



An integrated biomarker of PD-L1 expression and intraepithelial CD8⁺ T cell infiltration was associated with the prognosis of lung cancer patients after intracranial resection of brain metastases

Lin-Lin Li^{1,2}  | De-Xiang Zhou³ | Ming Lu⁴ | Dong Zhou³ | Xiao-Feng Lin³ | Yu Chen^{2,5} | Kai Yin^{1,2} | Hui-Bo Feng^{2,5} | Wei-Bang Guo² | Zhi Xie² | Wen-Qing Yan² | Zhi-Yi Lv² | Dan-Xia Lu² | Shui-Lian Zhang² | Xu-Chao Zhang^{1,2,5} 

¹The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China

²Guangdong Lung Cancer Institute, Medical Research Center, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

³Department of Neurosurgery, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

⁴Guangdong 999 Brain Hospital, Guangzhou, China

⁵Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

Correspondence

Xu-Chao Zhang, Guangdong Lung Cancer Institute, Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Medical Research Center, Cancer Center, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China; The Second School of Clinical Medicine, Southern Medical University, Guangzhou 510515, China.
Email: zhuchao3000@126.com

Funding information

GDPH Dengfeng Program, Grant/Award Number: DFJH201903 & KJ012019444 & 8197103306; General Research Project of Guangzhou Science and Technology Bureau, Grant/Award Number: 201607010391; Guangdong Provincial Key Lab of Translational Medicine in Lung Cancer, Grant/Award Number: 2017B030314120; Guangdong Provincial Natural Science Program, Grant/Award Number: 2019A1515010900; National Natural Science Foundation of China Program, Grant/Award Number: 82173202; National Natural Science Foundation of China

Abstract

Background: Brain metastases (BM) are common in lung cancer. However, data on the status of immune biomarkers in BM lesions remain limited.

Methods: We retrospectively analyzed PD-L1 expression and infiltration levels of CD3⁺, CD4⁺, CD8⁺ T cells as biomarkers by immunohistochemistry in both BM lesions and primary lung cancer (PL) lesions of 29 lung cancer (LC) patients. In addition, the correlations between these biomarkers and the clinical outcome were analyzed using log-rank test.

Results: Intratumoral heterogeneous expression of PD-L1 was observed on tumor cells (TCs) in 11 cases and on immune cells (ICs) in 10 cases with BM samples from multiple regions. There was a disagreement in PD-L1 expression on TCs between paired BM and PL lesions in 15 cases and on ICs in seven cases. Intraepithelial CD3⁺ and CD8⁺ T cell infiltration levels in BM samples were lower than those in the paired PL samples. PD-L1 positivity on both TCs and ICs was associated with a better post-BM-surgery prognosis ($p = 0.010$; $p = 0.041$). Notably, PD-L1 positivity on TCs and a high level of intraepithelial CD8⁺ T cell infiltration could serve as an integrated biomarker that indicates longer survival time ($p = 0.004$) in LC patients.

Conclusion: The heterogeneity in PD-L1 expression was common in both stromal and intraepithelial regions in BM lesions of LC patients, suggesting the need for multi-regional PD-L1 testing in clinical practice. More importantly, a combination of PD-L1 expression on TCs with intraepithelial CD8⁺ T cell infiltration might predict better post-BM-surgery outcomes.

KEYWORDS

brain neoplasms, PD-L1, survival analysis, tumor-infiltrating lymphocyte

Lin-Lin Li, De-Xiang Zhou, Ming Lu, Dong Zhou, and Xiao-Feng Lin authors have contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Thoracic Cancer* published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd.

INTRODUCTION

Brain metastases (BM) are the most common complication of lung cancer (LC).^{1,2} For LC patients with BM, the therapeutic options remain limited and the prognosis is very poor.³ Recently, immune checkpoint inhibitors (ICIs), especially programmed-death (ligand) 1 (PD-1/PD-L1) antibodies, have been demonstrated to exhibit significant efficacy in a series of trials and have become the standard of care for LC patients.⁴⁻⁷ Higher PD-L1 expression on tumor cells (TCs) has been reported to be significantly associated with longer progression-free survival (PFS) or overall survival (OS) in patients with non-small cell lung cancer (NSCLC) treated with ICIs versus those treated with chemotherapies.⁸⁻¹⁰ Preliminary observations indicated that the responses to ICIs were associated with tumor-infiltrating lymphocytes (TILs).¹¹⁻¹³

However, patients with BM are usually excluded from pivotal ICI trials. As a result, only a few trials have explored the efficacy of ICIs in patients with BM. One prospective phase II trial with pembrolizumab has explored the efficacy of ICIs in NSCLC patients with BM: an intracranial response was achieved in 33.3% (6/18) of cases.¹⁴ Updated data of this trial released in 2020 revealed that none of the patients with PD-L1-negative expression had an intracranial response, while 29.7% of patients with PD-L1-positive expression achieved an intracranial response in BM lesions. Discordant responses of brain versus extracranial lesions after ICI treatment in six cases have been observed.¹⁵ However, in that study, the researchers did not perform PD-L1 testing specifically in BM lesions. Thus, the predictive and prognostic roles of PD-L1 expression and TIL levels in patients with BM remain controversial.¹⁶⁻¹⁹ Moreover, Teng et al. showed that the tumor microenvironment could be classified into four types based on the presence of TIL and PD-L1 expression. Among them, type I tumors (PD-L1 positive with TILs driving adaptive immune resistance) are most likely to benefit from ICIs, as these tumors have been demonstrated to have pre-existing intratumor T cells turned off by PD-L1 engagement.²⁰ As for NSCLC cases, PD-L1 expression on TCs combined with CD8⁺ T cell infiltration was found to play a significant prognostic role.^{21,22} Although PD-L1 expression has been tested in BM lesions,^{16-18,23} there are scarce data on the prognostic significance of the combination of PD-L1 and TILs in patients with BM.

In this study, we retrospectively analyzed both PD-L1 expression and immune cell infiltration status in paired samples of primary lesions and BM lesions in a cohort of LC patients. Intratumoral heterogeneity and the potential prognostic role of these biomarkers in LC patients with BM were investigated.

METHODS

Patients

This was a retrospective study of LC patients histologically diagnosed with BM between 2005 and 2019 in Guangdong

Lung Cancer Institute (GLCI) at Guangdong Provincial People's Hospital. Clinical and genomic data were obtained from the electronic medical record database of GLCI. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), fluorescent in situ hybridization (FISH), SNaPshot sequencing, real-time PCR, immunohistochemistry (IHC), and next-generation sequencing (NGS) assays were performed to generate genomic profiles. Gene fusions (e.g., of ALK and ROS1) and amplifications (e.g., of MET and EGFR) were assessed by FISH, real-time PCR or NGS assays. MET amplification was defined by both a copy number gain (CNG) ≥ 5 and ratio of MET to centromeric portion of chromosome 7 (CEP7) ≥ 1.8 . EGFR amplification was defined by an EGFR/chromosome 7 copy number ratio of ≥ 2 or the presence of clusters (≥ 15 copies of EGFR per cell) in $\geq 10\%$ of cells. The relevant features of BM lesions were recorded by the latest magnetic resonance imaging (MRI) before BM resection. All patients provided written informed consent. Patients who had clinical and histological data about paired PL lesions, for whom adequate histological BM specimens containing abundant tumor cells had been available, were eligible for inclusion. A total of 29 patients with formalin-fixed paraffin-embedded (FFPE) BM samples were enrolled. All BM samples were obtained by surgical resection. Twenty-three patients had matched and available primary lung cancer (PL) samples for making wax blocks for preservation. Among them, 15 patients provided PL tissues obtained by surgery, while the remaining eight cases provided the biopsy specimens of PL tissues. Some specimens were divided into two to five sections, thus contributing several samples to our analyses. In this way, a total of 83 BM samples and 55 PL samples were finally obtained. OS was defined as the duration between the date of BM surgery and the date of death of any cause or the last follow-up

Immunohistochemistry

Immunohistochemistry (IHC) was performed using 4- μm -thick sections of FFPE samples and the standard protocols on the automated immunostaining system Omnis (DAKO). The following antibodies were used for IHC assays: PD-L1 (22C3, DAKO), CD3 (F7.2.38, DAKO), CD4 (4B12, DAKO), CD8 (clone CD8/144B, DAKO). Normal tonsil tissue was used as a positive control and normal tonsil tissue incubated with rabbit anti-human IgG (dilution 1:200; DAKO) was used as a negative control. These controls were included in every IHC assay.

