

company

SNP Based Allele-

Selective Editing

Target Product Profile

Mode of Action

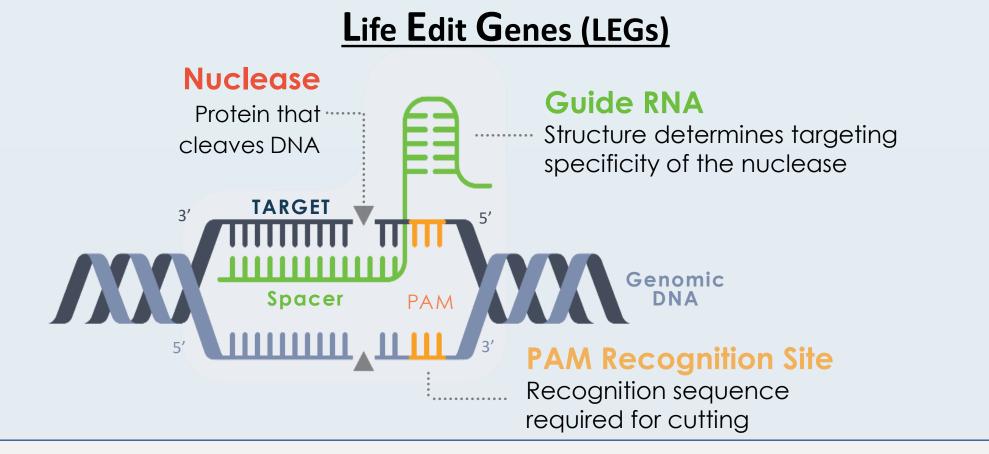
Utilizing PAM diversity for allele-specific knockdown of mut-HTT in vivo

life edit[®] an elevatebia company

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Life Edit Therapeutics

OUR PLATFORM Life Edit's genome editing platform offers a large and diverse collection of novel RNA guided nucleases (LEGs), base editors that provide flexible editing strategies and unprecedented access to genomic loci of interest. Our platform is derived from both an ever-growing proprietary collection of microbes and extensive mining of metagenomic data.

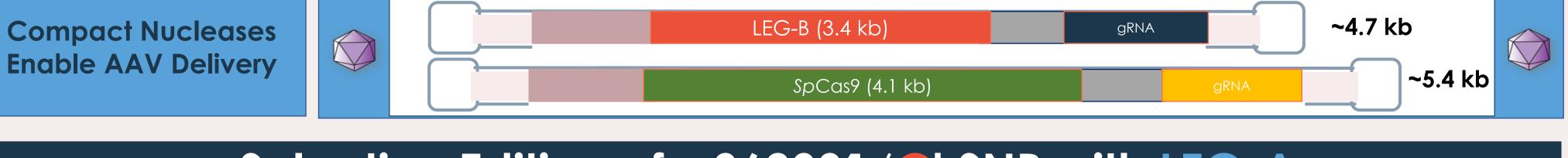


Life Edit nucleases (Life Edit Genes or LEGs) have unique PAM recognition sequences enabling flexible target strategies for diverse genomic targets, including many disease-linked genes.

Life Edit Lead Nucleases				
Nuclease	Base Pairs	Amino Acids	MW (kDa)	PAM
LEG-A	3213	1071	126	NNNNCC
LEG-B	3450	1150	133	NNRYA
LEG-C	3156	1052	124	NNGRR
LEG-D	3390	1130	130	NNGG
SpCas9	4104	1368	158	NGG

The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of HTT alleles with Life Edit nucleases based on the presence of 'C' (LEG-A) or 'T' (LEG-B) nucleotide AAV5-delivered Life Edit nuclease (LEG-B) and guide RNA (SGN2) targeting Huntingtin's Exon50 rs362331 'T' allele, to be expressed in at least 50% of striatal neurons, resulting in ≥ 40% knockdown of mutHTT Allele-specific DSBs activate NHEJ repair pathway, form INDELs that cause frameshift leading to mRNA containing

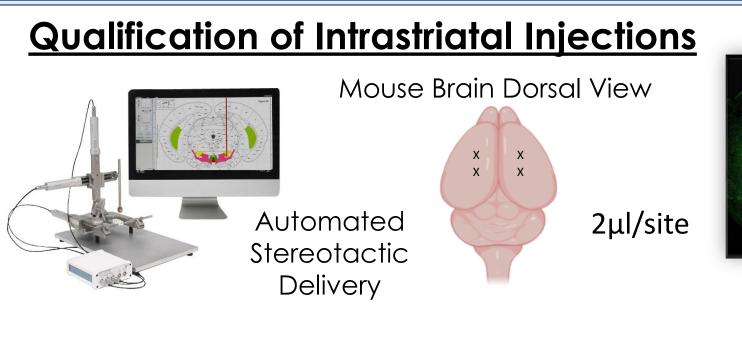
premature-stop codons which are degraded by non-sense mediated decay



