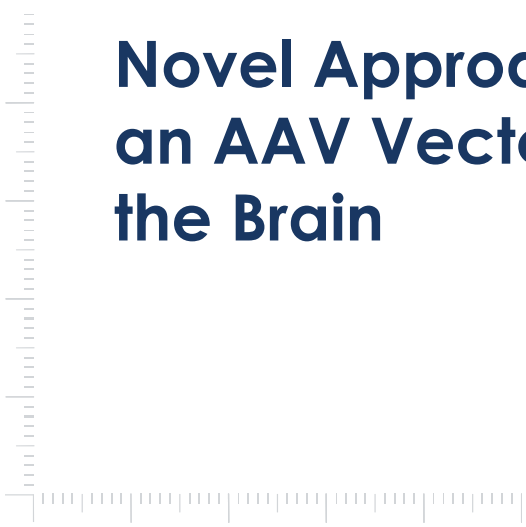
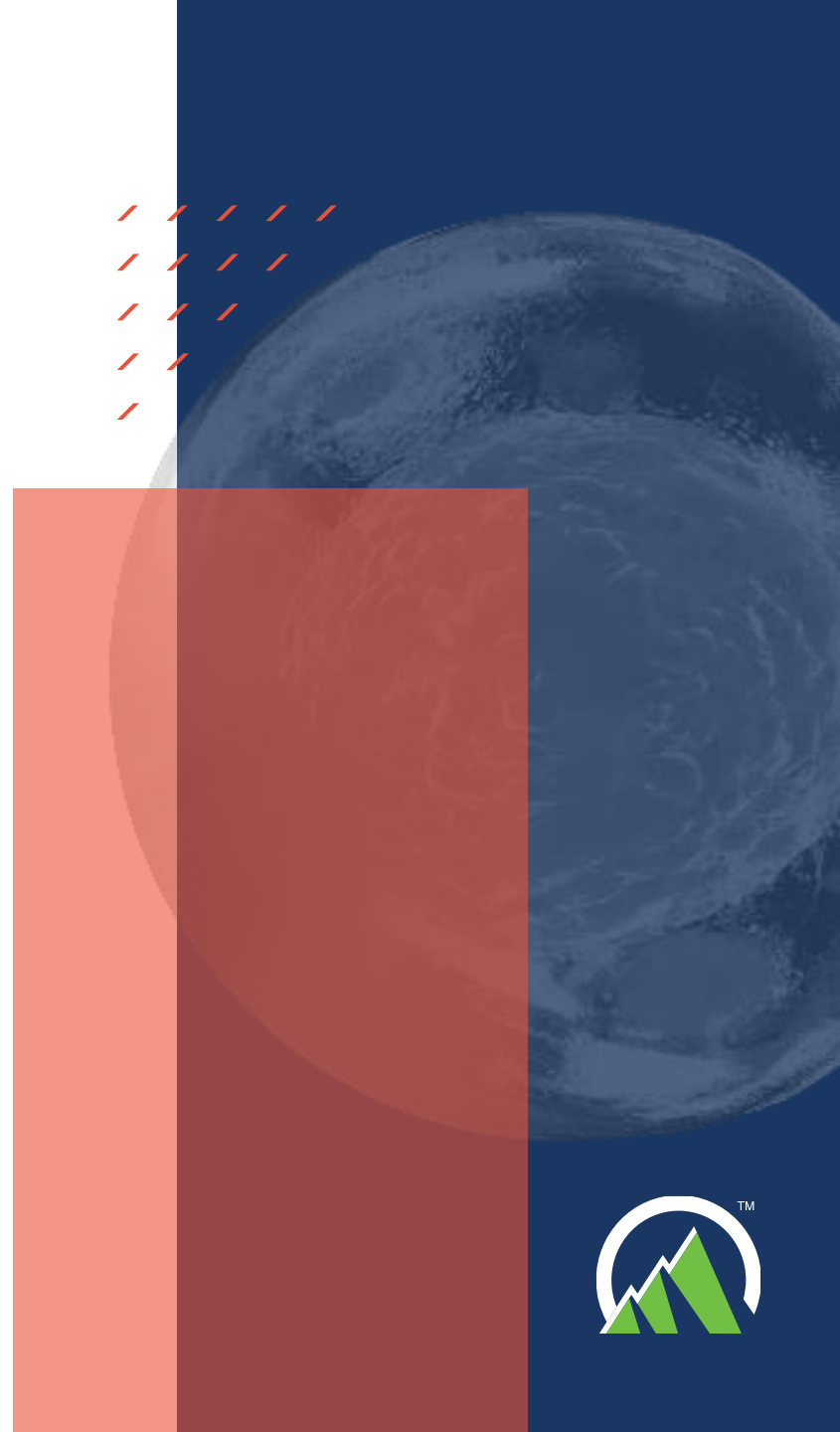




Novel Approach to Treating Rare Disease Using an AAV Vector to Deliver Gene Editing Tools to the Brain



ELEVATE.BIO
//LIFE EDIT



Contents



1. Provide overview of the development of LETI-101, a preclinical stage AAV5-delivered Life Edit® nuclease and guide RNA for mutant allele-specific editing in Huntington's Disease
2. Share approach for assessing manufacturability of LETI-101 through linking preclinical and CMC efforts
3. Discuss the importance of biotech “owning” the future CMC problems at an early stage of pharmaceutical development to de-risk clinical trials and commercialization

Life Edit is Developing an Allele-Selective Editing Approach to Treat Huntington's Disease (HD)

