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Original Article

Effect on Non-Small Cell Lung Cancer After Combination of Driver Gene Mutations and Anti-PD -1/PD-L1 Immunotherapy as Well as Chemotherapy

Zhengming Huang, Long Yu, *Weisong Chen, Dan Zhu, *Hui Chen

Pulmonary and Critical Care Medicine, Jinhua Municipal Central Hospital, Zhejiang University, Zhejiang, China

***Corresponding Author:** Email: zhudan4252@sina.com

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Abstract

Background: We aimed to reveal the correlation between pathological indicators and PD-L1, between gene mutation status in lung cancer through clinico-pathological data and lung cancer-related gene mutation and PD-L1 expression analysis.

Methods: The study was conducted in Jinhua Municipal Central Hospital, Zhejiang, China from 2017 to 2022. PD-L1 testing and targeted gene mutations detection were evaluated. The clinical characteristics of these non-small cell lung cancer (NSCLC) samples have been obtained. The groups (LUAD, n=142; LUSC, n=143) were grouped according to clinico-pathological features and PD-L1 expression (Yes/No or High/Low), and the clinico-pathological and genetic and molecular features and correlation with PD-L1 expression were compared across the above groups. Comparisons and analyses were made between different treatment schemes.

Results: Lung adenocarcinoma (LUAD, n=142) and lung squamous carcinoma (LUSC, n=143) samples were enrolled (median age: 64 years old). Pleural invasion and M staging were significantly different from PD-L1 alterations (P<0.05). The percentage of patients with PD-L1 tumor proportion score (TPS)≥50% was 36.24% and the percentage of patients with PD-L1 TPS<50% was 29.53%. The percentage of patients with PD-L1 high-expressed and treated by immunotherapy was 75.93% and 63.41% experienced Partial Response/Complete Response. The mutations ratio of *EGFR*, *ALK*, *KRAS*, *MET*, *RET* and *TP53* were 28.86%, 1.34%, 6.04%, 0.67%, 1.34% and 0.67%, respectively. *KRAS* mutation was significantly different from PD-L1 alterations (P<0.01).

Conclusion: There are individual differences in PD-L1 expression, which can also vary depending on the different clinical features. Specific molecular features correlate with differential PD-L1 expression and may influence the response to therapy.

Keywords: Non-small cell lung cancer (NSCLC); Gene mutations; Prognostic analysis; Immunotherapy

Introduction

NSCLC accounts for 80-85% of lung cancers and is the most common histological type of lung cancer (1). Tumor cells ≥50% were defined as high PD-L1 expression, which correlates with

immune checkpoint inhibitor (ICI) response; for advanced lung adenocarcinoma patients, PD-L1 detection was the only biomarker (approved by FDA) used for anti-PD-1 therapy (2,3). ICI Treatment has been shown to improve tumor response and survival rate in advanced patients, however, patients with high PD-L1 expression did not respond to ICI (4). Therefore, it is urgent to solve the relationship through clinico-pathological data and lung cancer-related gene mutation and PD-L1 expression analysis.

Pembrolizumab has been reported to produce impressive clinical outcomes in PD-L1-positive advanced NSCLC patients (5). For example, patients with NSCLC treated with PD-1 inhibitors such as nivolumab and pembrolizumab had improved ORR and survival rates, as did patients with strong PD-L1 positivity to pembrolizumab (6). However, most studies excluded some specific patients, such as the case of *EGFR* mutations (7). This suggests us that there are still many gaps in whether *EGFR* mutated NSCLC patients with high PD-L1 expression are associated with ICI response, and whether other mutations are associated to NSCLC? remains unclear.

Along with the increasing development of medical technology and the improvement of detection methods of genes, the research on tumor driver genes is being gradually studied and confirmed (8). For advanced lung adenocarcinoma population, testing mutations and rearrangements of *EGFR*, *ALK*, and *ROS1* have been included the routine assessment analysis (9). Notably, studies on driver-gene have also identified PD-L1 expression associated with *EGFR*, *ALK* and *KRAS* mutation in lung cancer (10).

Therefore, we aimed to explore the correlation with NSCLC immunotherapy and chemotherapy by analyzing PD-L1 and typing analysis of *EGFR*, *ALK* and *ROS1* gene mutations. Our study contributes to the development of treatment regimens in groups with high PD-L1 levels and gene mutations, tracking the course of the disease and exploring variations in survival.

Patients and Methods

Samples Source and treatment

Advanced NSCLC patients at the Jinhua Municipal Central Hospital, Jinhua, China, from 2017 to 2022, were enrolled (among them those receiving anti-PD-1 therapy). Pathological data (including age, gender, histology, disease status, mutated genes, treatment and survival) were collected about the included cases.

The first line treatment/second line treatment/after line treatment: tislelizumab or pembrolizumab; 74 NSCLC cases. The treatment plan is as follows: a. metastatic NSCLC; TPS score of PD-L1 ≥ 50%; continuous Tislelizumab, Pembrolizumab or other therapy (including surgical treatment, radiotherapy, chemotherapy and targeting therapy).

Next-Generation Sequencing and Analysis

The genes that were tested include: *AKT1*, *BRAF*, *CTNNB1*, *EGFR*, *FGFR1*, *KRAS*, *MAP2K1*, *PIK3CA*, *PTEN*, *SMAD4* and so on. The assay was high-throughput sequencing, genomic DNA was obtained from tissues of NSCLC samples, and then sequenced and analyzed after library construction. Mutation profiles were mapped by analyzing somatic cell substitutions, single nucleotide change occupancy and base coverage status. Annotation, DNA translocation analysis and CNVs analysis were performed according to the SNP database using Tophat2 and Factera and sequencing depth, respectively.

