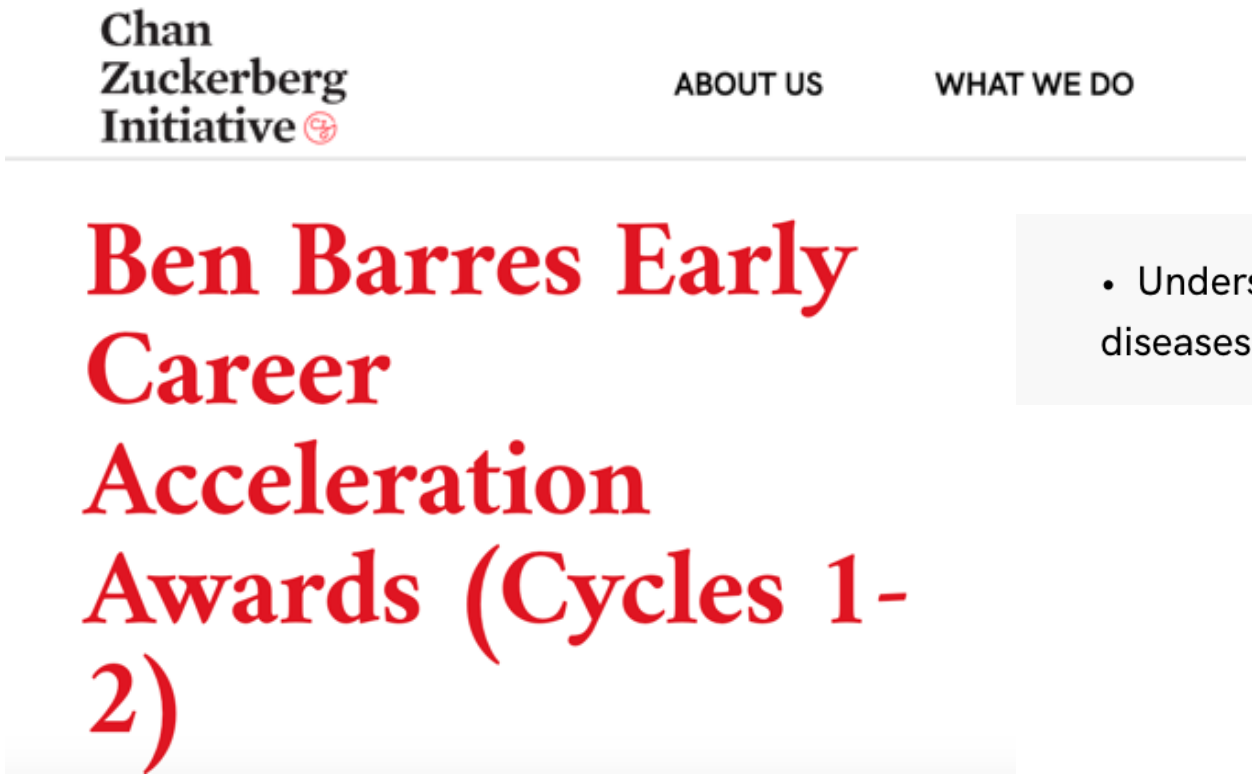
Rational drug design for prion disease and how this informs other ADRDs

- Sonia Vallabh, Broad Institute of MIT and Harvard
- Ken Chan, Broad Institute of MIT and Harvard
- Holly Kordasiewicz, Ionis Pharmaceuticals

November 14, 2024

From Eric's presentation on Tuesday: is "cross cutting therapeutic mechanisms" a real thing?



- Understanding common disease mechanisms that cut across diseases and that may point to common avenues for intervention.

https://chanzuckerberg.com/rfa/ben-barres-early-career-acceleration-awards/
https://cziscience.medium.com/a-new-approach-to-solving-neurodegeneration-2aa50654ed04

If the drug targets are not the same, then what do Alzheimer’s disease, prion disease, etc. have in common?

If the drug targets are not the same, then what do Alzheimer's disease, prion disease, etc. have in common?

- Prion mechanism
  - Seeding assays for diagnosis
  - Strain typing to predict clinical phenotypes
  - Challenge-based animal models
  - Decontamination & transmission concerns
- Neurodegeneration & neuroinflammation
  - NfL, T-tau, GFAP, etc. biomarkers for prognostication & monitoring
- At-risk, prodromal, and manifest disease stages
  - Need for longitudinal observational studies
  - Need for new clinical paths & regulatory flexibility

Platform technologies for targeting specific disease proteins

- DNA-targeted
  - Base editing
  - Epi-editing
  - Transcriptional repressors
- RNA-targeted
  - ASO
  - siRNA
  - ADAR
- Protein-targeted
  - mAbs
  - Secretory inhibitors

Platform technologies for delivery

- Engineered AAVs
- Engineered Fc mAbs
- mAb-RNA conjugates
- Conjugated / chemically stabilized oligonucleotides

Common needs for drug discovery

- Platform technologies to target specific genes
- Delivery systems for the human CNS

Eric Minikel

me

Divalent siRNA
UMass RTI

Anastasia Khvorova

Julia Alterman

AAV-CHARM
Deverman lab, Broad Institute

Ken Chan

Ben Deverman

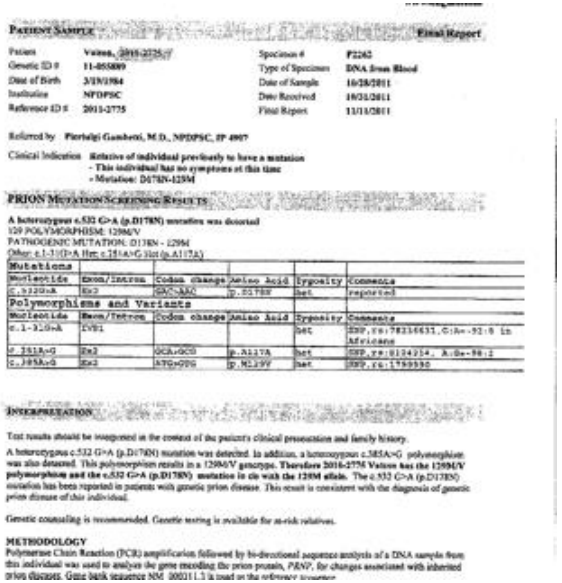
ASO ION717
Ionis Pharmaceuticals

Holly Kordasiewicz

Hien Zhao

Story #1: Towards a divalent siRNA for prion disease

The mission of our lab is a treatment in our lifetimes

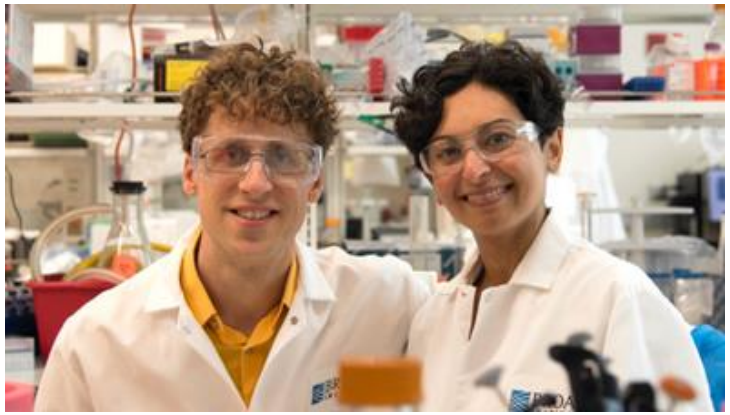


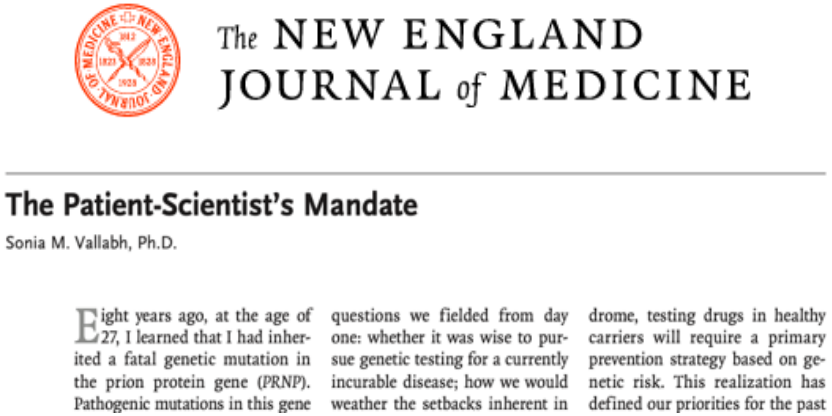
PATIENT SAMPLE — Final Report

PRION MUTATION SCREENING RESULTS

INTERPRETATION

METHODOLOGY





The NEW ENGLAND JOURNAL of MEDICINE

The Patient-Scientist's Mandate

Sonia M. Vallabh, Ph.D.

