Il futuro del genome editing

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UNIVERSITY
OF TRENTO - Italy
Department CIBIO

World map clinical trials using CRISPR-Cas9 technology

Selected trials from the world list:

Europe:
-Beta-Thalassemia
-Sickle Cell Disease

US:
-Edited T cells (TCR and PD1 ko)

•Multiple Myeloma
•Melanoma
•Synovial Sarcoma
•Myxoid/Round Cell Liposarcoma

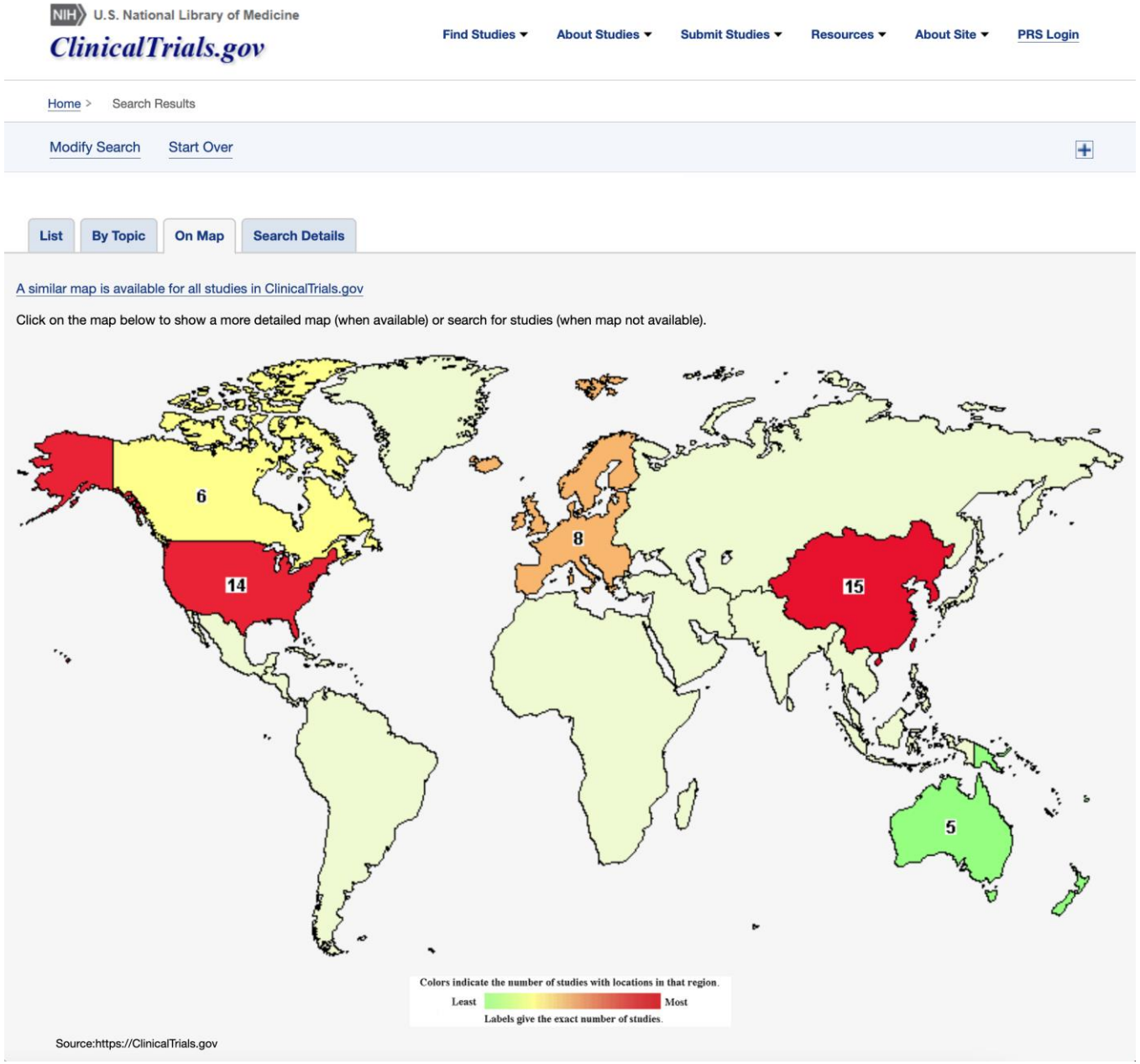
-Blindness (Leber Congenital Amaurosis)

China
-CAR-T cells (CD19, CD20, CD22)

•B Cell Leukemia
•B Cell Lymphoma

-CCR5 ko HIV-1-infection

-PD1 ko for EBV (Epstein Barr) malignancies (Gastric, Nasopharyngeal, Lymphoma)



October 2021: 37 studies

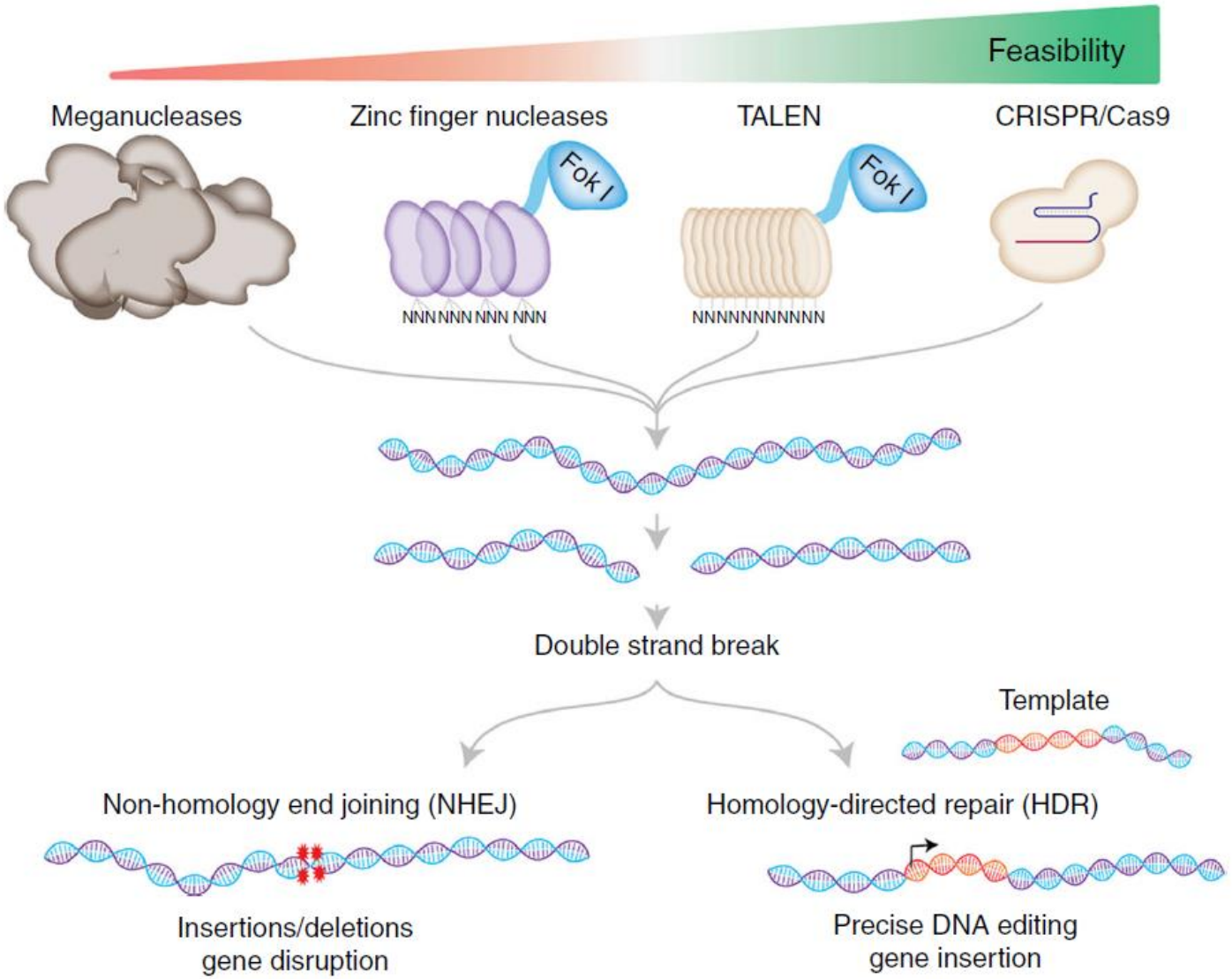
I. Background

- Avanzamenti e ostacoli del genome editing
- Proprietà in-vitro/in-vivo del genome editing
- Aggiornamenti di genome editing per la fibrosi cistica

II. Esempio di correzione di mutazione di splicing

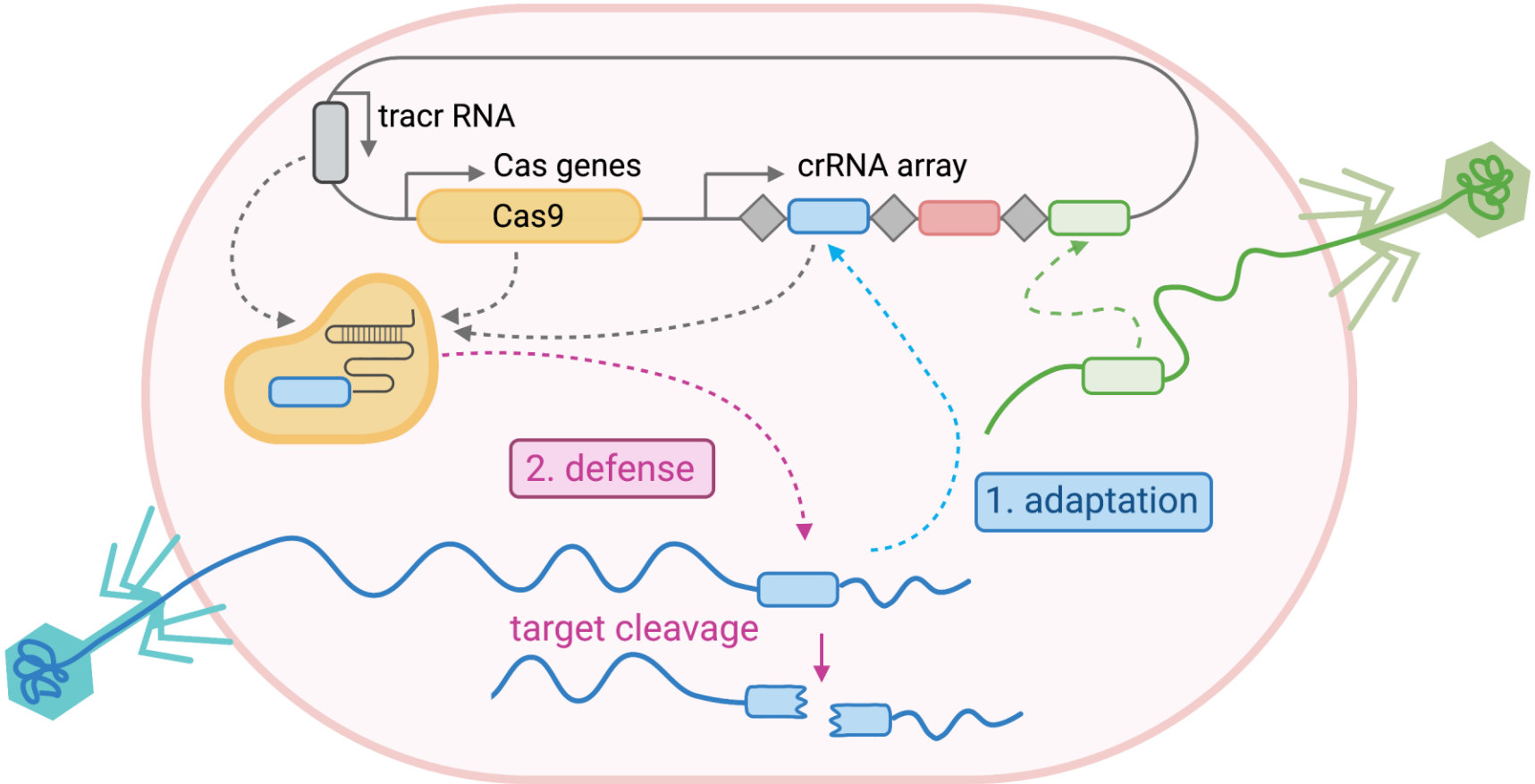
- Correzione della mutazione di splicing 3272-26A>G utilizzando CRISPR-Cas

