ImmunoTools *special* Award 2023

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‘X-TALK: In search for myelopoiesis perturbing cues that govern the bone marrow niche - lung cancer cross-talk’

Project rationale

Lung cancer remains the deadliest cancer in the world with over 1.7 million deaths in 2020 only, even though **immunotherapy (IT)** revolutionized the treatment paradigm for **non-small cell lung cancer (NSCLC)** patients. Both preclinical and clinical studies demonstrate that immunosuppressive **myeloid cells** within the lung tumor microenvironment (TME) play a detrimental role for effective IT. Startling, their main source, represented by **hematopoietic stem and progenitor cells (HSPCs)** in the **bone marrow niche (BMN)**, remains vastly understudied, leaving the field with major uncertainties about NSCLC-installed **myelopoiesis perturbing cues (MPCs)** that fuel lung cancer progression and resistance to curative therapies. Today, there is a plethora of suggested tumor induced MPCs yet no consensus is reached on which BMN-residing cells are the main tumor sensors that lead to the production of MPCs. While most studies focused on (often solely blood-derived) HSPCs, the stromal fraction of the BMN comprising amongst others endothelial and mesenchymal cells, is often entirely neglected while they support regulation of hematopoiesis and as such potentially the lung TME-BMN crosstalk. Moreover, MPCs have been reported to differ between lung cancer subtypes, cancer stage and cellular source. This lack of consensus is in part explained by the fact that in-depth analysis of the BMN of solid cancer patients is hampered by the inaccessibility of the BMN, the low fraction of stromal and HSPCs, and the heterogeneous and continuously differentiating character of hematopoiesis which also complicates its *in vitro* cultivation and evaluation.

We hypothesize that unravelling the BMN-lung tumor crosstalk holds ample fundamental information to aid the definition of new biomarkers and druggable targets that can be exploited to reduce the high attrition rate of IT.

Project set up

All ImmunoTools specific products are depicted in orange.

In close collaboration with Prof. Dr. Lore Decoster and surgeons Dirk Smets and Domien Vanhonacker from the University Hospital Brussels (UZ Brussel), we are currently collecting rib bone marrow (BM) aspirates and peripheral blood from NSCLC patients (5 patients included so far). This is part of the ongoing clinical trial (ClinicalTrials.gov ID: NCT05251805) in which costal bone marrow quantitity and quality is assessed of 4 healthy and 8 NSCLC patients. Next, we aim to define candidate MPCs by transcriptomic and proteomic analysis of these BM aspirates using single cell RNA sequencing and flow cytometry (FC). For the latter we plan to use the following antibodies: CD3-APC, CD11b-APC, CD14-APC, CD19-APC, CD235-APC, CD45RA-PerCP, CD127, CD123, CD34, CD38, CD90 and CD135.

To spatiotemporally study and functionally validate candidate MPCs, we are also optimizating an innovative 3D *in vitro* BMN – lung spheroid crosstalk assay. In brief, we aim to collect mesenchymal cells and $CD34^+$ HSPCs from donors and seed these on top of specifically designed scaffolds that mimic the honeycomb-like structure found within the BMN. These scaffols are currently provided by our collaborator Prof. Ahmed Eissa from Wolverhampton University (UK). To maintain balanced HSPC stemness and differentiation, their culture medium will contain SCF, G-CSF, IL-3, FLT3L, GM-CSF, and TPO. In order to study BMN – lung cancer crosstalk, these scaffolds will be co-cultured with human (H1650) lung cancer spheroids, separated by a 0,4 or 8µm transwell system. Upon co-cultivation with and without the addition of IT, the viability, phenotype, and location of the HSPCs, stromal and myeloid immune cells within the scaffolds and spheroids will be characterized using FC (panel above) and microscopy (CD34) while supernatants will be collected for multiplex cyto- and chemokine analysis.

As such we aim to deliver ground-breaking fundamental biological knowledge that could pave the way to novel lung cancer treatments and optimized treatment regimens.

ImmunoTools *special* AWARD for **Evelyn Calderon Espinosa**

includes 10 reagents

PerCP - conjugated anti-human CD45RA

APC - conjugated anti-human CD3-APC, CD11b-APC, CD14-APC, CD19-APC, CD235-APC, CD45RA-PerCP

unconjugated anti-human CD34

recombinant rh G-CSF, rh IL-3, rh SCF

DETAILS more AWARDS