SPK-10001 AAV-based microRNA mediates non-allele specific reduction of *HTT* mRNA through RNA interference, demonstrating its potential for further preclinical development.

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ABSTRACT

Rationale:
Huntington's disease (HD) is a fatal neurodegenerative disorder for which there are currently no disease-modifying therapies. Clinical symptoms of HD are caused by the accumulation of misfolded and aggregation-prone mutant huntingtin protein (mHTT). Reducing the amount of mHTT in neurons should in theory slow or halt the progression of disease, especially when the therapy is initiated before neurodegeneration is advanced. Advancement of mHTT-suppressing therapies has been impeded in part because many therapeutics do not efficiently penetrate the deep brain structures initially affected by disease pathology. AAV-based vectors circumvent this problem when directly administered to affected brain regions and can potently suppress mHTT via inclusion of HTT-targeting microRNA, zinc finger proteins or similar cargo.

Approach:

SPK-10001 comprises an engineered microRNA (HTT-miR) which binds to human *HTT* mRNA with 100% complementarity and targets it for degradation. HTT-miR expression is under control of a ubiquitous promoter and the transgene is vectorized in a proprietary AAV capsid. The HTT-miR also has 100% complementarity to macaque *HTT* mRNA which enables potency testing of SPK-10001 in non-human primates. SPK-10001 is designed to be delivered by direct injection to the caudate and putamen thereby providing durable and potent suppression of *HTT* mRNA and protein. The HTT-miR targets a region outside the exon 1 trinucleotide expansion and it leads to the reduction of both normal and expanded HTT protein in non-clinical species or human patients in which both mRNA are expressed.

REFERENCES

1. **Pfister EL**, Chase KO, Sun H, Kennington LA, Conroy F, Johnson E, Miller R, Borel F, Aronin N, Mueller C. Mol Ther Nucleic Acids. 2017 Jun 16;7:324-334.

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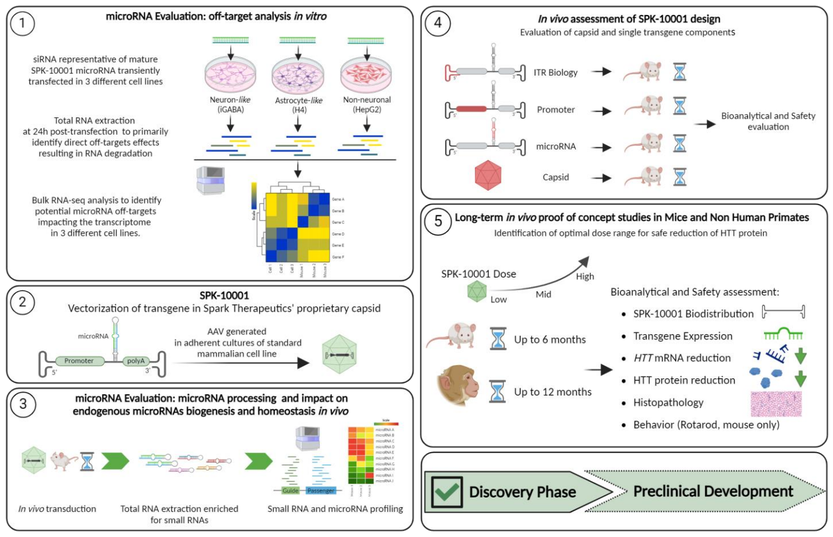
EXPERIMENTAL APPROACH



