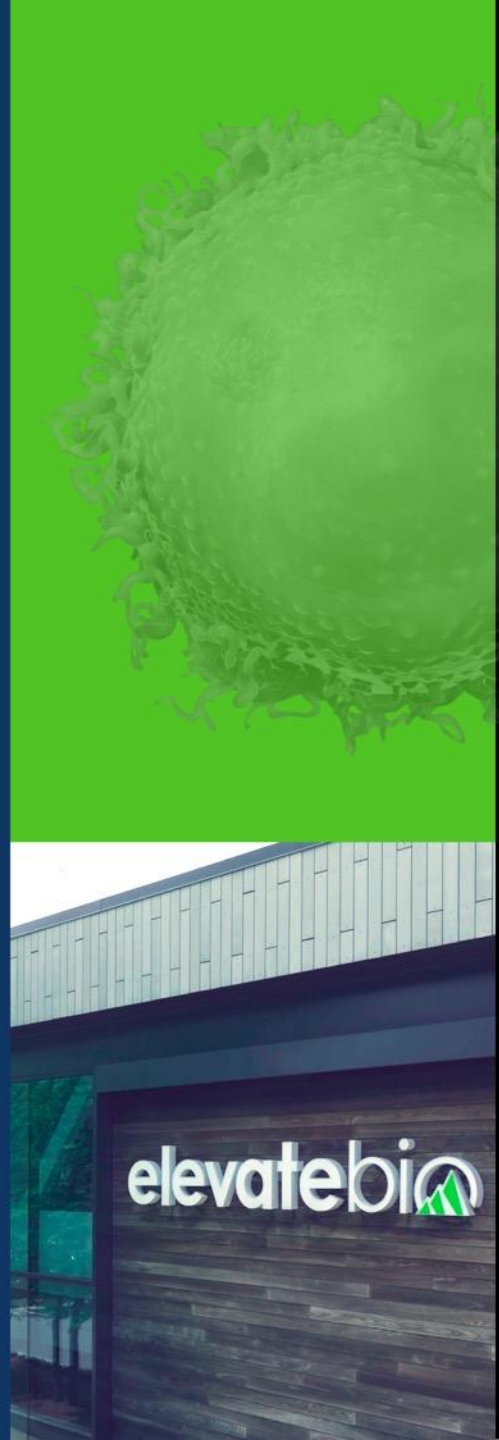
CMC considerations for CRISPR and next-gen technologies, including ElevateBio's LETI-101 as a case study

CASSS CGTP SYMPOSIUM 2026

DR. APRIL SENA, VP TECHNICAL OPERATIONS

elevatebio



JUNE 10, 2026

ElevateBio is a first-of-its kind CDMO with proprietary gene editing tools and services powering the development and manufacturing of advanced therapies

OUR VISION

To be the world's most indispensable advanced therapy technology company, changing how companies operate, how products are created, and how disease is treated

HOW WE WORK

We provide turnkey, scalable access across gene editing services and manufacturing capabilities to enable scalable, advanced therapeutic development.

