### Background
In the management of advanced Non-Small Cell Lung Carcinoma (NSCLC), PD-L1 Immune Checkpoint Inhibitors (ICIs) have been shown to increase Overall Survival (OS) over standard 2nd-line chemotherapy (CT). While this long-term increase in OS is driven by about 20% of patients, others display disease progression during the first weeks.

In clinical practice, high PD-L1 expression in tumor cells as well as high Tumor Mutational Burden provide an enriched population of PD-L1 inhibitors responders without being sufficiently precise to exclude patients from treatment.

### Challenge
Understand biological background behind resistances to PD-L1 ICIs through a comprehensive multiparametric biomarkers strategy (PIONeeR biomarkers program).

Identify a relevant predictive algorithm based on biomarkers combination adaptable to clinical practice.

### Strategy
450 patients prospectively collected with stage IV or recurrent NSCLC with first line anti-PD-L1 immunotherapy.

**Preliminary analysis** of more than 20 biomarkers on 11 patients at baseline as a proof of concept of Immunogram performances to identify relevant combinations of biomarkers to predict response to ICI treatment.

### PIONeeR study sampling workflow

**Blood sample**

- 20 FFPE slides
- Tissue microarray

- Longitudinal blood sampling

**Stool sample**

- 20 FFPE slides
- Tissue microarray

- Longitudinal stool sampling

### Technical workflow for immunoprofiling at baseline

**Immunoprofiling**
- through immunostaining & cytometry
- DNA/RNA extraction
- Endothelial activation through HACT & p-cytenometry
- Circulating endothelial cells (CEC, CD146+) & endothelial microcellulars (EMC, CD31+CD146+)

**DNA/RNA extraction**
- Targeted NGS on DNA
- Genomics - Targeted sequencing
- TMB: 700-1100 genes, 116M reads
- T cell clonality: TCR sequencing
- IHC staining + Digital pathology analysis

**Data acquisition and distribution across patients**

**Foreignness**
- T-cell clonality
- TMB per megagene relative to normal counterpart

**Immune infiltration**
- Immunofluorescence
- Tumor Mutational Burden
- Circulating lymphocytes

### Immune infiltration

- **CD3**
- **CD4**
- **CD8**
- **COX-2**
- **CD11b**

- **PD-L1 and immune checkpoints**

- **Immune suppressive cells**

- **Reference**

- **Conclusion**

- **Patient groups display heterogeneous immune profiles**

Variables were combined in five biological axes to define an immunogram; five arbitrary scores were calculated:

- Each test result was transformed to fit 0 – 100, where 100 = maximum value observed across the 11 samples
- Each normalized result was weighted as indicated in brackets below

**Foreignness**: TMB (1/2), TCR-clonality (1/2)

**Immune suppression within the tumor**: Treg (1/3), M-MDSC (1/3), PMN-MDSC (1/3)

**PD-L1 / Exhaustion**: PD-L1 cell density (1/2), PD1/LAG3/TIM-triple positive Tcells (1/4), PD1/LAG3/TMB-double positive Tcells (1/4)

**Infiltration**: Tumor Infiltrating Lymphocytes (1/2) and Tumor Infiltrating Monocytes (1/2)

**Circulating lymphocytes**: Total CD4+ T lymphocytes

**Anti-PD(L1) responders enriched population**

**Anti-PD(L1) treatment excluded population**

**Follow-up**

- 08-02-20
- 09-02-20
- 10-02-20
- 11-02-20
- 12-02-20
- 13-02-20
- 14-02-20
- 15-02-20
- 16-02-20
- 17-02-20

**Conclusion**

This preliminary profiling highlights immunological heterogeneity across patients not evaluated in current clinical practice.

Such analyses confronted to clinical parameters may highlight biological mechanisms explaining resistance to anti-PD(L1) ICIs.

These profiles will be expanded to additional biomarkers and optimized on more than 400 patients to identify reliable predictive biomarker combinations.

**Reference**

- Blank CU. et al., Science, 2016