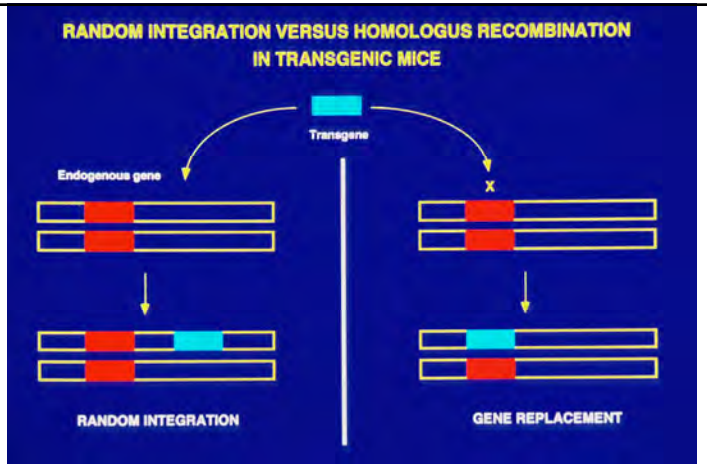
Universidad de Jaén

Terapias génicas hoy: CRISPR et al.

@LluisMontoliu
CNB-CSIC & CIBERER-ISCIII, Madrid

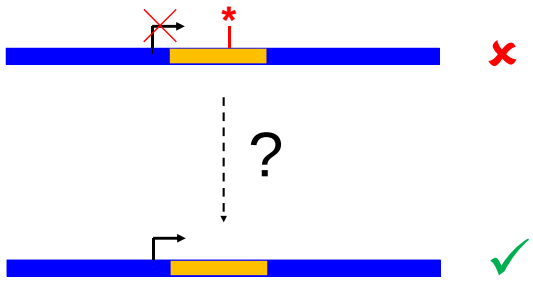


Gene Modification Random or Targeted

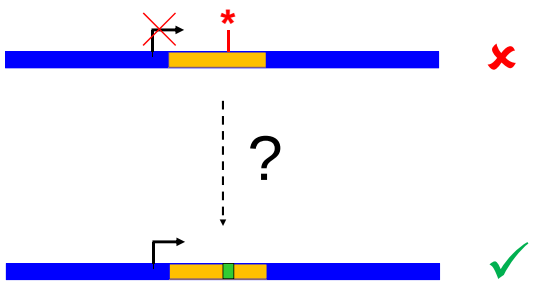
Challenges in Gene Therapy

1. To reach and fix the gene involved
2. To reach the cell(s) expressing that gene
3. To correct a significant amount of those cells
4. The correction should be therapeutically relevant
5. Not associated with toxicity / secondary effects
6. Therapy must be accesible and affordable (justice)

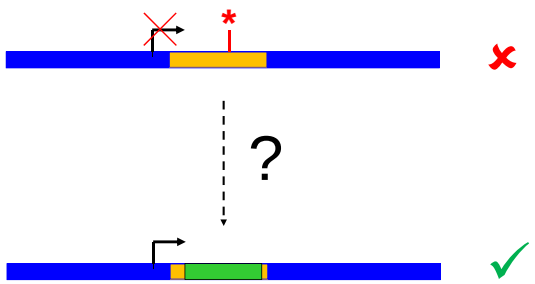
Gene Therapy



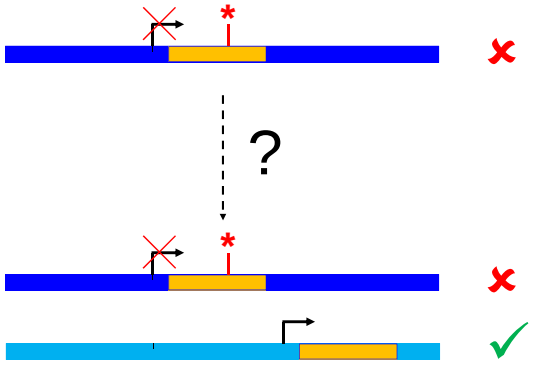
Gene Therapy



Gene Therapy



Gene Therapy



Lyfgenia (Bluebird Bio)

Lentivirus (one-time)

cDNA beta-globin gene

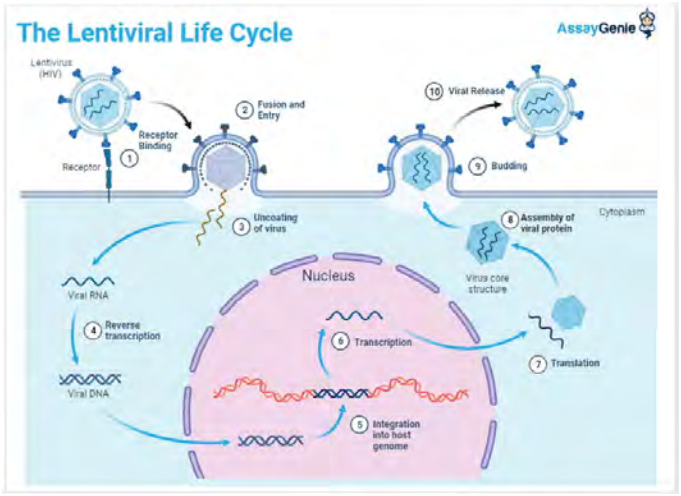
3,1 M$ / patient

Approved by FDA in December 2023

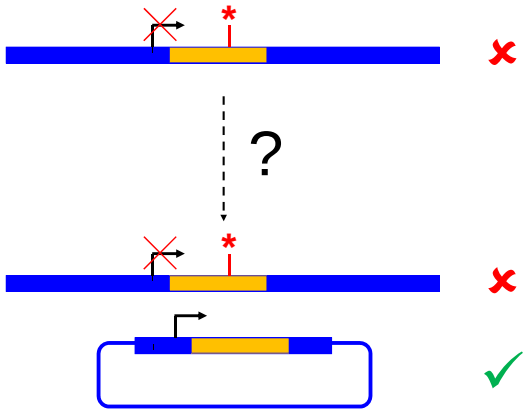
Patients aged 12 and older with Sickle Cell Disease and a history of vaso-occlusive events (VOE)

Results: severe VOE reduced in 30/32 patients, eliminated in 28/32

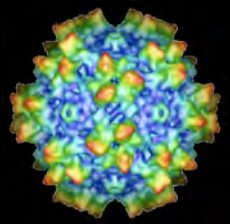
Risk of developing hematologic malignancies



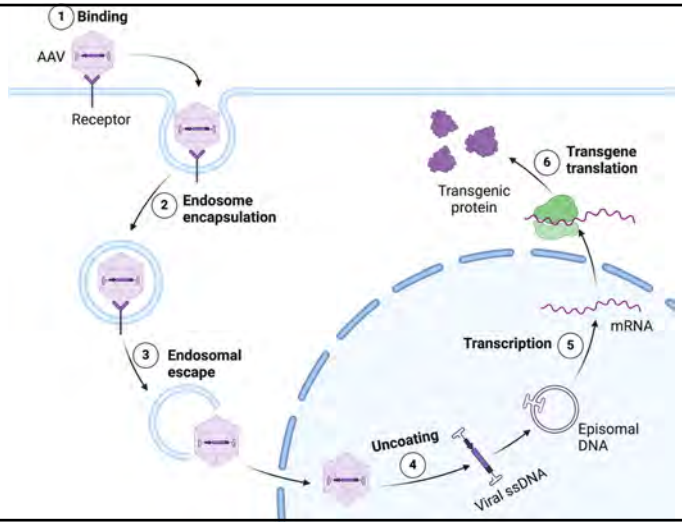
Gene Therapy



Adenoassociated virus (AAV)



AAV



Luxturna (Spark Therapeutics, Inc.)
Treatment of retinal degenerative diseases (RPE65 gene)

Approved by FDA in 2017
Approved by EMA in 2018
First administered in Spain in 2012 (12 Oct) – 345.000€/eye

Current list of gene therapies approved in the EU by EMA

15 products

Name	Company	Date of approval	Disease
Abecma	BMS Pharma	18 August 2021	Multiple Myeloma – CAR-T cells-LV
Breyanzi	BMS Pharma	4 April 2022	Different types of lymphomas – CAR-T cells-LV
Carvykti	Janssen-Cilag International	25 May 2022	Multiple Myeloma – CAR-T cells-LV
Casgevy	Vertex Pharmaceuticals	9 February 2024	CRISPR edited blood cells – SCD/β-Thal-AAV
Hemgenix	CSL Behring GmbH	20 February 2023	Haemophilia B (factor IX) – AAV cDNA
Imlygic	Amgen Europe	16 December 2015	Melanoma – HSV1
Kymriah	Novartis Europharm Ltd	23 August 2018	B-cell ALL and lymphomas – CAR-T cells LV
Libmeldy	Orchard Therapeutics	17 December 2020	Metchromatic Leukodystrophy – CD34+ cells LV
Luxturna	Novartis Europharm Ltd	22 November 2018	RP and LCA - AAV
Roctavian	BioMarin International Ltd	24 August 2022	Haemophilia A (factor VIII) – AAV cDNA
Strimvelis	Fondazione Telethon	26 May 2016	ADA – CD34+ cells RV
Tecartus	Kite Pharma	14 December 2020	Mantel Cell Lymphoma – CAR-T cells LV
Upstaza	PTC Therapeutics International Ltd	18 July 2022	L-amino acid decarboxylase deficiency - AAV
Yescarta	Kite Pharma	23 August 2018	Different types of lymphomas – CAR-T cells-LV
Zolgensma	Novartis Europharm Ltd	18 May 2020	Spinal Muscular Atrophy - AAV

Update: 3 June 2024 - https://www.pei.de/EN/medicinal-products/atmp/gene-therapy-medicinal-products/gene-therapy-node.html

CRISPR-Cas is the future

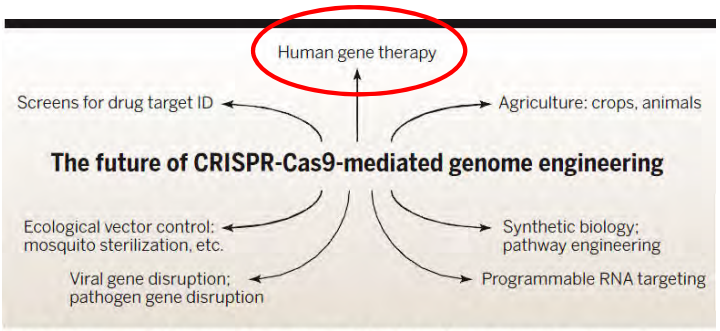
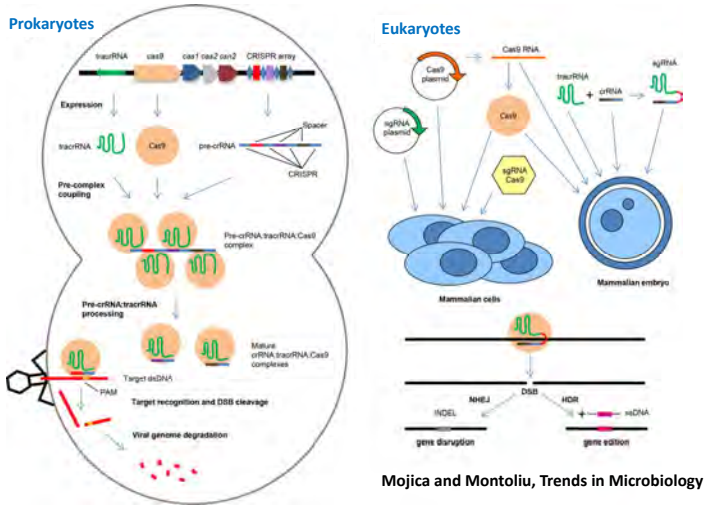
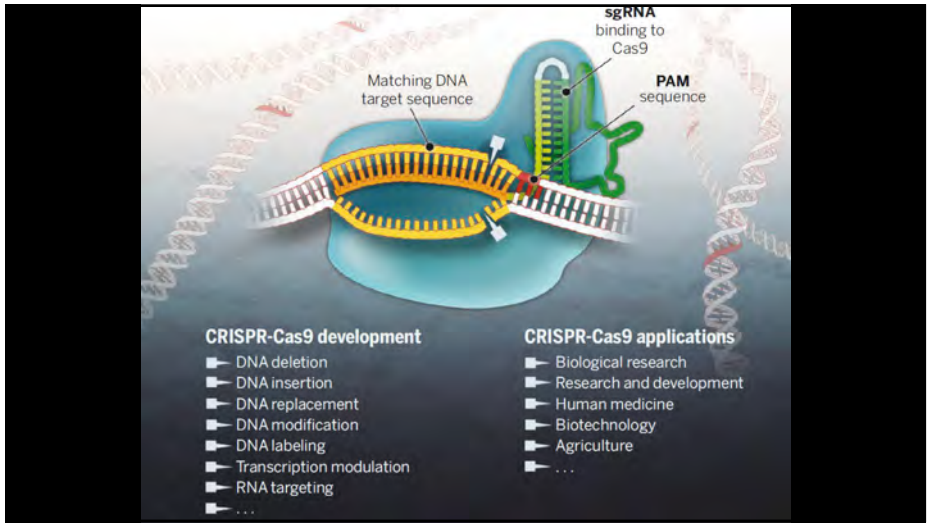


