



Correlations between peripheral blood biomarkers and clinical outcomes in advanced non-small cell lung cancer patients who received immunotherapy-based treatments

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Background: Peripheral blood-based biomarkers (PBB) predicting response, survival and immune-related adverse events (irAEs) in patients with advanced non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs) are still a matter of debate. Thus, we investigated the associations between PBB, the efficacy of ICIs and the incidence of irAEs.

Methods: Patients with advanced NSCLC, who had been treated at Peking Union Medical College Hospital and received ICIs or chemoimmunotherapy from January 2015 to December 2020, were retrospectively identified. PBBs results were retrieved from medical records. Associations with overall response rate, survival, and incidence of irAEs were assessed using Kruskal-Wallis, Kaplan-Meier analysis, Pearson's chi-squared and Student's *t*-tests as required. Cox proportional hazards and logistic regression models were used to determine independent risk factors. Analyses were performed on the whole population (n=103), patients receiving ICIs only (n=32), and patients receiving chemoimmunotherapy (n=71). Changes in pretreatment and on-treatment PBB were also analyzed.

Results: Among 103 patients, 38 (36.9%) developed irAEs. Pretreatment absolute lymphocyte count (ALC) was related to an increased risk of irAEs in the whole population [odds ratio (OR), 2.165; 95% confidence interval (CI): 1.040 to 4.509, P=0.039] and patients receiving ICIs only (OR, 6.461; 95% CI: 1.067 to 39.112; P=0.042). A low prognostic nutritional index (PNI ≤45) was associated with worse progression-free survival (PFS) and overall survival (OS) in the whole population, in patients receiving ICIs only, and in patients receiving chemoimmunotherapy. High pretreatment interleukin (IL)-6 was associated with both worse PFS and OS in the whole population (IL-6 >13.80 pg/mL), in patients receiving ICIs only (IL-6 >11.30 pg/mL), and in patients receiving chemoimmunotherapy (IL-6 >11.85 pg/mL). Increase of IL-6 during treatment was associated with inferior OS in the whole population (P<0.001).

Conclusions: Pretreatment ALC has the potential to predict irAEs in patients with advanced NSCLC treated with ICIs. Additionally, a low level of pretreatment PNI and high level of IL-6 may be associated

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with shorter survival.

Keywords: Immune checkpoint inhibitors (ICIs); advanced non-small cell lung cancer (NSCLC); peripheral blood biomarker; immune-related adverse events (irAEs)

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Introduction

Over the past decade, immune checkpoint inhibitors (ICIs) have emerged as a promising new treatment option in patients with cancer. ICIs, including anti-programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1), and anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibodies, have shown durable efficacy in subsets of patients with lung cancer (1-4). Except for their significant clinical benefits, ICIs are associated with a specific spectrum of autoimmune and autoinflammatory side effects which are collectively termed as immune-related adverse events (irAEs). These toxicities can lead to significant morbidity, treatment discontinuation, diminished quality of life, and even fatal events have been described. It has been reported that the incidence of irAEs varied between 24% and 38% in non-small cell lung cancer (NSCLC) patients treated with immunotherapy-based treatment (1,2,5-8). Thus, the identification of biomarkers predicting irAEs are desirable to render patients with increased susceptibility for ICI related toxicities. Previous studies have evaluated different serum-based biomarkers including interleukin (IL)-17, eosinophilia, and several cytokines. However, a sufficient tool predicting irAEs has yet to be determined (9-12).

Up to now, the most widely used stratification factors in clinical trials investigating ICIs are PD-L1 expression and microsatellite instability, since these factors have been associated with outcome (13,14). However, long term responses have also been described in NSCLC patients lacking PD-(L)1 expression or microsatellite instability (1,2). Peripheral blood biomarkers (PBB), including absolute blood cell counts (15) and calculated indexes, such as the neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) have also been investigated as predictive biomarkers. Furthermore, the prognostic nutritional index (PNI) seems also to predict outcome upon ICIs treatment (16-18).

Cytokines are important regulators of host immune activity that promote the recruitment of immune cells

into the tumor microenvironment (19) and may also serve as potential biomarkers. One of which is IL-6, which is a cytokine involved in immune regulation. Elevated level of IL-6 has been observed in various types of cancer such as melanoma (20), ovarian (21), and colorectal cancer (22), and it has been reported to play a role in tumor promotion by expansion and survival of malignant cells, neo-angiogenesis, and inflammation (23-26). In addition, monitoring long-term changes in peripheral blood cells and cytokine levels has been reported to facilitate the prediction of patient outcomes (12,27,28). However, the relationships between the above-mentioned circulating parameters and ICIs outcomes are not well established. Moreover, published literature has rarely enrolled both blood cells and cytokines. Thus, there is still a quest towards novel and easy to use biomarkers with either predictive or prognostic potential in NSCLC.

In this study, we investigated the relationship between pretreatment peripheral blood cell parameters and circulating cytokines and the objective response rate (ORR), onset of irAEs, progression-free survival (PFS), and overall survival (OS) in patients with advanced NSCLC. We compared the differences between patients receiving immunotherapy only and patients who received chemoimmunotherapy. Moreover, changes during course of disease were correlated with the respective outcome variables. We present the following article in accordance with the REMARK reporting checklist (available at <https://dx.doi.org/10.21037/tlcr-21-710>).

Methods

Patients

A total of 103 patients, aged 18 years or older, with pathologically confirmed stage III/IV advanced NSCLC, who received at least 2 doses of anti-PD-(L)1 monotherapy, or anti-PD-(L)1-based combination therapy at Peking Union Medical College Hospital (PUMCH) from January

2015 to December 2020, were retrospectively enrolled in this study. The pretreatment routine blood test results and at least 1 type of serum cytokine result were available for all patients. The pretreatment blood results were examined within 7 days prior to immunotherapy initiation. Of the 103 patients, on-treatment peripheral blood results that were examined 6 to 8 weeks after immunotherapy initiation, were available for 59 patients (see Figure S1). ICIs were administered intravenously every 21 days according to label. The follow-up period ended on December 31, 2020.

