SPK-10001 induces a durable and safe reduction of HTT protein supporting further development in Huntington's disease

Francesca Cargnin, PhD[1], Jeremy Brower, PhD[1], Christen Simon, PhD[2], Andy Carlson, BS[2], Haiyan Ma, PhD[3], Joan Wicks, PhD[1], Brad Bolon, DVM, MS, PhD[4], George Atkins, MS[1], Xue Wang, PhD[5], Charlie Li, PhD[1], Barbara Clark, BS[1], Heena Beck, MS[1], Edward Chroscinski, VMD[1], Rachel Wang, PhD[1], John White, BS[1], Mallory Becker, MS[1], Jenn Putman, BS[1], Jodi McBride, PhD[1], Kathila Alatsis, PhD[1], Elizabeth Ramsburg, PhD[1]

[1] Spark Therapeutics, Philadelphia PA; [2] Northern Biomedical Research, Norton Shores MI; [3] Northern Biomolecular Services, Portage MI; [4] GEMpath, Longmont CO; [5] Invicro, Boston MA

ABSTRACT

SPK-10001, an AAV-based gene therapy for Huntington's disease (HD), targets Huntingtin protein (HTT) accumulation in the brain's basal ganglia. Administered via bilateral intraparenchymal (IP) infusion using MRI-guided ClearPoint SmartFlow cannula, SPK-10001 aims for durable, dose-dependent reduction of HTT protein while minimizing brain tissue disruption and ensuring neuronal tolerance.

In cynomolgus macaques, increasing doses of SPK-10001 demonstrated lasting HTT reduction and safety over 12 months, with detectable transgene in the cerebrospinal fluid (CSF) serving as a durability biomarker. MRI scans showed resolution of surgery-related injuries within two months post-injection while CSF protein analysis and histopathology confirmed minimal neuronal damage. Overall, SPK-10001's preclinical data support its further development for Huntington's disease.

ACKNOWLEDGMENTS.

The Authors are grateful for the unconditional support and collaboration demonstrated by their colleagues at Spark Therapeutics.

Illustrations created with BioRender.com

BACKGROUND

- HD is a rare neurodegenerative disorder, caused by an autosomal dominant repeat expansion (CAG) in the *HTT* gene, which leads to the production of mutant HTT protein (mHTT) up on translation
- The striatum, and later the cerebral cortex, appear to be the brain regions most susceptible to mHTT, clinically translating to progressive motor and cognitive impairment and behavioral dysfunction
- Reduction of mHTT levels in these brain regions could ameliorate mHTT downstream pathogenic effects and halt or slow down disease progression
- SPK-10001 is an artificial microRNA vectorized in an AAV (adeno-associated virus) and its designed to reduce *HTT* mRNA and protein
- An effective, safe, and less invasive surgical procedure was developed and optimized to deliver SPK-10001 to the caudate and putamen nuclei

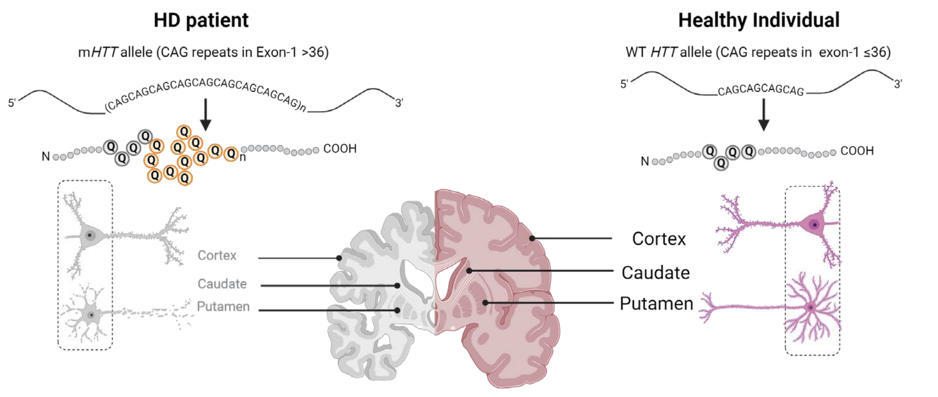


Figure 1. SCHEMATIC OF mHTT HUNTINGTON'S DISEASE PATHOLOGY AS RATIONALE FOR TARGETING THE CAUDATE AND PUTAMEN NUCLEI IN RESEARCH OF NOVEL THERAPIES FOR ITS TREATMENT

Although mHTT is produced in every brain region, the striatum and later the cortex are the most affected, suggesting that directly lowering mHTT in these brain regions could be a potential disease-modifying therapy for Huntington's disease

This study aimed to confirm the safety of surgical delivery of SPK-10001 to both the caudate and putamen, together with its durability and effectiveness at lowering HTT protein.

