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^{18}F -FDG PET/CT for prediction of PD-L1 expression and tumor mutational burden in non-small cell lung cancer: biomarkers prediction models development and immunotherapy responses verification

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Abstract

Background Whether metabolic parameters on PET/CT are noninvasive alternatives to programmed cell death ligand 1 (PD-L1) expression and tumor mutational burden (TMB) in adenocarcinoma (ADC) and squamous cell carcinoma (SCC) remains unknown. We identified predictors and developed PET/CT-based nomogram models for predicting PD-L1 expression and TMB, and tested the usefulness of models for stratifying immunotherapy responses.

Methods We enrolled 305 patients with non-small cell lung cancer from January 2017 to April 2024 as the primary cohort to investigate independent predictors of PD-L1 expression and TMB for ADC ($n = 183$) and SCC ($n = 122$). Clinicopathological characteristics, semantic CT features, and PET metabolic parameters were reviewed and analyzed. Significant predictors of ADC biomarkers were identified, and ADC were randomly assigned 7:3 to the training ($n = 128$) and validation ($n = 55$) cohorts to develop and validate predictive models for PD-L1 expression, TMB, and the combination of both. Clinical, SUL_{max} and clinical- SUL_{max} combined models were constructed using logistic regression analysis. Model discrimination, calibration, and clinical usefulness were assessed. An independent test cohort ($n = 29$) with ADC receiving neoadjuvant immunotherapy was used to validate the models in stratifying pathologic responses.

Results PD-L1 expression did not differ significantly between SCC and ADC; however, the TMB was significantly higher in SCC (10 vs. 5 mutations/Mb, $p < 0.001$). In the analysis of ADC, PD-L1 expression was associated with clinical stage, differentiation, and *EGFR* mutation status ($p < 0.05$). TMB was associated with age, gender, smoking

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history, and *EGFR* mutation status ($p < 0.05$). SUL_{max} was an independent predictor of both PD-L1-Pos and TMB-High ($p < 0.001$). The clinical- SUL_{max} combined models for predicting both PD-L1 expression and TMB demonstrated great performance (AUC = 0.805 for PD-L1-Pos and TMB-High; AUC = 0.798 for PD-L1-Neg and TMB-Low). The usefulness of models in stratifying pathologic responses was confirmed on the ADC test cohort receiving neoadjuvant immunotherapy ($p = 0.035$ for PD-L1-Pos and TMB-High model; $p = 0.001$ for PD-L1-Neg and TMB-Low model). However, no significant predictors were identified to develop models in the analysis of SCC.

Conclusion SUL_{max} is an independent predictor of both PD-L1-Pos and TMB-High in ADC. The clinical- SUL_{max} combined models effectively predicted PD-L1-Pos and TMB-High status, as well as PD-L1-Neg and TMB-Low status in ADC, indicating the clinical utility in patient selection and immunotherapy response stratification.

Keywords Non-small cell lung cancer, ^{18}F -FDG PET/CT, Programmed cell death ligand 1, Tumor mutational burden, Nomogram

Introduction

Immune checkpoint inhibitors are milestones in cancer therapy, yielding durable antitumor responses and improved clinical outcomes in non-small cell lung cancer (NSCLC). Identifying predictive biomarkers for patient selection remains a major clinical challenge [1, 2]. Programmed cell death ligand 1 (PD-L1) expression and tumor mutational burden (TMB), used as companion diagnoses in clinical trials, are currently decisive biomarkers for patient selection [3]. The combination use of TMB and PD-L1 expression, integrating both the upstream and downstream components of antitumor responses, has recently been recommended for its superior predictive relevance and maximum therapeutic benefits [1, 4]. Nonetheless, the availability of biomarkers is limited because of inherent deficiencies in invasive tissue sampling. Developing noninvasive, imaging-derived diagnostic frameworks to expand the application of biomarkers remains an unmet need.

Recently, PET/CT demonstrated a remarkable predictive role in the therapeutic efficacy of immunotherapy [5, 6], and we previously demonstrated that metabolic parameters could predict the major pathological response after neoadjuvant immunotherapy with 100% sensitivity, specificity, and accuracy [5]. As significant predictors, clear positive correlations have been identified between metabolic parameters and PD-L1 expression [7–9], as well as TMB [10, 11]. Based on these findings, PET/CT holds promise as a noninvasive alternative to biomarkers. Studies have developed models comprising PET/CT-based features [12–14], or radiomics features [15–17] for the noninvasive prediction of PD-L1 expression status with favorable performance. However, no models have been developed to predict TMB status, or concurrently predict both PD-L1 expression and TMB.

Given the distinct biological characteristics and heterogeneous immune landscapes of lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) [18], controversy remains regarding their response to immunotherapy [19, 20]. Specifically, compared to SCC, ADC

exhibits significant variability in treatment responses, even in cases with poor responses, which is attributed to higher intratumoral heterogeneity and specific driver gene mutations. There is insufficient evidence regarding predictive biomarkers for ADC and SCC, and patient selection is considered clinically more critical for ADC.

This study hypothesized that PET/CT metabolic parameters could serve as noninvasive alternatives to PD-L1 expression and TMB. The objective of this study was to identify independent predictors of PD-L1 expression and TMB and to develop PET/CT-based nomogram models, with a particular focus on ADC due to the high intratumoral heterogeneity. More importantly, we tested the models' ability to stratify pathologic responses after neoadjuvant immunotherapy in a real-world clinical setting, thereby bridging the gap between biomarkers predictions and clinical outcomes.

Materials and methods

Study participants

Patients with primary NSCLC who underwent PET/CT at our hospital were retrospectively included. The primary cohort consisted of consecutive patients underwent PD-L1 expression and TMB testing simultaneously from January 2017 to April 2024. The inclusion criteria were: (a) histological diagnosis of primary NSCLC, (b) PET/CT examination with measurable tumors and qualified images, (c) thorough PD-L1 expression and TMB test results of biopsy/surgery tumor samples, and (d) lesion pathology for cases with multiple primary lung cancers. Patients were excluded if they (a) had other types of NSCLC besides ADC and SCC, (b) received clinical treatment prior to PET/CT examination and pathological testing, (c) underwent pathological testing of metastatic lesions or lymph nodes, and (d) the interval between PET/CT examination and biopsy/surgery >3 month. The test cohort included patients receiving neoadjuvant immunotherapy in a real-world clinical setting, comprising patients from two clinical trials (ChiCTR-OIC-17013726 [Registration Date: December 6, 2017],

ChiCTR2000033588 [Registration Date: June 6, 2020]) and those admitted to the Department of Thoracic Surgery from January 2023 to April 2024. Patients were excluded if they (a) had other types of NSCLC besides ADC, (b) did not undergo ^{18}F -FDG PET/CT before neoadjuvant immunotherapy, or (c) did not undergo tumor resection after neoadjuvant immunotherapy due to their clinical conditions. Notably, if a patient did not undergo tumor resection but had radiographic or pathological progression, they were included, with pathologic response considered 0 [5].

