

# Prognostic significance of volume-based $^{18}\text{F}$ -FDG PET/CT parameters and correlation with PD-L1 expression in patients with surgically resected lung adenocarcinoma

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## Abstract

The aim of this study was to retrospectively analyze  $^{18}\text{F}$ -FDG positron emission tomography/computed tomography ( $^{18}\text{F}$ -FDG PET/CT) metabolic variables, programmed death-ligand 1 (PD-L1) and phosphorylated signal transducer and activator of transcription 3 (p-STAT3) tumor expression, and other factors as predictors of disease-free survival (DFS) in patients with lung adenocarcinoma (LUAD) (stage IA–IIIA) who underwent surgical resection. We still lack predictor of immune checkpoint (programmed cell death-1 [PD-1]/PD-L1) inhibitors. Herein, we investigated the correlation between metabolic parameters from  $^{18}\text{F}$ -FDG PET/CT and PD-L1 expression in patients with surgically resected LUAD.

Seventy-four patients who underwent  $^{18}\text{F}$ -FDG PET/CT prior to treatment were consecutively enrolled. The main  $^{18}\text{F}$ -FDG PET/CT-derived variables were primary tumor maximum standardized uptake value (SUVmax), metabolic tumor volume (MTV), and total lesion glycolysis (TLG). Surgical tumor specimens were analyzed for PD-L1 and p-STAT3 expression using immunohistochemistry. Correlations between immunohistochemistry results and  $^{18}\text{F}$ -FDG PET/CT-derived variables were compared. Associations of PD-L1 and p-STAT3 tumor expression,  $^{18}\text{F}$ -FDG PET/CT-derived variables, and other factors with DFS in resected LUAD were evaluated.

All tumors were FDG-avid. The cutoff values of low and high SUVmax, MTV, and TLG were 12.60, 14.87, and 90.85, respectively. The results indicated that TNM stage, PD-L1 positivity, and high  $^{18}\text{F}$ -FDG PET/CT metabolic volume parameters (TLG  $\geq 90.85$  or MTV  $\geq 14.87$ ) were independent predictors of worse DFS in resected LUAD. No  $^{18}\text{F}$ -FDG metabolic parameters associated with PD-L1 expression were observed (chi-square test), but we found that patients with positive PD-L1 expression have significantly higher SUVmax ( $P = .01$ ), MTV ( $P = .00$ ), and TLG ( $P = .00$ ) than patients with negative PD-L1 expression.

$^{18}\text{F}$ -FDG PET/CT metabolic volume parameters (TLG  $\geq 90.85$  or MTV  $\geq 14.87$ ) were more helpful in prognostication than the conventional parameter (SUVmax), PD-L1 expression was an independent predictor of DFS in patients with resected LUAD. Metabolic parameters on  $^{18}\text{F}$ -FDG PET/CT have a potential role for  $^{18}\text{F}$ -FDG PET/CT in selecting candidate LUAD for treatment with checkpoint inhibitors.

**Abbreviations:**  $^{18}\text{F}$ -FDG PET/CT =  $^{18}\text{F}$ -fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography, DFS = disease-free survival, IHC = immunohistochemistry, LUAD = lung adenocarcinoma, SUVmax = maximum standardized uptake value, NSCLC = non-small-cell lung cancer, OS = overall survival, MTV = metabolic tumor volume, TLG = total lesion glycolysis, PD-1 = programmed cell death-1, PD-L1 = programmed death-ligand 1, STAT3 = signal transducer and activator of transcription 3, ROC = receiver operating characteristic curve.

**Keywords:** FDG, lung adenocarcinoma, PET/CT, prognosis, programmed cell death-ligand 1

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## 1. Introduction

Lung cancer, as a prominent global health burden is the leading cause of malignancy and disease-related mortality worldwide. Lung adenocarcinoma (LUAD) is the most prevalent histological form of non-small-cell lung cancer (NSCLC), accounting for almost half of all lung cancer cases.<sup>[1]</sup>

Despite striking advances in treatment options, including surgical resection, chemotherapy, radiation, and targeted therapies, the prognosis of LUAD remains poor.<sup>[2]</sup> Thus, it is of great importance to identify novel prognostic methods to increase the predictability of outcomes in patients with LUAD. Positron emission tomography/computed tomography (PET/CT), with its advantages of non-invasive evaluation and accuracy, has been validated in the assessment of staging, recurrence, response, and prognosis in NSCLC.<sup>[3,4]</sup>

Previous studies have demonstrated that the tumor metabolism activity of fluoro-2-deoxy-D-glucose (FDG) uptake (the maximum standardized uptake value [SUVmax]) is a significant prognostic factor in NSCLC.<sup>[5,6]</sup> However, there are some variability and discrepancies in the measurement of SUVmax.<sup>[7]</sup> Recent reports have shown a superior correlation between primary tumor total lesion glycolysis (TLG) denoting the FDG uptake of the entire lesion correlates with progress free survival (PFS) in NSCLC patients compared with conventional SUVmax.<sup>[8]</sup> Further studies are required to verify whether the tumor volume metabolism (TLG) parameter is a better prognostic predictive factor in patients with surgically resected LUAD than conventional SUVmax.