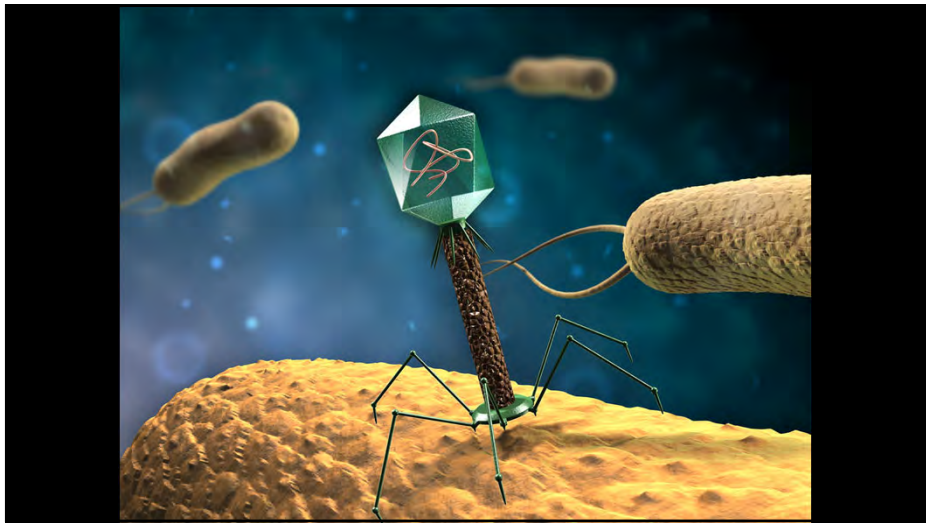
Fig. 6. Future applications in biomedicine and biotechnology. Potential developments include establishment of screens for target identification, human gene therapy by gene repair and gene disruption, gene disruption of viral sequences, and programmable RNA targeting.

Doudna & Charpentier (2014) *Science*

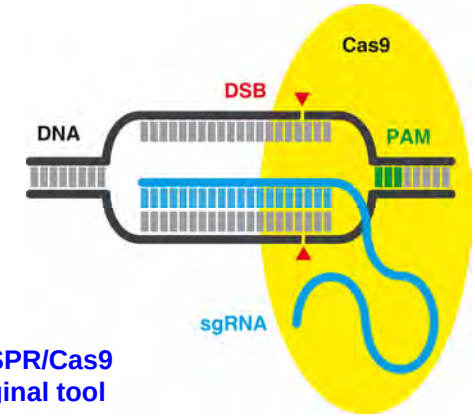


Mojica and Montoliu, Trends in Microbiology 2016





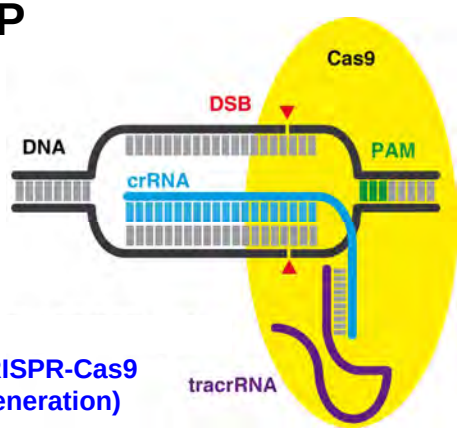
2012



CRISPR/Cas9
Original tool

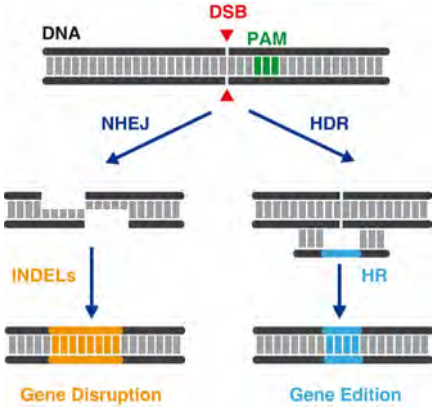
RNP

2024

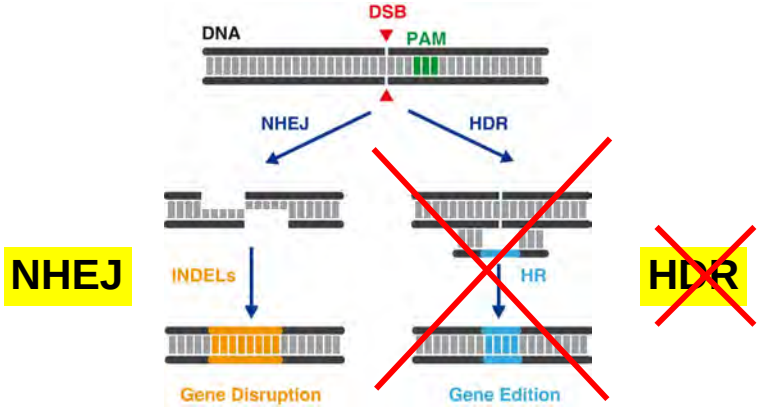


The current CRISPR-Cas9
System (1st generation)

CRISPR mechanism of action (first generation CRISPR tools)



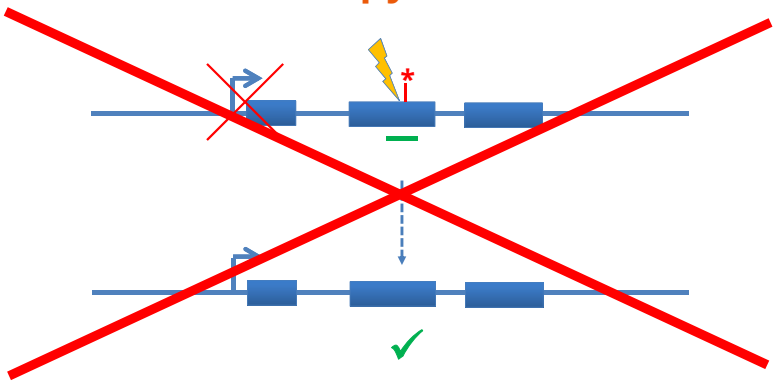
CRISPR mechanism of action (first generation CRISPR tools)



NHEJ

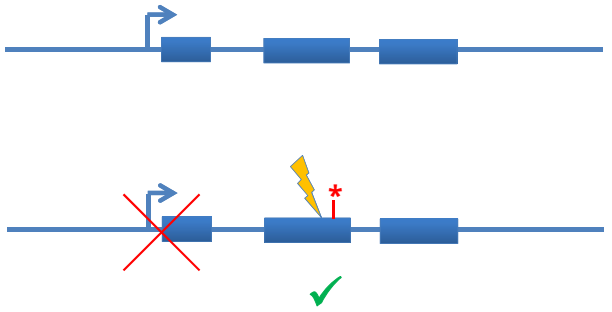
HDR

Gene Therapy with CRISPR

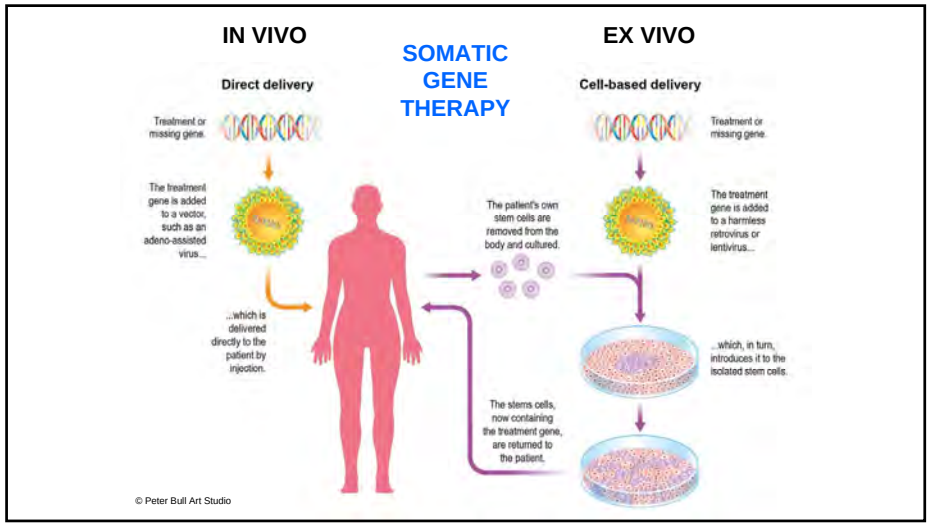


What we thought it would work

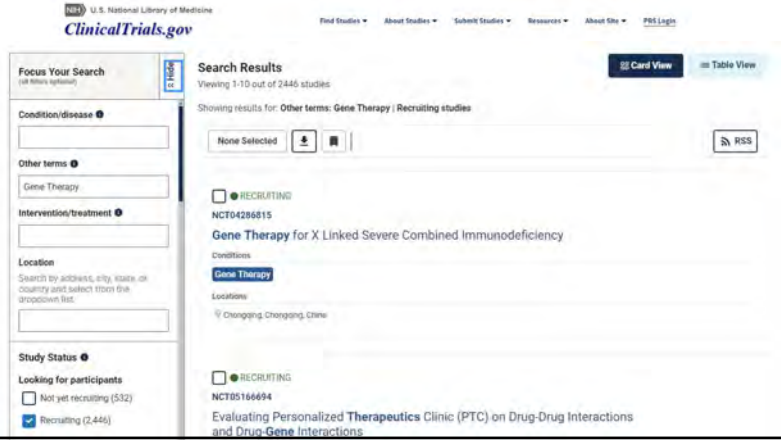
Gene Therapy with CRISPR



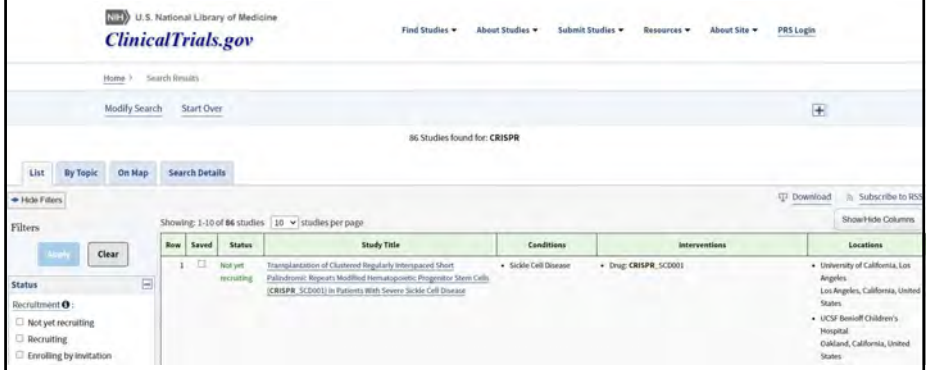
What we know it works



~2500 gene therapy clinical trials (recruiting)