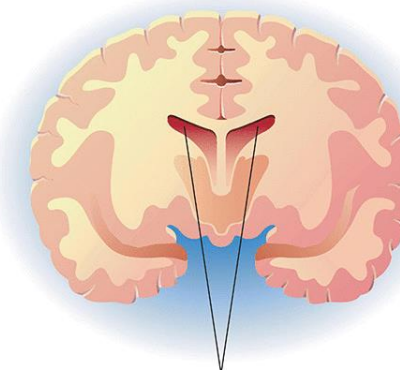
HD patients have severe, progressive, neurodegenerative disease, with no available disease modifying therapies

- HD is a rare, progressive, neurodegenerative disorder
 - Typical onset at 30-50 years; time from symptom emergence to death is ~10-30 years
 - Degeneration and atrophy of the striatum, and later the cerebral cortex

Targeting mutant huntingtin

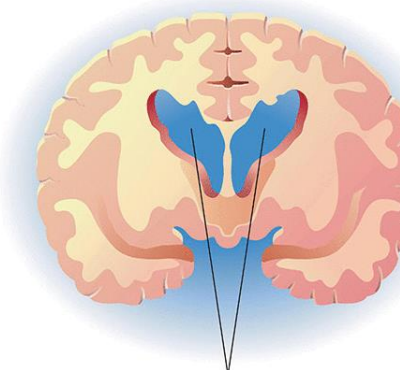
- HD is caused by the expansion of CAG repeats in the huntingtin gene (*HTT*)
 - Healthy brain: 10-35 CAG repeats; HD pts: 40 to >120 CAG repeats
- Expansion of CAG repeats leads to the production of the mutant HTT protein (mHTT) which ultimately leads to neuronal cell death
- The wtHTT protein supports a wide range of homeostatic functions including transcriptional regulation, axonal transport, endosomal trafficking, and vesicular recycling, thus, maintaining wtHTT is a high clinical priority

Normal brain section



Normal frontal horns of the lateral ventricles

Huntington's disease

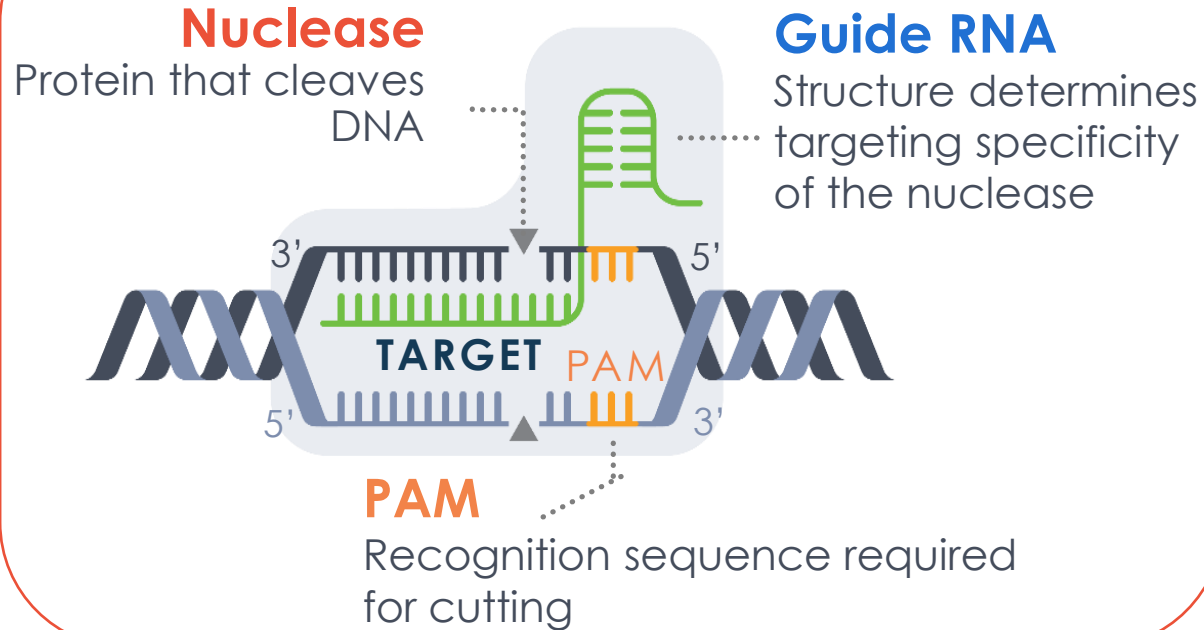


Enlargement of the frontal horns of the lateral ventricles

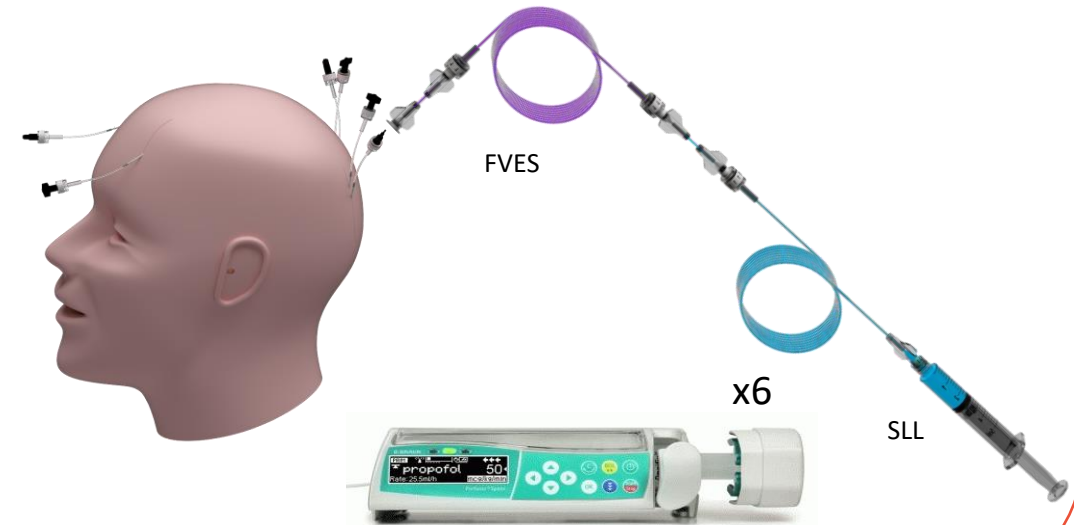


Overview of Combination Product LETI-101+neuroinfuse™ Intraparenchymal Drug Delivery System (Renishaw)