Genome editing technology

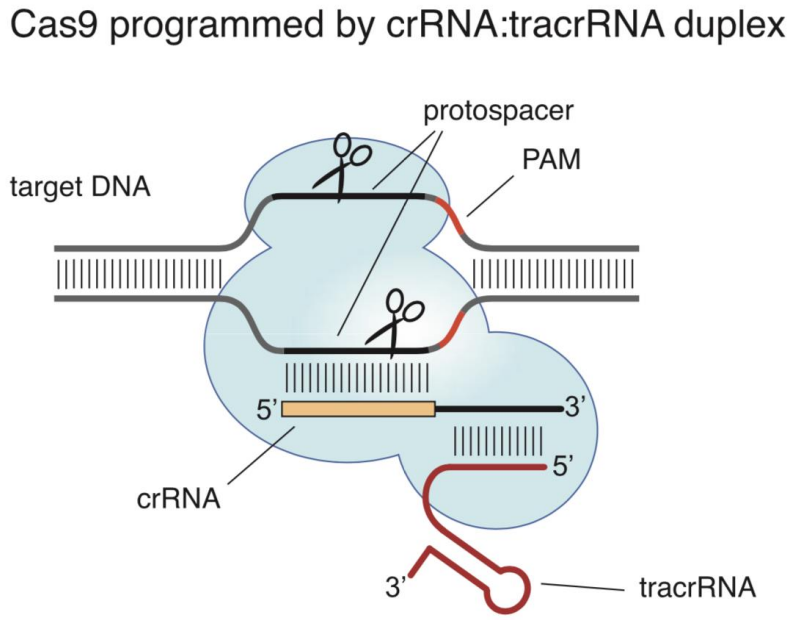


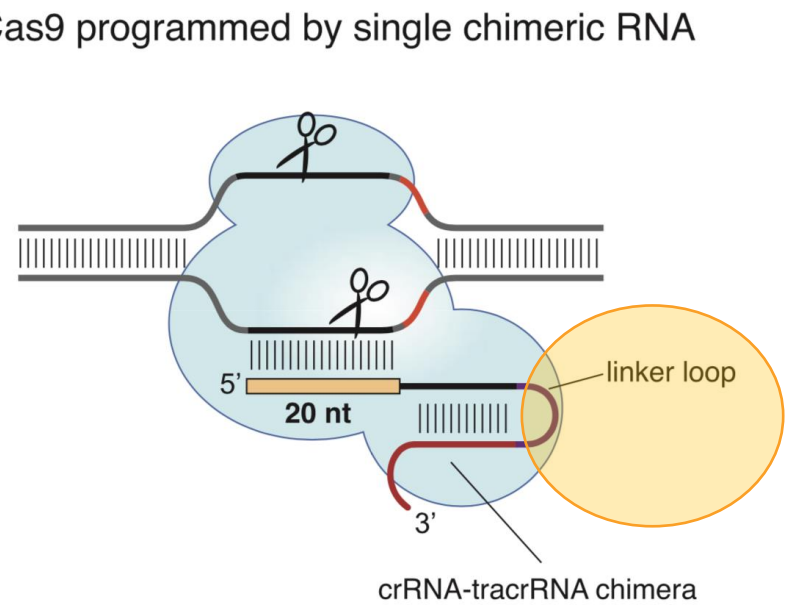
Mazhar Adli, Nature Comm 2018

Host defense in bacteria



Transforming bacterial CRISPR-Cas9 in a technology for genome editing

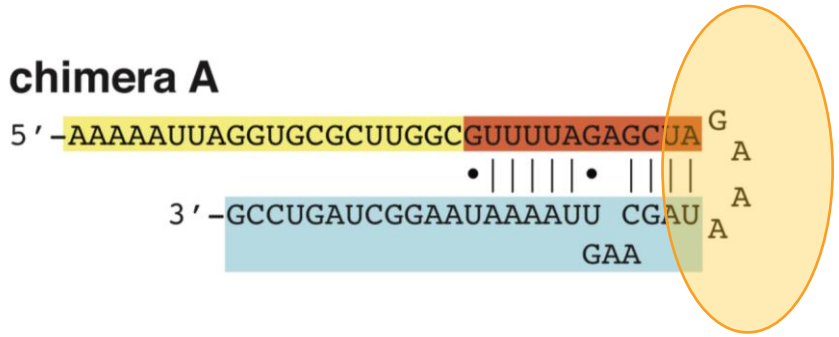




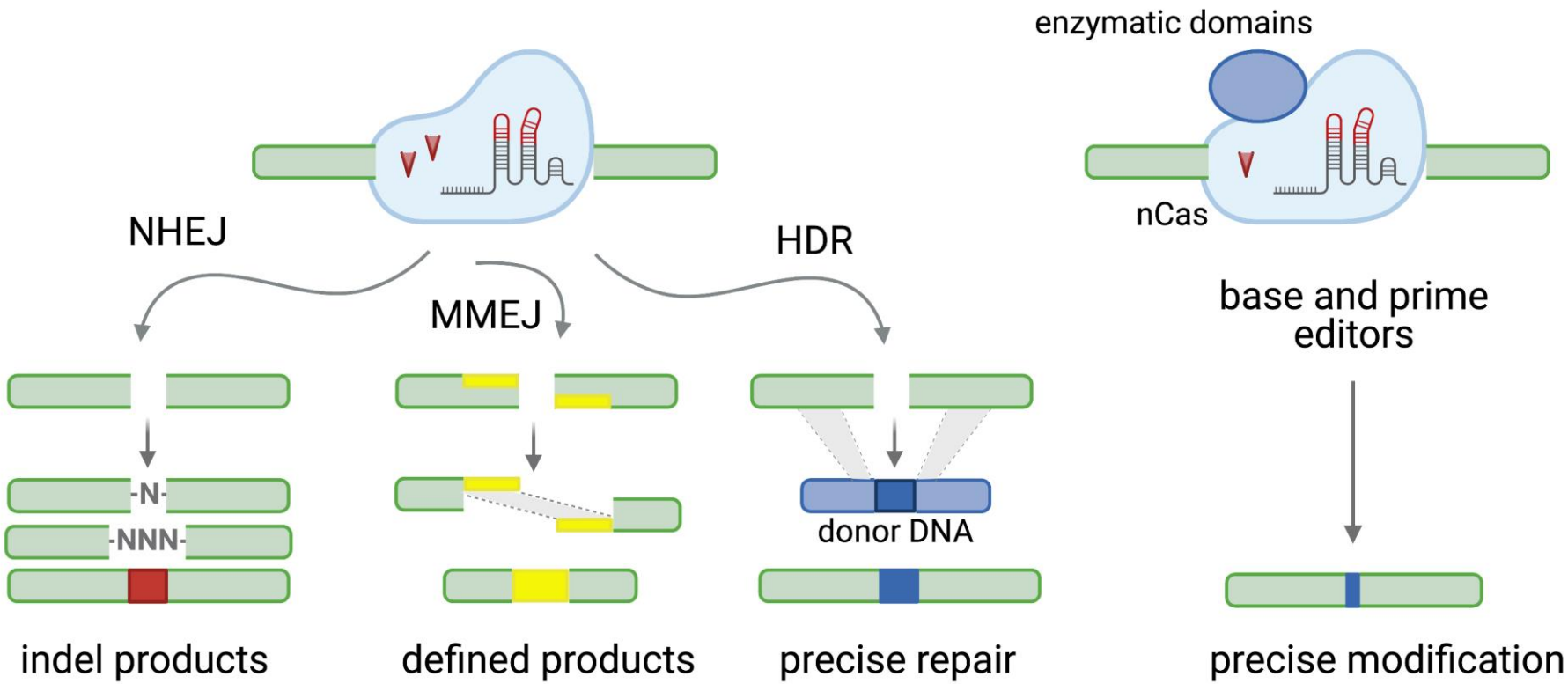
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

17 AUGUST 2012 VOL 337 SCIENCE www.sciencemag.org



Editing outcome



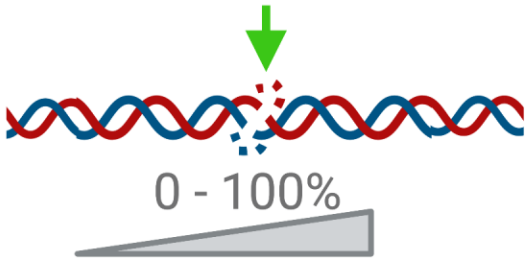
Le proprietà della tecnologia CRISPR-Cas

Considerazioni in-vitro

Properties of CRISPR genome editing technology

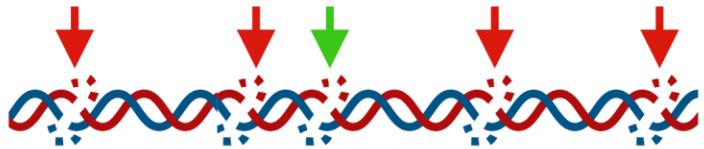
On target activity

efficiency?



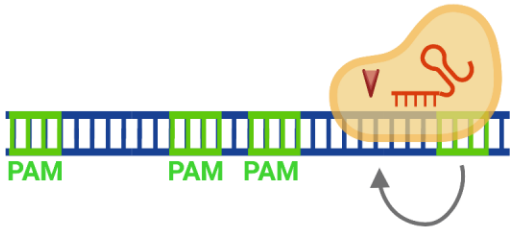
Off target activity

where else?



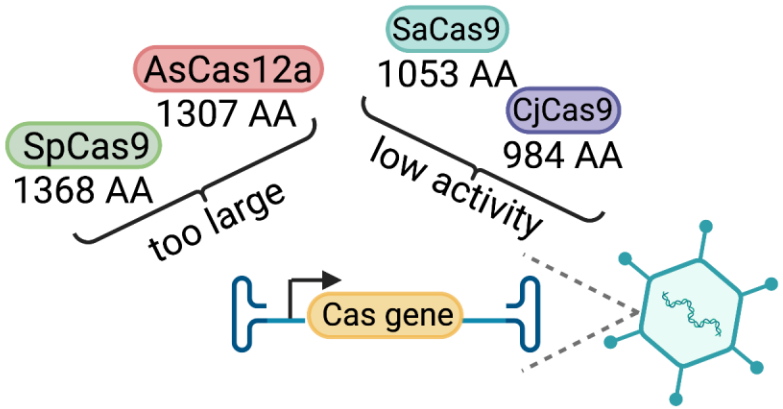
Targeting range (PAM constraints)

where?



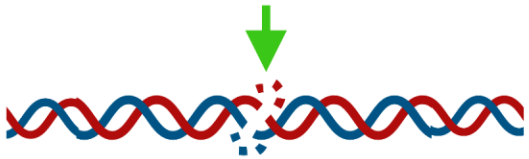
Delivery (Cas molecular weight)

how to deliver?