Eight years ago, at the age of 27, I learned that I had inherited a fatal genetic mutation in the prion protein gene (*PRNP*). Pathogenic mutations in this gene

questions we fielded from day one: whether it was wise to pursue genetic testing for a currently incurable disease; how we would weather the setbacks inherent in

drome, testing drugs in healthy carriers will require a primary prevention strategy based on genetic risk. This realization has defined our priorities for the past

Our lab's focus:

- Develop a therapy
- Race to the first drug – AND the best drug
- Make meaningful clinical trials possible
- Enable both treatment and prevention

BROAD INSTITUTE

MASSACHUSETTS GENERAL HOSPITAL
NEUROLOGY

The Vallabh/Minikel lab

vallabhminikel.org

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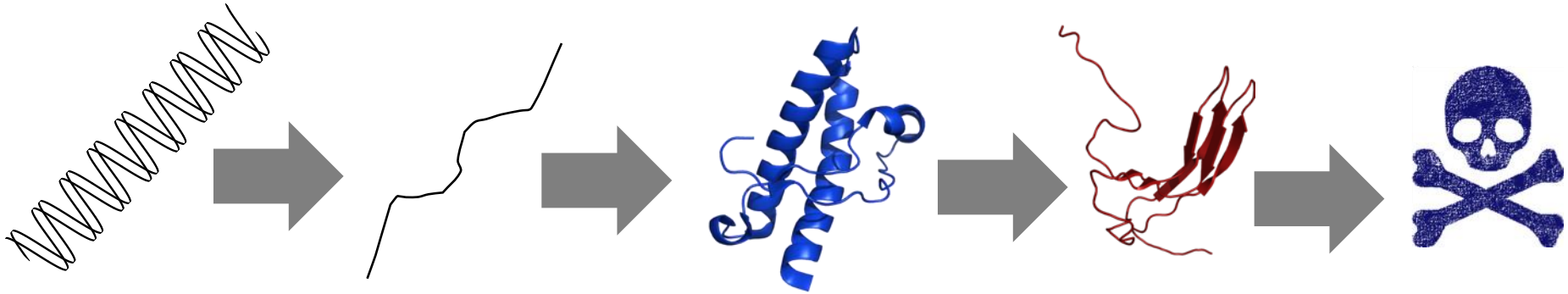
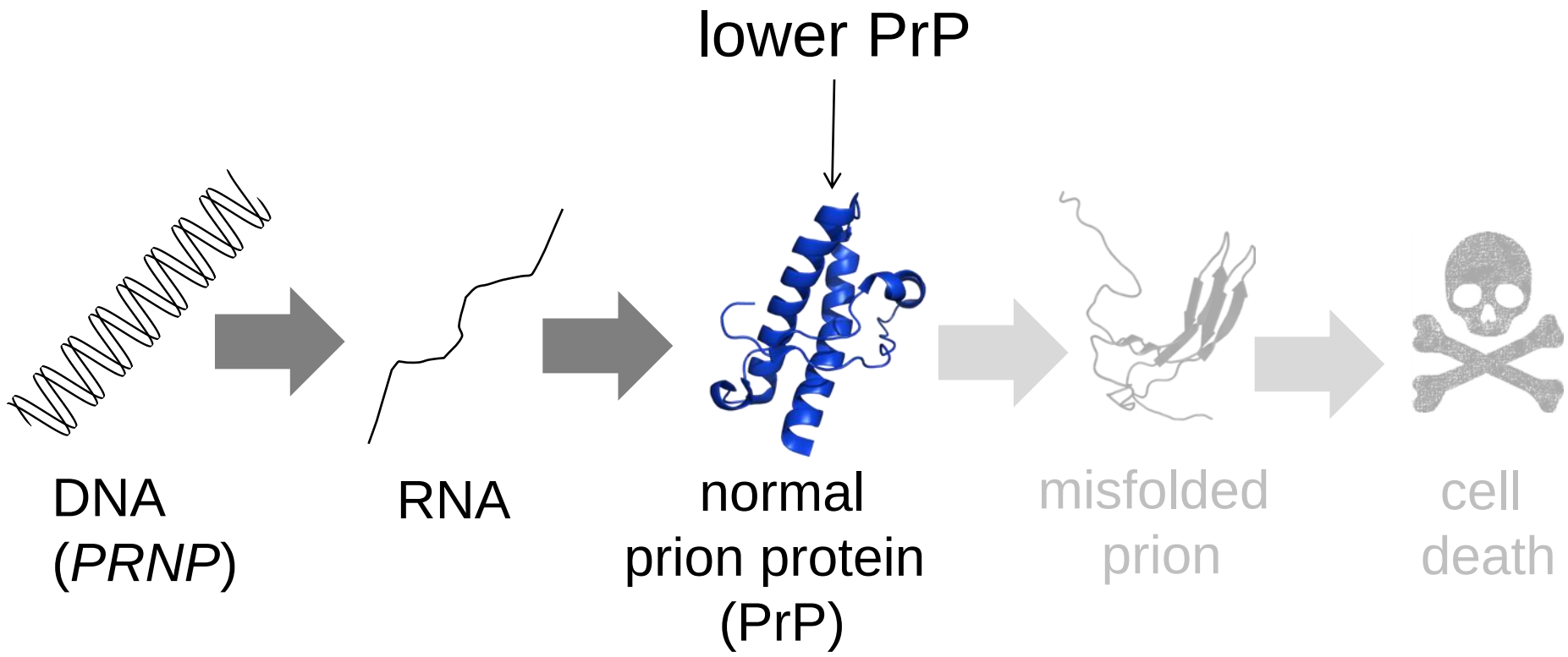
MGH 1811 MASSACHUSETTS GENERAL HOSPITAL
NEUROLOGY