Data collection

The clinicopathologic features and treatment history of the patients were reviewed. The peripheral blood test results retrieved included the absolute neutrophil count (ANC), absolute eosinophil count (AEC), absolute lymphocyte count (ALC), serum lactate dehydrogenase (LDH), serum hypersensitive C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), serum IL-6 level, serum IL-8 level, serum IL-10 level, serum tumor necrosis factor- α (TNF- α) level, and lymphocyte subset parameters, including B cell (cluster of differentiation CD3⁻CD19⁺ lymphocytes) count, natural killer (NK) cell (CD3⁻CD16⁺CD56⁺ lymphocytes) count, CD4⁺ T lymphocyte count, and CD8⁺ T lymphocyte count. Cytokines were examined by electrochemiluminescence immunoassay. Complex parameters were calculated as follows: (I) the NLR was calculated by dividing the ANC by the ALC; (II) the PLR was calculated by dividing the platelet count by the ALC; and (III) the PNI was calculated as $10 \times \text{albumin value (g/dL)} + 0.005 \times \text{ALC}$. The following cutoff values were set: (I) a NLR greater than 5 (29); and (II) a PNI greater than 45 (18).

In relation to the on-treatment peripheral blood parameters, including the ALC, AEC, NLR, PLR, B cell count, NK cell count, CD4⁺ T lymphocyte count, CD8⁺ T lymphocyte count, LDH level, ESR, and hsCRP level, we evaluated the relationship between the interquartile range (IQR) of changes between the pretreatment and on-treatment values. For cytokines, including IL-6, IL-8, IL-10, and TNF α , an increase or decrease of more than 40% in the pretreatment value was considered to represent significant biological variation (28).

This study was performed in accordance with the principles for Good Clinical Practice and the Declaration of Helsinki (as revised in 2013). The study was approved by the Internal Review Board of PUMCH on August 31, 2020

(protocol number SK-135) and individual consent for this retrospective analysis was waived.

Response assessments and recognition of irAEs

Response assessment was performed according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) by computed tomography scans every 6 to 8 weeks after the administration of the first dose of immunotherapy (30). Best overall response (BOR) refers to best percentage change from baseline in investigator-assessed tumor size during ICIs or chemoimmunotherapy for patients with measurable lesions. IrAEs were defined by physicians after alternative diagnoses had been excluded based on (I) pathologic evidence of irAEs; (II) multidisciplinary adjudication involving 2 or more oncologists; (III) clinical improvement with irAE-based treatment. The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE; version 4.0) was used to classify adverse events. PFS was defined as the time from the start of immunotherapy to the date of clinician-assessed radiographic progression, death, or last follow-up appointment, whichever occurred first. OS was defined as the date of the first dose of immunotherapy until death from any cause or the last follow-up appointment.

Statistical analysis

Patient characteristics are presented as descriptive statistics. Continuous variables are expressed as the median IQR, and categorical variables are expressed as numbers (%). Student's *t* and Kruskal-Wallis *H* tests were used to compare continuous variables. Chi-squared test was used to compare categorical variables. A logistic regression analysis was conducted to explore correlations between peripheral blood markers and the best overall response (BOR) and the onset of irAEs. A Kaplan-Meier analysis of PFS and OS was performed based on the cutoff values or IQRs, and differences between each group were assessed with the log rank test. The hazard ratios (HRs) and 95% confidence intervals (CI) were calculated with the multivariate Cox proportional hazard model. For all analyses, differences with a 2-tailed $P < 0.05$ were considered statistically significant. All statistical analyses were performed using the Statistical Analysis System Version 9.2 (SAS Institute, Cary, NC, USA). The figures were developed in GraphPad Prism (San Diego, CA, USA).

Table 1 Patients' clinical characteristics at baseline (n=103)

Characteristic	N (%)
Age (yrs) at ICI initiation, median (IQR)	66 (61, 71)
Gender	
Male	68 (66.0)
Female	35 (34.0)
BMI before ICI treatment, median (IQR)	22.3 (20.3, 24.1)
ECOG PS	
0–1	97 (94.2)
>1	6 (5.8)
Smoking status	
Never	45 (43.7)
Current	36 (35.0)
Former	22 (21.3)
Histology	
Non-squamous	66 (64.1)
Squamous	37 (35.9)
Driving mutation status	
Positive	38 (36.9)
EGFR	22 (21.4)
ALK	1 (1.0)
KRAS	9 (8.7)
Other	6 (5.8)
Negative	65 (63.1)
PD-L1 expression	
Negative	22 (21.4)
0–50%	13 (12.6)
≥50%	15 (14.6)
Not available	53 (51.4)
Stage at diagnosis	
III	20 (19.4)
IV	83 (80.6)
Number of metastatic sites	
<3	80 (77.7)
≥3	23 (22.3)

Table 1 (continued)**Table 1** (continued)

Characteristic	N (%)
Line of therapy	
1st	62 (60.2)
2nd	24 (23.3)
3rd or more	17 (16.5)
Treatment received	
Immunotherapy	32 (31.1)
Chemoimmunotherapy	71 (68.9)

ICI, immune checkpoint inhibitors; IQR, interquartile range; BMI, body mass index; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; KRAS, Kirsten rat sarcoma viral oncogene; PD-L1, programmed death-ligand 1; ECOG PS, Eastern Cooperative Oncology Group performance status.

Results

Patient characteristics, treatment, and efficacy

Pretreatment routine blood test results and clinicopathological data were available for all 103 patients. Among these patients, the following specific parameters were available: IL-6 for 103 patients (100%), the PNI for 101 patients (98.1%), lymphocyte subsets for 96 patients (93.2%), IL-8 for 91 patients (88.3%), IL-10 for 91 patients (88.3%), TNF- α for 77 patients (74.8%), LDH for 73 patients (70.9%), hsCRP for 62 patients (60.2%), and ESR for 55 patients (53.4%). Patient's clinical characteristics are summarized in *Table 1*. Of the 103 patients, 64.1% (n=66) were diagnosed with non-squamous NSCLC, while 35.9% (n=37) had a squamous NSCLC, 94.2% (n=97) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1, and 80.6% (n=83) of the whole cohort were diagnosed within a metastatic disease. In terms of treatment, 31.1% (n=32) received ICIs monotherapy, and 68.9% (n=71) received chemoimmunotherapy, 62 patients (60.2%) received ICI treatment in the first-line setting.

