Gene Therapy for SCD: Webinar program for patients

webinar

PATIENTS

Session 5: Safety of CRISPR/Cas9

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Bar-Ilan University / University Medical Centre Freiburg

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ERN | EuroBloodNet

Disclosure for conflict of interest

Ayal Hendel: No conflict of interest

Toni Cathomen: No conflict of interest

What we'll talk about today

1

Recap of the previous webinar
An overview on gene editing for SCD using CRISPR.

2

Off-targets: what they are and why they happen
Overview on CRISPR off-targets.

3

How off-target activity affects cells
The effects off-target activity can have on cells and how it is detected.

4

Off-target effects and how to find them
How scientists detect and measure off-target effects at different levels.

5

Making gene editing more precise
How researchers develop improved gene editing platforms to reduce off-targets and improve safety.

6

Clinical guidelines for CRISPR precision
Guidelines established by regulatory agencies for CRISPR precision in the clinical setting.

01

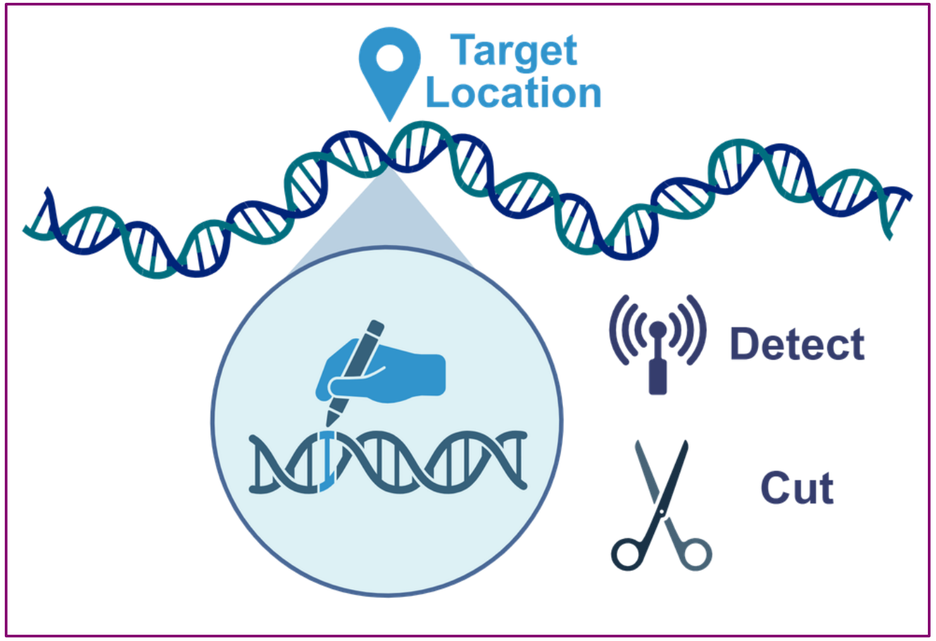
RECAP OF THE PREVIOUS WEBINAR

An overview on
genome editing for SCD using CRISPR.

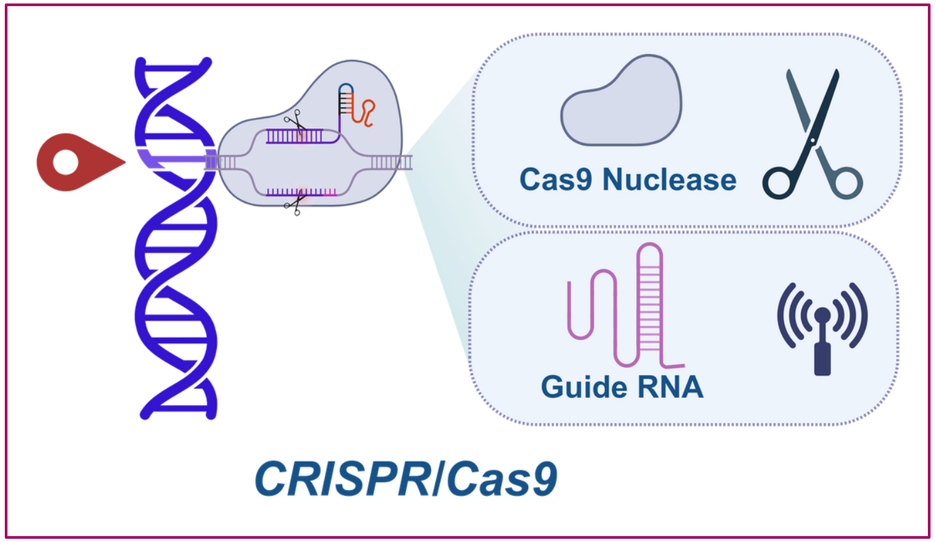
Toni Cathomen
University Medical Centre Freiburg

Gene Editing with CRISPR/Cas9

Gene editing addresses genetic mutations directly in the cell's DNA



Gene editing relies on CRISPR/Cas9
This "molecular scissor" cuts the DNA,
allowing scientists to edit the DNA sequence.



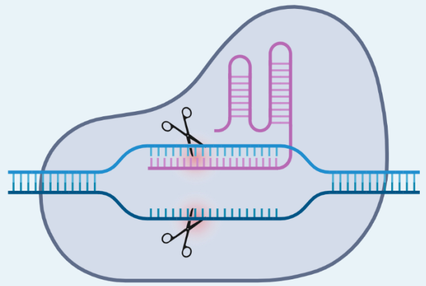
Gene editing addresses the genetic cause of the disease, trying to correct or by-pass a genetic mutation.

Base Editing can edit the DNA without cutting it

While this technology minimizes some of the problems associated with CRISPR-Cas9, it is <u>not</u> flawless.

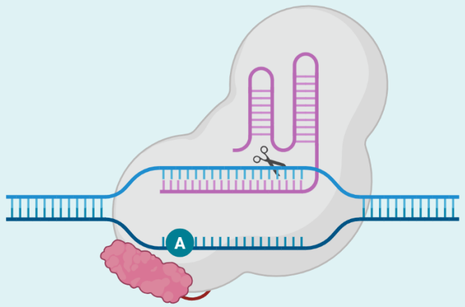
In both cases, assessing the potential risks is essential to ensure **safety of the product**.

CRISPR-Cas9



Molecular scissor
Cuts the DNA

Base Editing



Exchanges a single nucleotide
Does not cut the DNA

02

OFF-TARGETS
WHAT THEY ARE AND WHY THEY HAPPEN

Overview on CRISPR off-targets.