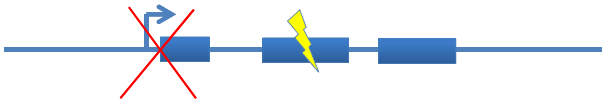
~101 CRISPR clinical trials



Most are EX-VIVO

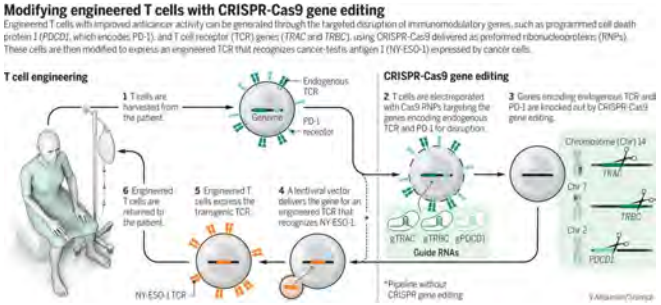
16 July 2024

Disrupting a gene: KO



First generation of CRISPR tools

Cancer Immunotherapy with CRISPR

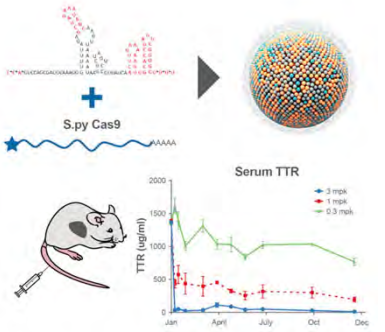


6 Feb 2020

Hamilton & Doudna (Science, 2020)
Stadtmauer et al. (Carl June Lab, Science 2020)

Transthyretine Amylodosis congenital (ATTR)
NANOTECNOLOGY - Nanoparticles

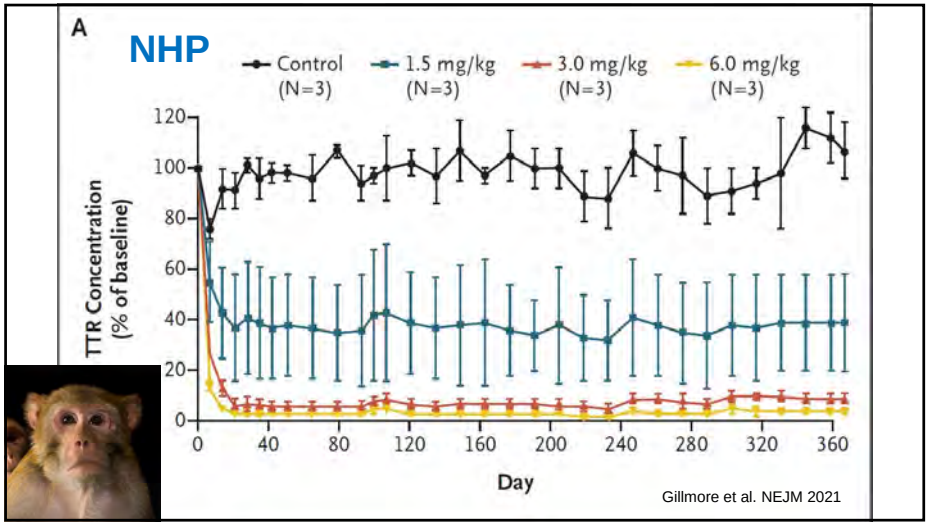
1:100.000



Cell PRESS

Finn et al. Cell Reports 2018 22, 2227-2235DOI: (10.1016/j.celrep.2018.02.014)

October 2020

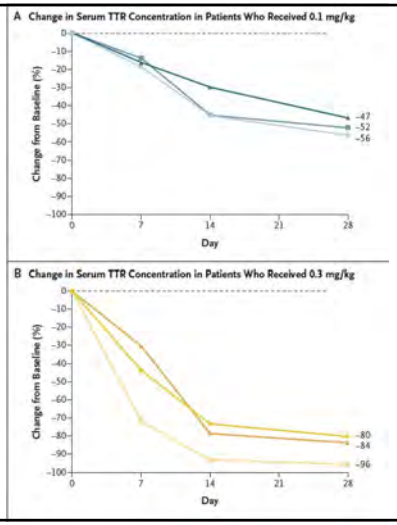


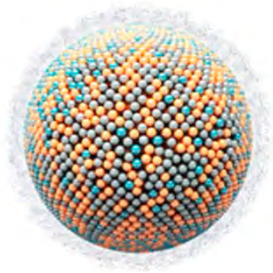
Gillmore et al. NEJM 2021

Paddy Doherty

CRISPR to treat Transthyretine Amylodosis congenital (ATTR)

Gillmore et al. NEJM 2021

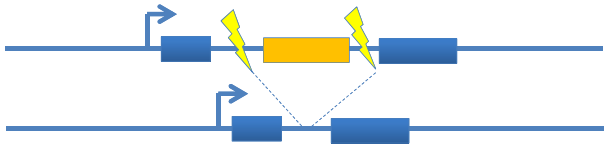




Lipid Nanoparticles (LNPs)

LNPs massively used in COVID-19 vaccines

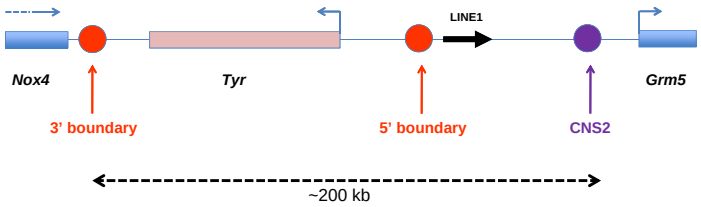
Deletions



First generation of CRISPR tools

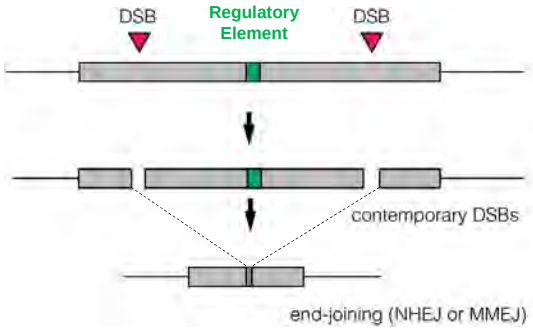
Tyr mutant mice as animal models of albinism

How can we functionally analyze the mouse *Tyr* DNA regulatory elements at the endogenous locations?



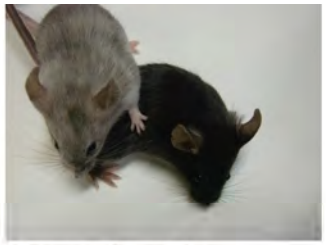
The known DNA regulatory elements at the mouse *Tyr* locus

Using CRISPR-Cas9 genome editing to target *Tyr* regulatory elements



CRISPR-Cas9 genome editing

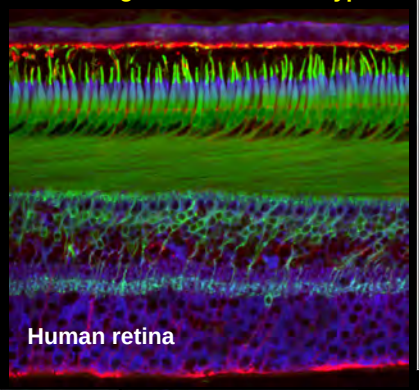
Deleting 5' *Tyr* regulatory elements with CRISPRs *in vivo*

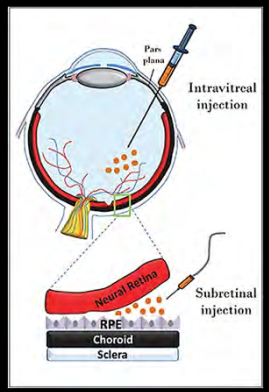


Seruggia et al. 2015 Nucleic Acids Res.
Seruggia et al. 2020 Scientific Reports
Seruggia et al. 2022 PCMR
Fernandez et al. 2022 PCMR

Correcting mutations in CEP290 gene with CRISPR

Leber Congenital Amaurosis type 10





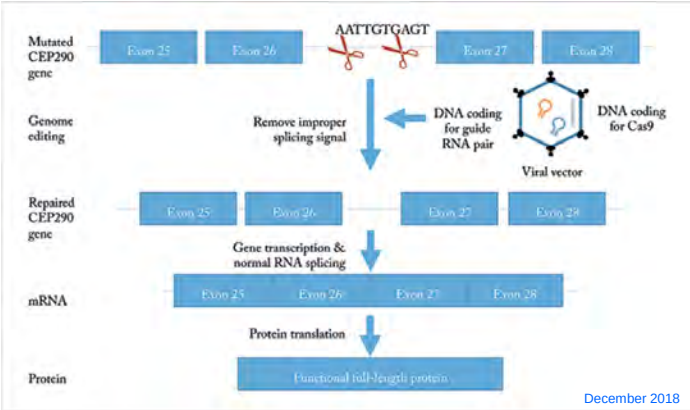
editas MEDICINE Allergan

In vivo

Dec 2018

Correcting a cryptic mutation in CEP290 gene with NHEJ CRISPR

Leber's congenital amaurosis type 10



December 2018

CRISPR to treat Leber Congenital Amaurosis type 10

Carlene Knight

9 out of 11 patients treated improved their vision
6 May 2024

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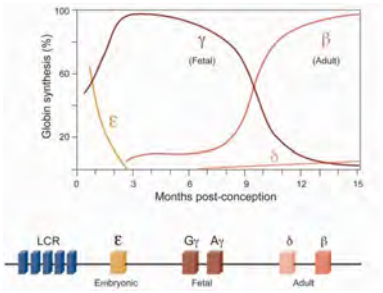
Treating sickle-cell disease and beta-thalassemia withn CRISPR



SCD in Spain:
~1: 33000

SCD in the world:
~400.000 newborn with SCD / year

SCD in Africa:
~300.000 newborn
with SCD / year

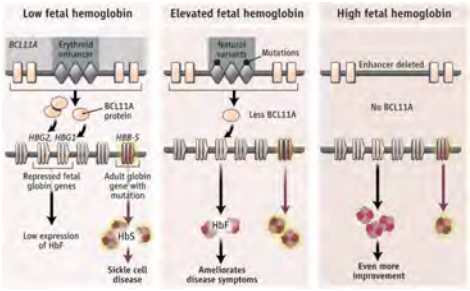
Bauer Lab (Dana Farber-Boston Children's)

CRISPR THERAPEUTICS VERTEX

First ex-vivo CRISPR therapy approved in USA and Europe

February 2024

Treating sickle-cell disease and beta-thalassemia withn CRISPR



Bauer Lab (Dana Farber-Boston Children's)