Hematoxylin-eosin (H & E) stain and Immunohistochemical examination

H&E staining is mainly a process of hematoxylin-eosin staining of sectioned specimens that have been sequentially treated with 10% neutral formalin buffer and fixed in paraffin. The above-prepared sections can be assayed for PD-L1 expression in NSCLC samples by the addition of PD-L1 antibody (22C3 pharmDx, Agilent Technologies) (set up positive control and negative control groups). IHC assay scoring settings for

PD-L1: from 0% to 100%, determined by the percentage of tumor cells stained by PD-L1 antibody. The definition of PD-L1 positive: at least 1% of tumor cells stained by PD-L1 antibody; the definition of PD-L1 high expression: at least 50% of tumor cells stained by PD-L1 antibody.

Statistical analysis

Clinical outcomes were measured by objective response rate (ORR) and response time, and univariate and multivariate analyses were performed mainly by stepwise selection of covariates. Different histopathological features were also compared. The enrolled samples were grouped by combining PD-L1 levels and mutated genes. Fisher's exact test was used for comparison of variables. $P < 0.05$ in the analysis was considered statistically significant.

Results

Clinical features

Basic clinical information of 300 lung cancer samples showed that ratio of men patients to women patients was nearly 3:1, the average age was 64.29 SD8.7, and the ratio of patients over 65 years old and under 65 years old was about 1:1 in this study. The number of patients with LUSC and LUAD was 143 and 142, respectively. Other pathological information, included smoking history, tumor size, tumor stage, and lymph node metastasis, was displayed in Table 1.

Table 1: The tumour and patient characteristics

Characteristic	*Value*
Age, years (Mean, range)	64.2 SD8.70 (39-90)
<65, n(%)	147 (49.00%)
≥65, n(%)	153 (51.00%)
Gender, n(%)	
Men	214 (71.33%)
Women	86 (28.67%)
Smoking History, n(%)	
Current smoker	122 (40.67%)
Ex-smoker	53 (17.67%)
Never smoker	125 (41.67%)
Histology, n(%)	
Adenocarcinoma	142 (47.33%)
Squamous cell carcinoma	143 (47.67%)
mixed adeno-squamous	6 (2.00%)
Large cell carcinoma	4 (1.33%)
Non-small cell lung cancer	1 (0.33%)
Lymphoepithelioma	1 (0.33%)
Neuroendocrine carcinoma	1 (0.33%)
Small-cell lung carcinoma	2 (0.67%)
Tumor size (cm), n(%)	
0.4*0.4	1 (0.33%)
<2*2	53 (17.67%)
<4*4	118 (39.33%)
<6*6	90 (30.00%)
≥6*6	25 (8.33%)
Other	13 (4.33%)
Stage, n(%)	
I	16 (5.33%)
II	39 (13.00%)

Table 1: Continued…

III	96 (32.00%)
IV	148 (49.33%)
Other	1 (0.33%)
T Stage, n(%)	
T1	65 (21.67%)
T2	81 (27.00%)
T3	53 (17.67%)
T4	90 (30.00%)
Tx	10 (3.33%)
Other	1 (0.33%)
N Stage, n(%)	
N0	39 (13.00%)
N1	61 (20.33%)
N2	124 (41.33%)
N3	68 (22.67%)
Tx	7 (2.33%)
Other	1 (0.33%)
M Stage, n(%)	
M0	150 (50.00%)
M1	149 (49.67%)
Other	1 (0.33%)
Pleural invasion, n(%)	
Absent	211 (70.33%)
Present	89 (29.67%)
Lymphatic invasion, n(%)	
Absent	48 (16.00%)
Present	252 (84.00%)
Meningeal invasion, n(%)	
Absent	266 (88.67%)
Present	34 (11.33%)

Specific characteristics of LUAD and LUSC

According to pathological types, 142 cases of LUAD and 143 cases of LUSC were counted (mean age: 63.37; 39-87 years). Briefly, of LUAD samples, there were a total of 70 men and 72 women. Approximately 62.68% of the patients were never smokers. Tumor size was mainly concentrated in <2*2 and <4*4. Patients in stage I/II/III/IV were 4, 6, 31 and 101. Up to 92% of patients in the III/IV stages. The proportion with lymphatic invasion was very high, followed by pleural invasion and meningeal invasion (Table 2). Then, of LUSC samples (mean age: 65.09; 39-90 years), there were a total of 134 men and 9 women. Approximately 20.98% of the patients were never smokers. Tumor size was mainly concentrated in <4*4 and <6*6. Patients in stage I/II/III/IV were 8, 32, 59 and 43. Up to 71% of patients in the III/IV stages. Also, the proportion with lymphatic invasion was very high, followed by pleural invasion and meningeal invasion (Table 2). The proportion of PD-L1+ in LUAD and LUSC was similar.