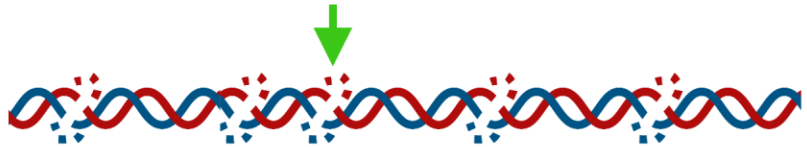
Properties of CRISPR genome editing technology

On target activity

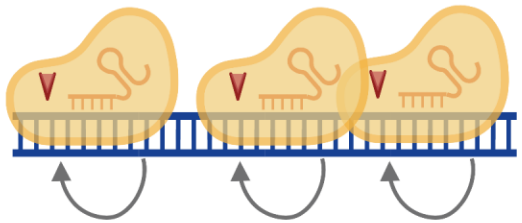


100%

Off target activity



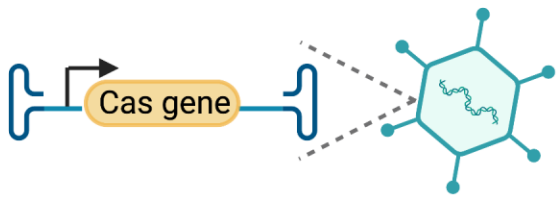
Targeting range (PAM constraints)

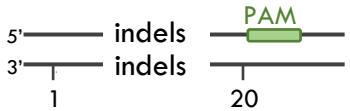
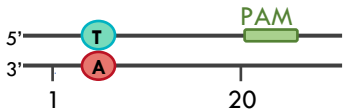
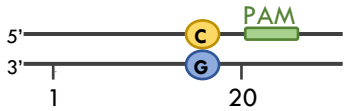
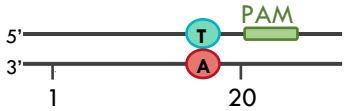
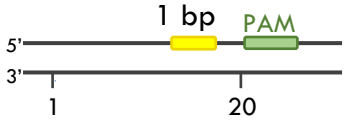
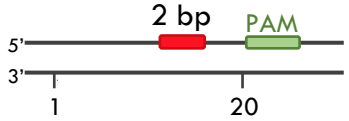
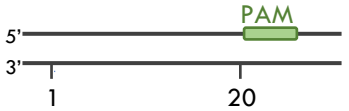
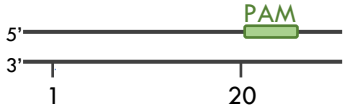
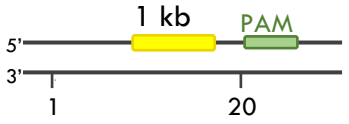
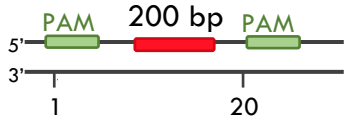
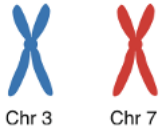
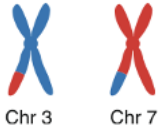


Delivery (Cas molecular weight)

Cas
<600 AA
} type 2?

small molecular weight



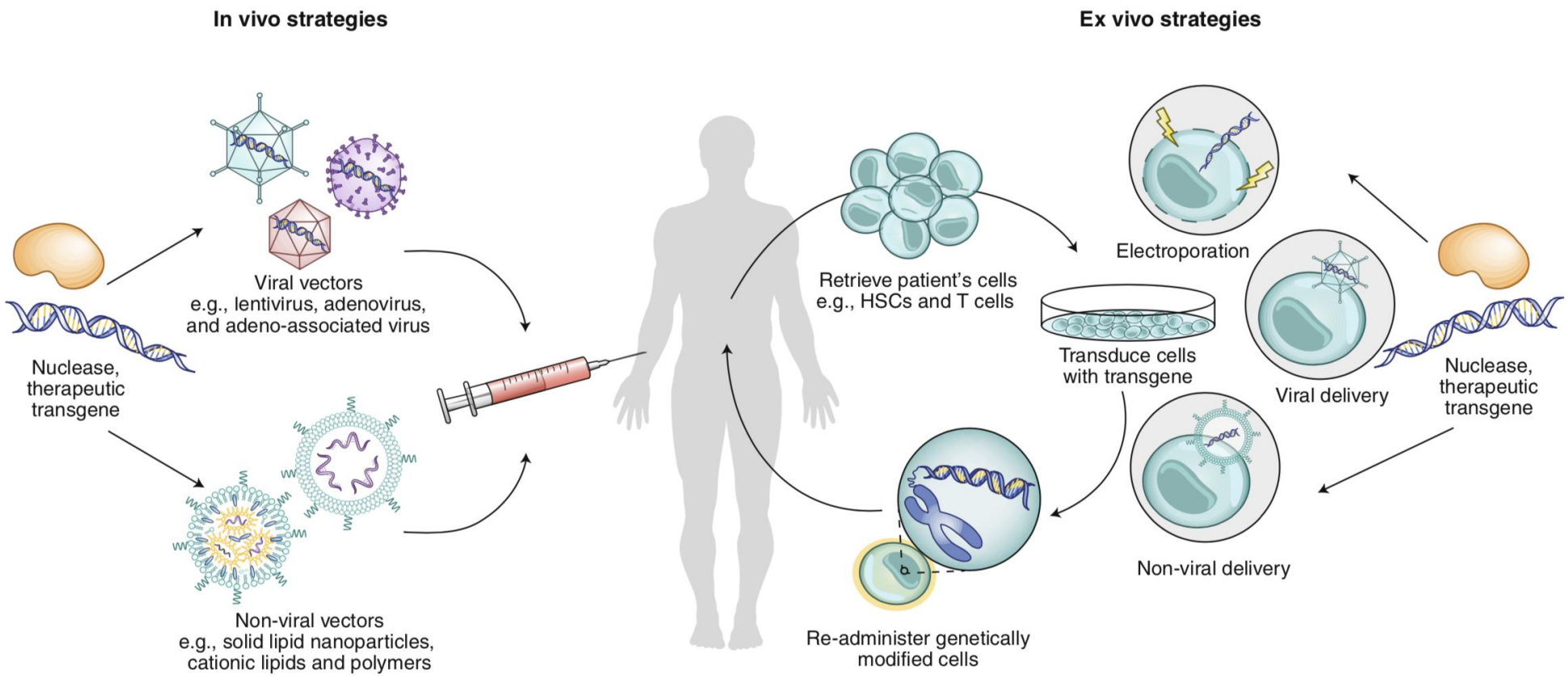
Edit types	Starting sequence	Desired product	Methods	Byproducts
Stochastic indels			Cas nuclease	Diverse indels not controllable
PAM-distal transition point mutations			Base editors	Bystander mutations
PAM-proximal transition point mutations			Cas nuclease HDR Prime editor	Extra indels
Small insertions (e.g. 1-40 bp)			Cas nuclease HDR Prime editor	Extra indels
Small deletions (e.g. 1-80 bp)			Cas nuclease HDR Prime editor	Extra indels
Large insertions (>30 bp)			Cas nuclease HDR Prime editor EJ Cas transp/recomb	Extra indels, wrong insert orientation, multiple insertions, vector insertions
Large deletions (>40 bp)			Cas nuclease EJ Cas nuclease HDR	Indels at cut sites, inverted sequence
Cromosomal translocations			Cas nuclease EJ Cas nuclease HDR	Indels at translocation junction, indels at sites without translocation

Adapted from Anzalone et al, Nat Biotech, 2020

Le proprietà della tecnologia CRISPR-Cas

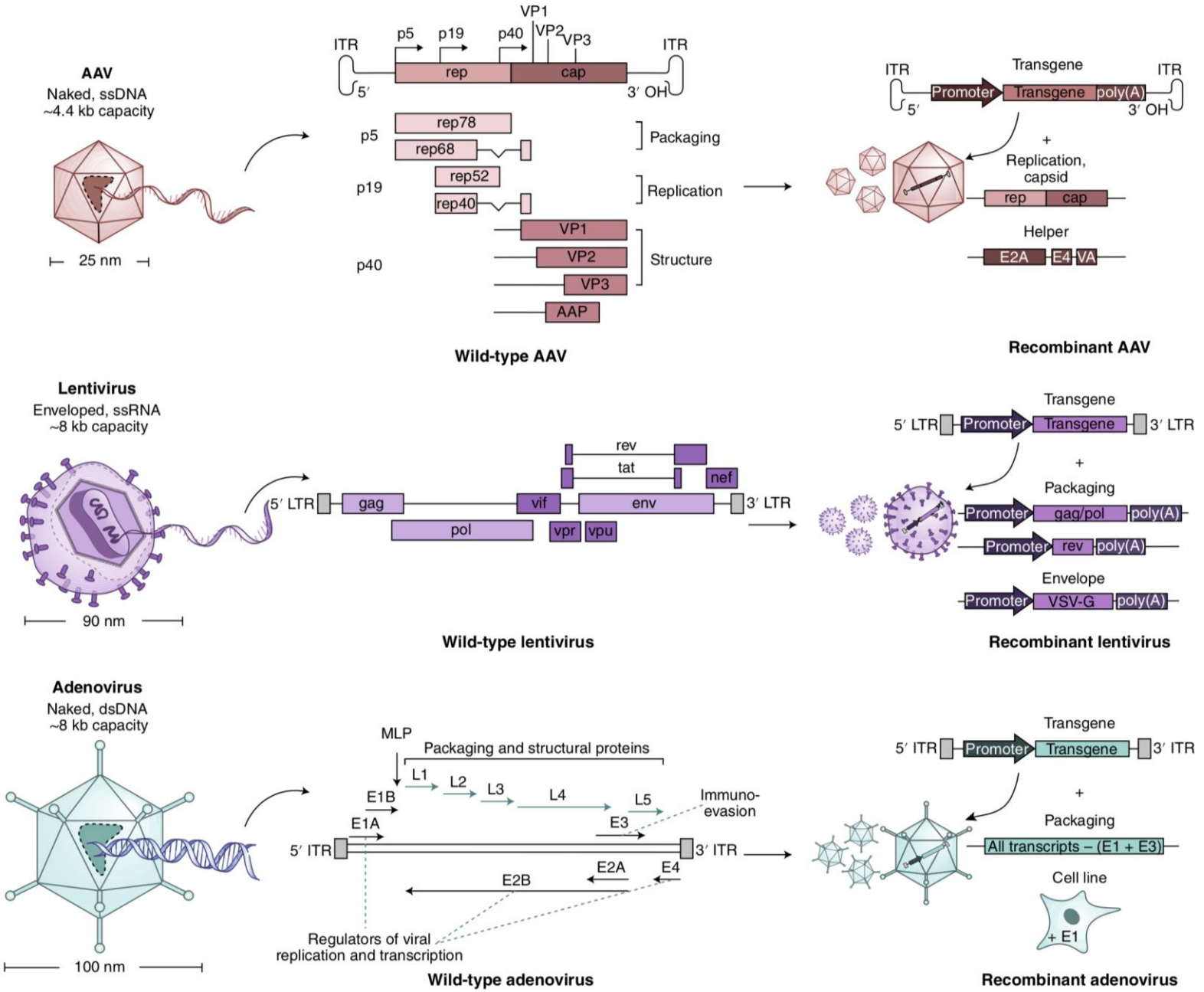
Considerazioni in-vivo

Therapeutic genome editing strategies

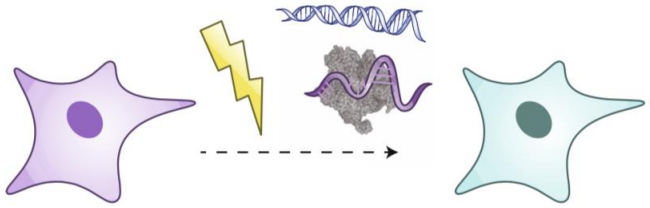


Nat Biotech, Haasteren et al 2020

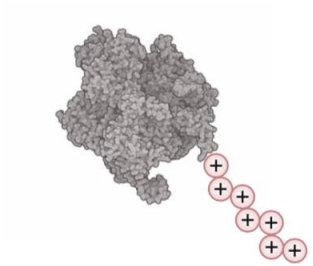
Viral methods to deliver genome editing cargo



