BMC Cancer

RESEARCH Open Access

Neutrophil-to-lymphocyte ratio-based prognostic score can predict outcomes in patients with advanced non-small cell lung cancer treated with immunotherapy plus chemotherapy

Shan Liao[1], Huiying Sun[1], Hao Lu[1], Jiani Wu[1], Jianhua Wu[1], Zhe Wu[1], Jingle Xi[1], Wangjun Liao[1,2*] and Yuanyuan Wang[1*]

Abstract

Backgroud Immune checkpoint inhibitor (ICI) plus chemotherapy has become the standard of care for advanced non-small cell lung cancer (NSCLC). Nonetheless, reliable efficacy biomarkers of ICI plus chemotherapy are lacking. In this research, we sought to explore efficacy biomarkers and construct robust prognostic models in NSCLC patients treated with ICI plus chemotherapy.

Methods We retrospectively analyzed 171 patients with advanced NSCLC treated with ICI plus chemotherapy. Clinical characteristics and peripheral blood inflammatory indexes were collected and prognostic models were constructed to explore efficacy and prognosis biomarkers of ICI plus chemotherapy.

Results In the cohort that received first-line ICI plus chemotherapy, pre-treatment neutrophil-to-lymphocyte ratio (NLR) > 3.3 and fibrinogen (FIB) > 3.196 were associated with worse efficacy and were independent risk factors of progression-free survival (PFS). Compared to programmed cell death ligand 1 (PD-L1), the derived NLR-FIB (NF) score had significantly improved accuracy in predicting efficacy and prognosis. In advanced NSCLC patients with targetable oncogenic driver alterations receiving second- or post-line ICI plus chemotherapy, pre-treatment NLR > 3.53 was associated with worse efficacy and was an independent risk factor of PFS and OS; Tyrosine kinase inhibitor (TKI)-PFS > 12 months were independent risk factors of overall survival (OS). Secondary epidermal growth factor receptor (EGFR)-T790M mutation, platelet-to-lymphocyte ratio (PLR) > 196.81 and albumin (ALB) < 40.25 were associated with worse PFS. Based on NLR and TKI-PFS, an NLR-TKI-PFS (NTP) score was constructed with three OS risk prognosis categories: favorable, intermediate, and poor (corresponding to a median OS of 21, 12, and 5.3 months).

*Correspondence:
Wangjun Liao
nfyyliaowj@163.com
Yuanyuan Wang
doc_wang@126.com

Full list of author information is available at the end of the article

Conclusions The noninvasive NF score, combining NLR > 3.3 and FIB > 3.196, was superior to PD-L1 estimated from tumor tissue in predicting the efficacy and prognosis of first-line ICI plus chemotherapy in advanced NSCLC patients. The noninvasive NTP score, combining NLR > 3.53 and TKI-PFS > 12 months, is a valuable tool for predicting OS and PFS in advanced NSCLC patients with targetable oncogenic driver alterations receiving second- or post-line ICI combination therapy.

Keywords Non-small cell lung cancer, Neutrophil lymphocyte ratio, Immunochemotherapy, Efficacy prediction, Prognostic biomarker

Backgrounds

Lung cancer is a malignant tumor with among highest morbidity and mortality rates [1]. The development of immune checkpoint inhibitor (ICI) targeting the PD-1/PD-L1 interaction has changed the treatment prospects for cancer patients, and ICI plus chemotherapy has become the first-line standard of care for patients with advanced non-small cell lung cancer (NSCLC) without targetable driver gene alterations [2–4]. However, only 15–30% of patients achieve long-term survival [5, 6]. Therefore, to improve the efficacy of ICI plus chemotherapy and achieve the goal of precision treatment, the exploration of ICI plus chemotherapy biomarkers has received considerable attention.

PD-L1 stands out as one of the most well-established and extensively validated predictive biomarkers for ICI in NSCLC [7]. Patients with a PD-L1 tumor cell proportion score (TPS) ≥ 50% are most likely to benefit from ICI and can be treated with ICI monotherapy [8, 9]. Nevertheless, people with high PD-L1 expression account for only 27% of the total population, most NSCLC patients have low PD-L1 expression and their 5-year survival rate with ICI plus chemotherapy remains below 20% [5]. Besides, some PD-L1-negative patients may still benefit from ICI plus chemotherapy [10, 11]. Therefore, the use of PD-L1 as a marker for ICI plus chemotherapy is not ideal. Similarly, current studies yield inconsistent results regarding the predictive value of tumor mutational burden (TMB) for ICI [12–14]. Moreover, according to the tumor microenvironment (TME), the immune cell status can be used to categorize tumor immune infiltration patterns, thereby enabling prediction of the efficacy of ICI [15]. Nonetheless, challenges remain in effectively applying this to clinical practice.

Current immunological predictive markers have been explored from various perspectives such as at cellular level [16], DNA level [17, 18], protein level [19–22], and patients' intrinsic factors (e.g., inflammation and nutritional indicators) [23]. Compared with tissue sample-based assays, patients' intrinsic factors have the advantages of being cost-effective, noninvasive, and easily accessible. Therefore, in this study, we primarily focused on exploring patients' intrinsic factors to find more robust markers that can predict the response to ICI plus chemotherapy than PD-L1 expression level.

Chronic inflammation and immune evasion are the main features of cancer occurrence and development [24]. Previous research has elucidated the molecular mechanisms of inflammation-related carcinogenesis and found several candidate biomarkers [18, 21]. Moreover, some peripheral blood inflammatory markers have been found to be related to the prognosis of ICI monotherapy in NSCLC, such as the neutrophil-to-lymphocyte ratio (NLR) and derived NLR (dNLR) [25–29]. However, their prognostic value in patients who receive ICI plus chemotherapy is still unknown. For example, based on the dNLR and lactate dehydrogenase (LDH), Mezquita et al. developed a lung immune prognostic index (LIPI) status for patients with advanced NSCLC treated with first-line ICI and showed that good LIPI status was associated with better clinical outcomes [29]. However, Wang et al. demonstrated that LIPI status could be a prognostic marker of the treatment response to ICI monotherapy but not to ICI plus chemotherapy [30]. Furthermore, studies that amalgamate multiple peripheral blood inflammatory markers in first-line ICI plus chemotherapy and offer cross-sectional comparisons with PD-L1 remain relatively scarce. Consequently, one objective of this study was to explore cost-effective, noninvasive, and easily accessible markers in combination with PD-L1 or to discover superior biomarkers, elucidating their value in predicting the efficacy and prognosis of first-line ICI plus chemotherapy.

In addition to first-line ICI plus chemotherapy for patients without targetable oncogenic driver alterations, we also addressed second-line immunotherapy for patients with targetable oncogenic driver alterations after developing resistance to targeted therapy. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have shown outstanding efficacy in the treatment of patients with EGFR-mutated NSCLC [31, 32]; however, nearly all patients eventually develop drug resistance. Among patients with resistance to first- or second-generation EGFR-TKIs, 50% have T790M resistance mutations, which is sensitive to third-generation EGFR-TKI therapy [33]. However, for patients with unknown resistance mechanisms who do not have T790M mutations or who have failed third-generation EGFR-TKI therapy, rescue treatment remains a challenging issue in clinical practice [34]. Chemotherapy has been

the standard of care for those patients with unknown resistance mechanisms after TKI treatment [35]. However, its effectiveness is limited, with median progression-free survival (mPFS) of only 4–5 months [36]. In addition, although ICI demonstrated greater effectiveness than chemotherapy in second-line treatment [37–39], second-line ICI monotherapy was not superior to standard chemotherapy regimens for patients harboring driver gene mutations, with an objective response rate (ORR) of only about 10–20% and a mPFS of only 2–3 months [40]. Our previous research also explained the poor efficacy of ICI monotherapy as a result of the immunosuppressive microenvironment from the perspective of the TME [41]. Preclinical studies have shown that chemotherapy can activate immune responses through various mechanisms and has a synergistic effect when used in combination with ICI [42]. Second-line ICI plus chemotherapy have yielded promising outcomes. A clinical study demonstrated a mPFS of up to 7 months in EGFR-TKI-resistant patients without T790M mutation treated with ICI plus chemotherapy [43]. Consistently, favorable findings in clinical trials such as IMPOWER 150 and ORIENT-31 also demonstrated higher efficacy with the addition of ICI to bevacizumab and chemotherapy than with the standard of care [44–46]. As a result, the combination immunotherapy obtained approval from the National Medical Products Administration for its indications, making it currently the highest-evidence treatment option in evidence-based medicine. Nonetheless, the results of the IMPOWER 151 study presented at the 2023 World Conference on Lung Cancer (WCLC) Annual Meeting did not find a clinical benefit of the four-drug regimen comprising bevacizumab plus chemotherapy containing a PD-L1 monoclonal antibody over bevacizumab in combination with chemotherapy. Taking these findings together, controversy remains regarding second-line treatment for patients with unexplained resistance mechanisms to EGFR-TKIs. Although ICI plus chemotherapy is a promising treatment strategy, clinical research data indicate limited benefits in the unscreened general population. Future development should move in a biomarker-driven direction to identify potential populations that would benefit from ICI combination therapy, thereby avoiding unnecessary medical expenses.

To date, predictive biomarkers for the outcomes of second-line ICI plus chemotherapy have not been identified [47]. The predictive value of the currently recognized immunotherapy biomarker PD-L1 is debatable in second-line immunology [48, 49]. Zhou et al. conducted an analysis of peripheral blood immune cells using full-spectrum flow cytometry and demonstrated that the expression type and level of immune checkpoint proteins on immune cells are related to the outcomes of subsequent ICI plus chemotherapy in patients with EGFR-TKI-resistant NSCLC [50]. Nevertheless, the value of more cost-effective and easily accessible peripheral blood parameters, such as NLR, in predicting the prognosis of TKI-resistant patients with NSCLC who are receiving ICI plus chemotherapy remains unclear. Consequently, another objective of this study was to explore potential prognostic biomarkers for NSCLC patients with targetable oncogenic driver alterations from the perspective of peripheral blood indexes to offer valuable insights for guiding treatment decisions regarding second- or post-line ICI combination therapy following the development of resistance to TKIs.