The median follow-up time was 12.9 (95% CI: 11.4 to 14.3) months. The median PFS was 12.8 (95% CI: 6.1 to 19.6) months, and the median OS was 23.8 (95% CI: 13.4 to 34.2) months in the whole cohort. The ORR was 43.7% [45/103, 0 complete response (CR), 45 partial response (PR)]. The disease control rate (DCR) was 91.3% [94/103,

0 CR, 45 PR, 49 stable disease (SD)]. In patients receiving ICIs only, the ORR was 34.4% (11/32, 11 PR) while for patients receiving chemoimmunotherapy, the ORR was 45.1% (32/71, 32 PR). Patients who received first-line chemoimmunotherapy showed better ORR than patients treated with ICI in subsequent lines: 58.5% (31/53, 31 PR) vs. 16.7% (3/18, 3 PR).

Baseline peripheral blood parameters related to the BOR

Patient stratification into three subgroups according to BOR [CR/PR; SD; progressive disease (PD)], showed that these sub-cohorts were characterized by different pretreatment NLR (P=0.043), and TNF- α levels (P=0.013) (Table S1).

In multivariate analysis, peripheral blood parameters failed to remain independent prognosticators (Table S2). In patients receiving immunotherapy only, there was a significant difference in ESR among the different response groups (the median ESR value was 25 in the CR/PR group, 24 in the SD group, and 84 in the PD group, P=0.029, Table S3); however, no significant difference was observed in the multivariate analysis. In patients receiving chemoimmunotherapy, there was a significant difference in TNF- α levels among the CR/PR, SD, and PD groups (the median TNF- α value was 9 in the CR/PR group, 8 in the SD group, and 7.45 in the PD group, P=0.009, Table S4). However, no significant difference was found in the logistic regression. No significant difference was observed in patients receiving first-line chemoimmunotherapy (n=53, Table S5).

irAEs

A total of 36.9% (38/103) patients experienced 57 irAEs. Twenty-three patients developed single-system irAEs, and 15 patients developed multiple-system irAEs across a maximum of four organ systems. The most observed irAEs were rash (22.8%), hepatic toxicity (14.0%), and hypothyroidism (12.3%). The most common severe irAEs (grade ≥ 3) was myocarditis (n=2). The detailed toxicity spectrum is provided in Table 2. Patients experiencing irAEs showed a longer PFS compared to patients without irAEs (498 vs. 269 days; Figure 1A; log rank P=0.124). Similarly, the median OS of irAE patients was better compared to patients without irAEs (1,198 vs. 484 days, Figure 1B, log rank P=0.071).

Baseline peripheral blood parameters and the incidence of irAEs

Patients with irAEs had higher baseline ALC (1.320 vs. 1.280, P=0.021), lower NLR (2.915 vs. 3.990, P=0.007) and lower PLR (159.560 vs. 207.780, P=0.032) compared to those without irAEs (Table 3). The univariate analysis revealed differences in the ALC (OR, 2.280, 95% CI: 1.089 to 4.775; P=0.029), NLR (OR, 0.823, 95% CI: 0.695 to 0.975; P=0.024), and PLR (OR, 0.996, 95% CI: 0.992 to 1.000; P=0.039). The multivariate analysis revealed that the ALC (OR, 2.165, 95% CI: 1.040 to 4.509; P=0.039) and the number of metastatic sites (OR, 0.196, 95% CI: 0.051 to 0.753; P=0.018) were independent risk factors for the occurrence of irAEs (Table 4).

In patients receiving immunotherapy only, the median pretreatment ALC was higher in the irAEs group compared to the non-irAEs group (P=0.038; Table 5). The univariate analysis also revealed a positive relationship between patients' pretreatment ALC and the incidence risk of irAEs (OR, 6.461, 95% CI: 1.067 to 39.112; P=0.042; Table 6). In the subgroups of patients receiving chemoimmunotherapy or patients receiving chemoimmunotherapy in the first line setting, no statistically significant associations were observed (Table S6; Table S7).

Relationship between pretreatment peripheral biomarkers and survival

Patients with high PNI (>45) tended to have superior PFS and OS than those with low PNI (log rank P=0.009 and log rank P<0.001, respectively; Figure 2A,2B). Additionally, patients with the highest (fourth quartile) IL-6 (>13.80 pg/mL) showed shorter PFS and OS compared to those with IL-6 in the other 3 quartiles (log rank P=0.007 and P=0.003, respectively; Figure 3A,3B). Moreover, a high NLR (>5; log rank P=0.013) and a high PLR (>median of 196.32; log rank P=0.025) were associated with inferior OS (Figure S2A,S2B). Thus, we set the lower limit of the fourth quartile of IL-6 (13.80 pg/mL) in the whole population as the cutoff value and performed further multivariate analysis. It revealed that IL-6 >13.80 pg/mL had a significant association with worse PFS (HR, 1.903, 95% CI: 1.055 to 3.433; P=0.032), whereas a PNI >45 had a significant association with better PFS (HR, 0.405, 95% CI: 0.184 to 0.892, P=0.025) and OS (HR, 0.294, 95% CI: 0.123 to 0.703; P=0.006; see Table 7).

In the subset of patients receiving immunotherapy only,

Table 2 Distribution of immune-related adverse events

Adverse event	N (%), total=57 events in 38 patients	Grade ≥ 3 irAEs
Cutaneous	16 (28.1)	
Pruritus	2 (3.5)	
Rash	13 (22.8)	1
Psoriasis	1 (1.8)	
Gastrointestinal	14 (24.5)	
Diarrhea	4 (7.0)	
Pancreatitis or lipase/amylase increase	2 (3.5)	
Alanine aminotransferase/bilirubin increase	8 (14.0)	
Constitutional symptoms	3 (5.3)	
Fatigue	2 (3.5)	
Arthralgia	1 (1.8)	
Endocrine disorders	12 (21.1)	
Hyperthyroidism	4 (7.0)	
Hypothyroidism	7 (12.3)	
Hypophysitis	1 (1.8)	
Pneumonitis	4 (7.0)	
Myocarditis/cardiac troponin I increase	3 (5.3)	2
Albuminuria	2 (3.5)	
Myositis	1 (1.8)	
Lactate dehydrogenase increase	1 (1.8)	
Eosinophilic granulocyte increase	1 (1.8)	

irAEs, immune-related adverse events.