Cells were considered to be PD-L1 positive if the membrane was partially or completely stained by the PD-L1 antibody. The percentages of PD-L1-positive tumor cells (TCs) and immune cells (ICs) among all the TCs and ICs were determined respectively. Samples with at least 1% PD-L1-positive cells were considered PD-L1-positive samples. We also investigated the amounts of CD3⁺, CD4⁺, and CD8⁺ T cells infiltrating into both stromal and

intraepithelial regions of tumors. T cells infiltrating into the stromal region of tumors were defined as sTCs, while T cells infiltrating into the intraepithelial region of tumors were denoted itTCs. The infiltration levels of sTC or itTC were evaluated by the percentage of the stained area among the stromal or intraepithelial region, respectively. The cutoff values determined by X-tile for CD3⁺, CD4⁺, CD8⁺ sTCs, and CD3⁺, CD4⁺, CD8⁺ itTCs were respectively set at 10%, 4%, 5%, and 5%, 1%, and 2%. Based on these thresholds, the infiltration levels of various T cells were divided into high and low density. The average values of variables among multiple samples were used to comparatively analyze the paired lesions.

Statistical analysis

The demographic characteristics of patients were summarized descriptively. Wilcoxon matched-pairs signed-rank test was used to compare different continuous variables between paired lesions. Group comparisons were performed using the Mann–Whitney U tests. Correlations among continuous variables were calculated using Spearman's correlation coefficient analysis. Correlations between categorical variables were studied using Fisher's exact test. Survival was presented with Kaplan–Meier plots and compared using the log-rank test. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 25.0 (SPSS, Inc.). Additional graphics were created with Prism 9.0 (GraphPad Software).

RESULTS

Patient characteristics

The demographic and clinical characteristics of the 29 LC patients with BM are listed in Table 1. The median age was 58 years old (range 39–72 years). The BM lesions of 26 patients were derived from the cerebrum, two from the cerebellum, and one from the brainstem. Of the 26 cases, 17 BM lesions were frontal lobe metastases (Table S1). Among the BM specimens, 24 were adenocarcinomas, two were small cell lung cancers (SCLC), two were adenosquamous carcinomas and one was adenocarcinoma combined with SCLC. In the PL specimens, 20 were adenocarcinomas, five were adenosquamous carcinomas, one was SCLC, and one was squamous carcinoma. Notably, inconsistent histological analysis results were observed between paired BM and PL lesions in six cases. We found nine cases with *EGFR* mutations, three with *ALK* rearrangements, seven with *cMET* amplifications or high expression, and one with *ROS1* rearrangement. Histological characteristics and genotypes of key LC driver genes in PL and BM lesions are also summarized in Table 1.

Intraepithelial heterogeneity in PD-L1 expression on TCs and ICs

Figures 1a,b show representative PD-L1 staining patterns. Among 19 patients with multiregional BM samples, 14 patients exhibited consistent PD-L1 expression patterns and five showed disagreements in PD-L1 expression on TCs among the BM samples. Of the 14 patients showing consistent PD-L1 expression patterns on TCs, all BM samples from seven patients were PD-L1-positive, whereas all BM samples from the other seven were PD-L1-negative (Figure 2a). The expression of PD-L1 in a BM sample was further divided into five levels by cutoffs at 1, 5, 10, and 50% PD-L1-positive TCs. In this way, BM samples from eight patients were found to have the same level of PD-L1 expression on TCs, whereas BM samples from the other 11 patients were found to have different levels of PD-L1 expression on TCs (Table S2).

Moreover, 10 patients showed consistent PD-L1 expression patterns and nine displayed disagreements in PD-L1 expression on ICs among their BM samples. Among the 10 patients showing consistent PD-L1 expression patterns on ICs, all samples from two patients were PD-L1-positive, whereas all samples from the other eight were PD-L1-negative (Figure 2b). The expression of PD-L1 in a sample was then divided into five levels using the cutoffs defined above. The results indicated that BM samples from nine patients had the same level of PD-L1 expression on ICs, whereas BM samples from the other 10 patients had different levels of PD-L1 expression on ICs (Table S2). The overall PD-L1 positivity rate on TCs was significantly higher than that on ICs (6.8% vs. 1.0%, $p = 0.003$) in BM samples.

Fifteen patients had multiregional PL samples. Among them, 13 showed consistent PD-L1 expression patterns and two displayed disagreements in PD-L1 expression on TCs among their PL samples. Of the 13 patients showing an agreement in PD-L1 expression on TCs, all PL samples from 11 were PD-L1-positive, whereas all PL samples from the other two were PD-L1-negative (Figure 3a). When the expression of PD-L1 on TCs in a sample was divided into five levels based on the cutoffs mentioned above, PL samples from six patients were found to have the same level of PD-L1 expression, whereas those from the other nine patients were found to have different levels of PD-L1 expression (Table S3).

Furthermore, nine patients displayed consistent PD-L1 expression patterns and six had disagreements in PD-L1 expression on ICs among their PL samples. Among the nine patients having consistent PD-L1 expression patterns on ICs, all PL samples from two were PD-L1-positive, while all PL samples from the other seven were PD-L1-negative (Figure 3b). We next divided PD-L1 expression on ICs into five levels using the cutoffs described above. This resulted in a same level of PD-L1 expression on ICs in PL samples from eight patients and different PD-L1 expression levels on ICs in PL samples from seven patients (Table S3). The overall

TABLE 1 Clinicopathological characteristics of 29 lung cancer patients with BM

ID	Gender (male, M or female, F)	Age (years)	Histology		Mutation type		PL	Therapy before BM surgery	Number of BM lesions	Synchronous (S) or metachronous (M)
			BM	PL	BM	PL				
1	M	63	AC	AC	<i>cMET</i> amp	WT	Targeted therapy	Single	S	
2	M	53	AC	NA	<i>cMET</i> amp, <i>EGFR</i> exon 19del	NA	Targeted therapy	Single	NA	
3	M	39	AC	AC	NA	<i>ALKr</i>	Radiotherapy	Multiple	M	
4	M	68	AC	AC	<i>ALKr</i>	<i>EGFR</i> exon 21 L858R mut	Targeted therapy	Multiple	M	
5	M	57	AC	AC	WT	WT	Chemotherapy	Single	M	
6	M	72	AC	AC	WT	WT	No therapy	Single	S	
7	M	59	AC	AC	<i>EGFR</i> exon 19del	<i>EGFR</i> exon 19del, <i>EGFR</i> exon 20 T790M	No therapy	Single	M	
8	M	63	AC	AC	NA	WT	No therapy	Multiple	M	
9	M	57	AC	AC	NA	<i>EGFR</i> exon 19del	Targeted therapy	Multiple	M	
10	M	58	AC	AC/SCC	NA	<i>EGFR</i> exon 19del	Chemotherapy	Multiple	M	
11	M	50	AC	NA	WT	NA	No therapy	Single	NA	
12	M	57	AC	AC	WT	<i>cMET</i> amp	No therapy	Single	M	
13	M	64	AC	AC	<i>EGFR</i> exon 19del	<i>EGFR</i> exon 19del	No therapy	Single	M	
14	M	40	AC	AC	NA	WT	No therapy	Single	M	
15	M	69	AC	AC	WT	WT	No therapy	Single	M	
16	M	67	AC	AC	WT	<i>cMET</i> amp	No therapy	Single	M	
17	M	48	AC	AC	NA	<i>ROS-1r, cMET</i> exp	No therapy	Multiple	M	
18	M	62	AC	AC	NA	<i>cMET</i> exp	Radiotherapy	Single	M	
19	M	65	SCLC	AC	NA	WT	No therapy	Multiple	M	
20	M	64	AC	SCC	NA	<i>cMET</i> amp	Chemotherapy	Multiple	M	
21	M	50	AC	AC	WT	WT	No therapy	Single	M	
22	M	60	AC	AC/SCC	WT	WT	No therapy	Single	M	
23	M	54	AC	AC	<i>ALKr</i>	WT	Chemotherapy	Single	M	
24	F	55	AC/SCC	AC/SCC	NA	WT	Chemotherapy	Single	S	
25	F	40	AC	AC	NA	<i>ALKr, cMET</i> exp	Targeted therapy	Multiple	M	
26	M	44	SCLC	SCLC	NA	WT	No therapy	Single	M	
27	F	50	AC/SCC	AC/SCC	NA	<i>EGFR</i> exon 19del	Chemotherapy plus Targeted therapy	Multiple	M	
28	M	66	AC	AC/SCC	<i>EGFR</i> exon 18 G719X, <i>EGFR</i> amp	<i>EGFR</i> exon 18 G719X	No therapy	Single	M	
29	M	65	SCLC_AC	AC	NA	<i>EGFR</i> exon 19del	Targeted therapy	Multiple	M	

Notes: Single lesion: only one lesion. Multiple lesions: more than one lesion. Synchronous specimens represent paired specimens obtained ≤ 1 month apart, otherwise the specimens are defined as metachronous specimens. Treatment within one year before BM surgery were recorded. Abbreviations: AC, adenocarcinoma; AC/SCC, adenocarcinoma; amp, amplification; BM, brain metastases; del, deletion; LCLC, large cell lung cancer; mut, mutation; N-r, N-arrangement; NA, not available; PL, primary lung cancer; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; SCLC_AC, small cell lung cancer combined with adenocarcinoma.

