PROJECT

Supervisor: VINCENZO RUSSO_____

Title: MD_Dissecting the immunesuppressive mechanisms limiting the efficacy of adoptive cell therapy in the tumor-draining lymph nodes

Curriculum: Basic and Applied Immunology and Oncology_____

Link to the personal page of the University or relevant hospital site website: https://research.hsr.it/en/divisions/experimental-oncology/immuno-biotherapy-of-melanoma-and-solid-tumors/vincenzo-russo.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Adoptive cell therapy (ACT) is an innovative therapeutic approach that promises to cure most types of cancer. However, there are still many obstacles hampering the efficacy of ACT (1, 2). The tumor/tumor-draining lymph node (TDLN) microenvironment inhibits the efficacy of antitumor T cells, including adoptively transferred cells, through multiple mechanisms, such as suppressive cytokines, aberrant ECM composition, metabolic competition, poor antigen presentation, and chronic TCR signaling (3, 4). We identified highly immunosuppressive myeloid subsets, exhausted T cells, and cancer-associated fibroblasts in the TDLNs of mice with progressing tumors compared to those rejecting tumors. These findings suggest that specific niches within the TDLNs may actively suppress adoptively transferred T cells, limiting ACT efficacy (preliminary results).

Rationale and hypothesis

Emerging data suggest that immune suppressive mechanisms also occur in the TDLNs, the primary site that supports the generation and expansion of antitumor T cell responses (3, 4); thus, suggesting that the harsh microenvironment of TDLNs restrain the full exploitation of ACT, especially in solid tumors. In this context, we recently found that mice with melanomas injected with antitumor T cells were able to control tumor growth much better than untreated mice. Despite this result, ACT mice eventually succumbed due to the low persistence and inefficiency of the T cells. Our results revealed that there are several immunosuppressive mechanisms in the TDLNs of mice with and without ACT that hinder the functionality of antitumor T cells. However, a detailed characterization of the dynamics underlying these phenomena remains to be performed in the ACT setting. Since many cancer therapies, including immunotherapy, now

include combinations of approaches, the long-term goal of this project is to improve the efficacy and persistence of adoptively transferred T cells, in particular the T stem cell memory (TSCM) cells (5), by modulating the cellular and molecular cues that currently hinder T cell performance in the TDLNs after ACT.

Objectives and specific aims

Therefore, we propose to characterize the cellular and molecular determinants responsible for the limited efficacy and long-term persistence of antitumor T cells through the following specific aims:

Aim 1. Dissect the spatial distribution of the cellular and molecular cues dampening TSCM antitumor T cells.

Aim 2. Target cellular and molecular determinants limiting the antitumor efficacy of T cells in vivo.

Expected outcomes

We aim to elucidate the phenotypic, molecular, and spatial distribution of cellular and molecular signals that suppress tumor antigen-specific TSCM in vivo, particularly within TDLNs. To achieve this, we will employ a single-cell spatial transcriptomics approach combined with immunofluorescence and flow cytometry analysis. Finally, we will use appropriate in vivo models to confirm and validate the effectiveness of strategies that inhibit immune regulatory mechanisms, ultimately restoring the function of antitumor T cells.

Skills that the student should acquire (max. 600 characters including spaces):

The candidate will develop a robust background in cellular and molecular tumor immunology. Specifically, they will gain proficiency in cell culture techniques for the activation and expansion of antigen-specific T cells under homeostatic conditions. Technical training will include in vivo methodologies (mouse handling, s.c., and i.v. injections) and molecular biology protocols for spatial transcriptomics. Furthermore, the student will master immunofluorescence and flow cytometry for advanced data analysis.

References (max. 15)

1) Rosenberg SA, Restifo NP. Science (New York, NY 2015;348(6230):62-8 doi 10.1126/science.aaa4967.

2) Anderson KG, Stromnes IM, Greenberg PD. Cancer cell 2017;31(3):311-25 doi 10.1016/j.ccell.2017.02.008.

3) van Krimpen A, et al. Cancer Cell. 2022. PMID: 35839777.

4) Zheng R, Shu S. J Surg Oncol. 2011 May 1;103(6):550-4. doi: 10.1002/jso.21692.

5) Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. Nat Med 2017;23(1):18-27 doi 10.1038/nm.4241.

6) Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, et al. CD8+ T cells. J Exp Med. 2003;198(4):569-80 doi 10.1084/jem.20030590.