Antisense Oligonucleotides to Delay or Prevent Onset of Prion Disease in Mice

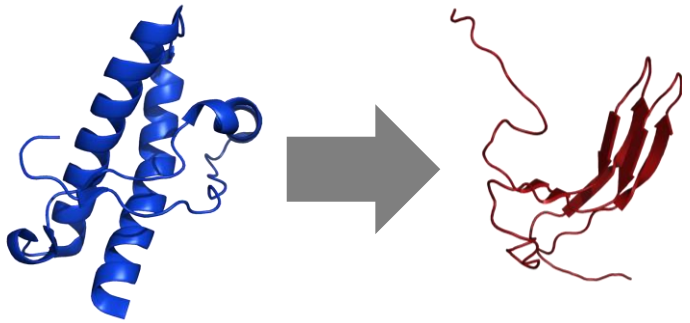
Byron Caughey

Slides for CJD Foundation Meeting July 2016

Outline

- What is a prion, and how can we stop it?
- Why target the healthy protein?
- Why antisense technology?
- Progress so far
- Plans for preclinical studies

What is prion disease?



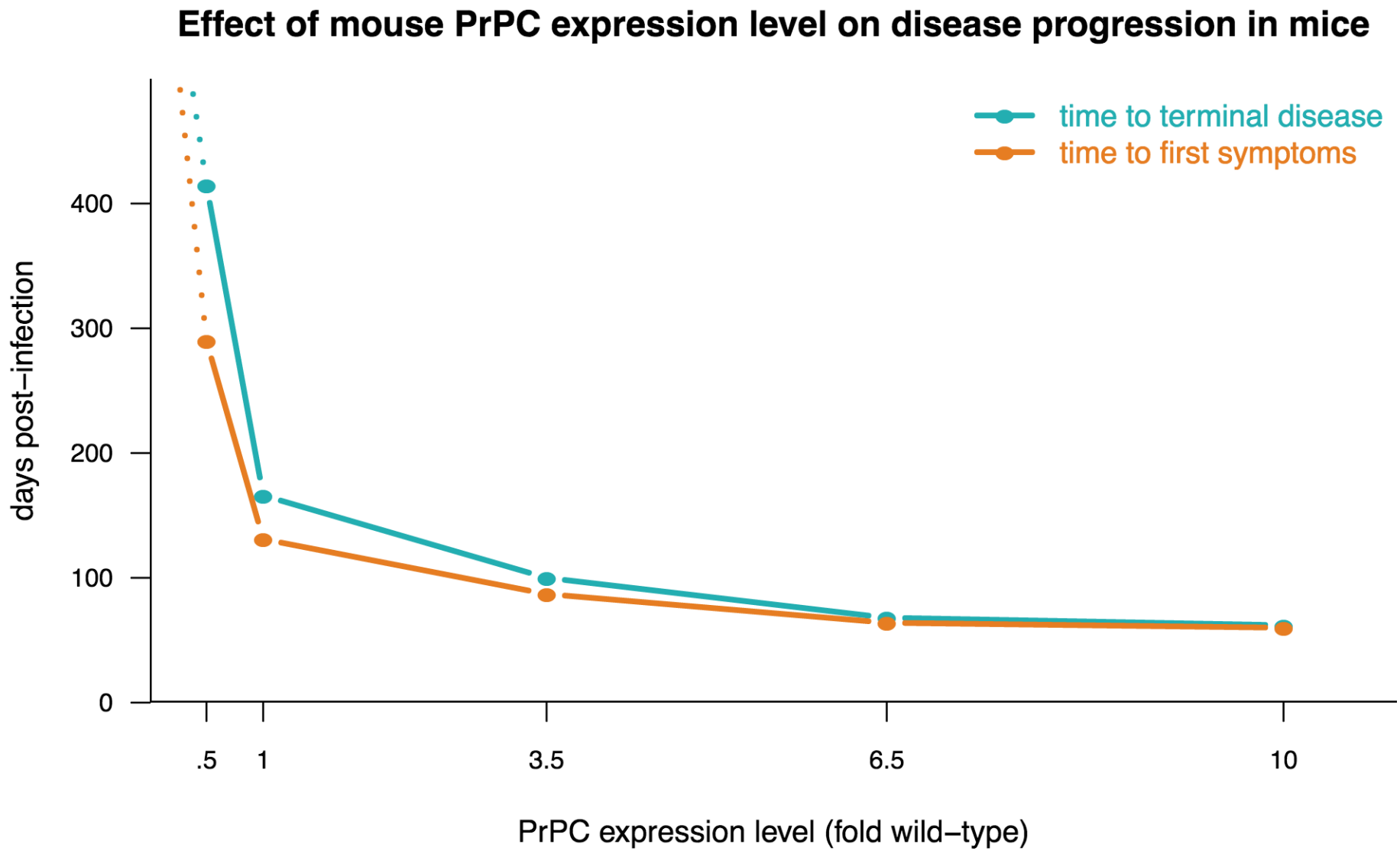
PrP^{C}

A healthy protein
