# CRISPR/Cas9-mediated genome editing in T-cells using non-viral nanovectors

**Bruna My**[1], Antonio Galeone[2], Gabriella Leccese[2], Gabriele Maiorano[2], Ilaria E. Palamà[2] and Giuseppe Gigli[2,3]

[1]Department of Mathematics and Physics, University of Salento, Monteroni Street, 73100 Lecce, Italy
[2]Nanotechnology Institute, CNR-NANOTEC, Monteroni Street, 73100 Lecce, Italy
[3]Department of Experimental Medicine, University of Salento, Monteroni Street, 73100 Lecce, Italy
bruna.my@unisalento.it (B.M.)
antonio.galeone@nanotec.cnr.it (A.G.)
gabriella.leccese@nanotec.cnr.it (G.L.)
gabriele.maiorano@nanotec.cnr.it (G.M.)
ilaria.palama@nanotec.cnr.it (I.E.P.)
giuseppe.gigli@unisalento.it (G.G.)

## Abstract

Immunotherapy using Chimeric Antigen Receptor (CAR)-T cells is one of the most exciting recent developments in cancer treatment [1]. The therapy involves genetic modifications of a patient's T-cells to improve immune activation against cancer cells (Figure 1) [2]. The process requires the use of viral vectors for efficient and stable DNA editing of T cells with relevant side effects related to unsafety procedures, potential for integration into the host genome, long-term effects in terms of mutagenesis and carcinogenesis [3]. This is leading to the necessity of using alternative delivery vectors for genome engineering of T-cells.
Nanovectors (NVs) are promising delivery system having unique physical, chemical, and biological characteristics. Some of them, made of lipids, ceramic, metallic, polymeric materials, find use in the biological world [4]. A class of carbon-based nanomaterials, with sizes typically less than 10 nm, is represented by Carbon Dots (CDs), emerged as promising nanomaterials for gene delivery due to their properties including biocompatibility, since CDs are generally non-toxic and well-tolerated by biological systems, tunable surface functionality, and cellular uptake [5]. Giving that, we are developing biocompatible and biodegradable nanovectors, to prevent the side effects of viral carriers, coupled with the CRISPR/Cas9 technology for modifying precisely the genome of T-cells (Figure 2). In particular, we have chosen one of relevant gene involved in escape of immunosurveillance as *PDCD1* (encoding PD-1), an inhibitory receptor that, through the binding to its ligand, PD-L1, promotes self-tolerance (Figure 3) [6]. Turning off PD-1 genetically means inducing autoimmunity, so is more appropriate to endogenously tune the binding affinity of PD-1 with PD-L1. We provide evidences that new synthetized polymeric CRIPSR/Cas9-nanovectors (Cas9_NVs) are able to target *PDCD1* at genomic and protein level. We are aiming to introduce site-specific modifications of *PDCD1* which lead to tune the affinity for its ligands. This may pave the way for new therapeutic avenues offering highly innovative and promising technology for immunotherapy of cancer at genomic level.

## Acknowledgements

This study was supported by EU funding within the MUR PNRR "National Center for Gene Therapy and Drugs based on RNA Technology", "Tecnopolo per la medicina di precisione" (TecnoMed Puglia) - Regione Puglia and Hub Life Science – Terapia Avanzata (LSH--TA) PNC-E3-2022-23683269, EU funding within the PNC Italian Health Ministry.

## References

[1] Del Bufalo, Francesca, et al. "GD2-CART01 for relapsed or refractory high-risk neuroblastoma." New England Journal of Medicine 388.14 (2023): 1284-1295.
[2] Kershaw, Michael H., Jennifer A. Westwood, and Phillip K. Darcy. "Gene-engineered T cells for cancer therapy." Nature Reviews Cancer 13.8 (2013): 525-541.
[3] Lundstrom, Kenneth. "Viral vectors in gene therapy." Diseases 6.2 (2018): 42.
[4] Wilczewska, Agnieszka Z., et al. "Nanoparticles as drug delivery systems." Pharmacological reports 64.5 (2012): 1020-1037.
[5] Hashemzadeh, Iman, et al. "Polyethylenimine-functionalized carbon dots for delivery of CRISPR/Cas9 complexes." ACS Applied Bio Materials 4.11 (2021): 7979-7992.
[6] Kythreotou, Anthousa, et al. "PD-L1." Journal of clinical pathology 71.3 (2018): 189-194.

## Figures

**Figure 1.** A schematic representation of CAR-T cell therapy. Peripheral blood is collected from a patient, T-cells are separated by apheresis from all other blood components and are genetically engineered to express a CAR receptor. Then, CAR-T cells are expanded and put back into the patient's bloodstream.

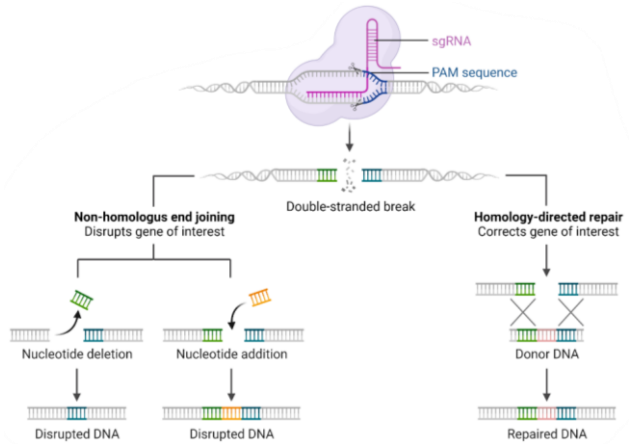


**Figure 2.** CRISPR-Cas9 genome editing system. This system targets the gene of interest by a single-guide RNA (sgRNA) that is designed to recognize the target DNA site via Watson–Crick base pairing. During the CRISPR editing process on successful recognition of the target, Cas9 undergoes a conformational change that involves its two nuclease domains. The nuclease domains cleave both strands of the target DNA about three nucleotides before the Protospacer Adjacent Motif (PAM) sequence, generating double-stranded DNA break (DSB). There are two mechanisms to repair the DSB: non-homologous end joining (NHEJ) and homology-directed repair (HDR).

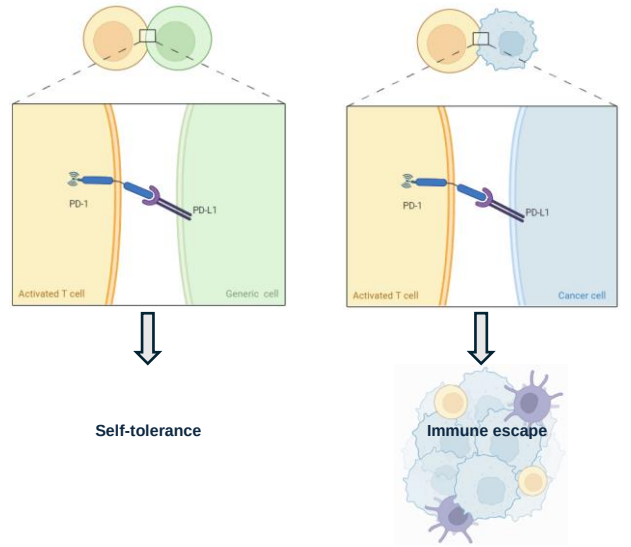


**Figure 3.** Biological role of PD-1 and its implication in cancer.