Epigenetic Regulation of Human Prion Expression as a Potential One-Time Treatment for Prion Disease

Shih-Wei Chou[1], Kimberly Marlen[1], David Ojala[1], Patrick Dunn[1], Giulia Cisbani[2], Finn Peters[2], Chiara Melis[2], Jing Hu[1], Qi Yu[1], Sarah Hinkley[1], Mohad Mehrabian[1], Toufan Parman[1], Marina Falaleeva[1], Alaric Falcon[1], Marian Glynn[1], Kathleen Meyer[1], Amy M Pooler[1], Bryan Zeitler[1]

[1]Sangamo Therapeutics Inc., 501 Canal Blvd, Richmond, CA 94804, USA [2]Evotec SE, Hamburg, Germany

Poster #1616

Introduction

- 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*.
- 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 (ZFRs) to achieve sustained and widespread reduction of PrP in the brain and rapid pharmacological effect.

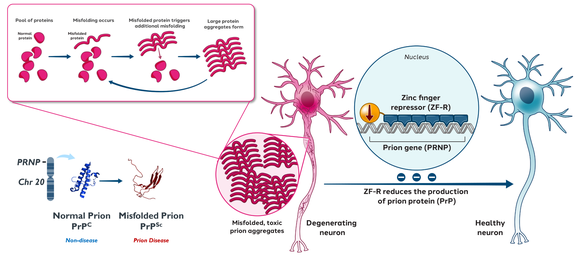


Figure 1. Schematic diagram of prion disease and PrP-lowing approach

ZF-Repressor genomic medicine for prion disease

ZFR packaged into STAC-BBB vector | *One-time IV administration* | *Stable PrP^C repression in the brain*

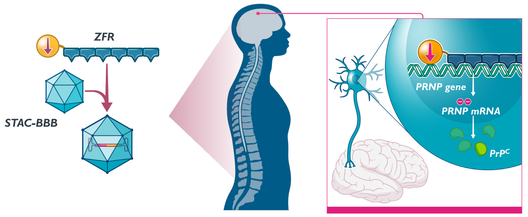


Figure 2. AAV ZFR genomic medicine for the treatment of prion disease

- A ZFR targeting the prion gene is packaged into STAC-BBB, which crosses the blood brain barrier (BBB) following a one-time IV administration.
- The ZFR represses *PRNP* transcription, resulting in specific depletion of neuronal PrP^C protein.

Disclosures

This work was funded by Sangamo Therapeutics. All listed Sangamo authors are current or former employees of Sangamo Therapeutic. Tg25109 mice were kindly provided by Dr. E. Minikel and Dr. S. Vallabh from the Broad Institute of MIT and Harvard.

Highly potent and specific ZFRs targeting *PRNP*

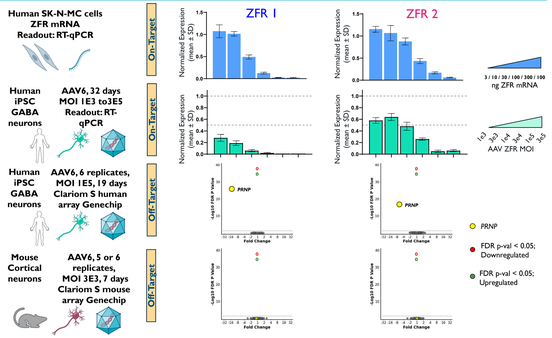
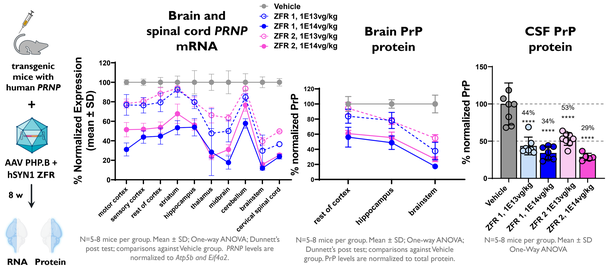


Figure 3. Characterization of potent and specific ZFRs targeting human *PRNP*

- Candidates ZFR 1 and 2 potently and specifically reduced *PRNP* in human neuroblastoma (SK-N-MC) cells and iPSC-derived GABA neuron culture.
- No off-target activity was detected in human iGABA nor mouse cortical neuron culture.

ZFRs achieve 50-90% prion reduction in the mouse brain, spinal cord and CSF following IV delivery



N=5-8 mice per group. Mean ± SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group. *PRNP* levels are normalized to *Atp5b* and *Eif4a2*.

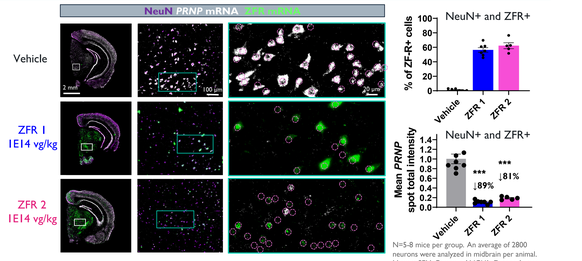
N=5-8 mice per group. Mean ± SD; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group. PrP levels are normalized to total protein.