Table 2: Clinicopathological characteristics by Histology

Variables		*Adenocarcinoma (LUAD)*		*Squamous cell carcinoma (LUSC)*	
		Number of Cases	%	Number of Cases	%
Age (yr)	Mean (range)	63.37 (39-87)		65.09 (39-90)	
Gender	Men	70	49.30	134	93.71
	Women	72	50.70	9	6.29
Smoking status	Current smoker	44	30.99	75	52.45
	Ex-smoker	9	6.34	38	26.57
	Never smoker	89	62.68	30	20.98
Tumor size (cm)	0.4*0.4	1	0.70	0	0
	<2*2	31	21.83	19	13.29
	<4*4	62	43.66	49	34.27
	<6*6	33	23.24	51	35.66
	≥6*6	8	5.63	20	14.08
	Other	7	4.93	4	2.80
Tumor Stage	I	4	2.82	8	5.60
	II	6	4.23	32	22.38
	III	31	21.83	59	41.26
	IV	101	71.13	43	30.07
	Other	0	0	1	0.70
T staging	T1	42	29.58	17	11.89
	T2	32	22.54	45	31.47
	T3	17	11.97	34	23.78
	T4	44	30.99	44	30.77
	Tx	7	4.93	2	1.40
	Other	0	0	1	0.70
N staging	N0	9	6.34	25	17.48
	N1	10	7.04	48	33.57
	N2	72	50.70	45	31.47
	N3	45	31.69	23	16.08
	Nx	6	4.23	1	0.70
	Other	0	0	1	0.70
M staging	M0	40	28.17	99	69.23
	M1	102	71.83	43	30.07
	Other	0	0	1	0.70
Pleural invasion	Absent	77	54.23	122	85.31
	Present	65	45.77	21	14.69
Lymphatic invasion	Absent	12	8.45	30	20.98
	Present	130	91.55	113	79.02
Meningeal invasion	Absent	118	83.10	135	94.41
	Present	24	16.90	8	5.60
PD-L1 expression	<1%	33	23.24	17	11.89
	1-49%	23	16.20	21	14.69
	≥50%	23	16.20	18	12.59
	Undetected	63	44.37	87	60.84

Relationship between clinical features and PD-L1 expression

According to PD-L1 level: high, intermediate and negative, the relationships among PD-L1 level and age, gender, smoking-history, pathological type, stage, metastasis, pleural invasion, venous invasion and lymphatic invasion were gathered (Table 3). The result uncovered that PD-L1 was

remarkable different with pleural invasion (P<0.05) and significantly different with M staging (P<0.01). PD-L1 level did not differ by age, gender, smoking-history, pathological type, T staging, N staging, lymphatic invasion and meningeal invasion. Of all lung tumor specimens detected for PD-L1 analyzed, PD-L1 TPS ≥50%: 54 (54/149=36.24%); PD-L1 TPS <50%: 44 (44/149=29.53%) (Table 3). The main treatment methods (Surgery; Chemotherapy; Targeted Therapy; Radiotherapy and Immunotherapy) and efficacy (c: Complete Response/PR: Partial Response/SD: Stable Disease/PD: Progressive Disease) in PD-L1 high group, PD-L1 intermediate group and PD-L1 negative group were analyzed. The results uncovered that 75.93% (41/54) cases with PD-L1 high-expressed was treated by immunotherapy and 63.41% (26/41) experienced PR or CR among them (Fig. 1A). About 29.55% (13/44) with PD-L1 intermediate-expressed was treated by immunotherapy and 69.23% (9/13) experienced PR or CR among them (Fig. 1B). There were 12 (12/51=23.53%) samples of patients with negative PD-L1 test results, and radiotherapy and chemotherapy combined with immune treatment were selected, among which 7 (7/12=58.33%) patients showed PR or CR (Fig. 1C).

Table 3: Clinical features and PD-L1 expression

PD-L1 Expression		***High (n=54)***		***Intermediate (n=44)***		***Negative (n=51)***		***P value***
n=149		No.	%	No.	%	No.	%	
Age (Mean, range)		65.17	39-90	64.80	48-83	63.62	43-83	0.726
Gender	Men	39	72.22	29	65.91	36	70.59	0.880
	Women	15	27.78	15	34.09	15	29.41	
Smoking status	Current	22	40.74	17	38.64	22	43.14	0.893
	Ex	12	22.22	8	18.18	9	17.65	
	Never	20	37.04	19	43.18	20	39.22	
Pathological type	LUAD	25	46.30	23	52.27	33	64.71	0.074
	LUSC	26	48.15	21	47.73	17	33.33	
	Other	3	5.56	0	0	1	1.96	
Tumor stage	I/II	6	11.11	4	9.09	2	3.92	0.181
	III/IV	48	88.89	40	90.91	49	96.08	
T staging	T1/T2	29	53.70	20	45.45	21	41.18	0.789
	T3/T4	25	46.30	24	54.55	30	58.82	
N staging	N0	4	7.41	7	15.91	1	1.96	0.181
	N1/N2/N3	50	92.59	37	84.09	50	98.04	
M staging	Absent	19	35.19	26	59.09	11	21.57	0.004**
	Present	35	64.81	18	40.91	40	78.43	
Pleural invasion	Absent	33	61.11	32	72.73	24	47.06	0.028*
	Present	22	40.74	12	27.27	27	52.94	
Lymphatic invasion	Absent	7	12.96	9	20.45	3	5.88	0.070
	Present	47	87.04	35	79.55	48	94.12	
Meningeal invasion	Absent	47	87.04	38	86.36	45	88.24	0.794
	Present	7	12.96	6	13.64	6	11.76	

