



# Predictive values of genomic variation, tumor mutational burden, and PD-L1 expression in advanced lung squamous cell carcinoma treated with immunotherapy

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**Background:** Immune checkpoint inhibitors (ICIs) prolong overall survival (OS) in patients with advanced lung squamous cell carcinoma (LUSC). However, predictive and prognostic factors related to ICIs in LUSC remain elusive. This study aimed to identify predictors that are related to better clinical benefit and outcomes in LUSC patients treated with immunotherapy.

**Methods:** Capture-based targeted sequencing was performed in 64 patients with advanced LUSC who underwent immunotherapy. Tumor mutational burden (TMB) was defined as the sum of nonsynonymous single nucleotide and indel variants. Programmed cell death ligand-1 (PD-L1) expression was evaluated by immunohistochemical analysis. Clinicopathological characteristics including age, sex, performance status, smoking history, body mass index (BMI), blood fat, brain metastases, liver metastases, previous thoracic radiotherapy, and treatment lines were analyzed.

**Results:** The most commonly mutated genes included *TP53*, *CDKN2A*, *KEAP1*, *CREBBP*, *KRAS*, *BIM*, *AMER1*, and *APC*. Copy number variations most frequently occurred in *AR*, *SOX2*, *PIK3CA*, *EGFR*, *RICTOR*, *FGFR1*, and *ZNF703*. The median and mean TMB was 9.35 and 10.62 mutations per megabase, respectively. Positive PD-L1 expression was detected in 29.7% patients. Patients with a history of heavy smoking ( $\geq 40$  pack-years) were more likely to have positive PD-L1 expression (35% *vs.* 16.7%,  $P=0.04$ ) and higher TMB (11.1 *vs.* 9.8 mut/Mb,  $P=0.04$ ). Gene alterations had no impact on PD-L1 expression or TMB level. The median progression-free survival (PFS) was 6.7 months and median OS was 13.7 months. Higher TMB was independently associated with longer PFS ( $P=0.01$ ) and OS ( $P=0.02$ ), and this correlation was more pronounced in patients treated with ICIs as a single agent ( $P=0.0001$ ). Higher TMB was also associated with better disease control rate (DCR) ( $P=0.02$ ). Compared with wild-type, patients with *KRAS* mutation and *EGFR* amplification had higher objective response rates (ORR,  $P=0.01$ ).

**Conclusions:** The predictive value of TMB is more significant in LUSC patients receiving ICI as a single agent than as a combination therapy. The combination of Eastern Cooperative Oncology Group performance status (ECOG-PS), smoking status, TMB, PD-L1, and genomic variation might be helpful for personalized immunotherapy decisions in clinical practice for advanced LUSC.

**Keywords:** Genome; immunotherapy; lung squamous cell carcinoma (LUSC); programmed cell death ligand-1 (PD-L1); tumor mutational burden (TMB)

Submitted Sep 15, 2020. Accepted for publication Nov 17, 2020.

doi: 10.21037/tlcr-20-1130

View this article at: <http://dx.doi.org/10.21037/tlcr-20-1130>

## Introduction

Lung squamous cell carcinoma (LUSC) accounts for 30% of all lung cancers (1). Although the therapeutic management for metastatic non-squamous-cell carcinoma has revolutionized from chemotherapy to molecular-targeted therapy in the past decade, platinum-based chemotherapy remains the first-line standard treatment for advanced LUSC (2), due to a lack of therapeutic targets. LUSC offers a very different genetic picture.

Recently, significant progress has been made in the development of immune checkpoint inhibitors (ICIs) (3,4), which target the programmed cell death-1 (PD-1)-programmed cell death ligand-1 (PD-L1) axis and elicit remarkable clinical efficacy in both treatment naïve and previously-treated advanced or metastatic non-small cell lung cancer (NSCLC) patients. According to the National Comprehensive Cancer Network (NCCN) guidelines, the recommended first-line treatment for advanced LUSC with PD-L1 expression levels of  $\geq 50\%$  is pembrolizumab monotherapy, and for patients with metastatic LUSC is pembrolizumab/carboplatin/paclitaxel (or albumin-bound paclitaxel) (4-6). Multiple trials have demonstrated a superior progression-free survival (PFS) and overall survival (OS) in advanced or metastatic LUSC, who received pembrolizumab either as a single agent or in combination with standard chemotherapy (4). Moreover, several ICIs including pembrolizumab, nivolumab, and atezolizumab, have also been approved as second-line treatment regimens for patients with advanced or metastatic LUSC (7-9).

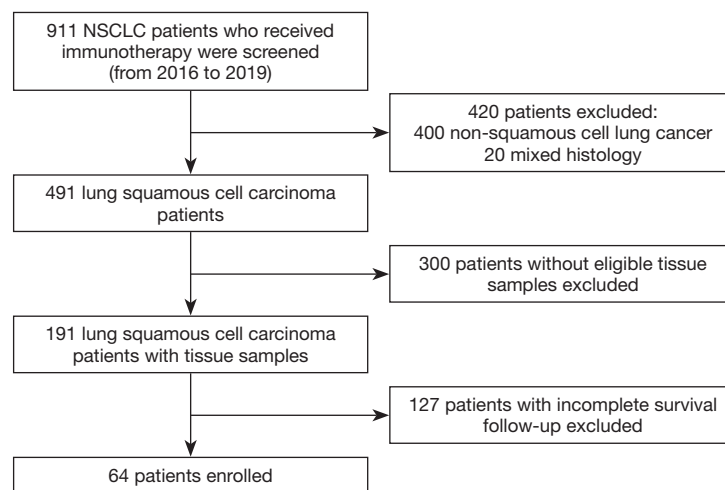
However, responses to ICIs therapies occur in only a minority of NSCLC patients. Identification of predictive biomarkers of response seems emergent. Biomarker studies have revealed some genomic features that are predictive of the efficacy of immunotherapy including (but not limited to) microsatellite instability (MSI) status, positive PD-L1 expression, and high tumor mutational burden (TMB) (10,11). However, unlike adenocarcinoma patients, a considerable proportion of LUSC patients with advanced stage refused to provide tumor tissue for molecular testing. Moreover, challenges in defining appropriate cut-off values and standardizing the testing platform have limited their clinical application.

Therefore, the ability of genomic features, TMB, PD-L1 expression, or other clinical characteristics to be used as predictive factors to guide immunotherapies in real-world clinical practice has attracted tremendous attention. Recent reports have indicated that gene profiling, cytokines, Eastern Cooperative Oncology Group performance status (ECOG-PS), and body mass index (BMI) were significant in predicting the response to immunotherapy (12,13), however each of these offers limited utility. A similar study has been carried out in NSCLC including adenocarcinoma and squamous cell carcinoma treated with combined anti-CTLA-4 and anti-PD-1 therapies (14). But the predictive value of mentioned indicators in the immunotherapy of LUSC is unclear. Studies have suggested that there are much more differences in the response of LUSC and adenocarcinoma to immunotherapy, as well as gene status and immune microenvironment. LUSC is not well characterized due to the poor understanding of the genomic evolution (15). Genomic alterations in the tumor characterize also various in different stages of LUSC progression. Thus, in particular, we focused on advanced LUSC. In this study, tissue samples and complete survival follow-up of patients with advanced LUSC who received immunotherapy in real-world clinical practice were examined to identify ideal factors related to better efficacy and prognosis. We present the following article in accordance with the REMARK reporting checklist (available at <http://dx.doi.org/10.21037/tlcr-20-1130>).

## Methods

### *Patients and samples*

A total of 911 patients with NSCLC who received anti-PD-L1 therapeutic agents at the Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital) between January 2016 and June 2019 were screened retrospectively. Only advanced and metastatic LUSC patients with sufficient archived tissue samples and complete survival data were included. The major inclusion criteria were as follows: >18 years old; an ECOG-PS of 0-2; have advanced or recurrent LUSC; treated with anti-PD-L1 therapy agents; radiologically evaluable according



**Figure 1** Trial profile. A total of 911 patients with NSCLC who underwent immunotherapy were screened. Major exclusion criteria were inadequate tissue samples, mixed histology, and incomplete follow-up data. Sixty-four patients were finally enrolled. NSCLC, non-small cell lung cancer.

to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; a minimum of 50% of tumor content was required. The major exclusion criteria were as follows: inadequate tissue samples; non-squamous histology; incomplete follow-up data. A total of 64 patients who met the inclusion criteria were enrolled.

