The road to preventing prion disease: opportunities, challenges, and my personal mandate

Sonia Vallabh

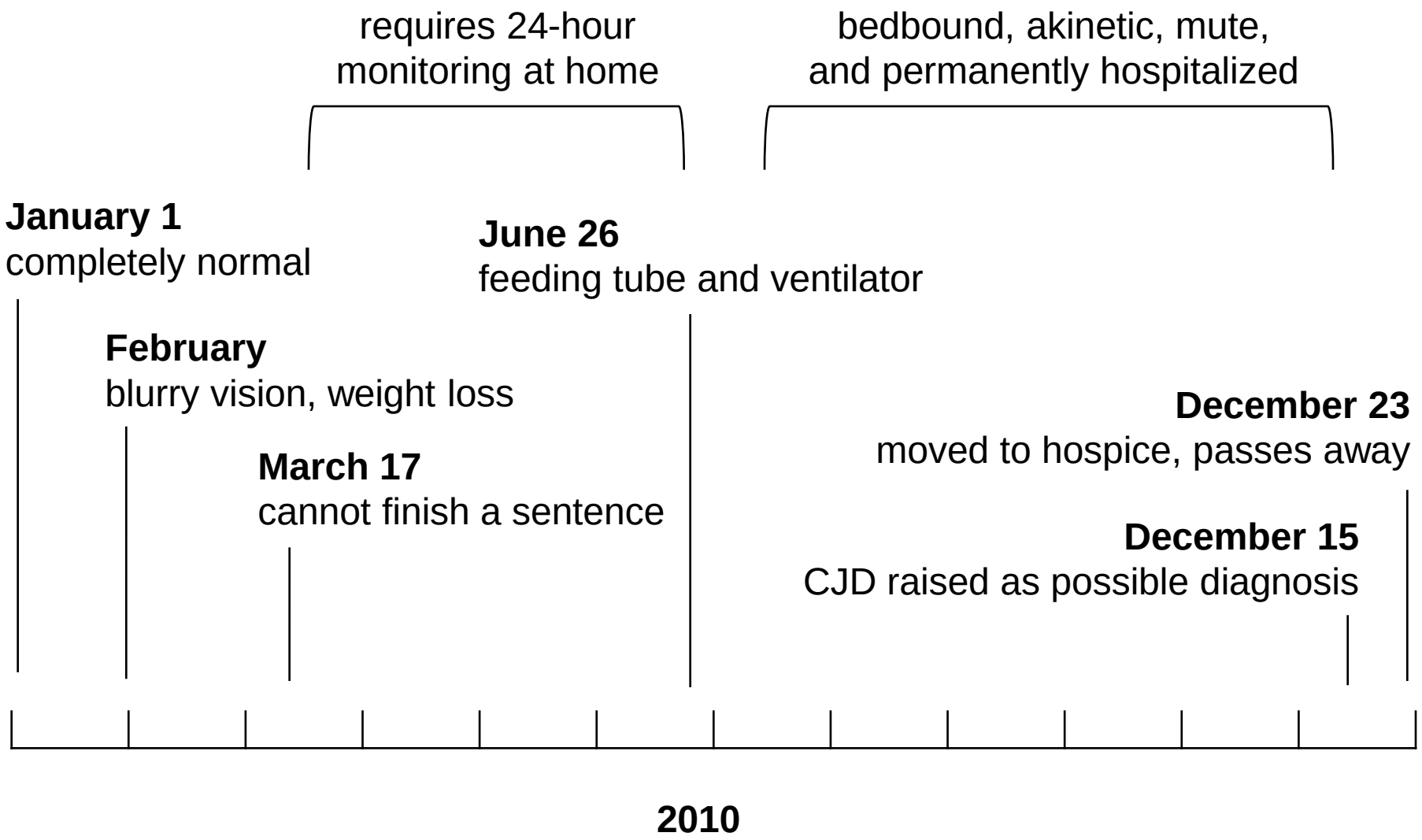
Senior Group Leader, Broad Institute of MIT and Harvard

Assistant Professor of Neurology, Massachusetts General Hospital

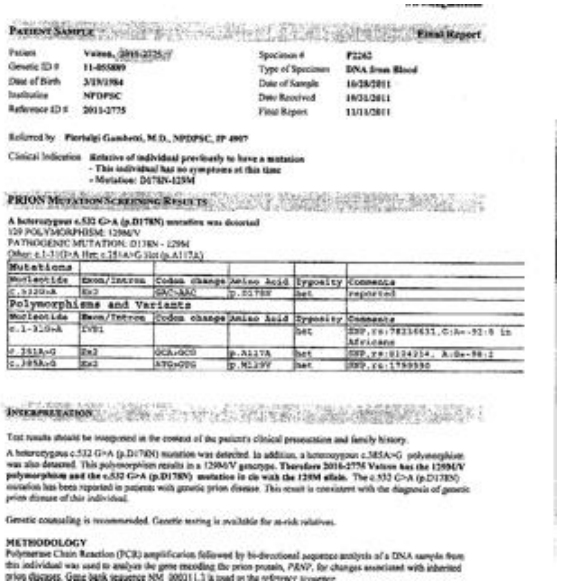
January 27, 2025

2009

2010: the timeline of my mom’s illness



The mission of our lab is a treatment in our lifetimes

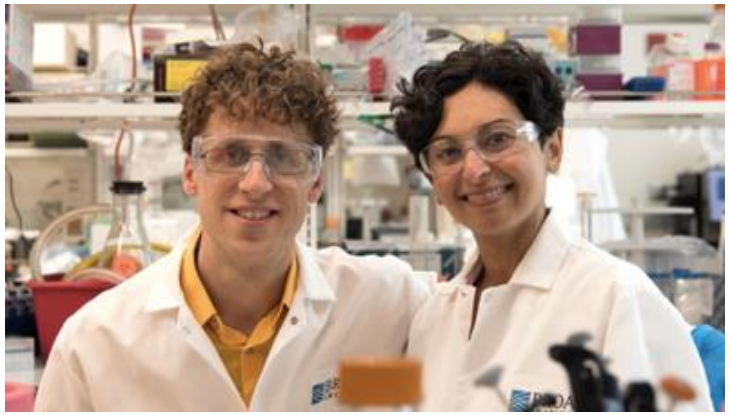


PATIENT SAMPLE — Final Report

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

The rest of today’s talk

- Introduction to prion disease
- Therapeutic proof of concept
- How to do the right clinical trials?
- Regulatory engagement
- What comes next?
- Closing thoughts

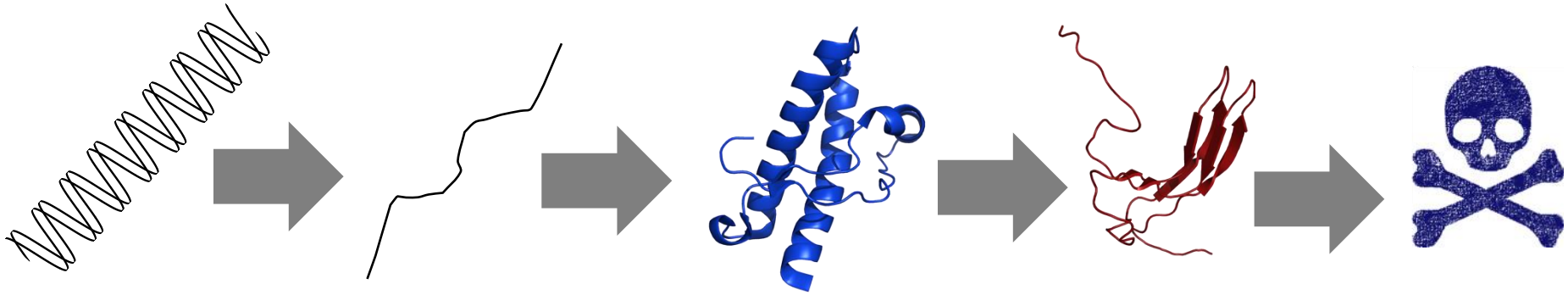
Introduction to prion disease

Despite many names, etiologies, and clinical presentations…

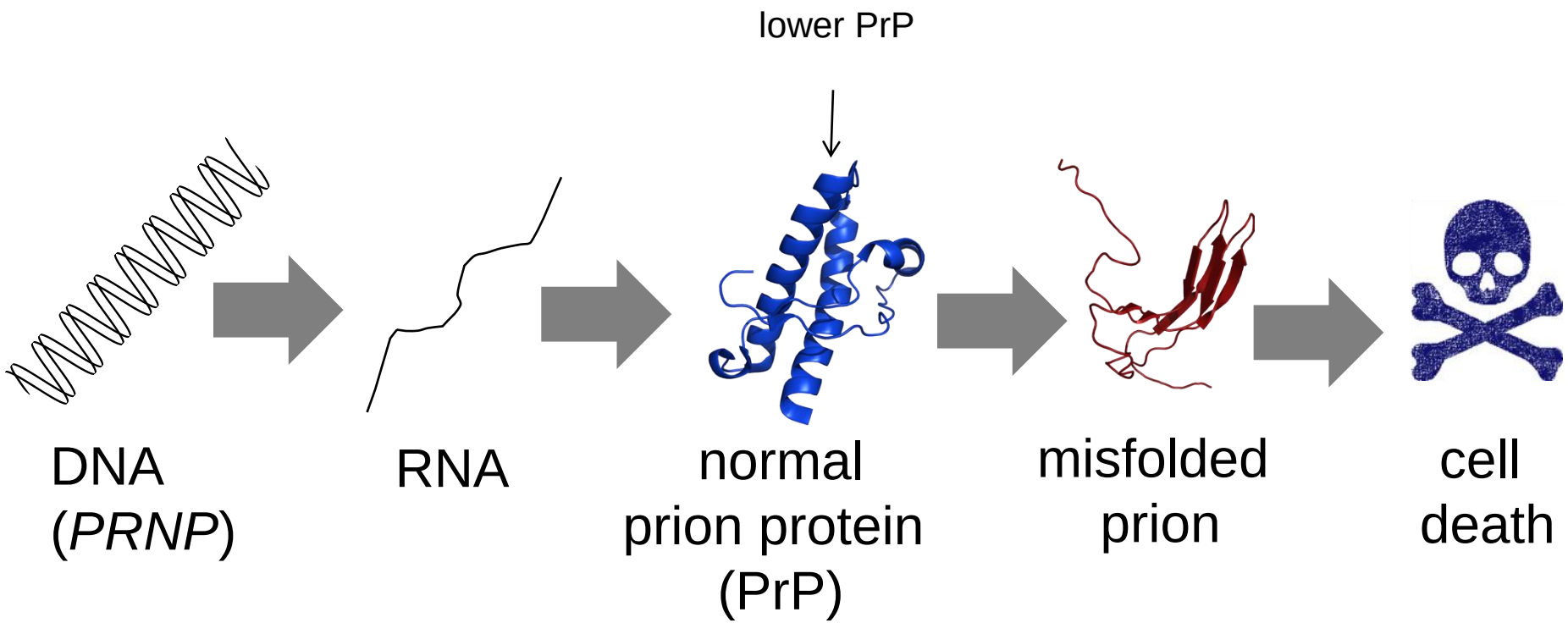
Major historical names for human prion disease
• CJD: Creutzfeldt-Jakob disease • FFI: fatal familial insomnia • GSS: Gerstmann-Straussler-Scheinker disease • vCJD: variant Creutzfeldt-Jakob disease