that your body
normally produces

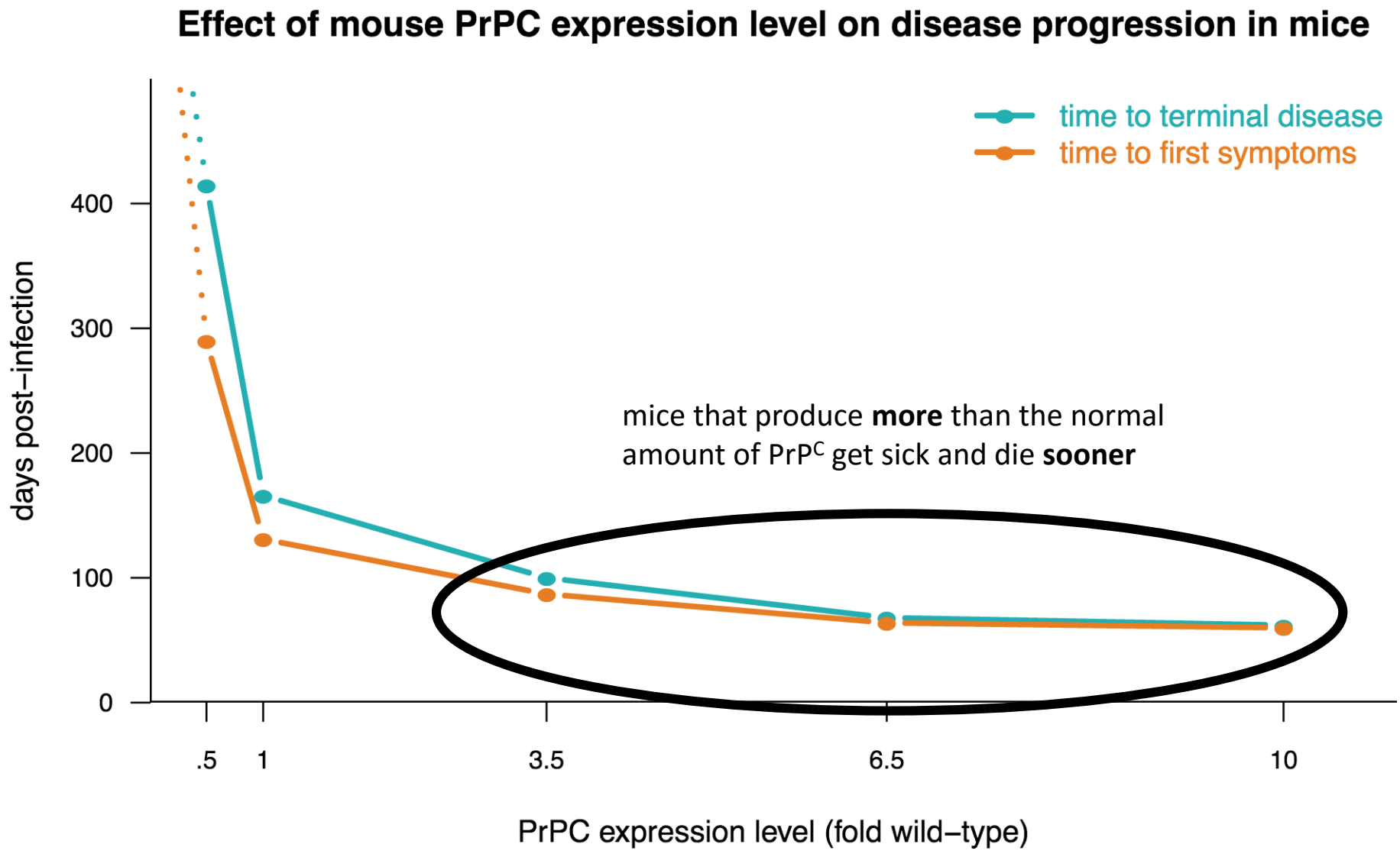
PrP^{Sc}

A misfolded protein
that kills brain cells

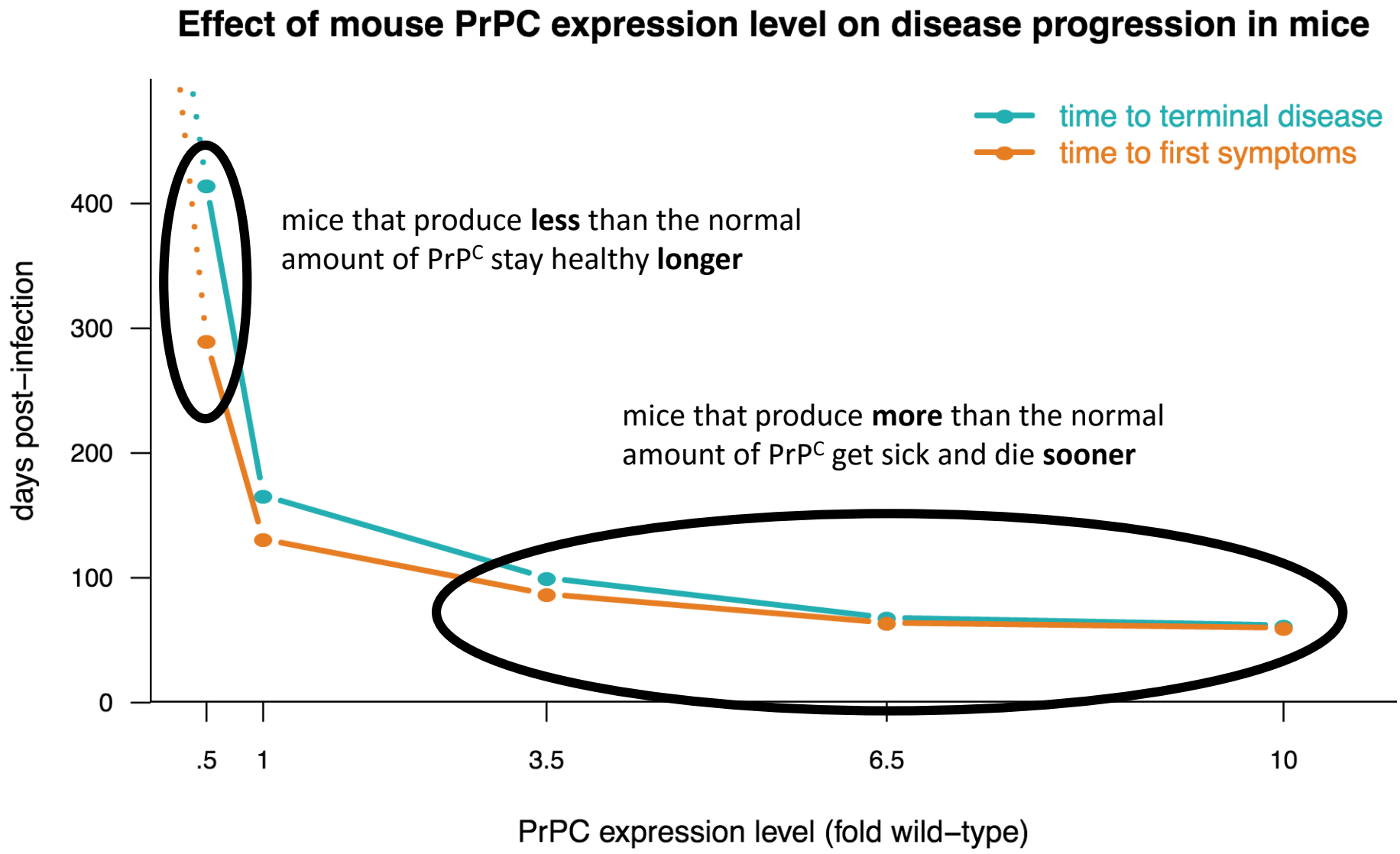
Why do we want to reduce PrP^C levels?