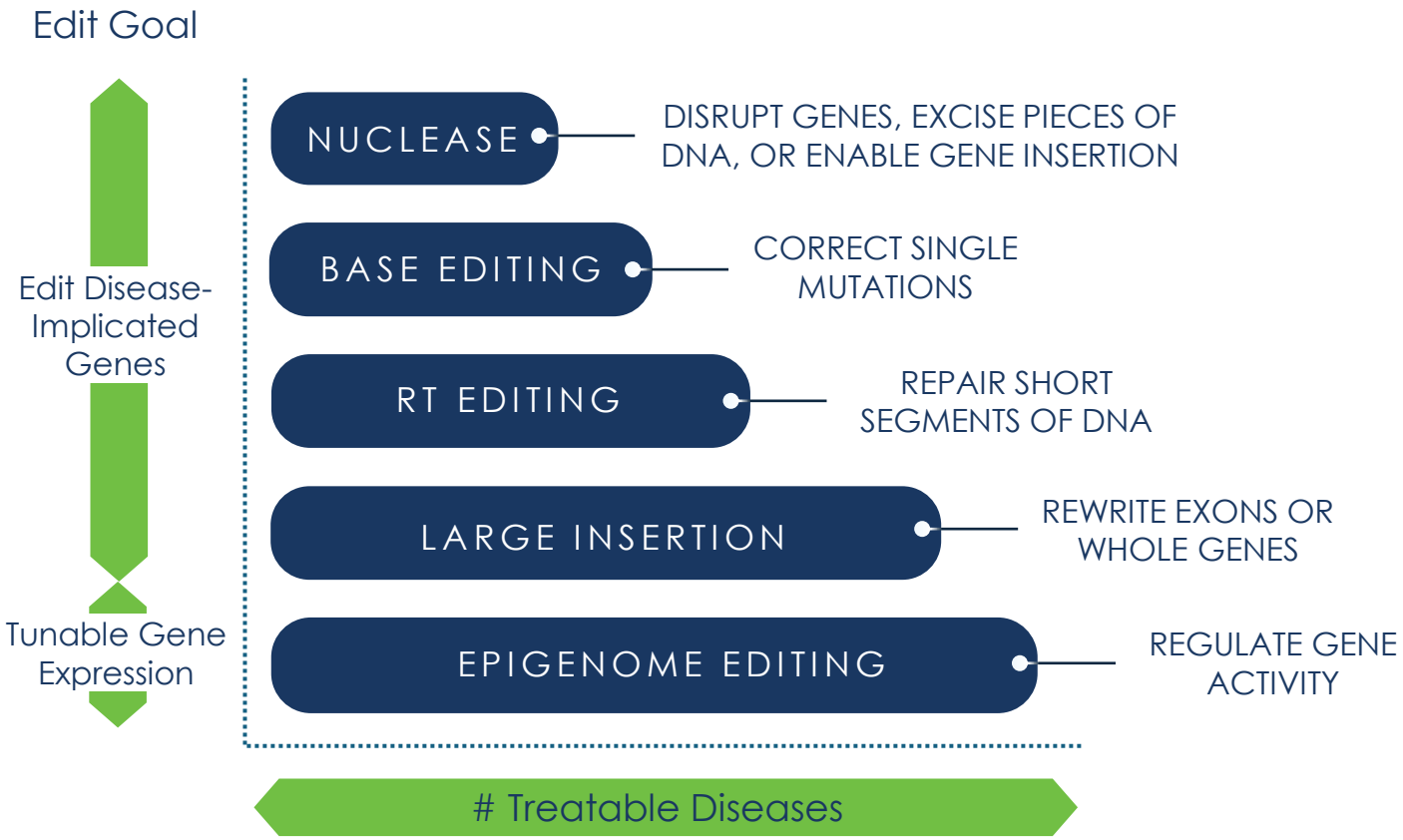
OUR SERVICES

Built for every stage of development, we support partners from early discovery and process development through technology transfer, clinical supply, and commercial cGMP manufacturing.

REDEFINING THE FUTURE OF MEDICINE

ElevateBio's comprehensive services span discovery to commercialization

With the technology and expertise to realize the promise of a new generation of medicines



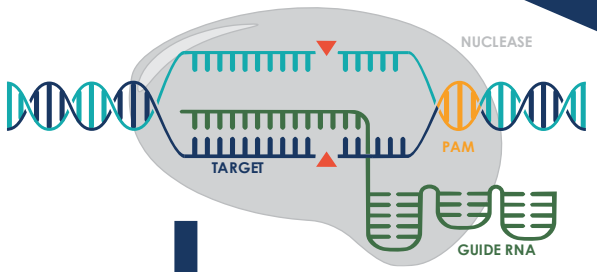
Original CRISPR nucleases recognize specific DNA sequences in the genome and **make a dsDNA break**

Next-gen CRISPR technologies **eliminate dsDNA break** capability and **add functional domains** to enable base, RT, or epigenome editing

CRISPR can assist Large Serine Recombinases with targeted **large gene insertion**

