ST-506 mediates brainwide lowering of prion protein in nonhuman primates via a conserved blood-brain-barrier crossing mechanism, as a potential one-time treatment for prion disease

[1] Sangamo Therapeutics, Inc., [2] Evotec SE, [3] Broad Institute of MIT and Harvard

Shih-Wei Chou[1], Kimberly Marlen[1], Matthew Tiffany[1], Toufan Parman[1], Patrick Dunn[1], Gillian Houlihan[1], Jing Hu[1], Marina Falaleeva[1], Jenny Hodges[1], Meredith A Pokatayev[3], Giulia Cisbani[2], Finn Peters[2], Qi Yu[1], Daniel Chung[1], Mihika Jalan[1], Jisoo Lee[1], Matthew Mendel[1], Satria Sajuthi[1], Madelena Nguyen[1], Yolanda Santiago[1], Tim Fieblinger[2], Chiara Melis[2], Sarah Mueller[1], Annemarie Ledeboer[1], Mohad Mehrabian[1], Lei Zhang[1], Sarah Hinkley[1], Hung Tran[1], Jason Eshleman[1], Carolyn Gasper[1], Alissa Coffey[3], Sonia M Vallabh[3], Eric Vallabh Minikel[3], Yanmei Lu[1], Amy M. Pooler[1], Kathleen Meyer[1], David Ojala[1], Bryan Zeitler[1]

Zinc finger repressor for prion protein (PrP) lowering

Prion disease is a progressive and fatal neurodegenerative disorder arising from misfolding of the normal cellular prion protein (PrP^C) into a pathogenic "Scrapie" form (PrP^{Sc}). While there is currently no approved treatment, the histopathological, biochemical, and genetic evidence all converge on PrP^C as the primary drug target for treating prion disease.

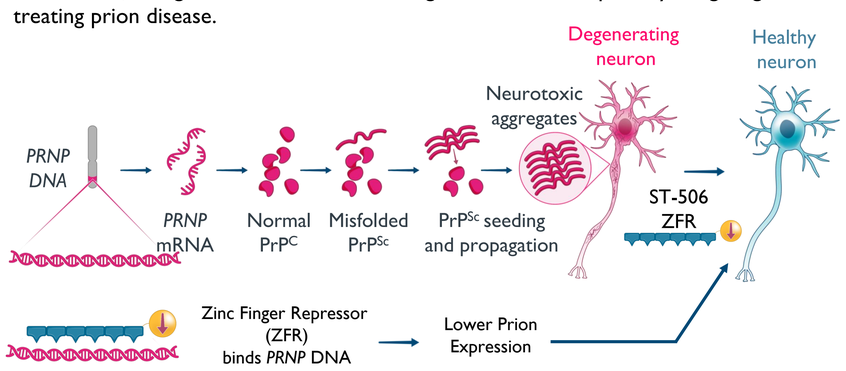

Figure 1. AAV-ZFR lowers prion protein expression to slow and prevent disease progression caused by misfolded, neurotoxic aggregates.

ST-506 for the treatment of prion disease

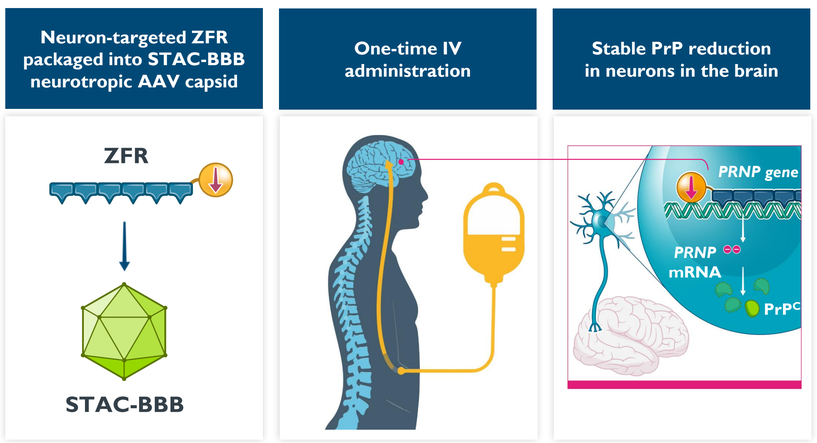

Figure 2. AAV ZFR genomic medicine for the treatment of prion disease
We are developing a one-time, intravenously (IV) administered AAV-based therapy using a ZFR, ST-506, to achieve sustained epigenetic downregulation of prion protein (PrP^C) expression in neurons throughout the brain as a potential therapeutic for prion disease. Our novel engineered AAV capsid, STAC-BBB, results in ~700x increased brain expression and up to 100x liver de-targeting compared to AAV9.

