

 <p>UniSR Università Vita-Salute San Raffaele</p>	<p>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</p>	<p>MO 20-5 ed. 02 of 16/01/2026 PO 20 Page 5 of 13</p>
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PROJECT

Supervisor: Marco De Giovanni -----

Title: High-Throughput analysis of newly recruited immune cells to tumors by sequential intravascular labeling, flow cytometry and scRNA-seq

Curriculum: BAIO -----

Link to the personal page of the University or relevant hospital site website:

<https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/units/immune-cell-migration-and-regionalization.html?ts=202407091420> -----

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

Background/gap of knowledge

The recruitment of immune cells into the tumor microenvironment (TME) plays a pivotal role in shaping anti-tumor immunity and influencing therapeutic responses. Immune cell infiltration requires leukocytes to extravasate from circulation and migrate into tumor tissue, guided by chemokines and adhesion molecules(1-5). Recent evidence suggests that immune cells undergo a rapid transcriptional shift as early as two days after entering the TME, complicating our ability to analyze early gene expression programs that govern recruitment(6). A major limitation in studying this process is the inability to distinguish newly recruited immune cells from those already residing in the tumor. Therefore, there is a critical need for methodologies that selectively identify and transcriptionally profile leukocytes during the earliest phases of tumor infiltration.

Rationale and hypothesis

To address this, here we set up Recruit-sequencing (Recruit-seq), a novel methodology that enables the specific labeling, isolation, and transcriptomic analysis of newly recruited immune cells in solid tumors. Recruit-seq combines sequential intravascular labeling(7-8), flow cytometry, and scRNA-seq to discriminate between three key leukocyte populations within the tumor: (i) intravascular leukocytes, which remain in circulation; (ii) parenchymal leukocytes, which have already infiltrated and adapted to the TME; and (iii) newly recruited leukocytes,



which have recently extravasated from the vasculature. By focusing on these newly recruited cells, Recruit-seq allows to capture the initial molecular signatures driving immune cell entry into tumors.

Objectives and specific aims

Aim 1: Profiling immune cells using Recruit-seq

We will employ Recruit-Seq to define transcriptional and protein signatures of immune cell subsets recently recruited to solid tumors. Specifically, we will analyze the expression of homing-related molecules, including GPCRs, integrins, and adhesion molecules. By integrating scRNA-seq with surface marker analysis, we aim to identify key molecular determinants regulating leukocyte entry into tumors.

Aim 2: Assessing the impact of immunotherapy on leukocyte homing

Immune checkpoint blockade (ICB) therapies have revolutionized cancer treatment(9,10), yet their effects on immune cell recruitment remain poorly understood. Given that only a subset of patients responds to ICB, we hypothesize that differences in immune cell homing contribute to treatment outcomes. To explore this, we will apply Recruit-Seq to tumors from responder and non-responder mice, comparing the transcriptional programs of newly recruited leukocytes in both groups.

Expected outcomes

This study will provide a comprehensive, high-resolution analysis of the molecular mechanisms governing leukocyte recruitment to solid tumors. By characterizing immune infiltration across different tumor models, time points, and treatment conditions, we aim to elucidate conserved pathways that enhance immune infiltration, potentially unveiling novel therapeutic targets.

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

- Proficiency in mouse handling, breeding and genotyping
- Proficiency in subcutaneous tumor cell line injections; intravenous and intraperitoneal injection of antibodies
- Proficiency in s.c. tumor processing for flow cytometry and scRNA-seq



- Training in confocal imaging sample preparation and analysis
- Training in multiparametric spectral flow cytometry analysis

References (max. 15)

1. Nicolas-Boluda, A., & Donnadieu, E. (2019). Obstacles to T cell migration in the tumor microenvironment. *Comparative Immunology, Microbiology and Infectious Diseases*, 63, 22–30. <https://doi.org/10.1016/j.cimid.2019.101595>
2. Barnes, T. A., & Amir, E. (2017). HYPE or HOPE: The prognostic value of infiltrating immune cells in cancer. *British Journal of Cancer*, 117(4), 451–460. <https://doi.org/10.1038/bjc.2017.220>
3. Du, W., et al. (2022). Cell trafficking at the intersection of the tumor-immune compartments. *Annual Review of Biomedical Engineering*, 24, 275–305. <https://doi.org/10.1146/annurev-bioeng-071221-105222>
4. Smith, A., et al. (2007). The role of the integrin LFA-1 in T-lymphocyte migration. *Immunological Reviews*, 218, 135–146. <https://doi.org/10.1111/j.1600-065X.2007.00536.x>
5. Mempel, T. R., Lill, J. K., & Altenburger, L. M. (2024). How chemokines organize the tumour microenvironment. *Nature Reviews Cancer*, 24(1), 28–50. <https://doi.org/10.1038/s41568-023-00512-9>
6. Kirschenbaum, D., et al. (2024). Time-resolved single-cell transcriptomics defines immune trajectories in glioblastoma. *Cell*, 187(1), 149–165.e23. <https://doi.org/10.1016/j.cell.2023.11.001>
7. Anderson, K. G., et al. (2014). Intravascular staining for discrimination of vascular and tissue leukocytes. *Nature Protocols*, 9(1), 209–222. <https://doi.org/10.1038/nprot.2014.005>
8. Chow, Y. H., et al. (2023). Intravascular leukocyte labeling refines the distribution of myeloid cells in the lung in models of allergen-induced airway inflammation. *ImmunoHorizons*, 7(12), 853–860. <https://doi.org/10.4049/immunohorizons.2300084>



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9. Mukherjee, A. G., et al. (2022). Role of immune cells and receptors in cancer treatment: An immunotherapeutic approach. *Vaccines*, 10(9), Article 9. <https://doi.org/10.3390/vaccines10091422>
10. Tan, S., Li, D., & Zhu, X. (2020). Cancer immunotherapy: Pros, cons and beyond. *Biomedicine & Pharmacotherapy*, 124, 109821. <https://doi.org/10.1016/j.biopha.2020.109821>