Single-cell characterization of ST-506, a BBB-penetrant epigenetic repressor of prion protein expression, in the nonhuman primate brain

Poster #1469

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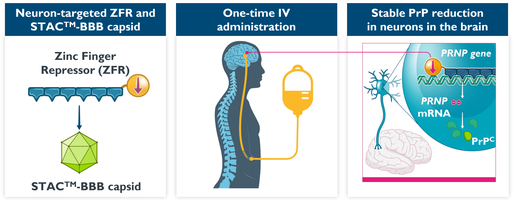
Introduction



Prion disease is a rapidly progressive and fatal neurodegenerative disorder driven by the conformational conversion of the normal cellular prion protein, PrP^{C}, into its misfolded, pathogenic isoform, PrP^{Sc}. There are over 1,500 new cases each year in the US, Europe, and Japan, and no currently available disease-modifying treatments. Genetic, biochemical, and pathological evidence supports PrP^{C} reduction as a primary therapeutic strategy. We are advancing our clinical candidate, ST-506, for the treatment of prion disease. ST-506 is a one-time intravenously delivered, AAV based neuron-specific gene therapy that employs a zinc finger repressor (ZFR) directed towards the human prion gene (*PRNP*) packaged in the blood-brain barrier (BBB) penetrant capsid STAC™-BBB, to achieve sustained brain-wide downregulation of PrP^{C}. Previously, we demonstrated that post-symptomatic treatment of RML-inoculated mice with a neuron-targeted surrogate ZFR profoundly extended survival (>494 days post-inoculation) and reduced PrP for >17 months **(Figure 1)**. Here, we evaluate the effect of ST-506 in nonhuman primates (NHP) in suppressing PrP expression at the single-cell level.

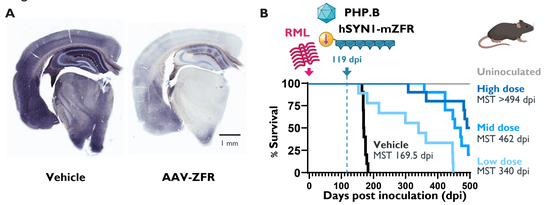


Figure 1. Persistent ZFR-mediated PrP reduction extends survival of RML-inoculated mice in a dose-dependent manner when given after symptom onset

(A) Immunohistochemistry (IHC) analysis showed reduced anti-PrP staining (black) in the brains of mice treated with PHP.B-hSYN1-mZFR (1E+14 vg/kg) after 518 days compared to vehicle treatment in wildtype mice. **(B)** 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.

ST-506 ZFR strongly reduces *PRNP* in human neurons

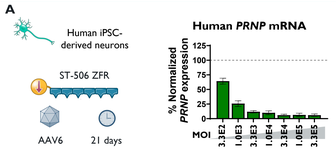


Figure 2. Potent lowering of human *PRNP* by >90% in iPSC-derived neurons

(A) The ST-506 ZFR induced *PRNP* mRNA repression across a wide dose range in human iGABA neurons.

Single-cell RNAscope spatial analysis reveals widespread neuronal ZFR expression in NHP brain

