Systemic immune index predicts tumor-infiltrating lymphocyte intensity and immunotherapy response in small cell lung cancer

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Background: Despite recent progresses in immune checkpoint blockade (ICB) in small-cell lung cancer (SCLC), a lack of understanding regarding the systemic tumor immune environment (STIE) and local tumor immune microenvironment (TIME) makes it difficult to accurately predict clinical outcomes and identify potential beneficiaries from ICB therapy.

Methods: We enrolled 191 patients with stage I–III SCLC and comprehensively evaluated the prognostic role of STIE by several quantitative measurements, and further integrate it with a local immune score system (LISS) established by eXtreme Gradient Boosting (XGBoost) machine learning algorithm. We also test the value of STIE in beneficiary selection in our independent advanced SCLC cohort receiving programmed cell death 1 ligand 1 (PD-L1) blockade therapy.

Results: Among several systemic immune markers, the STIE as assessed by prognostic nutritional index (PNI) was correlated with disease-free survival (DFS) and overall survival (OS), and remained as an independent prognostic factor for SCLC patients [hazard ratio (HR): 0.473, 95% confidence interval (CI): 0.241–0.929, P=0.030]. Higher PNI score was closely associated with inflamed SCLC molecular subtype and local tumor-infiltrating lymphocytes (TILs). We further constructed a LISS which combined top three important local immune biomarkers (CD8+ T-cell count, PD-L1 expression on CD8+ T-cell and CD4+ T-cell count) and integrated it with the PNI score. The final integrated immune risk system was an independent prognostic factor and achieved better predictive performance than Tumor Node Metastasis (TNM) stages and single immune biomarker. Furthermore, PNI-high extensive-stage SCLC patients achieved better clinical response and longer progression-free survival (PFS) (11.8 *vs.* 5.9 months, P=0.012) from PD-L1 blockade therapy.

Conclusions: This study provides a method to investigate the prognostic value of overall immune status by combining the PNI with local immune biomarkers in SCLC. The promising clinical application of PNI in efficacy prediction and beneficiary selection for SCLC immunotherapy is also highlighted.

Keywords: Small-cell lung cancer (SCLC); prognostic nutritional index (PNI); molecular subtype; tumor-infiltrating lymphocyte (TIL); programmed cell death 1 ligand 1 blockade therapy (PD-L1 blockade therapy)

Introduction

Background

Small-cell lung cancer (SCLC) is an exceptionally lethal malignancy and the limited survival improvement by conventional therapies has prompted the urgent search for innovative strategies (1,2). Despite that immune checkpoint blockade (ICB) therapy sheds light on a breakthrough of SCLC treatment recently, the survival benefits are still modest (3-6). In addition, although immune checkpoints such as programmed cell death 1 ligand 1 (PD-L1) are extremely valuable prognostic biomarkers across different cancer types including non-small-cell lung cancer (NSCLC), few biomarkers are available in SCLC for accurate survival prediction and ICB therapy selection.

Tumor immune microenvironment (TIME), compositing several immune cell groups such as tumor-infiltrating lymphocytes (TILs), has been well known for its key role on the tumor progression and local immunologic response to certain treatments (7). However, one must be kept in mind that both local tumor immune contexture and treatment response can also be influenced by systemic immune status, i.e., the host systemic tumor immune

environment (STIE) (8). STIE consists of circulating blood immune cells and molecules which can be easily assessed by routine blood tests. A prominent example is prognostic nutritional index (PNI), an indicator of nutritional status and systemic immune competence, the prognostic value of which has been revealed in various types of cancers (9-12). Moreover, various studies have illustrated the local TIME as determined by TIL status is significantly related to the PNI score (10,13).

Rationale and knowledge gap

For SCLC, it is still unknown regarding the complex interaction between STIE and TIME and whether they are valuable for predicting the long-term outcomes and immunotherapy responses of SCLC patients.

Objective

Therefore, we comprehensively evaluated the prognostic value of several systemic immune biomarkers in a relatively large SCLC cohort, determined their correlation with TILs, and further integrated systemic immune status with a local immune score system (LISS) constructed by an eXtreme Gradient Boosting (XGBoost) machine learning model to improve the prognostic accuracy in SCLC patients. In addition, another independent SCLC cohort receiving PD-L1 blockade therapy was used to test the value of PNI score in efficacy prediction and beneficiary selection. We present this article in accordance with the TRIPOD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-696/rc).

Methods

Patients

The study included two main SCLC patient cohorts (*Figure 1A*), the limited-stage surgically resected SCLC (LS-SCLC) cohort (N=191) from November 2006 to April 2021, and the extensive-stage immunotherapy SCLC (ES-SCLC) cohort (N=91) who received PD-L1 blockade combined with chemotherapy [atezolizumab 1,200 mg every three

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**Highlight box**

Key findings

- The prognostic nutritional index (PNI)-based immune risk system is an independent prognostic factor for small-cell lung cancer (SCLC) patients. The PNI-high extensive-stage SCLC patients also achieved better clinical response and survival from programmed cell death 1 ligand 1 blockade therapy.

What is known and what is new?

- Tumor immune microenvironment has been well known for its key role on the tumor progression and local immunologic response to certain treatments.
- We showed a strong relationship between systemic immune index and tumor-infiltrating lymphocyte intensity, patient survival and immunotherapy efficacy.

What is the implication, and what should change now?

- This study provides a method to investigate the prognostic value of overall immune status by combining the PNI with local immune biomarkers in SCLC.
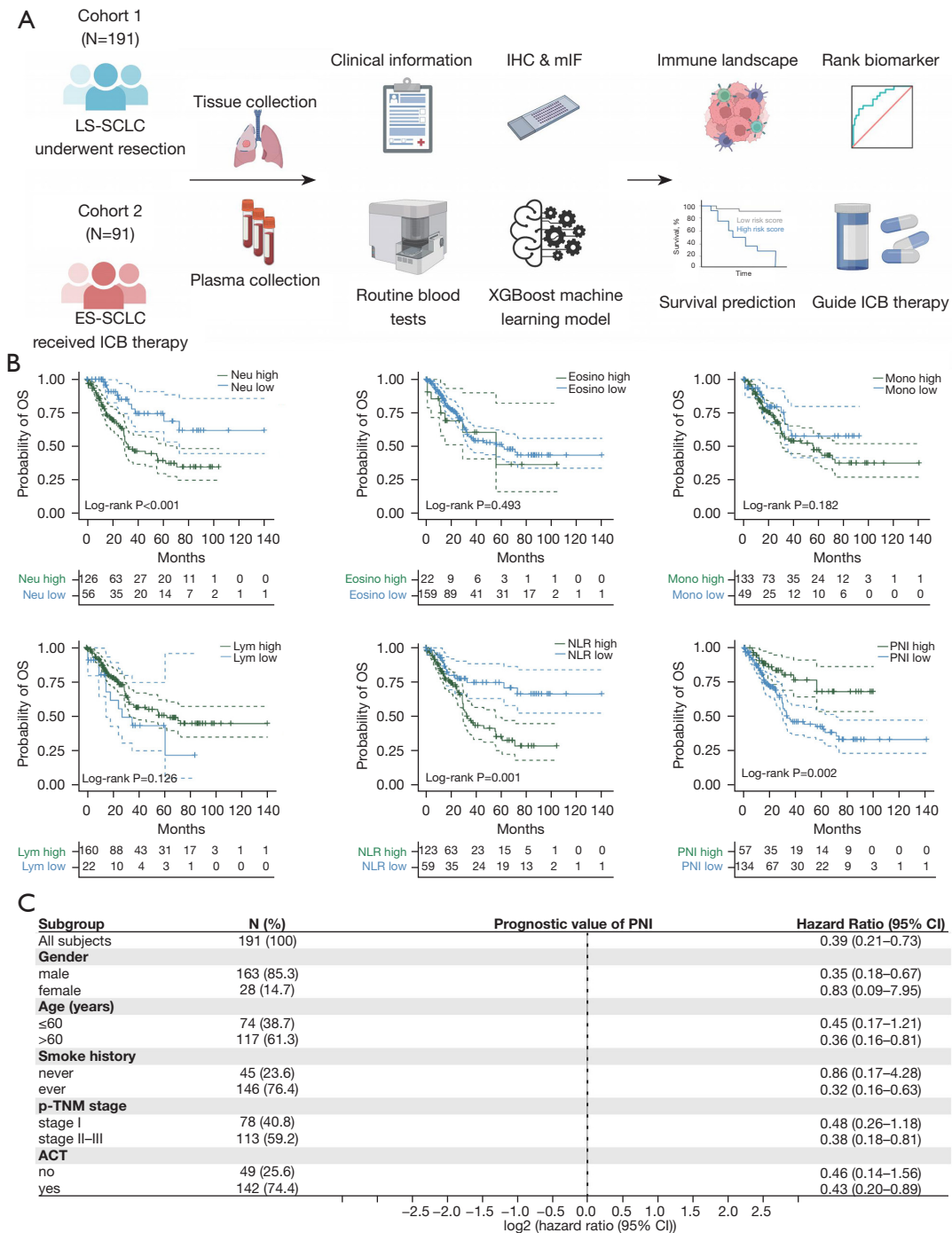
Figure 1 The prognostic value of several systemic immune biomarkers. (A) Overview of the study design. The illustration was created with BioRender.com. (B) The Kaplan-Meier analysis of each systemic immune biomarker in limited-stage SCLC cohort (N=191) with two-sided log-rank P value. (C) Stratification analysis by clinicopathological characteristic regarding the prognostic value of PNI in limited-stage SCLC cohort (N=191). LS-SCLC, limited-stage small-cell lung cancer; ES-SCLC, extensive-stage small-cell lung cancer; ICB, immune checkpoint blockade; IHC, immunohistochemistry; mIF, multiplex immunofluorescence; OS, overall survival; Neu, neutrophil; Eosino, eosinophil; Mono, monocyte; lymph, lymphocyte; NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; TNM, Tumor Node Metastasis; ACT, adjuvant chemotherapy; CI, confidence interval.

