PHYSICIAN SCIENTIST
LA MEDICINA INCONTRA LA RICERCA
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Principal Investigator	Zucali Paolo Andrea
Hosting institution	Humanitas Mirasole S.p.A.
Proposal title	Targeting pathogenesis and therapies of Thymic Epithelial Tumors at crossroad between cancer immunology and autoimmunity
Keywords	Microenvironment; Autoimmunity/Autoantibodies; Immune escape; Computational biology; Thymoma
PhD project description	Hypothesis Our preliminary data revealed that, other than ab and gd T cells, the tumor micro-environment (TME) of TETs is also home of other infiltrating immune cells such as Natural Killer (NK) and B lymphocytes as well as of Dendritic Cells (DCs). While all these immune cells have been described to play a major role in the pathogenesis of many solid cancers and in the onset of several ADs, a deep characterization of tumor-infiltrating and circulating immune cells of TETs could allow us to a better understanding of the cellular and molecular immunologic mechanisms associated with TETs and autoimmunity. Aims The main aim of this research proposal is to investigate the phenotypes and functional relevance of tumor-infiltrating and peripheral blood immune cells from TETs' patients either showing or not co-morbidities with ADs. Experimental Design This project is sub-divided in 3 main tasks: 1) Patient recruitment and sample collection. 2) Characterization of tumor-infiltrating and circulating immune cells as follow: a. Phenotypic and Functional Characterization of DCs, T and NK cells b. B cells, autoimmunity c. Transcriptomic profiles 3) Characterization of tumor infiltrating immune cells by tissue imaging Expected Results Enroll and collect samples of at least 100 adult patients diagnosed with TET (at least 50 with and 50 without ADs) and 10 patients affected by thymic hyperplasia as controls. Characterize tumor-infiltrating and circulating immune cells to disclose the pathogenic mechanisms of tumor escape from the immune-surveillance exerted by DCs, T-, B- and NK-cells. Reveal the precise contribution and mechanisms of T-, B- and NK-cells in the co-morbidity of ADs with TET. Identify tissue biomarkers and cellular infiltrates possibly predicting TETs' disease progression and survival and/or correlation with ADs by imaging mass cytometry.
Main topics of the lab	Characterization of tumour-infiltrating and circulating immune cells
Short description of the lab activity	Characterization of tumour-infiltrating and circulating immune cells Task 1.1: Flow cytometry. Mononuclear cells isolated from peripheral blood and tumour tissues will be stained under standard conditions and analysed by 28-color flow cytometry. The frequency and phenotype of the following innate and adaptive immune cell populations will be analysed: thymocytes (in tissues), αβT cells including Treg cells, γδT cells, B cells and plasmacells, DCs, NK cells.

	The state of activation of immune cells will be investigated by assessing the expression of proper stimulatory and inhibitory molecules on each cell type. Multiparameter flow cytometry data will be analyzed using different computational tools. Task 1.2: sc-RNAseq. Mononuclear cells isolated from peripheral blood and tumour tissues of 4 healthy donors and 4 patients with TH, 4 with each type of thymoma and 4 patients with TC, all further stratified according to the presence or absence of AD, will undergo transcriptomic profiling by sc-RNAseq. Task 1.3: functional assays. The cytotoxic, regulatory and proliferative activity of tumor-infiltrating and circulating immune cells, together with their production of cytokines, will be assessed to compare different TETs and to validate results obtained in Tasks 2.1 and 2.2. B cells, autoimmunity and transcriptomic profiles Task 2.1: screening of autoantibodies in the peripheral blood. In order to detect subclinical autoimmunity, a broad panel of IgM and IgG antibodies against self-antigens will be performed using auto-antibody array in 10 patients for each cohort (Core Facility, UT Southwestern Medical Center), and the results will be correlated with B cell paramaters resulting from flow cytometric analyses as well as scRNAseq and TCR/BCR sequencing. Task 2.2: TCR/BCR sequencing. In order to identify autoreactive T cell and B cell clones in thymoma patients with ADs, unbiased amplification of TCRs and BCRs will be performed using a novel dimer avoidance multiplexed polymerase chain reaction (PCR) next generation sequencing (NGS) assay (iR-RepSeq-plus 7-Chain Cassette, iRepertoire) [43]. NGS libraries covering all TCR and BCR chains including TCR-□, □, and BCR-IgH, IgK, IgL will be generated. Amplified libraries willl be multiplexed and pooled for sequencing on the Illumina NovaSeq platform, and raw data will be analyzed using Cell Ranger vdj pipeline (v6.1.2 10, 10XGenomics) and 'Scirpy' package. Characterization of tumor-infiltrating immune cells by tissue imaging Task 3.1: IHC. The composition of TME will be analysed by performing detailed IHC analyses. H&E staining will be used to check for basic histopathological changes and, in parallel, a pathological analysis will be done using a panel of primary antibodies to detect: innate and adaptive immune cells; cortical and medullary components; vascular and angiogenesis factors. Expression analysis of molecules involved in cellular checkpoint control will be performed with the final aim to identify prognostic biomarkers indicating disease progression and survival. Task 3.2: Imaging MC. In order to investigate the role of specific cellular interactions among different cell types in supporting AD development, paraffin-embedded tissue sections obtained from tumor tissues will be analyzed by imaging MC (Hyperion).
Main research area	Cancer biology
Group composition	PI and leader of the project: - Prof. Paolo Andrea Zucali. Associate Professor at Humanitas University; Head of Clinical Pharmacology Unit at Humanitas Research Hospital (HRH). PhD Students: - Dr. Sara Franzese. Ph.D. Student at Unit of Clinical and Experimental Immunology (UCEI) at HRH. - Dr. Nadia Cordua. Ph.D. Student at Oncological Unit at HRH. Early stage researchers: - Dr. Sara Terzoli.

	She is a bioinformatician and works within the Bionformatic facilitie of HRH Experienced Researchers: - Prof. Luca Di Tommaso. Assistant Professor of Pathology of Humanitas University - Prof. Silvia Della Bella. Prof. Silvia Della Bella is Associate Professor of General Pathology and Immunology at University of Milan – BioMeTra. - Dr. Laura Giordano. Statistician in the Statistician Unit at HRH. - Dr. Matteo Perrino. Assistant in the Oncological Unit at HRH. - Dr. Emanuele Voulaz. Assistant in the Thoracic Surgery Unit at HRH. Collaborators - Prof. Giuseppe Marulli. Head of Unit of Thoracic Surgery at HRH. - Prof. Domenico Mavilio. Associate Professor at Milan University; Head of the Unit of Clinical and Experimental Immunology (UCEI) at HRH. - Dr. Villa Anna. Director of Researcher at the National Research Council (NRC).
Institutional page link	https://www.humanitas.it/
Lab website link	
Social media link	
Lab bibliography	Resound Trial: A phase 2 study of regorafenib in patients with thymoma (type B2-B3) and thymic carcinoma previously treated with chemotherapy. Perrino M, De Pas T, Bozzarelli S, Giordano L, De Vincenzo F, Conforti F, Digiacomo N, Cordua N, D'Antonio F, Borea F, Santoro A, Zucali PA CANCER-AM CANCER SOC 2022 Feb; 128: 719