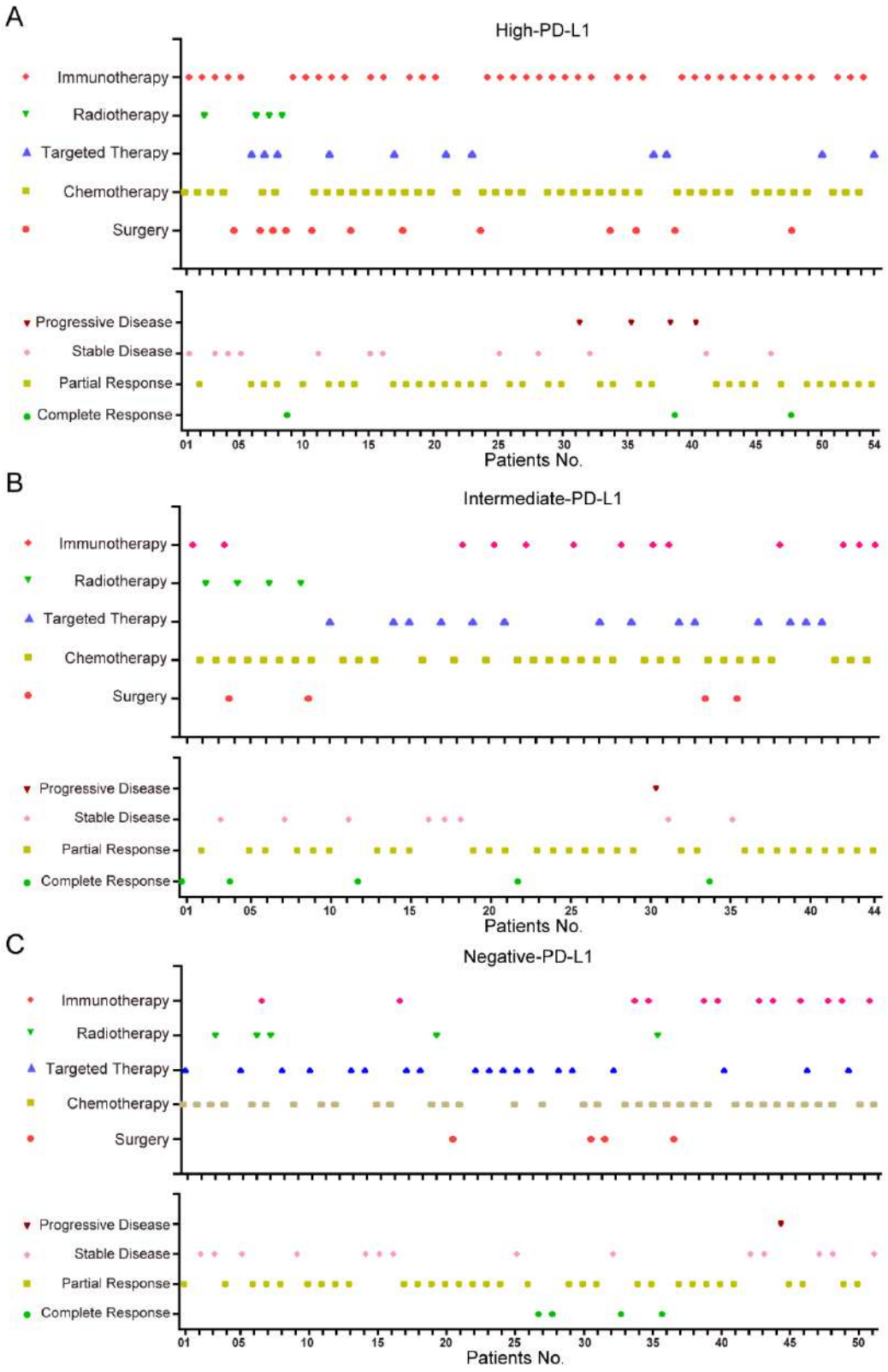

Fig. 1: The main treatment methods and efficacy in three PD-L1 expression group: High-PD-L1 (A), Intermediate-PD-L1 (B) and Negative-PD-L1 (C)

PD-L1 expression in LUAD and LUSC

PD-L1 level of the lung cancer samples tested by immunohistochemistry (IHC) varies from high to low. Grouping by LUAD, LUSC and advanced stage (III/IV stage), representative images of PD-L1 detected by IHC were presented according to different PD-L1. Fig. 2 showed the immunohistochemical identification results of negative PD-L1 expression, less than 20% PD-L1 expression, 20%-60% PD-L1 expression and PD-L1 expression > 60% in LUAD. Fig. 3 displayed the immunohistochemical identification results of negative PD-L1 expression, less than 20% PD-L1 expression and 20%-60% PD-L1 expression in LUSC. Fig. 4 showed the immunohistochemical identification results of negative PD-L1 expression, less than 20% PD-L1 expression, 20%-65% PD-L1 expression and more than 65% PD-L1 expression in advanced lung cancer samples.

