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

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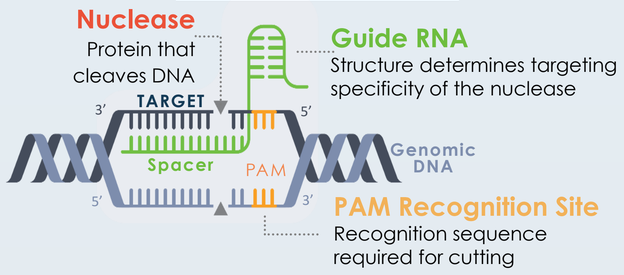
Life Edit Gene Editing Technology

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.

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	NNGRR
LEG145	3390	1130	130	NNGG
SpCas9	4104	1368	158	NGG

Life Edit nucleases (**L**ife **E**dit **G**enes or **LEG**s) have unique PAM recognition sequences enabling flexible target strategies for diverse genomic targets, including many disease-linked genes.

Allele Selective Strategy for Huntington's Disease

SNP Based Allele-Selective Editing

The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of mHTT allele with Life Edit nucleases based on the presence of 'C' or 'T' nucleotide

- Patient alleles must be heterozygous C/T (not C/C or T/T)
- CAG repeat expansion must be in-phase with targeted allele (T)
- 'T' allele projected to capture ~33% of U.S. HD patient population

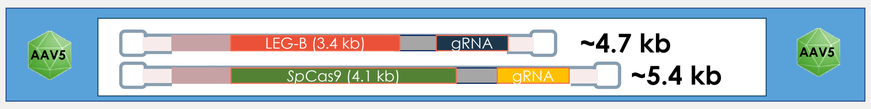
Target Product Profile

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

Mode of Action

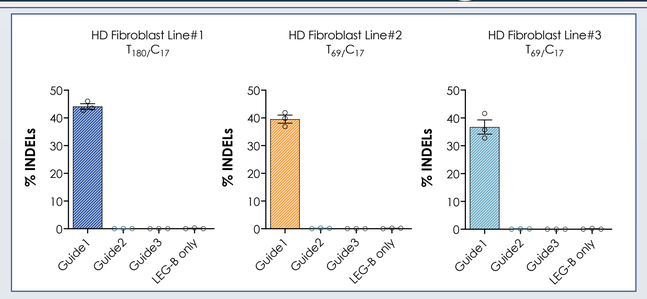
Allele-specific DSBs activate NHEJ repair pathway, form INDELs that cause frameshift leading to mRNA containing premature-stop codons which are degraded by non-sense mediated decay

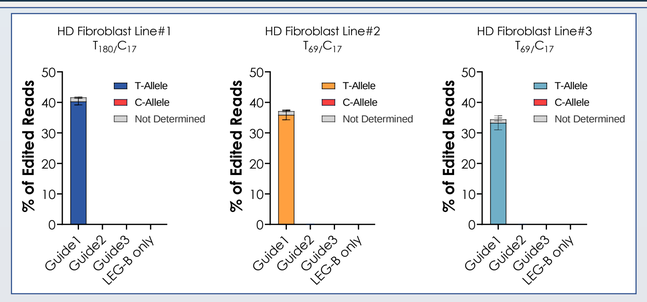
'All-in-one' Single AAV Delivery

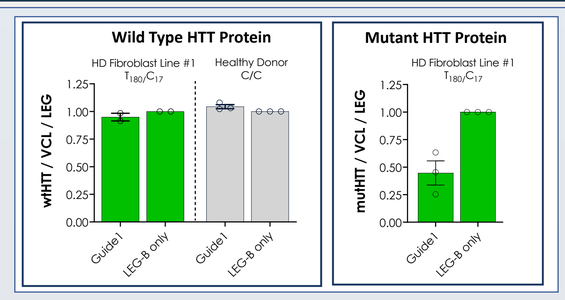


Allele Selective Editing & Reduction of mHTT in Patient Cells

- **Delivery by mRNA/RNA transfection**
- **Subtext denotes CAG repeat length of each allele**
  - Guide1 – Active; LETI-101 guide targeting HTT
  - Guide2 – Control; targeting HTT without PAM
  - Guide3 – Control; targeting mouse ROSA26 gene
  - LEG-B only – Control; nuclease without guide





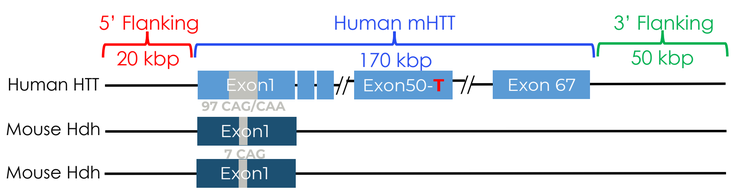


Results

1. Life Edit 'LEG-B-Guide1' system efficiently edits in three separate fibroblast lines derived from patients that are heterozygous for SNP rs362331 (C/T) with the 'T' allele in-phase with CAG trinucleotide repeat expansion
2. Life Edit 'LEG-B-Guide1' system selectively edits targeted mHTT allele when 'T' SNP rs362331 is present, and never edits the wtHTT allele containing 'C' SNP
3. Life Edit 'LEG-B-Guide1' system delivered to patient derived fibroblasts resulted in selective knockdown of the mHTT protein (~55%), while wtHTT protein is preserved