The relationship between <sup>18</sup>F-FDG uptake and the programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1) immune checkpoint is not well understood. The PD-1/PD-L1 immune checkpoint is a crucial mechanism underlying the immune escape of tumor cells from T cells.<sup>[9]</sup> The biological characteristics of patients with positive PD-L1 expression are dissimilar to those with negative expression; this results in the adoption of diverse treatment strategies and varying clinical outcomes in surgically resected LUAD patients. Several studies have shown concordance or discordance in the prognostic value of PD-L1 in surgically resected LUADs. Herein, further investigation is needed to confirm the prognostic value of PD-L1. PD-L1 expression assessed by immunohistochemistry (IHC) has been confirmed to be predictive of response to immune checkpoint inhibitors,<sup>[10]</sup> but the procedure requires an invasive biopsy. Alternative non-invasive strategies such as PET/CT that can predict PD-1/PD-L1 expression and inform treatment strategies involving anti-PD-1/PD-L1 antibodies in patients with LUAD would be of great value and could aid in seeking potential predictors of immunotherapy response.

Signal transducer and activator of transcription (STAT) 3 is activated by phosphorylation (p-STAT3) and plays an important role in regulating tumor invasion and metastasis.<sup>[11,12]</sup> However, the prognostic value of p-STAT3 expression in surgically resected LUAD is yet to be fully elucidated.

Thus, it is essential to investigate the prognostic value of PD-L1 and p-STAT3 expression and to develop a new non-invasive and practical method (PET/CT) for predicting outcomes in patients with surgically resected LUAD. We also investigated whether non-invasive strategies such as metabolic variables derived from PET/CT as predictors of immune checkpoint (PD-1/PD-L1) inhibitors.

## 2. Materials and methods

### 2.1. Patient characteristics

The Harbin Medical University Institutional Human Ethics Review Board approved this retrospective study (No. HMUIRB20160008), and the requirement for informed consent was waived. A total of 74 consecutive patients with LUAD were enrolled at the Affiliated Tumor Hospital of Harbin Medical University. All participants underwent <sup>18</sup>F-FDG PET/CT before radical surgical resection of lung cancer between August 2010 and September 2012. The inclusion criteria were as follows: <sup>18</sup>F-FDG PET/CT examination was performed within 4 weeks before surgery. Chest wall infiltration was not observed. The tumor had no sub-solid components. The tumor size (long diameter) was greater than 10 mm. Tumors on <sup>18</sup>F-FDG PET/CT showed an abnormal uptake of radioactivity. All patients who underwent curative resection were diagnosed with LUAD after surgery, and the pathological reports were confirmed by more than 2 experienced pathologists. All patients were treated according to National Comprehensive Cancer Network guidelines.

Pathological staging was determined according to the Tumor-Node-Metastasis (TNM) staging diagnosis of UICC/AJCC (7th edition). The survival time after surgery was greater than 90 days. Patients who received radiotherapy, chemotherapy, or chemoradiotherapy before surgery were excluded.

### 2.2. Survival data

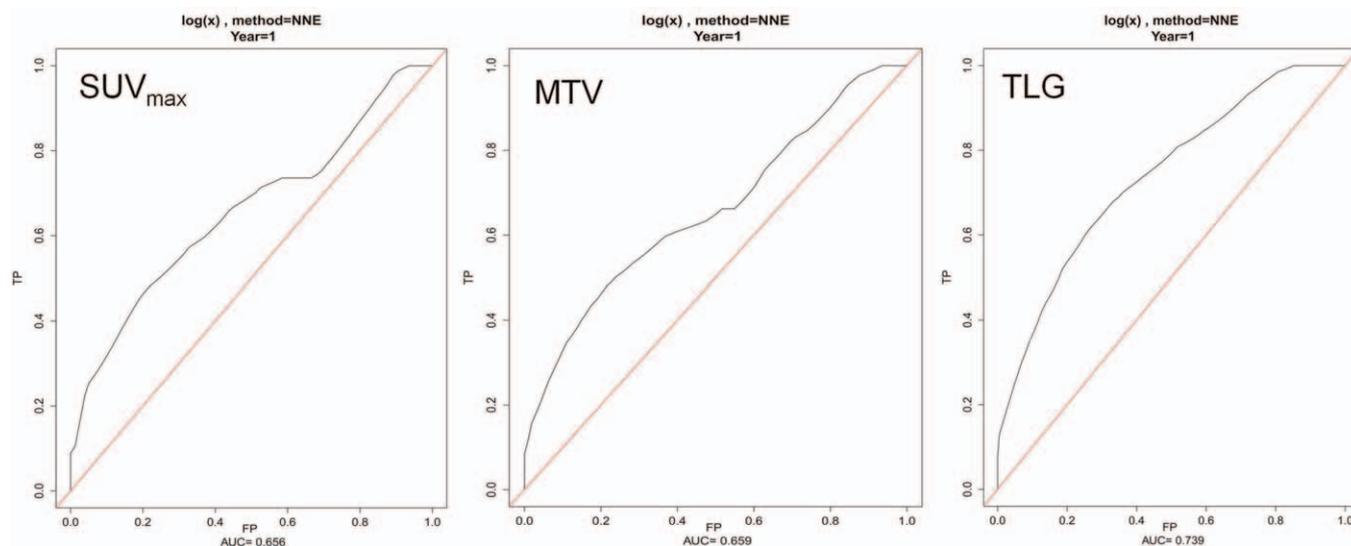
Survival data were obtained from medical records or telephone follow-ups. The follow-up period was 3 to 75 months, and 74 patients were followed up until death or until the cutoff date of November 25, 2016. Five-year disease-free survival (DFS) rate was selected as the study endpoint. DFS was defined as the time from the date of surgery to the first recurrence, metastasis, death, or cutoff date.