The genetic blueprint of prion disease

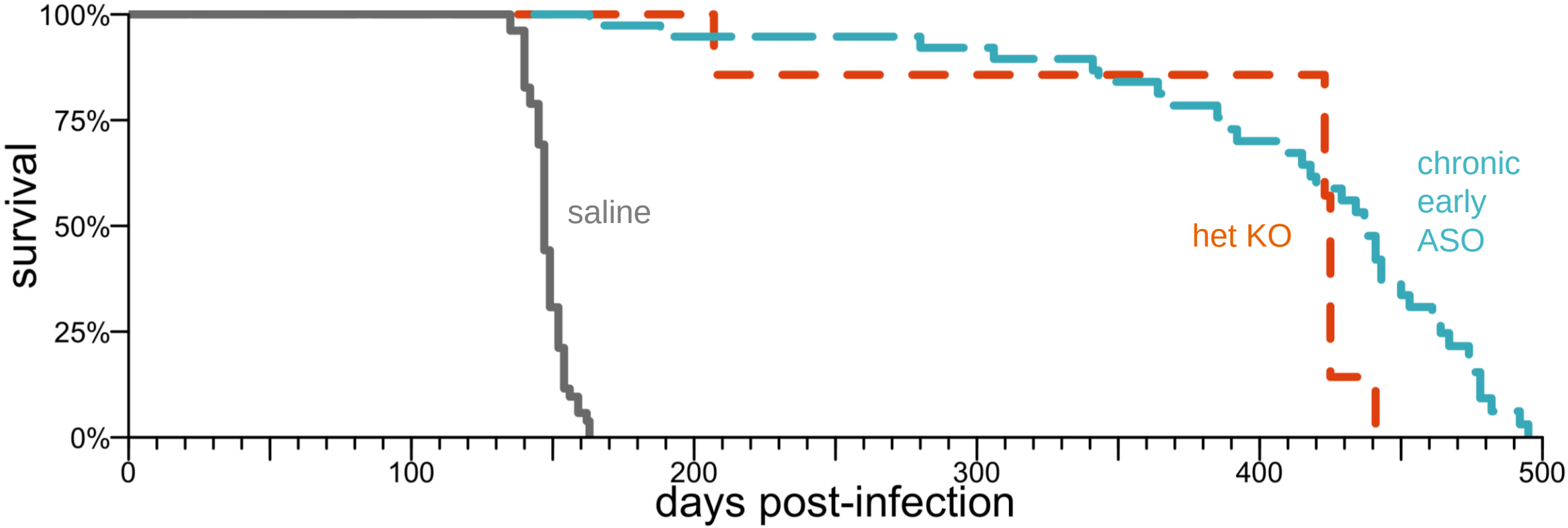


Our therapeutic strategy



- Not all prion disease is genetic – sporadic patients only have wild-type PrP
- Our strategy is non-allele specific -- goal is to maximally suppress <u>all</u> PrP

50% knockdown is an awesome start – but we need to go deeper



500 ug ASO
ICV delivery
Early ASO: chronic dosing q90d beginning -14d to 78dpi

Minikel 2020, PMID: 32776089

Development of a PrP-lower divalent siRNA

Broad Institute

Eric Minikel

Juliana Gentile

Taylor Corridon

Fiona Serack

Alissa Coffey

Kenney Lenz

UMass RTI

Anastasia Khvorova

Julia Alterman

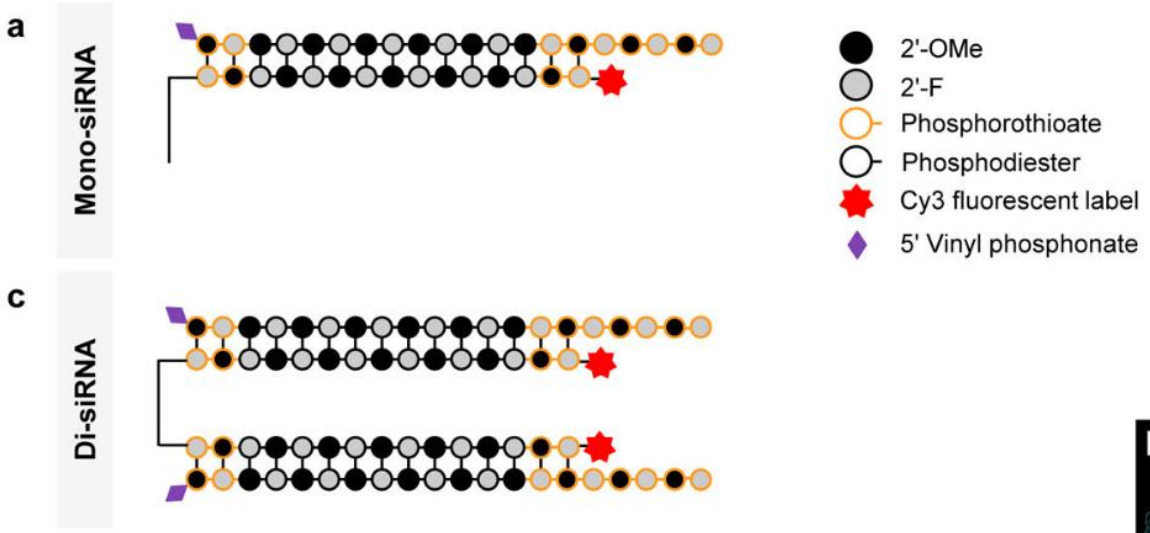
Dimas Echeverria Moreno

Ken Yamada

Zack Kennedy

Chapter 1: discovery

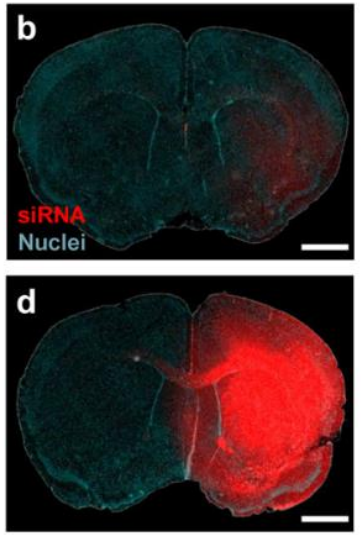
Aug 2, 2019



ARTICLES
https://doi.org/10.1038/s41587-019-0205-0
nature biotechnology

A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system

Julia F. Alterman[1,13], Bruno M. D. C. Godinho[1,13], Matthew R. Hassler[1,13], Chantal M. Ferguson[1,13], Dimas Echeverria[1], Ellen Sapp[2], Reka A. Haraszti[1], Andrew H. Coles[1], Faith Conroy[1,3], Rachael Miller[1,3], Loic Roux[1], Paul Yan[1], Emily G. Knox[1], Anton A. Turanov[1], Robert M. King[4,5], Gwladys Gernoux[6], Christian Mueller[6,7], Heather L. Gray-Edwards[4], Richard P. Moser[8], Nina C. Bishop[9], Samer M. Jaber[9,10], Matthew J. Gounis[4], Miguel Sena-Esteves[6,11], Athma A. Pai[1], Marian DiFiglia[2], Neil Aronin[1,3] and Anastasia Khvorova[1,12]*



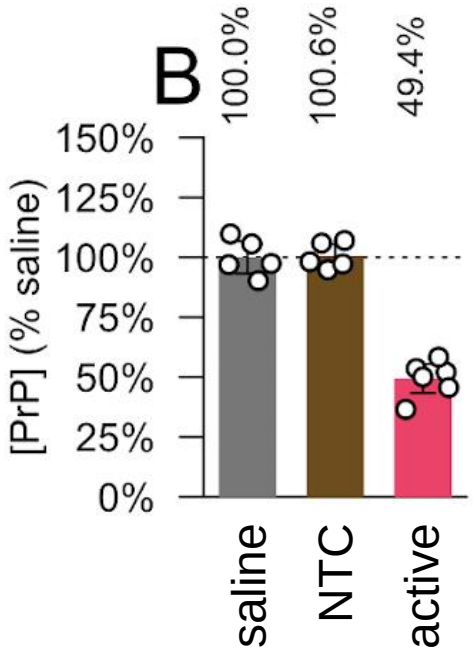
- Potent target RNA knockdown
- Durability out to 6 months
- Broad CNS distribution
- Not yet tested in humans