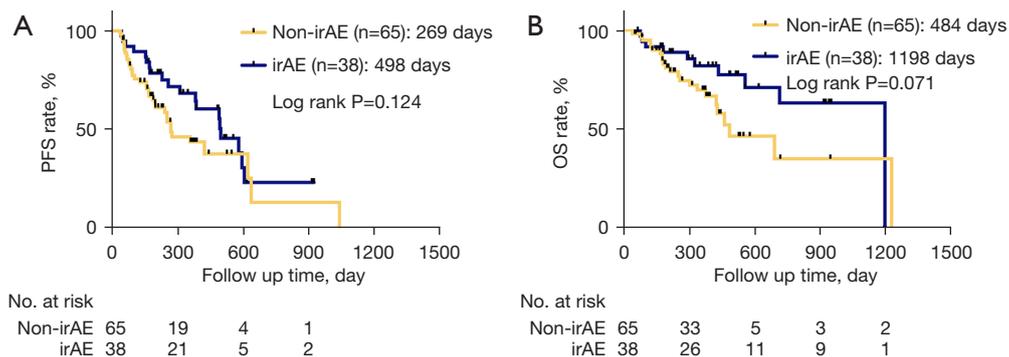


Figure 1 Kaplan-Meier analysis of PFS (A) and OS (B) for all patients (n=103) with ICI-treated advanced NSCLC according to the incidence of irAEs. The P values were calculated with the log rank test. PFS, progression-free-survival; OS, overall survival; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; irAEs, immune-related adverse events.

Table 3 Clinical-pathological characteristics and baseline peripheral blood parameters' and the risk irAEs

Clinical or biological marker	Without irAEs (n=65)	With irAEs (n=38)	P value
Age (years)	65 (61, 71)	67 (61, 72)	0.641
BMI (kg/m ²)	22.00 (20.30, 24.10)	22.30 (20.80, 23.90)	0.486
Blood routine test			
ANC	5.37 (3.98, 7.08)	4.81 (2.94, 5.74)	0.246
ALC	1.28 (0.86, 1.68)	1.32 (1.11, 1.88)	0.021
NLR	3.99 (2.81, 6.89)	2.92 (2.33, 4.76)	0.007
NLR <5	40 (61.5%)	29 (76.3%)	0.124
NLR ≥5	25 (38.5%)	9 (23.7%)	
AEC	0.13 (0.04, 0.23)	0.15 (0.06, 0.29)	0.071
PLR	207.78 (159.06, 281.32)	159.56 (124.65, 269.75)	0.032
PNI	N=64 47.33 (43.58, 49.88)	N=38 45.70 (43.10, 51.40)	0.830
Lymphocyte subsets test			
B cell	N=59 98.00 (66.00, 157.00)	N=37 107.00 (77.00, 183.00)	0.361
NK cell	247.00 (156.00, 419.00)	271.00 (194.00, 371.00)	0.978
CD4 ⁺ T lymphocyte	446.00 (263.00, 622.00)	542.00 (369.00, 703.00)	0.091
CD8 ⁺ T lymphocyte	338.00 (195.00, 465.00)	338.00 (285.00, 428.00)	0.219
LDH	N=46 221.00 (188.00, 254.00)	N=27 216.00 (177.00, 249.00)	0.527
hsCRP	N=39 9.61 (2.69, 41.48)	N=23 10.70 (2.27, 39.44)	0.973
ESR	N=38 25.00 (11.00, 51.00)	N=17 34.00 (14.00, 54.00)	0.653
IL-6	N=65 4.50 (2.00, 15.90)	N=38 5.00 (2.50, 11.70)	0.641
IL-8	N=63 25.00 (17.00, 57.00)	N=28 45.00 (16.00, 90.00)	0.256
IL-10	N=63 5.00 (5.00, 5.00)	N=28 5.00 (5.00, 5.00)	0.351
TNF α	N=56 8.10 (5.50, 10.50)	N=21 7.90 (6.20, 9.30)	0.130
ECOG PS			
0-1	61 (93.8%)	36 (94.7%)	0.852
≥1	4 (6.2%)	2 (5.3%)	
Number of metastases			
<3	45 (69.2%)	35 (92.1%)	0.007
≥3	20 (30.8%)	3 (7.9%)	

Table 3 (continued)

Table 3 (continued)

Clinical or biological marker	Without irAEs (n=65)	With irAEs (n=38)	P value
ICI treatment type			0.017
Anti-PD-(L)-1	65 (100.0%)	34 (89.5%)	
Anti-PD-(L)1 & anti-CTLA4	0	4 (10.5%)	
Line of therapy			0.197
1st	38 (56.9%)	25 (65.8%)	
2nd	14 (21.5%)	10 (26.3%)	
3rd or more	14 (21.5%)	3 (7.9%)	
Treatment received			0.333
Immunotherapy	18 (27.7%)	14 (36.8%)	
Chemoimmunotherapy	47 (72.3%)	24 (63.2%)	

irAEs, immune-related adverse events; BMI, body mass index; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; NLR, neutrophil-lymphocyte ratio; AEC, absolute eosinophils count; PLR, platelet-lymphocyte ratio; PNI, prognostic nutrition index; LDH, lactate dehydrogenase; hsCRP, hypersensitive C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; IL-8, interleukin-8; TNF- α , tumor necrosis factor- α ; ECOG PS, Eastern Cooperative Oncology Group performance status; ICI, immune checkpoint inhibitor; PD-(L)-1, programmed death-(ligand)-1; CTLA4, cytotoxic T-lymphocyte-associated protein 4.