The workflow is illustrated in Fig. 1. Finally, 305 NSCLC patients (183 ADC and 122 SCC) were included in the primary cohort to investigate the clinicopathological and imaging predictors of PD-L1 expression and TMB. Patients with ADC in the primary cohort were randomly assigned 7:3 to the training ($n=128$) and validation ($n=55$) cohorts to develop and validate predictive models of PD-L1 expression and TMB. An additional 29 patients with ADC receiving neoadjuvant immunotherapy served as an independent test cohort to assess the clinical usefulness of the models in stratifying pathological responses.

This study was conducted in accordance with the Declaration of Helsinki and approved by our Institutional Review Board. Written informed consent was waived because of the retrospective nature.

Clinical and demographic characteristics

Clinical and demographic characteristics were retrospectively collected from medical records, including age, sex, smoking history, T-N-M stage, clinical stage, histological subtype, differentiation, epidermal growth

factor receptor (*EGFR*) mutation status for ADC, and serum tumor markers. The tumor stage was determined according to the American Joint Committee on Cancer guideline, 8th edition. Serum tumor markers included carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), cytokeratin 19 fragment (Cyfra21-1), and squamous cell-associated antigen (SCC-Ag), with normal upper limits of 5.0 ng/mL, 35.0 U/mL, 3.3 ng/mL, and 1.5 ng/mL, respectively. A total of 246 patients enrolled in the primary cohort underwent serum tumor marker tests.

Histopathology and molecular pathology assessment

Histopathology and molecular pathology data were obtained from hospital records. Standard histological analysis was routinely conducted by two experienced pathologists (at least one with more than 20 years of experience in pathological diagnosis) according to institutional laboratory protocols. Disagreements were resolved by consensus or consultation with a third experienced pathologist.

In the primary cohort, tumor specimens were tested for PD-L1 expression and TMB based on the patient's clinical condition: 118 (38.7%) patients underwent surgical resection and 187 (61.3%) underwent bronchofibroscopy or CT-guided biopsy. PD-L1 expression was tested by immunohistochemistry using the antibodies 22C3, SP263, or SP142 in accordance with our institutional laboratory's standardized protocols and aligned with manufacturer recommendations for each clone. The tumor proportion score (TPS) was calculated as the percentage of PD-L1 positive tumor cells over all tumor cells. Patients were categorized as $\text{TPS} < 1\%$, $1\text{--}49\%$ or $\geq 50\%$.

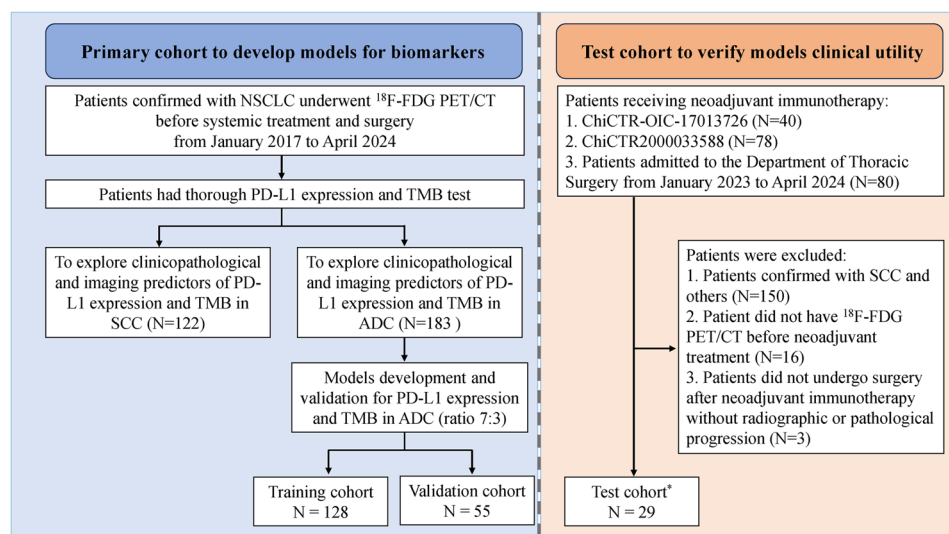


Fig. 1 Study flowchart.* Including 6 patients received neoadjuvant immune checkpoint inhibitor monotherapy and 23 patients received neoadjuvant immune checkpoint inhibitor combined with other treatments.

Abbreviations: PD-L1, programmed death ligand 1; TMB, tumor mutational burden; ADC, adenocarcinoma; SCC, squamous cell carcinoma

and further defined as PD-L1-Pos ($\geq 1\%$), PD-L1-Neg ($< 1\%$), PD-L1-High ($\geq 50\%$), and PD-L1-Low ($< 50\%$) groups. TMB was tested using next-generation sequencing and measured as mutations per megabase pair. Patients were subsequently categorized into TMB-High and TMB-Low groups according to the median TMB values for ADC and SCC.

In the test cohort, 24 (82.8%) patients underwent complete tumor resection after neoadjuvant immunotherapy, and routine hematoxylin and eosin staining was performed to assess the pathologic response as the percentage of viable residual tumors within the primary tumor. The degree of pathological regression was also recorded. Five (17.2%) patients did not undergo tumor resection but had radiological or pathological progression, including pleural metastasis, pulmonary metastasis, bone metastasis, retroperitoneal lymph node metastasis, and supraclavicular lymph node metastasis, with the pathological regression considered 0.

¹⁸F-FDG PET/CT acquisition

Whole-body PET/CT was performed according to standard hospital protocol using an integrated device (Discovery 690; GE Healthcare). After ≥ 6 h of fasting, approximately 3.70–4.44 MBq/kg ¹⁸F-FDG was administered intravenously, ensuring that the patient's blood glucose level was < 145 mg/dL. PET/CT scans were conducted from the head to the groin approximately 50–70 min after FDG administration. PET images were acquired in three-dimensional mode for 2 min per frame and reconstructed using the VPFx-S algorithm (two iterations, 24 subsets, 4 mm Gaussian post-filter). Spiral CT was performed without intravenous contrast administration (tube voltage, 120 kV; tube current, 150 mA; slice thickness, 3.75 mm; and rotation speed, 0.8 s). Subsequently, a breath-hold thoracic spiral CT scan was performed after the PET/CT scan, with image reconstruction from a thickness of 1.25 mm (tube voltage, 120 kV; tube current using automatic milliampere-second technology; slice thickness, 5 mm; and rotation speed, 0.5 s).