RESULTS

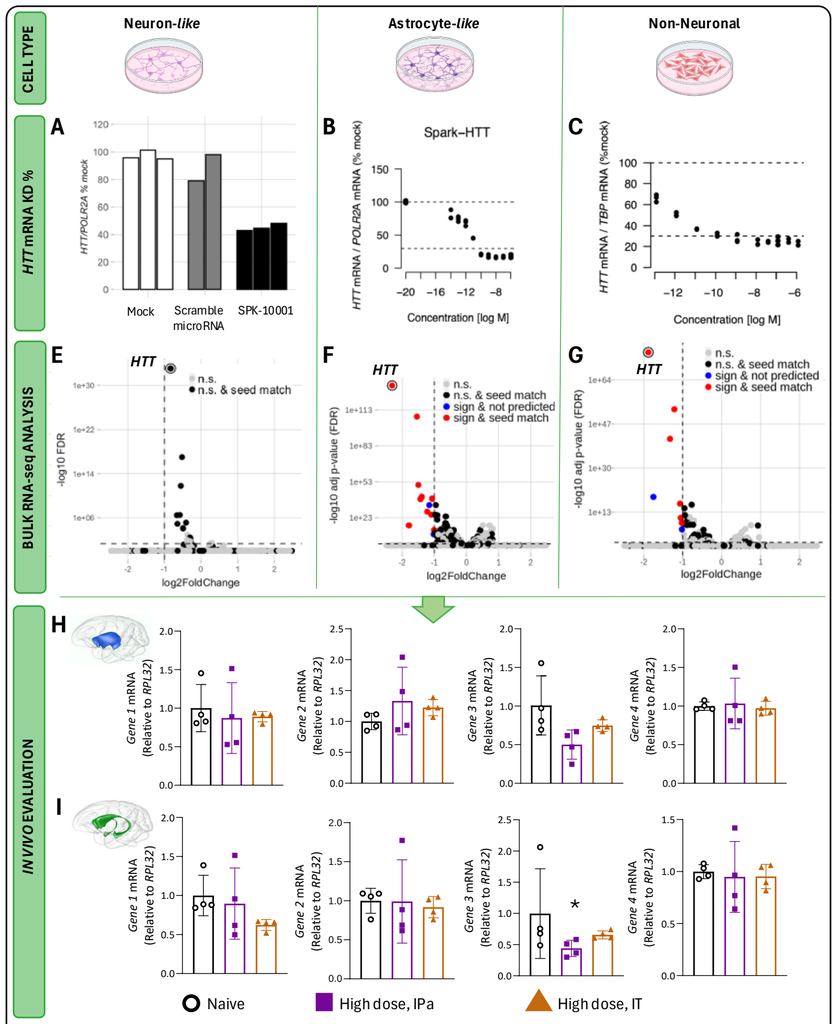


Figure 1. SPK-10001 microRNA HAS MINIMAL EFFECTS ON NON-TARGET mRNAs. Summary of dose response curves of a siRNA version of SPK-10001 microRNA *in vitro*, identification of potential off-targets, and evaluation of potential off-targets *in vivo*.

A-B-C. Effects of siRNA targeting *HTT* mRNA as measured by qRT-PCR in A) iGABA cell line, B) H4 cell line, C) HepG2 cell line. siRNA was transfected at 10 different concentrations ranging from 10^{-13} up to 10^{-6} M. Total RNA was extracted after 24h post-transfection and subjected to bulk sequencing to identify off-targets impacting the transcriptome and primarily identify direct off-target effects resulting in RNA degradation. Data on graphs in Panels A, B and C represents the average of three replicate experiments for each concentration. Dashed lines at 100% and 30% to help guide the eye.

E-F-G. Volcano plots displaying differentially regulated genes in D) iGABA cell line, E) H4 cell line, F) HepG2 cell line. Significantly de-regulated genes with adj p-value (FDR) <0.05 and log2 FC < -1 are color-coded to denote predicted seed match (red) or not predicted match (blue). Non significantly de-regulated genes are color-coded based on predicted (black) or not predicted (grey) seed match. *HTT* is marked with a black ring.

H, I. Evaluation of four potential off-target mRNAs (*Gene 1*, *Gene 2*, *Gene 3*, and *Gene 4*) identified from the *in vitro* screening in NHPs Putamen (H) and Caudate (I) after intraparenchymal (IPa) or intrathecal (IT) injection of SPK-10001.

Data plotted as individual NHP (*symbols*) and group mean ± SD (*bars*). Statistical pairwise comparisons between naïve and vector-injected NHPs were assessed by 1-way ANOVA followed by a Dunnett's test and reported as follows: *p<0.05.

