AAV5-delivered Life Edit® CRISPR system results in broad CNS biodistribution and allele selective editing and reduction of mHTT protein in critical HD brain regions

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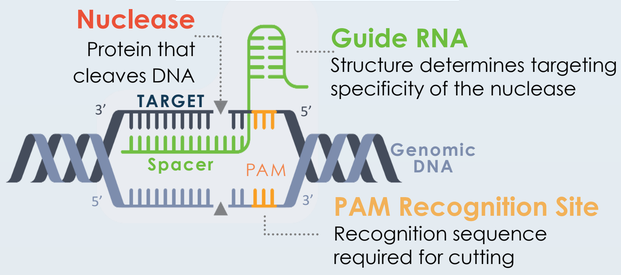
Life Edit Gene Editing Technology

OUR PLATFORM Life Edit's genome editing platform offers a large and diverse collection of novel RNA guided nucleases, base editors, & reverse transcriptase editors that provide flexible editing strategies and unprecedented access to genomic loci of interest.

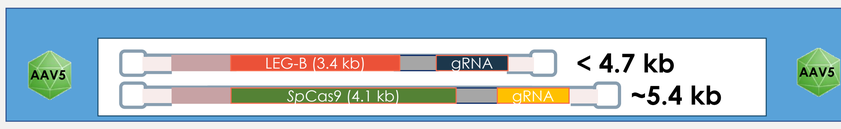
Life Edit Genes (LEGs)



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

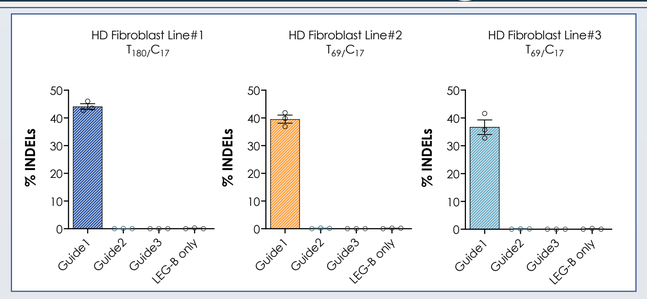
Life Edit nucleases (**L**ife **E**dit **G**enes or **LEG**s) have unique PAM recognition sequences enabling flexible target strategies for diverse genomic targets, including many disease-linked genes.

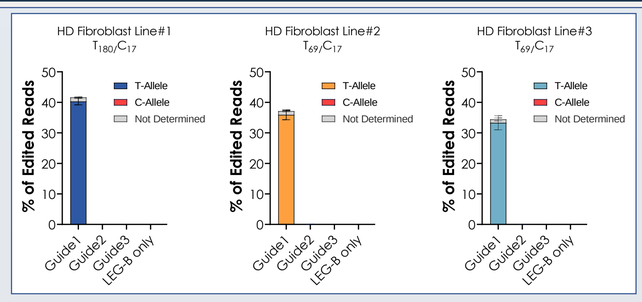
Allele Selective Strategy for Huntington's Disease

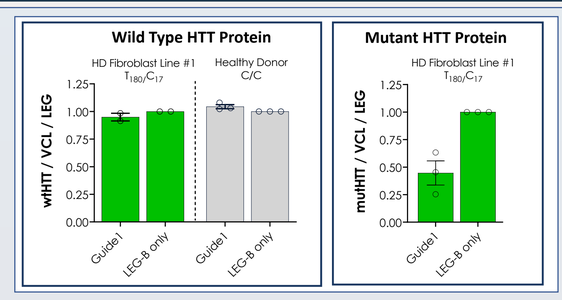
SNP Based Allele-Selective Editing	The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of mHTT allele with Life Edit nucleases based on the presence of 'C' or 'T' nucleotide ➢ Patient alleles must be heterozygous C/T (not C/C or T/T) ➢ CAG repeat expansion must be in-phase with 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 guideRNA (Guide1) targeting HTT exon50 rs362331 'T' allele, to be expressed in at least 50% of striatal neurons, resulting in ≥ 40% knockdown of mHTT protein
Mode of Action	Allele-specific DSB activates NHEJ repair pathway, forming INDELs that cause frameshift, leading to mRNA containing premature-stop codons which are degraded by the non-sense mediated decay pathway
'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





