CRISPR technology and its application in cancer

Maider Fagoaga Eugui
Bachelor's Degree in Biochemistry Final Project
Universitat Autònoma de Barcelona

Objectives

—Describe the **CRISPR system** in **prokaryotes**.
—Specify the adaptation for **gene editing**.
—Analyze **application** of CRISPR in **cancer**.

CRISPR technology for gene editing

How to stablish a CRISPR/Cas approach?

1. Guide RNA design

-Determine the **locus** to be mutated.
-Using bioinformatic programs analyze:
- ➢ **PAM** sites
- ➢ **Off-targets**

-Choose **20 nucleotides long** sequence.
*sgRNA: single-chain chimeric guide RNA (crRNA+tracrRNA)

2. Generation of gRNA (guide RNA) and Cas9

a) Two **DNA plasmids**.
-Appropriate promoters
-Cas9: codon-optimized
b) Two **mRNAs**.
-Cas9: 5' cap and 3 polyA + codon-optimized.
c) **RNA/protein** (RNP) complex.

3. Interference phase

-gRNA and Cas9 form a complex.
-Recognition and binding to the target sequence.
-Target DNA cleavage by Cas9.

Type II CRISPR/Cas9 immune system

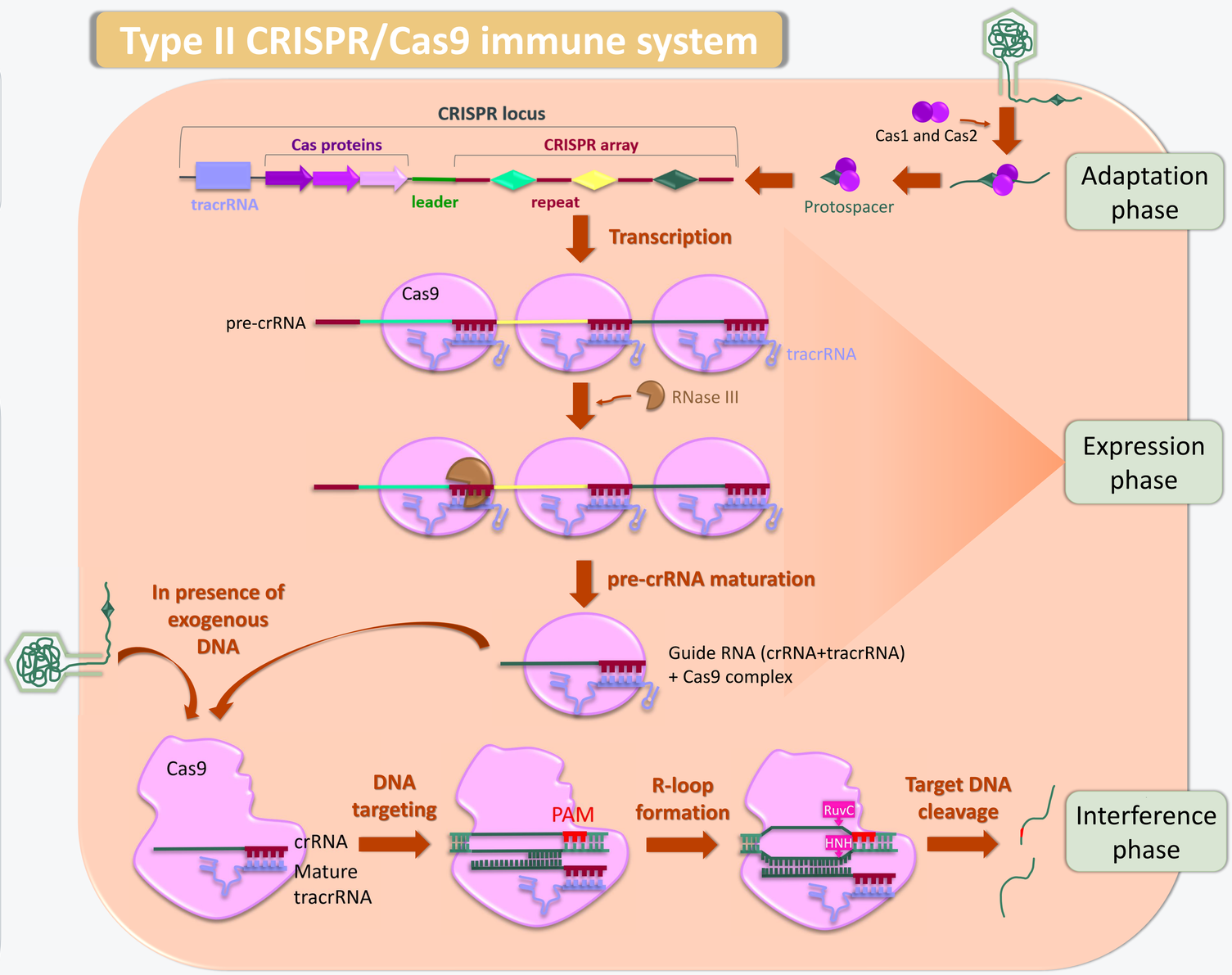

