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Circulating cytokines associated with clinical outcomes in advanced non-small cell lung cancer patients who received chemoimmunotherapy

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Abstract

Background: Pretreatment and on-treatment plasma cytokine levels in predicting clinical benefit in patients with advanced non-small cell lung cancer (NSCLC) treated with anti-programmed death-1 (PD-1)-based chemotherapy is still a matter of debate.

Methods: We measured 12 kind of plasma cytokines in patients with stage III/IV NSCLC before and during treatment with anti-PD-1 based chemotherapy. Associations with best overall response, and survival including progression-free survival (PFS) and overall survival (OS) were assessed using Chi-square test and Kaplan–Meier plots with log-rank test, respectively. Logistic regression and Cox regression were used to determine independent risk factors.

Results: Of a total of 60 patients, high-level of pretreatment interleukin-2 was associated with longer PFS (log rank $p = 0.049$), while high-level of pretreatment interleukin-8 was associated with shorter OS (log rank $p = 0.006$). Increased on-treatment interleukin-1β (IL-1β) was associated with both better response (odds ratio [OR] 6.233, 95% confidential interval [CI]: 1.451–26.344, $p = 0.013$) and longer PFS (hazard ratio [HR] 0.305, 95% CI: 0.127–0.730, $p = 0.008$). On the contrary, increased on-treatment interleukin-6 (IL-6) was associated with a worse response (OR 0.015, 95% CI: 0.001–0.400, $p = 0.012$), worse PFS (HR 2.639, 95% CI: 1.163–5.991, $p = 0.020$) and worse OS (HR 2.742, 95% CI: 1.063–7.074, $p = 0.037$). Increased interferon-γ (IFN-γ) was found to be associated with better PFS (HR 0.336, 95% CI: 0.153–0.745, $p = 0.007$).

Conclusions: In patients with advanced NSCLC who received chemoimmunotherapy, on-treatment increased IL-1β and IFN-γ may serve as positive indicator of efficacy, while on-treatment increased IL-6 might play a predictive role of worse clinical outcome.

KEYWORDS
advanced non-small cell lung cancer, chemoimmunotherapy, cytokines, interleukin-1β, interleukin-6

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have emerged as a promising new cancer treatment strategy over the past decade which can exhibit durable efficacy in subsets of patients with diverse tumor types and achieve disease control for extended periods. Clinicians have made efforts to search for biomarkers to predict not only clinical benefit but also immune-related adverse events (irAEs), which can affect almost any systems in the

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body and are sometimes even fatal. Up to now, several molecular biomarkers with predictive value for ICIs outcome have been reported including programmed death ligand-1 (PD-L1) expression and tumor mutational burden.[1] Peripheral blood biomarkers including neutrophil-lymphocyte ratio and lactic dehydrogenase have also been reported to have prognostic value in patients receiving ICI treatments.[2]

Cytokines are important regulators of host immune activity; they promote the recruitment of immune cells into the tumor microenvironment and may also serve as predictive biomarkers.[3] For instance, high pretreatment interleukin (IL)-6 and IL-8 has been reported as negative prognostic biomarker in patients receiving ICI treatments.[4,5] In addition, monitoring long-term changes in cytokine levels has been reported to facilitate the prediction of patient outcomes,[6,7] especially to differential diagnosis of pseudoprogression.[8] However, the relationships between plasma cytokines and anti-PD-1 treatment outcomes are not well established. Furthermore, most of the published data has been focused on ICI monotherapy. Chemoimmunotherapy has been approved by the United States Food and Drug Administration and the China National Medical Products Administration as a first-line treatment for patients with non-small cell lung cancer (NSCLC).[9–12]

In this study, we investigated the pretreatment plasma cytokine levels and changes of on-treatment plasma cytokine levels in relation to best overall response (BOR), progression-free-survival (PFS) and overall survival (OS) in patients with NSCLC who received anti-PD-1-based chemotherapy. Relationships between circulating cytokines and onset of irAEs were also analyzed.

METHODS

Patient enrollment and study assessments

Patients, aged 18 years or older, with pathologically-confirmed unresectable stage III/IV NSCLC, who received at least one dose of anti-PD-1-based chemotherapy at Peking Union Medical College Hospital (PUMCH) from January 1, 2015, to December 31, 2020, were retrospectively enrolled in this study. Anti-PD-1-based chemotherapy were administered intravenously every 21 days according to label. The data cutoff was on August 31, 2021.

Tumor assessment was performed before treatment, and every 6 to 8 weeks after injection of the first dose of chemoimmunotherapy. According to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1),[13] response of each patient was categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).

PFS was defined as the time from the start of chemoimmunotherapy 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 chemoimmunotherapy, or death from any cause or the last follow-up appointment.

The Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute (version 4.03) was used to assess adverse events in patients. The irAEs were defined as adverse events that reflected a disorder of the immune system.

