PROJECT 1

DoS: Monica Casucci

Title: Dissecting the pathophysiology of CAR-T cell related neurotoxicity to identify new actionable targets

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page: https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/innovative-immunotherapies.html

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

Beside extraordinary antitumor activity, CAR-T cell therapy also carries the risk of significant toxicities, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), a disorder of the central nervous system typically manifesting as toxic encephalopathy characterized by aphasia, confusion, impaired motor skills, somnolence, seizures and cerebral edema. Though reversible in most patients, ICANS can also be fatal (Morris et al. Nat Med 2021). ICANS has been reported with different CAR specificities, costimulatory domains, hematological tumors and recently also in patients with solid tumors treated with armored CAR T cell products. With increased effectiveness of CAR T-cell therapies we expect to witness a worsening of this toxicity. ICANS is commonly associated with CRS development and severity, but it lacks specific therapeutic strategies. Even if it is known that ICANS includes an inflammatory component associated with increased BBB permeability, ICANS pathophysiology *per se* is poorly understood. Preclinical models are limited, and current hypotheses mainly come from autopsies performed on brains of patients who suffered from fatal neurotoxicity without comparison with mild/reversible cases. Hence, several questions remain open regarding the role of CAR-T cell trafficking to the CNS, the site and cellular source(s) of inflammatory mediators and the impact of these mediators on glial cells and neurons (Gust J et al. Front Immunol 2020).
Hence, the aim of this PhD project is to identify ICANS pathophysiological mechanisms by exploiting a highly informative humanized mouse model recently developed in our Unit, where reconstitution with a human immune system allows tumor and CAR-T cells to dialogue with other immune cells and cytokines (Norelli M et al. Nat Med 2018). In this model, a significant proportion of mice treated with CD19 CAR-T cells develop brain hemorrhages reminiscent of neurotoxic manifestations observed in patients with high-grade ICANS. In addition to ICANS, the model permits to accurately monitor CRS development, antitumor activity, and CAR-T cell kinetics and phenotype, allowing correlations to be made with relevant parameters associated with the outcome of CAR T-cell therapy. Magnetic resonance imaging will be used to reveal changes in the permeability of the BBB and the occurrence of inflammatory events. Immunofluorescence and single cell RNA sequencing of the brains will be performed to identify key pathways and cell players involved in ICANS development, while behavioral tests will be exploited to give clinical relevance to the phenomena occurring in the brains of treated mice. In parallel, we will work to identify predictive biomarkers that will help decision-making in patients, for example by allowing early intervention in patients at high risk of developing severe ICANS. These biomarkers will be validated in a cohort of patients receiving CAR T-cell therapy at our institution.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

Hard skills: Design and cloning of lentiviral vectors and CAR constructs; 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 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.

References (max. 15)

Norelli M et al. Nat Med 2018. doi: 10.1038/s41591-018-0036-4.

Gust J et al. Front Immunol 2020. doi: 10.3389/fimmu.2020.577027.

Morris E et al. Nat Rev Immunol 2022. doi: 10.1038/s41577-021-00547-6.

Mezzanotte C et al. Pre-selected CAR TN/SCM Outperform CAR TBULK in Driving Tumor Eradication in the Absence of Cytokine Release Syndrome and Neurotoxicity [abstract]. Mol Ther 2020; 28, No 4S1:P42.