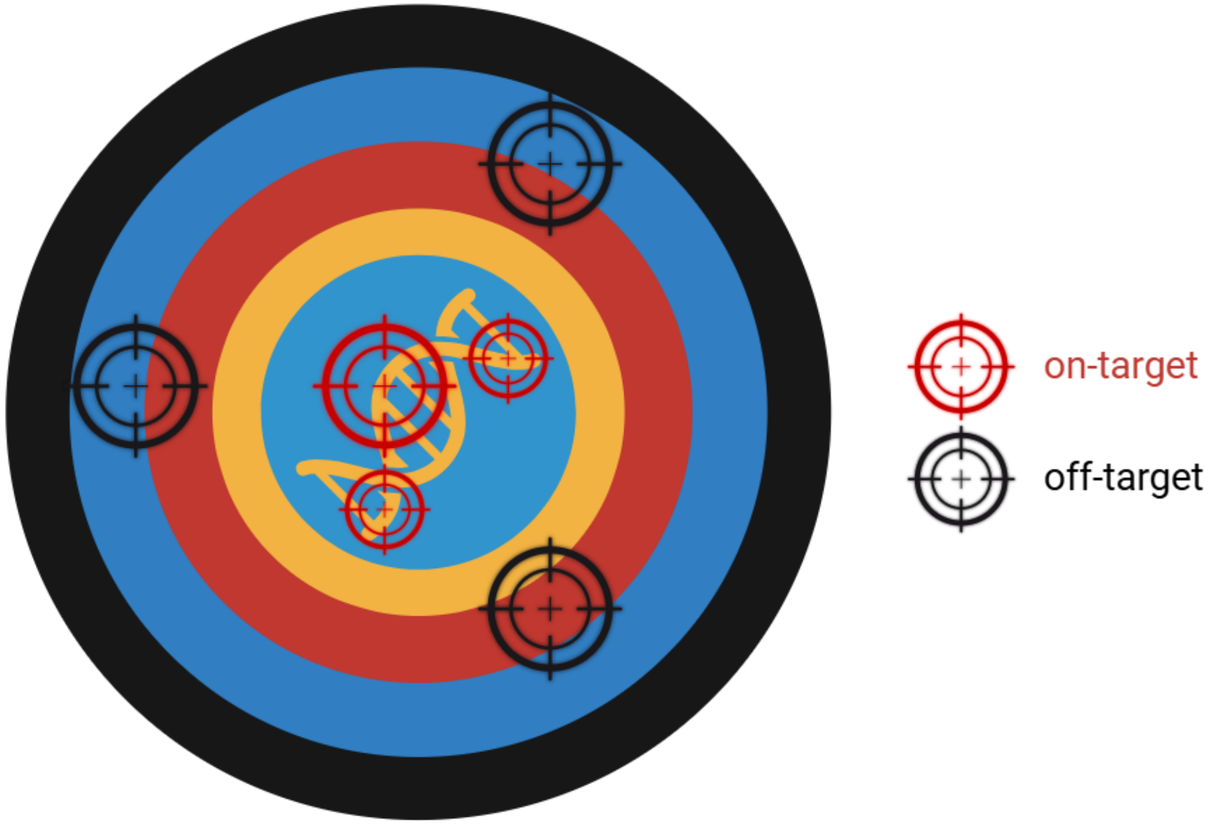
Ayal Hendel
Bar-Ilan University

What off-targets are

CRISPR/Cas9 can make mistakes

These mistakes are called off-target effects. All gene editing platforms can potentially produce off-target effects.

Detecting and measuring off-targets is essential to characterize the safety profile of a CRISPR therapy.



Why off-targets happen

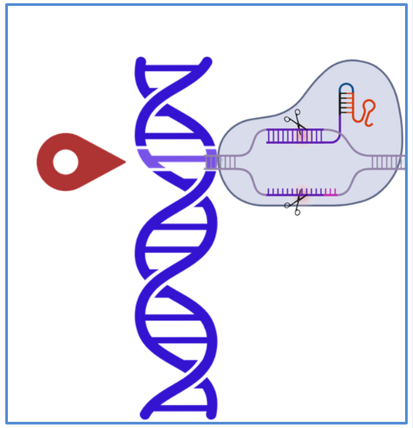
Off-targets occur when CRISPR cuts the wrong sequence

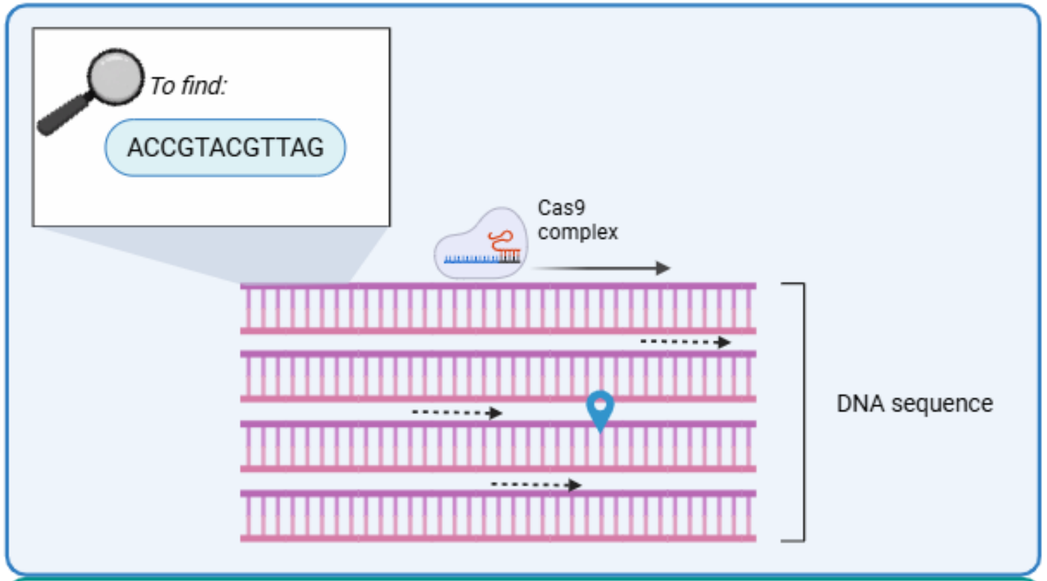
To cut the DNA, CRISPR acts like a GPS, searching for a specific DNA sequence like an “address”.

Even when the DNA is repaired, mistakes can happen, sometimes creating new problems that are more harmful than the original damage.

Especially when various double-strand breaks (DSBs) happen simultaneously, the wrong DNA fragments can be glued together creating chromosomal rearrangements and new, potentially dangerous genetic sequences.

However, DSB repair is very efficient and works properly in most cases.



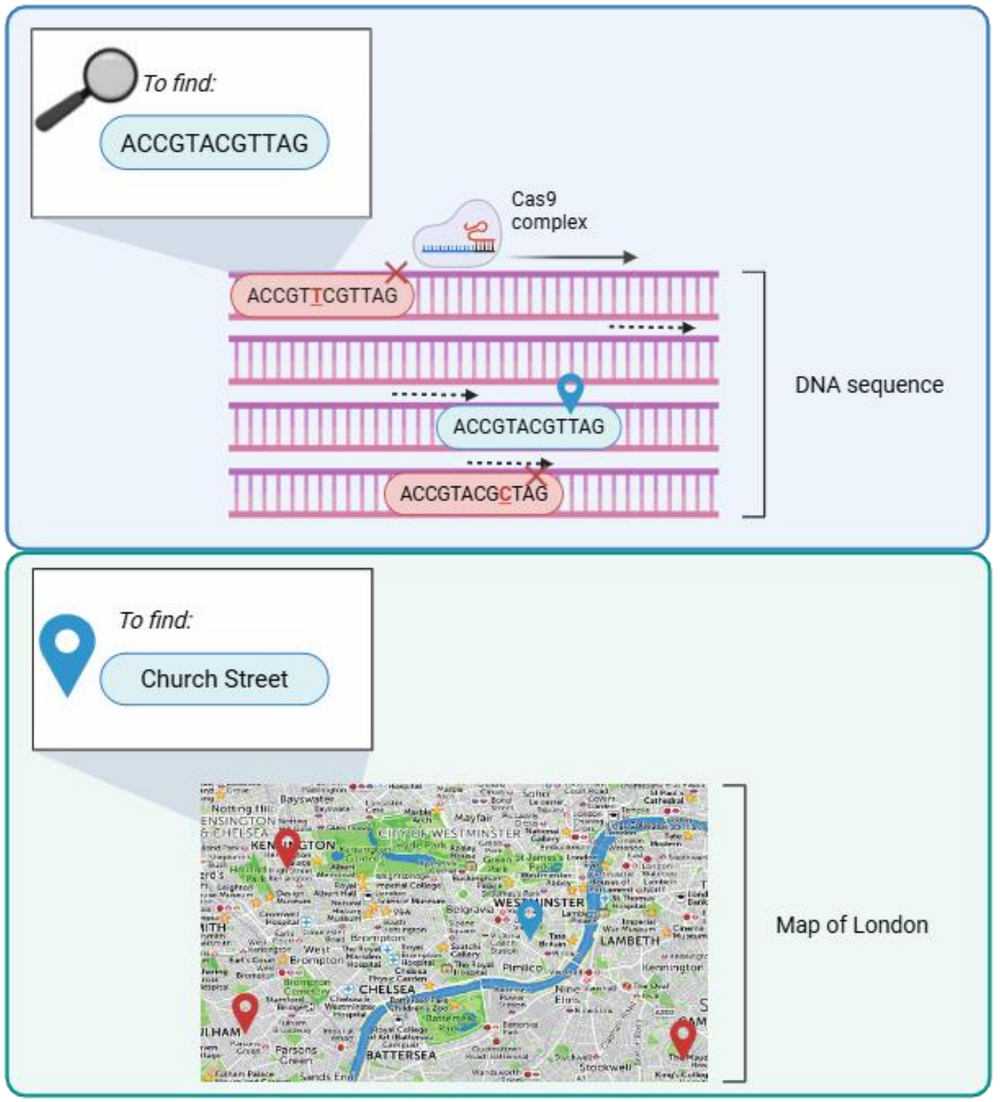


Why off-targets happen

DNA sequences can look very similar to each other

Just like a GPS can find streets with the same name in different towns, CRISPR can mistake a similar sequence for its target.

