# Neuron-Specific Expression of Zinc-Finger Repressors Mediate Widespread Prion Reduction in the Brain for the Potential Treatment of Prion Disease

Poster #XXX

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**Aims:** Misfolding of cellular prion protein, PrP, causes rapidly progressing and invariably fatal prion disease. We are investigating epigenetic regulation of the prion gene (*PRNP*) using a Zinc Finger Repressor (ZF-R) as a potential therapeutic strategy to achieve widespread, rapid, and sustained reduction of brain PrP. Cellular PrP is ubiquitously expressed and *PRNP* transcripts are abundant in neurons and glia cells. Several lines of evidence suggest that neuronal PrP is necessary and sufficient for neurotoxicity and disease progression. Our previous results showed substantial survival benefit in $PrP^{Sc}$-inoculated mice treated with a neuron-specific ZF-R at 60 and 122 dpi.

**Methods:** A surrogate ZF-R that represses murine *Prnp* expression >90% in mouse cortical neurons was paired with promoters that have known expression patterns: hSYN1 (neuronal), GfaABC1D (astrocytic), or CMV (ubiquitous). These promoter-ZF-R constructs were delivered to wildtype mice using a blood-brain-barrier (BBB) penetrating tool capsid (AAV.PHP.B). Prion mRNA and protein reduction were assessed in multiple brain regions.

## Repression of prion expression to slow or halt disease progression and neurodegeneration

- ZF-Rs utilize a human KRAB transcriptional repression domain to achieve specific gene knockdown at both the RNA and protein level.
- Several lines of evidence from prion-infected mouse models suggest that neuronal PrP expression is necessary and sufficient for neurotoxicity and disease progression.
- We investigated the cell-type specificity of different promotors paired with a *Prnp* ZF-R at the tissue and single-cell level.

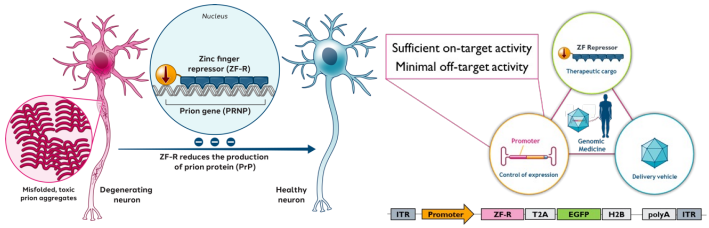


**Figure 1. Therapeutic Strategy using ZF-R for prion disease**

## hSYN1-ZF-R specifically reduces neuronal *Prnp* mRNA expression in mouse cortex

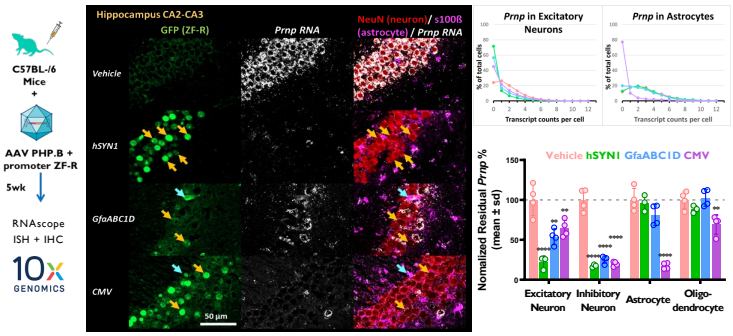


**Figure 2. RNAscope and transcriptomic analysis at the single-cell level**

- RNAscope ISH (ZF-R, *Prnp*) with immunohistochemistry (GFP, NeuN, S100β) showed that a strong negative correlation between ZF-R and *Prnp* expression was observed throughout the brain for all promoters. In all brain regions examined, the hSYN1 promoter resulted in neuron-specific expression (yellow arrows), the CMV promoter drove heterogenous expression primarily in neurons and astrocytes, and the GfaABC1D promoter showed minimal expression in astrocytes (blue arrows) and weak expression in neurons.
- Single nucleus 10x transcriptomic analysis of mouse cortex revealed promoter-dependent specificity of *Prnp* repression for neurons and glia. *Prnp* reduction was observed for all groups in both excitatory and inhibitory neurons, with the hSYN1 resulting in the most potent and selective effect. The GfaABC1D group displayed no significant reduction of *Prnp* in glial cells.