### 2.3. <sup>18</sup>F-FDG PET/CT acquisition

All patients fasted for 4 to 6 hours. Blood glucose levels <150 mg/dL were considered normal before the <sup>18</sup>F-FDG PET/CT examination (Discovery ST, GE Medical Systems, Milwaukee, WI). PET/CT images were obtained 60 minutes after intravenous administration of <sup>18</sup>F-FDG (5.55–7.40 MBq/kg). The PET/CT protocol mentioned in this study was described in our previous study.<sup>[13]</sup> In this study, the output results, including the SUVmax, metabolic tumor volume (MTV), TLG of lesions were evaluated by 2 imaging and nuclear medicine physicians who had more than 3 years of working experience with <sup>18</sup>F-FDG PET/CT imaging. Any disagreements between physicians were resolved by discussion, and consensus was achieved. The volume boundaries were automatically drawn to incorporate each tumoral lesion in <sup>18</sup>F-FDG PET/CT images using the software (PET-VCAR, GE Healthcare, Waukeha, WI, USA) using a 40% threshold of SUVmax. Manual adjustment of the SUVmax threshold was required when the defined tumor margin was not appropriate, relative to fused CT. TLG is the MTV multiplied by the mean SUV of the tumor.

### 2.4. Immunohistochemistry

IHC was performed using the standard indirect immunoperoxidase procedures. Briefly, 4  $\mu$ m-thick sections from a paraffin-embedded tissue block were processed. The slides were stained



**Figure 1.** ROC curve for the determination of the most discriminative cutoff point for SUVmax, metabolic tumor volume-MTV, and total lesion glycolysis-TLG in primary tumors. The optimal cutoff values for SUVmax, MTV, and TLG were 12.60, 14.87, and 90.58, respectively. SUVmax = maximum standardized uptake value.

**Table 1**  
**Correlation between PD-L1/p-STAT3 expression and clinicopathological parameters in patients with surgically resected lung adenocarcinoma.**

Characteristics	n (%)	PD-L1 expression		P	p-STAT3 expression		P
		-	+		-	+	
Age							
<60	45 (60.8)	16	29	.632	30	15	.445
≥60	29 (39.2)	12	17		22	7	
Gender							
Male	38 (51.35)1	10	28	.055	26	12	.802
Female	36 (48.65)0	18	18		26	10	
Smoking status							
+	28 (37.8)	12	16	.622	22	6	.297
-	46 (62.2)	16	30		30	16	
SUVmax							
<12.60	55 (74.3)	20	35	.785	39	16	1.000
≥12.60	19 (25.7)	8	11		13	6	
MTV							
<14.87	54 (73)	21	33	.795	40	14	.263
≥14.87	20 (27)	7	12		12	8	
TLG							
<90.58	49 (66.2)	21	28	.311	36	13	.430
≥90.58	25 (33.8)	7	18		16	9	
LDH							
<190	60 (81.1)	21	39	.364	40	20	.207
≥190	14 (18.9)	7	7		12	2	
CEA							
<5	43 (58.1)	18	25	.471	30	13	1.000
≥5	31 (41.9)	10	21		22	9	
Stage							
I	25 (33.8)	14	11	.026*	19	6	.592
II, IIIA	49 (66.2)	14	35		33	16	

CEA = carcinoembryonic antigen, LDH = lactic dehydrogenase, MTV = metabolic tumor volume, PD-L1 = programmed death-ligand 1, p-STAT3 = phosphorylated signal transducer and activator of transcription 3, SUVmax = maximum standardized uptake values, TLG = total lesion glycolysis.

\* P < .05 considered statistically significant.

with primary antibodies against PD-L1 (Abcam, Cambridge, UK) and p-STAT3 (Abcam, Cambridge, UK). The IHC assay results were independently interpreted by 2 experienced pathologists, and any discrepancies were resolved by consensus. PD-L1/p-STAT3 positivity was defined per specimen by a 5% expression threshold (positive tumor cells/total tumor cells) in cases where more than 5% were considered PD-L1/p-STAT3-positive.

### 2.5. Statistical analysis

The association between 2 continuous variables was analyzed by *t* test. The association between 2 categorical variables was evaluated by chi-square test. The measurement parameters of <sup>18</sup>F-FDG PET/CT were recorded as continuous variables. The cutoff values for the categorization of low and high SUVmax, TLG, and MTV were performed using R (version 3.3.2) with the package of survival receiver operating characteristic curve (ROC, version 1.0.3) (R Development Core Team, Vienna, Austria, <http://www.R-project.org>). The cutoff values for the categorization of low and high SUVmax, TLG, and MTV were calculated using the ROC curve. The cutoff value for each parameter was calculated by maximizing the Youden index. Univariate analysis of DFS was performed, and all variables with univariate significance ( $P < .05$ ) were selected for a multivariable Cox model. Spearman rank correlation coefficient was used to determine the multicollinearity

between parameters. SPSS19.0 (SPSS Inc., Chicago, IL) was performed using a two-tailed *P* value  $< .05$ , to indicate statistical significance.