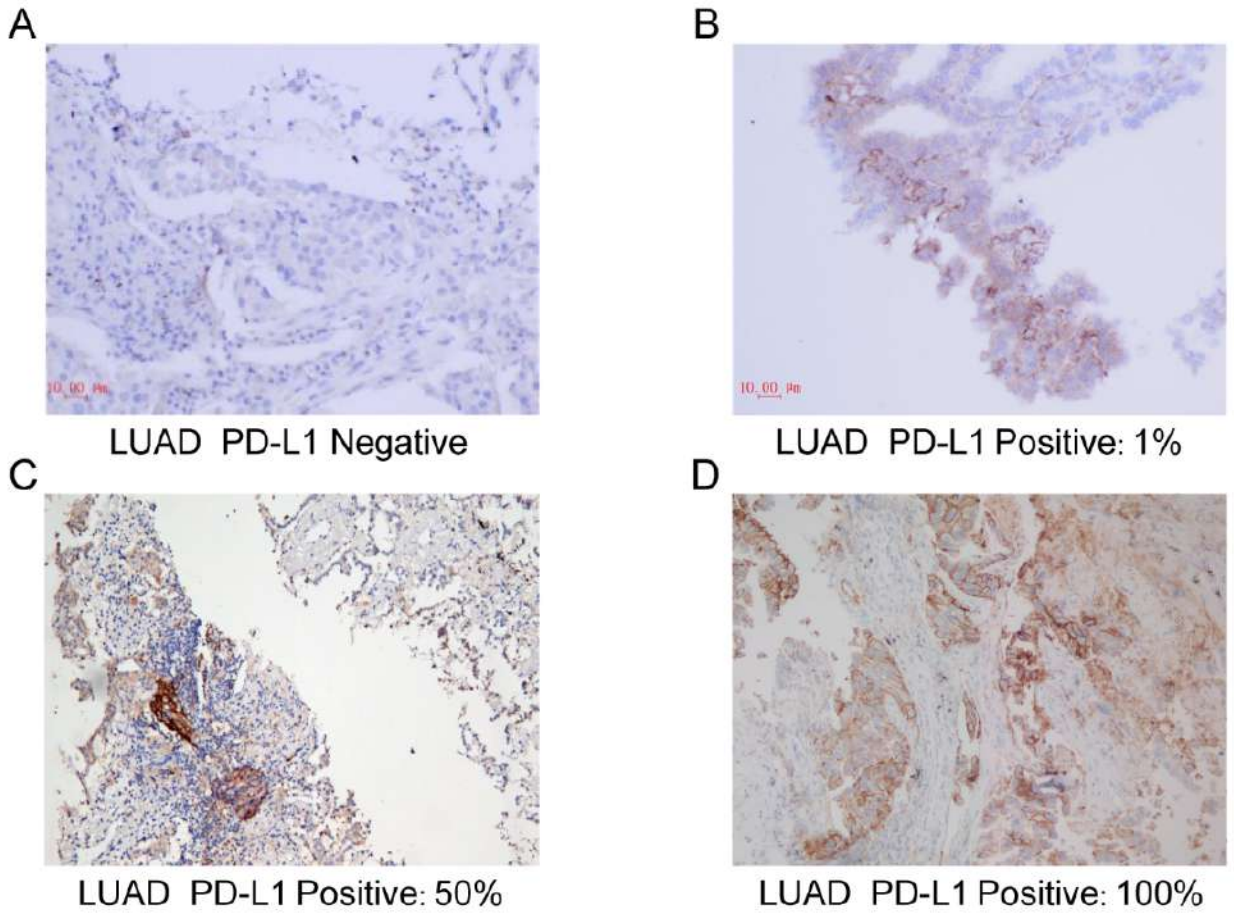

Fig. 2: IHC analysis of PD-L1 in patients with LUAD: negative PD-L1 expression (A), less than 20% PD-L1 expression (B), 20%-60% PD-L1 expression (C) and PD-L1 expression > 60% (D)

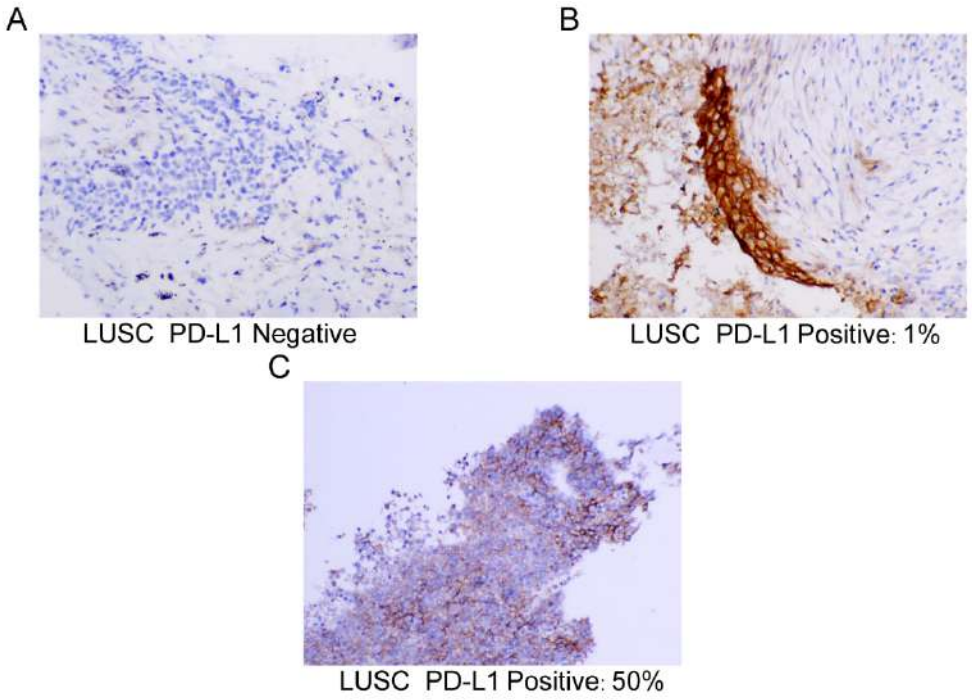

Fig. 3: IHC analysis of PD-L1 in patients with LUSC: negative PD-L1 expression (A), less than 20% PD-L1 expression (B) and 20%-60% PD-L1 expression (C)