ZFR-mediated potent *Prnp* mRNA repression in neurons of mouse brain

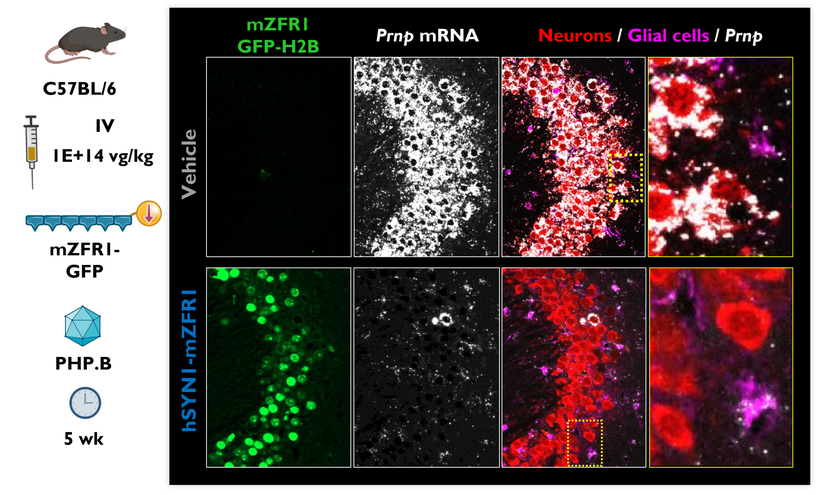

Figure 3. Selective reduction of *PRNP* in neurons *in vivo*
Multiplexed RNAscope (*PRNP*) + IHC (NeuN, GFP, s100β) revealed potent, selective *Prnp* mRNA repression in NeuN+ neurons but not S100β+ glial cells throughout the brain.

ZFR-mediated reduction of prion mRNA and protein persisted long-term (17 months) in the mouse brain

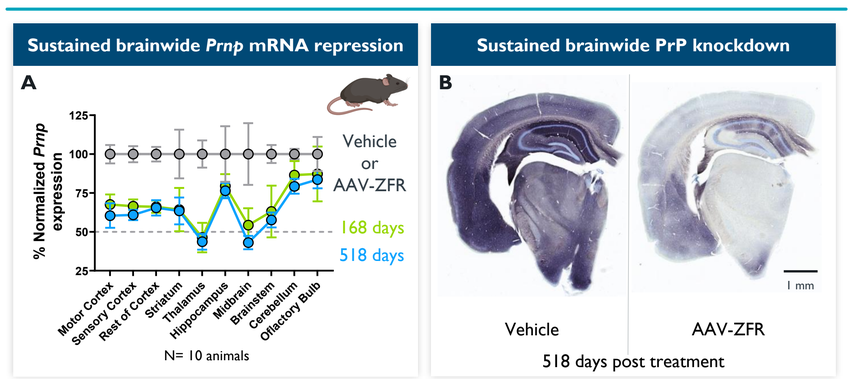

Figure 4. Effect of ZFR-mediated *Prnp* mRNA and PrP protein reduction is persistent and widespread in the mouse brain
(A) Mean % normalized *Prnp* mRNA reduction ranged from 17-57% as measured by RT-qPCR for 10 brain regions at 168 and 518 days after IV administration of PHP.B-hSYN1-mZFR1 at 3E+13 vg/kg. Vehicle animals were collected from 168 days post dosing. Repression at 518 days was equivalent or greater than that observed at 168 days for all regions. **(B)** Representative images from immunohistochemistry analysis showed reduced anti-PrP staining (black) in the brains of mice treated with PHP.B-hSYN1-mZFR1 (1E+14 vg/kg) after 518 days compared to vehicle treatment.

Post-symptomatic AAV-ZFR treatment profoundly extends survival of RML-inoculated mice