Sample processing and cytokine measurements

Plasma samples from patients were collected prospectively at baseline (collected 0–7 days prior to therapy initiation), and during treatment (after therapy initiation and before disease progression). Blood samples (∼10 ml) were collected in EDTA vacutainer tubes (BD vacutainer blood collection tubes) and processed within 4 h of collection. Samples were centrifuged at 1500 rpm (800 x *g*) for 10 min for further plasma clearance. Clarified plasma samples were stored at

TABLE 1 Patient clinical characteristics at baseline

Characteristic	Patients receiving chemoimmunotherapy ($n = 60$)
Age at chemoimmunotherapy initiation, median (IQR)	66 (61.2, 70.0)
Gender	
Male	42 (70.0)
Female	18 (30.0)
BMI before chemoimmunotherapy, median (IQR)	22.2 (20.8, 24.0)
ECOG PS	
0/1	55 (91.7)
≥ 2	5 (8.3)
Smoking status	
Never	30 (50.0)
Current	19 (31.7)
Former	11 (18.3)
Histology	
Nonsquamous	38 (63.3)
Squamous	22 (36.7)
Stage at diagnosis	
III	14 (23.3)
IV	46 (76.7)
Number of metastatic sites	
<3	45 (75.0)
≥3	15 (25.0)
Line of therapy	
1	42 (70.0)
2	8 (13.3)
≥3	10 (16.7)

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; PS, performance status.

TABLE 2 Pretreatment plasma cytokines and treatment response in patients receiving chemoimmunotherapy ($n = 56$)

Cytokines	Responders ($n = 30$) high-level (%)	Nonresponders ($n = 26$) high-level (%)	p-value
IL-1β	17 (56.7)	12 (46.2)	0.432
IL-2	18 (60.0)	11 (42.3)	0.186
IL-4	13 (43.3)	13 (50.0)	0.618
IL-5	15 (50.0)	14 (53.8)	0.774
IL-6	14 (46.7)	12 (46.2)	0.969
IL-8	14 (46.7)	14 (53.8)	0.592
IL-10	15 (50.0)	12 (46.2)	0.774
IL-12p70	14 (46.7)	14 (53.8)	0.592
IL-17	16 (53.3)	13 (50.0)	0.803
IFN-α	13 (43.3)	15 (57.7)	0.284
IFN-γ	12 (40.0)	15 (57.7)	0.186
TNF-α	16 (53.3)	12 (46.2)	0.592

Abbreviations: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12protein70; IL-17, interleukin-17; IFN-α, interferon-α; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.

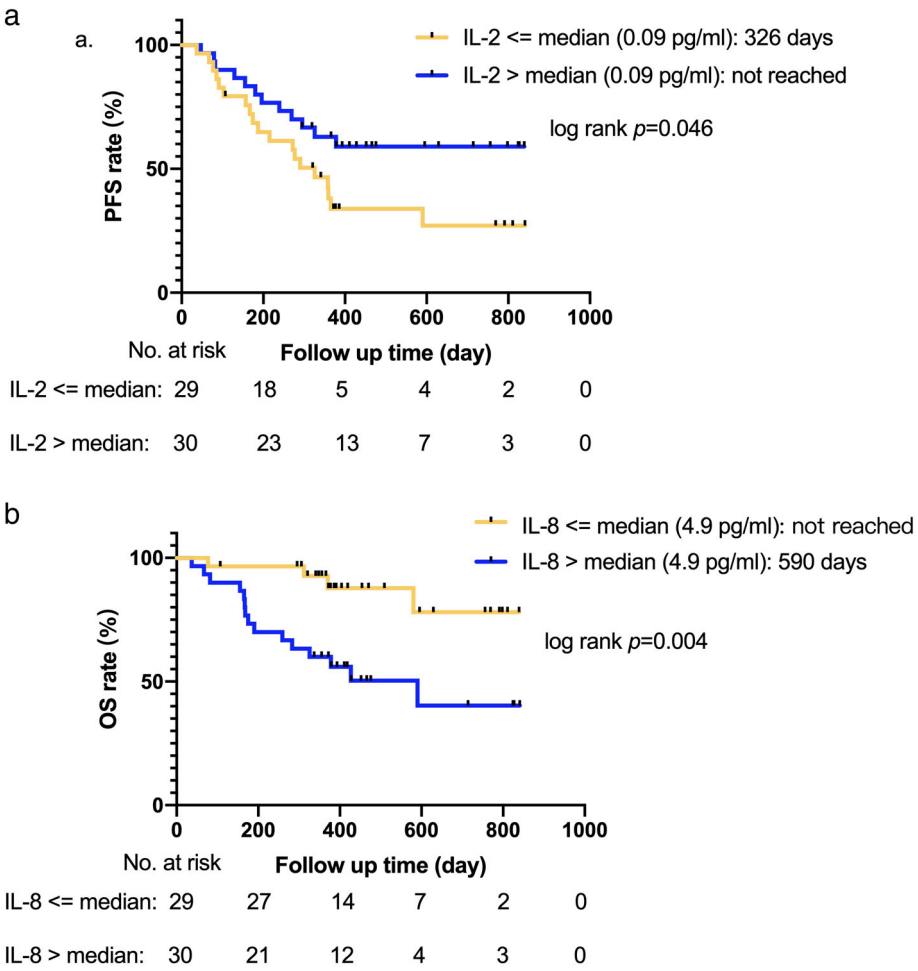

FIGURE 1 (a) Kaplan–Meier analysis of PFS for patients with NSCLC treated with chemoimmunotherapy according to pretreatment IL-2 (<= median vs. > median). (b) Kaplan–Meier analysis of OS for patients with NSCLC treated with chemoimmunotherapy according to pretreatment IL-8 (<= median vs. > median). The p-values were calculated with the log rank test. PFS, progression-free-survival; NSCLC, non-small cell lung cancer; IL-2, interleukin-2; OS, overall survival; IL-8, interleukin-8