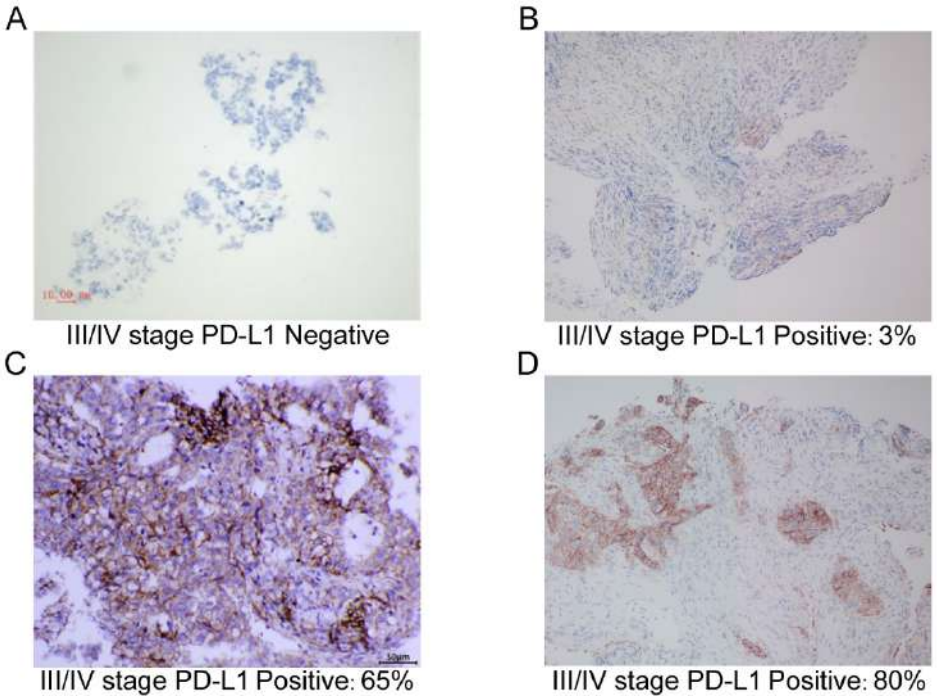

Fig. 4: IHC analysis of PD-L1 in III/IV stage lung cancer patients: negative PD-L1 expression (A), less than 20% PD-L1 expression (B), 20%-65% PD-L1 expression (C) and PD-L1 expression > 65% (D)

Association of PD-L1 expression and and Molecular Alterations

The association of PD-L1 and mutations were examined. Among all lung tumor specimens who assayed for PD-L1 expression, we identified 43 (28.86%) *EGFR* mutations, 2 (1.34%) *ALK* mutations, 9 (6.04%) *KRAS* mutations, 1 (0.67%) *MET* mutations, 2 (1.34%) *RET* mutations and 1 (0.67%) *TP53* mutations (Table 4). The correlation between gene mutations (*EGFR*, *KRAS*, *RET* and *TP53*, etc.) and PD-L1 levels was analyzed. The results showed that *KRAS* mutation was significantly correlated with PD-L1 ($P<0.01$). While, *EGFR*, *ALK*, *MET*, *RET* and *TP53* mutation status showed no association with PD-L1 level.

Table 4: Oncogenic aberration and PD-L1 expression in patients

Type of mutation	***PD-L1 Expression***			***No PD-L1 detection (n=151)***	***χ2***	***P value***	***OR*** 95% CI
	High (n=54)	Intermediate (n=44)	Negative (n=51)				
EGFR, n (%)							
Positive	10	15	18	41	0.108	0.742	1.088
Negative	44	29	33	110			0.657-1.802
ALK, n (%)							
Positive	2	0	0	7	2.795	0.095	0.280
Negative	52	44	51	144			0.057-1.370
KRAS, n (%)							
Positive	4	3	2	1	6.732	0.009**	9.643
Negative	50	41	49	150			1.206-77.095
MET, n (%)							
Positive	1	0	0	0	1.017	0.313	0.000
Negative	53	44	51	151			0.000-0.000
RET, n (%)							
Positive	0	1	1	2	0.000	0.989	1.014
Negative	54	43	50	149			0.141-7.292
TP53, n (%)							
Positive	0	0	1	1	0.000	0.992	1.014
Negative	54	44	50	150			0.063-16.356
ROS1, n (%)							
Positive	0	0	0	1	0.990	0.320	0.000
Negative	54	44	51	150			0.000-0.000

Efficacy in oncogenic aberration and PD-L1 expression patients

Classification of patients according to their status (CR, PR, SD and PD), the average PD-L1 level and gene mutations were analyzed. A small number of *EGFR*, *ALK* and *TP53* mutations appeared in patients with CR status and three had high expression of PD-L1 (Fig. 5A-5B).

