# A Translational Overview of Gene Editing as a Therapeutic Modality

Shravanthi Madhavan

Director, Translational Research Program and Portfolio Management

Nov 13, 2024

smadhavan@ultragenyx.com

# Considerations for Gene Editing as a Therapeutic Modality

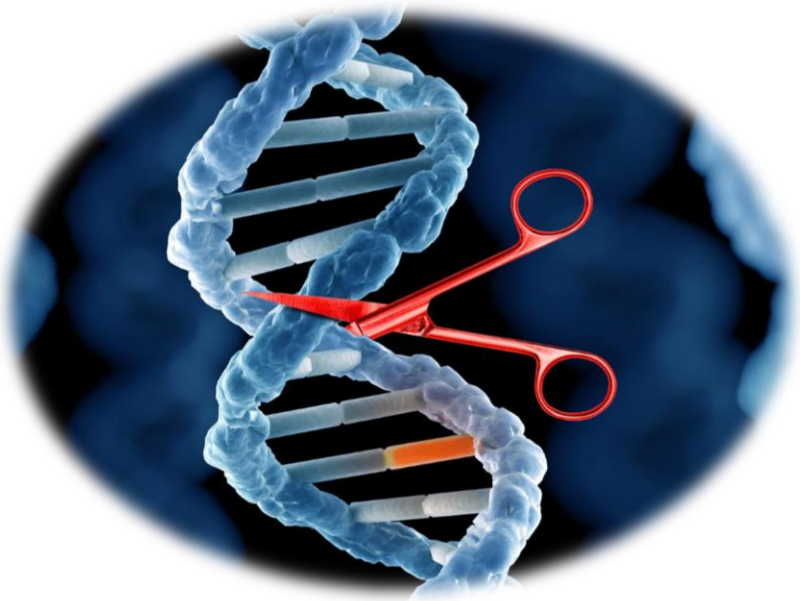
## BEFORE

DESIGN of editing complex

- **Type** of mutation/error
- **Size** of genetic error
- **Frequency** of error
- **Location** and **distribution** of error

DELIVERY into target tissue/cell

- Editing efficiency/% correction



## AFTER

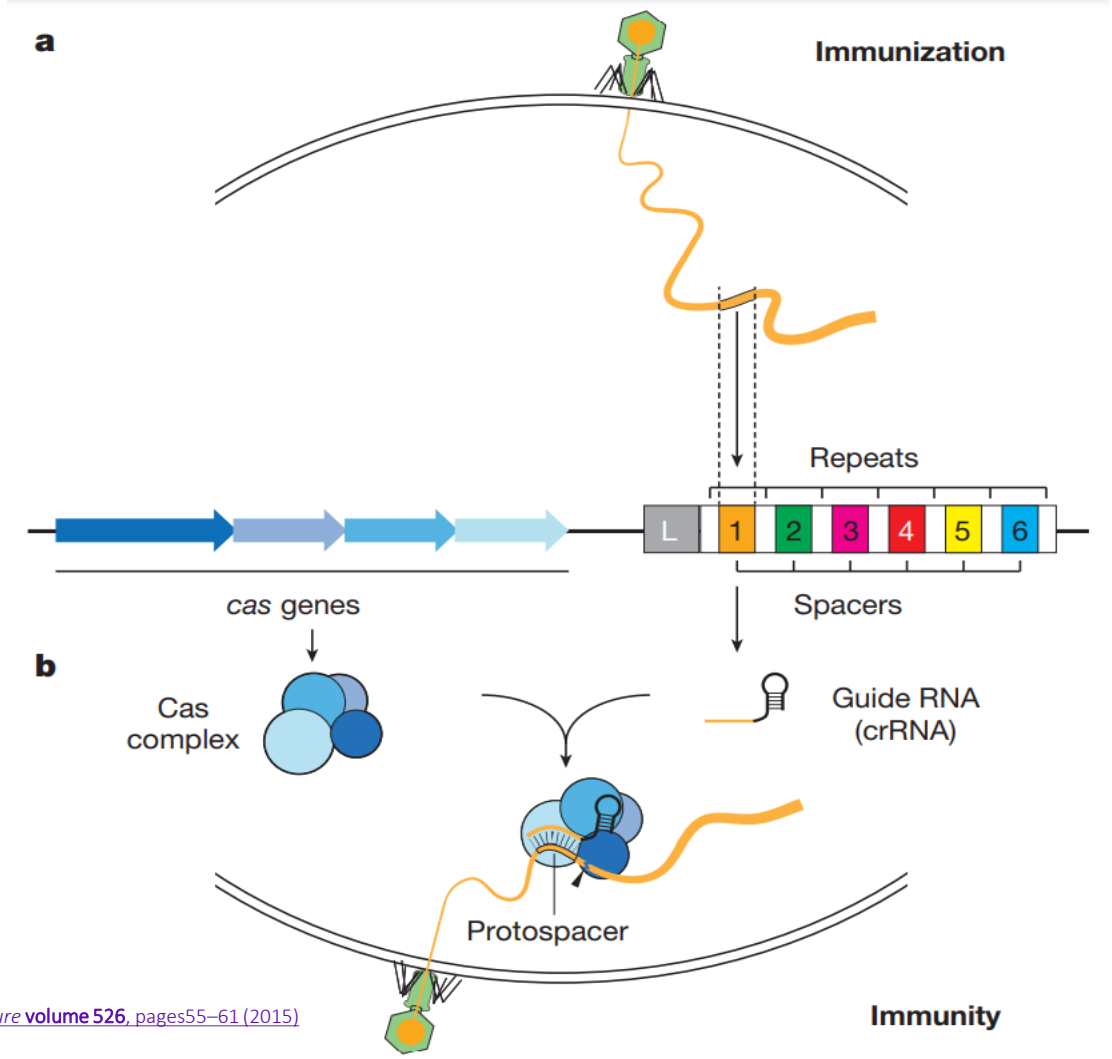
SPECIFICITY/OFF-TARGET effects

IMMUNE RESPONSE

# CRISPR/Cas: Bacterial Immune System to a Gene Editing Toolbox

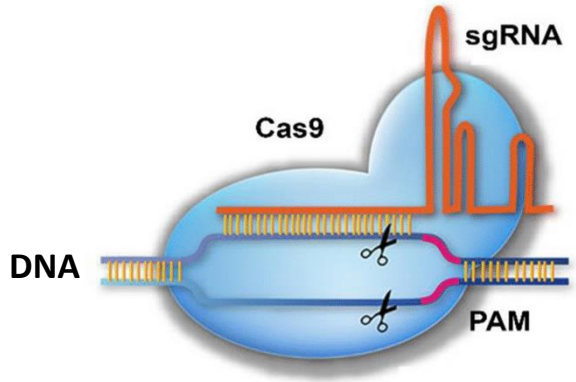
*Clustered Regularly Interspaced Short Palindromic Repeats*

## Natural CRISPR-Cas in Bacteria

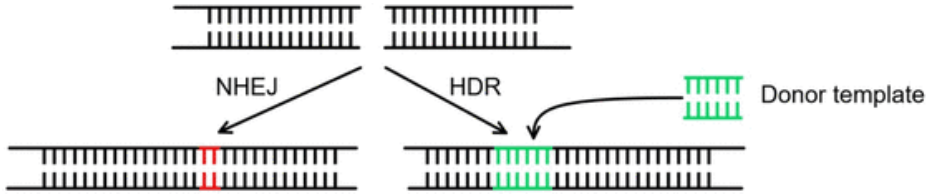



*Nature* volume 526, pages55–61 (2015)

## CRISPR-Cas for Gene Editing




| | |
|---|---|
| Recognition | PAM (Protospacer Adjacent Motif) |
| Binding | sgRNA (single guide RNA) |
| Cleavage | Cas Nuclease (CRISPR associated protein) |
| Repair | NHEJ (Non-homologous End Joining)<br>HDR (Homology Directed Repair) |