Anastasia Khvorova

Julia Alterman

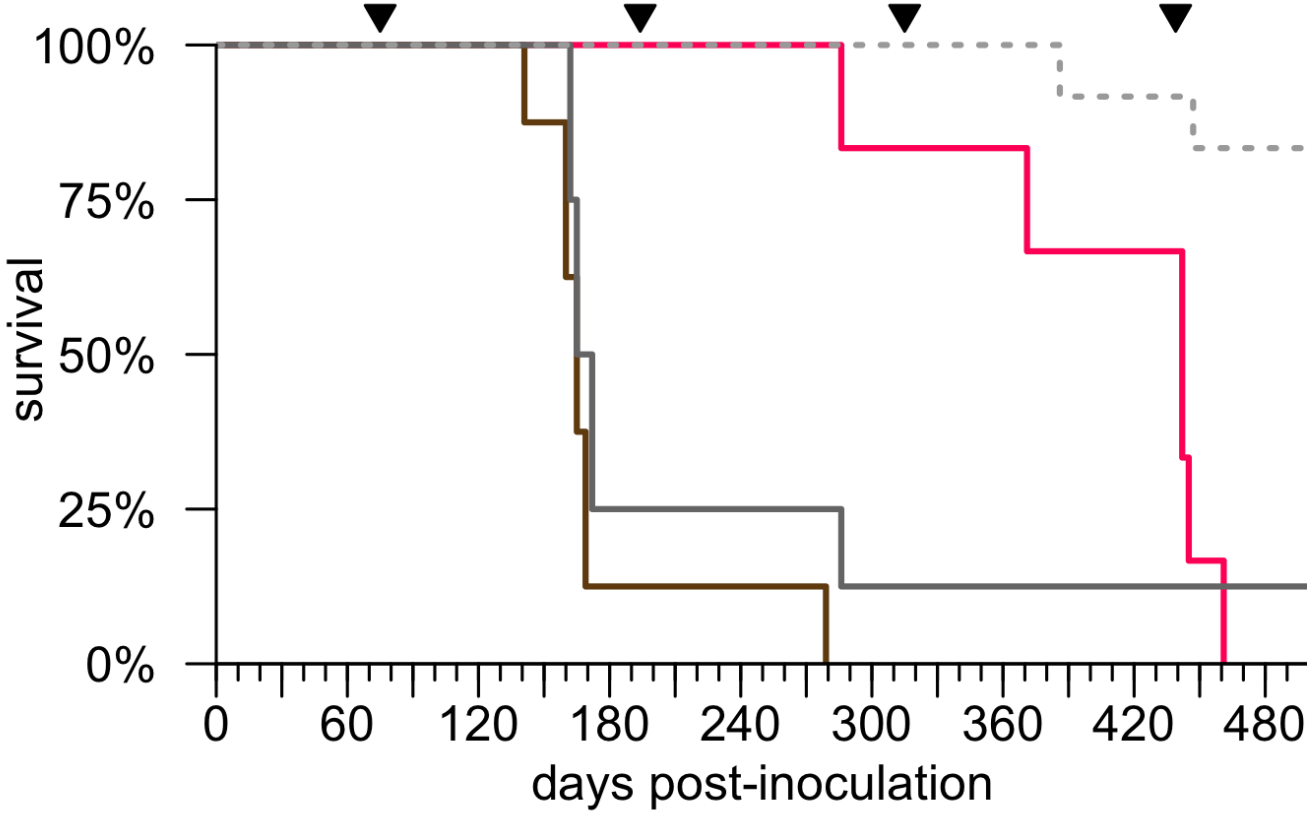
Alterman 2019, PMID: 31375812

Proof-of-concept in prion challenge model using tool di-siRNA against mouse *Prnp* - early treatment



Potency in naïve wild-type mice
Whole brain hemispheres, ELISA readout

348 µg, q120d from 75 dpi onward

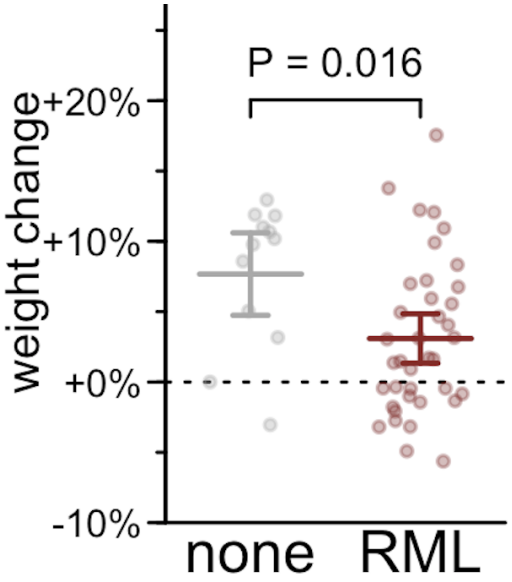


Wild-type mice challenged with RML prions at 0 days post-infection (dpi)

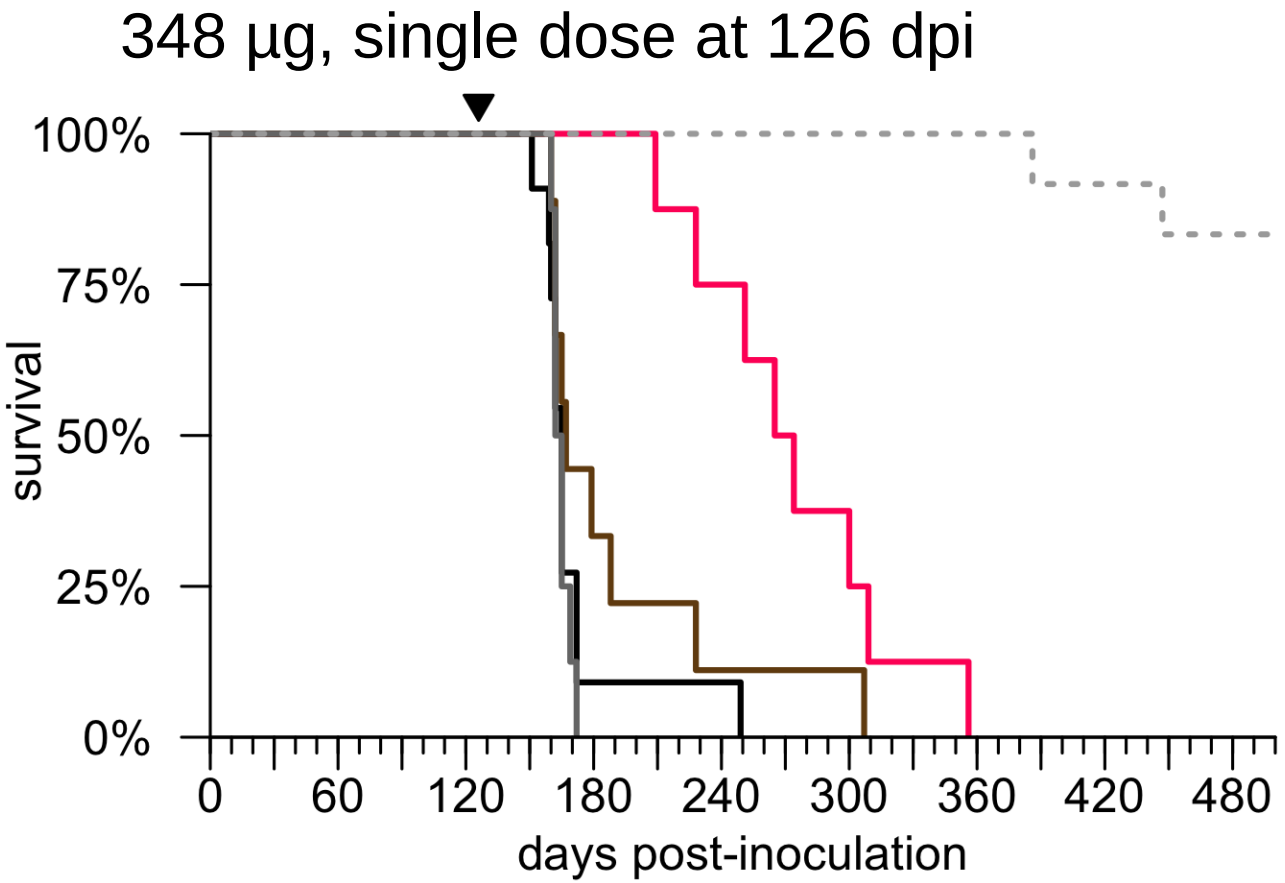
75 dpi: pathological timepoint — mice have elevated NfL and GFAP but no symptoms

Gentile 2024. In preparation.