Image analysis

Semantic CT features were evaluated on breath-hold thoracic spiral CT images in the lung window (level, -650 HU; width, 1500 HU) and mediastinal window (level, 40 HU; width, 400 HU) settings. Images were retrospectively reviewed by two radiologists (with 6 and 20 years of experience in thoracic tumor imaging) who were blinded to the clinical and pathological data. Any disagreements were resolved by consensus or consultation with a third experienced radiologist (with more than 40 years of experience in thoracic tumor imaging). The following semantic CT features were assessed: long-axis diameter,

short-axis diameter, location (peripheral or central), shape (round/oval or irregular), margin (well defined or poorly defined), presence of ground-glass opacity (GGO) (yes/no), lobulation sign (yes/no), spiculation sign (yes/no), bubble-like lucency (yes/no), calcification (yes/no), obstructive change (yes/no), pleural attachment (yes/no), pleural retraction (yes/no), and pleural effusion (yes/no).

Metabolic parameters derived from PET images were analyzed using PETVCAR (PET Volume Computerized Assisted Reporting) on an Advantage Workstation (version 4.6; GE Healthcare), which is an automated segmentation software system with an iterative adaptive threshold algorithm. The volume of interest of the primary tumor was auto-segmented using a three-dimensional cube to ensure the inclusion of all FDG PET-positive areas in the axial, coronal, and sagittal planes. The following standardized uptake values corrected by lean body mass (SUL) were calculated: SUL_{max} , SUL_{mean} , SUL_{peak} , metabolic tumor volume (MTV), and total lesion glycolysis (TLG).

Model construction and validation

In the primary cohort, clinicopathological factors, semantic CT features, and PET metabolic parameters were sequentially selected using univariate and multivariate logistic regression analyses to identify independent predictors, which were combined to establish predictive models for PD-L1 expression and TMB in ADC. Significant factors in the univariate analysis were included in the multivariate analysis following a backward stepwise selection method. Three models were constructed based on clinical, SUL_{max} , and combined clinical- SUL_{max} features to predict PD-L1 expression and TMB, both separately and jointly (PD-L1-Pos, PD-L1-High, TMB-High, PD-L1-Pos and TMB-High, PD-L1-Neg and TMB-Low). The coefficients of the model formulas were estimated using the maximum likelihood estimation method. The models were visualized as nomograms.

Statistical analysis

Continuous variables were tested for normality before analysis. Intergroup comparisons were conducted using the t-test or ANOVA test for normally distributed continuous variables, the Mann–Whitney U test or Kruskal–Wallis test for non-normally distributed continuous variables, and the chi-squared test or Fisher's exact test for categorical variables. A Bonferroni post hoc test was performed for multiple comparisons. The Spearman correlation method was used for correlation analyses. Univariate and multivariate logistic regression analyses were sequentially performed to identify independent predictors for PD-L1 expression and TMB. Univariate and multivariate linear regression analyses were sequentially performed to identify the independent predictors for

log10-transformed TMB, denoted as TMB (lg), as TMB distribution was highly skewed. Variables with $p < 0.05$ in the univariable analysis were entered into multivariable analysis using backward stepwise selection. The diagnostic performances of the models were assessed using the area under the receiver operating characteristic (ROC) curve (AUC). Sensitivity, specificity, and accuracy were determined at the threshold using the maximum Youden index. Differences between the ROC curves were evaluated using the DeLong test. The calibration curve and Hosmer-Lemeshow test were used to assess model calibration. Decision curve analysis (DCA) was conducted to evaluate the net benefit. All statistical analyses were conducted using SPSS (version 25.0; IBM Corp.) and R (version 4.0.3). A two-sided p value < 0.05 was considered statistically significant.

Results

Differences of PD-L1 expression and TMB between ADC and SCC

A total of 305 patients with NSCLC (225 males, 80 females; age, 61 ± 9 years) in the primary cohort were enrolled to investigate independent predictors and develop models for PD-L1 expression and TMB, including 183 (60.0%) with ADC and 122 (40.0%) with SCC. An

additional 29 patients with ADC receiving neoadjuvant immunotherapy served as an independent test cohort to test the clinical usefulness of the models. The demographic characteristics of enrolled patients are shown in Table 1. In the primary cohort, ADC and SCC showed significant differences in clinicopathological characteristics, semantic CT features, and PET metabolic parameters (Table S1). There was no significant difference in PD-L1 expression between ADC and SCC ($p = 0.341$ for categories, $p = 0.905$ for TPS; Fig. 2A and B). SCC had significantly higher TMB than ADC (10 vs. 5 mutations/Mb, $p < 0.001$, Fig. 2C). PD-L1 expression and TMB were independent of each other in both ADC and SCC (Spearman's rho $[r] = -0.042$, $p = 0.575$ for ADC; $r = 0.047$, $p = 0.605$ for SCC; Fig. 2D). For ADC, there were significant positive correlations between metabolic parameters and PD-L1 expression ($r = 0.15\text{--}0.31$; all $p < 0.05$), as well as TMB ($r = 0.18\text{--}0.30$; all $p < 0.05$); for SCC, metabolic parameters were not correlated with PD-L1 expression and TMB (Fig. 2E).

Predictors of PD-L1 expression and TMB for ADC

Among ADC patients in the primary cohort, PD-L1 expression accounted for 80 (43.7%), 70 (38.3%), and 33 (18.0%) in TPS $< 1\%$, $1\text{--}49\%$, $\geq 50\%$, respectively; 103 (56.3%) had PD-L1-Pos and 80 (43.7%) had PD-L1-Neg; 33 (18.0%) had PD-L1-High and 150 (82.0%) had PD-L1-Low. Patients with PD-L1-Pos were more likely to have advanced clinical stages ($p = 0.05$) and high PET metabolic parameters including SUL_{max} ($p < 0.001$), SUL_{peak} ($p < 0.001$), SUL_{mean} ($p < 0.001$), and TLG ($p = 0.002$). Patients with PD-L1-High were more likely to have poorly differentiation ($p = 0.007$), $EGFR-$ ($p = 0.014$), long short-axis diameter of primary tumor ($p = 0.033$), and high metabolic parameters including SUL_{max} ($p = 0.035$), SUL_{peak} ($p = 0.037$), SUL_{mean} ($p = 0.035$), and TLG ($p = 0.021$) (Table S2). Multivariate logistic regression analysis revealed that SUL_{max} (odds ratio [OR] = 1.157; 95% confidence interval [CI]: 1.074–1.247; $p < 0.001$) was an independent predictor of PD-L1-Pos; differentiation (OR = 0.382; 95% CI: 0.157–0.925; $p = 0.033$) was an independent predictor of PD-L1-High (Table 2).