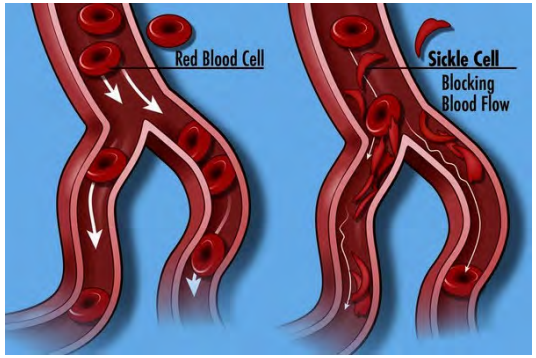
CRISPR THERAPEUTICS VERTEX

First ex-vivo CRISPR therapy approved in USA and Europe

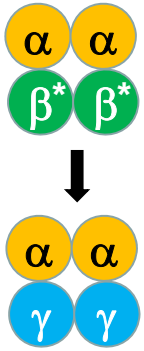
February 2024

Normal
red blood cell

Red blood cell
in SCD patients

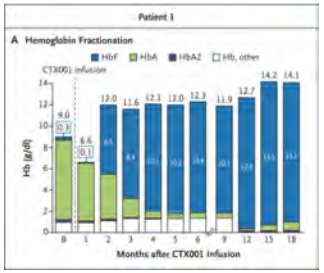


Hemoglobin
in SCD patients



Hemoglobin
cured with CRISPR

First CRISPR-treated Sickle Cell Disease patients cured (2020)

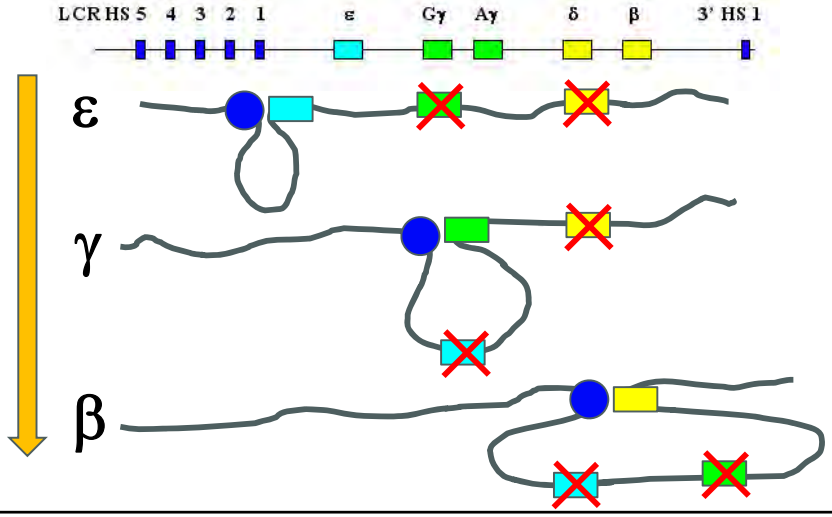


Frangoul *et al.* NEJM (5 December 2020)

Victoria Gray (treated July 2, 2019)

29/30 free of vaso oclusive crisis for at least 12 months

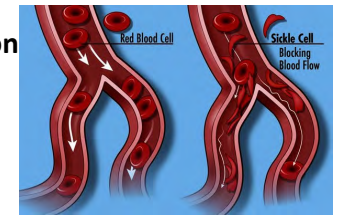
NPR



CASGEVY: First CRISPR therapy approved (sickle-cell disease & beta-thalassemia)

- 16 November– MHRA - UK
- 8 December – FDA – EE.UU.
- 15 December – EMA – recommendation
- 12 February – authorized CE/EMA
- Pending fixing price (Min. Health)

- In EEUU 2,2 million $ / patient



CASGEVY: First CRISPR therapy approved (sickle-cell disease & beta-thalassemia)

- **transfusion-dependent β-thalassemia (TDT) in patients 12 years of age and older for whom haematopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related HSC donor is not available**
- **severe sickle cell disease (SCD) in patients 12 years of age and older with recurrent vaso-occlusive crises (VOCs) for whom haematopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related HSC donor is not available.**

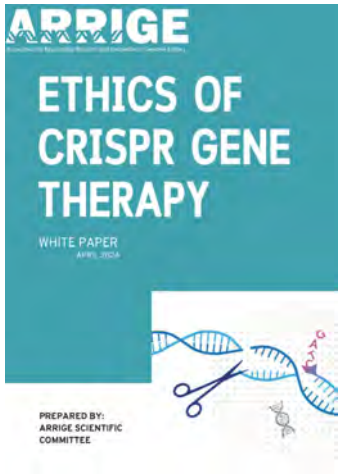
CASGEVY: First CRISPR therapy approved (sickle-cell disease & beta-thalassemia) in Spain?

- **Registro Español de Hemoglobinopatías y Anemias Raras (01/02/2024)**
- **Pacientes con Anemia Falciforme: ~1200**
- **Pacientes con Anemia Falciforme y seguimiento activo: 762**
- **Pacientes no sometidos a TPH con genotipo Sβ: 517**
- **Pacientes con crisis documentadas en 2022/23: 129**
- **Pacientes con al menos dos eventos vasooclusivos: 39**

BIOÉTICA SIBI

XII CONGRESO MUNDIAL DE BIOÉTICA
WORLD CONFERENCE ON BIOETHICS
Gijón, 13-15 mayo 2024

José Antonio Molina (Son Espases) y Elena Cela (Gregorio Marañon)



Innovative Genomics Institute

- **Accessibility**
- **Affordability**
- **Justice / Equity**

arrige.org

Strategies to lower the price and produce cheaper therapies

SPONSORED BY INTELLIA

TRANSTHYRETIN (ATTR) AMYLOIDOSIS

- MAGNITUDE: A Phase 3 Study of NTLA-2001 in Participants With Transthyretin Amyloidosis With Cardiomyopathy (ATTR-CM). Learn more on **clinicaltrials.gov**.
- Long-Term Follow-Up Study of Subjects Dosed with NTLA-2001. Learn more on **clinicaltrials.gov**.
- Phase 1 Study of NTLA-2001 in Patients with Hereditary Transthyretin Amyloidosis with Polyneuropathy and Transthyretin Amyloidosis-Related Cardiomyopathy. Learn more on **clinicaltrials.gov**.

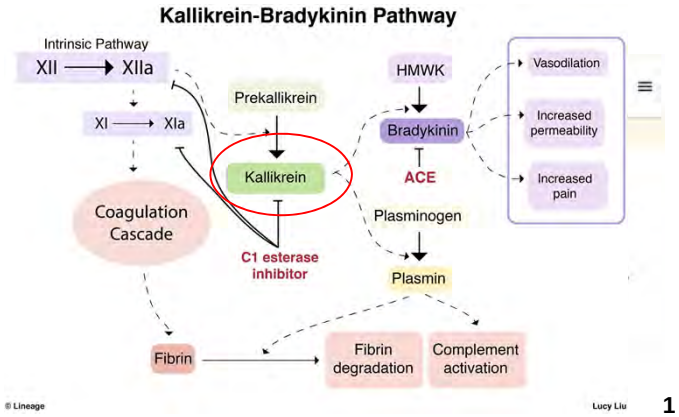
HEREDITARY ANGIOEDEMA

- Long-Term Follow-Up (LTFU) of Subjects Treated With NTLA 2002. Learn more on **clinicaltrials.gov**.
- Phase 1/2 Study of NTLA-2002 in Adults with Hereditary Angioedema. Learn more on **clinicaltrials.gov**.

Participation in a clinical trial is a decision that is made between a patient, their treating physician and the clinical trial site investigator. If you are interested in joining one of our trials, please consult with your physician.

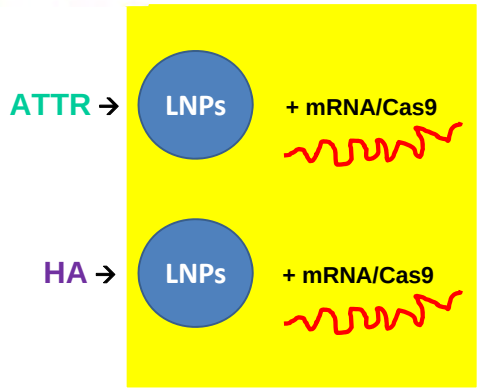
Angioedema congénito

1:100.000

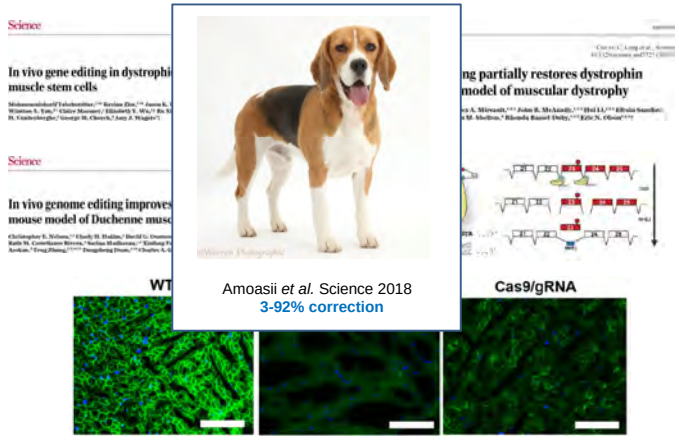


1 Feb 2024

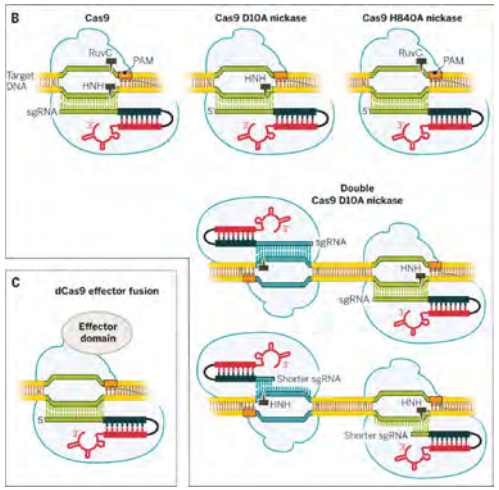
Intellia THERAPEUTICS — Strategies to lower the price and produce cheaper therapies



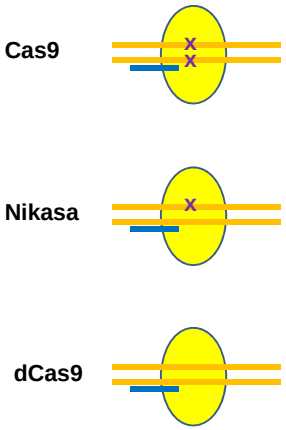
CRISPR-Cas9 and *in vivo* somatic gene therapy



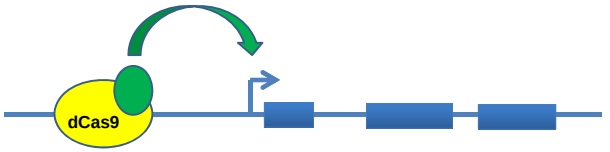
Amoasii *et al.* Science 2018
3-92% correction



Doudna & Charpentier (2014) Science



Activating a gene

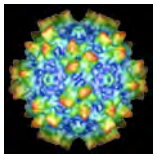


Epigenetic use (activation) of CRISPR tools

Single patient gene therapies

1 x 10^{14} AAVs / kg
7 x 10^{15} for 70 kg

Duchenne Muscular distrophy



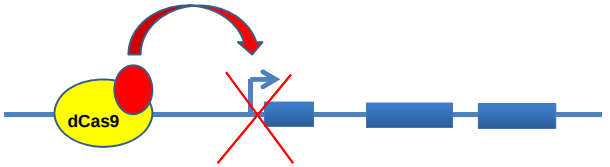
AAVs

N=1

Terry Horgan (1995-2022)
Cure Rare Diseases / dCas9-VP64

Lek et al. 2023 NEJM

Repressing a gene



Epigenetic use (silencing) of CRISPR tools

Nature

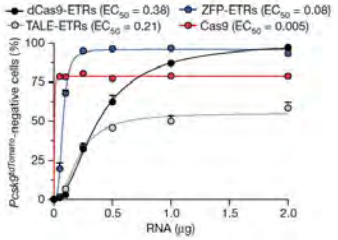
Article

Durable and efficient gene silencing in vivo by hit-and-run epigenome editing

https://doi.org/10.1038/s41586-024-07087-8
Received: 6 March 2023
Accepted: 17 January 2024
Published online: 28 February 2024
Open access
Check for updates