Proof-of-concept in prion challenge model using tool di-siRNA against mouse *Prnp* – late treatment



Weight loss 1 day prior to treatment

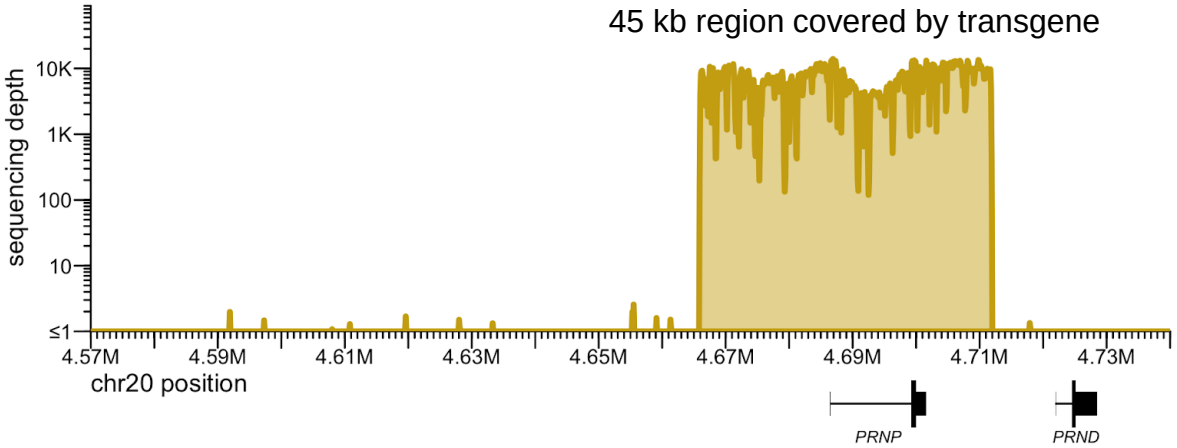


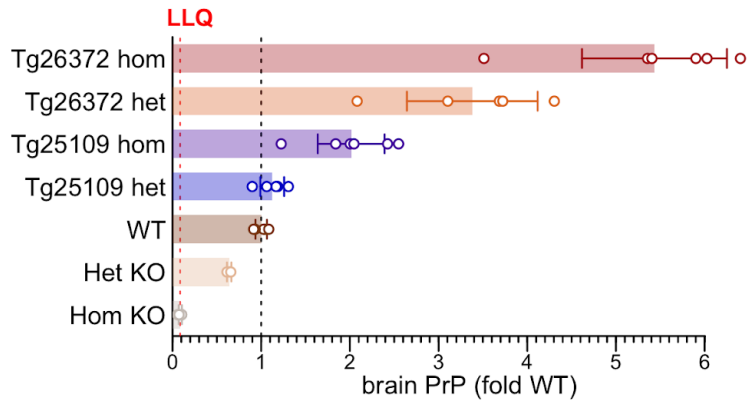
126 dpi: symptomatic timepoint – mice have lost weight relative to uninfected controls

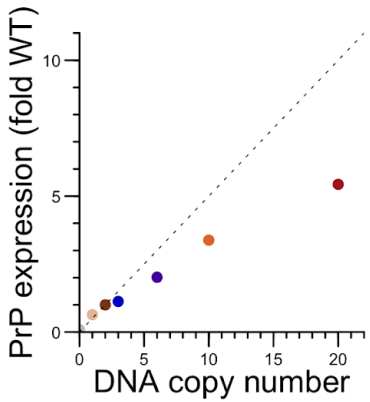
Gentile 2024. In preparation.

We created human *PRNP* BAC transgenic mice in order to test PrP-lowering drugs in vivo

Line	Integration site	Genes disrupted at integration site	Het copy number	Het PrP expression	Hom copy number	Hom PrP expression
Tg25109	chr12	*Frdm6*, *Tmx1*	3	1.1	6*	2.0*
Tg26372	chr18	*Dok6*	10	3.4	20	5.4