Molecular overview of CRISPR and next-gen technologies

Nuclease Editing



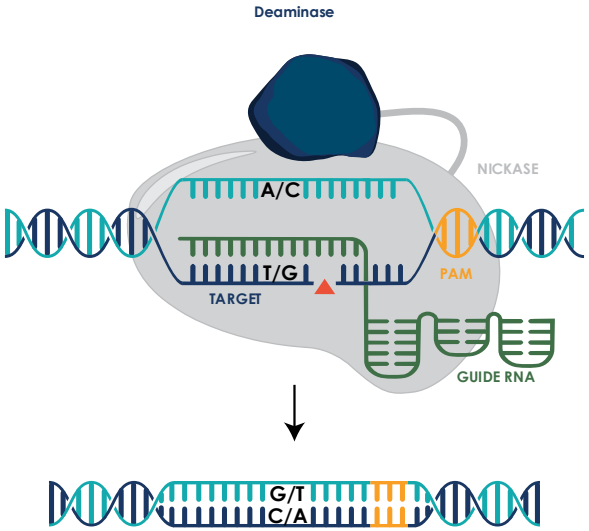
- **Type II CRISPR** nucleases, like Cas9 or **ElevateBio's LEG14, LEG95, LEG98, and LEG145** are primarily used in clinical products
- **Type V CRISPR** nucleases, like Cas12a or **ElevateBio's LEG238 and LEG241** are also of interest because they are much smaller
- **Type VI CRISPR** nucleases, like Cas13, edit RNA

From a CMC perspective, all comprise:

- **Protein(s) + guide RNA(s) = Ribonucleoprotein**
- Proteins with extra domains are harder to make as proteins in microbial fermentation systems
- Guide RNAs are much longer for RT editing

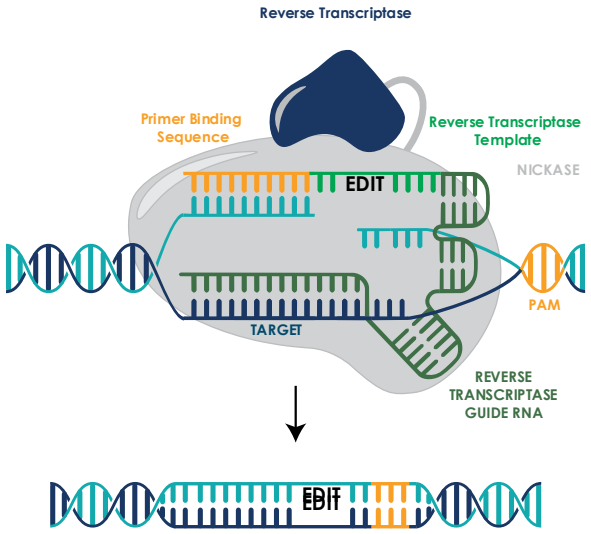
Next-Gen

Base Editing

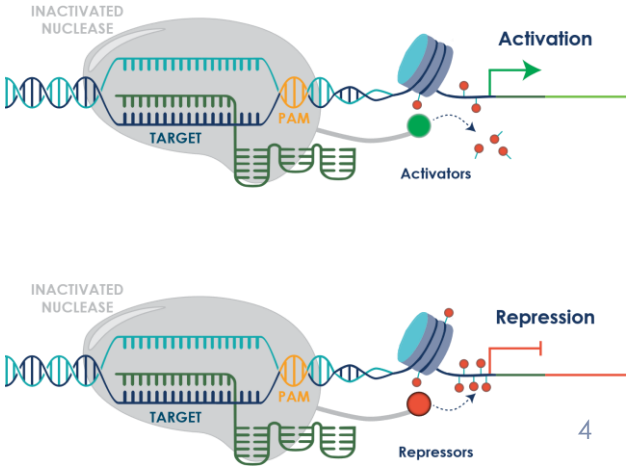


Types of deaminases:
adenosine deaminase (T•A→C•G)
cytidine deaminase (C•G→T•A)

Reverse Transcriptase Editing



Epigenetic Editing



PAM = Protospacer Adjacent Motif (~2-4 nucleotides in the genome recognized by the CRISPR enzyme)

