Deep CRISPR Nuclease Portfolio and Multiple Editing Modalities Accelerates Identification of Viable Clinical Candidates

Vasu Kommireddy, Anastasya Birger, Bikash Shakya, Allie Crawley, Matthew Nethery, Chuck Pepe-Ranney, Hui-Chia Yu-Kemp, Andy Chan, Tim Schwochert, Drew Kelso, Logan Brown, Julia Portocarrero, Lucas Ribeiro, Victor Bartsevich, Salem Faham, David Wiley, John Russell, Sarah Compton, Joel Parker, Philip Borden, Michael Coyle, Ron Chong, Tedd Elich

ElevateBio Life Edit, Durham, North Carolina, USA

life edit® an elevatebio company

Background

Extensive portfolio of validated CRISPR systems

- Novel RNA-guided CRISPR editors validated both *in vitro* and *in vivo*
- Compact systems fit for both AAV and LNP delivery
- Large collection of natural and engineered PAMs for full access of disease loci

DEPTH AND BREADTH OF GENE EDITING TECHNOLOGIES

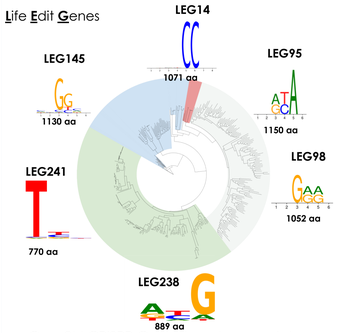
Comprehensive editing modalities

- Knock-out
- **A** and **C** base editing
- Gene writing with RT editing
- Expanding to additional modalities

Robust discovery engine allows more options for editing

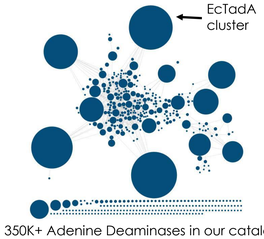
Type II and Type V collection

Life Edit Genes



Over 2M CRISPR-Cas systems in our catalog

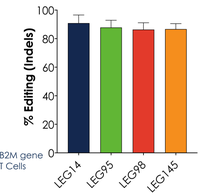
Deaminase and RT collection



350K+ Adenine Deaminases in our catalog

- Portfolio of characterized gene editing tools
- 20B+ protein catalog
- Rich collection of deaminase diversity fueling discovery and engineering
- Retroviral and non-retroviral RT collection

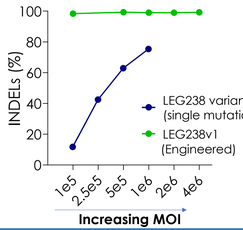
Nuclease Editing of B2M by Lead Type II Systems



- Robust and highly active naturally occurring Type II systems
- Low homology to established systems
- Discovery & Engineering further expanding PAM diversity

- 10 x improvement of engineered Type V systems
- Self processing guide RNAs enable multiplex editing
- Higher specificity of up to 17 nt of the spacer
- Delivery by a single AAV

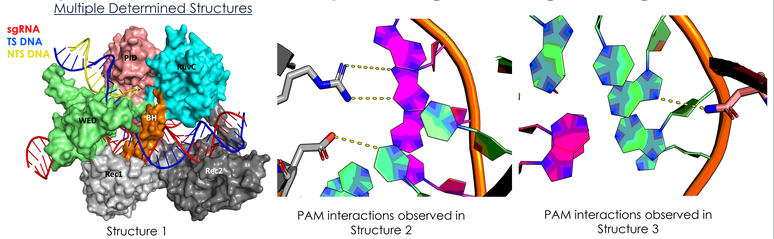
Editing of LDHA by Lead Type V Systems via AAV6 delivery



PAM engineering increases access to potential therapeutic targets

Cryo-EM structure of lead proteins guides engineering efforts

Multiple Determined Structures

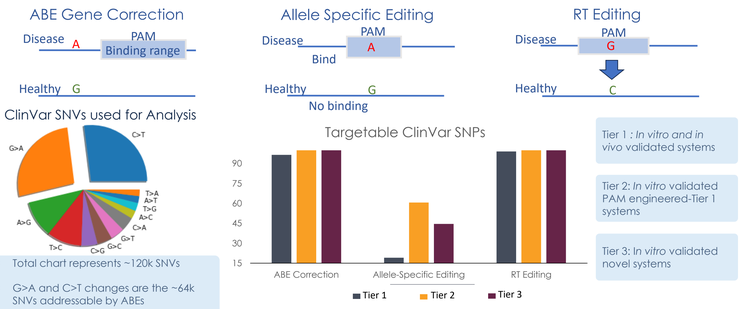


- Structures of leading nucleases have been determined
- Key PAM interactions have been identified