Transgenic Res (2021) 30:709–725

Rare Bootcamp™

# The Basic Gene Editing Toolbox

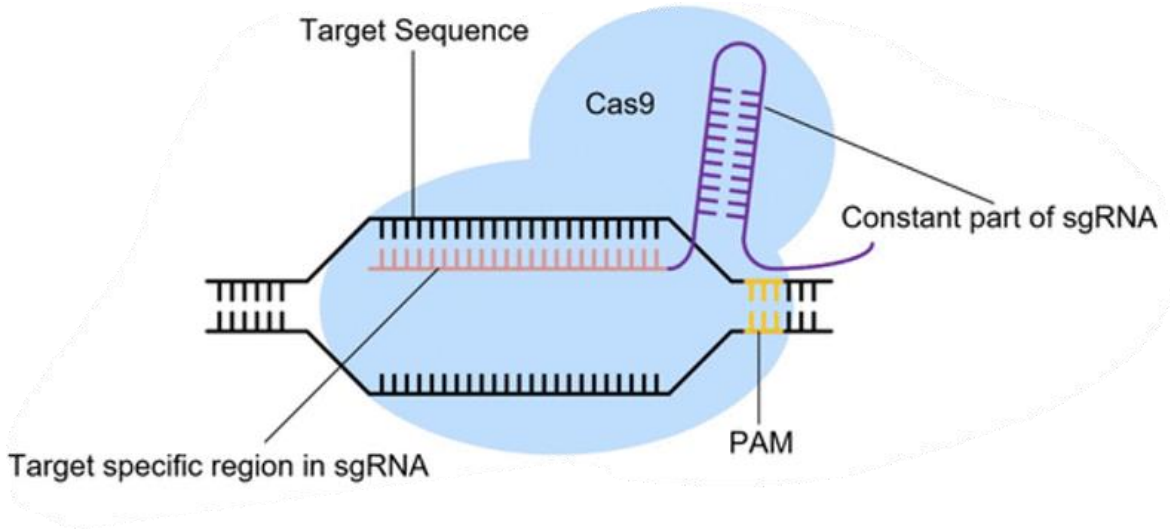
*PAM sequence near target DNA is key for Cas selection and sgRNA design*

## CRISPR Toolbox: The DRUG

| | | |
|---|---|---|
| 1 | Cas Nuclease/nickase (CRISPR-associated protein) | Recognition + Cleavage |
| 2 | sgRNA (single guide RNA) | Binding |



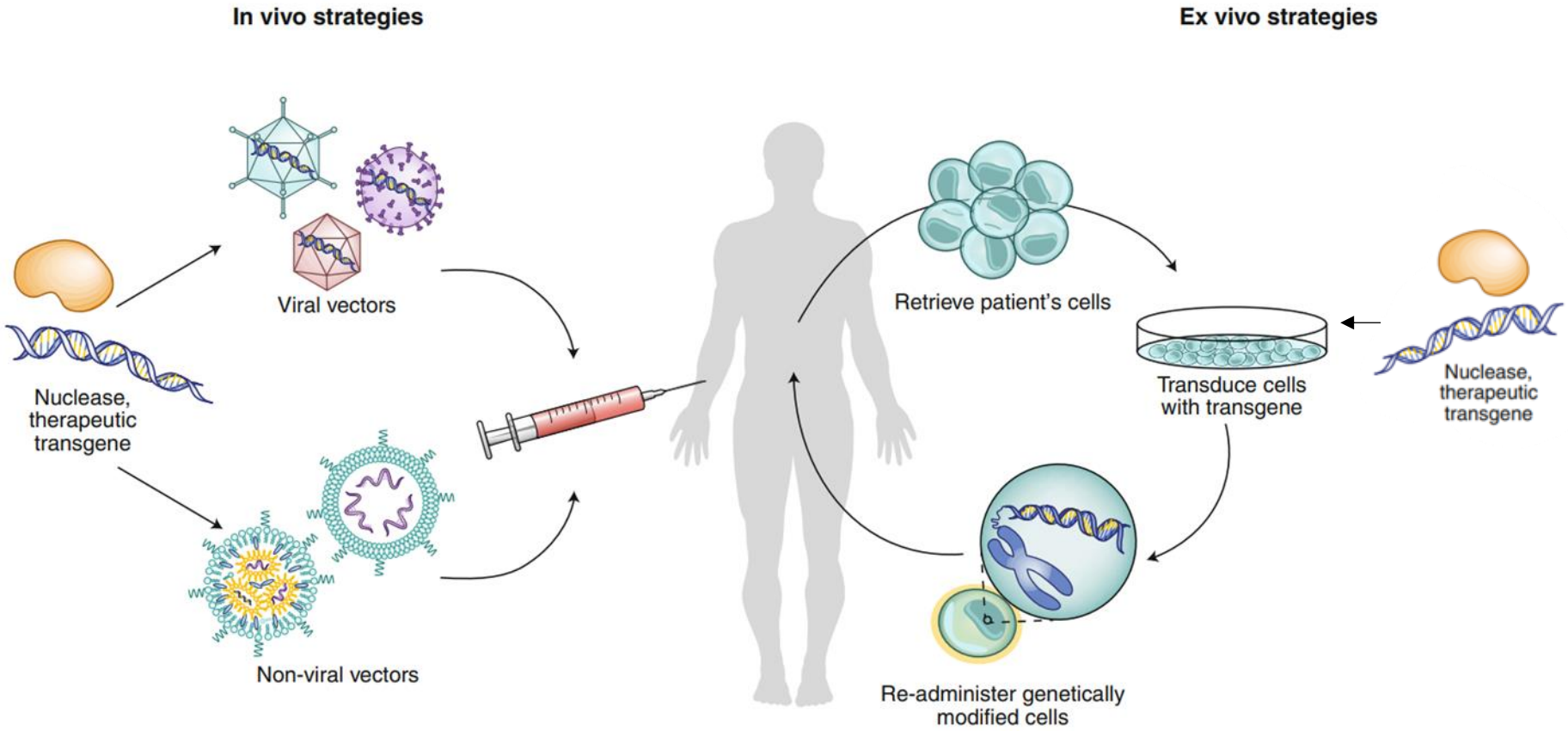

| Enzyme | Cas Size | PAM |
|---|---|---|
| SpCas9 | 1368 bp | NGG |
| FnCas9 | 1629 bp | NGG |
| SaCas9 | 1053 bp | NNGRRT |
| NmCas9 | 1082 bp | NNNNGATT |
| St1Cas9 | 1121 bp | NNAGAAW |
| St3Cas9 | 1409 bp | NGGNG |
| CjCas9 | 984 bp | NNNNACAC |
| AsCpf1 (Cas12a) | 1307 bp | TTTV |
| LbCpf1 (Cas12a) | 1228 bp | TTTV |






*Interdiscip Sci Comput Life Sci* **10**, 455–465 (2018)

# Delivery to target cells is a barrier to broad adaptation




## In vivo Gene Editing

- Delivery of editing payload to non-liver tissues can be challenging (e.g. CNS, muscle)
- Cost of manufacturing
- Risk of immune responses to AAV and payload

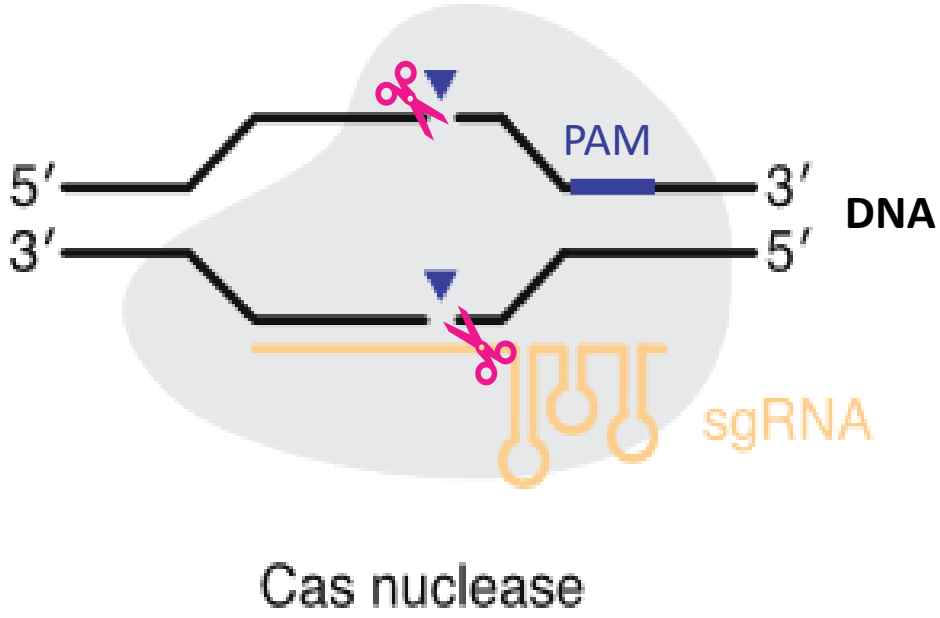
## Ex vivo Gene Editing

- Limited to certain disorders
- Greater technical control
- Difficult to develop process at scale
- Longer prep and treatment times