A target sequence for CRISPR is usually 20 bases long. Our genome contains 3.2 billion DNA bases. In this vast library, finding a 20-base sequence similar to our target DNA is quite probable.



03

HOW OFF-TARGET ACTIVITY AFFECTS CELLS

The effects of off-target activity on cells and how it is detected.

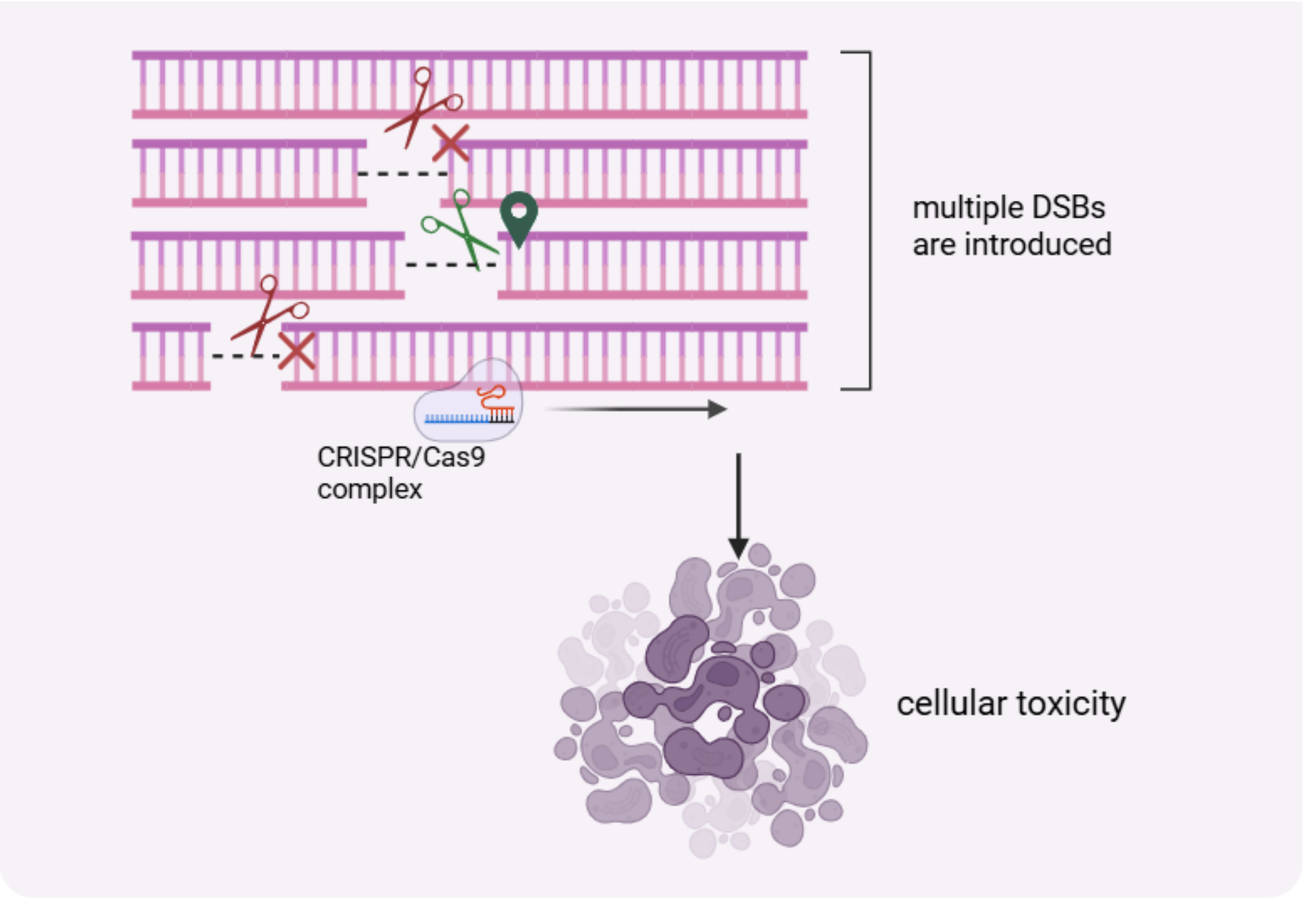
Toni Cathomen
University Medical Centre Freiburg

How off-target activity affects cells

Off-target activity leads to extra cuts in the cell's genome (the DNA)

DNA cuts (=DSBs) can be toxic for the cell and lead to cell death.

If not addressed, this issue could severely **limit CRISPR effectiveness** to treat SCD, as dying cells would reduce the chance of having enough cells for a transplant.



How off-target activity affects cells

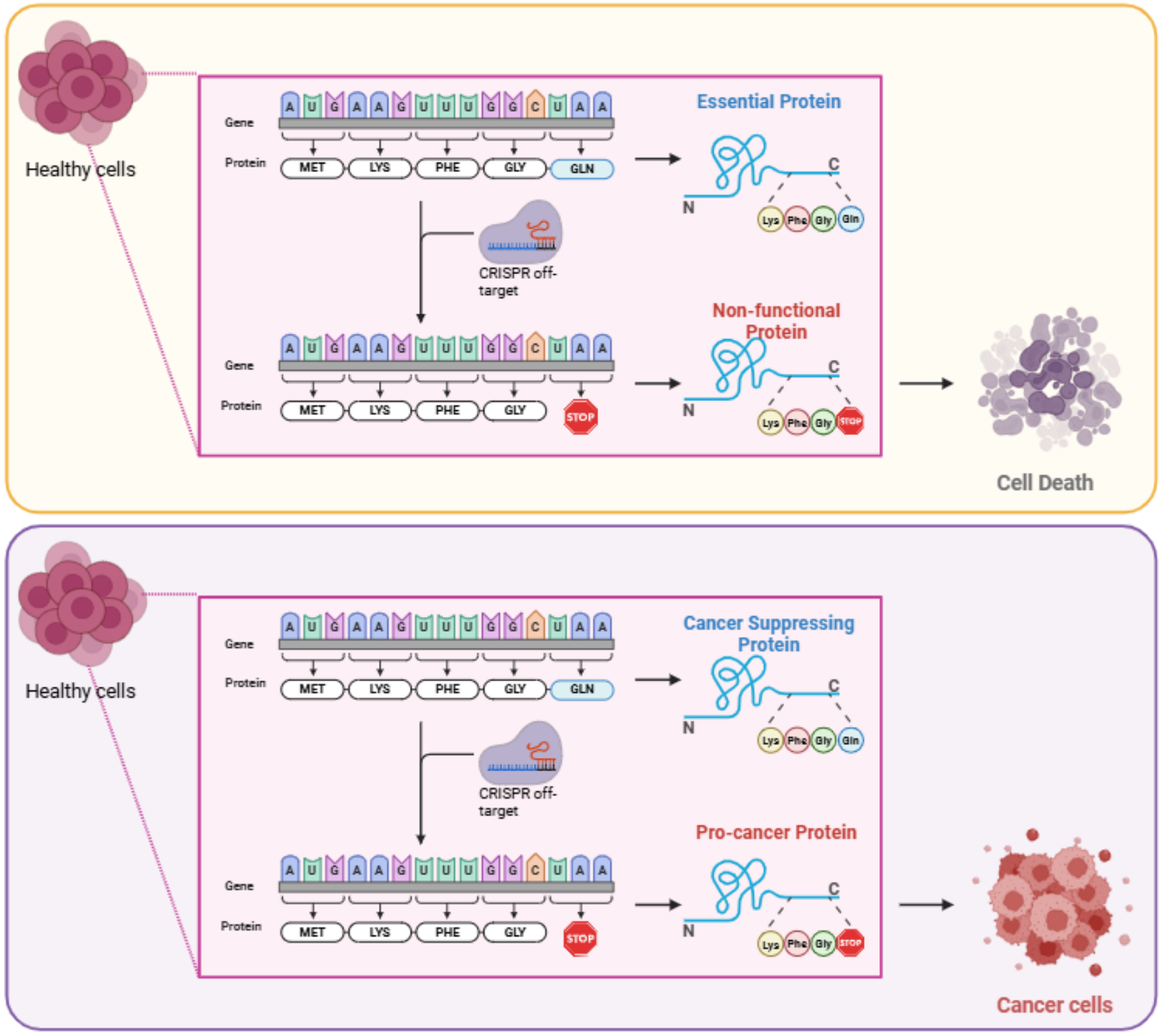
Off-target effects can affect genes that are key for cell survival. In that case, cells would die, so reducing the effectiveness of gene editing and the potential treatment.