Spacer RNA or crRNA = part of guide RNA that binds to the target DNA, ~20nt

tracrRNA = part of guide RNA that binds to the protein, varies in length

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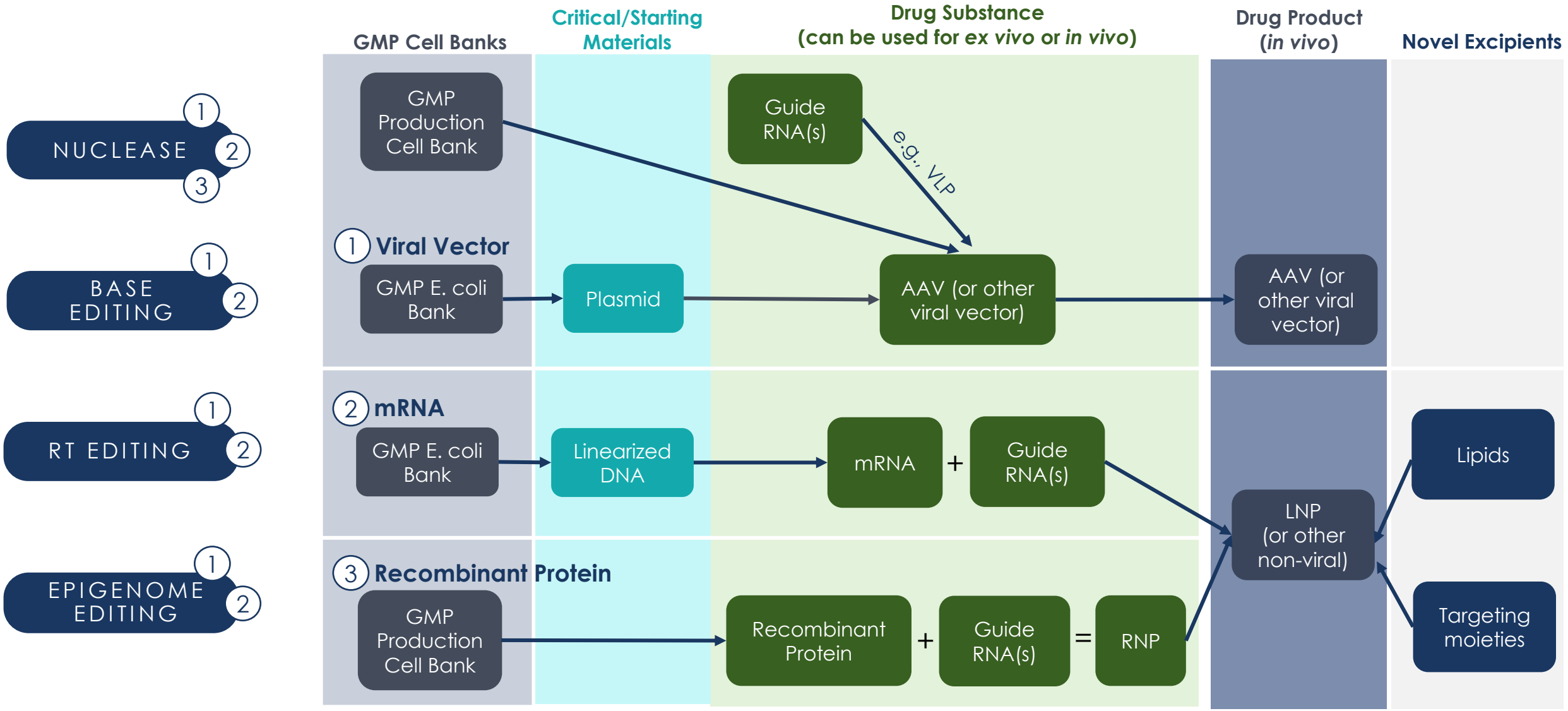
Delivery approaches for CRISPR and next-gen technologies



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Key CMC challenges with CRISPR and next-gen technologies