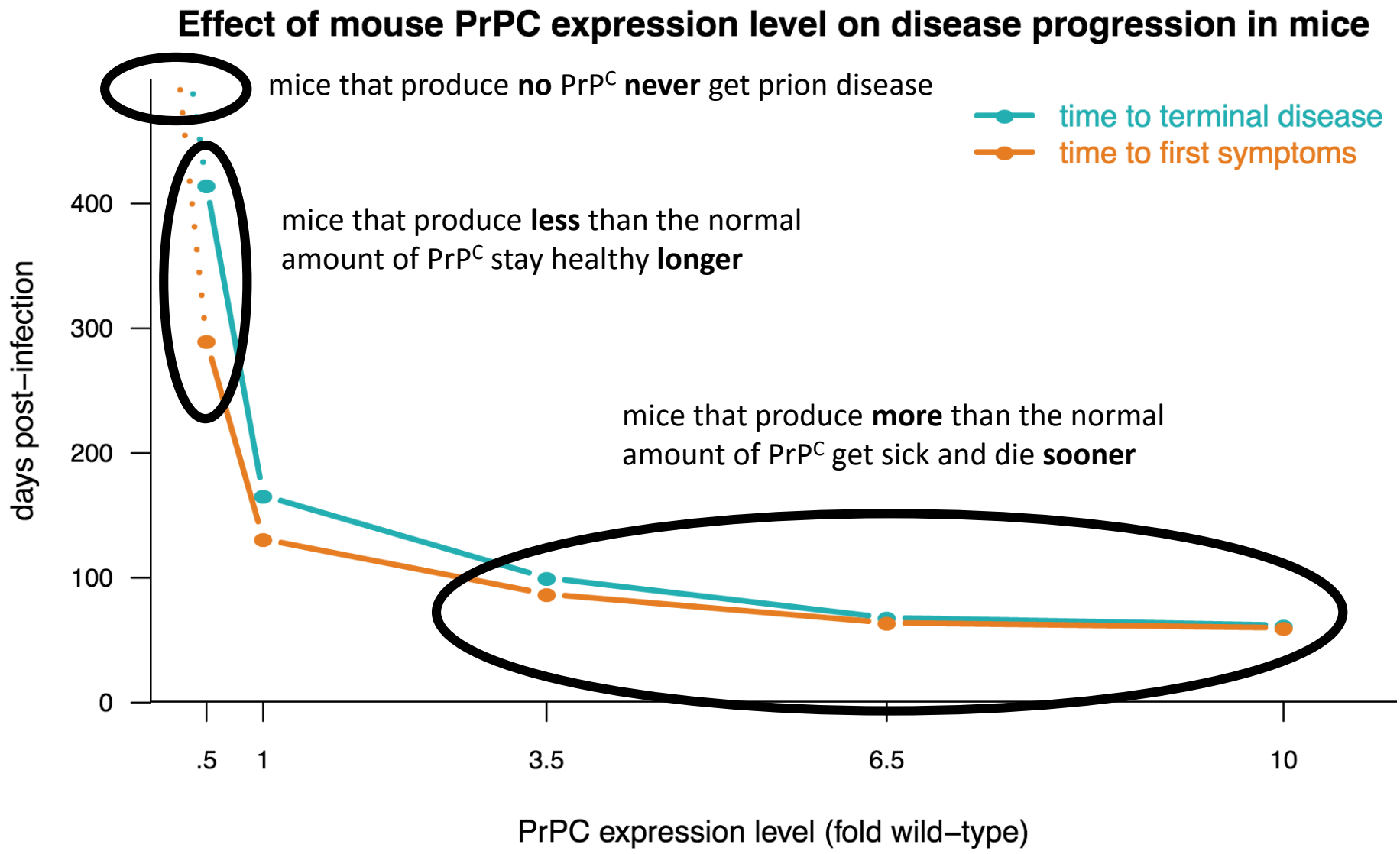
Why do we want to reduce PrP^C levels?



Why do we want to reduce PrP^C levels?



Why do we want to reduce PrP^C levels?



Is it safe to reduce PrP^C levels?

