Immune checkpoint modulators, such as antibodies targeting CTLA-4 or PD-1, are now being approved for treatment of patients with unresectable or metastatic melanoma and advanced squamous non small cell lung cancer (NSCLC) who have progressed on or after platinum-based chemotherapy. Efficacy was also evidenced on other tumor types (renal cell carcinoma, bladder, Hodgkin lymphoma, colorectal carcinoma (CRC) ...). However, there is still a need to identify predictive biomarkers of response in order to select patients who will benefit from treatments. PD-L1 expression was proposed to be a good candidate for NSCLC, even if PD-L1 expression is a difficult parameter due to its expression on both tumor cells and immune cells as well as technical challenges to use immunohistochromatic detection. The dynamic of the immune system as well as the site and time where interactions between tumor cells and immune cells take place, increase the complexity of having a solid biomarker identified. In addition, for other pathologies like colorectal genomic biomarkers were evidenced. For example, CRC patients with mismatch repair (MMR) deficiencies have an objective response rate of 62% compared with 0% in patients with MMR-proficient tumors.

We propose here the use of syngeneic models to address mechanism of action and biomarker-related questions for agent targeting efficacy. This is not the case for PD-1 or PD-L1 targeting therapies. It is well accepted that a key parameter that could help us understand efficacy of PD-1 /PD-L1 axis disruptors, intratumoral immune infiltrate was analyzed in depth using flow cytometry: regulatory T cells (Treg), effector T cells (Teff), M-MDCs, G-MDCs, TAMs were phenotypically quantified to its extent in each tumor setting, where Teff:Treg ratio was correlated to treatment efficacy, this is not the case for PD-1 or PD-L1 targeting therapies. It is now hypothesized that a more complex signature (e.g. detailed phenotype of CD8 positive T cells, tumor neoantigen expression...) will be needed to identify valuable biomarkers of response. Preliminary results using syngeneic models, both subcutaneously or orthotopically engrafted with tumors, will be presented.

**Abstract**

**Material and Methods**

In vivo experiments

Immune competent mice were obtained from Charles River (France). Animals were orthotopically (OT) or subcutaneously (SC) injected with syngeneic tumor cell lines on D0. The animals received repeated injections of antibodies directed against PD-1 or against PD-L1 at 10 mg/kg/inj. Tumor volume was determined by MRI. Representative intravesical instillations of BCG at 1.35 mg/kg/inj, with IP injection of isotype mAb or mAb against PD-1 at 10 mg/kg/inj.

**Conclusions and perspectives**

**Agents targeting PD-1/PD-L1 axis, alone or in combination with other molecules, may be evaluated using these syngeneic mouse models.**

**Biomarkers of response to immune checkpoint modulators are evaluated by flow cytometry as well as immunohistochemistry analyses.**

**Newly available RNA sequencing data will help to understand how genomics information could be used as biomarker of response for these new therapies.**