## Acknowledgement and Disclosure

This work was funded by Sangamo Therapeutics, the CJD Foundation, and the Prion Alliance.
All listed Sangamo authors are current or former employees of Sangamo Therapeutics.

## Widespread CNS-specific *Prnp* mRNA lowering and >50% PrP protein reduction in brain and CSF

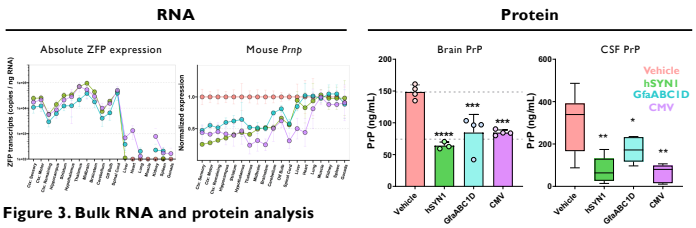


**Figure 3. Bulk RNA and protein analysis**

- Adult wildtype mice treated with different AAV-ZF-R constructs showed a significant reduction of *Prnp* mRNA expression at the bulk level in brain and spinal cord (hSYN1 ≥ CMV > GfaABC1D) via RT-qPCR analysis, depending on the region analyzed.
- hSYN1 promoter achieved specific ZF-R expression and *Prnp* repression in the brain and spinal cord; whereas CMV and GfaABC1D were expressed and active in peripheral tissues.
- The hSYN1-ZF-R achieved >50% of bulk PrP protein reduction in brain and CSF.

## hSYN1-ZF-R significantly extends survival, improves weight gain, and delays plasma NfL rise

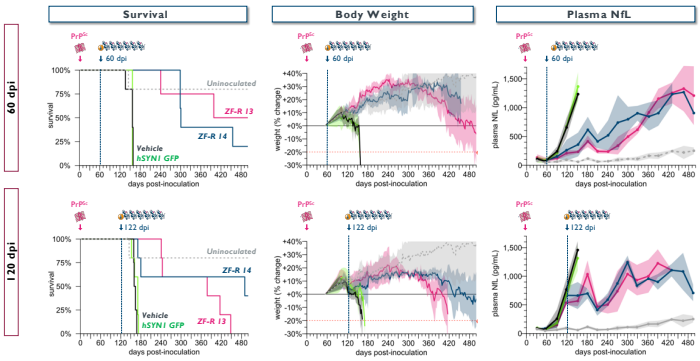


**Figure 4. Survival, body weight, and biomarker improvements following hSYN1-ZF-R administration at 60 dpi and 122 dpi**

- 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.

## hSYN1-ZF-R treatment reduces PrP deposition in surviving animals at 500 dpi

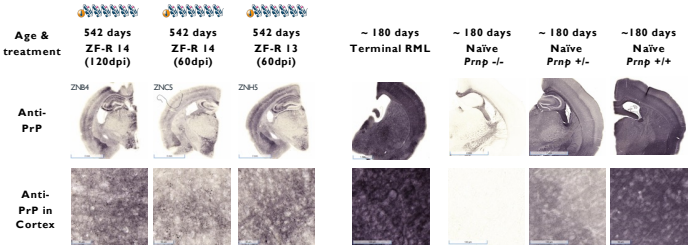


**Figure 5. Reduced $PrP^{Sc}$ levels in surviving hSYN1-ZF-R treated mice at 500 dpi**

- IHC staining for PrP on brain sections from 542-day old hSYN1-ZF-R-treated animals revealed a reduction in PrP deposition compared to untreated mice.
- Untreated ~180-day-old control mice were stained for PrP levels using the same conditions.

## Conclusions and next steps

- Neuronally restricted hSYN1-ZF-R expression and widespread reduction of *Prnp* RNA and PrP protein extended survival in RML-inoculated mice.
- We observed widespread CNS biodistribution and repression in nonhuman primates using a potential clinically translatable BBB-penetrant capsid to deliver a non-*PRNP* targeted tool hSYN1-ZF-R, supporting the potential clinical translation of the ZF-R approach.
- Highly specific human *PRNP* ZF-Rs are currently in late-stage preclinical development.