weeks (q3w) or durvalumab 1,500 mg q3w] from May 2020 to April 2021 at Fudan University Shanghai Cancer Center (FUSCC). This study was approved by the Institutional Review Board of FUSCC (IRB No. 2008223-9). Informed consents were waived because it was a retrospective study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data and tissue specimens

Data on clinicopathological variables were obtained by reviewing patient medical records specifically for this study purpose. These characteristics included age, sex, smoking history, surgery type, resection type, tumor size, pathological tumor stage, visceral pleural invasion (VPI) and lymphovascular invasion (LVI) status, treatment regimens, sodium level and lactate dehydrogenase (LDH) levels, and disease-free survival (DFS), progression-free survival (PFS) and overall survival (OS). All responses to ICBs were assessed using the Response Evaluation Criteria in Solid Tumors guidelines (14). Durable clinical benefit (DCB) was defined as the proportion of patients achieving objective response or stable disease (SD) lasting ≥6 months. The PNI was calculated as 10× serum albumin (g/dL) + 5× total lymphocytes count (/nL). The latest laboratory data within 1 month before operation or immunotherapy were used. Another inflammation-based prognostic score, the preoperative neutrophil-lymphocyte ratio (NLR) was also obtained. X-tile was used to determine the cut-off points. After independent review of all hematoxylin and eosin (HE) slides and assessment of formalin-fixed paraffin-embedded (FFPE) block quality, 129 cases in LS-SCLC cohort were constructed into tumor tissue microarray (TMA) and 16 cases were analyzed in slides. No cases in ES-SCLC cohort were evaluated with TMA.

TMA construction

SCLC TMA was constructed in the Department of Pathology, Fudan University Shanghai Cancer Center using the automated TMA Grand Master (3DHISTECH, Budapest, Hungary). The HE slides were reviewed to select the most viable tumor and uninvolved lung tissue areas. Finally, 260 cores (two or three cores per case) were punched in corresponding tumor areas with 2 mm in diameter.

Immunohistochemistry

TMAs and slides were stained for subtype-defined markers of SCLC including ASCL1 (clone 24B72D11.1, dilution 1:100, BD Biosciences, New York, USA), NEUROD1 (clone EPR17084, dilution 1:50, Abcam, Cambridge, UK), POU2F3 (clone 6D1, dilution 1:100, Santa Cruz, Texas, USA) and YAP1 (clone 63.7, dilution 1:2000, Santa Cruz) using Anti-mouse/rabbit Immunohistochemistry (IHC) Detection Kit (Cat No. PK10006, Proteintech, Chicago, USA) according to the manufacture protocol. Two independent pathologists reviewed the stained slides in a blinded fashion. The expression of each marker was assessed by histoscore (H-score, range, 0–300), which was calculated by multiplying the proportion of positive cells (0–100%) by the intensity of positive staining (no staining =0, weak staining =1, moderate staining =2, and strong staining =3). And the molecular subtype for a tumor was assigned based on the highest H-score among subtyping markers (15).

Multiplex immunofluorescence (mIF) staining

mIF analysis was performed on TMAs. TMA sections were stained using antibodies against CD4 (clone EP204, dilution 1:100, Cell Signaling Technologies, Boston, USA), CD8 (clone D8A8Y, dilution 1:200, Cell Signaling Technologies), CD68 (clone D4B9C, dilution 1:400, Cell Signaling Technologies), programmed cell death 1 (PD-1) (clone NAT105, dilution 1:100, Abcam), PD-L1 (clone E1L3N, dilution 1:100, Cell Signaling Technologies), CTLA-4 (clone CAL49, dilution 1:200, Abcam), and cytokeratins (clone C-11, dilution 1:250, Abcam). All antibodies were linked with one of the fluorophores from the Opal 7 IHC kit, (Cat No. NEL797001KT; Akoya Biosciences, MA, USA). Sections were scanned by Aperio Versa 8 tissue imaging system (Leica, Hesse, Germany). Imaging analysis was performed with a quantitative image analysis software (Halo, Indica Labs, New Mexico, USA). X-tile was used to determine the cut-off points for survival analysis.

RNA sequencing

The RNA sequencing process was performed for 67 patients with available tumor tissues as previously reported (16). Briefly, a total amount of 3 μg RNA was extracted from tumor tissues per sample using NucleoZOL (Macherey-

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Nagel, Dueren, Germany) and NucleoSpin RNA Set for NucleoZOL (Macherey-Nagel). The sequencing libraries were generated using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, USA) according to manufacturer's instructions and then sequenced on the Illumina HiSeq X Ten platform.

XGBoost and LISS

The XGBoost machine learning algorithm was adapted to construct LISS by various tumor immune biomarkers obtained by mIF. This machine learning method can discover non-linear relationships using the first and second derivatives, as well as controlling the overfitting and overcomplexity of one predictive model by employing regularization item, and scoring the importance of each measured variable (17,18).

The SCLC samples were randomly divided into training set and calibration set (7:3). After repeating 1,000 times for model construction, top three important features were selected for the LISS establishment. The obtained model formula was as follows: LISS = (0.1437 × CD8) − (0.1353 × PD-L1/CD8) + (0.1323 × CD4). We further performed time-dependent receiver operating characteristic (ROC) curve analysis and survival analysis to validate the model performance.

Statistical analysis

All data were processed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) and R Statistical Language (version 4.1.2). Correlations between two categorical variables were analyzed using Chi-squared test or Fisher's exact test. Student's t-test or Mann-Whitney test were used to compare continuous variables. The PFS and OS were investigated by log-rank test to compare differences between groups. Cox proportional hazard regressions were used to predict recurrence and death status. Variables with a P value less than 0.05 in univariate analysis were entered into multivariate survival analysis. All tests were two-tailed. Statistical significance was set at P<0.05.

Results

Prognostic value of systemic immune markers

We investigated the prognostic role of the host anti-tumor immunity, i.e., STIE in a cohort of 191 patients with

surgically resected stage I–III SCLC (*Figure 1B*), with a median follow-up time of 59.8 months. The cut-off points for several peripheral blood immune cells as well as NLR and PNI were shown in Table S1, and patients were divided into high- and low-risk groups accordingly.

As shown in *Figure 1B*, higher neutrophil count and NLR indicated a significantly worse prognosis (P<0.001 and P=0.001, respectively), while compared with low-PNI score group, patients in the high-PNI score group had a better OS (P=0.002, *Figure 1B*) as well as DFS (P=0.027, Figure S1).

Considering potential correlations between the three variables, they were included into multivariate Cox model separately. As summarized in *Table 1*, PNI remained a powerful and independent factor after adjustment for clinicopathological factors [hazard ratio (HR): 0.473, 95% confidence interval (CI): 0.241–0.929, P=0.030], whereas neutrophil count and NLR did not. Further stratification analysis by Tumor Node Metastasis (TNM) stage and receipt of adjuvant chemotherapy (ACT) confirmed the prognostic value of PNI, although not always statistically significant (*Figure 1C*).