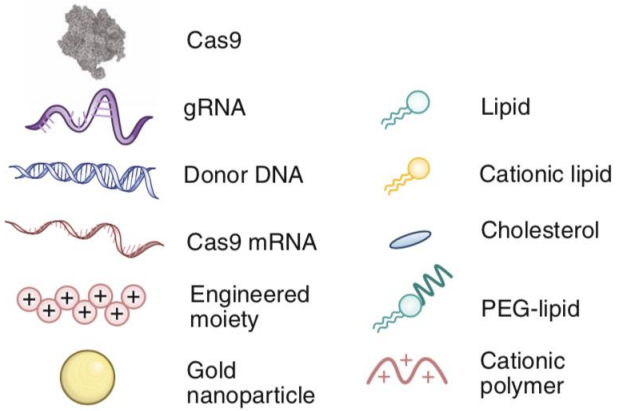
Non-viral methods to deliver genome editing cargo

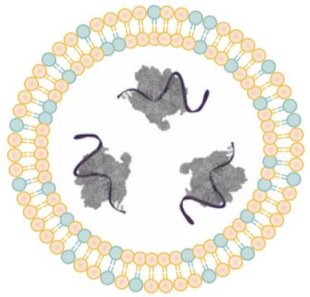


Electroporation

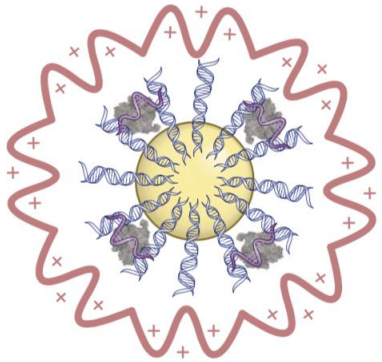


Engineered Cas9

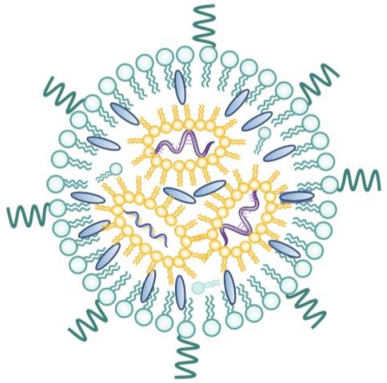




Lipofectamine



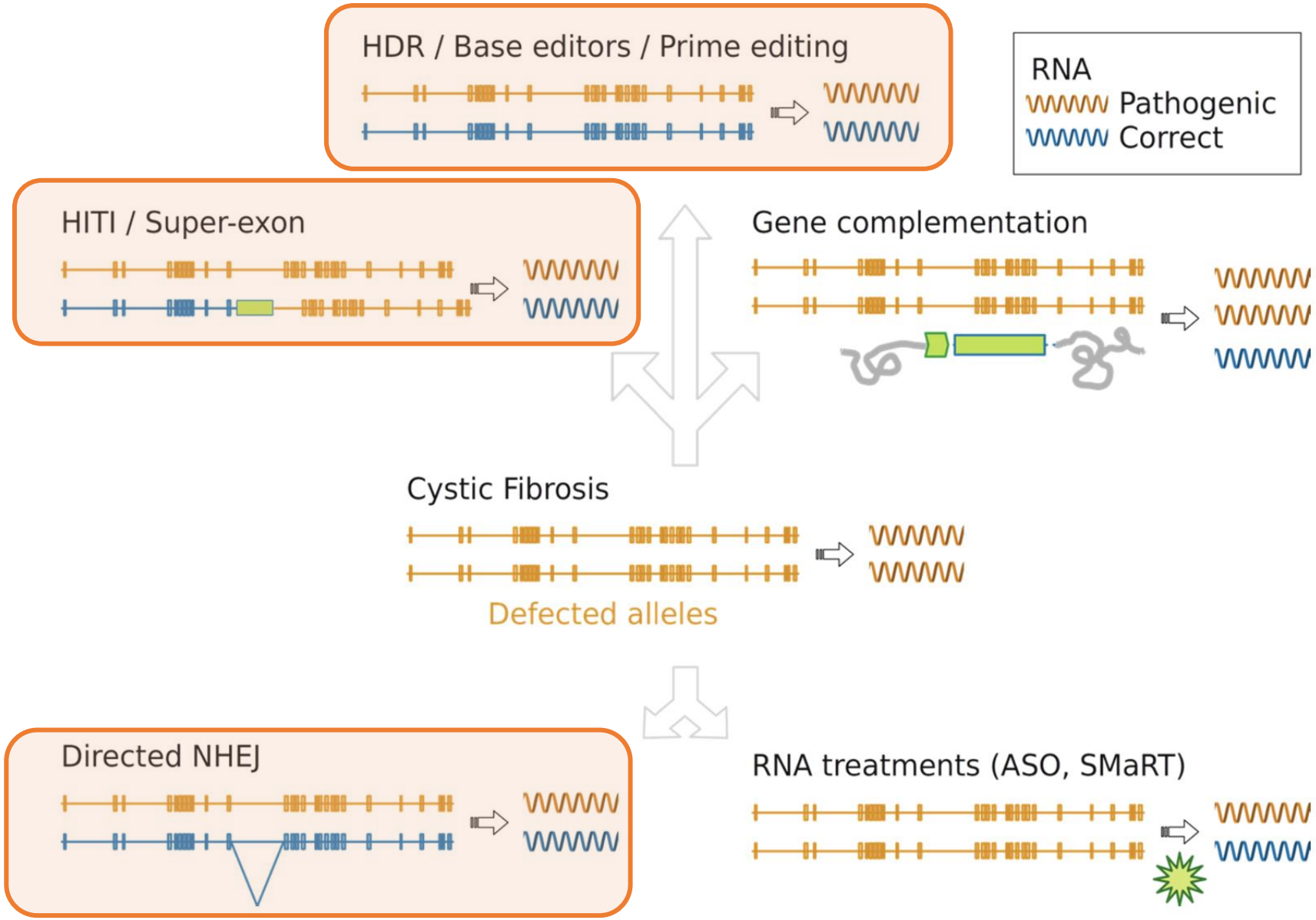
CRISPR Gold



Solid lipid nanoparticles

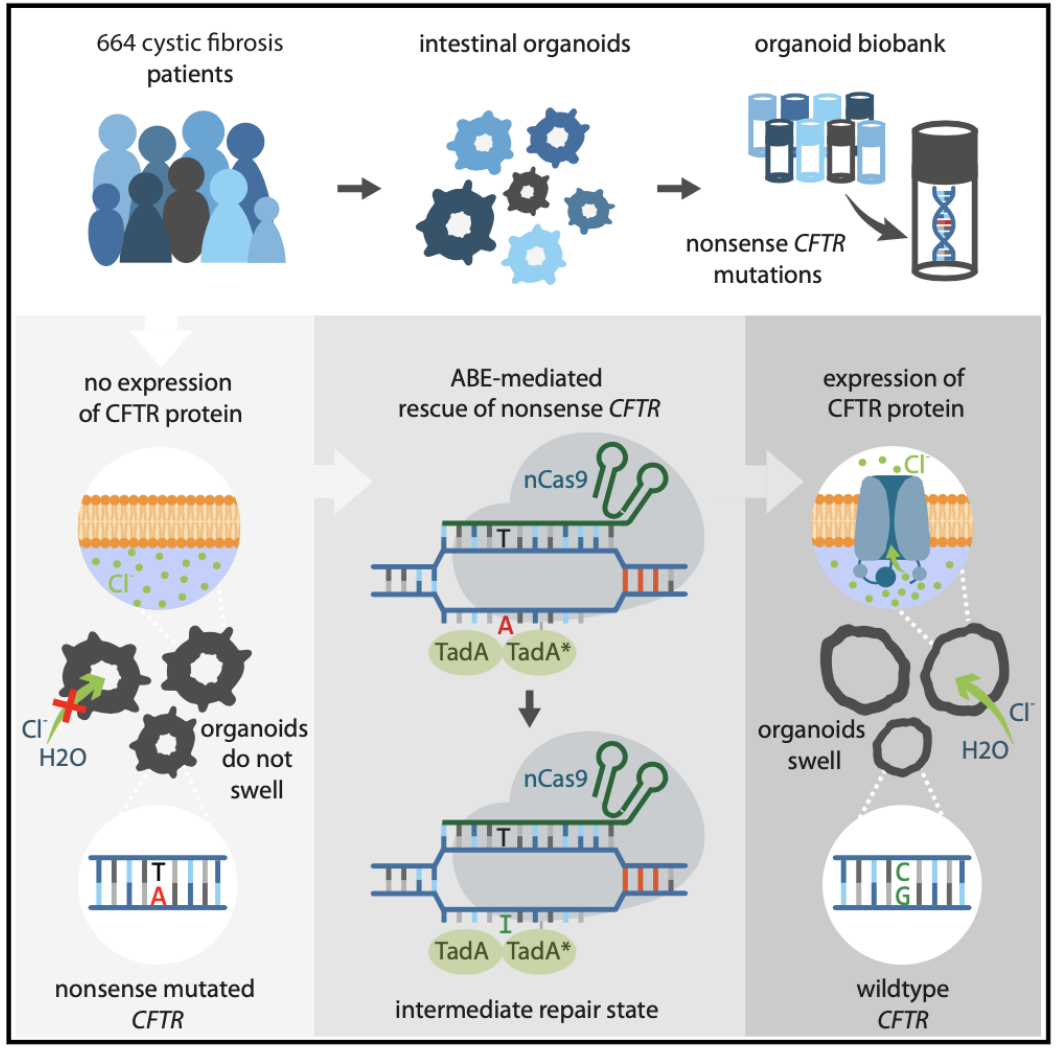
Nat Biotech, Haasteren et al 2020

Gene therapy for Cystic Fibrosis



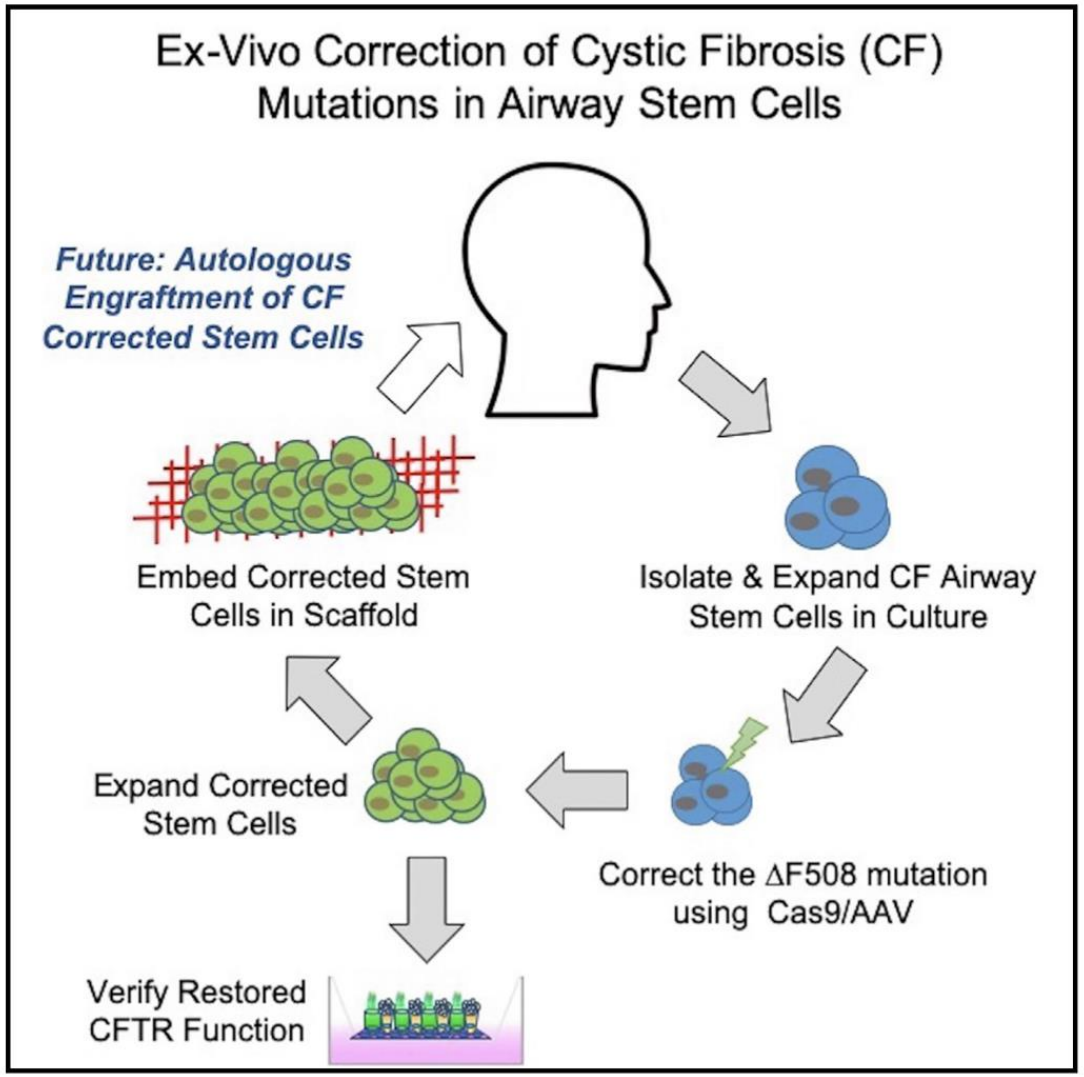
Maule, Arosio and Cereseto, IJMS 2020

Second generation CRISPR-Cas tools: Base-editors and prime editors



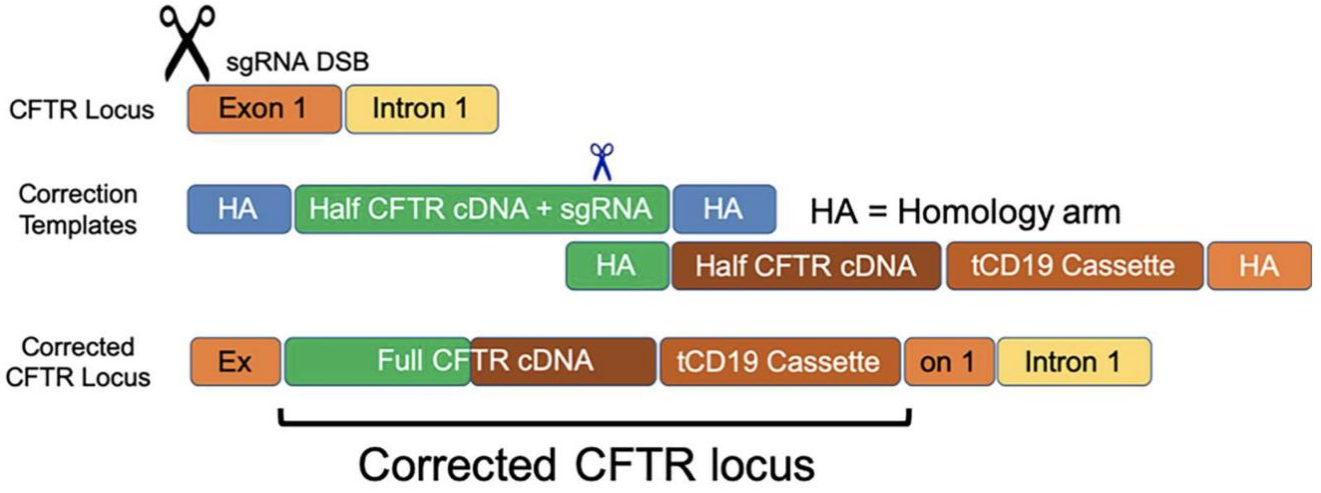
CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank- Cell Stem Cells, 2020-Hans Clevers

Ex-vivo gene replacement through CRISPR-Cas induced homologous recombination



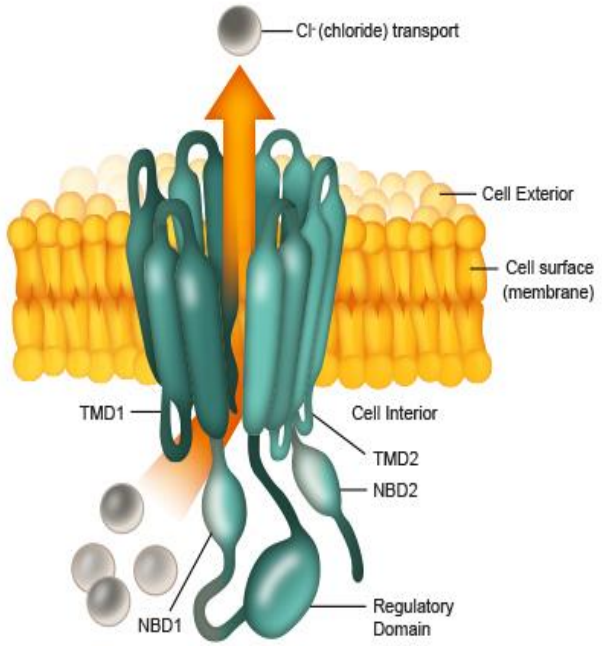
Targeted replacement of full-length CFTR in human airway stem cells by CRISPR/ Cas9 for pan-mutation correction in the endogenous locus – Mol Ther- 2021 M. Porteus