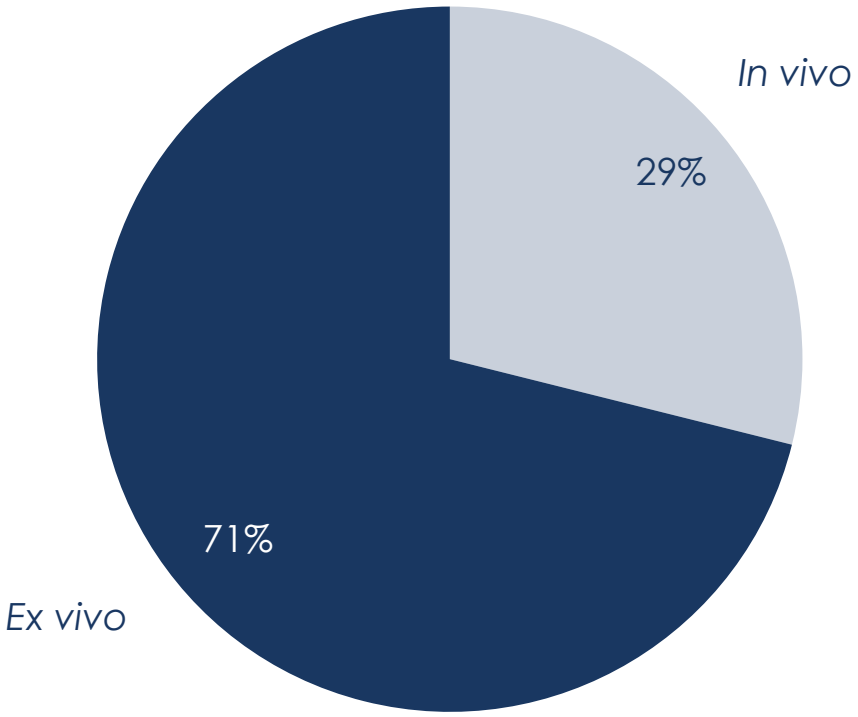
- CRISPR enzymes, added functional domains, and guide RNA can all be engineered, but keep a documented record of each step from wild-type
- Large chromosomal rearrangements are more likely the more dsDNA breaks, so consider next-gen CRISPR technology with multi-plex editing
- Cytidine deaminases are more promiscuous (changing C→A or C→G not C→T) and have more bystander edits than adenosine deaminases
- Established manufacturing platforms can be used, but still recommended to optimize structure for increased activity and manufacturability:
  - Type of CAP and length of PolyA tail for mRNA
  - Nucleotide modification scheme for guide RNA (particularly for *in vivo*)
  - Promoters for CRISPR enzyme and guide RNA in viral vectors
  - Size of transgene in viral vectors (e.g., would a Type V be helpful)
- Guide RNA purity is a challenge with solid phase synthesis; make sure you understand your purity assay and consider ways to increase purity:
  - Single guide RNA vs. double guide RNA
  - Guide manufacturer innovations in solid support, custom amidites, etc.
  - Ligating smaller pieces together

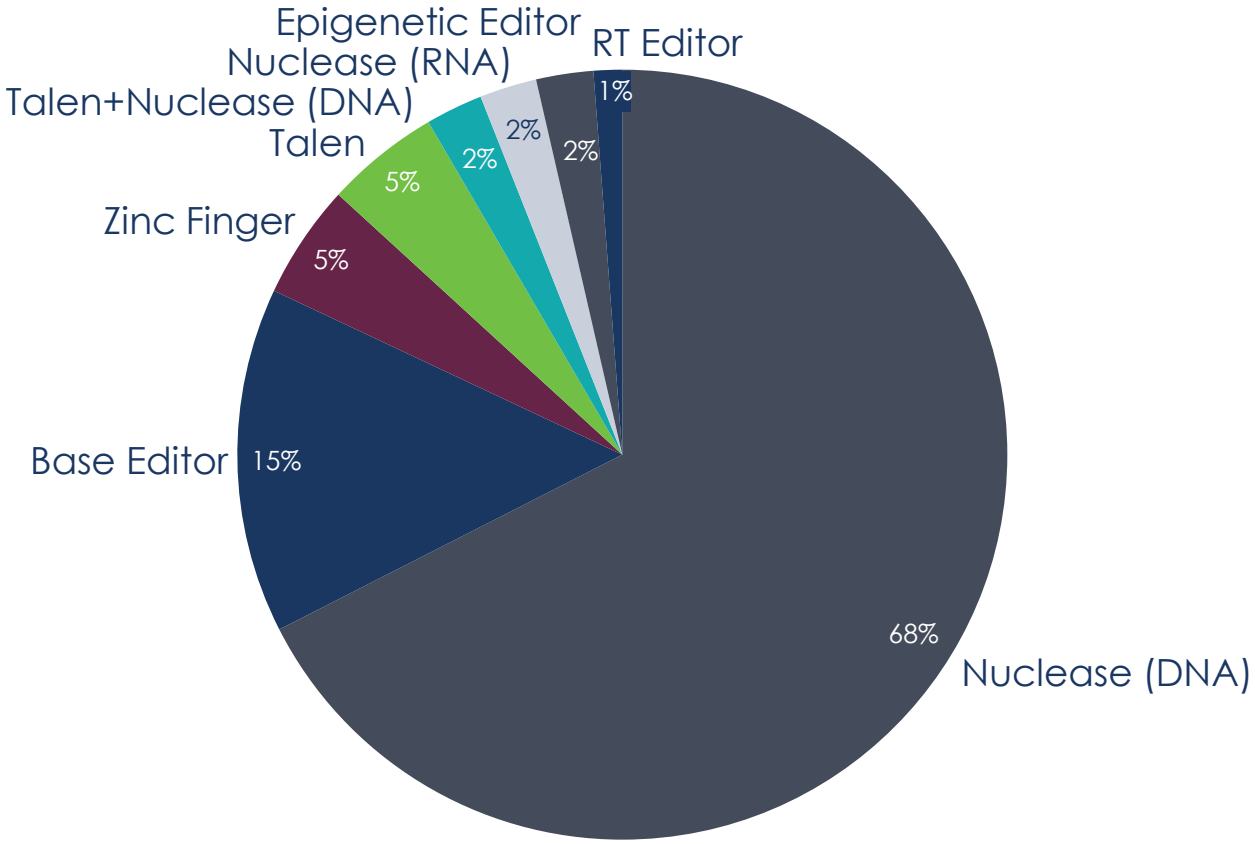
For more in-depth discussion on CMC for gene editing, please attend