Formalin-fixed and paraffin-embedded tissues were used for capture-based targeted sequencing and immunohistochemical (IHC) staining. The study scheme is summarized in *Figure 1*. The following clinicopathological features were analyzed: age, sex, ECOG-PS, smoking history, BMI, blood fat, brain metastases, liver metastases, history of thoracic radiotherapy, and treatment lines. PFS was defined as the time from the initiation of immunotherapy until disease progression. OS was calculated from the date of the initiation of immunotherapy until death of any cause or the last follow-up date. All procedures performed in studies involving human participants were in accordance with the Helsinki Declaration (as revised in 2013). The study was approved by the ethics committee of Zhejiang Cancer Hospital. Informed consent was obtained from each patient.

#### **Capture-based targeted sequencing**

DNA shearing was performed on tissue DNA using Covaris M220, followed by end repair, phosphorylation and adaptor ligation. Fragments of size 200–400 bp were

selected by bead (Agencourt AMPure XP Kit), followed by hybridization with capture probes baits, hybrid selection with magnetic beads and PCR amplification. Indexed samples were sequenced on Nextseq500 sequencer (Illumina, Inc., USA) with pair-end reads. Capture-based targeted sequencing for somatic mutation profiling was performed using a panel consisting of 520 cancer related genes, spanning 1.7 Mb of human genome.

#### **TMB calculation**

TMB was defined as the number of somatic single nucleotide variants (SNVs), coding, base substitution, and indels per megabase of genome examined, and was calculated as previously described (16). Briefly, all base substitutions, including non-synonymous and synonymous alterations, and indels in the coding region of targeted genes were considered with the exception of known hotspot mutations in oncogenic driver genes and truncations in tumor suppressors. Synonymous mutations were counted in order to reduce sampling noise.

#### **PD-L1 expression**

PD-L1 expression was tested by anti-human PD-L1 (Dako 22C3) according to the manufacturer's recommendations, using 4–5  $\mu$ m formalin-fixed and paraffin-embedded (FFPE) sections. The cutoff value was 1% for PD-L1 positivity or

negativity (PD-L1+/-). Representative IHC images have been provided as supplemented [Figure S1](#).

### Statistical analysis

Spearman's rank correlation was used to assess the correlations among gene variation, PD-L1 expression, TMB, and clinical parameters. Continuous variables were analyzed using analysis of variance and Tukey's multiple comparison tests. Discrete variables were analyzed using the Chi-square test or Fisher's exact test. The Kaplan-Meier curve was leveraged to assess the patients' survival curves. The Cox proportional-hazards model was used for univariate and multivariate survival analyses to calculate the hazard ratios (HR) and related 95% confidence intervals (CIs) for PFS and OS times. Logistic regression was used for univariate and multivariate analyses to calculate the odds ratios and related 95% CIs for overall response and disease control. A P value less than 0.05 was considered significant. All statistical analyses were performed using the SAS software, version 9.4 (SAS Institute Inc., NC, USA).

## Results

### Clinicopathological characteristics of advanced LUSC and their correlations with TMB and PD-L1 expression

A total of 64 patients with advanced and metastatic LUSC who were treated with anti-PD-L1 therapy agents at Zhejiang Cancer Hospital between January 2016 and November 2019 were included. All patients were stage IV at diagnosis with a median age of 62 years (range, 45–81 years) at the time of treatment initiation, and 92.2% were men. Also, 96.9% of patients had an ECOG-PS of between 0 and 1.

Thirteen (20.3%) patients received immunotherapy as first-line, 29 (45.3%) as second-line, and the remaining 34.4% of patients as > second-line treatment. Eight (12.5%) patients had brain metastasis, 14 (21.9%) had liver metastasis, and 35 (54.7%) had dyslipidemia. Thirty-five (54.7%) patients had a history of thoracic radiotherapy. Fifty-three (82.8%) patients received mono-immunotherapy. The median BMI was 22.24. The median and mean TMB was 9.35 and 10.62 mut/Mb, respectively. A PD-L1 tumor proportion score (TPS) >1% was considered as PD-L1 positivity and was identified in 29.7% of patients with LUSC ([Table 1](#)). No statistically significant difference was found in the clinicopathological characteristics, including age, sex, brain metastases, liver metastases, history

of thoracic radiotherapy, and treatment lines between patients with high (>10.62 mut/Mb) or low ( $\leq$ 10.62 mut/Mb) TMB or PD-L1 positivity.

Patients with a history of heavy smoking ( $\geq$ 40 pack-years) were more likely to have positive PD-L1 expression (35% vs. 16.7%,  $P=0.04$ ) and higher TMB (11.1 vs. 9.8 mut/Mb,  $P=0.04$ ). Patients with a BMI of 18.5–24 exhibited a trend of having a higher percentage of positive PD-L1 expression ( $P=0.02$ ) and a lower TMB level ( $P=0.04$ ). The baseline characteristics of patients in this cohort and their correlations with TMB and PD-L1 expression are summarized in [Table 1](#). The proportion of LUSC patients with positive PD-L1 and high TMB increases with the addition of smoking pack-years ([Table 1](#) and [Figure S2](#)). Positive PD-L1 expression was identified in 14.29% of never smokers, 23.53% of <40 pack-year smokers, and 35% of  $\geq$ 40 pack-year smokers. High TMB was identified in 28.57% of never smokers, 41.18% of <40 pack-year smokers, and 50% of  $\geq$ 40 pack-year smokers ([Figure S2](#)).

### Gene variations of advanced LUSC and their correlations with TMB and PD-L1 expression

Next, we investigated the correlations between frequently mutated genes and TMB/PD-L1 status. Eight genes with a somatic mutation frequency >5% were identified: *TP53*, *CDKN2A*, *KEAP1*, *CREBBP*, *KRAS*, *BIM*, *AMER1*, and *APC* ([Figure 2](#)). Genes with copy number variations occurring in >5% patients were also investigated, and seven genes were identified: *AR*, *SOX2*, *PIK3CA*, *EGFR*, *RICTOR*, *FGFR1*, and *ZNF703* ([Figure 2](#)). Mutation in the aforementioned genes had no impact on PD-L1 expression or TMB level ([Figure 2](#), [Table S1](#)).

### Predictive and prognostic value of clinicopathological characteristics

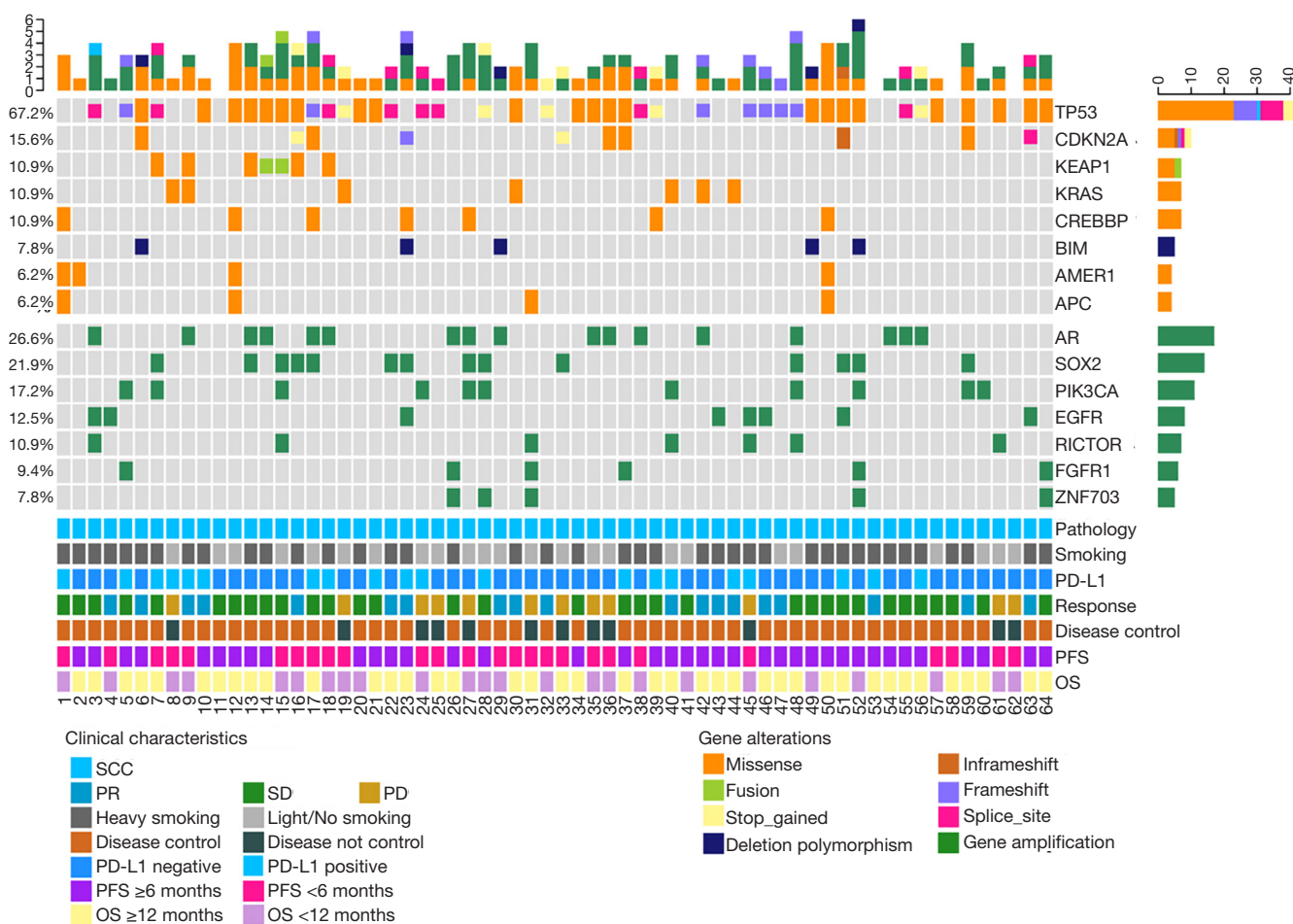
The median follow-up time was 17 months. The disease control rate (DCR) was 81.3% [partial response (PR) 29.7% + stable disease (SD) 51.6%]. The objective response rate (ORR) was 29.2%, with a median PFS of 6.7 months and a median OS of 13.7 months. The 6-month PFS rate was 57.8%, and the 1-year OS rate was 62.5% ([Figure 3](#)).

