Soluble PD-L1 and Circulating CD8+PD-1+ and NK Cells Enclose a Prognostic and Predictive Immune Effector Score in Immunotherapy Treated NSCLC patients

Soluble PD-L1 and Circulating CD8+PD-1+ and NK Cells Enclose a Prognostic and Predictive Immune Effector Score in Immunotherapy Treated NSCLC patients

Mazzaschi G, MD[1,2], Minari R, PhD[1*], Zecca A, PhD[3], Cavazzoni A, PhD[2], Ferri V, MD[1], Mori C, BS[3], Squadrilli A, BS[1], Bordi P, MD, PhD[1], Buti S, MD[1], PhD[1], Bersanelli M, MD[1], Leonetti A, MD[1], Cosenza A, Res Nurs[1], Ferri L, Res Nurs[1], Rapacchi E, BS[1], Missale G, MD, PhD[2,3], Petronini PG, PhD[2], Quaini F, MD[2], Tiseo M, MD, PhD[1,2].

Affiliations:

[1]Medical Oncology Unit, University Hospital of Parma, Parma, Italy

[2]Department of Medicine & Surgery, University of Parma

[3]Infectious Diseases and Hepatology Unit, Laboratory of Viral Immunopathology, University Hospital of Parma

Correspondence:

Dr. Roberta Minari, PhD

Affiliation: Medical Oncology Unit, University Hospital of Parma, Parma, Italy

Office: +39.0521.704466

Fax: +39.0521.995448

e-mail: rominari@ao.pr.it

Abstract

Introduction

Upfront criteria to foresee immune checkpoint inhibitors (ICIs) efficacy are far from being identified. Thus, we integrated blood descriptors of pro-inflammatory/immunosuppressive or effective anti-tumor response to non-invasively define predictive immune profiles in ICI-treated advanced non-small cell lung cancer (NSCLC).

Methods

Peripheral blood (PB) was prospectively collected at baseline from 109 consecutive NSCLC patients undergoing ICIs as first or more line treatment. Soluble PD-L1 (sPD-L1) (immunoassay), CD8+PD-1+ and NK (FACS) cells were assessed and interlaced to generate an Immune effector Score ($I_{eff}S$). Lung Immune Prognostic Index (LIPI) was computed by LDH levels and derived Neutrophil-to-Lymphocyte Ratio (dNLR). All these parameters were correlated with survival outcome and treatment response.

Results

High sPD-L1 and low CD8+PD-1+ and NK number had negative impact on PFS (P<0.001), OS (P<0.01) and ICI-response (P<0.05). Thus, sPD-L1^{high}, CD8+PD-1+^{low} and NK^{low} were considered as risk factors encompassing $I_{eff}S$, whose prognostic power outperformed that of individual features and slightly exceeded that of LIPI. Accordingly, the absence of these risk factors portrayed a favorable $I_{eff}S$ characterizing patients with significantly (P<0.001) prolonged PFS (median NR vs 2.3 months) and OS (median NR vs 4.1) and greater benefit from ICIs (P<0.01). We then combined each risk parameter composing $I_{eff}S$ and LIPI (LDH^{high}, dNLR^{high}), thus defining three distinct prognostic classes. A remarkable impact of $I_{eff}S$-LIPI integration was documented on survival outcome (PFS, HR=4.61; 95%CI=2.32-9.18; *P*<0.001; OS, HR=4.03; 95%CI=1.91-8.67; *P*<0.001) and ICI-response (AUC=0.90, 95%CI=0.81-0.97, P<0.001).

Conclusion

Composite risk models based on blood parameters featuring the tumor-host interaction might provide accurate prognostic scores able to predict ICI benefit in NSCLC patients.

Keywords: non-small cell lung cancer, immune checkpoint inhibitors, circulating biomarkers, prognostic scores

1. Introduction

Similar to the advent of target therapy, immune checkpoint inhibitors (ICIs) are rapidly shifting the oncological landscape and profoundly transforming clinical cancer care, in particular in non-small cell lung cancer (NSCLC) [1,2].

Anti PD-1/PD-L1 targeting agents have been established as the standard of care for advanced NSCLC in first and second or more lines [3], and have demonstrated a long-term survival benefit also in unresectable stage III setting [4]. However, issues concerning the tumor response and its evaluation criteria [5] and the occurrence of immune related side effects (iRAEs) [6] concurrently emerged with these promising outcomes.

In this context, the prediction of treatment benefit remains a crucial unsolved challenge to increase ICI overall efficacy rate and/or reduce unnecessary overtreatment [7]. Much attention has been paid to tissue biomarkers, among which tumor PD-L1 expression holds a primary role [8]. However, the accuracy of PD-L1 has been repeatedly questioned, since ICIs benefit has been documented also in PD-L1-negative NSCLC cases [2,3].

Strategies focusing on the complex interaction between tumor and its microenvironment such as somatic tumor mutational burden (TMB) [9], extent and phenotypes of tumor-infiltrating lymphocytes (TILs) [10–12] and immune gene signatures (i.e. Tumor Inflammation Signature – TIS) [13,14] are also endowed with predictive power.

Although tissue biopsy may be more representative of cancer biology, a single observation cannot express the dynamic nature of the tumor microenvironment and sample availability is often limited in advanced NSCLC setting. Methodological issues related to the standardization of different assays and cutoffs [15] and time-consuming laboratory procedures [8], further restrain the wide application of tissue derived predictive factors.

As immune checkpoint pathways include a substantial circulating phase, peripheral blood represents an easily available source of bio-humoral and cellular parameters potentially implicated in the response to immunotherapy. Indeed, both PD-1 and PD-L1 exist either as membrane bound and soluble forms [16]. Although the precise origin of soluble PD-L1 (sPD-L1) remains elusive, data in advanced stage NSCLC favor the view that most plasma protein derives from cancer cells [16–18]. In addition, circulating sPD-L1 may undergo dynamic changes, as following immunotherapy, potentially linked to disease response. Accordingly, low baseline sPD-L1 levels seemed to be associated to better response rate and clinical

benefit at six months in ICI treated NSCLC [17,19,20]. In addition, the association of low soluble Granzyme B with high sPD-L1 levels was coupled with poor response to nivolumab therapy [17].

Neutrophils are the most prevalent cell type in NSCLC immune landscape, being associated to ICI resistance [21]. A lung immune prognostic index (LIPI), generated from the combination of derived neutrophil to lymphocyte ratio (dNLR) with lactate dehydrogenase (LDH) levels, has been recently validated [22] showing strong correlation with NSCLC survival outcome independently from PD-L1 expression [23].

The quantitative and qualitative assessment of circulating phenotypes more explicitly implicated in the response to PD-1/PD-L1 targeting agents may upgrade the definition of predictive biomarkers. In this regard, the baseline number and function of peripheral blood cytotoxic Granzyme B+ and Perforin+ NK and CD8+ cells [24–26] have shown strong predictive power in advanced NSCLC patients.

Quickly accessible and reproducible parameters from blood samples and requiring simple techniques represent ideal candidate to foresee ICI benefit. This possibility was exploited here by interlacing multiple suppressive or effective immune features. Thus, the aim of our study was to determine whether the integrated analysis of sPD-L1 levels and distinctive subsets of circulating immune cells (CD8+PD-1+ and NK) may non-invasively predict the efficacy of immunotherapy in advanced NSCLC.