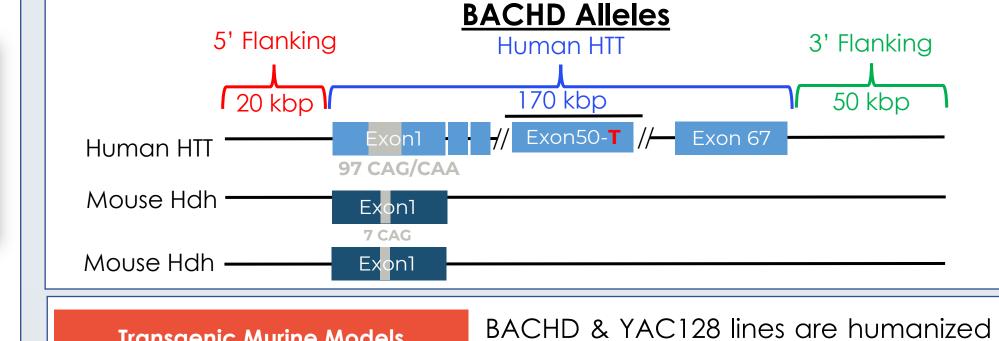
Transgenic Murine Models and Striatal Injections

AAV5-eGFP

DARPP32 (MSNs)



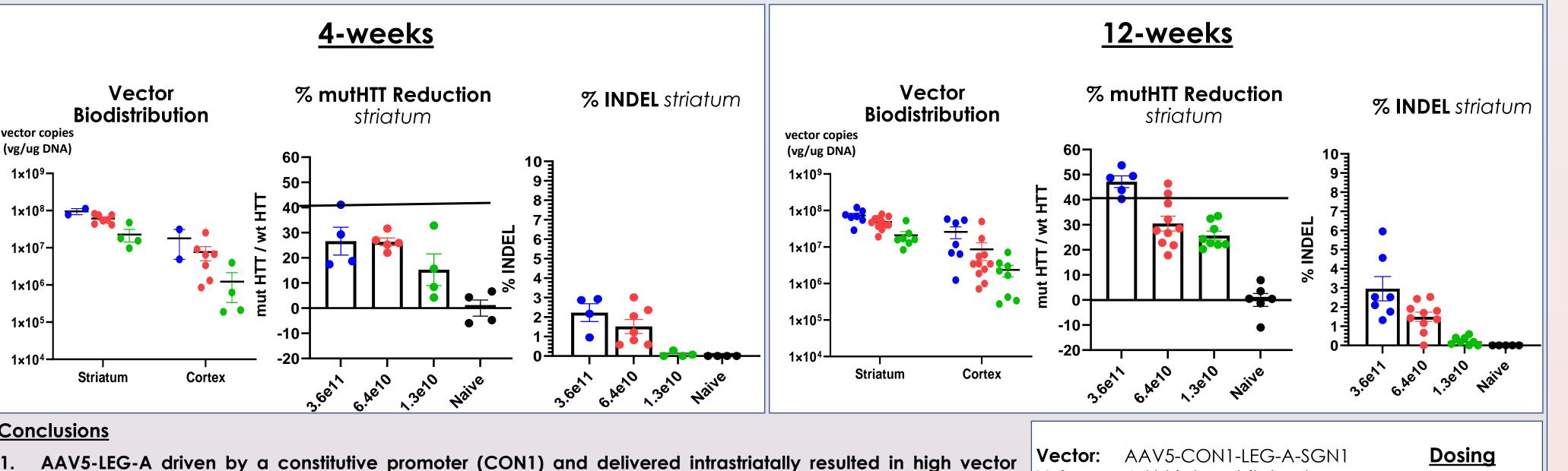
A fluorescent eGFP reporter driven by the CAG promoter was packaged into an AAV5 vector and bilateral injections were performed via automated stereotactic delivery. Brain sections subsequently underwent IHC using DARPP32 primary antibody conjugated to a fluorescent secondary for visualization using fluorescent microscope.



Transgenic Murine Models transgenic mice containing a fulllength human mutHTT allele in addition to the normal mouse Hdh alleles. The mutHTT transgene contains a mixed CAG/CAA repeat expansion and, importantly, either the Exon50 rs362331 'T' (BACHD) or 'C' SNP (YAC128).