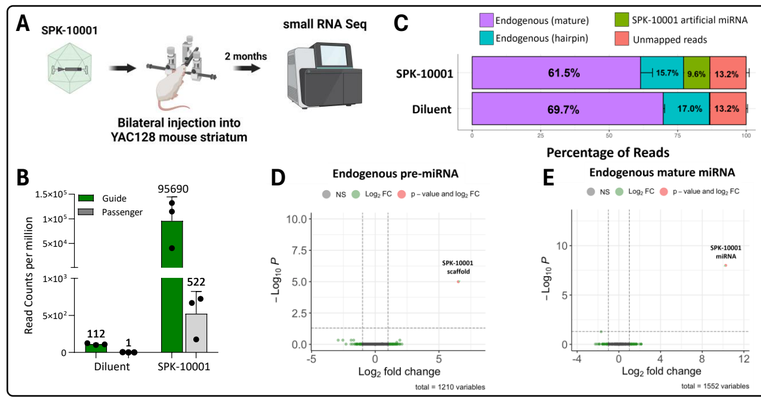
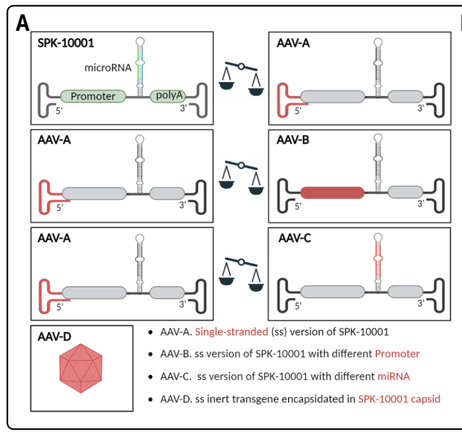


FIGURE 2. SUMMARY OF SPK-10001 microRNA PROCESSING AND EFFECTS ON ENDOGENOUS microRNA IN HD MICE.

A. Overview of 2-month study evaluating microRNA processing from SPK-10001 transgene. **B.** Guide and passenger read counts from YAC128 mice striatum injected with diluent or SPK-10001. N=3 mice/group. **C.** Read mapping of endogenous vs SPK-10001 artificial microRNA in YAC128 mouse striatum. N=3 mice/group.
D-E. Volcano plots showing changes in D) endogenous mouse pre-microRNA hairpins or E) endogenous mouse mature microRNAs after treatment with SPK-10001. N = 3 mice/group. No significant differences were found (adjusted $p < 0.05$ and $\log_2$ FC > 1).



B

HTT Protein Reduction	Impact on Behavior	Impact on histopathology	Conclusions
SPK-10001 induces higher HTT protein reduction at comparable doses	SPK-10001 induces behavior impairment **at high doses**	SPK-10001 impacts tissue integrity **at high doses**	SPK-10001 safety depends on **correct dose selection**
AAV-B reduces HTT protein **more** at comparable doses	Motor coordination **not impacted** by any AAV at any dose.	AAV-B impacts tissue integrity **at high doses**	SPK-10001 promoter is safer
AAV-C does NOT reduce HTT protein (by design. Scrambled microRNA)	Motor coordination **not impacted** by any AAV at any dose.	Tissue integrity **not impacted** by any AAV at any dose.	SPK-10001 RNA hairpin is safe
AAV-D does NOT reduce HTT protein (by design)	Motor coordination **not impacted** by AAV-D at any dose.	Tissue integrity **not impacted** by AAV-D at any dose.	SPK-10001 capsid is safe

FIGURE 3. ALL TRANSGENE COMPONENTS OF SPK-10001 ARE SAFE AND WELL TOLERATED IN YAC128 MICE. Summary of SPK-10001 capsid and single transgene components evaluation in HD mice to identify potential cause for behavioral and tissue integrity impairment after injection of high doses in POC studies.

A. Schematic of experimental comparisons between SPK-10001 and four additional AAVs (A-B-C-D) which differ from SPK-10001 for only one component (*red*). **B.** Summary of pharmacology and safety results 3 months after injection (N=8 mice/group).

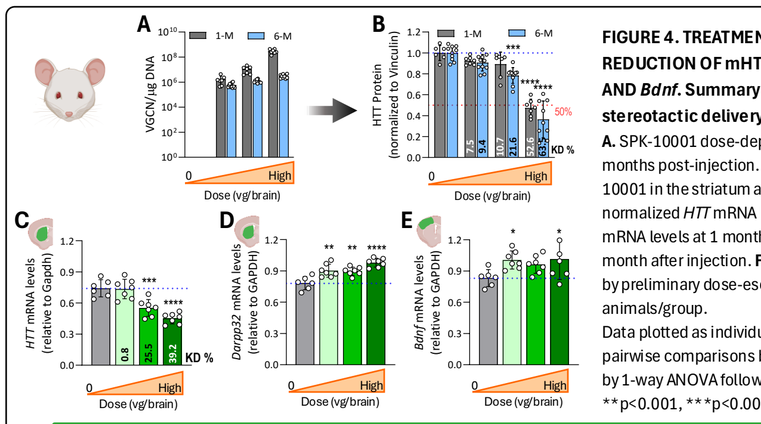


FIGURE 4. TREATMENT OF YAC128 MICE WITH SPK-10001 INDUCES DURABLE REDUCTION OF mHTT PROTEIN AND MAINTAINS THE EXPRESSION OF *Darpp32* AND *Bdnf*. Summary of SPK-10001 pharmaco-toxicology profile after IPa stereotactic delivery to HD mice striatum.