- Protein-sgRNA interactions analyzed for guide backbone modification
- Key domains identified for further engineering

PAM: Protospacer Adjacent Motif

CRISPR portfolio targeting >94% ClinVar disease mutations



- Total chart represents ~120k SNVs
- G>A and C>T changes are the ~64k SNVs addressable by ABEs

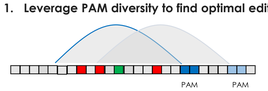
Tier 1 : *In vitro and in vivo* validated systems

Tier 2: *In vitro* validated PAM engineered-Tier 1 systems

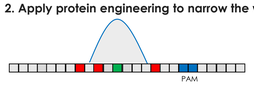
Tier 3: *In vitro* validated novel systems

PAM diversity enables optimal positioning of target edit

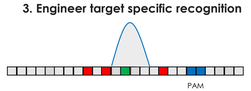
1. **Leverage PAM diversity to find optimal editing window**

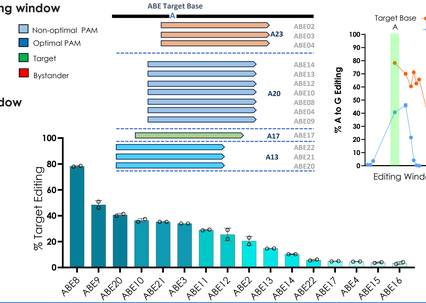


2. **Apply protein engineering to narrow the window**



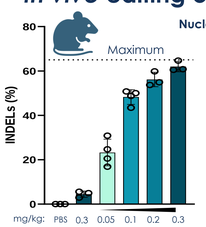
3. **Engineer target specific recognition**





Potent *in vivo* editing using proprietary LNPs for nuclease, base and RT editors

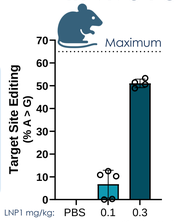
In vivo editing of *Hao1* with Life Edit LNPs



- Reproducible, robust editing at 0.3 mg/kg dose (62%)
- >20% editing from lowest dose tested (0.05 mg/kg)
- Steep inflection from 0.05 to 0.1 mg/kg doses

* Single IV dose in female C57BL/6 mice was well tolerated with no changes in clinical observations

In vivo Pcsk9 base editing



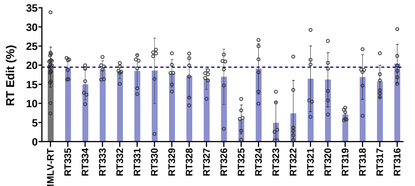
- High potency base editing at 0.3 mg/kg
- Treatment was well tolerated
- First in vivo study utilizing proprietary lipid and ABE to target PCSK9

- Single IV dose in female C57BL/6 mice was well tolerated with no changes in clinical observations

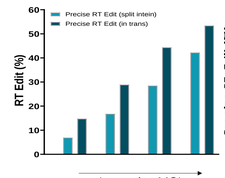
In vitro and *in vivo* demonstration of reverse transcriptase (RT) editing

Life Edit RT Platform:

- Utilizes proprietary LEGs in their nickase form
- Rewrite DNA with precise insertions, deletions and substitutions
- No DNA doble-strand breaks required
- Developing Dual RT editing strategies
- Multiple retroviral and non-retroviral RT systems that perform similar to or better than MMLV-RT system



Dual AAV Delivery *in vitro*



LNP1-RT Editor targeting in Mouse Liver

- 50% of the maximum possible RT editing in liver
- Minimal/no INDELs observed

Conclusions

- We have developed a large portfolio of compact RNA-guided nucleases with mammalian activity and with diverse PAM recognition allowing full genome access of human disease mutations
- We have validated, both *in vitro* and *in vivo*, a wide range of editing modalities, including RNA guided nucleases, base and RT editors, with multiple delivery methods
- We leverage AI/machine learning, structure guided rational design, automated HT screening and directed evolution to customize our editors for target-specific potency and specificity
- Our proprietary LNPs and AAV vectors plus *ex vivo* capabilities offer flexibility in delivery of genomic medicines to different organs and tissues
- Our technology platforms accelerate identification of initial hits and developing them into viable clinical candidates

Learn more

vkommireddy@lifeeditinc.com

life edit® an elevatebio company