Etiologies	Early symptoms
• Sporadic (85% of cases) • Genetic (15%) • Acquired (<1%)	• cognitive • motor • autonomic • psychiatric

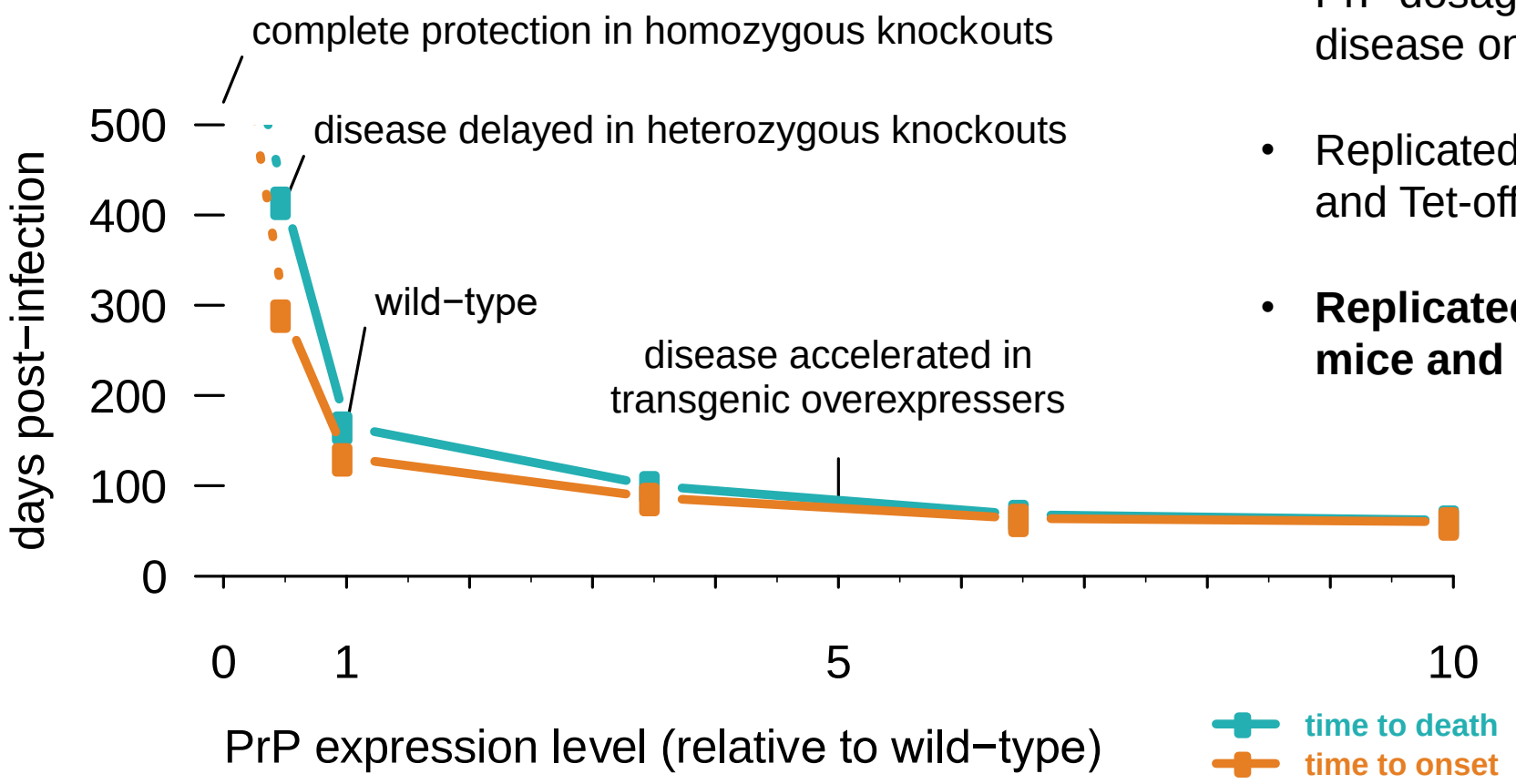
…all prion disease shares the same genetic blueprint



Therapeutic hypothesis



Lowering PrP should be effective



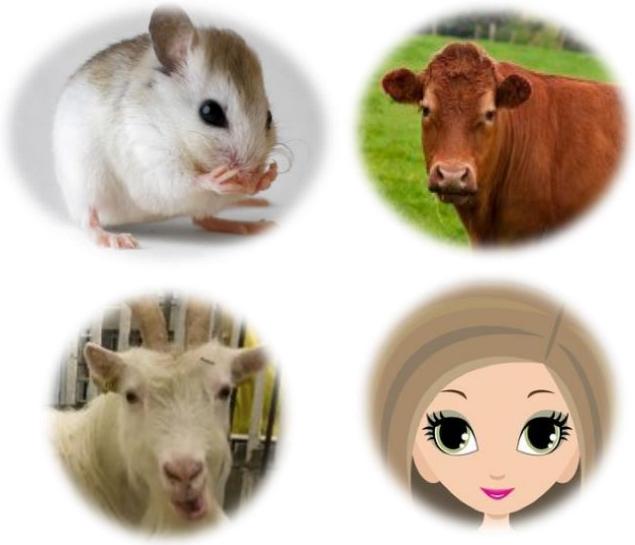
- PrP dosage dose-dependently determines disease onset and progression
- Replicated in conditional systems (NFH-Cre and Tet-off)
- **Replicated in hamsters, rats, humanized mice and spontaneous prion models**

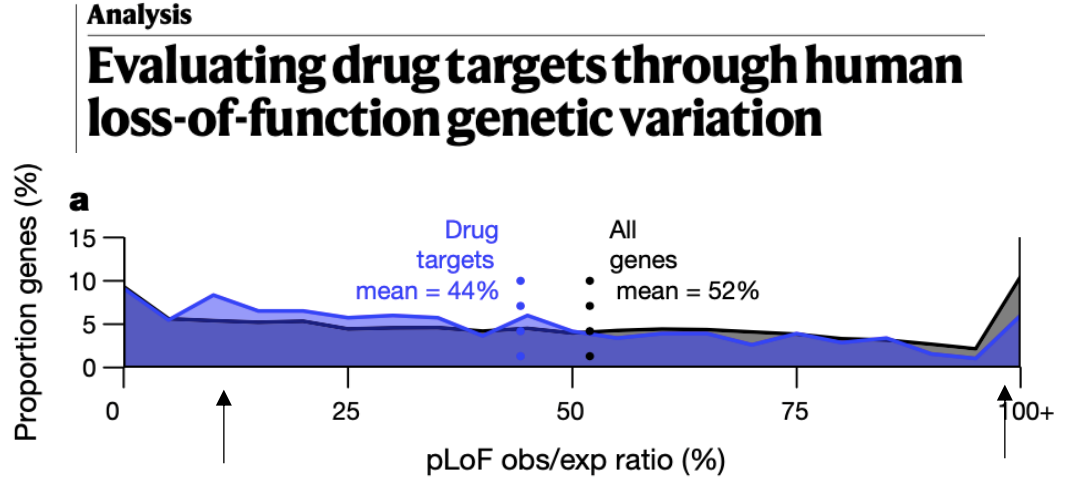
Fischer 1996, *Prion protein with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie*, PMID: 8635458

Mallucci 2003, *Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis*, PMID: 14593181

Safar 2005, *Prion clearance in bigenic mice*, PMID: 16186247

Lowering PrP should be tolerated





HMGCR: 12%
inactivating variants not tolerated

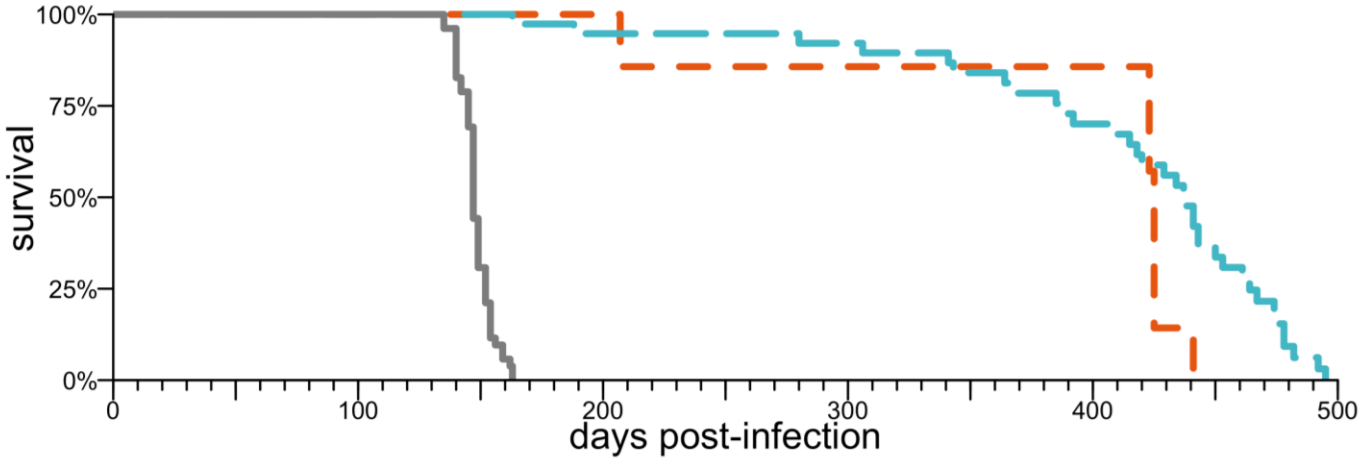
PRNP: 99%
inactivating variants tolerated

