BMC Pulmonary Medicine

RESEARCH

Open Access

Comprehensive characterization of PD-L1 expression and immunotherapy-related genomic biomarkers in early- versus advanced-stage non-small cell lung cancer

Yujie Chen[1†], Peiyuan Wang[1,2,3†], Rong Lian[4†], Mingming Yuan[4], Pengli Yu[4], Hao He[1], Peng Chen[1], Hang Zhou[1], Weijie Chen[1], Derong Zhang[1], Hui Lin[1], Shuoyan Liu[1,2,3*] and Feng Wang[1*]

Abstract

Background Programmed death-ligand 1 (PD-L1) expression is a key biomarker for predicting the efficacy of immune checkpoint inhibitors (ICIs). With the successful application of perioperative immunotherapy, understanding PD-L1-associated clinical and molecular characteristics in early-stage non-small cell lung cancer (NSCLC) patients is essential.

Methods We analyzed 3185 NSCLC patients undergoing targeted next-generation sequencing (NGS) and PD-L1 immunohistochemistry (IHC). Associations between PD-L1 expression and molecular profiles were compared across early- (I-III) and advanced-stage (IV) cohorts.

Results In early-stage NSCLC ($n=974$), high PD-L1 expression was less common than in advanced-stage patients (lung adenocarcinoma [LUAD]: 7.52% vs. 15.98%, $p<0.001$; lung squamous cell carcinoma [LUSC]: 18.33% vs. 20.84%, $p=0.058$). For LUAD, high PD-L1 expression was more frequent in older patients, males and smokers. Additionally, LUSC overall showed a higher rate of high PD-L1 expression than LUAD. In LUAD, early-stage patients had a lower proportion of tumor mutation burden-high (TMB-H) compared to advanced-stage patients ($p<0.001$), but no significant difference was observed in LUSC ($p=0.597$). Early-stage patients also had a lower proportion of immunotherapy resistance genes than advanced-stage (LUAD: 31.15% vs. 48.50%, $p=0.014$; LUSC: 13.64% vs. 45.24%, $p=0.0067$). Moreover, among LUAD patients with high PD-L1 expression and all LUSC patients, early-stage patients exhibited more significantly different genetic features compared to advanced-stage patients.

Conclusions This study provides a comprehensive analysis of immunotherapy-related biomarker rates in early-stage NSCLC patients, offering insights for perioperative immunotherapy research and biomarker analysis.

†Yujie Chen, Peiyuan Wang and Rong Lian are the co-first authors.

*Correspondence:
Shuoyan Liu
shuoyanliu2010@163.com
Feng Wang
wfmd120@163.com

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

Trial registration Not applicable.
Keywords PD-L1, Immunotherapy, LUAD, LUSC, Stage

Introduction

Immune checkpoint inhibitors (ICIs) targeting programmed death receptor 1 (PD-1) and programmed death-ligand 1 (PD-L1) have significantly improved outcomes for resectable non-small cell lung cancer (NSCLC). Key studies, including CheckMate 816 (nivolumab), IMpower010 (atezolizumab) and KEYNOTE-091 (pembrolizumab), have demonstrated durable clinical benefits of neoadjuvant/adjuvant immunotherapy in resectable NSCLC [1–4]. Despite these advances, many early-stage patients exhibit poor responses to immunotherapy, underscoring an urgent need for predictive biomarkers to optimize immunotherapy selection.

While molecular biomarkers such as PD-L1 expression [5], tumor mutation burden (TMB) [6], and genetic alterations (e.g., *EGFR/ALK* driver mutations, *STK11*/*KRAS* co-mutations, and *MDM*2/4 amplification) guide therapeutic decisions in advanced NSCLC, their predictive utility in early-stage disease remains poorly defined [7–9]. In this study, we retrospectively analyzed a cohort of 3185 Chinese NSCLC patients, including 974 with stage I-III disease. We investigated the correlations between PD-L1, TMB and multiple clinical features in adenocarcinoma and squamous cell carcinoma. Patients were stratified by PD-L1 expression levels to evaluate clinical characteristics, immunotherapy resistance genes and hyperprogression biomarkers. Additionally, we investigated genomic differences between early- and advanced-stage NSCLC patients with varying PD-L1 expression levels, hoping to provide some valuable information for clinical practice.

Materials and methods

Patients

This study retrospectively enrolled NSCLC patients who underwent NGS in Geneplus-Beijing (Beijing, China) for clinical molecular diagnosis and treatment at Fujian Cancer Hospital from May 2015 to November 2021. A total of 1021 cancer-related genes were tested in the samples from all patients, and immunohistochemical (IHC) staining was used to detect PD-L1 expression. The tumor node metastasis (TNM) stage was determined according to the guidelines mentioned in the 8th edition of the TNM classification. Patients whose PD-L1 could not be evaluated or whose disease stages could not be determined were excluded. Ultimately, 3185 patients were included in this study (Supplementary Fig. 1). The study was approved by the Ethics Committee of Fujian Cancer Hospital (No. SQ2022-116) and informed consents were obtained from all subjects involved in the study. For underage patients, informed consents were also obtained from their guardians.

Sample processing and DNA extraction

Comprehensive genomic analysis was performed using custom-designed NGS panels containing 1021 cancer-related genes. The somatic variants detected included single nucleotide variants (SNVs), small insertions and deletions (InDels), copy number variants (CNVs) and structural variants (SVs). Genomic DNA was isolated from FFPE tumor samples using the QIAamp DNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The 2100 Bioanalyzer (Agilent, Santa Clara, USA) spectra were used for quality control. DNA concentrations were measured using a Qubit fluorometer and a Qubit dsDNA HS (High Sensitivity) detection kit (Invitrogen, Carlsbad, CA, USA).

Sequencing and bioinformatics analysis

Index sequencing libraries were prepared using the protocol recommended by the Illumina TruSeq DNA Library Preparation Kit (Illumina, San Diego, CA). Libraries were hybridized to custom-designed biotinylated oligonucleotide probes (Roche NimbleGen, Madison, WI, USA) covering 1021 cancer-related genes. Sequencing was performed using Illumina HiSeq 3000 (Illumina). Cleaning reads were mapped and aligned to the reference human genome (hg19) with BWA (version 0.7.12) after the removal of end junction sequences and low-quality reads. SNVs were called using MuTect2 (3.4–46), InDels were called using GATK, CNVs were detected using Contra (2.0.8), and SVs were detected using BreakDancer. All final candidate variants were verified by the integrated genome browser.

Assessment of TMB

TMB was calculated using sequencing data from a 1021-gene panel. It was defined as the total number of somatic nonsynonymous single nucleotide variants and small insertions/deletions per megabase (Mb) in the coding region. In this study, a TMB value of ≥ 9 mutations per Mb (muts/Mb) was defined as TMB-high (TMB-H). This threshold was derived from a statistical analysis of a large cohort, utilizing the top quartile (> 25%) of the TMB distribution in 2000 lung cancer samples from the Geneplus database was used as the cutoff value, with clinical validation performed to confirm its prognostic utility [10, 11].

PD-L1 staining

PD-L1 expression was assessed by immunohistochemistry using 22C3 PharmDx assay. The Tumor Proportion Score (TPS) was used to determine PD-L1 expression, defined as the percentage of tumor cells exhibiting membranous PD-L1 staining. Based on previous studies [12, 13], PD-L1 expression was categorized as follows: negative (TPS < 1%), low (TPS 1–49%), or high (TPS ≥ 50%).