Off-target activity can have pro-cancer effects

By disrupting one of the proteins that safeguard the cell, they could promote cancer development.

This concern is particularly important after some conventional gene therapy (not gene editing) approaches in the past caused cancer in some patients.

Importantly, no evidence of off-target effects leading to cancer have been found in any of the many clinical trials performed with CRISPR to date.

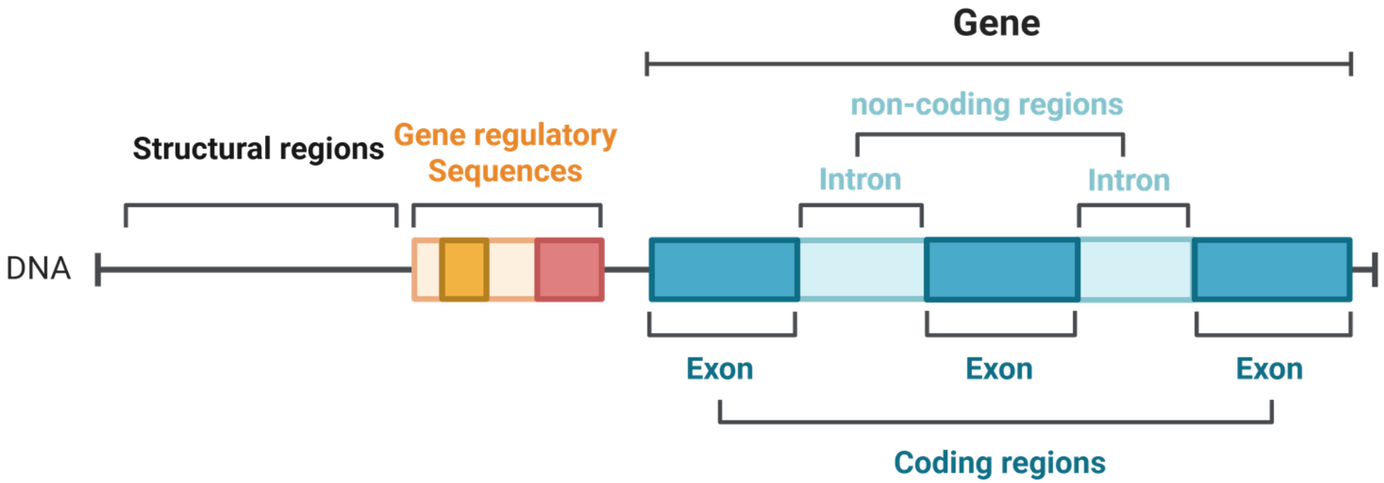


How off-target activity affects cells

Not all off-target effects affect protein production

Many regions in our genome (DNA) do not encode for genes. These regions are less sensitive to small mutations introduced by off-target activity.

On the other hand, many of these regions have important roles, like regulating gene expression. Thus, studying the potential off-targets in non-coding regions and what effects they could have is still important.



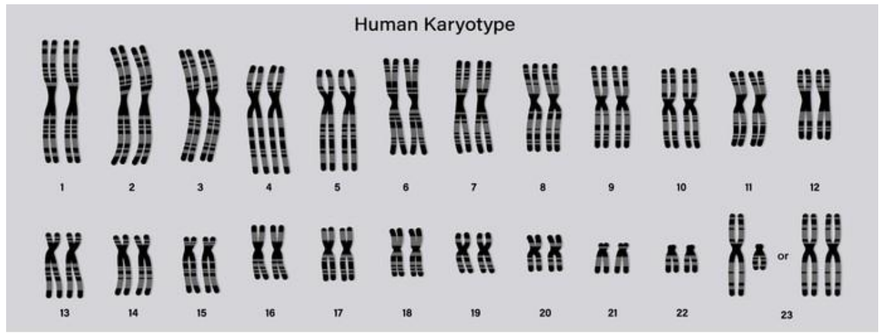
How off-target activity affects cells

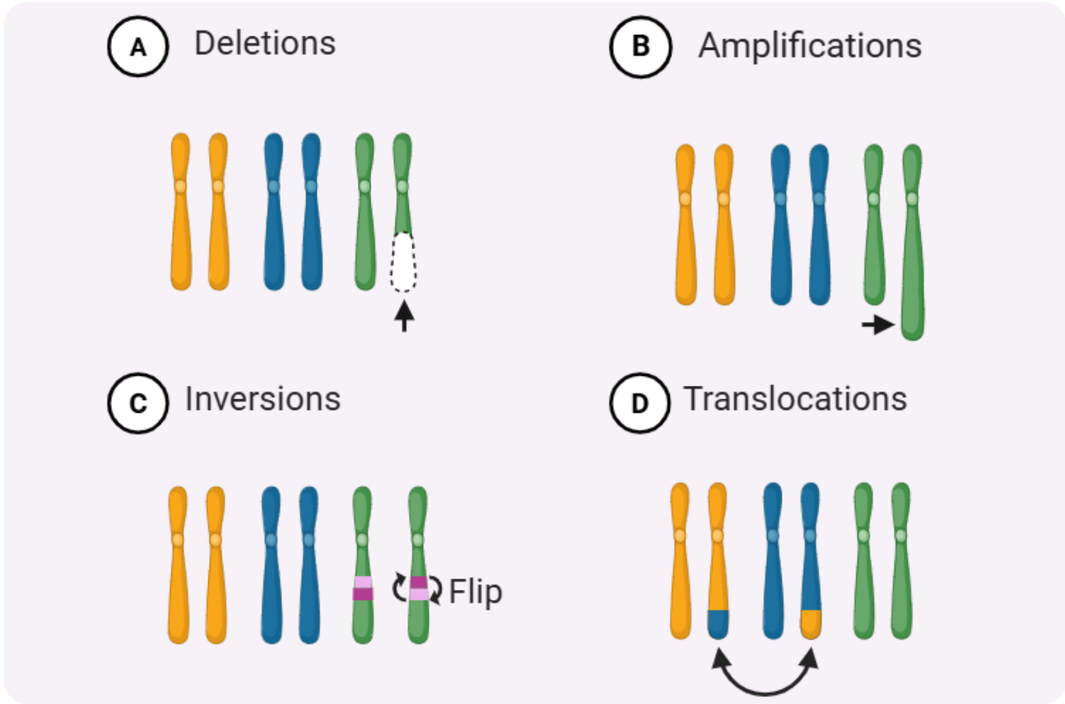
Off-target effects can alter the structure of the genome

Having multiple cuts on the DNA can lead to large rearrangements in chromosomes, the structures in which the DNA is packaged.

These major rearrangements can have severe effects, such as loss of essential genes, duplication of genetic sequences or formation of new, potentially **dangerous fusion genes**.

These effects are usually more drastic than single mutations, as they can affect hundreds of genes simultaneously. **Many cancers are caused by chromosomal rearrangements.**





04

OFF-TARGET EFFECTS AND HOW TO FIND THEM

How scientists detect and measure off-target effects at different levels.