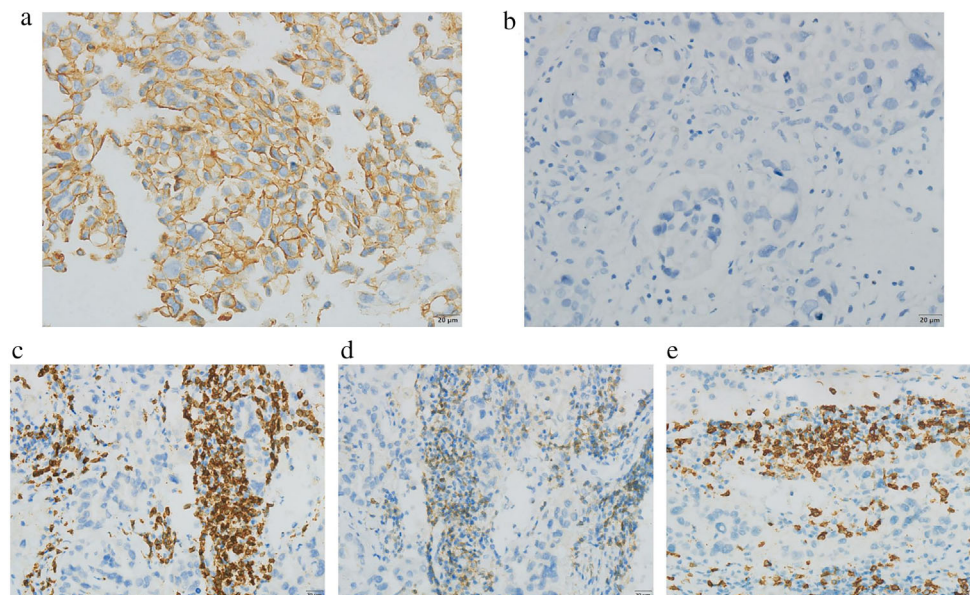


FIGURE 1 Representative IHC staining patterns for PD-L1 and T cells in BM samples. (a, b) IHC images under a 40 \times microscope showing BM samples with positive (the left panel) and negative (the right panel) PD-L1 staining. (c–e) IHC images under a 40 \times microscope showing BM samples with CD3 (the left panel), CD4 (the middle panel), and CD8 (the right panel) expression. BM, brain metastases; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1

PD-L1 positivity rate on TCs was significantly higher than that on ICs (15.6% vs. 0.6%, $p = 0.001$) in PL samples.

Heterogeneity in PD-L1 expression between paired PL and BM samples

Generally, in 20 LC patients that provided paired PL and BM tissues, there was no statistically significant difference in PD-L1 expression on TCs between the paired samples ($p = 0.25$, Figure 4a). Consistent PD-L1 expression patterns on TCs between paired PL and BM samples were observed in 15 of these 20 patients, while disagreements in PD-L1 expression on TCs between paired samples were observed in 5. Of the 15 patients exhibiting consistent PD-L1 expression patterns on TCs, the paired PL and BM samples were PD-L1-positive in 12 patients and PD-L1-negative in three cases (Figure 4b). In two cases, the TCs were PD-L1-positive in PL samples but PD-L1-negative in BM samples. In contrast, in three patients, the TCs were PD-L1-positive in BM samples but PD-L1-negative in PL samples. In four out of these five cases, the collection of BM and PL lesions was metachronous with a procurement time interval of more than 1 month. In some cases, strongly positive PD-L1 expression on TCs occurred in PL samples, but negative PD-L1 expression on TCs was observed in the matching BM samples.

In addition, consistent PD-L1 expression patterns on ICs between paired PL and BM samples were observed in 13 patients, while disagreements in PD-L1 expression on ICs between paired samples were observed in 7 cases. Among the 13 cases showing consistent PD-L1 expression patterns, the paired samples were PD-L1-positive in 5 patients and PD-L1-negative in 8 cases (Figure 4d). There was generally no statistically significant difference in PD-L1

expression on ICs between the paired PL and BM samples ($p = 0.27$, Figure 4c).

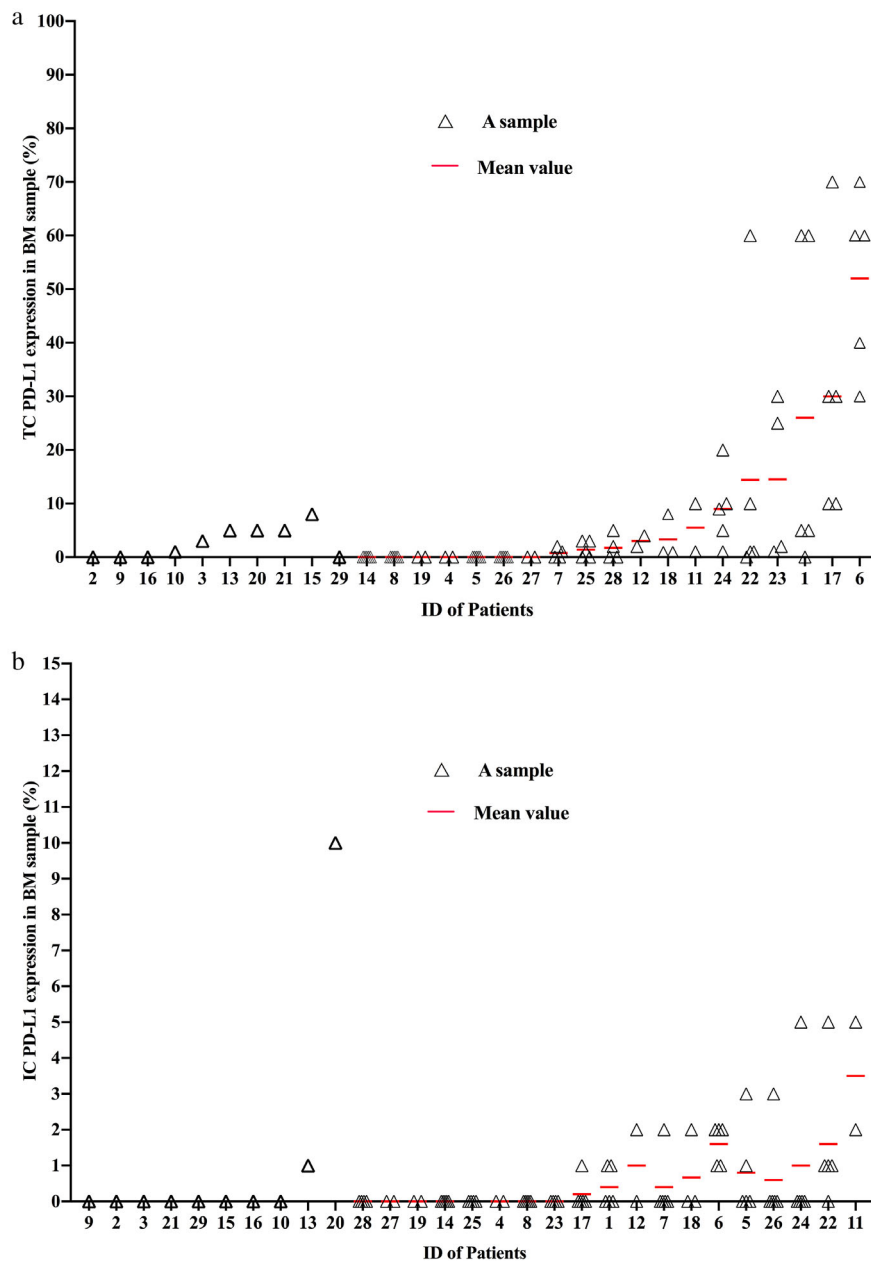
Differences in infiltration levels of T cells in the stromal and intraepithelial regions between PL and BM samples

Representative IHC staining patterns for CD3⁺, CD4⁺, CD8⁺ T cells are shown in Figure 1c–e. Overall, the percentages of CD3⁺, CD4⁺, CD8⁺ sTCs were unanimously higher than those of corresponding iTTCs in both BM (16.0% vs. 5.4%, $p < 0.001$; 4.4% vs. 0.6%, $p < 0.001$; 9.1% vs. 3.1%, $p < 0.001$, respectively) and PL (17.5% vs. 7.4%, $p < 0.001$; 2.9% vs. 0.6%, $p = 0.001$; 9.7% vs. 4.2%, $p < 0.001$, respectively) samples (Table 2). However, the percentages of CD3⁺, CD4⁺, CD8⁺ sTCs were not statistically different between paired PL and BM samples (16.0% vs. 17.5%, $p = 0.73$, Figure 5a; 4.4% vs. 2.9%, $p = 0.54$, Figure 5b; 9.1% vs. 9.7%, $p = 0.62$, Figure 5c, respectively). Notably, the percentages of CD3⁺ and CD8⁺ iTTCs in BM samples were significantly lower than those in the corresponding PL samples (5.4% vs. 7.4%, $p = 0.029$, Figure 5d; 3.1% vs. 4.2%, $p = 0.015$, Figure 5f, respectively), while CD4⁺ iTTC percentages were not significantly different between the paired lesions (0.6% vs. 0.6%, $p = 0.86$, Figure 5e).