Methods

Study design and participants

We conducted a retrospective analysis involving 131 patients with advanced NSCLC who attended Nanfang Hospital between January 2019 and October 2022 to receive first-line ICI plus chemotherapy. We also enrolled 40 advanced NSCLC patients with targetable oncogenic driver alterations who exhibited unexplained resistance to TKI treatment to receive second- or post-line ICI combination therapy. Among the patient cohort, individuals with comorbid hematologic or autoimmune diseases, co-infections, a history of hormone usage within 2 weeks prior to treatment initiation, or those lacking consistent follow-up were excluded from the study. The study was approved by the Ethics Committee of Nanfang Hospital.

Data collection

Data on patients' clinical characteristics and laboratory tests were anonymized and extracted from the electronic medical record system. The unit measures for the laboratory variables are as follows: FIB: g/L; LDH: U/L; D-Dimer: ug/mL; CRP: mg/L; ALB: g/L; HGB: g/L. All peripheral blood indicators were collected 1 week before immunotherapy. Moreover, the PD-L1 expression (tumor proportion score (TPS)) was evaluated by PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies China, Beijing, China). Therapeutic efficacy assessment adopts the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1), which includes categories of complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). Recent efficacy assessment is based on the ORR and disease control rate (DCR). The ORR is defined as the sum of the number of CR and PR cases divided by the total number of cases. DCR is defined as the sum of CR, PR, and SD cases divided by the total number of cases. Prognosis assessment is based on PFS and overall survival (OS). PFS is defined as the time from the start of treatment to the first determination of tumor progression or the follow-up endpoint. OS is defined as the time from the start

of treatment to death for any reason or the follow-up endpoint.

Statistical analysis

The NLR was calculated by dividing the absolute counts of neutrophils and lymphocytes measured in peripheral blood; the same applied to platelet-to-lymphocyte ratio (PLR). Analysis was conducted using the receiver operating characteristic (ROC) curve and area under the ROC curve (AUC), and the Youden Index was calculated based on the ROC curve. The maximum Youden Index are established as the optimal threshold values of NLR, PLR, fibrinogen (FIB), LDH, D-Dimer, C-reactive protein (CRP), albumin (ALB), and the CD4+/CD8 + T lymphocyte ratio, thereby dividing various peripheral blood indicators into two groups: high and low. The threshold for hemoglobin (HGB) in this study was set at 120 g/L according to the criteria for anemia.

We used chi-square tests or Fisher's exact probability method to compare the demographic characteristics of patients with count data or to compare the differences in treatment effectiveness between patient groups. Kaplan-Meier survival analysis was performed in the two patient groups, and differences between the groups were assessed using the log-rank test. Variables with $P < 0.1$ were included in the multivariate regression analysis. Multivariate regression analysis was conducted using the Cox proportional hazards model. Differences in continuous variables with a normal distribution among multiple groups were analyzed using one-way analysis of variance, and differences in continuous variables with a non-normal distribution among multiple groups were analyzed using nonparametric tests.

Table 1 Patient characteristics in first-line ICI plus chemotherapy

Characteristics		Total (*n*)	NLR ≤ 3.3	NLR>3.3	*P*
Age (years)	<60	68	52.1%	51.7%	0.959
	≥ 60	63	47.9%	48.3%	
Gender	Male	105	80.3%	80.0%	0.968
	Female	26	19.7%	20.0%	
Histology	Adenocarcinoma	82	60.6%	65.0%	0.701
	Squamous	40	31.0%	30.0%	
	Others	9	8.4%	5.0%	
Stage	III	28	32.4%	8.3%	**0.001***
	IV	103	67.6%	91.7%	
Smoking status	Yes	96	74.6%	71.7%	0.701
	Never	35	25.4%	28.3%	
Brain mets	No	111	88.7%	79.2%	0.166
	Yes	20	11.3%	20.8%	
Liver mets	No	105	83.1%	76.7%	0.358
	Yes	26	16.9%	23.3%	
PD-L1 TPS	<1%	24	18.9%	31.1%	0.160
	≥ 1%	74	81.1%	68.9%	

(PD-L1 TPS, programmed cell death ligand 1 tumor cell proportion score.)

All data were analyzed using SPSS 25.0 software (New York, USA) and GraphPad Prism (version 9.0, USA). All P-values were based on two-sided hypotheses, with statistical significance set at $P < 0.05$.

Results

Patient characteristics in first-line ICI plus chemotherapy

In this study, we enrolled 131 patients with advanced NSCLC who did not have targetable oncogenic driver alterations and who received first-line combination immunotherapy. The median age of the study population was 59 years. Most patients were men (105/131, 80.2%), had a history of smoking (96/131, 73.3%), and had positive PD-L1 expression (74/98, 75.5%) (Table 1).

According to the ROC curves (Supplementary material 1), the optimal cutoff values for NLR, FIB, LDH, D-Dimer, CRP, ALB, and the CD4+/CD8 + T lymphocyte ratio were 3.30, 3.92, 198.00, 0.53, 7.74, 43.95 and 1.73 respectively. In the high NLR group, the number of patients in stage IV was significantly higher than that in the low NLR group ($P = 0.001$). However, there were no significant differences between different pre-treatment NLR levels and clinical characteristics such as sex, age, smoking status, pathological tissue type, baseline liver metastasis, baseline brain metastasis, and PD-L1 expression (Table 1).

Analysis of factors affecting efficacy of first-line ICI plus chemotherapy

The results showed that squamous carcinoma was correlated with a better ORR than adenocarcinoma ($P = 0.041$) (Fig. 1A). In contrast, age, sex, liver metastasis, smoking status, PD-L1 expression, NLR, HGB, LDH, FIB, D-dimer, ALB, CRP, and the CD4/CD8 + T lymphocyte ratio were not associated with the ORR of first-line ICI plus chemotherapy (Table 2). As for the DCR, a low NLR ($P = 0.011$) and low FIB ($P = 0.024$) were correlated with a better DCR than a high NLR and high FIB (Fig. 1B, 1 C). In contrast, age, sex, histology, liver metastasis, smoking status, PD-L1 expression, HGB, CRP, ALB, LDH, D-Dimer, and the CD4/CD8 + T lymphocyte ratio were not associated with the DCR of first-line ICI plus chemotherapy (Table 2).

Univariate analysis revealed that only NLR and FIB were associated with DCR, in the pursuit of seek a more effective and comprehensive indicator, we further combined these two variables to establish the NLR-FIB score (hereafter referred to as NF score). A score of 1 was assigned for each risk factor when NLR > 3.3 or FIB > 3.915. These scores categorized patients according to three groups: [1] Group 0 with low NLR and low FIB [2], Group 1 with high NLR or high FIB [3], Group 2 with high NLR and high FIB. Fisher's exact test demonstrated that the DCR was 100% in Group 0, 97.9% in

Table 2 Relationship between clinical characteristics and peripheral blood indexes and the outcome of first-line ICI plus chemotherapy

Variables		Total (*n*)	ORR	*P*	DCR	*P*	mPFS	*P*
Age (years)	<60	68	36.8%	0.875	91.2%	0.495	11.0	0.832
	≥60	63	38.1%		95.2%		11.6	
Gender	Male	105	37.1%	0.901	92.4%	0.687	11.6	0.700
	Female	26	38.5%		96.2%		10.6	
Histology	Adenocarcinoma	82	29.3%	**0.041***	95.1%	0.134	11.3	0.500
	Squamous	40	52.5%		92.5%		10.0	
Stage	III	28	50.0%	0.120	100%	0.203	17.0	**0.004***
	IV	103	34.0%		91.3%		10.0	
Smoking status	Yes	96	38.5%	0.656	91.7%	0.443	12.0	0.849
	No	35	34.3%		97.1%		10.5	
Liver mets	No	111	39.6%	0.213	93.7%	0.626	12.3	0.120
	Yes	20	25.0%		90.0%		10.0	
PD-L1 TPS	<1%	27	33.3%	0.779	87.5%	0.401	7.6	0.077
	≥1%	79	36.5%		93.2%		12.3	
NLR	High	60	33.3%	0.376	86.7%	**0.011***	7.0	**<0.001***
	Low	71	40.8%		98.6%		17.7	
HGB (g/L)	High	89	37.4%	0.988	94.5%	0.454	15.0	**0.002***
	Low	40	37.5%		90.0%		7.6	
CRP (mg/L)	High	50	34.0%	0.554	92.0%	0.196	9.0	**0.001***
	Low	53	39.6%		98.1%		15.5	
ALB (g/L)	High	28	42.9%	0.501	96.4%	0.683	15.0	0.769
	Low	103	35.9%		92.2%		10.6	
FIB (g/L)	High	73	35.6%	0.935	89.0%	**0.024***	8.4	**<0.001***
	Low	44	36.4%		100%		17.0	
LDH (U/L)	High	41	41.5%	0.196	90.2%	0.183	10.0	**0.023***
	Low	46	28.3%		97.8%		13.0	
D-Dimer (ug/ml)	High	49	38.8%	0.744	89.8%	0.170	10.6	**0.013***
	Low	23	34.8%		100%		15.5	
CD4/8 + T	High	7	57.1%	0.650	85.7%	0.304	8.0	**0.015***
	Low	16	37.5%		100%		16.5	

(PD-L1 TPS, programmed cell death ligand 1 tumor cell proportion score; NLR, neutrophil-to-lymphocyte ratio; HGB, hemoglobin; CRP, C-reactive protein; ALB, albumin; FIB, fibrinogen; LDH, lactate dehydrogenase; CD4/8 + T, CD4/CD8 + T lymphocyte ratio.)