−80°C. Cytokines were measured by EasyMagPlex Human Cytokine 12 Plex Kit (Shenzhen Wellgrow Technology Inc.) in the department of clinical laboratory at PUMCH. The panel of measured cytokines included IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, interferon (IFN)-α, IFN-γ, and tumor necrosis factor (TNF)-α.

This study was performed in accordance with the principles for Good Clinical Practice and the Declaration of

TABLE 3 Changes of plasma cytokines related to best overall response ($n = 55$)

Cytokines	Responders ($n = 30$) increased (%)	Nonresponders ($n = 25$) increased (%)	*p*-value
IL-1β	18 (60.0)	7 (28.0)	0.018*
IL-2	13 (43.3)	11 (44.0)	0.960
IL-4	16 (53.3)	11 (44.0)	0.491
IL-5	21 (70.0)	12 (48.0)	0.097
IL-6	8 (26.7)	13 (52.0)	0.054
IL-8	8 (26.7)	7 (28.0)	0.912
IL-10	17 (56.7)	20 (80.0)	0.066
IL-12p70	15 (50.0)	13 (52.0)	0.883
IL-17	15 (50.0)	11 (44.0)	0.657
IFN-α	16 (53.3)	11 (44.0)	0.491
IFN-γ	19 (63.3)	12 (14.1)	0.254
TNF-α	12 (40.0)	12 (48.0)	0.551

Abbreviation: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12protein70; IL-17, interleukin-17; IFN-α, interferon-α; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
*means that the *p* value is statistically significant.

TABLE 4 Univariate and multivariate analysis of relationship between changes of increased plasma cytokines and response

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
IL-1β	3.857	1.236–12.040	0.020*	6.233	1.475–26.344	0.013*
IL-2	0.973	0.334–2.838	0.960			
IL-4	1.455	0.501–4.227	0.491			
IL-5	2.528	0.836–7.647	0.101	4.156	0.980–17.621	0.053
IL-6	0.336	0.109–1.036	0.058	0.105	0.020–0.537	0.007*
IL-8	0.935	0.284–3.075	0.912			
IL-10	0.327	0.097–1.104	0.327			
IL-12p70	0.923	0.319–2.670	0.883			
IL-17	1.273	0.438–3.695	0.657			
IFN-α	1.455	0.501–4.227	0.491			
IFN-γ	1.871	0.635–5.512	0.256			
TNF-α	0.722	0.247–2.110	0.552			

Abbreviations: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12protein70; IL-17, interleukin-17; IFN-α, interferon-α; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
*means that the *p* value is statistically significant.

Helsinki. The study was approved by the Internal Review Board of PUMCH on Sept 27th, 2021 (protocol number S-K1764).

Statistical analysis

Pretreatment cytokine level was determined as high or low according to median. In terms of on-treatment cytokine level, we chose >10% change over pretreatment cytokine level as the cutoff point to define increased on-treatment cytokine group and nonincreased on-treatment cytokine group. Patients with a BOR of CR or PR were defined as responders, while others with a BOR of SD or PD were defined as nonresponders. Categorical variables are expressed as numbers (%), which was compared by chi-squared test. Logistic regression was performed to examine the association of change of cytokines and clinical benefit of chemoimmunotherapy. A Kaplan–Meier analysis of PFS and OS was performed according to the median of pretreatment cytokine levels or changes between pretreatment and on-treatment cytokine levels. Differences between each group were assessed with the log rank test. The hazard ratios (HRs) and 95% confidence intervals (CI) were calculated with the Cox proportional hazard model. Cytokines with a *p*-value <0.2 were then entered into a multivariable model.

For all analyses, differences with a 2-tailed $p < 0.05$ were considered statistically significant.

All statistical analyses were performed using the SPSS 26.0 software. The figures were developed in GraphPad Prism 8.0.