- Mice engineered to produce **no** PrP^C have only mild health issues (Bueler et al 1992, Bremer et al 2010)
- Mice engineered to produce **half** the normal amount of PrP^C are indistinguishable from normal mice (Bremer et al 2010)
- Humans with only 1 functional copy of the prion protein gene, instead of 2, are healthy (Minikel et al 2016)

Therapeutic hypothesis

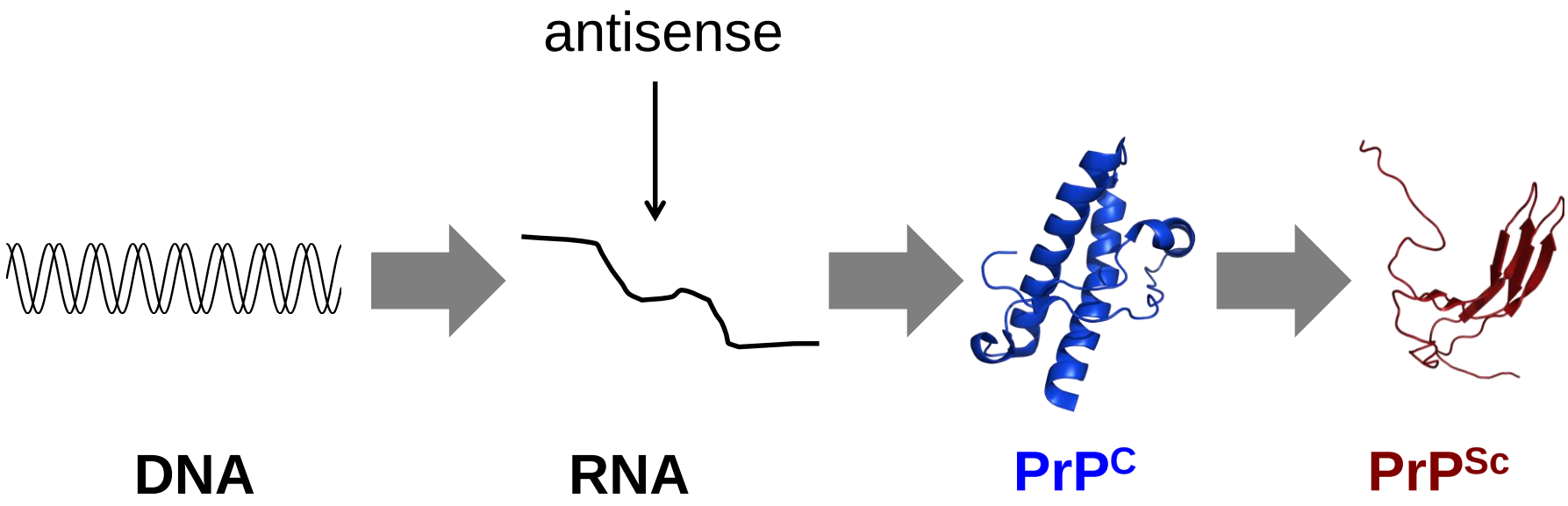
- If we can make people produce **less** PrP^{C}, they will stay healthy **longer**

Therapeutic hypothesis

- If we can make people produce **less** PrP^C, they will stay healthy **longer**

- …and how can we do that?

Antisense targets RNA before the protein is produced



What is antisense technology and how does it work?

- An **antisense oligonucleotide (ASO)** is a 20-mer of chemically modified DNA
- It is complementary to 20 bases of RNA sequence
- Binds to the RNA and causes the enzyme RNAse H to break down the RNA
- Reduces the amount of a specific RNA, thereby reducing the amount of a specific protein
- Different ASOs have been developed against many different RNAs for many different diseases

Antisense is already in clinical trials for other neurological diseases

- ASOs for brain disorders are usually dosed into intrathecal space (base of spine) once per four months
- Patient's experience is similar to undergoing a lumbar puncture (e.g. at Michael Geschwind's study at UCSF)
- Phase 1 trial in spinal muscular atrophy (nusinersen / SMNRx) found IT delivered ASOs to be safe and found preliminary evidence of efficacy (Chiriboga et al 2016), program is currently in phase 3
- Phase 1 trial in Huntington's disease launched last fall
  - Aims to reduce huntingtin RNA levels, similar to our goals for PrP