Group 1, and 81.6% in Group 2 ($P < 0.05$) (**Fig. 1D**). ROC curves were plotted for predicting the DCR (**Fig. 1E**). The AUC values for the NLR, FIB, NF score, PD-L1, and NFP score (which incorporates both the NF score and PD-L1) were 0.778, 0.781, 0.798, 0.528, and 0.731, respectively, with corresponding *P*-values of 0.015, 0.014, 0.007, 0.804, and 0.009. These findings suggest that the value of the NF score for accessing the efficacy of first-line ICI combination therapy was superior to that of single indexes and even PD-L1 (TPS ≥ 1%).

Multivariate cox regression analysis of factors related to PFS in response to first-line ICI plus chemotherapy

Kaplan-Meier survival curves showed that stage IV, NLR, FIB, HGB, LDH, CRP, D-Dimer, and the CD4/CD8 + T lymphocyte ratio were correlated with PFS (**Fig. 2A H**, Table 2). In multivariate Cox regression analysis, NLR (hazard ratio [HR] 3.834; 95% confidence interval [CI] 2.228–48.294, $P < 0.001$) and FIB (HR 2.043; 95% CI 1.755–3.552, $P = 0.011$) were independent prognostic factors for PFS (Table 3).

Table 3 Multivariate analysis of factors affecting PFS in first-line ICI plus chemotherapy

Variables	Univariate analysis		Multivariate analysis	
	χ^2	*P*	HR	*P*
Stage (IV VS III)	8.139	0.004		0.487
NLR (>3.3 VS ≤ 3.3)	51.605	<0.001	3.834	**<0.001***
HGB (<120 VS ≥ 120 g/L)	9.903	0.002		0.068
CRP (>7.735 VS ≤ 7.735 mg/L)	10.887	0.001		0.498
FIB (>3.915 VS ≤ 3.915 g/L)	14.317	<0.001	2.043	**0.011***
LDH (>198 VS ≤ 198 U/L)	5.153	0.023		0.626
PD-L1 TPS (<1% VS ≥ 1%)	3.136	0.077		0.492

(NLR, neutrophil-to-lymphocyte ratio; HGB, hemoglobin; CRP, C-reactive protein; FIB, fibrinogen; LDH, lactate dehydrogenase; PD-L1 TPS, programmed cell death ligand 1 tumor cell proportion score.)

After screening by multivariate Cox regression, only NLR and FIB were independent prognostic factors for

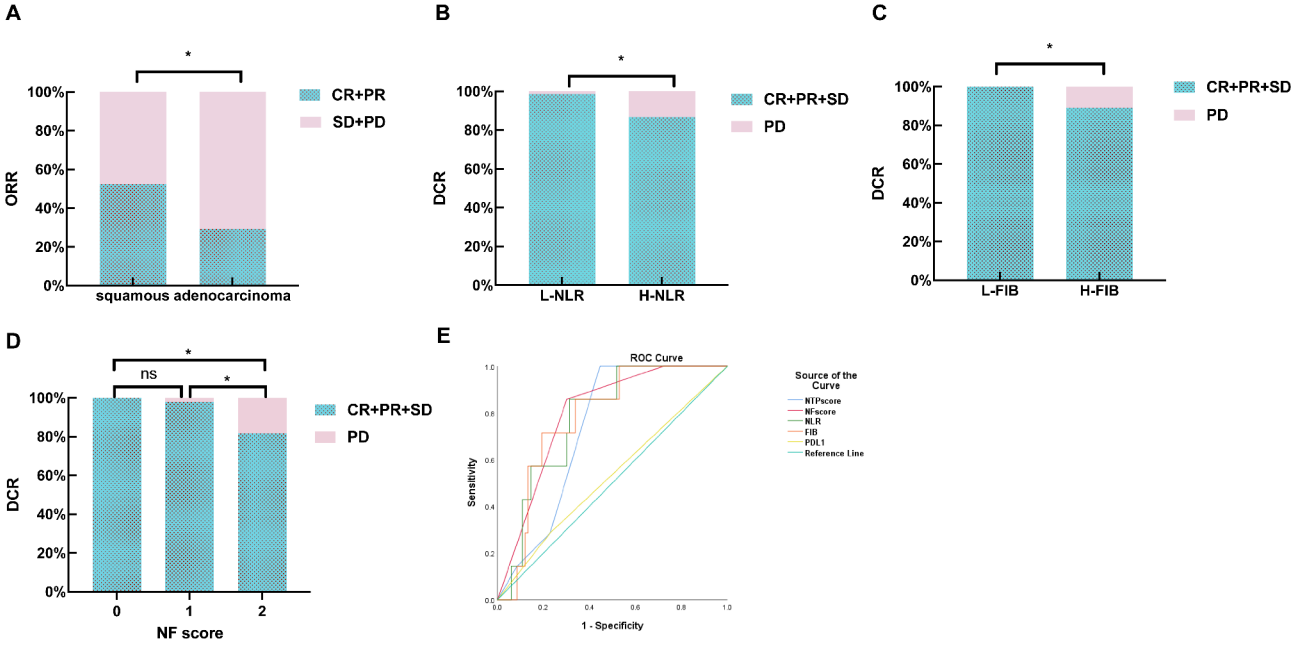

Fig. 1 Treatment response of first-line ICI plus chemotherapy according to **(A)** Histology; **(B)** NLR level; **(C)** FIB level; **(D)** NF score. **(E)** ROC curves of the NLR, FIB, PD-L1, NF score and NFP score to predict DCR

PFS. Therefore, the NF score was applied again and compared its prognostic value with that of PD-L1 expression. Based on the NF score, the median PFS for patients in the 0-score group was not reached; for patients in the 1-score group, the median PFS was 13.5 months; and for patients in the 2-score group, the median PFS was 6.5 months ($P < 0.05$) (**Fig. 2I**). The ROC curves were plotted for predicting PFS (**Fig. 2J**). The AUC values for NLR, FIB, NF score, PD-L1, and NFP score were 0.72, 0.698, 0.747, 0.54, and 0.664, respectively, with corresponding *P*-values of 0.001, 0.003, < 0.001, 0.548, and 0.013. These findings suggest that the prognostic value of the NF score was also better than that of single indexes and even PD-L1 (TPS ≥ 1%).

Dynamic changes in NLR and FIB during first-line ICI plus chemotherapy

In this study, NLR and FIB data were collected at three time points: baseline, RD (Response Disease, including Partial Response (PR) and Stable Disease (SD)), and PD. Among these, 89 patients had paired NLR data available at all three time points. NLR significantly decreased at the RD time point compared to the baseline ($P = 0.001$). Conversely, NLR increased again at the PD time point ($P < 0.001$), suggesting a substantial reduction in NLR levels during the treatment response phase followed by an elevation during immunotherapy resistance. Regarding FIB, 55 patients had paired data available at all three time points, and the results showed a similar trend to NLR; however, the differences did not reach statistical significance (Fig. 3).

Patient characteristics in second- or post-line ICI combination therapy

We enrolled 40 advanced NSCLC patients with targetable oncogenic driver alterations who received second- or post-line combination immunotherapy after developing resistance to targeted drugs. The median age of the study population was 60 years. Most patients were female (23/40, 57.5%), nonsmokers (27/40, 67.5%), had positive PD-L1 expression (12/20, 60%), and EGFR mutations (36/40, 90%). Thirteen patients (38.2%) developed EGFR-T790M mutations after developing resistance to EGFR-TKIs. Seventeen patients (42.5%) received second-line ICI combination therapy, and 23 patients (57.5%) received post-line ICI combination therapy (Table 4).

According to the ROC curves (Supplementary material 2), the optimal cutoff values for NLR, PLR, CRP, and ALB were 3.525, 196.805, 16.115, and 40.25, respectively. There was no significant correlation between different pre-treatment NLR levels and clinical characteristics such as sex, age, smoking history, number of treatment lines, baseline liver metastases, PD-L1 expression, TKI-PFS, and secondary EGFR-T790M mutation (Table 4).

Analysis of factors affecting efficacy of second- or post-line ICI combination therapy

The results showed that low NLR levels were associated with higher ORR ($P = 0.046$) and tended to be associated with higher DCR (Table 5). In contrast, age, sex, baseline liver metastases, smoking history, number of lines of therapy, secondary T790M mutation, PD-L1 ≥ 1%,