Better ECOG-PS was associated with longer PFS ( $P=0.019$ ) ([Table 2](#), [Figure 4](#)), and also with a better ORR ( $P<0.0001$ ) ([Table S2](#)). Our data revealed that patients with an ECOG-PS of 0 were more likely not to have disease progression at 6-month ( $P<0.005$ ). The 6-month PFS rate

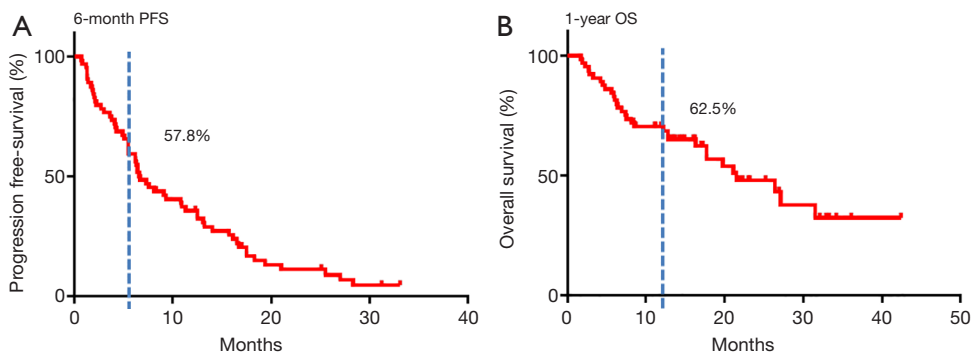
**Table 1** Baseline characteristics of included patients and their correlations with TMB and PD-L1 expression (N=64)

Characteristic	Total N (%)	PD-L1 (-)		PD-L1 (+)		P value	TMB low*		TMB high**		P value
		n	%	n	%		n	%	n	%	
<b>Age</b>											
<65	41 (64.1)	28	68.29	13	31.71	0.6368	23	56.1	18	43.90	0.7622
≥65	23 (35.9)	17	73.91	6	26.09		12	52.17	11	47.83	
<b>Sex</b>											
Male	59 (92.2)	41	69.49	18	30.51	0.3713	31	52.54	28	47.46	0.1992
Female	5 (7.8)	4	80	1	20		4	80	1	20	
<b>BMI</b>											
<18.5	7 (10.9)	5	71.43	2	28.57	0.0168	3	42.86	4	57.14	0.0436
18.5–24	37 (57.8)	23	62.16	14	37.84		22	59.46	15	49.54	
≥24	20 (31.3)	17	85	3	15		10	50	10	50	
<b>Smoking</b>											
Never	7 (10.9)	6	85.71	1	14.29	0.0443	5	71.43	2	28.57	0.0405
<b>Current/former</b>											
<40 pack-years	17 (26.6)	13	76.47	4	23.53		10	58.82	7	41.18	
≥40 pack-years	40 (62.5)	26	65	14	35		20	50	20	50	
<b>ECOG</b>											
0	18 (28.1)	12	66.67	6	33.33	0.1105	9	50	9	50	0.2325
1	44 (68.8)	31	70.45	13	29.55		26	59.09	18	40.91	
2	2 (3.1)	2	100	0	0		0	0	2	100	
<b>Dyslipidemia</b>											
Yes	35 (54.7)	27	77.14	8	22.86	0.0934	17	48.57	18	51.43	0.2802
No	29 (45.3)	18	62.07	11	37.93		18	62.07	11	37.93	
<b>Brain metastases</b>											
Yes	8 (12.5)	6	75	2	25	0.3147	5	62.5	3	37.5	0.268
No	56 (87.5)	39	69.64	17	30.36		30	53.57	26	46.43	
<b>Liver metastases</b>											
Yes	14 (21.9)	11	78.57	3	21.43	0.2055	7	50	7	50	0.6902
No	50 (78.1)	34	68	16	32		28	56	22	44	
<b>Radiotherapy</b>											
Yes	35 (54.7)	26	74.29	9	25.71	0.4447	17	48.57	18	51.43	0.2802
No	29 (45.3)	19	65.52	10	34.48		18	62.07	11	37.93	
<b>Lines of therapy</b>											
≤2	42 (65.6)	29	69.05	13	30.95	0.7596	23	54.76	19	45.24	0.9868
>2	22 (34.4)	16	72.73	6	27.27		12	54.55	10	45.45	

\*, TMB low, TMB ≤10.62 (Mean TMB for all 64 subjects is 10.62); \*\*, TMB high, TMB >10.62. ECOG PS, Eastern Cooperative Oncology Group performance score; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.



**Figure 2** Gene variations of advanced LUSC and their correlations with clinical characteristics. Eight genes with a somatic mutation frequency >5% were identified: *TP53*, *CDKN2A*, *KEAP1*, *CREBBP*, *KRAS*, *BIM*, *AMER1*, and *APC*. Seven genes with copy number variation frequency >5% were as follows: *AR*, *SOX2*, *PIK3CA*, *EGFR*, *RICTOR*, *FGFR1*, and *ZNF703*. Each column represents one patient. Clinical characteristics and genetic alterations of each patient were shown at the bottom. LUSC, lung squamous cell carcinoma; PR, partial response; SD, stable disease; PD, progressive disease.



**Figure 3** Kaplan-Meier estimates for progression-free survival and overall survival (N=64). (A) The median PFS was 6.7 months. The 6-month PFS rate was 57.8%. (B) The median OS was 13.7 months. The 1-year OS rate was 62.5%. PFS, progression-free survival; OS, overall survival.