Antisense meets all the requirements to be a sound therapeutic strategy

Requirement	Why ASOs
Safe for chronic use	• There is one ASO drug against a different RNA already approved and marketed (Kynamro, for high cholesterol) • Preliminary data from ASO trials for spinal muscular atrophy indicate ASOs are well-tolerated in the human brain
Gets into brain	• ASOs are stable for multiple months and can be injected into base of spine, directly into cerebrospinal fluid, a few times per year • Monkeys treated with ASOs for Huntington's disease have reduced huntingtin levels across many brain regions • Preliminary data from spinal muscular atrophy trials indicate good ASO brain distribution in humans
Likely to translate from mouse studies to humans	• By targeting RNA, ASOs slow the disease process upstream of species- and strain-specific problems • Should be effective in multiple species and against all subtypes of prion disease

Previous work on ASOs for prion disease

- In addition to targeting RNA, some ASOs happen to also interfere directly with prion formation in cells and in mice (Kocisko et al 2006, Karpuj et al 2007)
- An ASO against PrP RNA was moderately effective at delaying disease in mice (Nazor-Friberg et al 2012)
- Improvements in ASO chemistry and sequence screening offer opportunities for more effective, longer-lasting, and safer ASOs.

Goals of our preclinical study

- Develop ASOs against the mouse prion protein RNA
- Dose ASOs directly into mouse brain (intraventricular injection)

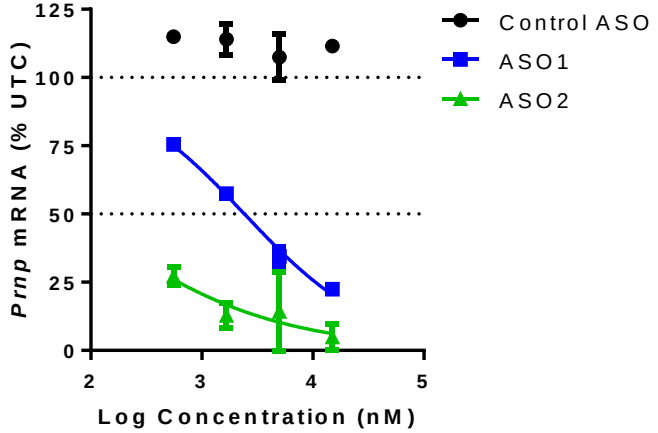
- **Potency**: How much we can reduce PrP levels in the mouse brain
- **Efficacy**: How much can ASOs extend the survival time of mice infected with prions?
- **Safety**: Are ASOs well tolerated in these mice?
- **Biomarkers**: Establish whether we can measure PrP levels and prion seeding activity levels in mouse brain as a predictor of efficacy.

- **Ultimate goal**: establish a proof of principle so that we can develop an ASO against the human prion protein RNA and get it into clinical trials

Identification of potent well-tolerated ASOs for use in preclinical studies

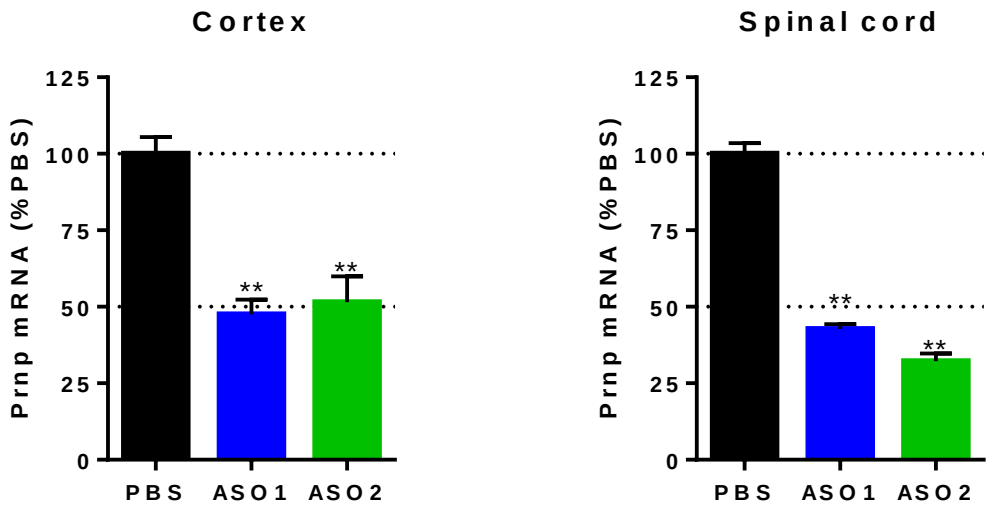
- 450+ ASOs screened in Hepa1-6 cells
- 26 of the most potent ASOs selected for large-scale synthesis and *in vivo* testing
- 11 of the most *in vivo* potent ASO selected for high dose, long-term tolerability screening
- The two most potent, with the longest duration of action and well-tolerated ASOs chosen for additional testing