Patients in PR status, the mutated genes were mainly *EGFR*, followed by *ALK*, *KRAS*, *RET*, and *TP53* mutations, of which PD-L1 was highly expressed in 1/2 (Fig. 5A, 5C). In addition, *EGFR* and *TP53* mutations were detected in SD status patients and high-expressed PD-L1 accounted for about half (Fig. 5A, 5D). Four PD patients with *EGFR*, *KRAS* and *TP53* mutations and high-expressed PD-L1 (Fig. 5A, 5E).

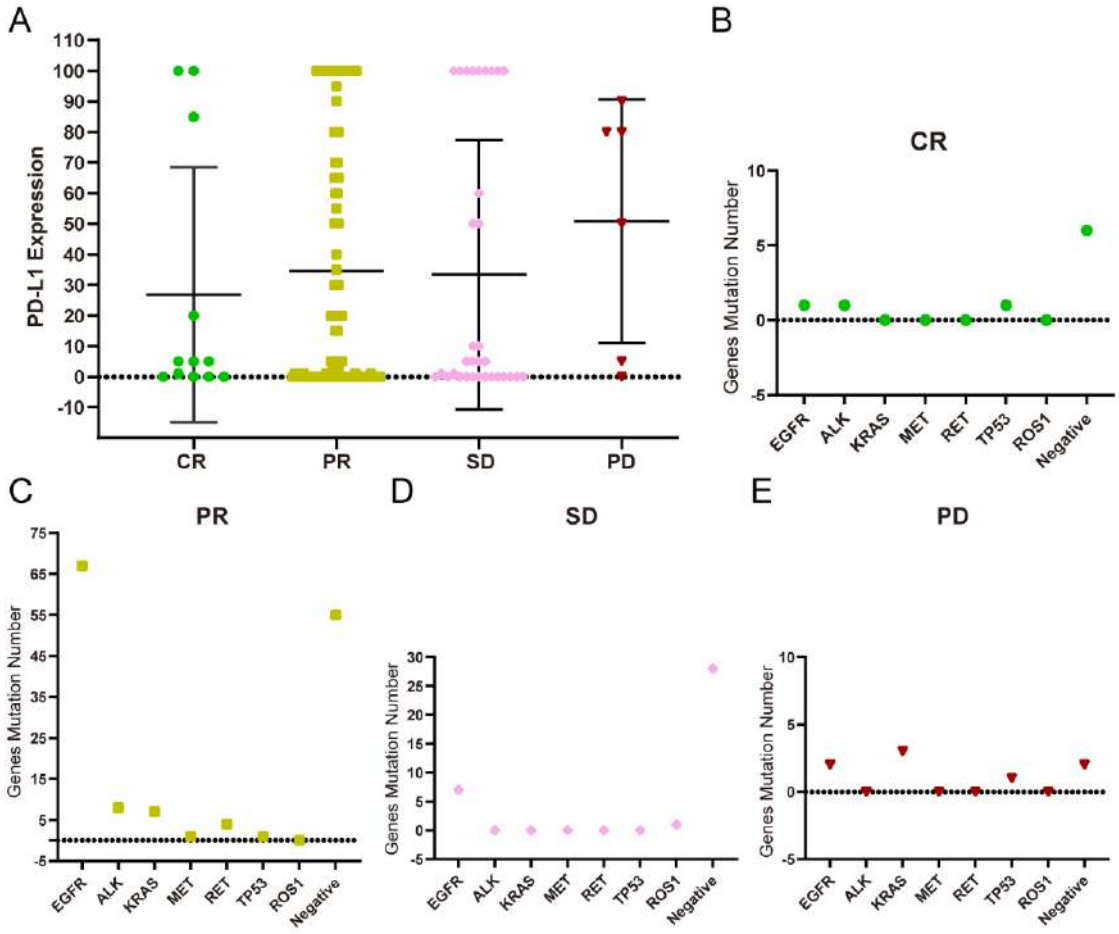

Fig. 5: Patients efficacy distribution and PD-L1 expression (A); Genes molecular alterations and patients efficacy distribution: CR (B), PR (C), SD (D) and PD (E)

Chemotherapy clinical outcomes analysis

The ORR of the samples included was 80.67%. Six groups, including chemotherapy, targeted therapy, chemotherapy + surgery, chemotherapy + targeted therapy, chemotherapy + radiotherapy and chemotherapy + immunotherapy, were divided and therapeutic effects were statistically analyzed. Table 5 summarized the univariate analysis of ORR, the results showed that 95% CI in chemotherapy group (95% CI: 62.07, 0.833-2.045) was significantly lower than that in chemotherapy + immunotherapy group (95% CI: 65.00, 0.805-1.930), targeted therapy group (95% CI: 89.06, 0.613-1.350), chemotherapy + targeted therapy group (95% CI: 90.91, 0.475-1.671), chemotherapy + radiotherapy group (95% CI: 94.44, 0.433-1.700) and chemotherapy + surgery group (95% CI: 98.67, 0.571-1.180). The same trend was displayed among the time to response, the median DOR in chemotherapy alone group was 7.00 months (range, 2-33 months) and it was shorter than that in other five groups (Table 5), showed significantly difference among these six groups (P<0.01). The best overall response showed significantly difference among these six groups (P<0.01).