- Homozygous knockout mice: mild peripheral neuropathy
- Heterozygous knockout mice: no phenotype
- Knockout cows & goats: healthy
- Humans with heterozygous loss-of-function mutations: healthy

Bremer 2010, PMID: 20098419; Richt 2007, PMID: 17195841;
Benestad 2012, PMID: 23249298; Minikel 2016, PMID: 26791950; Minikel 2020, PMID: 32461653;

Therapeutic proof of concept

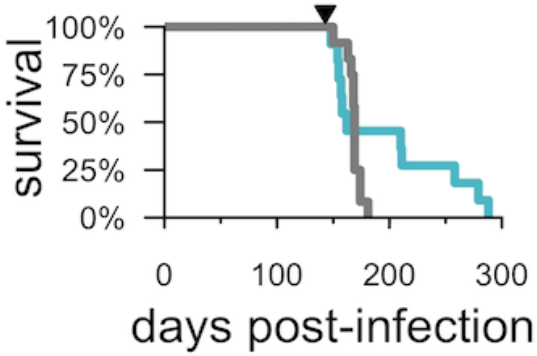
ASOs work – but when you treat matters



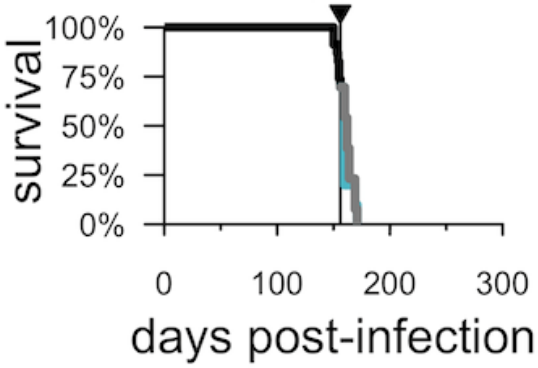
- 500 µg doses delivered by bolus ICV injection every 90 days
- Endpoint = 5 symptoms

Presymptomatic ASO, chronic dosing (q90d)

Heterozygous PrP knockout



Early symptomatic ASO



Advanced symptomatic ASO

- Single 500 µg dose of ASO 6 by bolus ICV injection
- Endpoint = terminal

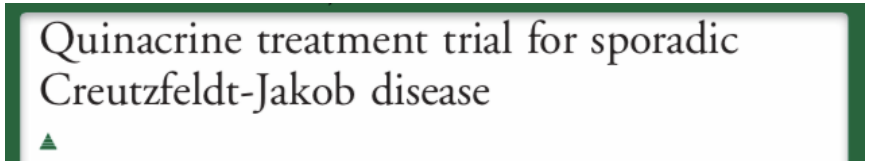
Repurposeable assets to support clinical trials of next gen PrP-lowering therapeutics

- Penetrance and age of onset data for *PRNP* variants
  - Minikel 2016, *Quantifying prion disease penetrance using large population control cohorts*, PMID: 26791950
  - Minikel 2019, *Age of onset in genetic prion disease and the design of preventive clinical trials.* PMID: 31171647
- Natural history of mutation carriers
  - Vallabh 2020, *Cerebrospinal fluid and plasma biomarkers in individuals at risk for genetic prion disease*, PMID: 32552681
- ELISA and mass spec assays for measuring PrP in CSF
  - Vallabh 2019, *Prion protein quantification in cerebrospinal fluid as a tool for prion disease drug development*, PMID: 30936307
  - Minikel and Kuhn 2019, *Domain-specific quantification of prion protein in CSF by targeted mass spectrometry*, PMID: 31558565
  - Mortberg 2022*, Regional variability and genotypic and pharmacodynamic effects on PrP concentration in the CNS*, PMID: 35133987
- *PRNP*-humanized mice
- Validation of efficacy of PrP-lowering in rats, hamsters and humanized mice
- Online prion disease patient and carrier registry: PrionRegistry.org
- Precedent for double-blind, randomized, placebo-controlled clinical trials in symptomatic prion disease patients with a survival endpoint
- National, centralized prion disease testing in US, Europe, Australia and Japan
- RT-QuIC: molecular diagnostic run on CSF, >95% sensitive and specific for sporadic prion disease

How to do the right clinical trials?

Two patient populations in prion disease

- **Symptomatic patients**
  - Live ~6 months from diagnosis
  - 300-500 per year in the US
  - Highest good: stabilize in symptomatic state
  - Precedent for trials: yes

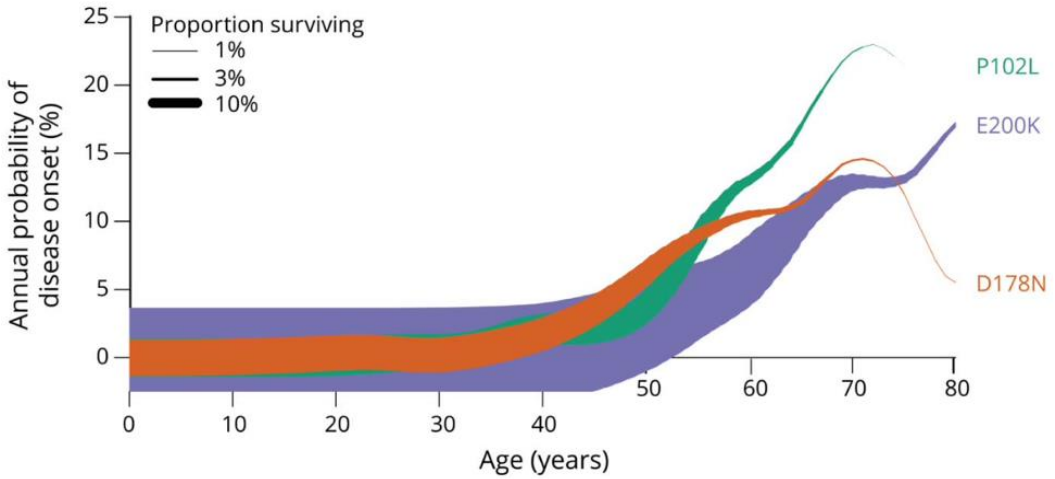

Quinacrine treatment trial for sporadic Creutzfeldt-Jakob disease


Doxycycline in Creutzfeldt-Jakob disease: a phase 2, randomised, double-blind, placebo-controlled trial

Stéphane Haik*, Gabriella Marcon*, Alain Mallet, Mauro Tettamanti, Arlette Welaratne, Giorgio Giaccone, Shohreh Azimi, Vladimiro Pietrini,

- **Pre-symptomatic patients**
  - Can be identified by predictive genetic testing years or decades before onset
  - Low thousands in the US
  - Highest good: preserve years of healthy life
  - Precedent for trials: no

What to measure in a pre-symptomatic trial?



- No phenotype → can't measure symptoms
- Age of onset is variable and unpredictable → can't randomize to onset

Conclusion: we need some sort of biomarker than inform on whether the drug is doing something useful in healthy carriers.

Option 1: **Drug activity biomarker** to show PrP lowering in the brain?

Option 2: **Disease biomarker** that reports on subclinical pathology?

Minikel 2019, *Age of onset in genetic prion disease and the design of preventive clinical trials.* PMID: 31171647

Our genetic prion disease at-risk cohort

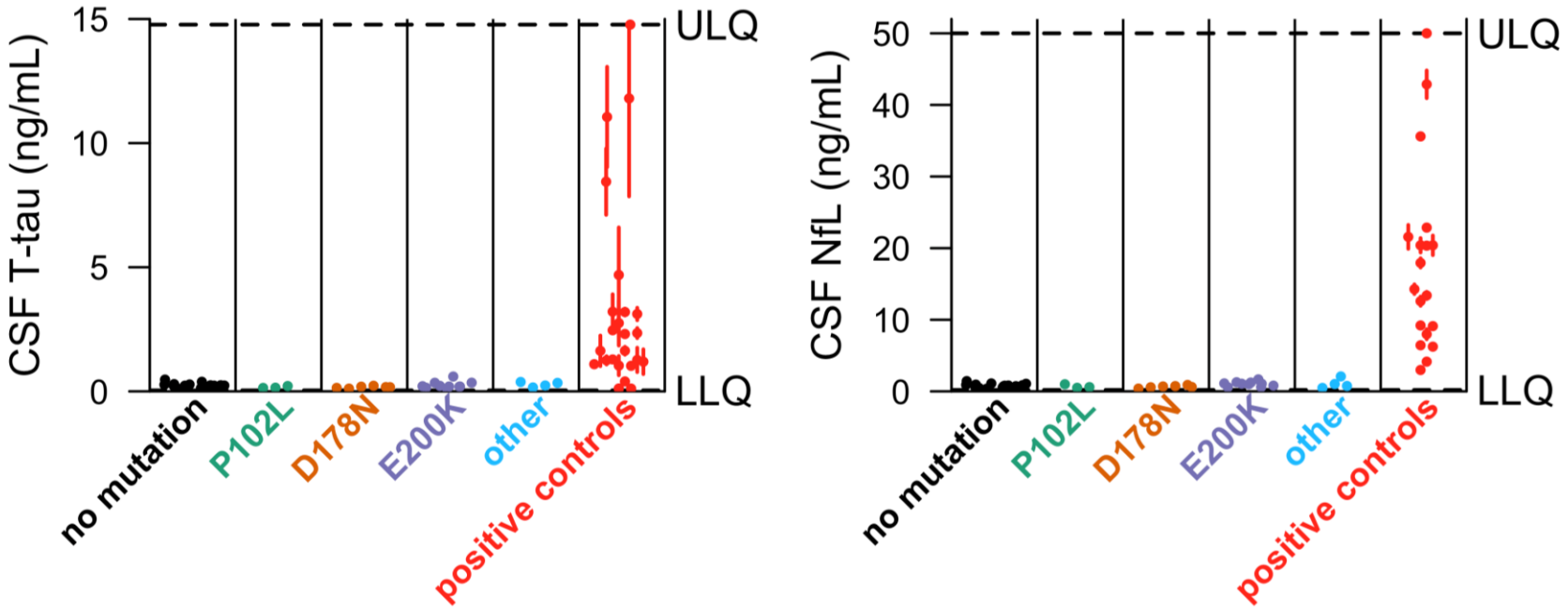
status	N	sex	age (mean±sd)	follow-up years (mean±sd)	study visits	CSF	plasma	mutations
carrier	41	13M / 28F	47.5±14.0	2.0±1.9	126	104	109	6 P102L, 7 D178N, 22 E200K, 6 other
control	21	6M / 15F	46.1±13.3	1.4±1.5	57	51	51	21 none