Toni Cathomen
University Medical Centre Freiburg

Off-target effects and how to find them

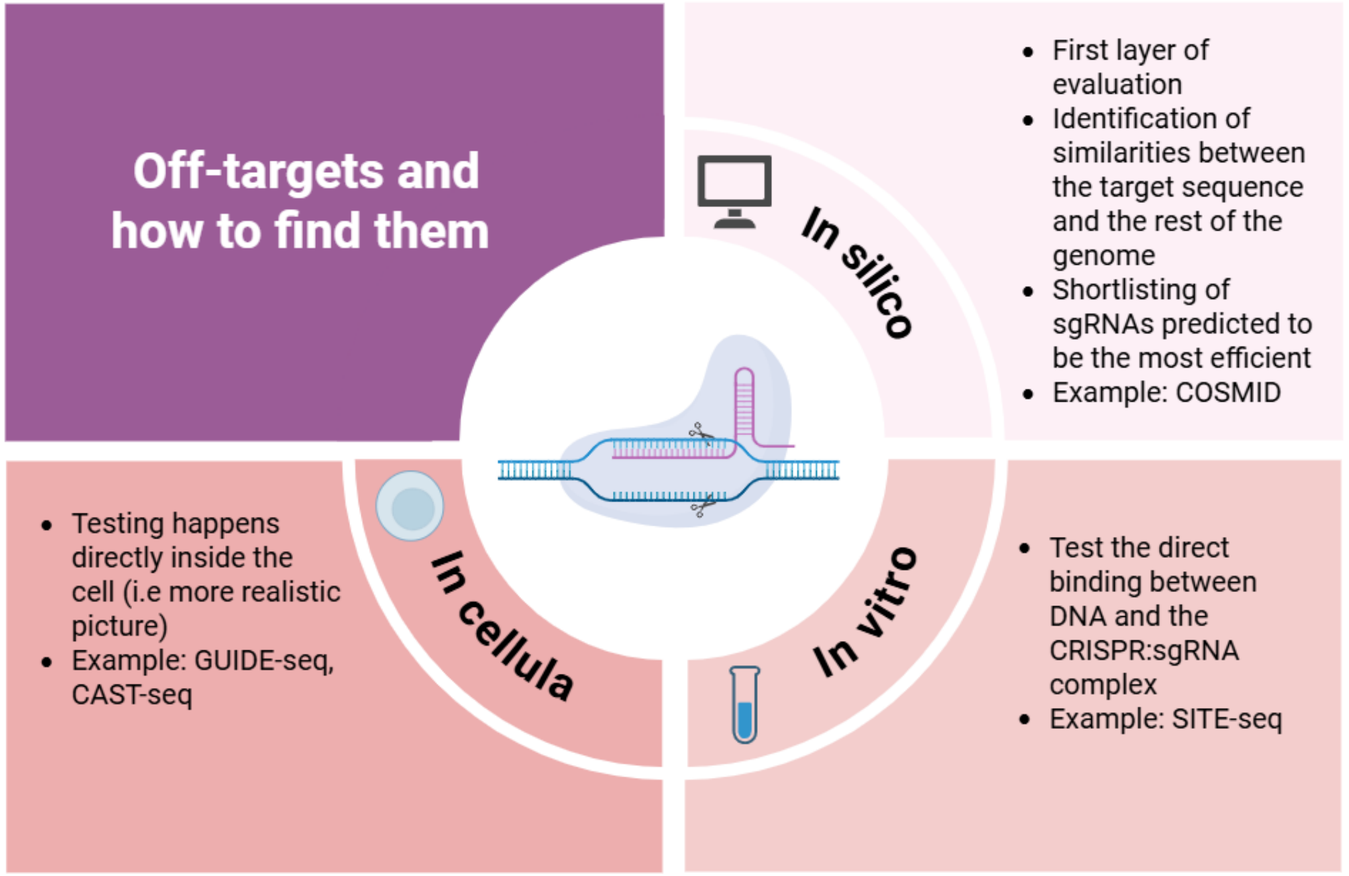
Scientists have developed many methods to detect and quantify off-target effects

Off-target assessment can be performed with different methods (different layers).

These various layers of control are essential to ensure that any off-target effect is caught at pre-clinical stages and never reach the patients.

These efforts aim to make **CRISPR products as safe as possible**, ensuring that any potential off-target effects are identified and quantified for a proper risk assessment.

These methods are a fantastic tool for designing **more specific CRISPR** strategies with fewer off-target effects.



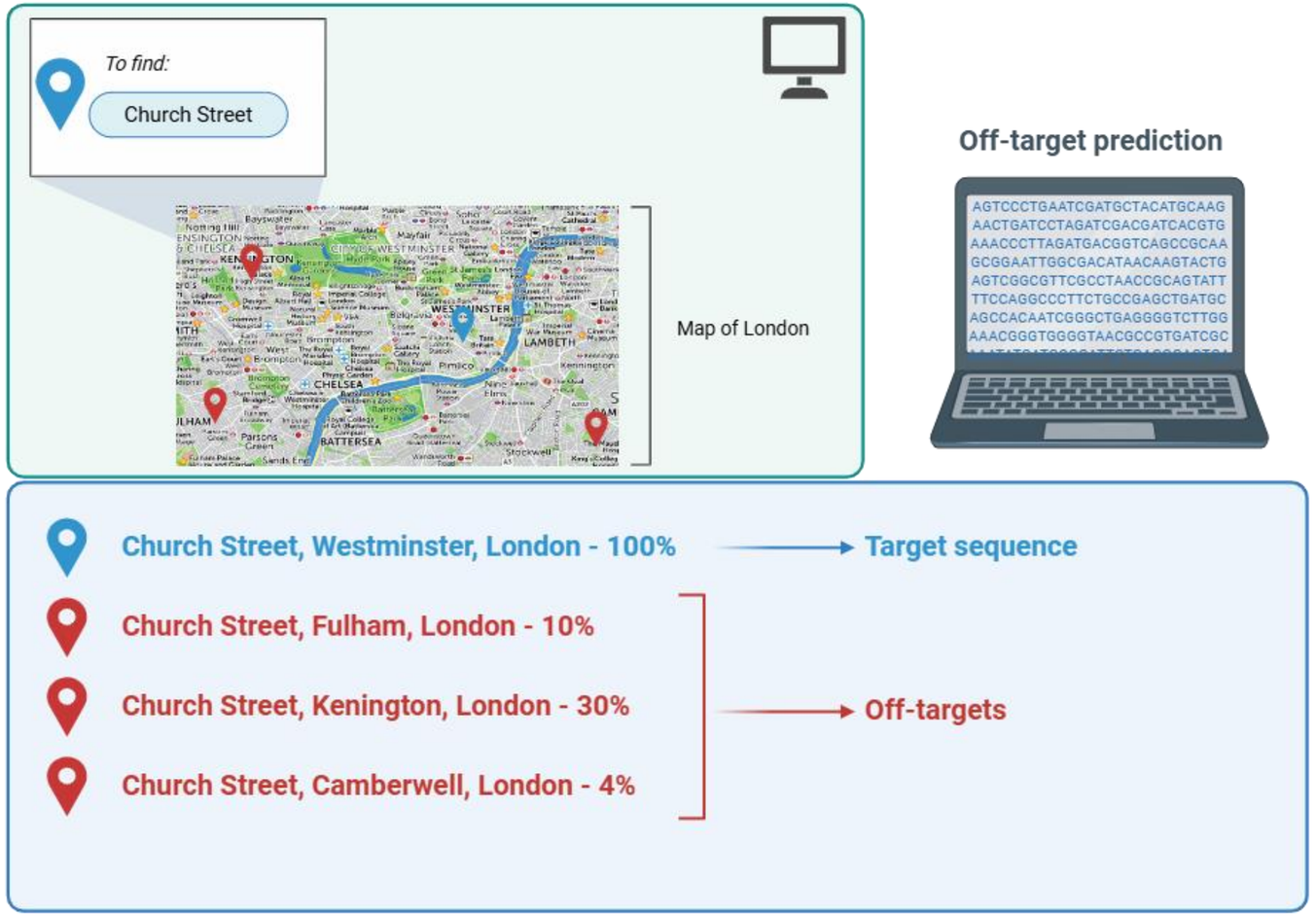
Off-target effects and how to find them

Off-target sites can be predicted using bioinformatics tools

Computer-based tools (such as CRISPRESSO) can predict locations in the DNA genome to which CRISPR editors can bind as well.

These tools are a great way to **predict potential off-target sites.** In a second step, the predicted off-target sites will be sequenced in the lab.

Furthermore, such predictions can help scientists to design guide RNAs with fewer potential off-target sites, so facilitating downstream processes.