2. Materials and Methods

2.1. Patient Population

NSCLC patients from Medical Oncology Unit of the University Hospital of Parma treated with ICIs in first or subsequent line were prospectively enrolled in this study. The clinico-pathological characteristics of this patient population are summarized in Table 1.

Inclusion and exclusion criteria are detailed in Supplementary (Suppl.) Material and Methods. According to RECIST criteria 1.1 [27], we categorized NSCLC patients in clinical benefit group (CB), including complete (CR) or partial (PR) response or stable disease (SD) lasting at least 6 months and non-responders (NR), including patients with disease progression and stable disease lasting less than 6 months as best response.

Patients were enrolled after informed consent and the study was performed following the approval from the ethical committee (155/2018/SPER/AOUPR) and in accordance with Helsinki principles.

2.2. Peripheral Blood Analysis

Peripheral blood (PB) samples were collected at baseline, right before the first ICI administration.

sPD-L1. sPD-L1 concentrations were measured, one replicate for each patients, in 200 ul of serum using the enzyme-linked immunosorbent assay (ELISA) Quantikine® Human/Cynomolgus Monkey PD-L1/B7-H1 Immunoassay kit (©2017 R&D Systems®, Inc) following the manufacturer's instructions.

ha eliminato: *Plasma*

ha eliminato:

Flow Cytometry (FC) of circulating immune cells. Fluorescence-Activated Cell Sorting (FACS) of peripheral blood samples involved the analysis of circulating CD3+, CD8+, CD4+ and NK. FACSCanto II flow cytometer (BD Biosciences) and FACSDiva Software were employed to compute the analyses.

A previously used gating strategy [24] to assess the frequency and absolute number of circulating CD8+PD-1+ lymphocytes and NK cells is detailed in Suppl. Material and Methods.

Immune Effector Score. Following an immune oriented approach, we intersected sPD-L1 values with the number of PB cytotoxic cells. Thus, to portray an immune effector score ($I_{eff}S$) able to define different prognostic classes, we combined sPD-L1 levels with the circulating number of cytotoxic CD8+PD-1+ and NKs. Specifically, we first applied univariate correlation test (Mann-Whitney U test), evaluating the clinical impact of individual parameters, to select circulating descriptors with significant level of correlation ($P<0.05$). The designated peripheral blood features (sPD-L1, CD8+PD-1+ and NKs), considered as continuous variables, were then challenged in a Cox proportional-hazard model. We categorized sPD-L1 values in high vs low subgroups according to cut-off established by Classification and regression tree (CART) analysis. Previous CART-based cut-offs of circulating CD8+PD-1+ and NK cells values were employed here to serve as validation of our reported data [24]. To provide a prognostic score, NSCLC patients were split in two different classes based on presence or absence of the three predetermined risk factors: sPD-L1^{high} vs sPD-L1^{low}, CD8+PD-1+^{low} vs CD8+PD-1+^{high} and NK^{low} vs NK^{high}.

LIPI Score. LDH levels were measured by conventional fluorometric assay and reported as Unit/litre. Values resulting within the normal range (0-248 U/L) or higher than the Upper Limit of Normality (>UNL) were separately considered. Complete blood cell count and leukocytes differential were obtained by automated routine hemocytometric analysis. Neutrophil to lymphocyte ratio (NLR) and derived neutrophil to lymphocyte ratio (dNLR; absolute neutrophil count/[white blood cell concentration - absolute neutrophil count]) were computed.
Lung immune prognostic index (LIPI) scores were calculated based on dNLR and LDH levels according to published criteria able to generate good (dNLR < 3 and LDH lower than UNL), intermediate (1 factor) and poor (2 factors) composite scores [22].

Integrated LIPI-$I_{eff}S$ Model. Adopting the methodological approach illustrated above, we combined the individual risk factors composing $I_{eff}S$ (sPD-L1^{high}, CD8+PD-1+^{low} and NK^{low}) and LIPI (LDH^{high}, dNLR^{high}) and three distinct risk categories were obtained: ≤ 1, 2-3, ≥ 4 risk factors.

2.3. Immunohistochemical analysis of tissue PD-L1

Five μm thick sections from formalin fixed and paraffin embedded biopsies from a subset (n: 61) of NSCLC patients were subjected to the immunohistochemical evaluation of PD-L1. PD-L1 levels were measured using anti PD-L1 antibody (clone SP263) by immunoperoxidase and expressed as % of cell surface labelling following established criteria [28]. Three distinct subgroups were defined according to tissue PD-L1 (tPD-L1) score: negative (<1%), intermediate (1-49%) and high (50-100%).

2.4. Statistical analysis

Overall survival (OS) and progression-free survival (PFS) were estimated by Kaplan Meier method (see Supplementary Material and Methods). Cut-off for survival analysis was set at December 30th, 2019. Median follow-up was calculated according to the so-termed "reverse Kaplan Meier" (Kaplan Meier estimate of potential follow-up) technique [29]. Log-rank test (Mantel-Cox) was applied to evaluate statistical differences in PFS and OS between groups. PFS and OS data were then analysed through Cox uni- and multivariate proportional hazards regression models and results were expressed as hazard ratios (HR), 95% CI and p values. The multivariate models were fitted including the covariates that resulted statistically significant in the univariate model. The Fisher's exact test was used to examine the differences between categorical variables and the Mann-Whitney U test or Kruskal Wallis to detect differences in continuous variables between groups of patients, given that the distribution of data was not normal (Kolmogorov–Smirnov test). ROC curves were used to test the sensitivity and specificity of a marker, with the area under the curve (AUC) being given with its 95% confidence interval (CI). In the case that more than one marker was used to create the ROC curve (marker pattern), the probability of being in a given group, as calculated by means of logistic regression, was used instead of marker values. Classification and regression tree (CART) analysis identified specific cut-off values that segregated patients by clinical outcomes.

P value of 0.05 was set as a threshold of statistical significance. IBM SPSS Statistics v 25.0 (IBM) and Stata 13 with Cart module (Statacorp) were used to perform all computational analyses.

3. Results

3.1 Patient Population

The patient population included 109 consecutive advanced NSCLC cases (97% Stage IV) undergoing immunotherapy. ICIs were administered as second line treatment in 67% of cases (n=73) and mostly consisted of single anti-PD-1 agents (n=80) (Table 1). The contralateral lung and lymph nodes were the predominant sites (>90%) of metastatic involvement followed by bone and pleura (≅ 40%), while brain metastases were present in 15% of patients. At data cut-off (December 30th, 2019), median follow-up was 17.3 months. The median PFS and OS in the overall population were 2.6 (95%CI, 0.76-4.36) and 7.9 months (95%CI 2.36-13.35), respectively (Supplementary Figure S1).