**Table 2** Univariate and multivariate analyses of clinical parameters on progression-free survival (PFS) and overall survival (OS) (Cox regression) (N=64)

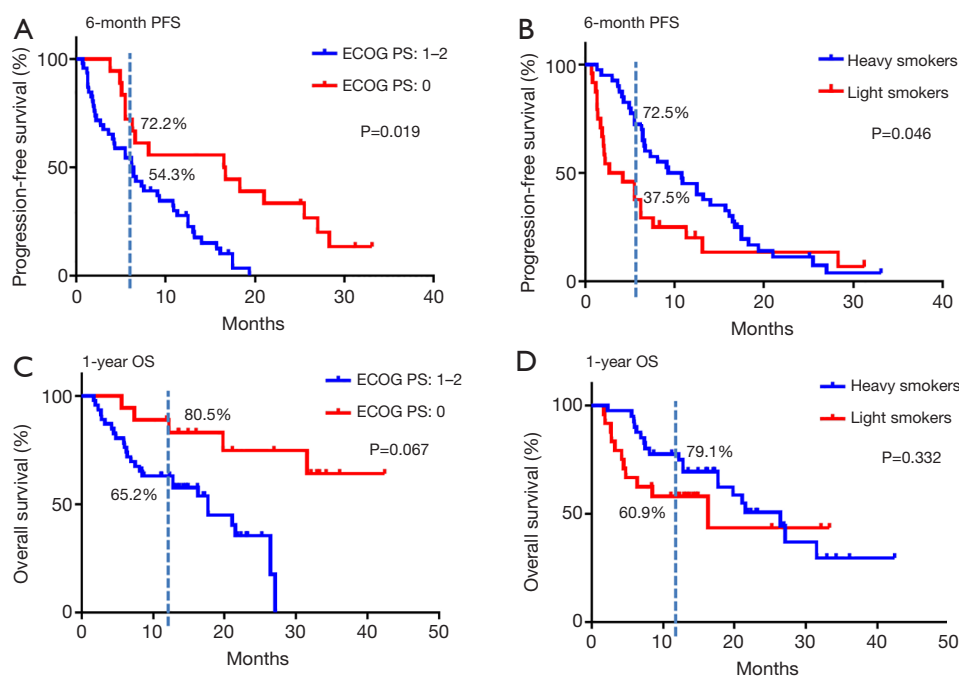
Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
Progression-free survival						
Sex (male/female)	1.501	0.539–4.181	0.4371			
Age ( $\geq 65$ / $< 65$ )	1.055	0.620–1.796	0.8435			
Smoker ( $< 40$ pack-years / $\geq 40$ pack-years)	2.368	0.913–6.142	0.0462			
BMI (18.5–24/ $< 18.5$ )	0.601	0.260–1.386	0.2322			
BMI ( $\geq 24$ / $< 18.5$ )	0.670	0.278–1.618	0.3734			
ECOG (1–2/0)	3.248	1.611–6.547	0.0010	2.614	1.175–5.816	0.0185
Dyslipidemia (yes/no)	1.079	0.641–1.815	0.7753			
Brain metastases (yes/no)	1.271	0.572–2.827	0.5562			
Liver metastases (yes/no)	1.337	0.718–2.491	0.3604			
Radiotherapy (yes/no)	1.793	1.024–3.141	0.0411	1.375	0.728–2.597	0.3258
Lines of therapy (3–4/1–2)	2.026	1.160–3.537	0.0130	1.368	0.738–2.533	0.3195
PD-L1 (positive/negative)	1.074	0.607–1.901	0.8061			
TMB ( $> 10.62$ / $\leq 10.62$ )	0.545	0.322–0.924	0.0241	0.461	0.264–0.803	0.0063
Overall survival						
Sex (male/female)	3,850,984	0.000	0.9889			
Age ( $\geq 65$ / $< 65$ )	0.976	0.466–2.042	0.9478			
Smoker ( $< 40$ pack-years / $\geq 40$ pack-years)	6.667	0.849–52.38	0.3321			
BMI (18.5–24/ $< 18.5$ )	1.068	0.315–3.620	0.9163			
BMI ( $\geq 24$ / $< 18.5$ )	0.911	0.245–3.393	0.8895			
ECOG (1–2/0)	4.790	1.575–14.57	0.0058	3.162	0.924–10.82	0.0665
Dyslipidemia (yes/no)	0.683	0.336–1.391	0.2936			
Brain metastases (yes/no)	0.825	0.249–2.733	0.7532			
Liver metastases (yes/no)	1.507	0.646–3.513	0.3423			
Radiotherapy (yes/no)	2.328	1.054–5.143	0.0366	1.518	0.622–3.703	0.3587
Lines of therapy (yes/no)	2.543	1.211–5.341	0.0137	1.645	0.709–3.819	0.2463
PD-L1 (positive/negative)	1.384	0.618–3.097	0.4298			
TMB ( $> 10.62$ / $\leq 10.62$ )	0.449	0.213–0.944	0.0348	0.383	0.177–0.831	0.0152

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

in patients with an ECOG-PS of 0 and 1–2 was 72.2% and 54.3%, respectively (Figure 4A). The 1-year OS rate showed a higher trend in patients with an ECOG-PS of 0 compared to patients with an ECOG-PS of 1–2 (80.5% vs. 65.2%,

$P=0.067$ ) (Figure 4C).

Considering the impact of smoking history on TMB and a high percentage of heavy smokers (62.5%, N=40) in this cohort, an analysis of smoking history and clinical outcomes



**Figure 4** Prognostic value and effectiveness of clinicopathological characteristics. (A) The 6-month PFS rate in patients with an ECOG PS of 0 and 1–2 was 72.2% and 54.3%, respectively ( $P=0.019$ ). (B) The 6-month PFS rate was 72.5% and 37.5% in heavy smokers ( $\geq 40$  pack-years) and light smokers, respectively ( $P=0.046$ ). (C) The 1-year OS rate in patients with an ECOG PS of 0 and 1–2 was 80.5% and 65.2%, respectively ( $P=0.067$ ). (D) No statistically significant difference was found in the relationship of smoking status with OS ( $P=0.332$ ). The 1-year OS rate was 79.1% and 60.9% in heavy smokers ( $\geq 40$  pack-years) and light smokers, respectively. ECOG PS, Eastern Cooperative Oncology Group performance score; PFS, progression-free survival; OS, overall survival.

was conducted. Patients with a history of heavy smoking ( $\geq 40$  pack-years) were more likely to have a longer PFS ( $P<0.005$ ), but were not statistically different in OS (Figure 4B,D). The 6-month PFS rates for heavy smokers and non-heavy smokers were 72.5% and 37.5%, respectively ( $P=0.046$ ). The 1-year OS rates for heavy smokers and non-heavy smokers were 79.1% and 60.9%, respectively ( $P=0.332$ ). However, patients with a history of heavy smoking had a better DCR (97.5% vs. 54.2%,  $P=0.01$ ) (Table S2).

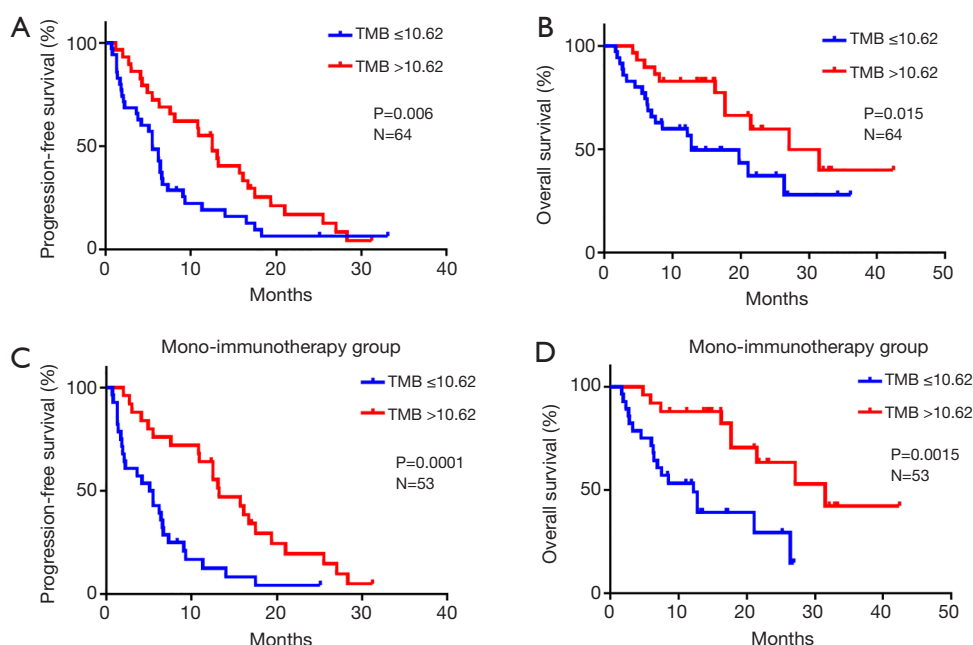
Moreover, the univariate analysis revealed that a history of thoracic radiotherapy was associated with a better ORR ( $P=0.001$ ) (Table S2). However, according to the multivariate analysis, the difference was not statistically significant. Similar results were observed between lines of therapy and DCR (Table S2). In the total population, there were 53 (82.8%) patients who received immunotherapy as a single agent (mono-immunotherapy group,  $N=53$ ). According to the multivariate analysis of this mono-immunotherapy group, BMI (18.5–24 vs.  $<18.5$ ,  $P=0.023$ ) and lines of therapy (3–4 vs. 1–2,  $P=0.005$ ) were found to be

associated with PFS (Table S3).