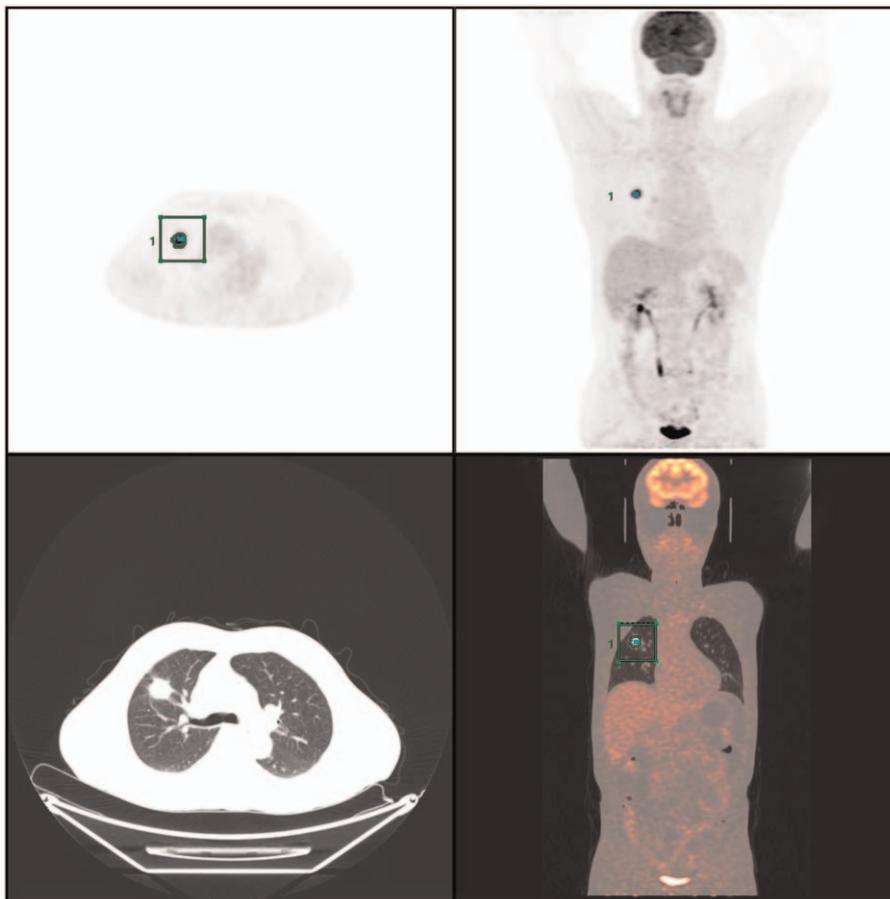
## 3. Results

### 3.1. Patient characteristics

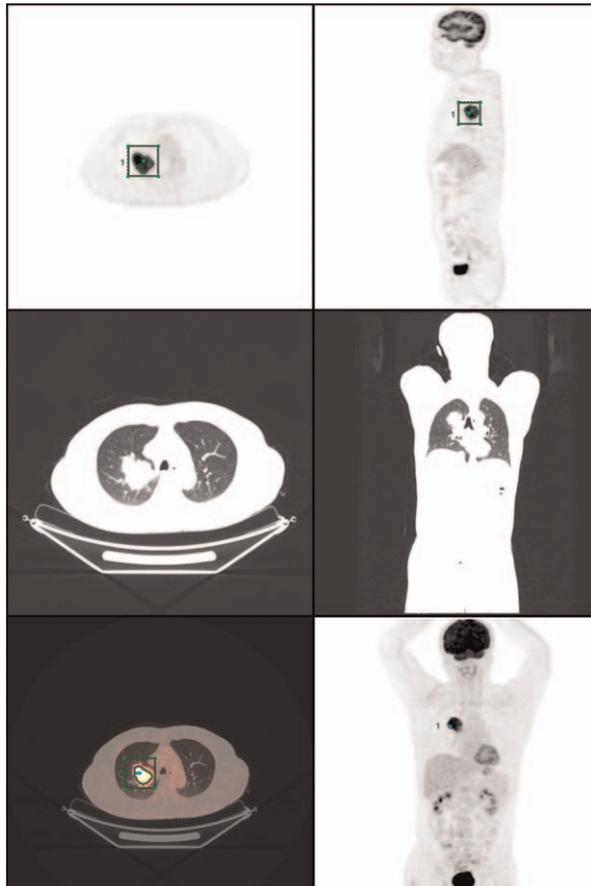
Seventy-four patients with LUAD were enrolled in the study, consisting of 38 men and 36 women with a median age of 57 (range, 38–83) years. Eleven patients were diagnosed with stage IA, 14 with stage IB, 17 with stage IIA, 4 cases with stage IIB, and 28 with stage IIIA. Based on the time-dependent ROC analysis results, the cutoff points for the categorization of low and high SUVmax, MTV, and TLG were 12.60, 14.87, and 90.58, respectively (Fig. 1). The main clinicopathological data of all patients are shown in Table 1. Selected <sup>18</sup>F-FDG PET/CT imaging cases of surgically resected LUAD are shown in Figures 2 and 3.

### 3.2. Follow-up data

The follow-up endpoint of the entire cohort was November 25, 2016 and there were 67 cases of local recurrence or distant metastasis. The tumor-free survival rates at 1, 2, 3, 4, and 5 years were 83.78%, 48.69%, 25.67%, 18.91%, and 13.51%, respectively, and the total median DFS was 23.60 months (range, 3–75



**Figure 2.** A typical case of PET/CT volumetric metabolic parameters. The patient was a 55-year-old male with stage IIA moderately differentiated adenocarcinoma. PET/CT metabolic parameters were: SUVmax, 13.45; metabolic tumor volume (MTV), 5.61; and total lesion glycolysis (TLG), 34.68. Disease-free survival (DFS) was 50.5 months. PET/CT = positron emission tomography/computed tomography, SUVmax = maximum standardized uptake value.



