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RESEARCH

Clinical and dynamic circulating cytokines profile features of long-term progression-free survival benefit to immune checkpoint inhibitors in advanced non–small cell lung cancer

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Abstract

Background Immune checkpoint inhibitors (ICIs) offer durable progression-free survival (PFS) benefit in a subset of patients with advanced non-small cell lung cancer (NSCLC). However, the predictors of long-term PFS (LTPFS) remain unclear.

Methods Advanced NSCLC patients receiving first-line ICIs monotherapy at Guangdong Lung Cancer Institute between December 2017 and August 2022 were identified. Predictive value of different characteristics was evaluated in LTPFS (PFS ≥ 24 months) compared with short-term PFS (STPFS, PFS ≤ 3 months). Circulating cytokine levels were evaluated in paired peripheral blood samples collected before and after ICIs treatment.

Results Among 202 patients identified and 171 included (median follow-up: 41.0 months), 44 (25.7%) experienced LTPFS, associated with a 5-year overall survival (OS) rate of 81.2%. Squamous NSCLC, intermediate or poor lung immune prognostic index (LIPI) score, and liver metastases, were negatively associated with LTPFS. High tumor mutational burden (TMB, ≥ 10 mutations/megabase) was enriched in LTPFS compared to STPFS ($P = 0.002$). Patients with both high TMB and PD-L1 demonstrated the greatest survival benefit from first-line ICIs monotherapy (median PFS: 24.5 months, median OS: 67.0 months). Thirty-eight peripheral blood samples were collected before and after ICIs treatment from 10 patients with LTPFS and 9 with STPFS, which revealed increased CCL11 ($P = 0.013$) and decreased IL1RA ($P = 0.001$) and IL17A ($P = 0.003$) levels in LTPFS after ICIs treatment.

Conclusion Distinct clinical characteristics, including TMB, PD-L1, pathologic subtypes, LIPI score, number of organs involved, metastatic sites, and dynamic circulating cytokines profile features, can distinguish NSCLC patients achieving LTPFS from those with STPFS following first-line ICIs monotherapy.

Keywords Immunotherapy · Non-small cell lung cancer · Predictive · Cytokine · Survival · Long-term

Introduction

Immune checkpoint inhibitors (ICIs) targeting programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) have become the cornerstone of treatment for advanced non-small cell lung cancer (NSCLC) since their approval. Current guidelines recommend first-line ICIs, either as monotherapy or in combination with chemotherapy, based on PD-L1 expression levels in patients without druggable oncogenes [1]. Phase III trials have demonstrated significant survival benefits of first-line pembrolizumab monotherapy compared with platinum-based chemotherapy in PD-L1-positive advanced NSCLC without druggable genomic alterations, particularly for patients with a PD-L1 tumor proportion score (TPS) ≥ 50% [2, 3].

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Recently, KEYNOTE-024 and KEYNOTE-042 studies reported 5-year overall survival (OS) rates of 31.9% and 21.9%, respectively, for patients with high PD-L1 expression, highlighting the potential for long-term survival and even curability in advanced NSCLC [2, 3]. These durable responses underscore a unique advantage of ICIs-based treatments. However, the majority of patients either fail to respond to ICIs or develop resistance within 12 to 24 months of initial disease control [4]. Furthermore, a subgroup of patients experiences early disease progression or death within the first 3 to 6 months of chemotherapy-free immunotherapy strategies [5, 6]. Identifying clinical and biological markers predictive of long-term ICIs benefits is critical for selecting patients who may achieve durable responses without requiring intensified upfront treatments, such as chemotherapy.

Currently, no biomarkers other than PD-L1 have been established to guide immunotherapy strategies for NSCLC in China [7]. While patients with PD-L1 TPS ≥ 50% are more likely to benefit from ICIs monotherapy without combination chemotherapy, the role of PD-L1 in predicting long-term survival remains controversial across studies [8, 9]. In clinical practice, clinicians often rely on comprehensive assessments of clinical characteristics, such as age, performance status, pathological type, and baseline tumor size (BTS) [10]. Additional biomarkers, including tumor mutational burden (TMB), tumor-infiltrating lymphocytes, circulating cytokine signatures, neutrophil–lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), lung immune prognostic index (LIPI) score, circulating tumor DNA, and the microbiome, require further investigation before clinical application [10–13].

The characteristics that predict long-term ICIs benefits are still not fully determined, mainly due to the general rarity of long-term responders and the long follow-up required for identification. More data about the frequency, predictive clinical features, and long-term outcomes of long-term ICIs benefits are needed. In this study, we characterize the clinical factors of patients according to the progression-free survival (PFS) following first-line ICIs monotherapy in a long follow-up cohort. Additionally, we explored dynamic circulating cytokine profiles before and after ICIs treatment to identify features of NSCLC patients who may derive sustained benefits from ICIs.

Methods and materials

Study design

The clinical analysis cohort comprised consecutive NSCLC patients who received first-line ICIs monotherapy at Guangdong Lung Cancer Institute between December 2017 and August 2022, which were identified from an electronic database. No specific ICIs molecules were specified as inclusion criteria in this study; all frontline patients treated with various ICIs were included. The analysis of specific drugs administered and their proportions was conducted post-enrollment. The circulating cytokines analysis cohort prospectively included advanced NSCLC patients receiving ICIs between October 2020 and November 2021. Patients without available and paired peripheral blood samples obtained before and at 6 weeks after the initiation of ICIs were excluded. As of September 30, 2024, the median follow-up time for the clinical analysis cohort and the circulating cytokines analysis cohort were 37.1 months and 35.7 months, respectively.

Efficacy evaluation and grouping

Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 was used to assess efficacy to ICIs. PFS was defined as the time from the first ICIs prescription date to the first documented disease progression or death due to any cause. OS was defined as the time from the first ICIs prescription date to either the date of death or the final follow-up date. The data of patients who survived were censored.

According to the results of previous studies, long-term PFS (LTPFS) was defined as PFS ≥ 24 months, as patients without progressive disease (PD) at 24 months after ICIs treatment were likely to remain progression-free at 5 years and survive for more than 5 years, which was identified as long-term ICIs benefits population [8, 9]. Short-term PFS (STPFS) was defined as PFS ≤ 3 months, which included those experienced early death (deaths within the first 3 months) or early progression (PD within 3 months) to ICIs [6, 14]. At the last follow-up, patients in the clinical analysis cohort who did not reach the PFS endpoints and had follow-up durations of less than 2 years were excluded from subsequent grouping analysis. The remaining patients were divided into LTPFS, non-LTPFS/non-STPFS (defined as 3 < PFS < 24 months), and STPFS groups for further analysis (Fig. 1).The circulating cytokines analysis cohort only included those with LTPFS or STPFS.