Steven E. Arnold

MASSACHUSETTS GENERAL HOSPITAL

Vallabh 2020, *Cerebrospinal fluid and plasma biomarkers in individuals at risk for genetic prion disease*, PMID: 32552681
+ unpublished updates 2023

Pathological biomarker findings as of 2020

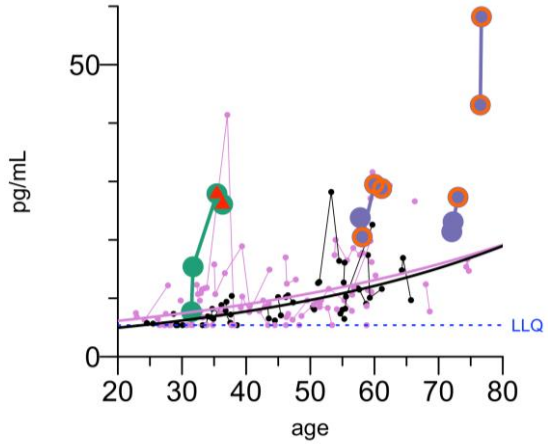


- Everyone healthy
- Normal T-tau and NfL levels

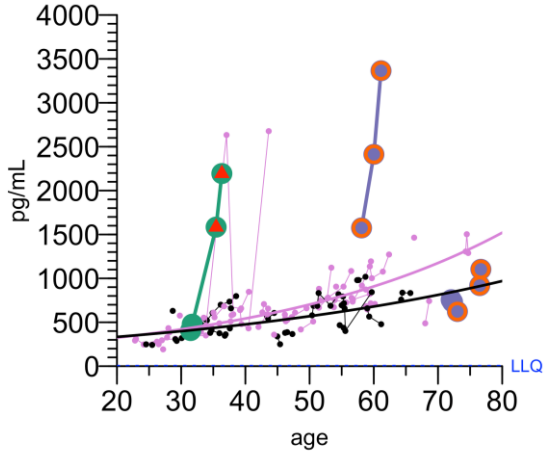
Vallabh 2020, *Cerebrospinal fluid and plasma biomarkers in individuals at risk for genetic prion disease*, PMID: 32552681