**Figure 3.** A typical case of PET/CT volumetric metabolic parameters. A 61-year-old male with stage IIa poorly differentiated adenocarcinoma. PET/CT metabolic parameters were: SUVmax, 14.26; metabolic tumor volume (MTV), 36.90; and total lesion glycolysis (TLG), 319.23. DFS was 20.8 months. DFS = disease-free survival, PET/CT = positron emission tomography/computed tomography, SUVmax = maximum standardized uptake value.

months). The median DFS in stage I patients was 37.00 months, while that in patients with stage II and IIIA was 18.70 months.

### 3.3. IHC results

IHC was used to detect PD-L1 expression in LUAD tissues and its relationship with clinicopathological parameters. Positive PD-L1 expression was observed in 62.2% of LUAD specimens, and PD-L1 was positively expressed in LUAD specimens (Fig. 4-A). The correlations between PD-L1 expression and various  $^{18}\text{F}$ -FDG PET/CT parameters are shown in Table 1. The positive expression of PD-L1 in stage II–IIIA disease was higher than that in stage I, and the difference between the 2 groups was significant (chi-squared test,  $P=.026$ ), and there was no significant correlation between PD-L1 positivity and  $^{18}\text{F}$ -FDG PET/CT parameters. p-STAT3 was positively but weakly expressed in 29.7% of LUAD cases, as shown in Figure 4-B. There was no significant correlation between the expression of p-STAT3 and other characteristics (Table 1).

### 3.4. $^{18}\text{F}$ -FDG PET/CT metabolic parameters of primary tumors

$^{18}\text{F}$ -FDG PET/CT imaging showed abnormal FDG uptake in all lesions. The average SUVmax of all patients was  $10.49 \pm 4.84$ ,

and the median value was 9.45 (2.50–30.20). The mean MTV of the primary tumors was  $14.51 \pm 20.97$ , and the median value was 7.20 (1.26–142.20). The mean TLG of the primary tumors was  $118.67 \pm 169.43$ , with a median value of 50.09 (2.91–993.34). The patients with positive PD-L1 expression have significantly higher SUVmax ( $P=.01$ ), MTV ( $P=.00$ ), and TLG ( $P=.00$ ) than patients with negative PD-L1 expression. The mean  $\pm$  SD (PD-L1<sup>-</sup> vs PD-L1<sup>+</sup>) of SUVmax, MTV, and TLG were  $8.63 \pm 3.78$  vs  $12.35 \pm 5.10$ ,  $4.11 \pm 2.93$  vs  $24.92 \pm 25.69$ , and  $21.42 \pm 14.25$  vs  $215.90 \pm 196.39$ , respectively.

### 3.5. Survival analysis

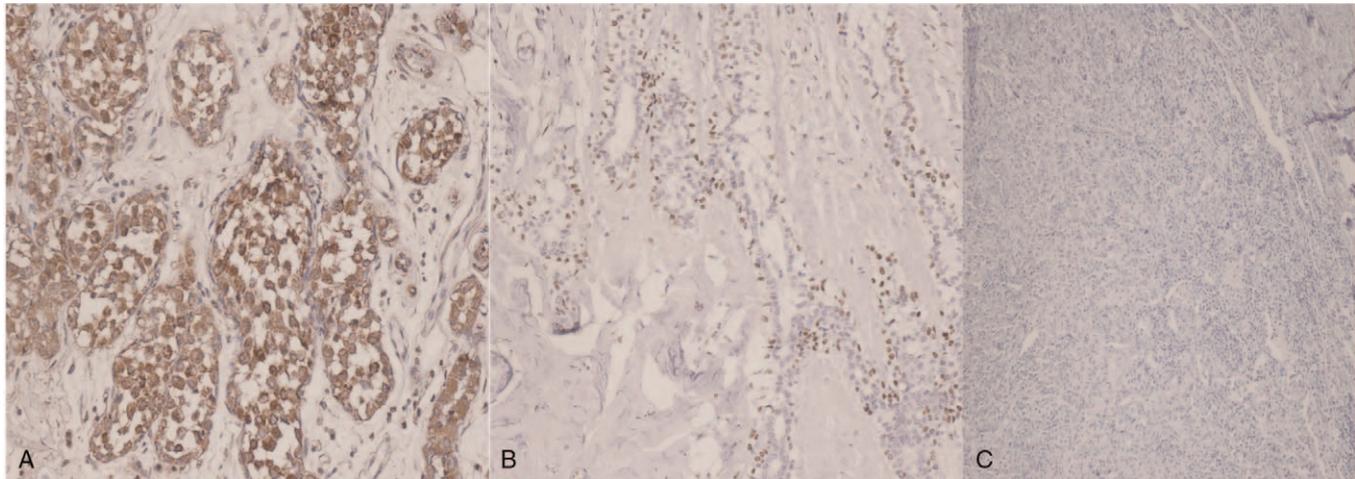
The results of univariate analysis of prognosis (DFS) in all patients with surgically resected LUAD are shown in Table 2; univariate analysis of DFS prognosis showed that sex, PD-L1 expression, MTV, TLG, and stage were statistically significant ( $P=.023$ ,  $P=.010$ ,  $P=.007$ ,  $P=.000$ , and  $P=.000$ , respectively), while age, smoking, p-STAT3, SUVmax, lactic dehydrogenase, carcinoembryonic antigen, and postoperative adjuvant therapy were not statistically significant. The statistical analyses of the Kaplan-Meier survival curves for stage, PD-L1, TLG, and MTV are shown in Figure 5.