Clinical data collection

Pre-treatment clinical characteristics data, including age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG PS), smoking history, pathology, PD-L1 expression, TMB, blood cell count (absolute neutrophil count, absolute lymphocyte count, absolute platelet count, and white blood cell concentration), lactate dehydrogenase (LDH) level, driver alterations, and sites of disease at diagnosis, were retrieved from the electronic medical records. Additional data included the

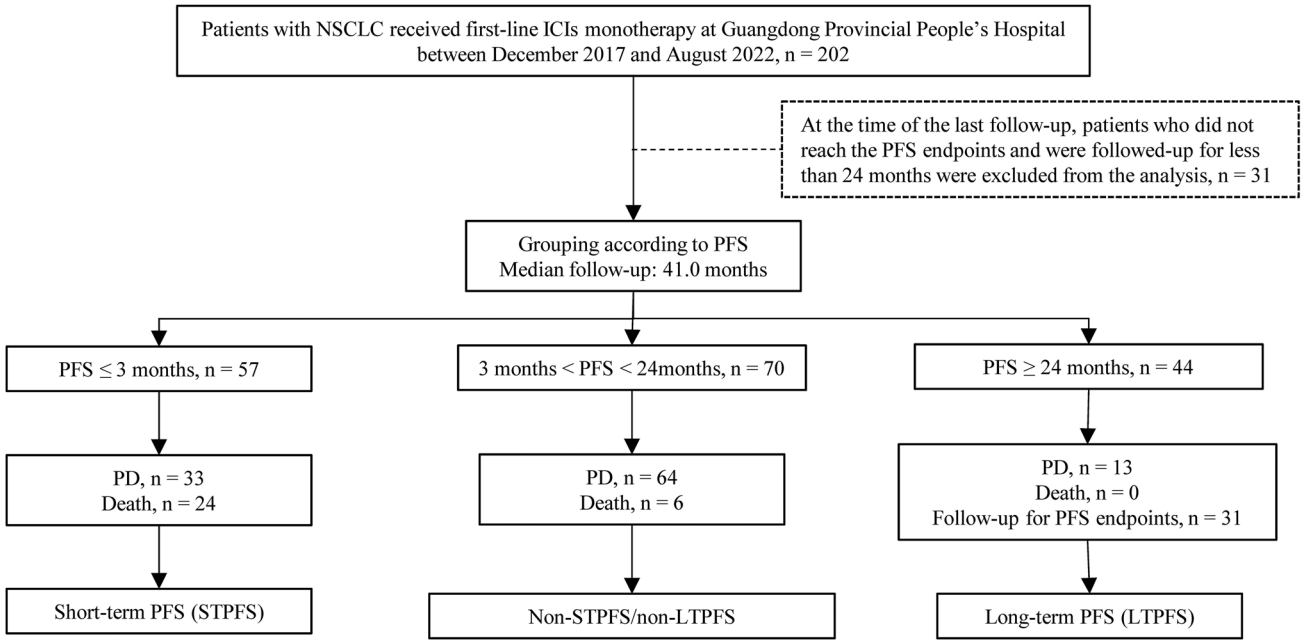

Fig. 1 Flow diagram and grouping of the clinical analysis cohort. NSCLC: non-small cell lung cancer; PFS: progression-free survival; PD: progressive disease

best response and the incidence of adverse events (AEs) during the treatment. Driver-negative status was defined as the absence of *EGFR*, *ALK*, *ROS1*, *RET*, *MET*, *BRAF*, *KRAS*, *HER2*, and *NTRK* alterations [15]. BTS was assessed using RECIST 1.1 and calculated as the max BTS (longest diameter of target lesions) and total BTS (sum of target lesion diameters) [10]. NLR was defined by [absolute neutrophil count]/[absolute lymphocyte count]. PLR was defined by [absolute platelet count]/[absolute lymphocyte count]. LIPI score was calculated from LDH and derived NLR (dNLR; [absolute neutrophil count]/[white blood cell concentration—absolute neutrophil count]) values [16].

Analyses of circulating cytokines

Peripheral blood samples were collected before and at 6 weeks after the initiation of therapy based on the first time of efficacy assessment in clinical trials and previous studies [17]. All samples were heparinized and centrifuged at 3000 rpm for 5 min within 30 min of collection. The plasma supernatants were transferred to new tubes and stored at − 80 °C immediately until measurement. For this assay, the circulating cytokines test was conducted using Luminex Human Magnetic Assay Kit (R&D Systems, Minneapolis, USA) at the KingMed Diagnostics Laboratories (Guangzhou, China) in January 2022.

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) software (version 23) and GraphPad Prism software (Version 8). The Kaplan–Meier method was used to analyze the survival probability, and the log-rank test was used to calculate the significance of differences. For normally distributed data, differences between two groups were analyzed using the t-test, while the Mann–Whitney U test was applied for non-normally distributed data. Categorical variables were compared using the chi-square test or Fisher's exact test.

Logistic regression was performed for the univariable and multivariable analyses to calculate the odds ratio (OR) and 95% confidence interval (95% CI). Pre-treatment characteristic associated with LTPFS based on the univariate analyses were considered for the multivariate analysis. Variables with more than 10% missing data (like TMB) were excluded from the multivariate analysis. Only cases with complete data for all collected variables are analyzed in the multivariate analysis [18]. For circulating cytokine measurements below the lower limit of detection (LLOD), values were replaced with LLOD/$\sqrt{2}$ [19, 20]. All reported P values are two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

The pre-treatment characteristics of the study participants are summarized in Table 1. A total of 202 patients with advanced NSCLC receiving first-line ICIs were identified in the clinical analysis cohort, with 171 patients included for grouping. All patients received anti-PD-1 inhibitors alone, including pembrolizumab (200 mg i.v. every 3 weeks), sintilimab (200 mg i.v. every 3 weeks), and nivolumab (3 mg/kg i.v. every 2 weeks) (Supplementary