Collectively, compared with other peripheral blood immune cells and index, the PNI score might be a better indicator representing systemic immune competence, as well as a strong independent prognostic factor in SCLC, which was vital for subsequent analyses.

Correlation between PNI, clinicopathological factors and molecular subtypes

The clinicopathological features of all surgically resected SCLC patients stratified by PNI score are summarized in *Table 2*. This cohort included 163 men (85.3%) and 28 women (14.7%), with a median age of 63 years [interquartile range (IQR), 57–68 years]. Out of 191 cases, 134 (70.2%) presented with low PNIs and 57 (29.8%) presented with high PNIs. There were no significant differences in age, sex, tobacco use, operative procedure, tumor stages, VPI and LVI status, ACT, or hyponatremia status and LDH levels between PNI-high and PNI-low groups, which also suggested the innate prognostic value of PNI but not distribution bias and cachexia conditions.

Recently, a novel molecular subtype model of SCLC defined by four transcription factors (ASCL1, NEUROD1, POU2F3 and YAP1, designated as SCLC-A, N, P, Y, respectively) has been proposed and these subtypes are reported to have distinct therapeutic vulnerabilities (19). Therefore, we next evaluated whether PNI correlated

Table 1 Cox regression analysis of OS in the entire cohort

Variables	Univariable		Multivariate (model 1)		Multivariate (model 2)		Multivariate (model 3)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age (year) (≥60 vs. <60)	1.165 (0.713, 1.905)	0.542						
Gender (female vs. male)	0.256 (0.093, 0.706)	0.008	0.130 (0.031, 0.541)	0.005	0.148 (0.033, 0.655)	0.012	0.148 (0.033, 0.655)	0.012
Smoking history (ever vs. never)	2.840 (1.351, 5.967)	0.006	1.427 (0.512, 3.976)	0.496	0.891 (0.295, 2.693)	0.838	0.885 (0.293, 2.675)	0.829
Operative procedure (sublobar vs. lobectomy)	0.595 (0.187, 1.895)	0.38						
p-TNM stage (II–III vs. I)	2.378 (1.387, 4.078)	0.002	2.705 (1.461, 5.010)	0.002	4.277 (1.684, 10.87)	0.002	4.277 (1.684, 10.87)	0.002
LVI (presence vs. absence)	1.921 (1.129, 3.267)	0.016	1.272 (0.659, 2.455)	0.474	1.212 (0.439, 3.350)	0.711	1.453 (0.560, 3.770)	0.443
VPI (presence vs. absence)	2.463 (1.369, 4.431)	0.003	2.476 (1.265, 4.846)	0.008	3.780 (1.155, 12.37)	0.028	3.780 (1.155, 12.37)	0.028
Adjuvant chemotherapy (presence vs. absence)	0.324 (0.197, 0.532)	<0.001	0.254 (0.129, 0.500)	<0.001	0.183 (0.076, 0.442)	<0.001	0.183 (0.076, 0.442)	<0.001
PNI status (high vs. low)	0.391 (0.209, 0.728)	0.003	0.473 (0.241, 0.929)	0.03				
NLR status (high vs. low)	2.369 (1.324, 4.238)	0.004			1.772 (0.743, 4.224)	0.197		
Neutrophil (high vs. low)	2.628 (1.406, 4.910)	0.002					1.335 (0.472, 3.778)	0.586

OS, overall survival; HR, hazard ratio; CI, confidence interval; TNM, Tumor Node Metastasis; LVI, lymphovascular invasion; VPI, visceral pleural invasion; PNI, prognostic nutritional index; NLR, neutrophil-lymphocyte ratio.

**Table 2** Relationship between clinicopathologic characteristics and PNI in all patients

Variables	Total patients (N=191)	PNI		P
		Low (≤55.5), N=134	High (>55.5), N=57	
Age (year)				0.271
Median	63	64	61	
IQR	57–68	58–68	55–68	
Gender				0.874
Male	163 (85.3)	114 (85.1)	49 (86.0)	
Female	28 (14.7)	20 (14.9)	8 (14.0)	
Smoking history				0.594
Never	45 (23.6)	33 (24.6)	12 (21.1)	
Ever	146 (76.4)	101 (75.4)	45 (78.9)	

Table 2 (*continued*)

Table 2 (*continued*)

Variables	Total patients (N=191)	PNI		P
		Low (≤55.5), N=134	High (>55.5), N=57	
Operative procedure				0.484
Lobectomy	175 (91.6)	124 (92.5)	51 (89.5)	
Sublobar resection	16 (8.4)	10 (7.5)	6 (10.5)	
p-TNM stage				0.631
I	78 (40.8)	52 (38.8)	26 (45.6)	
II	44 (23.0)	31 (23.1)	13 (22.8)	
III	69 (36.1)	51 (38.1)	18 (31.6)	
Tumor stage				0.493
pT1	108 (56.5)	74 (55.2)	34 (59.6)	
pT2	63 (33.0)	45 (33.6)	18 (31.6)	
pT3	15 (7.9)	10 (7.5)	5 (8.8)	
pT4	5 (2.6)	5 (3.7)	0	
Nodal status				0.420
pN0	90 (47.1)	59 (44.0)	31 (54.4)	
pN1	36 (18.8)	27 (20.1)	9 (15.8)	
pN2	65 (34.0)	48 (35.8)	17 (29.8)	
*VPI				0.528
Present	31 (17.2)	23 (18.4)	8 (14.5)	
Absent	149 (82.8)	102 (81.6)	47 (85.5)	
*LVI				0.467
Present	78 (44.8)	56 (46.7)	22 (40.7)	
Absent	96 (55.2)	64 (53.3)	32 (59.3)	
ACT				0.094
Present	142 (74.3)	95 (70.9)	47 (82.5)	
Absent	49 (25.7)	39 (29.1)	10 (17.5)	
Hyponatremia				0.203
Present (<135 mmol/L)	8 (4.2)	4 (3.0)	4 (7.0)	
Absent (≥135 mmol/L)	183 (95.8)	130 (97.0)	53 (93.0)	
LDH level				0.443
>230 U/L	7 (3.7)	4 (3.0)	3 (5.3)	
≤230 U/L	184 (96.3)	130 (97.0)	54 (94.7)	