Off-target effects and how to find them

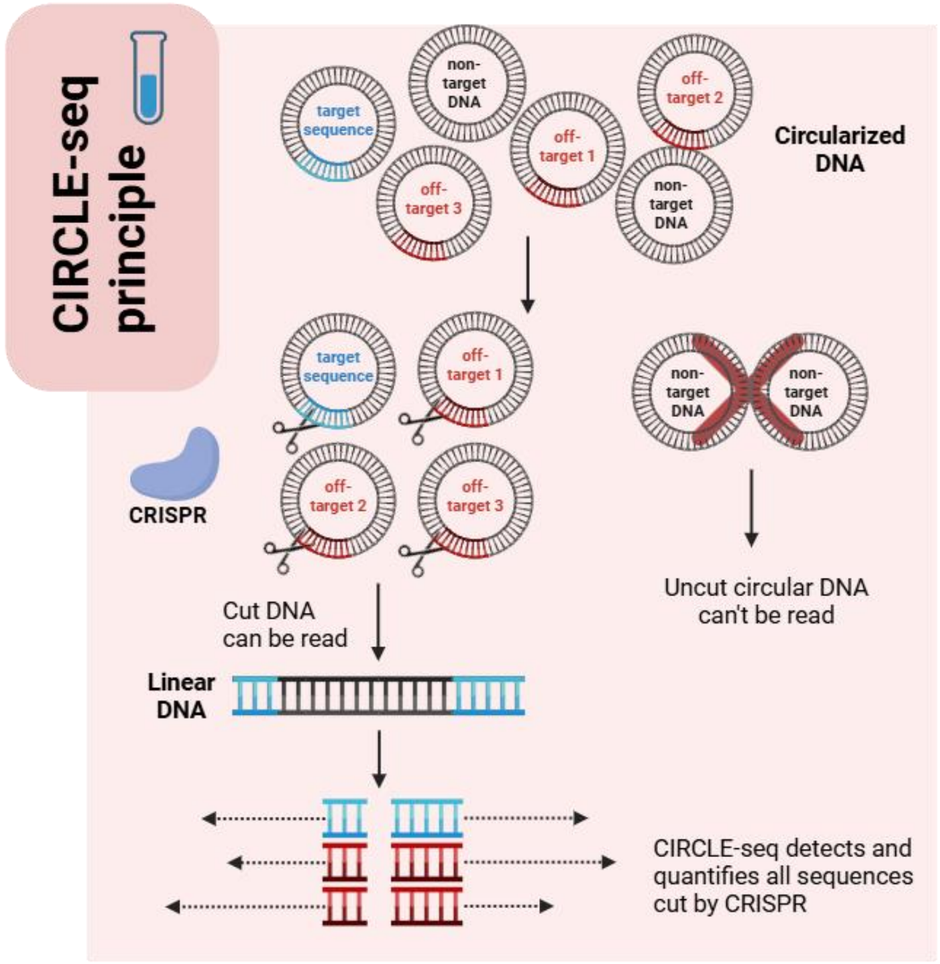
In vitro methods detect off-target sites in a test tube

For this, naked cellular DNA is exposed to CRISPR and the guide RNA, and any cuts are analyzed.

CIRCLE-seq is a commonly-used tool to identify off-target sites in a test tube.

(1) DNA from human cells is isolated, (2) broken down into fragments, (3) processed to small circular DNA molecules, (4) exposed to CRISPR/Cas (on- and off-target sites are cut), (5) and linearized circles will be read (sequenced) to identify the position of off-target sites.

This approach has a **high sensitivity**, allowing scientists to detect even extremely **rare potential off-targets**.



Off-target effects and how to find them

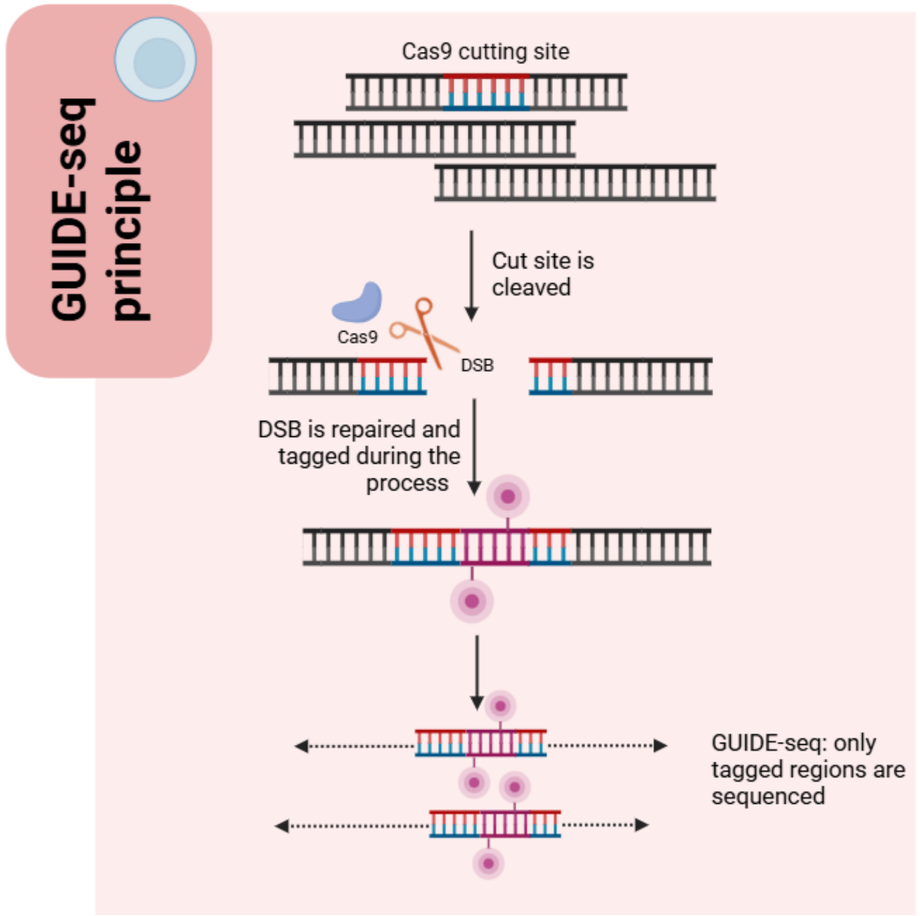
Cell-based methods identify off-target sites directly in relevant cell type

In these approaches, scientists introduce CRISPR and the guide RNA into cultured cells, then extract and study the cell's DNA to search for off-target sites.

GUIDE-seq is a commonly-used method to identify off-target sites directly in cells.

Aside from the CRISPR / guide RNA complex, scientists use a small **DNA tag** that inserts itself into the cut (DSB) made by CRISPR.

This tag acts like a flag, allowing scientists to identify the location of these cuts with high precision.