3.2 Baseline Circulating Parameters

Individual values of sPD-L1, CD8+PD-1+, NK, dNLR and LDH, in the overall patient population are reported in Supplementary Figure S2A. The distribution of sPD-L1 levels ranged from 10.02 pg/ml to 261.26 pg/ml, resulting in an average value of 84.07 ± (standard error, SE) 5.04 pg/ml. The average absolute number of circulating CD8+PD-1+ (117.98 ± 13.94/μl) and NK (265.43 ± 20.26/μl) cells was in line with our previous observations in advanced setting of NSCLC patients [24].

ha formattato

We also observed internal correlations among the investigated variables. Specifically, dNLR inversely correlated with the number of CD8+PD-1+ (P<0.005) and NK (P=0.001) cells, while a trend of a direct correlation with sPD-L1 (P=0.09) was apparent (Supplementary Figure S2B).

3.3 Correlations Between Clinico-pathological and Circulating Parameters

Among clinico-pathological features, poor performance status (ECOG PS 2) significantly correlated to high sPD-L1 levels (P=0.014) and dNLR (P=0.014) and low number of PB CD8+PD-1+ (P=0.05) and NK (P=0.026) cells (Table 2). The trend of circulating parameters according to the number and site of metastatic involvement showed that cases with more than 4 sites and liver metastasis had significantly increased sPD-L1 and LDH values (Table 2). In addition, bone involvement was associated with low PB CD8+PD-1+ (P=0.004) and NK

(*P*=0.05) cells and high dNLR (*P*=0.004) and LDH (*P*=0.011). Higher levels of sPD-L1 were documented in NSCLC patients displaying liver (*P*=0.041) and brain (*P*=0.03) metastases. Sex and histotype did not appear to affect circulating parameters, while advanced age conditioned increased levels of LDH (656 vs 435 U/L, *P*=0.009, Table 2).

We did not document significant association between the expression of tPD-L1 (tPD-L1) assessed on primary tumors with blood parameters (data not shown).

3.4 Impact of Individual Parameters on Survival Outcome and Response to ICIs

3.4.1 Clinico-pathological Features

In addition to ECOG PS 2, previous administration of steroids and antibiotics to ICI treated patients negatively conditioned PFS and OS at univariate analysis (Table 3), while only steroids had a significant impact on PFS at multivariate level (HR 3.91, 95%CI 2.76-5.64, *P*=0.033).

Steroid therapy appeared to negatively impact also on disease response, since 79% (n=26/33) of patients on steroids before ICIs belonged to NR group (*P*<0.001) vs 21% (n=7/33) of patients without steroids before ICIs (data not shown).

Significantly prolonged PFS and OS were documented at both univariate (PFS: HR 0.69, 95%CI 0.52-0.92, *P*=0.010; OS: HR 0.72, 95%CI 0.52-0.99, *P*=0.046) and multivariate (PFS: HR 0.45, 95%CI 0.32-0.78, *P*=0.004; OS: HR 0.54, 95%CI 0.531-0.95, *P*=0.031) analysis in NSCLC patients whose primary tumor displayed high tPD-L1 score. In addition, distinctive survival curves were obtained following the stratification of patients according to negative (< 1%), intermediate (1-49%) and high (50-100%) tPD-L1 score (Supplementary Figure S3A*i-ii*).

The positive clinical impact of tPD-L1 was also confirmed in terms of tumor response, since 89% (n=16/18) of cases displaying ≥ 50% tPD-L1 expression had CB vs 37% (n=10/27) and 54% (n=6/11) of patients with intermediate and negative expression, respectively (*P*=0.002; Supplementary Figure S3B). The corresponding ROC curve showed an AUC value of 0.68 (95%CI 0.53-0.81, *P*=0.027), thus demonstrating a moderate discrimination ability.

3.4.2 Peripheral Blood Parameters

The clinical relevance of serum (sPD-L1 and LDH) and PB phenotypic (CD8+PD-1+, NK and dNLR) parameters was clearly highlighted by Cox proportional hazard models (Table 3).

sPD-L1. According to a cutoff value set at 113 pg/ml by CART tree analysis, patients with low vs high sPD-L1 had a median PFS of 11.9 vs 3.8 months (HR 2.55, 95%CI 1.50-4.32, *P*<0.001) and a median OS of 15.0 vs 5.8 (HR 2.53, 95% CI1.42-4.51, *P*=0.001), respectively (Figure 1A*i-ii*).

A trend toward higher sPD-L1 levels was detected in patients who failed to respond to ICIs compared to CB group (86.04 ± 7.86 pg/ml vs 77.02 ± 6.58 pg/ml) although not reaching statistical significance (Figure 1B*i*, *P*=0.379). This was confirmed by ROC curve showing the lack of sPD-L1 in discriminating NR from CB patients (Figure 1C*i*).

CD8+PD-1+ and NKs. In strong agreement with our previous findings [24] FACS analysis documented that cases with high vs low number of PB CD8+PD-1+ (Figure 1 A*iii-iv*) and NK (Figure 1 A*v-vi*) cells had longer PFS (CD8+PD-1+, median PFS 9.8 vs 1.2 months, *P*<0.001; NK, median PFS 10.2 vs 3.8 months, *P*<0.001) and OS (CD8+PD-1+, median OS 12.1 vs 2.8 months, *P*=0.001; NK, median OS 12.8 vs 6.1 months, *P*=0.001).

A 2.2-fold and 1.4-fold increase in baseline CD8+PD-1+ (*P*=0.003) and NK (*P*=0.043), respectively, was also detected in patients responsive to ICIs (Figure 1B*ii-iii*). Importantly, the number of CD8+PD-1+ (AUC 0.80, 95%CI 0.67-0.93, *P*<0.001) and NK (AUC 0.67, 95%CI 0.56-0.68, *P*=0.005) cells accurately predicted ICI benefit, as disclosed by the corresponding ROC curve (Figure 1C*ii-iii*).

dNLR and LDH. A dNLR greater than 3 clearly characterized patients with poor survival outcome (median PFS 5.7 months vs 12.6 months, *P*=0.001; median OS 8.0 months vs 15.6 months, *P*=0.002) (Figure 2A*i-ii*). Similarly, elevated LDH levels (>ULN) conditioned significantly reduced PFS and OS (median PFS 3.5 months vs 11.9 months, *P*<0.001; median OS 7.5 months vs 14.6 months, *P*<0.001) (Figure 2A*iii-iv*).

Higher values of both dNLR and LDH also characterized patients NR to ICIs compared to CB group (Figure 2B*i-ii*). Importantly, as shown by the respective ROC curve AUC, the specificity and sensitivity of dNLR (AUC 0.69, 95%CI 0.58-0.79, *P*=0.001) and of LDH (AUC

0.67, 95%CI 0.57-0.78, *P*=0.002) in discerning the differential ICI response was documented (Figure 2C*i-ii*).

3.5 Integrated Prognostic and Predictive Scores

The clinical relevance of individual PB parameters prompted us to generate potential prognostic scores through the combination of the most significant serum and immunophenotypic characteristics (see details in Material and Methods).

Immune Effector Score. Plasma sPD-L1 levels associated with circulating CD8+PD-1+ and NKs were able to portray an immune effector score ($I_{eff}S$) with significant prognostic impact. As shown in Figure 3A*i-ii*, while median PFS and OS were not reached in patients carrying low sPD-L1 and high number of CD8+PD-1+ and NKs, the presence of at least one risk factor was sufficient to markedly affect survival outcome (PFS, HR 5.72, 95%CI 2.17-15.04, P<0.001; OS, HR 5.07, 95%CI 1.71-14.97, P<0.001).

