Comprehensive biomarkers analysis to explain resistances to PD1/L1 ICIs: The **P**recision **I**mmuno-**O**ncology for advanced Non-Small C**E**ll Lung Canc**E**R (PIONeeR) trial

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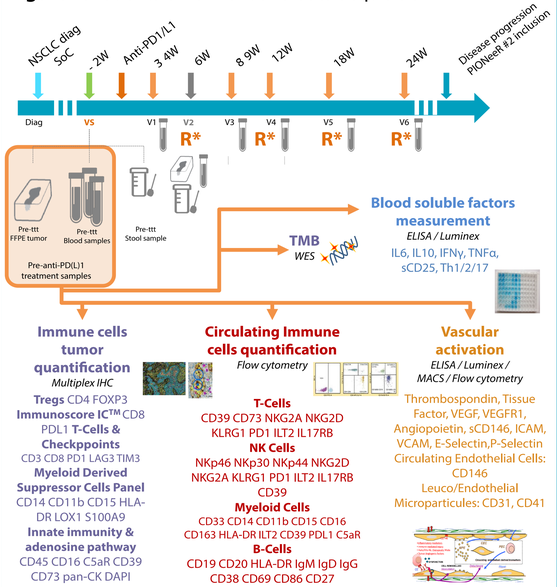
Introduction

Resistance to PD1/L1 immune checkpoint inhibitors (ICIs) in advanced NSCLC patients is observed in about 80% of individuals with no robust predictive biomarker yet. The PIONeeR trial (NCT03493581) aims to predict such resistances through a comprehensive multiparametric biomarkers analysis.

Among the >350 enrolled patients, this study focuses on the first **137 patients** treated in **second line or more with anti-PD1/L1 in monotherapy** and aims at predicting Early Progressors (EP), here defined as relapse before 3,77 months.

Material and methods

Figure 1: Patients enrolment and data acquisition



*R : RECIST 1.1 evaluation every 6 weeks

Enrolled patients description:

- **≥2nd line,** treated with **single-agent** nivolumab, pembrolizumab or atezolizumab
- **ECOG 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 (for progression-free survival, PFS) were used. Control variables for multivariate models were sex, age, ECOG, 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.

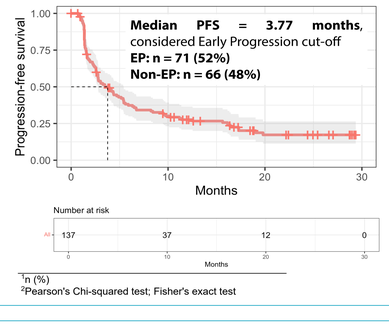
Cohort description

Characteristic	Total n = 137[1]	Early Progressors n = 71[1]	Non-Early Progressors n = 66[1]	p-value[2]
Sex				0.8
F	49 (36%)	26 (37%)	23 (35%)	
M	88 (64%)	45 (63%)	43 (65%)	
Age				0.5
< 70	92 (68%)	50 (70%)	42 (65%)	
≥ 70	44 (32%)	21 (30%)	23 (35%)	
Unknown	1	0	1	
ECOG				0.048
0	39 (28%)	15 (21%)	24 (36%)	
1	98 (72%)	56 (79%)	42 (64%)	
Tobacco history				0.9
current	45 (34%)	22 (31%)	23 (36%)	
former	78 (58%)	42 (60%)	36 (56%)	
never	11 (8.2%)	6 (8.6%)	5 (7.8%)	
Unknown	3	1	2	
PD-L1 expression				0.004
< 1%	66 (64%)	41 (77%)	25 (50%)	
≥ 1%	37 (36%)	12 (23%)	25 (50%)	
Unknown	34	18	16	
Histology				0.3
ADK	95 (73%)	44 (67%)	51 (80%)	
Squamous	27 (21%)	17 (26%)	10 (16%)	
Others	8 (6.2%)	5 (7.6%)	3 (4.7%)	
Unknown	7	5	2	
TREATMENT				0.7
anti-PD1	94 (69%)	50 (70%)	44 (67%)	
anti-PDL1	40 (29%)	19 (27%)	21 (32%)	
Pembro+Chemo	3 (2.2%)	2 (2.8%)	1 (1.5%)	
RECIST				<0.001
CR	1 (0.9%)	0 (0%)	1 (1.7%)	
PD	29 (26%)	28 (55%)	1 (1.7%)	
PR	22 (20%)	3 (5.9%)	19 (32%)	
SD	59 (53%)	20 (39%)	39 (65%)	
Unknown	26	20	6	

[1]n (%)
[2]Pearson's Chi-squared test; Fisher's exact test

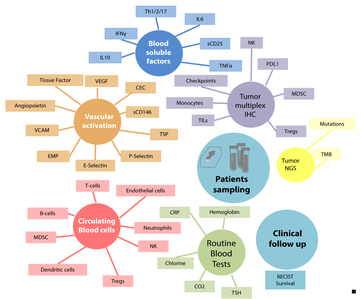
Table 1: Description of the 137 ≥2nd line patient treated with single-agent nivolumab, pembrolizumab or atezolizumab

