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RESEARCH

Tertiary lymphoid structures gene signature predicts response to immunotherapy plus chemotherapy in advanced non-small cell lung cancer

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Abstract

Background Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality, with chemoimmunotherapy (CIT) as the first-line standard for advanced NSCLC without driver mutations. However, predictive biomarkers for CIT response are limited. Tertiary lymphoid structures (TLS) play a critical role in antitumor immunity and may serve as potential biomarkers. This study aimed to screen and validate a TLS-derived gene signature to predict responses to first-line CIT in advanced NSCLC.

Methods Data from three randomized trials (ORIENT-11, OAK, POPLAR) and The Cancer Genome Atlas—NSCLC were analyzed. TLS scores were computed via ssGSEA based on 17 TLS-related gene signatures. Patients were stratified into TLS-high and TLS-low groups. The predictive value was assessed by survival analysis, nomograms, and receiver operating characteristic curve. Correlations with programmed cell death 1 ligand (PD-L1) and ImmuneScore were evaluated.

Results TLS signature 3 was identified as a predictive biomarker. In ORIENT-11, high signature 3 scores correlated with longer progression-free survival (PFS) (9.92 vs 6.77 months, $p = 0.001$) and overall survival (OS) (not reached vs 17.60 months, $p < 0.001$). Multivariate analysis confirmed signature 3 as an independent predictor for both outcomes (PFS HR = 2.14, $p = 0.006$; OS HR = 2.24, $p = 0.002$). A nomogram integrating signature 3 and clinicopathologic factors showed strong discriminative power. Signature 3 also correlated with PD-L1 expression and 'hot' immune phenotypes, enhancing prediction in PD-L1-negative subsets.

Conclusions TLS signature 3 predicts CIT response independently of PD-L1, improving outcomes in PD-L1-negative patients and complementing PD-L1 testing. Integration into clinical practice may refine treatment decisions, warranting further mechanistic and clinical validation.

Keywords Non-small cell lung cancer · Platinum-based chemotherapy combined with immunotherapy · First-line · Tertiary lymphoid structures

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Introduction

Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality worldwide, with advanced-stage disease accounting for most diagnoses and presenting significant therapeutic challenges [1]. Immune checkpoint inhibitors (ICIs), particularly those targeting the programmed cell death 1 (PD-1)/programmed cell death 1 ligand (PD-L1), have revolutionized treatment paradigms. Although the combination of ICIs with platinum-based chemotherapy (chemoimmunotherapy, CIT) is the first-line standard for NSCLC patients without driver mutations [2],

many patients derive limited benefit, highlighting the need for predictive biomarkers [3].

Current biomarkers such as PD-L1 expression and tumor mutational burden (TMB) exhibit conservative predictive utility, reflecting the complexity of tumor-immune interactions [4]. Tertiary lymphoid structures (TLS), ectopic lymphoid aggregates that develop in non-lymphoid tissues, have emerged as promising indicators of antitumor immunity [5]. In NSCLC, the presence of TLS has been associated with improved survival and enhanced responses to ICIs, likely attributable to their role in coordinating tumor-specific T-cell and B-cell responses [6]. Nevertheless, the predictive value of TLS in the context of CIT remains insufficiently explored.

Several TLS-related gene signatures have been proposed across various cancer types, reflecting either TLS neogenesis or function [7]. A robust TLS gene signature could serve as a functional biomarker to guide personalized treatment selection. In this study, we aim to screen and validate a TLS-derived gene signature for predicting clinical responses to first-line CIT in advanced NSCLC.

Methods

Enrolled study

Our analysis incorporated three independent randomized controlled trials (RCTs): the internal ORIENT-11 study (NCT03607539), along with the OAK (NCT02008227) and POPLAR (NCT01903993) trials, supplemented by The Cancer Genome Atlas (TCGA)—Lung adenocarcinoma cohort. ORIENT-11 was a multicenter, randomized, double-blind, phase 3 trial conducted across 47 Chinese centers, enrolling treatment-naïve patients with locally advanced or metastatic non-squamous NSCLC lacking sensitizing epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) rearrangements. Participants were randomized (2:1) to receive either sintilimab plus pemetrexed-platinum chemotherapy or placebo with chemotherapy.

POPLAR (phase II) and OAK (phase III) were both global, multicenter trials enrolling NSCLC patients progressing after platinum-based chemotherapy. Participants underwent 1:1 randomization to receive either atezolizumab or docetaxel. Detailed methodologies for the three RCTs have been previously published [8–10].

Data source

From 397 intent-to-treat (ITT) patients in ORIENT-11, we analyzed 171 with available PD-L1 tumor proportion score (TPS) and RNA sequencing data (113 in the combination therapy group and 58 in the chemotherapy-only group).

Internal RNA data from ORIENT-11 trial: RNA extraction from FFPE tumor samples used RNeasy FFPE Kit (Qiagen) and rRNA depletion with NEBNext kit. Complementary DNA (cDNA) libraries were prepared using NEBNext Ultra II Kit, involving divalent cation fragmentation and strand-specific library construction. Sequencing on NovaSeq 6000 followed by FastQC quality check, Trimmomatic filtering and STAR mapping to GRCh38. Samples required a $\geq 30\%$ unique mapping rate and $\leq 90\%$ duplication rate. Gene expression was quantified via HTSeq-count and TPM normalization based on raw read counts.