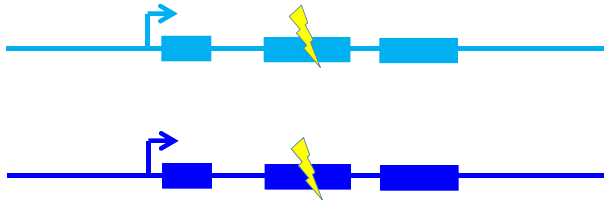
Martino Alfredo Cappelluti[1], Valeria Mollica Poeta[1], Sara Valsoni[1], Pierluigi Quarato[1], Simone Merlin[1], Ivan Merelli[1,2] & Angelo Lombardo[1,3]

Permanent epigenetic silencing using programmable editors equipped with transcriptional repressors holds great promise for the treatment of human diseases[1–3]. However, to unlock its full therapeutic potential, an experimental confirmation of durable epigenetic silencing after the delivery of transient delivery of editors in vivo is



Epigenetic use (silencing) of ZFP tools & LNPs

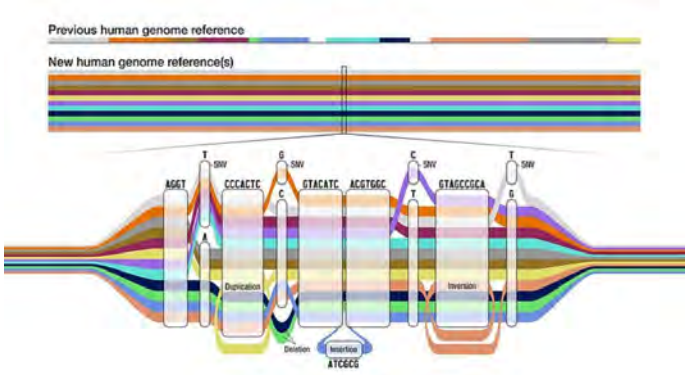
Inactivating similar genes



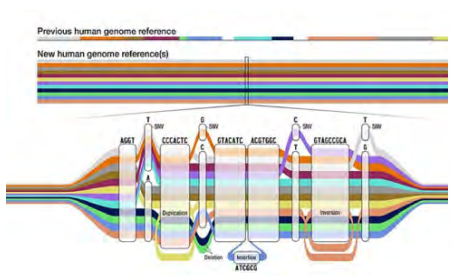
off target effects

Pangenome

AI

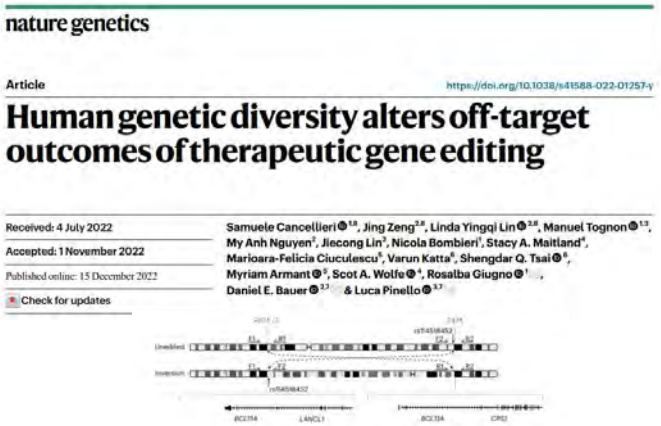


Pangenoma



Inteligencia Artificial

sgRNA guides targeting BCL11A enhancers can also target another gene on the same chrosomome 2 hence promoting deletions/inversions…



nature genetics

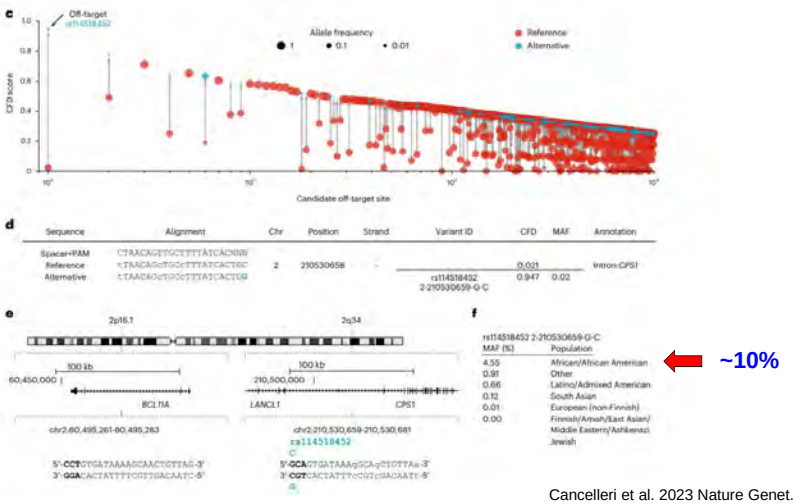
Article

https://doi.org/10.1038/s41588-022-01257-y

Human genetic diversity alters off-target outcomes of therapeutic gene editing

Received: 4 July 2022
Accepted: 1 November 2022
Published online: 15 December 2022
Check for updates

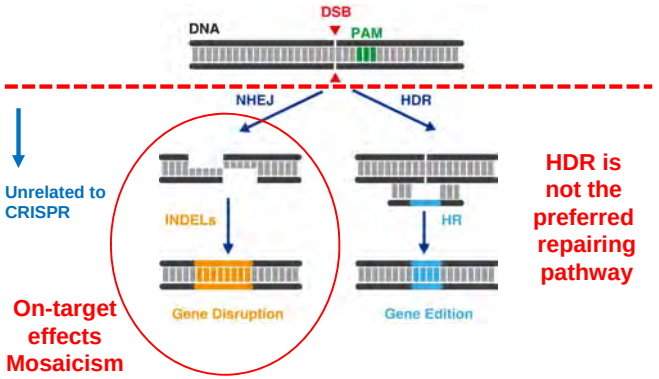
Samuele Cancellieri[1,8], Jing Zeng[2,8], Linda Yingqi Lin[2,8], Manuel Tognon[1,3], My Anh Nguyen[2], Jiecong Lin[2], Nicola Bombieri[1], Stacy A. Maitland[4], Marioara-Felicia Ciuculescu[5], Varun Katta[6], Shengdar Q. Tsai[6], Myriam Armant[5], Scot A. Wolfe[4], Rosalba Giugno[1], Daniel E. Bauer[2,3] & Luca Pinello[3,7]



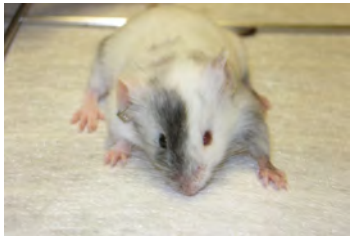
~10%

Cancelleri et al. 2023 Nature Genet.

on target effects - mosaicism

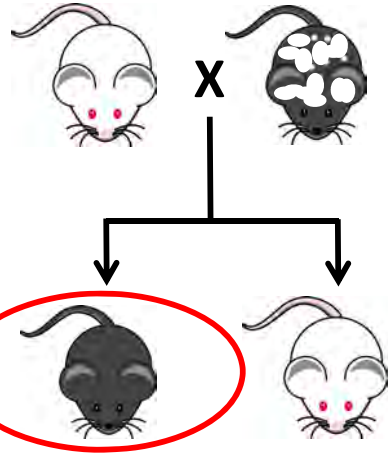


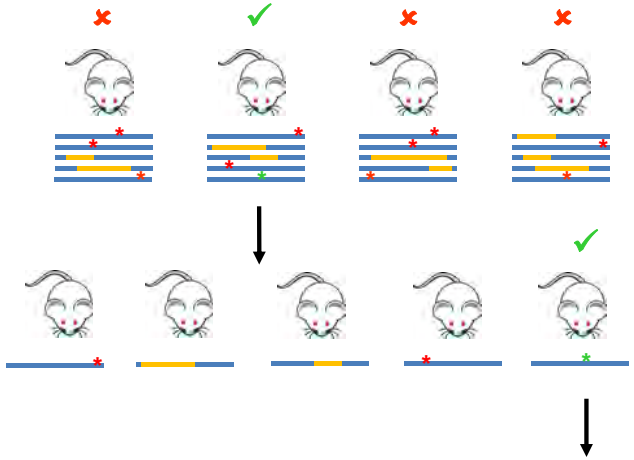
On-targets: the real problem



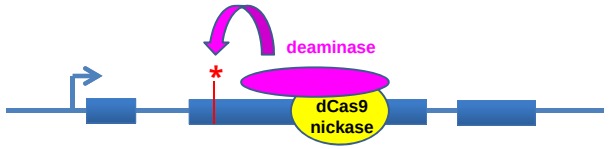
- Founder animals are nearly always complex mosaic
- Many different alleles can be present
- Not all of them might transmit through germline

One 8-cell embryo = 16 possible alleles





Base editing a gene



Second generation of CRISPR tools

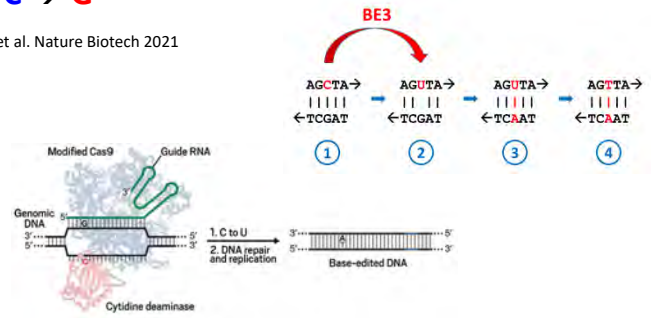
CBE: C → U → T
ABE: A → I → G

Komor et al. Nature 2016

C → G

Koblan et al. Nature Biotech 2021

CRISPR-derived BASE EDITORS (BE3) CBE and ABE



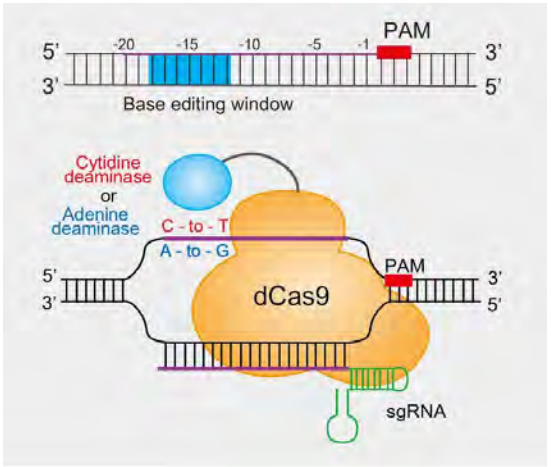
C → T

A → G

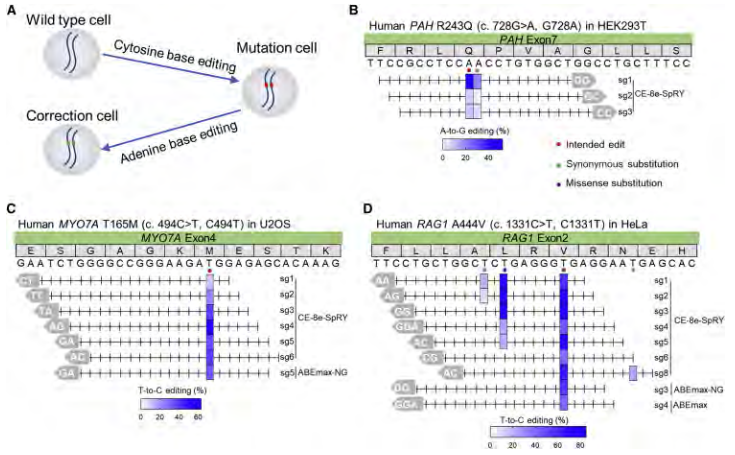
C → G

AGGATTAGAGCCGATAGCATACGATCAGTACGAT

How to design RNA guides for CRISPR base editing use?

