PROJECT 1

DoS: Monica Casucci

Title: Identification of a novel CAR specificity to tackle solid malignancies

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page:

http://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/innovative-immunotherapies.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

CAR T cell therapy has recently changed the landscape of treatment options for refractory B-cell malignancies, leading to the recent approval of the first CAR T cell products targeting CD19. Despite the enthusiasm, however, first-in-man studies against solid tumors revealed unique hurdles contributing to the lack of a sharp demonstration of efficacy. Among others, the identification of a suitable target antigen is particularly challenging because surface molecules expressed on tumor cells are generally shared with normal tissues and often intratumorally heterogeneous.

Within this project, we propose to develop a novel CAR specificity based on the data emerging from the AIRC 5x1000 project running at our institution. We will start by analyzing RNAseq data of liver metastasis samples derived from patients with colorectal cancer (CRC) and pancreatic adenocarcinoma (PDAC). A sequential process will lead to identify potential candidate proteins, by selecting genes encoding for surface proteins and with low expression on healthy tissues. The expression of selected target antigens will then be validated by FACS analysis from fresh patient samples derived from the LIMET Protocol. Once a suitable antigen will be identified, we will take advantage of our consolidated expertise to generate and validate the new CAR molecule. Single-chain variable fragments (scFv) from one or more available specific antibodies will be used to generate a panel of CAR constructs. Antigen binding moieties will be combined to extracellular spacers of different lengths, which confer different flexibility to bind epitopes placed in different positions. The different combinations will be built on backbones carrying the CD3 zeta chain alone or in combination with CD28 or 41BB endodomain regions. The resulting panel of constructs will be tested for correct folding and expression on the cell membrane. Afterwards, the constructs showing correct folding will be screened for ability to induce transcriptional activation in T cells, and CAR-T cell functionality will be deeply investigated in vitro, both in terms of efficacy and toxicity profile against heathy cells.

After in vitro validation, the new CAR construct will be tested for antitumor activity in immunodeficient mice engrafted with human tumor cell lines. Finally, we will take advantage of a more sophisticated mouse model we recently developed (Norelli M, Nat Med 2018), in which malignant cells co-exist with human healthy hematopoietic cells, to study not only efficacy but also CAR-related toxicities, including the cytokine release syndrome and neurotoxicity.

Skills to be acquired by the student:

Hard skills: Design and cloning of lentiviral vectors; Synthetic biology competences for CAR construction; Basic and advanced in vitro studies with CAR-T cells (flow-cytometry characterization and functional testing); efficacy and safety studies with CAR-T cells in complex xenograft in vivo models (including or not reconstitution with human HSCs).

Soft skills: Critical analysis of scientific literature; Design and management of scientific projects; Data presentation and discussion at internal lab meetings; Scientific presentations at Institutional progress reports and at International conferences; Thesis and manuscript writing; Tutoring of younger undergraduate students.

References (max. 3)

Norelli M, Camisa B, Falcone L et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine release syndrome and neurotoxicity by CAR-T cells. Nat Med 2018.

Casucci M, Falcone L, Camisa B et al. Extracellular NGFR spacers allow efficient tracking and enrichment of fully functional CAR-T cells co-expressing a suicide gene. Front Immunol 2018.

Casucci M, Nicolis di Robilant B, Falcone L et al. CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. Blood 2013.