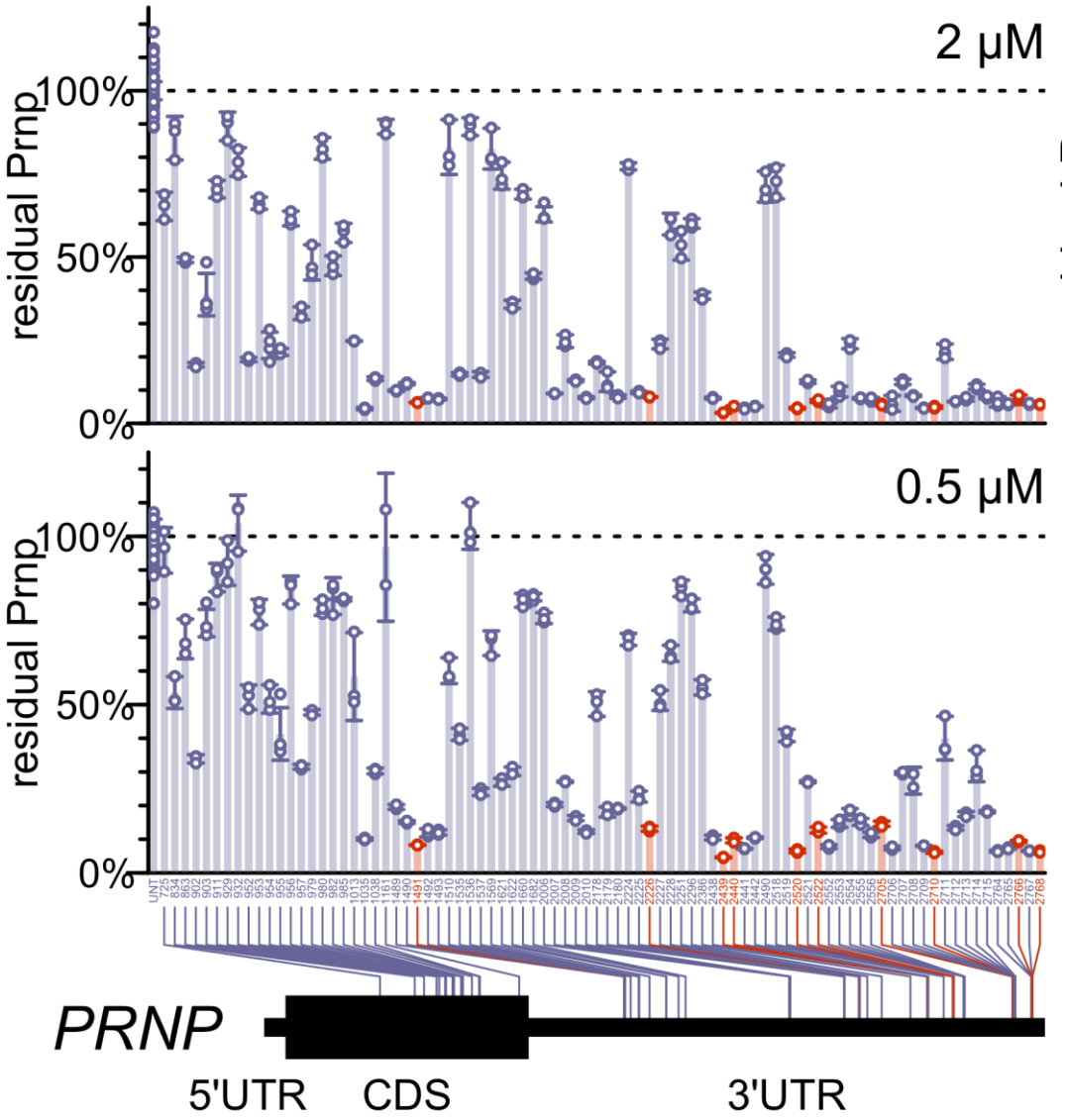


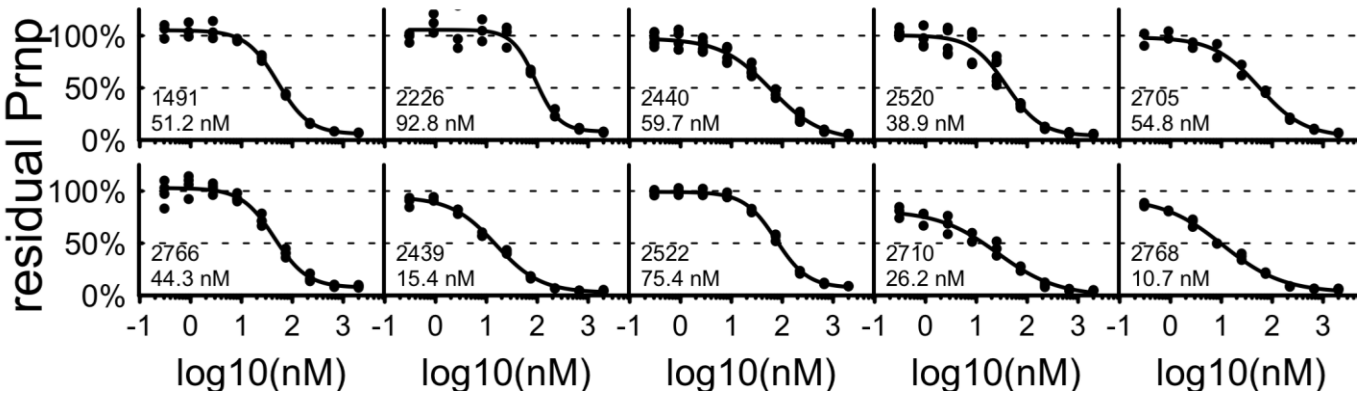




Gentile 2024. In preparation.

Search for the best human *PRNP*-targeting sequence



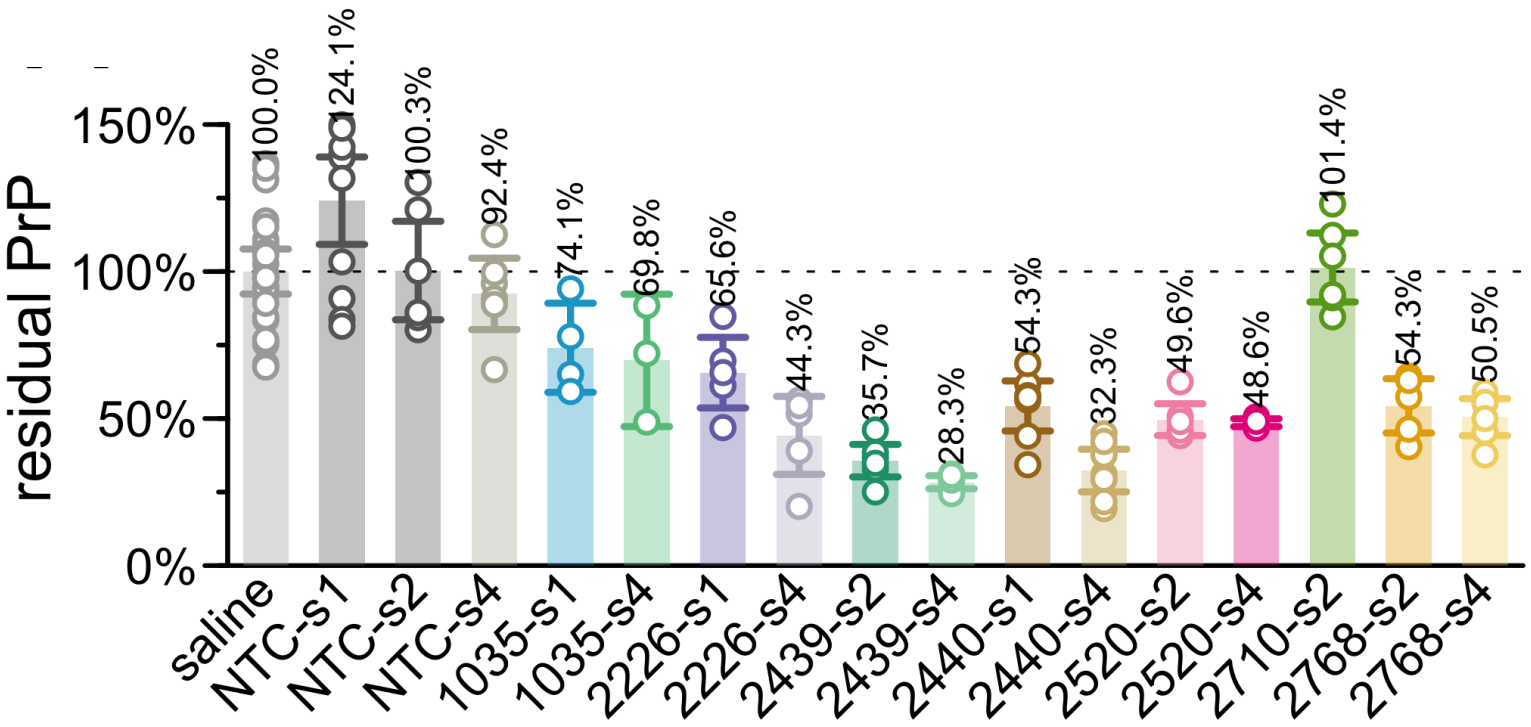


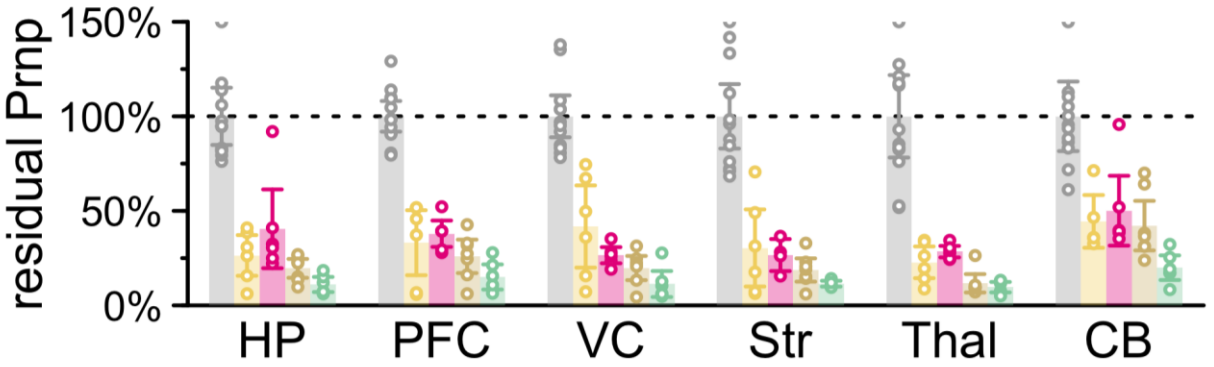
Juliana Gentile

Taylor Corridon

Gentile 2024. In preparation.

