Intravenous Delivery of AAV Gene Therapy Provides Broad SOD1 Knockdown in the Spinal Cord and Robust Efficacy in a Mouse Model of SOD1-ALS

#1061

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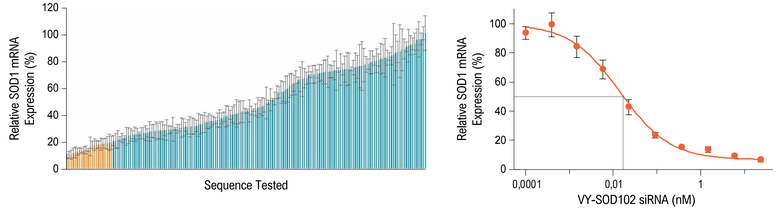
SUMMARY

- Mutations in SOD1 are responsible for a toxic gain of function and result in one of the most common forms of familial ALS
- Reduction of SOD1 in the spinal cord is thought to be a viable treatment approach, but conventional AAV therapies lack broad knockdown due to delivery limitations
- Using a capsid suitable for IV delivery in rodents, we have demonstrated robust knockdown of SOD1 in all levels of the spinal cord resulting in significant improvements in motor performance and survival in a mouse model of ALS
- Voyager's novel AAV capsids now allow for evaluation of this treatment approach in primates

INTRODUCTION

Mutations in superoxide dismutase 1 (SOD1) result in progressive motor neuron loss through toxic gain-of-function properties and are responsible for up to 20% of familial amyotrophic lateral sclerosis (ALS), or 2-4% of all ALS patients in the U.S. Studies using transgenic mice expressing SOD1 mutations have demonstrated reduced neuropathology, improved motor behavior, and extension of survival following several methods of SOD1 reduction. While these approaches have demonstrated varying degrees of efficacy, they often rely on direct spinal cord delivery and fail to achieve the broad SOD1 reduction throughout the CNS thought to be necessary for maximal clinical benefit. Voyager's novel AAV capsids now allow for intravenous delivery that is expected to provide appropriate biodistribution to motor neurons along the entire primate spinal cord, a key translational step for successful therapy. These novel AAV vectors carry transgenes encoding artificial pri-miRNAs using RNA interference (RNAi), a naturally occurring process that mediates gene silencing, to selectively reduce SOD1. Here, we report the results of an AAV gene therapy targeting SOD1 with RNAi, using IV delivery with a BBB-penetrant capsid, in studies in a G93A mouse model of ALS. These studies demonstrated robust SOD1 knockdown throughout the rostral-caudal extent of the spinal cord that correlated with vector genome levels and significant improvements in motor performance and survival extension, beyond what we have previously reported with intraparenchymal, intrathecal, or intracisternal delivery. These results suggest that the combination of potent and tolerable AAV-siRNA mediated knockdown with intravenous dosing of a BBB-penetrant capsid can demonstrate substantial phenotypic rescue in an SOD1-ALS mouse model and support its continued development and translation into the clinic.

Figure 1. Primary Screen of SOD1 siRNA Sequences in HeLa Cells



Primary screen of SOD1 siRNA sequences in HeLa cells. 169 sequences selectively targeting SOD1 were designed, synthesized and evaluated in HeLa cells. 24hr after transfection of 100pM SOD1 siRNA, cells were harvested, and SOD1 and GAPDH mRNA were quantified by RT-qPCR. SOD1 mRNA levels were normalized to GAPDH mRNA levels, and then expressed relative to a negative control. Data shown represent mean ± SD. 22 SOD1 siRNAs (yellow bars) resulted in >80% SOD1 mRNA knockdown.

G93A Efficacy Study

Mice: ~56-day old B6SJL-Tg(SOD1*G93A)1Gur/J (male and female)