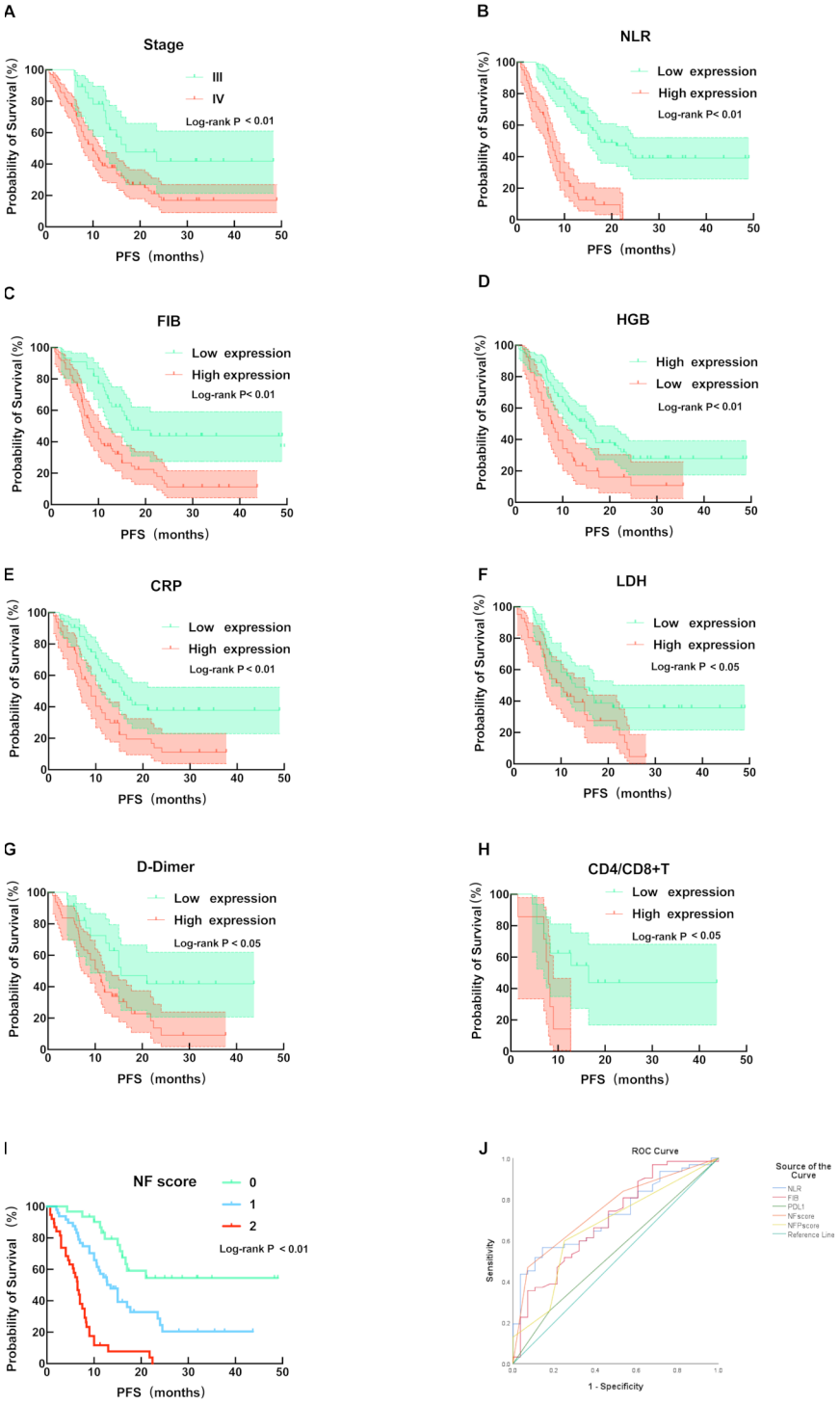

Fig. 2 Kaplan–Meier curves for progression-free survival (PFS) of first-line ICI plus chemotherapy according to **(A)** Clinical stage; **(B)** NLR level; **(C)** FIB level; **(D)** HGB level; **(E)** CRP level; **(F)** LDH level; **(G)** D-Dimer level; **(H)** CD4/CD8 + T lymphocyte ratio; **(I)** NF score. **(J)** ROC curves of the NLR, FIB, PD-L1, NF score and NFP score to predict PFS

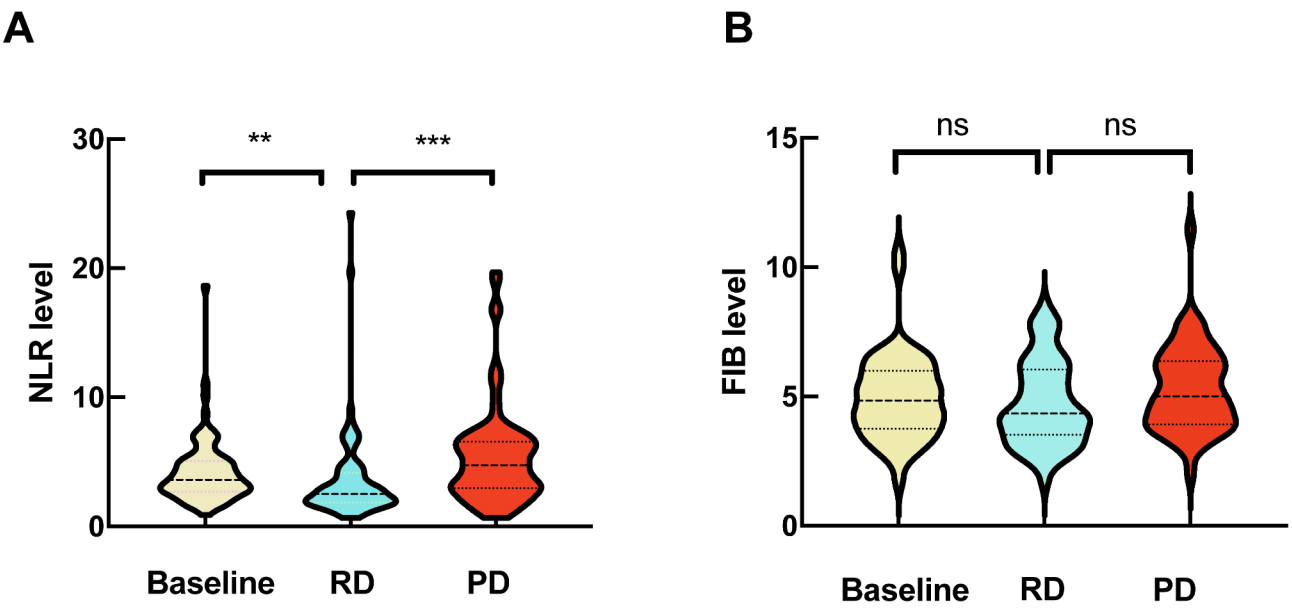

Fig. 3 Dynamic changes in NLR level **(A)** and FIB level **(B)** before (pre) and after (post) first-line ICI plus chemotherapy for patients with paired blood samples

Table 4 Clinical characteristics of patients in second- or post-line ICI plus chemotherapy

Variables		Total (*n*)	NLR ≤ 3.53	NLR>3.53	*P*
Age (years)	≤60	18	12(57.1%)	6(31.6%)	0.105
	>60	22	9(42.9%)	13(68.4%)	
Gender	Male	17	8(38.1%)	9(47.4%)	0.554
	Female	23	13(61.9%)	10(52.6%)	
Lines of therapy	2	17	10(47.6%)	7(36.8%)	0.491
	≥2	23	11(52.4%)	12(63.2%)	
TKI-PFS (months)	≤12	23	14(66.7%)	8(42.1%)	0.119
	>12	17	7(33.3%)	11(57.9%)	
T790M mutation	+	13	4(23.5%)	9(50.0%)	0.105
	-	21	13(76.5%)	9(50.0%)	
Smoking status	Yes	13	5(23.8%)	8(42.1%)	0.217
	Never	27	16(76.2%)	11(57.9%)	
Liver mets	No	25	14(66.7%)	11(57.9%)	0.567
	Yes	15	7(33.3%)	8(42.1%)	
PD-L1 TPS	<1%	8	5(50.0%)	3(30.0%)	0.650
	≥1%	12	5(50.0%)	7(70.0%)	

(TKI-PFS, tyrosine kinase inhibitor progression-free survival; PD-L1 TPS, programmed cell death ligand 1 tumor cell proportion score.)

TKI-PFS, HGB, PLR, ALB, and CRP levels were not associated with ORR and DCR (Table 5).

Multivariate cox regression analyses of factors related to OS and PFS in response to second- or post-line ICI combination therapy

Kaplan-Meier survival curves showed that TKI-PFS and NLR were correlated with OS ($P=0.008$, $P<0.001$, respectively) (Fig. 4A and B). Age, sex, number of lines of treatment, history of smoking, liver metastasis, secondary EGFR-T790M mutation, PD-L1 expression, HGB, CRP, ALB, and PLR levels were not significantly correlated with OS (Table 5). In multivariate Cox regression analysis, NLR (HR 11.918; 95% CI 2.941–48.294, $P=0.001$) and TKI-PFS (HR 5.398; 95% CI 1.518–19.2, $P=0.009$) were independent prognostic factors for OS (Table 6).

In terms of PFS, secondary EGFR-T790M mutation, NLR, PLR, and ALB were associated with PFS ($P=0.029$, <0.001, 0.026, and 0.012, respectively) (Fig. 4C and F; Table 5). In multivariate Cox regression analysis, only NLR (HR 6.95; 95% CI 2.068–23.355, $P=0.002$) was an independent prognostic factor for PFS (Table 6).

After screening by multivariate Cox regression, only NLR and TKI-PFS were independent prognostic factors for OS, in the pursuit of seek a more effective and comprehensive indicator, we further combined these two variables to establish the NLR-TKI-PFS score (hereafter referred to as NTP score), where risk factors such as high NLR or long TKI-PFS were each assigned a score of (1) Fourteen (35%) patients had an NTP score of 0, 16 (40%) had an NTP score of 1, and 10 (25%) had an NTP score of (2) The median OS (mOS) of patients with scores of 0, 1, and 2 were 21, 12, and 5.3 months, respectively ($P<0.001$) (Fig. 4G). The median PFS times of patients with scores of 0, 1, and 2 were 12.5, 7, and 4.1 months, respectively ($P=0.001$) (Fig. 4H). The ROC curves were plotted for predicting OS (Fig. 4I). The AUC values for NLR, TKI-PFS, and NTP score were 0.72, 0.698, and 0.747 (P values of 0.001, 0.003, and $P<0.001$, respectively), which indicated that the NTP score had better prognostic value than a single index.