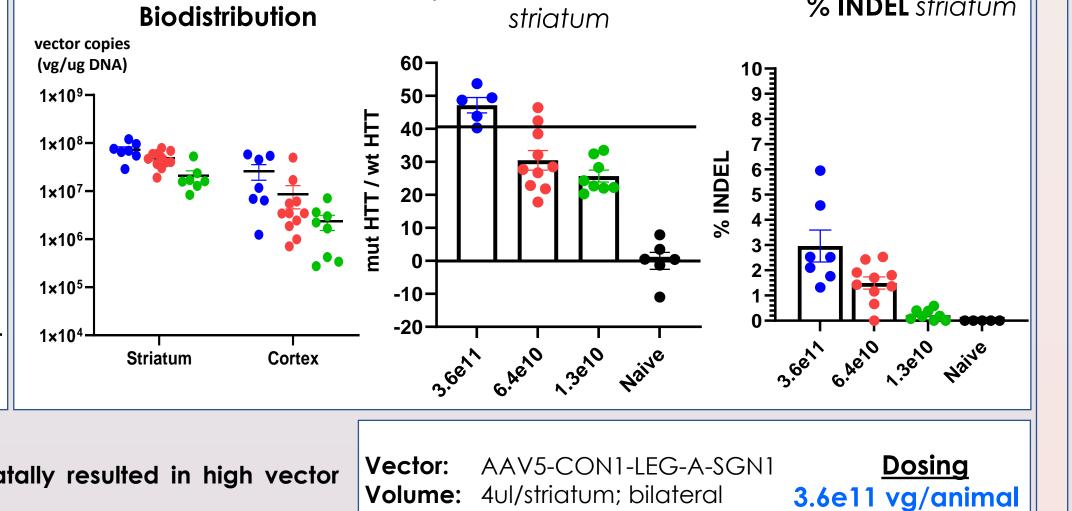
Selective Editing of rs362331 'C' SNP with LEG-A

Therapeutic Strategy



Conclusions

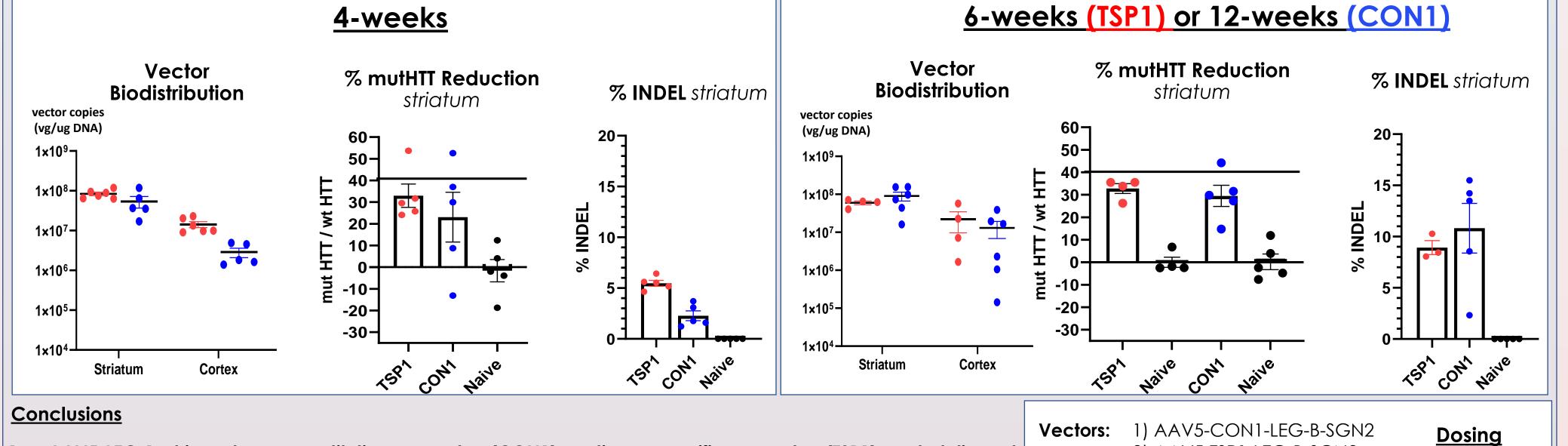
- copy number in YAC128 striatum and cortex
- Clinically relevant reduction of mutHTT protein observed in striatum after 12-weeks in-life
- INDELs detected at target HTT Exon50 site; low rates may be due to other potential editing outcomes not detectable by short amplicon-based next generation sequencing (NGS)



Subjects: YAC128 mice

Duration: 4-weeks or 12-weeks

Selective Editing of rs362331 'T' SNP with LEG-B



- AAV5-LEG-B driven by a constitutive promoter (CON1) or tissue specific promoter (TSP1) and delivered intrastriatally resulted in high vector copy number in BACHD striatum and cortex
- Reduction of mutHTT protein observed in striatum after 4-, 6- and 12-weeks in-life with two different promoters INDELs detected at target HTT Exon50 site; observed increased rates after 6- and 12-weeks in-life relative to 4-

