Deciphering the cellular and molecular mechanisms leading to immune exclusion in lung cancer:

IMMUNEX - Pierre-Alexis Da Costa

Non-small-cell lung cancer (NSCLC) is characterized by a complex and heterogeneous tumor microenvironment (TME) that includes various immune cells, cancer-associated fibroblasts (CAFs), and endothelial cells, in addition to tumor cells. Immune cells are organized in tertiary lymphoid structure (TLS), which leads to a coordinated *in situ* anti-tumoral immune response, that is associated with a good prognosis. Moreover, recent studies have shown that TLS are necessary to achieve an objective response to immunotherapy. However, only a fraction of NSCLC patients shows TLS in the TME, and some of them are devoid of immune cells. The objectives of my M2 internship are 1/ to confirm that the organization of the immune infiltrate impacts the prognosis and to what extent, and 2/ to decipher the cellular and molecular mechanisms of the TME that influence this organization in TLS or not. In sum, our goal is to define the TME signature associated with distinct patterns of immune infiltrates.

This study includes a cohort of 213 patients operated for a NSCLC in 2018 at Cochin Hospital. The histology of NSCLC has already been characterized: 130 adenocarcinomas (61%), 65 squamous cell carcinomas (31%), and 18 other histological types (8%). This cohort will be further characterized using multiplex immunofluorescence (IF) and transcriptomics analysis.

One of the first steps was to complete the cohort database. Next, the data had to be wrangled using R software and language. For the moment, we have selected around 80 variables and excluded the ones containing more than 30% of missing values. A stricter exclusion could be performed. Survival analyses were performed for this cohort as described in Figure 1. Odds Ratio, Risk Ratio, and Hazard Ratio were also calculated for univariate regression.

Characteristic	N = 213[1]
age	66 (60, 72)
sex	
female	69 (32%)
male	144 (68%)
smoking	
non_smoker	21 (9.9%)
smoker	60 (28%)
ancient_smoker	132 (62%)
pack_years	40 (20, 50)

[1] n (%); Median (IQR); Range
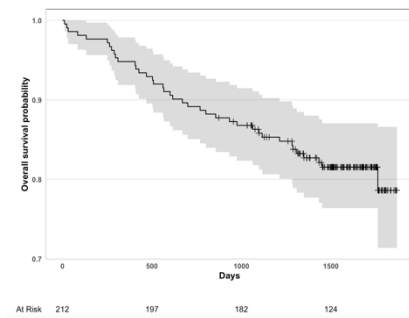
Table 1. Cohort description.

Figure 1. Kaplan Meyer survival curve.

Furthermore, to visualize and describe the TME, we designed a panel of 6 antibodies considering multiple variables such as order or association with a tyramide-fluorophore. The staining is performed using the LEICA Bond automat on the CHIC platform at CRC. Once this panel will be fully optimized, we will be able to stain the whole cohort and quantify it using Halo Software.
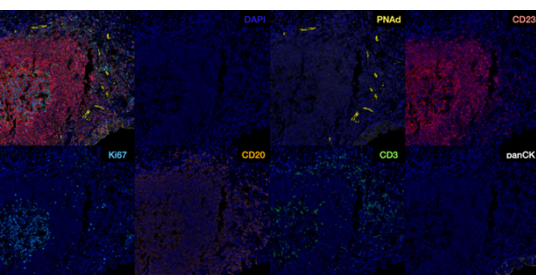

Figure 2. Lung multiplex IF staining image of the panel. (DAPI, PNAD, CD20, CD3, and pan-cytokeratin respectively stain the DNA, the high endothelium venules, the B cells, the T cells, and the tumor cells. CD23 and Ki67 are respectively markers for TLS maturity and cellular proliferation.)

Transcriptomics analysis and optimization of a second multiplex IF panel — for fibroblasts, lymphatic vessels, and endothelial cells — are envisaged by the end of the internship.

We expect that the presence of immune infiltration and TLS will be associated with a better prognosis and that the cohort will be split into two groups: a/No or low immune infiltrate; b/Presence of immune infiltrate (organized in TLS or not). Finally, we expect to identify TME characteristics linked with these two distinct subgroups.

I interact with researchers at the Research Center of Cordeliers such as Isabelle Cremer — who is my supervisor — and Catherine Monnot, with MD Ph.D. at Cochin Hospital (Diane Damotte, Marco Alifano), with post-doctoral students like Yoan Velut, research engineers like Antoine Bougouïn or Julien Lavergne and other Master students (Mathilde Prieto).