Correlations between immune biomarkers in BM lesions and clinical factors

As shown in Table 3, PD-L1 positivity on TCs was significantly associated with the location of intracranial lesions, with significantly higher PD-L1 expression being observed in BM lesions located in the cerebrum ($p = 0.045$) than in

FIGURE 2 Overview of PD-L1 expression on TCs or ICs in BM samples. (a) Scatter plot for PD-L1 expression on TCs in BM samples for each patient. (b) Scatter plot for PD-L1 expression on ICs in BM samples for each patient. Each triangle means one sample. Each red horizontal line means the average value of PD-L1 expression among several samples of one patient determined by IHC scoring. BM, brain metastases; IC, immune cells; IC PDL1, PD-L1 expression on immune cells; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; TCs, tumor cells; TC PDL1, PD-L1 expression on tumor cells



those located in other sites. However, PD-L1 positivity on TCs was not significantly associated with age, gender, smoking history, and pathological type, and the numbers and maximum diameter of BM lesions. PD-L1 expression levels on ICs in LC patients with only a single BM lesion were significantly higher than those in patients with multiple BM lesions ($p = 0.047$). Compared with patients with multiple BM lesions (≥ 2 lesions), the patients with a single BM lesion also had significantly higher percentages of CD3⁺, CD4⁺, and CD8⁺ sTCs ($p = 0.009$, $p = 0.003$, and $p = 0.035$, respectively). The percentages of CD3⁺, CD4⁺, and CD8⁺ sTCs were also negatively correlated with the maximal diameter of BM lesions ($r = -0.41$, $p = 0.029$; $r = -0.61$, $p < 0.001$; $r = -0.49$, $p = 0.006$, respectively). The percentages of CD3⁺ and CD8⁺ itTCs were found to be

correlated with the histological diagnosis of adenocarcinoma ($p = 0.048$ and $p = 0.004$, respectively) (Table S4).

Correlations between immune biomarkers in BM lesions and survival time after BM surgery

At the last follow-up, 16 patients were alive, 12 died and one was censored. The median survival time after BM surgery was 38.5 months (95% confidence interval [CI]: 21.1–55.9). LC patients with less than 2 months of survival time after BM surgery were excluded from the following survival analysis.

Patients with PD-L1-positive TCs or ICs in their BM samples had longer OS than those with PD-L1-negative TCs

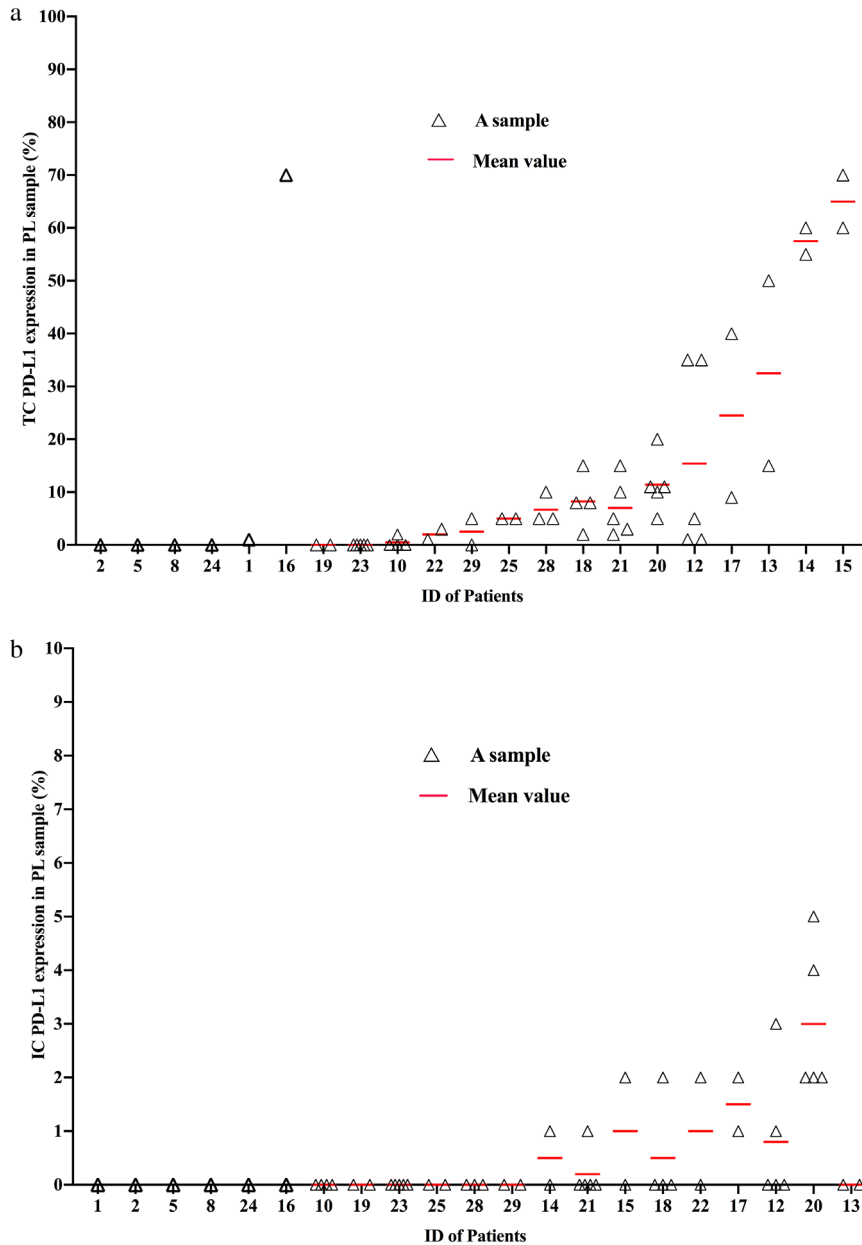


FIGURE 3 Overview of PD-L1 expression on TCs or ICs in PL samples. (a) Scatter plot for PD-L1 expression on TCs in PL samples for each patient. (b) Scatter plot for PD-L1 expression on ICs in PL samples for each patient. Each triangle means one sample. Each red horizontal line means the average value of PD-L1 expression among several samples of one patient determined by IHC scoring. ICs, immune cells; IC PDL1, PD-L1 expression on immune cells; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; PL, primary lung cancer; TCs, tumor cells; TC PDL1, PD-L1 expression on tumor cells

or ICs in their BM samples (undefined vs. 27.27 months, $\chi^2 = 6.59$, $p = 0.010$; undefined vs. 22.80 months, $\chi^2 = 4.16$, $p = 0.041$, respectively) (Figure 6). Patients with a high level of CD3⁺ itTC infiltration tended to have longer OS than those with a low level of CD3⁺ itTC infiltration (undefined vs. 27.27 months, $\chi^2 = 3.84$, $p = 0.050$), although the difference was not statistically significant. The level of CD3⁺ sTC infiltration was not significantly associated with OS (39.17 vs. 38.50 months, $\chi^2 = 0.24$, $p = 0.62$). Patients with a high level of CD8⁺ sTC or itTC infiltration had longer OS than those with a low level of CD8⁺ sTC or itTC infiltration (undefined vs. 22.80 months, $\chi^2 = 0.96$, $p = 0.33$; undefined vs. 27.27 months, $\chi^2 = 1.25$, $p = 0.26$, respectively), although the differences were not statistically significant. Patients with a high CD4⁺ sTC or itTC infiltration level had a longer survival time after BM resection than

those with a low CD4⁺ sTC or itTC infiltration level (undefined vs. 27.27 months, $\chi^2 = 1.84$, $p = 0.18$; undefined vs. 27.27 months, $\chi^2 = 1.38$, $p = 0.24$, respectively), although the differences were not statistically significant (Figure S1).