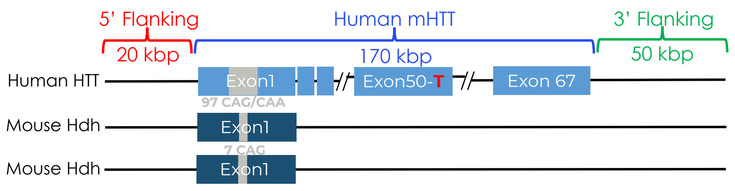


Results

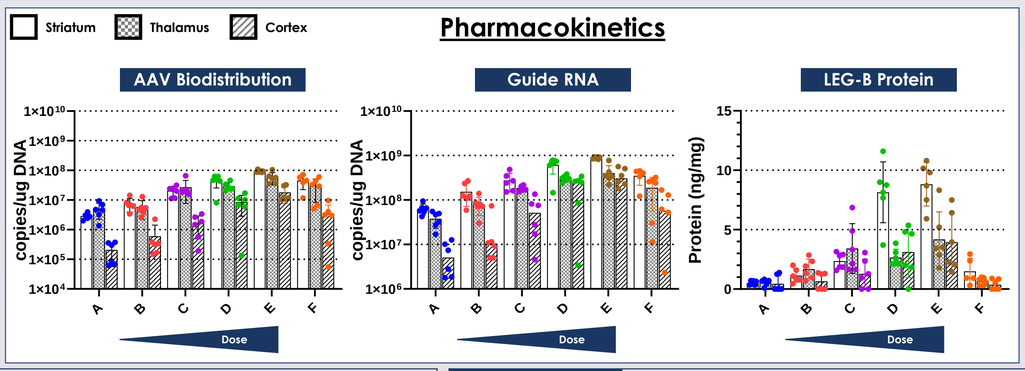
1. **Life Edit 'LEG-B-Guide1' system efficiently edits in three separate fibroblast lines derived from patients that are heterozygous for SNP rs362331 (C/T) with the 'T' allele in-phase with CAG trinucleotide repeat expansion**
2. **Life Edit 'LEG-B-Guide1' system selectively edits targeted mHTT allele when 'T' SNP rs362331 is present, with no editing of the wtHTT allele containing 'C' SNP observed**
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**

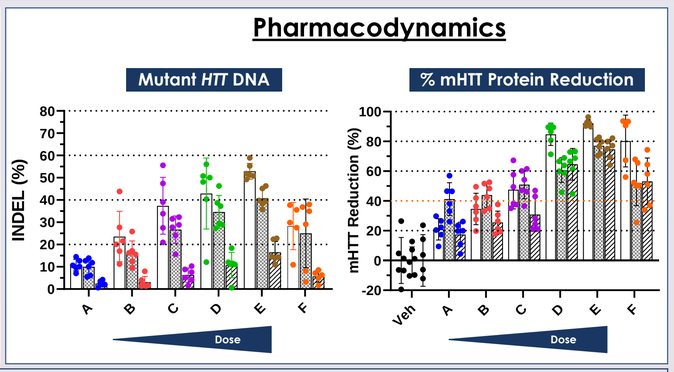
In Vivo Editing & Reduction of mHTT in CNS of BACHD Transgenic Mice

BACHD Transgenic Mouse



BACHD animals are humanized transgenic mice containing a full-length human mHTT allele in addition to the endogenous mouse Hdh alleles. The mHTT 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.





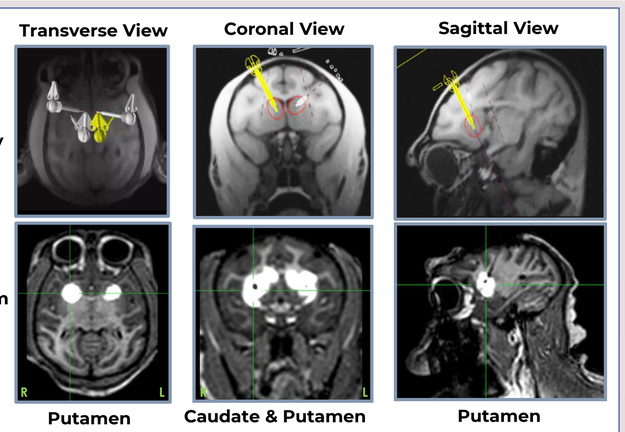
Study Design

- ❖ **LETI-101 (AAV5-LEG-B-Guide1) was administered intrastriatally (2µl/site; 2 sites/hemisphere for a total 8µl/animal) at six ascending doses (A-F) in BACHD mice which contain a full-length human mHTT transgene and express the full-length mutant protein**
- ❖ **6-months following administration, brain tissues were harvested and bulk tissue samples from striatum, thalamus, and cortex were assessed for AAV vector copy number, transgene expression, on-target editing, and mHTT protein reduction**

Conclusions

1. **LETI-101 delivered intrastriatally resulted in high AAV vector copy number, transgene expression, and activity in the striatum, cortex, and thalamus**
2. **Dose-dependent editing of the targeted mHTT exon50 site, and a clinically relevant reduction of mHTT protein (>40%), was observed in the striatum, cortex, and thalamus at the 2nd and 3rd highest dose levels (groups D and E)**
3. **At the highest dose level (group F) reduced transgene expression was observed in all brain regions leading to lower activity, suggesting possible silencing of expression from AAV episomes at the highest dose level evaluated**