Figure 1. CRISPR/Cas9 immunity is based in three major phases: adaptation, expression and interference. In the adaptive phase, the protospacer sequence near a specific protospacer adjacent motif (PAM) is integrated by Cas1 and Cas2 into the CRIPSR locus. In the expression and interference phase, precursor CRISPR RNA (pre-crRNA) is synthesized and processed by RNase III into crRNAs, which forms a complex with tracrRNA and Cas9. When this complex pairs with exogenous DNA by recognition of the PAM by Cas9 and complementarity between crRNA and the protospacer, Cas9 cuts the foreign DNA.

Novel Cas proteins

The most commonly used Cas9 in genome editing is the one of *S. pyogenes* (**SpCas9**) that recognizes 5'-NGG-3' PAM.

Cas9n (nickase enzyme)	**dCas9** (nuclease deficient Cas9)	**Cas9-HF1** (high fidelity Cas9)	**SaCas9**	**Cas12a**
⬇	⬇	⬇	⬇	⬇
Single-stranded cut (nick).	Target modifications of loci.	No off-targets.	Encoded by a 3,2Kb sequence.	Staggered DNA breaks.

Application of CRISPR in CAR T cell therapy

Why is programmed cell death protein-1 (PD-1) knocked-out?

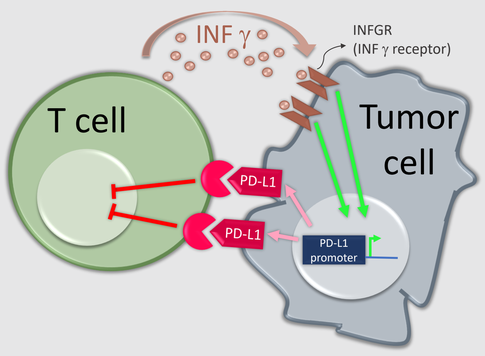

Figure 3. Inhibition of T cell antitumor response. Activated CD8+ T cells secrete interferon γ (INF γ) that upregulates programmed cell death ligand-1 (PD-L1) expression in tumor cells. Binding of PD-L1 to PD-1 inhibits CD8+ T cell cytotoxic activity.