Statistical analysis

Unpaired Student's t tests were used to compare age and TMB between groups. Chi-square and Fisher's exact tests were used to assess mutational differences between groups. Pearson correlation (r) was selected to assess the linear relationship between TMB (continuous variable) and PD-L1 TPS expression (continuous percentage range 0–100%, but typically categorized as < 1%, 1–49%, and ≥ 50% in clinical practice). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). The results were considered statistically significant when *p* values were < 0.05.

Results

Clinical characteristics and PD-L1 expression in stage I-III and stage IV NSCLC patients

A total of 3185 patients were enrolled, including those with pathologically confirmed adenocarcinoma ($n = 2476$, 77.7%), squamous cell carcinoma ($n = 523$, 16.42%), or others ($n = 186$, 5.8%). Of these patients (Table 1), 1892 (59.4%) were male and 1068 (33.53%) were smokers. The median patient age was 61 years, and the median TMB was 4 muts/Mb. Among these patients, 974 were stage I-III (30.58%), and 2211 were stage IV (69.42%). The clinical characteristics of all patients with stage I-III and stage IV NSCLC were compared. Compared with stage IV NSCLC cohort, stage I-III cohort were younger ($p < 0.001$), showed higher proportion of female patients ($p < 0.001$), non-smokers ($p < 0.001$), and adenocarcinoma patients ($p < 0.001$), and had a lower rate of high PD-L1 expression ($p < 0.001$) (Supplementary Table 1).

Considering the large differences in clinical and molecular characteristics between patients with lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), we analyzed PD-L1 expression in patients with LUAD and LUSC, separately. In LUAD, compared with stage IV patients, stage I-III patients were diagnosed at a younger age ($p < 0.001$, Table 1), and had higher percentage of females ($p = 0.0075$), and non-smokers ($p < 0.001$). In LUSC, there was no statistical difference in age or sex between stage I-III patients and stage IV patients, while a higher proportion of smokers was observed in stage I-III patients ($p = 0.046$). In addition, patients with stage I-III LUAD demonstrated a significantly lower percentage of high PD-L1 expression than patients with stage IV LUAD ($p < 0.001$, Table 1). A similar trend was observed for LUSC, but the difference was not statistically significant ($p = 0.058$).

Correlation between clinical characteristics and PD-L1 expression in LUAD and LUSC at stages I-III and IV

Next, we stratified the clinical characteristics of patients by PD-L1 expression (high, low, negative) in stages I-III and IV of LUAD and LUSC (Table 2), respectively. We observed that the percentages of patients with high PD-L1 expression in stages I-III and IV were 7.52% (61/811) and 15.98% (266/1665) in LUAD, and 18.33%

Table 1 Clinical characteristics of LUAD patients and LUSC patients

Clinical characteristics	Overall	LUAD (*N* = 2476)		*P*	LUSC (*N* = 523)		*P*
	(*N* = 3185)	I-III (*N* = 811)	IV (*N* = 1665)		I-III (*N* = 120)	IV (*N* = 403)	
	n (%)	*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Age (median, range)	61 (11–90)	58 (11–85)	61 (25–90)	< 0.001	64 (33–85)	65 (30–87)	0.359
Sex							
Male	1892 (59.40)	393 (48.46)	902 (54.17)	0.0075	110 (91.67)	358 (88.83)	0.375
Female	1293 (40.60)	418 (51.54)	763 (45.83)		10 (8.33)	45 (11.17)	
Smoking status				< 0.001			0.046
Ever	1068 (33.53)	154 (18.99)	408 (24.50)		105 (87.50)	324 (80.40)	
Never	1427 (44.71)	463 (57.09)	857 (51.47)		7 (5.83)	49 (12.16)	
Unknown	690 (21.66)	194 (23.92)	400 (24.02)		8 (6.67)	30 (7.44)	
PD-L1 expression				< 0.001			0.058
High	457 (14.35)	61 (7.52)	266 (15.98)		22 (18.33)	84 (20.84)	
Low	1131 (35.51)	270 (33.29)	582 (34.95)		63 (52.50)	163 (40.45)	
Negative	1597 (50.14)	480 (59.19)	817 (49.07)		35 (29.17)	156 (38.17)	
TMB				< 0.001			0.597
TMB-H	747 (23.45)	95 (11.71)	284 (17.06)		68 (56.67)	241 (59.80)	
TMB-L	2438 (76.55)	716 (88.29)	1381 (82.94)		52 (43.33)	162 (40.20)	
TMB (median, range)	4 (0-995)	2 (0-121.91)	3.84 (0-995)	0.012	10.56 (0-935.04)	10.56 (0-312)	0.123

Table 2 Clinical characteristics of different PD-L1 expression cohorts in LUAD and LUSC

PD-L1 expression level	LUAD (*n* = 2476)							
	I-III (*n* = 811)				IV (*n* = 1665)			
	High(*n* = 61)	Low(*n* = 270)	Negative (*n* = 480)	*P*	High(*n* = 266)	Low(*n* = 582)	Negative (*n* = 817)	*P*
	n (%)	*n* (%)	*n* (%)		*n* (%)	*n* (%)	*n* (%)	
Age (median, range)	64 (26–82)	61 (11–84)	61 (25–85)	0.037	61 (29–90)	61 (28–90)	61 (28–90)	0.493
Sex				< 0.001				< 0.001
Male	43 (70.49)	149 (55.19)	201 (41.88)		179 (67.29)	311 (53.44)	412 (50.43)	
Female	18 (29.51)	121 (44.81)	279 (58.13)		87 (32.71)	271 (46.56)	405 (49.57)	
Smoking status				< 0.001				< 0.001
Ever	17 (27.87)	61 (22.59)	76 (15.83)		85 (31.95)	140 (24.05)	183 (22.40)	
Never	20 (32.79)	140 (51.85)	299 (62.29)		108 (40.60)	308 (52.92)	441 (53.98)	
Unknown	24 (39.34)	69 (25.56)	105 (21.88)		73 (27.44)	13 (23.02)	193 (23.62)	
PD-L1 expression level	**LUSC (*n* = 523)**							
	I-III (*n* = 120)				**IV (*n* = 403)**			
	High(*n* = 22)	**Low(*n* = 63)**	**Negative (*n* = 35)**	***P***	**High(*n* = 84)**	**Low(*n* = 163)**	**Negative (*n* = 156)**	***P***
	n (%)	**n (%)**	**n (%)**		**n (%)**	**n (%)**	**n (%)**	
Age (median, range)	62 (33–75)	62 (35–85)	61 (44–81)	0.1276	61 (32–87)	61 (30–85)	62 (40–84)	0.5766
Sex				0.6770				0.5475
Male	20 (90.91)	59 (93.65)	31 (88.57)		72 (85.71)	145 (88.96)	141 (90.38)	
Female	2 (9.09)	4 (6.35)	4 (11.43)		12 (14.29)	18 (11.04)	15 (9.62)	
Smoking status				0.6422				0.7484
Ever	19 (86.36)	56 (88.89)	30 (85.71)		67 (79.76)	132 (80.98)	125 (80.13)	
Never	2 (9.09)	4 (6.35)	1 (2.86)		8 (9.52)	22 (13.50)	19 (12.18)	
Unknown	1 (4.55)	3 (4.76)	4 (11.43)		9 (10.71)	9 (5.52)	12 (7.69)	

(22/120) and 20.84% (84/403) in LUSC. In the stage IV cohort of LUAD patients, PD-L1 expression did not significantly differ by age ($p = 0.493$). PD-L1 expression was significantly higher in males and smokers ($p < 0.001$). In the stage I-III cohort of LUAD patients, PD-L1 expression was significantly higher in older patients($p = 0.037$), males, and smokers ($p < 0.001$). In LUSC, there were no significant differences in patients with different PD-L1 expression levels in terms of age, gender, or smoking status (Table 2).