One Month Tolerability & Biodistribution in adult Cynomolgus Monkeys

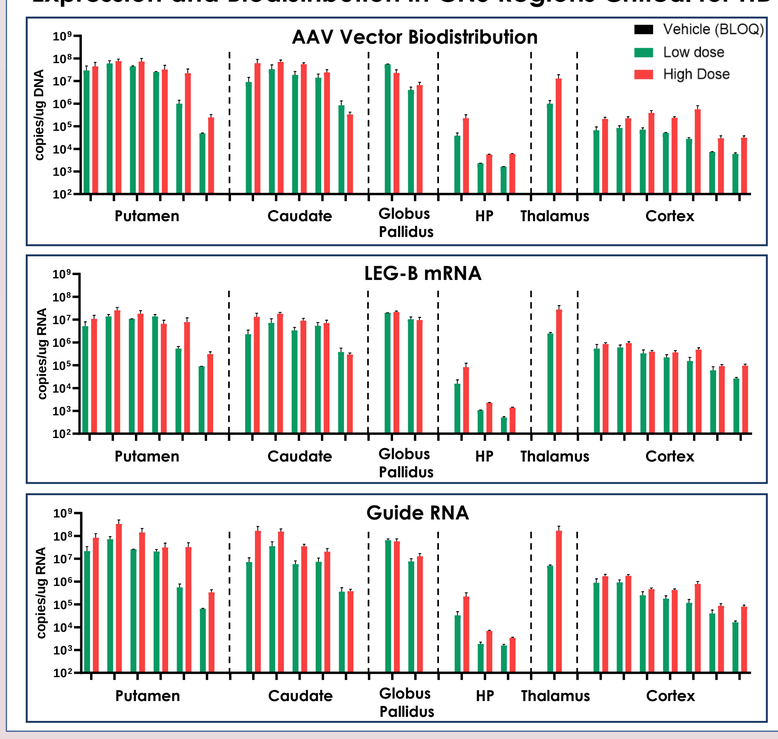


Study Design

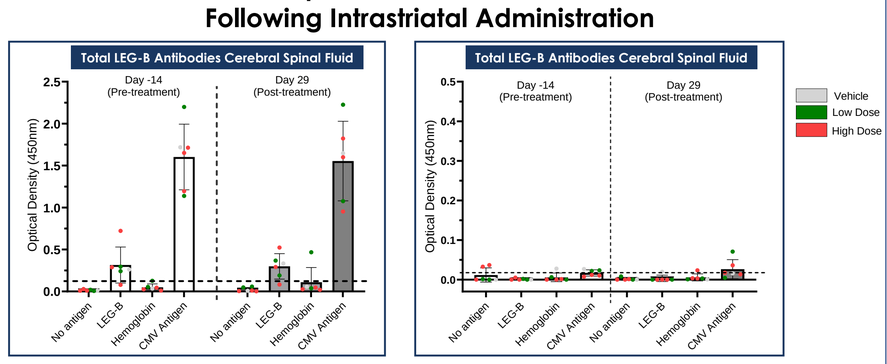
Treatment Group	Dose (vg/brain)	Animals	Injection Vol.
Vehicle	0 vg	N=1	75µl/caudate 150µl/putamen
Low Dose	2e12 vg	N=2	75µl/caudate 150µl/putamen
High Dose	1e13 vg	N=3	75µl/caudate 150µl/putamen

- ❖ **Objective: One month study to evaluate the tolerability and biodistribution of LETI-101 (AAV5-LEG-B-Guide1) 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



Conclusions

1. **Bilateral intrastriatal administration of LETI-101 utilizing CED was well-tolerated with a NOAEL obtained for the highest evaluated dose. No untoward clinical observations occurred based on evaluation of body weight, food consumption, ophthalmic examination, functional observational battery[1], clinical chemistries, and histopathologic evaluation**
2. **Bilateral intrastriatal administration of LETI-101 by CED resulted in high vector copy number and transgene expression across brain regions that are critically vulnerable in HD**
3. **Minimal AAV vector was observed systemically, with nothing detected in the gonads (data not shown)**
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 of 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 (LETI-101) 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, cortex, and thalamus of BACHD transgenic mice which carry a full-length human mHTT transgene**
- **Bilateral intrastriatal delivery of LETI-101 (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) NOAEL obtained for the highest dose level evaluated including no untoward clinical observations based on body weight, food consumption, ophthalmic examination, functional observational battery[1], clinical chemistries, and histopathologic evaluation.**
  - **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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