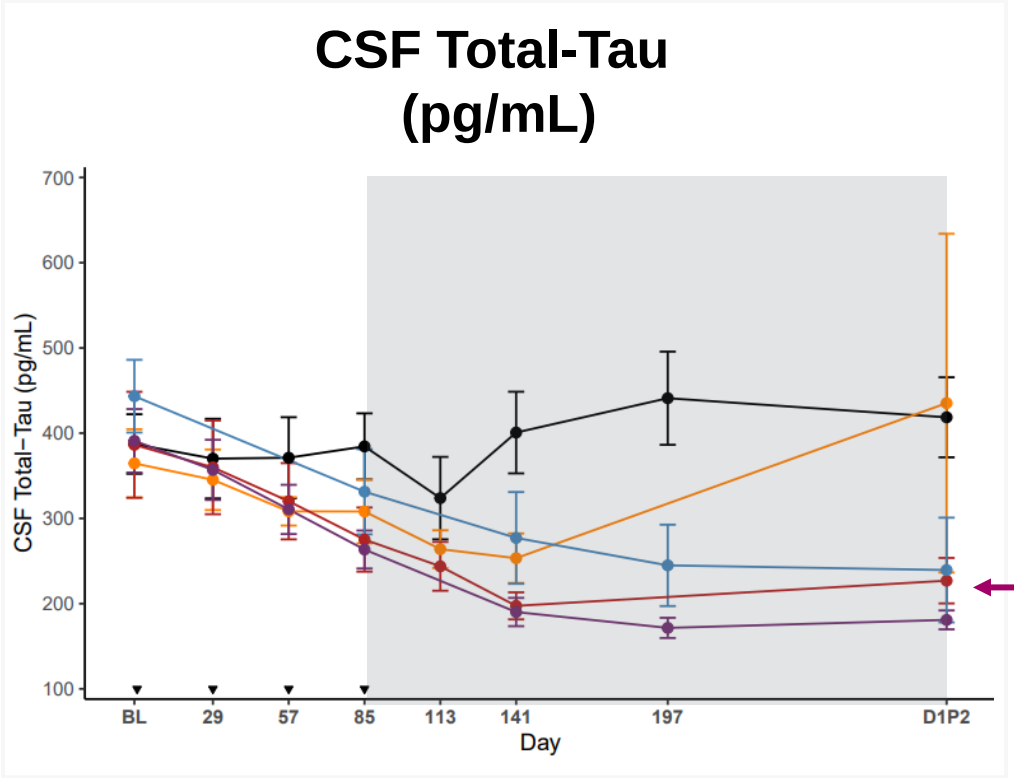
DeVos, S. L. et al. (2013) J. Neurosci.

Design of first in human MAPT ASO clinical trial



Durable reduction in CSF Tau levels in ASO treated patients





- Low dose Q4W
- Medium dose Q4W
- High dose Q4W
- Q12W dose

Target reduction sustained for >6 months

Treatment phase

Recovery Phase (6-24 months)