#### Predictive and prognostic value of TMB and PD-L1 expression

The median and mean TMB in this cohort was 9.35 and 10.62 mut/Mb, respectively. Higher TMB was defined as  $>10.62$  mut/Mb (Table 1), which was found to be independently associated with longer PFS ( $P=0.006$ ) and OS ( $P=0.015$ ) (Table 2, Figure 5A,B). This correlation was more pronounced in patients treated with mono-immunotherapy ( $P=0.0001$ ) (Figure 5C,D, Table S3). Patients with a higher TMB level had a better DCR in both the total population and the mono-immunotherapy group ( $P=0.02$ ) (Table S2). Univariate analyses of combined TMB and PD-L1 on OS and PFS showed that TMB has a greater predictive value for PFS in PD-L1 negative populations than PD-L1 positive populations, and were also more significant in the mono-immunotherapy group than the combined therapy group (Table S4).





**Figure 5** TMB correlate with PFS and OS in advanced LUSC receiving immunotherapy. (A) Improved PFS in all patients with high TMB (>10.62) (N=64, P=0.006). (B) Improved OS in patients with high TMB (>10.62) (N=64, P=0.015). A more significant trend toward increased PFS (N=53, P=0.0001) (C) and OS (N=53, P=0.0015) (D) was also observed in high TMB patients receiving mono-immunotherapy. TMB, tumor mutational burden; PFS, progression-free survival; OS, overall survival; LUSC, lung squamous cell carcinoma.

### Predictive and prognostic value of gene mutation and amplification

The univariate and multivariate analyses revealed no statistically significant correlations between gene alterations and PFS or OS (Figure 2, Table S5). Moreover, *KRAS* mutation and *EGFR* amplification were found to be associated with better ORR (P=0.01) (Figure 2, Figure S3, Table S6).

### Discussion

This retrospective analysis explored potential predictive and prognostic biomarkers of ICIs in stage IV LUSC patients treated with immunotherapy as a single agent or a combination therapy. The median PFS and OS were 6.7 and 13.7 months in this cohort, respectively. The 6-month PFS rate was 57.8% and the 1-year OS rate was 62.5%. Thirty-three people survived until the last follow-up.

In the phase III study (CheckMate 017) of nivolumab *vs.* docetaxel in LUSC, the median PFS and OS for patients treated with nivolumab as the second-line were 3.5 and 9.2 months, respectively (7). After 1 year, the OS rate

was 42% with nivolumab (7). In the double-blind phase 3 trial (Keynote-407) of pembrolizumab plus chemotherapy for LUSC, the median PFS was 6.4 months in the pembrolizumab combination group (17).

In contrast, 79.7% of the patients in the present study received immunotherapy as a second-line (or beyond) treatment; their median PFS, OS, and 1-year OS rates were comparable to those receiving nivolumab as a second-line treatment. It might not be appropriate to compare the data of a retrospective study directly with that of prospective clinical trials. However, these results could be explained by the fact that most of the patients included in the present analysis had an ECOG-PS score of 0–1 (96.9%), and 40 (62.5%) patients were heavy smokers. A real-world study in Canada indicated that patients with an ECOG-PS score of 0–1 benefited more significantly compared with those with an ECOG-PS score of 2, with median OS times of 12.91 and 6.77 months, respectively (18). Patients with an ECOG-PS of 2 might have been excluded from most immunotherapy trials. The available data suggested that patients with an ECOG-PS score of 2 had a worse prognosis, and that an ECOG-PS of 2 might be a predictor for shorter PFS and OS (18–22).

When considering smoking history, previous studies have shown that tobacco was a major cause of genomic instability and heterogeneity in cancer, and smokers had a higher TMB (23). The EAP study in Italy showed a higher ORR in patients with long-term smoking history, and prolonged PFS was observed in patients with a smoking history of >5 pack-years compared with patients with a smoking history of ≤5 pack-years (24). Moreover, objective responses were observed in 27% of never smokers, 40% of light smokers, and 40% of heavy smokers with NSCLC treated with immunotherapies (24). The 29.2% ORR in this study was closer to that achieved in the aforementioned studies using monotherapy in patients with LUSC. In the present study, patients with a history of heavy smoking tended to have a higher PD-L1 positive rate and higher TMB. The proportion of LUSC patients with positive PD-L1 and high TMB increases with the addition of smoking pack-years. Variables associated with a better ORR or DCR in the multivariate analysis included an ECOG-PS of 0, a history of heavy smoking (≥40 pack-years), and a high TMB. These results might partially explain the similarity between the present study and previous data.

In fact, treatment strategies for lung adenocarcinoma have significantly revolutionized, owing to the identification of targetable mutations (known as driver mutations) (25). However, less evidence is available regarding druggable genetic targets in LUSC. Attempts have been made using the next-generation sequencing (NGS) approach to identify driver mutations and identify those of clinical relevance to LUSC. Frequent mutations in *TP53* (64.5% of analyzed patients), *PIK3CA* (28.5%), *CDKN2A* (24.4%), *SOX2* (17.7%), and *CCND1* (15.8%) have been identified (26). The Cancer Genome Atlas (TCGA) has identified 10 genes (*TP53*, *CDKN2A*, *PTEN*, *PIK3CA*, *KEAP1*, *MLL2*, *HLA-A*, *NFE2L2*, *NOTCH1*, and *RBI*) with a Q value of <0.1 (27). In the present study, eight genes with a mutation rate of >5% were identified: *TP53*, *CDKN2A*, *KEAP1*, *CREBBP*, *KRAS*, *BIM*, *AMER1*, and *APC*. Seven genes had significant copy number variations: *AR*, *SOX2*, *PIK3CA*, *EGFR*, *RICTOR*, *FGFR1*, and *ZNF703*. These results differed slightly from TCGA findings and the published data. However, no significant correlations were observed between gene variations and clinicopathological features, DCR, PFS, and OS in the multivariate analysis. Moreover, our study indicated that genomic alterations did not correlate with the TMB level.

Also, mutations associated with a better ORR in our study included the *KRAS* mutation and *EGFR* amplification.

Some recent studies suggested that patients with *KRAS* mutations might benefit from immunotherapy due to the association between smoking and *KRAS* mutations (28,29). Other studies showed that co-occurring mutations in *TP53* and *KRAS* might be associated with the activation of antitumor immunity (30,31). Actually, the underlying mechanisms of *TP53* and/or *KRAS* co-occurrence have not been explored yet. *EGFR* amplification is considered to be a negative prognostic factor in immunotherapy (32,33). Contrary to previous studies, our results may be due to the small sample size and other confounding factors. The relationship between *EGFR* amplification and the efficacy of immunotherapy remains to be explored.

In addition, this study explored the relationships between clinical features, genomic alterations, PD-L1 expression, and also TMB. The mean TMB was 10.62 mut/Mb and the median TMB was 9.35 mut/Mb, which were slightly higher than TCGA findings (mean TMB, 8.1 mut/Mb; median TMB, 8.4 mut/Mb) and similar to the CHOICE (mean TMB, 11.8 mut/Mb) studies (27,34). This may be explained due to a higher rate of heavy smokers in our cohort. LUSC was considered to have higher TMB because of tobacco exposure. A significant association was observed between smoking history and TMB in the present study with a large proportion of heavy smokers (34-37).

Currently, TMB and PD-L1 expression are identified to be the two major predictive biomarkers for anti-PD-1/PD-L1 therapy. In our study, we found no association between TMB level and PD-L1 expression, which was consistent with previous data (38). Interestingly, our findings indicated that high TMB (>10.62 mut/Mb) was correlated with significantly longer PFS and OS in advanced LUSC and especially in patients who received mono-immunotherapy, which was also consistent with previous reports (11,39-41). TMB >10.62 mut/Mb identified LUSC patients who may potentially benefit from mono-immunotherapy irrespective of PD-L1 expression and genomic alterations. Patients with a higher TMB level had a better DCR in both the total population and the mono-immunotherapy group. Moreover, univariate analyses of TMB and PD-L1 on OS and PFS showed that TMB has greater predictive value for PFS in PD-L1 negative populations and the mono-immunotherapy group.

## Conclusions

TMB is predictive factor of improved clinical outcomes in advanced LUSC patients receiving immunotherapy,

and the predictive value is more pronounced in patients treated with immunotherapy as a single agent. TMB seems to have greater predictive value for PFS in PD-L1 negative populations than in PD-L1 positive populations. Although the small sample size and cohort heterogeneity might limit the conclusions made in this study, the combination of ECOG-PS, smoking status, TMB, PD-L1, and genomic alterations might be helpful for personalized immunotherapy decisions in clinical practice for advanced LUSC.