### 3.6. Multivariate analysis of prognosis (DFS) in all patients with surgically resected lung adenocarcinoma

The results of the multivariate analysis of the prognosis (DFS) of all patients with surgically resected LUAD are shown in Table 3. Because MTV and TLG have a correlation coefficient of 0.85, there may be multiple collinearity; therefore, multivariate analysis revealed that PD-L1 expression, MTV, and staging were independent prognostic indicators for patients with surgically resected LUAD ( $P=.029$ ,  $P=.026$ , and  $P=.003$ , respectively) when TLG was not included. Multivariate analysis revealed that PD-L1 expression, TLG, and staging were independent prognostic indicators for patients with surgically resected LUAD ( $P=.046$ ,  $P=.022$ , and  $P=.005$ , respectively) when MTV was not included.

## 4. Discussion

LUAD accounts for almost half of all lung cancer cases. Further research to explore the prognosis of patients with LUAD has important clinical significance.  $^{18}\text{F}$ -FDG PET/CT is an important tool for calculating the degree of tumor metabolism in NSCLC prognosis research. The main metabolic index currently used is the SUVmax. In this study, new  $^{18}\text{F}$ -FDG PET/CT volume metabolic parameters (MTV and TLG) were included. As the most commonly used  $^{18}\text{F}$ -FDG PET/CT metabolic parameter, the prognostic value of SUVmax for NSCLC has been controversial. The univariate and multivariate prognostic analyses in this study showed that there was no statistical difference between the SUVmax of pre-operative primary lesions and prognosis (DFS). This can be attributed to inter-study variations in tumor SUVmax that do not reflect actual changes in the metabolic uptake rate. Compared with SUVmax, MTV and TLG are new parameters that can reflect both the degree of FDG uptake and its scope. It is considered to be more accurate and stable in response to tumor burden, invasion, metastasis, and prognosis, and the results of our study are similar to recent findings,<sup>[14,15]</sup> in which high  $^{18}\text{F}$ -FDG PET/CT volume metabolic parameters (MTV or TLG) of



**Figure 4.** IHC staining for PD-L1/p-STAT3 in pulmonary adenocarcinoma specimens. (A) Positive expression of PD-L1 in pulmonary adenocarcinoma. (B) Positive expression of p-STAT3 in pulmonary adenocarcinoma. (C) Negative. The magnification is  $\times 200$ . PD-L1 = programmed death-ligand 1, p-STAT3 = phosphorylated signal transducer and activator of transcription 3.

**Table 2**  
DFS according to univariate analysis in 74 patients with surgical resection of lung adenocarcinoma.

Factor	No. of patients	DFS Median survival time (months $\pm$ SE)	P value
Age			
<60	45 (60.8)	23.00 $\pm$ 1.65	.486
$\geq 60$	29 (39.2)	24.90 $\pm$ 1.83	
Gender			
Male	38 (51.35)	22.23 $\pm$ 3.40	.023*
Female	36 (48.65)	25.07 $\pm$ 2.83	
Smoking history			
+	28 (37.8)	21.77 $\pm$ 2.12	.232
-	46 (62.2)	24.90 $\pm$ 3.47	
PD-L1			
+	46 (62.2)	18.13 $\pm$ 1.78	.010*
-	28 (37.8)	30.1 $\pm$ 2.47	
p-STAT3			
+	22 (29.7)	23.00 $\pm$ 5.53	.372
-	52 (70.3)	24.00 $\pm$ 3.11	
SUVmax			
<12.60	55 (74.3)	24.00 $\pm$ 2.98	.096
$\geq 12.60$	19 (25.7)	24.90 $\pm$ 6.46	
MTV			
<14.87	54 (73)	26.80 $\pm$ 2.47	.007*
$\geq 14.87$	20 (27)	18.00 $\pm$ 3.96	
TLG			
<90.58	49 (66.2)	27.87 $\pm$ 3.20	.000*
$\geq 90.58$	25 (33.8)	16.30 $\pm$ 2.48	
LDH			
<190	60 (81.1)	23.93 $\pm$ 1.43	.619
$\geq 190$	14 (18.9)	27.87 $\pm$ 13.56	
CEA			
<5	43 (58.1)	24.10 $\pm$ 1.86	.922
$\geq 5$	31 (41.9)	23.33 $\pm$ 5.02	
Stage			
I	25 (33.8)	42.20 $\pm$ 10.21	.000*
II, IIIA	49 (66.2)	19.65 $\pm$ 1.65	
Adjuvant therapy			
-	35	18.13 $\pm$ 2.19	.583
+	39	29.47 $\pm$ 3.66	

CEA = carcinoembryonic antigen, DFS = disease free survival, LDH = lactic dehydrogenase, MTV = metabolic tumor volume, PD-L1 = programmed death-ligand 1, p-STAT3 = phosphorylated signal transducer and activator of transcription 3, SE = standard error, SUVmax = maximum standardized uptake values, TLG = total lesion glycolysis.

