

# The CRISPR era: Clinical Trials - current and future perspectives

John Murphy

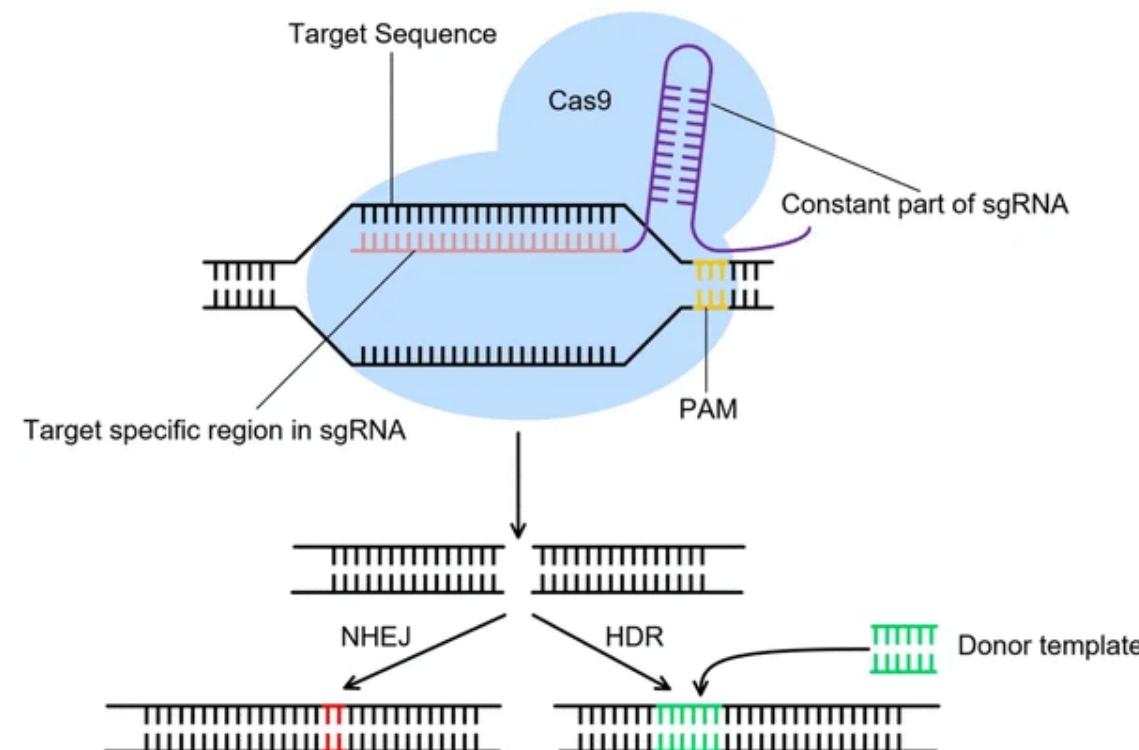
# Lecture plan;

- Our experience and research using CRISPR-Cas9
- CRISPR strategies in cancer research
- CRISPR strategies in cancer treatments - clinical trials
- CRISPR strategies for other diseases – clinical trials
- CRISPR treatment strategies – challenges and limitations

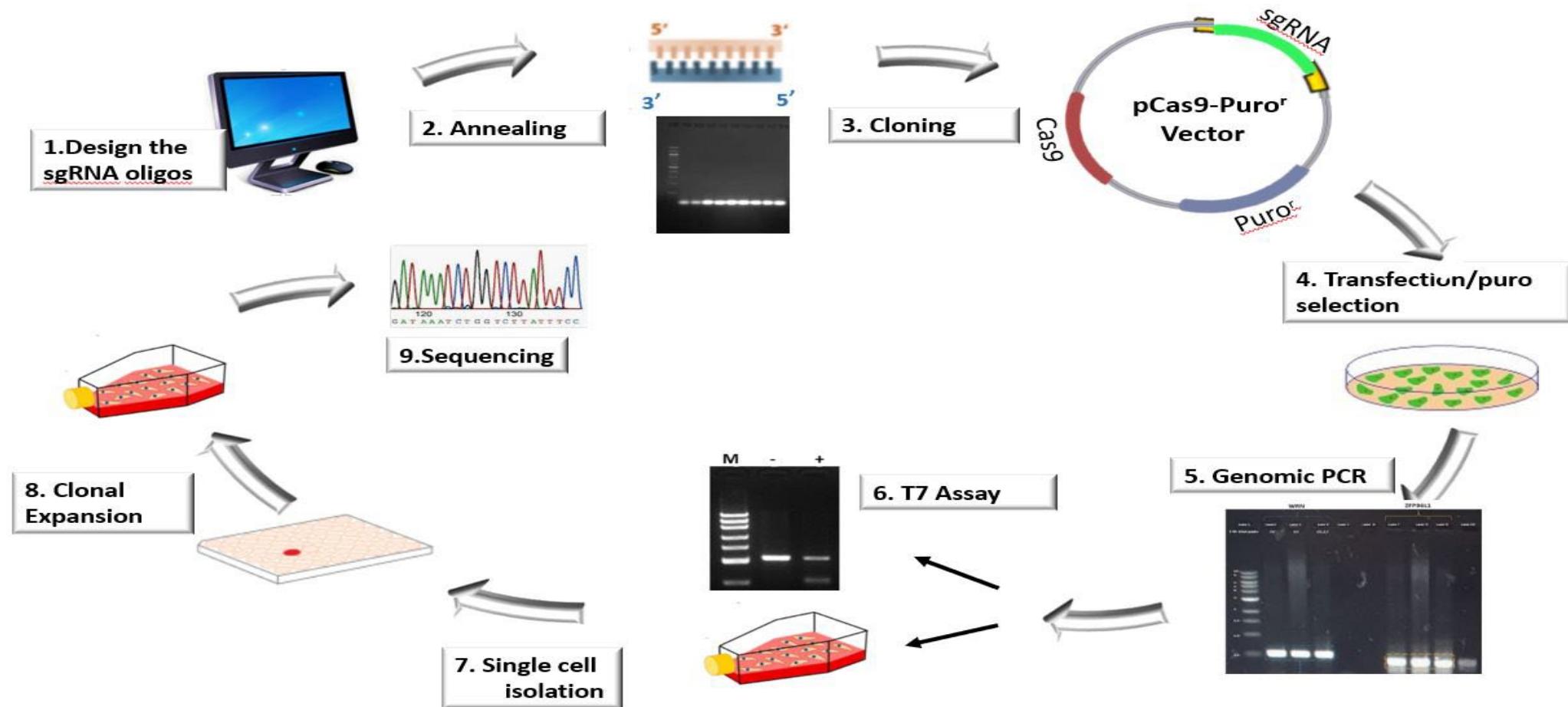
- A two component system for gene editing – Type II CRispr

## 1. Single guide RNA (sgRNA)

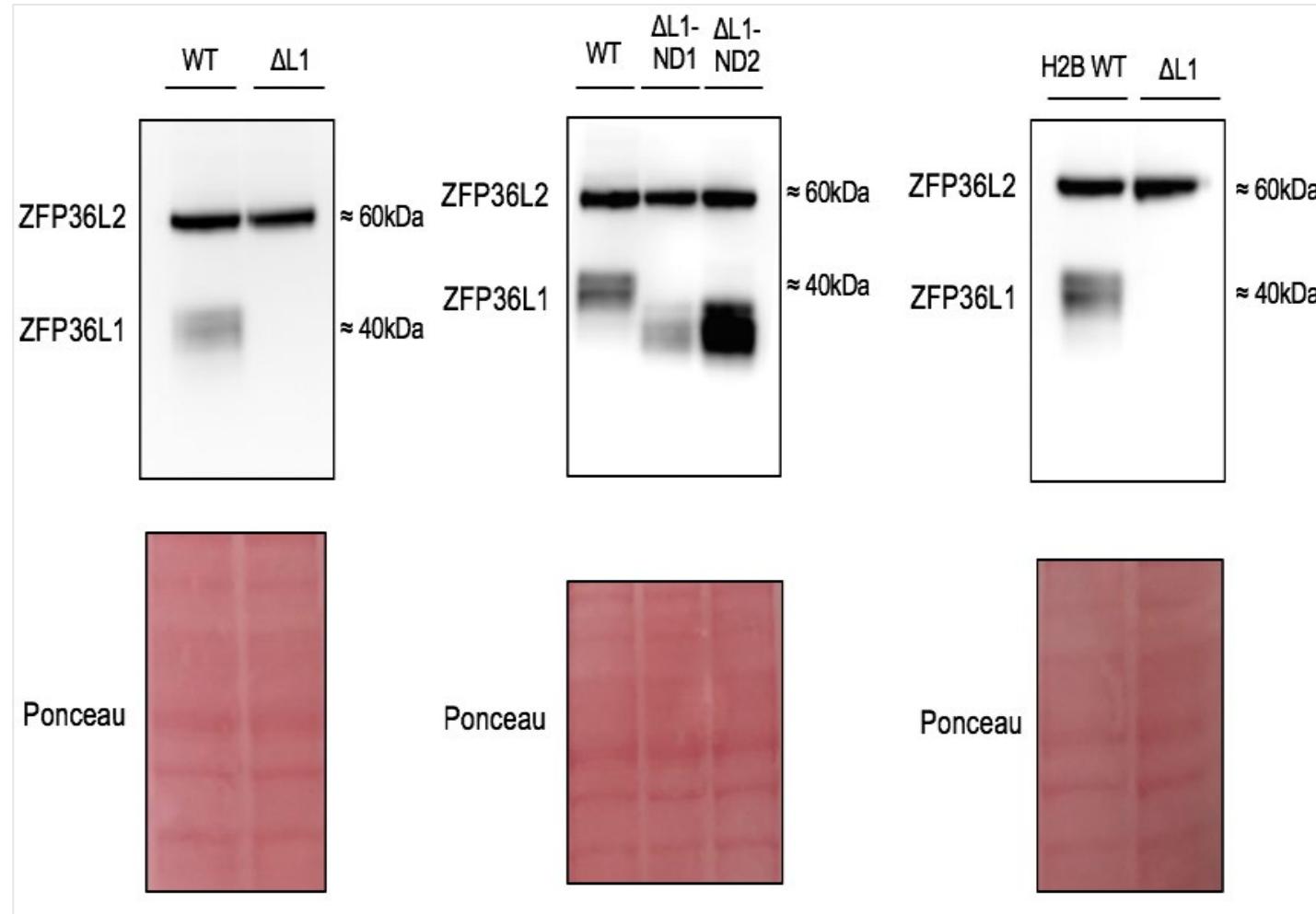
## 2. Cas9 enzyme



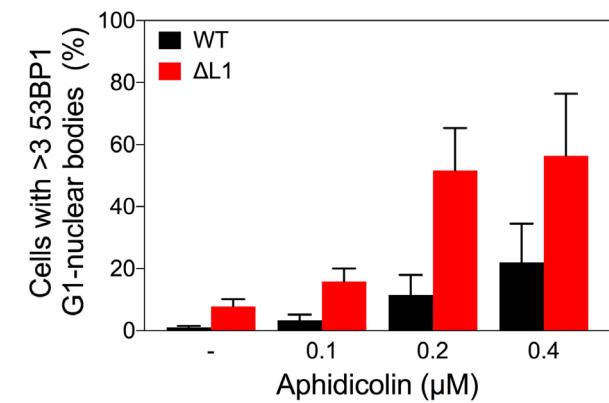
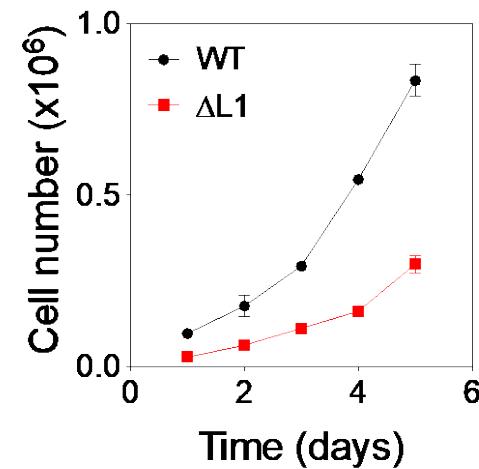
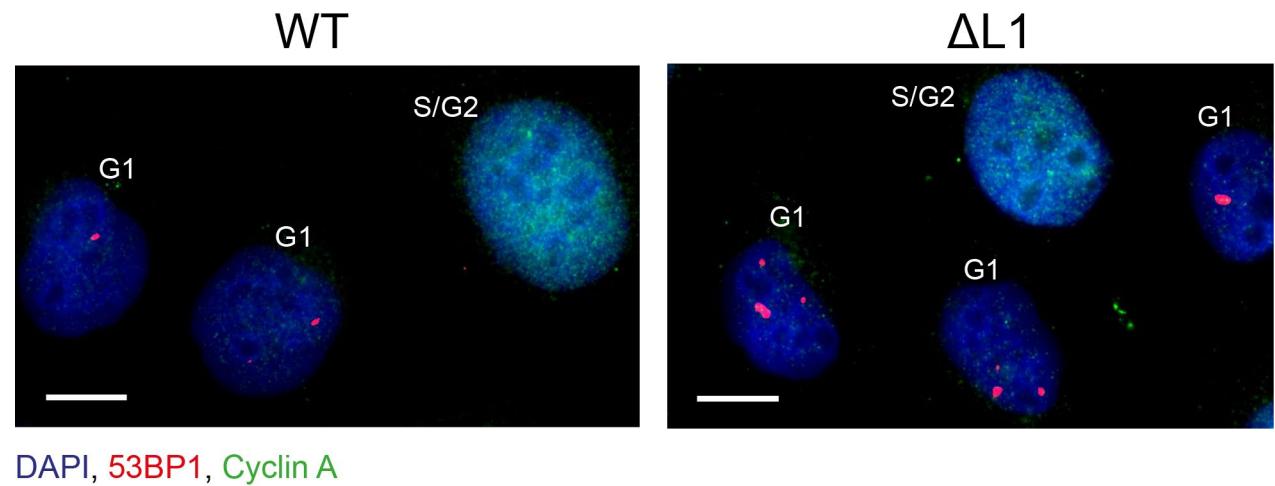
# Our CRISPR experience – Pipeline for generation of ZFP36L1 knockout cell models