We next stratified the patients based on a combination of PD-L1 expression on TCs and T cell infiltration to analyze the prognosis of LC patients after BM surgery. Of note, patients with both PD-L1-positive TCs and a high infiltration level of CD3⁺ or CD8⁺ itTCs exhibited longer OS (undefined vs. 27.27 months, $\chi^2 = 6.26$, $p = 0.012$, Figure 7a; undefined vs. 27.27 months, $\chi^2 = 8.37$, $p = 0.004$, Figure 7c, respectively). In addition, patients who had both PD-L1-positive TCs and a high infiltration level of CD3⁺ or CD8⁺ sTCs also showed a better prognosis after BM resection (undefined vs. 22.80 months, $\chi^2 = 5.27$, $p = 0.022$,

FIGURE 4 Comparison of PD-L1 expression on TCs and ICs between PL and BM samples of LC patients with BM. (a, c) PD-L1 expression levels on TCs and ICs in BM samples and matched PL samples were compared. The connecting line means the comparison of PD-L1 expression between paired BM and PL samples from one patient. (b, d) PD-L1 expression status on TCs and ICs in BM samples and matched PL samples for every patient are shown. Dark and light blue boxes respectively represent the positive and negative status of PD-L1 expression, with the cutoff value set at 1%. BM, brain metastases; ICs, immune cells; PD-L1, programmed death-ligand 1; PL, primary lung cancer; TCs, tumor cells

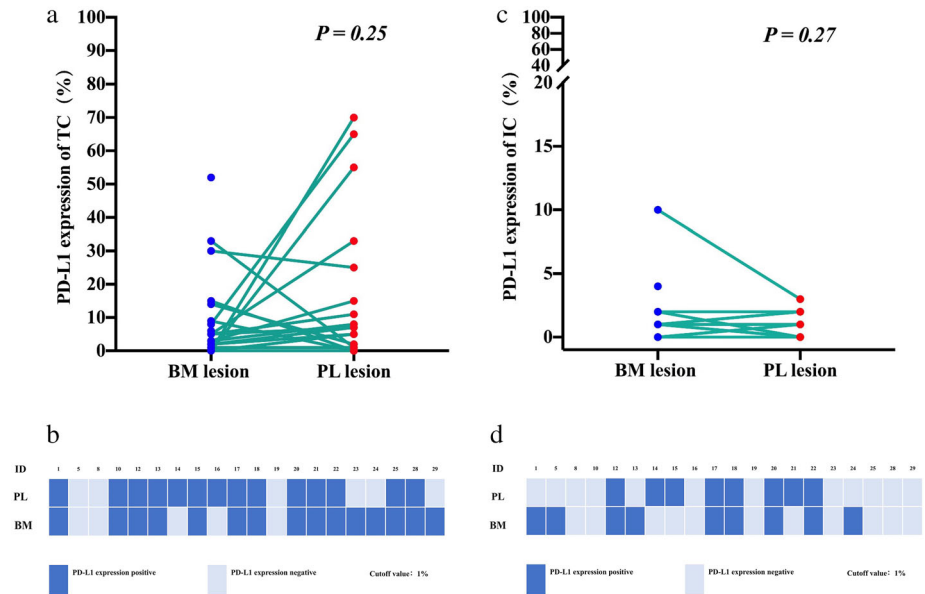


TABLE 2 PL and BM lesions with different T cell infiltration levels in stromal and intraepithelial regions

		CD3 ⁺ T cell			CD4 ⁺ T cell			CD8 ⁺ T cell		
		Low (<15%)	High (≥15%)	p-value	Low (<10%)	High (≥10%)	p-value	Low (<5%)	High (≥5%)	p-value
PL	Stromal	9	14	<0.001	14	9	0.001	19	4	<0.001
	Intraepithelial	22	1		22	1		23	0	
BM	Stromal	13	10	<0.001	17	6	<0.001	18	5	<0.001
	Intraepithelial	22	1		22	1		23	0	

Note: p-values were obtained by Wilcoxon matched-pairs signed-rank test. Abbreviations: BM, brain metastases; PL, primary lung cancer.

Figure 6d; undefined vs. 27.27 months, $\chi^2 = 4.31$, $p = 0.038$, Figure 7f, respectively). Furthermore, patients with both PD-L1-positive TCs and a high infiltration level of CD4⁺ iTTCs had statistically longer OS (undefined vs. 27.27 months, $\chi^2 = 4.40$, $p = 0.036$, Figure 7b). Finally, those patients with both PD-L1-positive TCs and a high infiltration level of CD4⁺ sTTCs tended to have a better prognosis after BM surgery (undefined vs. 27.27 months, $\chi^2 = 3.64$, $p = 0.056$, Figure 7d), although the difference was not statistically significant.

Correlations between immune biomarkers in PL lesions and survival time after BM surgery

We also performed KM analysis to investigate the correlations between immune biomarkers in PL lesions and LC patient survival after BM surgery. These immune biomarkers included PD-L1 expression, CD3⁺ T cell infiltration, CD4⁺ T cell infiltration, CD8⁺ T cell infiltration, and combinations of PD-L1 expression/CD3⁺ T cell infiltration, PD-L1 expression/CD4⁺ T cell infiltration, and PD-L1 expression/CD8⁺ T cell infiltration. The data showed that these biomarkers in PL lesions were not significantly

associated with the survival of LC patients after BM surgery (Figure S2).

DISCUSSION

The therapeutic effects of targeted therapies and ICIs in BM patients have been investigated in clinical trials, revealing their efficacy in some but not all patients.^{24–28} Thus, the cellular and molecular characteristics of BM lesions need to be extensively studied to improve our understanding of the tumor microenvironment and facilitate precision treatment.

We collected both PL and BM lesions from 29 LC patients and compared PD-L1 expression levels in multiple samples from the same patient. PD-L1 was found to be heterogeneously expressed on TCs and ICs in multiple BM samples from 26.3% (5/19) and 47.4% (9/19) cases, respectively. In addition, heterogeneous PD-L1 expression on TCs and ICs were observed in PL samples from 13.3% (2/15) and 40.0% (6/15) LC patients, respectively. Moreover, by comparing paired BM and PL lesions, we found heterogeneous PD-L1 expression on TCs and ICs in 25.0% (5/20) and 35.0% (7/20) cases, respectively. These results suggested that there was heterogeneity in PD-L1 expression on TCs and

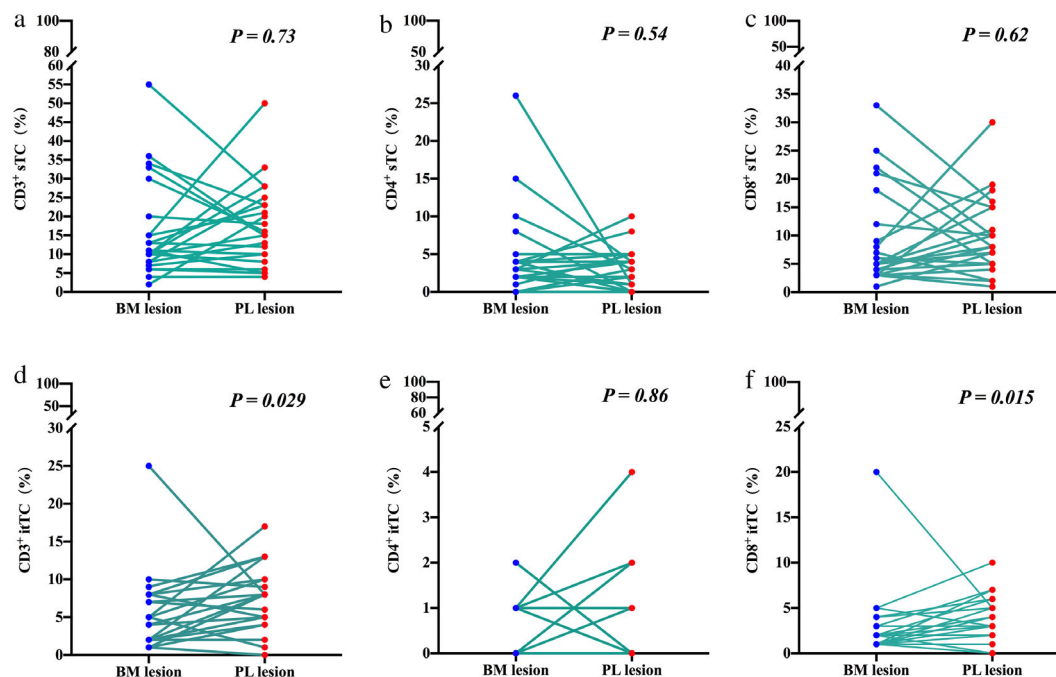


FIGURE 5 Comparison of proportions of infiltrating T cells between PL and BM samples of LC patients with BM. (a–c) Stromal CD3⁺, CD4⁺, CD8⁺ T cell percentages in BM samples and matched PL samples were compared. (d–f) Intraepithelial CD3⁺, CD4⁺, CD8⁺ T cell percentages in BM samples and matched PL samples were compared. The connecting line means the comparison between paired BM and PL samples from one patient. sTC, T cells infiltrating into the stromal region of tumors; iTTC, T cells infiltrating into the intraepithelial region of tumors. BM, brain metastases; LC, lung cancer; PL, primary lung cancer