The median TMB of ADC was 5 mutations/Mb. TMB was categorized as TMB-High (≥ 5 mutations/Mb) and TMB-Low (< 5 mutations/Mb) for 91 (49.7%), and 92 (50.3%), respectively. Patients with TMB-High were more likely to have old age ($p = 0.024$), male ($p = 0.002$), smoker ($p < 0.001$), $EGFR-$ ($p < 0.001$), and high PET metabolic parameters including SUL_{max} ($p < 0.001$), SUL_{peak} ($p < 0.001$), SUL_{mean} ($p < 0.001$), and TLG ($p = 0.013$) (Table S2). Multivariate logistic regression analysis revealed age (OR = 1.039; 95% CI: 1.004–1.076; $p = 0.030$), $EGFR$ mutation status (OR = 0.330; 95% CI: 0.158–0.692; $p = 0.003$), and SUL_{max} (OR = 1.165; 95% CI: 1.073–1.265;

Table 1 Clinicopathological characteristics of all patients

	The primary cohort with PD-L1 expression and TMB data (N=305)	The test cohort re- ceiving neoadjuvant immunotherapy (N=29)
Age (years)	61 ± 9	58 ± 11
Gender		
Male	225 (73.8)	21 (72.4)
Female	80 (26.2)	8 (27.6)
Smoking history		
Smoker	194 (63.6)	20 (69.0)
Non-smoker	111 (36.4)	9 (31.0)
Histological subtype		
ADC	183 (60.0)	29 (100.0)
SCC	122 (40.0)	NA
TNM stage*		
I/II/III/IV	62/81/102/60 (20.3/26.6/33.4/19.7)	3/11/15/0 (10.3/37.9/51.7/0.0)
T1/T2/T3/T4	72/148/59/26 (23.6/48.5/19.3/8.5)	8/15/6/0 (27.6/51.7/20.7/0.0)
N0/N1/N2/N3	113/60/91/41 (37.0/19.7/29.8/13.4)	4/11/14/0 (13.8/37.9/48.3/0.0)
M0/M1	244/61 (80.0/20.0)	29/0 (100.0/0.0)
PD-L1 expression data	305 (100.0)	20 (69.0)
TMB data	305 (100.0)	17 (58.6)

* Based on the criteria of the American Joint Committee on Cancer, eighth edition

Abbreviations PD-L1 programmed cell death ligand 1, TMB tumor mutational burden, ADC adenocarcinoma, SCC squamous cell carcinoma

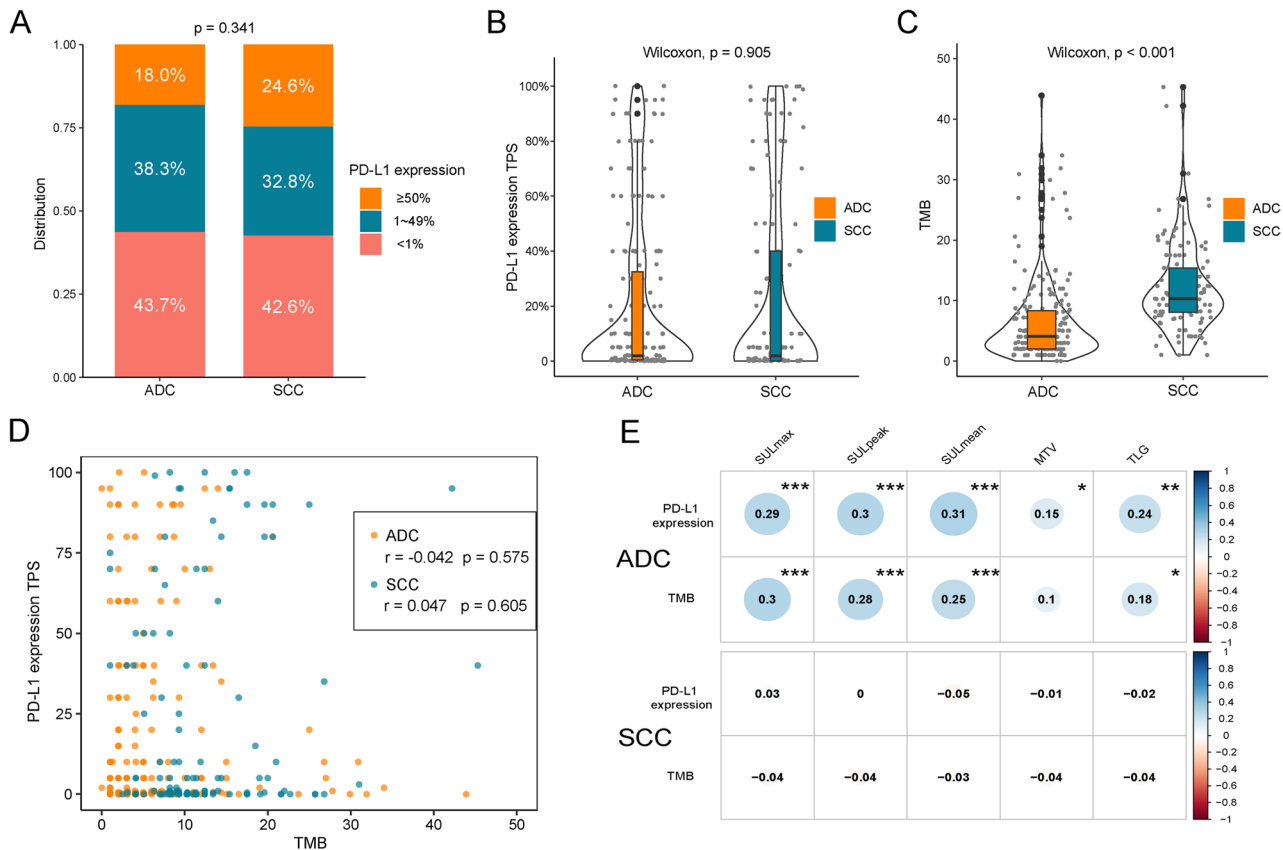


Fig. 2 Comparison of PD-L1 expression and TMB between ADC and SCC. **A–B** Comparison of PD-L1 expression between ADC and SCC. **C** Comparison of TMB between ADC and SCC. **D** Correlations between PD-L1 expression and TMB in ADC and SCC. **E** Correlations between PET/CT metabolic parameters with PD-L1 expression and TMB in ADC and SCC. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Spearman's correlation)

$p < 0.001$) were independent predictors of TMB-High (Table 2). Linear regression analysis identified age ($\beta = 0.259$; 95% CI: 0.131–0.387; $p < 0.001$), smoking history ($\beta = 0.151$; 95% CI: 0.014–0.288; $p = 0.031$), *EGFR* mutation status ($\beta = -0.249$; 95% CI: -0.383–0.114; $p < 0.001$), and SUL_{max} ($\beta = 0.245$; 95% CI: 0.113–0.376; $p < 0.001$) as independent predictors of TMB (lg), with age and SUL_{max} showing positive linear correlations (Tables S3 and S4).