$I_{eff}S$ was also highly associated with disease control rate (*P*=0.002, Figure 3B*i*), and the ROC curve, obtained by plotting the regression coefficients of $I_{eff}S$ parameters, displayed an AUC value of 0.80 (95%CI 0.66-0.92, *P*=0.001).

LIPI. In our cohort of NSCLC, good LIPI score was present in 38% (n=41) of cases, intermediate in 47% (n=51) and poor in 15% (n=17). Patients with good LIPI score had a median PFS of 23 months compared to 5.1 and 2.9 months of cases with intermediate and poor LIPI score, respectively (*P*<0.001, Figure 3A*iii*). A good LIPI score was also associated with longer OS (median not reached) compared to intermediate (median 4.8 months) and poor (median 1.3 months) scores (HR 2.42; 95%CI, 1.70-3.45; *P*<0.001; Figure 3A*iv*).

Moreover, 66% of ICI responders had good LIPI, while 77% of NR displayed poor/intermediate LIPI (P<0.001, Figure 3B*ii*). The high predictive power of LIPI score was also confirmed by ROC curve AUC value (AUC 0.75, 95%CI 0.66-0.92, *P*<0.001; Figure 3C*ii*).

Integrated LIPI-$I_{eff}S$ Score. Finally, we integrated $I_{eff}S$ and LIPI parameters in a comprehensive multiparametric model, delineating three risk categories with distinct survival outcome. Significantly prolonged PFS and OS were detected in patients displaying 0-1 risk factors (at median follow-up, nearly 80% of cases were not progressed, nor death) compared

to both intermediate (2-3 risk factors) and poor (4-5 risk factors) risk groups (PFS: HR 4.61, 95%CI, 2.32-9.18, *P*<0.001; OS: HR 4.03, 95% CI, 1.91-8.67, *P*<0.001; Figure 3A*v-vi*).

Accordingly, we documented a remarkable impact of I_{eff}S-LIPI integration also in terms of tumor response, as none of the patients with poor risk score showed response to immunotherapy while up to 61% of NSCLC cases belonging to CB group displayed a good score (*P*<0.001, Figure 4B*iii*). As illustrated by the corresponding ROC curve, the discrimination ability of the integrated model was clearly superior to individual I_{eff}S and LIPI scores, reaching an AUC of 0.90 (95%CI 0.81-0.97, *P*<0.001; Figure 3C*iii*).

4. Discussion

Research strategies to predict the response to immunotherapy have been recognized by the American Society of Clinical Oncology as one of the top nine priorities to advance progress against cancer [30].

Approximately 20% to 40% of lung cancer patients benefits from ICI [31] with an expected growing population eligible for immunotherapy alone or in combination with chemotherapy [32].

In this context, the current evidence-based approach to biomarker assessment needs to be urgently revisited [33]. While tissue PD-L1 expression successfully served as a criteria of NSCLC patient selection [8], its definite role as predictor of the response to ICI is more debated. Molecular analysis of TMB [9,34] and TIS [13] at tissue and blood levels (i.e. liquid biopsy), although displaying predictive impact, have not reached sufficient standardization for a wide clinical application.

Due to the heterogeneous nature of cancer, individual biomolecular benchmarks have generally failed to meet the criteria of a valid biomarker. Suitable predictive scores of ICI efficacy may be achieved by a multiparametric strategy involving easily accessible and non-invasive samples, such as peripheral blood [35]. Importantly, the circulation gathers the systemic immune response and may be more informative and timely reflecting cancer immunity and tumor heterogeneity than the local tissue context.

In the attempt to foresee ICI benefit, peripheral blood was used here as a source of common players of the inflammatory response (dNLR, LDH) and more immune-specific biomolecules (sPD-L1) and phenotypes (CD8+PD-1+ and NK) critically implicated in PD-1/PD-L1 pathway.

Increasing attention has been paid to the soluble form of PD-L1 protein, as evidences suggest the negative impact of high sPD-L1 circulating levels on patients prognosis and response to treatment [17,19,20]. PD-L1 is present in the circulation and other biofluids, such as in pleural effusion of lung cancer patients [18], as a result of proteolytic cleavage or mRNA alternative splicing of the cell surface protein. The precise contribution of different cell types to the actual plasmatic concentration of sPD-L1 remains uncovered. This is a relevant issue since it has been shown in melanoma patients that, depending on the source, differences in sPD-L1 concentrations might condition either favorable or unfavorable clinical outcome [36].

In agreement with tumor cells as major contributors to the circulating protein, we observed a direct correlation between sPD-L1 levels and disease burden in a large cohort of advanced NSCLC.

The real function of sPD-L1 is not completely clear yet, although several biological effects have been proposed. Tumor cell-derived sPD-L1 appeared to induce apoptosis in T cells of advanced renal carcinoma patients [37]. An additional mechanistic hypothesis comes from the observation that sPD-L1 might inactivate tumoricidal circulating T cells, reducing antitumor immune activity [19]. This assumption may be supported here, at least in part, by the documentation of the negative impact of the association of high sPD-L1 levels with low number of cytotoxic T and NK cells, thereby enclosing the proposed prognostic and predictive Immune effector score ($I_{eff}S$). Moreover, sPD-L1 could compete and saturate PD-1 binding sites therefore eluding the activity of anti PD-1 agents [16].

Circulating effector cells are fundamental to generate a natural and therapy induced anti-cancer response. CD8+ T and NK cells act recruiting adaptive immune cells, directly and indirectly, through the production of chemokines and the stimulation of Antigen Presenting Cells (APCs), in order to trigger an efficient cancer immune surveillance [26,38]. In the present immunotherapy scenario, these mechanistic events are translated into relevant variables affecting disease response. Indeed, as suggested by our [24] and other laboratories, the availability of a significant number of circulating cytotoxic phenotypes increases patient sensitivity to ICI [35]. This finding was validated here by the evidence of prolonged OS ad PFS in NSCLC cases displaying high levels of CD8+PD-1+ and NK cells.

Several reports indicate that, independently from the absolute number of white blood cells, an increased relative proportion of circulating neutrophils with respect to lymphocytes (dNLR) might be considered a marker of unfavorable immune response [39–41].

dNLR, seemingly expressing an inflammatory state, is combined with LDH levels, reflecting *bona fide* cell turnover, to constitute LIPI [22]. As supported by our data, LIPI score represents a successful attempt to merge multiple circulating parameters able to reproducibly predict the outcome of metastatic NSCLC patients irrespective of treatment modality [23].

However, LIPI parameters just scratch the surface of profound immuno-inflammatory events and may be affected by tumor-independent variables, such corticosteroids, infections or co-morbidities, partly limiting their biological and clinical significance. Thus, to provide immune profiles more representative of the multifaceted aspects of cancer immunity, we

combined LIPI with sPD-L1 and circulating immunophenotypes encompassing $I_{eff}S$. Intriguingly, the power of this integrated model overcame that of individual scores. Moreover, in our cohort of NSCLC, the score resulting from $I_{eff}S$-LIPI integration was able to sharply define three distinct prognostic categories and, at variance with LIPI, the increment in risk factors was translated in parallel reduction in PFS and OS duration. This finding strongly supports the notion that multiparametric strategies may improve our ability to anticipate the clinical response to immunotherapy and to prospectively select the best patient- and disease-specific therapeutic option. In addition, similar approaches own the translational significance of comprehensively decoding cancer immune landscape by simple blood sampling.