ICs within PL and BM samples and between paired samples as well. This is in line with the findings of previous studies,^{18,23,29} suggesting PD-L1 testing in multiple regions or wax blocks of each resected cancer tissue is necessary. A trial conducted at the Yale University¹⁵ reported that inconsistent responses appeared in systemic and BM sites. In most of the previous studies, PD-L1 expression status was determined in cancer tissues from different sites. Notably, in some patients with ICI-nonresponsive BM, PD-L1 expression or T cell infiltration was positive in PL lesions, but the status of these biomarkers remains unknown in BM. The intertumoral heterogeneity of PD-L1 also suggests that caution should be taken when the immune status in the BM microenvironment based simply on data obtained from PL lesions is evaluated.

At present, there are four PD-L1 IHC assays using four PD-L1 antibodies (22C3, 28–8, SP263, SP142) approved by the US Food and Drug Administration (FDA).³⁰ Among them, only the SP142-based assay has been used to evaluate PD-L1 expression on ICs in NSCLC clinical trials to date.³¹ However, the Blueprint (BP) 2 Project has demonstrated that the SP142 antibody appeared to stain fewer TCs and ICs compared with the 22C3, 28–8, and SP263 antibodies.³² Therefore, the 22C3-based assay, which has been chosen for exploratory PD-L1 staining on ICs in some studies,^{15,33,34} was adopted in our study. Yet, we agree that the 22C3-based PD-L1 staining on ICs needs further evaluation by clinical trials.

We also analyzed the infiltration levels of sTCs and iTTCs in PL and BM lesions. Overall, the percentages of CD3⁺, CD4⁺, CD8⁺ sTCs were unanimously higher than those of corresponding iTTCs in PL and BM lesions, which is concordant with the findings reported previously.³⁵ However, we did not find any differences in the percentages of sTCs between paired samples. Notably, CD3⁺ and CD8⁺ iTTC percentages in BM lesions were significantly lower than those in PL lesions. These results indicated lower CD8⁺ T cell infiltration levels in BM lesions, which might be helpful to explain the immune status in BM, although the overall PD-L1 expression levels were not significantly different between BM and PL.

We further briefly analyzed the relationships between clinical factors and immune biomarkers in BM lesions. The associations between intracranial tumor location and immune biomarkers remain unknown so far. In the present study, 89.7% of BM samples were originated from the cerebrum, especially the frontal lobe, which is consistent with the findings by Wang et al.³⁶ Zhang et al. reported that low expression of PD-L1 may be associated with the location of primary diffuse gliomas.³⁷ Our results also exhibited significantly higher PD-L1 expression on TCs in BM samples derived from the cerebrum. In addition, we found that the number and largest diameter of BM lesions were negatively correlated with the percentage of CD8⁺ T cells and PD-L1 expression on ICs in BM lesions. Therefore, in clinical practice, when testing PD-L1 expression in BM lesions, the

TABLE 3 Correlations between clinical factors and PDL1 expression on TCs and CD8⁺ T cell infiltration level in BM samples

Factor	Total	TC PD-L1 <1%	TC PD-L1 ≥1%	<i>p</i> -value	CD8 ⁺ sTC low	CD8 ⁺ sTC high	<i>p</i> -value	CD8 ⁺ itTC low	CD8 ⁺ itTC high	<i>p</i> -value
Age (years)										
<65	22	7	15	0.375 ^a	8	14	0.403 ^a	7	15	0.375 ^a
≥65	7	4	3		4	3		4	3	
Gender										
Male	26	10	16	1.000 ^a	10	16	0.553 ^a	9	17	0.939 ^a
Female	3	1	2		2	1		2	1	
Smoking status										
Never	19	5	14	0.114 ^a	8	11	1.000 ^a	7	12	1.000 ^a
Current/former	10	6	4		4	6		4	6	
Histology										
AC	24	7	17	0.054 ^a	9	15	0.622 ^a	6	18	0.004^a
Not AC	5	4	1		3	2		5	0	
BM position										
Cerebrum	26	8	18	0.045^a	11	15	1.000 ^a	9	17	0.135 ^a
Cerebellum	2	2	0		1	1		2	0	
Brainstem	1	1	0		0	1		0	1	
Number of BM lesions										
Only one lesion	18	5	13	0.079 ^b	5	13	0.035^b	4	14	0.121 ^b
≥2 lesions	11	6	5		7	4		7	4	
Maximum diameter of BM samples (cm)										
<3.0	11	4	7	0.928 ^c	1	10	0.006^c	3	8	0.838 ^c
≥3.0	18	7	11		11	7		8	10	

Note: The significant *P* values were highlighted by bold form.

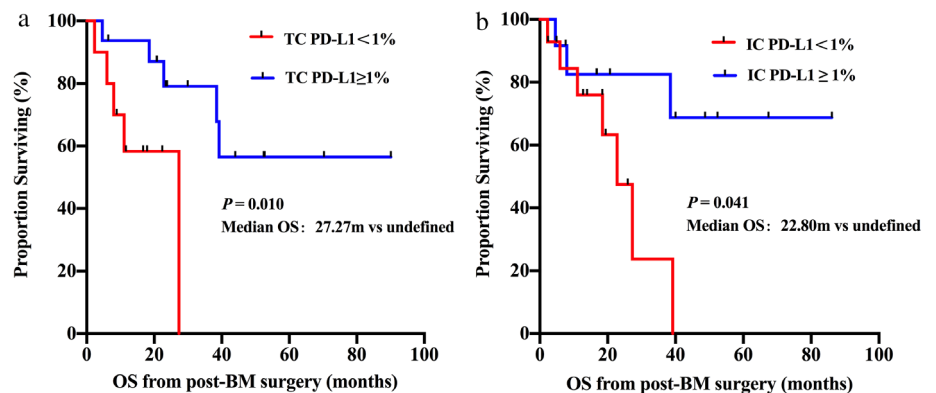
Abbreviations: AC, adenocarcinoma; BM, brain metastases; sTC, T cells infiltrating into the stromal region of tumors; itTC, T cells infiltrating into the intraepithelial region of tumors; TC PDL1, PD-L1 expression on tumor cells.

^aFisher's exact test.

^bMann-Whitney U test.

^cSpearman's correlation test.

FIGURE 6 Kaplan–Meier analysis of the associations of PD-L1 expression on TCs and ICs in BM lesions with post-BM-surgery survival of LC patients. (a, b) Kaplan–Meier survival analysis of the associations of PD-L1 expression on TCs and ICs in BM lesions with OS after BM surgery, respectively. The cutoff value for PD-L1 expression was set at 1%. *p*-values were calculated using the log-rank test. BM, brain metastases; ICs, immune cells; LC, lung cancer; OS, overall survival; PD-L1, programmed death-ligand 1; PL, primary lung cancer; TCs, tumor cells



location, size, and number of BM lesions should also be taken into account.