Pathological biomarker findings as of 2024

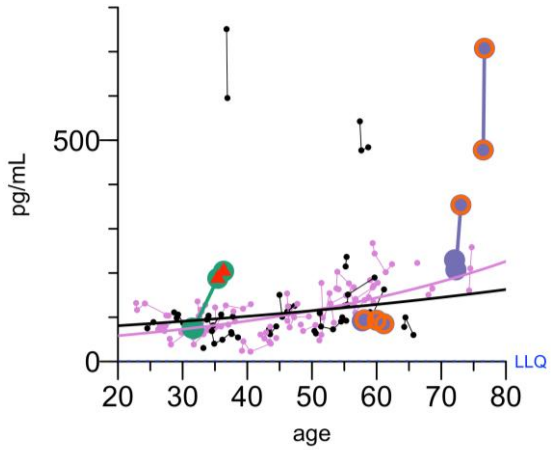
plasma neurofilament



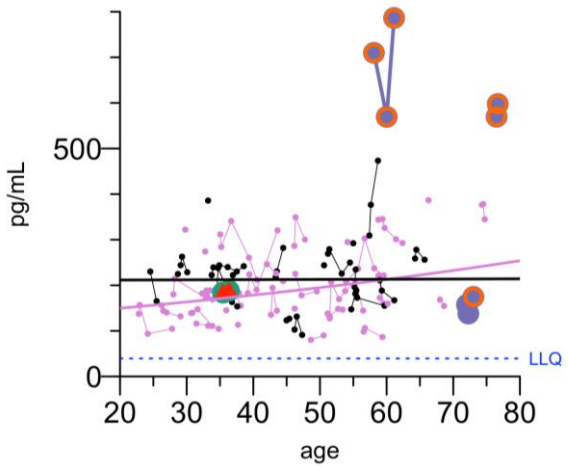
CSF neurofilament



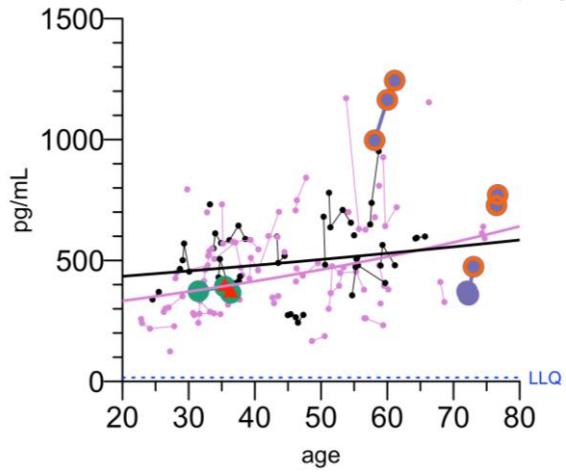
Plasma GFAP

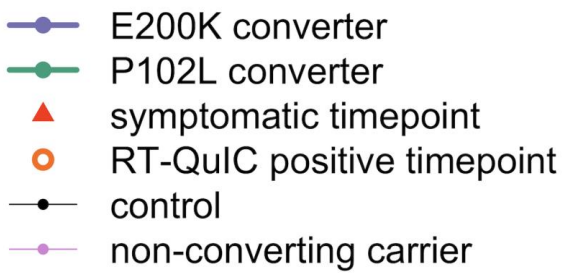


CSF total tau

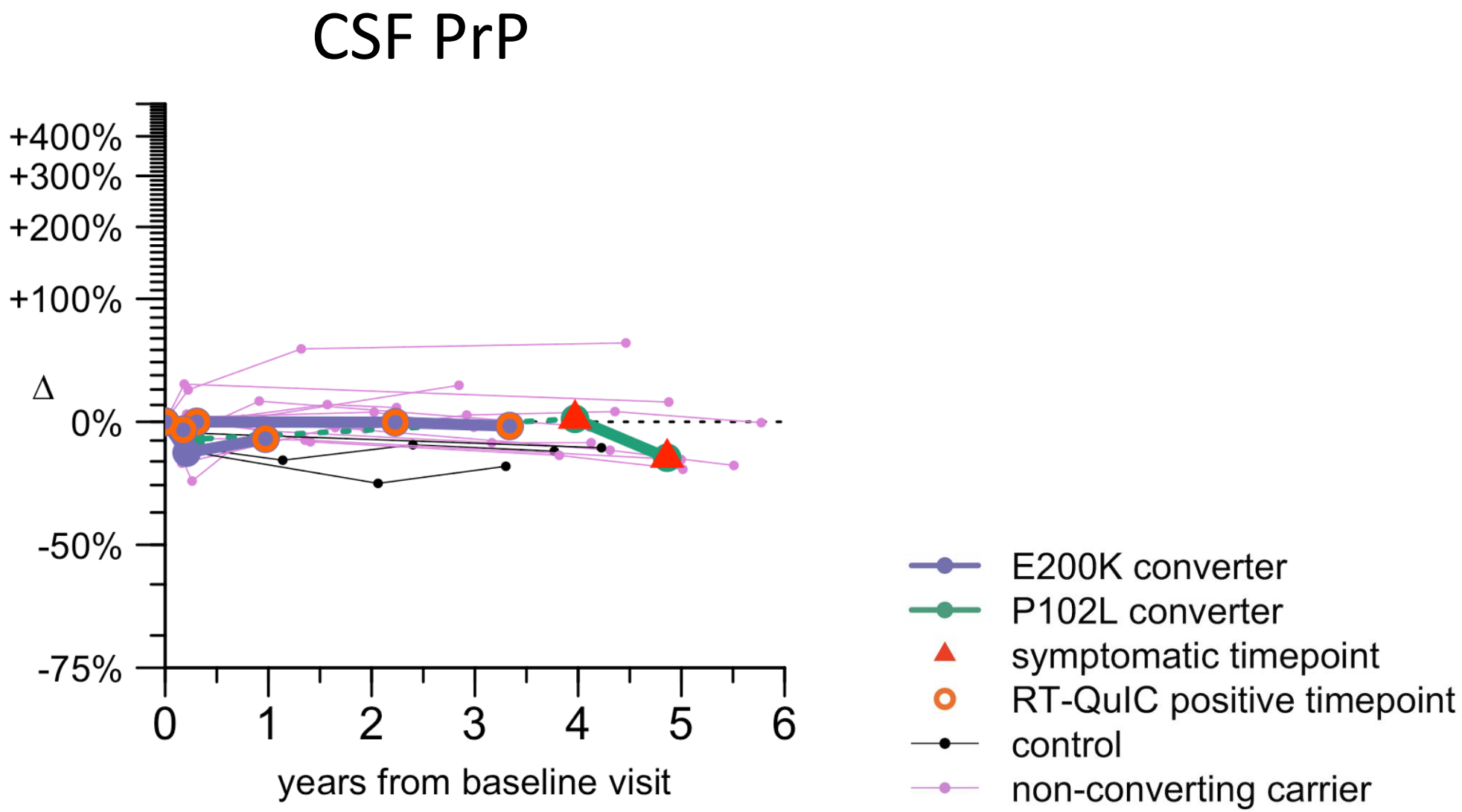


CSF β-synuclein

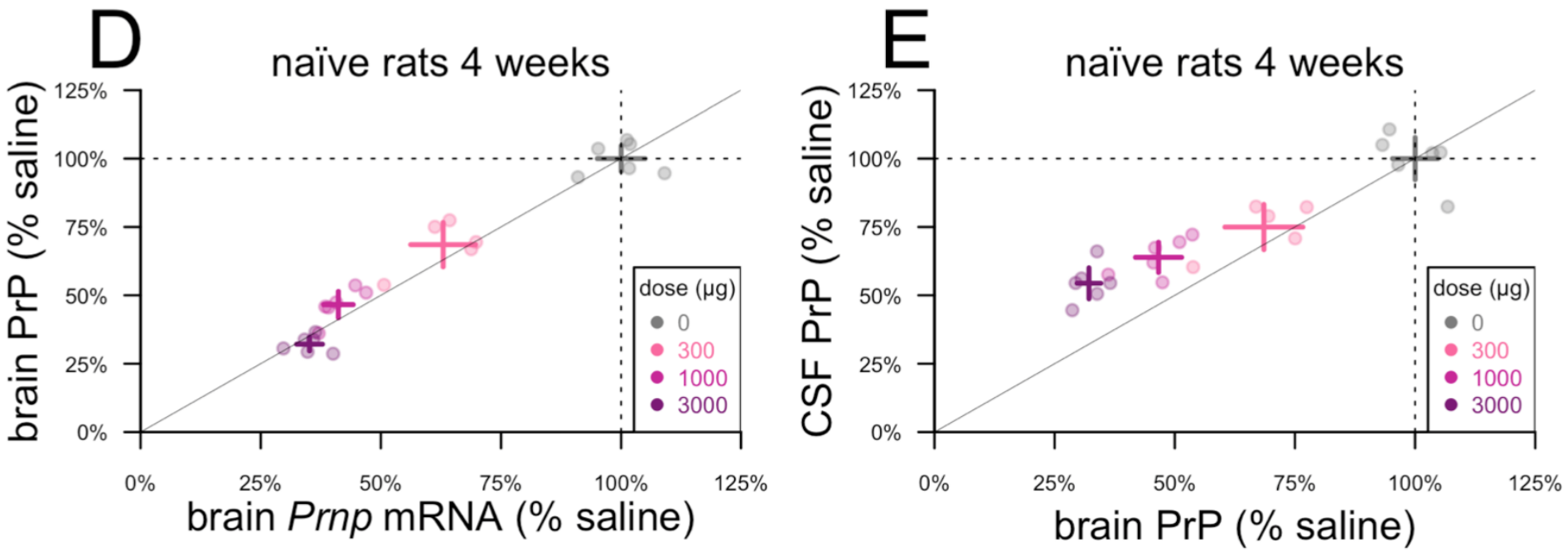




Drug activity biomarker: longitudinally stable over years



CSF PrP responds to pharmacologic intervention in rats



- PrP-lowering ASO administered ICV to Sprague-Dawley rats, whole hemispheres harvested 4 weeks post dose
- Dose-dependent knockdown of RNA is reflected in brain parenchyma PrP and in turn in CSF PrP

Mortberg 2021, *Regional variability, genotypic effects, and pharmacodynamic impact on prion protein concentration in the central nervous system*, medRxiv 2021.11.01.21265619

Conclusions from natural history so far

- The average carrier who you could recruit today is **healthy – on the molecular level.**
- We **can’t reliably predict onset** for a given individual based on prodromal biomarkers.
- CSF PrP appears to have a **stable baseline** and to **respond to therapy**.
- And let’s not forget: we saw the best efficacy in mice when treatment started **before any sign of neuropathology.**

Our mandate: primary prevention.

Regulatory engagement

Background on Accelerated Approval

Types of clinical trial endpoints for demonstrating efficacy of a drug

Mechanism	**Type of endpoint**	**Standard**	**Example**
Traditional clinical trial	clinical endpoint	"an effect on how a patient **feels, functions, or survives**"	survival
	validated surrogate endpoint	"**known to predict clinical benefit**"	blood pressure for cardiovascular disease
Accelerated Approval trial	novel surrogate endpoint	**"reasonably likely to predict clinical benefit"**	plasma HIV viral load in AIDS

Federal Food, Drug and Cosmetic Act, U.S.C., Sect. Title 21, Chapter 9, Subchapter V, Part A.

Background on Accelerated Approval

Program requirements

- Same **efficacy and safety** standards as in any drug trial
  - Efficacy: “substantial evidence based on adequate and well-controlled clinical investigations”
  - Safety: “sufficient information to determine whether the drug is safe for use under conditions prescribed, recommended, or suggested in the proposed labeling”
- Evidence that the surrogate endpoint is **reasonably likely to predict clinical benefit**
- “**Phase 4**” post-marketing study to confirm clinical benefit following provisional biomarker-based drug approval

U.S. FDA Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics

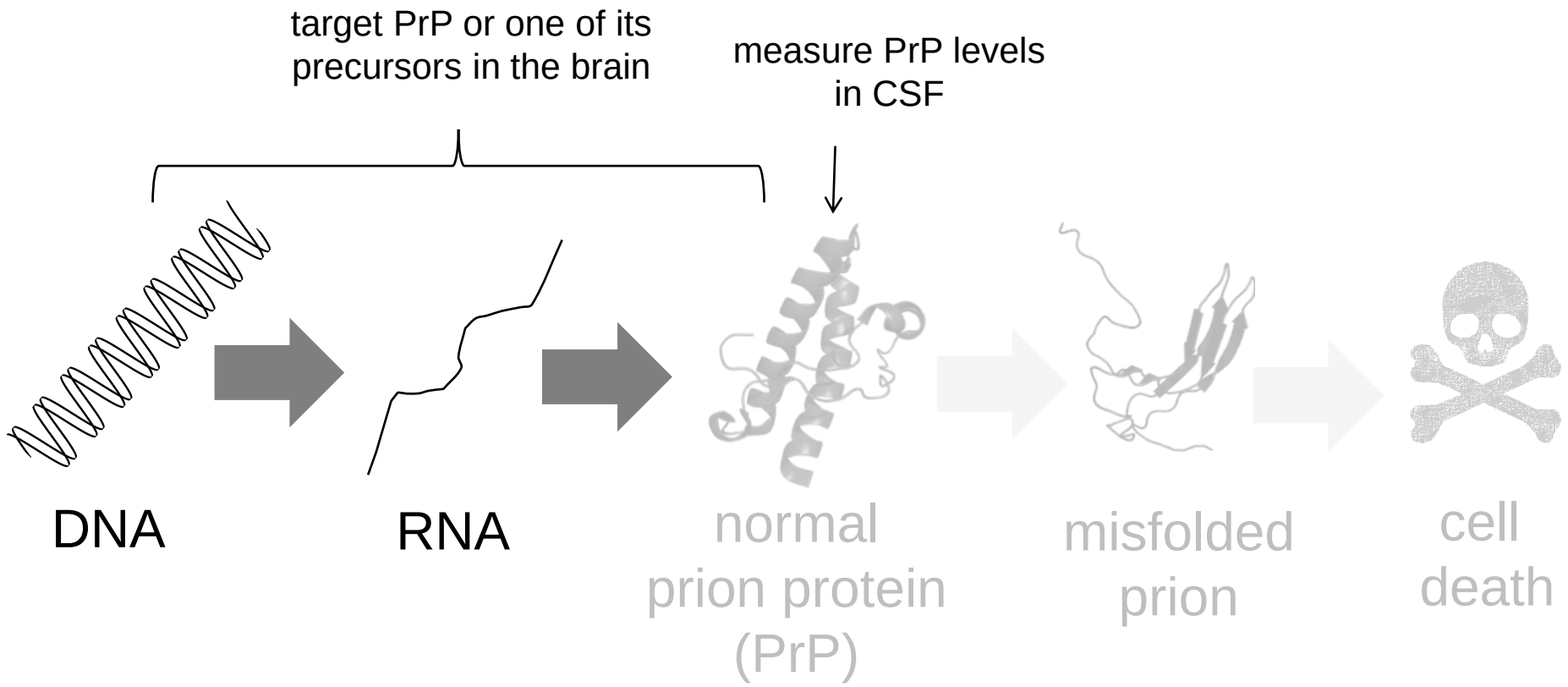
Background on Accelerated Approval

When is Accelerated Approval appropriate to consider?

- ✓ Serious or life-threatening disease
- ✓ Unmet medical need/ lack of available treatments
- ✓ Sufficient understanding of the disease process
- ✓ Biomarker is on-pathway with clear causal role
- ✓ Reasonable risk/benefit (disease severity, safety profile of drug modality, potential for off-label use)
- ✓ Added by Congress in 2012: **disease rarity** makes other trial designs infeasible

U.S. FDA Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics

Clinical strategy for trials in carriers



Lowered CSF PrP — a pharmacodynamic biomarker — needs to be a **primary endpoint** and **basis for provisional approval**

Vallabh 2020, *Towards a treatment for genetic prion disease: trials and biomarkers*, PMID: 32199098

FDA Critical Path Innovation Meeting, Nov 2017

Core message: if we can show that an ASO reduces PrP in the CSF of healthy carriers, those data could support an application for Accelerated Approval.

FDA scientists are supportive!

What comes next?

The first clinical trial of a PrP-lowering drug is now fully enrolled, top line results expected July 2025

- Ionis PrProfile Phase 1/2a trial of ION717
- 16 international sites
- Recruited 56 patients from Dec 2023 – Dec 2024

PrProfile: A Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ION717.

ClinicalTrials.gov ID NCT06153966

Sponsor Ionis Pharmaceuticals, Inc.

Information provided by Ionis Pharmaceuticals, Inc. (Responsible Party)

Last Update Posted 2024-07-30

Contacts and Locations

This section provides the contact details for those conducting the study, and information on where this study is being conducted.