We then compared PD-L1 expression between LUAD and LUSC at stages I-III and IV, respectively. There was a significant difference in PD-L1 expression between LUAD and LUSC in both the I-III and IV phases. The percentage of positive PD-L1 (TPS ≥ 1%) in LUSC was significantly higher than that in LUAD in both the early-stage (LUAD vs. LUSC: 40.81% vs. 70.83, $p < 0.001$) and advanced stage (LUAD vs. LUSC: 50.93% vs. 61.29%, $p < 0.001$) (Supplementary Fig. 2).

Immunotherapy-related genomic characteristics of stage I-III and stage IV NSCLC patients

We next analyzed the immunotherapy relevant genomic characteristics in the LUAD and LUSC cohorts with different stages. In the LUAD cohort, the percentage of TMB-H and median TMB in stage IV LUAD were significantly higher than those in stage I-III patients (TMB-H: 17.06% vs. 11.71%, $p < 0.001$, TMB-median: 3.84 vs. 2 muts/Mb, $p = 0.012$, Table 1). In the LUSC group, 56.67% of patients with stage I-III and 59.8% of patients with stage IV were TMB-H ($p = 0.597$). The median TMB was consistent in early- and advanced-stage patients (stage I-III vs. stage IV = 10.56 vs. 10.56 mutations/Mb, $p = 0.123$, Table 1). Compared with those in the LUAD cohort, LUSC cohort had higher TMB values (stage I-III: 10.56 vs. 2.00 muts/Mb, $p < 0.001$; stage IV: 10.56 vs. 3.84 muts/Mb, $p < 0.001$; Fig. 1A). In addition, there was no significant correlation between PD-L1 expression and TMB (Pearson's correlation coefficient = 0.1043, 95% CI: 0.68 – 0.14, $p < 0.001$; Fig. 1B).

Next, we calculated the incidence of negative immune-related and hyperprogressive factors in the overall cohort. According to previous literature, several genes were analyzed, including genes *EGFR*, *ALK*, *KRAS* + *STK11* comutation, *PTEN*, *JAK1/2*, *MDM2/4*, and *CCND1*. In the entire cohort, negative immunotherapy-related variants were identified in a total of 1700 (53.38%) patients, and the incidence of these mutations was not low, the incidence of *EGFR* was the highest, at 40.78% (1299/3185). The proportions of patients with other immunotherapy-related negative mutations were 4.68% (149/3185) for *CCND1* amplification, 4.4% (140/3185) for *PTEN* mutation, 4.21% (134/3185) for *ALK* mutation, 1.6% (51/3185) for *KRAS* + *STK11* comutations, 1.13% (36/3185, 1.13%)

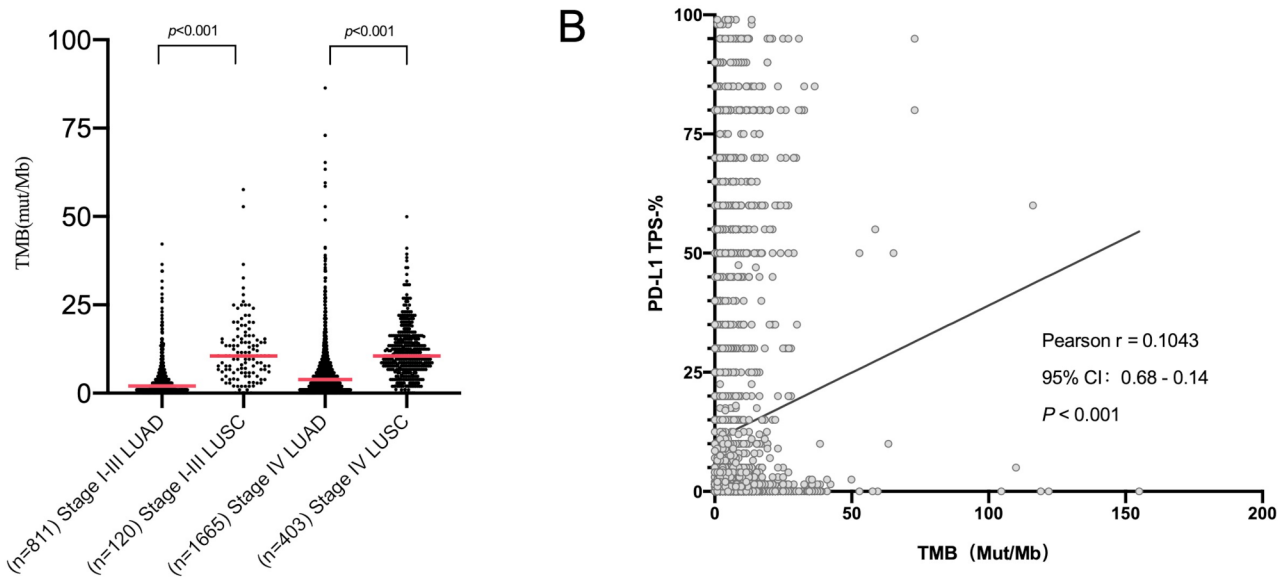

Fig. 1 Characteristics of PD-L1 expression and the TMB in patients. **A**. TMB in patients with different stages of LUAD and LUSC. The LUSC cohort exhibited significantly higher TMB than the LUAD cohort across all stages (stage I-III: median 10.56 vs. 2.00 muts/Mb, $p < 0.001$; stage IV: 10.56 vs. 3.84 muts/Mb, $p < 0.001$). **B**. Correlation between TMB and PD-L1 expression. No significant correlation was observed (Pearson's correlation coefficient = 0.1043, 95% CI: 0.68 – 0.14, $p < 0.001$)

for *MDM2/4* amplification and 0.53% (17/3185) for *JAK1/2* mutations (Supplementary Table 2).

We further calculated the incidence of genetic biomarkers that were negatively related to immunotherapy efficacy in LUAD and LUSC patients with different PD-L1 expression levels at stages I-III and stage IV, respectively. As shown in Table 3, there were large differences in the genetic biomarkers between LUAD and LUSC. Mutations in *EGFR* and *ALK* were enriched in LUAD patients. Among patients with stage I-III LUAD, the percentage of *EGFR*-mutated patients with high PD-L1 expression, low PD-L1 expression and negative PD-L1 expression were 18.03 (11/61), 48.89% (132/270) and 62.29% (299/811), respectively ($p < 0.001$). Among patients with stage IV LUAD, the percentage of *EGFR*-mutated patients with high PD-L1 expression, low PD-L1 expression and negative PD-L1 expression were 30.08% (80/266), 51.03% (297/582) and 53.24% (435/817), respectively ($p < 0.001$). A negative correlation was observed between the percentage of patients with *EGFR* mutations and PD-L1 expression in the LUAD cohort, regardless of stage ($p < 0.001$, Table 3). Among the other negative immune-related factors in LUAD, the most common mutation was *ALK*. Interestingly, it seemed that *ALK* mutation was more common in patients with high PD-L1 expression than low PD-L1 expression and negative PD-L1, regardless of stage. However, the differences were not significant, possibly because of the limited number of patients with *ALK* mutations. Other factors accounted for a relatively small proportion.