https://www.nature.com/articles/s41587-020-0565-5

# 1st Generation CRISPR-Cas Editing

*Edit Type: PAM proximal point mutations, small gene deletions and insertions*




**DRUG** = sgRNA + Cas enzyme +/- donor template

**Recognition**

**sgRNA-Cas** complex recognizes PAM

**Cleavage**

**sgRNA** guides **Cas** to bind target DNA to generate a double strand cut

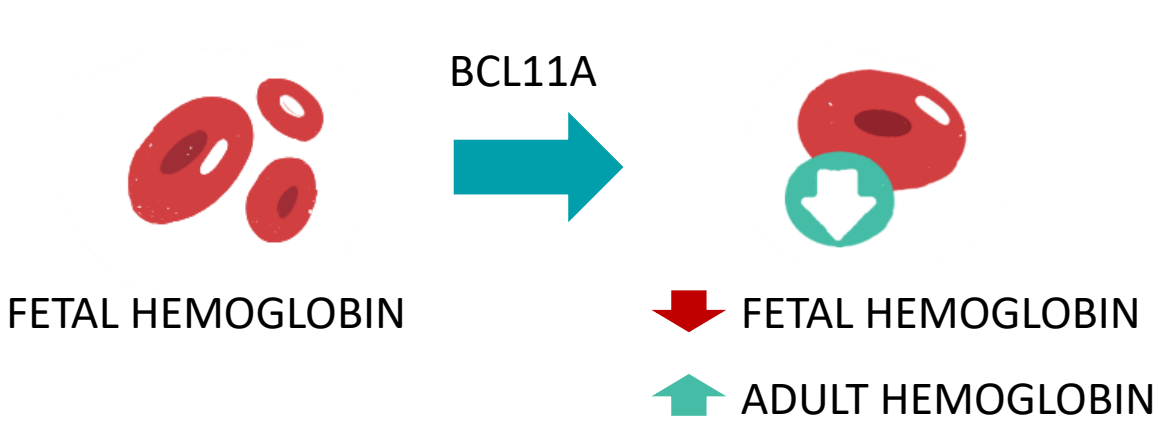
**Repair**

Non-Homologous End Joining (**NHEJ**)
Gene knockouts
Homology Directed Repair (**HDR**)
Gene insertions with **donor DNA** template into break site

# CASGEVY: Autologous cell therapy for Sickle Cell Disease

*ex vivo CRISPR/Cas9 gene editing of BCL11A in patient's own cells*

## DISEASE BIOLOGY



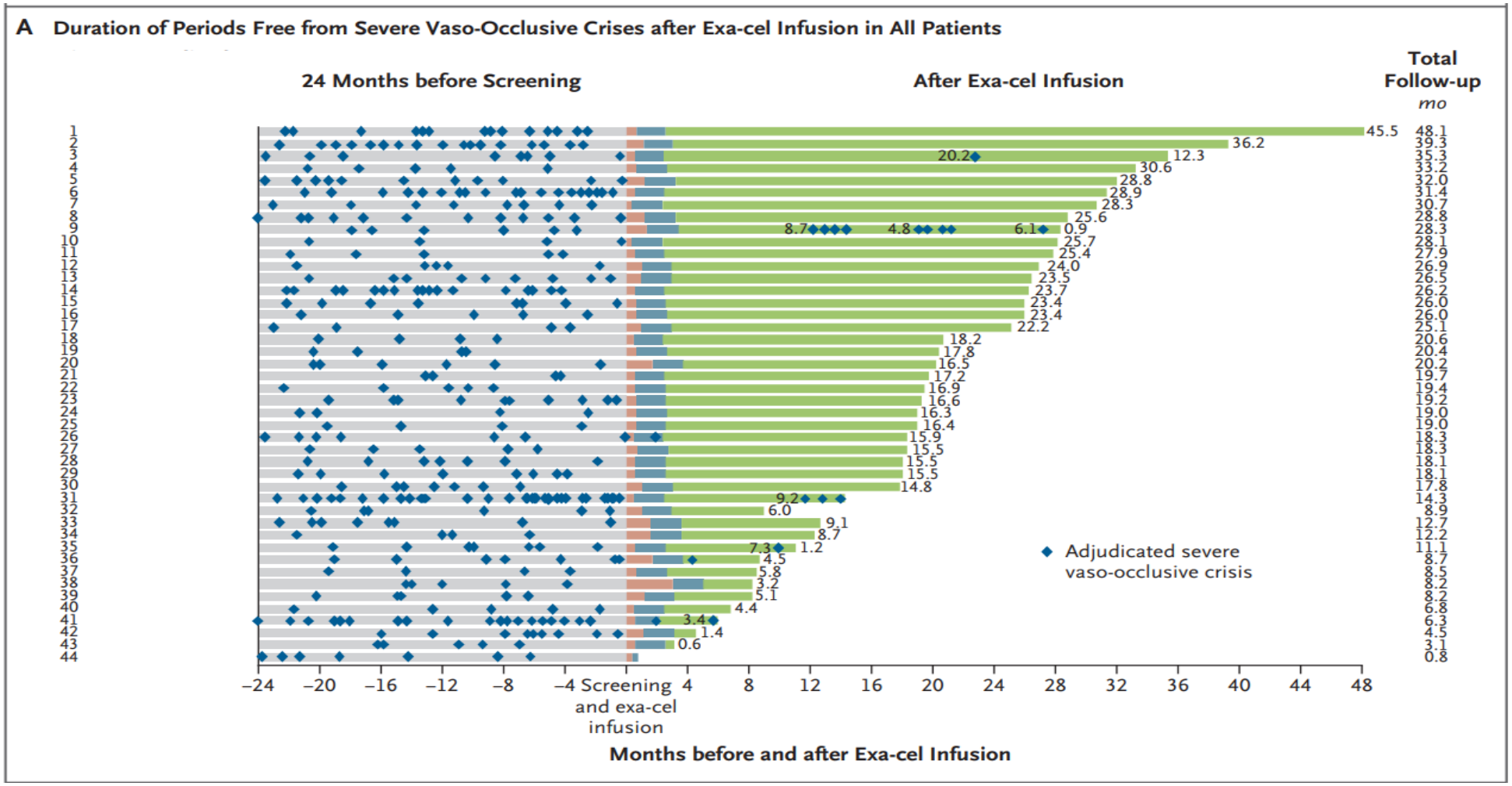

## THERAPEUTIC APPROACH

Ex vivo CRISPR-Cas BCL11A knockdown to increase FETAL HEMOGLOBIN production and produce normal blood cells

# CASGEVY: Autologous cell therapy for Sickle Cell Disease

*ex vivo CRISPR/Cas9 gene editing of BCL11A in patient's own cells*

*Elevated levels of fetal hemoglobin which eliminated vaso-occlusive crises in 97% of patients with sickle cell disease for a period of 12 months or more*