# Targeting the ZFP36L1 gene by CRISPR –Cas9



# ZFP36L1 RNA binding protein is involved in maintaining genomic stability



# CRISPR in cancer research strategies

Identifying **cancer cell fitness genes** and cancer **vulnerabilities** which could provide new therapeutic targets

- Cancer specific fitness genes
- Pan-cancer fitness genes

Identifying **synthetic lethality interactions** in cancer which could provide new therapeutic targets

# Example: CRISPR strategies for identifying novel targets in cancer

## Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens

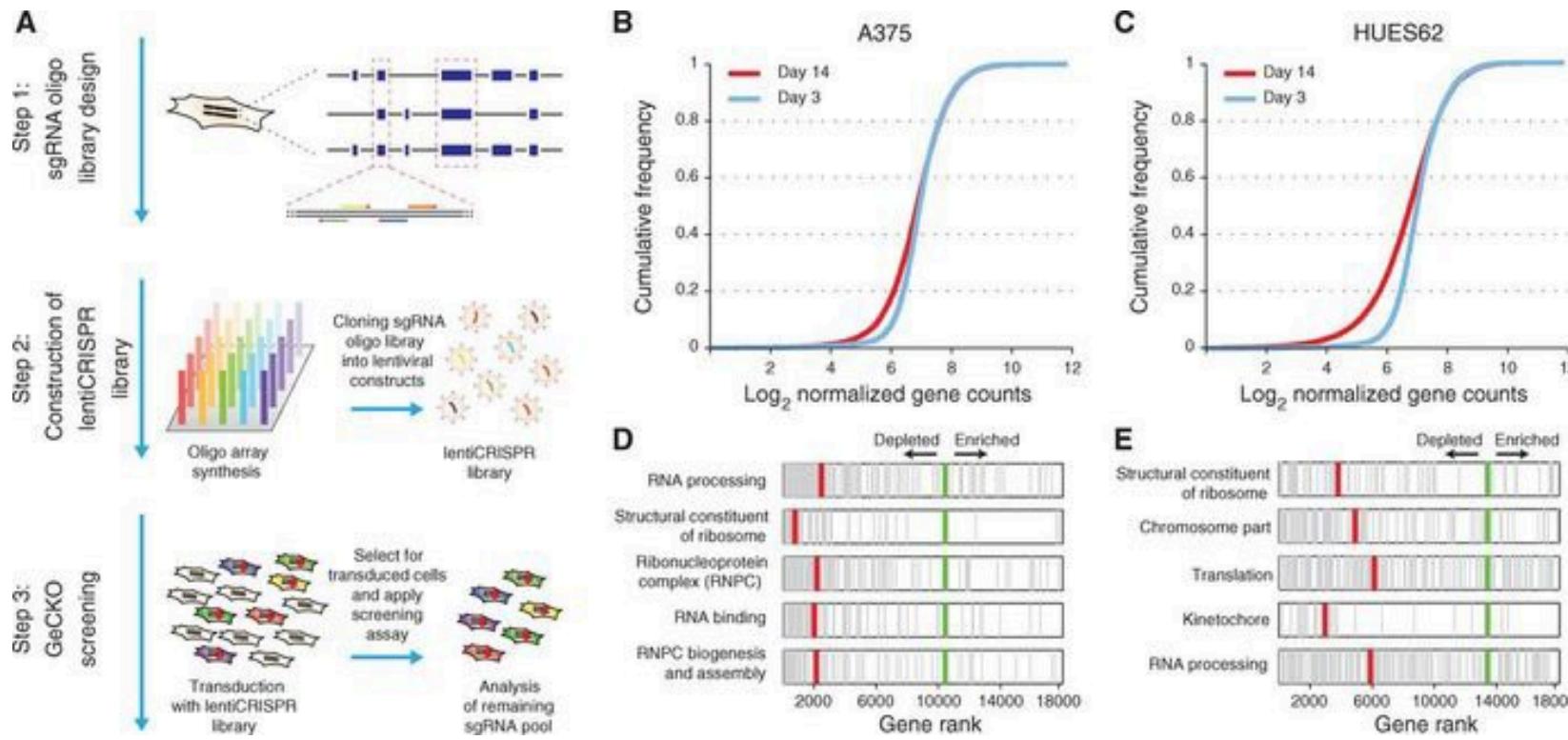
Fiona M. Behan<sup>1,2,12</sup>, Francesco Iorio<sup>1,2,3,12</sup>, Gabriele Picco<sup>1,12</sup>, Emanuel Gonçalves<sup>1</sup>, Charlotte M. Beaver<sup>1</sup>, Giorgia Migliardi<sup>4,5</sup>, Rita Santos<sup>6</sup>, Yanhua Rao<sup>7</sup>, Francesco Sassi<sup>4</sup>, Marika Pinnelli<sup>4,5</sup>, Rizwan Ansari<sup>1</sup>, Sarah Harper<sup>1</sup>, David Adam Jackson<sup>1</sup>, Rebecca McRae<sup>1</sup>, Rachel Pooley<sup>1</sup>, Piers Wilkinson<sup>1</sup>, Dieudonne van der Meer<sup>1</sup>, David Dow<sup>2,6</sup>, Carolyn Buser–Doepner<sup>2,7</sup>, Andrea Bertotti<sup>4,5</sup>, Livio Trusolino<sup>4,5</sup>, Euan A. Stronach<sup>2,6</sup>, Julio Saez–Rodriguez<sup>2,3,8,9,10</sup>, Kosuke Yusa<sup>1,2,11,13\*</sup> & Mathew J. Garnett<sup>1,2,13\*</sup>

Behan et al., 2019, *Nature* 568, 511-516.

genome-scale CRISPR–Cas9 screens in 324 human cancer cell lines  
from 30 cancer types

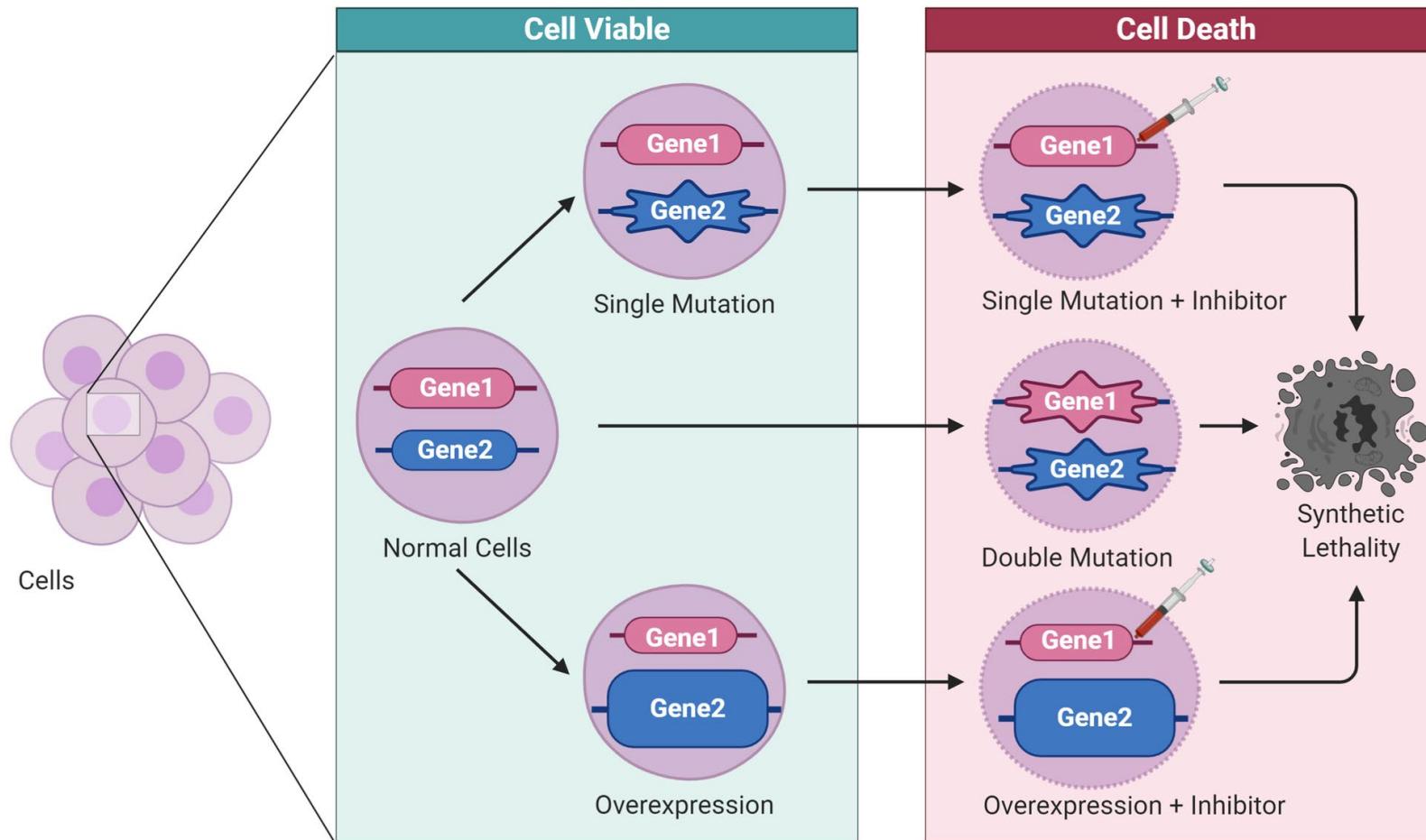
*Aim to identify **novel** cancer therapeutic targets*

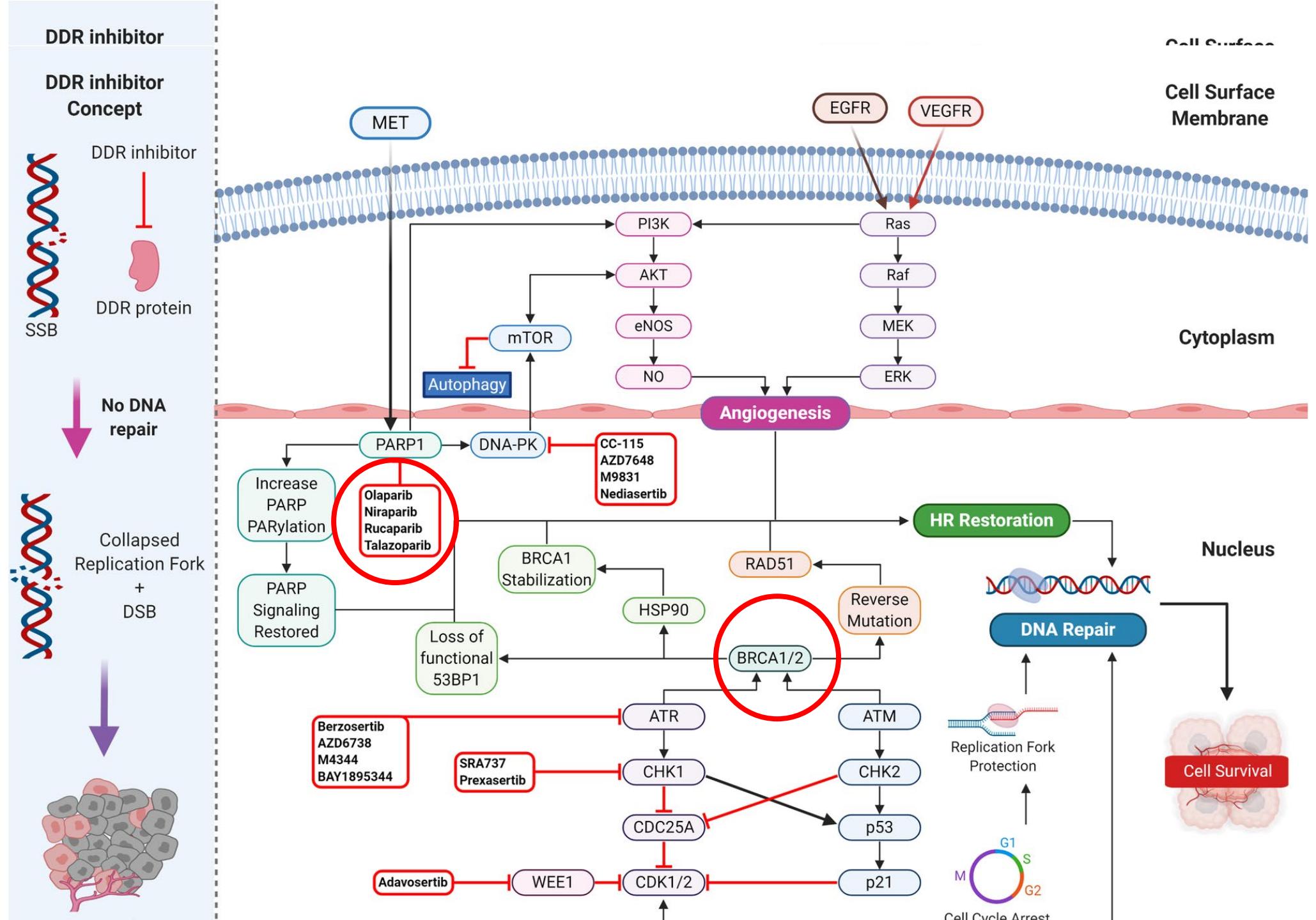
# CRISPR library screen –GeCKO library screen



