

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 02 of 16/01/2026 PO 20 Page 5 of 12</p>
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## PROJECT

**Supervisor:** Dr. Francesco Andreata

**Title:** Targeting RNA programs to restore cytotoxic T-cell function in liver cancer

**Curriculum:** Basic and Applied Immunology and Oncology

Link to the personal page of the University or relevant hospital site website: <https://www.unisr.it/docenti/a/andreata-francesco>

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

### **Background/gap of knowledge**

Cytotoxic CD8<sup>+</sup> T cells are key mediators of anti-tumor immunity and a determinant of response to cancer therapies, including immune checkpoint blockade. However, in many tumors these cells progressively lose effector function and enter dysfunctional states that limit therapeutic efficacy. Increasing evidence indicates that these states are not solely determined by antigen persistence but are profoundly shaped by the tumor microenvironment (TME). The liver represents a particular context, as hepatic tumors frequently display strong immunoregulatory properties that constrain cytotoxic T-cell activity. Preliminary data from our lab indicate that liver tumors harbor a distinctive T-cell dysfunctional state that differs from canonical exhaustion and is conserved across human patients and mouse models. Transcriptomic analyses suggest that this state is driven by coordinated RNA programs involving coding and non-coding RNAs. However, the functional role of these programs in shaping cytotoxic T-cell function remains poorly defined.

### **Rationale and hypothesis**

We hypothesize that the hepatic TME imposes specific RNA programs that actively drive cytotoxic T-cell dysfunction and limit immunity. Because RNA molecules can be directly targeted using novel RNA-based therapeutics, identifying these programs may reveal new strategies to restore T-cell function cancer. This project integrates fundamental investigation of immune regulation with a translational perspective aimed at identifying therapeutically actionable RNA targets. The study will combine single-cell and spatial transcriptomics, high-dimensional immune profiling by spectral flow cytometry, and preclinical mouse models of liver cancer to dissect the molecular circuits governing cytotoxic T-cell states within the TME.



### **Objectives and specific aims**

*1. Identify RNA programs associated with cytotoxic T-cell dysfunction in liver tumors.*

Candidate RNA programs will be identified through integrative analysis of transcriptomic datasets generated by the laboratory, including single-cell and spatial transcriptomics of human and mouse liver tumors, together with publicly available datasets to identify conserved signatures.

*2. Functionally interrogate candidate RNA drivers.*

Prioritized RNA candidates will be perturbed using RNA-targeting approaches such as antisense oligonucleotides or CRISPR-based systems. Functional effects will be assessed in tumor and immune cell systems to determine their impact on cytotoxic T-cell activation and effector function.

*3. Evaluate therapeutic potential in vivo.*

Selected targets will be tested in preclinical mouse models of liver cancer to determine whether modulation of these RNA programs can restore cytotoxic T-cell activity and improve anti-tumor responses.

### **Expected outcomes**

This project will identify RNA programs that causally contribute to cytotoxic T-cell dysfunction in liver tumors and determine whether these programs represent therapeutically tractable targets. By integrating transcriptomic technologies, high-dimensional immune profiling, and in vivo experimentation, the study aims to uncover mechanisms through which the TME shapes anti-tumor immunity and to identify innovative RNA-based strategies to restore cytotoxic T-cell function.

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

The PhD candidate will acquire advanced experimental skills in tumor immunology, including spectral flow cytometry for immune profiling, confocal and intravital microscopy to study cellular interactions, and spatial transcriptomics and RNA sequencing for gene expression analysis. The project involves preclinical mouse models, including handling of transgenic strains. Within the dynamic and international environment of the lab, the student will also develop skills in experimental design, data interpretation, and scientific communication.

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**References** (max. 15)

- 1.** Andreata, F., Laura, C., Ravà, M., Krueger, C.C., Fitch, X., Kawashima, K., Beccaria, C.G., et al. (2024). Therapeutic potential of co-signaling receptor modulation in hepatitis B. 187, 4078-4094. 10.1016/j.cell.2024.05.038.  
**Cell.**
- 2.** Andreata, F., Moynihan, K.D., Fumagalli, V., Lucia, P.D., Pappas, D.C., Kawashima, K., Ni, I., Bessette, P.H., Perucchini, C., Bono, E., et al. (2024). CD8 cis-targeted IL-2 drives potent antiviral activity against hepatitis B virus. 16. 10.1126/scitranslmed.adil1572.  
**Sci Transl. Med.**
- 3.** Iannacone, M., and Guidotti, L.G. (2022). Immunobiology and pathogenesis of hepatitis B virus infection. 22, 19-32. 10.1038/s41577-021-00549-4.  
**Nat. Rev. Immunol.**
- 4.** Kawashima, K., Andreata, F., Beccaria, C.G., and Iannacone, M. (2024). Priming and Maintenance of Adaptive Immunity in the Liver. 42. 10.1146/annurev-immunol-090122-041354.  
**Annu. Rev. Immunol.**
- 5.** Bénéchet, A.P., Simone, G.D., Lucia, P.D., Cilenti, F., Barbiera, G., Bert, N.L., Fumagalli, V., Lusito, E., Moalli, F., Bianchessi, V., Andreata, F., et al. (2019). Dynamics and genomic landscape of CD8<sup>+</sup> T cells undergoing hepatic priming. 574, 200-205. 10.1038/s41586-019-1620-6.  
**Nature**
- 6.** Guidotti, L.G., Inverso, D., Sironi, L., Di Lucia, P., Fioravanti, J., Ganzer, L., Fiocchi, A., Vacca, M., Aiolfi, R., Sammicheli, S., et al. (2015). Immunosurveillance of the Liver by Intravascular Effector CD8<sup>+</sup> T Cells. 161, 486-500. 10.1016/j.cell.2015.03.005.  
**Cell**
- 7.** Simone, G.D., Andreata, F., Bleriot, C., Fumagalli, V., Laura, C., Garcia-Manteiga, J.M., Lucia, P.D., Gilotto, S., Ficht, X., Ponti, F.F.D., et al. (2021). Identification of a Kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming. 54, 2089-2100.e8. 10.1016/j.immuni.2021.05.005.  
**Immunity**
- 8.** Fumagalli, V., Venzin, V., Di Lucia, P., Moalli, F., Ficht, X., Ambrosi, G., Giustini, L., Andreata, F., et al. (2022). Group 1 ILCs regulate T cell-mediated liver immunopathology by controlling local IL-2 availability. 10.1126/sciimmunol.abi6112.  
**Sci Immunol.**