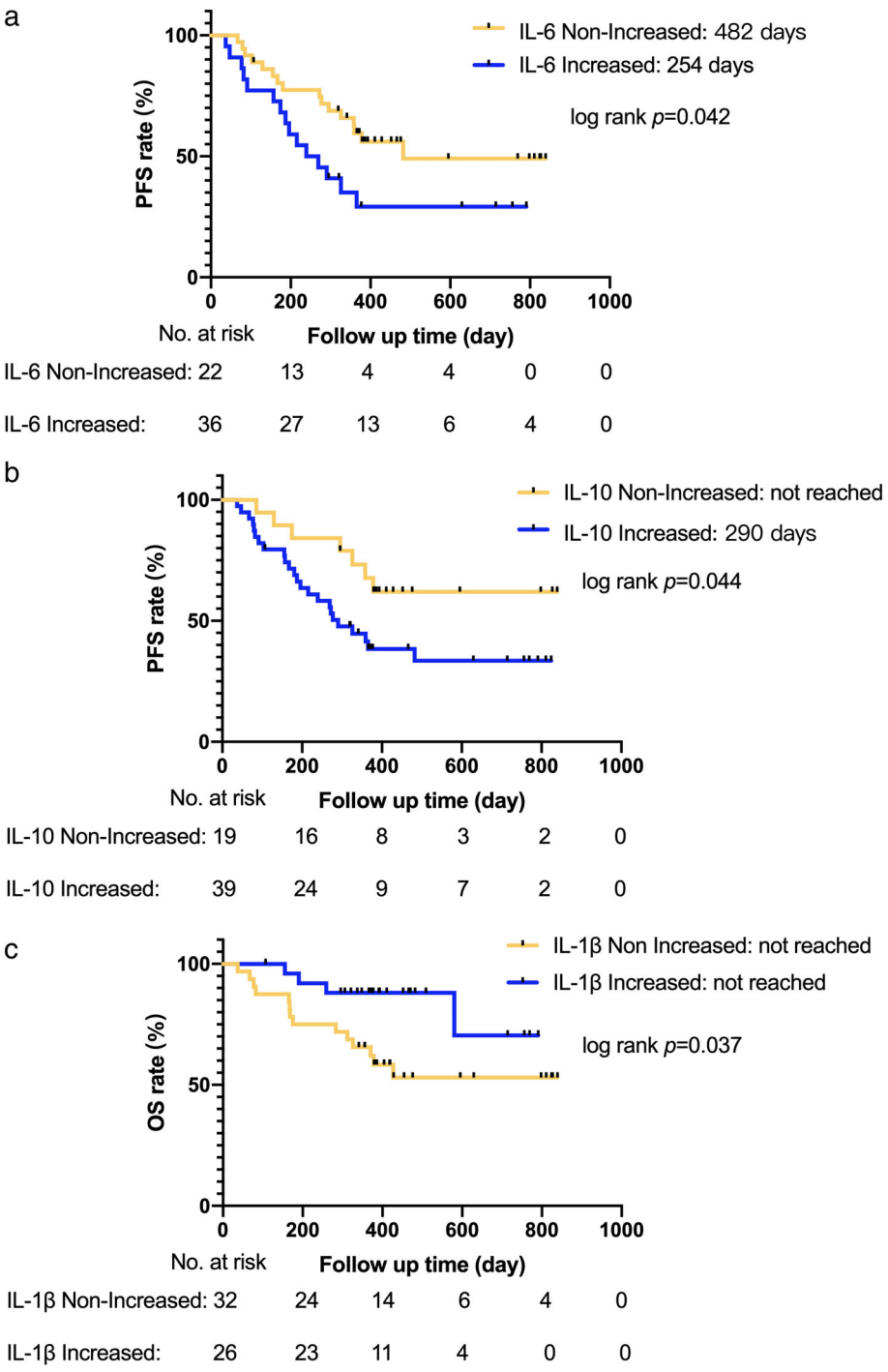

FIGURE 2 (a) Kaplan–Meier analysis of PFS for patients with NSCLC treated with chemoimmunotherapy according to on-treatment increased or nonincreased IL-6. The *p*-values were calculated with the log rank test. (b) Kaplan–Meier analysis of PFS for patients with NSCLC treated with chemoimmunotherapy according to on-treatment increased or nonincreased IL-10. The *p*-values were calculated with the log rank test. (c) Kaplan–Meier analysis of OS for patients with NSCLC treated with chemoimmunotherapy according to on-treatment increased or nonincreased IL-1β. The *p*-values were calculated with the log rank test. PFS, progression-free-survival; NSCLC, non-small cell lung cancer; IL-6, interleukin-6; IL-10, interleukin-10; OS, overall survival; IL-1β, interleukin-1β

RESULTS

Patient characteristics, treatment, and efficacy

We identified 60 patients with advanced NSCLC treated with PD-1 based chemoimmunotherapy. Of these 60 patients, 63.3% ($n = 38$) were diagnosed with adenocarcinoma, while 36.7% ($n = 22$) had a squamous NSCLC. A total of 91.7% ($n = 55$) of the patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1, and 76.7% ($n = 46$) had metastatic disease. A total of 70.0% ($n = 48$) received chemoimmunotherapy in the first-line setting. The patient clinical characteristics are summarized in Table 1.