In Vivo Editing & Reduction of mHTT in Striatum of BACHD Transgenic Mice

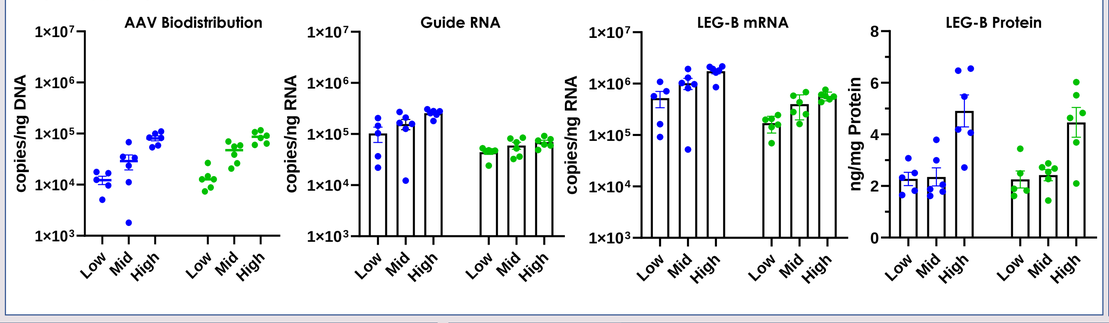
BACHD Transgenic Mouse



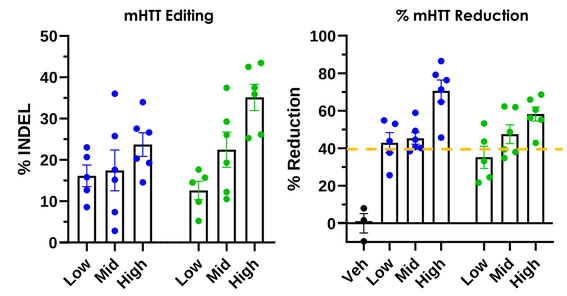
BACHD animals are humanized transgenic mice containing a full-length human mutHTT allele in addition to the normal mouse Hdh alleles. The mutHTT transgene contains a mixed CAG/CAA repeat expansion and the Exon50 rs362331 'T' SNP required for allele selective editing utilizing Life Edit's LEG-B nuclease.

Pharmacokinetics

AAV5-Promoter1-LEG-B
AAV5-Promoter2-LEG-B



Pharmacodynamics



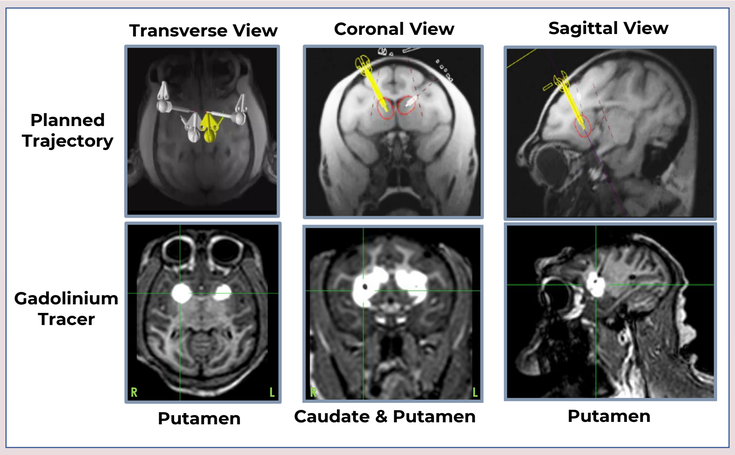
Study Design

- Two AAV constructs utilizing different promoters were packaged in AAV5 and administered intrastriatally (8ul/animal) at three doses (2e10, 7e10, 3e11 vg/animal) to BACHD mice which contain a full-length human mHTT transgene and express the full-length mutant protein
- 12-weeks following AAV administration, brain tissues were harvested and bulk lysate tissue samples from striatum were assessed for AAV vectors, transgene expression, on-target editing, and mHTT protein reduction

Conclusions

1. AAV5-LEG-B-SGN1 delivered intrastriatally resulted in high vector copy number and dose-dependent expression of guide RNA and LEG-B mRNA and protein in the BACHD striatum
2. Observed dose-dependent editing of the targeted mHTT exon50 site as measured by insertion-deletions (INDELs)
3. A clinically relevant reduction of mHTT protein (>40%) was observed in the BACHD striatum at all dose levels evaluated as measured by capillary electrophoresis using the HTT antibody 2B7.