Start of LTE

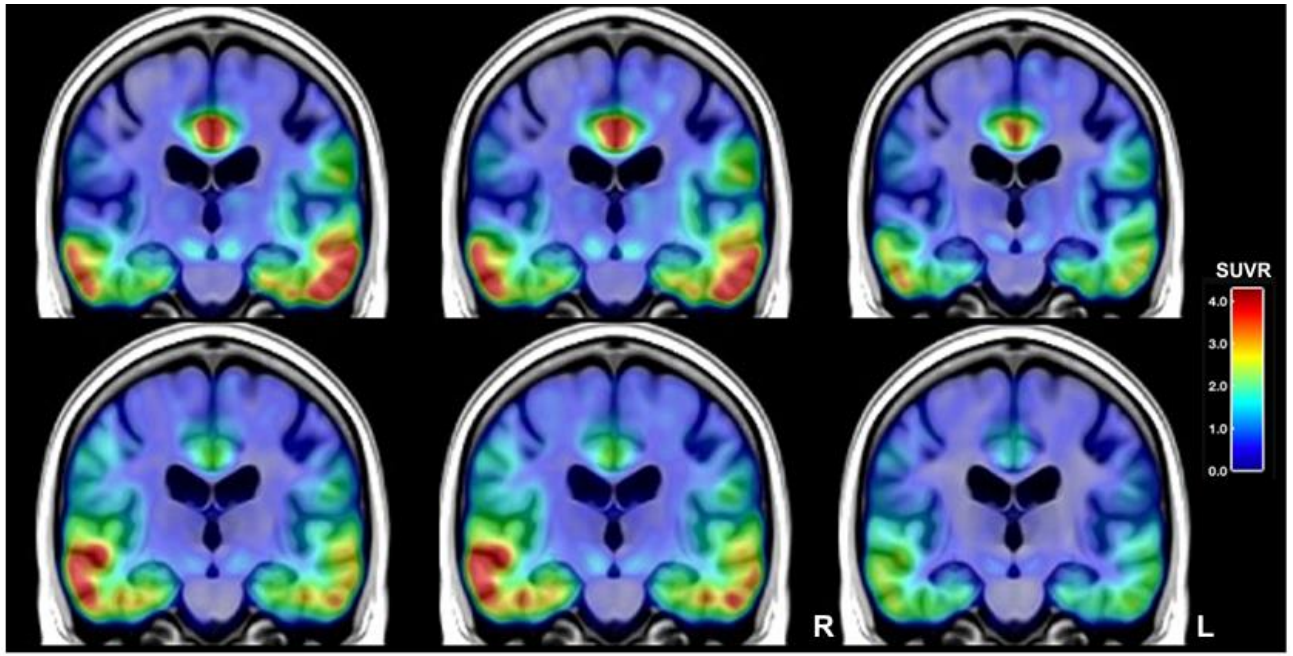
Mummery et al. Nature Medicine 2023

Consistent reduction in Tau burden across all brain regions following MAPT ASO treatment

Screening —Placebo→ **Week 25** —115mg Q12W→ **Week 100**

2380-4011
67 y/o
Male
CDR= 0.5
MMSE= 26