The median follow-up time was 14.3 (95% CI: 12.6 to 16.0) months. The median PFS was 12.0 (95% CI: 7.0 to 17.3) months, and the median OS was not reached. A total of 56 patients were available for response assessment. The objective response rate (ORR) was 53.6% (30/56, 0 CR, 30 PR), and the disease control rate (DCR) was 94.6% (53/56, 0 CR, 30 PR, 23 SD). As for patients who received chemoimmunotherapy as first-line treatment ($n = 42$), the

TABLE 5 Cox regression of cytokine changes and PFS ($n = 58$)

	Univariate Cox regression			Multivariate Cox regression		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
IL-1β	0.578	0.277–1.209	0.146	0.305	0.127–0.730	0.008*
IL-2	1.161	0.578–2.373	0.661			
IL-4	0.830	0.408–1.686	0.606			
IL-5	0.808	0.395–1.650	0.558			
IL-6	2.059	1.012–4.189	0.046	2.639	1.163–5.991	0.020*
IL-8	1.590	0.744–3.398	0.231			
IL-10	2.324	0.997–5.418	0.051	2.768	0.932–8.215	0.067
IL-12p70	0.972	0.480–1.970	0.938			
IL-17	1.005	0.495–2.041	0.990			
IFN-α	1.609	0.790–3.277	0.190	1.681	0.721–3.972	0.236
IFN-γ	0.587	0.289–1.193	0.141	0.336	0.152–0.745	0.007*
TNF-α	1.868	0.913–3.823	0.087	1.375	0.615–3.076	0.438

Abbreviations: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12protein70; IL-17, interleukin-17; IFN-α, interferon-α; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
*means that the *p* value is statistically significant.

TABLE 6 Cox regression of cytokine changes and OS ($n = 58$)

	Univariate Cox regression			Multivariate Cox regression		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
IL-1β	0.325	0.107–0.989	0.048	0.307	0.093–1.013	0.052
IL-2	0.768	0.297–1.986	0.586			
IL-4	0.694	0.268–1.794	0.451			
IL-5	0.470	0.185–1.192	0.112	0.553	0.206–1.487	0.241
IL-6	1.885	0.744–4.776	0.181	2.742	1.063–7.074	0.037*
IL-8	1.816	0.699–4.718	0.221			
IL-10	1.160	0.435–3.097	0.767			
IL-12p70	0.672	0.260–1.740	0.413			
IL-17	0.888	0.350–2.254	0.803			
IFN-α	1.209	0.479–3.053	0.688			
IFN-γ	0.465	0.180–1.204	0.465			
TNF-α	1.304	0.510–3.332	0.580			

Abbreviations: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12protein70; IL-17, interleukin-17; IFN-α, interferon-α; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
*means that the *p* value is statistically significant.

ORR was 65.0% (26/40, 0 CR, 26PR), and DCR was 100.0% (40/40, 0 CR, 26 PR, 14 SD).

Pretreatment plasma cytokines related to clinical outcome

Patients were stratified into two subgroups according to whether they were responders (whose BOR was CR or PR) or nonresponders (whose BOR was SD or PD) to chemoimmunotherapy. Pretreatment plasma cytokines were defined as high-level group and low-level group according to the median value of each cytokine. No significant association was noticed between pretreatment plasma cytokine and response (Table 2).

In terms of survival analysis, patients with high pretreatment IL-2 (> median, which is 0.09 pg/ml) showed longer PFS compared to those with low IL-2 (log rank $p = 0.046$) (Figure 1a). Moreover, patients with high pretreatment IL-8 (> median, which is 4.9 pg/ml) showed shorter OS in patients receiving chemoimmunotherapy (log rank $p = 0.004$) (Figure 1b). A similar tendency was also noticed in pretreatment IL-2 and its relationship with OS, as well as pretreatment IL-8 and its relationship with PFS (Supplementary Figure S1a and S1b), although no significant relationship was found.

TABLE 7 Distribution of immune-related adverse events

Adverse event	N (%), total = 31 events in 21 patients	Grade ≥ 3 irAEs
Cutaneous	8 (25.8)	
Pruritus	3 (9.7)	
Rash	5 (16.1)	1
Gastrointestinal	7 (22.6)	
Pancreatitis or lipase/amylase increase	4 (12.9)	
alanine aminotransferase/bilirubin increase	3 (9.7)	
Endocrine disorders	9 (29.0)	
Hyperthyroidism	3 (9.6)	
Hypothyroidism	3 (9.7)	
Hypophysitis	1 (3.2)	
Hyperglycemia	2 (6.5)	
Pneumonitis	2 (6.5)	
Myocarditis/cardiac troponin I increase	3 (9.7)	2
Albuminuria	1 (3.2)	
Drug induced sarcoidosis-like reaction	1 (3.2)	

Abbreviation: irAEs, immune-related adverse events.

Changes between pretreatment and on-treatment plasma cytokines related to treatment outcome

On-treatment plasma cytokines were available in 59 patients. A total of 55 patients were available with an assessment of response. Significant biological variation was predetermined as ≥10% change compared to the pretreatment level. Increased IL-1β was significantly higher in the responder's group compared to the nonresponder's group (60.0% vs. 28.0%, $p = 0.018$, Table 3). Increased IL-5 was also higher in the responder's group (70.0% vs. 48.0%, $p = 0.097$, Table 3). To the contrary, increased IL-6 was higher in the nonresponder's group compared to the responder's group (52.0% vs. 26.7%, $p = 0.054$, Table 3). Moreover, increased IL-10 was also higher in the nonresponder's group (80.0% vs. 56.7%, $p = 0.066$, Table 3). Multivariate analyses result showed that increased IL-6 (HR 0.015, 95% CI: 0.001–0.400, $p = 0.012$) was a negative risk factor for treatment response, while increased IL-1β (HR 6.233, 95% CI: 1.451–26.344, $p = 0.013$) was a positive risk factor for treatment response in patients receiving chemoimmunotherapy (Table 4).