SUL_{max} enables the stratification of both PD-L1 expression and TMB in ADC (Fig. 3). Patients were subsequently classified into four groups based on the combined benefits of PD-L1 expression and TMB: (1) PD-L1-Neg and TMB-Low; (2) PD-L1-Pos and TMB-Low; (3) PD-L1-Neg and TMB-High; and (4) PD-L1-Pos and TMB-High. The PD-L1-Pos and TMB-High group was considered the highest potential for immunotherapy benefits, whereas the PD-L1-Neg and TMB-Low group was considered the lowest. The differences in the clinicopathological and imaging features among the four groups are summarized in Table S5. Multivariate logistic regression analysis revealed age (OR = 1.042; 95% CI: 1.001–1.085; $p = 0.045$) and SUL_{max} (OR = 1.229; 95% CI: 1.123–1.344; $p < 0.001$) were independent predictors of

PD-L1-Pos and TMB-High; smoking history (OR = 0.379; 95% CI: 0.147–0.977; $p = 0.045$) and SUL_{max} (OR = 0.769; 95% CI: 0.676–0.876; $p < 0.001$) were independent predictors of PD-L1-Neg and TMB-Low (Table 2).

Predictors of PD-L1 expression and TMB for SCC

Among SCC patients in the primary cohort, PD-L1 expression accounted for 52 (42.6%), 40 (32.8%), and 30 (24.6%) in TPS $< 1\%$, 1–49%, $\geq 50\%$, respectively; 70 (57.4%) had PD-L1-Pos and 52 (42.6%) had PD-L1-Neg; 30 (24.6%) had PD-L1-High and 92 (75.4%) had PD-L1-Low. No significant clinicopathological or imaging features were associated with PD-L1 expression (Table S6).

The median TMB of SCC was 10 mutations/Mb. TMB was categorized as TMB-High (≥ 10 mutations/Mb) and TMB-Low (< 10 mutations/Mb) for 68 (55.7%) and 54 (44.3%), respectively. Patients with TMB-High were more likely to have poorly differentiation ($p = 0.033$) (Table S6). Linear regression analysis identified gender ($\beta = 0.197$; 95% CI: 0.023–0.371; $p = 0.027$) and differentiation ($\beta = -0.205$; 95% CI: -0.381–0.032; $p = 0.021$) as independent predictors of TMB (lg) (Tables S3 and S4).

Table 2 Predictors of PD-L1 expression and TMB by multivariate logistic regression analysis in ADC

Predictors	OR	95% CI	β coefficient	p
PD-L1-Pos				
SUL _{max}	1.157	1.074–1.247	0.146	<0.001
PD-L1-High				
Differentiation (Highly or moderately/Poorly)	0.382	0.157–0.925	−0.964	0.033
EGFR mutation status (EGFR+/EGFR−)	0.412	0.145–1.170	−0.887	0.096
SUL _{max}	1.077	0.990–1.171	0.074	0.084
TMB-High				
Age	1.039	1.004–1.076	0.039	0.030
Smoking history (Smoker/Non-smoker)	1.809	0.915–3.578	0.593	0.088
EGFR mutation status (EGFR+/EGFR−)	0.330	0.158–0.692	−1.107	0.003
SUL _{max}	1.165	1.073–1.265	0.153	<0.001
PD-L1-Pos and TMB-High				
Age	1.042	1.001–1.085	0.041	0.045
EGFR mutation status (EGFR+/EGFR−)	0.436	0.189–1.008	−0.830	0.052
SUL _{max}	1.229	1.123–1.344	0.206	<0.001
PD-L1-Neg and TMB-Low				
Smoking history (Smoker/Non-smoker)	0.379	0.147–0.977	−0.969	0.045
Lobulation sign (Yes/No)	0.434	0.178–1.058	−0.834	0.066
SUL _{max}	0.769	0.676–0.876	−0.262	<0.001

AbbreviationsPD-L1 programmed cell death ligand 1, TMB tumor mutational burden, ADC adenocarcinoma, EGFR epidermal growth factor receptor, SUL standardized uptake value corrected by lean body mass, OR odds ratio, CI confidence interval

clinicopathological or imaging features among the PD-L1 expression and TMB combined groups (Table S7).

Models construction and validation for predicting PD-L1 expression and TMB in ADC

The above results identified significant predictors for PD-L1 expression and TMB in ADC, but not in SCC. Consequently, predictive models have been established to identify biomarkers of ADC. A total of 183 patients with ADC in the primary cohort were randomly assigned to the training cohort ($n=128$) and validation cohort ($n=55$) at a ratio of 7:3 to develop and validate predictive models for PD-L1 expression and TMB separately and jointly (PD-L1-Pos, PD-L1-High, TMB-High, PD-L1-Pos and TMB-High, and PD-L1-Neg and TMB-Low). Three models were developed: clinical, SUL_{max}, and clinical-SUL_{max} combined. The nomogram, ROC, calibration, and DCA curves are shown in Fig. 4 and Fig. S1. The diagnostic performances of the ROC curves are summarized in Table 3, and the differences between the ROC curves using the DeLong test are provided in Table S8. Discrete clinical data account for the low sensitivity and specificity of clinical models. After incorporating the SUL_{max}, the clinical-SUL_{max} combined models exhibited superior performance. The models predicting PD-L1 expression and TMB separately had relatively limited performance (training cohort: AUC=0.655, 0.692, and 0.765 for PD-L1-Pos, PD-L1-High, and TMB-High; validation cohort: AUC=0.689, 0.733, and 0.705 for PD-L1-Pos, PD-L1-High, and TMB-High). The models predicting PD-L1 expression and TMB jointly demonstrated the best performance (training cohort: AUC=0.805 for PD-L1-Pos and TMB-High, AUC=0.798 for PD-L1-Neg and TMB-Low; validation cohort: AUC=0.724 for PD-L1-Pos and TMB-High, AUC=0.744 for PD-L1-Neg and TMB-Low), indicating the potential to predict events involving both PD-L1 expression and TMB occurring in a single patient.

There were no significant differences in the

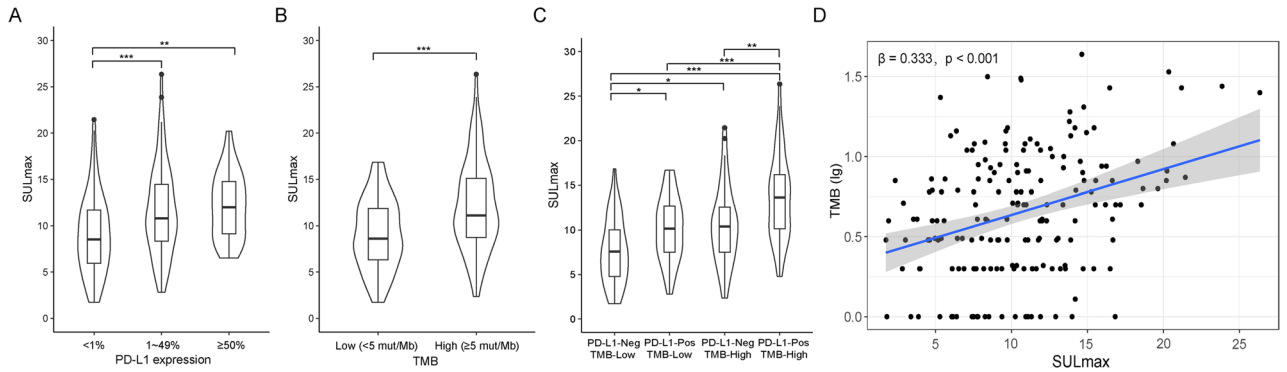


Fig. 3 SUL_{max} with PD-L1 expression and TMB in ADC **A–C** Difference of SUL_{max} in PD-L1 expression (**A**), TMB (**B**), and the combined (**C**) groups. **D** Significant linear positive correlation between SUL_{max} and TMB (lg). (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