The prognostic value of PD-L1 expression in PL lesions has been previously investigated.^{4,5,7,38} However, the correlations of PD-L1 expression and T cell infiltration with the survival time of BM patients remain to be investigated. In our study, BM patients with PD-L1-positive TCs had longer survival after intracranial surgery. Hulsbergen et al. showed

that PD-L1 positivity on TCs predicted favorable OS in NSCLC patients with BM receiving ICIs. However, in their study, only eight of 49 samples subjected to PD-L1 testing were derived from BM lesions.³⁹ Some other studies showed that PD-L1 expression in BM lesions had no significant correlation with survival in patients with cancers.^{15,17–19} These inconsistent findings may be caused by the use of different PD-L1 IHC antibodies, varied cutoff values for PD-L1

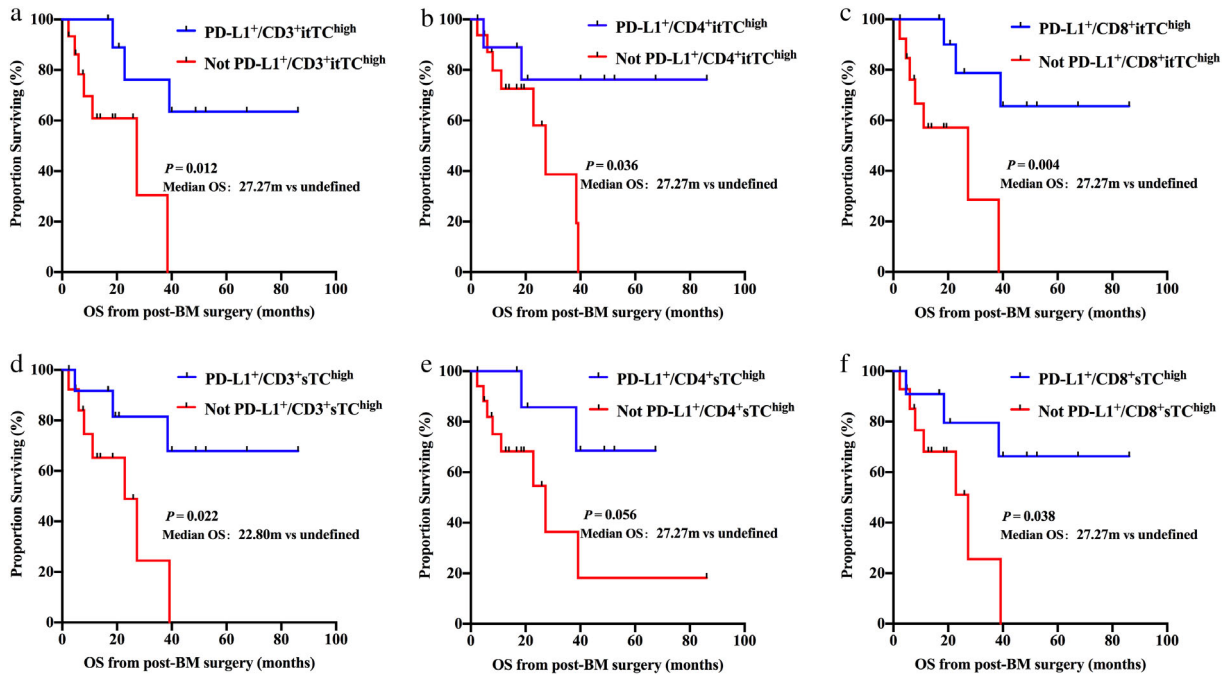


FIGURE 7 Correlations between the combinations of PD-L1 expression on TCs and T cell infiltration with post-BM-surgery survival of LC patients. (a–c) Kaplan–Meier analysis of OS after BM surgery based on the combinations of PD-L1 expression on TCs with CD3⁺, CD4⁺, and CD8⁺ iTTC infiltration. (d–f) Kaplan–Meier analysis of OS after BM surgery based on the combinations of PD-L1 expression on TCs with CD3⁺, CD4⁺, and CD8⁺ sTC infiltration. The cutoff value for PD-L1 expression was set at 1%. p -values were calculated using the log-rank test. BM, brain metastases; iTTC, T cells infiltrating into the intraepithelial region of tumors; PD-L1, programmed death-ligand 1; sTC, T cells infiltrating into the stromal region of tumors; TCs, tumor cells; TC PD-L1, PD-L1 expression on tumor cells

positivity, and various sampling sites for PD-L1 testing. In fact, PD-L1 expression varies substantially across anatomic sites and PD-L1 expression at different sites may have different prognostic values.⁴⁰ Therefore, to precisely reflect the PD-L1 expression status in BM lesions, future studies should both adopt standardized PD-L1 testing methods and collect various specimens at different anatomic sites.

The activation of the PD-1/PD-L1 axis results in peripheral T cell tolerance. Berghoff et al. found a correlation between the infiltration of CD3⁺ or CD8⁺ T cells in BM samples and patient survival.¹⁶ The case series presented by Zhou¹⁸ et al. showed that a higher level of stromal CD8⁺ T cell infiltration in BM samples was associated with longer OS in patients with NSCLC. In our study, the higher proportion of CD8⁺ sTCs in BM samples was also correlated with a longer survival time after BM surgery in LC patients. All the results suggest that TILs may play a prognostic role in NSCLC patients with BM.

Zhou et al. explored the prognostic value of the combination of PD-L1 expression and CD8⁺ T cell infiltration in PL-resected LC patients. The longest and shortest OS values were respectively observed in patients with PD-L1⁺/CD8^{high} and PD-L1⁺/CD8^{low}.⁴¹ However, Tokito et al. found that the PD-L1⁺/CD8^{low} group had the shortest survival time, whereas the PD-L1⁻/CD8^{high} group had the longest survival time in patients with stage III NSCLC.²¹ In our study, the combination of PD-L1 positivity on TCs and a high infiltration level of CD3⁺ or CD8⁺ T cells in BM lesions, rather

than in PL lesions, was associated with a longer survival time in LC patients after BM surgery. Our study also emphasized that PD-L1 expression and T cell infiltration level in BM lesions might be superior to those in PL lesions in terms of predicting the prognosis of LC patients with BM. Due to the limited number of cases, we did not further classify the LC patients into four subgroups according to the PD-L1 positivity and the infiltration level of T cells in BM lesions. Overall, testing both PD-L1 expression and TILs may be a better approach for predicting the prognosis of LC patients with BM and can help to precisely select the population that can benefit from ICIs.

Our study has several limitations. First, it was a retrospective study with a potential bias of selection for patients and samples. Second, LC patients with BM had some clinical symptoms before BM resection. Whether prior hormone use for relieving the symptoms can affect the analysis of immune biomarkers in BM samples remains unexplored due to the lack of relevant data. Third, the correlations between immune biomarkers and responses to ICIs could not be investigated due to the lack of LC patients treated by ICIs. Finally, the small sample size of our study limited the multivariate regression analysis.

In conclusion, our data exhibited the stromal, intraepithelial, and intertumoral heterogeneity in PD-L1 expression and T cell infiltration level in LC patients with BM. The combination of PD-L1 positivity on TCs and infiltration of CD3⁺ or CD8⁺ T cells in BM lesions might be an effective

prognostic biomarker to estimate LC patients' survival time after BM surgery. This study provided preliminary but important data on immune biomarkers in the microenvironment of BM lesions in LC patients, which may help us select LC patients who can respond better to ICIs. Our findings need to be verified by future studies with larger sample sizes.

ACKNOWLEDGMENTS

The authors are grateful to TopEdit (www.topedit.com) for its linguistic assistance. This study was supported by the following grants: Guangdong Provincial Natural Science Program (no. 2019A1515010900, X.Z.); National Natural Science Foundation of China Program (no. 82173202, X.Z.); GDPH Deng Feng Program (nos. DFJH201903 & KJ012019444 and 8197103306, X.Z.); Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer (No.2017B030314120, Y.W.); General Research Project of Guangzhou Science and Technology Bureau (No.201607010391, X.Z.).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ORCID