### Acknowledgments

The authors appreciate technical support from Mr. Yonggang Ding from Jiangsu Hengrui Medicine Co.

*Funding:* This study was supported by the Natural Scientific Foundation of China (No. 81972718), the Natural Scientific Foundation of Zhejiang Province, China (No. LY19H160007 and LGF18H160017), and the Science and Technology Program for Health and Medicine in Zhejiang Province, China (2021419564).

### Footnote

*Reporting Checklist:* The authors have completed the REMARK reporting checklist. Available at <http://dx.doi.org/10.21037/tlcr-20-1130>

*Data Sharing Statement:* Available at <http://dx.doi.org/10.21037/tlcr-20-1130>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tlcr-20-1130>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the Helsinki Declaration (as revised in 2013). The study was approved by the ethics committee of Zhejiang Cancer Hospital. Informed consent was obtained from each patient.

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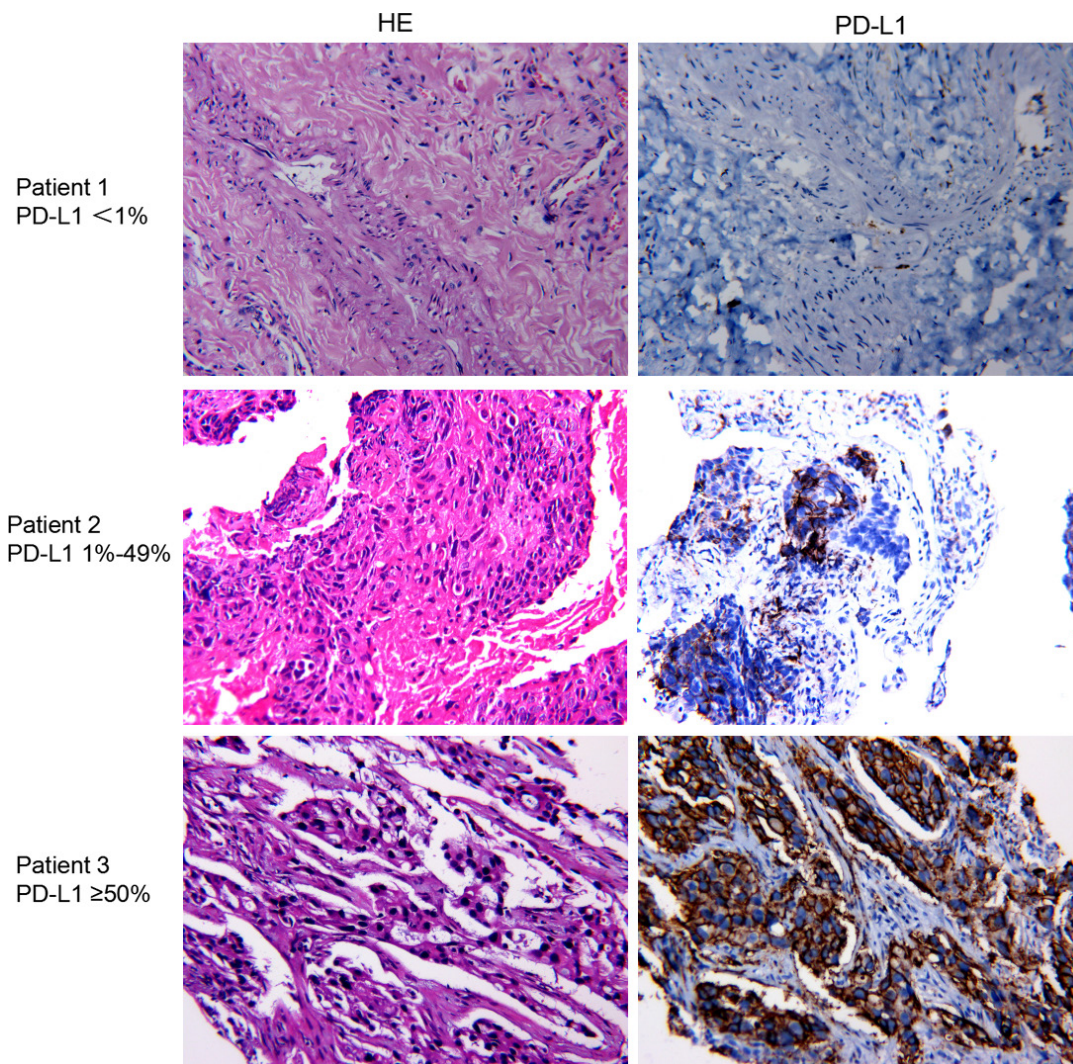
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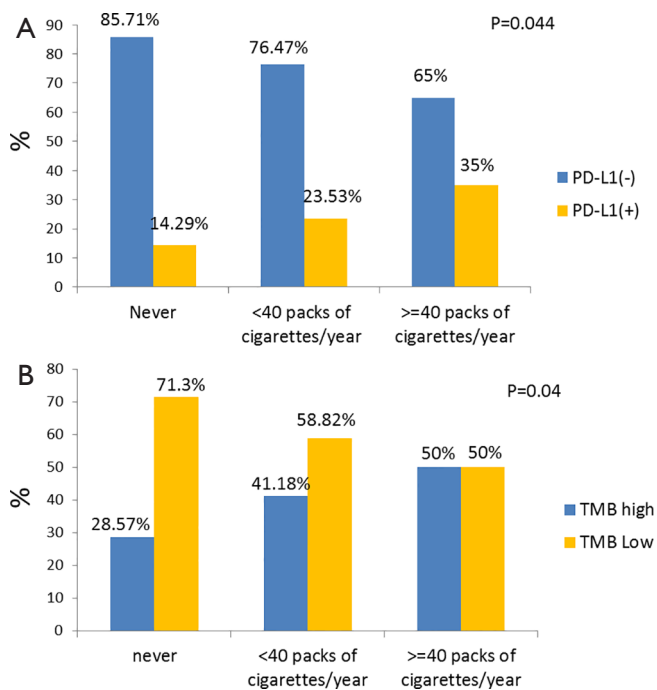
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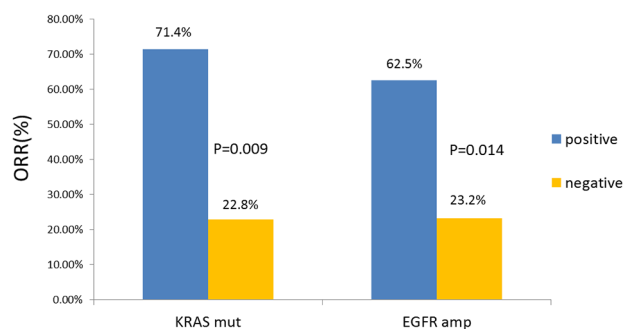
**Cite this article as:** Xu Y, Li H, Huang Z, Chen K, Yu X, Sheng J, Zhang HH, Fan Y. Predictive values of genomic variation, tumor mutational burden, and PD-L1 expression in advanced lung squamous cell carcinoma treated with immunotherapy. *Transl Lung Cancer Res* 2020;9(6):2367-2379. doi: 10.21037/tlcr-20-1130



**Figure S1** Representative IHC images. PD-L1 expression was tested by anti-human PD-L1 (Dako 22C3). The cutoff value was <1% for PD-L1 positivity or negativity. The cutoff value was  $\geq 50\%$  for high expression level. IHC, immunohistochemical; PD-L1, programmed cell death ligand-1.



**Figure S2** The relationship between smoking status, PD-L1 positivity, and TMB. (A) Patients with a history of heavy smoking ( $\geq 40$  pack-years) were more likely to have a higher PD-L1 positive rate ( $P=0.044$ ). The proportion of LUSC patients with positive PD-L1 increases with the addition of smoking pack-years. Positive PD-L1 expression was identified in 14.29% of never smokers, 23.53% of <40 pack-year smokers, and 35% of  $\geq 40$  pack-year smokers. (B) Patients with a history of heavy smoking ( $\geq 40$  pack-years) were more likely to have a higher TMB ( $P=0.04$ ). The proportion of LUSC patients with high TMB increases with the addition of smoking pack-years. High TMB were identified in 28.57% of never smokers, 41.18% of <40 pack-year smokers, and 50% of  $\geq 40$  pack-year smokers. PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden; LUSC, lung squamous cell carcinoma.



**Figure S3** Correlation of gene mutation and amplification with immunotherapy response. Compared with wild-type, patients with KRAS mutation had higher ORR (71.4% *vs.* 22.8%,  $P=0.009$ ). Compared with wild-type, patients with EGFR amplification had higher ORR (62.5% *vs.* 23.2%,  $P=0.014$ ). ORR, objective response rates.