# Synthetic lethal interaction in cancer

Example: PARP inhibitor (**Olaparib**) and BRCA1/2 mutations in certain breast cancers





# CRISPR-based patient treatment strategies in clinical trials

**The challenge** – treatments for diseases for which CRISPR strategies might provide benefits but need to be introduced into humans

- **ex vivo** and **in vivo** strategies (**cell and gene therapies**)

**Potential benefits** – improved treatments for **cancers**

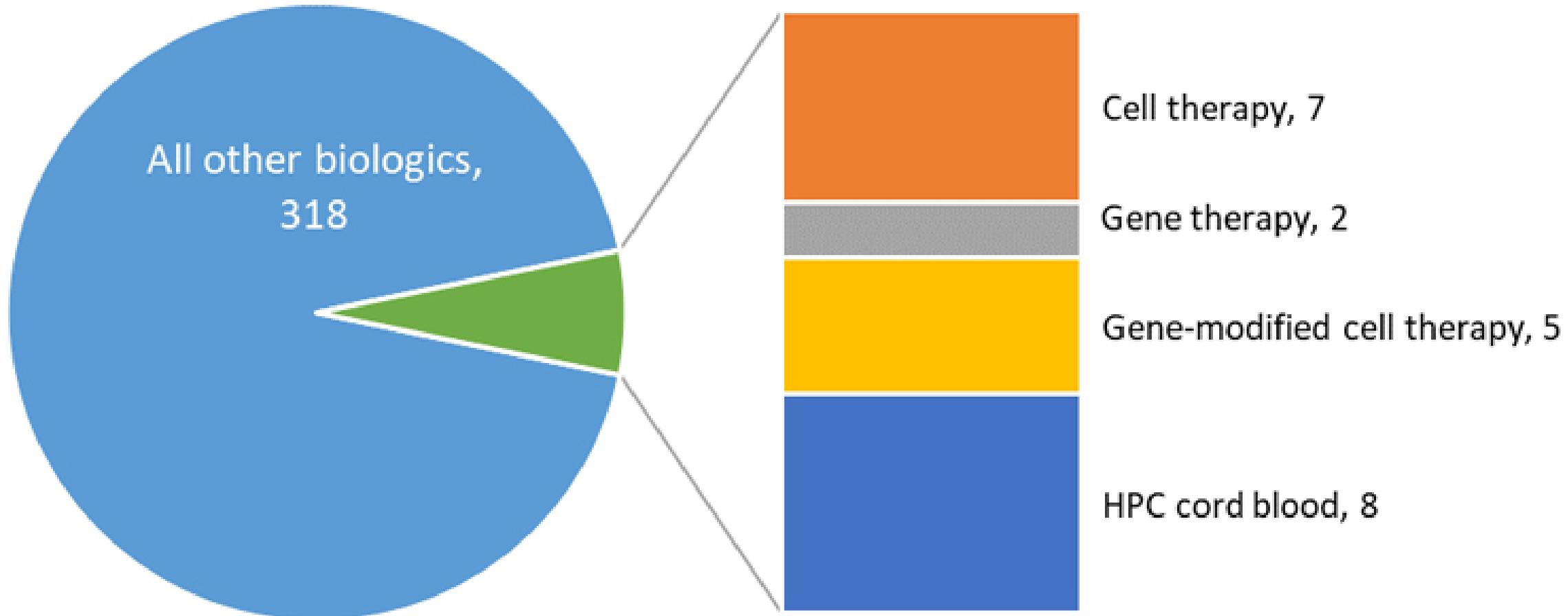
**Potential benefits** – potential **cures** for certain genetic diseases

- Sickle cell anaemia
- Beta thalassemia
- Leber Congenital Amaurosis
- Transthyretin Amyloidosis

USA Food and Drug Administration (FDA) predominant is approving new drugs/therapies worldwide

## FDA approved cell and gene therapies

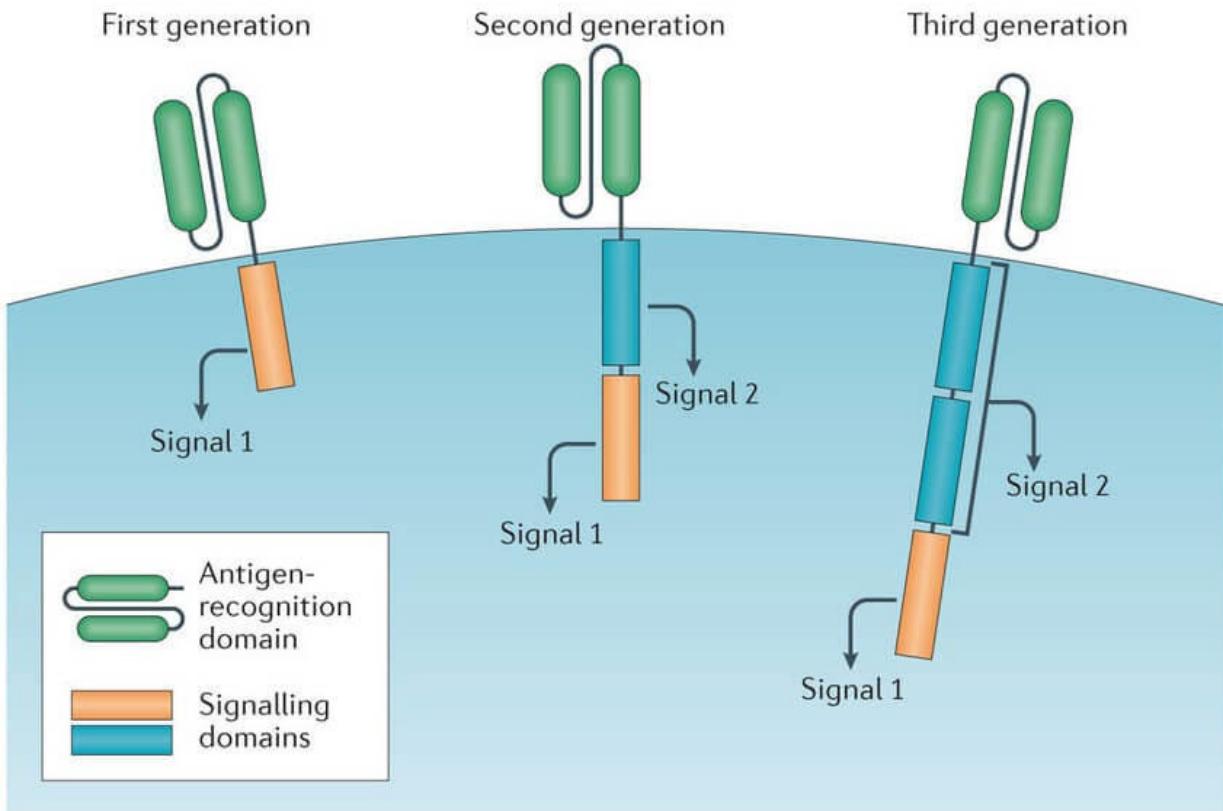
There are not that many (update August 2024 – 38 total) !



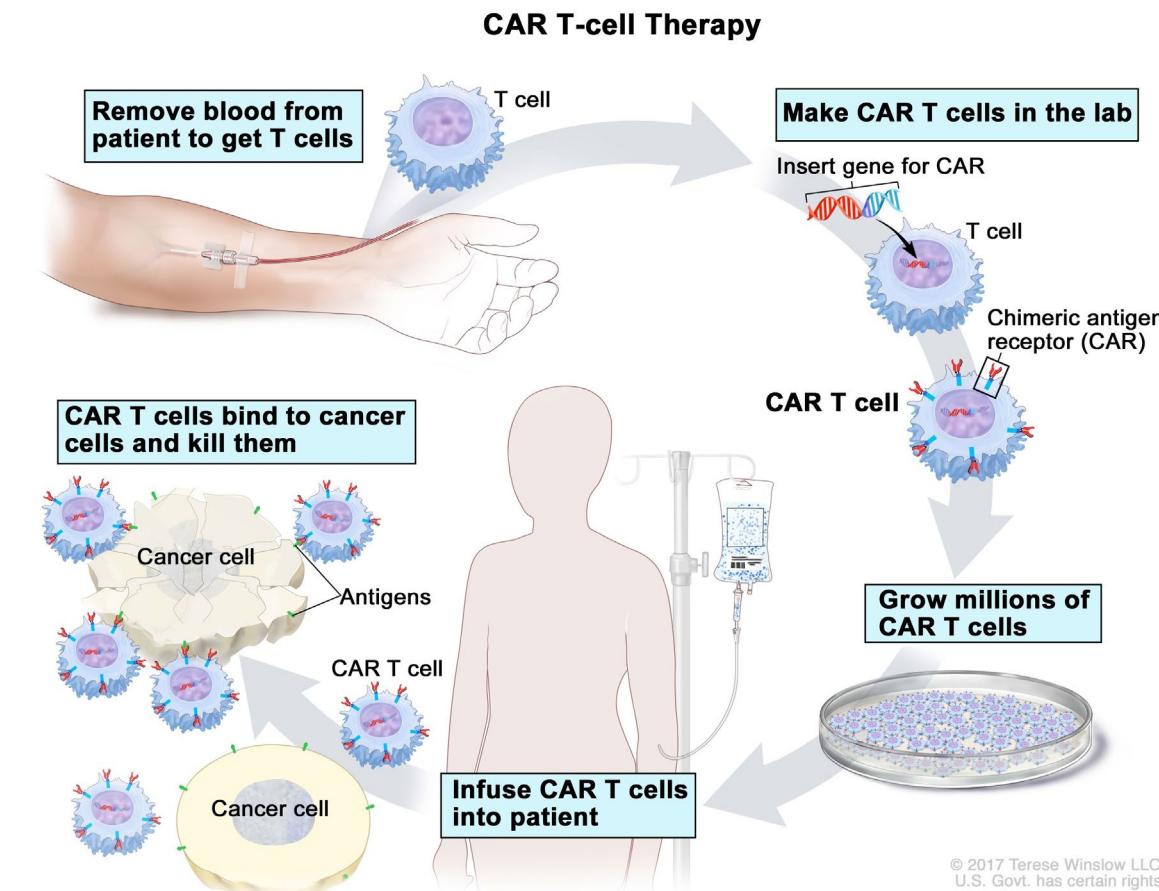
**CRISPR** cancer therapies are currently focussing on generating improved strategies for **CAR-T** treatments or tumour infiltrating lymphocytes (**TILs**)

Mostly CAR-T - so what is **CAR-T** therapy  
.....???

# Chimeric antigen receptor T (CAR-T) therapies – redirecting patients T cells to fight their cancer



Nature Reviews | Clinical Oncology



© 2017 Terese Winslow LLC  
U.S. Govt. has certain rights

Chimeric antigen receptor T (CAR-T) cells  
are promising approach in cancer treatment

**6 CAR-T therapies** approved by FDA – all directed to treatment  
of B cell leukaemia/lymphomas

4 directed against CD19 receptor on B lymphocytes  
2 directed against BCMA receptor on B lymphocytes

**CRISPR** cancer therapies are currently focussing on  
generating improved strategies for **CAR-T** strategies

## FDA-Approved CAR T-Cell Therapies

Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

For new cell and gene therapies to be approved as therapies successful clinical trials have to be carried out .....