Table 4 Univariate and multivariate analyses of risk factors for irAEs (n=103)

Variable	Category	Univariate			Multivariate		
		OR	95% CI	P value	OR	95% CI	P value
ALC	–	2.280	1.089–4.775	0.029	2.165	1.040–4.509	0.039
NLR	–	0.823	0.695–0.975	0.024			NS
AEC	–	3.185	0.970–10.454	0.056			
PLR	–	0.996	0.992–1.000	0.039			NS
PNI	>45	1.740	0.758–3.995	0.191			
TNF- α	–	0.958	0.868–1.056	0.385			
IL-6	–	0.993	0.964–1.023	0.638			
IL-8	–	1.002	0.999–1.005	0.184			
Number of metastases	≥ 3	0.193	0.053–0.702	0.012	0.196	0.051–0.753	0.018
ICI treatment type	With anti-CTLA4	3.088E+9	<0.001; >999.999	0.999			NS

irAEs, immune-related adverse events; ALC, absolute lymphocyte count; NLR, neutrophil-lymphocyte ratio; NS, not significant; AEC, absolute eosinophils count; PLR, platelet-lymphocyte ratio; PNI, prognostic nutrition index; IL-6, interleukin-6; IL-8, interleukin-8; ICI, immune checkpoint inhibitors.

high NLR and low PNI were associated with poor survival compared to the low NLR group and the high PNI group in terms of both: PFS (log rank $P < 0.001$, and log rank $P = 0.006$, respectively) and OS (log rank $P = 0.011$, and log rank $P = 0.019$, respectively; Figure S3A-S3D). Patients with

a high PLR (>median, which was 179.94) had worse PFS (log rank $P = 0.022$; Figure S3E) compared to the low PLR group. Moreover, patients with IL-8 higher than the 3rd number of IQR, which is 76 pg/mL, showed worse PFS (log rank $P < 0.001$, Figure S3F) compared to patients with

Table 5 Baseline peripheral blood parameters' the incidence of irAEs in patients only receiving immunotherapy (n=32)

Clinical or biological marker	Without irAEs (n=18)	With irAEs (n=14)	P value
Blood routine test	N=18	N=14	
ANC	4.16 (3.28, 6.20)	4.15 (2.74, 5.08)	0.527
ALC	1.23 (0.81, 1.45)	1.53 (1.11, 1.98)	0.038
NLR	3.23 (2.69, 6.45)	2.42 (2.01, 3.17)	0.067
AEC	0.15 (0.06, 0.32)	0.21 (0.08, 0.30)	0.356
PLR	209.41 (168.61, 292.31)	130.41 (112.86, 210.81)	0.052
PNI	N=18 47.08 (44.05, 48.95)	N=14 49.78 (43.10, 53.15)	0.572
Lymphocyte subsets	N=18	N=14	
B lymphocyte	83.00 (44.00, 124.00)	98.00 (81.00, 194.00)	0.159
NK cell	220.00 (99.00, 290.00)	275.50 (194.00, 317.00)	0.226
CD4 ⁺ T lymphocyte	421.00 (352.00, 606.00)	571.50 (440.00, 703.00)	0.093
CD8 ⁺ T lymphocyte	331.50 (165.00, 439.00)	356.00 (285.00, 441.00)	0.227
LDH	N=13 191.00 (170.00, 242.00)	N=8 216.00 (181.00, 263.00)	0.324
hsCRP	N=10 17.92 (1.45, 41.48)	N=10 6.88 (2.27, 53.92)	0.520
ESR	N=10 30.50 (14.00, 51.00)	N=9 17.00 (10.00, 42.00)	0.268
IL-6	N=18 6.55 (2.00, 23.20)	N=14 5.05 (3.60, 11.30)	0.275
IL-8	N=17 33.00 (19.00, 80.00)	N=5 37.00 (18.00, 61.00)	0.142
IL-10	N=17 5.00 (5.00, 5.00)	N=5 5.00 (5.00, 5.00)	NA
TNF- α	N=13 8.50 (6.40, 10.50)	N=3 8.30 (4.80, 9.30)	0.171
ECOG			0.370
0-1	17 (94.4%)	14 (100%)	
>1	1 (5.6%)	0	
Number of metastases			0.360
0-2	13 (72.2%)	12 (86.7%)	
≥ 3	5 (27.8%)	2 (13.3%)	
ICI treatment type			0.015
Anti-PD-(L)1	18 (100%)	10 (71.4%)	
Anti-PD-(L)1 & anti-CTLA-4	0	4 (28.6%)	

Table 4 (continued)

Table 4 (continued)

Clinical or biological marker	Without irAEs (n=18)	With irAEs (n=14)	P value
Line of therapy			0.096
1st	4 (22.2%)	5 (33.3%)	
2nd	9 (50.0%)	9 (60.0%)	
3rd or more	5 (27.8%)	0	

irAEs, immune-related adverse events; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; NLR, neutrophil-lymphocyte ratio; AEC, absolute eosinophils count; PLR, platelet-lymphocyte ratio; PNI, prognostic nutrition index; LDH, lactate dehydrogenase; hsCRP, hypersensitive C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; TNF- α , tumor necrosis factor- α ; ECOG PS, Eastern Cooperative Oncology Group performance status; ICI, immune checkpoint inhibitors; PD-(L)-1, programmed death-(ligand)-1; CTLA4, cytotoxic T-lymphocyte-associated protein 4.

Table 6 Univariate analysis of risk factors for irAEs in patients receiving ICIs treatment (n=32)

Variable	Category	Univariate		
		OR	95% CI	P value
ALC	–	6.461	1.067–39.112	0.042
NLR	–	0.718	0.481–1.070	0.104
AEC	–	3.249	0.264–40.008	0.358
PLR	–	0.992	0.983–1.001	0.073
PNI	–	1.035	0.921–1.163	0.560
TNF- α	–	0.881	0.516–1.504	0.642
IL6	–	0.977	0.933–1.023	0.319
IL8	–	0.993	0.973–1.014	0.498
Number of metastases	≥ 3	0.433	0.070–2.669	0.367
ICI treatment type	With anti-CTLA4	2.908E+9	<0.001; >999.999	0.999

irAEs, immune-related adverse events; ALC, absolute lymphocyte count; NLR, neutrophil-lymphocyte ratio; AEC, absolute eosinophils count; PLR, platelet-lymphocyte ratio; PNI, prognostic nutrition index; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; IL-8, interleukin-8; ICI, immune checkpoint inhibitors.

