PROJECT

DoS: Angelo Lombardo

Title: Development of multifunctional editing strategies for adoptive T-cell immunotherapy of cancer.

Curriculum: Gene and Cell Therapy (GCT)

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/epigenetic-regulation-and-targeted-genome-editing.html

Project description:

Adoptive transfer of genetically engineered T lymphocytes has provided remarkable clinical benefits for the treatment of several types of cancer (1). In this context, the targeting specificity of autologous or allogenic T cells is redirected against tumor-related antigens through de novo expression of a transgenic TCR or a chimeric antigen receptor (CAR). Although endowed with such new powerful properties, transplanted cells often fail to exert their biological function, because limited tumor trafficking, premature exhaustion and/or deactivation in the tumor microenvironment. Molecular dissection of these roadblocks in periclinal models and clinical samples is providing a wealth of novel actionable targets for more effective cancer immunotherapy. These advances, however, pose the challenge of implementing innovative tools able to perform orthogonal engineering (e.g., targeted transgene insertion, gene knock-out and activation) at multiple genomic sites (i.e., multiplexing) without perturbing T cell effector function and survival. To cope with this, the field is rapidly moving towards genome editing (2), given its versatility in terms of genetic engineering outcomes and potential scalability. However, a notable safety concern related to the use of genome editing for multiplexing is induction of genetic aberrations, both at and among the targeted genes. Indeed, clinical and preclinical studies of multiplex editing in CAR-T cell products reported alarming levels of chromosomal translocations (3). Base editing technologies promise to solve this issue, but their targeting precision awaits further investigations. Yet, as currently designed, this type of editors can alter single or few proximal bases, reducing the range of T cell engineering opportunities. Based on these premises, the goal of this project is to develop multifunctional editing platforms for the generation of safe multiplexed T-cell products. This will be achieved by iterative rounds of optimization of state-of-the-art editing platforms based on the CRISPR/Cas9 system, coupled with tailored refinements of T cell manipulation procedures to maximize multiplexing efficiency while reducing genotoxicity. These studies will be complemented by high-throughput target gene discovery activities, together with in-depth characterization of the mechanism of action of the novel editors, their targeting specificity and cellular responses to treatments. Functional in vitro assays will be used to indicate the most effective platform(s) among those developed. Potency and safety of the so generated T cells will be evaluated in ad hoc mouse models of cancer. Eventually, process development activities for advanced therapy medicinal products based on the engineered T cells will be launched, thanks also to an already established industrial partnership. If successful, these studies will deliver innovative editing strategies, paving the way to safe multiplexing in T cells and beyond.

Skills to be acquired by the student:

Technical skills: advanced molecular biology techniques; multiparametric flow cryometry analyses; gene and epigenetic editing; T cell culture, manipulation and functional assays; in vivo assays of cancer immunotherapy; genome-wide screens; epigenetic and transcriptomic analyses.

Other expertise: design, perform, interpret and conduct research independently; present data at national and international venues; draft manuscripts.

References (max. 3)

1- Genetic engineering of T cells for immunotherapy. Nat Rev Genet. 2021 Feb 18. PMID: 33603158
2- The next generation of CRISPR-Cas technologies and applications. Nat Rev Mol Cel Biol. 2019 Aug;20(8):490-507. PMID: 31147612
3- CRISPR-engineered T cells in patients with refractory cancer. Science. 2020 Feb 28;367(6481). PMID: 32029687