Table 5 Relationship between clinical characteristics and peripheral blood indexes and the outcome of second- or post-line ICI plus chemotherapy

Variables		Total (*n*)	ORR	*P*	DCR	*P*	mOS	*P*	mPFS	*P*
Age (years)	≤60	18	16.7%	0.709	94.4%	0.197	15	0.731	7.0	0.682
	>60	22	22.7%		77.3%		13		10.0	
Gender	Male	17	11.8%	0.428	88.2%	0.686	16	0.496	10.0	0.554
	Female	23	26.1%		82.6%		13		7.6	
Lines of therapy	2	17	17.6%	>0.999	88.2%	0.686	15	0.083	14.3	0.166
	>2	23	21.7%		82.6%		12		6.5	
TKI-PFS (months)	>12	18	11.1%	0.258	77.8%	0.381	11	**0.008***	6.5	0.067
	≤12	22	27.3%		90.9%		21		11.5	
Smoking status	Yes	13	15.4%	0.700	84.6%	1.000	10	0.794	6.5	0.582
	Never	27	22.2%		85.2%		15		10.3	
Liver mets	No	25	20.0%	>0.999	84.0%	1.000	15	0.224	10.0	0.481
	Yes	15	20.0%		86.7%		10		7.0	
T790M mutation	-	25	24.0%	0.686	90.9%	0.166	13	0.361	11.5	**0.029***
	+	15	13.3%		69.2%		15		5.0	
PD-L1 TPS	<1%	8	12.5%	>0.999	75.0%	0.537	10	0.782	4.5	0.966
	≥1%	12	8.3%		91.7%		11		5.7	
NLR	High	22	5.3%	**0.046***	73.7%	0.085	6	**<0.001***	12.5	**<0.001***
	Low	18	33.3%		95.2%		21		5.0	
HGB (g/L)	High	24	20.8%	>0.999	87.5%	0.668	13	0.900	10.0	0.841
	Low	16	18.8%		81.3%		15		6.5	
CRP (mg/L)	High	11	18.2%	>0.999	72.7%	0.288	10	0.236	5.0	0.697
	Low	14	21.4%		92.9%		15		11.5	
ALB (g/L)	High	26	23.1%	0.689	92.3%	0.159	15	0.051	11.5	**0.012***
	Low	14	14.3%		71.4%		12		4.1	
PLR	High	20	10.0%	0.235	80.0%	0.661	10	0.054	5.0	**0.026***
	Low	20	30.0%		90.0%		15		12.0	

(TKI-PFS, tyrosine kinase inhibitor progression-free survival; PD-L1 TPS, programmed cell death ligand 1 tumor cell proportion score; NLR, neutrophil-to-lymphocyte ratio; HGB, hemoglobin; CRP, C-reactive protein; ALB, albumin; PLR, platelet-to-lymphocyte ratio.)

Dynamic changes in NLR during second- or post-line ICI combination therapy

In the second- or post-line immunotherapy cohort, NLR data were collected again at three time points: baseline, RD, and PD. Among these, 15 patients had paired NLR data available at all three time points. NLR decreased at the RD time point compared to the baseline ($P=0.029$). NLR increased again at the PD time point ($P=0.012$), suggesting a substantial reduction in NLR levels during the treatment response phase followed by an elevation during immunotherapy resistance (Fig. 5).

Discussion

Advanced NSCLC can be categorized into two groups based on gene mutation status. For patients without targetable oncogenic driver alterations, ICI plus chemotherapy is now the first-line standard of care recommended in the guidelines. PD-L1 expression estimated from tumor tissue has gained approval from the US Food and Drug Administration as a biomarker for predicting the response to first-line immunotherapy. However, the therapeutic activity of ICI is the result of a complex interplay between multiple immune cells and factors in the TME [51], it is difficult for a single PD-L1 indicator to precisely predict responses to immunotherapy, especially in terms of the efficacy of ICI plus chemotherapy. Therefore, it remains imperative to discover more robust biomarkers for ICI plus chemotherapy.

Research has shown that the inflammatory environment substantially impacts the effectiveness of immunotherapy. Furthermore, peripheral blood inflammatory markers such as NLR and FIB have gained recognition for their relevance in predicting immunotherapy outcomes [25–29, 52]. Neutrophils contain a subpopulation that promotes tumor growth and metastasis, stimulates angiogenesis, and mediates immunosuppression [53]. Inflammatory processes can stimulate the migration of neutrophils to the tumor periphery, where they release reactive oxygen species (ROS), causing DNA damage in adjacent cells. Moreover, neutrophils induce the secretion of various chemokines, which contribute to tumor growth, neovascularization, and the acceleration of distant metastasis. In addition, lymphocytes can differentiate into tumor-infiltrating lymphocytes (TILs), which play an essential role in the host immune response by effectively inhibiting tumor growth, thus contributing

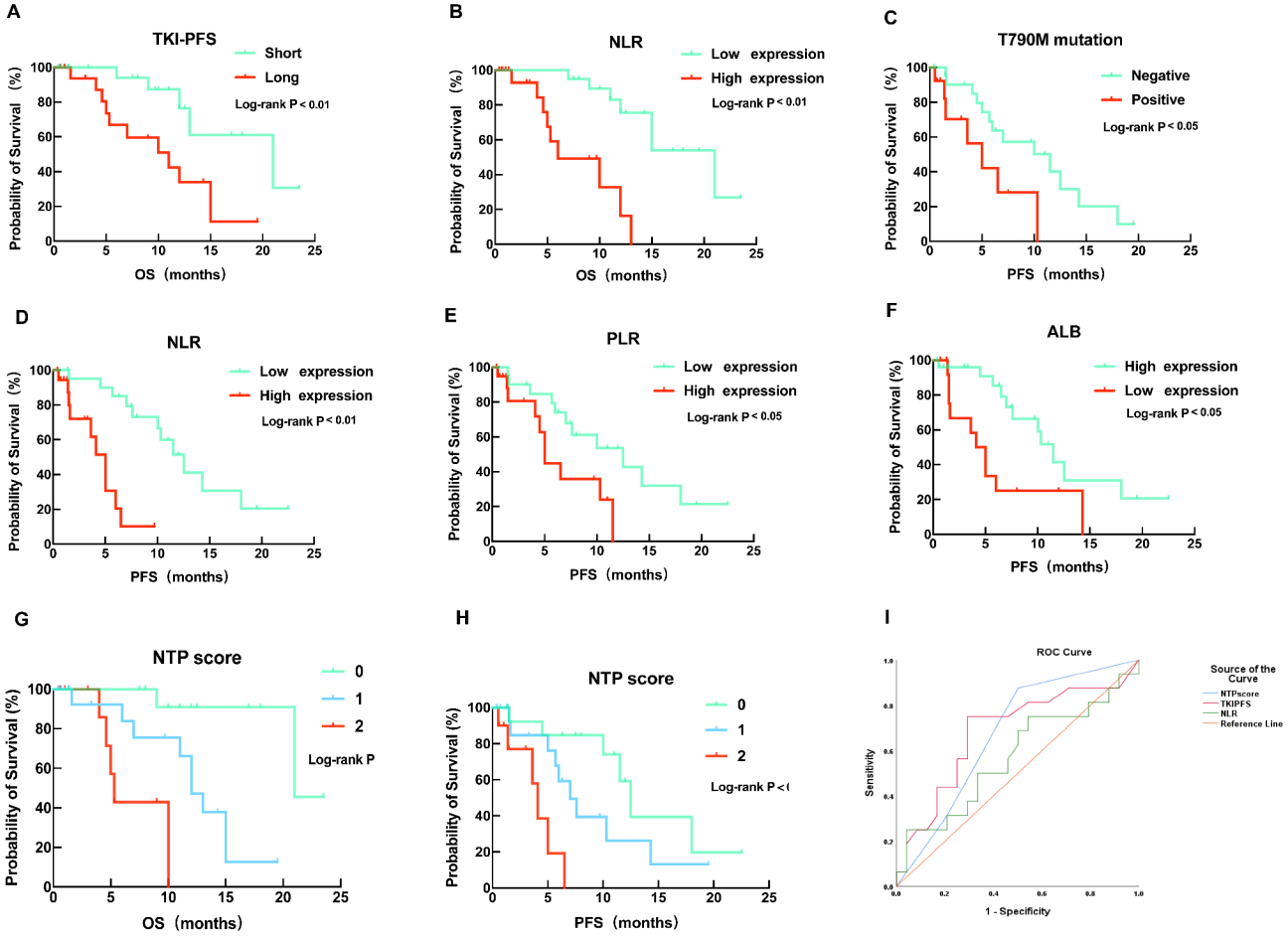

Fig. 4 Kaplan–Meier curves for Overall survival (OS) of second- or post-line ICI combination therapy according to **(A)** TKI-PFS; **(B)** NLR level. Kaplan–Meier curves for PFS according to **(C)** Secondary T790M mutation; **(D)** NLR level; **(E)** PLR level; **(F)** ALB level. **(G)** Kaplan–Meier curves for OS according to NTP score. **(H)** Kaplan–Meier curves for PFS according to NTP score. **(I)** ROC curves of the NLR, TKI-PFS and NTP score to predict OS

Table 6 Multivariate analysis of factors affecting OS and PFS in second- or post-line ICI plus chemotherapy

Prognosis	Variables	Univariate analysis		Multivariate analysis	
		χ^2	*P*	HR	*P*
OS	TKI-PFS (>12 VS ≤ 12 months)	7.077	0.008	5.398	**0.009***
	Lines of treatment (>2 vs. 2)	3.007	0.083		0.116
	NLR (>3.53 VS ≤ 3.53)	16.029	<0.001	11.918	**0.001***
	ALB (<40.25 VS ≥ 40.25 g/L)	3.821	0.051		0.298
	PLR (>196.81 VS ≤ 196.81)	3.711	0.054		0.760
PFS	T790M mutation (+VS -)	4.795	0.029		0.181
	TKI-PFS (>12 VS ≤ 12 months)	3.347	0.067		0.417
	PLR (>196.81 VS ≤ 196.81)	4.982	0.026		0.735
	NLR (>3.53 VS ≤ 3.53)	16.127	<0.001	6.950	**0.002***
	ALB (<40.25 VS ≥ 40.25 g/L)	6.309	0.012		0.058

(TKI-PFS, tyrosine kinase inhibitor progression-free survival; NLR, neutrophil-to-lymphocyte ratio; ALB, albumin, PLR, platelet-to-lymphocyte ratio.)

significantly to anti-tumor immunity. A tumor microenvironment characterized by high neutrophil infiltration and low lymphocyte presence promotes angiogenesis, inhibits apoptosis, ultimately resulting in a poor prognosis. Existing research suggests that the expression of NLR was a strong indicator of survival in NSCLC patients [54] and was associated with poor response to ICI monotherapy in NSCLC patients [55]. Other published study also revealed the potential value of the dynamic change of NLR during treatment as a real-time indicator of ICI monotherapy [56, 57]. However, there are few reports on whether NLR and its dynamic changes can predict the