*, only cases with available data were reported. PNI, prognostic nutritional index; IQR, interquartile range; TNM, Tumor Node Metastasis; VPI, visceral pleural invasion; LVI, lymphovascular invasion; ACT, adjuvant chemotherapy; LDH, lactate dehydrogenase.
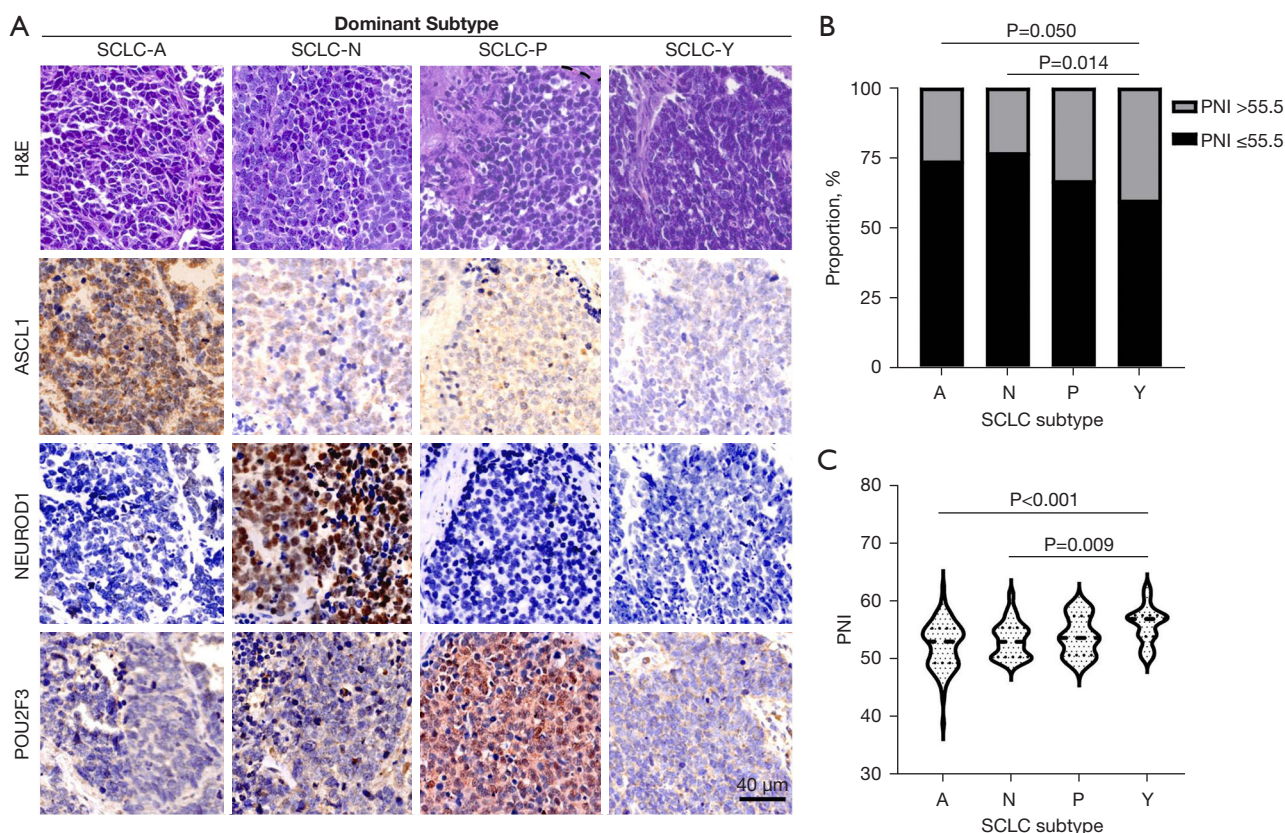
Figure 2 The correlation between PNI and SCLC dominant molecular subtypes in limited-stage SCLC cohort. (A) Representative images of IHC staining in primary SCLC tumors. Scale bars for all images, 40 μm. (B) The proportion of low- and high-PNI tumors in each dominant molecular subtype. Fisher's exact test was used. (C) Differential PNI level across four SCLC dominant molecular subtypes. Mann-Whitney test was used. PNI, prognostic nutritional index; SCLC, small-cell lung cancer; IHC, immunohistochemistry.

with different molecule subtype of SCLC. Using IHC in available TMAs and slides, 145 cases were successfully classified as one of these subtypes (*Figure 2A*).

As illustrated in *Figure 2B,2C*, the proportion of high-PNI score cases in SCLC-Y subtype was significantly higher than other subtypes (P=0.043). The mean value of PNI score in SCLC-Y was also significantly higher than that in other subtypes when considered as a continuous variable (P=0.003).

In summary, our results revealed a certain degree of intrinsic association of systemic immune and nutritional status with tumor molecular subtypes in SCLC. This demonstrates that tumor heterogeneity can be reflected in systemic immunity, and noninvasive PNI assessment can hint at the molecular subtyping of SCLC to a certain extent.

PNI, local immune contexture and immune risk system establishment

To evaluate the association between systemic immune environment and local TIME, we performed mIF and comprehensively analyzed the tumor-infiltrating lymphocytes (CD4$^+$ T-cell, CD8$^+$ T-cell, CD68$^+$ macrophage) and immune checkpoint expression (PD-1, PD-L1, CTLA-4) on TMA slides (*Figure 3A*).

Quantification using mIF demonstrated that CD4$^+$ T-cell (*Figure 3B*) and CD8$^+$ T-cell counts (*Figure 3C*) were relatively higher in high-PNI group when compared to low-PNI group (P=0.010 and P=0.002, respectively). However, the CD68$^+$ cell count and expression levels of immune checkpoints whether on tumor cells or lymphocytes had no association with PNI score (*Figure 3D-3G*). PD-1

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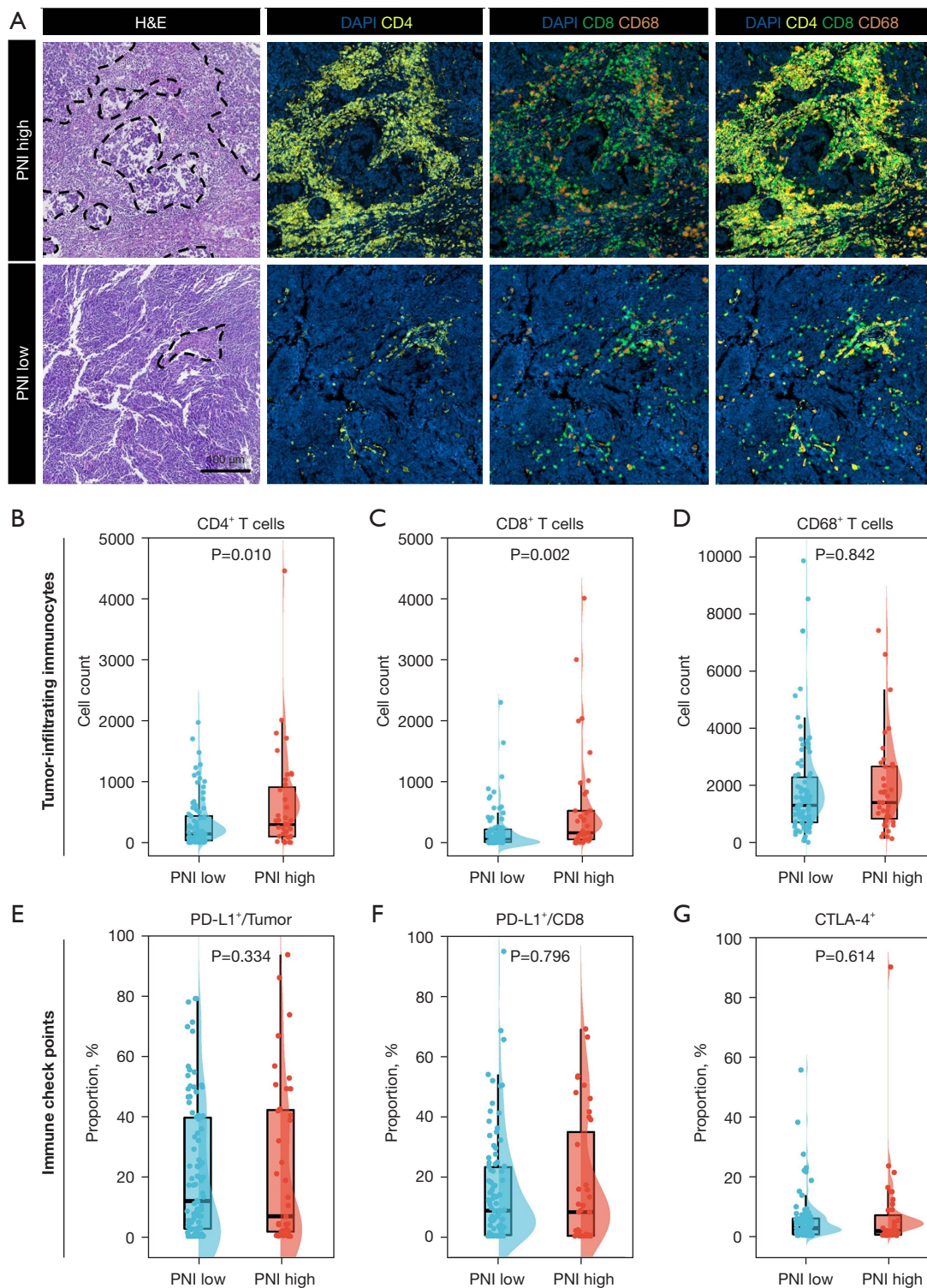
Figure 3 The correlation between PNI score and local immune contexture in limited-stage SCLC cohort. (A) Representative images of mIF staining in low- and high-PNI tumor tissues. The dashed line represents the tumor boundary. Scale bars for all images, 100 µm. (B-D) Quantification of counts of CD4^{+} T-cells, CD8^{+} T-cells, CD68^{+} cells in low- and high-PNI tumors. (E-G) Proportions of PD-L1^{+} tumor cells, PD-L1^{+}CD8^{+} cells, and CTLA-4^{+} cells in low- and high-PNI tumors. PNI, prognostic nutritional index; H&E, hematoxylin and eosin; PD-L1, programmed cell death 1 ligand 1; SCLC, small-cell lung cancer; mIF, multiplex immunofluorescence.

expression was not detected in most cases, which was therefore not included in subsequent analyses.