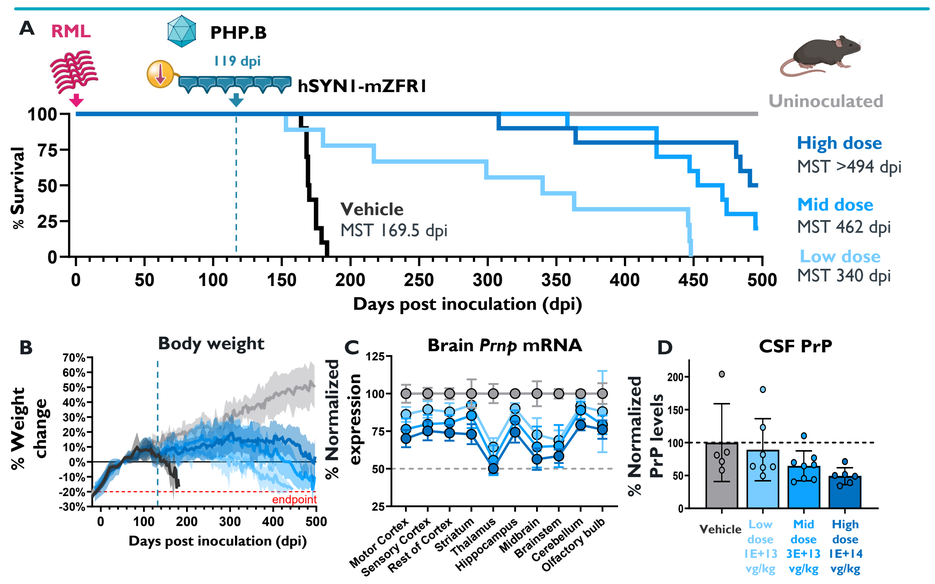

Figure 5. Dose-dependent prion repression by ZFR in wildtype mice correlated with the extension of median survival time in ZFR-treated RML-infected mice
(A) Median survival time (MST) of RML-inoculated mice was extended from 169.5 dpi in vehicle-treated animals to 340, 462, and >494 dpi for low (1E+13 vg/kg), mid (3E+13 vg/kg), and high (1E+14 vg/kg) dose treated animals, respectively. **(B)** In ZFR-treated mice, body weight loss after RML inoculation was regained and subsequently maintained or declined at a slower rate. Similar dose-dependent effects were observed in wildtype mice for the repression levels of **(C)** *Prnp* mRNA across 10 brain regions and **(D)** PrP protein in cerebrospinal fluid (CSF).