Parallel Session 6: Control Strategies for Gene Editing Across Platforms

(13:45 – 15:30 in Salons A-C)

Gene editing products in the clinic (from 2025)





Clinical trial list from: Cetin B, Erendor F, Eksi YE,Sanlioglu AD and Sanlioglu S (2025).Advancing CRISPR genome editing into genetherapy clinical trials: progress and futureprospects. *Expert Reviews in Molecular Medicine*, **27**, e16, 1–33 https://doi.org/10.1017/erm.2025.10

Note: two clinical trials using epigenetic editing initiated in 2025 were added

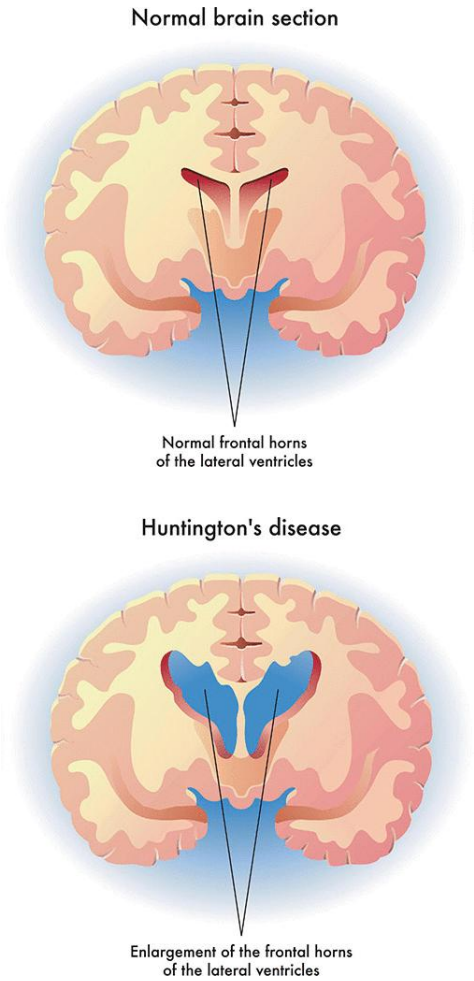
Huntington's Disease (HD) and treatment approach

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

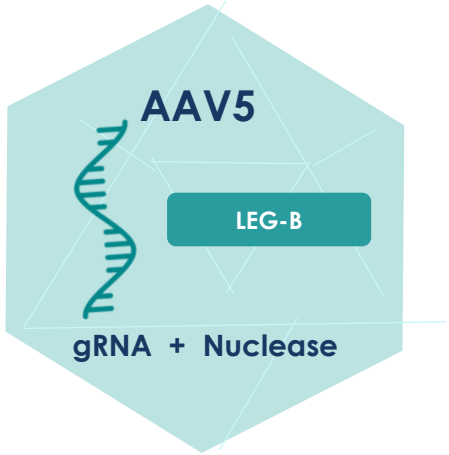
Targeting mutant huntingtin

- 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
- Maintaining wtHTT is a high clinical priority
  - wtHTT protein supports a wide range of homeostatic functions including transcriptional regulation, axonal transport, endosomal trafficking, and vesicular recycling