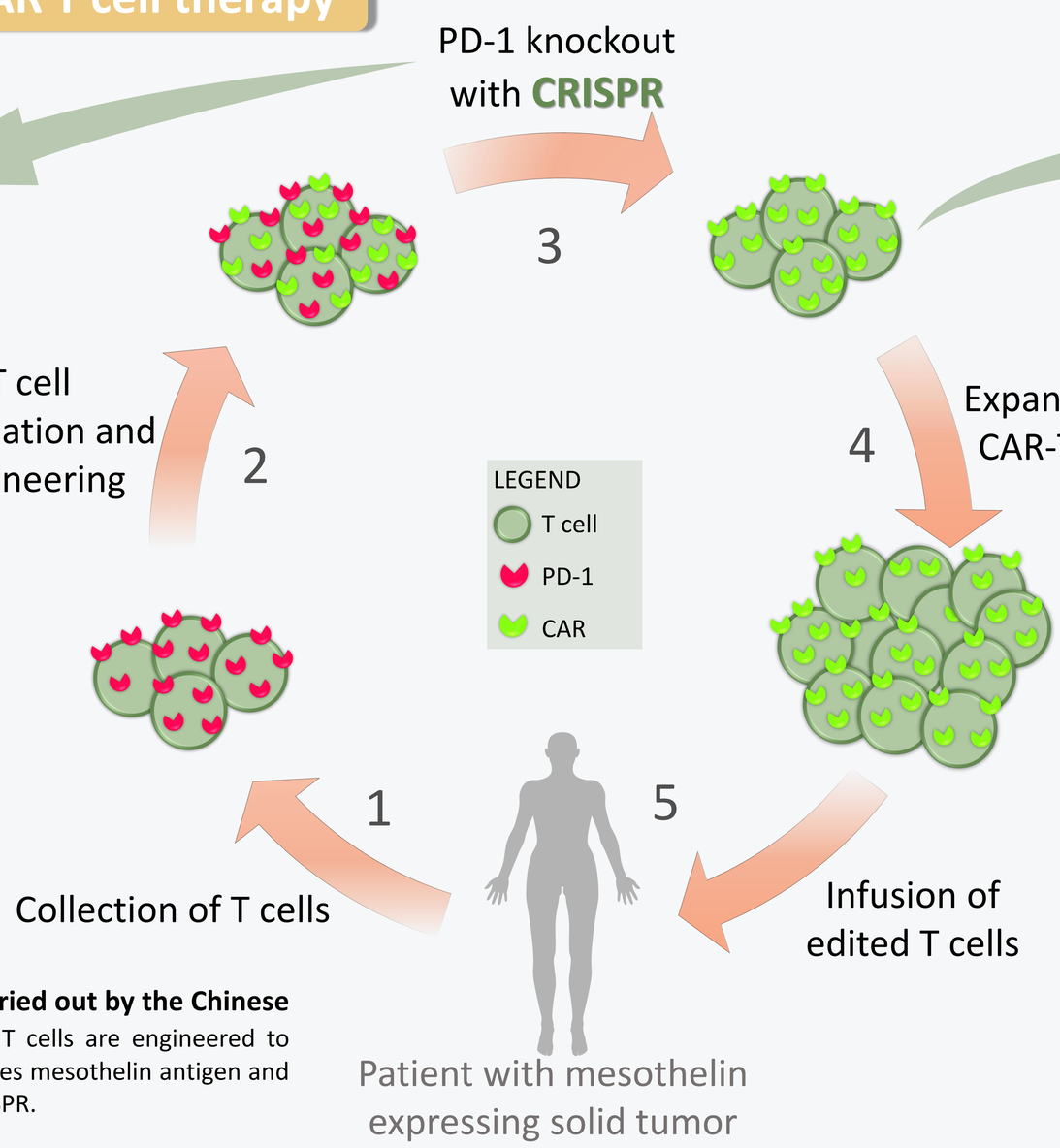

Figure 2. Workflow of a Phase I clinical trial carried out by the Chinese PLA General Hospital (NCT03747965). Patient T cells are engineered to express CARs whose antigen binding domain recognizes mesothelin antigen and endogenous PD-1 expression is knocked-out with CRISPR.

Chimeric Antigen Receptor (CAR)

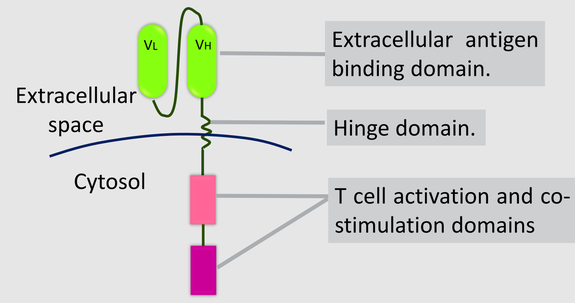

Figure 4. Receptor structure.

Conclusions

1. CRISPR technology is **faster**, **simpler** and **cheaper** comparing with the design of Zinc Fingers or Transcription activator-like effector nucleases (TALENs).
2. Improvements in CRISPR **efficiency** and **off-targets** effects due to **Cas9 variants.**
3. PD-1 knockout in CAR-T cell therapy **decreases** T cell **exhaustion** and **increases** their **persistence**.

References. **(1)** Jinek, M. *et al.* A Programmable Dual-RNA – Guided. *Science* 337, 816–822 (2012). **(2)** Doudna, J. A. & Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, (2014). **(3)** Kleinstiver, B. P. et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529, 490–495 (2016). **(4)** Rafiq, S. *et al.* Targeted delivery of a PD-1-blocking scFV by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat. Biotechnol.* 36, 847–858 (2018). **(5)** https://www.clinicaltrials.gov/