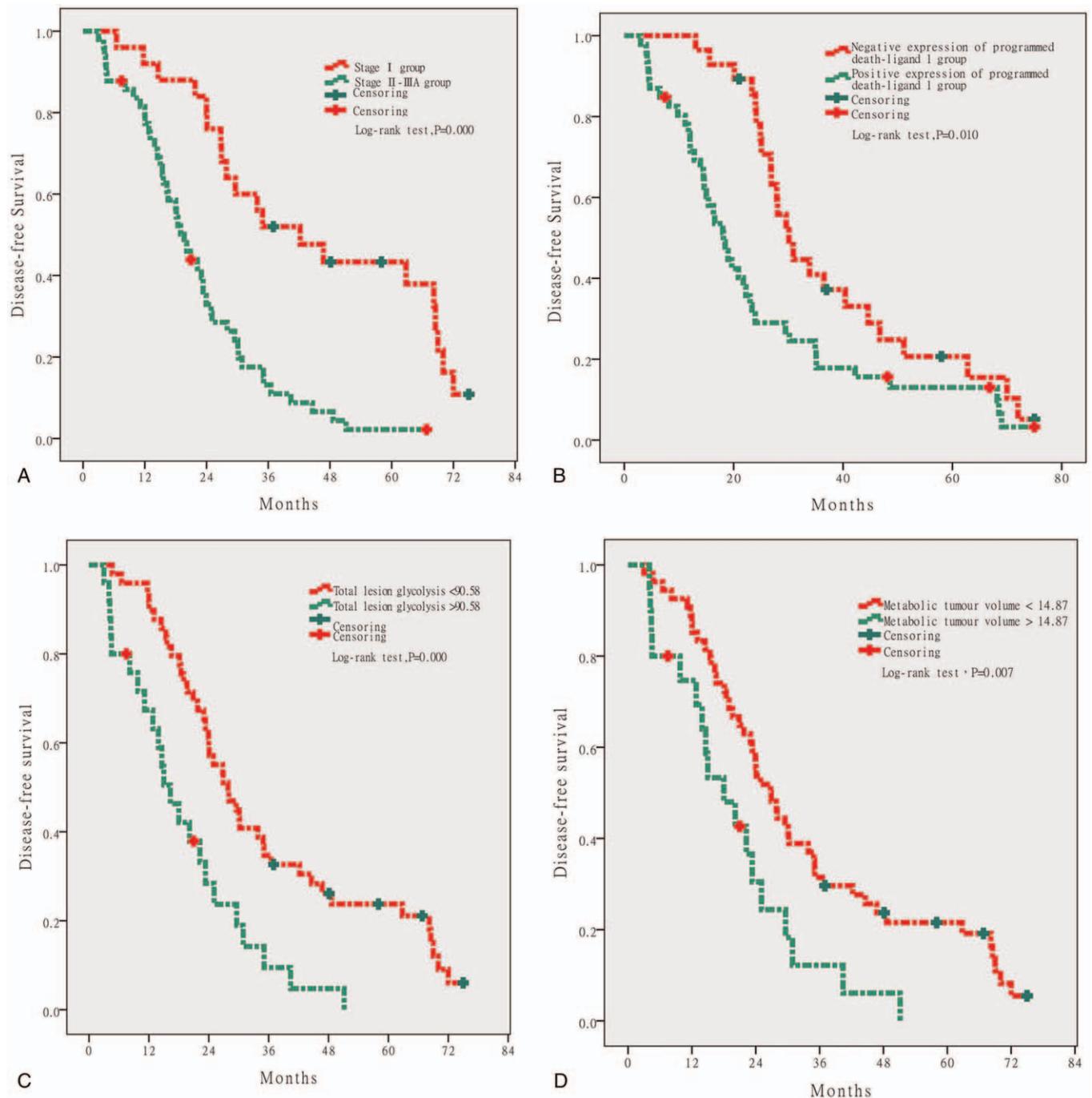
\*  $P < .05$  considered statistically significant.

primary tumors were independent factors affecting the poor prognosis (DFS) of LUAD with surgical resection.

Immunotherapy targeting PD-1/PD-L1 has been successfully used to treat a variety of malignant tumors, including LUAD. PD-L1 possesses prognostic capacities, and NSCLC patients with positive PD-L1 expression exhibit poor prognosis.<sup>[16]</sup> However, some studies did not show similar results,<sup>[17,18]</sup> and PD-L1 expression in NSCLC prognosis is controversial. Zhang et al<sup>[19]</sup> analyzed 143 cases of surgically resected LUAD (stage I–III) and found that PD-L1-positive patients had significantly poorer relapse-free survival ( $P = .001$ ) and overall survival (OS) ( $P = .002$ ). Song et al<sup>[20]</sup> studied the prognosis of 385 patients with surgically resected LUAD, and univariate analysis revealed that PD-L1 expression was associated with DFS, while multivariate analysis was not an independent prognostic factor for DFS and OS. Wu et al<sup>[21]</sup> analyzed the prognosis of 133 cases of surgically removed LUAD (stage I–IV) and found that PD-L1 expression was an independent prognostic factor for recurrence (relapse-free survival) and OS ( $P = .000$  and  $P = .000$ , respectively). Our research showed that positive PD-L1 expression as a target for immunotherapy in LUAD possessed poor prognostic capacity for DFS. Thus, our results showed that  $^{18}\text{F}$ -FDG PET/CT metabolism parameters and PD-L1 expression may provide new information for prognosis and insights into clinical treatment options.

The high expression of PD-L1 in tumor tissues and its interaction with its receptor PD-1 weakened the immunogenicity of tumor cells and inhibit the production of tumor immune responses. In current clinical trials (CheckMate-057), an anti-PD-1/PD-L1 monoclonal antibody has been shown to be a potential drug for advanced NSCLC therapy, but not all patients are responsive.<sup>[22]</sup> The clinical trial results indicated that high PD-L1 expression was positively correlated with the efficacy of anti-PD-1/PD-L1 monoclonal antibodies.