Table 1 Patient characteristics

Characteristics	STPFS ($n=57$)	non-LTPFS/non-STPFS ($n=70$)	LTPFS ($n=44$)	Grouping population ($n=171$)	Total population ($n=202$)
Age, median (range)	65 (24, 86)	66 (39, 82)	66 (38, 82)	66 (24, 86)	66 (24, 86)
Gender, No. (%)					
Female	7 (12.3)	12 (17.1)	3 (6.8)	22 (12.9)	24 (11.9)
Male	50 (87.8)	58 (82.9)	41 (93.2)	149 (87.1)	178 (88.1)
Smoking history, No. (%)					
Current/ Former	42 (73.7)	48 (68.6)	35 (79.5)	125 (73.1)	144 (71.3)
Never	15 (26.3)	22 (31.4)	9 (20.5)	46 (26.9)	58 (28.7)
ECOG PS, No. (%)					
0–1	44 (77.2)	69 (98.6)	42 (95.5)	155 (90.6)	184 (91.1)
≥2	13 (22.8)	1 (1.4)	2 (4.5)	16 (9.4)	18 (8.9)
Histology, No. (%)					
Adenocarcinoma	25 (43.9)	43 (61.4)	29 (65.9)	97 (56.7)	120 (59.4)
Squamous	27 (47.4)	22 (31.4)	11 (25.0)	60 (35.1)	68 (33.7)
others	5 (8.7)	5 (7.2)	4 (9.1)	14 (8.2)	14 (6.9)
Brain metastases					
With	14 (24.6)	15 (21.4)	11 (25.0)	40 (23.4)	47 (23.3)
Without	43 (75.4)	55 (78.6)	33 (75.0)	131 (76.6)	155 (76.7)
Liver metastases					
With	19 (33.3)	8 (11.4)	1 (2.3)	28 (16.4)	29 (14.4)
Without	38 (66.7)	62 (88.6)	43 (97.7)	143 (83.6)	173 (85.6)
Total BTS, median (range)	85.0 (12.0, 236.0)	69.0 (12.0, 249.0)	61.0 (12.0, 182.0)	72.0 (12.0, 249.0)	71.0 (12.0, 249.0)
Max BTS, median (range)	52.0 (12.0, 114.0)	46.0 (12.0, 109.0)	45.0 (12.0, 99.0)	49.0 (12.0, 114.0)	50.0 (12.0, 114.0)
LIPI score, n (%)					
Good	15 (26.3)	30 (42.9)	25 (56.8)	70 (40.9)	85 (42.1)
Intermediate	28 (49.1)	28 (40.0)	13 (29.5)	69 (40.4)	75 (37.1)
Poor	14 (24.6)	12 (17.1)	6 (13.6)	32 (18.7)	42 (20.8)
Number of organs involved, No. (%)					
≤3	18 (31.6)	42 (60.0)	25 (34.1)	85 (49.7)	108 (3.5)
>3	39 (68.4)	28 (40.0)	19 (43.2)	86 (50.3)	94 (46.5)
PD-L1, No. (%)					
TPS ≥ 50%	37 (64.9)	51 (72.9)	35 (79.5)	123 (71.9)	144 (71.3)
TPS < 50%, ≥ 1%	13 (22.8)	9 (12.9)	5 (11.4)	27 (15.8)	33 (16.3)
TPS < 1%	3 (5.3)	5 (7.1)	1 (2.3)	9 (5.3)	10 (5.0)
Not available	4 (7.0)	5 (7.1)	3 (6.8)	12 (7.0)	15 (7.4)
TMB, No. (%)					
≥ 10 mut/Mb	13 (22.8)	20 (28.6)	20 (45.5)	53 (31.0)	61 (30.2)
< 10 mut/Mb	15 (26.3)	17 (24.3)	3 (6.8)	35 (20.5)	39 (19.3)
Not available	29 (50.9)	33 (47.1)	21 (47.7)	83 (48.5)	102 (50.5)
NLR, median (range)	4.9 (1.7, 34.2)	3.6 (0.3, 15.6)	3.1 (1.1, 12.1)	4.1 (1.1, 34.2)	3.9 (0.3, 34.2)
PLR, median (range)	209.5 (48.8, 1475.9)	180.3 (52.3, 488.7)	153.0 (70.4, 409.6)	188.9 (48.8, 1475.9)	189.2 (48.8, 1475.9)

Table 1).The median age was 66 years (range: 24–86), with 87.1% ($n=149$) being male, and 73.1% ($n=125$) having a smoking history. Most patients had an ECOG PS of 0–1 (90.6%, $n=155$). Adenocarcinomas accounted for 56.7% ($n=97$) of the cases, with 71.9% ($n=123$) demonstrating PD-L1 TPS ≥ 50%. The median total and max BTS for all patients were 71.0 mm (range: 12.0–249.0) mm, and 50.0 mm (range: 12.0–114.0), respectively. None of the patients harbored *EGFR* or *ALK* alterations. The driver alterations involved *KRAS* ($n=44$), *BRAF* ($n=3$), *RET* (n = 1), *HER2* ($n=1$), while 99 patients were driver-negative (Supplementary Table 2). The proportion of patients whose best response was partly response (PR), stable disease (SD), and PD were 38.6%, 34.5%, and 14.6%, respectively. Grade ≥ 3 AEs occurred in 19.3% of patients, with 13.5% experiencing pneumonia of any grade (Supplementary Table 3).

Frequency and clinical outcomes of LTPFS

With a median follow-up of 37.1 months, the median PFS and OS in the clinical analysis cohort were 8.6 months and 20.6 months, respectively (Fig. 2a and b). After excluding 31 patients who had not reached the PFS endpoints and were followed up for less than 2 years, LTPFS occurred in 44 patients (25.7%) and STPFS in 57 (33.3%) patients (Fig. 2c). The median PFS for LTPFS, non-STPFS/non-LTPFS, and STPFS groups were 55.0 months, 8.6 months, and 1.8 months, respectively (Fig. 2d). In the LTPFS group, the median OS was not reached during the 41.0-month median follow-up, and the 5-year OS rate was 81.2%. In contrast, patients with STPFS had a median OS of 3.0 months, while those in the non-STPFS/non-LTPFS group had a median OS of 21.6 months (Fig. 2e).