Survival data was available in 58 patients, although one patient developed grade IV irAE and stopped anti-PD-1 treatment permanently. Kaplan–Meier analysis showed increased IL-6 (log rank $p = 0.042$, Figure 2a) and increased IL-10 (log rank $p = 0.044$, Figure 2b) were related to shorter PFS. Patients with on-treatment increased IL-1β had longer OS (log rank $p = 0.037$, Figure 2c). Multivariate Cox regression analysis revealed that an increased IFN-γ and IL-1β were associated with longer PFS (IFN-γ: HR 0.336, 95% CI: 0.153–0.745, $p = 0.007$; IL-1β: HR 0.305, 95% CI: 0.127–0.730, $p = 0.008$), while increased IL-6 was a negative risk factor for better PFS (HR 2.639, 95% CI: 1.163–5.991, $p = 0.020$, Table 5). As for OS, multivariate analysis showed increased IL-6 remained a negative risk factor for better OS (HR 2.742, 95% CI: 1.063–7.074, $p = 0.037$, Table 6).

Onset of irAEs and its relationship with circulating cytokines

A total of 21 patients (35.0%) experienced 31 irAEs, 13 patients developed single-system irAEs, while eight patients developed multiple-system irAEs with a maximum of three systems. The most observed irAEs were thyroid dysfunction (19.4%), rash (16.1%) and pancreatic dysfunction (12.9%). The most common severe irAE (grade > =3) was myocarditis (n = 2). One patient developed Steven-Johnson syndrome after the first injection of PD-1 antibody and stopped anti-PD-1 treatment permanently. The irAEs are listed in Table 7.

Comparison between patients who developed irAEs and patients without irAEs are listed in supplementary Table S1. No significant differences were found between the two groups on cytokine changes.

DISCUSSION

This retrospective study involved analyses of pretreatment and on-treatment cytokine concentrations on anti-PD-1-based chemotherapy. We found that pretreatment levels of IL-2 and IL-8 as well as on-treatment increased IL-6, increased IFN-γ, and increased IL-1β were associated with clinical outcome from chemoimmunotherapy.

Among cytokines analyzed pretreatment, we observed a positive association between IL-2 and PFS as well as a negative relationship between IL-8 and OS. IL-2 is recognized as having the ability to promote natural killer cell differentiation and T cell clonal expansion after antigen exposure.[14] Moreover, a high dose of IL-2 has been used to interfere with the activity of endothelial cells.[15] It has been reported higher pretreatment IL-2 was associated with a better clinical response and longer OS and time to treatment failure NSCLC receiving chemotherapy.[16] Pretreatment IL-8 was found to be a negative prognostic factor in patients receiving chemoimmunotherapy in our study. IL-8 is a chemokine with multiple protumorigenic roles within the tumor microenvironment, including stimulating tumor cell proliferation, migration, angiogenesis and recruiting of immunosuppressive cells. IL-8 has been observed to be associated with worse disease prognosis in pancreatic cancer, ovarian cancer, breast cancer and NSCLC.[17–20] Our results contribute to the literature in the era of chemoimmunotherapy.

As for on-treatment cytokines, increased IL-6 was correlated with a worse response, shorter PFS and shorter

OS. Previous studies have also observed an inverse relationship between clinical benefit and levels of IL-6 in multiple cancer types treated with ICIs, including malignant melanoma,[4] NSCLC,[6] and renal cell carcinoma.[21] One of the key biological pathways governing this phenomenon is the IL-6/JAK/STAT3 axis, which potentiates tumor proliferation and cellular metabolism on upregulation.[22] In addition to STAT3, IL-6 also activates Ras, MAPK, COX-2, Wnt and PI3K/AKT pathways which are related to protumorigenic activities.[23,24] Moreover, IL-6 can promote tumor survival through recruiting mesenchymal stem cells, which are non-immune cells and can be induced by IL-6 to suppress tumor-infiltrating lymphocytes in the tumor environment.[25–27] In recent years, anti-IL-6 receptor antibody tocilizumab and anti-IL-6 antibody siltuximab have shown potential clinical benefits in treating various human cancers.[28] Our results on IL-6 are consistent with the published literature that IL-6 serves as a negative prognostic factor in NSCLC patients treated with chemoimmunotherapy, which indicates to clinicians the importance of monitoring IL-6 levels before and during systemic anticancer treatment. However, since IL-6 is not measured routinely in clinical practice, the predictive role of IL-6 might be replaced by monitoring C-reactive protein, which is a serum protein that is elevated in acute phases of inflammation and changes in correlation with serum IL-6, both pre-ICI-treatment and after ICI treatment in cancer patients.[4]