# Clinical Trials Phase 1

A phase of research to describe clinical trials that focus on the safety of a drug. They are usually conducted with healthy volunteers, and the goal is to determine the drug's most frequent and serious adverse events and, often, how the drug is broken down and excreted by the body. These trials usually involve a small number of participants.

# Clinical Trials Phase 2

A phase of research to describe clinical trials that gather preliminary data on whether a drug works in people who have a certain condition/disease (that is, the drug's effectiveness). For example, participants receiving the drug may be compared to similar participants receiving a different treatment, usually an inactive substance (called a placebo) or a different drug. Safety continues to be evaluated, and short-term adverse events are studied.

# Clinical Trials Phase 3

A phase of research to describe clinical trials that gather more information about a drug's safety and effectiveness by studying different populations and different dosages and by using the drug in combination with other drugs. These studies typically involve more participants.

# Clinical Trials Phase 4

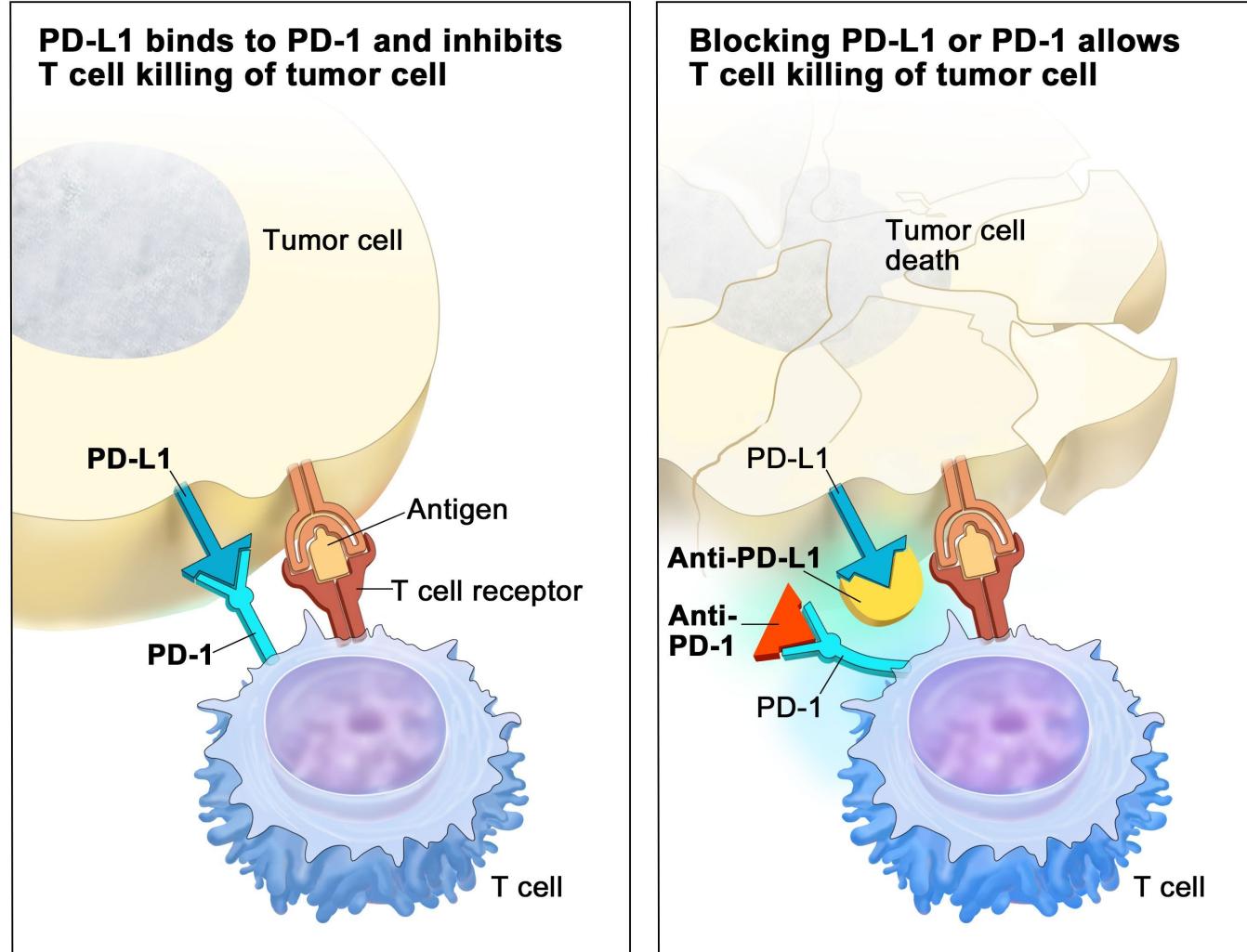
A phase of research to describe clinical trials occurring after FDA has approved a drug for marketing. They include postmarket requirement and commitment studies that are required of or agreed to by the study sponsor. These trials gather additional information about a drug's safety, efficacy, or optimal use.

There are no CRISPR therapies in Phase 4 trials yet

# 19 Registered US Food and Drug Administration (FDA) CRISPR/CAS9 cancer clinical trials, from clinicaltrials.gov, accessed 24<sup>th</sup> August 2020

Identifier	Condition	Phase	Treatment and status of clinical trial
NCT03081715	Esophageal cancer	II	PD-1 knockout T cells (completed)
NCT02863913	Bladder cancer	I	PD-1 knockout T cells (withdrawn)
NCT02867345	Hormone refractory prostate cancer	I	PD-1 knockout T cells (withdrawn)
NCT02867332	Renal cell carcinoma	I	PD-1 knockout T cells (withdrawn)
NCT02793856	Non small cell lung cancer	I	PD-1 knockout T cells (active not recruiting)
NCT03044743	EBV positive advanced stage malignancies	I/II	PD-1 knockout EBV-CTL cells (recruiting)
NCT03166878	B cell lymphoma / leukemia	I/II	CRISPR-Cas9 edited CAR-T cells targeting CD19 (recruiting)
NCT03057912	HPV related cervical intraepithelial neoplasia	I	CRISPR/Cas9-sg HPV E6/E7 gel to disrupt HPV DNA (unknown)
NCT03399448	Multiple myeloma, synovial sarcoma, myxoid/round cell liposarcoma, melanoma	I	autologous T cells targeting tumor antigen NY-ESO-1, edited with CRISPR-Cas9 to disrupt endogenous TCR $\alpha$ , TCR $\beta$ and PD-1 (NYCE T Cells) (terminated)
NCT03398967	B cell lymphoma / leukemia	I/II	edited CAR-T Cells Targeting CD19 and CD20 or CD22 (recruiting)
NCT04426669	Metastatic Gastrointestinal Epithelial Cancer	I/II	Tumor-Infiltrating Lymphocytes gene Encoding CISH knockout (recruiting)
NCT03545815	Mesothelin Positive Multiple Solid Tumors	I	PD-1 and TCR Gene-knocked Out Chimeric Antigen Receptor (CAR) T Cells (recruiting)
NCT04037566	CD19+ B cell leukemia / lymphoma	I	CD19-specific CAR-T cells with edited endogenous HPK1 (XYF19 CAR-T cells) (recruiting)
NCT04244656	Multiple Myeloma	I	Anti-BCMA Allogeneic CRISPR-Cas9-Engineered T Cells (CTX120) (recruiting)
NCT04438083	Renal cell carcinoma.	I	CTX130 CD70-directed T-cell immunotherapy comprised of allogeneic T cells (recruiting)
NCT04502446	T cell lymphoma	I	Anti-CD70 Allogeneic Engineered T Cells (CTX130) (recruiting)
NCT04035434	B cell malignancies	I/II	CTX110 CD19-directed T-cell immunotherapy comprised of allogeneic T cells (recruiting)
NCT03747965	Mesothelin Positive Multiple Solid Tumors	I	PD-1 knockout Mesothelin-directed CAR T cells (recruiting)
NCT04417764	Hepatocellular Carcinoma	I	PD-1 knockout engineered T cells (recruiting)

# CRISPR cancer therapies directed to knocking out the immune checkpoint molecule **programmed cell death protein 1 (PD-1)**



<https://www.clinicaltrials.gov/ct2/home>

update 26/11/22

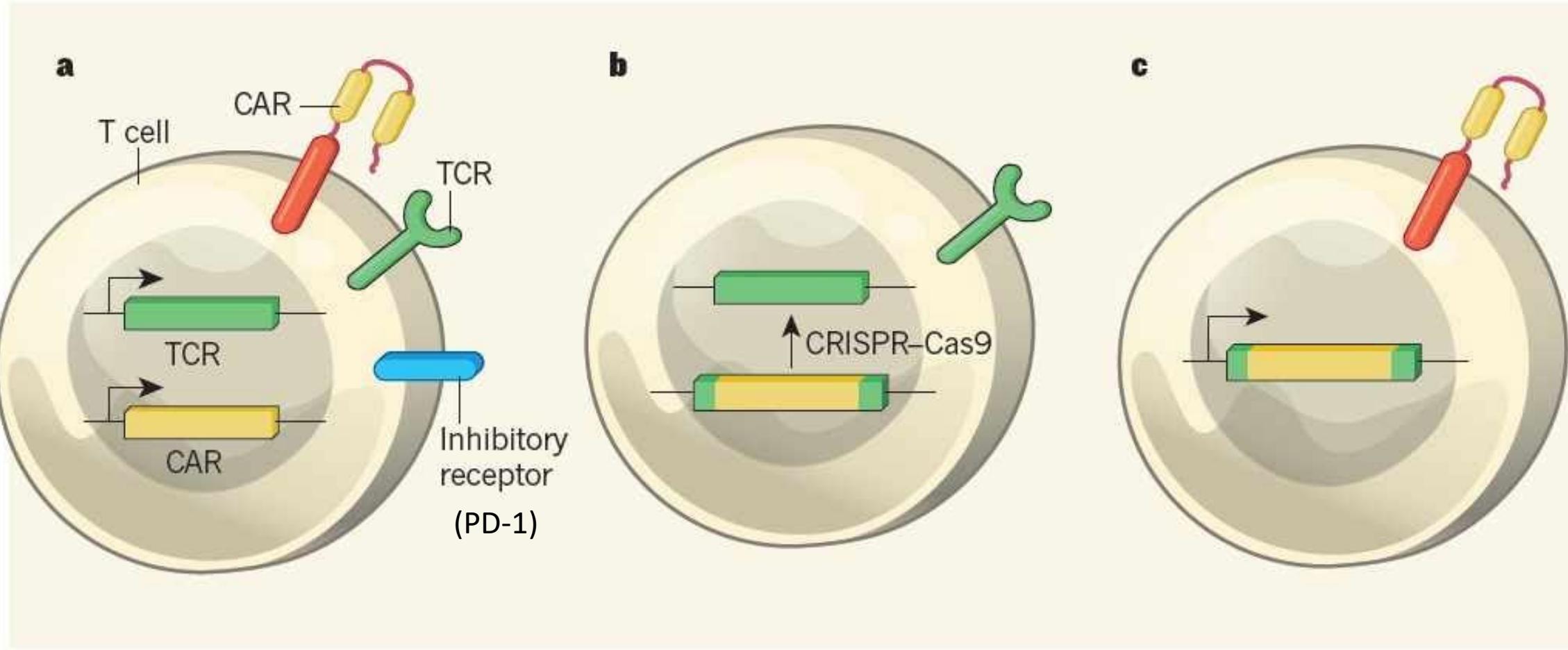
- **71 clinical trials with “CRISPR”** (most treatments (**58**) a few diagnostics (**13**))
- 34 **CRISPR** cancer treatments clinical trials
- CURRENT STATUS OF CRISPR CLINICAL TRIALS
  - 29 recruiting
  - 9 not yet recruiting
  - 4 active
  - 8 completed**
  - 5 withdrawn
  - 3 terminated
  - 12 unknown (all in China)