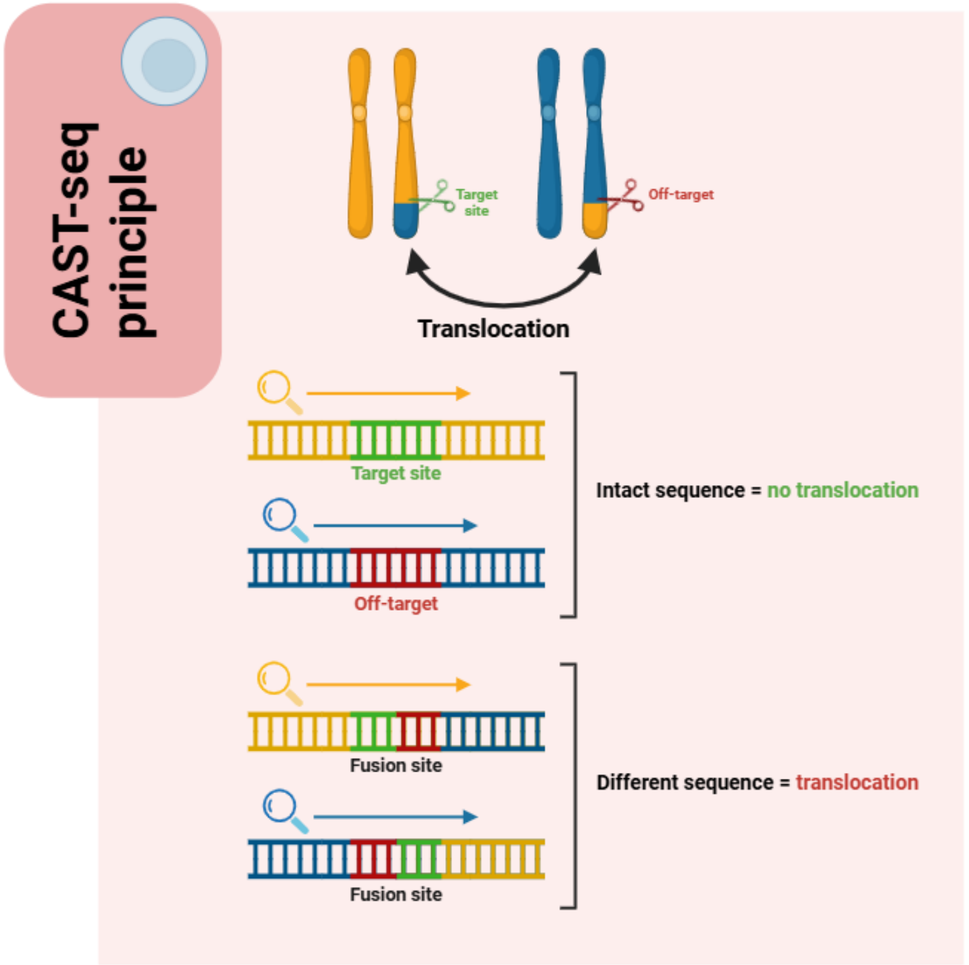
Off-target effects and how to find them

Cell-based methods to detect chromosomal rearrangements

In CAST-seq, scientists probe the DNA in close proximity of the target site. This allows them to detect aberrant sequences resulting from chromosomal translocations.

CAST-seq uses the sequences surrounding the target cut sites as a “bait” to fish out any potential translocations. This is done by reading the DNA sequence that has been fused to the target site, allowing the scientists to identify the location where the DNA originally came from.

This approach is essential to inform about **large-scale effects** of off-target CRISPR cutting.



05

MAKING GENE EDITING MORE PRECISE

How researchers develop improved gene editing platforms to reduce off-targets and improve safety.

Ayal Hendel
Bar-Ilan University

Evolving CRISPR to make it safer

Scientists can evolve the CRISPR system to make it safer

Through this process, they can tweak how the Cas9 protein works to improve its specificity. This reduces the potential for off-targets and makes CRISPR approaches safer.

However, improving Cas9 is not the only option to make CRISPR safer: other improvements such as using Cas9 orthologs, improving guide design or using nickase pairs, can also improve CRISPR safety.

In the next slides we'll explore these various strategies.

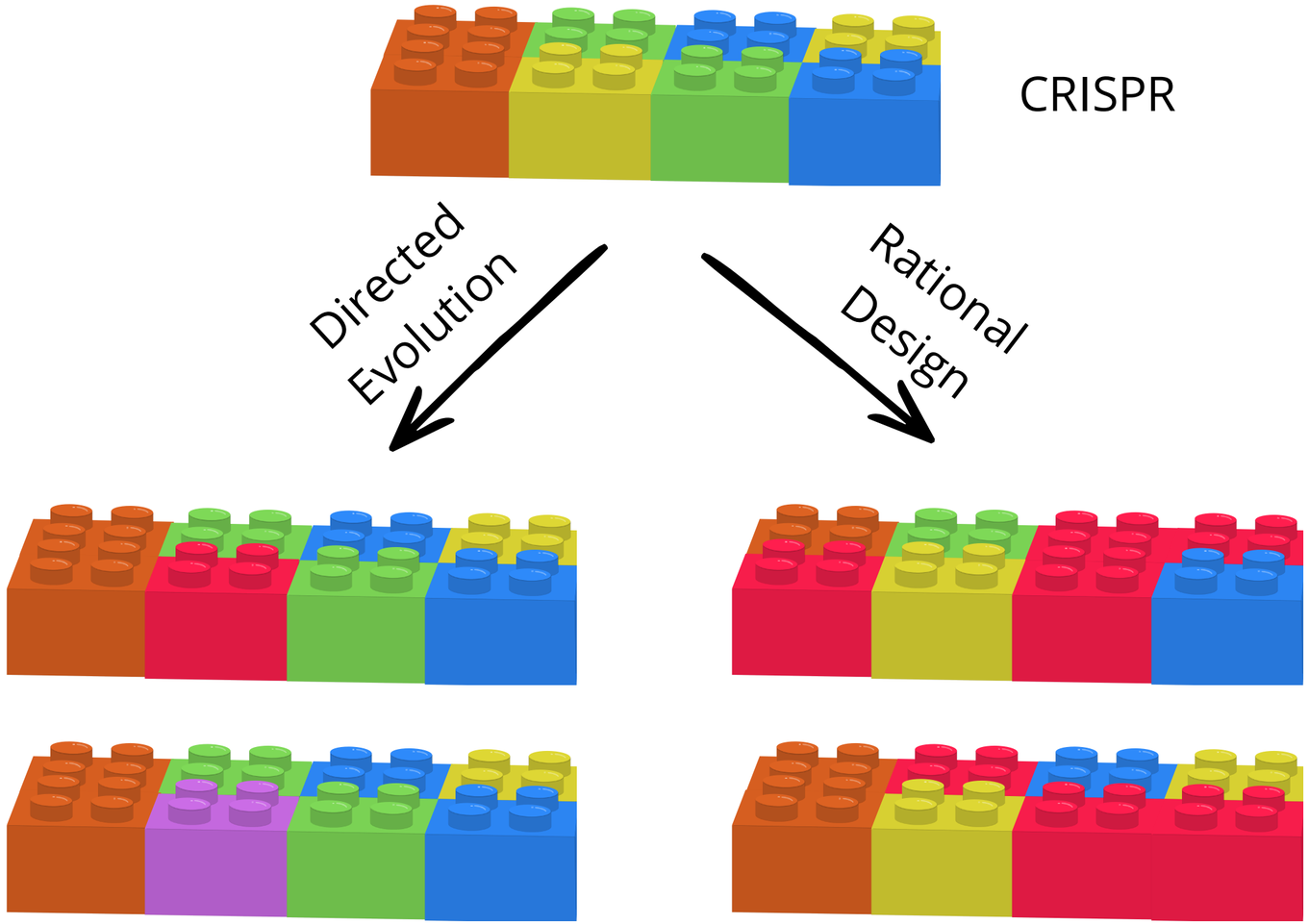
CRISPR/Cas9

Evolved CRISPR

Evolving CRISPR to make it safer

Changing CRISPR's building blocks can give it new properties

The new CRISPR variants generated can then be tested and compared with the original CRISPR. To measure their specificity, scientists use the standard off-target detection tools we previously discussed, such as CIRCLE-seq and GUIDE-seq.