The Innovation 5(3): 100619, May 6, 2024

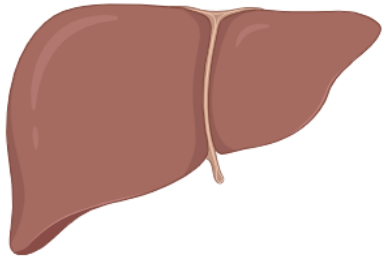
N Engl J Med 2024;390:1649-1662

# CRISPR/Cas9 to inactivate the TTR gene in the liver causing ATTR amyloidosis

*NTLA-2001 demonstrated proof-of-concept in small group of patients*

## DISEASE BIOLOGY

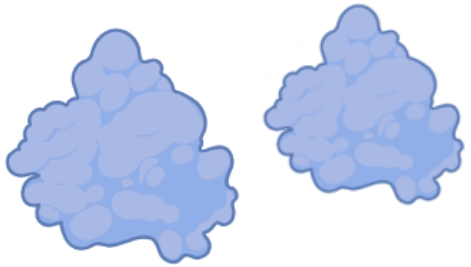
Liver



TTR protein

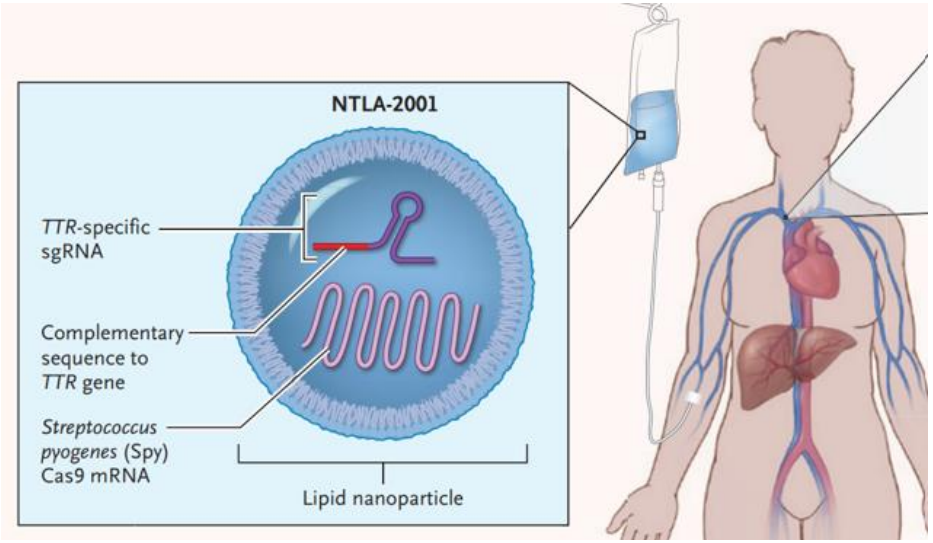
Misfolded TTR protein

Aggregates



Build up of plaque in multiple organs

## THERAPEUTIC APPROACH

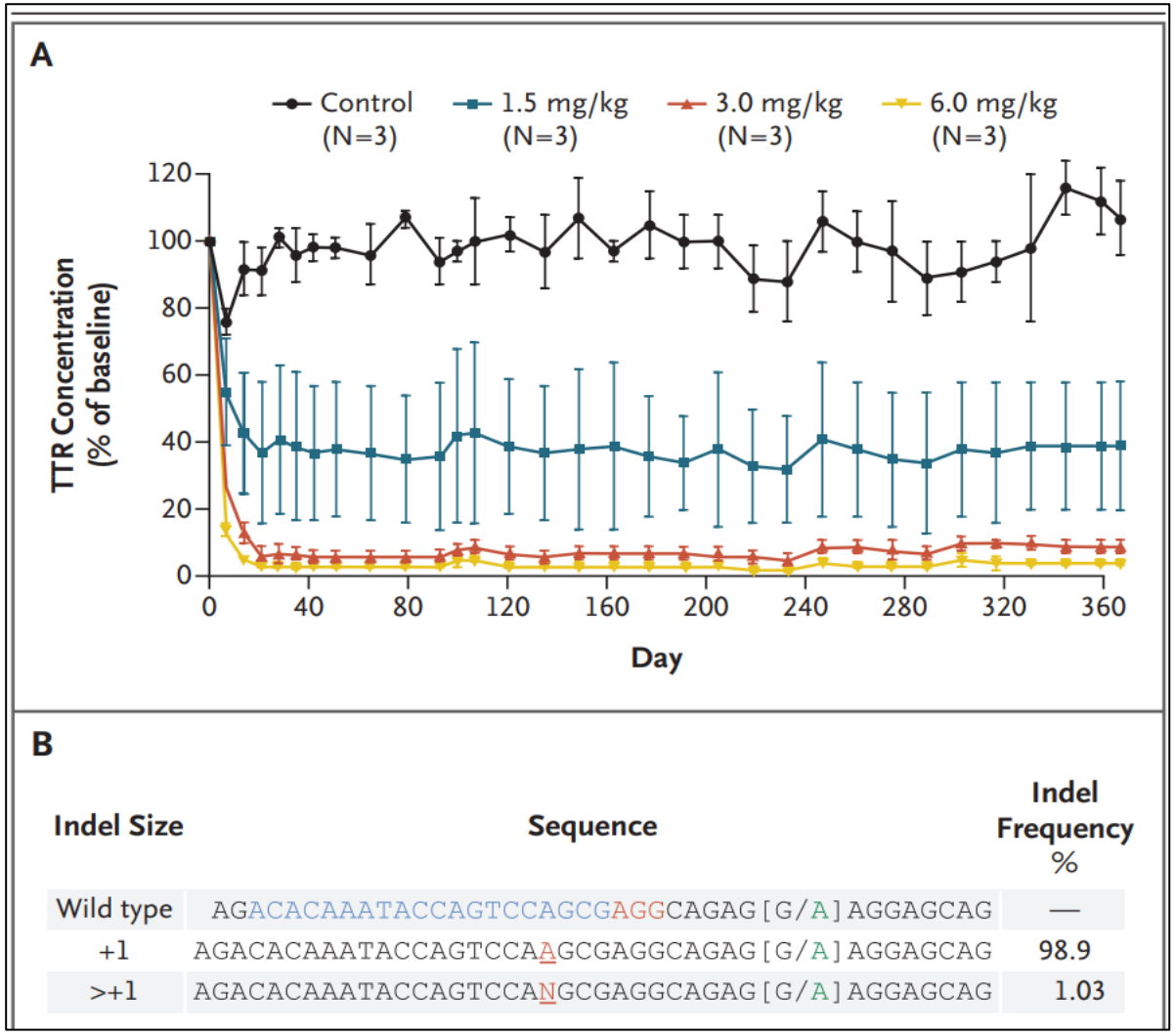



In vivo CRISPR-Cas to inhibit TTR levels

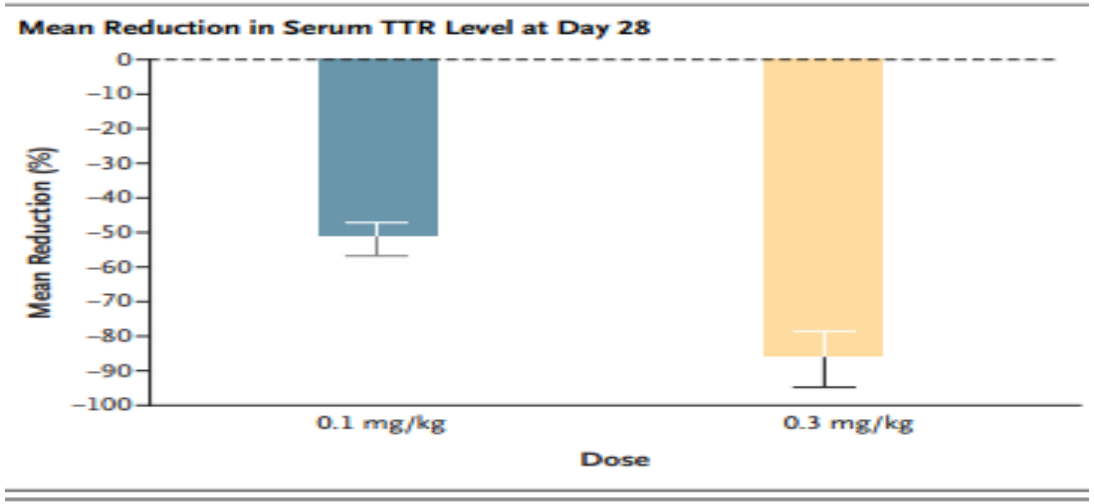
# CRISPR/Cas9 to inactivate the TTR gene in the liver causing ATTR amyloidosis