One Month Tolerability & Biodistribution in adult Cynomolgus Monkeys

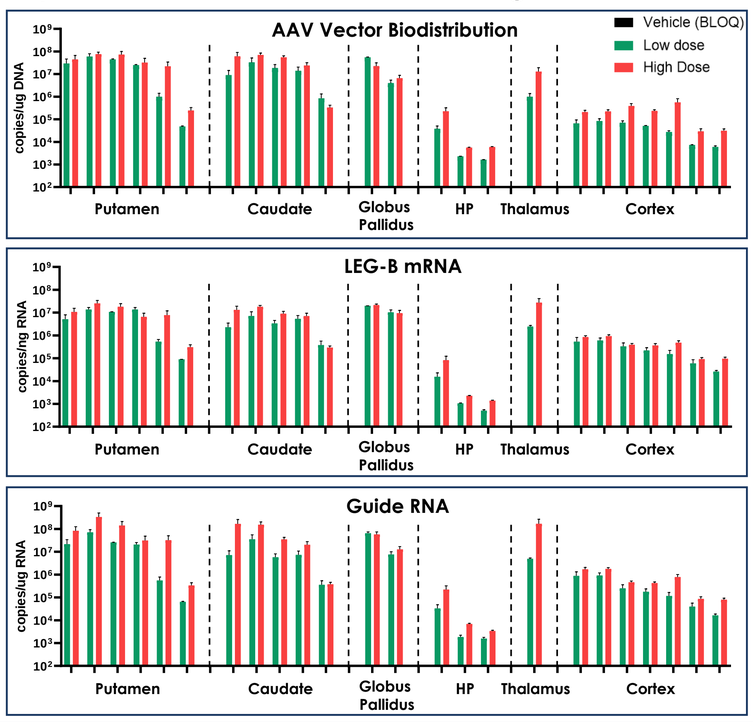


Study Design

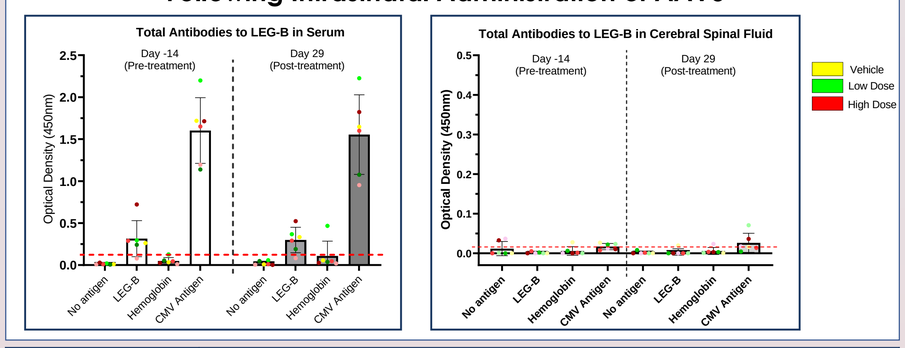
Treatment Group	Dose (vg/brain)	Animals	Injection Vol.
Vehicle	0 vg	N=1	75µl/caudate 150µl/putamen
Low Dose	2e12 vg	N=2	75µl/caudate 150µl/putamen
High Dose	1e13 vg	N=3	75µl/caudate 150µl/putamen

- Objective: One month study to evaluate the tolerability and biodistribution of AAV5-LEG-B-SGN1 following intrastriatal administration in adult cynomolgus monkeys
- Route of Administration: Bilateral intrastriatal delivery of AAV5 was performed using MRI guided convection enhanced delivery (CED) with the Neuroninfuse™ (Renishaw, UK) cannula

Expression and Biodistribution in CNS Regions Critical for HD



No Immune Response to LEG-B Nuclease Observed Following Intrastriatal Administration of AAV5



Conclusions

1. Detailed clinical observations including body weight and food consumption monitoring, ophthalmic examination, and functional observational battery[1], revealed no untoward outcomes*
   **Pending clinical chemistries and histopathologic evaluation*
2. Bilateral intrastriatal administration of AAV5-LEGB-Guide1 by CED resulted in high vector copy number, LEG mRNA, and guide RNA expression across brain regions that are critically vulnerable in HD
3. Minimal AAV vector was observed systemically, with nothing detected in the gonads
4. No change in immune response to the LEG-B nuclease was observed

[1] Gauvin DV, Baird TJ. "*A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments.*" J. Pharmacol Toxicol Methods. 2008 Sep-Oct;58(2):88-93.

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 nuclease LEG-B enables selective targeting of the mutant HTT allele, and reduction in mHTT protein, in patient-derived cells based on the PAM generated by HTT exon50 SNP rs362331
- Life Edit nuclease and guide RNA targeting mHTT can be packaged into a single AAV5 vector and delivered to CNS *in vivo* resulting in dose-dependent expression of guide RNA and LEG-B protein, leading to clinically relevant reduction of mHTT protein (>40%) in the striatum of BACHD transgenic mice which carry a full-length human mHTT transgene
- Bilateral intrastriatal delivery of AAV5-LEG-B-Guide1 in adult cynomolgus monkeys using the Neuroinfuse™ (Renishaw, UK) device resulted in…
  1. High vector copy number, LEG mRNA, and guide RNA expression across brain regions known to be critically vulnerable in HD
  2. No untoward clinical observations including body weight, food consumption, and functional observational battery
  3. No change in immune response to the LEG-B nuclease and minimal systemic vector distribution, including nothing detected in the gonads

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