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

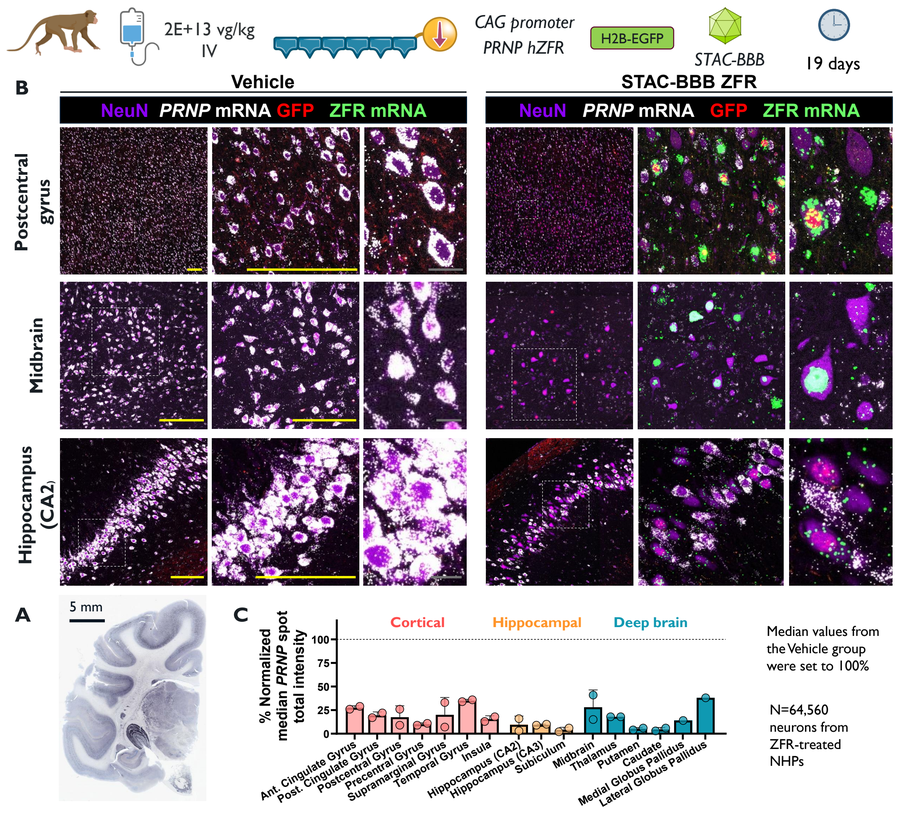

Figure 6. Widespread and robust *PRNP* mRNA repression at the single-neuron level following IV administration of STAC-BBB hZFR
(A) IHC (Anti-GFP) demonstrated broad transgene expression in the NHP brain 19 days after IV administration of STAC-BBB at a 2E+13 vg/kg dose. **(B)** Multiplexed RNAscope (*PRNP*, ZFR) combined with IHC (NeuN, GFP) demonstrated that prion transcripts were repressed in transduced neurons across multiple brain regions in STAC-BBB–treated animals, but not in vehicle-treated controls. Despite using a non-specific CAG promoter, STAC-BBB showed neurotropism. **(C)** Quantification of median *PRNP* transcript signal in 16 different brain regions revealed an average knockdown ranging from 62-96% in ZFR+ NeuN+ cells (n = 64,560 cells).

Cross-species performance and utilization of a conserved receptor support the translational potential of STAC-BBB in humans

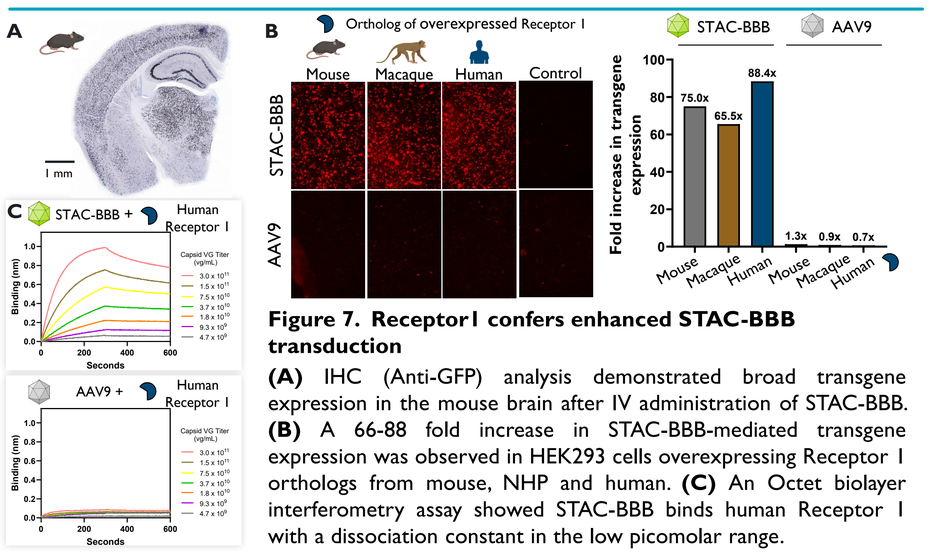

Figure 7. Receptor1 confers enhanced STAC-BBB transduction
(A) IHC (Anti-GFP) analysis demonstrated broad transgene expression in the mouse brain after IV administration of STAC-BBB. **(B)** A 66-88 fold increase in STAC-BBB-mediated transgene expression was observed in HEK293 cells overexpressing Receptor 1 orthologs from mouse, NHP and human. **(C)** An Octet biolayer interferometry assay showed STAC-BBB binds human Receptor 1 with a dissociation constant in the low picomolar range.

Clinical candidate ST-506 ZFR enables potent and specific neuronal *PRNP* repression

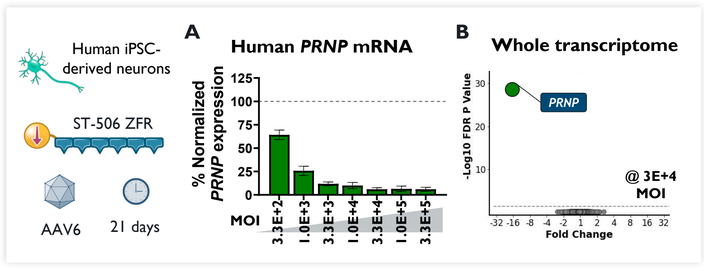

Figure 8. Potent lowering of human *PRNP* by >90% with no detectable off-targets in iPSC-derived neurons
(A) The ST-506 clinical candidate ZFR induced *PRNP* mRNA repression across a wide dose range in human iGABA neurons. **(B)** Specificity analysis by Clariom S gene arrays showed no off-target genes were dysregulated using a False Discovery Rate (FDR) *P* value <0.05.