INF-γ also stands out as one of the meaningful cytokines in our study. We found that increased levels of INF-γ were highly predictive of a good and durable response to chemo-immunotherapy. IFN-γ is a proinflammatory cytokine released from tumor suppressor cells and is believed to have a positive relationship with increased PD-L1 expression.[29] Meanwhile, it can enhance cancer immunogenicity[30] and induce lymphocyte activation.[31] IFN-γ facilitates cell-mediated adaptive immune response through the IFN-γ/JAL/STAT1 pathway to promote T cell-mediated antitumor immune responses.[32] Another study also found a positive effect of high INFγ levels both before and during treatment on the outcome of ICI treatment.[33] Damage of IFN-γ stimulus response is correlated with both primary and acquired resistance to ICI therapy.[34,35]

Our results showed that increased IL-1β is a positive factor correlated with both clinical response and PFS. IL-1β is produced and secreted by both immune and tumor cells. Usually it has a protumor role, but it can act as an antitumor cytokine in some cases depending on both cancer and treatment types.[36] Preclinical studies showed that IL-1β increases the antitumor effect through granzyme B production in mice harboring B16 melanoma.[37] Furthermore, chemotherapeutic agents promote caspase-1 activation and pyroptotic cell death of mesothelioma cells, leading to the release of IL-1β from cancer cells.[38] Our study also suggests that on-treatment increased IL-1β was positively associated with clinical benefit. However, most of the published data showed that IL-1β served as a negative prognostic factor. Further studies are awaited to investigate this discrepancy.

This study had 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 chemoimmunotherapy at different lines of therapy. Thus, the degree of baseline inflammation may have been affected by previous treatments. Third, cytokines from multiple timepoints should be examined especially at time of disease progression to better understand cytokine evolution.

In conclusion, in the present study, we evaluated the associations between peripheral cytokine concentrations and treatment outcome to PD-1-based chemotherapy in patients with advanced NSCLC. On-treatment increased IL-1β and IFN-γ may serve as a positive indicator of efficacy, while on-treatment increased IL-6 might play a predictive role of worse clinical outcome. Cytokine is therefore of predictive significance for clinical outcome in patients receiving chemoimmunotherapy.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

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REFERENCES

1. Negrao MV, Lam VK, Reuben A, Rubin ML, Landry LL, Roarty EB, et al. PD-L1 expression, tumor mutational burden, and cancer gene mutations are stronger predictors of benefit from immune checkpoint blockade than HLA class I genotype in non-small cell lung cancer. J Thorac Oncol. 2019;14(6):1021–31.
2. Peng L, Wang Y, Liu F, Qiu X, Zhang X, Fang C, et al. Peripheral blood markers predictive of outcome and immune-related adverse events in advanced non-small cell lung cancer treated with PD-1 inhibitors. Cancer Immunol Immunother. 2020;69(9):1813–22.
3. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res. 2009;69(7):3077–85.
4. Laino AS, Woods D, Vassallo M, Qian X, Tang H, Wind-Rotolo M, et al. Serum interleukin-6 and C-reactive protein are associated with survival in melanoma patients receiving immune checkpoint inhibition. J Immunother Cancer. 2020;8(1):e000842.
5. Yuen KC, Liu LF, Gupta V, Madireddi S, Keerthivasan S, Li C, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26(5):693–8.
6. Keegan A, Ricciuti B, Garden P, Cohen L, Nishihara R, Adeni A, et al. Plasma IL-6 changes correlate to PD-1 inhibitor responses in NSCLC. J Immunother Cancer. 2020;8(2):e000678.
7. Lim SY, Lee JH, Gide TN, Menzies AM, Guminski A, Carlino MS, et al. Circulating cytokines predict immune-related toxicity in melanoma patients receiving anti-PD-1-based immunotherapy. Clin Cancer Res. 2019;25(5):1557–63.
8. Sanmamed MF, Perez-Gracia JL, Schalper KA, Fusco JP, Gonzalez A, Rodriguez-Ruiz ME, et al. Changes in serum interleukin-8 (IL-8)

levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Ann Oncol. 2017; 28(8):1988–95.
9. Rodriguez-Abreu D, Powell SF, Hochmair MJ, Gadgeel S, Esteban E, Felip E, et al. Pemetrexed plus platinum with or without pembrolizumab in patients with previously untreated metastatic nonsquamous NSCLC: protocol-specified final analysis from KEYNOTE-189. Ann Oncol. 2021;32(7):881–95.
10. Paz-Ares L, Vicente D, Tafreshi A, Robinson A, Soto Parra H, Mazieres J, et al. A randomized, placebo-controlled trial of pembrolizumab plus chemotherapy in patients with metastatic squamous NSCLC: protocol-specified final analysis of KEYNOTE-407. J Thorac Oncol. 2020;15(10):1657–69.
11. Yang Y, Wang Z, Fang J, Yu Q, Han B, Cang S, et al. Efficacy and safety of sintilimab plus pemetrexed and platinum as first-line treatment for locally advanced or metastatic nonsquamous NSCLC: a randomized, double-blind, phase 3 study (oncology pRogram by InnovENT anti-PD-1-11). J Thorac Oncol. 2020;15(10):1636–46.
12. Zhou C, Chen G, Huang Y, Zhou J, Lin L, Feng J, et al. Camrelizumab plus carboplatin and pemetrexed versus chemotherapy alone in chemotherapy-naive patients with advanced non-squamous non-small-cell lung cancer (CameL): a randomised, open-label, multicentre, phase 3 trial. Lancet Respir Med. 2021;9(3):305–14.
13. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47.
14. Martini DJ, Liu Y, Shabto JM, Lewis C, Kline MR, Collins H, et al. Clinical outcomes of advanced stage cancer patients treated with sequential immunotherapy in phase 1 clinical trials. Invest New Drugs. 2019;37(6):1198–206.
15. Majidpoor J, Mortezaee K. Interleukin-2 therapy of cancer-clinical perspectives. Int Immunopharmacol. 2021;98:107836.
16. Orditura M, Romano C, De Vita F, Galizia G, Lieto E, Infusino S, et al. Behaviour of interleukin-2 serum levels in advanced non-small-cell lung cancer patients: relationship with response to therapy and survival. Cancer Immunol Immunother. 2000;49(10):530–6.
17. Feng L, Qi Q, Wang P, Chen H, Chen Z, Meng Z, et al. Serum levels of IL-6, IL-8, and IL-10 are indicators of prognosis in pancreatic cancer. J Int Med Res. 2018;46(12):5228–36.
18. Sanguinete MMM, Oliveira PH, Martins-Filho A, Micheli DC, Tavares-Murta BM, Murta EFC, et al. Serum IL-6 and IL-8 correlate with prognostic factors in ovarian cancer. Immunol Invest. 2017; 46(7):677–88.
19. Keskin S, Kutluk AC, Tas F. Prognostic and predictive role of angiogenic markers in non- small cell lung cancer. Asian Pac J Cancer Prev. 2019;20(3):733–6.
20. Tiainen L, Hamalainen M, Luukkaala T, Tanner M, Lahdenpera O, Vihinen P, et al. Low plasma IL-8 levels during chemotherapy are predictive of excellent long-term survival in metastatic breast cancer. Clin Breast Cancer. 2019;19(4):e522–33.
21. Chehrazi-Raffle A, Meza L, Alcantara M, Dizman N, Bergerot P, Salgia N, et al. Circulating cytokines associated with clinical response to systemic therapy in metastatic renal cell carcinoma. J Immunother Cancer. 2021;9(3):e002009.
22. Chang Q, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, et al. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. Neoplasia. 2013;15(7):848–62.
23. Ara T, Declerck YA. Interleukin-6 in bone metastasis and cancer progression. Eur J Cancer. 2010;46(7):1223–31.
24. Jee SH, Chu CY, Chiu HC, Huang YL, Tsai WL, Liao YH, et al. Interleukin-6 induced basic fibroblast growth factor-dependent angiogenesis in basal cell carcinoma cell line via JAK/STAT3 and PI3-kinase/Akt pathways. J Invest Dermatol. 2004;123(6): 1169–75.
25. Ling W, Zhang J, Yuan Z, Ren G, Zhang L, Chen X, et al. Mesenchymal stem cells use IDO to regulate immunity in tumor microenvironment. Cancer Res. 2014;74(5):1576–87.
26. Liotta F, Querci V, Mannelli G, Santarlasci V, Maggi L, Capone M, et al. Mesenchymal stem cells are enriched in head neck squamous cell carcinoma, correlates with tumour size and inhibit T-cell proliferation. Br J Cancer. 2015;112(4):745–54.
27. Sun Z, Wang S, Zhao RC. The roles of mesenchymal stem cells in tumor inflammatory microenvironment. J Hematol Oncol. 2014;7:14.
28. Yao X, Huang J, Zhong H, Shen N, Faggioni R, Fung M, et al. Targeting interleukin-6 in inflammatory autoimmune diseases and cancers. Pharmacol Ther. 2014;141(2):125–39.
29. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 2012;4(127):127ra137.
30. Chen A, Sceneay J, Godde N, Kinwel T, Ham S, Thompson EW, et al. Intermittent hypoxia induces a metastatic phenotype in breast cancer. Oncogene. 2018;37(31):4214–25.
31. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002; 2(11):850–61.
32. Zaidi MR, Merlino G. The two faces of interferon-gamma in cancer. Clin Cancer Res. 2011;17(19):6118–24.
33. Kanai T, Suzuki H, Yoshida H, Matsushita A, Kawasumi H, Samejima Y, et al. Significance of quantitative interferon-gamma levels in non-small-cell lung cancer patients' response to immune checkpoint inhibitors. Anticancer Res. 2020;40(5):2787–93.
34. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016;375(9):819–29.
35. Gao J, Shi LZ, Zhao H, Chen J, Xiong L, He Q, et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell. 2016;167(2):397–404 e399.
36. Rebe C, Ghiringhelli F. Interleukin-1beta and cancer. Cancers (Basel). 2020;12(7):1791.
37. Lee PH, Yamamoto TN, Gurusamy D, Sukumar M, Yu Z, Hu-Li J, et al. Host conditioning with IL-1beta improves the antitumor function of adoptively transferred T cells. J Exp Med. 2019;216(11): 2619–34.
38. Westbom C, Thompson JK, Leggett A, MacPherson M, Beuschel S, Pass H, et al. Inflammasome modulation by chemotherapeutics in malignant mesothelioma. PLoS One. 2015;10(12):e0145404.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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