Engineered Zinc Finger Transcriptional Regulators Specifically Reduce Prion Expression and Extend Survival in an Aggressive Prion Disease Model

Abstract #490

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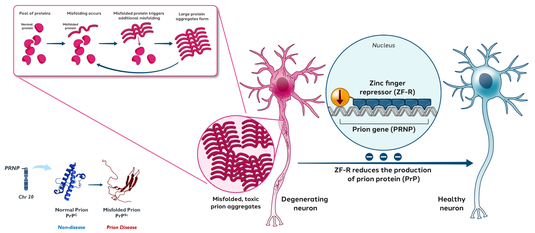
Introduction and background

- Prion disease is a rapidly progressing, invariably fatal neurodegenerative disorder caused by misfolding of the cellular prion protein, PrP^C, encoded by the *PRNP* gene.
- Most cases are sporadic or caused by inherited dominant mutations in *PRNP*, with an estimated 500 patients diagnosed per year in the US.
- There are currently no approved or clinical-stage disease-modifying therapies for the prevention or treatment of prion disease.
- Here, we investigated a single-administration epigenetic regulation approach using AAV-delivered Zinc Finger Repressors (ZF-Rs) to achieve sustained and widespread reduction of PrP in the brain and rapid pharmacological effect.



ZF-repressor genomic medicine for prion disease

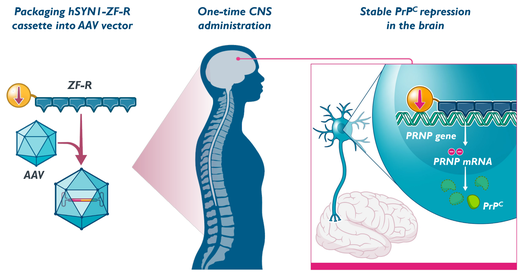


Figure 1. AAV ZF-R genomic medicine for the treatment of prion disease

- A ZF-R targeting the prion gene is packaged into an AAV vector for delivery to the CNS.
- The ZF-R represses *PRNP* transcription, resulting in specific depletion of neuronal PrP^C protein.

Disclosures

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Highly potent and specific ZF-Rs targeting *Prnp*

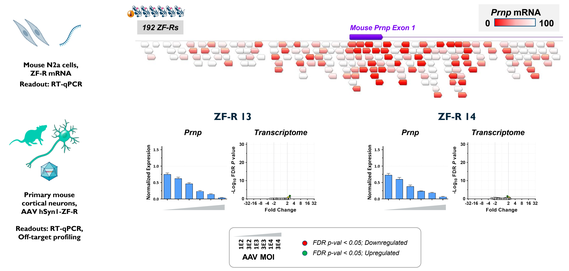


Figure 2. Characterization of potent and specific ZF-Rs targeting mouse *Prnp*

- 192 ZF-Rs were designed against the mouse *Prnp* gene and screened in N2a cells, with ~30% of ZF-Rs achieving at least 50% repression of the *Prnp* transcript.
- Candidates ZF-R 13 and 14 potently and specifically reduced *Prnp* in primary mouse cortical neurons, resulting in >90% repression with no detectable off-target activity.

PrP^{Sc} RML mouse model recapitulates the major hallmarks of human prion disease

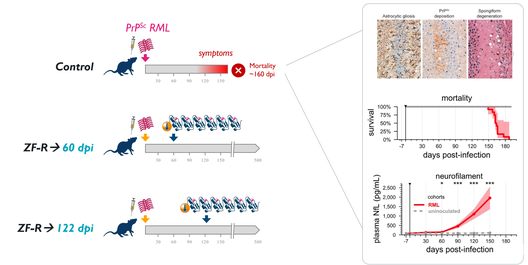
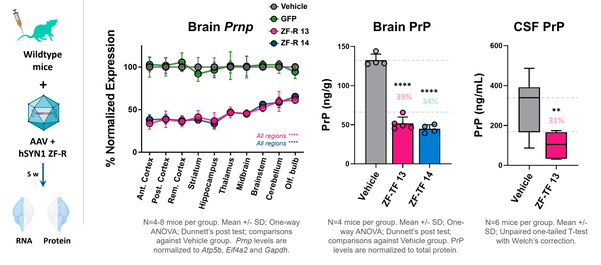


Figure 5. PrP^{Sc} RML inoculation model of prion disease and experimental design

- Wildtype mice intracerebrally injected with the Rocky Mountain Laboratory (RML) strain of prions (PrP^{Sc}) develop prion disease symptoms and inevitable mortality approximately 160 days post inoculation (dpi).
- This well-studied and aggressive model exhibits key human pathological disease hallmarks, including astrocytic gliosis, PrP^{Sc} deposition, spongiform degeneration, and plasma neurofilament light chain (NfL) increase[1].
- We investigated the potential survival benefit of AAV ZF-R treatment at either 60 dpi or 122 dpi and measured body weight and monthly plasma NfL levels out to 500 dpi.