High-Efficiency, Selection-free Gene Repair in Airway Stem Cells from Cystic Fibrosis Patients Rescues CFTR Function in Differentiated Epithelia – Cell Stem Cell 2020 M Porteus



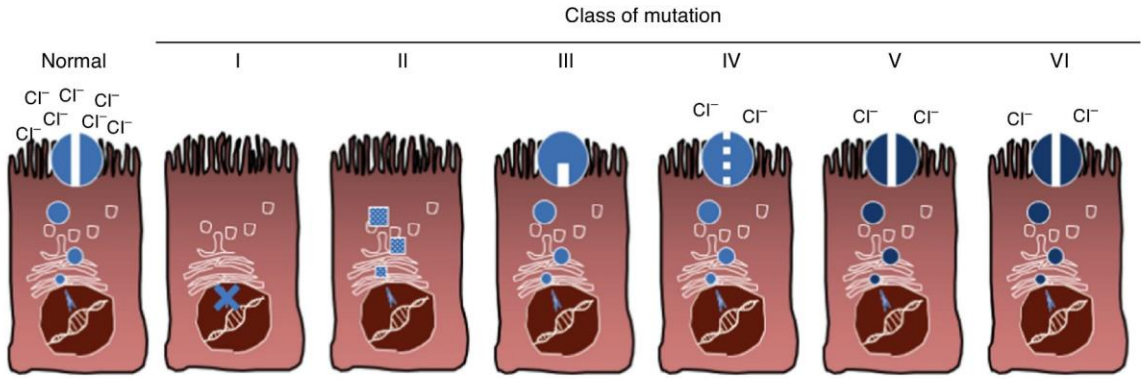
Correzione di una mutazione di splicing 3272-26A>G in CFTR tramite genome editing con CRISPR-Cas

Cystic fibrosis



***C**ystic*
***F**ibrosis*
***T**ransmembrane conductance*
***R**egulator*

- autosomal recessive monogenetic disease caused by mutations in the ***CFTR*** gene
- ABC transporter-class ion channel that conducts chloride ions across epithelial cell membranes



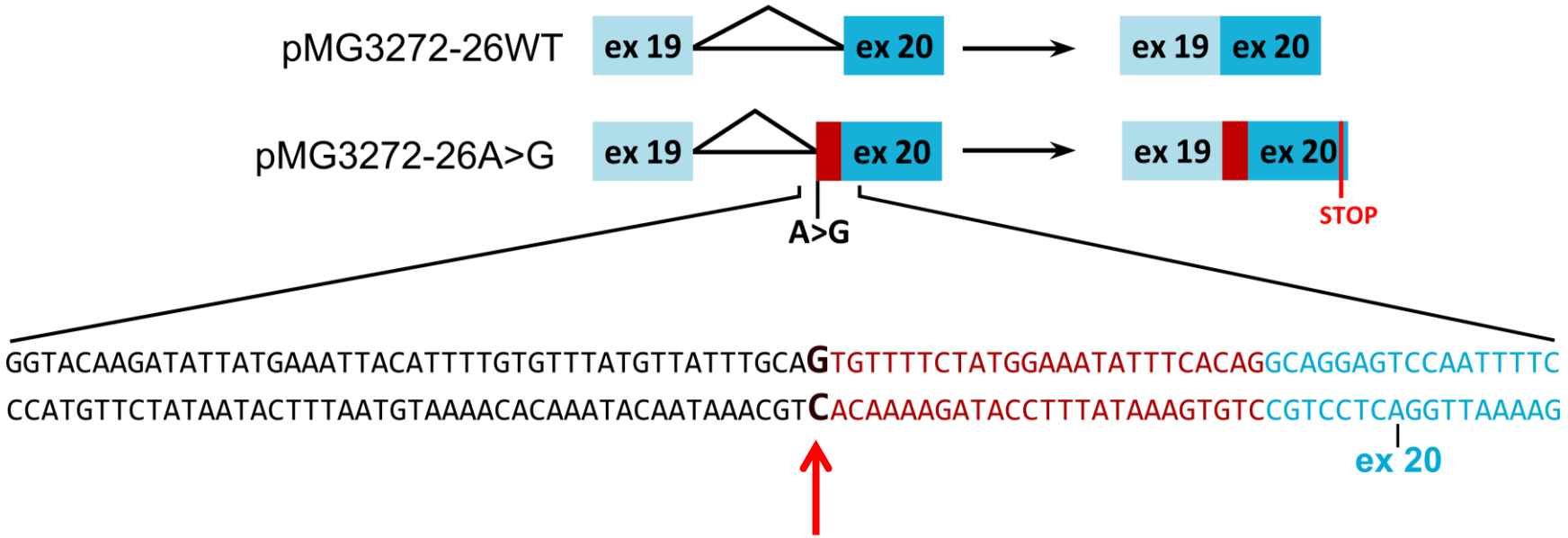
	Normal	I	II	III	IV	V	VI
Molecular defect		No synthesis	Block in processing	Block in regulation	Reduced conductance	Reduced synthesis	Reduced half-life
Functional abnormality		Protein is not synthesized	Folding defect	Channel opening defect	Ion transport defect	Decreased protein synthesis	Decreased half-life of the protein
Main mutations		Gly542X Trp128X Arg553X 621+1G→T	Phe508del Asn1303Lys Ile507del Arg560Thr	Gly551Asp Gly178Arg Gly551Ser Ser549Asn	Arg117His Arg347Pro Arg117Cys Arg334Trp	3849+10kbC→T 2789+5G→A 3272-26A →G 5T	4326delTC Gln1412X 4279insA

