Background Immune checkpoint inhibitors (ICIs) are associated with long-term survival in ~20% of advanced NSCLC patients while biological mechanisms triggering resistance are not fully elucidated. The PIONeR project (NCT03493583) aims to predict the response/resistance to PD1/L1 ICIs in advanced NSCLC patients through comprehensive agnostic multiparametric biomarkers assessment. The immune system is crucial for tumor evolution and is composed of different subsets of immune cells that can be activating (T-, B- lymphocytes, ...) or regulating such as regulatory T-cells (Treg) linked to Tregs’ ability to suppress general inflammation that itself triggers cell proliferation and metastasis.

Methods Tumor was sampled at diagnosis from 101 advanced pretreated NSCLC patients, ECOCO P01, treated with standard PD1/L1 ICIs monotherapy. Complete database of ≥2nd line PIONeR patients was released in July 2023. Overall Response Rate was assessed by RECIST 1.1. Multiplex IHC Brightplex® T-cells exhaustion quantifies cytotoxic (Te) (CD3 + CD8+) and helper (Th) (CD3 + CD8+) T-lymphocytes in both tumor parenchyma and stroma. This quantification allows stratification into 4 tumor groups: Hot, Parenchyma Hot, Cold and Stroma Tumor Infiltrating Lymphocytes (TILs). 1 2 Dual staining CD4 FOXP3 quantifies Treg density in parenchyma and stroma. Correlation analyses: spearman non-parametric test. Samples’ classification: unsupervised neural-network-based machine learning algorithm Self-Organizing Maps (SOM). Statistical significance of progression-free/overall survival (PFS/OS) differences: log-rank test. Response distribution differences: Fisher’s test.

Results Patients were mainly male (65%), current/previous smoker (92%), <70yrs (68%) with median PFS=4.4months. Across the 101 tumors, Treg were not strongly correlated to any other cell type (Te: R=0.54; Th: R=0.59). As expected, Brightplex® TCE identified 4 patient groups based on Th/Te infiltration revealing outcome differences: Hot (N=32), Cold (N=19), Parenchyma Hot (N=15) and Stroma TILs (N=35). Each group was stratified according to Th/Treg infiltration. The 35 Stroma TILs patients (median PFS=6.4) were split into 4 groups (SOM): low Th/Treg-infiltration (N=10); Th-only parenchyma-infiltration (N=7); intermediate Treg+Th infiltration in both compartments (N=9); Treg+Th high infiltration (N=9). The two lowest infiltrated groups had poorer outcome (median PFS=1.6/1.8; median OS=6.9/7.4 respectively) than both infiltrated groups (median PFS=17.3/14.1; median OS=17.3/not reached respectively), p=4.1e-4. 10/11 responders were part of both infiltrated groups (p=2.6e-3) regardless of PDL1 status.

Conclusions Treg infiltration evaluation improved previous lymphocyte-associated NSCLC classification regarding response to anti-PD1/L1 ICIs. 1 2 Absence of Treg, regardless of Th cells infiltration, in the Stroma TILs patient subset, was associated with early progression and poor survival. These unexpected results were already described in some cancers and could be linked to Treg’s ability to suppress general inflammation that itself triggers cell proliferation and metastasis.

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Trial Registration NCT03493583

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Ethics Approval The study is conducted in accordance with Good Clinical Practice and the French applicable regulatory requirements (Public Health Code, article L.1121-1/La loi n° 2012–300 du 5 mars 2012 relative aux recherches impliquant la personne humaine (dite loi Jardé), the applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki. The study was approved by the French Ethic Committee, CPP Ouest II - Angers, ref. CPP: 2028/08, Ref ANSM (French competent authority) 2018020500208, 2018072600120, 2019083000148. Freely given written informed consent was signed and obtained from each individual participating in the study, before any study specific procedure was undertaken and after the provision of information about the study by the investigator during a physician-patient consultation and sufficient time for reflection.

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