In contrast, the proportions of patients with *PTEN* and *CCND1* mutations were higher in those with LUSC (Table 3). The proportions of *PTEN* mutation, *CCND1* amplification, *ALK* mutation, *KRAS* + *STK11* co-mutation and *MDM2/4* amplification in the high PD-L1 expression, low PD-L1 expression and negative PD-L1 expression populations were no significantly difference at either stage I-III or stage IV. However, the detection rates of *EGFR* and *JAK1/2* mutations were significantly higher in stage IV patients with high PD-L1 expression. We combined the incidence of these immune-related biomarkers and found that in the high PD-L1 expression group, the proportion of patients with genes associated with poor immunotherapy efficacy in phase IV patients was higher than that in stage I-III (stage I-III vs. IV: 31.15% vs. 48.50%, $p = 0.014$, LUAD; 13.64% vs. 45.24%, $p = 0.0067$, LUSC). Moreover, in LUAD, as the expression of PD-L1 increased, the occurrence of immune-negative related genes decreased (I-III: $p < 0.001$, IV: $p < 0.001$; Table 3). In early-stage LUSC, the incidence of genes associated with poor immunotherapy efficacy decreased with the increasing of PD-L1 expression but was not significant ($p = 0.318$, Table 3). However, in stage IV LUSC, the expression of PD-L1 was positively correlated with the occurrence of genes associated with poor immunotherapy efficacy ($p = 0.023$, Table 3).

Differentially mutated genes among patients with different disease stages and PD-L1 expression

To identify other differences in genomic features, we compared the genomic variants among different PD-L1

Table 3 Occurrence of negative correlations and hyperprogression factors in LUAD and LUSC patients with different PD-L1 expression levels

PD-L1 expression level	LUAD (*n* = 2476)							
	I-III (*n* = 811)			*P*	IV (*n* = 1665)			*P*
	High (*n* = 61)	Low (*n* = 270)	Negative (*n* = 480)		High (*n* = 266)	Low (*n* = 582)	Negative (*n* = 817)	
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
EGFR	11 (18.03)	132 (48.89)	299 (62.29)	< 0.001	80 (30.08)	297 (51.03)	435 (53.24)	< 0.001
ALK	6 (9.84)	22 (8.15)	24 (5.00)	0.126	24 (9.02)	29 (4.98)	24 (2.94)	< 0.001
KRAS + STK11	0 (0.00)	3 (1.11)	8 (1.67)	0.521	5 (1.88)	10 (1.72)	22 (2.69)	0.437
PTEN	0 (0.00)	4 (1.48)	13 (2.71)	0.262	4 (1.50)	17 (2.92)	23 (2.82)	0.447
JAK1/2	2 (3.28)	2 (0.74)	2 (0.42)	0.049	3 (1.13)	1 (0.17)	0 (0.00)	< 0.01
MDM2/4	0 (0.00)	4 (1.48)	4 (0.83)	0.491	3 (1.13)	11 (1.89)	11 (1.35)	0.613
CCND1	0 (0.00)	8 (2.96)	10 (2.08)	0.348	10 (3.76)	18 (3.09)	29 (3.55)	0.851
Total	19 (31.15)	175 (64.81)	360 (75.00)	< 0.001	129 (48.50)	383 (65.81)	544 (66.59)	< 0.001
PD-L1 expression level	**LUSC (*n* = 523)**							
	I-III (*n* = 120)			*P*	IV (*n* = 403)			*P*
	High (*n* = 22)	Low (*n* = 63)	Negative (*n* = 35)		High (*n* = 84)	Low (*n* = 163)	Negative (*n* = 156)	
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
EGFR	0 (0.00)	2 (3.17)	0 (0.00)	0.399	9 (10.71)	2 (1.23)	6 (3.85)	< 0.01
ALK	0 (0.00)	0 (0.00)	0 (0.00)	-	1 (1.19)	0 (0.00)	1 (0.64)	0.428
KRAS + STK11	0 (0.00)	0 (0.00)	0 (0.00)	-	1 (1.19)	1 (0.61)	1 (0.64)	0.867
PTEN	2 (9.09)	7 (11.11)	6 (17.14)	0.596	11 (13.10)	21 (12.88)	14 (8.97)	0.472
JAK1/2	0 (0.00)	1 (1.59)	0 (0.00)	0.634	4 (4.76)	0 (0.00)	0 (0.00)	< 0.001
MDM2/4	0 (0.00)	0 (0.00)	0 (0.00)	-	1 (1.19)	1 (0.61)	0 (0.00)	0.440
CCND1	1 (4.55)	6 (9.52)	5 (14.29)	0.483	11 (13.10)	24 (14.72)	23 (14.74)	0.930
Total	3 (13.64)	16 (25.39)	11 (31.43)	0.318	38 (45.24)	49 (30.05)	45 (28.84)	0.023

expression subgroups in early- and advanced-stage LUAD and LUSC, respectively. In the high PD-L1 expression group, rare genes, such as *NTRK2* and *ERBB3*, were more significantly present in I-III LUAD (Fig. 2A). In the low and negative PD-L1 expression group, most genes, such as *TP53*, *ATRX*, *ARID1A*, *RET*, *KEAP1*, *LRP1B*, and *RB1*, were more common in stage IV LUAD, while a few genes, such as *SETD2*, *RBM10*, and *EGFR*, were more significantly observed in I-III LUAD (Fig. 2B and C, Supplementary Table 3). In LUSC, most genes, such as *EPHA3*, *AFAF*, and *PIK3R2*, were more significantly expressed among different PD-L1 expression subgroups in I-III LUSC (Fig. 2D and F, Supplementary Table 3). Early-stage LUAD and LUSC patients with different PD-L1 expression levels had distinct genomic profiles compared with those of advanced patients.

Furthermore, we compared the genetic differences among different PD-L1 expression subgroups at different stages. We observed some similarities in differential genes between early- and advanced-stage LUAD patients. In both the I-III and IV phases of LUAD, *EGFR* was more prevalent in the PD-L1 negative group, and *TP53* was more prevalent in the high PD-L1 expression group (Fig. 3A and B). In addition, in the group with high PD-L1 expression in LUAD, driver gene variants such as *KRAS*, *MET*, and *BRAF* were more common, and the percentages of other genes, such as *LRP1B* and *ARID1A*, were also significantly higher. In stage I-III LUSC, the proportion of *NFE2L2* and *EPHA3* genes was higher in the group with high PD-L1 expression. In stage IV LUSC, *ARID1A* and *ERBB2* genes were more significantly observed in the group with high PD-L1 expression, while *MLL2* genes was more significantly observed in the group with negative PD-L1 expression. (Figure 3C and D). There were differences of differentially genes in LUSC between the early- and advanced-stage. The proportions of genes occurring in different PD-L1 expression subgroups at different stages are detailed in Supplementary Table 4.