Further survival analyses of these local immune markers indicated different degrees of prognostic value (*Figure 4A*). For example, higher CD8^{+} T-cell count correlated significantly with better prognosis (P<0.001) while patients with higher percentage of PD-L1^{+} cell in CD8^{+} T-cell had a significantly worse prognosis (P=0.016). And several local immune markers were associated with patient pathological TNM stages (Figure S2A). Spearman's correlation analysis showed that there were positive correlations among these indicators (Figure S2B).

When the variables were correlated, the traditional Cox model was not applicable. Therefore, we adapted XGBoost machine learning algorithm for importance assessment and feature selection. This method can discover non-linear relationships by working with data of the first and second derivatives, as well as controlling the overfitting and overcomplexity of one predictive model. The importance feature map demonstrated CD8^{+} T-cell count as the first rank, followed by PD-L1 expression on CD8^{+} T-cell and CD4^{+} T-cell count (*Figure 4B*). To construct LISS for patients with SCLC, we combined these top three biomarkers and the obtained model formula was as follows: LISS = (0.1437 × CD8) − (0.1353 × PD-L1/CD8) + (0.1323 × CD4). We performed time-dependent ROC curve analysis in the calibration set to validate the model performance and it showed well-predictive ability [area under the curve (AUC) at 3-year: 0.76; 5-year: 0.78, *Figure 4C*]. We further used X-tile to determine the LISS cut-off point (0.13) (Table S1), and divided patients into a high-LISS group and a low-LISS group. As shown in the survival diagram by Kaplan-Meier analysis, patients with higher LISS score had significantly better OS (P<0.001, *Figure 4D*) and DFS (P<0.001, Figure S2C).

To establish a comprehensive immune risk system integrating systemic tumor immune macroenvironment and local TIME, we further reclassified SCLC patients based on PNI score and LISS. The survival curve showed that the stratification based on the PNI and LISS can better distinguish patients with poor OS (P<0.001, *Figure 4E*) and DFS (P<0.001, Figure S2D). Another multivariate Cox regression model was built (Table S2) and this immune risk system was independently and significantly correlated with OS in SCLC [HR: 1.429, 95% confidence interval (CI): 1.028–1.986, P=0.034]. To further confirm the prognostic role of this immune risk system in SCLC, we compared the predictive perf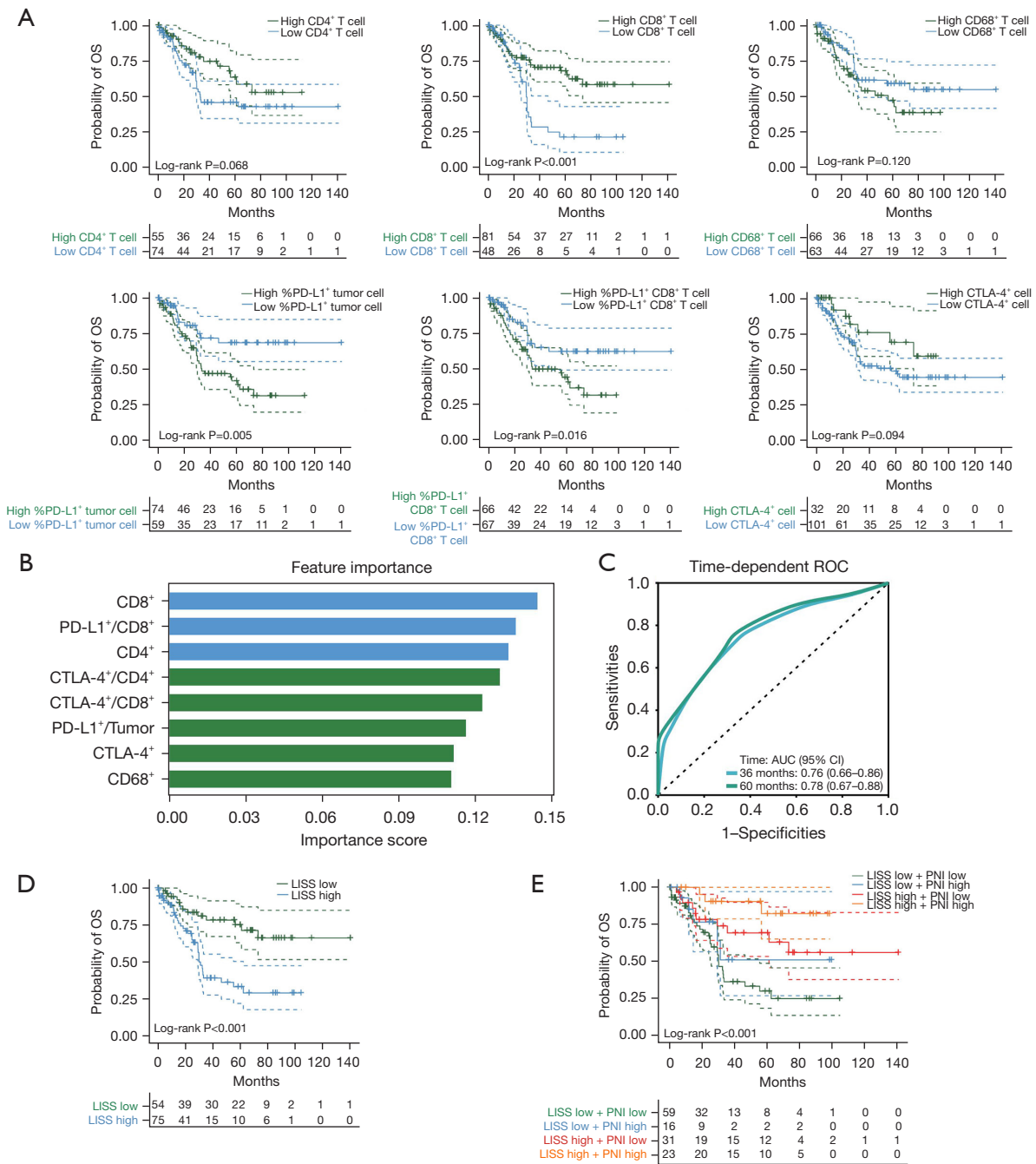ormance between integrated immune risk system, TNM stages and single indicators. Among different indicators, this integrated immune risk system obtained the best AUC value of 0.75, which performed better than either TNM stages or single immune markers (Figure S2E).

### High PNI score predicts PFS benefit from PD-L1 blockade

Local TIME has been well known for its key role on the responses to ICB therapies. However, the predictive value of systemic tumor immunity for immunotherapy was merely illustrated previously. It was recently reported that clinical benefit from immunotherapy is associated with tumor capacity of antigen presentation in SCLC. Therefore, we first assessed the correlations between antigen presentation machinery (APM) related gene (*HLA-A*, *HLA-B*, *HLA-C*, *B2M*, *TAP1*, *TAP2*) expressions and PNI and LISS. No correlation was observed between PNI score and APM related gene expressions (Figure S3A). However, high expression of APM related genes were associated with LISS-high groups (Figure S3B). We next attempt to validate the predictive power of the PNI score in an independent cohort of 91 patients with ES-SCLC received anti-PD-L1 immunotherapy (atezolizumab or durvalumab). Individual PFS of 91 patients with SCLC coupled with their clinical benefit, gender, age, smoking status, and immunotherapy was summarized in *Figure 5A*. There were no significant differences in age, sex, tobacco use or immunotherapy strategies between PNI-high and PNI-low groups (Table S3).

PNI-high tumors showed significantly more patients who experienced DCB, defined as best overall response of complete response (CR), partial response (PR), or SD (≥6 months) compared with PNI low tumors (P=0.003, *Figure 5B*). In addition, we also observed that the median PFS in PNI-high patients (11.8 months) was significantly higher than that in PNI-low patients (5.9 months) (P=0.012, *Figure 5C*). Last, we identified a patient with high-PNI SCLC. This patient was diagnosed with ES-SCLC and malignant pleural effusion in June 2021. The patient then began treatment with 1,200 mg of atezolizumab every 3 weeks combined with six cycles of carboplatin and etoposide and showed a durable PR for nine months, with no evidence of progression or active disease at the latest follow-up in March 2022 (*Figure 5D*).