This study has 16 locations

United States

California Locations

Los Angeles, California, United States, 90095
UCLA Neurology Clinic

Colorado Locations

Denver, Colorado, United States, 80204
University of Colorado Hospital

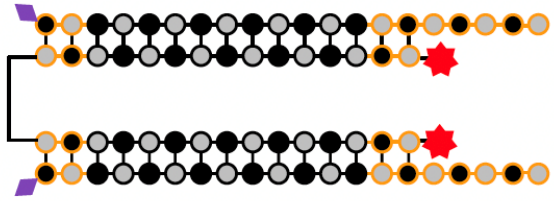
Massachusetts Locations

Orienting goals for our lab right now

- **Develop, and advance as fast as possible, next-gen PrP-lowering drugs**
  - Deeper knockdown
  - Less frequent dosing and/or less invasive RoA
  - More uniform brain distribution
- **Continue to fight for prevention in at-risk individuals**
  - Enable acceptance of CSF PrP — a pharmacodynamic biomarker — as primary endpoint for Accelerated Approval

Two next gen stragies for lowering PrP

Divalent siRNA



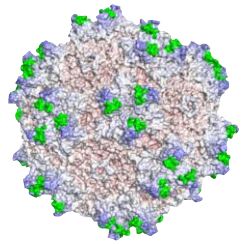
What it is:
Two siRNA molecules connected by a linker, delivered naked into CSF

Goal: lower PrP in the brain by degrading its precursor RNA

Anastasia Khvorova

Julia Alterman

CHARM



What it is:
An epigenetic editor programmed to methylate DNA, delivered via an engineered CNS-tropic AAV

Goal: lower PrP in the brain by turning off the prion protein gene

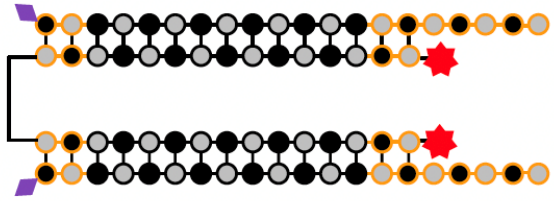
Jonathan Weissman

Ben Deverman

Ken Chan

Two next gen stragies for lowering PrP

Divalent siRNA



What it is:
Two siRNA molecules connected by a linker, delivered naked into CSF

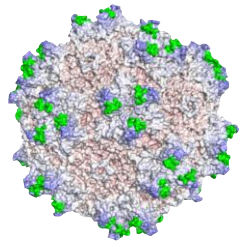
Goal: lower PrP in the brain by degrading its precursor RNA

Anastasia Khvorova

Julia Alterman

No commercial pharma partners –
we are the sponsor

CHARM



What it is:
An epigenetic editor programmed to methylate DNA, delivered via an engineered CNS-tropic AAV

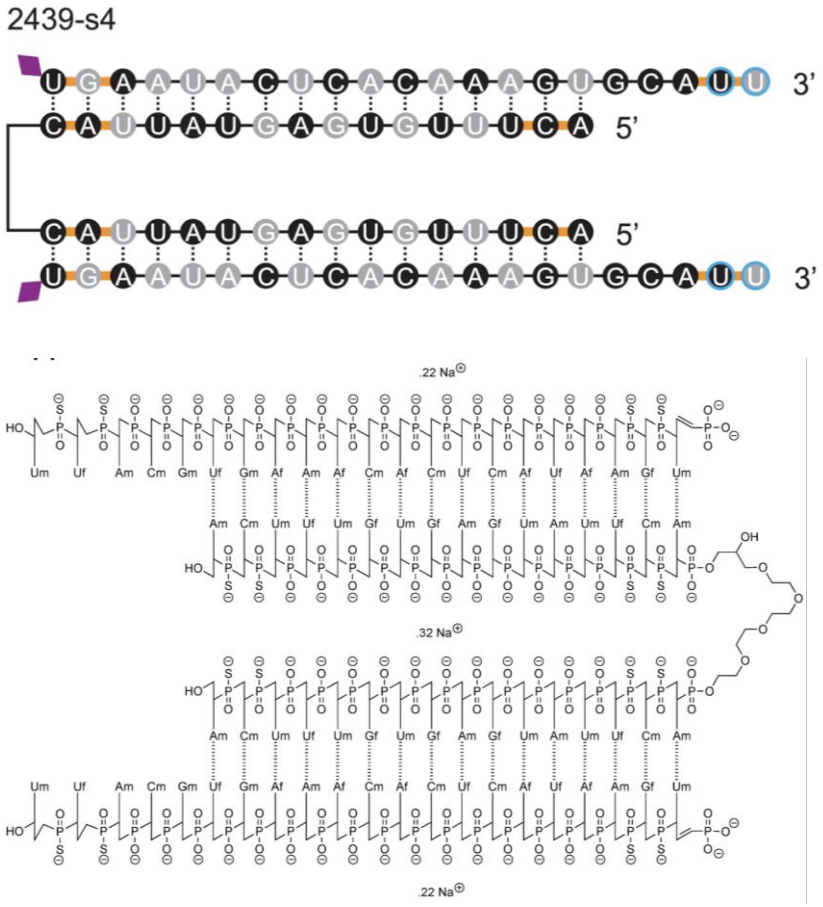
Goal: lower PrP in the brain by turning off the prion protein gene

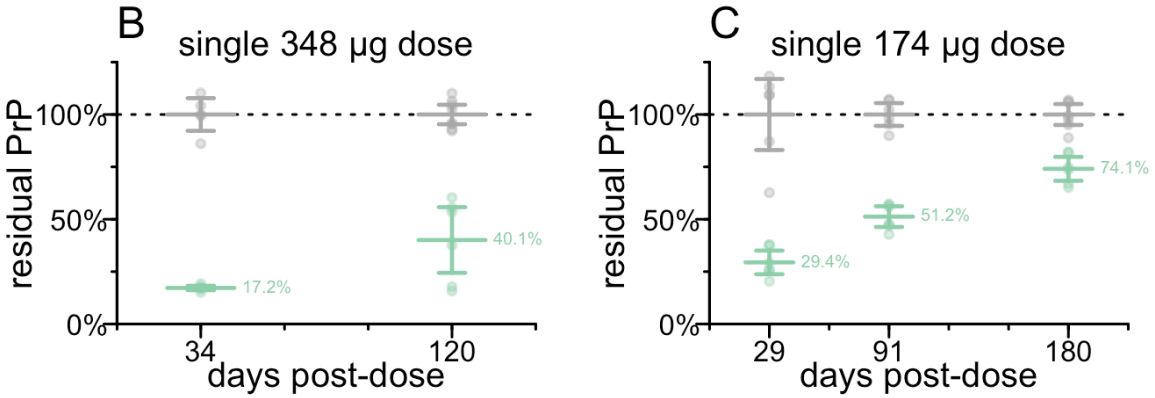
Jonathan Weissman

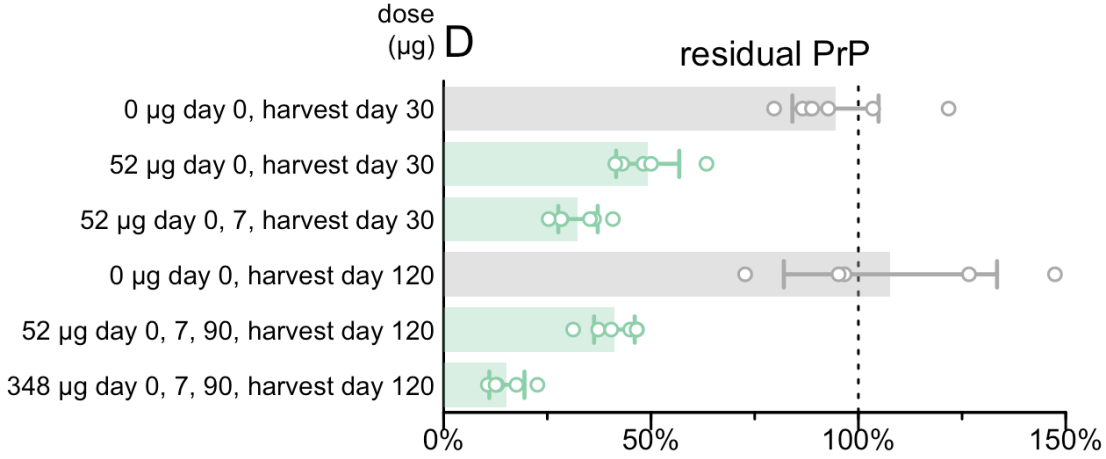
Ben Deverman

Ken Chan

Divalent siRNA may be the 2nd PrP-lowering drug in humans



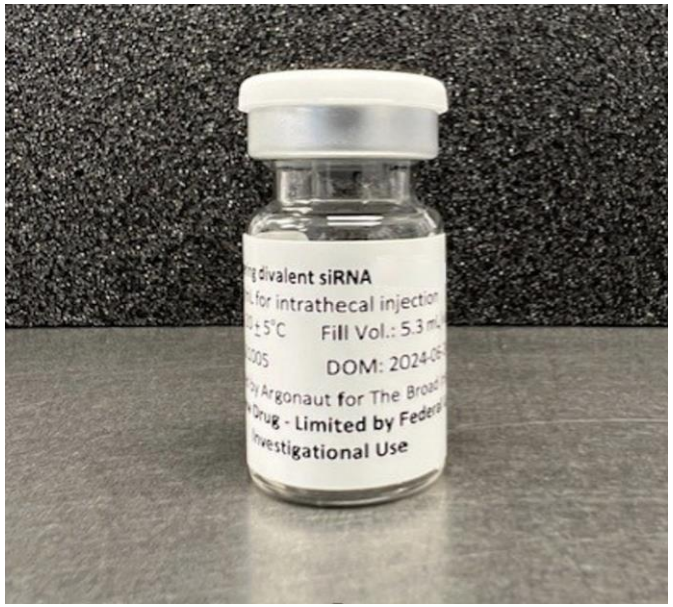




Gentile 2024, Divalent siRNA for prion disease. https://doi.org/10.1101/2024.12.05.627039

Manufacturing and GLP toxicology studies for divalent siRNA are complete

biodistribution in dogs



Funded by NIH U01 & philanthropic donations

Genotox, DDI, rat & dog intrathecal GLP tox all complete

Awaiting rat PK and revised tox reports

Goal: submit IND by end of Jan 2025

Gentile 2024, Divalent siRNA for prion disease. https://doi.org/10.1101/2024.12.05.627039

How would divalent siRNA differentiate from ASO?