CRISPR System Components in AAV5



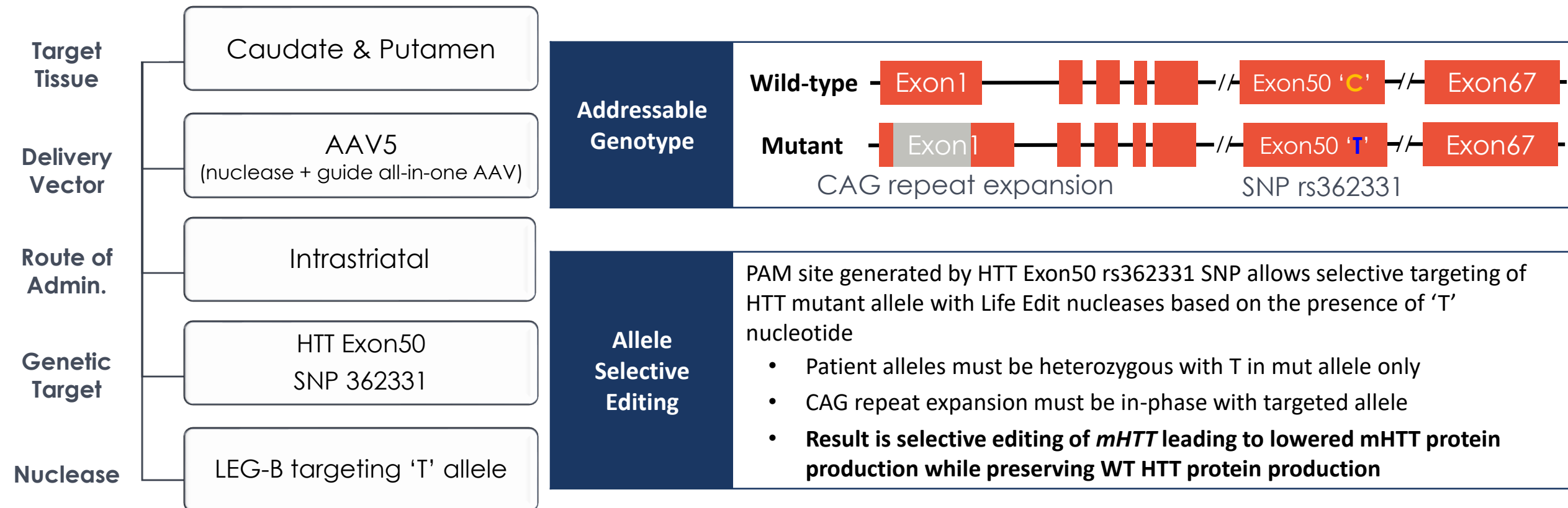
neuroinfuse Drug Delivery System



- Life Edit Genes (LEGs) have diverse PAM recognition sequences enabling broad genome targeting
- Life Edit nucleases are compact, enabling efficient delivery with a single AAV vector

- Reflux inhibiting feature facilitates convection enhanced delivery to the interstitial space
- Implantation of catheters outside MRI
- Quick, simultaneous infusion while patient is awake, reducing exposure to anesthesia

LETI-101 Allele Selective Strategy for Huntington's Disease



- LETI-101 is selective for mHTT allele based on the PAM generated by SNP rs362331 in exon 50
- LETI-101 is a one-time treatment that permanently modifies mHTT DNA for lasting therapeutic effect

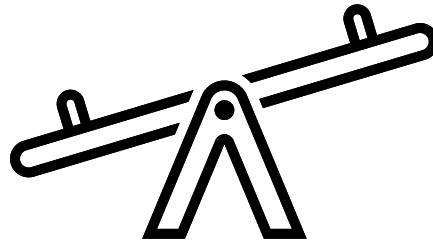


Collaboration on LETI-101 Candidate Selection in R&D

RESEARCH

Can it treat the disease?

Nuclease + sgRNA combination in an AAV5 vector for $\geq 40\%$ reduction in mHTT protein in mice





DEVELOPMENT

Can we manufacture it?

AAV5 vector with high productivity from HEK suspension cells and high proportion of full capsids

