Immuno-monitoring within the context of the ProfileR and MOST clinical studies

The **I**mmuno-monitoring and **I**mmunotherapy **I**nnovation platform (PI^3) is a translational research platform dedicated to the development of innovative strategies in "Immunotherapy/Biotherapy" and their transfer into clinical studies. One of the key issues of this PI^3 platform is to demonstrate the importance of the role played by the anti-tumour immune response in the therapeutic response to treatment, in human clinical studies.

Within the ProfileR trial (n=2000), an ancillary study is conducted in order to identify whether there is a correlation between constitutional or somatic genetic alterations identified in the genetic profile of a patient's tumour, and **immune system abnormalities detected at the systemic level** (particularly T-CD4 lymphopenia) in 10-20% of patients with advanced cancers, **or at the intra-tumoral level** where we detect an over representation of regulatory T cells (Treg) and macrophages, alteration of dendritic cell subsets (pDC), and over expression of inhibitory ligands (PD-L1, ...).

We developed a whole blood assay for **the phenotypic analysis, allowing evaluation of the different circulating immune cell populations,** by multi-colour flow cytometry stainings. We are thus able to simultaneously quantify B lymphocytes, naïve or effector T $CD4^+$ or $CD8^+$ lymphocytes, Treg and innate immune lymphocytes (NK, NKT, $\gamma\delta$ T cells). We will also be able to measure different DC subsets (pDC, mDC), as well as monocytes, and evaluate their level of activation. In parallel, through a close collaboration with the transfer research team of Isabelle Treilleux, we will assess by immuno-histochemical analyses on paraffin-embedded tumour tissues, the expression level of different markers, for which the prognostic value (or predictive value) is known or suspected and for which therapeutic strategies are currently being validated (CSF-1R, PDL1, PD-1, …).

With regards to the phase II, randomised, multi-centre study, MOST (n=500), the analyses cited above will be combined with an **innovative analysis carried out using whole blood, with the aim to assess the functionality of the different subsets of circulating immune cells, using multi-parametric flow cytometry analyses (based on 11 colours). These analyses will particularly evaluate** the ability of immune cell subsets to secrete specific cytokines (intra-cytoplasmic stainings) after a short activation period, and will especially focus on DC subsets, monocytes, NK cells and subsets of T lymphocytes.
The combined use of these different analyses, as well as the monitoring of patients during their treatment, will improve our knowledge of the biology of advanced solid cancers and will allow us to:

- assess the impact of patients' peripheral immune system disorders on the immune response to targeted therapies,
- measure the link between these bio-markers and the efficiency and toxicity of targeted therapies assessed in this trial and / or the therapeutic impact of targeted therapies,
- identify targets and / or therapeutic strategies to restore anti-tumour immune responses.