Potential advantages:

- Clean tox so far — dose higher
- More potent (lower ED50)
- More durable – maybe q90d dosing
- Deeper maximal knockdown
- We control the program and can be aggressive about asking to dose presymptomatic

But:

- Still chronic intrathecal dosing
- Still below IC50 in deep brain at plausible clinical doses

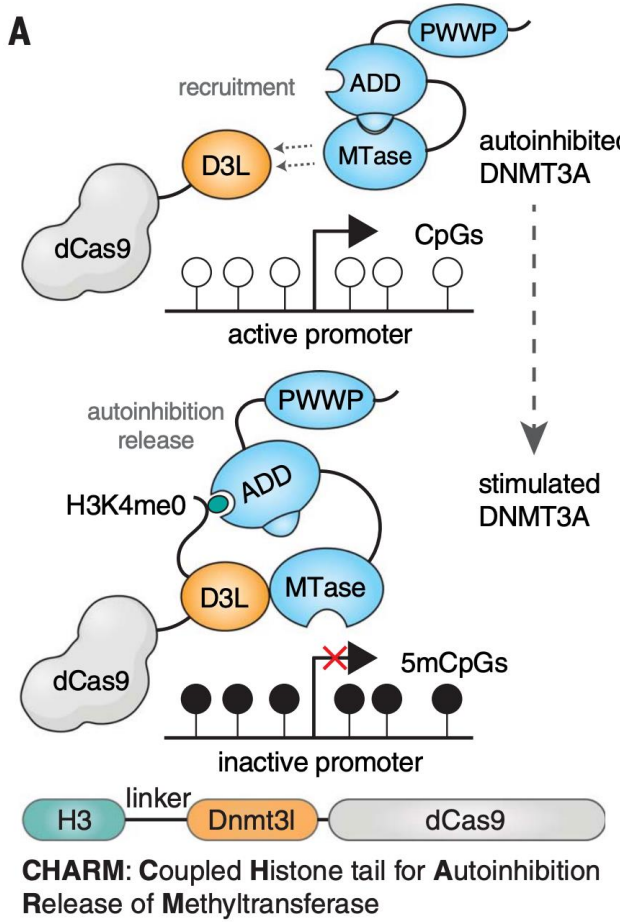
CHARM is a one-time epigenetic therapy to methylate the *PRNP* promoter

- Use **DNA methylation** (C → 5mC) to silence genes

- 3 pieces:

1. DNA-binding domain (CRISPR or zinc finger) – target gene of interest
2. D3L – recruit host DNA methylation machinery
3. Histone tail – turn on DNA methylation machinery (release autoinhibition)

Optional: KRAB domain – repress transcription



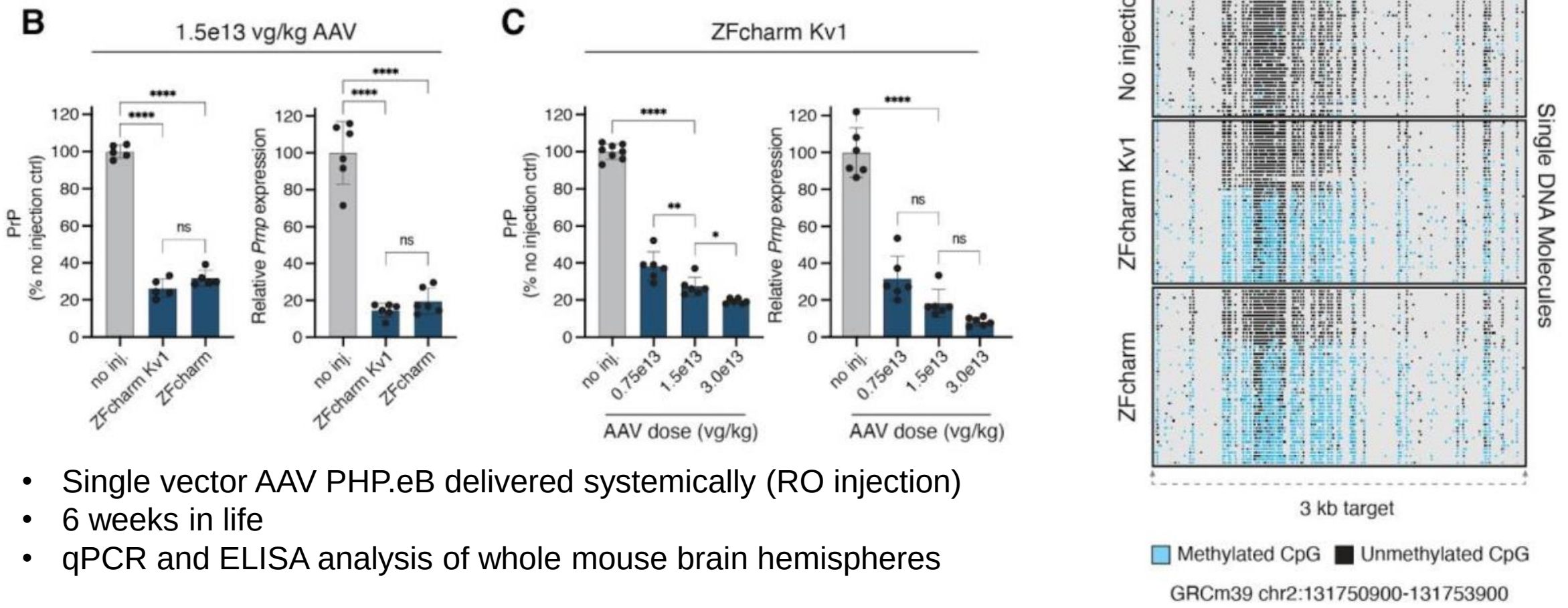
Neumann & Bertozzi 2024. *Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor.* PMID: 38935715.

CHARM potently methylates the *Prnp* promoter and knocks down *Prnp* RNA, PrP

KRAB vs. KRABless

dose response

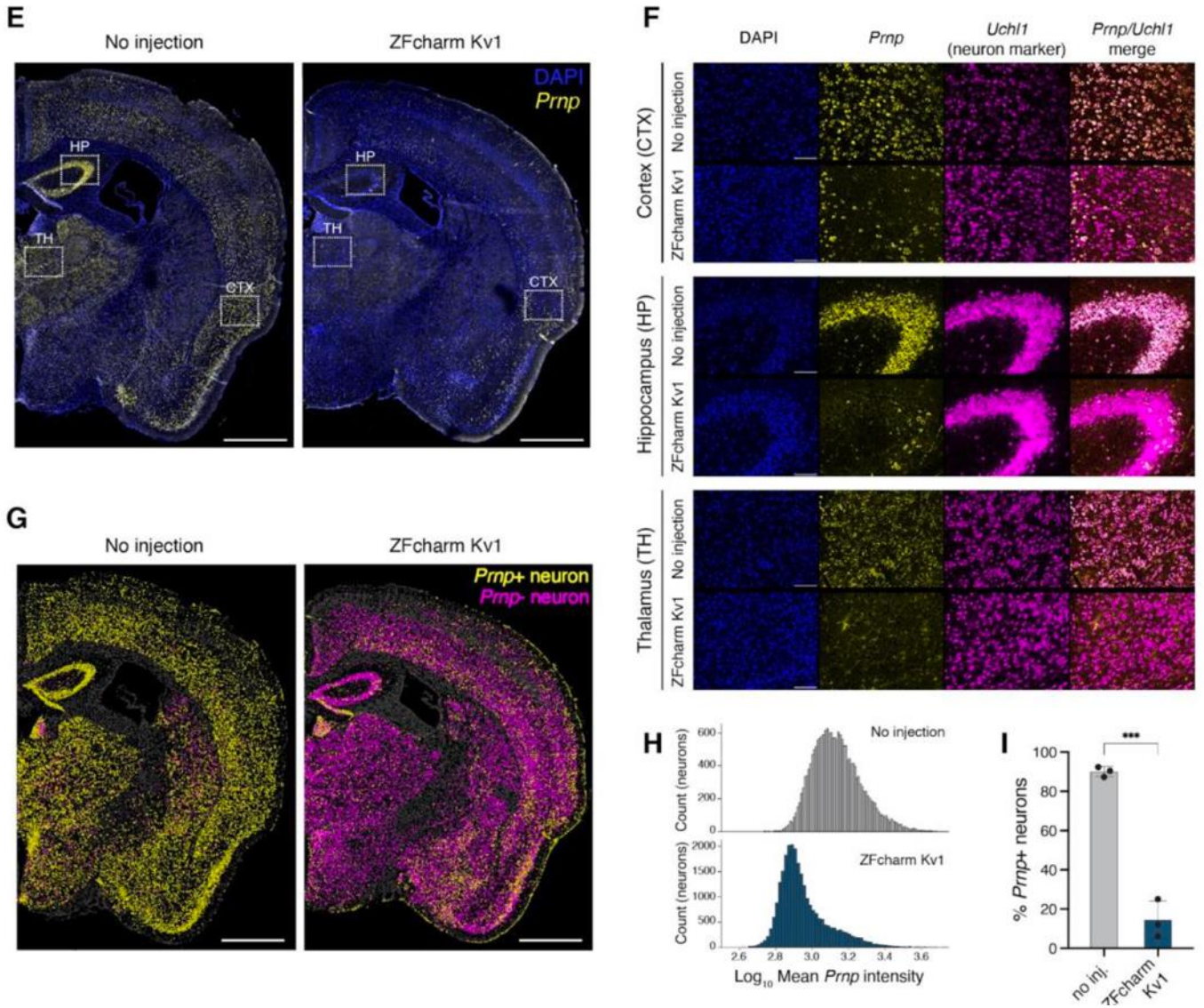
installation of 5-methylC at promoter



- Single vector AAV PHP.eB delivered systemically (RO injection)
- 6 weeks in life
- qPCR and ELISA analysis of whole mouse brain hemispheres

Neumann & Bertozzi 2024. *Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor.* PMID: 38935715.

Single cell HCR RNA FISH analysis shows that cells transduced by CHARM do not express *Prnp*



Neumann & Bertozzi 2024. *Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor.* PMID: 38935715.

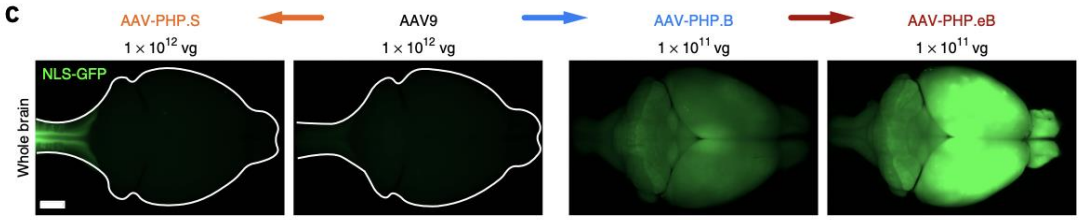
Editors need a vector for delivery to the brain; engineered viral vectors provide a solution.