Research Batches

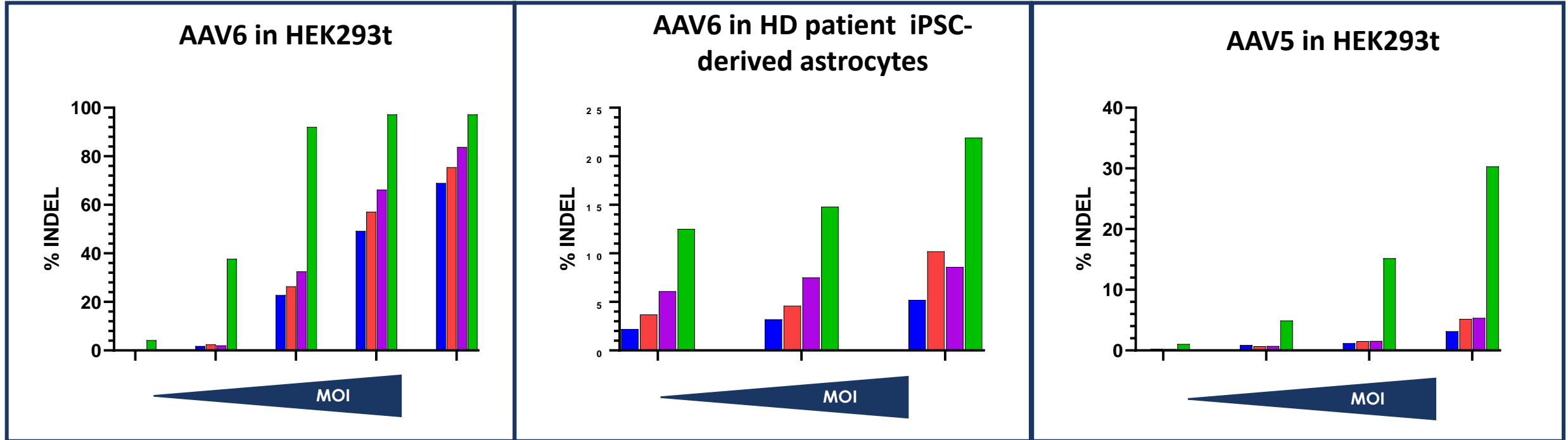
Development Batches

Vector Construct	sgRNA Promotor	Nuclease NLS	Transgene Size	Genome titer vg/mL	% Capsid protein : genome titer	Upstream Genome titer vg/mL	% Full after Affinity Capture
1	A	A	X	9.95e12	60	1.20e11	7.2 
2	A	A	X - 98	8.96e12	58	1.12e11	10.8
3	B	A	X - 167	1.22e13	56	1.17e11	11.8
4	B	B	X - 146	1.25e13	54	1.62e11	12.9 

Vector Construct Screening in Research – *In Vitro*

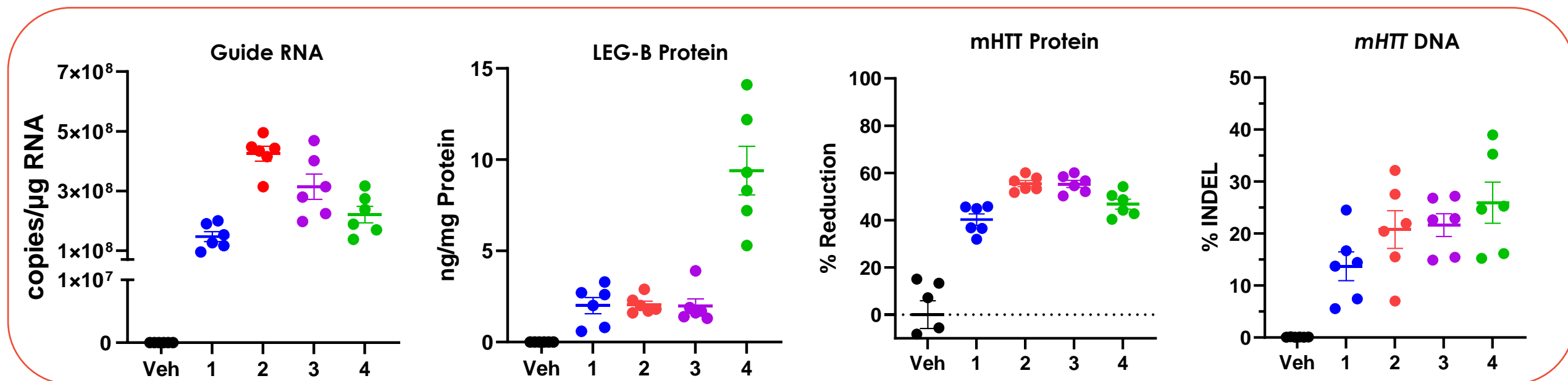


Vector construct #
1 2 3 4
■ ■ ■ ■
■ ■ ■ ■



- Construct #4 resulted in improved on-target editing rates relative to previous constructs
- The same rank order was observed among constructs with AAV5 as with AAV6, and in both HEK293t and HD patient iPSC-derived astrocytes

Vector Construct Screening in Research – *In Vivo*



Each point represents individual mice with mean \pm SD shown

Study Design

- ❖ BACHD transgenic mice (carry HTT rs362331'T' SNP)
- ❖ Intrastratial injections of AAV5 with 4 vector construct designs at single dose
- ❖ 6-weeks in-life duration \rightarrow brain tissues harvested and striatum analyzed

- NLS option B exhibited higher LEG-B protein expression
- High editing activity levels maintained with all design iterations

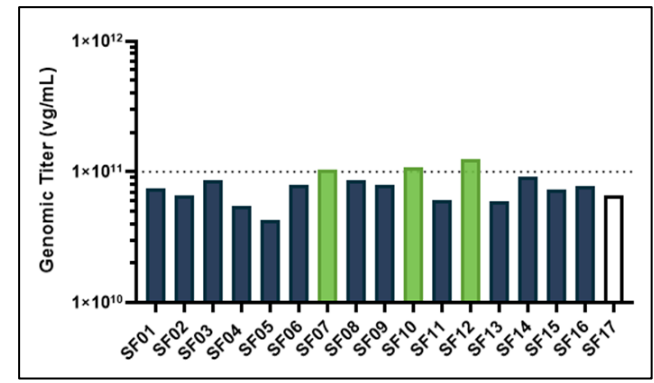
DoE Optimization of AAV Transfection Conditions