Vector: PHP.eB.VY-SOD102 siRNA

Dose: 100µl, IV administration 2e12, 6.3e12, and 2e13 vg/kg

Endpoints: Motor performance, body weight, survival, IHC in spinal cord

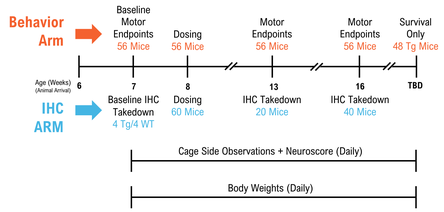
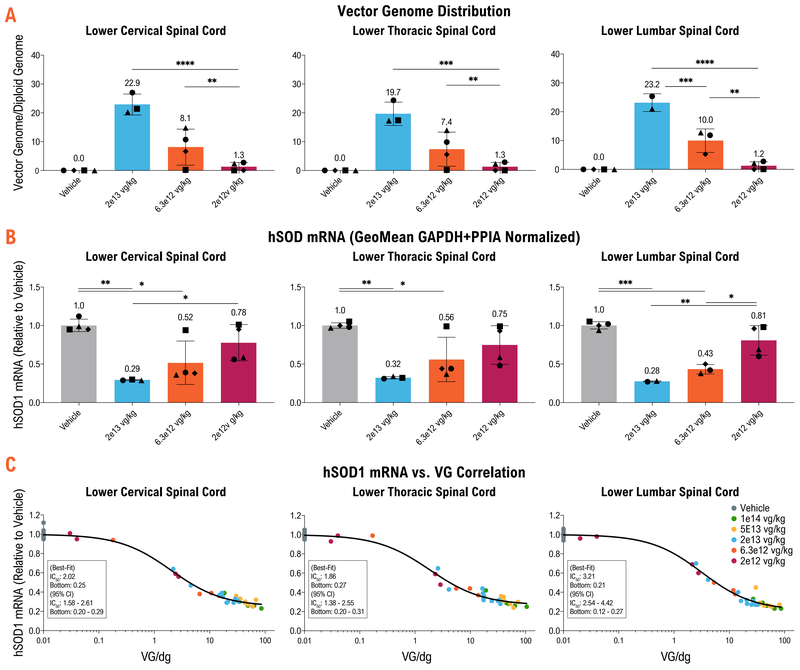
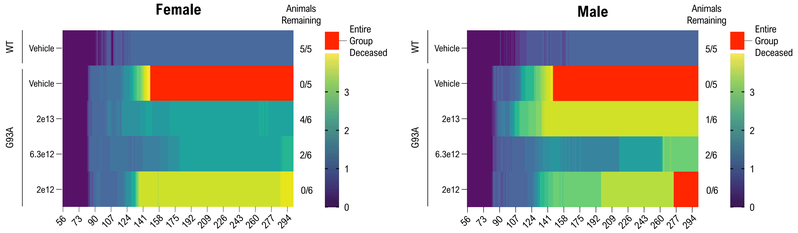


Figure 2. Vector Genome Distribution in Mouse Spinal Cord Following IV Delivery



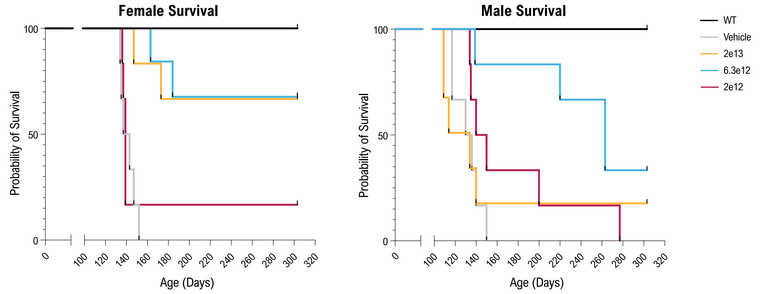
Vector genome distribution and huSOD1 knockdown in the cervical, thoracic, and lumbar spinal cord of G93A mice 32 days following AAV administration. A) Vector genome distribution was analyzed using a multiplex ddPCR assay against transgene and host targets in multiple regions of the spinal cord. B) huSOD1 expression measured using multiplexed RT-qPCR with huSOD1 expression level normalized to 2 host reference gene transcripts with vehicle control group as the comparator. C) Correlation of vector genome to huSOD1 knockdown in the mouse spinal cord. *p<0.05, **0.01, ***0.001, 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 3. Improvement in Neuroscore Composite Rating Following IV Delivery



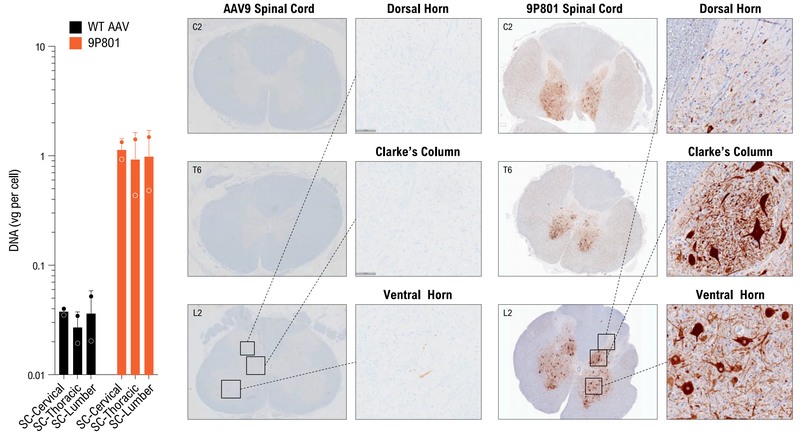
Neuroscore composite rating assessment in G93A mice. Mice were assessed for motor performance using the Neuroscore rating scale ~3x/week for the duration of the study. The scale ranges from 0-4, with 0 = no deficit, 1 = first symptoms, 2 = onset of paresis, 3 = paralysis, 4 = humane endpoint. Animals currently remaining are shown for each treatment group.

Figure 4. Increase in Survival in G93A Mice



Survival analysis of male and female G93A mice. Median survival could not be calculated for female 2e13 or 6.3e12 vg/kg dose groups due to over half of the cohort remaining.

Figure 5. Capsid 9P801 Mediates Strong Transduction in Macaque Spinal Cord



TRACER AAV9-derived capsid mediates widespread transgene expression in NHP brain. Previously disclosed data depicts enhanced spinal cord transduction compared to WT AAV9 when administered intravenously. Data shown was collected following an intravenous dose of 2e13 vg/kg.

CONCLUSIONS

- Robust knockdown of SOD1 in all levels of the spinal cord is observed following IV administration of and AAV gene therapy targeting SOD1 with RNAi
- Significant improvements in motor performance, body weight, and survival are observed in the G93A mouse model of ALS
- These data support the continued development of IV delivered RNAi using a novel BBB-penetrant capsid for use in primates

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