Limitations of our study have to be acknowledged. The relatively low number of NSCLC patients in different lines of treatment and the absence of chemo-immuno combinatory regimens might not allow definitive conclusions. In addition, the lack of both external validation cohort and control group not treated with ICIs limits the definition of predictive significance of our score. However, the current validation of the predictive role of CD8+PD-1+ and NK cells [24], the internal consistency of all evaluated parameters as well as the robustness of our score estimates could represent the strengths of the present study.

5. Conclusion

In conclusion, our findings suggest that the integrated analysis of blood descriptors of the inflammatory response (dNLR, LDH) and immune-relevant biomolecules/phenotypes (sPD-L1) and phenotypes (CD8+PD-1+ and NK), critically implicated in PD-1/PD-L1 axis, might define prognostic and predictive immune scores in ICI-treated advanced NSCLC.

Funding

This work was supported in part by COST BIONECA 2016-CA16122 to FQ.

Acknowledgment

M.T. is currently supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) under Investigator Grant (IG) No. IG2017-20074.

References

[1] J.J. Havel, D. Chowell, T.A. Chan, The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy, Nat. Rev. Cancer. 19 (2019) 133–150. https://doi.org/10.1038/s41568-019-0116-x.

[2] R.S. Herbst, D. Morgensztern, C. Boshoff, The biology and management of non-small cell lung cancer, Nature. 553 (2018) 446–454. https://doi.org/10.1038/nature25183.

[3] D. Planchard, S. Popat, K. Kerr, S. Novello, E.F. Smit, C. Faivre-Finn, T.S. Mok, M. Reck, P.E. Van Schil, M.D. Hellmann, S. Peters, Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 29 (2018) iv192–iv237. https://doi.org/10.1093/annonc/mdy275.

[4] J.E. Gray, A. Villegas, D. Daniel, D. Vicente, S. Murakami, R. Hui, T. Kurata, A. Chiappori, K.H. Lee, B.C. Cho, D. Planchard, L. Paz-Ares, C. Faivre-Finn, J.F. Vansteenkiste, D.R. Spigel, C. Wadsworth, M. Taboada, P.A. Dennis, M. Özgüroğlu, S.J. Antonia, Three-Year Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC—Update from PACIFIC, J. Thorac. Oncol. 15 (2020) 288–293. https://doi.org/10.1016/j.jtho.2019.10.002.

[5] L. Seymour, J. Bogaerts, A. Perrone, R. Ford, L.H. Schwartz, S. Mandrekar, N.U. Lin, S. Litière, J. Dancey, A. Chen, F.S. Hodi, P. Therasse, O.S. Hoekstra, L.K. Shankar, J.D. Wolchok, M. Ballinger, C. Caramella, E.G.E. de Vries, RECIST working group, iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics., Lancet. Oncol. 18 (2017) e143–e152. https://doi.org/10.1016/S1470-2045(17)30074-8.

[6] M.A. Postow, R. Sidlow, M.D. Hellmann, Immune-related adverse events associated with immune checkpoint blockade, N. Engl. J. Med. 378 (2018) 158–168. https://doi.org/10.1056/NEJMra1703481.

[7] S. Zhang, X. Bai, F. Shan, The progress and confusion of anti-PD1/PD-L1 immunotherapy for patients with advanced non-small cell lung cancer, Int. Immunopharmacol. 80 (2020) 106247. https://doi.org/10.1016/j.intimp.2020.106247.

[8] R. Buttner, J.R. Gosney, B.G. Skov, J. Adam, N. Motoi, K.J. Bloom, M. Dietel, J.W. Longshore, F. Lopez-Ríos, F. Penault-Llorca, G. Viale, A.C. Wotherspoon, K.M. Kerr, M.S. Tsao, Programmed death-ligand 1 immunohistochemistry testing: A review of analytical assays and clinical implementation in non-small-cell lung cancer, J. Clin.

Oncol. 35 (2017) 3867–3876. https://doi.org/10.1200/JCO.2017.74.7642.

[9] N.A. Rizvi, M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, W. Lee, J. Yuan, P. Wong, T.S. Ho, M.L. Miller, N. Rekhtman, A.L. Moreira, F. Ibrahim, C. Bruggeman, B. Gasmi, R. Zappasodi, Y. Maeda, C. Sander, E.B. Garon, T. Merghoub, J.D. Wolchok, T.N. Schumacher, T.A. Chan, Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science (80-.). 348 (2015) 124–128. https://doi.org/10.1126/science.aaa1348.

[10] G. Corredor, X. Wang, Y. Zhou, C. Lu, P. Fu, K. Syrigos, D.L. Rimm, M. Yang, E. Romero, K.A. Schalper, V. Velcheti, A. Madabhushi, Spatial architecture and arrangement of tumor-infiltrating lymphocytes for predicting likelihood of recurrence in early-stage non–small cell lung cancer, Clin. Cancer Res. 25 (2019) 1526–1534. https://doi.org/10.1158/1078-0432.CCR-18-2013.

[11] G. Mazzaschi, D. Madeddu, A. Falco, G. Bocchialini, M. Goldoni, F. Sogni, G. Armani, C.A. Lagrasta, B. Lorusso, C. Mangiaracina, R. Vilella, C. Frati, R. Alfieri, L. Ampollini, M. Veneziani, E.M. Silini, A. Ardizzoni, K. Urbanek, F. Aversa, F. Quaini, M. Tiseo, Low PD-1 expression in cytotoxic CD8 þ tumor-Infiltrating lymphocytes confers an immune-privileged tissue microenvironment in NSCLC with a prognostic and predictive value, Clin. Cancer Res. 24 (2018) 407–419. https://doi.org/10.1158/1078-0432.CCR-17-2156.

[12] S. Hu-Lieskovan, A. Lisberg, J.M. Zaretsky, T.R. Grogan, H. Rizvi, D.K. Wells, J. Carroll, A. Cummings, J. Madrigal, B. Jones, J. Gukasyan, I.P. Shintaku, D. Slamon, S. Dubinett, J.W. Goldman, D. Elashoff, M.D. Hellmann, A. Ribas, E.B. Garon, Tumor Characteristics Associated with Benefit from Pembrolizumab in Advanced Non–Small Cell Lung Cancer, Clin. Cancer Res. (2019). https://doi.org/10.1158/1078-0432.ccr-18-4275.

[13] P. Danaher, S. Warren, R. Lu, J. Samayoa, A. Sullivan, I. Pekker, B. Wallden, F.M. Marincola, A. Cesano, Pan-cancer adaptive immune resistance as defined by the Tumor Inflammation Signature (TIS): Results from The Cancer Genome Atlas (TCGA), J. Immunother. Cancer. 6 (2018) 1–17. https://doi.org/10.1186/s40425-018-0367-1.