Clinical characteristics of LTPFS

A higher proportion of patients with LTPFS achieved PR as best response ($P=0.003$) (Fig. 3a). Non-squamous NSCLC was significantly associated with LTPFS (univariate: OR 2.76, $P=0.018$; multivariate: OR 3.14, $P=0.047$) compared to squamous NSCLC. Patients with fewer than three organs involved had a higher likelihood of LTPFS than those with three or more organs involved (univariate: OR 3.13, $P=0.006$; multivariate: OR 4.13, $P=0.022$). Conversely, patients with intermediate or poor LIPI scores had a lower probability of LTPFS (univariate: OR 0.15, $P=0.001$; multivariate: OR 0.17, $P=0.040$) compared to those with good scores. Liver metastases were negatively associated with LTPFS in both univariate (OR 0.05, $P=0.003$) and multivariate analyses (OR 0.09, $P=0.041$). ECOG PS ≥ 2 and large total BTS (> 71.0 mm) showed a significant and negatively association with LTPFS in univariate analysis but not in multivariate analysis. No

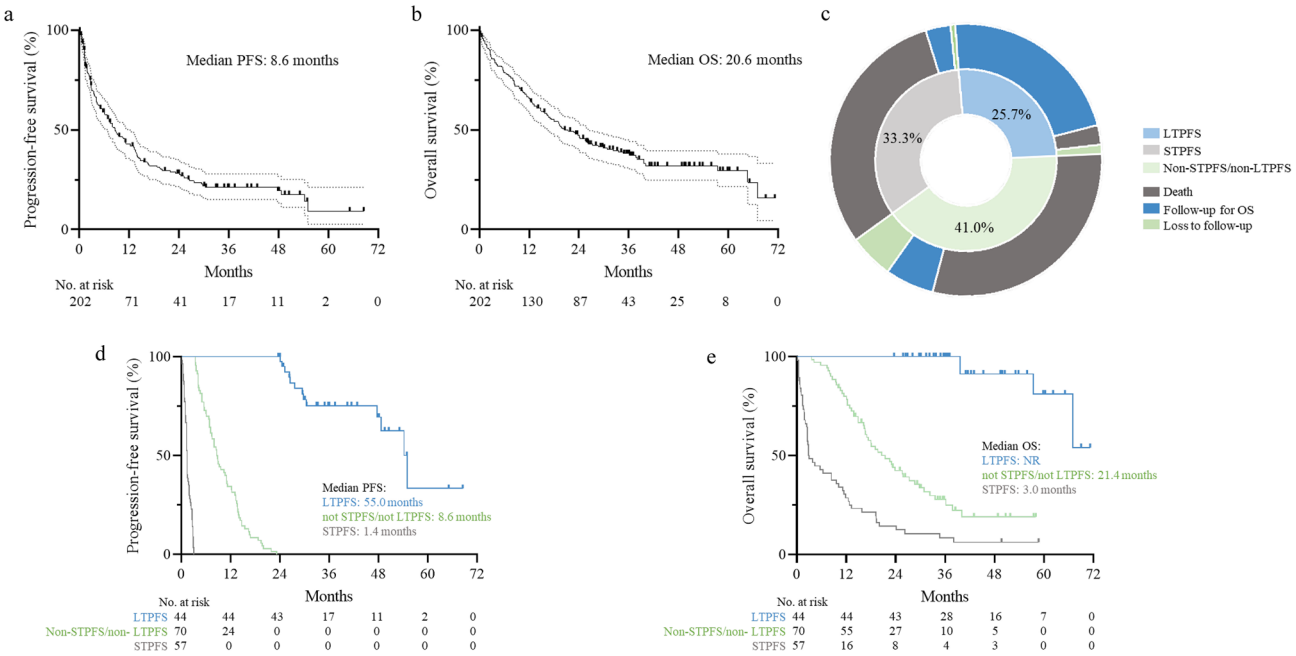

Fig. 2 Clinical outcomes in advanced NSCLC receiving first-line immunotherapy monotherapy. **a** Kaplan–Meier curves for PFS in the total population. **b** Kaplan–Meier curves for OS in the total population. **c** Percentage of patients with LTPFS, STPFS, and non-STPFS/non-LTPFS. **d** Kaplan–Meier curves for PFS among patients with LTPFS, STPFS, and non-STPFS/non-LTPFS. **e** Kaplan–Meier curves for OS among patients with LTPFS, STPFS, and non-STPFS/non-LTPFS

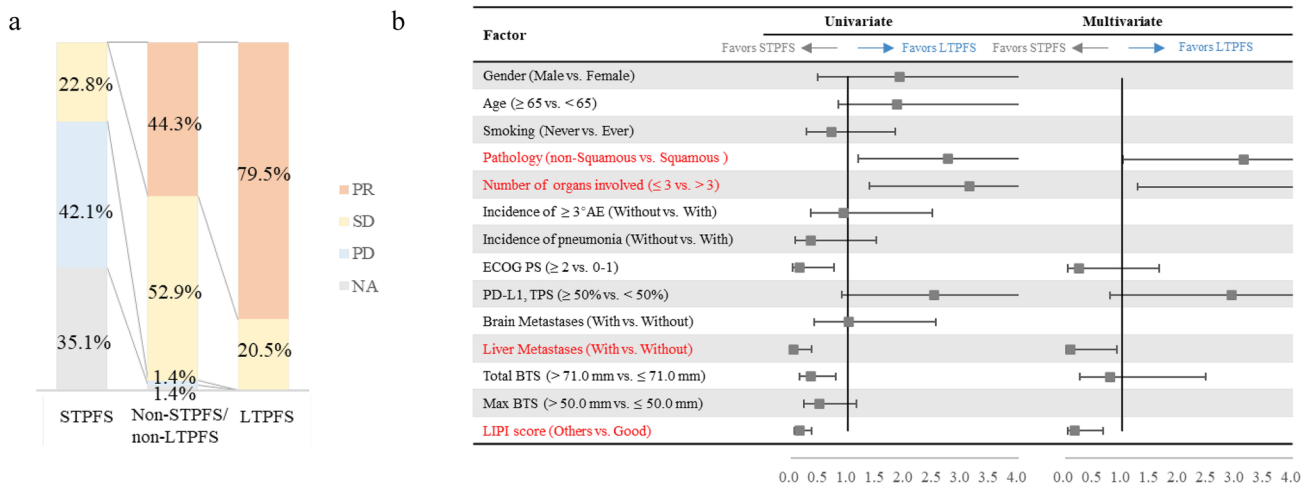

Fig. 3 Clinical characteristics of LTPFS. **a** Stacked bar graphs showing the percentage of patients with PR, SD, PD, and NA as the best response in the LTPFS, STPFS, and non-STPFS/non-LTPFS groups. **b** Forest plots of univariate and multivariate analyses assessing the association of clinical characteristics with LTPFS compared to STPFS. PR: partly response; SD: stable disease; PD: progressive disease; NA: not available

significant differences were observed concerning other clinical parameters, including age, gender, smoking history, incidence of any grade ≥ 3 AEs or any grade pneumonia, max BTS, or brain metastases (Fig. 3b, Supplementary Table 4).