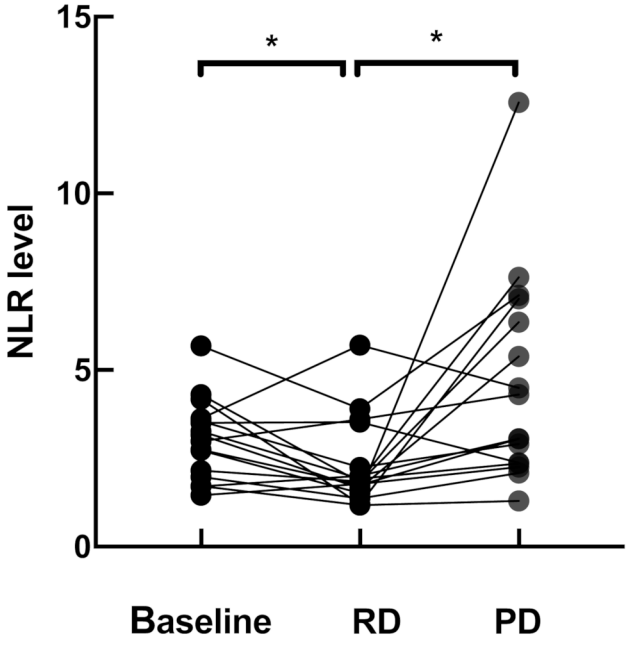

Fig. 5 Dynamic changes in NLR level before (pre) and after (post) second- or post-line ICI combination therapy for patients with paired blood samples

efficacy of ICI plus chemotherapy. One study by Miriam et al. enrolled 12 patients with advanced NSCLC undergoing ICI plus chemotherapy and demonstrated that low NLR can be a predictive marker for therapy responses and outcomes [58]. However, the sample size in that study was relatively small; further research is needed to explore the prognostic value of NLR and its dynamic changes in ICI plus chemotherapy. FIB is an important plasma coagulation factor and a marker of a systemic inflammatory response. FIB can be deposited in the extracellular matrix and serves as a substrate for binding with the growth factors of tumor cells, thereby fostering tumor cell proliferation and neovascularization [59, 60]. Consequently, FIB is considered to have an association with poor prognosis in a variety of tumors [61–64]. Yuan et al. revealed that the pretreatment FIB-ALB ratio (FAR) was an independent predictor for the treatment response and an independent prognostic factor in patients with advanced NSCLC treated with first-line ICI plus chemotherapy [65]. However, the prognostic value of FIB in combination with NLR in advanced NSCLC patients treated with first-line ICI plus chemotherapy is still unknown.

In our study, we found that high pretreatment NLR and FIB levels were independent prognostic factors for poorer PFS in first-line ICI plus chemotherapy. Based on these two independent prognostic factors, we created a unique score called the NF score with three PFS risk prognosis categories: favorable, intermediate, and poor (corresponding mPFS: not reached, 13.5 and 6.5 months). Compared to single indicators that are susceptible to external influences, the more robust NF score demonstrated a better prognostic value. Interestingly, similar studies have not conducted comparisons with tumor PD-L1 expression, which may lead to an underestimation of the relationship between PD-L1 expression and the antitumor effects of ICI plus chemotherapy. Surprisingly, we further found that the NF score detected in blood samples had better performance than PD-L1 estimated from tumor tissue as a reliable efficacy and prognosis biomarkers of first-line ICI plus chemotherapy, with an impressive 79.8% accuracy rate in predicting efficacy (AUC = 0.798), outperforming the value of PD-L1 (AUC = 0.528), and a remarkable 74.7% accuracy rate in predicting prognosis (AUC = 0.747), which was also superior to that of PD-L1 (AUC = 0.54). While PD-L1 expression (TPS ≥ 1%) is a commonly used biomarker for patient selection in immunotherapy, its prognostic value is often limited by variability in its expression and the complex relationship with immune response, and is often not sufficient by itself to predict the outcome of ICI combination therapy. The NF score, which combines NLR and FIB, offers a more dynamic and comprehensive reflection of the systemic inflammatory and coagulation environment, which may be more directly associated with immune modulation and are integral to patient response to ICI plus chemotherapy. Our research suggest that the NF score may serve as a more reliable and specific predictor of ICI plus chemotherapy compared to PD-L1 alone, as it incorporates both immune and inflammatory components that influence response to ICI plus chemotherapy.

In NSCLC patients with targetable oncogenic driver alterations, resistance is inevitably developed after standard targeted therapy. As an emerging antitumor treatment method, the efficacy of immunotherapy in TKI-resistant patients is still a subject of debate. The results of the IMPOWER 150 and ORIENT 31 trials indicated that the PFS of TKI-resistant patients treated with ICI in combination with chemotherapy and bevacizumab are extended in comparison to patients treated with chemotherapy combined with anti-angiogenesis therapy. However, the KEYNOTE 789 (NCT03515837), CHECKMATE 722 (NCT02864251), and IMPOWER 151 trials did not yield positive outcomes. Therefore, the efficacy of ICI combination therapy in NSCLC patients with TKI resistance remains controversial, suggesting that only a subset of patients with unclear TKI resistance mechanisms can benefit from ICI combination therapy. How to utilize existing clinical data and laboratory indicators to select patients who are most likely to benefit from subsequent ICI combination therapy, thereby avoiding unnecessary waste of medical resources, is a challenging issue in clinical practice.

In this study, we further explored the currently challenging issue of whether NSCLC patients with unclear mechanisms after TKI resistance can benefit from second- or post-line ICI combination therapy. Our research results indicated that for TKI-resistant NSCLC patients: [1] ICI combination therapy achieved an ORR of 20%, and a median PFS of 5.85 months; [2] the factor associated with better ORR and prognosis was NLR ≤ 3.53; [3] the factors associated with worse PFS was with secondary T790M mutation, PLR > 196.81 and ALB < 40.25; [4] the factors related to better OS included NLR ≤ 3.53 and TKI-PFS ≤ 12 months. Additionally, based on NLR and TKI-PFS, an NTP score was constructed with three OS risk prognosis categories. Our research innovatively identified a group of low-risk patients (with NLR ≤ 3.53 and TKI-PFS ≤ 12 months), who had longer mOS and mPFS than those in the high-risk group. This was the first study to develop a comprehensive model that incorporates clinical characteristics and easily accessible peripheral blood parameters to predict the prognosis of TKI-resistant NSCLC patients treated with second- or post-line ICI combination therapy. Previous studies have reported that fewer than 60% of patients are willing to undergo invasive re-biopsy after disease progression [66]. Hence, our noninvasive NTP score will be of great importance in providing a reference to screen TKI-resistant NSCLC patients who may benefit from subsequent ICI combination therapy, especially those who are reluctant to undergo re-biopsy following disease progression.

The potential mechanisms underlying the association between TKI-PFS and the benefits of immunotherapy are not yet fully understood. Research by Liu et al. found that patients with shorter TKI-PFS have significantly prolonged PFS and higher ORR in subsequent immune therapy compared to patients with longer TKI-PFS [48]. Their study revealed that patients with shorter TKI-PFS had a higher proportion of CD8 + effector T cells and proliferating T cells; in the longer TKI-PFS cohort, there was a higher ratio of M2-like macrophages to M1-like macrophages. This may lead to a more suppressive tumor microenvironment, potentially impairing the clinical efficacy of immunotherapy. Further basic research is needed to explore these mechanisms in greater depth.

In addition to TKI-PFS, Haratani et al. reported that secondary T790M mutation was negatively correlated with immunotherapy efficacy following EGFR-TKI treatment [67]. In the present study, we also found that patients without secondary EGFR-T790M mutations were more likely to benefit from subsequent ICI combination therapy in terms of PFS than patients with secondary EGFR-T790M mutations. Our research results support the conclusions of Haratani et al., indicating that for patients with secondary EGFR-T790M mutations, subsequent ICI combination therapy may not be the optimal treatment choice.

Cheng et al. found that for EGFR-TKI-resistant patients with NSCLC, liver metastasis is a negative prognostic factor for subsequent ICI combination therapy [68]. In this study, we did not find a relationship between liver metastasis and the effectiveness of subsequent ICI combination therapy. Notably, however, we found that NLR can serve as a prognostic marker for TKI-resistant patients receiving subsequent ICI combination therapy. To our knowledge, previous findings have demonstrated that NLR is a promising biomarker to predict the survival of ICI in advanced NSCLC patients without targetable genomic alterations. However, patients with NSCLC carrying targetable genomic alterations have a unique TME [69, 70], and whether NLR has prognostic value for ICI combination therapy in NSCLC patients with sensitive driver gene mutations remains unclear. Our research elucidates the value of NLR in patients harboring sensitive gene mutations who are undergoing ICI combination therapy. We further integrated NLR and TKI-PFS; the established composite biomarker was more robust, further enhancing the predictive efficacy. Our study can serve as a reference for the selection of TKI-resistant NSCLC patients who may benefit from ICI plus chemotherapy.

Peripheral blood inflammatory biomarkers have shown great promise in predicting responses to ICI therapies due to easy specimen accessibility and the opportunity for serial monitoring. In the two cohorts in our study, it is noteworthy that with an enhanced immune response or ongoing tumor progression, the peripheral blood parameters of NLR also exhibited a corresponding decline or increase. Our study underscores the continued effectiveness of NLR as a reliable indicator of the body's antitumor immune status over the course of treatment. Moreover, by closely tracking NLR fluctuations before and after treatment, clinicians can gain valuable insights to assess treatment efficacy and predict patient outcomes.