<https://www.clinicaltrials.gov/ct2/home>

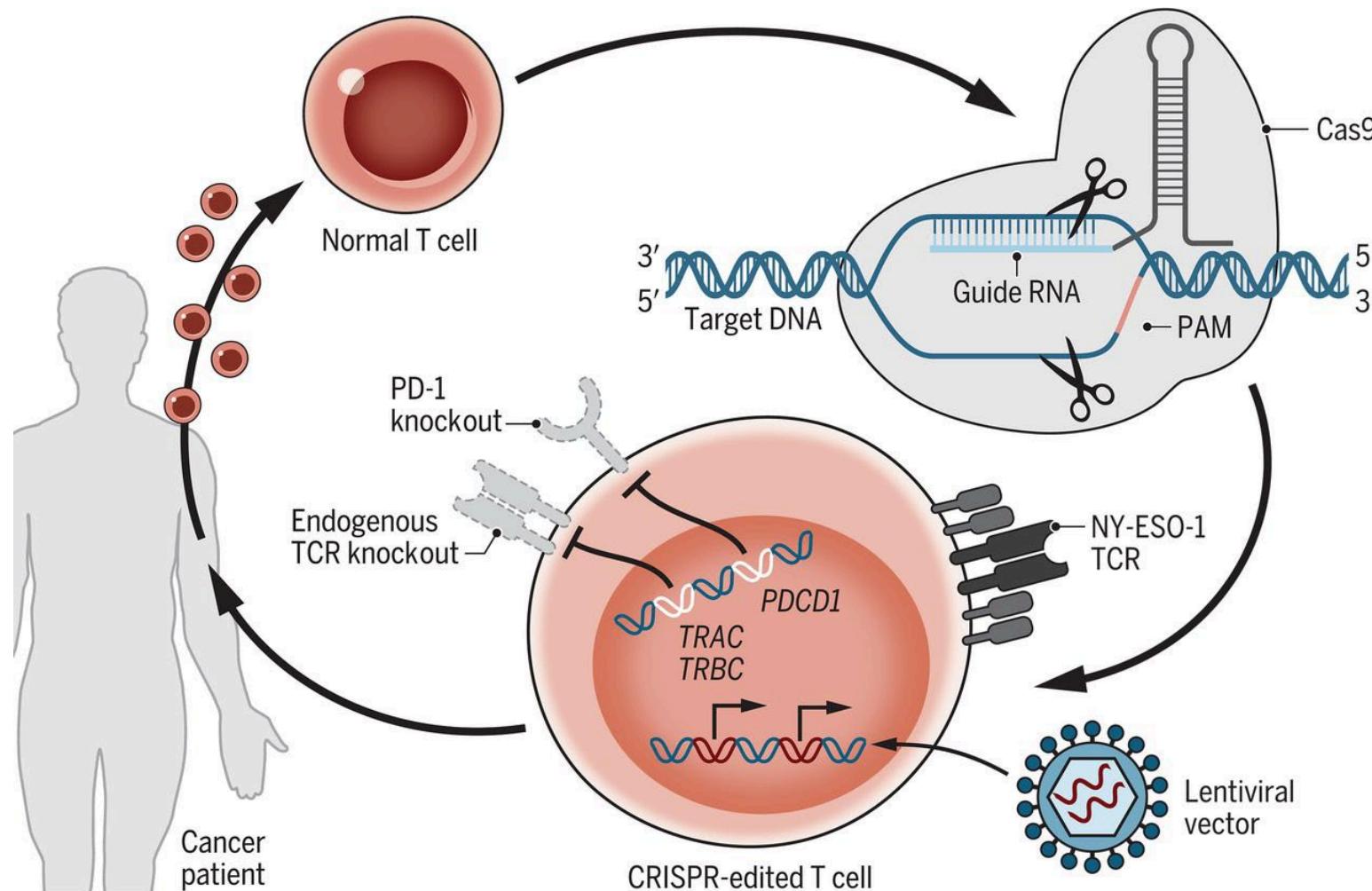
update 29/08/24

- **102 clinical trials with “CRISPR”** (most treatments (**60**) and some diagnostic (**32**))
- 30 CRISPR cancer treatments clinical trials
  - CURRENT STATUS OF ALL CRISPR CLINICAL TRIALS
    - 32 recruiting
    - 13 not yet recruiting
    - 14 active not recruiting
    - 11 completed**
    - 7 withdrawn
    - 5 terminated
    - 15 unknown (most in China)

## CRISPR – modify CAR-T knockout PD-1 inhibitory receptor and remove endogenous TCR



# Example: strategy for clinical application of CRISPR – *ex vivo*

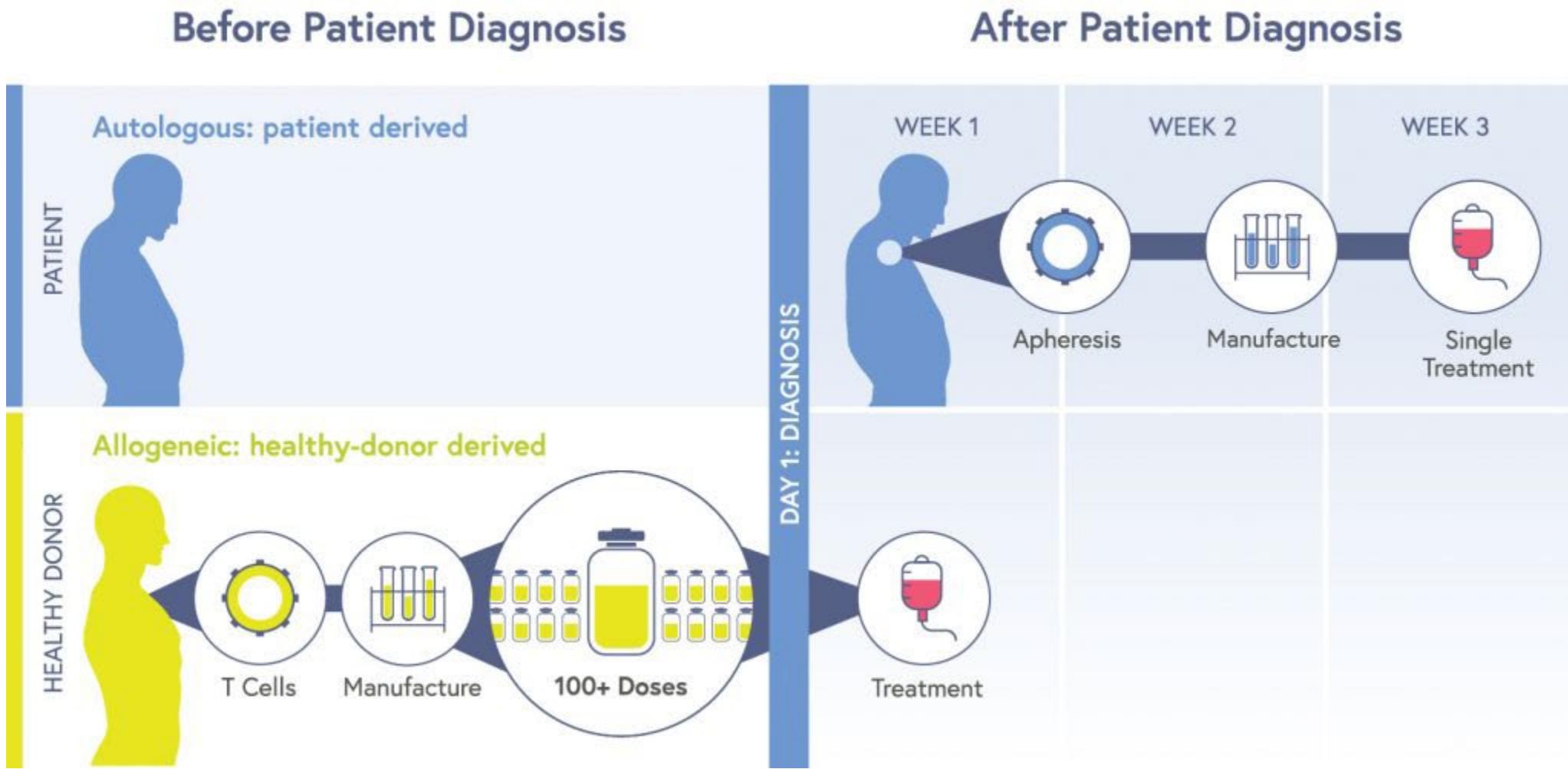


# Some issues with *ex vivo* CRISPR treatments

- Individual design and production needed for each patient
- Requires a number of specialised procedures
- Very expensive!
- Lack of availability to all patients

# Autologous vs Allogeneic treatments

<https://innovativegenomics.org/news/crispr-clinical-trials-2022/>



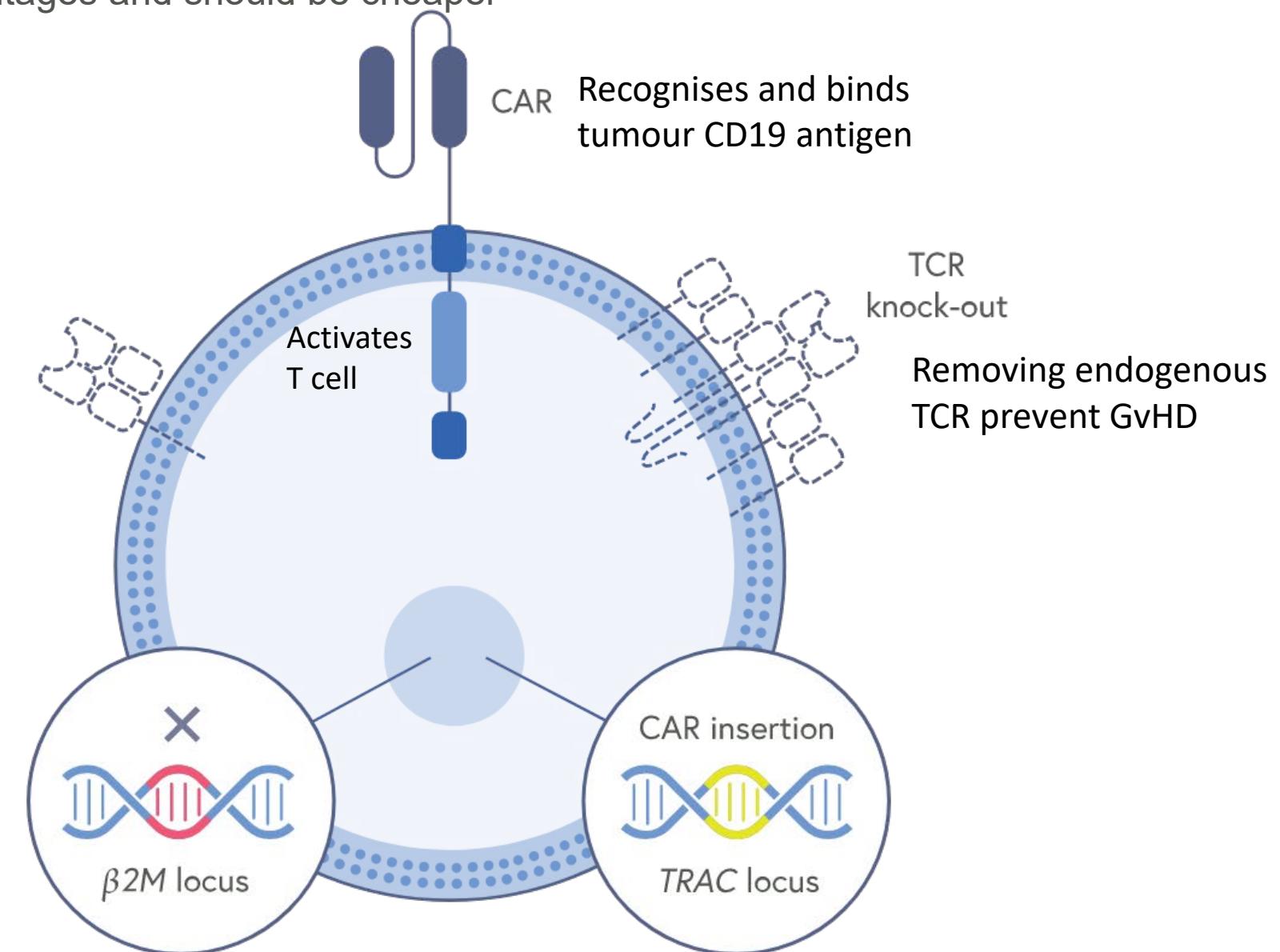
# CRISPR Therapeutics Announces FDA Regenerative Medicine Advanced Therapy (RMAT) Designation Granted to CTX110™ for the Treatment of Relapsed or Refractory CD19+ B-cell malignancies

PRESS RELEASE Nov. 22, 2021 CRISPR Therapeutics

**ALLOGENEIC** CRISPR gene-edited T cells Phase 1 clinical study  
Estimated completion date July 2026

**CTX110™** - Allogenic CRISPR treatment – so if it works – it will have “off-the-shelf” availability and therefore has significant advantages and should be cheaper

Removing MHC 1 should help with preventing Immune rejection in recipients



# Other allogeneic CRISPR Strategies.....

**NCT06321289** [Allogeneic TRAC Locus-inserted CD19-targeting STAR T Cell Therapy in r/r B-NHL](#)

**2024 recruiting** above to remove HLA A,B, TRAC alpha, CIITA and PD-1 – from T cells of Healthy donors - for resistant B NHL – China

Previous NCT05631912 before above (Allogeneic CD19-STAR T cell)

**NCT05066165 (terminated) Intellia Therapeutics**

[Study Investigating NTLA-5001 in Subjects With Acute Myeloid Leukemia](#)

Autologous WT1-directed TCR T cells engineered ex vivo using CRISPR/Cas9 as intravenous infusion after pre-conditioning chemotherapy.