In vitro dose response of 2 lead mouse *Prnp* ASOs



Two lead *in vivo* active rodent *Prnp* ASOs selected

- Study #1: Single 300μg ICV bolus injection in C57bl6 mice of ASO or PBS and tissue collection 2 weeks post-treatment



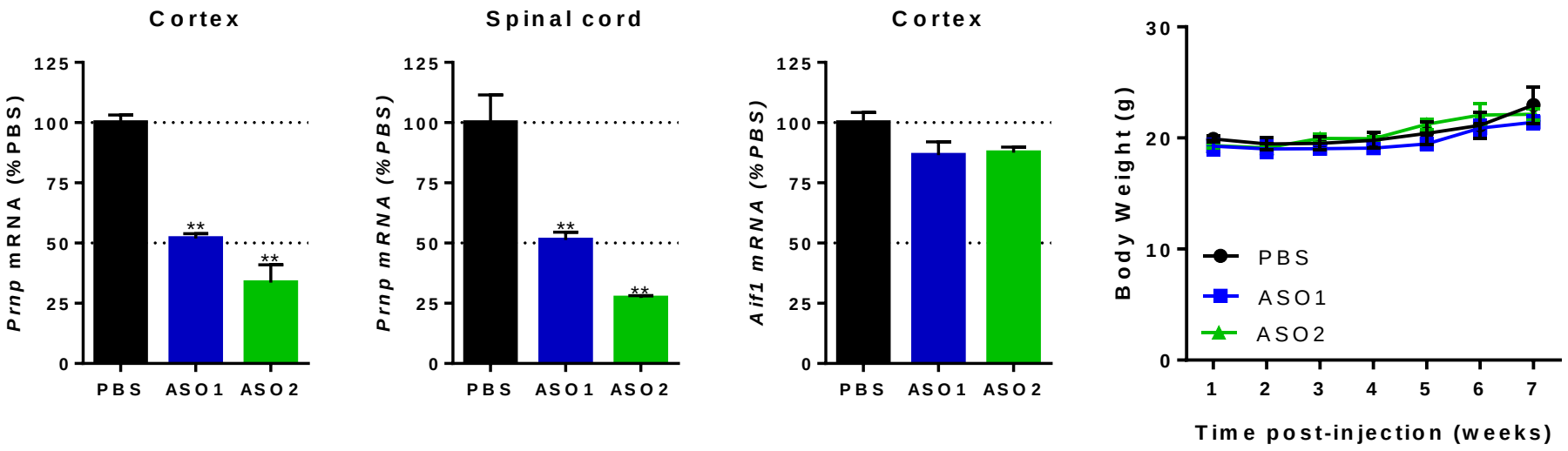
Lead *in vivo* ASOs are active, well-tolerated and have long duration of action

- Study #2: Single 700μg ICV bolus injection in C57bl6 mice of ASO or PBS. Animals weighed and subject to neurological exam weekly. Tissue collection 8 weeks post-treatment

RNA 8 week post-injection

No microglial activation

No body weight change



No adverse events on neurological exams and no histopathological findings

Plans for preclinical studies

Question	Experiments planned	Progress
Potency	Screen 100s of ASOs in cells.	Complete
	Test 26 ASOs in mice.	Complete
	Re-test best ASOs for 4 or 8 weeks in two different varieties of mice to identify single most potent ASO.	In progress
Safety and efficacy	Inject mice at 4 different timepoints at 5 different doses to identify highest tolerated dose.	Planned
	Dose prion-infected mice with ASOs at four different timepoints: prophylactic, immediately after infection, early infection, and late stages. Determine survival times and tolerability.	Planned
Biomarkers	Determine reduction in PrP levels in mouse brain	Planned
	Quantify prion seeding activity (RT-QuIC) in brains of mice treated or not treated with ASO	Planned
	Establish ability to measure PrP levels in human cerebrospinal fluid as a potential biomarker	Planned

Collaborators

Rocky Mountain Labs

Byron Caughey

Greg Raymond

Ionis Pharmaceuticals

Holly Kordasiewicz

Hien Zhao

Eric Swayze

Broad Institute

Sonia Vallabh

Eric Minikel

Western Washington University

Jeff Carroll