It is important to acknowledge the limitations of this study. This study was a retrospective analysis with a relatively small sample size from a single Chinese cancer institution, which may have resulted in potential objective bias for data collection and analysis. Also, there is currently no unified standard for the critical values of indicators like NLR. Moreover, this study lacks external data to verify the prognostic model. Therefore, future research should focus on establish unified critical thresholds for NLR and other inflammatory indices through large-scale prospective clinical trials, and should aim to conduct multicenter, large-sample, long-term follow-up clinical trials to validate and assess the prognostic ability of the NF and NTP scores. Moreover, future studies should investigate the molecular mechanisms linking

NLR fluctuations to immune modulation and treatment resistance in NSCLC.

Conclusions

The NF score we established has the advantages of being noninvasive, easy to access and monitor, economical, and can discriminate potential patients benefit from ICI combined with chemotherapy, while PD-L1 underperformed in predicting response to combination immunotherapy. More importantly, the noninvasive NTP score constructed in this study may provide new insights for screening TKI-resistant NSCLC patients who may benefit from subsequent ICI combination therapy, especially those who are reluctant to undergo re-biopsy following disease progression.

Future perspectives

Future research should focus on establish unified critical thresholds for NLR and other inflammatory indices through multicenter collaborations to enhance reproducibility and clinical applicability, and conduct multicenter, prospective trials with diverse NSCLC cohorts to validate the generalizability of the NF and NTP scores.

Abbreviations

NSCLC	Non-small cell lung cancer
ICI	Immune checkpoint inhibitor
PD-L1 TPS	Programmed cell death ligand 1 tumor cell proportion score
NLR	Neutrophil-to-lymphocyte ratio
FIB	Fibrinogen
PFS	Progression-free survival
OS	Overall survival
ORR	Objective response rate
DCR	Disease control rate
EGFR	Epidermal growth factor receptor
TKI	Tyrosine kinase inhibitor
NF score	NLR-FIB score
NTP score	NLR-TKI-PFS score
TME	Tumor microenvironment
TMB	Tumor mutation burden
LDH	Lactate dehydrogenase
HGB	Hemoglobin
CRP	C-reactive protein
ALB	Albumin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13811-y.

Supplementary Material 1

Acknowledgements

The author would like to thank all the patients involved in this study and to all of the research staff who participated in this study.

Author contributions

Yuanyuan Wang and Wangjun Liao were responsible for the conception and design of this study. Shan Liao conducted research and performed statistical analysis. Huiying Sun, Hao Lu, Jiani Wu, Jianhua Wu, Zhe Wu and Jingle Xi collected and analyzed the data and provided critical input. Shan Liao interpreted the data. Shan Liao wrote the manuscript. All authors reviewed the manuscript and approved the final version of manuscript.

Funding

This work was supported by the Wu Jieping Medical Foundation (Grant Number: 320.6750.2022-22-21) and the Chinese Thoracic Oncology Group Lung Cancer Research Foundation (Grant Number: CTONG-YC20220107).

Data availability

The datasets used and analysed during the current study available from the corresponding author (Yuanyuan Wang: doc_wang@126.com) on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Nanfang Hospital (NFEC-BPE-098). This is an observational and retrospective study that has obtained exemption from informed consent from the Ethics Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Oncology, Nanfang Hospital, Southern Medical University, 1838 North Guangzhou Avenue, Guangzhou 510515, Guangdong, China
[2]Cancer Center, the Sixth Affiliated Hospital, School of Medicine, South China University of Technology, Foshan, China

Received: 21 October 2024 / Accepted: 25 February 2025
Published online: 15 April 2025

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–86. https://doi.org/10.1002/ijc.29210.
2. Pardoll DM. The Blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64. https://doi.org/10.1038/nrc3239.
3. Elanany MM, Mostafa D, Hamdy NM. Remodeled tumor immune microenvironment (TIME) parade via natural killer cells reprogramming in breast cancer. Life Sci. 2023;330:121997. https://doi.org/10.1016/j.lfs.2023.121997.
4. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic Non-Small-Cell lung Cancer. N Engl J Med. 2018;378:2078–92. https://doi.org/10.1056/NEJMoa1801005.
5. Garon EB, Hellmann MD, Rizvi NA, et al. Five-Year overall survival for patients with advanced Non–Small-Cell lung Cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study. J Clin Oncol. 2019;37:2518–27. https://doi.org/10.1200/jco.19.00934.
6. Garassino MC, Gadgeel S, Speranza G, et al. Pembrolizumab plus pemetrexed and platinum in nonsquamous Non-Small-Cell lung cancer: 5-Year outcomes from the phase 3 KEYNOTE-189 study. J Clin Oncol. 2023;41:1992–8. https://doi.org/10.1200/jco.22.01989.
7. Xu Y, Wan B, Chen X, et al. The association of PD-L1 expression with the efficacy of anti-PD-1/PD-L1 immunotherapy and survival of non-small cell lung cancer patients: a meta-analysis of randomized controlled trials. Transl Lung Cancer Res. 2019;8:413–28. https://doi.org/10.21037/tlcr.2019.08.09.
8. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-Positive Non-Small-Cell lung Cancer. N Engl J Med. 2016;375:1823–33. https://doi.org/10.1056/NEJMoa1606774.
9. Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393:1819–30. https://doi.org/10.1016/s0140-6736(18)32409-7.
10. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic Non-Small-Cell lung Cancer. N Engl J Med. 2018;378:2078–92. https://doi.org/10.1056/NEJMoa1801005.

11. Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus chemotherapy for squamous Non-Small-Cell lung Cancer. N Engl J Med. 2018;379:2040–51. https://doi.org/10.1056/NEJMoa1810865.
12. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 Inhibition. N Engl J Med. 2017;377:2500–1. https://doi.org/10.1056/NEJMc1713444.
13. Carbone DP, Reck M, Paz-Ares L, et al. First-Line nivolumab in stage IV or recurrent Non-Small-Cell lung Cancer. N Engl J Med. 2017;376:2415–26. https://doi.org/10.1056/NEJMoa1613493.
14. Klein O, Kee D, Markman B, et al. Evaluation of TMB as a predictive biomarker in patients with solid cancers treated with anti-PD-1/CTLA-4 combination immunotherapy. Cancer Cell. 2021;39:592–3. https://doi.org/10.1016/j.ccell.2021.04.005.
15. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541:321–30. https://doi.org/10.1038/nature21349.
16. Hashemi S, Fransen MF, Niemeijer A, et al. Surprising impact of stromal TIL's on immunotherapy efficacy in a real-world lung cancer study. Lung Cancer. 2021;153:81–9. https://doi.org/10.1016/j.lungcan.2021.01.013.
17. Shi Y, Lei Y, Liu L, et al. Integration of comprehensive genomic profiling, tumor mutational burden, and PD-L1 expression to identify novel biomarkers of immunotherapy in non-small cell lung cancer. Cancer Med. 2021;10:2216–31. https://doi.org/10.1002/cam4.3649.
18. Hammad R, Eldosoky MA, Elmadbouly AA, et al. Monocytes subsets altered distribution and dysregulated plasma hsa-miR-21-5p and hsa-miR-155-5p in HCV-linked liver cirrhosis progression to hepatocellular carcinoma. J Cancer Res Clin Oncol. 2023;149:15349–64. https://doi.org/10.1007/s00432-023-05313-w.
19. Bar J, Leibowitz R, Reinmuth N, et al. Biological insights from plasma proteomics of non-small cell lung cancer patients treated with immunotherapy. Front Immunol. 2024;15:1364473. https://doi.org/10.3389/fimmu.2024.1364473.
20. Eissa S, Swellam M, el-Mosallamy H, et al. Diagnostic value of urinary molecular markers in bladder cancer. Anticancer Res. 2003;23:4347–55.
21. Hammad R, Aglan RB, Mohammed SA, et al. Cytotoxic T cell expression of Leukocyte-Associated Immunoglobulin-Like Receptor-1 (LAIR-1) in viral hepatitis C-Mediated hepatocellular carcinoma. Int J Mol Sci. 2022;23. https://doi.org/10.3390/ijms232012541.
22. El-Mesallamy HO, Hamdy NM, Rizk HH, et al. Apelin serum level in Egyptian patients with chronic hepatitis C. Mediators Inflamm. 2011;2011:703031. https://doi.org/10.1155/2011/703031.
23. Bodor JN, Boumber Y, Borghaei H. Biomarkers for immune checkpoint Inhibition in non-small cell lung cancer (NSCLC). Cancer. 2020;126:260–70. https://doi.org/10.1002/cncr.32468.
24. Mantovani A. The inflammation - cancer connection. Febs J. 2018;285:638–40. https://doi.org/10.1111/febs.14395.
25. Diem S, Schmid S, Krapf M, et al. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Lung Cancer. 2017;111:176–81. https://doi.org/10.1016/j.lungcan.2017.07.024.
26. Liu J, Li S, Zhang S, et al. Systemic immune-inflammation index, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio can predict clinical outcomes in patients with metastatic non-small-cell lung cancer treated with nivolumab. J Clin Lab Anal. 2019;33:e22964. https://doi.org/10.1002/jcla.22964.
27. Li Y, Zhang Z, Hu Y, et al. Pretreatment Neutrophil-to-Lymphocyte ratio (NLR) May predict the outcomes of advanced Non-small-cell lung Cancer (NSCLC) patients treated with immune checkpoint inhibitors (ICIs). Front Oncol. 2020;10:654. https://doi.org/10.3389/fonc.2020.00654.
28. Chen Y, Wen S, Xia J, et al. Association of dynamic changes in peripheral blood indexes with response to PD-1 Inhibitor-Based combination therapy and survival among patients with advanced Non-Small cell lung Cancer. Front Immunol. 2021;12:672271. https://doi.org/10.3389/fimmu.2021.672271.
29. Mezquita L, Auclin E, Ferrara R, et al. Association of the lung immune prognostic index with immune checkpoint inhibitor outcomes in patients with advanced Non-Small cell lung Cancer. JAMA Oncol. 2018;4:351–7. https://doi.org/10.1001/jamaoncol.2017.4771.
30. Wang W, Huang Z, Yu Z, et al. Prognostic value of the lung immune prognostic index May differ in patients treated with immune checkpoint inhibitor monotherapy or combined with chemotherapy for Non-small cell lung Cancer. Front Oncol. 2020;10:572853. https://doi.org/10.3389/fonc.2020.572853.
31. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012;13:239–46. https://doi.org/10.1016/s1470-2045(11)70393-x.
32. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of Afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 2013;31:3327–34. https://doi.org/10.1200/jco.2012.44.2806.
33. Goss G, Tsai CM, Shepherd FA, et al. Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol. 2016;17:1643–52. https://doi.org/10.1016/s1470-2045(16)30508-3.
34. Passaro A, Jänne PA, Mok T, et al. Overcoming therapy resistance in EGFR-mutant lung cancer. Nat Cancer. 2021;2:377–91. https://doi.org/10.1038/s43018-021-00195-8.
35. Dong L, Lei D, Zhang H. Clinical strategies for acquired epidermal growth factor receptor tyrosine kinase inhibitor resistance in non-small-cell lung cancer patients. Oncotarget. 2017;8:64600–6. https://doi.org/10.18632/oncotarget.19925.
36. Liao X, He T, Wan X, et al. Clinical management in NSCLC patients with EGFR mutation after osimertinib progression with unknown resistance mechanisms. Clin Respir J. 2024;18:e70025. https://doi.org/10.1111/crj.70025.
37. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced Squamous-Cell Non-Small-Cell lung Cancer. N Engl J Med. 2015;373:123–35. https://doi.org/10.1056/NEJMoa1504627.
38. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous Non-Small-Cell lung Cancer. N Engl J Med. 2015;373:1627–39. https://doi.org/10.1056/NEJMoa1507643.
39. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387:1540–50. https://doi.org/10.1016/s0140-6736(15)01281-7.
40. Piper-Vallillo AJ, Sequist LV, Piotrowska Z. Emerging treatment paradigms for EGFR-Mutant lung cancers progressing on osimertinib: A review. J Clin Oncol. 2020;Jco1903123. https://doi.org/10.1200/jco.19.03123.
41. Fang Y, Wang Y, Zeng D, et al. Comprehensive analyses reveal TKI-induced remodeling of the tumor immune microenvironment in EGFR/ALK-positive non-small-cell lung cancer. Oncoimmunology. 2021;10:1951019. https://doi.org/10.1080/2162402x.2021.1951019.
42. Champiat S, Ileana E, Giaccone G, et al. Incorporating immune-checkpoint inhibitors into systemic therapy of NSCLC. J Thorac Oncol. 2014;9:144–53. https://doi.org/10.1097/jto.0000000000000074.
43. Jiang T, Wang P, Zhang J, et al. Toripalimab plus chemotherapy as second-line treatment in previously EGFR-TKI treated patients with EGFR-mutant-advanced NSCLC: a multicenter phase-II trial. Signal Transduct Target Ther. 2021;6:355. https://doi.org/10.1038/s41392-021-00751-9.
44. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-Mutated Non-Small cell lung Cancer-A Meta-Analysis. J Thorac Oncol. 2017;12:403–7. https://doi.org/10.1016/j.jtho.2016.10.007.
45. Lu S, Wu L, Jian H, et al. Sintilimab plus bevacizumab biosimilar IBI305 and chemotherapy for patients with EGFR-mutated non-squamous non-small-cell lung cancer who progressed on EGFR tyrosine-kinase inhibitor therapy (ORIENT-31): first interim results from a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol. 2022;23:1167–79. https://doi.org/10.1016/s1470-2045(22)00382-5.
46. Reck M, Mok TSK, Nishio M, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. Lancet Respir Med. 2019;7:387–401. https://doi.org/10.1016/s2213-2600(19)30084-0.
47. Cortinovis D, Gregorc V, Migliorino MR, et al. New perspectives in the second-line treatment of Non squamous NSCLC patients: results from a large Italian lung Cancer working group. Crit Rev Oncol Hematol. 2017;109:35–41. https://doi.org/10.1016/j.critrevonc.2016.11.015.
48. Liu S, Wu F, Li X, et al. Patients with short PFS to EGFR-TKIs predicted better response to subsequent Anti-PD-1/PD-L1 based immunotherapy in EGFR common mutation NSCLC. Front Oncol. 2021;11:639947. https://doi.org/10.3389/fonc.2021.639947.
49. Masuda K, Horinouchi H, Tanaka M, et al. Efficacy of anti-PD-1 antibodies in NSCLC patients with an EGFR mutation and high PD-L1 expression. J Cancer