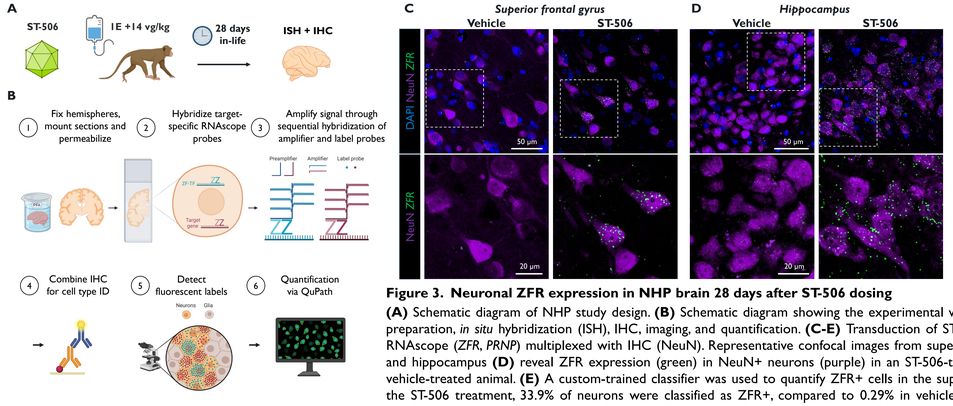


Figure 3. Neuronal ZFR expression in NHP brain 28 days after ST-506 dosing

(A) Schematic diagram of NHP study design. **(B)** Schematic diagram showing the experimental workflow from sample preparation, *in situ* hybridization (ISH), IHC, imaging, and quantification. **(C-E)** Transduction of ST-506 was assessed by RNAscope (*ZFR*, *PRNP*) multiplexed with IHC (NeuN). Representative confocal images from superior frontal gyrus **(C)** and hippocampus **(D)** reveal ZFR expression (green) in NeuN+ neurons (purple) in an ST-506-treated animal but not vehicle-treated animal. **(E)** A custom-trained classifier was used to quantify ZFR+ cells in the superior frontal gyrus. In the ST-506 treatment, 33.9% of neurons were classified as ZFR+, compared to 0.29% in vehicle treatment. The total NeuN+ cells analyzed were 5128 and 7466 for ST-506 and vehicle treatments, respectively.

ST-506 mediates potent *PRNP* mRNA repression in neurons throughout the NHP brain

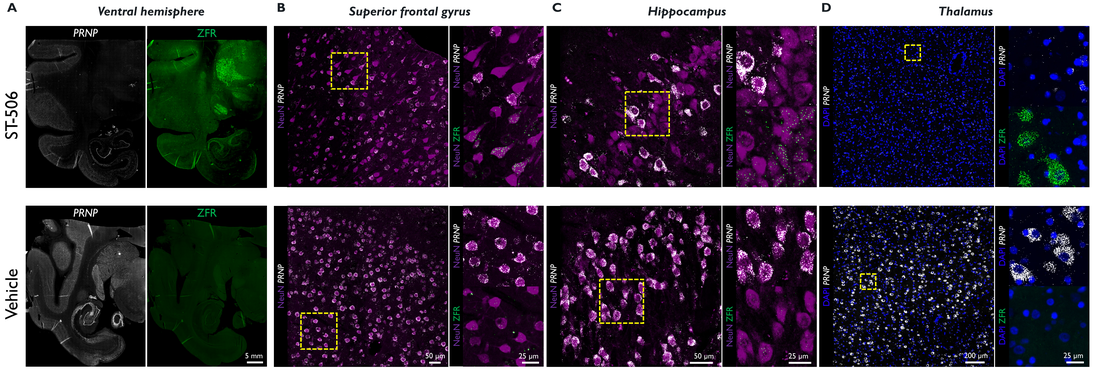
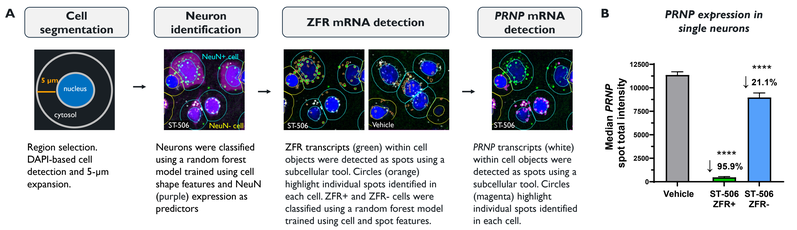


Figure 4. *PRNP* mRNA expression is potently reduced in ST-506 transduced NHP neurons

Representative fluorescent images from ST-506- (top panel) and vehicle- (bottom panel) treated NHP brain sections stained for ZFR transcripts (green), *PRNP* transcripts (white), NeuN neuronal marker (purple), and DAPI (blue) demonstrate widespread ST-506-mediated *PRNP* reduction. **(A)** Ventral coronal section and enlarged views of **(B)** superior frontal gyrus, **(C)** hippocampus, and **(D)** thalamus[1] show neurons expressing ZFR (green dots) lack detectable *PRNP* (white dots) in an ST-506-treated animal, but not in the vehicle control. [1]NeuN staining in the thalamus was below detection and requires further optimization.

