PROGETTO

Supervisore:	Monica Casucci
Titolo/*Title*:	Optimizing the therapeutic index of CD44v6 CAR T cells
Curriculum:	Gene and Cell Therapy
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	_https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/innovative-immunotherapies.html_

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

T cells engineered to express chimeric antigen receptors (CAR-T cells) represent a potent weapon against B-cell tumors. This success has fueled the search for additional CARs to expand these result to other tumor indications. In this regard, we have selected CD44v6 as a promising target for adoptive cancer immunotherapy approaches. CD44v6 is a membrane glycoprotein over-expressed in hematological tumors, including multiple myeloma (MM), and solid malignancies, including carcinoma and sarcoma. In the past, we have tested different CAR designs and used several in vitro and in vivo models to demonstrate the therapeutic efficacy of CD44v6 CAR-T cells products against MM, acute myeloid leukemia[1,2] and different types of carcinomas[3]. Preliminary expriments suggest that CD44v6 CAR-T cells can cause cytokine release syndrome (CRS) similarly to CD19 CAR-T cells, but with a different kinetics due to CD44v6 expression on monocytes. Despite CD44v6 expression in both monocytes and keratinocytes, the only on-target off-tumor toxicity predicted in preclinical models was monocytopenia. However, despite this encouraging results, the potential risk for severe skin reactions in patients prompted the addition of a suicide gene to ablate CD44v6 CAR-T cells at need.

Our mission is to build upon on these results to expand the applicability of the CD44v6 CAR-T cells. We plan to further optimize CAR design and apply combination strategies to enhance efficacy, especially in solid tumors, which poses unique barriers to CAR-T cell function. At the same time, however, the main focus of this project will be to develop a strategy for skin de-targeting without the need of induce CAR-T cell suicidal that may restrain antitumor efficacy. Hence, we plan to develop a new inhibitory CAR (iCAR), capable of suppressing T-cell activation when the target cell expresses selected ligands associated with non-malignant tissues[6]. The first step for reaching this objective is to search for a suitable antigen expressed on healthy squamous epithelia but not malignant cells. For this reason, we will establish a proof-of-concept construct on MM as a model disease, to then shift toward more challenging settings like

sarcomas o adenocarcinomas. Novel CAR-T cell products will be challenged both in vitro and in vivo to test their potential to recognize cells expressing the CAR antigen with or without the target of the iCAR. In addition, the new cell products will be infused in HPSC-humanized animals for a more precise testing of antitumor potential in the presence of a competent human immune system and a stringent profiling of CRS occurrence[4,5].

The continuous innovation carried by the development of CAR design with a safe therapeutic profile will deliver a new generation of CAR-T cell products with the potential of greatly impact therapeutic choices for a wide range of patients. Indeed, the novel design for an iCAR developed in the context of CD44v6 has the potential to be expanded for a wide array of CAR specificities.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

Hard skills: Design and cloning of lentiviral vectors and CAR constructs; gene disruption with Crispr-Cas9 technology; CAR-T cell production and functional testing in vitro and in complex xenograft mouse models (HPSCs-humanized immunodeficient mice); Advanced transcriptomics and proteomics analysis.

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.

Bibliografia (max. 15)

1. 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;122(20):3461–3472.

2. 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;9:507.

3. Greco B, Malacarne V, De Girardi F, et al. Disrupting N-glycan expression on tumor cells boosts chimeric antigen receptor T cell efficacy against solid malignancies. Sci Transl Med. 2022;14(628):eabg3072.

4. Arcangeli S*, Bove C*, Mezzanotte C*, et al. CAR T-cell manufacturing fromnaive/stem memory T lymphocytes enhances antitumor responses while curtailing cytokine release syndrome. J Clin Invest. 2022 Jun 15;132(12):e150807. doi: 10.1172/JCI150807.

5. Bove C, Arcangeli S et al. CAR-T cells targeting CD19 play a key role in exacerbating cytokine release syndrome, while maintaining long-term responses. J Immunother Cancer. 2023 Jan;11(1):e005878. doi: 10.1136/jitc-2022-005878.

6. Flugel CL, Majzner RG, Krenciute G, et al. Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. Nat Rev Clin Oncol. 2023;20(1):49–62.