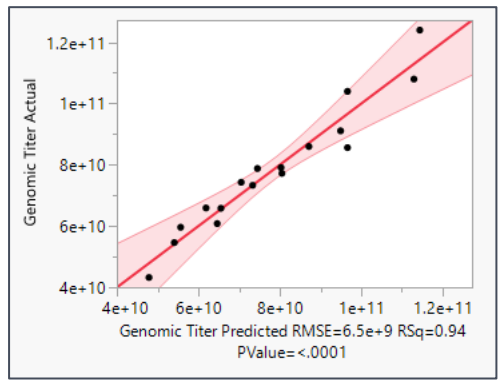
AAV upstream titer

(DoE process inputs)

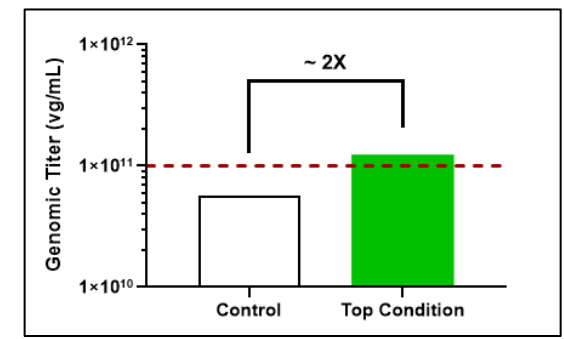
- DNA/cell
- Cell Density
- DNA/Reagent Ratio



AAV5 transfection optimization



Predictive Model

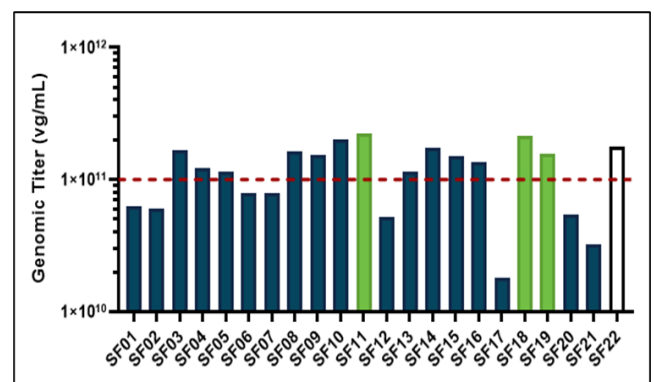


~2X titer improvement

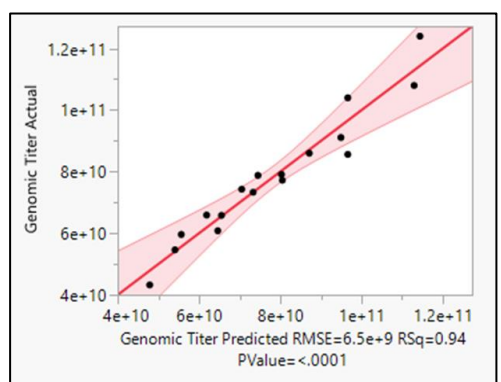
AAV upstream % Full

(DoE process inputs)

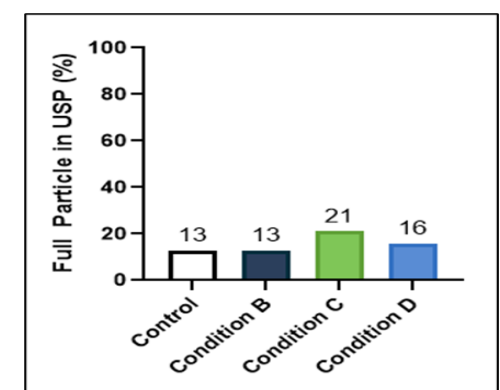
- 3- Plasmid ratios
 - RepCap
 - Helper
 - GOI



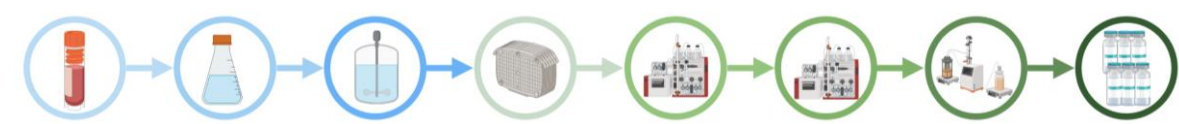
AAV5 transfection optimization



Predictive Model



~2X % Full AAV capsid improvement





DoE Optimization of AAV AEX Downstream Process

Chromatography parameters

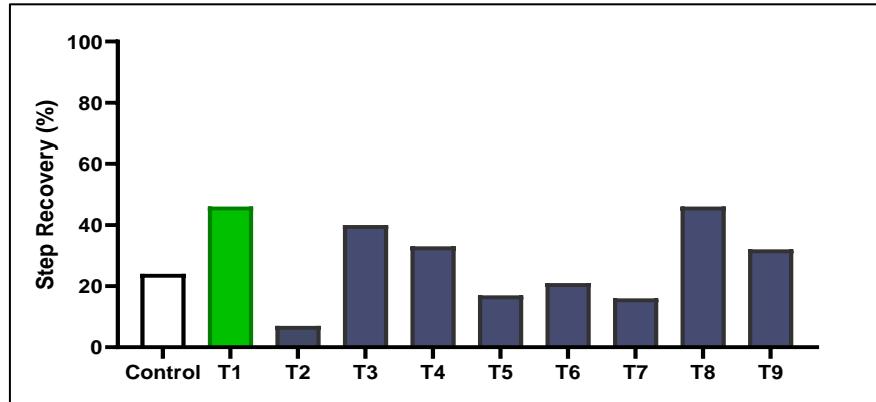
(DoE process inputs)