N=5-8 mice per group. Mean ± SD One-Way ANOVA

Figure 4. Widespread reduction of prion mRNA and protein in the CNS of a humanized *PRNP*-expressing transgenic mouse model (Tg25109)

- Dose-dependent prion repression was observed for both ZFRs. At 1E14 vg/kg, AAV hSYN1-ZFR 1 or ZFR 2 delivered to Tg mice resulted in 50-90% repression of bulk *Prnp* mRNA levels across ten Central Nervous System (CNS) regions compared to vehicle controls.
- Both constructs led to >70% bulk PrP protein knockdown in the brainstem.
- In the CSF, ~70% PrP protein knockdown was observed, suggesting the majority of CSF PrP is derived from neurons.

Potent single-neuron *PRNP* repression in the brain



N=5-8 mice per group. An average of 2800 neurons were analyzed in midbrain per animal. Mean ± SEM; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group.

Figure 5. Selective reduction of *PRNP* in neurons *in vivo*

- Multiplexed RNAscope (*PRNP, ZFR*) + IHC (NeuN) revealed potent and selective prion transcript repression in Neun+ neuron through out the brain.
- In the midbrain region, efficient ZFR expression was detected in ~60% of NeuN+ cells. For both test articles, ZFR+ midbrain neurons showed significantly lower *PRNP* transcript expression (~90% repression at single-cell level) compared to abundant expression observed in vehicle-treated mice.

STAC-BBB efficiently delivers a prion-targeted ZFR to the adult NHP brain following IV delivery

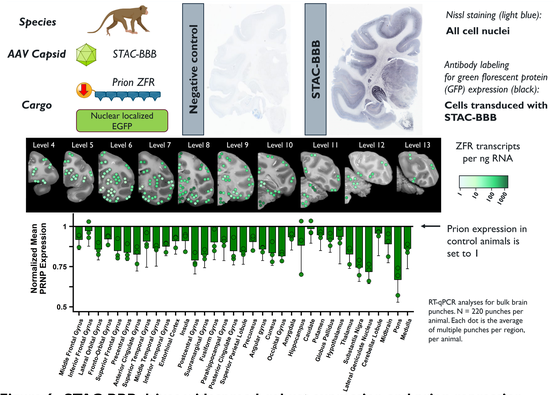


Figure 6. STAC-BBB drives widespread, robust expression and prion repression throughout the adult NHP brain

- IHC (Anti-GFP) demonstrated broad transgene expression in the NHP brain 19 days after IV administration of STAC-BBB at a 2E13 vg/kg dose.
- RT-qPCR analyses revealed ZFR expression and *PRNP* repression in all 35 brain regions tested, with the greatest reduction observed in the pons, lateral geniculate nucleus, and substantia nigra.

Effective *PRNP* repression in ZFR transduced neurons of adult non-human primates

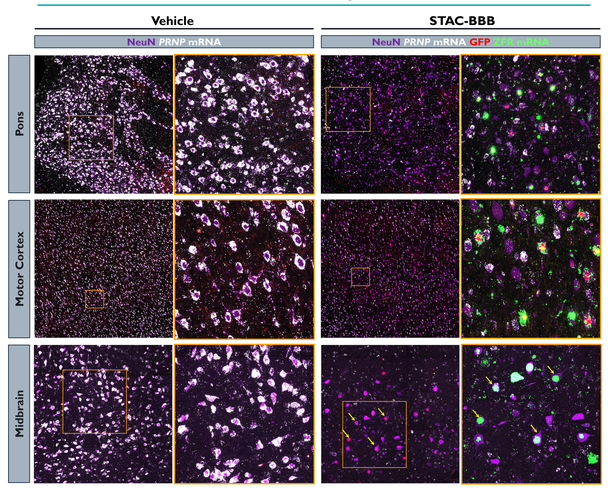


Figure 7. STAC-BBB efficiently transduces the NHP CNS following IV administration and enables ZFR-mediated *PRNP* repression in neurons throughout the brain

- Multiplexed RNAscope (*PRNP, ZFR*) with IHC (NeuN, GFP) revealed that prion transcripts were repressed in transduced neurons across multiple brain regions in animals treated with STAC-BBB encoding a *PRNP*-targeted ZFR, but not the vehicle treated animals.
- Examples of transduced neurons (yellow arrows) in representative images of the midbrain.
- Despite the use of a non-specific promoter (CAG) in this study, *PRNP* repression and ZFR expression was predominantly observed in Neun+ cells, suggesting that STAC-BBB is primarily neurotropic.

Conclusion and next steps

- ZFRs potently and specifically repress human *PRNP in vitro* in human iPSC-derived neurons.
- Neuronal promoter driven ZFRs potently reduce prion mRNA and protein expression in human *PRNP* transgenic mice. The ZFRs were well tolerated following systemic administration and showed no signs of pathology or toxicity in brain.
- Intravenous administration of STAC-BBB delivering a prion-targeted ZFR to adult NHPs resulted in widespread neuronal ZFR expression. Repression of *PRNP* mRNA was detected at the bulk tissue and single-cell level throughout the brain.
- Ongoing quantification will characterize % transduction of neuronal vs non-neuronal cells and *PRNP* repression at the single-cell level in different brain regions.
- This work supports the initiation of an IND-enabling toxicology study for a STAC-BBB delivered ZFR for the potential treatment of prion disease.

Presented at ASGCT 2024