Table 5: Summary of ORR/Best overall response/Time to response

Variable	*Chemotherapy Alone (n=58)*	*Targeted therapy Alone (n=64)*	*Chemotherapy+Surgery (n=75)*	*Chemotherapy+Targeted therapy (n=22)*	*Chemotherapy+Radiotherapy (n=18)*	*Chemotherapy+Immunotherapy (n=60)*	*χ2*	*P value*
ORR No. of Patients, % (95% CI)	36 62.07 (0.833-2.045)	57 89.06 (0.613 - 1.350)	74 98.67 (0.571-1.180)	20 90.91 (0.475-1.671)	17 94.44 (0.433-1.700)	39 65.00 (0.805-1.930)	4.787	0.442
Best overall response, No. (%)							101.531	0.000**
Complete response	2	2	30	0	1	5		
Partial response	34	55	44	20	16	34		
Stable disease	19	6	1	1	1	17		
Progressive disease	3	1	0	1	0	4		
Time to response, Mo median (range)	7.00 (2-33)	14.00 (2-84)	26.00 (6-109)	14.00 (5-25)	11.50 (4-37)	9.00 (2-45)	21.449	0.001**

Discussion

The current study was retrospective. We statistically summarized a number of clinicopathological indicators and variation of biomarkers including PD-L1 level and molecular mutations, so that explore the clinical relevance and the role of treatment methods and efficacy status in NSCLC patients. Our results displayed that the PD-L1 was significantly different from tumor stage, lymphatic invasion and metastasis. PD-L1 high-expressed patients were treated by immunotherapy and 63.41% experienced PR/CR. We also found that EGFR mutation status was significantly associated with PD-L1 level in NSCLC. PD-L1-IHC evaluation is of great significance for patients with PD-1 or PD-L1 blockade immunotherapy (11). At present, many clinical trials have performed IHC evaluation of PD-L1 on surgical specimens (12). Our study also combined the correlation analysis with genomic abnormalities and the correlation analysis with clinical treatment/response. Such a comprehensive assessment is helpful for clinical patient benefit.

Most studies reported that clinicopathological features did not correlate with whether PD-L1 was expressed, but presented a correlation with high PD-L1 expression in patients with, for example, metastatic tumors or advanced tumors (13,14). PD-L1 expression was measured by TPS

or CPS, as shown in our results: 36.24% had PD-L1≥50% and 29.53% had PD-L1 TPS<50%. There are studies on pembrolizumab that confirm its benefit to patients with high PD-L1 expression: 50% TPS patients had longer PFS than those who with 1-49% TPS or TPS<1% (PFS in the previous 2 months were comparable among the three groups) (15). This was consistent with our research. It was previously reported that pembrolizumab plus chemotherapy provided clinically significant benefits in PD-L1-negative NSCLC patients (16). The current study further revealed the value of pembrolizumab in combination with chemotherapy for the survival of PD-L1-positive patients.

In CheckMate's exploratory analysis, nivolumab plus chemotherapy was reported to improve PFS in PD-L1-negative NSCLC patients; Nivolumab+ipilimumab improved OS (HR=0.62; 95% CI: 0.48-0.78) (17). Angelis et al. found that the PD-L1 high group (TPS: 90-100%) had a higher response rate than the PD-L1 suboptimal group (TPS: 50-89%), consistent with the response rate of patients receiving pembrolizumab plus chemotherapy (TPS: 50% or more) (18). The present study found that the ORR of only chemotherapy, only targeted therapy, chemotherapy + surgery, targeted therapy, radiotherapy or immunotherapy cases showed that 95% CI in chemotherapy group was lower and time to response was shorter than that in other combined groups. In addition, the best overall response was difference among six groups (P<0.05). The ORR comparing of different treatment groups confirmed that the chemotherapy group had the lowest ORR; the DOR comparing of different treatment groups found that the DOR in chemotherapy group was only 7 months, which was shorter than the other five groups.

As traditional chemoradiotherapy, targeted therapy and immunotherapy are being combined and actively investigated, it is urgent and necessary to clarify the level of the immune indicator PD-L1 and the relationship with tumor target genes such as *EGFR*, *ALK* and *KRAS* (19,20). It has been reported that PD-L1 levels were not significantly correlated with the presence or absence of mutations in *EGFR*, *ALK* and *ROS1*, and only 1 sample had an *ALK* mutation and the TPS of PD-L1 ≥50% (21). However, a higher rate of PD-L1 positivity in NSCLC with mutations in *EGFR* has also been reported (22). We summarized the correlation between high frequency mutated genes and PD-L1 expression or not in NSCLC in present study: among who assayed for PD-L1 expression, 43 (28.86%) *EGFR* mutations, 2 (1.34%) *ALK* mutations, 9 (6.04%) *KRAS* mutations, 1 (0.67%) *MET* mutations, 2 (1.34%) *RET* mutations and 1 (0.67%) *TP53* mutations were identified. *KRAS* mutations significantly related with PD-L1 level (P<0.01). Targeted therapies, immunotherapy was an important tool for personalized treatment, combined the mutation analysis data with PD-L1 detection data can provide the safest and most effective treatment plans for NSCLC patients.

The present results analyzed with a sample size close to 300 cases can enriched clinical studies. However, there are still shortcomings: although the basic clinical information and clinicopathological data have been comprehensively analyzed, individual differences still exist. A larger sample size analysis was needed to determine the generality of personalized treatment planning by detecting mutation + PD-L1 for NSCLC patients.

Conclusion

All in all, PD-L1 and its response to were different in tissue samples in present study. In addition, specific molecular characteristics were related to the differential PD-L1 level, and affected the responses of PD-L1 to treatment.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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