**Table S1** Gene variations of included patients and their correlations with TMB and PD-L1 expression (N=64).

Gene	Total, N (%)	PD-L1 (-)		PD-L1 (+)		P value	TMB low*		TMB low**		P value
		n	%	n	%		n	%	N%		
TP53 mutation											
Yes	44 (68.8%)	30	68.18	14	31.82	0.5800	22	50.00	22	50.00	0.2638
No	20 (31.2%)	15	75.00	5	25.00		13	65.00	7	35.00	
CDKN2A mutation											
Yes	9 (14.1%)	6	66.67	3	33.33	0.2866	3	33.33	6	66.67	0.1129
No	55 (85.9%)	39	70.91	16	29.09		32	58.18	23	41.82	
KEAP1 mutation											
Yes	8 (12.5%)	4	50.00	4	50.00	0.1788	2	25.00	6	75.00	0.0639
No	56 (87.5%)	41	73.21	15	26.79		33	58.93	23	41.07	
CREBBP mutation											
Yes	7 (10.9%)	3	42.86	4	57.14	0.0885	3	42.86	4	57.14	0.2502
No	57 (89.1%)	42	73.68	15	26.32		32	56.14	25	43.86	
KRAS mutation											
Yes	7 (10.9%)	4	57.14	3	42.86	0.2324	5	71.43	2	28.57	0.2122
No	57 (89.1%)	41	71.93	16	28.07		30	52.63	27	47.37	
BIM mutation											
Yes	5 (7.8%)	4	80.00	1	20.00	0.3713	3	60.00	2	40.00	0.3485
No	59 (92.2%)	41	69.49	18	30.51		32	54.24	27	45.76	
AMER1 mutation											
Yes	4 (6.2%)	3	75.00	1	25.00	0.4243	1	25.00	3	75.00	0.2013
No	60 (93.8%)	42	70.00	18	30.00		34	56.67	26	43.33	
APC mutation											
Yes	4 (6.2%)	3	75.00	1	25.00	0.4243	2	50.00	2	50.00	0.3802
No	60 (93.8%)	42	70.00	18	30.00		33	55.00	27	45.00	
AR amplification											
Yes	17 (26.6%)	13	76.47	4	23.53	0.5167	9	52.94	8	47.06	0.8660
No	47 (73.4%)	32	68.09	15	31.91		26	55.32	21	44.68	
SOX2 amplification											
Yes	14 (21.9%)	9	64.29	5	35.71	0.5766	6	42.86	8	57.14	0.3144
No	50 (78.1%)	36	72.00	14	28.00		29	58.00	21	42.00	
PIK3CA amplification											
Yes	11 (17.2%)	6	54.55	5	45.45	0.2085	8	72.73	3	27.27	0.1157
No	53 (82.8%)	39	73.58	14	26.42		27	50.94	26	49.06	
EGFR amplification											
Yes	8 (12.5%)	5	62.50	3	37.50	0.2675	2	25.00	6	75.00	0.0639
No	56 (87.5%)	40	71.43	16	28.57		33	58.93	23	41.07	
RICTOR amplification											
Yes	7 (10.9%)	5	71.43	2	28.57	0.3363	5	71.43	2	28.57	0.2122
No	57 (89.1%)	40	70.18	17	29.82		30	52.63	27	47.37	
FGFR1 amplification											
Yes	6 (9.4%)	4	66.67	2	33.33	0.3398	3	50.00	3	50.00	0.3190
No	58 (90.6%)	41	70.69	17	29.31		32	55.17	26	44.83	
ZNF703 amplification											
Yes	5 (7.8%)	4	80.00	1	20.00	0.3713	3	60.00	2	40.00	0.3485
No	59 (92.2%)	41	69.49	18	30.51		32	54.24	27	45.76	
PIK3CA mutation or amplification											
Yes	23 (35.9%)	15	65.22	8	34.78	0.5040	14	60.87	9	39.13	0.4568
No	41 (64.1%)	30	73.17	11	26.83		21	51.22	20	48.78	

\*TMB low, TMB  $\leq$ 10.62 (Mean TMB for all 64 subjects is 10.62); \*\*TMB high, TMB >10.62. TMB, tumor mutational burden; PD-L1, programmed death ligand 1.



**Table S2** Univariate and multivariate analyses of clinical parameters on overall response and disease control (Logistic regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Overall response rate						
Sex (male/female)	0.607	0.093–3.962	0.6020			
Age ( $\geq 65$ / $<65$ )	0.760	0.243–2.376	0.6373			
Smoker ( $<40$ pack-years/no)	0.156	0.012–2.108	0.0603			
Smoker ( $\geq 40$ pack-years/no)	1.667	0.287–9.664	0.0512			
BMI (18.5–24/ $<18.5$ )	0.722	0.140–3.731	0.5085			
BMI ( $\geq 24$ / $<18.5$ )	0.235	0.034–1.631	0.0883			
ECOG (1–2/0)	0.009	0.001–0.057	0.0000	0.027	0.005–0.142	$<0.0001$
Dyslipidemia (yes/no)	0.889	0.303–2.606	0.8300			
Brain metastases (yes/no)	0.765	0.140–4.180	0.7569			
Liver metastases (yes/no)	0.933	0.252–3.452	0.9176			
Radiotherapy (yes/no)	0.120	0.034–0.429	0.0011	0.241	0.045–1.280	0.0949
Lines of therapy (yes/no)	$<0.001$	$<0.001 \rightarrow 999.999$	0.9402			
PD-L1 (positive/negative)	0.880	0.275–2.815	0.8297			
TMB ( $>10.62/\leq 10.62$ )	1.520	0.518–4.466	0.4459			
Disease control rate						
Sex (male/female)	$<0.001$	$<0.001 \rightarrow 999.999$	0.9661			
Age ( $\geq 65$ / $<65$ )	1.152	0.306–4.338	0.8349			
Smoker ( $<40$ pack-years/no)	0.117	0.011–1.195	0.0005	0.093	0.005–1.632	0.0036
Smoker ( $\geq 40$ pack-years/no)	6.500	0.357–118.4	0.0121	6.327	0.298–134.5	0.0134
BMI (18.5–24/ $<18.5$ )	0.604	0.063–5.773	0.5214			
BMI ( $\geq 24$ / $<18.5$ )	0.945	0.082–10.91	0.8188			
ECOG (1–2/0)	$<0.001$	$<0.001 \rightarrow 999.999$	0.9565			
Dyslipidemia (yes/no)	1.261	0.359–4.432	0.7178			
Brain metastases (yes/no)	0.652	0.114–3.715	0.6299			
Liver metastases (yes/no)	0.293	0.076–1.135	0.0756			
Radiotherapy (yes/no)	1.261	0.359–4.432	0.7178			
Lines of therapy (yes/no)	0.184	0.048–0.709	0.0139	0.425	0.050–3.640	0.4345
PD-L1 (positive/negative)	0.412	0.081–2.091	0.2845			
TMB ( $>10.62/\leq 10.62$ )	5.400	1.076–27.09	0.0404	13.109	1.429–120.2	0.0229

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

**Table S3** Univariate and multivariate analyses of immunotherapy-only patients on Progression-Free Survival and Overall Survival (Cox regression) (N=53)