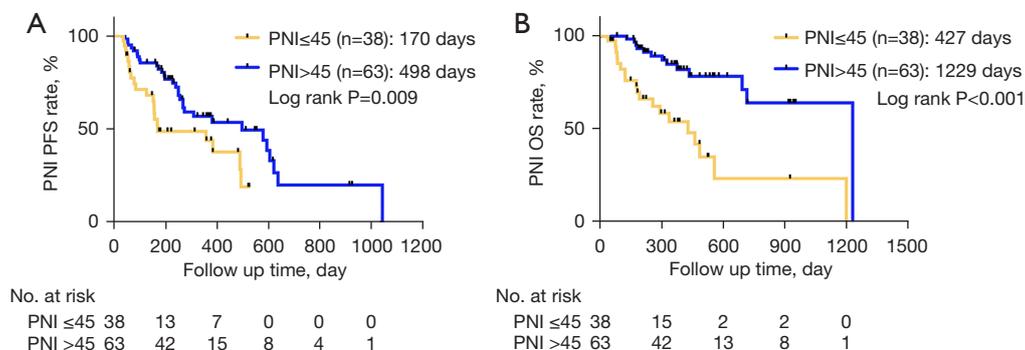


Figure 2 Kaplan-Meier analysis of PFS (A) and OS (B) for all patients according to the pretreatment PNI (≤ 45 vs. > 45) (n=103, PNI was available in 101 patients). The P values were calculated with the log rank test. PFS, progression free survival; OS, overall survival; PNI, prognostic nutritional index.

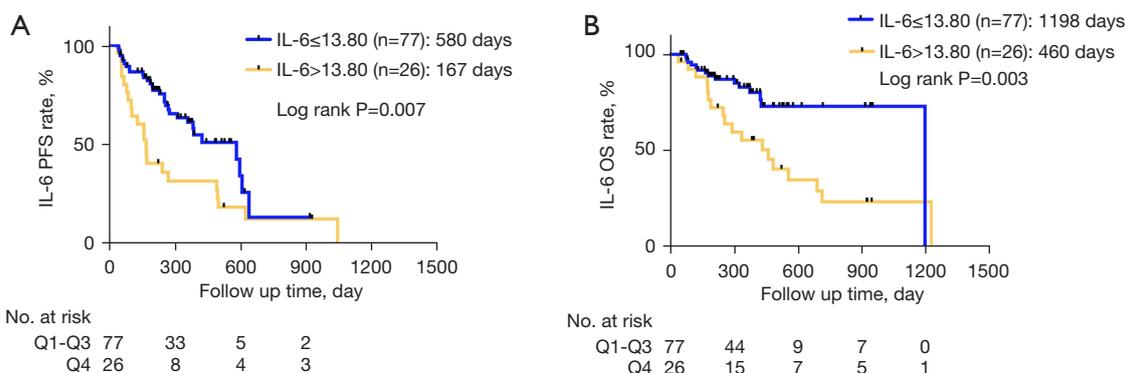


Figure 3 Kaplan-Meier analysis of PFS (A) and OS (B) for all patients (n=103) according to pretreatment IL-6 level (≤13.80 vs. >13.80 pg/mL). The P values were calculated with the log rank test. PFS, progression free survival; OS, overall survival; IL-6, interleukin-6.

Table 7 Multivariate analyses of PFS and OS for all patients (n=103, with 101 patients' data available)

Variable	Category	PFS			OS		
		HR	95% CI	P value	HR	95% CI	P value
NLR	>5	1.071	0.457–2.508	0.874	1.133	0.386–3.330	0.820
PLR	>196.52	0.603	0.258–1.412	0.244	0.877	0.297–2.594	0.813
PNI	>45	0.405	0.184–0.892	0.025	0.294	0.123–0.703	0.006
IL-6	>13.80	1.903	1.055–3.433	0.032	2.117	0.993–4.514	0.052

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PNI, prognostic nutritional index; IL-6, interleukin-6.

low IL-8. IL-6=11.30 pg/mL was a meaningful cutoff of PFS (log rank P=0.028) and OS (log rank P=0.038) for patients receiving immunotherapy only (Figure 4). In the multivariate Cox regression yielded a correlation between high PNI was associated with better OS (HR 0.068, 95% CI: 0.005 to 0.876; P=0.039; Table 8).

In the subset of patients receiving chemoimmunotherapy, with IL-6 >11.85 pg/mL (3rd number of IQR) was associated with inferior PFS (log rank P=0.015, Figure 5A) and OS (log rank P=0.047, Figure 5B) compared to patients with IL-6 ≤11.85 pg/mL. Patients with PNI >45 showed improved OS (log rank P=0.001; Figure S4A). Conversely, patients with high PLR (>median, which was 200) had worse OS (log rank P=0.044, Figure S4B). In multivariate Cox regression analysis, high PLR (HR 3.594, 95% CI: 1.096 to 11.789, P=0.035), low PNI (HR 0.273, 95% CI: 0.082 to 0.907, P=0.034) and high IL-6 (HR 2.195, 95% CI: 1.008 to 4.779, P=0.048) remained independent poor prognostic factors for PFS. In multivariate analysis for OS including the same covariates, no independent risk factor was detected

(Table 9). In patients receiving chemoimmunotherapy as first-line treatment, high PLR (>214.4, log rank P=0.009, Figure S5A) and low PNI (≤45, log rank P=0.047, Figure S5B) were associated with worse OS. In patients with high IL-6 (>11.30 pg/mL, 3rd number of IQR), there was a trend towards poor OS (log rank P=0.056, Figure S5C). The multivariate analysis failed to reveal any independent risk factors in this subgroup of patients (Table S8).

Changes between pretreatment and on-treatment peripheral blood parameters

Pretreatment and on-treatment blood results were available for 59 patients. Of those, 13 received ICIs and 46 received chemoimmunotherapy. Routine blood and IL-6 results were available for all 59 patients. Other parameters were available as follows: 57 patients had PNI results, 55 patients had IL-8 results, 46 patients had lymphocyte subset results, 45 patients had TNF-α results, 37 patients had LDH results, 27 patients had had CRP results, and 26 patients