Splicing defects

From: labiotech.eu/galapagos-and-abbvie-expand-their-cystic-fibrosis-collaboration/

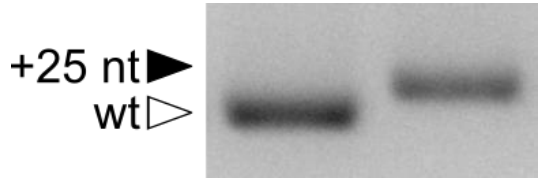
3272-26A>G CF splicing mutation

CFTR intron 19 MINIGENE models



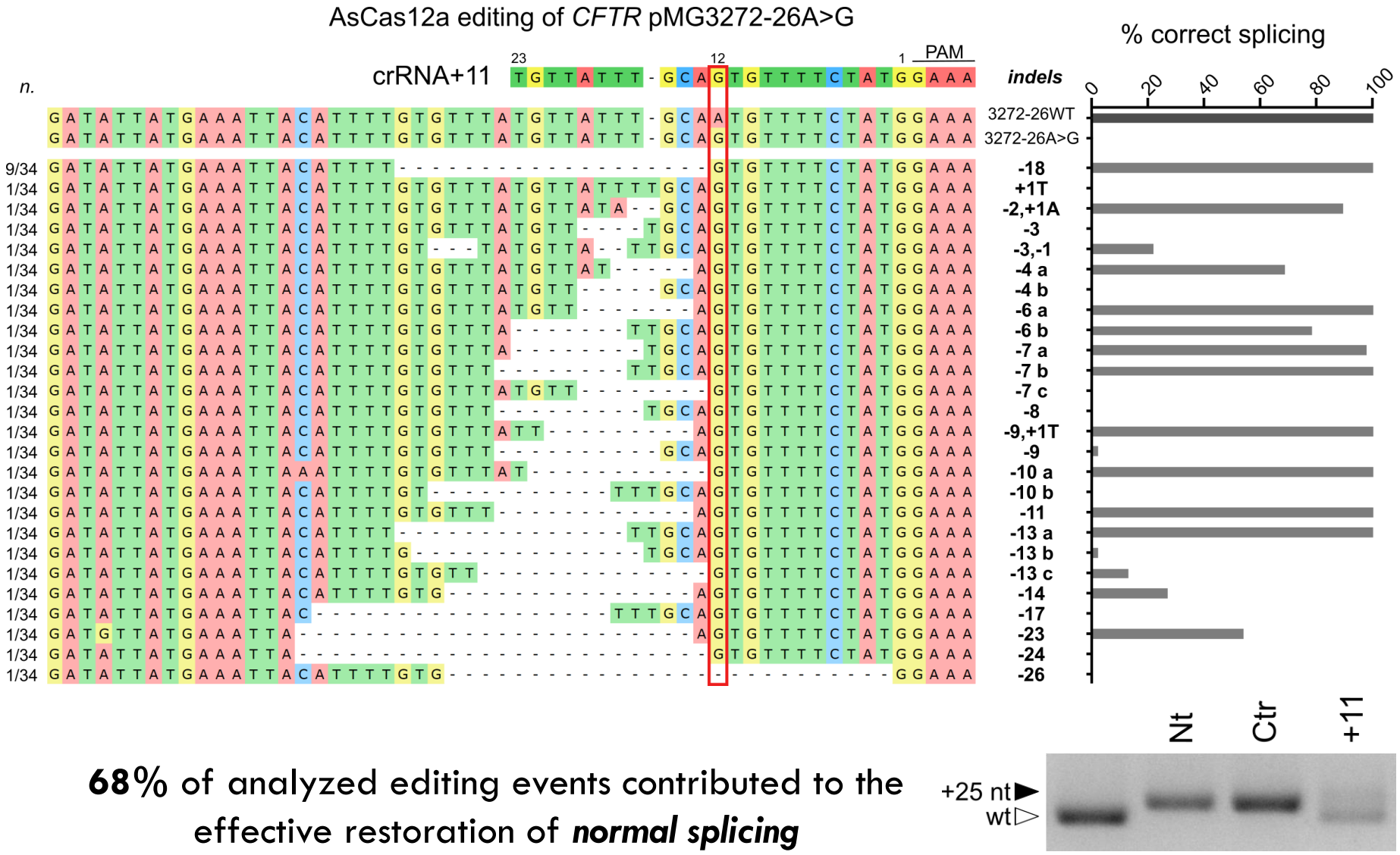
Alternative acceptor splice site

RNA splice products



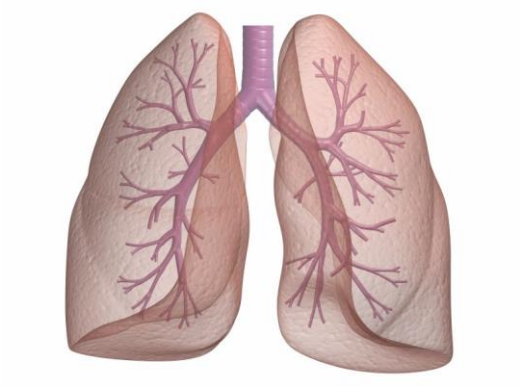
Maule et al, Nature Comm 2019

AsCas12a-crRNA +11 editing

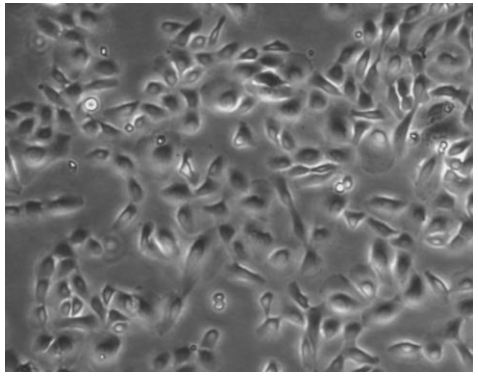


68% of analyzed editing events contributed to the effective restoration of ***normal splicing***

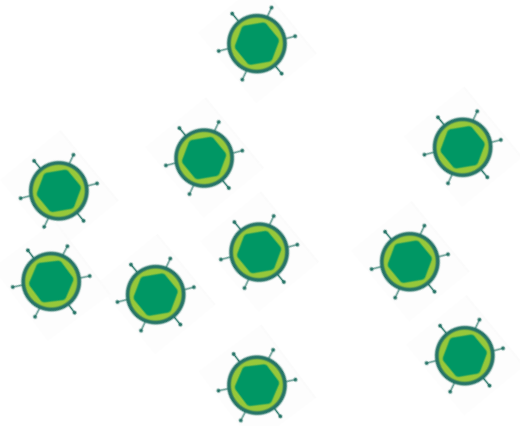
CFTR splicing repair in primary airway cells



CF patient

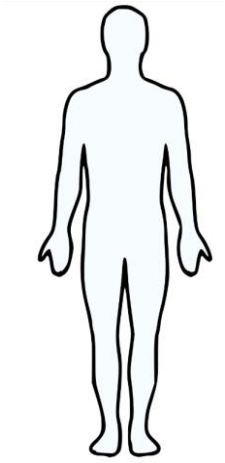


Primary
airway cells

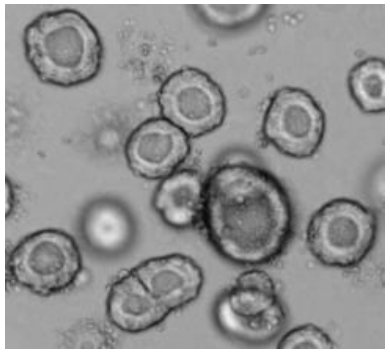


Lentiviral transduction with
CRISPR/AsCas12a
+ crRNA

…and in intestinal organoids

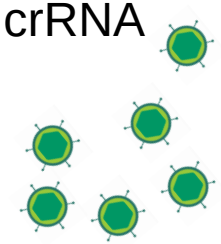


CF patient

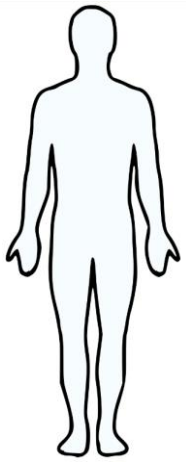


Intestinal organoids

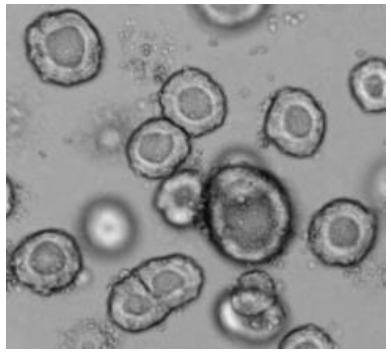
Transduction with CRISPR/AsCas12a + crRNA