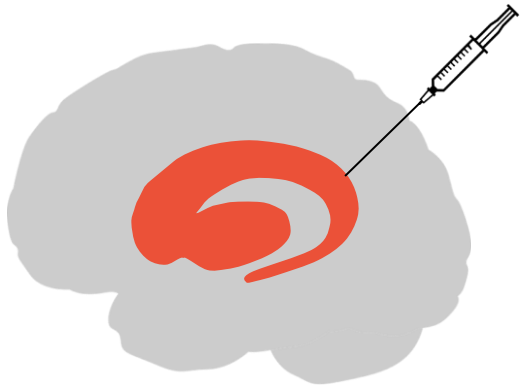


LETI-101: A precision editing approach as potential one-time treatment for HD (pre-clinical stage)

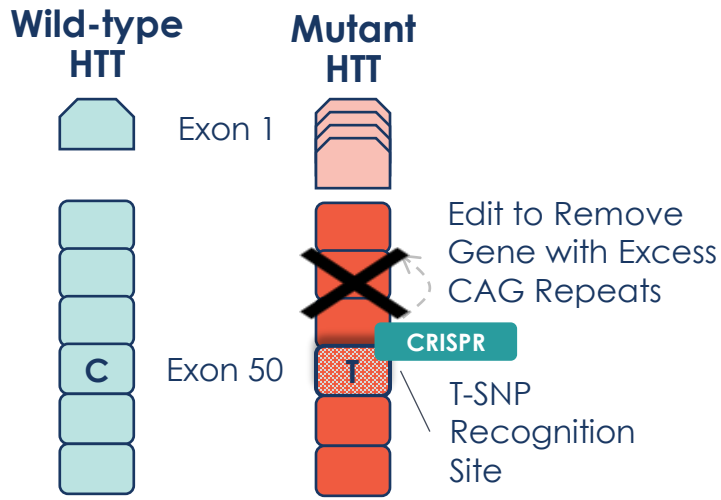
NOVEL CRISPR SYSTEM | TARGETED CNS DELIVERY | ALLELE-SELECTIVE EDITING



Proprietary, compact CRISPR system, packaged in AAV5 vector



One-time, bilateral intrastriatal administration



Potent and selective reduction in mutant while preserving wild-type; selective approach made possible by diverse genomic recognition sites

LETI-101 OFFERS POTENTIAL FOR A DURABLE, **ONE-TIME TREATMENT** WITH AN IMPROVED SAFETY PROFILE THROUGH SELECTIVE TARGETING

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Collaboration on LETI-101 candidate selection in R&D

RESEARCH
Can it treat the disease?

AAV5 (sgRNA+nuclease) results in potentially clinically relevant ≥40% reduction in mHTT protein in striatum of BACHD mice

DEVELOPMENT
Can we manufacture it?

AAV5 from HEK suspension cells achieving ≥1.00e11 vg/mL and ≥20% full capsids upstream and ~70% full capsids in final drug substance

Further vector construct optimization

Initial candidate

DOEs to optimize transfection and chromatography

				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 Capsid 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

LETI-101 Scientific Advice meeting with MHRA (Sep 2024)

Questions

MHRA Feedback

Quality: Comparability plan, test panel/specifications, potency assay, device compatibility

- Accepted potency test for early phase (ddPCR for INDEL formation)
- For comparability, required full IPC and release testing on batches used for preclinical safety studies
- Required targets for all impurities and putting on release panel
- For device compatibility, required sterility and particulates

Preclinical: overall pharm/tox strategy, off-target study design, GLP tox study design

- Preclinical data package deemed "sufficient and comprehensive"

Clinical: Phase 1b/2a study population and trial design, including endpoints

- Overall concurrence on clinical trial design
- Allow patients to cross-over within disease treatment window to facilitate recruitment

Summary

CMC FOR CRISPR AND NEXT-GEN

- Original CRISPR enzymes have functional domains added for next-gen base editing, RT editing, and epigenetic editing
- CRISPR-based products can all be made in similar ways, with key differences:
  - Next-gen may not manufacture well as recombinant proteins in microbial systems due to extra functional domains
  - Guide RNA for RT editing is longer
  - Guide RNA for *in vivo* require mods to stabilize
- Benefit/risk of different editing approaches should be considered when choosing the best editor for a given disease

LETI-101

- Collaboration in R&D to improve % Full capsids resulted in a candidate that also had:
  - Higher editing in HD patient iPSC-derived astrocytes
  - Higher productivity, resulting in higher yield
- Selection of the UK for the first in human clinical trial based on ability to perform bilateral administration and include a sham control
- MHRA SA feedback:
  - Preclinical data packaged deemed "sufficient and comprehensive"
  - Concurrence with overall clinical trial design and CMC strategy

Thank you