[14] M. Binnewies, E.W. Roberts, K. Kersten, V. Chan, D.F. Fearon, M. Merad, L.M. Coussens, D.I. Gabrilovich, S. Ostrand-Rosenberg, C.C. Hedrick, R.H. Vonderheide, M.J. Pittet, R.K. Jain, W. Zou, T.K. Howcroft, E.C. Woodhouse, R.A. Weinberg, M.F. Krummel, Understanding the tumor immune microenvironment (TIME) for effective

therapy, Nat. Med. 24 (2018) 541–550. https://doi.org/10.1038/s41591-018-0014-x.

[15] M.S. Tsao, K.M. Kerr, M. Kockx, M.B. Beasley, A.C. Borczuk, J. Botling, L. Bubendorf, L. Chirieac, G. Chen, T.Y. Chou, J.H. Chung, S. Dacic, S. Lantuejoul, M. Mino-Kenudson, A.L. Moreira, A.G. Nicholson, M. Noguchi, G. Pelosi, C. Poleri, P.A. Russell, J. Sauter, E. Thunnissen, I. Wistuba, H. Yu, M.W. Wynes, M. Pintilie, Y. Yatabe, F.R. Hirsch, PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project, J. Thorac. Oncol. 13 (2018) 1302–1311. https://doi.org/10.1016/j.jtho.2018.05.013.

[16] T. Abu Hejleh, M. Furqan, Z. Ballas, G. Clamon, The clinical significance of soluble PD-1 and PD-L1 in lung cancer, Crit. Rev. Oncol. Hematol. 143 (2019) 148–152. https://doi.org/10.1016/j.critrevonc.2019.08.009.

[17] A. Costantini, C. Julie, C. Dumenil, Z. Hélias-Rodzewicz, J. Tisserand, J. Dumoulin, V. Giraud, S. Labrune, T. Chinet, J.F. Emile, E. Giroux Leprieur, Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab, Oncoimmunology. 7 (2018). https://doi.org/10.1080/2162402X.2018.1452581.

[18] S. Vecchiarelli, F. Passiglia, A. D'Incecco, M. Gallo, A. De Luca, E. Rossi, F. D'Incà, G. Minuti, L. Landi, C. Bennati, M. Spreafico, M. D'Arcangelo, V. Mazza, N. Normanno, F. Cappuzzo, Circulating programmed death ligand-1 (cPD-L1) in non-smallcell lung cancer (NSCLC), Oncotarget. 9 (2018) 17554–17563. https://doi.org/10.18632/oncotarget.24785.

[19] Y. Okuma, Y. Hosomi, Y. Nakahara, K. Watanabe, Y. Sagawa, S. Homma, High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer, Lung Cancer. 104 (2017) 1–6. https://doi.org/10.1016/j.lungcan.2016.11.023.

[20] Y. Okuma, H. Wakui, H. Utsumi, Y. Sagawa, Y. Hosomi, K. Kuwano, S. Homma, Soluble Programmed Cell Death Ligand 1 as a Novel Biomarker for Nivolumab Therapy for Non–Small-cell Lung Cancer, Clin. Lung Cancer. 19 (2018) 410-417.e1. https://doi.org/10.1016/j.cllc.2018.04.014.

[21] J. Kargl, S.E. Busch, G.H.Y. Yang, K.H. Kim, M.L. Hanke, H.E. Metz, J.J. Hubbard, S.M. Lee, D.K. Madtes, M.W. McIntosh, A.M. McGarry Houghton, Neutrophils dominate the immune cell composition in non-small cell lung cancer, Nat. Commun. 8 (2017) 1–11. https://doi.org/10.1038/ncomms14381.

[22] L. Mezquita, E. Auclin, R. Ferrara, M. Charrier, J. Remon, D. Planchard, S. Ponce, L.P. Ares, L. Leroy, C. Audigier-Valette, E. Felip, J. Zerón-Medina, P. Garrido, S. Brosseau, G. Zalcman, J. Mazieres, C. Caramela, J. Lahmar, J. Adam, N. Chaput, J.C. Soria, B. Besse, Association of the lung immune prognostic index with immune checkpoint inhibitor outcomes in patients with advanced non-small cell lung cancer, JAMA Oncol. 4 (2018) 351–357. https://doi.org/10.1001/jamaoncol.2017.4771.

[23] D. Kazandjian, Y. Gong, P. Keegan, R. Pazdur, G.M. Blumenthal, Prognostic Value of the Lung Immune Prognostic Index for Patients Treated for Metastatic Non-Small Cell Lung Cancer, JAMA Oncol. 5 (2019) 1481–1485. https://doi.org/10.1001/jamaoncol.2019.1747.

[24] G. Mazzaschi, F. Facchinetti, G. Missale, D. Canetti, D. Madeddu, A. Zecca, M. Veneziani, F. Gelsomino, M. Goldoni, S. Buti, P. Bordi, F. Aversa, A. Ardizzoni, F. Quaini, M. Tiseo, The circulating pool of functionally competent NK and CD8+ cells predicts the outcome of anti-PD1 treatment in advanced NSCLC, Lung Cancer. 127 (2019) 153–163. https://doi.org/10.1016/j.lungcan.2018.11.038.

[25] L.N. Bodduluru, E.R. Kasala, R.M.R. Madhana, C.S. Sriram, Natural killer cells: The journey from puzzles in biology to treatment of cancer, Cancer Lett. 357 (2015) 454–467. https://doi.org/10.1016/j.canlet.2014.12.020.

[26] A.O. Kamphorst, R.N. Pillai, S. Yang, T.H. Nasti, R.S. Akondy, A. Wieland, G.L. Sica, K. Yu, L. Koenig, N.T. Patel, M. Behera, H. Wu, M. McCausland, Z. Chen, C. Zhang, F.R. Khuri, T.K. Owonikoko, R. Ahmed, S.S. Ramalingam, Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 4993–4998. https://doi.org/10.1073/pnas.1705327114.

[27] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1), Eur. J. Cancer. 45 (2009) 228–247. https://doi.org/10.1016/j.ejca.2008.10.026.

[28] S. Lantuejoul, M. Sound-Tsao, W.A. Cooper, N. Girard, F.R. Hirsch, A.C. Roden, F. Lopez-Rios, D. Jain, T.Y. Chou, N. Motoi, K.M. Kerr, Y. Yatabe, E. Brambilla, J. Longshore, M. Papotti, L.M. Sholl, E. Thunnissen, N. Rekhtman, A. Borczuk, L. Bubendorf, Y. Minami, M.B. Beasley, J. Botling, G. Chen, J.H. Chung, S. Dacic, D.

Hwang, D. Lin, A. Moreira, A.G. Nicholson, M. Noguchi, G. Pelosi, C. Poleri, W. Travis, A. Yoshida, J.B. Daigneault, I.I. Wistuba, M. Mino-Kenudson, PD-L1 Testing for Lung Cancer in 2019: Perspective From the IASLC Pathology Committee, J. Thorac. Oncol. 15 (2020) 499–519. https://doi.org/10.1016/j.jtho.2019.12.107.