Organoids experiments

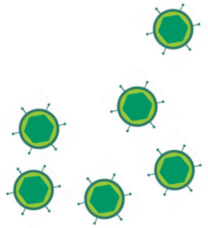


CF patient



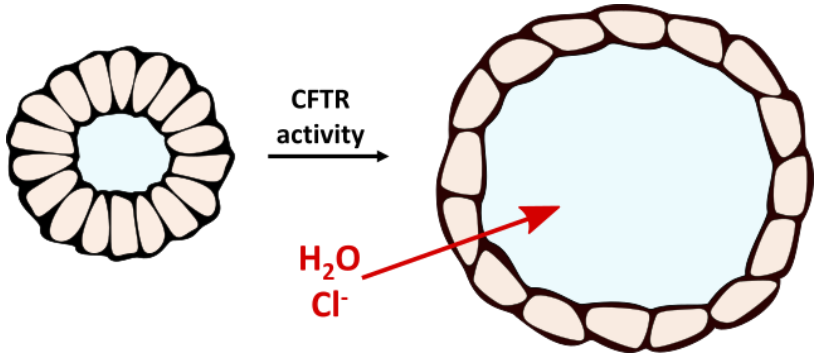
Intestinal organoids

Transduction with CRISPR/AsCas12a + crRNA

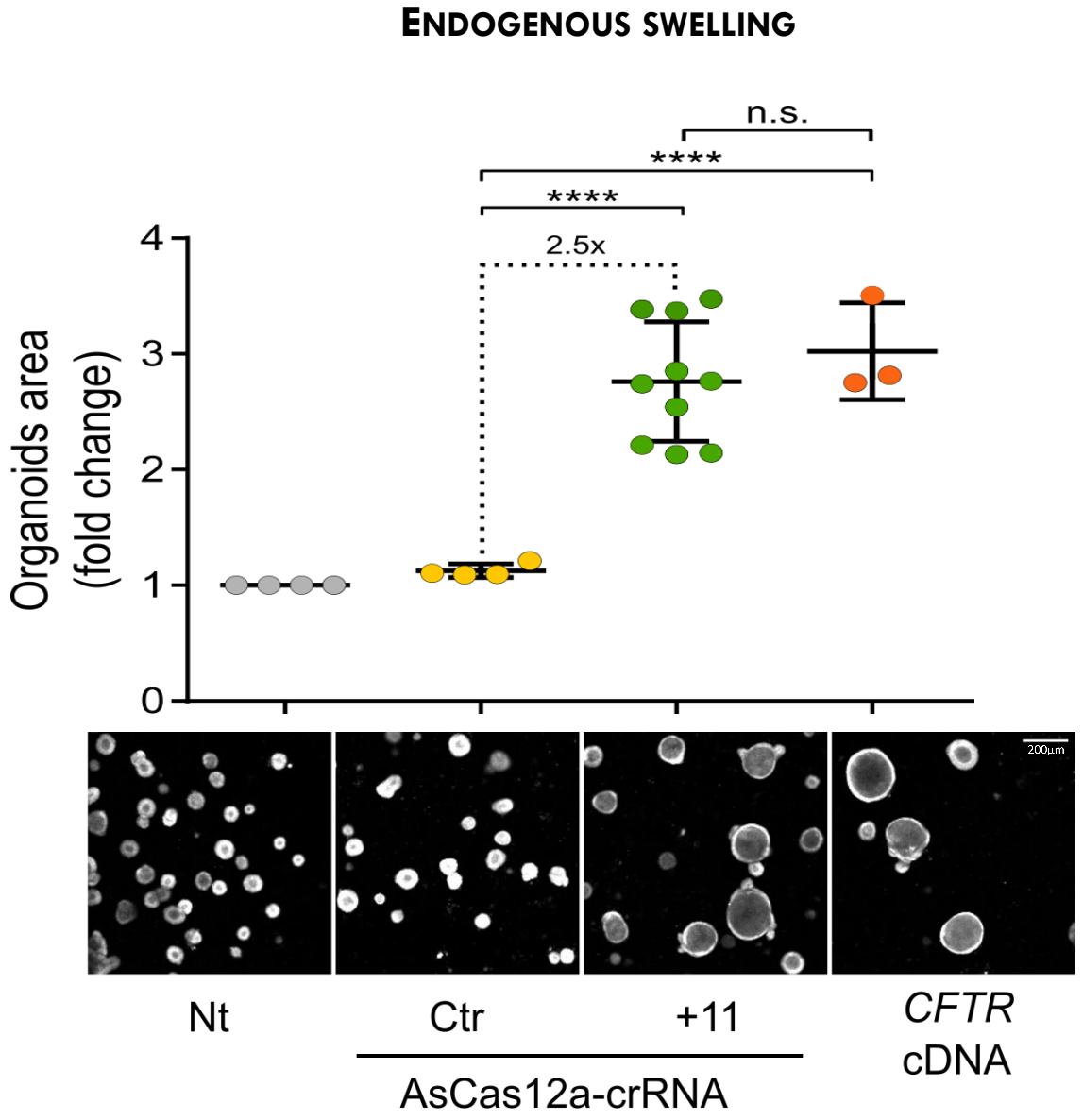


15 days

ORGANOIDS ENDOGENOUS SWELLING:
Measurements of **organoids area** as an evaluation of CFTR restored activity



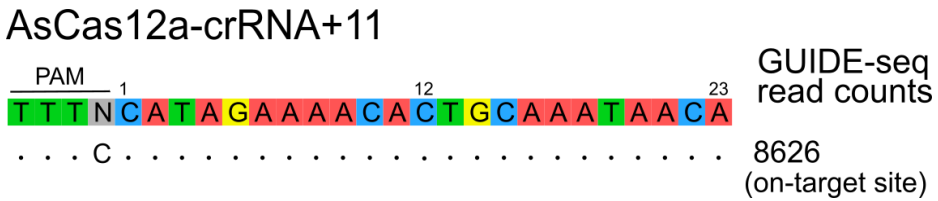
AsCas12a editing in 3242-26A>G organoids



**** P<0,0001

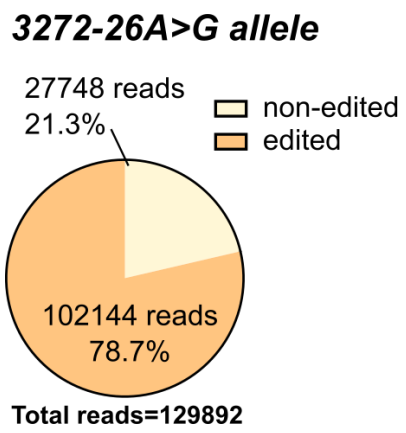
crRNA OFF target analysis

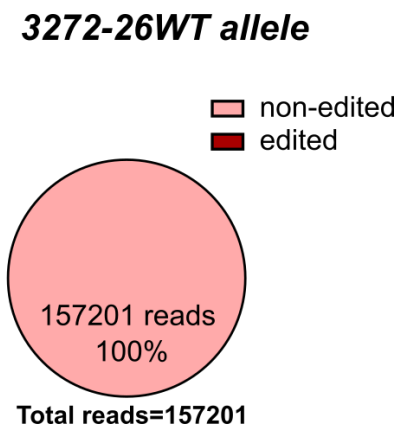
GUIDE-SEQ METHOD



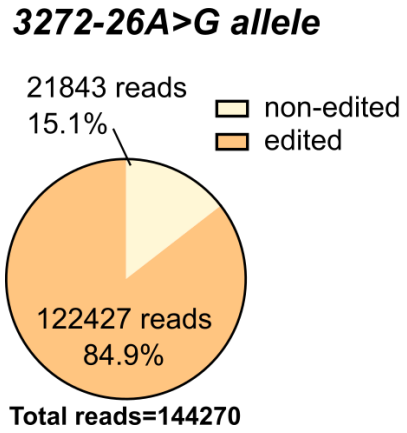
TARGETED DEEP-SEQUENCING

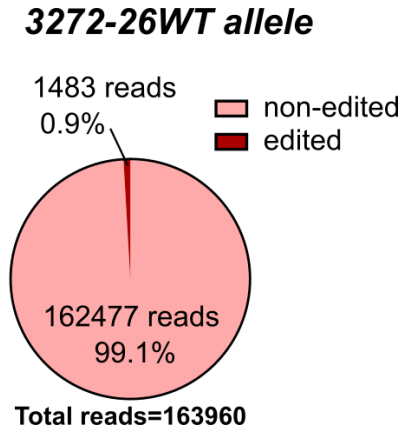
Primary airway cells





Organoids





Genome editing per fibrosi cistica. Dove siamo:

La tecnologia CRISPR-Cas permette di correggere in-vitro la maggioranza (tutte) le mutazioni del gene *CFTR*.

Il successo in clinica della tecnologia dipende da:

1) **DELIVERY**
2) Precisione
3) Accessibilità del locus in cellule primarie (a seconda delle mutazioni)

UNIVERSITY OF TRENTO - Italy
Department CIBIO

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italian cystic fibrosis research founda

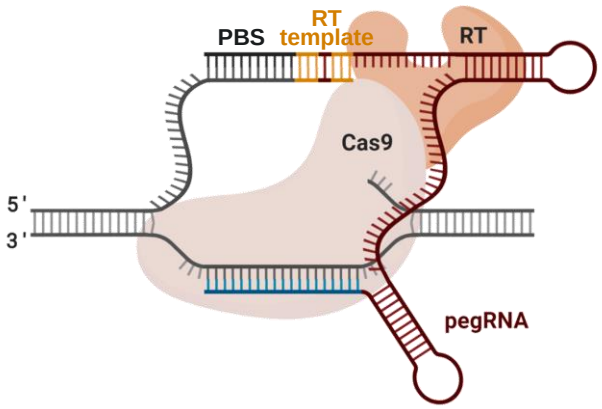
CYSTIC FIBROSIS FOUNDATION®

Horizon 2020

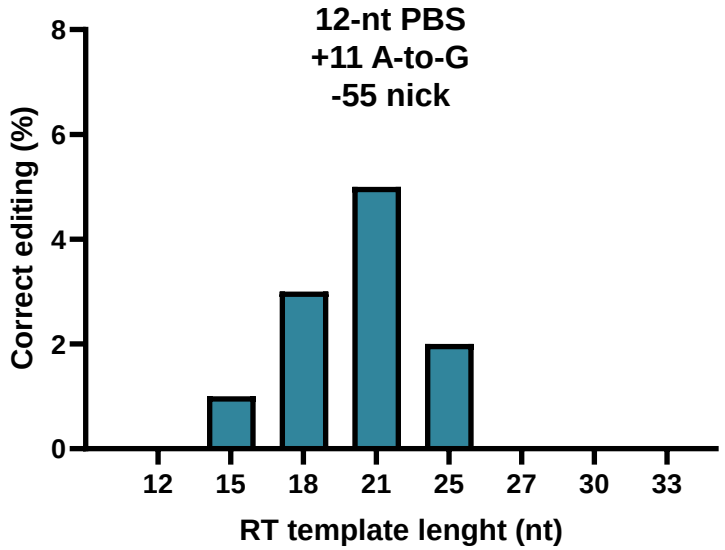
UPGRADE
Unlocking Precision Gene Therapy

FONDAZIONE CARITRO
CASSA DI RISPARMIO DI TRENTO E ROVERETO

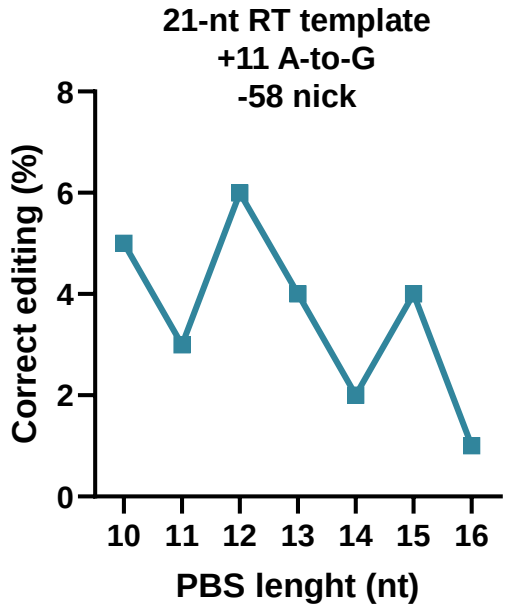
Prime editing: 2789+5G>A: pegRNA optimization