In total, these data suggest that PNI-high tumors may portend survival benefit in patients with ICBs. The PNI score was not only a prognostic factor for early resected SCLCs but also a predictor for immunotherapy benefit in advanced tumors, which may better assist surgeons in identifying

Figure 4 The prognostic value of local tumor immunophenotypic signatures and the integrated immune risk system establishment in limited-stage SCLC cohort. (A) The prognostic value of immune cell infiltration and checkpoints expression for overall survival with two-sided log-rank P value. (B) The importance feature map of local immune biomarkers depicted by XGBoost machine learning model. (C) Validation of the LISS performance by time-dependent ROC curve analysis in the calibration set. (D) Survival analysis on the basis of the LISS score with two-sided log-rank P value. (E) Four groups were created according to the cut-off points of the PNI and the LISS, and the overall survival in SCLC patients of these four groups was evaluated by Kaplan-Meier curves. OS, overall survival; PD-L1, programmed cell death 1 ligand 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4; ROC, receiver operating characteristic; AUC, area under curve; CI, confidence interval; LISS, local immune score system; PNI, prognostic nutritional index; SCLC, small-cell lung cancer; XGBoost, eXtreme Gradient Boosting.
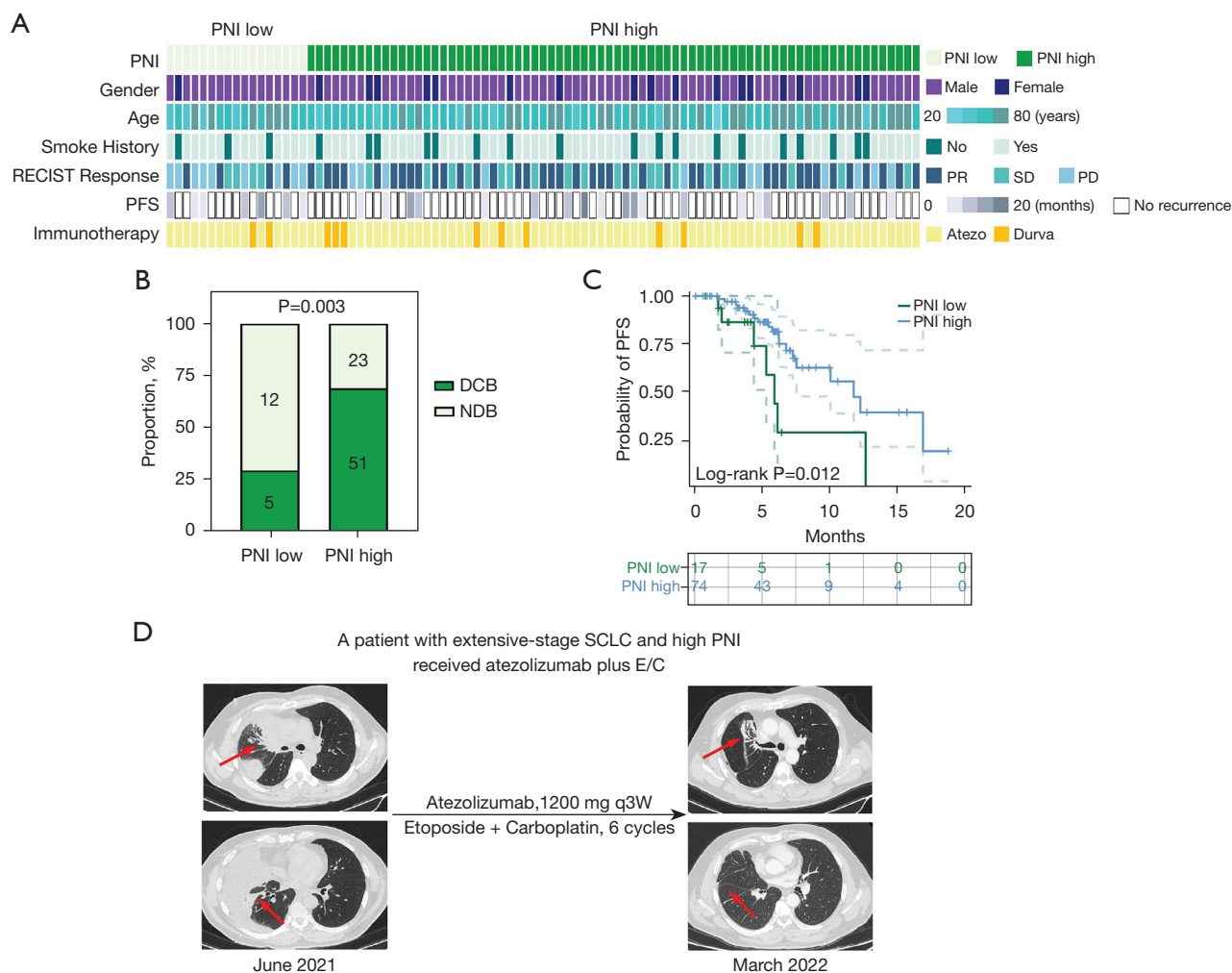
Figure 5 The predictive value of the PNI in PD-L1 blockades treated ES-SCLC cohort (N=91). (A) Clinical parameters of this study cohort were indicated in the heatmap. (B) The durable clinical benefit rates stratified by the PNI score with Chi-square test P value. The numbers in the boxes represented the patient number of each group. (C) Kaplan-Meier curve predicting the progression-free survival of SCLC patients stratified by the PNI score with two-sided log-rank P value. (D) A case with high-PNI ES-SCLC received anti-PD-L1 therapy and showed a durable partial response for 9 months. The red arrows represented the locations of tumors. PNI, prognostic nutritional index; PFS, progression-free survival; PR, partial response; SD, stable disease; PD, progressive disease; DCB, durable clinical benefit; NDB, no-durable benefit; SCLC, small-cell lung cancer; E/C, etoposide/carboplatin.

potential beneficiaries of ICB therapy. However, the lack of local tumor immune features of this immunotherapy cohort, mainly due to unavailable tumor tissues, hindered the construction of integrated immune system for efficacy prediction, which should be further studied.

Discussion

Despite the use of multimodal treatments, including surgery, postoperative chemotherapy and radiotherapy, and prophylactic cranial irradiation (PCI), the overall prognosis of SCLC patients is still unsatisfactory. In recent years, ICBs have significantly altered survival outcomes in many tumor types (20,21). The Food and Drug Administration (FDA) has approved PD-L1 inhibitors for use in more than 10 cancer types (22) including advanced SCLC. However, the number of SCLC patients who respond to the PD-L1 inhibitors is few and the survival benefit gained by

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responders is minimal (23-26). One explanation could be low or absent expression of checkpoint molecules and rare TILs infiltration in SCLC (6,27-29), and the application of ICBs in SCLC patients is still unfavorable. To date, there are no guidelines surrounding the administration of ICBs in SCLC and no predictive biomarkers have been established. In this study, therefore, we aimed to provide the comprehensive immune contexture by considering both systemic and local tumor immunity, and investigated their predictive value for prognosis and treatment efficacy.

We demonstrated that the PNI score was an outstanding, independent prognostic factor in patients with resectable SCLC, which was superior to other systemic immune cells and markers such as neutrophil and NLR. As an indicator for systemic immune competence and nutritional status, the PNI was initially designed to evaluate surgical complications and postoperative mortality in patients with gastrointestinal cancers (30,31). Since then, several studies have revealed that PNI is significantly associated with prognosis in various types of cancers including NSCLC (9,11,12,32). Nevertheless, the prognostic value of PNI in SCLC has not been reported, possibly hampered by small sample size. Our study first assessed PNI status in a much larger cohort of surgically resected patients with SCLC, and revealed that PNI-low was associated with an unfavorable clinical outcome. Moreover, considering that postoperative ACT could significantly impact the survival of limited-resected SCLC patients, the result that PNI remained as an independent prognostic factor regardless of ACT significantly increased its extensiveness of clinical applicability. Given that the PNI can be measured easily from clinical tests (10,33), it might be suitable as a routine prognostic marker for SCLC patients.