The predictive value of PD-L1 and TMB in LTPFS

In univariate and multivariate analyses, no significant difference in PD-L1 levels were observed between the STPFS and LTPFS groups by a cut-off value of 50%. We found a numerically higher fraction of patients with LTPFS accompanied by PD-L1 TPS ≥ 50%. No significant differences in PFS or OS were identified between those with PD-L1 TPS ≥ 50% and those with PD-L1 TPS < 50% (Supplementary Fig. 1). Then we analyzed the association of TMB level and LTPFS. Patients with LTPFS were significantly enriched for TMB ≥ 10 mut/Mb compared to those with STPFS ($P = 0.002$) and non-STPFS/non-LTPFS ($P = 0.004$) (Fig. 4a). Patients with TMB ≥ 10 mut/Mb showed extended PFS and OS compared to those with TMB < 10 mut/Mb (median PFS: 11.1 vs. 6.1 months, $P < 0.001$; median OS: 32.3 vs. 11.0 months, $P < 0.001$) (Fig. 4b and c). When combining TMB level with PD-L1 status in 93 patients with available data in both TMB and PD-L1, patients with LTPFS were significantly enriched for TMB ≥ 10 mut/Mb as well as PD-L1 TPS ≥ 50% ($P = 0.001$) (Fig. 4d). These patients demonstrated the greatest survival benefit from first-line ICIs monotherapy, with a median PFS of 24.5 months and a median OS of 67.0 months (Fig. 4e and f).

Association of NLR and PLR with LTPFS

Pre-treatment NLR and PLR have been reported as prognostic or predictive biomarkers for immunotherapy. These immune function parameters, derived from peripheral blood cell classification and counts, reflect the interaction between systemic inflammation and overall immune function. In our cohort, patients with STPFS exhibited higher pre-treatment NLR compared to those with LTPFS (median: 4.87 vs. 3.11, $P = 0.005$) and non-STPFS/non-LTPFS ($P = 0.006$). Similarly, pre-treatment PLR was elevated in patients with STPFS compared to LTPFS (median: 209.49 vs 153.00, $P = 0.015$) and non-STPFS/non-LTPFS ($P = 0.043$) (Fig. 5a and b).

Dynamic circulating cytokines features of LTPFS

Thirty-eight peripheral blood samples were collected from 19 advanced NSCLC patients before and after the administration of ICIs, comprising 10 patients with LTPFS and 9 with STPFS from the circulating cytokines analysis cohort. The median age was 64 years (range: 50–82), with 78.9% being male, 68.4% having a smoking history, and 94.7% having an ECOG PS status of 0 to 1. Most tumors were adenocarcinomas (63.2%) with PD-L1 TPS ≥ 1% (58.0%) (Fig. 5c, Supplementary Table 5). No significant differences in detected circulating cytokines were observed between the STPFS and LTPFS groups at the single time point of baseline or 6 weeks. The detected cytokines were shown in Fig. 5c. However, following ICIs treatment, CCL11 levels increased in most LTPFS patients while decreasing in STPFS patients (median: 5.67 vs. − 1.21 pg/mL,

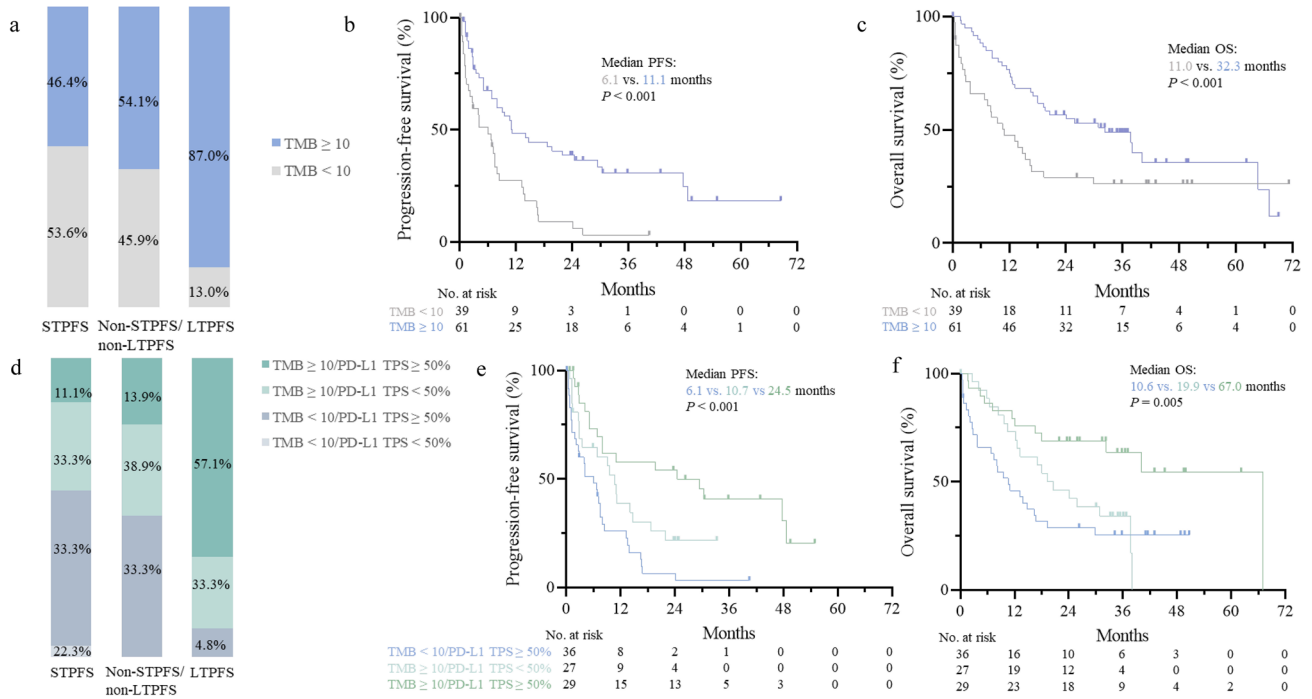

Fig. 4 Predictive value of PD-L1 and TMB in LTPFS. **a** Stacked bar graphs showing the percentage of patients with TMB ≥ 10 mut/Mb and < 10 mut/Mb in the LTPFS, STPFS, and non-STPFS/non-LTPFS groups. **b** Kaplan–Meier curves for PFS stratified by TMB. **c** Kaplan–Meier curves for OS stratified by TMB. **d** Stacked bar graphs showing the percentage of patients with different TMB and PD-L1 statuses in the LTPFS, STPFS, and non-STPFS/non-LTPFS groups. **e** Kaplan–Meier curves for PFS stratified by the combination of TMB and PD-L1 status. **f** Kaplan–Meier curves for OS stratified by the combination of TMB and PD-L1 status