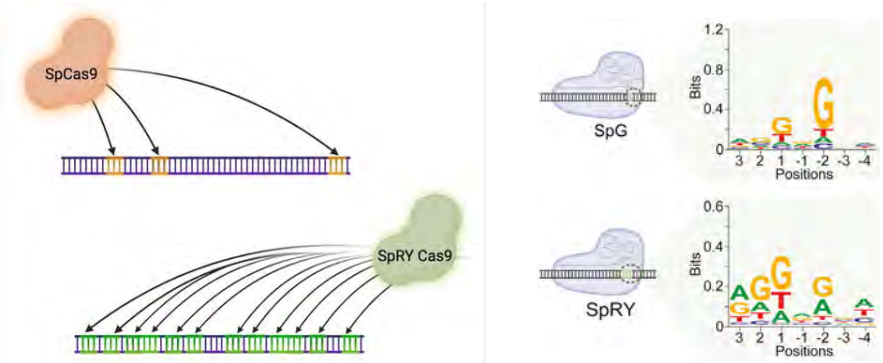


Uncertainty / variation of target base edited positions



Cao et al. 2022

PAM-less Cas9 or near PAM-less Cas9 nucleases

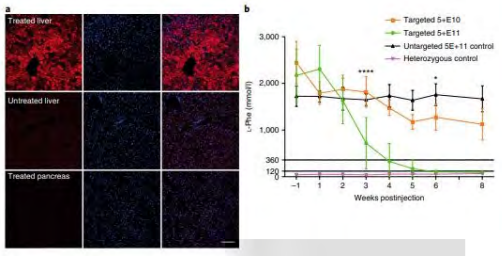


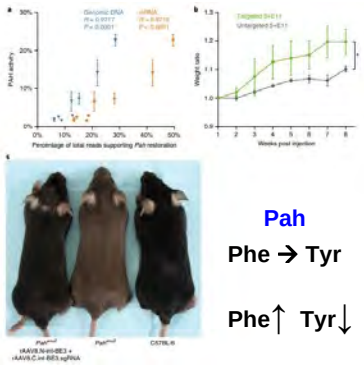
nature medicine | LETTERS

Octubre 2018

Treatment of a metabolic liver disease by in vivo genome base editing in adult mice

Lukas Villiger[1], Hiu Man Grisch-Chan[2], Helen Lindsay[2,6], Femke Ringnalda[1], Chiara B. Pogliano[3], Gabriella Allegri[3], Ralph Fingerhut[3,6], Johannes Häberle[4,6,7], Joao Matos[5], Mark D. Robinson[5], Beat Thöny[2,7] and Gerald Schwank[1]✉





Pah

Phe → Tyr

Phe↑ Tyr↓

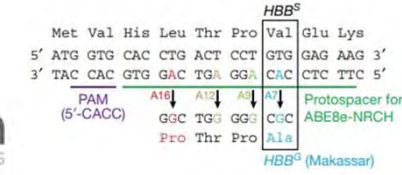
Nature (2021)

Article

Base editing of haematopoietic stem cells rescues sickle cell disease in mice

https://doi.org/10.1038/s41586-021-03609-w
Received: 10 January 2021
Accepted: 4 May 2021
Published online: 2 June 2021
Check for updates

Gregory A. Newby[1,2,3,12], Jonathan S. Yen[4,12]✉, Kaitly J. Woodard[4,12], Thiyagaraj Mayuranathan[4,12], Cicera R. Lazzarotto[4], Yichao Li[4], Heather Sheppard-Tillman[5], Shaina N. Porter[6], Yu Yao[5], Kalin Mayberry[4], Kelcee A. Everette[1,2,3], Yoonjeong Jang[4], Christopher J. Podracky[1,2,3], Elizabeth Thaman[7], Christophe Lechauve[4], Akshay Sharma[4], Jordana M. Henderson[5], Michelle F. Richter[1,2,3], Kevin T. Zhao[1,2,3], Shannon M. Miller[1,2,3], Tina Wang[1,2,3], Luke W. Koblan[1,2,3], Anton P. McCaffrey[8], John F. Tisdale[10], Theodosia A. Kalfa[7,11], Shondra M. Pruett-Miller[6], Shengdar Q. Tsai[4], Mitchell J. Weiss[4,9]✉ & David R. Liu[1,2,3,5]✉



Beam THERAPEUTICS

Sickle-cell disease mutation in the β-globin gene
p.Glu6Val

Makassar allele in the β-globin gene
p.Glu6Ala

Base editing the sickle-cell disease mutation in the β-globin gene
p.Val6Ala

CAG → TAG

Glu STOP

Article

In vivo CRISPR base editing of *PCSK9* durably lowers cholesterol in primates

Nature (2021)

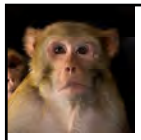
https://doi.org/10.1038/s41586-021-03534-y
Received: 6 December 2020
Accepted: 11 April 2021
Published online: 19 May 2021
Check for updates

Kiran Musunuru[1,2,3], Alexandra C. Chadwick[4], Taiji Mizoguchi[4], Sara P. Garcia[4], Jamie E. DeNizio[4], Caroline W. Reiss[4], Kui Wang[4], Sowmya Iyer[4], Chaitali Dutta[4], Victoria Clendaniel[4], Michael Amaonye[4], Aaron Beach[4], Kathleen Berth[4], Souvik Biswas[4], Maurine C. Braun[4], Huei-Mei Chen[4], Thomas V. Colace[4], John D. Ganey[4], Soumyashree A. Gangopadhyay[4], Ryan Garrity[4], Lisa N. Kasiewicz[4], Jennifer Lavoie[4], James A. Madsen[4], Yuri Matsumoto[4], Anne Marie Mazzola[4], Yusuf S. Nasrullah[4], Joseph Nneji[4], Huilan Ren[4], Athul Sanjeev[4], Madeleine Shay[4], Mary R. Stahley[4], Steven H. Y. Fan[5], Ying K. Tam[5], Nicole M. Gaudelli[6], Giuseppe Ciaramella[6], Leslie E. Stolz[4], Padma Malyala[4], Christopher J. Cheng[4], Kailanthottathil G. Rajeev[4], Ellen Rohde[4], Andrew M. Bellinger[4] & Sekar Kathiresan[4,7]✉

PCSK9 ⊣ LDL receptor ↑ LDL ↓

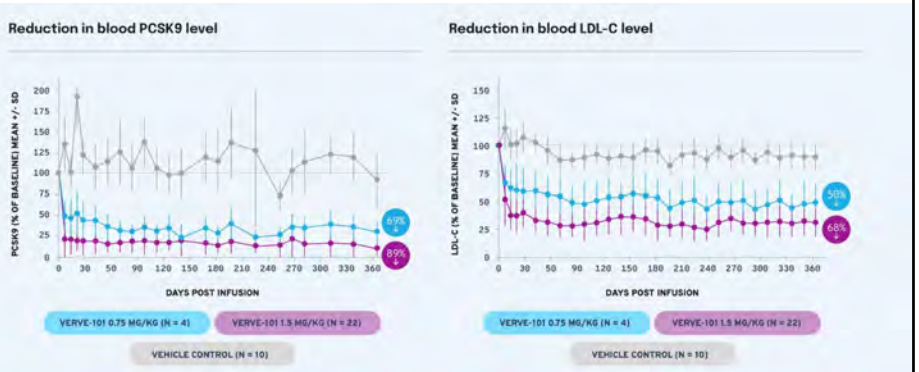
Reduced risk of atherosclerosis & cardiovascular diseases

verve THERAPEUTICS



↓PCSK9 ➔ ↓LDL-C

verve THERAPEUTICS



Base editing to cure a ALL (acute lymphoblastic leukemia)

- **TCR inactivated**
- **CD7 inactivated**
- **CD52 inactivated**
- **universal CAR-T added**

NEWS RELEASE 10-DEC-2022

World-first use of base-edited CAR T cells to treat resistant leukemia at Great Ormond Street Hospital

Clinical Trial | People

Reports and Proceedings

UNIVERSITY COLLEGE LONDON

Alyssa May 2022

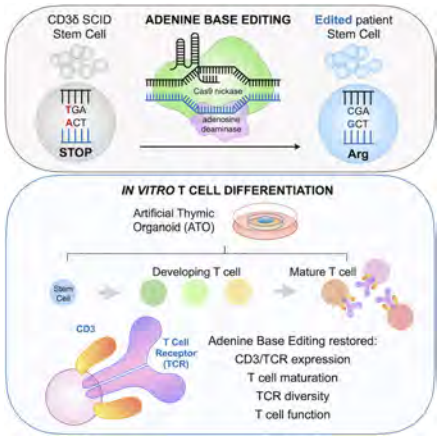
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia

Robert Chiesa, M.D., Christos Georgiadis, Ph.D., Farhatullah Syed, Ph.D., Hong Zhan, Ph.D., Annie Etuk, Ph.D., Soragia Athina Gkazi, Ph.D., Roland Preece, Ph.D., Giorgio Ottaviano, M.D., Toni Braybrook, M.Bio., Jan Chu, M.Sc., Agnieszka Kubat, B.Sc., Stuart Adams, Ph.D., Rebecca Thomas, Ph.D., Kimberly Gilmour, Ph.D., David O'Connor, M.B., Ch.B., Ajay Vora, M.B., B.S., and Waseem Qasim, M.B., B.S., Ph.D., for the Base-Edited CAR T Group*

14 June 2023



ABE base editor to correct SNP in *CD3D* gene

Severe Combined Immunodeficiency (SCID)

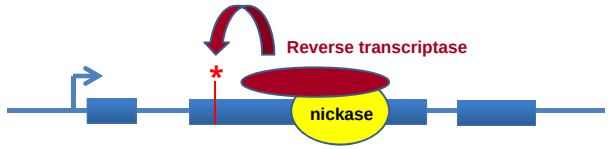
Done with hHESc in mice

McAuley et al. Cell, 2023

High-fidelity PAMless base editing of hematopoietic stem cells to treat chronic granulomatous disease

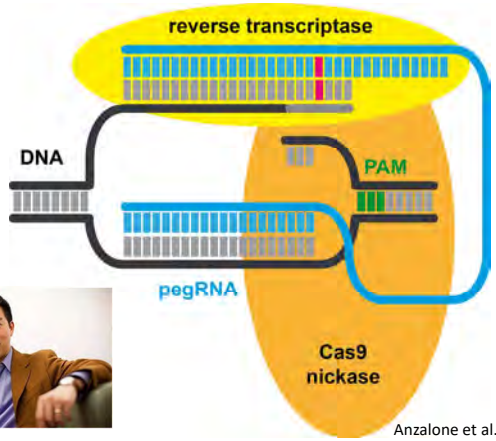
- **X-linked chronic granulomatous disease (X-CGD) is an inborn error of immunity (IEI) resulting from genetic mutations in the cytochrome b-245 beta chain (CYBB) gene.**
- **For the prototypical X-CGD mutation CYBB c.676C>T, ABE8e-SpRY achieved up to 70% correction**
- **ABE base editor + SpRY pamless Cas9**