A. SPK-10001 dose-dependent biodistribution in the striatum at 1 (grey) and 6 (blue) months post-injection. **B.** Dose-dependent mHTT protein reduction induced by SPK-10001 in the striatum at 1 (grey) and 6 (blue) months post-injection. **C.** Striatal normalized *HTT* mRNA levels at 1 month after injection. **D.** Striatal normalized *Darpp32* mRNA levels at 1 month after injection. **E.** Cortical normalized *Bdnf* mRNA levels at 1 month after injection. **F.** Overall SPK-10001 pharmaco-toxicology profile as determined by preliminary dose-escalation studies. N=6 (1-month study) -15 (6-month study) animals/group.
Data plotted as individual mouse (*symbols*), and group mean ± SD (*bars*). Statistical pairwise comparisons between naïve and vector-injected YAC128 mice were assessed by 1-way ANOVA followed by a Dunnett's test and reported as follows: *p<0.05, **p<0.001, ***p<0.001, and ****p<0.0001.

F

Pharmacology and toxicology drug profile assessments are purely indicative in mice due to drug delivery limitations:

- AAV Biodistribution evaluated in the striatum corresponds to persistent dose-dependent reduction of mHTT protein for up to 6 months after SPK-10001 administration.
- Behavioral impairment proportional to progressively increasing high AAV doses and independent of mHTT lowering rate.
- Histopathological findings proportional to progressively increasing high AAV doses, independent of mHTT lowering rate, and compatible with stereotactic intraparenchymal delivery.

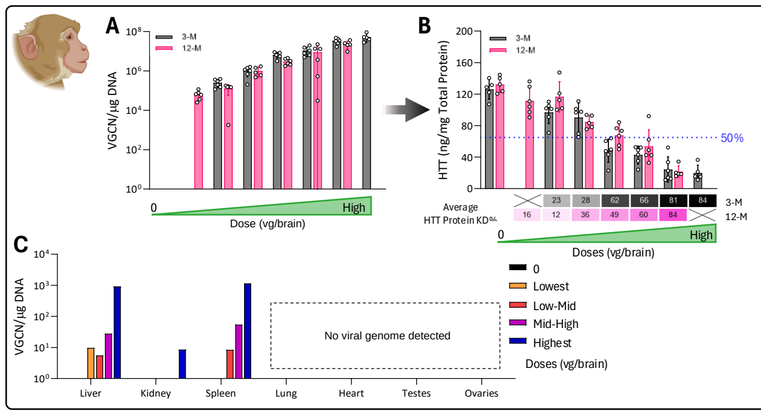


FIGURE 5. SPK-10001 INDUCES AN EFFICIENT AND DURABLE REDUCTION OF HTT PROTEIN IN NHPs. Summary of SPK-10001 pharmaco-toxicology profile after intraparenchymal CED delivery to putamen

A. SPK-10001 dose dependent biodistribution in the putamen at 3 (grey) and 12 (pink) months post-injection. **B.** Dose-dependent HTT protein reduction induced by SPK-10001 in the putamen at 3 (grey) and 12 (pink) months post-injection. **C.** SPK-10001 biodistribution in representative peripheral tissues 12 months post-injection. N=5-6 animals/group per timepoint. Data plotted as individual NHP (*symbols*) and group mean ± SD (*bars*). SPK-10001 biodistribution and HTT protein values were analyzed with statistical linear models. All pairwise comparisons were performed using Tukey's correction. No significant difference of SPK-10001 biodistribution and HTT protein reduction between same doses at different timepoints.

CONCLUSIONS

- **SPK-10001 is an engineered adeno-associated virus expressing an artificial microRNA which targets the human *HTT* mRNA for degradation**
- **SPK-10001 has minimal effects on non-target mRNAs**
- **microRNA overexpression induced by SPK-10001 did not affect endogenous microRNA biogenesis and homeostasis**
- **Durable reduction of HTT protein up to 6 months (mice) or up to 12 months (NHPs) was well tolerated**
- **High SPK-10001 vector loads used in pilot studies were associated with behavioral (mice) and histopathological (mice and NHPs) adverse events**
- **These data support further preclinical development of SPK-10001**