[1] *Minikel EV, et al. Nucleic Acids Res. 2020;48(19):10615-10631.*

40-70% prion reduction in the mouse brain and CSF



N=4-8 mice per group. Mean +/- SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group. *Prnp* levels are normalized to *Atp5b*, *Eif4a2* and *Gapdh*.

N=4 mice per group. Mean +/- SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group. PrP levels are normalized to total protein.

N=6 mice per group. Mean +/- SD; Unpaired one-tailed T-test with Welch's correction.

Figure 3. Widespread reduction of prion mRNA and protein in the mouse CNS

- AAV hSYN1-ZF-R 13 or 14 delivered to wildtype mice resulted in 40-70% repression of bulk *Prnp* mRNA levels across ten CNS regions compared to hSYN1-GFP and vehicle controls.
- Both constructs also led to >60% bulk PrP protein knockdown in homogenized hemispheres.
- In the CSF, ~70% PrP protein knockdown was observed, suggesting the majority of PrP is derived from neurons.

ZF-R treatment dramatically extends survival, improves weight gain, and delays plasma NfL rise

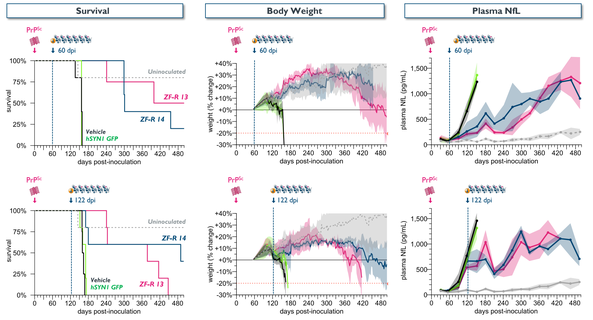


Figure 6. Survival, body weight, and biomarker improvements following ZF-R administration at 60 dpi and 122 dpi

- As expected, AAV GFP and vehicle groups reached terminal endpoint at 160±8 dpi (mean±sd).
- A majority of AAV-ZF-R treated mice (n=10/19) were alive 1 year after inoculation, with attendant improvements in body weight and plasma NfL.
- In total, 5/19 mice treated with AAV ZF-Rs survived to the scheduled necropsy date (500 dpi).
- The PrP^{Sc}-induced plasma NfL rise was delayed following ZF-R treatment.

Potent *Prnp* repression in transduced neurons

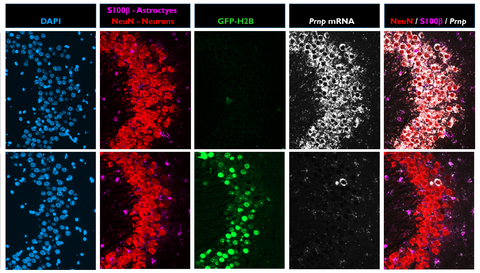


Figure 4. Selective reduction of *Prnp* in neurons in vivo

- Multiplexed RNAscope (*Prnp*) + IHC (NeuN, S100β, GFP-H2B) revealed potent and selected prion transcript repression in the CA3 hippocampal region.
- Neurons positive for ZF-R expression (H2B-GFP+) had no detectable *Prnp* transcript compared to abundant expression observed in a vehicle-treated mouse or H2B-GFP negative neurons and astrocytes in a ZF-R13 treated mouse.

PrP^{Sc} reduction in surviving ZF-R animals at 500 dpi

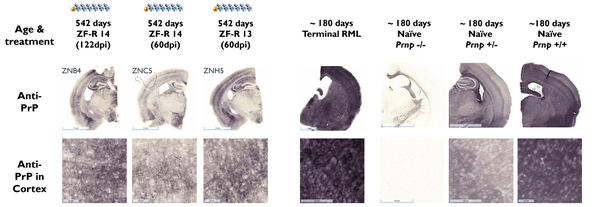


Figure 7. Reduced PrP^{Sc} levels in surviving ZF-R treated mice 500 days after inoculation

- IHC staining for PrP deposition on brain sections from 542-day old ZF-R-treated animals revealed a striking reduction in PrP pathology compared to untreated mice.
- Untreated ~180-day-old control mice were stained for PrP levels using the same conditions.

Conclusion

- ZF-Rs potently and specifically repress mouse *Prnp* in vitro and in vivo.
- ZF-R treatment at 60- or 122-dpi significantly extends survival in RML-inoculated mice.
- ZF-R treatment delays plasma NfL rise and body weight decline in RML-inoculated mice.
- Highly specific ZF-Rs targeting human *PRNP* are currently in late-stage preclinical development.
- These results support the further development of AAV ZF-Rs for the potential treatment of prion disease.

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