2) AAV5-TSP1-LEG-B-SGN2 **Volume:** 4ul/striatum; bilateral **Subjects:** BACHD mice **Duration:** 4, 6, or 12-weeks

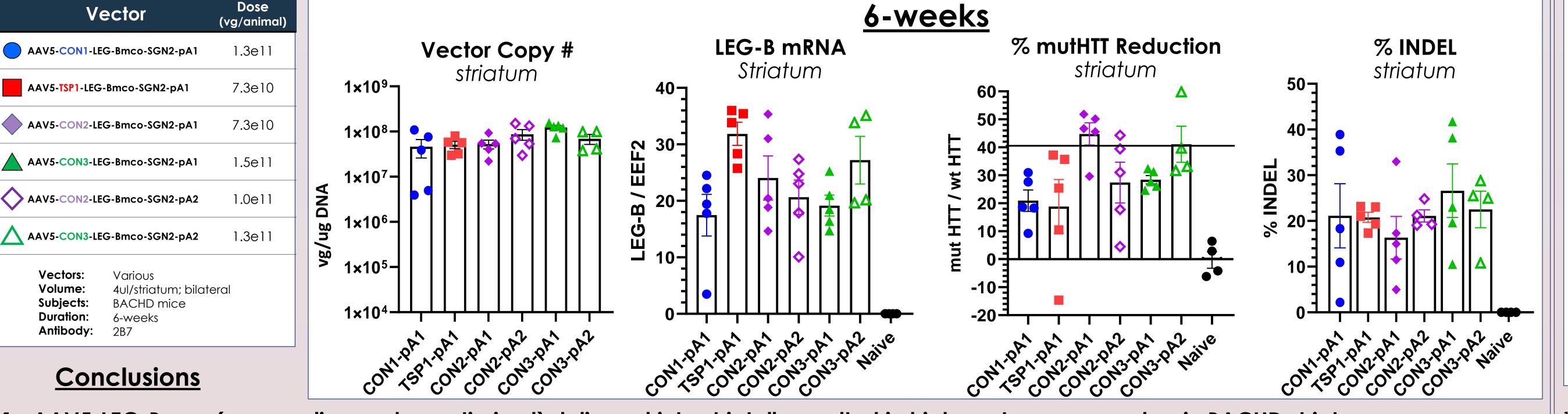
Antibody: 2B7

2.8e10 vg/anima (TSP1) 1.7e11 vg/animal (CON1)

Optimization of Selective Editing of rs362331 'T' SNP with LEG-B

6.4e10 vg/animal

1.3e10 vg/animal



Conclusions

- AAV5-LEG-Bmco (mammalian codon optimized) delivered intrastriatally resulted in high vector copy number in BACHD striatum
- 2. Clinically relevant reduction of mutHTT protein was observed in BACHD striatum after 6-weeks in-life with CON2-pA1 & CON3-pA2 constructs
- 3. INDEL rates at target HTT Exon50 site improved with LEG-B transgene that has been codon optimized for expression in mammalian cells

12-weeks dose-response % mutHTT Reduction **Vector Copy #** 1×10⁹ striatum striatum 의 1×10⁸

Both AAV5-LEGB constructs delivered intrastriatally resulted in high vector copy number in BACHD Clinically relevant, dose-dependent reduction in

mutHTT protein observed in Striatum at mid & high doses for both constructs tested

Vectors: AAV5-CON2-LEG-Bmco-SGN2-pA1 AAV5-CON3-LEG-Bmco-SGN2-pA2 Volume: 4ul/striatum; bilateral **Subjects:** BACHD mice **Duration:** 12-weeks Antibody: 2B7

H= 2.94e11 vg/animal (CON2) **H=** 2.05e11 vg/animal (CON3) **M=** 7.28e10 vg/animal **L=** 2.05e10 vg/animal

Summary

- Life Edit nucleases are compact, facilitating all-in-one delivery with a single AAV vector, and characterized by diverse PAM recognition sequences, enabling flexible targeting of genomic loci including many disease-linked genes
- Life Edit nucleases LEG-A and LEG-B enable allele specific targeting of mutant HTT based on the PAM generated by HTT Exon50 SNP rs362331
- Life Edit gene editing systems packaged into AAV5 efficiently transduce CNS tissue in vivo with high vector copy numbers detected up to 12-weeks post injection (furthest time point tested)
- AAV5-delivered Life Edit gene editing systems targeting mutant HTT allele resulted in clinically relevant reduction of mutant HTT protein in the striatum of YAC128 and BACHD transgenic mice which contain a full-length human mutant HTT gene
- Optimization of vectors using different combinations of promoters, polyA signals, and codon optimized transgenes resulted in improved editing rates and reduction of mutant HTT protein
- Life Edit is an ElevateBio company, enabling access to ElevateBio's proven expertise in gene therapy development including vector engineering, process development, and GMP manufacturing

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