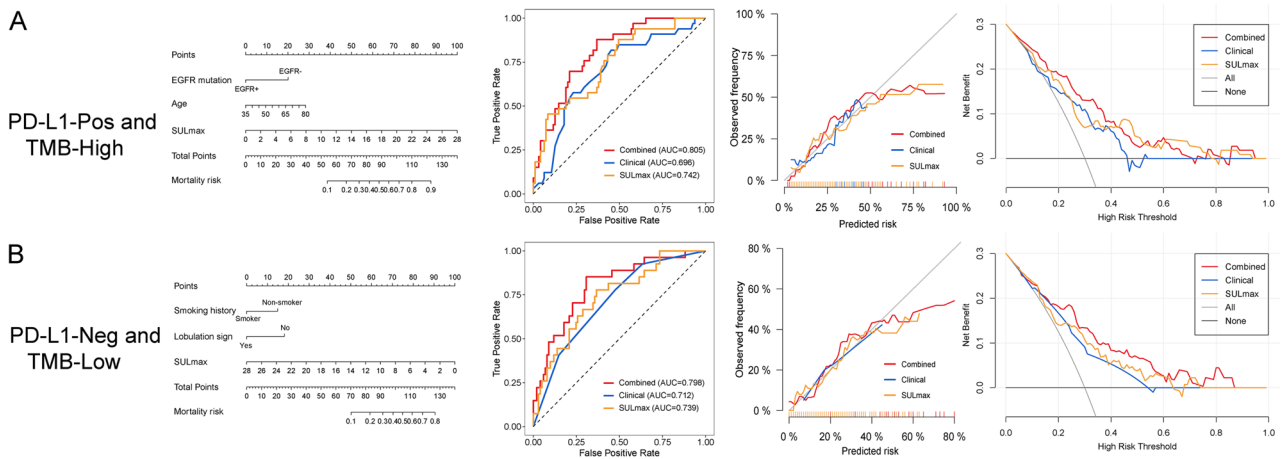


Fig. 4 Models construction for predicting PD-L1 expression and TMB simultaneously in ADC. **A-B** Nomogram, ROC, calibration, and decision curves for predicting PD-L1-Pos and TMB-High (**A**), and PD-L1-Neg and TMB-Low (**B**). * PD-L1-Pos and TMB-High model: $Y = -5.790 - 1.292 \times EGFR \text{ mutation} + 0.040 \times \text{Age} + 0.229 \times SUL_{\text{max}}$ PD-L1-Neg and TMB-Low model: $Y = 1.785 - 0.944 \times \text{Smoking history} - 1.150 \times \text{Lobulation sign} - 0.226 \times SUL_{\text{max}}$

Table 3 Diagnostic performance of models for predicting PD-L1 expression and TMB in ADC

Models	Training cohort (N = 128)					Validation cohort (N = 55)				
	AUC	95% CI	Sen.	Spe.	Acc.	AUC	95% CI	Sen.	Spe.	Acc.
PD-L1-Pos										
SUL _{max}	0.655	0.559–0.750	0.729	0.534	0.641	0.689	0.540–0.839	0.515	0.864	0.655
PD-L1-High										
Clinical	0.673	0.563–0.783	0.682	0.632	0.641	0.661	0.504–0.818	0.636	0.636	0.636
SUL _{max}	0.596	0.477–0.716	0.955	0.264	0.383	0.692	0.544–0.840	0.818	0.591	0.636
Combined	0.692	0.586–0.798	0.727	0.689	0.695	0.733	0.576–0.891	0.636	0.795	0.764
TMB-High										
Clinical	0.720	0.631–0.809	0.797	0.594	0.695	0.703	0.563–0.843	0.556	0.821	0.691
SUL _{max}	0.691	0.600–0.782.600.782	0.656	0.656	0.656	0.669	0.526–0.813	0.741	0.536	0.636
Combined	0.765	0.683–0.846	0.891	0.547	0.719	0.705	0.565–0.845	0.704	0.679	0.691
PD-L1-Pos and TMB-High										
Clinical	0.696	0.593–0.800.593.800	0.818	0.547	0.617	0.573	0.401–0.745	0.235	0.974	0.745
SUL _{max}	0.742	0.645–0.838	0.879	0.505	0.602	0.716	0.567–0.865	0.647	0.789	0.745
Combined	0.805	0.726–0.884	0.879	0.632	0.695	0.724	0.564–0.885	0.706	0.789	0.764
PD-L1-Neg and TMB-Low										
Clinical	0.712	0.611–0.812	0.778	0.525	0.578	0.585	0.414–0.756	0.833	0.326	0.436
SUL _{max}	0.739	0.635–0.842	0.778	0.634	0.664	0.744	0.573–0.915	0.667	0.767	0.745
Combined	0.798	0.704–0.892	0.852	0.693	0.727	0.744	0.585–0.903	0.667	0.791	0.764

AbbreviationsPD-L1 programmed cell death ligand 1, TMB tumor mutational burden, ADC adenocarcinoma, SUL standardized uptake value corrected by lean body mass, AUC area under the receiver operating characteristic curve, CI confidence interval, Sen sensitivity, Spe specificity, Acc accuracy

Models test for clinical usefulness

A total of 29 patients with ADC receiving neoadjuvant immunotherapy in the test cohort were used to evaluate the clinical usefulness of the models. Biomarkers data were incomplete, with PD-L1 expression available for 20 (69.0%) patients, TMB available for 17 (58.6%) patients, and both PD-L1 expression and TMB available for 12 (41.4%) patients. The models effectively stratified pathological responses: for the PD-L1-Pos and TMB-High model, patients predicted to be PD-L1-Pos and TMB-High exhibited significantly higher pathological regression than those predicted otherwise ($p=0.035$); for the

PD-L1-Neg and TMB-Low model, patients predicted to be PD-L1-Neg and TMB-Low showed significantly lower pathological regression than those predicted otherwise ($p=0.001$) (Fig. 5).