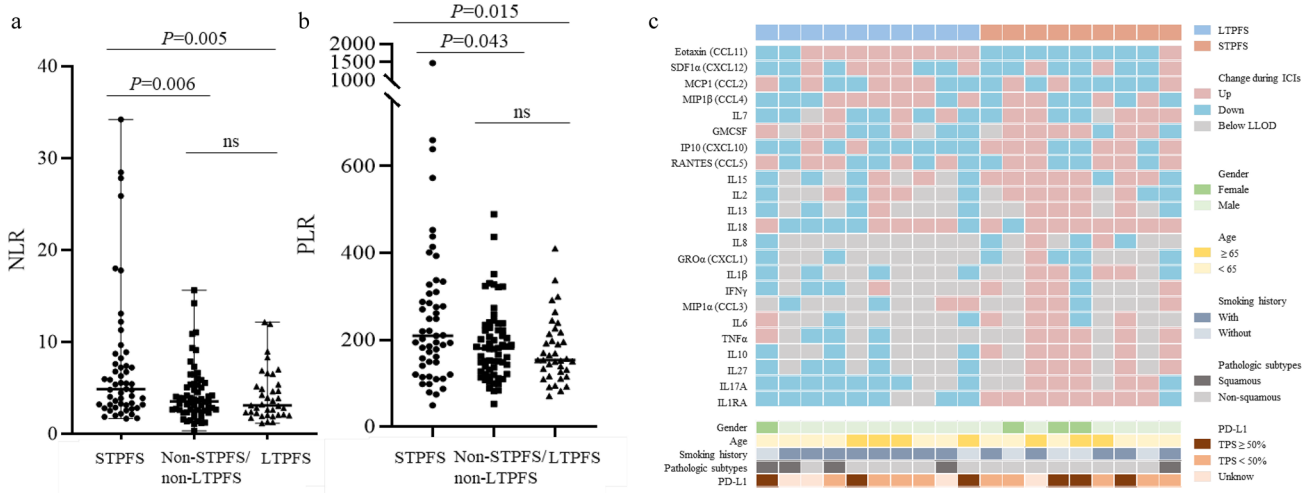

Fig. 5 Circulating biomarkers in predicting LTPFS. **a** Pre-treatment neutrophil–lymphocyte ratio in the LTPFS, STPFS, and non-STPFS/non-LTPFS groups. **b** Pre-treatment platelet-lymphocyte ratio in the LTPFS, STPFS, and non-STPFS/non-LTPFS groups. **c** Changes in circulating cytokines after ICIs treatment between STPFS and LTPFS

$P = 0.017$). Conversely, IL1RA and IL17A levels declined in LTPFS patients ([IL1RA] median: −175.30 vs. 269.60 pg/mL, $P = 0.001$; [IL17A] median: −2.00 vs. 3.76 pg/mL, $P = 0.004$) (Fig. 5c, Supplementary Fig. 2).

Discussion

In clinical practice, identifying patients who can achieve durable therapeutic effects with ICIs without the need for intensified upfront treatment through chemotherapy or other approaches is crucial. In this long follow-up clinical cohort, a relatively homogeneous population of patients receiving first-line ICIs alone was included. We described the frequency, predictive value of different characteristics, and long-term clinical outcomes of patients with LTPFS, who might represent those benefiting most from first-line ICIs monotherapy without additional intensive treatments.

Our results indicated that approximately one-quarter of patients treated with first-line ICIs monotherapy could achieve PFS exceeding 2 years, accompanied by a 5-year OS rate of more than 80.0%. These real-world data align with results from clinical trials, where approximately 25.8% and 16.0% of patients completed 2 year of first-line pembrolizumab monotherapy, with a 5-year OS rate of 81.4% in KEYNOTE-024 and a 6-year OS rate of 61.8% in KEYNOTE-042 [2, 3]. The durable responses represent a distinctive benefit of ICIs treatment. The identification of clinical and other biomarkers capable of discriminating LTPFS patients implies identifying those who truly benefit from first-line ICIs monotherapy. Here, we reported that patients with squamous NSCLC, high tumor burden (> 3 disease organs involved at baseline), intermediate or poor LIPI score, and liver metastases were negatively associated with LTPFS, which is consistent with published data [6, 8, 10, 16, 21]. It was suggested that patients with these clinical features might not benefit from ICIs monotherapy, and more aggressive ICIs-based combination strategies might be recommended. BTS was identified as a prognostic factor for worse PFS in patients treated with ICIs monotherapy [10]. In our cohort, a large total BTS was significantly and negatively associated with LTPFS in univariate analysis but not in multivariate analysis, which might be explained by the increased number of affected organs from non-target lesions.

PD-L1 expression is commonly recommended as a predictive biomarker for identifying patients who derive the most benefit from ICIs monotherapy. However, in this study, the predictive value of PD-L1 for LTPFS was overshadowed by TMB. Patients with high PD-L1 expression combined with high TMB experienced the most durable survival benefits from first-line ICIs monotherapy. The limited predictive effect of PD-L1 in this cohort may be partially due to the small number of patients with PD-L1 < 50%. Whereas, consistent with our results, several studies have reported that high PD-L1 expression does not necessarily associated with durability of response to ICIs monotherapy [8, 9]. The Food and Drug Administration has approved pembrolizumab for unresectable or metastatic TMB-high (≥ 10 mut/Mb) solid tumors without satisfactory alternative treatment options and progression after prior therapy [22]. Several studies have described an association between high TMB and long-term survival benefits from ICIs. In a large real-world data set, higher TMB was concluded as a potential biomarker for prolonged benefit from ICIs, even in patients with low PD-L1 levels, where ICIs without chemotherapy could be considered [23]. Additionally, increasing TMB levels have been associated with greater immune cell infiltration [24]. In a prospective trial, TMB ≥ 10 mut/Mb was identified as a predictive marker for ICIs monotherapy, yielding prolonged PFS and OS even in patients with PD-L1 TPS < 50% [25].