Clinical candidate ST-506 drives broad *PRNP* and PrP lowering across the NHP brain

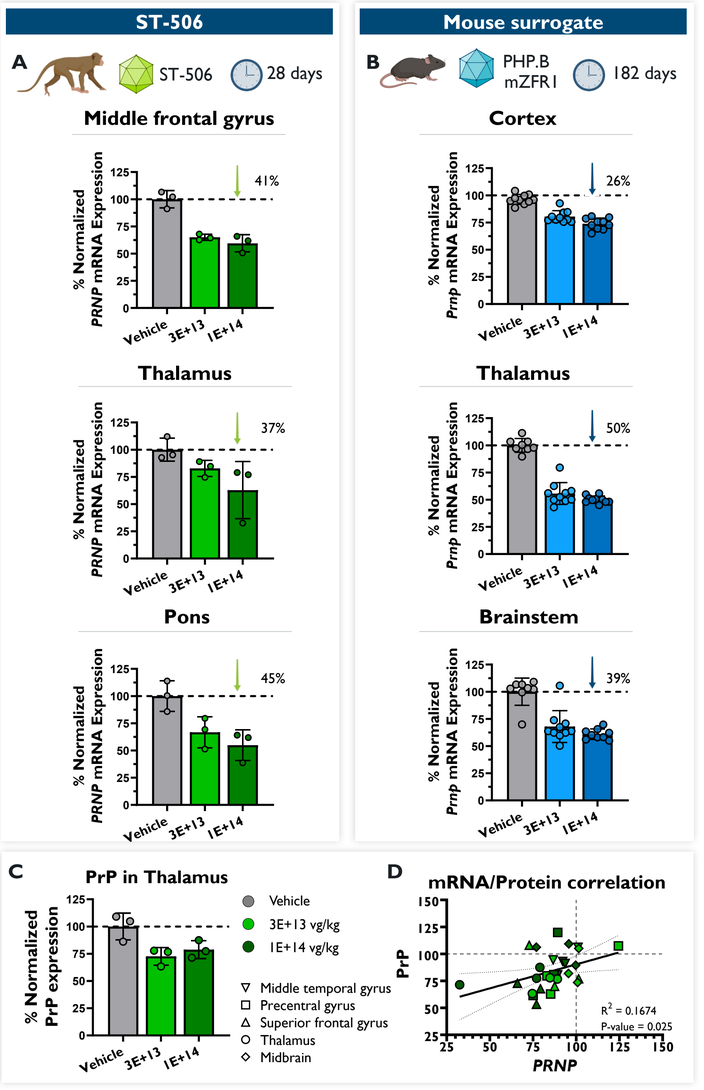

Figure 9. ST-506 mediates prion repression in NHPs at levels comparable to, or greater than, those associated with substantial survival extension in mice
(A) Relative to the vehicle control group, NHPs administered ST-506 at a dose of 1E+14 vg/kg exhibited mean *PRNP* mRNA repression of approximately 41%, 37%, and 45% in middle frontal gyrus, thalamus, and pons, respectively. N=3 NHP per group; multiple punches from each brain region. Across all 35 brain regions analyzed, the average of mean *PRNP* mRNA repression is 21 ± 11.6% (mean ± SD) **(B)** RT-qPCR data from the mouse study (Fig. 5) is replotted for individual brain regions (N = 10 mice), demonstrating ST-506 achieves similar or greater *PRNP* knockdown levels in NHPs to those observed in mZFR1-treated mice with profound survival extension. **(C)** ST-506 mediated PrP protein reduction in the thalamus at both dose levels 28 days after dosing (N = 3 NHP per group). **(D)** PrP protein levels were measured in 4 other brain regions: middle temporal gyrus, precentral gyrus, superior frontal gyrus and midbrain. PrP protein reduction correlated with *PRNP* mRNA levels in these regions. Finally, ST-506 was safe at both dose levels with no adverse microscopic pathology findings, including liver and DRG.

Conclusion

- ZFRs mediate potent, sustained, and specific prion gene repression.
- Neuronal promoter driven ZFRs potently reduced *Prnp* and PrP expression for at least 17 months and profoundly extended survival in RML-infected mice when dosed post-symptomatically.
- The STAC-BBB capsid enables widespread brain delivery and *PRNP* reduction in NHPs at the bulk and single-cell levels.
- Cross-species activity of STAC-BBB and utilization of a conserved receptor underscores the clinical potential for treating human prion disease.
- Clinical candidate ST-506 mediates widespread *PRNP* mRNA and PrP protein reduction in the nonhuman primate brain with a robust safety profile.
- We anticipate a clinical trial application (CTA) submission for the ST-506 clinical study as early as mid-2026.

Presented at Prion 2025 Conference, November 3-7, 2025

Chou et al, 2025
Zinc Finger Repressors mediate widespread *PRNP* lowering in the nonhuman primate brain and profoundly extend survival in prion disease mice
bioRxiv