Discussion

PD-L1 expression and TMB are currently recognized as decisive biomarkers for guiding immunotherapy; however, the inherent deficiencies of invasive tissue sampling limit their feasibility in challenging situations (e.g., lack of available specimens or biopsy contraindications). Based on the evidence that metabolic parameters on PET/

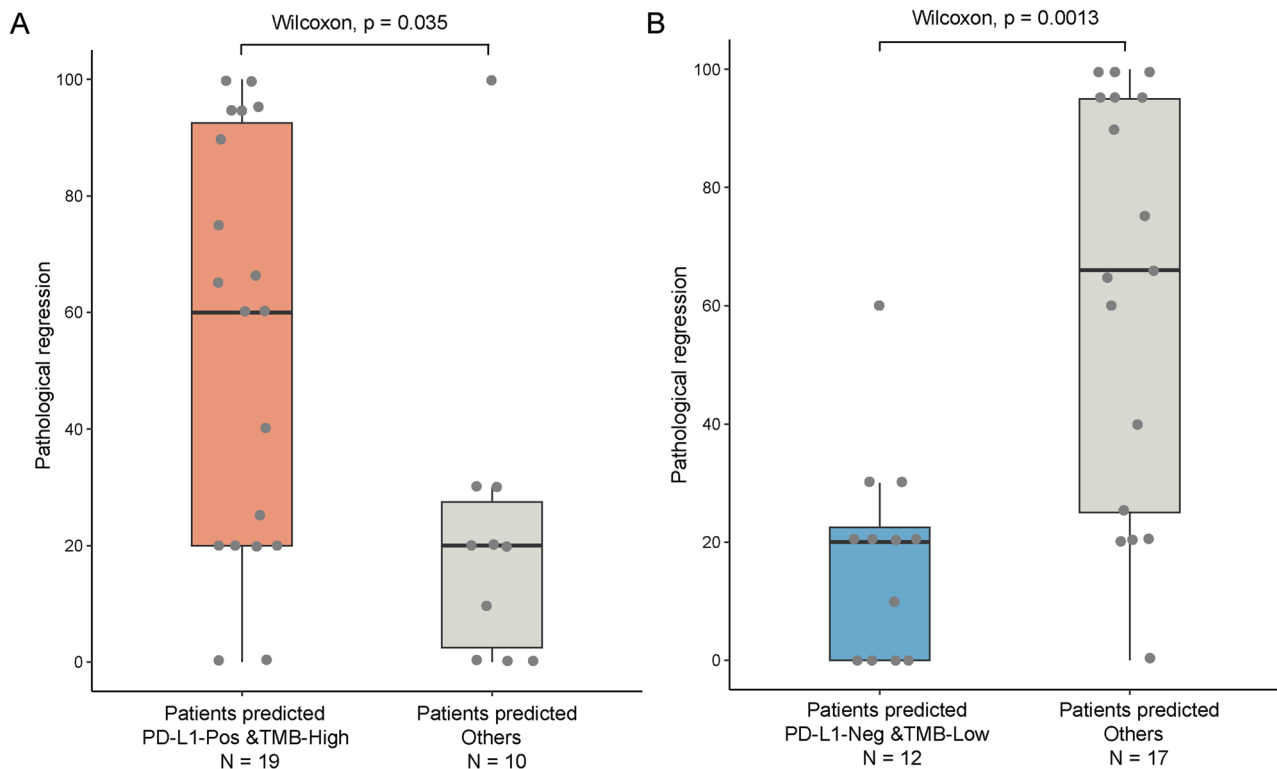


Fig. 5 Models test for pathological regression after neoadjuvant immunotherapy of ADC. **A-B** Models test by patients grouped by PD-L1-Pos and TMB-High model (**A**), and patients grouped by PD-L1-Neg and TMB-Low model (**B**)

CT have demonstrated remarkable predictive value in response to immunotherapy [5, 6], PET/CT holds promise as a non-invasive alternative for biomarkers that integrate both PD-L1 expression and TMB. In this study, we identified independent predictors and developed PET/CT-based nomogram models to predict PD-L1 expression and TMB. The results demonstrated that PET/CT had a significant predictive value for biomarkers in ADC but not in SCC. SUL_{max} is an independent predictor of both PD-L1-Pos and TMB-High in ADC. The clinical- SUL_{max} combined models demonstrated excellent performance in predicting PD-L1-Pos and TMB-High status (AUC = 0.805 in the training cohort, AUC = 0.724 in the validation cohort), as well as PD-L1-Neg and TMB-Low status (AUC = 0.798 in the training cohort, AUC = 0.744 in the validation cohort). More importantly, the models were useful for stratifying immunotherapy responses in a real-world clinical setting ($p = 0.035$ for the PD-L1-Pos and TMB-High model, $p = 0.001$ for the PD-L1-Neg and TMB-Low model), bridging the gap between biomarkers prediction and clinical outcomes.

Given the distinct biological differences and heterogeneous immune landscapes of ADC and SCC [18], it is not surprising that PD-L1 expression and TMB differ between the two histological subtypes. Our results indicated that ADC and SCC had similar levels of PD-L1 expression, whereas SCC exhibited significantly higher

mutation burden than ADC, which is consistent with the results of previous studies [21–23]. The independence of PD-L1 expression and TMB ensures the cumulative effect of combined biomarkers in immunotherapy [1, 4]. It is also not unexpected that the clinicopathological and imaging predictors of PD-L1 expression and TMB in ADC and SCC showed completely different patterns. In the analysis of ADC, PD-L1 expression was associated with clinical stage, differentiation, and *EGFR* mutation status, and TMB was associated with age, gender, smoking history, and *EGFR* mutation status, all of which were consistent with the results of previous studies [11, 24, 25]. Previous studies have suggested that some morphological features were associated with PD-L1 expression, such as presence of GGO, and pleural indications [13, 26]. However, no morphological features were associated with PD-L1 expression in our study, which may be due to observer bias and variability between studies. Analysis of SCC has shown that there are no significant clinicopathological and PET/CT imaging predictors of PD-L1 expression [25, 27] or TMB [11, 28], but conclusions are inconsistent. However, the available evidence does not support the development of a predictive diagnostic framework for SCC.