EXPERIMENTAL APPROACH

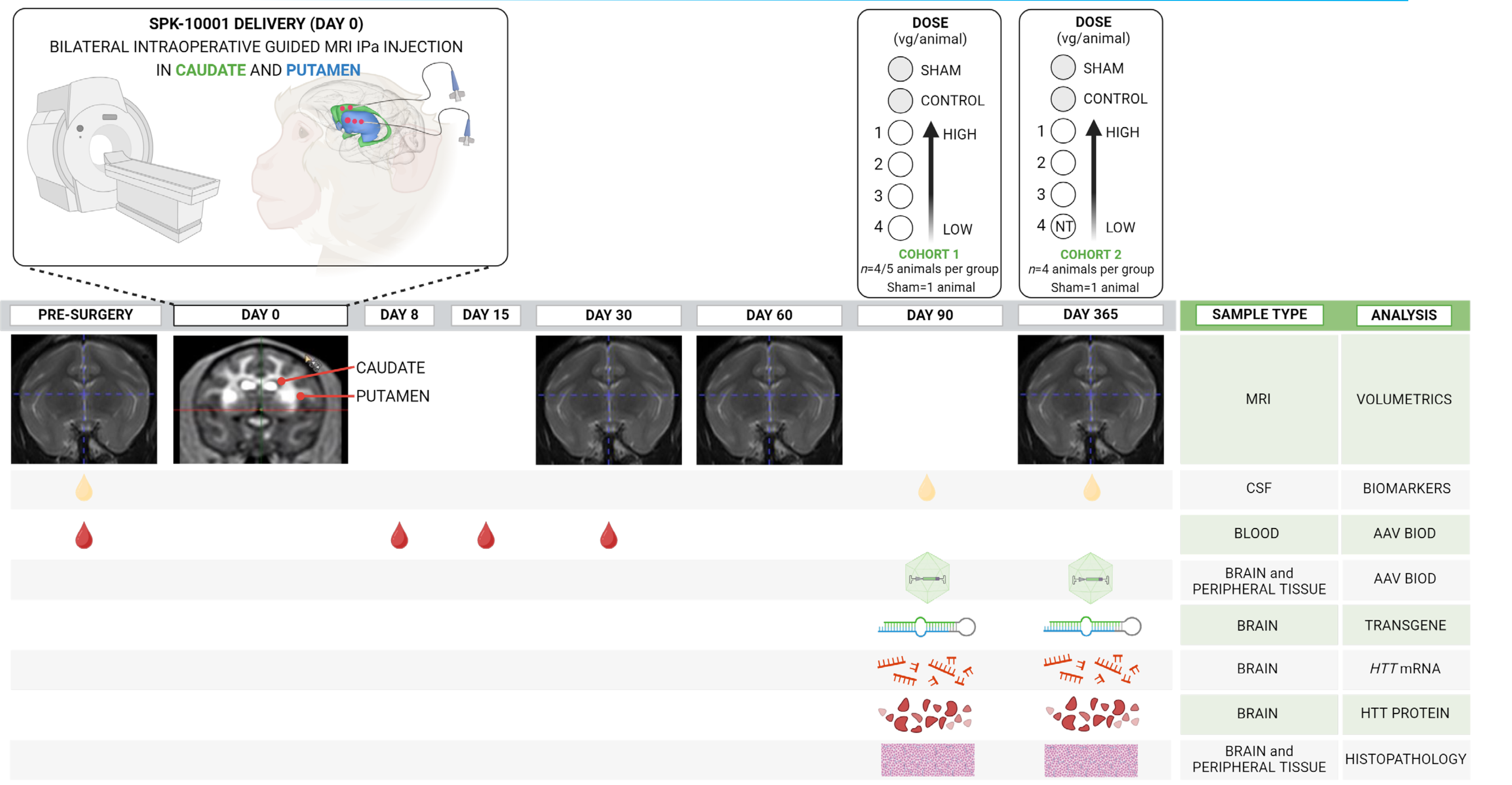
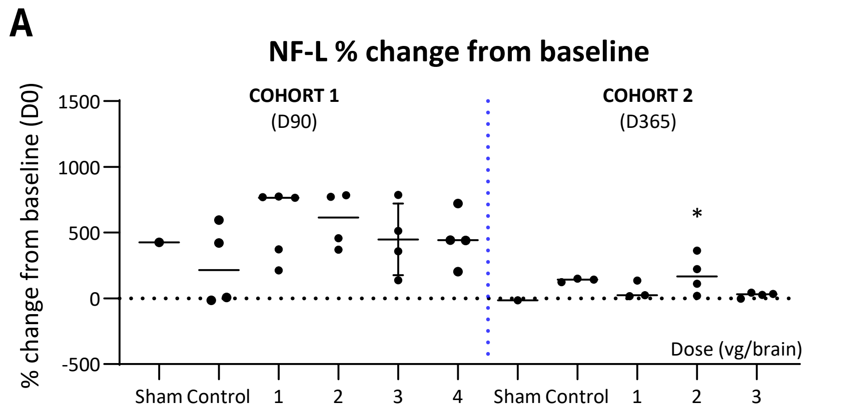