Interesting reason for terminating the study – “Pivoting to an **allogeneic version** of this program currently in preclinical development.” Started 2021-12-17; Terminated 2022-08-31 – only 6 out estimated 54 participants recruited

CRISPR showing genuine promise  
in possibility of cures for certain  
genetic diseases!

<https://www.clinicaltrials.gov/ct2/home>

update 26/11/22

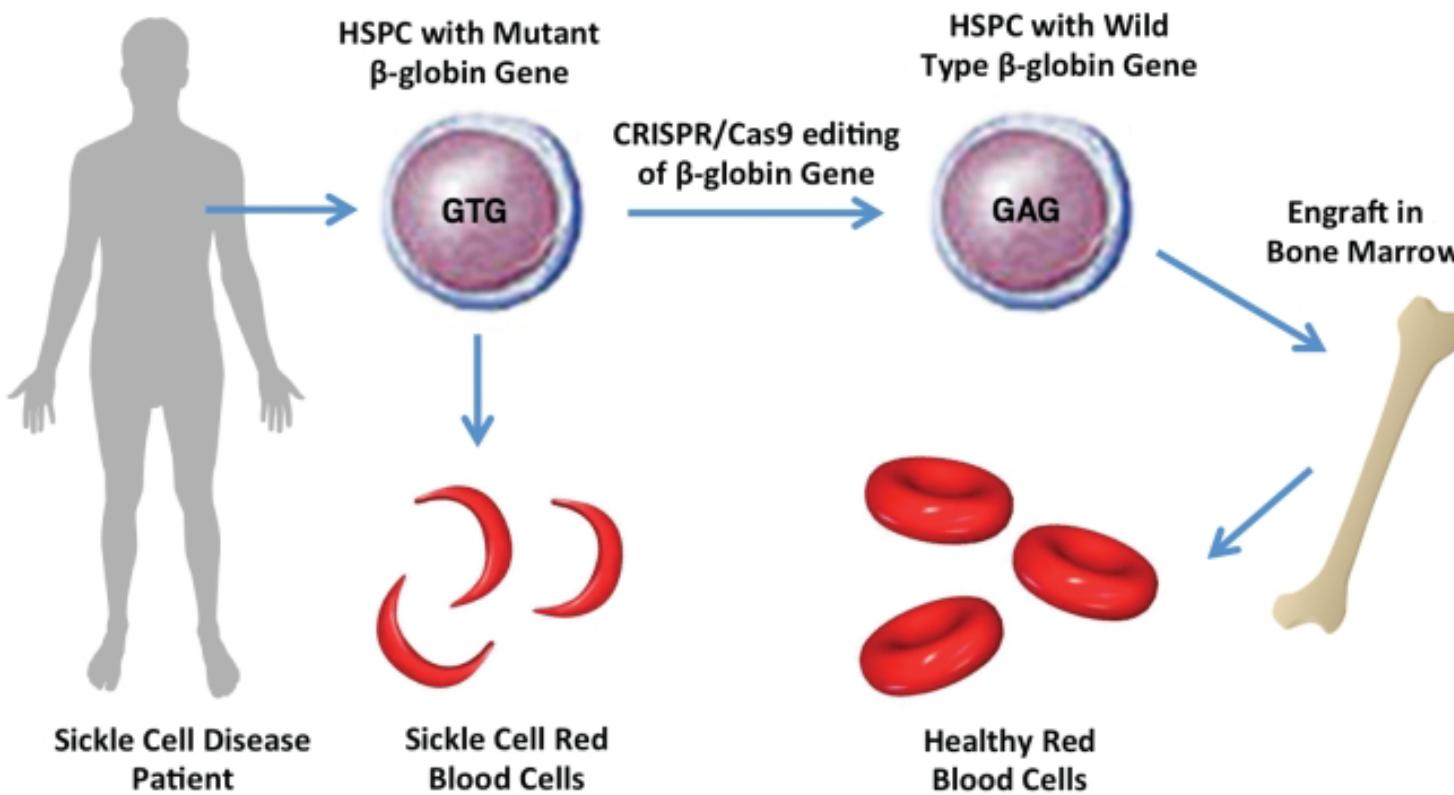
- **5 Phase 3 clinical trials** – all CTX001 – sickle cell anaemia and beta-thalassemia
- 18 phase 2 clinical trials
- 43 Phase1 clinical trials
- 7 trials not applicable

<https://www.clinicaltrials.gov/ct2/home>

update 29/08/24

- 7 Phase 3 clinical trials – all 7 CTX001 (Exa-cel, Casgevy) – sickle cell anaemia and beta-thalassemia
- 25 phase 2 clinical trials
- 52 Phase1 clinical trials
- 15 trials not applicable

# CRISPR/Cas9 Cellular Therapeutics – The Next Generation of Cures – Sickle Cell Anaemia



# CRISPR/Cas9 Cellular Therapeutics – Haemoglobinopathies – a work-around approach

Significant worldwide burden

ANNUAL BIRTHS



300k

Sickle cell disease

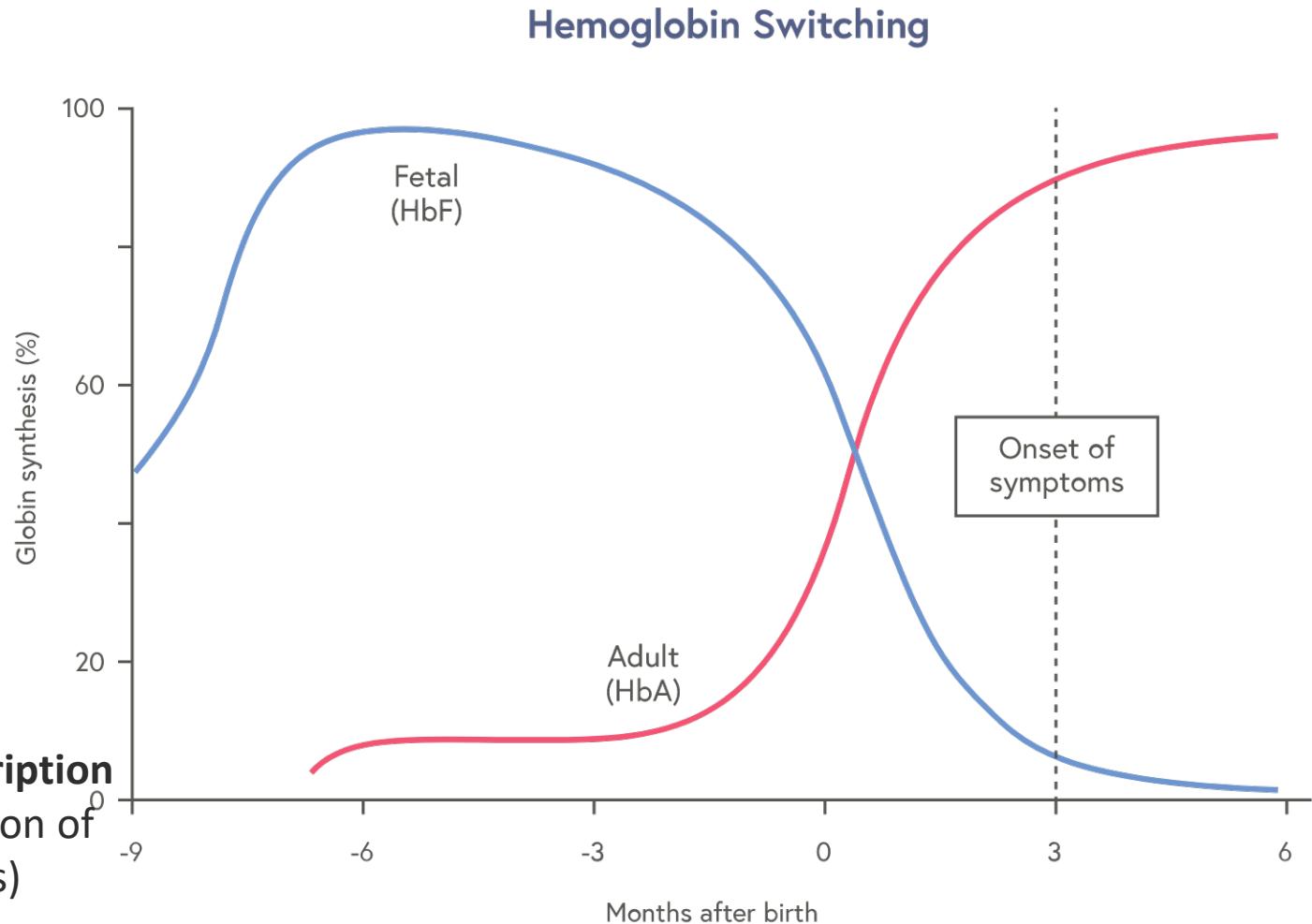
60k

$\beta$ -thalassemia

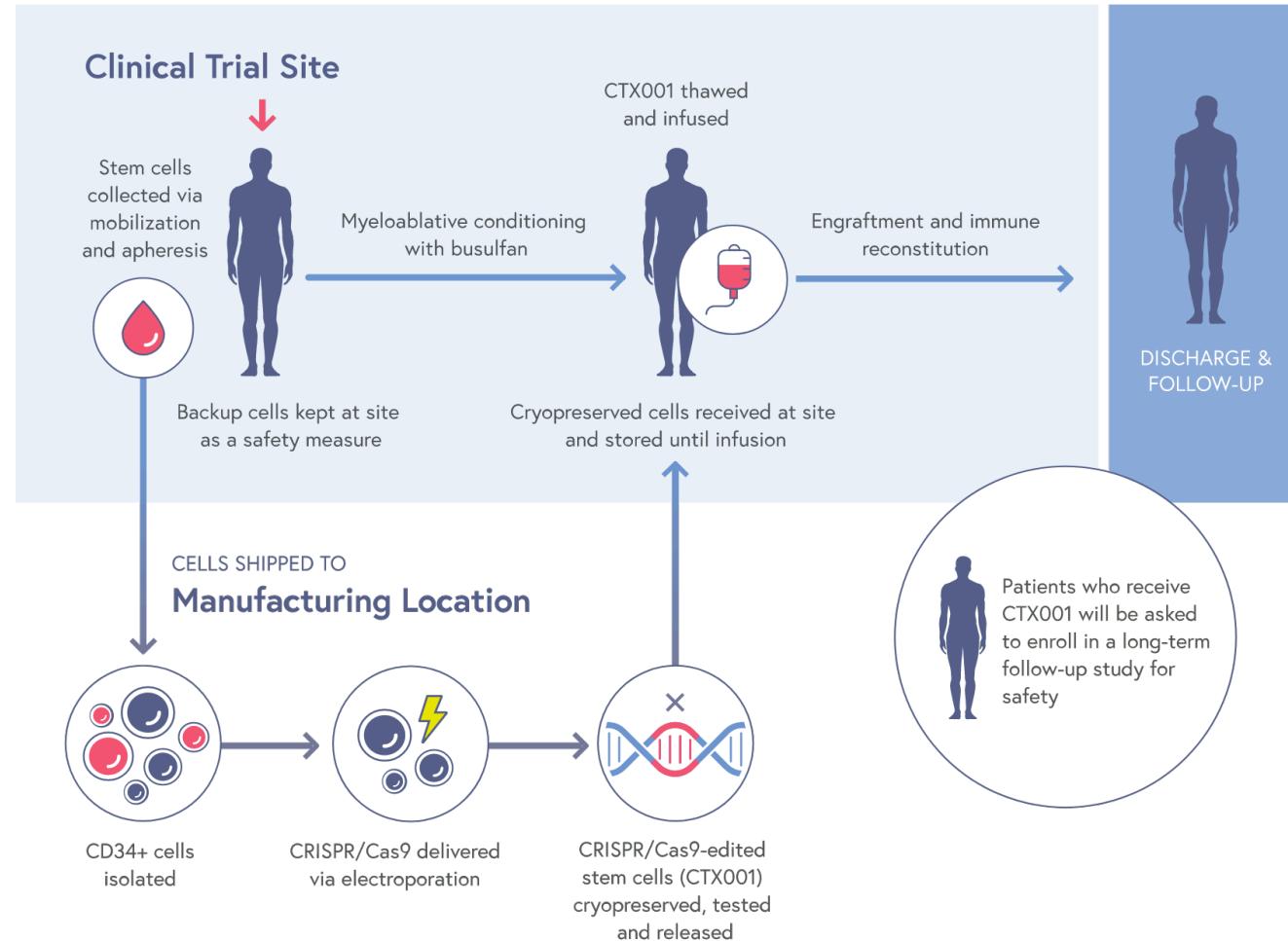
CTX001- CRISPR THERAPEUTICS

CRISPR targeting (knockout) of **BCL11A Transcription**

**Repressor factor** that suppresses the production of fetal hemoglobin (HbF) in red blood cells (RBCs)



# CRISPR/Cas9 Cellular Therapeutics (CTX001) – The Next Generation of Cures – Sickle Cell Anaemia



CRISPR Therapeutics: ClinicalTrials.gov Identifier:  
NCT03655678

Source: <https://innovativegenomics.org/news/crispr-clinical-trials-2022/>

- Patients treated for sickle cell disease (SCD) or beta thalassemia show normal to near-normal hemoglobin levels, where at least 30% (SCD) or 40% (beta thalassemia) of hemoglobin is fetal hemoglobin.
- Patients with beta thalassemia are free from needing blood transfusions. Patients with SCD are free from transfusions and disabling pain crises.
- Molecular tests on bone marrow from each of six patients a year or more after treatment show the continued presence of genome-edited cells
- Medical Regulatory Authority UK – announced CRISPR **Casgevy (CTX001)** approved for sickle cell anaemia – 16/11/23 – **first approved CRISPR therapy in the world** (£1.65 million per patient treatment!) – approx. 2,000 suitable patients
- National Institute for Health and Care Excellence (NICE) UK approved Casgevy for older children and adults with a severe form of thalassaemia – estimated approx. 430 suitable patients - 08/08/24