Biomarkers obtained through minimally invasive methods are expected to distinguish LTPFS. We identified pre-treatment NLR and PLR as candidate predictive features of LTPFS warranting further exploration in larger studies. Elevated pre-treatment NLR and PLR have been associated with poorer outcomes in ICIs, possibly due to reduced circulating lymphocytes correlating with lower tumor-infiltrating lymphocyte levels [26, 27]. Circulating cytokines are identified as soluble mediators of host immune activity, which play critical roles in cancer immunotherapy [17, 28]. Our findings highlighted dynamic changes in circulating cytokines, including CCL11, IL-1Ra, and IL-17A, as promising predictive features to distinguish LTPFS from STPFS. Cytokines within the human body and tumor microenvironment exhibit diverse roles, with some exerting immunostimulatory effects and others promoting tumor activity through inflammatory inhibition [29]. CCL11 could induce eosinophils migration and activation, exerting tumoricidal effects by limiting angiogenesis and causing severe necrosis [30]. IL-1Ra, an anti-inflammatory cytokine, naturally antagonizes the IL-1 receptor, counteracting the IL-1 signaling pathway. It has been reported that IL-1Ra levels in serum or tumor tissues, as well as their relationship with survival prognosis, vary across different types of cancer. This underscores the dual nature of IL-1Ra in tumor biology, which is highly dependent on the specific context and interactions within the tumor microenvironment. Given the intricate nature of the tumor microenvironment, it is likely that specific regulatory mechanisms or cellular interactions enable IL-1Ra to predominantly demonstrate either tumor-promoting or tumor-suppressive functions [31]. A recent study observed that those who respond to ICIs experience more pronounced decreases in IL-1Ra levels compared to non-responders, suggesting that an early decrease in serum IL-1Ra level could serve as a prognostic biomarker for ICIs treatment [32]. IL-17A has been reported to be associated with promoting cancer cell invasion and resistance to anti-PD-1 therapy by increasing PD-L1 expression in various cancers [33]. It is important

to note that some circulating cytokines change significantly during treatment, and their dynamic changes, rather than static values at a single time point, may be more relevant to efficacy [34]. According to our findings, it was suggested that patients with elevated plasma IL-1Ra and IL-17A levels early during ICIs treatment may require more aggressive combination regimens, which might serve as potential biomarkers for adaptive treatment.

This study included only patients receiving first-line ICIs monotherapy to enhance population homogeneity and focus on identifying those who benefit most from ICIs. However, predictive features may differ between monotherapy and combination therapy. In addition, given the nature of a retrospective study, TMB and PD-L1 expression data were unavailable for some patients. In particular, TMB data were not available for more than half of the patients in this study. The small sample size for cytokine measurements necessitates cautious interpretation, and further studies are required to validate these findings.

Conclusions

LTPFS occurred in approximately one-quarter of patients receiving first-line ICIs monotherapy, associated with a 5-year OS rate of more than 80.0%. Distinct pre-treatment clinical characteristics, including TMB, PD-L1, pathologic subtypes, LIPI score, number of organs involved, and metastatic sites, along with biomarkers such as pre-treatment NLR and PLR, and dynamic circulating cytokines profile features, can distinguish NSCLC patients with LTPFS.

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Data availability Data is provided within the manuscript or supplementary information files. All other relevant data are available from the corresponding author of this study (Qing Zhou, gzzhouqing@126.com) upon reasonable request.

Declarations

Conflict of interests Prof. Q. Zhou reports honoraria from AstraZeneca, Boehringer Ingelheim, BMS, Eli Lilly, MSD, Pfizer, Roche, and Sanofi outside the submitted work. The other authors have no competing interests to declare.

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References

1. Liu SM, Zheng MM, Pan Y, Liu SY, Li Y, Wu YL (2023) Emerging evidence and treatment paradigm of non-small cell lung cancer. J Hematol Oncol 16:40. https://doi.org/10.1186/s13045-023-01436-2
2. Reck M, Rodriguez-Abreu D, Robinson AG et al (2021) Five-year outcomes with pembrolizumab versus chemotherapy for metastatic non-small-cell lung cancer with PD-L1 tumor proportion score >/= 50. J Clin Oncol 39:2339–2349. https://doi.org/10.1200/JCO.21.00174
3. de Castro G, Jr., Kudaba I, Wu YL, et al (2023) Five-year outcomes with pembrolizumab versus chemotherapy as first-line therapy in patients with non-small-cell lung cancer and programmed death ligand-1 tumor proportion score >/= 1% in the KEYNOTE-042 study. J Clin Oncol 41:1986–1991. https://doi.org/10.1200/JCO.21.02885
4. Perol M, Felip E, Dafni U et al (2022) Effectiveness of PD-(L)1 inhibitors alone or in combination with platinum-doublet chemotherapy in first-line (1L) non-squamous non-small-cell lung cancer (Nsq-NSCLC) with PD-L1-high expression using real-world data. Ann Oncol 33:511–521. https://doi.org/10.1016/j.annonc.2022.02.008
5. Mok TSK, Wu YL, Kudaba I et al (2019) 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 393:1819–1830. https://doi.org/10.1016/S0140-6736(18)32409-7