2176-4009
71 y/o
Male
CDR= 0.5
MMSE= 26



CELIA Phase 2 Study underway in patients with early AD

Phase 1b Tau PET Results

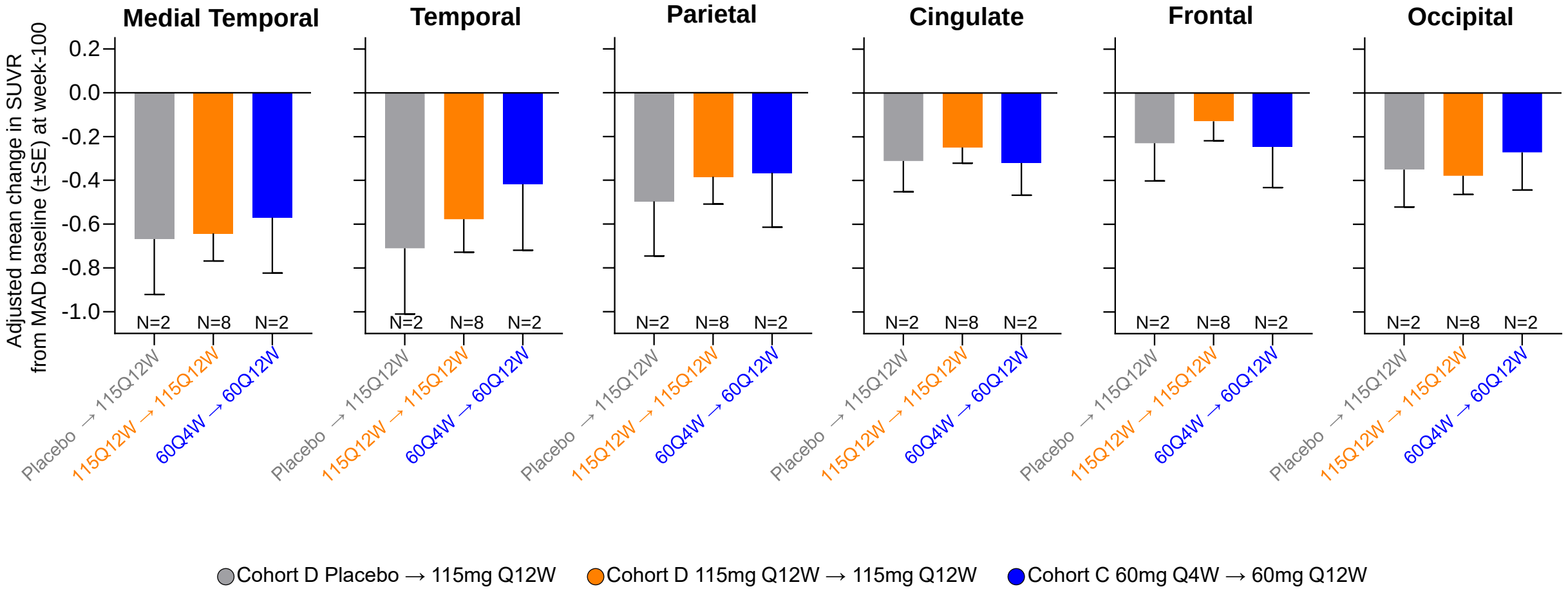
Patients initially on placebo then $MAPT_{Rx}$ (BIIB080) showed **reduced tau burden following treatment**