Discussion

This is the largest study to date to comprehensively examine the clinical and molecular characteristics associations with PD-L1 expression levels in early-stage NSCLC. We revealed a significant relationship between PD-L1 expression and factors such as disease stage, pathologic subtype, age, gender, and smoking history. Additionally, early-stage patients showed lower rates of immunotherapy resistance genes, especially those with high PD-L1 expression. These findings highlight the high heterogeneity of NSCLC across stages and PD-L1 expression levels.

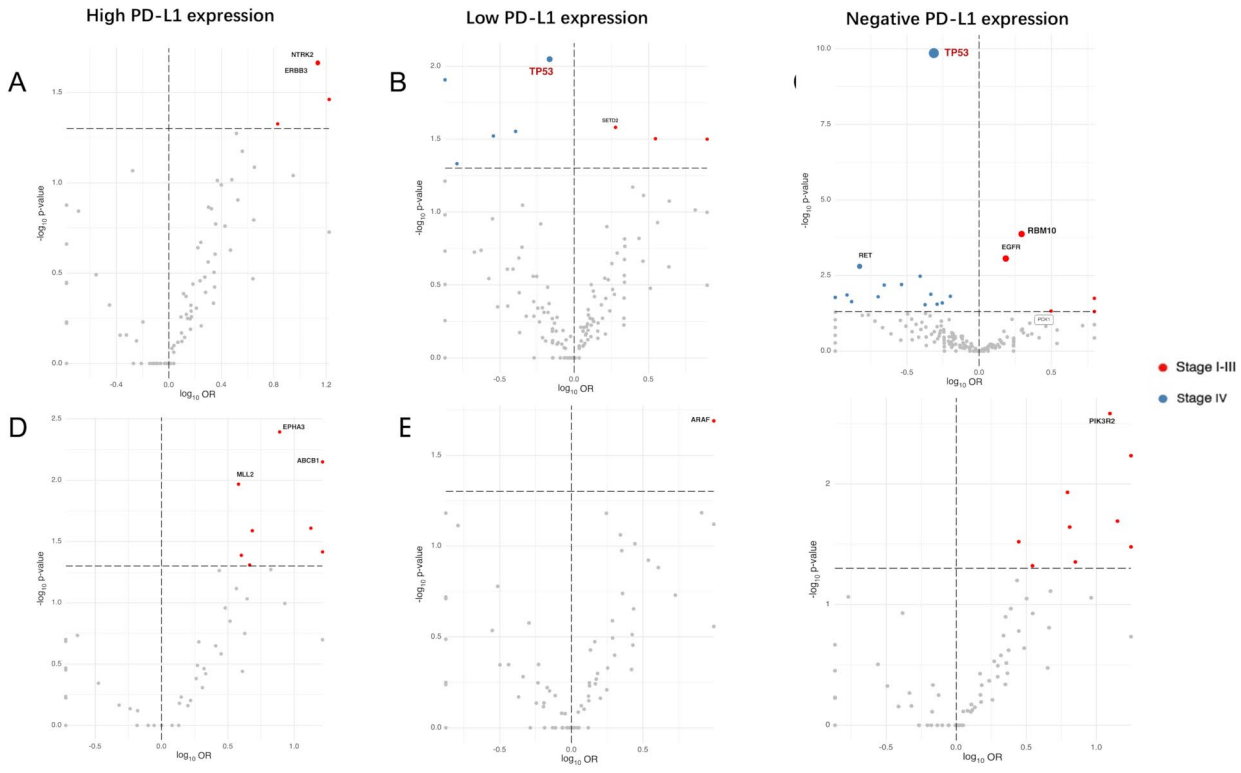

Fig. 2 Stage-specific genomic features of PD-L1-stratified LUAD and LUSC. **LUAD Subgroups**. **A**. High PD-L1 expression (TPS ≥ 50%). Stage I-III LUAD showed enrichment of *NTRK2* (I-III vs. IV: 4.92% vs. 0.38%, $p = 0.022$) and *ERBB3* (I-III vs. IV: 4.92% vs. 0.38%, $p = 0.022$) compared to stage IV. **B**. Low PD-L1 expression (TPS 1–49%). Stage IV tumors exhibited higher *TP53* mutations (I-III vs. IV: 49.62% vs. 59.51%, $p = 0.009$), while *SETD2* was more enrichment in Stage I-III (I-III vs. IV: 9.77% vs. 5.41%, $p = 0.026$). **C**. Negative PD-L1 expression (TPS < 1%). *TP53* (I-III vs. IV: 32.48% vs. 51.05%, $p < 0.001$) and *RET* (I-III vs. IV: 0.43% vs. 2.58%, $p = 0.001$) were more common in stage IV LUAD, while *RBM10* (I-III vs. IV: 16.45% vs. 9.29%, $p < 0.001$) and *EGFR* were more significant in I-III LUAD (I-III vs. IV: 64.74% vs. 55.14%, $p = 0.001$). **LUSC Subgroups**. **D**. High PD-L1 expression (TPS ≥ 50%). *EPHA3* (I-III vs. IV: 28.57% vs. 4.76%, $p = 0.004$), *ABCB1* (I-III vs. IV: 14.29% vs. 0.00%, $p = 0.007$) and *MLL2* were more significantly expressed in stage I-III (I-III vs. IV: 47.62% vs. 19.05%, $p = 0.011$). **E**. Low PD-L1 expression (TPS 1–49%). *AFAF* was more significantly expressed in stage I-III (I-III vs. IV: 4.84% vs. 0.00%, $p = 0.02$). **F**. Negative PD-L1 expression (TPS < 1%). *PIK3R2* and other genes were enriched in stage I-III (I-III vs. IV: 14.29% vs. 1.29%, $p = 0.003$). TPS, tumor proportion score; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma

Early-stage patients had fewer cases of high PD-L1 expression compared to advanced-stage patients. This might be because early tumors have not yet developed immune-evasion mechanisms. While PD-L1 expression is known to be influenced by oncogenic driver activation and tumor heterogeneity [14–16], emerging evidence highlights its dynamic evolution during disease progression. A longitudinal study ($n = 402$) demonstrated that 40.1% of patients exhibited increased PD-L1 expression at recurrence, while 32.8% showed significant decreases, while molecular alterations such as *STK11/B2M* copy number gains driving upregulation and *JAK2*/*CD274* loss causing downregulation [17]. These expression changes directly influence immunotherapy selection. In addition, studies have shown that in the latest test, patients with PD-L1 ≥ 1% had longer progression-free survival (PFS) compared to patients with PD-L1 < 1% (PFS: 3.7 vs. 1.6 months, $p = 0.01$) [17]. Studies such as IMpower010 have confirmed the predict value of PD-L1 status for immunotherapy outcomes [4]. These findings underscore the critical of reassessing PD-L1 status through rebiopsy at recurrence to enable personalized therapeutic strategies. For patients exhibiting PD-L1 upregulation (PD-L1 ≥ 1%), treatment should be tailored by integrating molecular features such as *STK11* and *JAK2* alterations, which may guide immunotherapy optimization. Conversely, those with PD-L1 downregulation require comprehensive genomic profiling to determine alternative therapies (targeted drugs or combination regimens).