Search for the best human *PRNP*-targeting sequence





Protein by ELISA in whole brain hemisphere

Gentile 2024. In preparation.

exNA modification

Article

https://doi.org/10.1038/s41587-024-02336-7

Enhancing siRNA efficacy in vivo with extended nucleic acid backbones

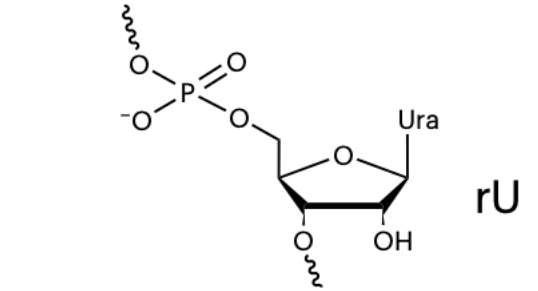
Received: 26 May 2023

Accepted: 25 June 2024

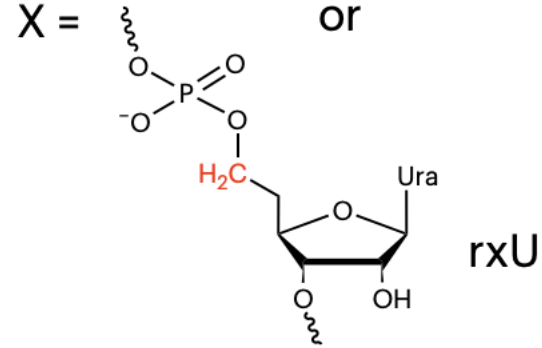
Published online: 01 August 2024

Check for updates

Ken Yamada[1] ✉, Vignesh N. Hariharan[1], Jillian Caiazzi[1], Rachael Miller[1,2], Chantal M. Ferguson[1,2], Ellen Sapp[3], Hassan H. Fakih[1], Qi Tang[1], Nozomi Yamada[1], Raymond C. Furgal[1], Joseph D. Paquette[1,2], Annabelle Biscans[1], Brianna M. Bramato[1], Nicholas McHugh[1], Ashley Summers[1], Clemens Lochmann[1], Bruno M. D. C. Godinho[1], Samuel Hildebrand[1], Samuel O. Jackson, Dimas Echeverria[1], Matthew R. Hassler[1], Julia F. Alterman[1], Marian DiFiglia[3], Neil Aronin[1,2] & Anastasia Khvorova[1,4] ✉



X = or

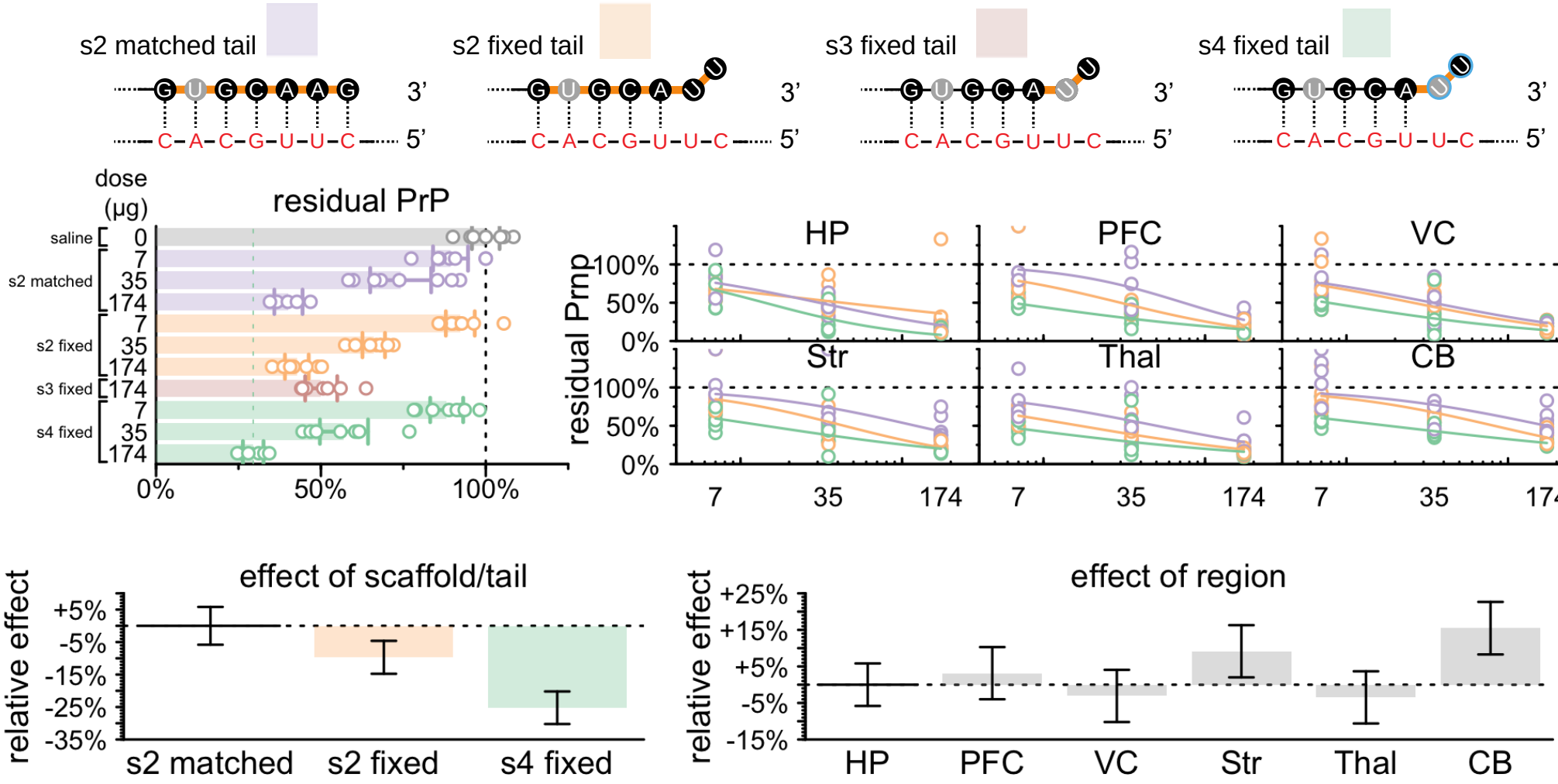


Ken Yamada

exNA enhances stability, allowing us to the number of PS linkages at the 3’ end of the AS strand from 7 to 2

Yamada 2024. *Enhancing siRNA efficacy in vivo with extended nucleic acid backbones.* PMID: 39090305