Prime editing a gene



Third generation of CRISPR tools

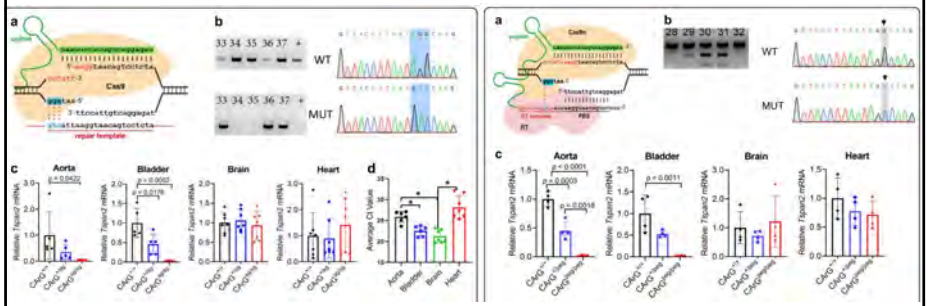
Prime editing a gene



David Liu Lab

Anzalone et al. Nature 2019

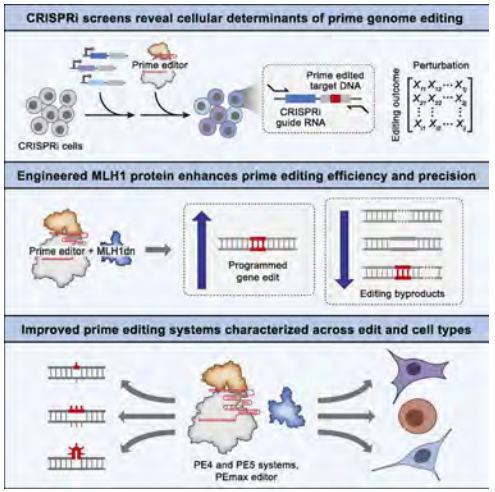
CRISPR-Cas9 mediated gene editing **vs** **Prime-mediated gene editing**



56% correct on-targeting
40% INDELS (mosaicism)

21% correct on-targeting
0% INDELS (mosaicism)

Gao et al. 2021 Genome Biol



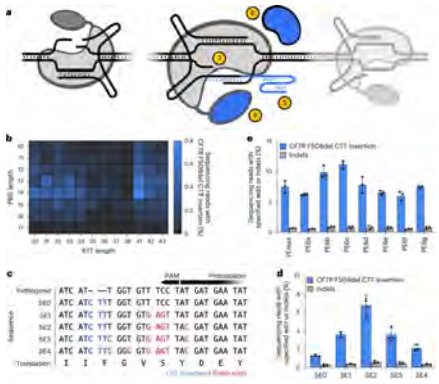
DNA mismatch repair (MMR) inhibits prime editing

Prime editing can be enhanced with MLH1dn (transiently inhibiting MMR) → PE4 and PE5

PEmax = PE4/5 + epegRNA

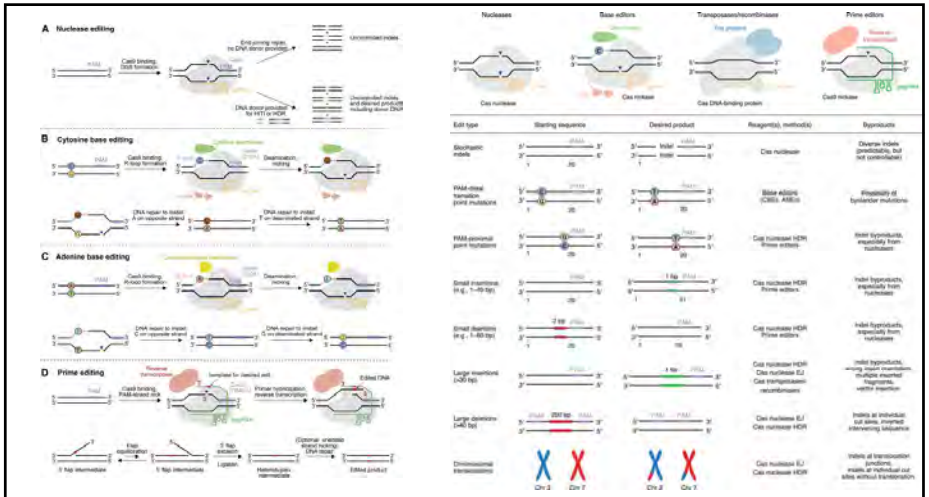
David Liu's Lab, 28 october
Chen et al. Cell (2021)

Optimized Prime Editing corrects CF F508Δ mutation



- **epegRNA (3' protective structure)**
- **Co-expression of MLH1dn**
- **translationally silent edits to evade cellular mismatch repair (MMR)**
- **engineered and evolved prime editor proteins**
- **works with PEmax and PE6**
- **doesn't work with PE2 or PE3**

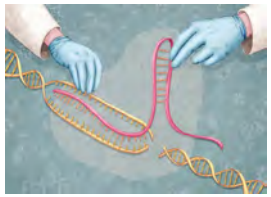
Sousa et al. Nature Biomed. Eng. (2024)



David Liu (Broad) Anzalone et al. Nature Biotech 2020

CRISPR & gene therapy (today) – clinical trials

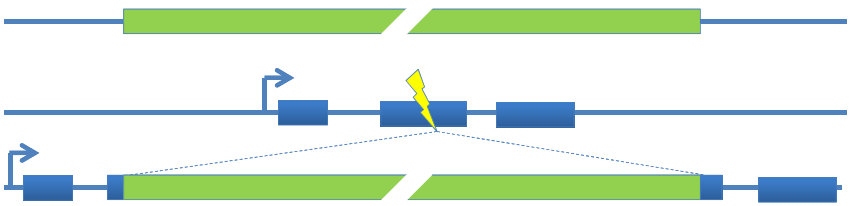
- **Inactivating genes** → Cas9 (1st), base editors (2nd) or epigenetic editing (dCas9/ZP/TALE)
- **Correcting genes** → Base editors (2nd), prime editors (3rd)
- **Delivery technologies** → AAVs, VLPs, mRNA, EV, LNPs



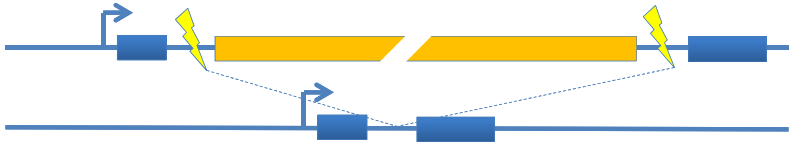
Not everything is solved in genome editing

CRISPR-Cas systems suffer from known limitations

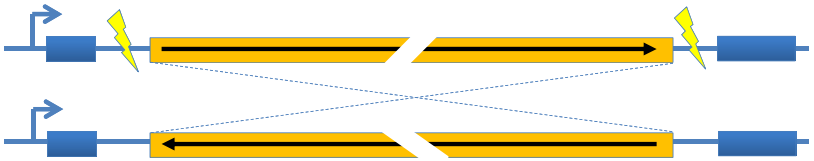
Large Insertions are challenging with CRISPR



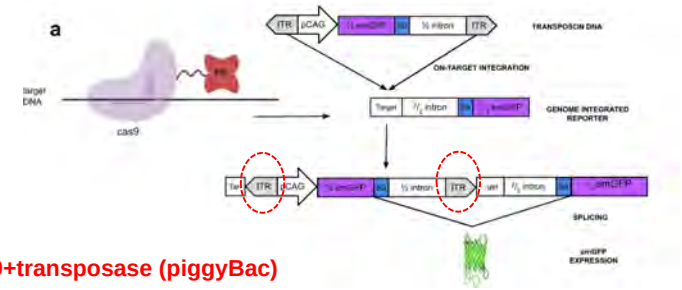
Large Deletions are challenging with CRISPR



Large Inversions are challenging with CRISPR



Find and cut-and-transfer (FiCAT) mammalian genome engineering



Cas9+transposase (piggyBac)

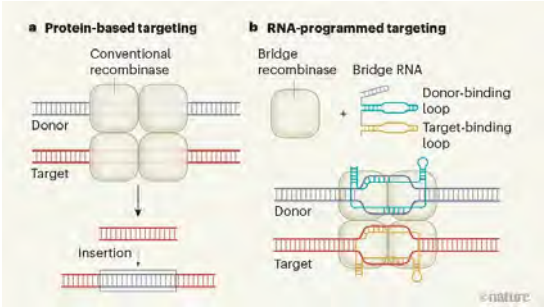
Duplication of ends / not clean recombination

Integra therapeutics

Marc Güell lab

Pallarés-Masmitjà et al. Nature Comm. (2021)

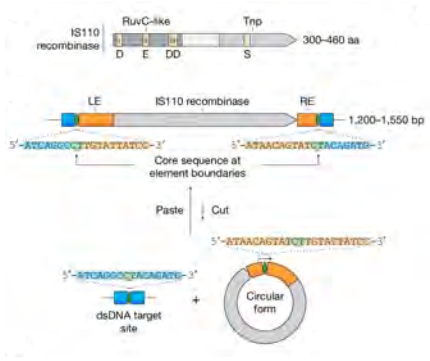
Bridge editing (RNA-programmed targeting)



Tou & Kleinstiver, Nature (2024)

Patrick Hsu lab

Bridge editing (RNA-programmed targeting)



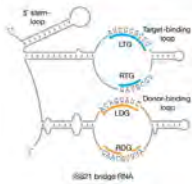
The IS110 family of mobile genetic elements (transposons)

IS = Insertion Sequences

Small recombinase (300-460 aa)

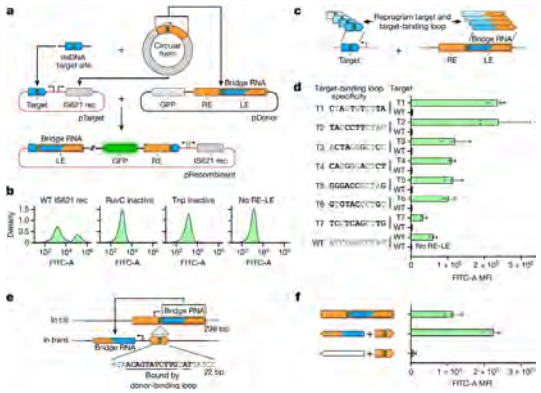
Small bridge RNA (150-250 nt) with two loops, for target and donor DNA

Minor duplications of ends (CT)



Durrant et al. Nature (2024)

Bridge editing (RNA-programmed targeting)



It is possible to program the RNA sequences in the donor and target loops of the bridge RNA

Durrant et al. Nature (2024)

Bridge editing versus FiCAT

- **can insert up to ~5 kb**
- **>85% efficiency**
- **Tested only in bacteria so far**
- **Duplicates only "TC" at target sites**
- **~30% off targets**

Durrant et al. Nature (2024)

- **can insert up to ~10 kb**
- **25-30% efficiency**
- **Works in mouse and human cells**
- **Duplicates ITR at target sites**
- **Low or absent off targets**

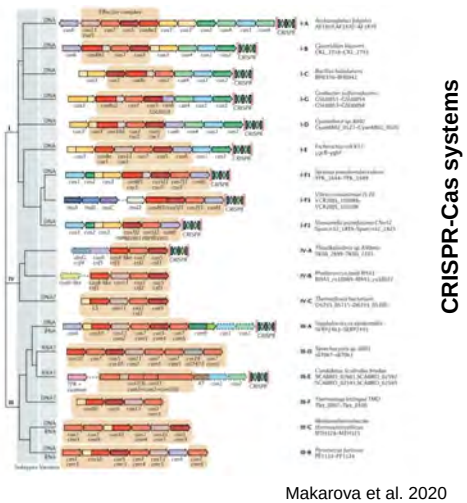
Pallarés-Masmitjà et al. Nature Comm. (2021)