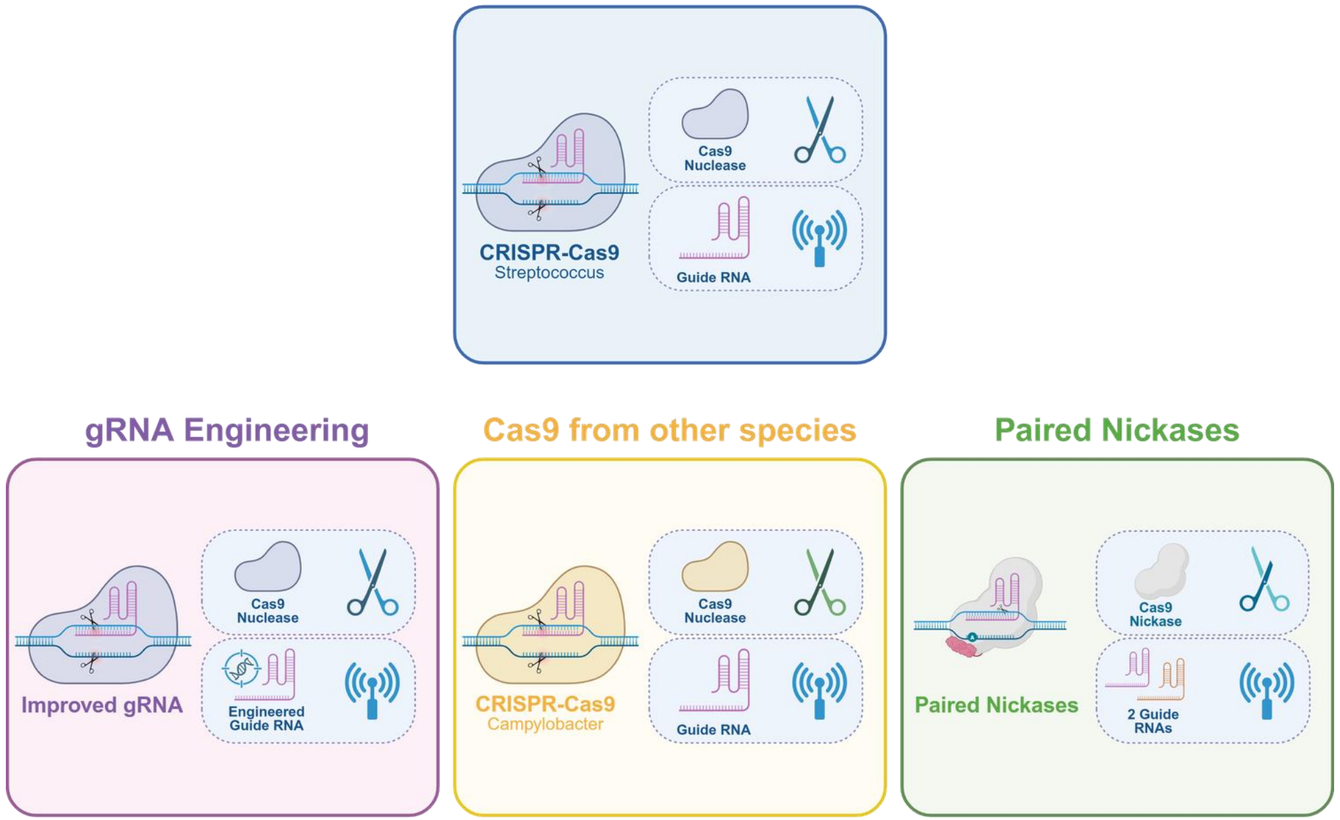


Alternatives to CRISPR evolution

CRISPR off-targets can also be reduced by other methods

Improving guide RNA design, using Cas9 variants from different species, or using nickases can all reduce off-targets

These different approaches can also be combined to build more specific CRISPR strategies. As with CRISPR evolution, tools to detect and quantify off-targets are essential to benchmark these improved strategies.



06

CLINICAL GUIDELINES FOR CRISPR PRECISION

Guidelines established by regulatory agencies for CRISPR precision in the clinical setting.

Ayal Hendel
Bar-Ilan University

Current Guidelines for CRISPR precision in the clinic

New guidelines require using at least two different methods to nominate off-targets

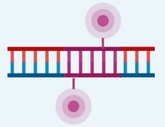
Nominated off-target nomination must be confirmed in clinically relevant cell types.

In addition, the potential effects of any confirmed off-targets must be studied. This includes whether the off-targets fall into coding regions of the DNA, and their potential effects on cell survival and reproduction.

Step 1: Nomination

At least 2 different methods

CIRCLE-seq



GUIDE-seq

Step 2: Confirmation

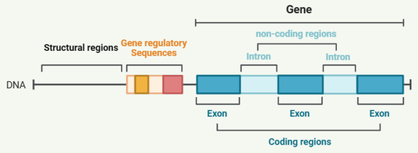
In relevant cell lines

rhAmpSeq

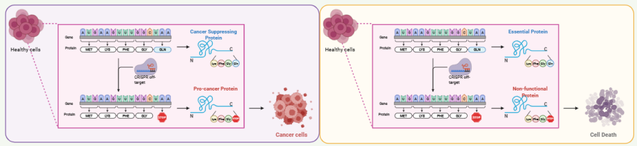
CAST-seq

Step 3: Risk evaluation

For confirmed off-targets



coding/non coding



survival/proliferation effects

webinar

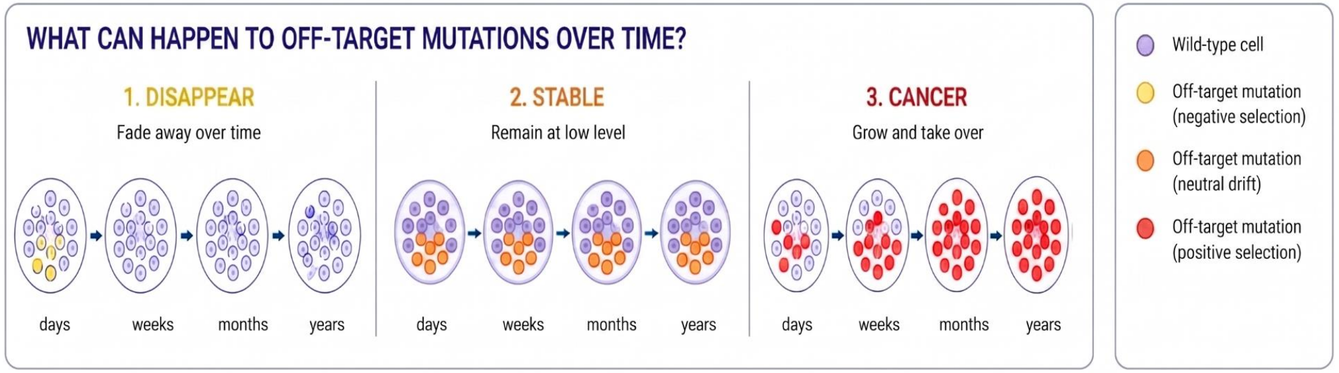
Long-term follow up after treatment with CRISPR

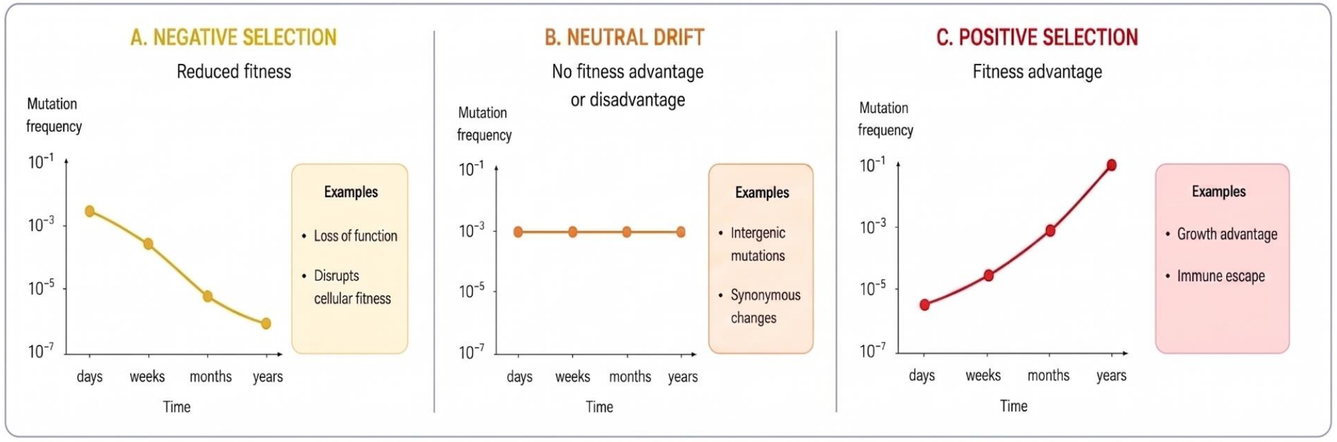
Clinical trials require long-term monitoring of patients once a year

The same panel of off-target assessment tools are used once per year on each patient for up to 15.

This allows scientists and doctors to monitor the potential long-term effects of known and unknown off-targets, especially when it comes to promoting cancer.

Tests are performed on peripheral blood mononuclear cells (PBMCs), which are easily collected from the blood and don't require invasive procedures.





Lessons from 10 years of CRISPR clinical trials

No negative effects from CRISPR off-targets have been detected so far

While longer-term follow-up will be important, the data until now suggests that CRISPR approaches are safe and effective in clinical settings, with over 300 patients treated safely worldwide.

The clinical trial for CASGEVY included 250 patients, who were followed for at least 3 years without any relevant negative effects.

This suggests that CRISPR approaches are safe enough to avoid genotoxic effects like those observed with the first gene therapy approaches. Still, the community is strongly committed to long-term follow-ups to ensure each patient's safety.

Emily Whitehead: The First Child Successfully Treated with CAR-T Cell Therapy

Future Webinars

Session 6: Future Developments and CRISPR/Cas9 for SCD
Annarita Miccio
July 2026

Session 7: Regulatory path to the clinic
David Morrow
September 2026

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