Contribution of exNA and fixed tail to best scaffold

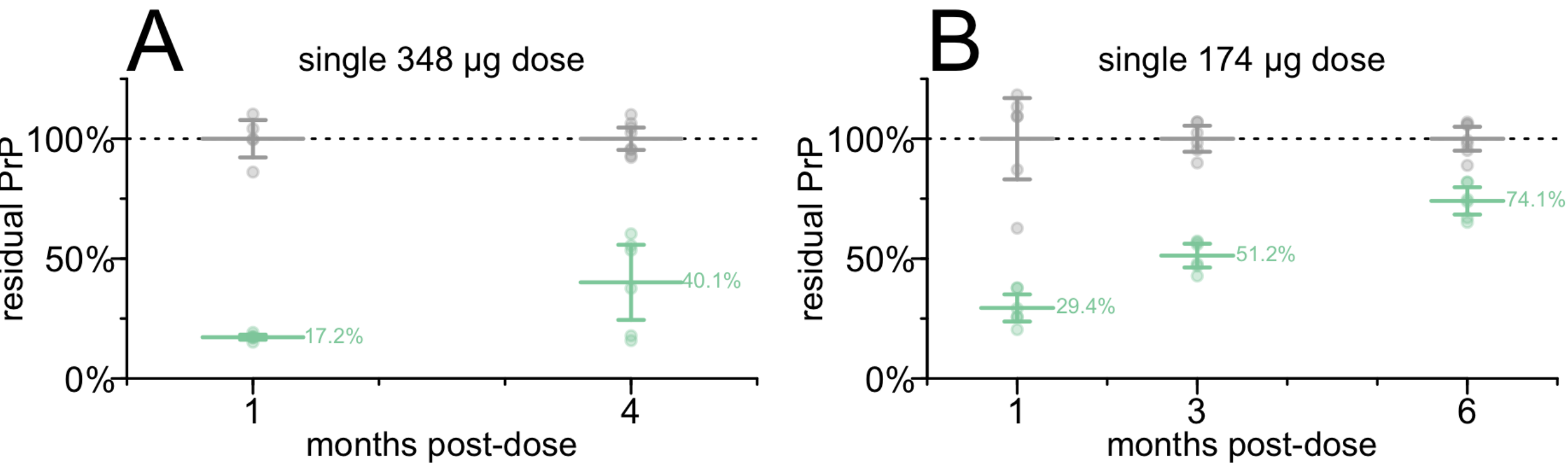


ED_{50} varies by region from 5 to 18 µg

fixed tail contributes 9.7% knockdown
exNA contributes another 15.5% knockdown

Gentile 2024. In preparation.

Potency and durability



Gentile 2024. In preparation.

Chapter 2: how to get to an investigator-initiated trial of a novel drug modality??

Structure of the program

- Academic led
- No pharma partner

- **Funding**: 2-year NINDS U01/ URGenT award

  - **Scope**: Support IND enabling studies to enable a small investigator-initiated clinical trial, up to IND filing.

  - **Structure:**
    - ~$1M direct costs to our lab
    - Access to NIH contractor network to contract studies that NIH pays for
    - Access to NIH consultants (CMC, pharmtox, regulatory)

Chris Boshoff
NIH

Overall development plan

How can we get to patients as soon as possible?

Accelerate activities required for whole program: GMP manufacturing of drug substance, fill-finish of drug product, DDI, Genotox

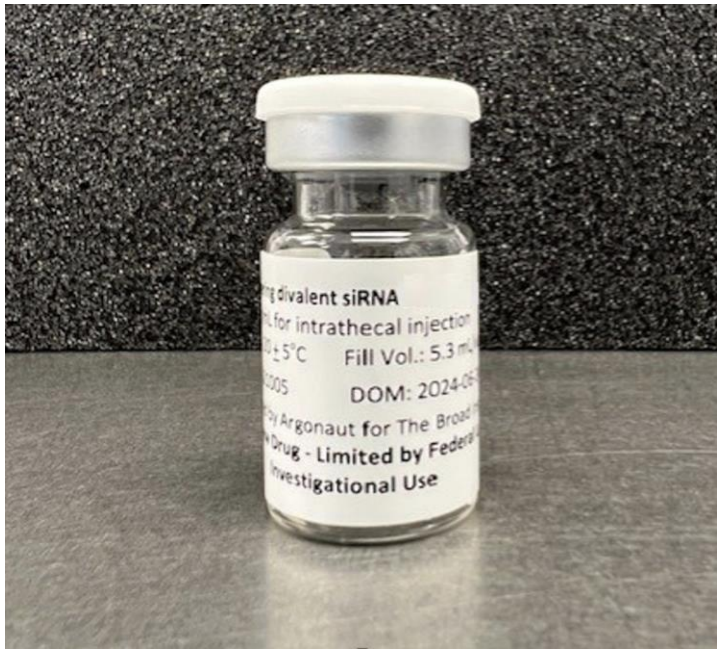
Race to single dose IND: Single dose tox and PNA PK assay

Backfill the requirements for repeat dose IND: Repeat dose tox, validated PK and PD assays for clinic

Rationale for racing to single dose IND:

- Single dose has activity 6 months
- Most patients live 1-2 months from diagnosis

GMP drug substance manufactured by Hongene Biotech



Actual drug product vial

Who and when

contracted activity	who	status
GMP manufacturing	Hongene	completed March 2024
Courier/ importation	Marken	completed May 2024
Sterile fill/finish	Argonaut	completed June 2024
Stability	Argonaut	ongoing since June 2024
GLP toxicology	Amplify-Bio	in-life completed August 2024, histopathology ongoing
Genotox	ITR Canada	completed June 2024
Bioanalytical	Axolabs	assays validated September 2024 study sample analysis ongoing
Drug-drug interaction	BioIVT	completed June 2024
GMP storage	Xerimis	received September 2024
IND filing	Us + NIH URGenT network	targeting end of 2024

Alissa Coffey

June 2023 pre-IND meeting

Questions:

Can we reduce the amount of material retained for regulatory testing? If not, 90% of our drug product will not reach patients.

Do we have any meaningful adjacency to the ASO N-of-1 guidance? Can we do rodent only tox?

June 2023 pre-IND meeting

Questions:

Can we reduce the amount of material retained for regulatory testing? If not, 90% of our drug product will not reach patients.

→ YES

Do we have any meaningful adjacency to the ASO N-of-1 guidance? Can we do rodent only tox?

June 2023 pre-IND meeting

Questions:

Can we reduce the amount of material retained for regulatory testing? If not, 90% of our drug product will not reach patients.

→ YES

Do we have any meaningful adjacency to the ASO N-of-1 guidance? Can we do rodent only tox?

→ NO

Rat intrathecal

Group	N	Dose level	Harvest timepoint
1	10F/10M	0	24h
2	10F/10M	300 µg	24h
3	10F/10M	1 mg	24h
4	10F/10M	3 mg	24h
5	5F/5M	0	1 month
6	5F/5M	300 µg	1 month
7	5F/5M	1 mg	1 month
8	5F/5M	3 mg	1 month

Dog intrathecal

Group	N	Dose level	Harvest timepoint
1	4F/4M	0	24h
2	4F/4M	20 mg	24h
3	4F/4M	60 mg	24h
4	4F/4M	200 mg	24h
5	2F/2M	0	1 month
6	2F/2M	20 mg	1 month
7	2F/2M	60 mg	1 month
8	2F/2M	200 mg	1 month

Note: These single-dose safety studies will enable us to give a single dose of drug to patients (expected to last ~6 months.)
FDA specified that to be able to re-dose patients, we will need to conduct repeat-dose animal safety studies.

Clinical plan

- Small number of early symptomatic patients
- No placebo arm
  - Rapidly progressive fatal disease with no standard of care
  - Phase I will not be a pivotal trial anyway – primary endpoint is adverse events, secondary endpoint is target engagement
  - Placebo is not a $15 bottle of saline, it’s $100Ks to do a whole placebo “fill”
  - Substantial funding from patients & families affected by prion disease
  - Can we ask them for more money in order to *not* give everyone the drug?
- Whenever we're not able to dose drug, ask patients to participate in longitudinal observational study

Concrete goals

- Help people here and now
- Symptomatic population
  - Who can we recruit, what stage, what rate of decline
  - Trajectory of CSF PrP and can we modify it
- Pre-symptomatic
  - Lever for discussion with regulators about pivot to this population

What will the broader ADRD field have gained from NINDS's investment in di-siRNA for prion disease?

- Swift first-in-human data for a novel oligonucleotide modality for the CNS
  - Safety
  - Biodistribution
  - Potency
  - Durability
- Capacity-building for this novel modality
  - Manufacturing/CMC
  - Regulatory
  - Publicly shared IND-enabling data package

Thank you!

NOTES

- What ADRD have in common – not exact genetic targets – ability to leverage platform tech and learnings
- Develop platform tech
- Show that it can be done – POC

- Panel: what are the common wins that we should all be steering toward; bring NIA along