*NTLA-2001 demonstrated proof-of-concept in small group of patients*

## *Reduced serum TTR concentrations in non-human primates*




## *Reduced serum TTR concentrations in patients*



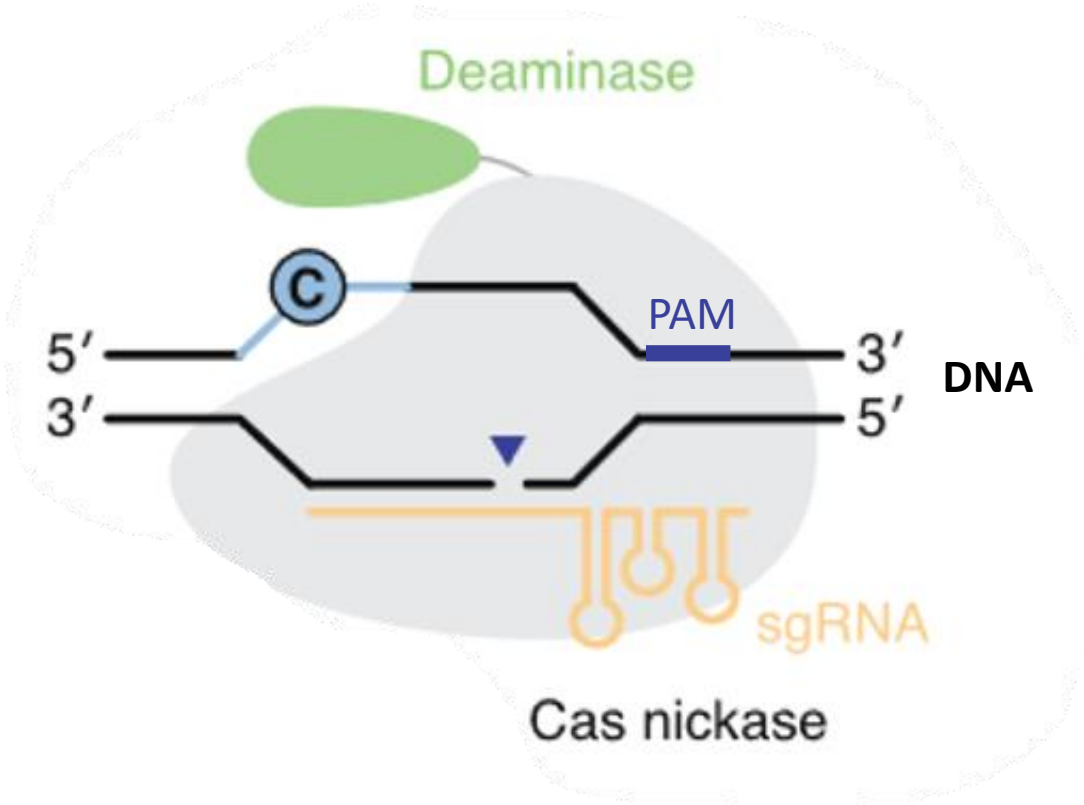

| Company/Drug | Disease | Gene | Edit | Tissue | Delivery |
|---|---|---|---|---|---|
| Intellia (NTLA-2001) | ATTR amyloidosis | TTR | Deletion | Liver | IV; LNP |



N Engl J Med 2021;385:493-502

# Base Editing to introduce or correct point mutations

*Edit Types: Gene knockouts and single nucleotide modifications (activation, silencing)*