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

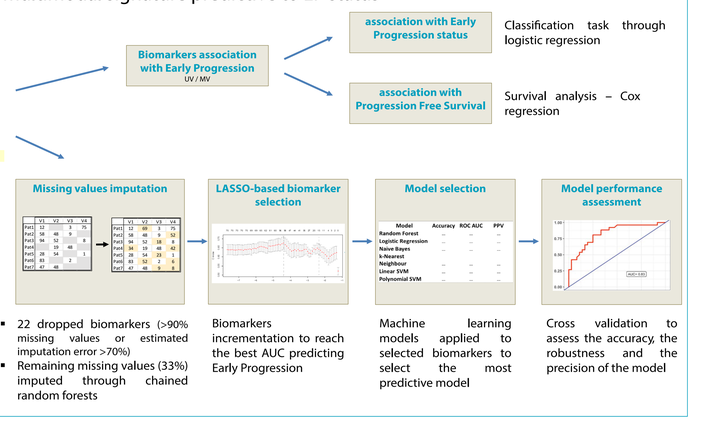


Analyses workflow

Figure 3: Analysis workflow to highlight multimodal signature predictive to EP status



374 biomarkers



- 22 dropped biomarkers (>90% missing values or estimated imputation error >70%)
- Remaining missing values (33%) imputed through chained random forests

Biomarkers incrementation to reach the best AUC predicting Early Progression

Machine learning models applied to selected biomarkers to select the most predictive model

Cross validation to assess the accuracy, the robustness and the precision of the model

RESULTS

Biomarkers association with Early Progression

Among the 374 biomarkers submitted to univariate and multivariate logistic regression models to predict Early Progression, 27 were significant (UV and MV)

Table 2: 12 biomarkers predictive for Early Progression with AUC < 0,4 or > 0,6, Odd Ratio < 0,85 or > 1,15 and both UV and MV p < 0.05

Biomarker	AUC	UV Odd Ratio	UV p-value	MV p-value
Circulating Activated T-cells	0,68	2,1 (1,4 - 3,5)	***	***
Circulating Cytotoxic T-cells	0,66	1,9 (1,3 - 3)	**	**
PDL1 TC % - cut off 1%	NA	0,29 (0,12 - 0,67)	**	**
Circulating ILT2+ NK cells	0,3	0,44 (0,24 - 0,74)	**	*
Circulating NKG2D+ NK cells	0,3	0,45 (0,25 - 0,75)	**	*
Hemoglobin concentration	0,35	0,59 (0,39 - 0,85)	**	*
Circ. PDL1+ Inflam. Monocytes	0,63	1,8 (1,2 - 2,9)	*	*
Parenchymal CD3+ T-cells	0,34	0,56 (0,35 - 0,86)	*	*
Alkaline Phosphatase	0,61	1,6 (1,1 - 2,5)	*	*
Circulating NKp80+ NK cells	0,32	0,52 (0,29 - 0,85)	*	*
Parenchymal PD1+ Cytotoxic T-cells	0,32	0,56 (0,33 - 0,88)	*	*
Circulating Regulatory T-cells	0,36	0,57 (0,34 - 0,91)	*	*

* p < 0.05
** p < 0.01
*** p <0.001

Figure 4: Box plot representation of three biomarkers in EP and Non-EP patients respectively, associated T-test result

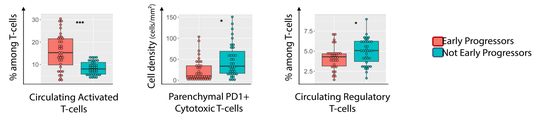
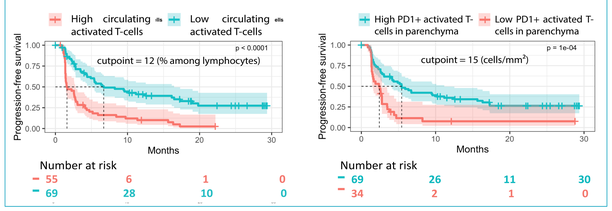


Figure 5: PFS Kaplan Meier representation of two biomarkers predictive of EP status



Multimodal data integration through supervised machine learning

Figure 6: EP biomarkers selection through LASSO

LASSO is a regression analysis method that also performs biomarker selection through L^1 penalization. Decreasing the penalization coefficient λ generates an increasing number of biomarkers with non-zero coefficient.
Ten-fold cross-validation for each λ was then performed and the value and corresponding biomarkers with highest AUC are selected (37 biomarkers).

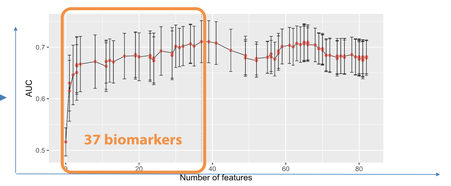
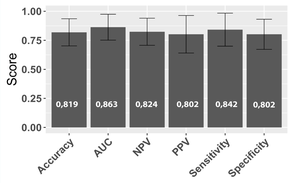
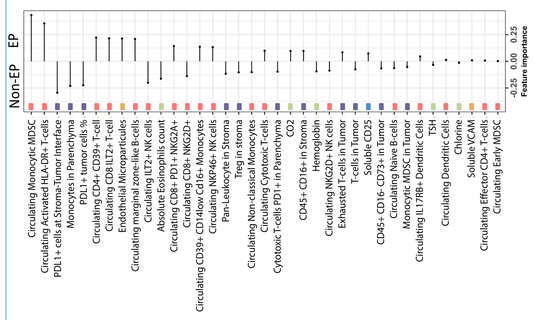


Figure 7: EP signature performance evaluation



Ten-fold cross-validation was performed on the data with the 37 selected biomarkers selected with LASSO. A logistic regression outperformed a random forest algorithm.
1 = EP, 0 = non-EP hence PPV is the probability of the model to correctly predict Early Progression.

Figure 8: EP signature description



Color code
- Circulating blood cells
- Tumor biomarker
- Vascular origin
- Routine blood tests
- Soluble immune marker

The best predictive model includes markers of diverse origins: immune, vascular, biochemical.
It highlights the complexity of ICI-response and the complementarity of blood and tumor samples for its prediction.

Discussion

- PDL1 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 PIONeeR 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.

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