While PD-L1 and TMB are both used to predict immunotherapy responses [18, 19], our study found no strong correlation between them. This finding aligns with previous reports in lung and other cancers, though some studies suggest a weak positive correlation in NSCLC [20]. A retrospective multicenter study demonstrated that NSCLC patients with PD-L1 expression ≥ 50% and TMB-H exhibited superior objective response rate (ORR) (57% vs. 8.7% in dual-low) and OS (47.7 vs. 10.4 months) with immunotherapy [21]. These data indicate that combined biomarker analysis (PD-L1 + TMB) may better

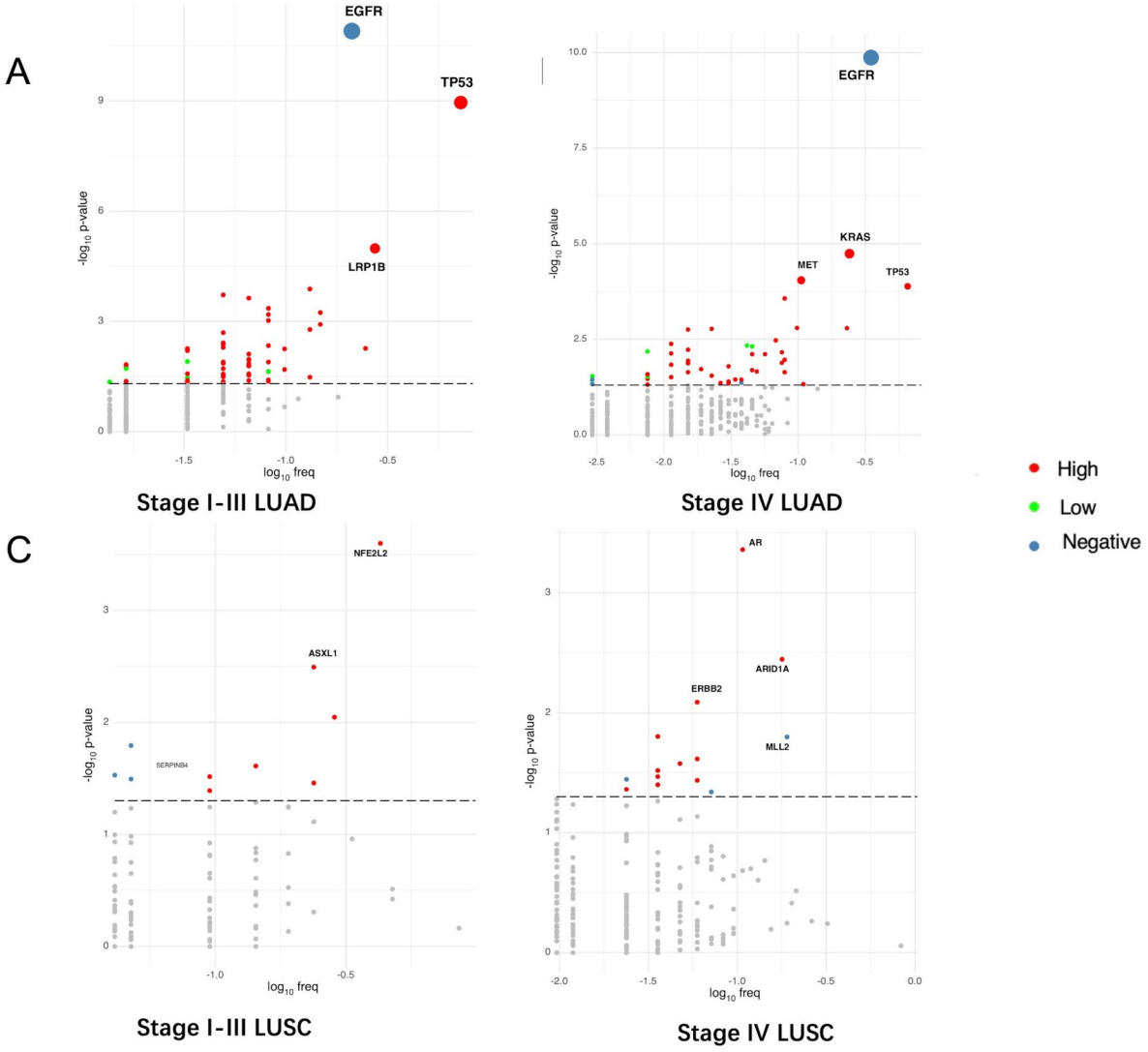

Fig. 3 PD-L1-associated genomic signatures across NSCLC subtypes and stages. **LUAD Subgroups**. **A**. Early-stage (I-III). *TP53* and *LRP1B* were more significantly expressed in high PD-L1 expression subgroup (*TP53*: High: 68.85% vs. Low: 49.62% vs. Negative: 32.48%, $p < 0.001$; *LRP1B*: High: 27.87% vs. Low: 17.29% vs. Negative: 8.97%, $p < 0.001$), while *EGFR* was more common in negative PD-L1 expression subgroup (High: 19.67% vs. Low: 51.13% vs. Negative: 64.74%, $p < 0.001$). **B**. Advanced stage (IV). *TP53*, *KRAS* and *MET* were more significantly expressed in high PD-L1 expression subgroup (*TP53*: High: 64.15% vs. Low: 59.51% vs. Negative: 51.05%, $p < 0.001$; *KRAS*: High: 23.02% vs. Low: 13.44% vs. Negative: 11.03%, $p < 0.001$; *MET*: High: 10.19% vs. Low: 4.19% vs. Negative: 3.22%, $p < 0.001$), while *EGFR* was also observed to be associated with negative PD-L1 expression (High: 31.70% vs. Low: 52.53% vs. Negative: 55.14%, $p < 0.001$). **C**. Early-stage (I-III). The proportion of *NFE2L2* and *ASXL1* genes was higher in the group with high PD-L1 expression (*NFE2L2*: High: 42.86% vs. Low: 11.29% vs. Negative: 2.86%, $p < 0.001$; *ASXL1*: High: 23.81% vs. Low: 1.61% vs. Negative: 11.43%, $p = 0.003$). **D**. Advanced stage (IV). *AR* and *ARID1A* genes were more significantly expressed in the group with high PD-L1 expression (*AR*: High: 10.71% vs. Low: 2.47% vs. Negative: 0.65%, $p < 0.001$; *ARID1A*: High: 17.86% vs. Low: 8.64% vs. Negative: 4.52%, $p = 0.004$), while *MLL2* genes was more significantly expressed in the group with negative PD-L1 expression (*MLL2*: High: 19.05% vs. Low: 30.25% vs. Negative: 36.77%, $p = 0.016$). Circles represent PD-L1 expression levels: red (high), green (low), blue (negative). NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma

stratify patients for immunotherapy, particularly among dual-high patients.

