The complexity of response to ICIs is also a major concern. The PIONEEER trial (NCT03500430) aims to predict such resistances through a comprehensive multiparametric biomarker analysis. Among the 3,350 enrolled patients, this study focuses on the first 137 patients treated in second line or more with anti-PD1/L1 in monotherapy and at predicting Early Progression (EP), here defined as relapse before 3.77 months.

**Material and methods**

**Figure 1:** Patients enrolment and data acquisition

**Blood soluble factors** measured:
- Hb, WBC, PLT
- CRP, TSH, PTH, COP, CO2
- Carcinoembryonic antigen, Procalcitonin, CA125

**Immunocytes**
- T cells: CD45+ CD16+ in Stroma
- Monocytes: CD45+ CD16- CD73+ in Tumor
- Circulating CD8+ NKG2D+ T-cell
- Circulating Cytotoxic T-cells
- Cytotoxic T-cells PD1+ in Parenchyma
- Exhausted T-cells in Tumor
- Circulating CD4+ CD39+ T-cell
- Circulating CD8 ILT2+ T-cell
- Circulating margin zone-like B-cells
- Monocytic MDSC in Tumor
- Monocytes
- Activated platelets
- Neutrophils
- Circulating basophils

**Table 1:** Description of the 137 2nd line patient treated with single-agent nivolumab, pembrolizumab or atezolizumab

**Figure 2:** Progression Free Survival Kaplan Meier representation of the 137 patients

**Table 2:** 12 biomarkers predictive for Early Progression with AUC ≥ 0.6 For association of biomarkers with Early Progression, Student's t and nonparametric tests were used. Controls variable for multiparametric models were sex, age, ECOS, smoking status, histology and PD-L1 expression. Benjamini-Hochberg p-value adjustment was performed on all candidate biomarkers for EP prediction and led to no significance.

**Results**

- **Introduction**
  - Resistance to PD1/L1 immune checkpoint inhibitors (ICIs) in advanced NSCLC patients is observed in about 80% of individuals with no robust biomarkers analysis to explain resistances to PD1/L1 ICIs.
  - The PIONEEER trial (NCT03500430) aims to predict such resistances through a comprehensive multiparametric biomarker analysis.

**Cohort description**

- Among 3,350 enrolled patients, this study focuses on the first 137 patients treated in second line or more with anti-PD1/L1 in monotherapy and at predicting Early Progression (EP), here defined as relapse before 3.77 months.

**Table 1**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC</th>
<th>95% CI</th>
<th>p-value</th>
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<tbody>
<tr>
<td>ICIs efficacy / resistance</td>
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**Enrolled patients description:**
- 2nd line, treated with single-agent nivolumab, pembrolizumab or atezolizumab
- ECOS PS-0-1
- Mandatory archived pre-ICI tumor block available

**Statistical methods**

- For association of biomarkers with Early Progression, Student’s t and Wilcoxon tests were used. In addition, univariate (UV) and multivariate (MV) logistic regressions for EP and proportional hazard Cox models, the progression-free survival (PFS) were used. Controls variables for multiparametric models were sex, age, ECOS, smoking status, histology and PD-L1 expression. Benjamini-Hochberg p-value adjustment was performed on all candidate biomarkers for EP prediction and led to no significance.

**Biomarkers association with Early Progression**

- Among the 374 biomarkers submitted to univariate and multivariate logistic regressions Models to predict Early Progression, 27 were significant (UV and MV).

**Figure 3**

**Figure 6**

**Figure 7**

**RESULTS**

**Multimodal data integration through supervised machine learning**

- LASSO is a regression analysis method that also performs biomarker selection through L1 penalization. Decreasing the penalization coefficient generates an increasing number of biomarkers with non-zero coefficient.
- Ten-fold cross-validation for each AUC was then performed and a biomarker subset with the highest AUC was selected (37 biomarkers).

**Figure 8**

**Discussion**

- **PD1/L1 evaluation in tumor is necessary but not enough to stratify patients and predict response to anti-PD1/L1 ICIs.**
- **The 37 biomarkers signature identifies patients resistant to anti-PD1/L1 ICIs prior to treatment initiation.**
- **Our predictive algorithm achieves good performances for all classification metrics (e.g. >80% accuracy).**
- **This study highlights that Blood and Tumor samples are complementary for ICIs efficacy/ resistance prediction.**
- **The complexity of response to ICIs is also highlighted by the diversity of markers’ origin integrated to the predictor: immune, biochemical, vascular.**

**Perspectives**

- The predictive model will be validated on a test set of ≥2nd line patients also enrolled in the PIONEEER project.
- It will also be tested in 1st line patients.
- It will be dissected at the physiopathological level to disentangle complex biological mechanisms associated with resistance.