Figure 2. SCHEMATIC OF EXPERIMENTAL DESIGN TO CONFIRM SAFE DELIVERY AND EFFICACY OF SPK-10001.

39 cynomolgus macaques were subdivided in 2 groups, and they were either sham-operated, or diluent-injected, or SPK-10001-injected and treated at 4 (Cohort 1) or 3 (Cohort 2) increasing doses. Brain MRI scans were acquired and biofluids were collected for baseline readouts before the surgery procedure. Intraparenchymal (IPa) injections to the caudate and putamen were guided by intraoperative MRI and Gadoteridol admixed to formulations, which also helped the estimation of the percentage of target structure coverage and AAV distribution in post-dosing MR scans. Brain volumes were analyzed and biofluids were collected at several intermediate timepoints up to scheduled necropsy at Day 90 (Cohort 1) and Day 365 (Cohort 2) post-injection to monitor the surgery procedure as well treatment safety. Brain, other central nervous system structures, and peripheral organs were collected and analyzed to evaluate both treatment safety and vector biodistribution, vector persistence, and target engagement.

RESULTS

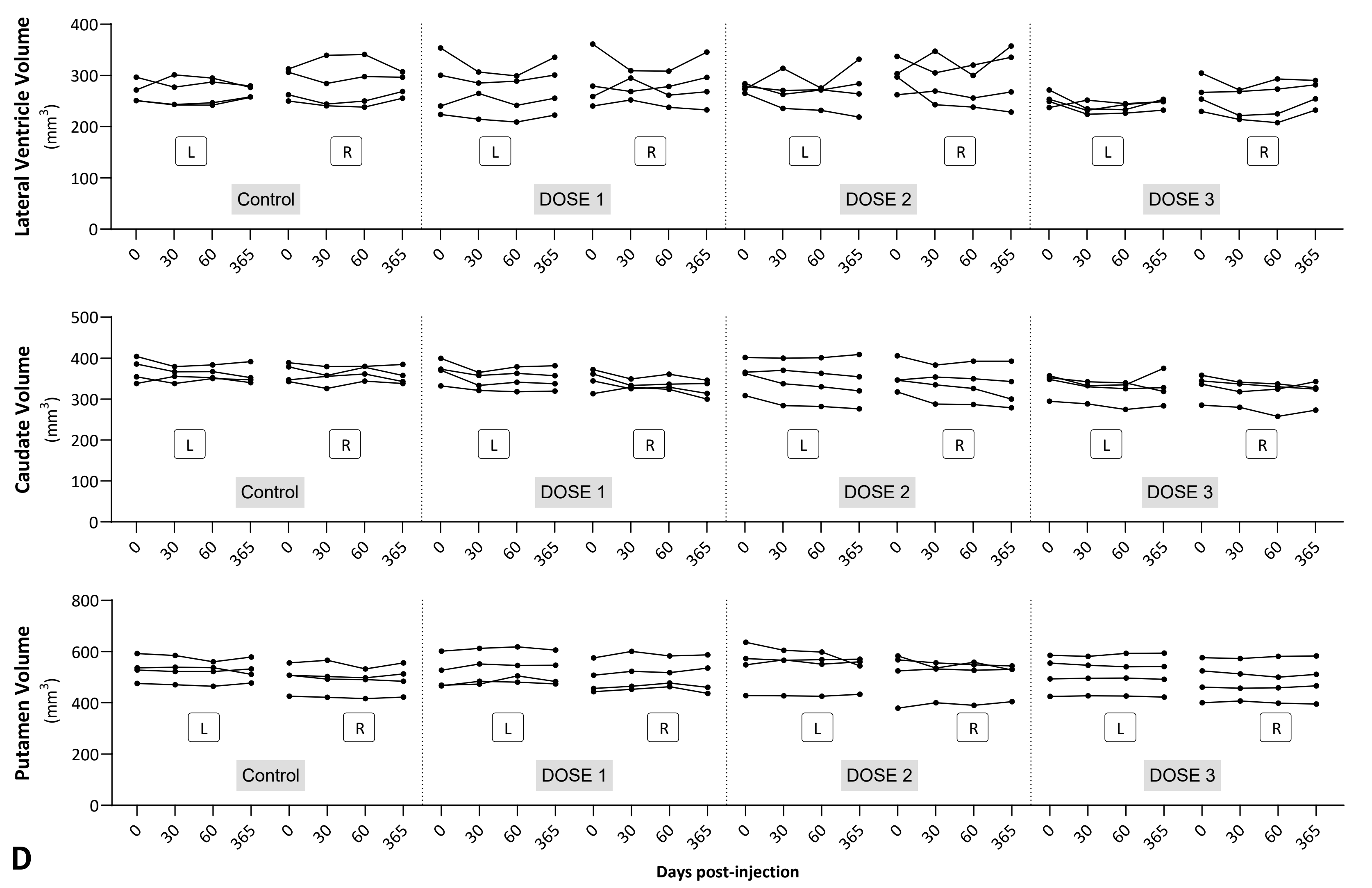
A



B

Study Cohort	Average of Structure % Coverage (± SD)			
	CAU-L	CAU-R	PUT-L	PUT-R
1	33 ± 16	35 ± 13	51 ± 15	49 ± 16
2	44 ± 7	45 ± 11	49 ± 18	52 ± 17