EGFR activating mutations are the most common driver mutations in NSCLC, particularly in LUAD [22]. Our study showed that *EGFR* mutations were more frequent in patients with negative PD-L1 expression, aligning with previous research that supports targeted therapies may be a preferable option for this subgroup [23]. Furthermore, multiple clinical trials (CheckMate-012, KEYNOTE-001) have demonstrated that *EGFR*-mutant NSCLC have shown poor response to PD-1/PD-L1 monotherapy [24, 25]. This may occur because *EGFR* mutations weaken immune responses by upregulating PD-L1 expression and suppressing T-cell activity [26]. These findings validate guideline recommendations that prioritize targeted therapy regardless

of PD-L1 status. In contrast, *TP53* mutations correlate with elevated PD-L1 expression and better immunotherapy responses, demonstrated by a 51.2% ORR in *TP53*-mutant NSCLC versus 20.7% in wild-type patients [27–29]. Other actionable genomic alterations including *KRAS and MET* also exhibit significant association with PD-L1 upregulation. In the KRYSTAL-7 study, patients with *KRAS* G12-mutant advanced NSCLC receiving combined adagrasib and pembrolizumab demonstrated a robust ORR of 63% in the PD-L1 ≥ 50% subgroup ($n = 51/148$) [30]. Notably, evidence indicates that *KRAS*-mutant tumors with co-occurring *TP53* mutations exhibit significantly improved immunotherapy efficacy (ORR: 55.4% vs. 39.5%) [31]. Furthermore, *MET*-mutant NSCLC exhibits heightened immunogenicity through increase tumor-infiltrating lymphocytes (TILs) [32]. While *MET* tyrosine kinase inhibitors (TKIs) remain first-line therapy for *METex14* mutations, the dual targeting of *MET* and PD-1 demonstrated clinical activity in pretreated patients with advanced *EGFR* wild-type NSCLC, independent of *MET* status [33]. These findings suggesting that incorporating information on these genetic variations into clinical practice may enable more precise selection of patients suitable for immunotherapy, particularly those with PD-L1 ≥ 50%.

The widespread adoption of NGS in clinical practice has revealed extensive genetic heterogeneity in NSCLC, with co-occurring mutations significantly influencing response to immune checkpoint inhibitors (ICIs). While most studies historically focused on single-gene biomarkers (e.g., *EGFR*, *TP53*), emerging evidence highlights the critical role of multi-gene interactions in shaping ICIs outcomes [34]. A retrospective analysis of 1745 patients showed that co-mutations such as *KRAS* + *TP53* predict ICIs efficacy more better than individual biomarkers, independent of TMB and PD-L1 expression [35]. To address this, multi-gene profiling (e.g., *TP53*, *KRAS* and *MDM2/4*) is essential for risk stratification. For instance, a phase 2 trial in resectable *EGFR*-mutant NSCLC revealed that 44% patients achieved major pathological response (MPR) with neoadjuvant immunotherapy plus chemotherapy, with *TP53* missense mutations, *RB1* and *RBM10* mutations enriched in responders [36]. Conversely, *MDM2/4* amplifications correlate with an increased risk of hyperprogressive disease (HPD) receiving immune checkpoint inhibitors [8, 37]. Notably, our study revealed that 13.64–31.15% of early-stage cases with high PD-L1 expression harbored immunotherapy resistance genes such as *MDM2*/*4* amplifications. These findings collectively emphasize the critical need of integrating multi-gene molecular signatures (e.g., *TP53* and *RBM10* for immunotherapy sensitivity, *MDM2/4* for resistance) into clinical practice. Future research should prioritize prospective multi-center, randomized controlled trials (RCTs) to validate their predictive utility of co-mutation profiles (*KRAS* + *TP53*) and resistance markers (*MDM2/4* amplifications), particularly in PD-L1-high NSCLC subgroups. Given the high prevalence of immunotherapy resistance mechanisms adaptive therapeutic strategies integrating ICIs must be developed to overcome therapy resistance. Concurrently, implementing real-time resistance surveillance frameworks—combining serial liquid biopsy (ctDNA) and standardized multi-gene panels (*TP53*/*KRAS*/*STK11*/*MDM2/4*)—will enable dynamic mapping of clonal trajectories and early detection of acquired alterations during perioperative immunotherapy, thereby closing the loop between biomarker discovery and precision intervention.

Our research has several limitations. First, the retrospective design introduces potential selection bias, particularly in sample selection due to heterogeneous treatment regimens across patients. Future prospective multi-center studies should implement standardized therapeutic protocols with centralized biomarker assessment. Second, detailed clinical outcome data (e.g., PFS, OS or ORR) were not systematically recorded, limiting direct correlations between genomic features and immunotherapy efficacy. Additionally, the sample size for early-stage NSCLC, particularly PD-L1-high subgroups, remains modest, necessitating validation in larger cohorts. Multi-institutional consortia could aggregate larger early-stage NSCLC cohorts. Despite these constraints, to our knowledge, this is the first real-world study to comprehensively compare the genomic characteristics of NSCLC patients with varying PD-L1 expression leves across early- and advanced stage. Prospective multi-center studies integrating standardized treatment protocols and longitudinal outcome tracking are warranted to validate and refine predictive biomarkers for immunotherapy in early-stage NSCLC. In conclusion, it is hoped that our results provide a comprehensive understanding of the genomic landscape of early-stage NSCLC.

Conclusions

In summary, significant differences in PD-L1 expression, TMB, and clinical characteristics are observed between early-stage and advanced NSCLC patients. Furthermore, the prevalence of immune-related genes and genomic profiles vary substantially among patients with different PD-L1 expression levels. These findings underscore the importance of conducting comprehensive genetic evaluations before immunotherapy in both early-stage and advanced NSCLC patients.

Abbreviations

CNVs	Copy number variants
CPS	Combined positive score
DFS	Disease free survival
FDA	Food and Drug Administration

ICIs	Immune checkpoint inhibitors
IHC	Immunohistochemical
InDels	Insertions and deletions
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
NCCN	National Comprehensive Cancer Network
NSCLC	Non-small cell lung cancer
ORR	Objective response rates
PD-1	Programmed death receptor 1
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
SNVs	Single nucleotide variants
SVs	Structural variants
TCs	Tumor cells
TMB-H	Tumor mutation burden-high
TNB	Tumor neoantigen burden
TNM	Tumor node metastasis
TPS	Tumor proportion score

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-025-03687-w.

Supplementary Material 1

Acknowledgements
We thank all the medical staff who contributed to the maintenance of the medical record database.

Author contributions
All authors have contributed to the study conception and design. FW and SL: Conceptualization, Project administration. YC: writing—original draft preparation, Project administration. PW: Data curation, formal analysis. RL: Data curation, writing—review and editing. MY and PY: Methodology. HH, PC, HZ, WC, DZ, HL: Software, Data curation, Visualization. All the authors have browsed and agreed with the final manuscript.

Funding
This study was supported by the National Natural Science Foundation of China (Grant No. 82002497) and the Science and Technology Program of Fujian Province (Nos. 2020J05072, 2020J011125, and 2021J01442).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Institutional review board statement
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Fujian Cancer Hospital (No. SQ2022-116).

Informed consent
Informed consents were obtained from all subjects involved in the study.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Thoracic Oncology Surgery, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, No. 420 Fuma Road, Jin'an District, Fuzhou 350011, Fujian Province, China
[2]Fujian Key Laboratory of Translational Cancer Medicine, Fuzhou 350011, China
[3]Fujian Provincial Key Laboratory of Tumor Biotherapy, Fuzhou 350011, China
[4]Beijing GenePlus Technology Co., Ltd., Beijing, China

Received: 30 November 2023 / Accepted: 28 April 2025
Published online: 07 May 2025