In our study we found that patients with positive PD-L1 expression have significantly higher SUVmax ( $P = .01$ ), MTV ( $P = .00$ ), and TLG ( $P = .00$ ) than patients with negative PD-L1 expression. We further analyzed the correlation between PD-L1 expression and  $^{18}\text{F}$ -FDG PET/CT parameters (SUVmax, MTV, and TLG) with chi-square test. There was no correlation between



**Figure 5.** Kaplan-Meier curve analyses of disease-free survival (DFS) of 74 patients with resected lung adenocarcinoma. (A) DFS according to staging. (B) Kaplan-Meier analysis of DFS according to PD-L1 expression. (C) Kaplan-Meier analysis of DFS according to the total lesion glycolysis (TLG). (D) Kaplan-Meier analysis of DFS according to the metabolic tumor volume (MTV). PD-L1 = programmed death-ligand 1.

PD-L1 expression and  $^{18}\text{F}$ -FDG PET/CT parameters (chi-square test), which is inconsistent with the findings of previous studies<sup>[23]</sup> that showed that SUVmax was correlated with PD-L1 and DFS in NSCLC. This may be related to the heterogeneous expression of PD-L1, such as its expression not only in tumor cells but also in the interstitial tumor microenvironment. As a glucose analogue, FDG is transported into tumor cells and tumor-associated active immune cells such as TILs and TAMs<sup>[24,25]</sup>; thus, PET can provide useful information on the metabolic state of the tumor

microenvironment. Glucose consumption in the tumor immune microenvironment can strictly limit metabolic T cells by inhibiting their effector function,<sup>[25,26]</sup> but a limitation of our study was that we did not analyze the expression of PD-L1 in the interstitial tumor microenvironment. The results may also be related to the selected cutoff value for PD-L1-positive expression; our IHC assay for PD-L1 utilized a cutoff of 5% TPS. Previous studies<sup>[27-29]</sup> reported PD-L1 staining with 1%, 5%, and 10% as expression thresholds, but no uniform threshold was defined and

**Table 3**  
**Multivariate analyses of prognostic factors for DFS in patients with surgical resection of lung adenocarcinoma.**

Factor	Hazard ratio (exp. B)	DFS	
		95%CI	P value
Gender	1.571	0.920–2.061	2.680
PD-L1	1.859	1.066–3.240	.029*
MTV	2.045	1.090–3.839	.026*
Stage	2.653	1.397–5.038	.003*
Factor	DFS		
	Hazard ratio (exp. B)	95%CI	P value
Gender	1.478	0.872–2.507	.147
PD-L1	1.736	1.011–2.981	.046*
TLG	1.950	1.099–3.461	.022*
Stage	2.550	1.324–4.914	.005*

CI = confidence interval, DFS = disease free survival, MTV = metabolic tumor volume, PD-L1 = programmed death-ligand 1, TLG = total lesion glycolysis.

\*  $P < .05$  considered statistically significant.

the scoring standards were different. Thus, the diversity of PD-L1 examination methods resulted in different conclusions. FDG hypermetabolism is not unique to malignant tumors, as it is also present in active inflammatory cells.<sup>[19,30]</sup> Thus, this non-specific uptake may be the reason why correlations between PET/CT parameters and PD-L1 expression were not observed in this study. Furthermore, this study was limited by its retrospective design and small sample size. Therefore, our study only suggested a potential role for <sup>18</sup>F-FDG PET/CT in selecting candidate LUAD patients for treatment with checkpoint inhibitors. Further studies or new methods (Quantitative Lung Remodelling for Reliable Pulmonary Dosimetry<sup>[31]</sup> etc) are needed for confirming our findings or exploring new strategies.

STAT3 is most closely associated with tumors among members of the STAT family. p-STAT3 was found to play a crucial role in the occurrence and development of many carcinomas, and is also a critical regulator of immunosuppression<sup>[32]</sup> and has been shown to be associated with poor prognosis in various carcinomas<sup>[33]</sup>; however, its prognostic value in lung cancer has not yet been clarified. In our study, univariate and multivariate survival analyses showed that p-STAT3 was not an independent factor affecting the poor prognosis (DFS) of LUAD, similar to the results of Jiang et al,<sup>[34]</sup> who showed that high expression of p-STAT3 is not an independent prognostic factor for DFS and OS in NSCLC. Our study showed that the positive expression rate of p-STAT3 in LUAD was low (29.7%), which was significantly lower than that in previous reports, with 65%<sup>[35]</sup> and 54%<sup>[36]</sup> expression in NSCLC. Studies have shown that<sup>[37]</sup> EML4–ALK directly binds to the *PDL1* promoter by p-STAT3 to increase the expression of PD-L1 in LUAD cell lines, suggesting that p-STAT3 may be associated with PD-L1 expression. It is beneficial to screen populations receiving targeted therapy, but no correlation between p-STAT3 and PD-L1 protein expression was observed in this study. There was also no statistically significant correlation between p-STAT3 expression and other parameters in this study.

## 5. Conclusion

<sup>18</sup>F-FDG PET/CT metabolic volume parameters (TLG  $\geq 90.85$  or MTV  $\geq 14.87$ ) were more helpful in prognostication than the conventional parameter (SUVmax), PD-L1 expression was an independent predictor of DFS in patients with resected LUAD.

The present study did not show a direct association between metabolic parameters on <sup>18</sup>F-FDG PET/CT and PD-L1 expression (chi-square test), but we found that patients with positive PD-L1 expression have significantly higher SUVmax ( $P = .01$ ), MTV ( $P = .00$ ), and TLG ( $P = .00$ ) than patients with negative PD-L1 expression, which suggest a potential role for <sup>18</sup>F-FDG PET/CT in selecting candidate LUAD patients for treatment with checkpoint inhibitors.

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