Reduced tau burden at all doses and dose frequencies in the long-term extension study

Generally **well-tolerated at all doses and dose frequencies**

ADD REF CDR Clinical Dementia Rating scale; MMSE Mini Mental State Examination; SUVR standard uptake value ratio;
CELIA Study (Biogen conducting): Clinialtrials.gov/NCT05399888
Edwards et al. JAMA Neurology 2023

MAD + LTE tau PET results: MAPT ASO reduces Tau burden at the end of the LTE following drug administration in all treatment groups



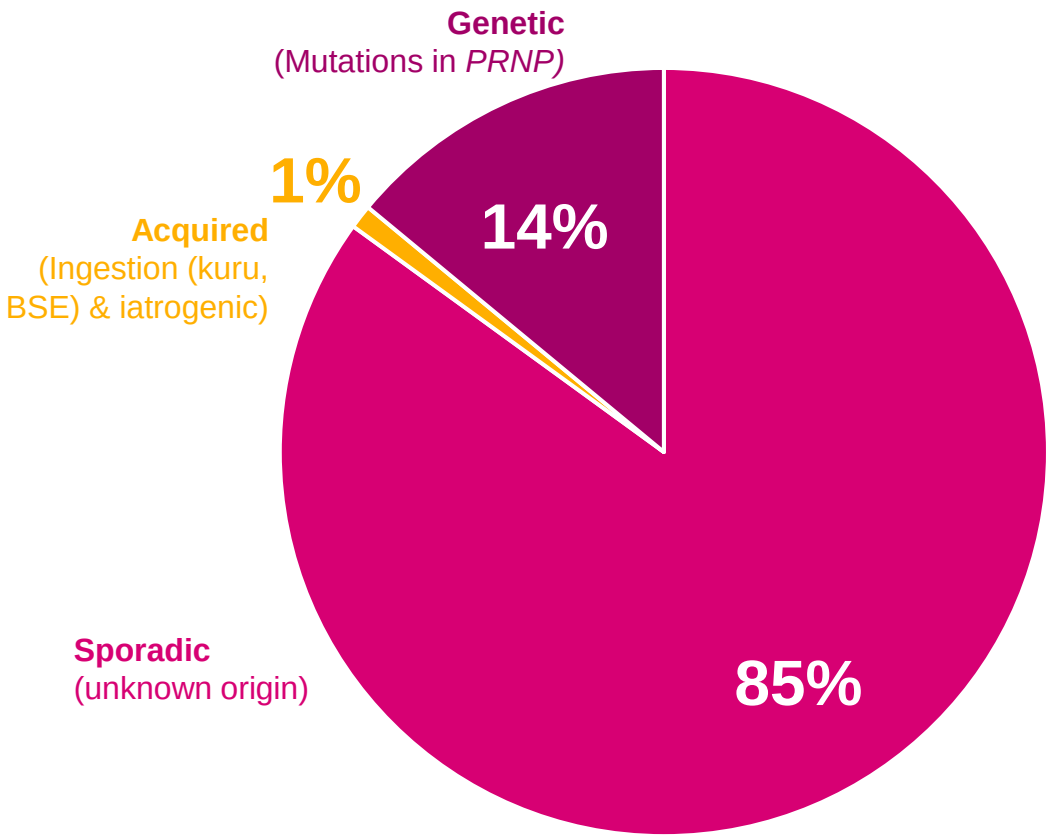
Edwards et al. JAMA Neurology 2023

[^{18}F]-MK6240 standard uptake value ratios (SUVRs) were calculated with inferior cerebellum as the reference region. Adjusted mean change from MAD baseline based on ANCOVA model with fixed effects of categorical treatment and baseline tau PET SUVR, Error bars reflect standard error of the mean

LTE = long term extension; MAD = multiple ascending dose; PET = positron emission tomography

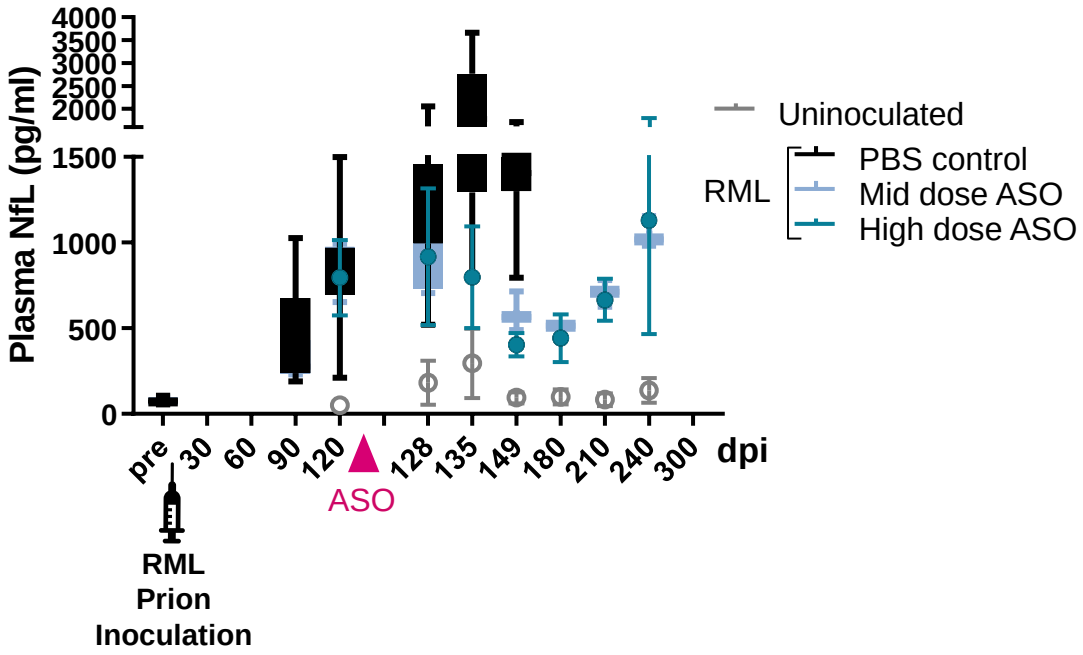
Rationale for targeting PRNP for the treatment of prion disease

- **Prion disease** is a fatal dementia caused by misfolding of prion protein (PrP)
- PrP is the **root cause** of all **forms of prion disease**
- An ASO targeting PRNP mRNA will **decrease prion protein** levels and has the potential to be beneficial in all forms of prion disease