[29] M. Schemper, T.L. Smith, A note on quantifying follow-up in studies of failure time, Control. Clin. Trials. 17 (1996) 343–346. https://doi.org/10.1016/0197-2456(96)00075-X.

[30] Research Priorities to Accelerate Progress Against Cancer | ASCO, (n.d.). https://www.asco.org/research-guidelines/reports-studies/clinical-cancer-advances-2020/research-priorities-accelerate (accessed May 1, 2020).

[31] N.H. Hanna, B.J. Schneider, S. Temin, S. Baker, J. Brahmer, P.M. Ellis, L.E. Gaspar, R.Y. Haddad, P.J. Hesketh, D. Jain, I. Jaiyesimi, D.H. Johnson, N.B. Leighl, T. Phillips, G.J. Riely, A.G. Robinson, R. Rosell, J.H. Schiller, N. Singh, D.R. Spigel, J.O. Stabler, J. Tashbar, G. Masters, Therapy for Stage IV Non–Small-Cell Lung Cancer Without Driver Alterations: ASCO and OH (CCO) Joint Guideline Update, J. Clin. Oncol. (2020) JCO.19.03022. https://doi.org/10.1200/jco.19.03022.

[32] A. Leonetti, B. Wever, G. Mazzaschi, Y.G. Assaraf, C. Rolfo, F. Quaini, M. Tiseo, E. Giovannetti, Molecular basis and rationale for combining immune checkpoint inhibitors with chemotherapy in non-small cell lung cancer, Drug Resist. Updat. 46 (2019). https://doi.org/10.1016/j.drup.2019.100644.

[33] N. Tray, J.S. Weber, S. Adams, Predictive biomarkers for checkpoint immunotherapy: Current status and challenges for clinical application, Cancer Immunol. Res. 6 (2018) 1122–1128. https://doi.org/10.1158/2326-6066.CIR-18-0214.

[34] J.D. Fumet, C. Truntzer, M. Yarchoan, F. Ghiringhelli, Tumour mutational burden as a biomarker for immunotherapy: Current data and emerging concepts, Eur. J. Cancer. 131 (2020) 40–50. https://doi.org/10.1016/j.ejca.2020.02.038.

[35] C. Hernandez, H. Arasanz, L. Chocarro, A. Bocanegra, G. Kochan, Systemic Blood Immune Cell Populations as Biomarkers for the Outcome of Immune Checkpoint Inhibitor Therapies, (n.d.) 1–13.

[36] S. Ugurel, D. Schadendorf, K. Horny, A. Sucker, S. Schramm, J. Utikal, C. Pföhler, R. Herbst, B. Schilling, C. Blank, J.C. Becker, A. Paschen, L. Zimmer, E. Livingstone, P.A. Horn, V. Rebmann, Elevated baseline serum PD-1 or PD-L1 predicts poor outcome of

PD-1 inhibition therapy in metastatic melanoma, Ann. Oncol. 31 (2020) 144–152. https://doi.org/10.1016/j.annonc.2019.09.005.

[37] X. Frigola, B.A. Inman, C.M. Lohse, C.J. Krco, J.C. Cheville, R.H. Thompson, B. Leibovich, M.L. Blute, H. Dong, E.D. Kwon, Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma, Clin. Cancer Res. 17 (2011) 1915–1923. https://doi.org/10.1158/1078-0432.CCR-10-0250.

[38] L. Schmidt, B. Eskiocak, R. Kohn, C. Dang, N.S. Joshi, M. DuPage, D.Y. Lee, T. Jacks, Enhanced adaptive immune responses in lung adenocarcinoma through natural killer cell stimulation, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 17460–17469. https://doi.org/10.1073/pnas.1904253116.

[39] F. Facchinetti, M. Veneziani, S. Buti, F. Gelsomino, A. Squadrilli, P. Bordi, M. Bersanelli, A. Cosenza, L. Ferri, E. Rapacchi, G. Mazzaschi, F. Leonardi, F. Quaini, A. Ardizzoni, G. Missale, M. Tiseo, Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab, Immunotherapy. 10 (2018) 681–694. https://doi.org/10.2217/imt-2017-0175.

[40] J. Kargl, X. Zhu, H. Zhang, G.H.Y. Yang, T.J. Friesen, M. Shipley, D.Y. Maeda, J.A. Zebala, J. McKay-Fleisch, G. Meredith, A. Mashadi-Hossein, C. Baik, R.H. Pierce, M.W. Redman, J.C. Thompson, S.M. Albelda, H. Bolouri, A. McGarry Houghton, Neutrophil content predicts lymphocyte depletion and anti-PD1 treatment failure in NSCLC, JCI Insight. 4 (2019) 1–16. https://doi.org/10.1172/jci.insight.130850.

[41] K. Takada, S. Takamori, Y. Yoneshima, K. Tanaka, I. Okamoto, M. Shimokawa, T. Oba, A. Osoegawa, T. Tagawa, M. Takenoyama, Y. Oda, Y. Nakanishi, M. Mori, Serum markers associated with treatment response and survival in non-small cell lung cancer patients treated with anti-PD-1 therapy, Lung Cancer. 145 (2020) 18–26. https://doi.org/10.1016/j.lungcan.2020.04.034.

Table 1. Clinico-pathological Characteristics

Patient Population (n = 109)		
Age, years (Median, range)		72 (41-85)
		n (%)
Histotype	SCC	32 (30)
	ADC	70 (64)
	NSCLC NOS	7 (6)
Sex	Male	73 (67)
	Female	36 (33)
Smoking status	Smokers	36 (33)
	Ex-Smokers	48 (44)
	Non Smokers	25 (23)
ECOG PS	0-1	95 (87)
	2	14 (13)
Stage	IIIB	3 (3)
	IV	106 (97)
Metastatic Involvement	Lymph nodes	100 (92)
	Liver	17 (16)
	Bone	45 (41)
	Adrenal	20 (18)
	Brain	16 (15)
	Contralateral lung	102 (94)
	Pleura	32 (39)
	Others	28 (26)
Mutational status	KRAS	19 (17)
	EGFR	6 (5)
	ALK	1 (1)
	Other	5 (4)
ICI Treatment	I line	15 (14)
	II line	73 (67)
	≥ III line	21 (19)
ICI molecule	Nivolumab	66 (61)
	Pembrolizumab	21 (19)
	Atezolizumab	22 (20)

SCC: Squamous Cell Carcinoma; ADC: Adenocarcinoma; NSCLC: Non Small Cell Lung Cancer; NOS: Not Otherwise Specified, ICI: Immune Checkpoint Inhibitor