Single-cell detection of ZFR and *PRNP* mRNA levels reveals >95% *PRNP* transcript knockdown within individual neurons transduced with ST-506



Region selection. DAPI-based cell detection and 5-µm expansion.

Neurons were classified using a random forest model trained using cell shape features and NeuN (purple) expression as predictors

ZFR transcripts (green) within cell objects were detected as spots using a subcellular tool. Circles (orange) highlight individual spots identified in each cell. ZFR+ and ZFR- cells were classified using a random forest model trained using cell and spot features.

PRNP transcripts (white) within cell objects were detected as spots using a subcellular tool. Circles (magenta) highlight individual spots identified in each cell.

Figure 5. Workflow and quantification of ST-506 mediated neuronal ZFR and *PRNP* expression

(A) Step-wise single-cell quantification using Qupath. **(B)** Median *PRNP* spot total intensity in NeuN+ cells classified as ZFR+ or ZFR-. N = 7422, 1736, 3392 NeuN+ cells for vehicle, ST-506 ZFR+, and ST-506 ZFR-, respectively. **** $P < 0.0001$ with Mann Whitney test. Plotted values correspond to fluorescent intensity measurements from cells in superior frontal gyrus. Median ± 95% CI.

ST-506 achieves >95% *PRNP* repression across a wide range of ZFR expression levels within individual neurons

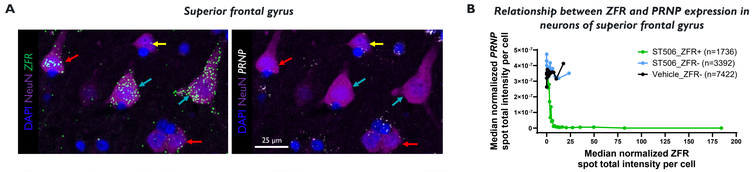


Figure 6. Robust *PRNP* repression by ST-506 across neuronal ZFR expression levels

(A) Representative ISH/IHC images show heterogeneous ZFR expression with uniformly low *PRNP* levels in individual neurons. Arrows in blue, red, and yellow are cells with high, low, and no ZFR detection, respectively. **(B)** *PRNP* mRNA expression in the superior frontal gyrus is shown as the median normalized *PRNP* spot total intensity per cell (y-axis) after correcting for imaging batch effects and cell size. Values were binned into 20 equal quantiles. In ZFR- neurons, *PRNP* expression is comparable between vehicle- (black line) and ST-506-treated (blue line) samples, indicating minimal *PRNP* repression in the absence of detectable ZFR. In contrast, ZFR+ neurons from ST-506-treated samples (green line) exhibit robust *PRNP* repression at nearly all measured ZFR expression levels. Notably, over 55% of ST-506-treated ZFR+ neurons display near-zero *PRNP* expression, regardless of increasing ZFR spot total intensity per cell, demonstrating potent *PRNP* suppression across neuronal ZFR expression levels.

Conclusion

- ZFRs mediated potent PrP reduction and significantly extended survival in RML-inoculated mice when dosed after symptom onset.
- Previously, we showed that ST-506 mediates bulk mRNA and protein knockdown throughout the brain in NHPs at levels comparable to those associated with substantial survival extension in RML mice.
- Using our in-house ISH/IHC assay to enable single-cell assessment of ST-506 biodistribution and prion repression in the NHP brain, we demonstrated *PRNP* expression was reduced by >95% in ST-506 expressing neurons.
- Widespread ST-506 expression was observed throughout the NHP brain.
- ST-506 dosed at 1E+14 vg/kg was well tolerated in NHP over 28 days of follow up.
- The potent and specific *PRNP* repression in NHP combined with the good tolerability support the potential of ST-506 for the treatment of symptomatic prion disease in the clinic.

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