

# Allele Selective SNP Editing Utilizing AAV5-delivered Life Edit® Nuclease and guide RNA Resulting in Meaningful Reduction of mutant HTT Protein

Logan Y. Brown PhD, Alexandra Crawley PhD, Nancy Cheng PhD, Helen Mao PhD, Jamie Moy PhD, Ariel Vitenzon PhD, and Kathryn Woodburn PhD  
Life Edit Therapeutics, Inc. Durham, North Carolina, USA

## Life Edit Therapeutics

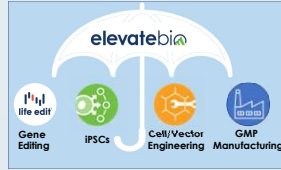
**OUR VISION** To pave the way to faster, safer, gene editing therapeutics that cure disease and improve patient lives

**OUR VALUES** We are guided by curiosity, rigor, and integrity. We believe in discovery and unlimited potential. We care about science and the lives of patients.

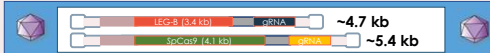
**OUR MISSION** To re-write the future by curing disease, making any edit, anywhere

### Life Edit® and ElevateBio®

Life Edit is an ElevateBio company, enabling access to ElevateBio's proven expertise in gene therapy development including cell, vector, and protein engineering, GMP manufacturing, process development, and induced pluripotent stem cells (iPSCs) technology.

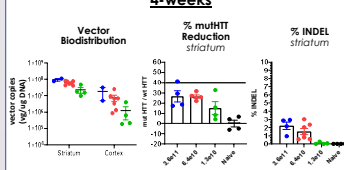


## Therapeutic Strategy

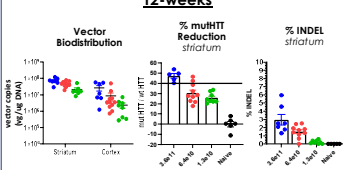
<b>SNP Based Allele-Selective Editing</b>	The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of HTT alleles with Life Edit nucleases based on the presence of 'C' (LEG-A) or 'T' (LEG-B) nucleotide
<b>Target</b>	AAV5-delivered Life Edit nuclease (LEG-B) and guide RNA (SGN2) targeting Huntingtin's Exon50 rs362331 'T' allele, to be expressed in at least 50% of striatal neurons, resulting in ≥ 40% knockdown of muHTT
<b>Mode of Action</b>	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
<b>Compact Nucleases Enable AAV Delivery</b>	

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

**4-weeks**



**12-weeks**



**Conclusions**

- AAV5-LEG-A driven by a constitutive promoter (CON1) and delivered intrastrially resulted in high vector copy number in YAC128 striatum and cortex
- Clinically relevant reduction of muHTT 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)

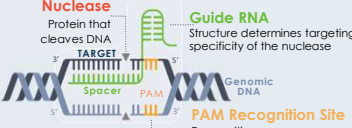
**Vector:** AAV5-CON1-LEG-A-SGN1  
**Volume:** 4µl/striatum; bilateral  
**Subjects:** YAC128 mice  
**Duration:** 4-weeks or 12-weeks  
**Antibody:** 2B7

**Dosing**  
 3.6e11 vg/animal  
 4.4e10 vg/animal  
 1.3e10 vg/animal

## Life Edit Gene Editing Technology

**OUR PLATFORM** Life Edit's genome editing platform offers a large and diverse collection of novel RNA guided nucleases (LEGs), base editors, & reverse transcriptase editors that provide flexible editing strategies and unprecedented access to genomic loci of interest. Our platform is derived from AgBiome's ever-growing collection of tens of thousands of proprietary, non-pathogenic microbes.

**Life Edit Genes (LEGs)**

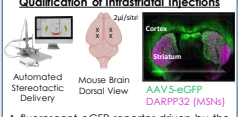


Life Edit Lead Nucleases				
Nuclease	Base Pairs	Amino Acids	MW (kDa)	PAM
LEG14	3213	1071	126	NNNNCC
LEG95	3450	1150	133	NNRYA
LEG98	3156	1052	124	NNRRR
LEG145	3390	1130	130	NNGG
SpCas9	4104	1368	158	NGG

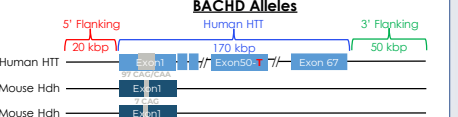
Life Edit nucleases (Life Edit Genes or LEGs) have unique PAM recognition sequences enabling flexible target strategies for diverse genomic targets, including many disease-linked genes.

## Transgenic Murine Models and Striatal Injections

**Qualification of Intrastriatal Injections**



**BACHD Alleles**



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

Transgenic Murine Models			
Model	rs362331 SNP	CAG/CAA Repeats	Transgene Copy #
BACHD	T	97	5x
YAC128	C	92 & 118	3x

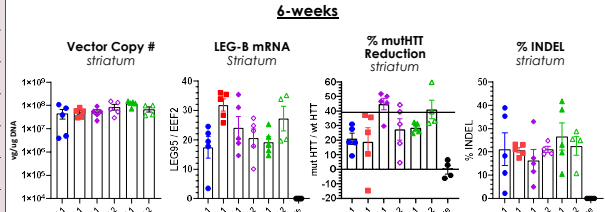
## Optimization of Selective Editing of rs362331 'T' SNP with LEG-Bmco

**Vector**

AAV5-CON1-LEG-Bmco-SGN2-pA1	1.3e11
AAV5-TSP1-LEG-Bmco-SGN2-pA1	7.3e10
AAV5-CON2-LEG-Bmco-SGN2-pA1	7.3e10
AAV5-CON3-LEG-Bmco-SGN2-pA1	1.5e11
AAV5-CON2-LEG-Bmco-SGN2-pA2	1.0e11
AAV5-CON3-LEG-Bmco-SGN2-pA2	1.3e11

**Vectors:** Various  
**Volume:** 4µl/striatum; bilateral  
**Subjects:** BACHD mice  
**Duration:** 6-weeks  
**Antibody:** 2B7

**6-weeks**



**Conclusions**

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

**Vector:** AAV5-TSP1-LEG-Bmco-SGN2-pA1  
**Volume:** 4µl/striatum; bilateral  
**Subjects:** BACHD mice  
**Duration:** 6-weeks  
**Antibody:** 2B7

**Dosing**  
 2.7e11 vg/animal  
 7.3e10 vg/animal  
 2.7e10 vg/animal

## Summary

- Life Edit nucleases are 1. compact facilitating all-in-one delivery with a single AAV vector and 2. characterized by diverse PAM recognition sequences that enable 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