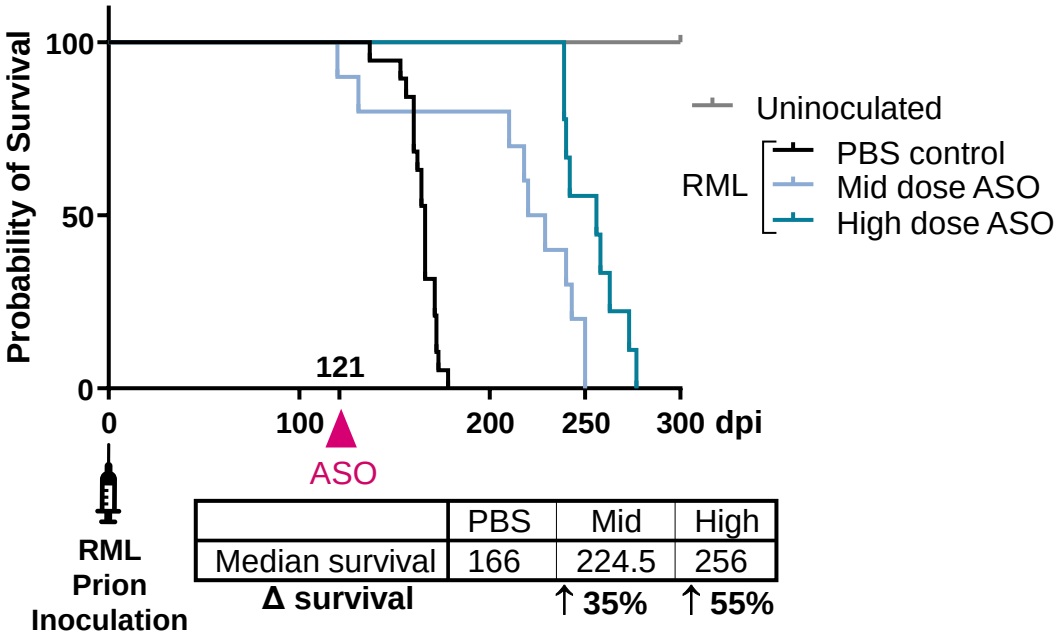


Dose-dependent reversal of plasma NfL levels and extended survival with ASO treatment initiated in late-stage disease in a prion mouse model

Plasma NfL Reversal in Model of Prion Disease



Extended Survival in Model of Prion Disease



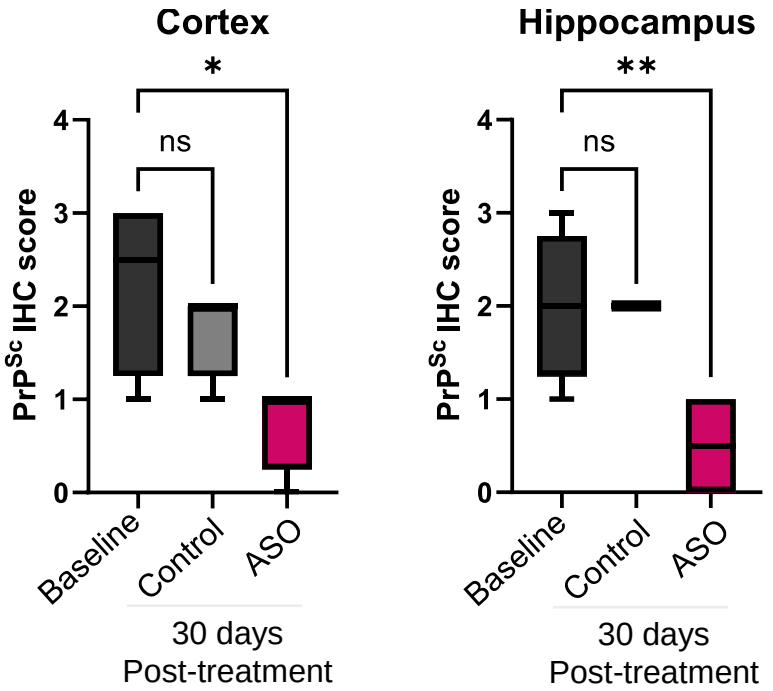
	PBS	Mid	High
Median survival	166	224.5	256
Δ survival		↑ 35%	↑ 55%

Minikel et al. NAR, 2020; Ionis, unpublished

Reversal of PrP deposits after ASO treatment initiated in late-stage disease model

PrP deposits (PrP^{SC}) as determined by PrP IHC following proteinase K treatment

RML Injected Mice
with PrP ASO at 120 Days Post-RML prion inoculation (dpi), pathology performed at 150 dpi