There are extensive interactions between systemic immune status (i.e., STIE) and TIME through capillary vessels and lymphatic drainage system in the tumor (34). Thus, we speculated that the PNI might influence patients prognosis via local tumor immunity. Okadome *et al.* (10) confirmed the relationship between high-PNI score and abundant CD8⁺ TILs in patients with esophageal cancer. Kitahara *et al.* (35) also showed that the absolute number of TILs positive for CD3, CD4 and CD8 in the high-PNI lung squamous cell carcinoma patients was significantly increased compared with the low-PNI group. These are similar to our results. Furthermore, we additionally revealed the complex interrelationship between STIE, TIME and tumor heterogeneity. We found that the median PNI score in SCLC-I subtype, a recently proposed infiltrated-

inflamed "hot" subtype (19), was higher than that in other subtypes. Given that the SCLC-I subtype was observed deriving greater benefit from ICB therapy, our observations are likely to have clinical implications. In fact, the predictive power of PNI in our PD-L1 inhibitors-treated SCLC cohort was confirmed. High-PNI patients achieved longer PFS and better overall response than low-PNI group, which may assist surgeons in identifying potential beneficiaries of ICB therapy.

Nevertheless, a single indicator is far from accurate prognostic assessment and appropriate regimens selection. To establish a simple and practical tool incorporating both systemic and local tumor immunity features, we first constructed a XGBoost-based local immune risk model which consisted of top three local immune markers, and further integrated it with the PNI to provide a personalized system for patients with SCLC to predict outcomes. It turned out that in comparison with single biomarkers as well as TNM stages, this integrated immune risk system exhibited the best prediction performance with the maximal AUC value (Figure S2), and might provide the possibility for patients with the same TNM stage to receive different treatments to improve their long-term prognosis.

Our study has several limitations. First, no validation cohort was included in this study. The two cohorts were distinct in tumor stages and treatments, and these findings must be treated with caution and validated in future multi-center studies with larger sample size. Second, although most patients draw blood tests within 7 days before the surgery or immunotherapy, the longest interval was up to 15 days and the patient's nutritional status could change significantly over days. Third, this was a retrospective, single-institution study. Thus, selection bias and time-trend bias are inevitable.

Conclusions

These findings show a strong relationship between systemic immune index and TIL intensity, patient survival and immunotherapy efficacy, suggesting its potential use as biomarker and shedding light on potential mechanistic relationships between the systemic response and local TME, pointing the way to further studies.

Acknowledgments

We thank the Department of Pathology, Fudan University Shanghai Cancer Center for providing the SCLC tissue

samples and related anonymous clinical data.

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-696/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-696/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-696/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-696/coif). C.D. reports funding from Shanghai Anticancer Association EYAS PROJECT (No. SACA-CY21B07). J.W. reports funding from the Shanghai Health Commission Collaborative Innovation Project (No. 2020CXJQ02). H.C. reports funding from the National Natural Science Foundation of China (No. 81930073) and Shanghai Technology Innovation Action Project (No. 20JC1417200). Y.Z. reports funding from Shanghai Rising-Star Program (No. 21QC1400600). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Review Board of FUSCC (IRB No. 2008223-9). Informed consents were waived because it was a retrospective study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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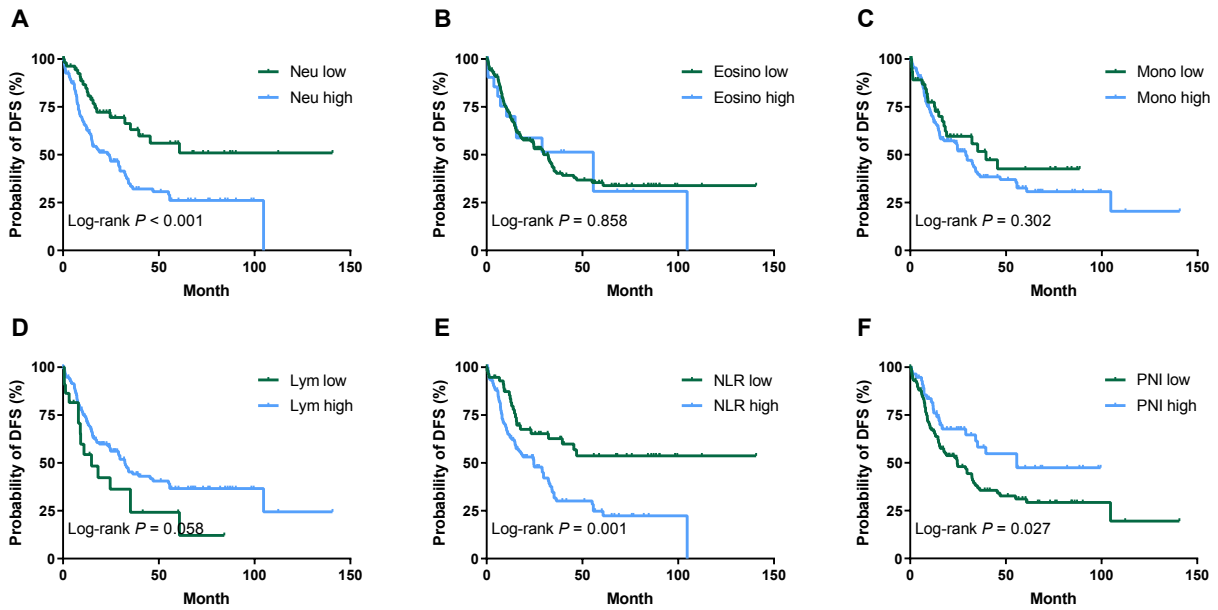
Figure S1 The disease-free survival of each systemic immune biomarkers in limited-stage SCLC cohort. (A) The Kaplan-Meier analysis of the neutrophil level. (B) The Kaplan-Meier analysis of the eosinophil level. (C) The Kaplan-Meier analysis of the monocyte level. (D) The Kaplan-Meier analysis of the lymphocyte level. (E) The Kaplan-Meier analysis of the NLR level. (F) The Kaplan-Meier analysis of the PNI level. DFS, disease-free survival; Neu, neutrophil; Eosino, eosinophil; Mono, monocyte; Lymph, lymphocyte; NLR, neutrophil-lymphocyte ratio; PNI, prognostic nutritional index; SCLC, small-cell lung cancer.
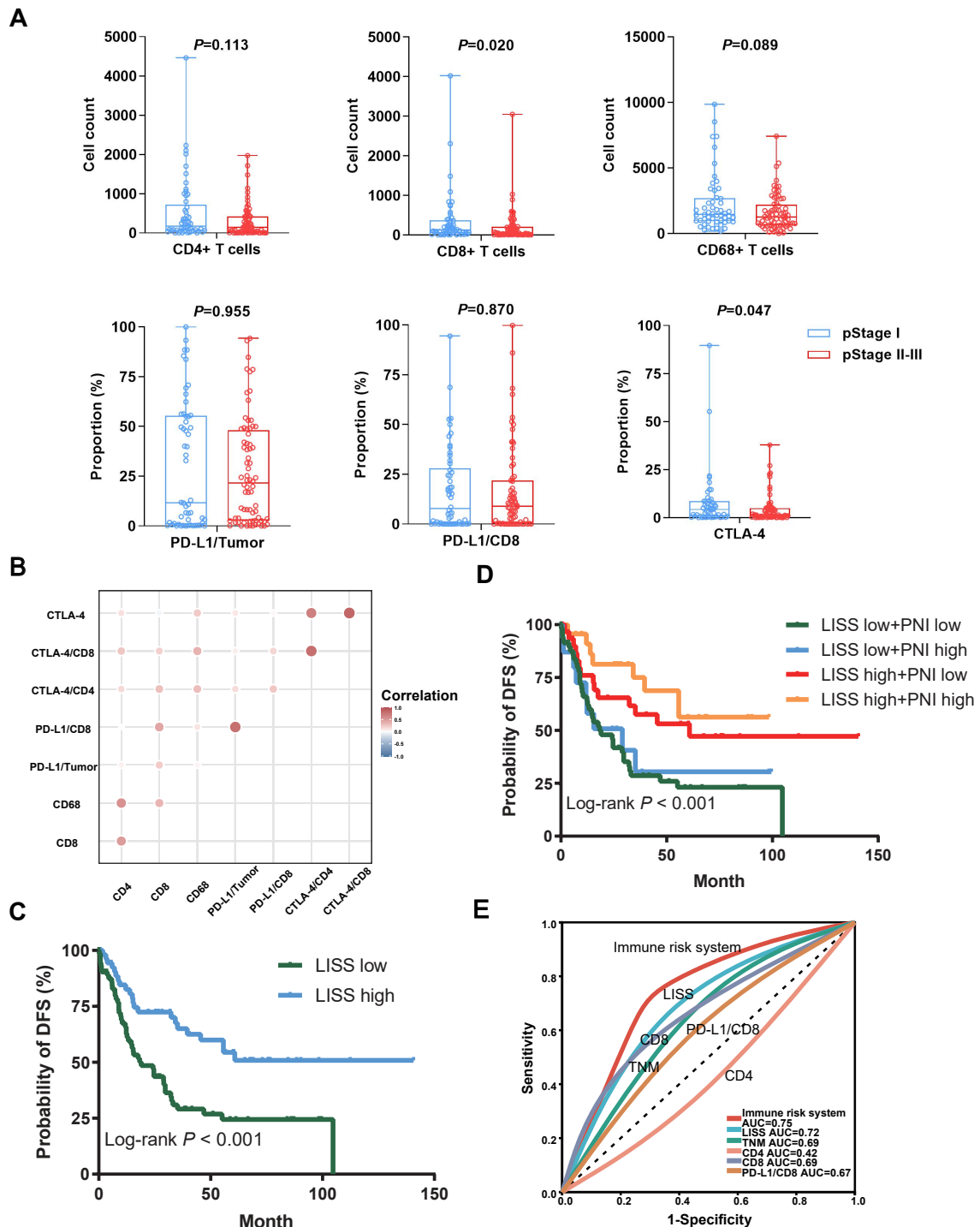
Figure S2 The prognostic performance of the integrated immune risk system in limited-stage SCLC cohort. (A) The correlation between several local immune markers and pathological TNM stages. (B) The correlation between several local immune markers. (C) The DFS stratified by LISS. (D) The DFS stratified by four groups based on PNI and LISS. (E) Validation of the integrated immune risk system performance by time-dependent ROC curve analysis. PD-L1, programmed cell death 1 ligand 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4; LISS, local immune score system; PNI, prognostic nutritional index; DFS, disease-free survival; TNM, Tumor Node Metastasis; AUC, area under curve; ROC, receiver operating characteristic; SCLC, small-cell lung cancer.
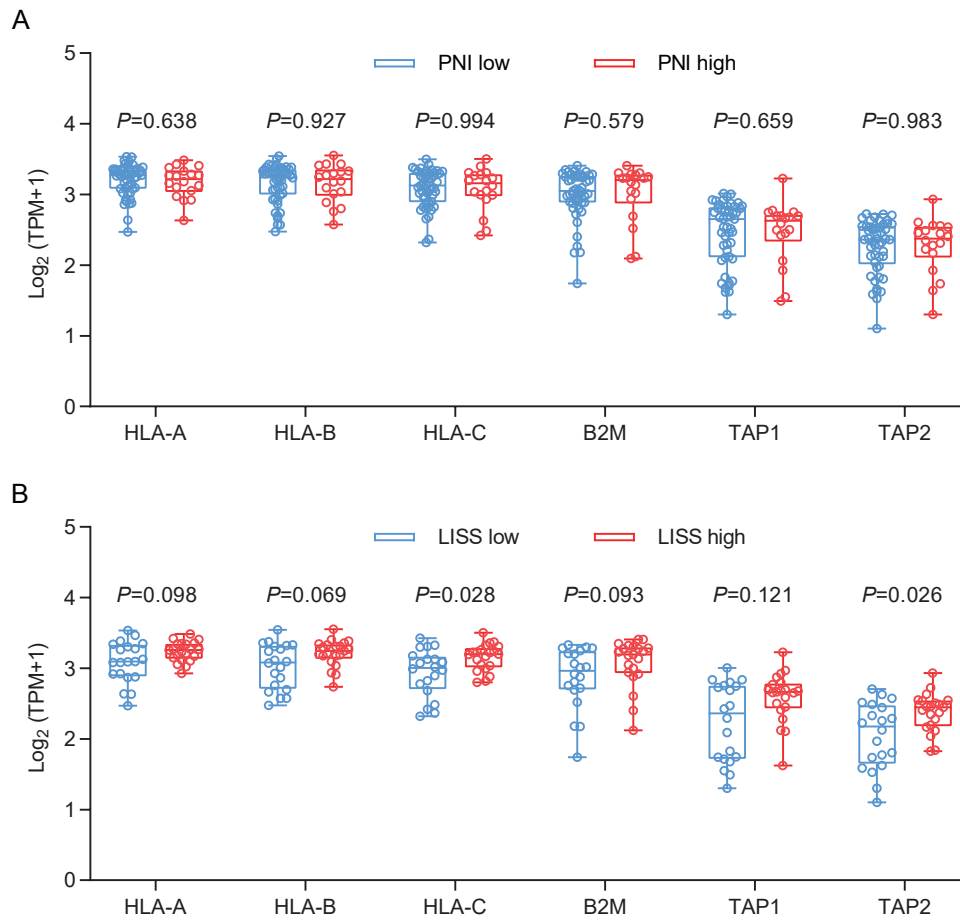
Figure S3 The correlations between antigen presentation machinery related gene expressions and PNI (A) and LISS (B) in limited-stage SCLC cohort. PNI, prognostic nutritional index; LISS, local immune score system; SCLC, small-cell lung cancer.