C



D

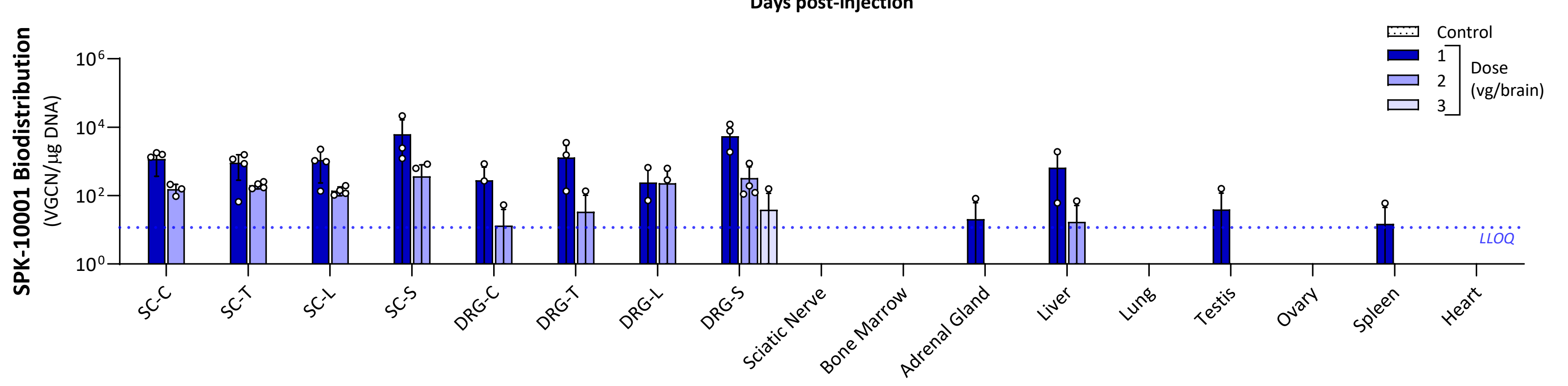


Figure 3. EVALUATION OF SPK-10001 SAFETY AFTER IPa DELIVERY. Quantifying the neurodegeneration marker neurofilament light chain (NF-L) in the CSF of animals from Cohort 1 and 2, both before CED infusion and at the end of the study, confirmed that the changes were acute and solely due to surgery rather than chronic and attributable to gene therapy. Neither surgery nor exposure to varying doses of AAV caused any significant change in the volumes of the target structures throughout the entire treatment period. These results were corroborated by the lack of adverse histopathological changes at any dose tested. SPK-10001 safety was also supported by minimal spread of the viral genomes outside of the brain and to the periphery. No trace of SPK-10001 was detected in blood 8, 15, and 30 days post-injection.

A. Percentage (%) change in NF-L levels in CSF from baseline (prior to injection) to scheduled necropsy (D92, Cohort 1; D365, Cohort 2). Only Dose 2 animals in Cohort 2 showed a significant (*, p=0.017) difference between baseline and terminal NF-L levels. **B.** Summary of the infusion coverage percentage (%) of caudate and putamen after intraparenchymal injection, calculated as the portion of the entire target structure volume occupied by Gadoteridol. *L=left; R=right*. **C.** Longitudinal assessment of changes in the lateral ventricles (*top*), caudate (*middle*) and putamen (*bottom*) volumes for each animal in Cohort 2 measured at 30-, 60-, and 365-days post-injection, compared to pre-injection volumes. *L=left; R=right*. **D.** Quantification of vector genome copy numbers (VGCN) outside of the brain and in the periphery (representative organs) in Cohort 2 animals. *SC= Spinal Cord; DRG= Dorsal Root Ganglion; C=cervical; T=thoracic; L= lumbar; S= sacral. LLOQ= Lower Limit of Quantification.*