Table 2. Correlations between clinical and peripheral blood parameters

	sPD-L1 pg/mL			CD8+ PD-1+, n/uL			NK, n/uL			dNLR, n			LDH, U/L		
	n	**Mean ± SD**	***P***	**n**	**Mean ± SD**	***P***	**n**	**Mean ± SD**	***P***	**n**	**Mean ± SD**	***P***	**n**	**Mean ± SD**	***P***
Sex															
Male	73	84.2 ± 52.3	0.986	38	112 ± 62	0.202	38	270±175	0.915	73	3.44 ± 2.20	0.176	73	544 ± 383	0.678
Female	36	82.7 ±48.6		20	127 ±95		20	257 ±134		36	2.82 ±1.93		36	528 ± 349	
Age															
< 70	49	87.4 ±7.3	0.540	28	99 ±17	0.203	28	275 ±29	0.640	51	3.58 ±0.34	0.098	51	435 ±19	**0.009**
≥ 70	60	81.2 ±6.9		30	135 ±21		30	256 ±28		58	2.89 ±0.22		58	656 ±85	
ECOG PS															
0-1	95	79.9 ±19.8	**0.019**	39	124 ±40	**0.050**	39	285 ±125	**0.026**	95	3.04 ± 2.0	**0.014**	95	519 ±142	0.111
2	14	114.7 ±20.6		19	80 ±25		19	157 ±83		14	4.41 ±1.54		14	672 ±137	
Smoking status															
Never	25	74.9 ±33.9	0.145	11	185 ±46	0.146	11	234 ±53	0.632	25	3.33 ±1.41	0.396	25	498 ±146	0.510
Ex	48	78.8 ±51.1		23	134 ±74		23	272 ±88		48	3.51 ±1.31		48	563 ±196	
Current	36	97.7 ±54.1		24	95 ±37		24	274 ±95		36	2.79 ±1.38		36	542 ±148	
Histotype															
SCC	32	77.8 ±31.7	0.551	17	131 ±49	0.866	17	237 ±115	0.433	32	3.56 ±2.22	0.249	32	399 ±99	0.141
ADC	70	85.8 ±31.2		37	114 ±87		37	114 ±87		70	3.18±2.26		70	610 ±232	
N metastatic sites															
< 3	44	83.9 ±38.2	**0.001**	21	119 ±92	0.568	21	249 ±133	0.628	44	2.82 ±1.60	0.143	44	407 ±109	**0.007**
3-4	39	63.7 ±37.5		19	136 ±74		19	307 ±185		39	2.96 ±1.85		39	582 ±188	
> 4	26	117.2 ±42.3		18	95 ±44		18	233 ±126		26	4.12 ±2.07		26	754 ±296	
Bone metastasis															
Yes	45	90.7 ±54.4	0.282	24	77±22	**0.004**	24	226 ±161	**0.050**	45	4.07 ±2.69	**0.004**	45	718 ±235	**0.011**
No	64	79.8 ±48.6		34	146 ±81		34	292 ±145		64	2.63 ±1.34		64	413 ±121	
Liver metastasis															
Yes	17	99.9 ±45.5	**0.041**	10	96 ±63	0.666	10	271 ±154	0.484	17	3.93 ±1.99	0.402	17	771 ±526	**0.043**
No	92	81.1 ±51.6		48	122 ±81		48	264 ±155		92	3.11 ±1.83		92	496 ±314	
Brain metastasis															
Yes	16	101.6 ±26.2	**0.030**	12	119 ±84	0.701	12	281 ±153	0.490	16	3.70 ±2.98	0.971	16	637 ±382	0.325
No	93	70.8 ±42.8		46	117 ±82		46	261 ±155		93	3.14 ±1.93		93	522 ±236	

Table 3. Explanatory prognostic factors in Cox proportional hazard models

PFS	Univariate[a]				Multivariate[b]			
	HR	CI (95%)	χ^2	p value	HR	CI (95%)	χ^2	p value
Sex	0.62	0.38-1.02	3.42	0.067				
Age	0.98	0.96-1.06	2.05	1.146				
ECOG PS	2.99	1.62-5.51	9.79	**< 0.001**	0.93	0.25-3.43		0.917
Smoking	1.14	0.85-1.53	0.77	0.380				
Steroids before ICIs	2.59	1.54-4.09	7.78	**< 0.001**	3.91	2.76-5.64		**0.033**
Antibiotics before ICIs	2.05	1.12-3.74	4.64	**0.020**	1.01	0.26-3.95		0.979
Histotype	0.99	0.67-1.04	0.03	0.953				
tPD-L1	0.69	0.52-0.92	6.91	**0.010**	0.45	0.32-0.78		**0.004**
LDH	2.01	1.21-2.45	9.57	**< 0.001**	3.57	1.89-6.36		**0.002**
dNLR	1.33	1.24-1.47	4.93	**0.007**	1.46	1.12-1.89		**0.004**
CD3+	0.89	0.78-1.00	4.63	0.062				
CD8+	0.87	0.69-0.99	4.22	0.088				
CD4+	0.99	0.78-1.13	2.91	0.090				
CD8+ PD-1+	0.61	0.32-1.45	8.18	**0.005**	0.18	0.02-0.37		**0.001**
NK	0.57	0.28-1.02	10.42	**0.001**	0.26	0.07-0.86		**0.029**
sPD-L1	2.14	2.00-2.72	5.87	**0.020**	3.51	1.13-5.91		**0.030**

OS	Univariate[a]				Multivariate[b]			
	HR	CI (95%)	χ^2	p value	HR	CI (95%)	χ^2	p value
Sex	0.92	0.55-1.52	0.11	0.743				
Age	0.98	0.85-1.09	1.44	0.113				
ECOG PS	2.72	1.44-5.13	9.45	**0.002**	1.38	0.35-5.40		0.640
Smoking	1.01	0.76-1.37	0.01	0.904				
Steroids before ICIs	2.58	1.59-4.19	15.99	**0.001**	2.60	1.19-3.73		0.280
Antibiotics before ICIs	2.01	1.05-3.86	4.60	**0.032**	1.32	0.27-6.47		0.731
Histotype	1.15	0.76-1.76	0.44	0.506				
tPD-L1	0.72	0.52-0.99	4.25	**0.046**	0.54	0.31-0.95		**0.031**
LDH	2.01	1.61-3.02	7.95	**< 0.001**	2.95	0.98-4.87		0.053
dNLR	1.30	1.19-1.42	8.09	**0.003**	1.48	1.14-1.92		**0.003**
CD3+	0.99	0.79-2.09	3.59	0.181				
CD8+	0.86	0.69-0.99	4.76	0.072				
CD4+	1.03	0.99-1.17	1.96	0.164				
CD8+ PD-1+	0.49	0.28-0.98	9.82	**0.015**	0.40	0.07-2.96		**0.002**
NK	0.56	0.39-1.23	9.25	**0.022**	0.74	0.19-2.94		0.076
sPD-L1	3.04	2.69-3.76	7.54	**0.031**	3.03	0.87-5.54		**0.008**

PFS: progression free survival; OS: overall survival; Sex (Male=0, Female=1), Age (continue variable), Eastern Cooperative Oncology Group performance status (ECOG PS, 0-1 vs 2), Smoking (negative smoking history=0, positive smoking history=1), Steroids before ICIs (0= no, 1= yes), Antibiotics before ICIs (0= no, 1= yes), Histotype (ADC=0, SCC=1), tissue PD-L1 (tPD-L1, 0=negative, 1=1-49%, 2= ≥50%); continue variables: Lactate Dehydrogenase (LDH), derived Neutrophil-to-Lymphocyte Ratio (dNLR), CD3+, CD8+, CD4+, CD8+PD-1+, NK. Statistical results with $P < 0.05$ are bolded.

a Univariate analysis is carried out without any adjustment.