DoE 1 Elution

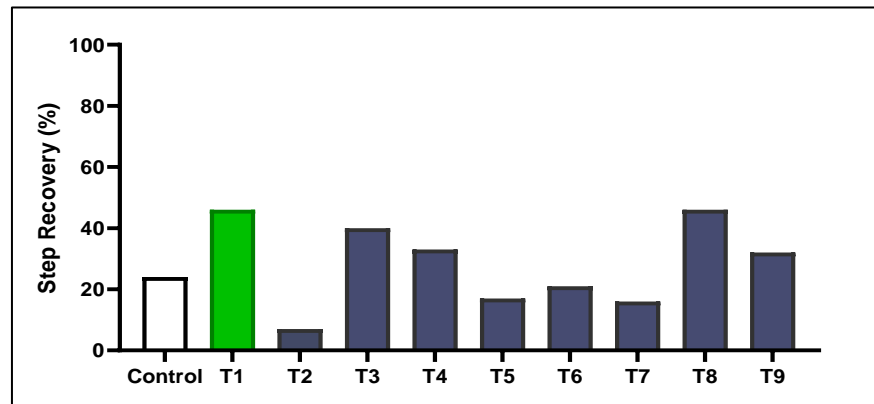
- Salt type
- pH
- Supplements

DoE 2 Binding

- pH
- Conductivity



AAV5 DoE 1 Elution parameter optimization



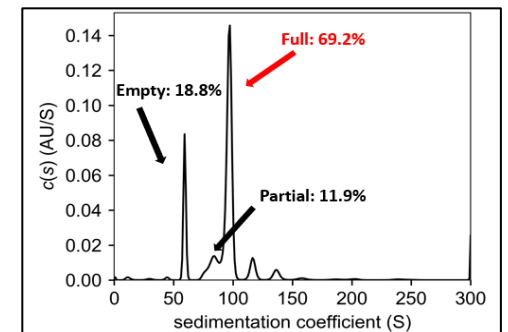
AAV5 DoE 2 Loading adjustment

Downstream AEX %Full optimization



AAV5 - AEX 2-step elution profile

AAV5 AUC



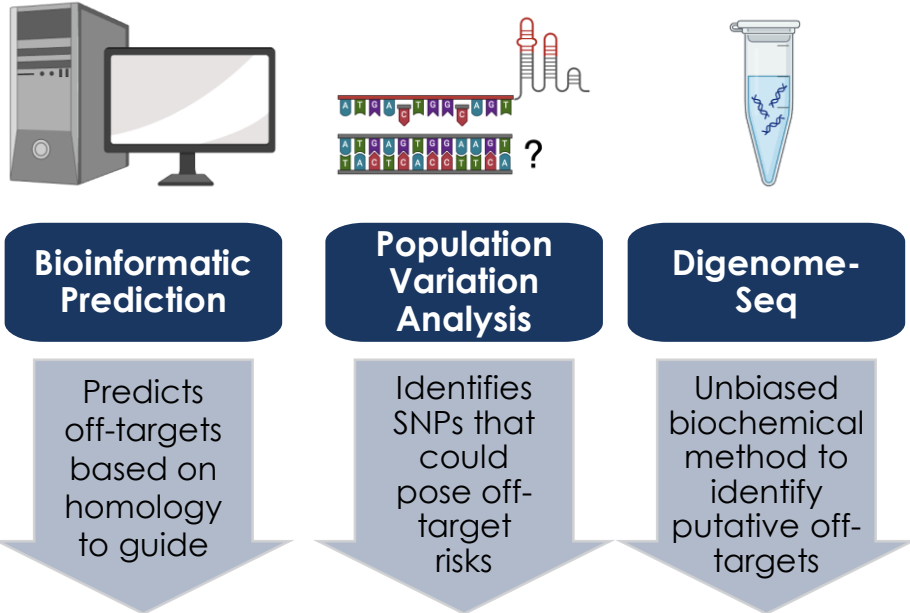
Full content 69.2%



Off-Target Analysis Reveals Specificity of LETI-101

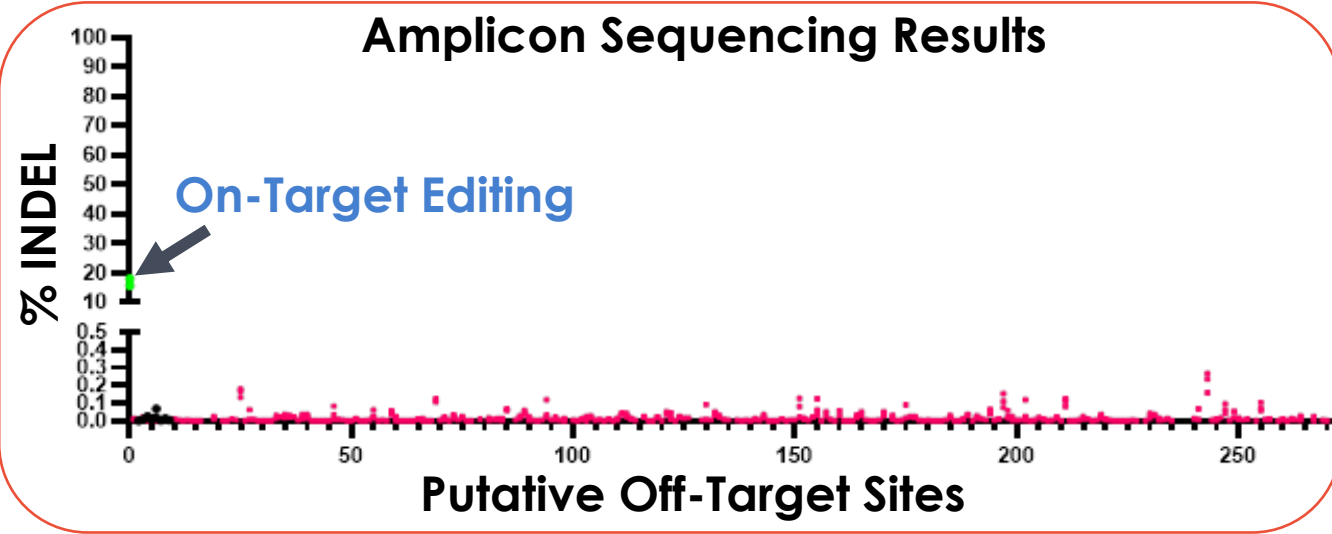


Off-Target Identification Strategy



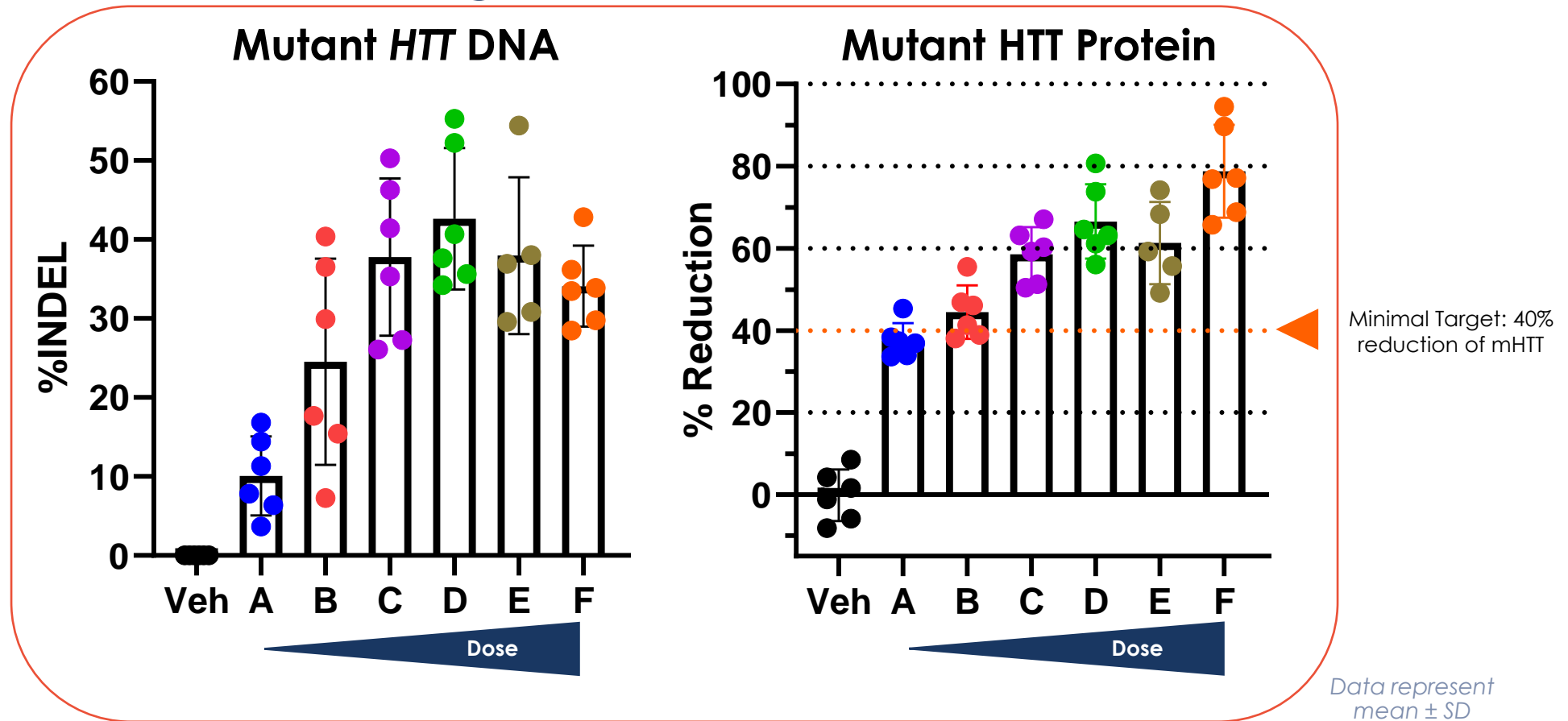
~300 putative off-target sites identified and profiled via amplicon sequencing using genomic material from HD patients edited with mRNA/RNA delivery

Amplicon Sequencing Results



No off-target editing observed at sequenced sites & no off-target liabilities identified

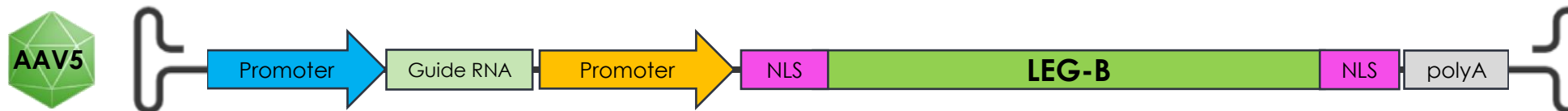
LETI-101 Dose-Dependent Expression & Activity in Striatum of BACHD Transgenic Rodent Model



- Intrastriatal injections of **LETI-101** in BACHD mice at six ascending doses (cohorts A-F)
- **3-month in-life duration** → bulk striatal tissue harvested and analyzed

Dose-dependent, on-target editing of mHTT allele and up to 80% reduction of mHTT protein