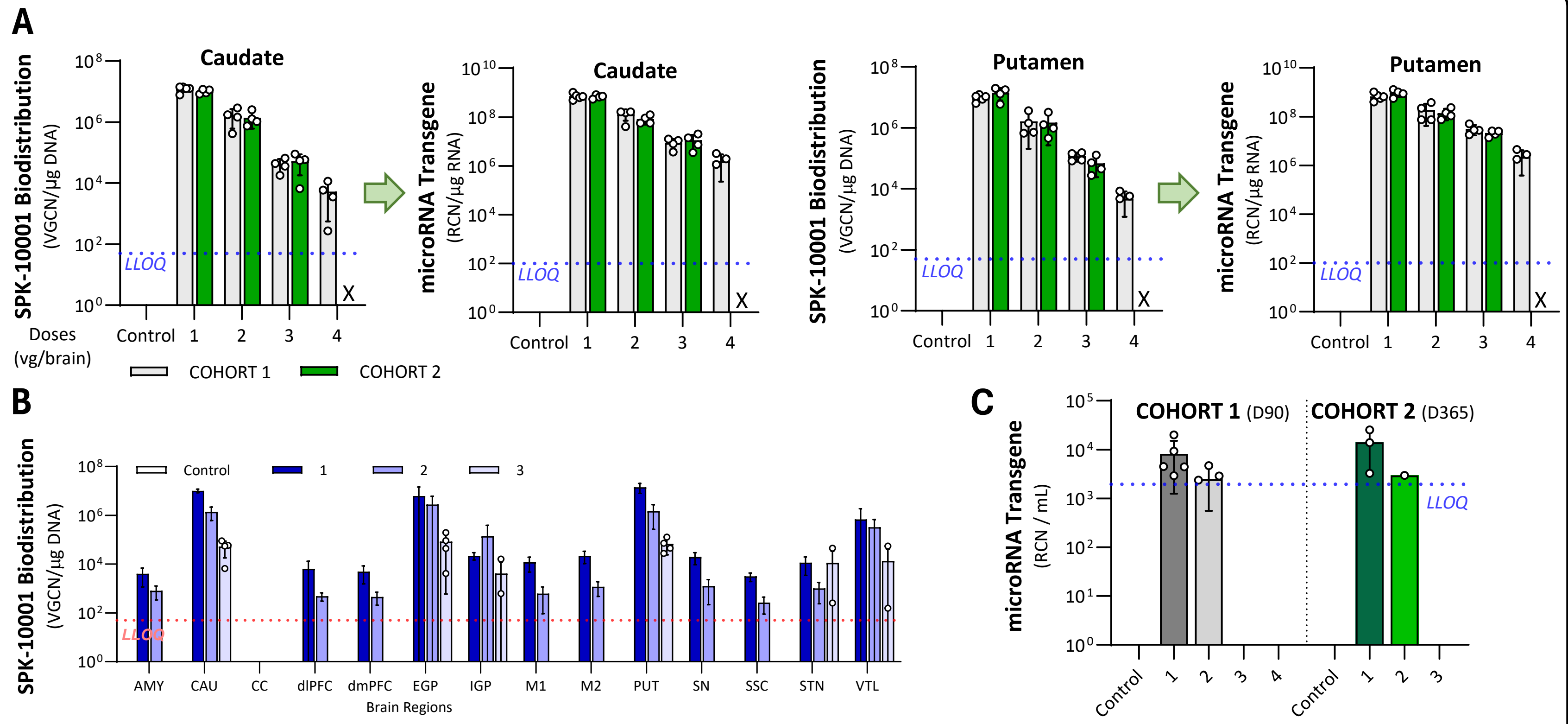


Figure 4. EVALUATION OF SPK-10001 DURABILITY AFTER IP DELIVERY. SPK-10001 vector genomes and the expression of its transgene were measured 90- and 365-days post-injection in various brain regions and CSF. Results from terminal tissue analysis confirmed SPK-10001 durability and good dose-response throughout treatment, while quantification of SPK-10001 transgene in CSF identified a valuable biomarker for monitoring SPK-10001 durability in live animals.

A. SPK-10001 biodistribution and relative transgene expression in the caudate and putamen of Cohort 1 and 2 animals. No significant differences were identified between groups dosed at the same level but evaluated at different timepoints. *X=dose not-tested (NT) in Cohort 2.* **B.** SPK-10001 biodistribution throughout the brain. **C.** SPK-10001 artificial microRNA transgene detected in the CSF of NHPs 90- and 365-days post-injection. *AMY= Amygdala; CAU= caudate; CC= Cerebellar Cortex; dlPFC= dorsolateral Prefrontal Cortex; dmPFC= dorsomedial Prefrontal Cortex; EGP= External Globus Pallidus; IGP= Internal Globus Pallidus; M1= Motor Cortex M1; M2= Motor Cortex M2; PUT= putamen, SN= Substantia Nigra; SSC= Somatosensory Cortex; STN= Subthalamic Nucleus; VTL= ventrolateral Thalamus; VGCN=vector genome copy number; RCN=RNA copy number; LLOQ= Lower Limit of Quantification.*