Matthew Porteus **2019** ***Nature Med.***

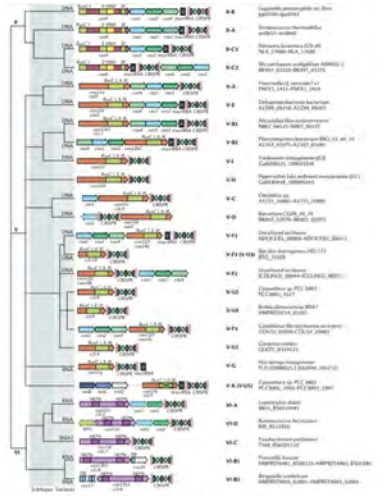
nature medicine

- Cas9 antibodies found in human serum
- Anti-Cas9 T lymphocites found in human blood
- 79% individuals have antibodies against SaCas9
- 65% individuals have antibodies against SpCas9
- 46% individuals have anti-Cas9 T cells
- Immunosupression or alternative Cas proteins

Current CRISPR tools are derived from pathogenic bacteria

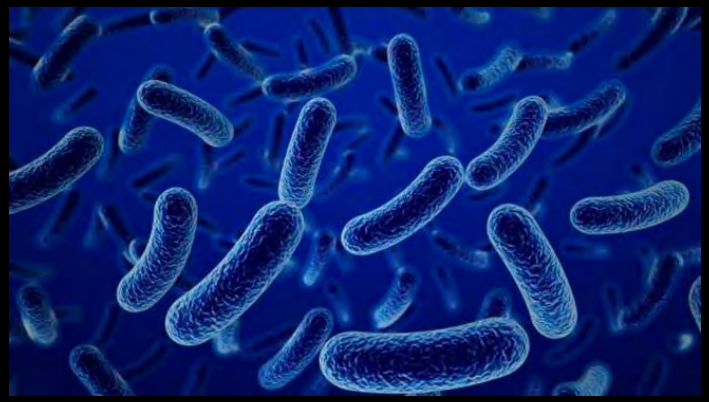


CRISPR-Cas systems



Makarova et al. 2020

Finding out new CRISPR systems in nature



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RESEARCH ARTICLE

MICROBIAL BIOTECHNOLOGY

Identification of the EH CRISPR-Cas9 system on a metagenome and its application to genome engineering

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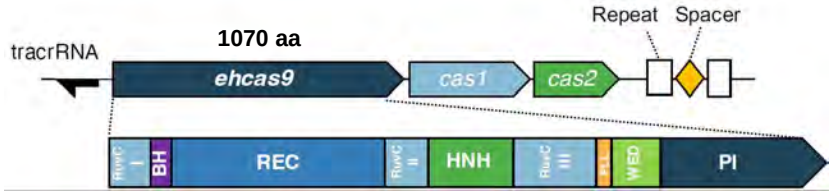
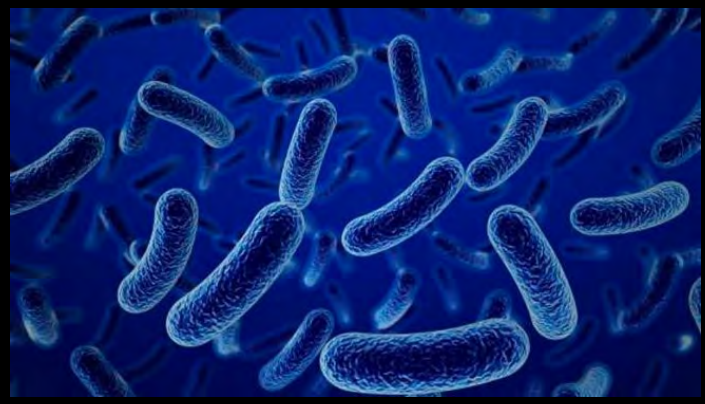
Almudena Fernandez[2,3] | Lluis Montoliu[2,3] | Francisco J. M. Mojica[1,4]





What about CRISPR systems from ancestral bacteria?

Svante Pääbo (~10.000 to ~400.000 years old DNA)

Article

A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA

https://doi.org/10.1038/s41586-022-05453-y
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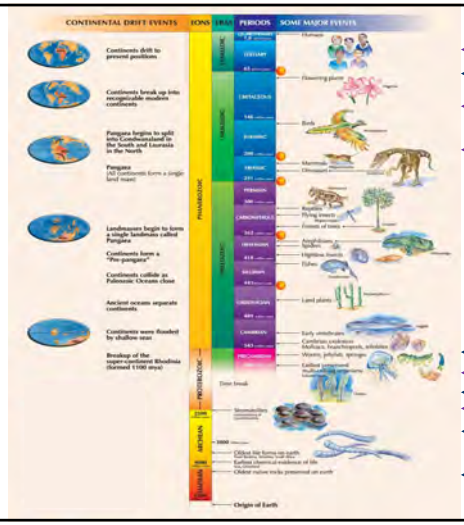
Open access
Check for updates

Kurt H. Kjær[1,27], Mikkel Winther Pedersen[1,27], Bianca De Sanctis[2,3], Binia De Cahsan[4], Thorfinn S. Korneliussen[1], Christian S. Michelsen[1,5], Karina K. Sand[1], Stanislav Jelavić[1,6], Anthony H. Ruter[7], Astrid M. A. Schmidt[7,8], Kristian K. Kjeldsen[9], Alexey S. Tesakov[10], Ian Snowball[11], John C. Gosse[12], Inger G. Alsos[13], Yucheng Wang[1,2], Christoph Dockter[14], Magnus Rasmussen[14], Morten E. Jørgensen[14], Birgitte Skadhauge[14], Ana Prohaska[1,2], Jeppe Å. Kristensen[15,16], Morten Bjerager[17], Morten E. Allentoft[1,18], Eric Coissac[13,19], PhyloNorway Consortium*,**, Alexandra Rouillard[1,21], Alexandra Simakova[10], Antonio Fernandez-Guerra[1], Chris Bowler[20], Marc Macias-Fauria[22], Lasse Vinner[1], John J. Welch[3], Alan J. Hidy[23], Martin Sikora[1], Matthew J. Collins[24,25], Richard Durbin[3], Nicolaj K. Larsen[1] & Eske Willerslev[1,2,26]



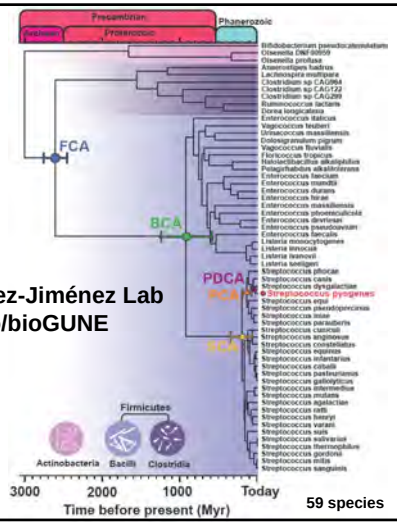
-37 PDCA
-65
-137 PCA
-200 SCA

Resurrecting ancestral Cas9

Raúl Pérez-Jiménez Lab
CIC nanoGUNE/bioGUNE

-580
-1.000 BCA
-1.850
-2.600 FCA
-3.500
-4.500
My

Alonso –Lerma et al.
Nature Microbiol. 2023



Raúl Pérez-Jiménez Lab
CIC nanoGUNE

59 species

Ancestral Sequence Reconstruction (ASR) technique

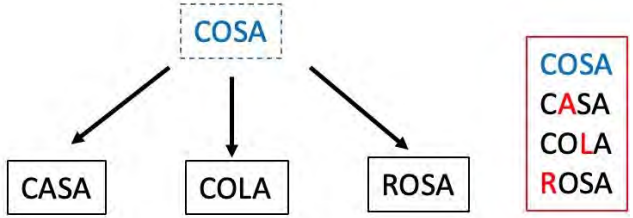
maximum likelihood method

Ancestral Cas9 (anCas)

PDCA ~ 37 Myr
PCA ~ 137 Myr
SCA ~ 200 Myr
BCA ~ 1000 Myr
FCA ~ 2600 Myr

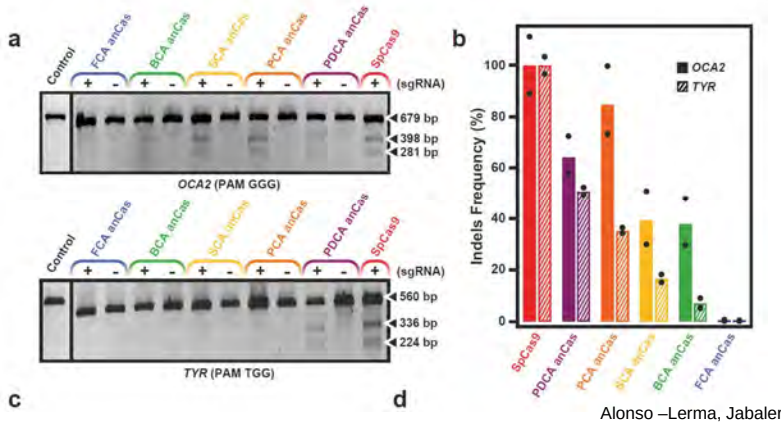
53-96% AA identities

Alonso–Lerma, Jabalera et al. Nature Microbiol. 2023



Gemma Marfany (El Nacional, 2023)

Resurrected ancestral Cas9 can be used for genome editing in human cells



Alonso –Lerma, Jabalera et al. Nature Microbiol. 2023

Ancestral CRISPR-Cas9 can be used as genoma editing tools

nature microbiology

Article

Evolution of CRISPR-associated endonucleases as inferred from resurrected proteins

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Borja Alonso-Lerma, Ylenia Jabalera, Sara Samperio, Matías Morin, Almudena Fernandez, Logan T. Hille, Rachel A. Silverstein, Ana Quesada-Gumuza, Antonio Reifs, Sergio Fernández-Peñalver, Yolanda Benítez, Lucía Soletto, Jose A. Gavira, Adrian Diaz, Wim Vranken, Avencia Sanchez-Mejías, Marc Güell, Francisco J. M. Mojica, Benjamin P. Kleinstiver, Miguel A. Moreno-Pelayo, Lluís Montoliu & Raul Perez-Jimenez

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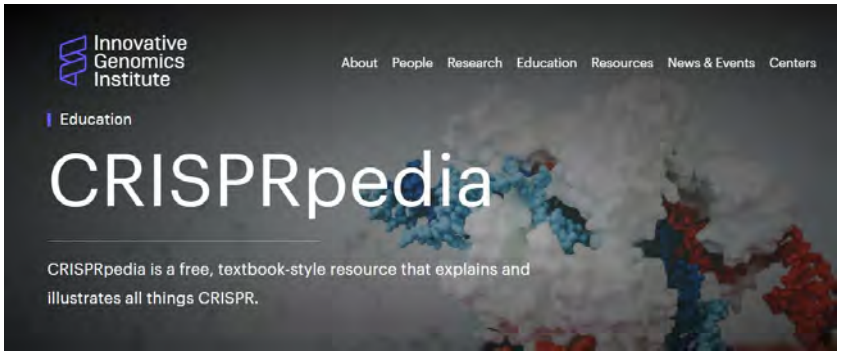
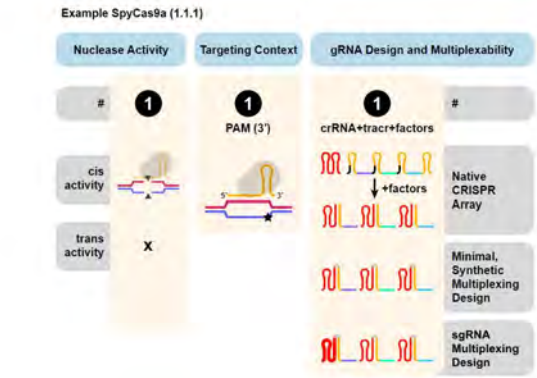
- **MA Moreno-Pelayo**
- **Ben Kleinstiver**
- **Marc Güell**
- **et al.**

2019-2023



https://innovativegenomics.org/crisprpedia/



http://caspedia.org/

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