AAV viral vectors can deliver up to 4.7 kb of DNA payloads to cells

2017: brain AAV engineered for **mice** (PHP.B/eB)
Receptor: *Ly6a*

Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems

Ken Y Chan, Min J Jang, Bryan B Yoo, Alon Greenbaum, Namita Ravi, Wei-Li Wu, Luis Sánchez-Guardado, Carlos Lois, Sarkis K Mazmanian, Benjamin E Deverman & Viviana Gradinaru

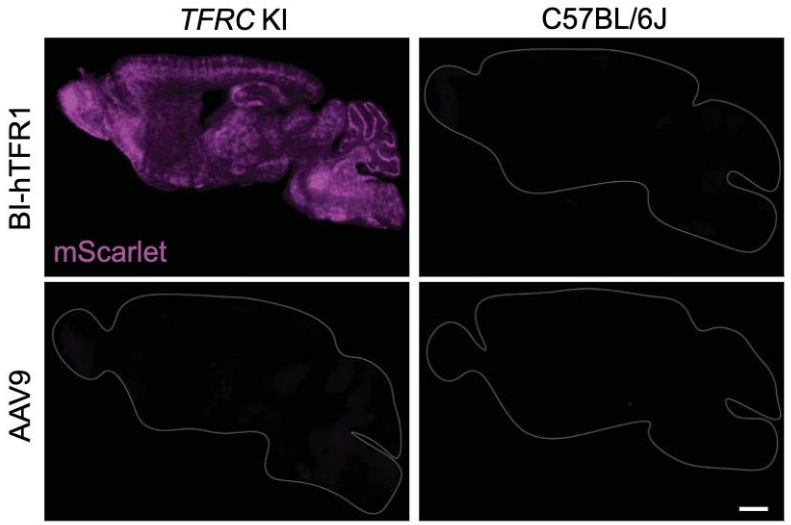


2024: brain AAV engineered for **humans**
Receptor: *TFRC*

GENE THERAPY

An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery

Qin Huang[1]†, Ken Y. Chan[1]†, Jason Wu[1], Nuria R. Botticello-Romero[1], Qingxia Zheng[1], Shan Lou[1],



Ben Deverman

Chan 2017. Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. PMID: 28671695
Huang & Chan 2024. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. PMID: 38753766

Reflections on leading drug development ourselves

- The “IND enabling studies industrial complex” is built for pharma – not for small sponsors.
  - Anyone other than us would have given up by now.
- Guidances and precedent are anchoring people in the wrong tech.
  - High dose AAV9 isn’t going to work – but we keep doing it.
  - There now exist better knockdown modalities for CNS than MOE gapmers – but we keep using them.
- NHP toxicology does not make sense in a disease where patients die in < 6 months and are
- Billions of dollars are being spent on neurology trials rendered uninterpretable by the question, “Did we just treat to late?”

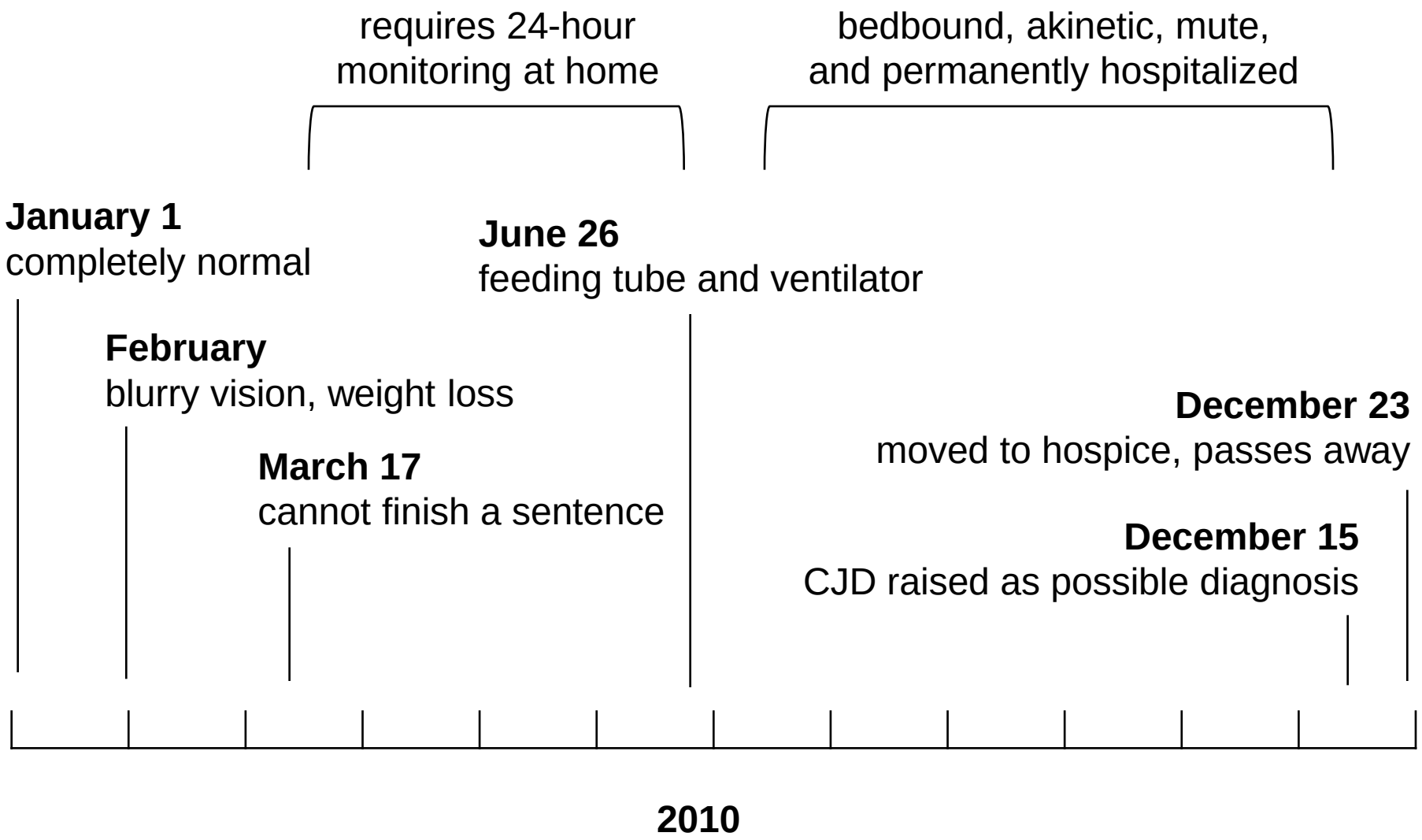
If not for prion disease – **when**?

- Single gene, single protein target
  - Essential for disease
  - Expendable for healthy life
  - Reduction dose-dependently protects against disease.
  - Can be knocked down in brain with existing tech and monitored in CSF.
- Known high penetrance variants.
- Damage irreversible once it begins.
- Fatal in weeks to months once symptoms strike.
- Organized, highly motivated carrier base.

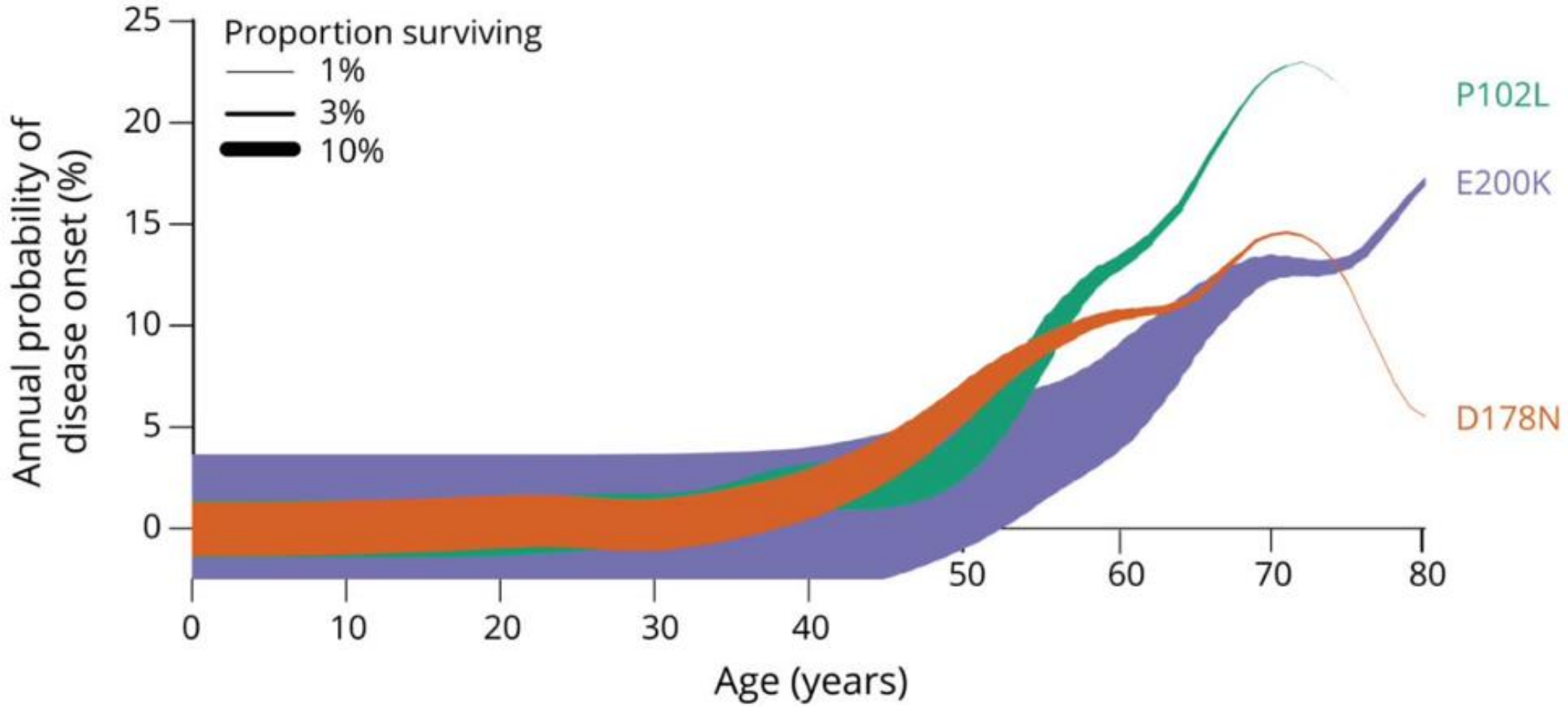
We are the consummate case for primary prevention.

Closing thoughts

2010: the timeline of my mom’s illness



12% of universes



Thank you for your time and attention!

svallabh@broadinstitute.org

prionalliance.org

\The Vallabh/Minikel Lab → vallabhminikel.org