**DRUG** = sgRNA + Cas enzyme + Deaminase

**Recognition**

**sgRNA-Cas** complex recognizes **PAM**

**Cleavage**

**sgRNA** guides **Cas** to bind target DNA to generate a single strand nick

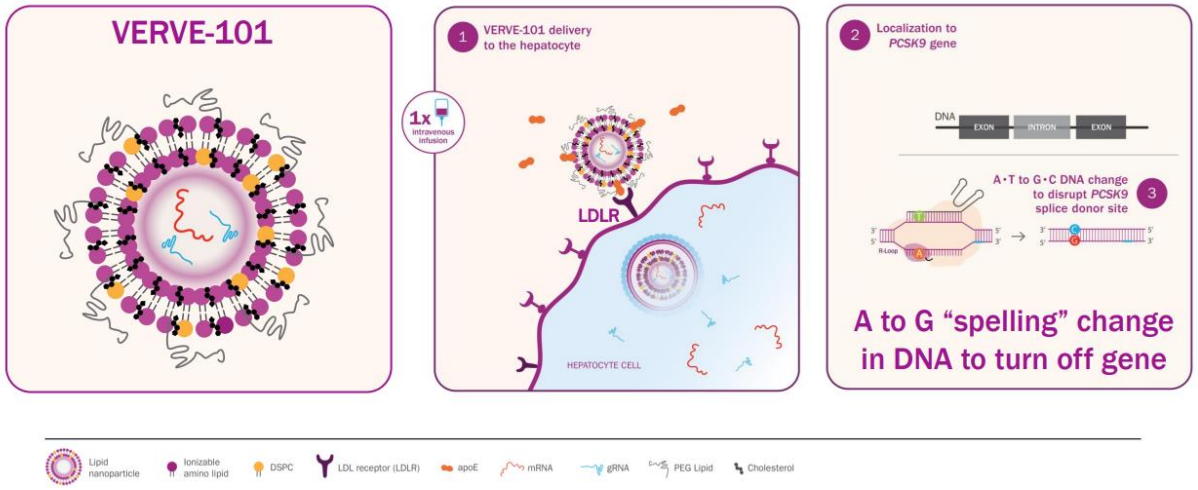
**Repair**

**Deaminase** replaces nucleotide
Adenine Base Editors (ABEs) = A→G or T→C
Cytosine Base Editors (CBEs) = C→T or G→A

# *in vivo* base editing proof-of-concept in NHP and humans

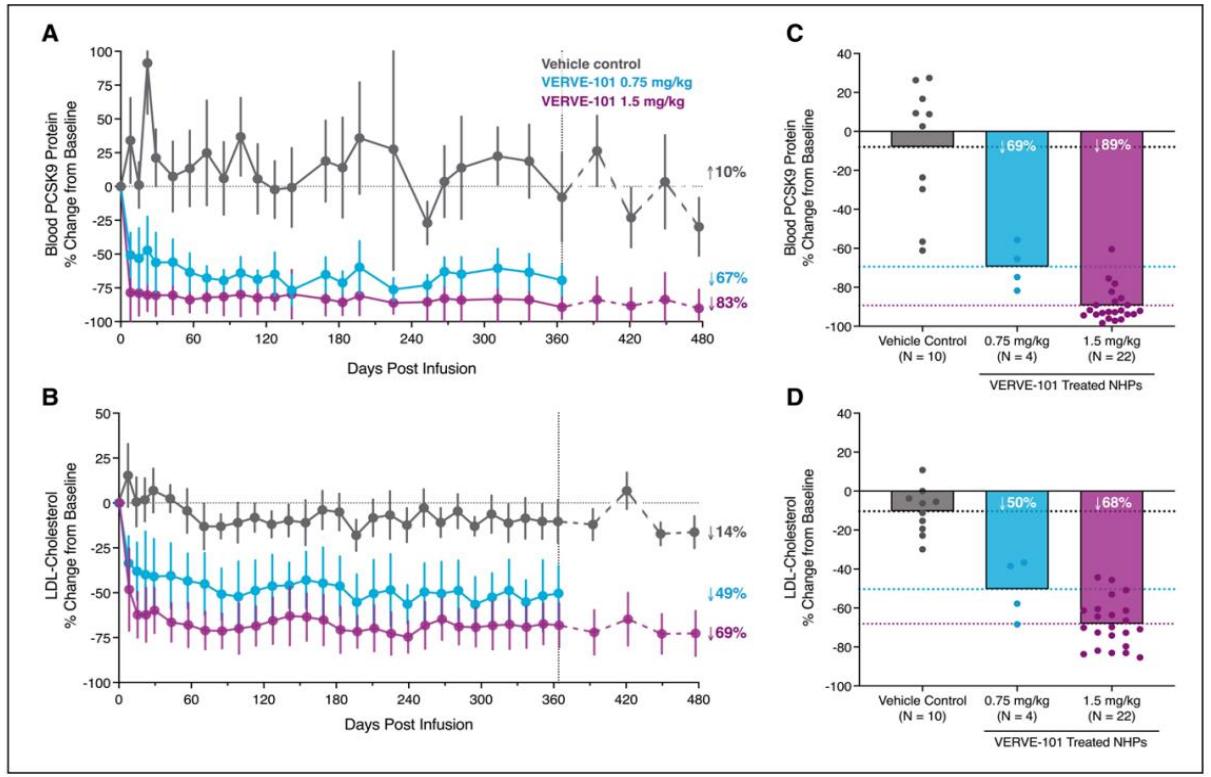
## DISEASE BIOLOGY AND THERAPEUTIC APPROACH

**VERVE-101: designed to inactivate liver *PCSK9* and lower LDL-C with a single DNA base pair change**




TIDES, 2024

## PRECLINICAL DATA: Single IV infusion in NHP -> 83% lower PCSK9 protein and 69% lower LDL-C up to 476 days post-dose

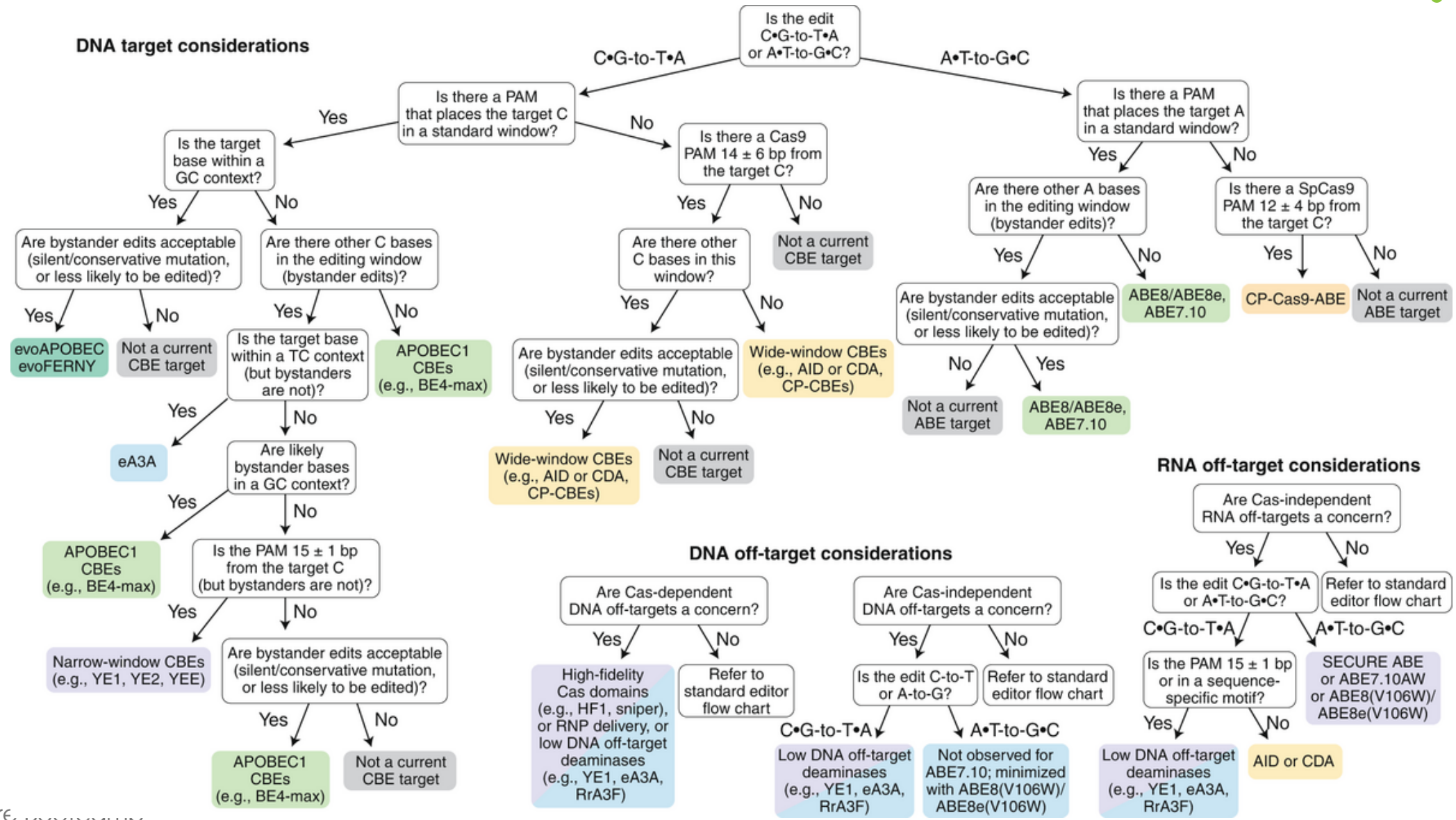



Circulation. 2023;147:242–253

## CLINICAL UPDATE:

- *Clinical trial demonstrated cholesterol reduction of up to 73%.*
- *Enrollment **paused** due to adverse effects in 1 patient.*