Table S1 The cutoff points of immune cells and markers

No.	Marker	Cutoff point
1	PNI (for OS)	55.5
2	PNI (for PFS)	56.5
3	NLR	1.8
4	Neutrophil count ($\times 10^9$/L)	3.1
5	Eosinophil count ($\times 10^9$/L)	0.2
6	Monocyte count ($\times 10^9$/L)	0.3
7	Lymphocyte count ($\times 10^9$/L)	1.1
8	CD4$^+$ T (cell/mm^2)	223.5
9	CD8$^+$ T (cell/mm^2)	40.7
10	CD68$^+$ (cell/mm^2)	1,275.8
11	PD-L1$^+$ tumor cell (%)	10.0
12	PD-L1$^+$ CD8$^+$ cell (%)	8.0
13	CTLA-4$^+$ cell (%)	5.8
14	CTLA-4$^+$ CD4$^+$ cell (%)	4.5
15	CTLA-4$^+$ CD8$^+$ cell (%)	9.6
16	LISS score	0.13

PNI, prognostic nutritional index; OS, overall survival; PFS, progression-free survival; NLR, neutrophil-lymphocyte ratio; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4; LISS, local immune score system.

Table S2 Multivariate Cox regression analysis for integrated immune risk system

Variables	Univariable		Multivariate (model 1)	
	HR (95% CI)	P	HR (95% CI)	P
Age (year) (≥60 vs. <60)	1.165 (0.713, 1.905)	0.542		
Gender (female vs. male)	0.256 (0.093, 0.706)	0.008	0.220 (0.052, 0.940)	0.041
Smoking history (ever vs. never)	2.840 (1.351, 5.967)	0.006	0.924 (0.275, 3.102)	0.899
Operative procedure (sublobar vs. lobectomy)	0.595 (0.187, 1.895)	0.380		
p-TNM stage (II-III vs. I)	2.378 (1.387, 4.078)	0.002	3.108 (1.462, 6.606)	0.003
LVI (presence vs. absence)	1.921 (1.129, 3.267)	0.016	1.436 (0.654, 3.150)	0.367
VPI (presence vs. absence)	2.463 (1.369, 4.431)	0.003	2.495 (1.137, 5.473)	0.023
ACT (presence vs. absence)	0.324 (0.197, 0.532)	<0.001	0.239 (0.114, 0.503)	<0.001
Immune risk system (low vs. high)	1.737 (1.320, 2.285)	<0.001	1.429 (1.028, 1.986)	0.034

HR, hazard ratio; CI, confidence interval; TNM, Tumor Node Metastasis; LVI, lymphovascular invasion; VPI, visceral pleural invasion; ACT, adjuvant chemotherapy.

Table S3 Relationship between clinicopathologic characteristics and PNI in immunotherapy cohort

Variables	Total Patients	PNI		P
	N=91	Low (≤47)	High (>47)	
		N=17	N=74	
Age (year)				0.051
Median	63	66	63	
IQR	57-66	63-72	60-64	
Gender				0.111
Male	73 (80.2)	16 (94.1)	57 (77.0)	
Female	18 (19.8)	1 (5.9)	17 (23.0)	
Smoking history				0.556
Never	21 (23.1)	3 (17.6)	18 (24.3)	
Ever	70 (76.9)	14 (82.4)	56 (75.7)	
Immunotherapy				0.848
Atezolizumab	79 (86.8)	15 (88.2)	64 (86.5)	
Durvalumab	12 (13.2)	2 (11.8)	10 (13.5)	

PNI, prognostic nutritional index; IQR, interquartile range.