# CRISPR/Cas9 Cellular Therapeutics – The Next Generation of Cures - *in vivo*

- First *in vivo* CRISPR clinical trial- Sept 2019 start
- Leber's congenital amaurosis 10 (LCA10) trial for genetic blindness condition
  - direct injection into the eye with;
- Adeno-associated virus (AAV) vector carrying sgRNA and CAS 9 enzyme designed to eliminate the mutation in CEP290 gene that encodes a protein important for vision.
- 2 to 3 per 100,000 newborns incidence

# CRISPR editing *in vivo* - Leber Congenital Amaurosis (LCA) clinical trial

## LCA10 Photoreceptor

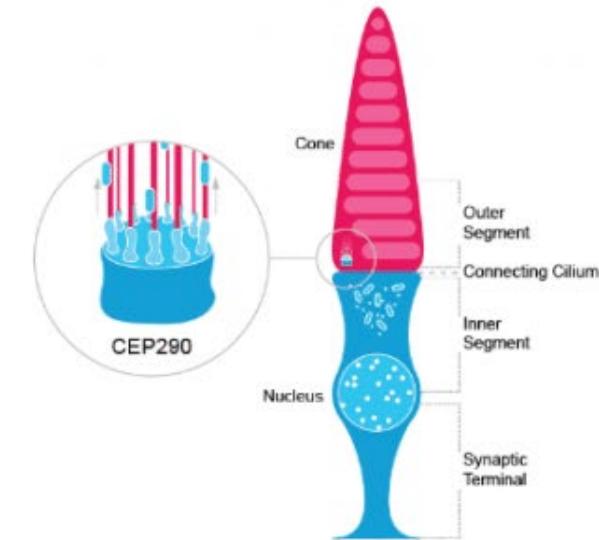
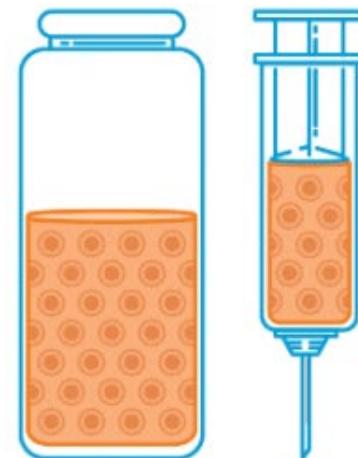
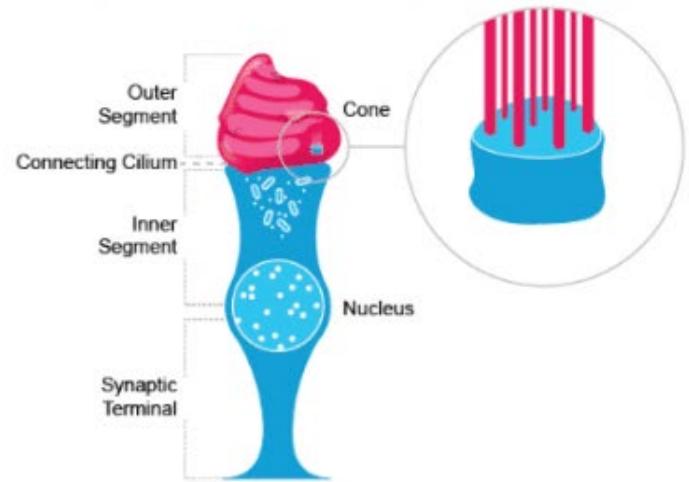
Degenerates because CEP290 lacking

## EDIT-101

Removes disease-causing mutation

## Rescued Photoreceptor

By correcting CEP290 protein



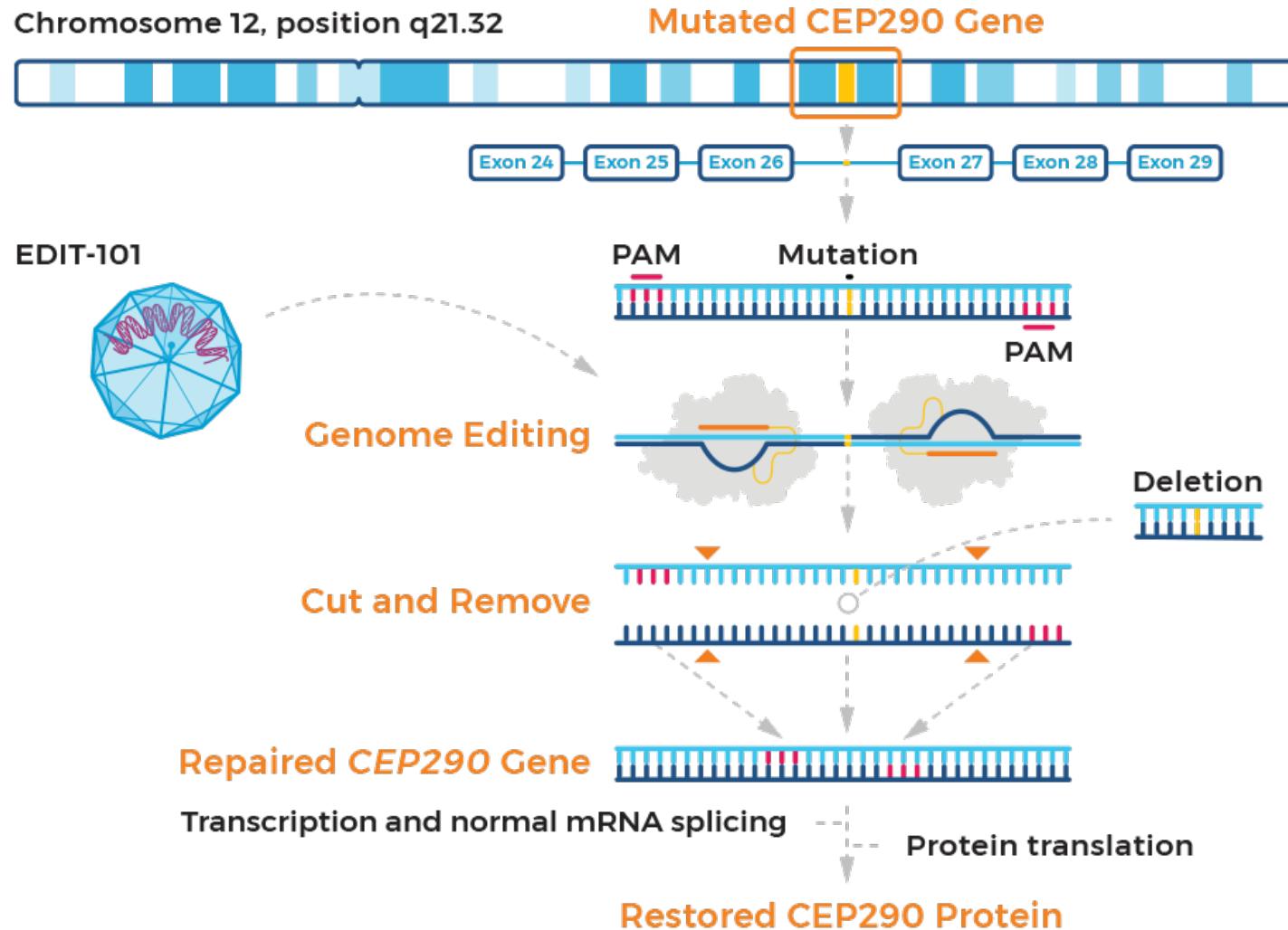
Degeneration of outer segment but cell body remains intact

EDIT-101 subretinal injection to remove disease-causing mutation

Restoration of full-length protein and rebuilding of outer segment

# Leber Congenital Amaurosis (LCA) clinical trial

<https://www.editasmedicine.com/gene-editing-pipeline/>

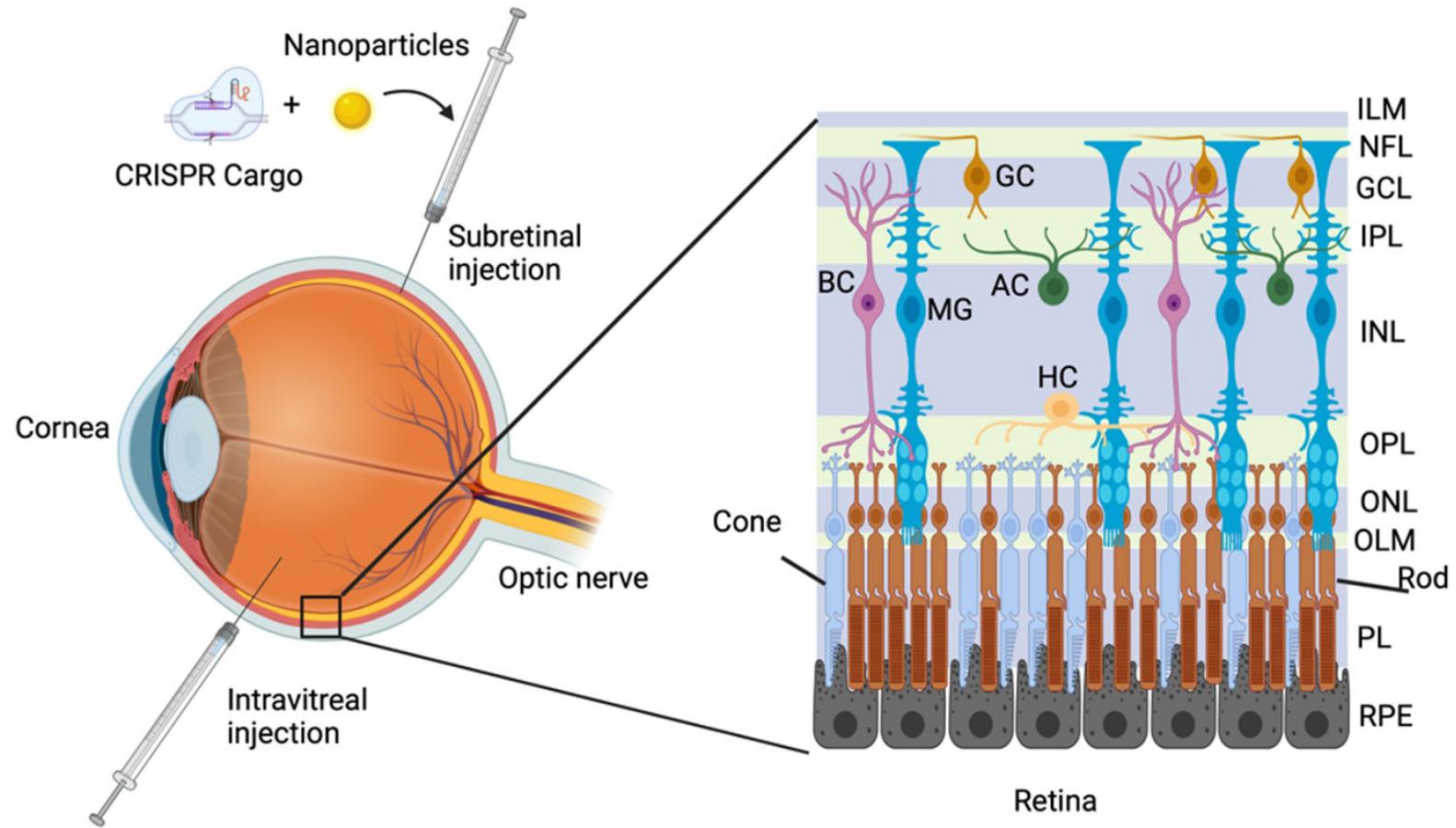


## Editas Medicine Announces Clinical Data Demonstrating Proof of Concept of EDIT-101 from Phase 1/2 BRILLIANCE Trial November 17, 2022

"Three out of 14 treated subjects met a responder threshold having experienced clinically meaningful improvements in best corrected visual acuity (BCVA) (LogMAR >0.3) and demonstrated consistent improvements in two of the following three additional endpoints: full field sensitivity test (FST), visual function navigation course (VFN), or the visual function quality of life (VFQ)."