Res Clin Oncol. 2021;147:245–51. https://doi.org/10.1007/s00432-020-03329-0.
50. Zhou J, Chu X, Zhao J, et al. Full spectrum flow cytometry-powered comprehensive analysis of PBMC as biomarkers for immunotherapy in NSCLC with EGFR-TKI resistance. Biol Proced Online. 2023;25:21. https://doi.org/10.1186/s12575-023-00215-0.
51. Genova C, Dellepiane C, Carrega P, et al. Therapeutic implications of tumor microenvironment in lung cancer: focus on immune checkpoint Blockade. Front Immunol. 2021;12:799455. https://doi.org/10.3389/fimmu.2021.799455.
52. Wang GX, Huang ZN, Ye YQ, et al. Prognostic analysis of the plasma fibrinogen combined with neutrophil-to-lymphocyte ratio in patients with non-small cell lung cancer after radical resection. Thorac Cancer. 2023;14:1383–91. https://doi.org/10.1111/1759-7714.14883.
53. Jaillon S, Ponzetta A, Di Mitri D, et al. Neutrophil diversity and plasticity in tumour progression and therapy. Nat Rev Cancer. 2020;20:485–503. https://doi.org/10.1038/s41568-020-0281-y.
54. Yap WK, Shih MC, Kuo C, et al. Development and validation of a nomogram for assessing survival in patients with metastatic lung Cancer referred for radiotherapy for bone metastases. JAMA Netw Open. 2018;1:e183242. https://doi.org/10.1001/jamanetworkopen.2018.3242.
55. Bagley SJ, Kothari S, Aggarwal C, et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. Lung Cancer. 2017;106:1–7. https://doi.org/10.1016/j.lungcan.2017.01.013.
56. Passiglia F, Galvano A, Castiglia M, et al. Monitoring blood biomarkers to predict nivolumab effectiveness in NSCLC patients. Ther Adv Med Oncol. 2019;11:1758835919839928. https://doi.org/10.1177/1758835919839928.
57. Hwang M, Canzoniero JV, Rosner S, et al. Peripheral blood immune cell dynamics reflect antitumor immune responses and predict clinical response to immunotherapy. J Immunother Cancer. 2022;10. https://doi.org/10.1136/jitc-2022-004688.
58. Möller M, Schütte W, Turzer S, et al. Blood immune cells as biomarkers in Long-Term surviving patients with advanced Non-Small-Cell lung Cancer undergoing a combined immune/Chemotherapy. Cancers (Basel). 2023;15. https://doi.org/10.3390/cancers15194873.
59. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. Blood Rev. 2015;29:17–24. https://doi.org/10.1016/j.blre.2014.09.003.
60. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. Blood. 2019;133:511–20. https://doi.org/10.1182/blood-2018-07-818211.
61. Wen J, Yang Y, Ye F, et al. The preoperative plasma fibrinogen level is an independent prognostic factor for overall survival of breast cancer patients who underwent surgical treatment. Breast. 2015;24:745–50. https://doi.org/10.1016/j.breast.2015.09.007.
62. Jiang HG, Li J, Shi SB, et al. Value of fibrinogen and D-dimer in predicting recurrence and metastasis after radical surgery for non-small cell lung cancer. Med Oncol. 2014;31:22. https://doi.org/10.1007/s12032-014-0022-8.
63. Kim KH, Park TY, Lee JY, et al. Prognostic significance of initial platelet counts and fibrinogen level in advanced non-small cell lung cancer. J Korean Med Sci. 2014;29:507–11. https://doi.org/10.3346/jkms.2014.29.4.507.
64. Zhu JF, Cai L, Zhang XW, et al. High plasma fibrinogen concentration and platelet count unfavorably impact survival in non-small cell lung cancer patients with brain metastases. Chin J Cancer. 2014;33:96–104. https://doi.org/10.5732/cjc.012.10307.
65. Yuan C, Huang M, Wang H, et al. Pretreatment Fibrinogen-Albumin ratio (FAR) associated with treatment response and survival in advanced Non-Small cell lung Cancer patients treated with First-Line Anti-PD-1 therapy plus Platinum-Based combination chemotherapy. Cancer Manag Res. 2022;14:377–86. https://doi.org/10.2147/cmar.S347547.
66. Fukui T, Ishihara M, Kasajima M, et al. Questionnaire survey on patient awareness of invasive rebiopsy in advanced non-small cell lung cancer. Thorac Cancer. 2019;10:501–7. https://doi.org/10.1111/1759-7714.12964.
67. Haratani K, Hayashi H, Tanaka T, et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. Ann Oncol. 2017;28:1532–9. https://doi.org/10.1093/annonc/mdx183.
68. Cheng Y, Yang B, Ouyang W, et al. Is ICI-based therapy better than chemotherapy for metastatic NSCLC patients who develop EGFR-TKI resistance? A real-world investigation. Front Oncol. 2022;12:920047. https://doi.org/10.3389/fonc.2022.920047.
69. Dong ZY, Zhang JT, Liu SY, et al. EGFR mutation correlates with uninflamed phenotype and weak immunogenicity, causing impaired response to PD-1 Blockade in non-small cell lung cancer. Oncoimmunology. 2017;6:e1356145. https://doi.org/10.1080/2162402x.2017.1356145.
70. Gettinger SN, Choi J, Mani N, et al. A dormant TIL phenotype defines non-small cell lung carcinomas sensitive to immune checkpoint blockers. Nat Commun. 2018;9:3196. https://doi.org/10.1038/s41467-018-05032-8.

Publisher's note