PROJECT 1

DoS: CHIARA BONINI

Title: **Exploiting cutting edge technologies to hunt novel T cell receptors for the immune gene therapy of acute myeloid leukemia**

Curriculum: Cell and Gene Therapy

Link to OSR/UniSR personal page: https://research.hsr.it/en/divisions/division-of-immunology-transplantation-and-infectious-diseases/experimental-hematology/chiara-bonini.html

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

Empowering T cells with new T cell receptors (TCR) can effectively redirect some of the most potent players of our immune system against virtually all tumor antigens. Due to the low frequency of cancer-specific T cells, the identification of the ideal tumor-specific TCRs remains the major bottleneck for the broad use of TCR-based therapies in clinical practice. Even when cancer-specific T cells are retrieved and infused to cancer patients, their anti-tumor efficacy depend on the ability to recognize the tumor with high avidity and to persist long-term.

Focusing on acute myeloid leukemia (AML), hematological malignancies sensitive to immunotherapy still harboring a high mortality rate, in this research plan we aim at: a) gaining a comprehensive understanding of the immunological signature of tumor-specific T cells in patients experiencing different disease outcomes, (b) identifying tumor-specific TCRs with antigen "biased" and "unbiased" approaches; (c) boosting TCR-based immunotherapies.

For the first 2 aims, high throughput sequencing technologies and immune repertoire profiling[1] will be employed to identify novel tumor-specific TCRs from AML patients' samples cryopreserved at the San Raffaele Hospital biobank. In particular, we will identify tumor-specific T cells (and TCRs) and characterize their immune-signature (i.e. homing molecules, differentiation markers, exhaustion markers, transcription factors) by employing barcoded multimers[2] (specific for a panel of relevant tumor antigens) and single cell RNA sequencing. We will select peptides restricted to the most frequent HLA class I and class II alleles in the Caucasian population. Novel markers identified by the gene expression analysis and specifically associated with anti-tumor T lymphocytes will be validated at the protein level. In addition, we will search for tumor-specific TCRs, without prior knowledge of the recognized antigens, by focusing on the examination of the TCR sequences features potentially associated with tumor antigen recognition. With these approaches, we envisage to generate a collection of tumor-specific TCRs able to recognize target cells with a wide range of functional avidities. The strength used by those receptors to recognize the cognate antigens and the persistence/expansion of newly generated TCR-transgenic T cells will be further addressed in the last aim of the project by exploiting genetic engineering (e.g. by transducing T cells with lentiviral vectors encoding for selected cytokines) and pharmacological approaches. The relevance in cancer immunotherapy, the safety, and the efficacy of the TCR-engineered T cells[3] will be evaluated in vitro and in vivo.

Results obtained in this project will lead to the generation of cellular products to be tested as novel therapeutic options for AML patients.

Skills to be acquired by the student:

In this 3-years training program the PhD student will learn several skills in cellular and molecular biology, including gene transfer technologies, high throughput sequencing of the immune repertoire, flow cytometry protocols and use of in vivo models. We expect that the student will acquire knowledge on tumor immunology and genetic engineering of T cells.

The student will be encouraged to acquire a critical attitude towards scientific literature, also by actively participating at journal clubs organized in the hosting lab, and to refine the abilities required for the proper design and interpretation of generated data.

Furthermore, the student will participate at scientific meetings and seminars, thus fostering scientific communication skills development.

References (max. 3)

1. Ruggiero, E. et al. High-resolution analysis of the human T-cell receptor repertoire. Nat. Commun. 6, 8081 (2015).
2. Bentzen, A. K. et al. Large-scale detection of antigen-specific T cells using peptide-MHC-I multimers labeled with DNA barcodes. Nat. Biotechnol. (2016). doi:10.1038/nbt.3662
3. Provasi, E. et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat. Med. 18, 807 (2012).