6. Passaro A, Novello S, Giannarelli D, Bria E, Galetta D, Gelibter A, Reale ML, Carnio S, Vita E, Stefani A, Pizzutilo P (2021) Early progression in non-small cell lung cancer (NSCLC) with high PD-L1 Treated with pembrolizumab in first-line setting: a prognostic scoring system based on clinical features. Cancers 13(12):2935. https://doi.org/10.3390/cancers13122935
7. Maggie Liu SY, Huang J, Deng JY et al (2024) PD-L1 expression guidance on sintilimab versus pembrolizumab with or without platinum-doublet chemotherapy in untreated patients with advanced non-small cell lung cancer (CTONG1901): a phase 2, randomized, controlled trial. Sci Bull (Beijing) 69:535–543. https://doi.org/10.1016/j.scib.2023.12.046
8. Thummalapalli R, Ricciuti B, Bandlamudi C et al (2023) Clinical and molecular features of long-term response to immune checkpoint inhibitors in patients with advanced non-small cell lung cancer. Clin Cancer Res 29:4408–4418. https://doi.org/10.1158/1078-0432.CCR-23-1207
9. Jo H, Yoshida T, Yagishita S et al (2023) Clinical characteristics and pharmacokinetics change of long-term responders to anti-programmed cell death protein 1 inhibitor among patients with advanced NSCLC. JTO Clin Res Rep 4:100474. https://doi.org/10.1016/j.jtocrr.2023.100474
10. Uehara Y, Hakozaki T, Kitadai R, Narita K, Watanabe K, Hashimoto K, Kawai S, Yomota M, Hosomi Y (2022) Association between the baseline tumor size and outcomes of patients with non-small cell lung cancer treated with first-line immune checkpoint inhibitor monotherapy or in combination with chemotherapy. Transl Lung Cancer Res 11:135–149. https://doi.org/10.21037/tlcr-21-815
11. Yu Y, Zeng D, Ou Q et al (2019) Association of survival and immune-related biomarkers with immunotherapy in patients with non-small cell lung cancer: a meta-analysis and individual patient-level analysis. JAMA Netw Open 2:e196879. https://doi.org/10.1001/jamanetworkopen.2019.6879
12. Mino-Kenudson M, Schalper K, Cooper W et al (2022) Predictive biomarkers for immunotherapy in lung cancer: perspective from the international association for the study of lung cancer pathology committee. J Thorac Oncol 17:1335–1354. https://doi.org/10.1016/j.jtho.2022.09.109
13. Bodor JN, Boumber Y, Borghaei H (2020) Biomarkers for immune checkpoint inhibition in non-small cell lung cancer (NSCLC). Cancer 126:260–270. https://doi.org/10.1002/cncr.32468
14. Ferrara R, Mezquita L, Texier M et al (2019) Fast-progression (FP), hyper-progression (HPD) and early deaths (ED) in advanced non-small cell lung cancer (NSCLC) patients (pts) upon PD-(L)-1 blockade (IO). J Clin Oncol 37:9107. https://doi.org/10.1200/JCO.2019.37.15_suppl.9107
15. Uehara Y, Watanabe K, Hakozaki T, Yomota M, Hosomi Y (2022) Efficacy of first-line immune checkpoint inhibitors in patients with advanced NSCLC with KRAS, MET, FGFR, RET, BRAF, and HER2 alterations. Thorac Cancer 13:1703–1711. https://doi.org/10.1111/1759-7714.14448
16. Mezquita L, Auclin E, Ferrara R et al (2018) Association of the lung immune prognostic index with immune checkpoint inhibitor outcomes in patients with advanced non-small cell lung cancer. JAMA Oncol 4:351–357. https://doi.org/10.1001/jamaoncol.2017.4771
17. Wei F, Azuma K, Nakahara Y, Saito H, Matsuo N, Tagami T, Kouro T, Igarashi Y, Tokito T, Kato T, Kondo T (2023) Machine learning for prediction of immunotherapeutic outcome in non-small-cell lung cancer based on circulating cytokine signatures. J ImmunoTher Cancer 11(7):e006788. https://doi.org/10.1136/jitc-2023-006788
18. Burton A, Altman DG (2004) Missing covariate data within cancer prognostic studies: a review of current reporting and proposed guidelines. Br J Cancer 91:4–8. https://doi.org/10.1038/sj.bjc.6601907
19. Mu S, Echouffo-Tcheugui JB, Ndumele CE et al (2023) NT-proBNP reference intervals in healthy U.S. children, adolescents, and adults. J Appl Lab Med 8:700–712. https://doi.org/10.1093/jalm/jfad024
20. Beal SL (2001) Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn 28:481–504. https://doi.org/10.1023/a:1012299115260
21. Deng JY, Gou Q, Yang L, Chen ZH, Yang MY, Yang XR, Yan HH, Wei XW, Liu JQ, Su J, Zhong WZ (2023) Immune suppressive microenvironment in liver metastases contributes to organ-specific response of immunotherapy in advanced non-small cell lung cancer. J Immunother Cancer 11(7):e007218. https://doi.org/10.1136/jitc-2023-007218
22. Marcus L, Fashoyin-Aje LA, Donoghue M et al (2021) FDA approval summary: pembrolizumab for the treatment of tumor mutational burden-high solid tumors. Clin Cancer Res 27:4685–4689. https://doi.org/10.1158/1078-0432.CCR-21-0327
23. Huang RS, Carbone DP, Li G, Schrock A, Graf RP, Zhang L, Murugesan K, Ross JS, Tolba K, Sands J, Oxnard GR (2023) Durable responders in advanced NSCLC with elevated TMB and treated with 1L immune checkpoint inhibitor: a real-world outcomes analysis. J ImmunoTher Cancer 11(1):e005801. https://doi.org/10.1136/jitc-2022-005801
24. Ricciuti B, Wang X, Alessi JV et al (2022) Association of high tumor mutation burden in non-small cell lung cancers with increased immune infiltration and improved clinical outcomes of PD-L1 blockade across PD-L1 expression levels. JAMA Oncol 8:1160–1168. https://doi.org/10.1001/jamaoncol.2022.1981
25. Liu SM, Zhou Q, Du Y et al. (2024) 75P PD-L1 expression and TMB guided first-line sintilimab monotherapy for advanced NSCLC in a multi-center, phase II umbrella trial (CTONG 1702). ESMO Open. 9
26. Valero C, Lee M, Hoen D et al (2021) Pretreatment neutrophil-to-lymphocyte ratio and mutational burden as biomarkers of tumor response to immune checkpoint inhibitors. Nat Commun 12:729. https://doi.org/10.1038/s41467-021-20935-9
27. Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, Templeton AJ, Fruh M (2017) 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 111:176–181. https://doi.org/10.1016/j.lungcan.2017.07.024
28. Berraondo P, Sanmamed MF, Ochoa MC et al (2019) Cytokines in clinical cancer immunotherapy. Br J Cancer 120:6–15. https://doi.org/10.1038/s41416-018-0328-y
29. Propper DJ, Balkwill FR (2022) Harnessing cytokines and chemokines for cancer therapy. Nat Rev Clin Oncol 19:237–253. https://doi.org/10.1038/s41571-021-00588-9
30. Xing Y, Tian Y, Kurosawa T, Matsui S, Touma M, Yanai T, Wu Q, Sugimoto K (2016) CCL11-induced eosinophils inhibit the formation of blood vessels and cause tumor necrosis. Genes Cells 21:624–638. https://doi.org/10.1111/gtc.12371
31. Fang Z, Jiang J, Zheng X (2023) Interleukin-1 receptor antagonist: an alternative therapy for cancer treatment. Life Sci 335:122276. https://doi.org/10.1016/j.lfs.2023.122276
32. Lu Z, Zou J, Hu Y et al (2019) Serological markers associated with response to immune checkpoint blockade in metastatic gastrointestinal tract cancer. JAMA Netw Open 2:e197621. https://doi.org/10.1001/jamanetworkopen.2019.7621
33. Song M, Liang J, Wang L et al (2023) IL-17A functions and the therapeutic use of IL-17A and IL-17RA targeted antibodies for cancer treatment. Int Immunopharmacol 123:110757. https://doi.org/10.1016/j.intimp.2023.110757

34. Agullo-Ortuno MT, Gomez-Martin O, Ponce S, Iglesias L, Ojeda L, Ferrer I, Garcia-Ruiz I, Paz-Ares L, Pardo-Marques V (2020) Blood predictive biomarkers for patients with non-small-cell lung cancer associated with clinical response to nivolumab. Clin Lung Cancer 21:75–85. https://doi.org/10.1016/j.cllc.2019.08.006