# Is base editing the right approach for a given mutation?

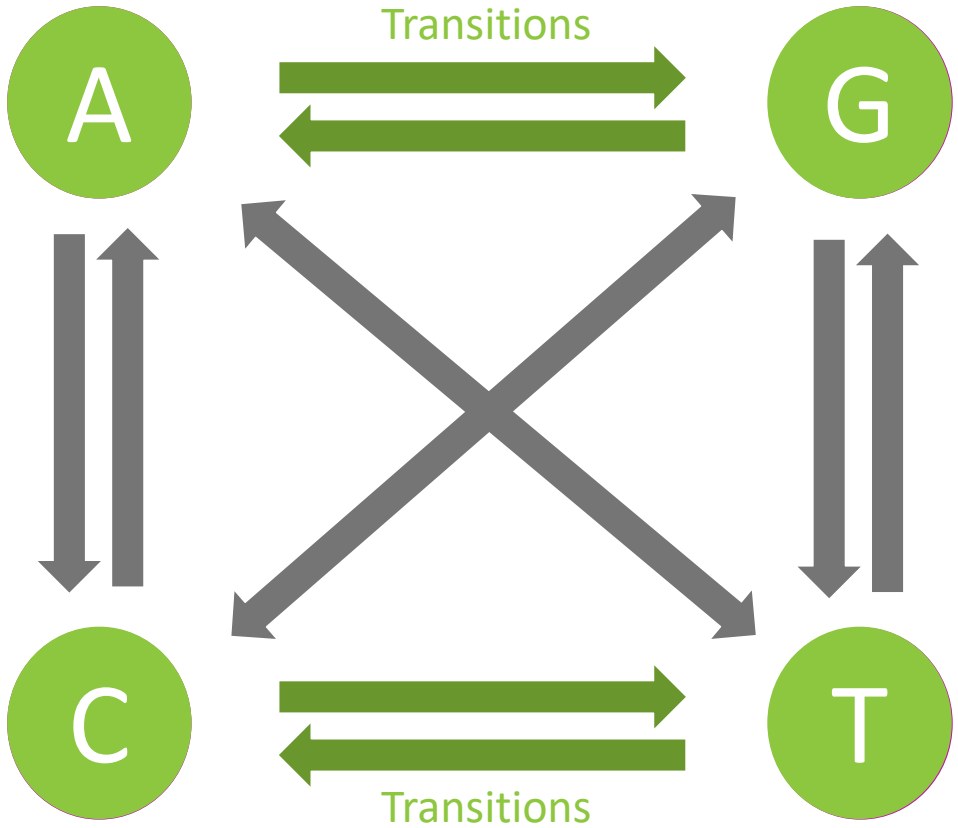





Nature Biotechnology | VOL 38 | July 2020 | 824–844

# Is base editing the right approach for a given mutation?

## 1. Base to be edited




## 2. PAM in target DNA

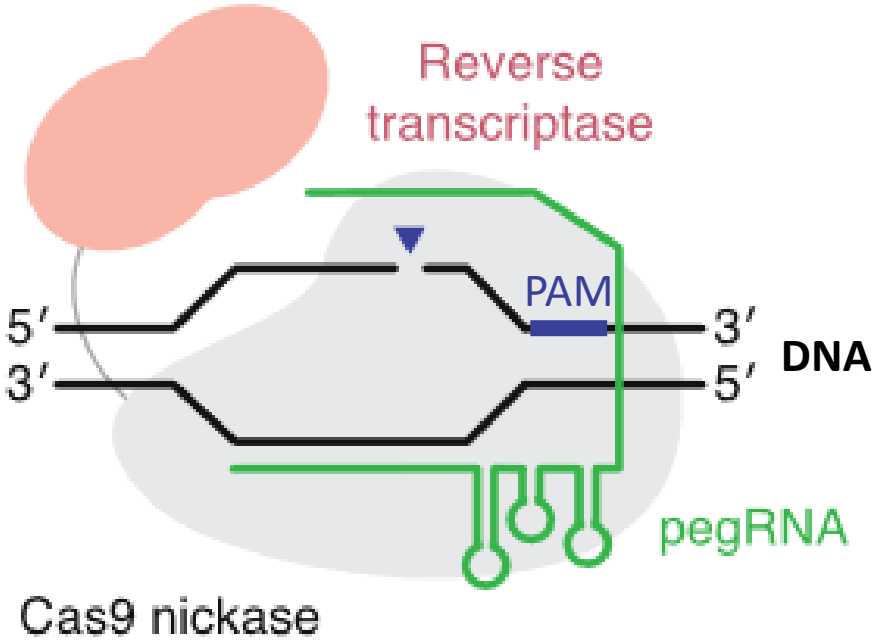
PAM is key

PAM defines the editing window

# Prime Editing is the future if we can overcome size and delivery

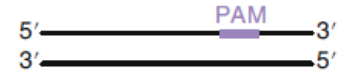
*Edit type: Search and replace editing without double strand breaks or donor DNA*

DRUG: pegRNA (sgRNA + primer binding site + template) + Cas enzyme + Reverse transcriptase enzyme




**Recognition**

**pegRNA**: sgRNA-Cas complex recognizes PAM

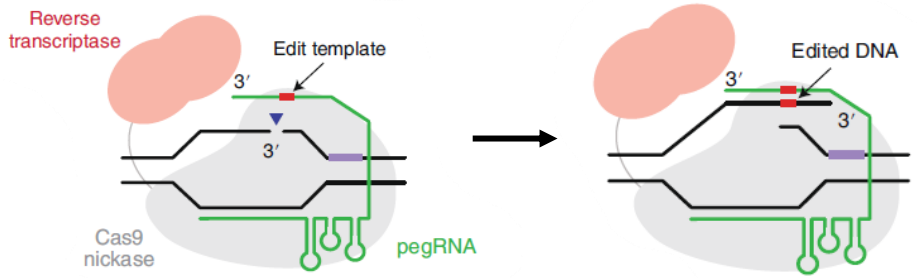



**Cleavage**

**sgRNA** guides **Cas** to bind target DNA to generate a single strand nick

**Repair**

Nicked DNA strand pairs to the pegRNA, to synthesize desired edit directly into the target DNA site



# **Prime Editing Today:** Promising preclinical data paves path for first ex vivo human trial, **but in vivo delivery still remains a challenge**

- In vivo AAV-mediated prime editing currently requires the use of **two or more AAVs.**
- Most published preclinical studies use **complex delivery platforms** and **size reduction** technologies to deliver the prime editing machinery into the desired cell.
- Prime Editing is still **several years of R&D away from being a therapeutic reality**.

April 29, 2024 11:29 AM EDT *Updated May 3, 12:27 PM* | R&D, Cell/Gene Tx, FDA+

**Prime Medicine receives FDA clearance to run first prime editing clinical trial**

| Company/Drug | Disease | Gene | Edit | Tissue | Delivery |
|---|---|---|---|---|---|
| Prime Medicine (PM359) | Chronic granulomatous disease (CGD) | NCF1 | Prime edit | Bone marrow | Ex vivo cell therapy |



*Nature Biotechnology* volume 42, pages253–264 (2024)

# CRISPR-Cas has been evaluated extensively in vitro and in vivo in various disease models

| Disorder | Target (HGVS name) | Model | Approach | R |
|---|---|---|---|---|
| β-Thalassemia | *HBB*$^{-28\ (A>G)}$ (HBB:c.-78A>G) | iPSC<br>Fibroblasts<br>NR embryos | HDR<br>BE | H<br>B |
| | *HBB*$^{CD\ 14/15\ (+G)}$ (HBB:c.45_46insG) | HEK293T<br>*Hs* zygotes | HDR | N<br>H |
| | *HBB*$^{CD\ 17\ (AAG>TAG)}$ (HBB:c.52A>T) | iPSC<br>HEK293T<br>*Hs* zygotes | HDR<br>HDR | N<br>N |
| | *HBB*$^{CD\ 41/42\ (-TTCT)}$ (HBB:c.124_127delTTCT) | HEK293T<br>*Hs* zygotes<br>iPSC<br>iPSC<br>iPSC in NSG mice | HDR<br>HDR<br>HDR<br>HDR | N<br>H<br>H<br>1 |
| | *HBB*$^{IVS2-654\ (C>T)}$ (HBB:c.316-197C>T) | iPSC | HDR | 1 |
| Chronic granulomatous disease, X-linked | *CYBB*$^{R226*}$ *(CYBB:c.676C>T)* | *Hs* CD34$^+$ | HDR | N<br>de<br>co |
| Frontotemporal dementia with Parkinsonism linked to Chromosome 17 | *MAPT*$^{IVS10+16}$ | iPSC | RNA knockdown (based on CasRx) | n/ |
| Fanconi anemia | *FANCC*$^{IVS4+4A>T}$ (FANCC: c.456+4A>T) | Fibroblasts | HDR (based on Cas9 and *Cas9*$^{D10A}$ nickase) | H<br>H |
| | *FANCC*$^{Q506*}$ (FANCC c. 1517G>A) | HEK293FT expressing *FANCC*$^{Q506*}$ cDNA | RNA A>I editing (based on Cas13) | 23 |
| | *FANCD1 886delGT* | Fibroblast | HDR | HDR 23% (7/30) corrected cells |
| Hemophilia A | *F8* Int1 and Int22 inversions | iPSC | Inversion/Deletion by double NHEJ | Inversion 6.7% ( |
| Hemophilia B | *F9*$^{R226W}$ | iPSC | HDR TI (*AAVSI*) | 100% (6/6) clona of integrants |
| Huntington disease | *HTT* $(CAG)_n$ | Fibroblast | Inversion/Deletion by double NHEJ | NHEJ 97% for fi shown; |
| | | Fibroblast | Inversion/Deletion by double NHEJ | n/a; reduction of down to 1/3 of n |