In this study, SUL_{max} was identified as an independent predictor of PD-L1 expression and TMB in ADC, but not in SCC. The potential explanations for the differences

primarily lie in their heterogeneous biological characteristics. ADC and SCC exhibit distinct driver gene profiles that directly modulate their tumor metabolism [29]. The immune function-related pathways, including programmed cell death and innate immune system were differentially enriched between the two subtypes, and metabolism-related gene sets were downregulated in SCC [30]. Besides, ADC exhibited a more active tumor immune microenvironment characterized by more abundant and diverse tumor-infiltrating immune cells compared to SCC [30]. The metabolic reprogramming of the tumor microenvironment also differs in ADC and SCC [31]. Genes correlated to glucose uptake were predominately overexpressed in a single cell-type comprising the tumor microenvironment. In SCC, most of these genes were expressed by malignant cells, whereas in ADC they were predominately expressed by stromal cells [31]. In previous studies, SUL_{max} has been consistently confirmed as an independent and imaging-derived predictor of PD-L1 expression [15, 16, 24, 25, 27, 32] and TMB [10, 11, 33] in ADC. The present study, for the first time, further proposes that SUL_{max} serves as an independent predictor of events involving both upstream TMB and downstream PD-L1 expression in a single patient with ADC. SUL_{max} holds promise as a non-invasive alternative for integrating both PD-L1 expression and TMB. ADC with high SUL_{max} were prone to PD-L1-Pos and TMB-High, whereas those with low SUL_{max} were prone to PD-L1-Neg and TMB-Low. The intrinsic mechanism linking tumor metabolism with PD-L1 expression and TMB has been proven to be a complex and dynamic process, including hypoxic microenvironment, activation of the mammalian target of rapamycin signaling pathway, and expression of glucose transporters, thus promoting glucose metabolism [34–37].

Previous studies have developed models comprising PET/CT-based features to predict PD-L1 expression status with AUCs ranging from 0.77 to 0.85 [12, 13], and some studies have further incorporated radiomics features with AUCs ranging from 0.76 to 0.97 [15–17, 38]. Despite the superior performance of radiomics models, the complexity of image segmentation, poor reliability associated with small sample sizes, and lack of interpretability owing to the “black box” effect significantly constrain their practical application. Besides, these studies have certain limitations. First, studies have primarily focused on NSCLC or specifically ADC, with few discussing the histological subtypes separately. Second, no models have been developed to predict TMB status, or models that concurrently consider both PD-L1 expression and TMB. More importantly, although these models performed well in predicting biomarkers, few studies have tested their usefulness in predicting immunotherapy responses in clinical settings. In this study, we aimed

to develop predictive models with practical applications using easily accessible and reliable clinical data. The clinical- SUL_{max} combined models demonstrated excellent performance in predicting PD-L1 expression and TMB in ADC. The combined models consistently outperformed the clinical and SUL_{max} models, illustrating a characteristic fusion trend. The models that predicted PD-L1 expression and TMB jointly exhibited superior performance than separately, indicating that clinical and SUL_{max} features comprehensively captured the multi-molecular characteristics of tumor. The models prioritizing SUL_{max} over other PET parameters (SUL_{peak} , SUL_{mean} , MTV, and TLG) focuses on model parsimony and clinical applicability. The PET parameters exhibited high collinearity. Using the backward stepwise selection method, SUL_{max} was retained as it contributed most to the multivariate logistic regression model, while the other PET parameters were excluded as collinear variables would not improve model discrimination. Furthermore, SUL_{max} is the most widely used and interpretable PET parameter in clinical practice. Its measurement, requiring only identification of the single highest uptake voxel in the tumor without specialized post-processing tools. In contrast, SUL_{peak} and SUL_{mean} are sensitive to partial-volume effects in small tumors, and MTV and TLG depend on thresholds that vary across clinical centers.

This study tested the clinical usefulness of the models by demonstrating their effectiveness in stratifying pathological responses in patients with ADC receiving neoadjuvant immunotherapy, which provides a unique window to intuitively and accurately evaluate the treatment effects of immunotherapy at an early time point [39], thus avoiding the complexity and misleading of clinical evaluation that sometimes does not necessarily reflect actual therapeutic effects [40, 41]. Ideally, PD-L1 expression and TMB should be tested first, followed by the response to immunotherapy. However, biomarkers data are often incomplete in real-world clinical settings, and patients are carefully selected before receiving immunotherapy with inevitable bias. As an assistive tool for patient selection, clinical- SUL_{max} combined models transcend the testing of biomarkers and directly stratify clinical outcomes, thereby approaching the ultimate goal of the studies.

This study has some limitations. First, this was a retrospective study relying on nearly 5 years of data from a single Asian center, so selection bias is inevitable. Second, the independent test cohort had small sample size. It included patients receiving neoadjuvant immunotherapy for the most accurate efficacy via pathological regression, but faced selection constraints: neoadjuvant immunotherapy remains investigational and not the standard first-line regimen, and lung adenocarcinoma with driver mutations prioritize targeted therapy over

immunotherapy. Due to rigorous selection, the small sample size limited the statistical power for validating the model's efficacy. Besides, incomplete data of failed PD-L1 expression and TMB testing precluded biomarkers validation in the test cohort, further impeding detection of associations among models, biomarkers and clinical outcomes. Additionally, the majority of patients in the test cohort were selected from specific clinical trial populations, this may restrict the models' generalizability to broader real-world clinical populations, or cohorts receiving different immunotherapies. Future prospective, large-scale, multicenter studies are indeed essential for further validating the robustness, generalizability, and clinical applicability of the proposed models. Third, owing to the retrospective design of this study, PD-L1 expression was evaluated using three antibody clones (22C3, SP263, and SP142), and variability across these assays may introduce bias. Additionally, beyond the *EGFR* mutation status of ADC, other mutations with low incidence were not analyzed. Finally, artificial intelligence and machine learning have revolutionized medical imaging by enabling the advanced analysis and interpretation of high-throughput data. The ability to characterize tumor heterogeneity through "digital biopsy" holds promise with the rapid advancement of technology.

Conclusion

In conclusion, SUL_{max} is a key independent predictor of both PD-L1-Pos and TMB-High in ADC. The clinical- SUL_{max} combined models offer practical application value in an easy-to-use manner, effectively predicting biomarkers status and further stratifying clinical outcomes for immunotherapy. SUL_{max} is considered an assistive tool for ADC selection in conjunction with or as a substitute for additional tests and extensive diagnostic workups, thus contributing to personalized treatment strategies.

Abbreviations

NSCLC	Non-small cell lung cancer
ADC	Adenocarcinoma
SCC	Squamous cell carcinoma
PD-L1	Programmed cell death ligand 1
TPS	Tumor proportion score
TMB	Tumor mutational burden
^{18}F -FDG PET/CT	^{18}F -fluorodeoxyglucose positron emission tomography/computed tomography
SUL	Standardized uptake value corrected by lean body mass
MTV	Metabolic tumor volume
TLG	Total lesion glycolysis
ROC	Receiver operating characteristic
AUC	Area under the ROC curve
EGFR	Epidermal growth factor receptor

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

XLT and NW designed the study, supervised the research, secured funding and obtained approval from the ethics committee. QZ, PY, and ZWZ organized the database. QZ, PY, and ZWZ performed the statistical analysis. XLT, JMY, LG, NL, SHW, JL, YL, WG performed the data interpretation. QZ, PY, and ZWZ wrote the initial manuscript. XLT and NW reviewed the initial manuscript. All authors contributed to the article and approved the submitted version.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Institutional Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences (Approval Number: 22/491–3693), with an approved date of 5 December 2022. The requirement for informed consent was waived because of the retrospective and anonymous nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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