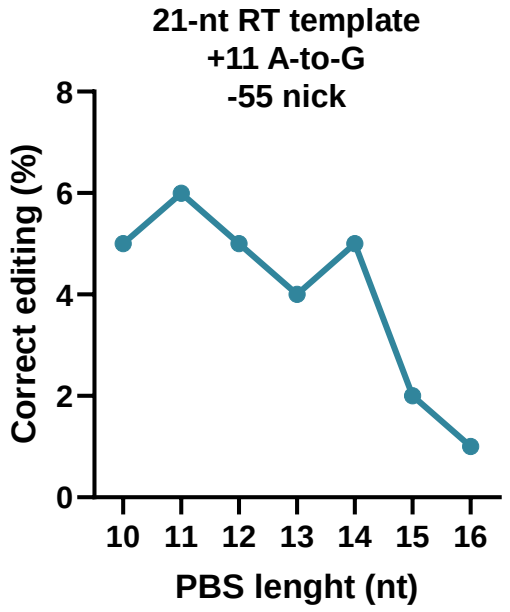


RT template length



PBS length

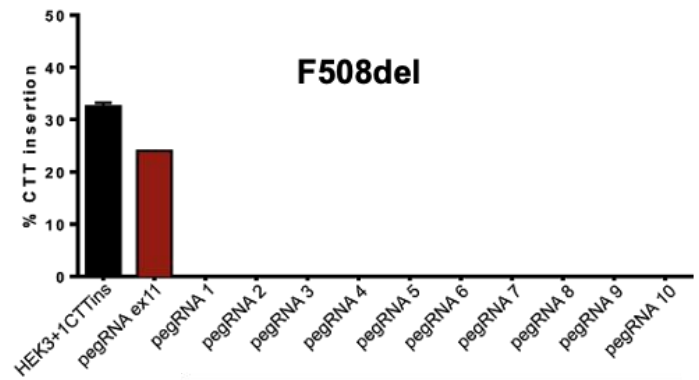




A

pegRNAs	spacer sequence	3' extension	gRNA for PE3 strategy
HEK3 +1CTTins	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	GTCAACCAGTATCCCGGTGC
pegRNA ex11	GGGAGAACTGGAGCCTTCAG	TTACCCTCTGAAGAAGGCTCCAGTTC	TGGAGATGTCCTCTTCTAGT
pegRNA1	TCTGTATCTATATTCATCAT	TCTTTGGTGTTTCCTATGATGAATATAG	CATTCTGTTCTCAGTTTTCC
pegRNA2	TCTGTATCTATATTCATCAT	TCTTTGGTGTTTCCTATGATGAATATAGAT	
pegRNA3	TCTGTATCTATATTCATCAT	TCTTTGGTGTTTCCTATGATGAATATAGATACA	
pegRNA4	TCTGTATCTATATTCATCAT	TCTTTGGTGTTTCCTATGATGAATATAGATACAGA	
pegRNA5	ACCATTAAAGAAAATATCAT	AGGAAACACCAAAGATGATATTTTCTT	TGGAGATGTCCTCTTCTAGT
pegRNA6	ACCATTAAAGAAAATATCAT	AGGAAACACCAAAGATGATATTTTCTTTA	
pegRNA7	ACCATTAAAGAAAATATCAT	AGGAAACACCAAAGATGATATTTTCTTTAAT	
pegRNA8	ACCATTAAAGAAAATATCAT	AGGAAACACCAAAGATGATATTTTCTTTAATGGT	
pegRNA9	CAGTTTTCCTGGATTATGCC	ACCAAAGATGATATTTTCTTTAATGGTGCCAGGCATAATCCAGGAAAAC	TGGAGATGTCCTCTTCTAGT
pegRNA10	CAGTTTTCCTGGATTATGCC	ACCAAAGATGATATTTTCTTTAATGGTGCCAGGCATAATCCAGGAAAACTG	

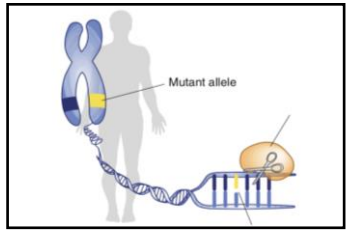
B



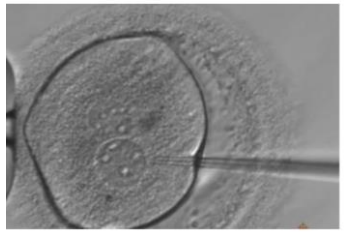
Use of CRISPR in biomedical field

Life science reserch
Genome editing for gene ko, screenings (drug), cell marking etc

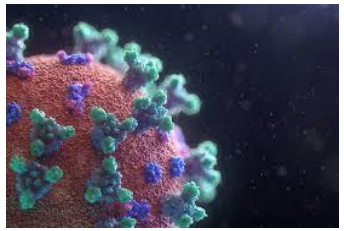
Animal model
Disease model to understand pathogenic mechanisms and therapeutic development.



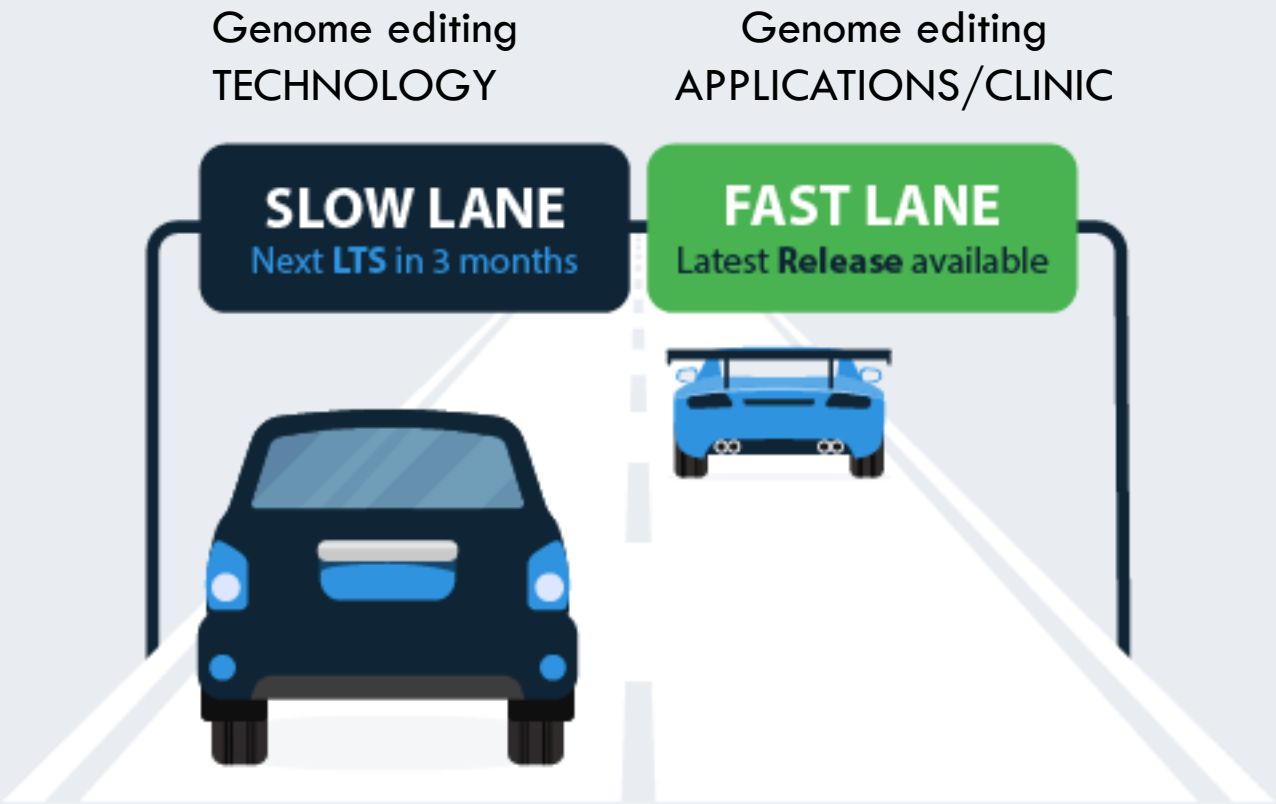
Gene therapy
Not just gene complementation but genes repair or ko of gain of function mutations



Embrio genome editing
Treatments of genetic disease



Virus detection and control
Identification and cleavage of viral nucleic acids or neutralization of viral co-factors (e.g. CCR5)


Genome editing
TECHNOLOGY
Genome editing
APPLICATIONS/CLINIC
SLOW LANE
Next LTS in 3 months
FAST LANE
Latest Release available

Use of CRISPR

De-extinction
Woolly mammoths (*Mammuthus primigenius*) to extinction.

Disease control
Disease resistance is one of the most popular applications for CRISPR help to stem the dramatic loss of honeybees.

Better food production
The US Food and Drug Administration approved the first transgenic animals for human consumption: fast-growing salmon.

Making drugs
chickens with components required for CRISPR integrated directly into their genomes (CRISPR chickens) to edit chicken DNA: 'farmaceuticals' — drugs created using domesticated animals.

Vector control
genetically modifying mosquitos to prevent the spread of diseases such as dengue or malaria.

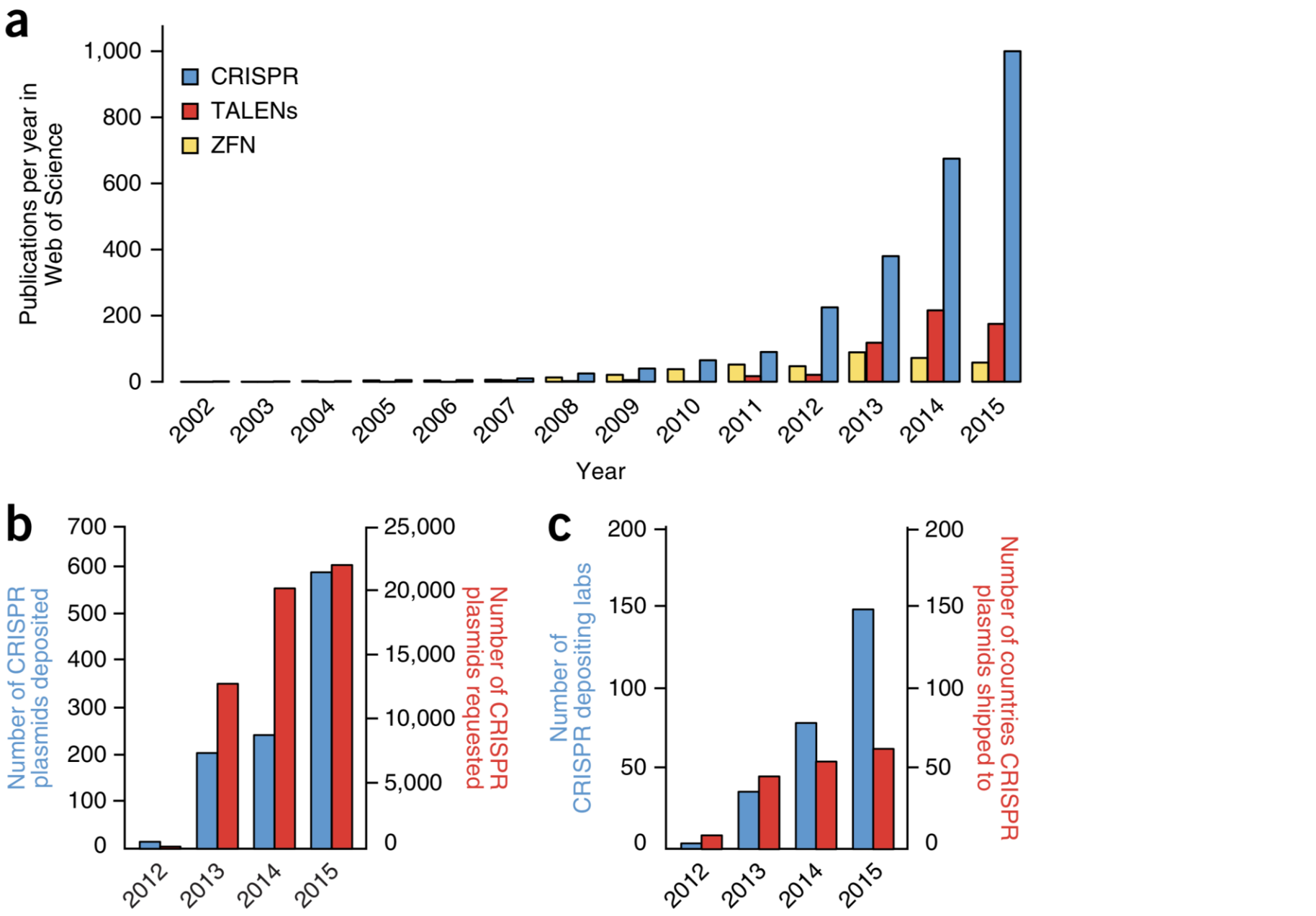
Take-home message

Genome editing for cystic fibrosis:

Technological opportunities through CRISPR-Cas:
the majority/all mutations can be corrected this technology

More advancements needed:
1) **DELIVERY**
2) Precision
3) Genomic locus accessibility

Genome-editing in the literature



R. Barrangou & J.A. Doudna Nature Biotech 2016