Development of advanced genetic tools in combination with nanovectors for T-cell engineering

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Gene therapy has evolved into a powerful tool for cancer treatment (fig.1). Although viral vector-mediated gene therapies are the most widely used due their high transfection efficiency, safety risks and immunogenicity still make their use questionable [1]. Meanwhile, non-viral vector could bypass the intrinsic limitation of viral vector as they offer higer biosafety and simpler large-scale production methods [2]. Despite these potentials, several obstacles to their applications are still present, thus limiting their clinical applications. In particular, their inefficient nuclear targeting represents a critical issue, mainly due to inevitable trapping in lysosomes with subsequent degradation. For this reason, several strategies have been adopted in order to overcome the low gene expression of non-viral nanovectors. In this context, the aim of our research is the design and validation of biocompatible, non-viral gene vectors able to deliver the CRISPR/Cas9 biomacromolecular machine for editing target genes by ensuring targeting, uptake and lysosomal escape (fig.2). This is made possible by the use of a polymeric nanocarrier named CAS-NV based on the proper assebly of cystamine as stimuli responsive mojety, agmatine [Agm] for the binding of nucleic acids, and benzenesulfonamide [BS]derivative for specifically targeting the endoplasmic reticulum (ER). By this strategegy, three functional domains aim to improve the critical issues encountered during cell transfection (fig.3). BS promotes the binding of complexes to the endoplasmic reticulum which, being the organelle closest to the nucleus, contributes to the nuclear uptake of genes. Agm is involved in the binding of the biologically active cargo, and, additionally, in the lysosomal escape, while cystamine acts as a glutathione GSH-sensitive linker to control the release of CRISPR/Cas9 payload, as high intracellular levels of GSH cause a sensitive disruption of disulphide bonds, resulting in gene release in a controlled manner [3]. The polymer was synthesised using a Michael addition and finally analysed using different techniques. The polymer thus obtained was used to complex a plasmid coding for Cas9 that was named ‘Multiplex’, into which we cloned two single guides RNA (sgRNAs) that direct the Cas9 nuclease against the Regnase-1 and PCD1 genes respectively. These are two crucial master genes in immune activation, involved in T-cell exhaustion, a dysfunctional state in which T-cells show low persistence in circulation and at the tumour site. Editing these genes should alter the expression of factors involved in modulating T-cell activity, inducing a downregulation of their cell cycle, reducing proliferation and, consequently, increasing persistence at the tumour site, thus improving therapeutic efficacy [4]. The non-viral CRISPR/Cas9 nanocarriers generated have been characterized by employing different techniques. First results indicate that the proper design of non-viral gene vectors could pave the way for new advanced approaches in cancer immunotherapy also allowing the intrinsic obstacles of intracellular pathways to be circumvented, making it a promising tool in gene therapy (Fig. 4) [5].

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Figures

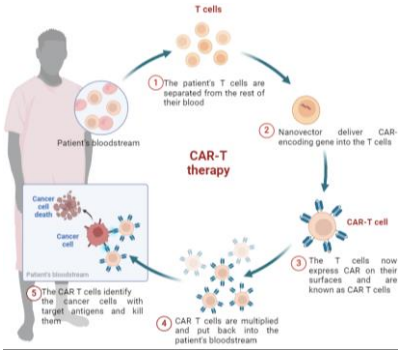

Figure 1. Representation of the production and administration of CAR-T therapy.

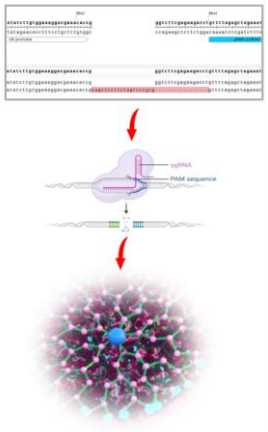

Figure 2. Cloning of the single guide RNA for CRISPR/Cas9 system in a plasmid vector to be encapsulated in polymeric nanocarriers.

Figure 3. Illustration of CAS-NV gene vector, with the respective functions of the domains of which it is composed.

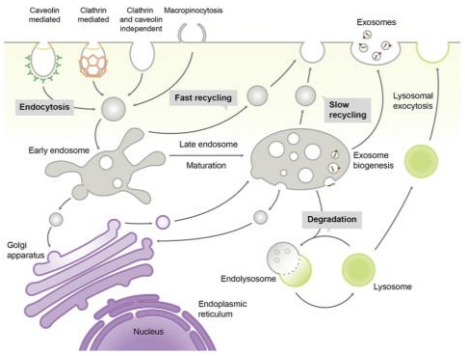

Figure 4. Targeted ER strategy to improve gene transfer and simplify non-viral vector-based gene transfection [The Dynamic Nature of the Nuclear Envelope Paola De Magistris and Wolfram Antonin Current Biology 28, R487–R497, April 23, 2018ª Elsevier Ltd].