Lin-Lin Li  <https://orcid.org/0000-0001-5440-4797>

Xu-Chao Zhang  <https://orcid.org/0000-0002-4138-8115>

REFERENCES

- Fenske DC, Price GL, Hess LM, John WJ, Kim ES. Systematic review of brain metastases in patients with non-small-cell lung cancer in the United States, European Union, and Japan. *Clin Lung Cancer*. 2017; 18(6):607–14.
- Ostrom QT, Wright CH, Barnholtz-Sloan JS. Brain metastases: epidemiology. *Handb Clin Neurol*. 2018;149:27–42.
- Schuler M, Wu YL, Hirsh V, O'Byrne K, Yamamoto N, Mok T, et al. First-line afatinib versus chemotherapy in patients with non-small cell lung cancer and common epidermal growth factor receptor gene mutations and brain metastases. *J Thorac Oncol*. 2016;11(3):380–90.
- Wu YL, Lu S, Cheng Y, Zhou C, Wang J, Mok T, et al. Nivolumab versus docetaxel in a predominantly chinese patient population with previously treated advanced NSCLC: CheckMate 078 Randomized Phase III Clinical Trial. *J Thorac Oncol*. 2019;14(5):867–75.
- Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823–33.
- Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2):123–35.
- Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627–39.
- Reck M, Taylor F, Penrod JR, DeRosa M, Morrissey L, Dastani H, et al. Impact of nivolumab versus docetaxel on health-related quality of life and symptoms in patients with advanced squamous non-small cell lung cancer: results from the CheckMate 017 study. *J Thorac Oncol*. 2018;13(2):194–204.
- Reck M, Brahmer J, Bennett B, Taylor F, Penrod JR, DeRosa M, et al. Evaluation of health-related quality of life and symptoms in patients with advanced non-squamous non-small cell lung cancer treated with nivolumab or docetaxel in CheckMate 057. *Eur J Cancer*. 2018;102:23–30.
- De Lima LG, Wu Y-L, Sadowski S, Zhang J, Rangwala R, Kush D, et al. P2.43: pembrolizumab vs platinum-based chemotherapy for PD-L1+ NSCLC: phase 3, randomized, open-label KEYNOTE-042 (NCT02220894). *J Thorac Oncol*. 2016;11(10):S244–S5.
- Kim M-Y, Koh J, Kim S, Go H, Jeon YK, Chung DH. Clinicopathological analysis of PD-L1 and PD-L2 expression in pulmonary squamous cell carcinoma: comparison with tumor-infiltrating T cells and the status of oncogenic drivers. *Lung Cancer*. 2015;88(1):24–33.
- Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, et al. Subsets of exhausted CD8⁺ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol*. 2019;20(3):326–36.
- Gide TN, Quek C, Menzies AM, Tasker AT, Shang P, Holst J, et al. Distinct immune cell populations define response to anti-PD-1 monotherapy and anti-PD-1/anti-CTLA-4 combined therapy. *Cancer Cell*. 2019;35(2):238–55.e6.
- Goldberg SB, Gettinger SN, Mahajan A, Chiang AC, Herbst RS, Sznol M, et al. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. *Lancet Oncol*. 2016; 17(7):976–83.
- Goldberg SB, Schalper KA, Gettinger SN, Mahajan A, Herbst RS, Chiang AC, et al. Pembrolizumab for management of patients with NSCLC and brain metastases: long-term results and biomarker analysis from a non-randomised, open-label, phase 2 trial. *Lancet Oncol*. 2020;21(5):655–63.
- Berghoff AS, Fuchs E, Ricken G, Mlecnik B, Bindea G, Spanberger T, et al. Density of tumor-infiltrating lymphocytes correlates with extent of brain edema and overall survival time in patients with brain metastases. *Onco Targets Ther*. 2016;5(1):e1057388.
- Teglasi V, Reiniger L, Fabian K, Pipek O, Csala I, Bago AG, et al. Evaluating the significance of density, localization, and PD-1/PD-L1 immunopositivity of mononuclear cells in the clinical course of lung adenocarcinoma patients with brain metastasis. *Neuro-Oncology*. 2017;19(8):1058–67.
- Zhou J, Gong Z, Jia Q, Wu Y, Yang ZZ, Zhu B. Programmed death ligand 1 expression and CD8(+) tumor-infiltrating lymphocyte density differences between paired primary and brain metastatic lesions in non-small cell lung cancer. *Biochem Biophys Res Commun*. 2018; 498(4):751–7.
- Camy F, Karpathiou G, Dumollard JM, Magne N, Perrot JL, Vassal F, et al. Brain metastasis PD-L1 and CD8 expression is dependent on primary tumor type and its PD-L1 and CD8 status. *J Immunother Cancer*. 2020;8(2):e000597.
- Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying cancers based on T-cell infiltration and PD-L1. *Cancer Res*. 2015;75(11):2139–45.
- Tokito T, Azuma K, Kawahara A, Ishii H, Yamada K, Matsuo N, et al. Predictive relevance of PD-L1 expression combined with CD8⁺ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy. *Eur J Cancer*. 2016;55:7–14.
- Gennen K, Kasmann L, Taugner J, Eze C, Karin M, Roengvoraphoj O, et al. Prognostic value of PD-L1 expression on tumor cells combined with CD8⁺ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy. *Radiat Oncol*. 2020;15(1):1–12.
- Mansfield AS, Aubry MC, Moser JC, Harrington SM, Dronca RS, Park SS, et al. Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer. *Ann Oncol*. 2016;27(10):1953–8.
- Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med*. 2017;376(7):629–40.
- Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013;368(25):2385–94.

26. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2012;13(3):239–46.
27. Ahmed KA, Kim S, Arrington J, Naghavi AO, Dilling TJ, Creelan BC, et al. Outcomes targeting the PD-1/PD-L1 axis in conjunction with stereotactic radiation for patients with non-small cell lung cancer brain metastases. *J Neuro-Oncol*. 2017;133(2):331–8.
28. Hendriks LEL, Henon C, Auclin E, Mezquita L, Ferrara R, Audigier-Valette C, et al. Outcome of patients with non-small cell lung cancer and brain metastases treated with checkpoint inhibitors. *J Thorac Oncol*. 2019;14(7):1244–54.
29. Takamori S, Toyokawa G, Okamoto I, Takada K, Kozuma Y, Matsubara T, et al. Discrepancy in programmed cell death-ligand 1 between primary and metastatic non-small cell lung cancer. *Anticancer Res*. 2017;37(8):4223–8.
30. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol*. 2017;12(2):208–22.
31. Ancevski Hunter K, Socinski MA, Villaruz LC. PD-L1 testing in guiding patient selection for PD-1/PD-L1 inhibitor therapy in lung cancer. *Mol Diagn Ther*. 2018;22(1):1–10.
32. Tsao MS, Kerr KM, Kockx M, Beasley MB, Borczuk AC, Botling J, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of blueprint phase 2 project. *J Thorac Oncol*. 2018;13(9):1302–11.
33. Batur S, Dulger O, Durak S, Yumuk PF, Caglar HB, Bozkurtlar E, et al. Concordance of PD-L1 expression and CD8⁺ TIL intensity between NSCLC and synchronous brain metastases. *Bosn J Basic Med Sci*. 2020;20(3):329–35.
34. Bonanno L, Pavan A, Dieci MV, Di Liso E, Schiavon M, Comacchio G, et al. The role of immune microenvironment in small-cell lung cancer: distribution of PD-L1 expression and prognostic role of FOXP3-positive tumour infiltrating lymphocytes. *Eur J Cancer*. 2018;101:191–200.
35. Berghoff AS, Ricken G, Widhalm G, Rajky O, Dieckmann K, Birner P, et al. Tumour-infiltrating lymphocytes and expression of programmed death ligand 1 (PD-L1) in melanoma brain metastases. *Histopathology*. 2015;66(2):289–99.
36. Wang G, Xu J, Qi Y, Xiu J, Li R, Han M. Distribution of brain metastasis from lung cancer. *Cancer Manag Res*. 2019;11:9331–8.
37. Zhang L, Zhang B, Dou Z, Wu J, Iranmanesh Y, Jiang B, et al. Immune checkpoint-associated locations of diffuse gliomas comparing pediatric with adult patients based on voxel-wise analysis. *Front Immunol*. 2021;12:582594.
38. Velcheti V, Patwardhan PD, Liu FX, Chen X, Cao X, Burke T. Real-world PD-L1 testing and distribution of PD-L1 tumor expression by immunohistochemistry assay type among patients with metastatic non-small cell lung cancer in the United States. *PLoS One*. 2018;13(11):e0206370.
39. Hulsbergen AFC, Mammi M, Nagtegaal SHJ, Lak AM, Kavouridis V, Smith TR, et al. Programmed death receptor ligand one expression may independently predict survival in non-small cell lung carcinoma brain metastases patients receiving immunotherapy. *Int J Radiat Oncol Biol Phys*. 2020;108(1):258–67.
40. Hong L, Negrao MV, Dibaj SS, Chen R, Reuben A, Bohac JM, et al. Programmed death-ligand 1 heterogeneity and its impact on benefit from immune checkpoint inhibitors in NSCLC. *J Thorac Oncol* 2020;15(9):1449–59.
41. Yang H, Shi J, Lin D, Li X, Zhao C, Wang Q, et al. Prognostic value of PD-L1 expression in combination with CD8⁺ TILs density in patients with surgically resected non-small cell lung cancer. *Cancer Med* 2018;7(1):32–45.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Li L-L, Zhou D-X, Lu M, Zhou D, Lin X-F, Chen Y, et al. An integrated biomarker of PD-L1 expression and intraepithelial CD8⁺ T cell infiltration was associated with the prognosis of lung cancer patients after intracranial resection of brain metastases. *Thorac Cancer*. 2022;13(13):1948–60. <https://doi.org/10.1111/1759-7714.14473>