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
<b>Progression-free survival</b>						
Sex (male/female)	0.953	0.530–1.713	0.8713			
Age ( $\geq 65$ / $<65$ )	1.943	0.589–6.410	0.2753			
Smoker ( $<40$ pack-years/no)	0.676	0.257–1.783	0.4292			
Smoker ( $\geq 40$ pack-years/no)	0.708	0.256–1.959	0.5056			
BMI (18.5–24/ $<18.5$ )	4.638	1.750–12.29	0.0020	0.288	0.098–0.845	0.0234
BMI ( $\geq 24$ / $<18.5$ )	2.395	0.856–6.705	0.0963	0.414	0.143–1.199	0.1041
ECOG (1–2/0)	1.099	0.420–2.881	0.8471			
Dyslipidemia (yes/no)	1.036	0.582–1.843	0.9052			
Brain metastases (yes/no)	1.273	0.564–2.873	0.5606			
Liver metastases (yes/no)	1.263	0.639–2.496	0.5012			
Radiotherapy (yes/no)	1.764	0.952–3.266	0.0711			
Lines of therapy (3–4/1–2)	2.044	1.115–3.746	0.0207	2.527	0.129–0.512	0.0045
PD-L1 (positive/negative)	0.898	0.486–1.661	0.7318			
TMB ( $>9.76$ / $\leq 9.76$ )	0.339	0.183–0.629	0.0006	0.256	0.129–0.512	0.0001
<b>Overall survival</b>						
Sex (male/female)	3850984	0.000–.	0.9893			
Age ( $\geq 65$ / $<65$ )	0.859	0.385–1.917	0.7104			
Smoker ( $<40$ pack-years/no)	5.348	0.672–42.58	0.1132			
Smoker ( $\geq 40$ pack-years/no)	2.963	0.394–22.31	0.2916			
BMI (18.5–24/ $<18.5$ )	1.573	0.363–6.809	0.5447			
BMI ( $\geq 24$ / $<18.5$ )	1.030	0.212–4.993	0.9711			
ECOG (1 – 2/0)	7.068	1.545–32.34	0.0117	3.117	0.598–16.25	0.1772
Dyslipidemia (yes/no)	0.595	0.276–1.286	0.1869			
Brain metastases (yes/no)	0.774	0.231–2.593	0.6775			
Liver metastases (yes/no)	1.328	0.534–3.303	0.5412			
Radiotherapy (yes/no)	2.712	1.140–6.455	0.0241	2.459	0.838–7.213	0.1013
Lines of therapy (yes/no)	2.210	0.995–4.905	0.0513	1.196	0.482–2.964	0.6995
PD-L1 (positive/negative)	1.074	0.470–2.457	0.8654			
TMB ( $>10.62$ / $\leq 10.62$ )	0.245	0.100–0.597	0.0020	0.210	0.080–0.551	0.0015

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

**Table S4** Univariate analyses of TMB and PD-L1 on overall survival and progression-free survival (Cox regression)

Factor	HR (log rank)	95% CI	P value
Total population (n=64)			
Overall survival			
TMB $\leq$ 10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.897	0.343–2.342	0.8240
TMB >10.62 and PD-L1 (-) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.528	0.226–1.232	0.1394
TMB >10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.240	0.054–1.062	0.0600
Progression-free survival			
TMB $\leq$ 10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.783	0.357–1.718	0.5424
TMB >10.62 and PD-L1 (-) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.494	0.262–0.928	0.0284
TMB >10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.531	0.235–1.200	0.1282
Mono-immunotherapy group (n=53)			
Overall survival			
TMB $\leq$ 10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	1.309	0.486–3.525	0.5946
TMB >10.62 and PD-L1 (-) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.307	0.112–0.839	0.0213
TMB >10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.175	0.037–0.832	0.0284
Progression-free survival			
TMB $\leq$ 10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	1.210	0.520–2.817	0.6587
TMB >10.62 and PD-L1 (-) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.345	0.168–0.710	0.0038
TMB >10.62 and PD-L1 (+) vs. TMB $\leq$ 10.62 and PD-L1 (-)	0.384	0.155–0.952	0.0388

TMB, tumor mutational burden; PD-L1, programmed cell death ligand-1;

**Table S5** Univariate and multivariate analyses of gene variations on progression-free survival and overall survival (Cox regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
Progression-free survival						
TP53 mutation (yes/no)	1.061	0.599–1.877	0.8394			
CDKN2A mutation (yes/no)	0.658	0.308–1.405	0.2791			
KEAP1 mutation (yes/no)	1.848	0.857–3.986	0.1175			
CREBBP mutation (yes/no)	1.433	0.642–3.200	0.3797			
KRAS mutation (yes/no)	0.615	0.255–1.483	0.2787			
BIM mutation (yes/no)	0.644	0.233–1.785	0.3980			
AMER1 mutation (yes/no)	0.782	0.243–2.514	0.6795			
APC mutation (yes/no)	1.798	0.639–5.059	0.2663			
AR amplification (yes/no)	1.478	0.830–2.631	0.1846			
SOX2 amplification (yes/no)	1.209	0.660–2.213	0.5392			
PIK3CA amplification (yes/no)	1.233	0.638–2.384	0.5339			
EGFR amplification (yes/no)	0.671	0.314–1.432	0.3024			
RICTOR amplification (yes/no)	1.154	0.517–2.578	0.7263			
FGFR1 amplification (yes/no)	1.110	0.474–2.602	0.8102			
ZNF703 amplification (yes/no)	1.417	0.560–3.585	0.4615			
PIK3CA mutation or amplification (yes/no)	1.153	0.670–1.985	0.6073			
Overall survival						
TP53 mutation (yes/no)	1.551	0.667–3.605	0.3081			
CDKN2A mutation (yes/no)	0.631	0.219–1.818	0.3934			
KEAP1 mutation (yes/no)	2.094	0.842–5.209	0.1120			
CREBBP mutation (yes/no)	1.307	0.455–3.752	0.6187			
KRAS mutation (yes/no)	0.678	0.200–2.300	0.5334			
BIM mutation (yes/no)	0.330	0.045–2.432	0.2769			
AMER1 mutation (yes/no)	1.080	0.255–4.564	0.9170			
APC mutation (yes/no)	1.252	0.293–5.343	0.7618			
AR amplification (yes/no)	1.253	0.575–2.732	0.5702			
SOX2 amplification (yes/no)	0.939	0.404–2.182	0.8840			
PIK3CA amplification (yes/no)	0.985	0.378–2.567	0.9746			
EGFR amplification (yes/no)	0.698	0.240–2.031	0.5098			
RICTOR amplification (yes/no)	1.194	0.415–3.435	0.7416			
FGFR1 amplification (yes/no)	0.559	0.133–2.361	0.4291			
ZNF703 amplification (yes/no)	0.422	0.057–3.108	0.3968			
PIK3CA mutation or amplification (yes/no)	0.805	0.368–1.762	0.5880			

**Table S6** Univariate and multivariate analyses of gene variations on overall response and disease control (Logistic regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Overall response rate						
TP53 mutation (yes/no)	0.696	0.224–2.163	0.5315			
CDKN2A mutation (yes/no)	3.661	0.860–15.58	0.0790			
KEAP1 mutation (yes/no)	0.765	0.140–4.180	0.7569			
CREBBP mutation (yes/no)	0.361	0.040–3.225	0.3619			
KRAS mutation (yes/no)	7.678	1.338–44.06	0.0222	11.111	1.851–66.691	0.0085
BIM mutation (yes/no)	4.031	0.616–26.39	0.1459			
AMER1 mutation (yes/no)	<0.001	<0.001–>999	0.9756			
APC mutation (yes/no)	<0.001	<0.001–>999	0.9756			
AR amplification (yes/no)	0.415	0.104–1.659	0.2136			
SOX2 amplification (yes/no)	0.933	0.252–3.452	0.9176			
PIK3CA amplification (yes/no)	0.471	0.092–2.419	0.3668			
EGFR amplification (yes/no)	5.000	1.057–23.65	0.0424	7.407	1.490–36.822	0.0144
RICTOR amplification (yes/no)	0.361	0.040–3.225	0.3619			
FGFR1 amplification (yes/no)	<0.001	<0.001–>999	0.9756			
ZNF703 Amplification (yes/no)	<0.001	<0.001–>999	0.9756			
PIK3CA mutation or amplification (yes/no)	0.365	0.104–1.276	0.1144			
Disease control rate						
TP53 mutation (yes/no)	1.762	0.483–6.433	0.3913			
CDKN2A mutation (yes/no)	0.778	0.140–4.319	0.7738			
KEAP1 mutation (yes/no)	>999	<0.001–>999	0.9569			
CREBBP mutation (yes/no)	1.434	0.156–13.16	0.7498			
KRAS mutation (yes/no)	0.532	0.090–3.142	0.4861			
BIM mutation (yes/no)	>999	<0.001–>999	0.9661			
AMER1 mutation (yes/no)	>999	<0.001–>999	0.9698			
APC mutation (yes/no)	0.673	0.064–7.098	0.7419			
AR amplification (yes/no)	1.105	0.261–4.680	0.8919			
SOX2 amplification (yes/no)	1.499	0.288–7.803	0.6302			
PIK3CA amplification (yes/no)	1.047	0.195–5.613	0.9577			
EGFR amplification (yes/no)	1.711	0.190–15.39	0.6317			
RICTOR amplification (yes/no)	0.250	0.048–1.312	0.1012			
FGFR1 amplification (yes/no)	1.170	0.124–11.05	0.8909			
ZNF703 amplification (yes/no)	0.917	0.093–9.026	0.9406			
PIK3CA mutation or amplification (yes/no)	1.152	0.306–4.338	0.8349			