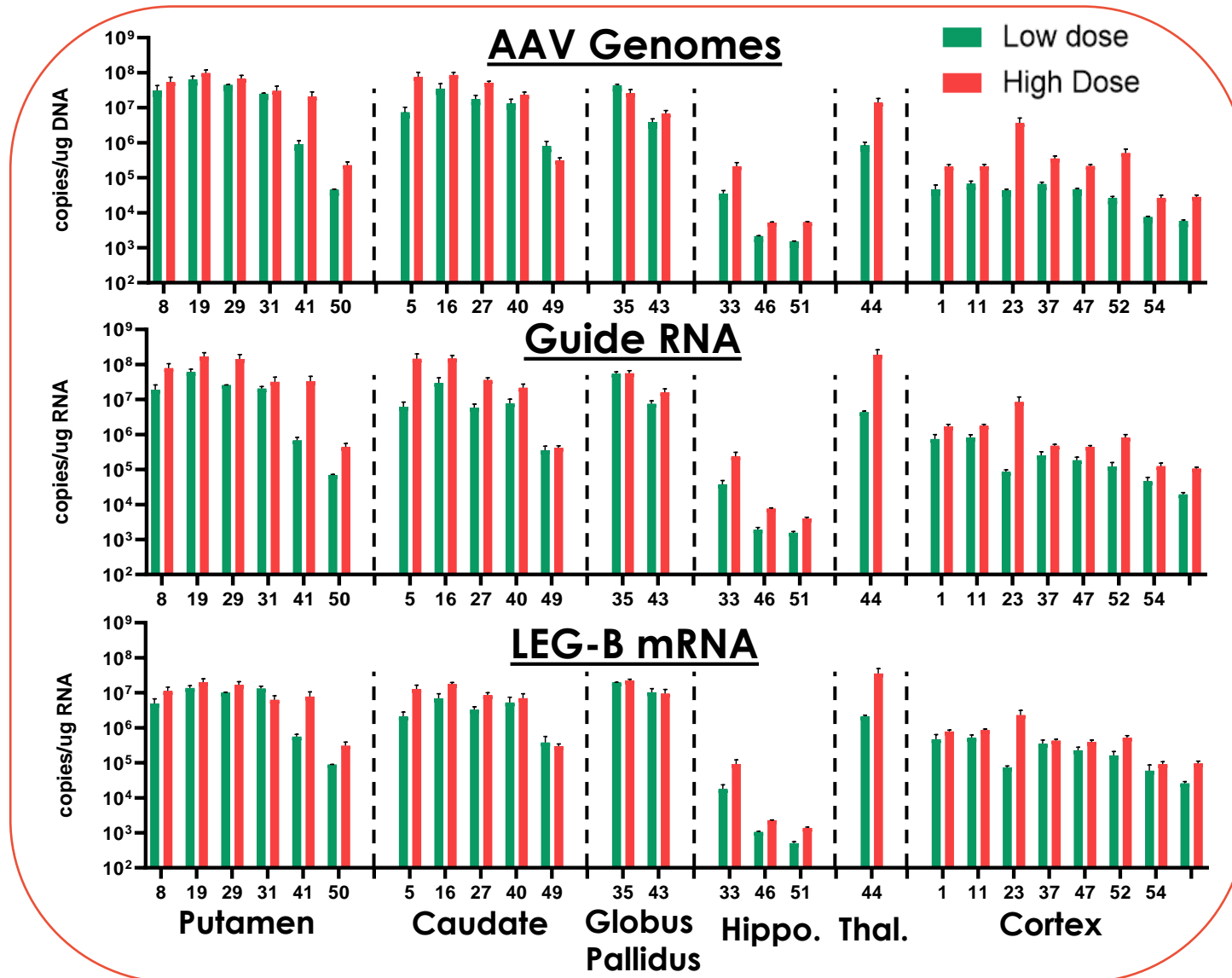
One Month Tolerability and Biodistribution of LETI-101 in Adult Cynomolgus Monkeys



Group	Subjects	Treatment	Dose (vg/brain)	Volume per Hemisphere (μL)
Vehicle	1M	Vehicle	0	Caudate: 75, Putamen: 150
Low Dose	1M / 1F	LETI-101	Low Dose	Caudate: 75, Putamen: 150
High Dose	1M / 2F	LETI-101	High Dose	Caudate: 75, Putamen: 150

- Administration performed using the Renishaw Acute Drug Delivery system with Neuroinfuse™ catheters
- No LEG-B PAM in NHP *HTT* exon 50 homologous region

LETI-101 Dose-Dependent Biodistribution in HD Critical Brain Regions of Cynomolgus Macaque



- Intrastriatal injections of LETI-101 in Cynomolgus monkeys at 2 dose levels
- X-axis denotes tissue punch #
- 1-month in-life duration
- Data represent mean \pm SD

- **Intrastriatal delivery of LETI-101 in cynomolgus macaques was well-tolerated; all animals survived to scheduled necropsy with no noted untoward clinical observations**
- **Dose-dependent vector biodistribution, guide RNA expression, & LEG-B expression in the striatum was observed 1-month following bilateral intrastriatal CED administration**
- **NOAEL obtained for highest dose level evaluated**



Biotech Can “Own” Future CMC Problems and De-Risk Clinical Trials and Commercialization





LETI-101 Summary

1. Delivery of editing tools with a single AAV5 vector to CNS
2. Completing late-stage discovery while evaluating manufacturability saved time and improved yield and editing outcomes
3. Successful scaleup to 50 L (for both upstream and downstream steps)
4. Allele specific targeting of *mHTT* with clinically relevant reduction of mHTT protein in BACHD transgenic mice and biodistribution and transgene expression across brain regions that are critically vulnerable in HD in NHPs

Life Edit Therapeutics met with MHRA in September 2024 to review LETI-101

- Preclinical data package well received; deemed "sufficient and comprehensive" including off-target characterization strategy
- Concurrence with overall clinical trial design and CMC strategy

THANK YOU
FOR YOUR
ATTENTION!