**Table 1.** Applications of CRISPR-based tools for fighting rare diseases.

| Diseases Type | Diseases | Gene | Strategy | Animal Model or Cells | Delivery | Outcome | References; Clinical Trials |
|---|---|---|---|---|---|---|---|
| Blood disorder | SCD/b-thalassemia | *HBB* | ABE8e-NRCH-mediated point mutation correction | HSPCs from patients with SCD | RNP; electroporation | 80% editing efficiency, 72% decrease in the pathogenic protein | [124] |
| | | *HBB* | IBE-mediated point mutation correction | CD34$^+$ HSPCs/erythroid differentiated cells | mRNA; electroporation | 77% editing efficiency, 80% Makassar editing | [125] Clinical trials |
| | | *HBG1, HBG2* | ABE8s-mediated point mutation to create "British mutation" and increase levels of γ-globin | CD34$^+$ cells, human T cells | RNP; electroporation | 80% editing efficiency, 60% protein knockdown efficiency | [129] Clinical trials |
| | | *HBB* (−28) | A3A(N57Q)-BE3-mediated BCL11A enhancer disruption to reproduce γ-globin | CD34$^+$ HSPCs | RNP; electroporation | >20% editing efficiency | [47] |
| | | *BCL11A* (+58) | A3A(N57Q)-BE3-mediated BCL11A erythroid-specific enhancer disruption to reproduce γ-globin | CD34$^+$ HSPCs | RNP; electroporation | 93.3% editing efficiency, restoring >60% of γ-globin | [130] |
| | | *BCL11A* | NHEJ-mediated BCL11A erythroid-specific enhancer disruption | CD34$^+$ HSPCs | RNP; electroporation | 80% editing efficiency, 30% decrease in sickle hemoglobin | [131] Clinical trials |
| | | *HBB* | NHEJ-mediated mRNA splicing | CD34$^+$ HSPCs | Cas12a RNP; electroporation | >30% editing efficiency, restoring >60% of γ-globin | [132] |
| | | *BCL11A* | NHEJ-mediated BCL11A erythroid-specific enhancer disruption | HSPCs | AsCas12a/Cpf1 RNP; electroporation | ~80% editing efficiency | [133] |
| | | *HBG* | NHEJ-mediated HBG disruption | CD34+ cells | AsCas12a/Cpf1 RNP; electroporation | >80% editing efficiency | [134] Clinical trials |
| Eye diseases | LCA10 | *CEP290* | HDR-mediated IVS26 mutation deletion | HEK293FT/Mice | Dual AAV5 | 7.5–26.4% editing efficiency | [135] |
| | | *CEP290* | NHEJ-mediated IVS26 mutation deletion | iPSC | AAV5 | >50% editing efficiency | [136] |
| | | *Cep290* | HDR-mediated IVS26 mutation deletion or inversion | Mice | AAV5 | ~30% editing efficiency | [137] Clinical trials |

| | | | | | Efficiencies; Ref. |
|---|---|---|---|---|---|
| | *F9 nullizygous (-/-) mice* | Liver by homing; intrasplenic injection of *ex vivo*-corrected differentiated iPSC-derived hepatocyte-like cells | HDR TI (cDNA knock in) | Not reported; iPSC-HLCs viable and functional for 9–12 months, modest increases in the clotting efficiency (from less than 10% to ~25% of wt activity) | [74] |
| | *Human F9*$^{R226W}$ *in NOD/SCID mice* | Liver by homing; splenic injection of *ex vivo* corrected iPSCs | HDR TI (*AAVS1*) | Four-fold induction of human F9 plasma levels | [68] |
| Hereditary tyrosinemia type I | *Fah5981SB mice* | Liver by hemodynamics; tail-vein injection of plasmid with ssDNA donor | HDR | 1/250 hepatocytes, increased to 33% after withdrawal of medication | [124] |
| | | Liver by hemodynamics; tail-vein injection of lipid-nanoparticles with AAV donor | HDR | 6% in hepatocytes; full rescue of weight loss and liver damage | [125] |
| | *Fah*$^{-/-}$ *mice* | Liver by hemodynamics; tail vein injection of RNPs | *Hpd* gene excision by double NHEJ | 92% in hepatocytes | [126] |
| Huntington disease | *Human HTT* $(CAG)_n$*-transgenic mice* | Brain; injection of AAV | Excision by double NHEJ | n/a; reduction of mutant HTT expression in injected hemisphere to <40% of control hemisphere | [55] |
| Hyperammonemia/Ornithine transcarbamylase deficiency | *spf*$^{ash}$ *mice* | Liver by hemodynamics; intravenous infusion of AAV-encoded RGN and | HDR | 10% correction and 1% inadvertent ablation of *OTC* in 10% of hepatocytes in neonates; lower | [127] |

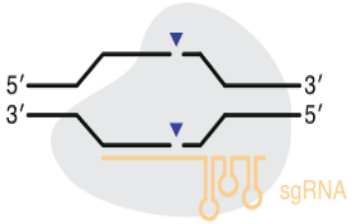
***Tables linked to source***

# Current state of editing technologies

*Utility is highly specific, and not all diseases are treatable with the current technology*

| Preclinical | Phase 1 | Phase 2/3 | Approved |
|---|---|---|---|

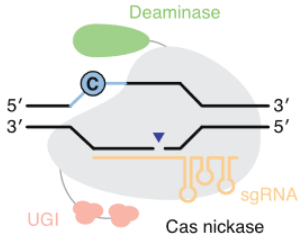
*1st gen Cas nuclease*




Ex vivo 1st gen Cas Nuclease

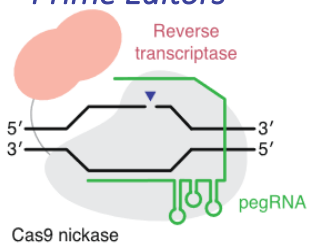
In vivo 1st gen Cas Nuclease

*Base Editors*




In vivo Base editing

*Prime Editors*

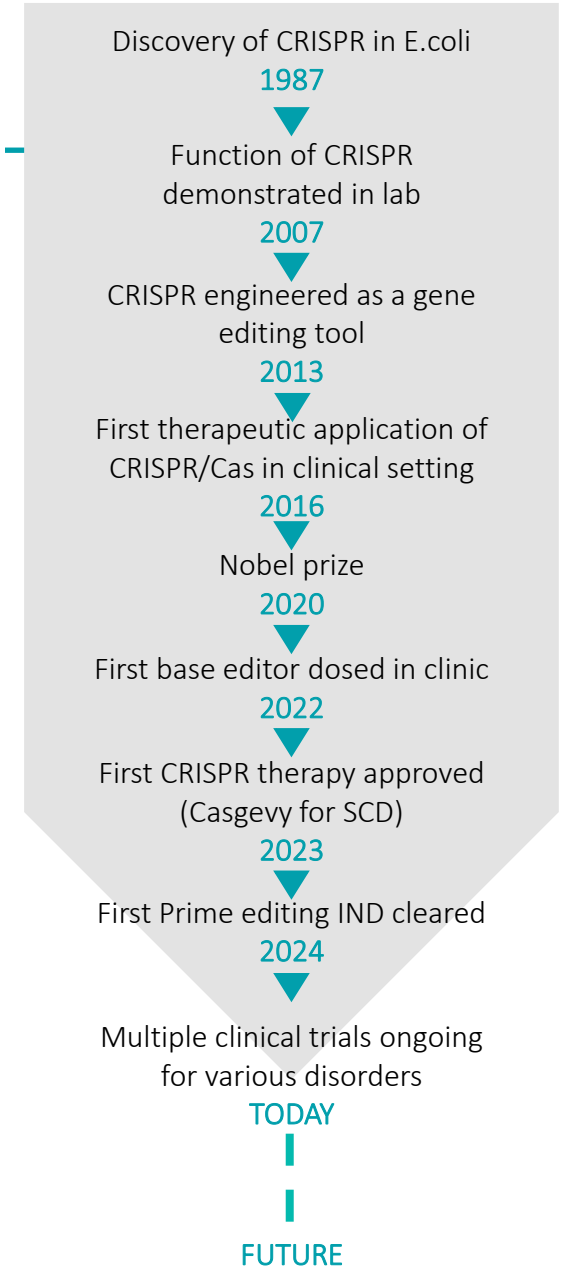



Prime Editing

# Key Takeaways

- THE POTENTIAL: Gene editing offers great therapeutic potential to edit the genome **permanently** to correct disease-causing mutations.
- THE CHALLENGE: Delivery, specificity, and immunogenicity considerations limit broad utility and adaptation.
- THE NOVELTY: The field is in its infancy in terms of therapeutic development.
  - Limited CMC/manufacturing experience
  - Limited regulatory precedence
  - High Cost (e.g. Casgevy $2.2M)
- THE PROMISE: Several editing programs in preclinical and clinical development will pave the path towards our evolving understanding of the technology, its utility and limitations.

*"Healing is a matter of time, but it is sometimes also a matter of opportunity"*
***- Hippocrates***



Rare Bootcamp™

Sponsored by Ultragenyx

# Thank You