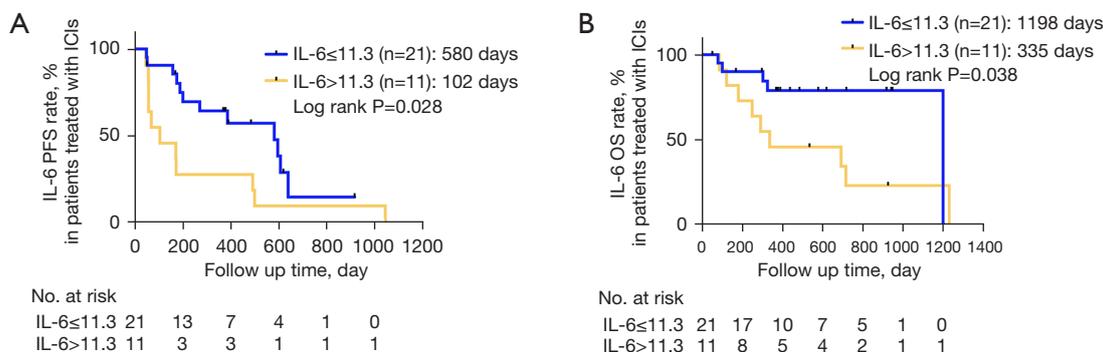


Figure 4 Kaplan-Meier analysis of PFS (A) and OS (B) for patients receiving immunotherapy only (n=32) according to pretreatment level of IL-6 (≤ 11.30 vs. > 11.30 pg/mL). The P values were calculated with the log-rank test. PFS, progression free survival; OS, overall survival; IL-6, interleukin-6.

Table 8 Multivariate analyses of PFS and OS for patients receiving immunotherapy alone (n=32, with 21 patient's data available)

Variable	Category	PFS			OS		
		HR	95% CI	P value	HR	95% CI	P value
NLR	>5	1.524	0.194–11.970	0.689	67933.508	0.000–9.517E+126	0.938
PLR	>179.94	2.089	0.269–16.198	0.481	0.000	0.000–1.310E+117	0.936
PNI	>45	1.040	0.223–4.845	0.960	0.068	0.005–0.876	0.039
IL-6	>11.3	5.171	0.486–55.010	0.173	1.031	0.083–12.787	0.981
IL-8	>76	4.454	0.598–33.154	0.145	4.227	0.483–36.991	0.193

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PNI, prognostic nutritional index; IL-6, interleukin-6; IL-8, interleukin-8.

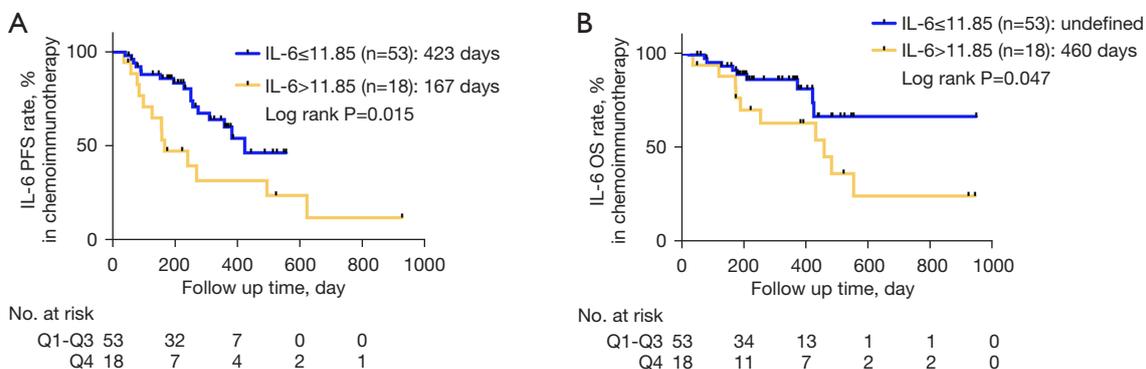


Figure 5 Kaplan-Meier analysis of PFS (A) and OS (B) for patients receiving chemoimmunotherapy (n=71) according to pretreatment level of IL-6 (≤ 11.85 vs. > 11.85 pg/mL). The P values were calculated with the log rank test. PFS, progression free survival; OS, overall survival; IL-6, interleukin-6.

Table 9 Multivariate analyses of PFS and OS for patients receiving chemoimmunotherapy (n=73, with 71 patient's data available)

Variable	Category	PFS			OS		
		HR	95% CI	P value	HR	95% CI	P value
PLR	>200	3.594	1.096–11.789	0.035	1.223	0.330–4.532	0.764
PNI	>45	0.273	0.082–0.907	0.034	0.252	0.061–1.045	0.058
IL-6	>11.85	2.195	1.008–4.779	0.048	1.713	0.614–4.780	0.304

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; PLR, platelet-to-lymphocyte ratio; PNI, prognostic nutritional index; IL-6, interleukin-6.

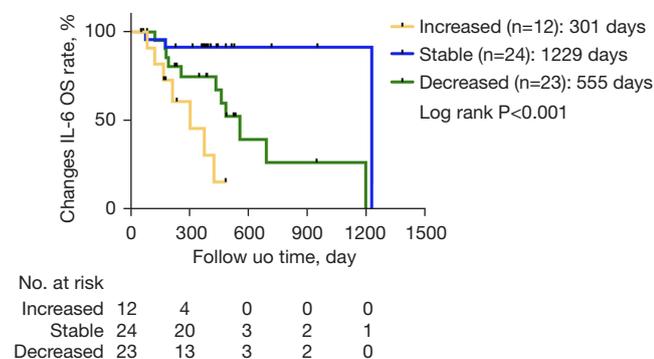


Figure 6 Kaplan-Meier analysis of OS for all patients (n=59) according to changes between pretreatment and on-treatment levels of IL-6 [increased (>40%), stable (decreased ≤40% or increased ≤40%), vs. decreased (>40%)]. The P value was calculated with the log-rank test. OS, overall survival; IL-6, interleukin-6.

had ESR results.

Analyses were conducted to examine changes between pretreatment and on-treatment peripheral blood parameters and the BOR, incidence of irAEs, and survival. There was a significant difference in the IQR changes between pretreatment and on-treatment CD4+ T lymphocyte counts that was related to the BOR (patients with Q2 of CD4+ T lymphocyte changes more likely to be in the PD group, P=0.043; Table S9). No parameter was found to be related to irAE incidence (Table S10). In terms of the analyses of patients receiving immunotherapy only and patients receiving chemoimmunotherapy, no significant difference was found between either BOR or irAE incidence.