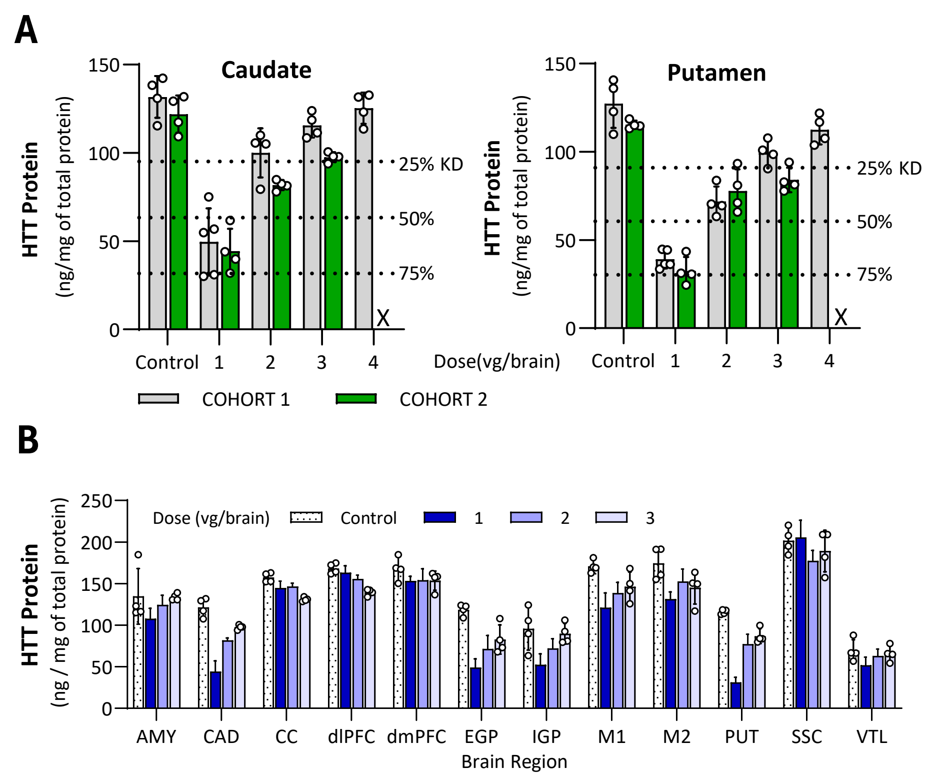
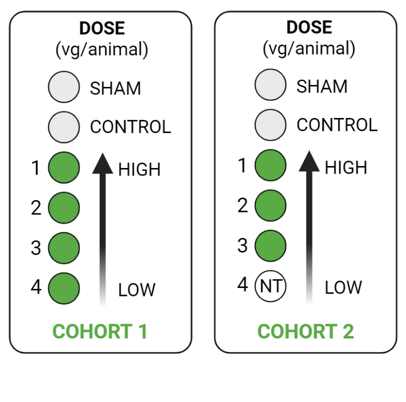


Figure 5. EVALUATION OF SPK-10001 POTENCY TO INDUCE A LONG-LASTING REDUCTION OF HTT PROTEIN. IPa infusion of SPK-10001 formulation in the caudate and putamen induced a durable and robust reduction of HTT protein up to 365-days post-injection. SPK-10001 genome distribution throughout the brain also resulted in effective protein reduction in corresponding brain regions.

A. HTT protein in the caudate (left) and putamen (right) of cynomolgus macaques 90- and 365-days post-injection. No significant differences were identified between groups dosed at the same level but evaluated at different timepoints. *X=dose not-tested (NT) in Cohort 2.* Dotted lines mark 25-50-75% protein reduction compared to average of Controls. *KD= knockdown.* **B.**HTT protein reduction induced by SPK-10001 throughout the brain 365-days post-injection. *AMY= Amygdala; CAU= caudate; CC= Cerebellar Cortex; dlPFC= dorsolateral Prefrontal Cortex; dmPFC= dorsomedial Prefrontal Cortex; EGP= External Globus Pallidus; IGP= Internal Globus Pallidus; M1= Motor Cortex M1; M2= Motor Cortex M2; PUT= putamen, SN= Substantia Nigra; SSC= Somatosensory Cortex; STN= Subthalamic Nucleus; VTL= ventrolateral Thalamus.*

CONCLUSIONS



- **SPK-10001 reduction of HTT protein is POTENT and durable throughout the brain up to 12 months post injection**
- **SPK-10001 delivery and presence in the brain is SAFE at every tested dose up to 365-days post-injection (*green dots, left*)**
- **SPK-10001 transgene durability can be measured in CSF and it's a potentially valuable biomarker for further development**
- **SPK-10001 vector genome and transgene expression are DURABLE up to 12 months post injection**
- **Overall, these data support the further development of SPK-10001 for Huntington's disease**

CONTACT

Francesca Cargnin, PhD
francesca.cargnin@sparktx.com

Liz Ramsburg, PhD
elizabeth.ramsburg@sparktx.com

Spark Therapeutics, Inc.

3025 / 3737 Market Street
Philadelphia, PA 19104