Raymond et al., JCII, 2019; Ionis, unpublished

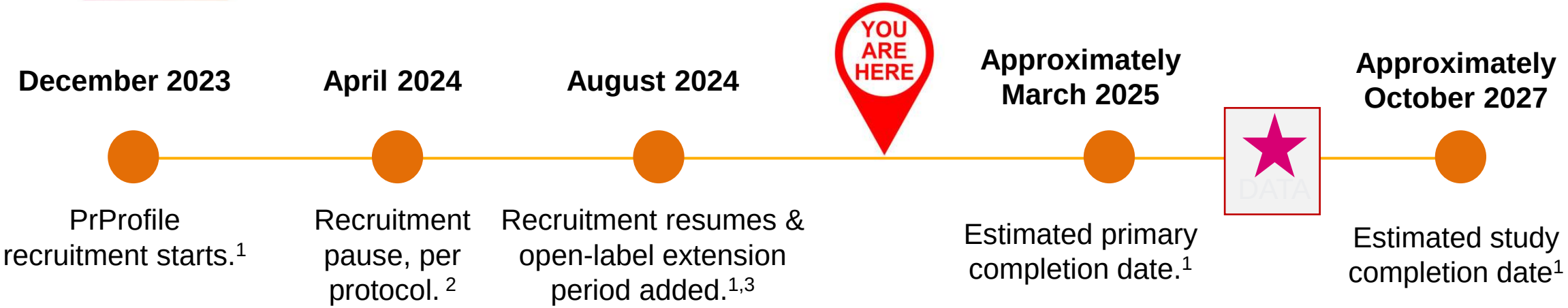
PrPROFILE : An ongoing clinical trial of ION717 in people with prion disease[1]

- **ION717:** an investigational RNA-targeted therapy that is designed to reduce the production of prion protein
- **Trial Purpose:** Phase 1/2a evaluation the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecal (IT) delivery of ION717
- **Study Design:**
  - **Treatment period** (30 wks): everyone receives ION717 and placebo; order of doses randomized & blinded; multiple dose levels tested
  - **Open-label extension period** (OLE; 70 wks): everyone receives ION717 (no placebo)
  - **Post-study period** (32 wks): no ION717 or placebo
- **Outcomes measures:**
  - **Primary**: incidence of treatment-emergent adverse events (i.e., safety & tolerability)
  - **Secondary**: change in the amount of ION717 (i.e., pharmacokinetics) as well as change in the amount of prion protein (i.e., pharmacodynamics)
- **Locations:** 16 sites in 9 countries (USA, Australia, Canada, France, Germany, Israel, Italy, Japan, Spain)

The safety and efficacy of ION717 has not been established. For more information speak with your doctor or visit clinicaltrials.gov

Sources: 1. Clinical Trial Page https://clinicaltrials.gov/study/NCT06153966 (Accessed August 29, 2024. 2. Qualitative caregiver interviews and PAG engagements, data on file

PrPROFILE : Timeline



- The ***estimated primary study completion date*** is when the last person enrolled in PrProfile completes their final assessment for the primary outcome measure
  - date is subject to change
  - initial analysis of data in the months after this date; results shared with the community when appropriate
- Study continues until October 2027 (projected). Data collected during the OLE will be important for understanding the safety and tolerability of ION717.

The safety and efficacy of ION717 has not been established. For more information speak with your doctor or visit clinicaltrials.gov

Sources: 1. Clinical Trial Page https://clinicaltrials.gov/study/NCT06153966 (Accessed August 29, 2024), 2. Community Statement, Apr 2024 3. Community Statement, Aug 2024

Conclusions

- ASO-mediated Tau suppression reverses aggregates after pathology is established
- MAPT ASO is in Phase II clinical trials
- Rodent PRNP-targeted ASOs can suppress PRNP mRNA and pathology, and extend survival in a dose responsive manner
- NfL is reversed after ASO treatment in PRNP mouse model
- An ASO targeting human *PRNP* is in the clinic in a Phase I/II study

Acknowledgements

Patients and families who participated in our clinical studies

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- Ionis Neurology Team, Ionis medicinal chemistry team, with a special thanks to Hien Zhao, Rob Pulido, Anne Smith, Frank Bennett, Eric Swayze, and Roger Lane
- Countless physicians and academic collaborators