Further, an increased IL-6 (>40% compared to pretreatment) was associated with inferior OS in the whole population (log rank P<0.001; Figure 6). The analysis of pretreatment and on-treatment blood parameters of patients receiving immunotherapy only and those receiving chemoimmunotherapy showed no significant differences

(Table S11).

Discussion

In this cohort, high levels of pretreatment absolute lymphocytes were associated with an increased risk for irAEs. Moreover, a higher baseline level of PNI (>45) was associated with better survival, while a higher IL-6 (>13.80 pg/mL in the whole population, >11.30 pg/mL in the immunotherapy group, and >11.85 pg/mL in the chemoimmunotherapy group) was associated with worse survival. Increased IL-6 levels after immunotherapy initiation were associated with worse OS in the whole population. Our study adds additional evidence on the prognostic relevance of blood-based biomarkers in NSCLC patients treated with ICIs.

Our results revealed a correlation between high pretreatment ALC and irAE incidence both in the whole population and patients receiving ICI only. These results are consistent with those reported by Diehl *et al.*, who found that an ALC >2×10⁹/L at baseline was associated with grade 2 or higher irAEs (31). IrAEs are autoimmune and autoinflammatory disorders caused by a hyper-reactive immune system after the upregulation of T cell immune function after treatment. It is possible that a higher level of pretreatment ALC improves the potential antitumor ability of T cells and their hyper-reactive ability to immunotherapy (32). Similarly, lower ALC has been reported to be associated with reduced multiple sclerosis disease activity, which is consistent with our cognition of irAEs and autoimmune diseases (33). As a biomarker, ALC value is directly presented in the lab reports and needless to calculate compared to PLR or NLR, which is easier to be used by clinicians.

Our results showed negative relationship between high NLR, high PLR and survival, which is consistent with former studies (34,35). We also showed that a high

pretreatment PNI is related to improved OS. Additionally, a high pretreatment PNI was also related to improved PFS in patients receiving chemoimmunotherapy. The PNI is a simple way to assess perioperative nutritional conditions and immunological status (36). In a study of Peng *et al.*, baseline PNI ≥ 45 was independently associated with both better PFS and longer OS in advanced lung cancer patients treated with PD-1 inhibitors (35). Johannet *et al.* found that a low baseline PNI was associated with a decreased response rate, shorter PFS and OS among patients with various types of cancer, including melanoma, lung cancer, and others malignancies treated with anti-PD-(L)1 or anti-CTLA-4 antibodies (37). Our results provide further evidence that PNI represents a nutritional condition predicting response to chemoimmunotherapy. PNI also serves as a measure of a patient's immunological status (38), which plays an important role in a patient's response to immunotherapy.

IL-6 is closely involved in tumor immune-evasion and negatively correlated with prognosis in cancer patients (39). In line with this finding, our results showed that a high pretreatment IL-6 level was associated with shortened OS. There is a body of evidence that suggests that high pretreatment IL-6 is associated with a poor survival in patients undergoing treatment with ipilimumab (40-42). In the Checkmate-064, -066, and -067 trials, higher pretreatment IL-6 levels were associated with shortened OS in melanoma patients (43). Notably, the cutoff value of the Checkmate-064 study (15.1 and 12.7 pg/mL) was quite close to the cutoff value used in our study (13.8, 11.3 and 11.85 pg/mL). This further supports the credibility of our findings and suggests that an IL-6 level around this interval is of prognostic significance. Low pretreatment IL-6 serum levels were also shown to be an independent risk factor for irAEs in malignant melanoma patients treated with the anti-CTLA-4 antibody (44). The potential role of serum IL-6 in patients with advanced lung cancer receiving ICI might be related to a stimulation of tumor cell proliferation, survival, and invasiveness through the IL-6/JAK/STAT3 pathway. Moreover, IL-6 and JAK/STAT3 signaling is often hyperactivated in tumor-infiltrating immune cells thus leading to downmodulation of antitumor immunity and contributing to a highly immunosuppressive tumor microenvironment (45).

An increased NLR (≥ 2) during immunotherapy was reported to be a negative prognostic factor in patients with advanced cancers (46). Our results showed an association of increased on-treatment IL-6 and poor OS in the whole population. Moreover, the KM analysis showed best OS

in patients with stable IL-6 group. This may be due to the small sample of patients. A decrease in IL-6 levels was earlier found to be associated with improved PFS in advanced lung cancer patients treated with PD-1 antibodies (28). In the Checkmate-064 study, increased serum IL-6 correlated with reduced survival (43). We failed to identify any relationship between changes in peripheral blood cell parameters and survival. This may be because our cohort comprised patients treated with both immunotherapy and chemoimmunotherapy. Chemotherapy is known to affect peripheral blood cell counts due to hematotoxicity. In our cohort, no information was available regarding the administration of granulocyte colony stimulating factor (GCSF) or thrombopoietin and blood products. Thus, we cannot exclude this potential confounder. Therefore, prospective studies are clearly needed to clarify this issue.

This study has several limitations. First, its retrospective nature and limited number of patients may have introduced case selection bias and restricted the generalizability of the results. Second, the patients received immunotherapy-based treatment at different lines of therapy. Thus, the degree of baseline inflammation may have been affected by previous treatments. Despite these limitations, an interesting finding of this study was the similarity of risk factors for survival in patients treated with both immunotherapy and immunochemotherapy. These results extend our understanding of the relationship between peripheral blood parameters and clinical outcomes after immunotherapy-based treatment. Further studies are needed to validate our results and better establish cut-points for rigorous clinical implementation.

Conclusions

A high pretreatment ALC can be a predictor of increased risk of irAEs in patients with advanced NSCLC receiving immunotherapy-based treatments. In addition, high PNI and low IL-6 are correlated with improved survival. Blood-based markers may serve as reliable predictors of immune-related toxicity and survival outcomes in patients with advanced NSCLC treated with immunotherapy or chemoimmunotherapy.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://dx.doi.org/10.21037/tlcr-21-710>). Dr. JJ reported that he attained consulting fees from AstraZeneca, BMS, MSD, Pfizer, Roche, and got travel support from Roche, Pfizer, Boehringer. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed in accordance with the principles for Good Clinical Practice and the Declaration of Helsinki (as revised in 2013). The study was approved by the Internal Review Board of PUMCH on August 31, 2020 (protocol number SK-135) and individual consent for this retrospective analysis was waived.

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