# Delivery of CRISPR therapeutics to the eye

surgically accessible; low immunogenicity

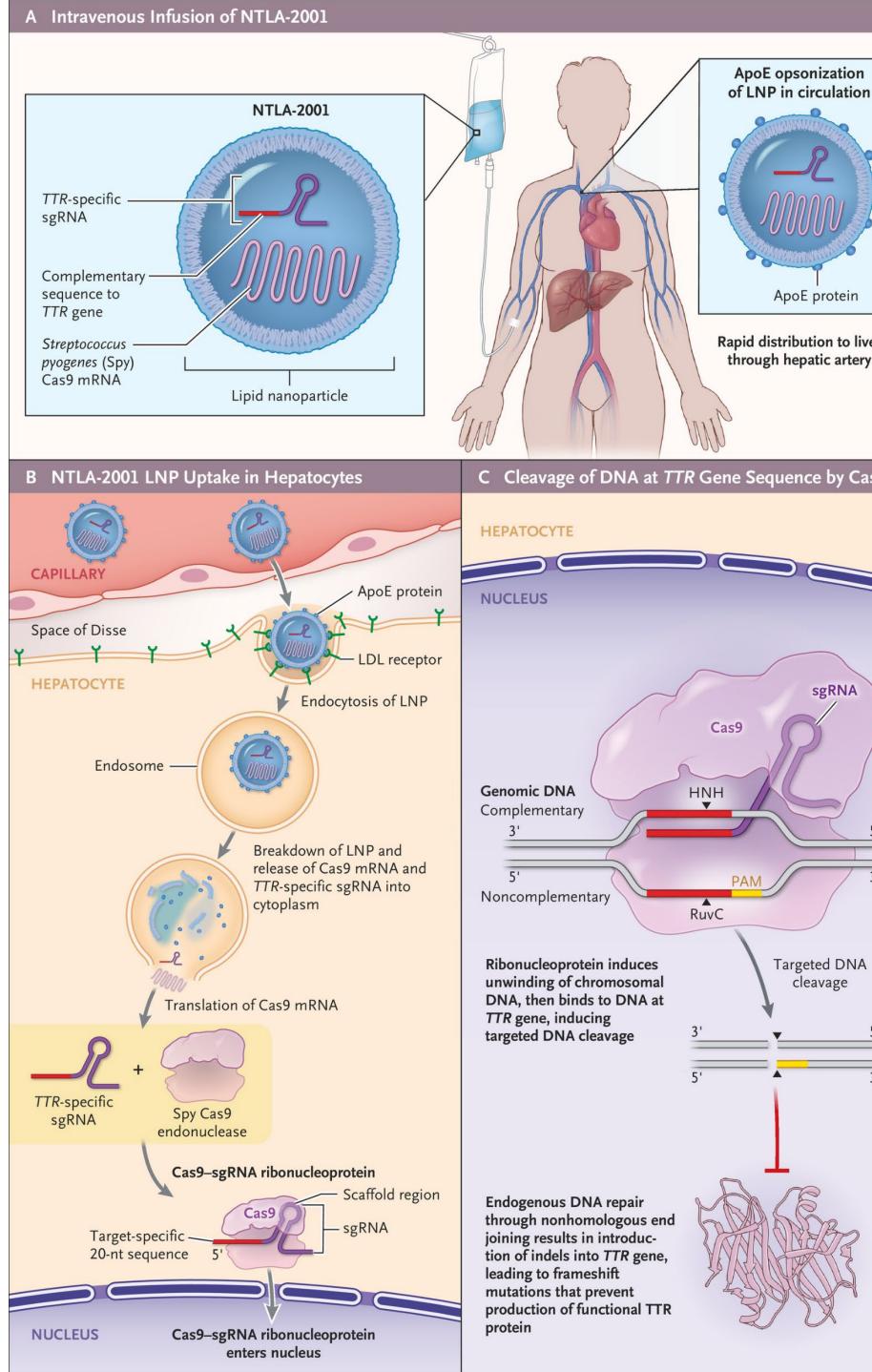


# CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

Gillmore et al., N Engl J Med 2021; 385:493-502 DOI: 10.1056/NEJMoa2107454

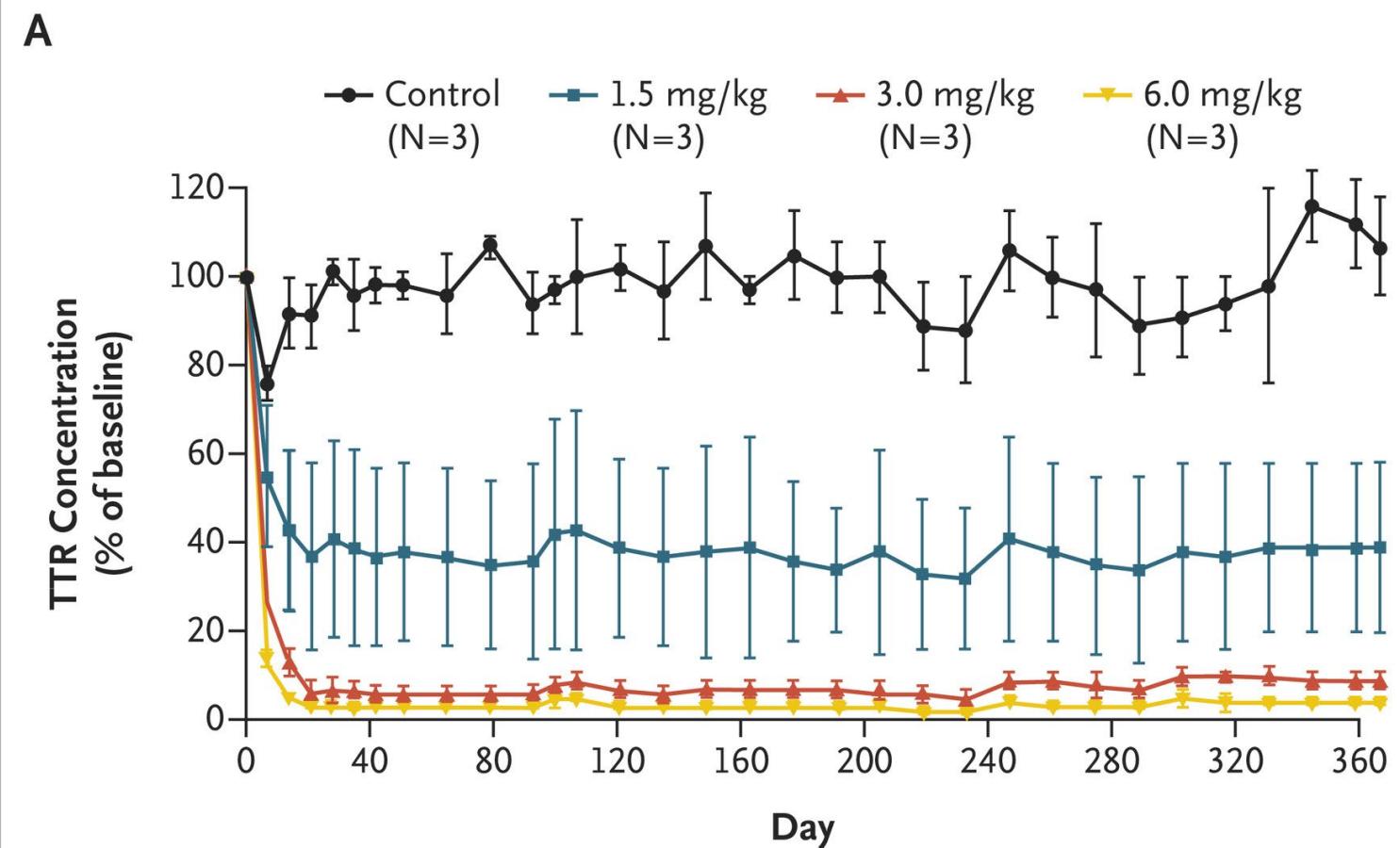
- **Transthyretin amyloidosis** – is a progressive fatal disease characterized by accumulation in tissues of amyloid fibrils composed of misfolded **transthyretin (TTR)** protein.
  - It can be inherited but also can also be acquired.
  - Over 100 different TTR pathogenic mutations described cause the disease
  - It is progressive fatal disease that causes cardiac failure.
  - **transthyretin (TTR)** is a transport protein produced in the liver

# Figure 1. Mechanism of Action of NTLA-2001 – CRISPR for TTR amyloidosis



Gillmore et al., N Engl J Med 2021;  
385:493-502 DOI:  
10.1056/NEJMoa2107454

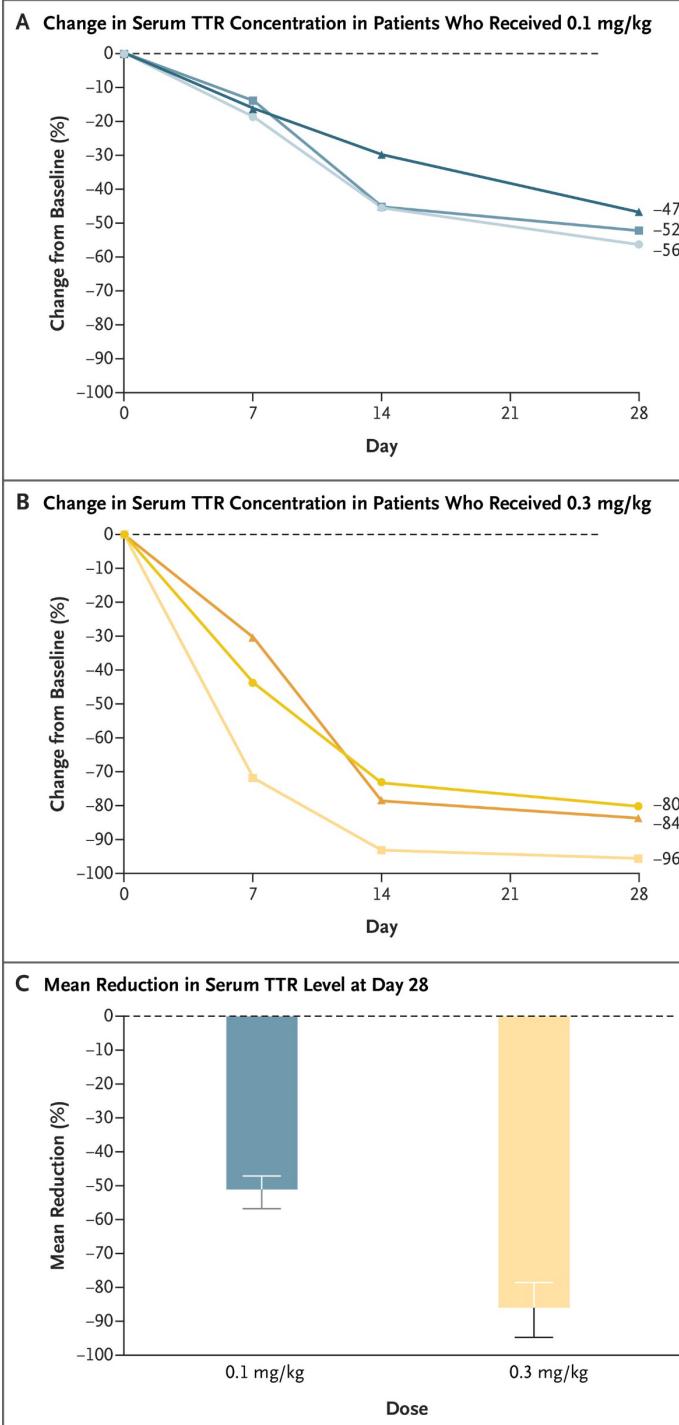
**Figure 3. In Vivo Pharmacologic Properties of Cyn-LNP, the Nonhuman Primate Surrogate of NTLA-2001.**



**B**

Indel Size	Sequence	Indel Frequency %
Wild type	AGACACAAATACCAGTCCAGCGAGGAGAG [ G / A ] AGGAGCAG	—
+1	AGACACAAATACCAGTCCA <del>A</del> GCGAGGCAGAG [ G / A ] AGGAGCAG	98.9
>+1	AGACACAAATACCAGTCCAN <del>G</del> CGAGGCAGAG [ G / A ] AGGAGCAG	1.03

## Figure 4. Reductions from Baseline in Serum TTR Protein Concentration after Infusion of NTLA-2001 in Humans.



**Intellia Therapeutics Receives U.S. FDA Orphan Drug Designation for NTLA-2001, an Investigational CRISPR Therapy for the Treatment of Transthyretin (ATTR) Amyloidosis**

**21/10/21**

**Phase 3 study started 13/12/23  
Trial no. NCT06128629**

# SUMMARY

- CRISPR has potential to identify new therapeutic targets in cancer research
- CRISPR cancer therapies showing promise but still a long way to go and are expensive at present
- The prospect of CRISPR strategies “curing” some genetic and other diseases is very exciting
- Some significant challenges still remain to be overcome for future success of CRISPR therapeutic strategies