References

1. Forde PM, Spicer J, Lu S, Provencio M, Mitsudomi T, Awad MM, et al. Neoadjuvant nivolumab plus chemotherapy in resectable lung cancer. N Engl J Med. 2022;386(21):1973–85.
2. Felip E, Altorki N, Zhou C, Vallieres E, Martinez-Marti A, Rittmeyer A, et al. Overall survival with adjuvant atezolizumab after chemotherapy in resected stage II-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase III trial. Ann Oncol. 2023;34(10):907–19.
3. O'Brien M, Paz-Ares L, Marreaud S, Dafni U, Oselin K, Havel L, et al. Pembrolizumab versus placebo as adjuvant therapy for completely resected stage IB-IIIA non-small-cell lung cancer (PEARLS/KEYNOTE-091): an interim analysis of a randomised, triple-blind, phase 3 trial. Lancet Oncol. 2022;23(10):1274–86.
4. Felip E, Altorki N, Zhou C, Csoszi T, Vynnychenko I, Goloborodko O, et al. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. Lancet. 2021;398(10308):1344–57.
5. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823–33.
6. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med. 2017;376(25):2415–26.
7. Gainor JF, Shaw AT, Sequist LV, Fu X, Azzoli CG, Piotrowska Z, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. Clin Cancer Res. 2016;22(18):4585–93.
8. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after immunotherapy: analysis of genomic alterations associated with accelerated growth rate. Clin Cancer Res. 2017;23(15):4242–50.
9. Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. Cancer Discov. 2018;8(7):822–35.
10. Wang S, Fang Y, Jiang N, Xing S, Li Q, Chen R, et al. Comprehensive genomic profiling of rare tumors in China: routes to immunotherapy. Front Immunol. 2021;12:631483.
11. Li H, Meng L, Wang H, Cui L, Sheng H, Zhao P et al. Precise identification of somatic and germline variants in the absence of matched normal samples. Brief Bioinform. 2024;26(1).
12. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, 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(10183):1819–30.
13. Lin G, Fan X, Zhu W, Huang C, Zhuang W, Xu H, et al. Prognostic significance of PD-L1 expression and tumor infiltrating lymphocyte in surgically resectable non-small cell lung cancer. Oncotarget. 2017;8(48):83986–94.
14. Zens P, Bello C, Scherz A, von Gunten M, Ochsenbein A, Schmid RA, et al. The effect of neoadjuvant therapy on PD-L1 expression and CD8 + lymphocyte density in non-small cell lung cancer. Mod Pathol. 2022;35(12):1848–59.
15. Jardim DL, Murugesan K, Elvin JA, Huang RSP, Kurzrock R. PD-L1 gene amplification and focality: relationship with protein expression. J Immunother Cancer. 2023;11(2).
16. John N, Schlintl V, Sassmann T, Lindenmann J, Fediuk M, Wurm R et al. Longitudinal analysis of PD-L1 expression in patients with relapsed NSCLC. J Immunother Cancer. 2024;12(4).
17. Di Federico A, Alden SL, Smithy JW, Ricciuti B, Alessi JV, Wang X, et al. Intrapatient variation in PD-L1 expression and tumor mutational burden and the impact on outcomes to immune checkpoint inhibitor therapy in patients with non-small-cell lung cancer. Ann Oncol. 2024;35(10):902–13.
18. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-Ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol. 2018;36(7):633–41.
19. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell. 2018;33(5):843–52. e4.

20. Yarchoan M, Albacker LA, Hopkins AC, Montesion M, Murugesan K, Vithayathil TT et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight. 2019;4(6).
21. Error in figure axis label. JAMA Oncol. 2022;8(11):1702.
22. Shi Y, Li J, Zhang S, Wang M, Yang S, Li N, et al. Molecular epidemiology of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology - Mainland China subset analysis of the PIONEER study. PLoS ONE. 2015;10(11):e0143515.
23. Li N, Zuo R, He Y, Gong W, Wang Y, Chen L, et al. PD-L1 induces autophagy and primary resistance to EGFR-TKIs in EGFR-mutant lung adenocarcinoma via the MAPK signaling pathway. Cell Death Dis. 2024;15(8):555.
24. Gettinger S, Rizvi NA, Chow LQ, Borghaei H, Brahmer J, Ready N, et al. Nivolumab monotherapy for first-line treatment of advanced non-small-cell lung cancer. J Clin Oncol. 2016;34(25):2980–7.
25. Garon EB, Hellmann MD, Rizvi NA, Carcereny E, Leighl NB, Ahn MJ, 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(28):2518–27.
26. Dong Y, Khan L, Yao Y. Immunological features of EGFR-mutant non-small cell lung cancer and clinical practice: a narrative review. J Natl Cancer Cent. 2024;4(4):289–98.
27. Assoun S, Theou-Anton N, Nguenang M, Cazes A, Danel C, Abbar B, et al. Association of TP53 mutations with response and longer survival under immune checkpoint inhibitors in advanced non-small-cell lung cancer. Lung Cancer. 2019;132:65–71.
28. Yu J, Ling S, Hong J, Zhang L, Zhou W, Yin L et al. TP53/mTORC1-mediated bidirectional regulation of PD-L1 modulates immune evasion in hepatocellular carcinoma. J Immunother Cancer. 2023;11(11).
29. Saw SPL, Ng WP, Zhou S, Lai GGY, Tan AC, Ang MK, et al. PD-L1 score as a prognostic biomarker in Asian early-stage epidermal growth factor receptor-mutated lung cancer. Eur J Cancer. 2023;178:139–49.
30. Garassino MC, Theelen WSME, Jotte R, Laskin J, de Marinis F, Aguado C, et al. LBA65 KRYSTAL-7: efficacy and safety of adagrasib with pembrolizumab in patients with treatment-naïve,advanced non-small cell lung cancer (NSCLC) harboring a KRASG12C mutation. Annals of Oncology. 2023;34:S1309-S10.
31. Bischoff P, Reck M, Overbeck T, Christopoulos P, Rittmeyer A, Luders H, et al. Outcome of first-line treatment with pembrolizumab according to KRAS/TP53 mutational status for nonsquamous programmed death-ligand 1-high (>/=50%) NSCLC in the German National Network Genomic Medicine Lung Cancer. J Thorac Oncol. 2024;19(5):803–17.
32. Yoshimura K, Inoue Y, Tsuchiya K, Karayama M, Yamada H, Iwashita Y, et al. Elucidation of the relationships of MET protein expression and gene copy number status with PD-L1 expression and the immune microenvironment in non-small cell lung cancer. Lung Cancer. 2020;141:21–31.
33. Felip E, Metro G, Tan DSW, Wolf J, Mark M, Boyer M, et al. Capmatinib plus nivolumab in pretreated patients with EGFR wild-type advanced non-small cell lung cancer. Lung Cancer. 2024;192:107820.
34. Budczies J, Romanovsky E, Kirchner M, Neumann O, Blasi M, Schnorbach J, et al. KRAS and TP53 co-mutation predicts benefit of immune checkpoint blockade in lung adenocarcinoma. Br J Cancer. 2024;131(3):524–33.
35. Zhang F, Wang J, Xu Y, Cai S, Li T, Wang G, et al. Co-occurring genomic alterations and immunotherapy efficacy in NSCLC. NPJ Precis Oncol. 2022;6(1):4.
36. Zhang C, Sun YX, Yi DC, Jiang BY, Yan LX, Liu ZD, et al. Neoadjuvant sintilimab plus chemotherapy in EGFR-mutant NSCLC: phase 2 trial interim results (NEOTIDE/CTONG2104). Cell Rep Med. 2024;5(7):101615.
37. Song Q, Han L, Mao Y, Huang T. An investigation on biomarkers of hyperprogressive disease after PD-1/PDL-1 therapies in Chinese non-small cell lung cancer patients. J Clin Oncol. 2021;39(15suppl):e21173–e.

Publisher's note