The PD-L1 TPS, representing the proportion of tumor cells with membrane staining, was evaluated on baseline tumor tissue via the 22C3 pharmDx assay.

External RNA data from OAK and POPLAR trials: data were formally requested from Genentech/Roche at the European Genome-phenome Archive.

Definition of TLS levels

Seventeen tertiary lymphoid structures gene signatures as previously reported [11–25] were included in the analysis. Detailed gene signatures were provided in Supplementary Table 1. The TLS scores were calculated using the single-sample gene set enrichment analysis (ssGSEA) via the GSVA package. Patients were categorized into TLS-high and TLS-low groups based on the median value of the TLS scores.

TLS gene signature screening and validation

We utilized RNA-seq data from the OAK and POPLAR trials for screening, with the process shown in Fig. 1. Multiple testing correction was performed using the Benjamini & Hochberg (BH) method to adjust the *P* values. Candidate signatures from OAK/POPLAR screening required: 1) Predictive capacity for immunotherapy response ($p.adjust < 0.05$); 2) No prognostic value in chemotherapy-only arm ($p > 0.05$). ORIENT-11 (CIT cohort) tested the screened signatures for CIT-specific prediction.

Definition of tumor immune infiltration levels

The scores for tumor purity, the level of stromal cells present, and the infiltration level of immune cells in tumor tissues (ImmuneScore) were calculated using the ESTIMATE package. Patients were classified as hot (above the median value) or cold tumors (below the median value) group based on the median ImmuneScore value.

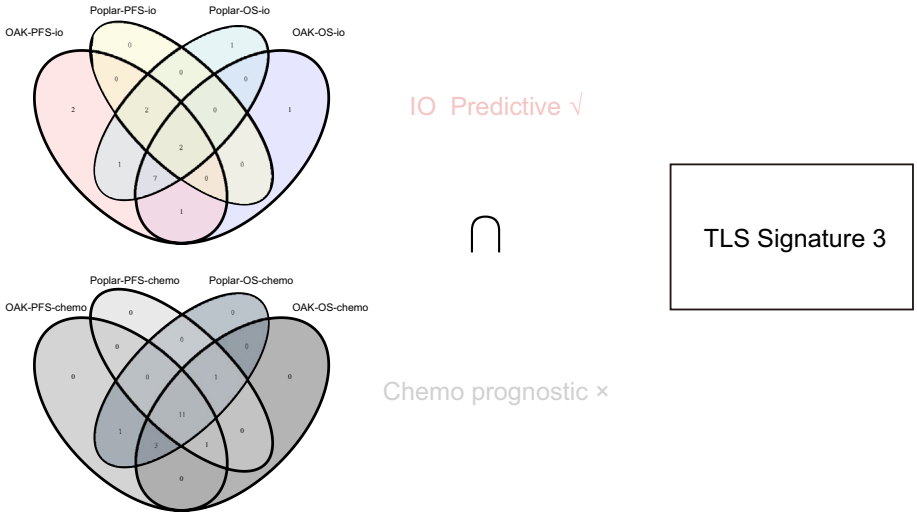


Fig. 1 Schematic diagram of the screening process. OS, overall survival; PFS, progression-free survival; IO, immunotherapy; chemo, chemotherapy

Statistical analysis

Categorical variables were presented as counts (percentages), and continuous variables as median ± range. Differences in continuous variables were assessed using the Wilcoxon Rank Sum or Kruskal–Wallis test, while associations between categorical variables were evaluated with Fisher's exact test.

Kaplan–Meier survival curves were plotted and median survival was estimated. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using the Cox regression model to compare overall survival (OS) and progression-free survival (PFS) between groups, with significance determined by the log-rank test.

Univariable analyses initially explored the impact of baseline clinicopathological factors (age, sex, smoking status, stage, brain metastasis, Eastern Cooperative Oncology Group performance status [ECOG PS], PD-L1 expression, and TLS signature 3). Variables with $p < 0.05$ were included in a backword-stepwise multivariable Cox analysis to adjust for confounding.

Nomograms incorporating the TLS gene signature and other clinicopathological predictors were constructed to forecast 1- and 2-year OS and 1-year PFS probabilities using the R "rms" and regplot packages. Discrimination ability of nomograms model was evaluated by receiver operating characteristic (ROC) curves using the area under the curve (AUC) value. All tests were two-sided, with significance set at $p < 0.05$. Analyses were conducted in R (version 4.2.2).

Results

TLS gene signature screening

For patients receiving immunotherapy in the OAK cohort, TLS signature 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17 predicted PFS, while TLS signature 3, 4, 6, 8, 9, 10, 11, 12, 13, 14 and 15 predicted OS. Similarly, in the POPLAR cohort, TLS signature 2, 3, 4 and 17 predicted PFS, while TLS signature 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14 and 17 predicted OS (Supplementary Table 2).

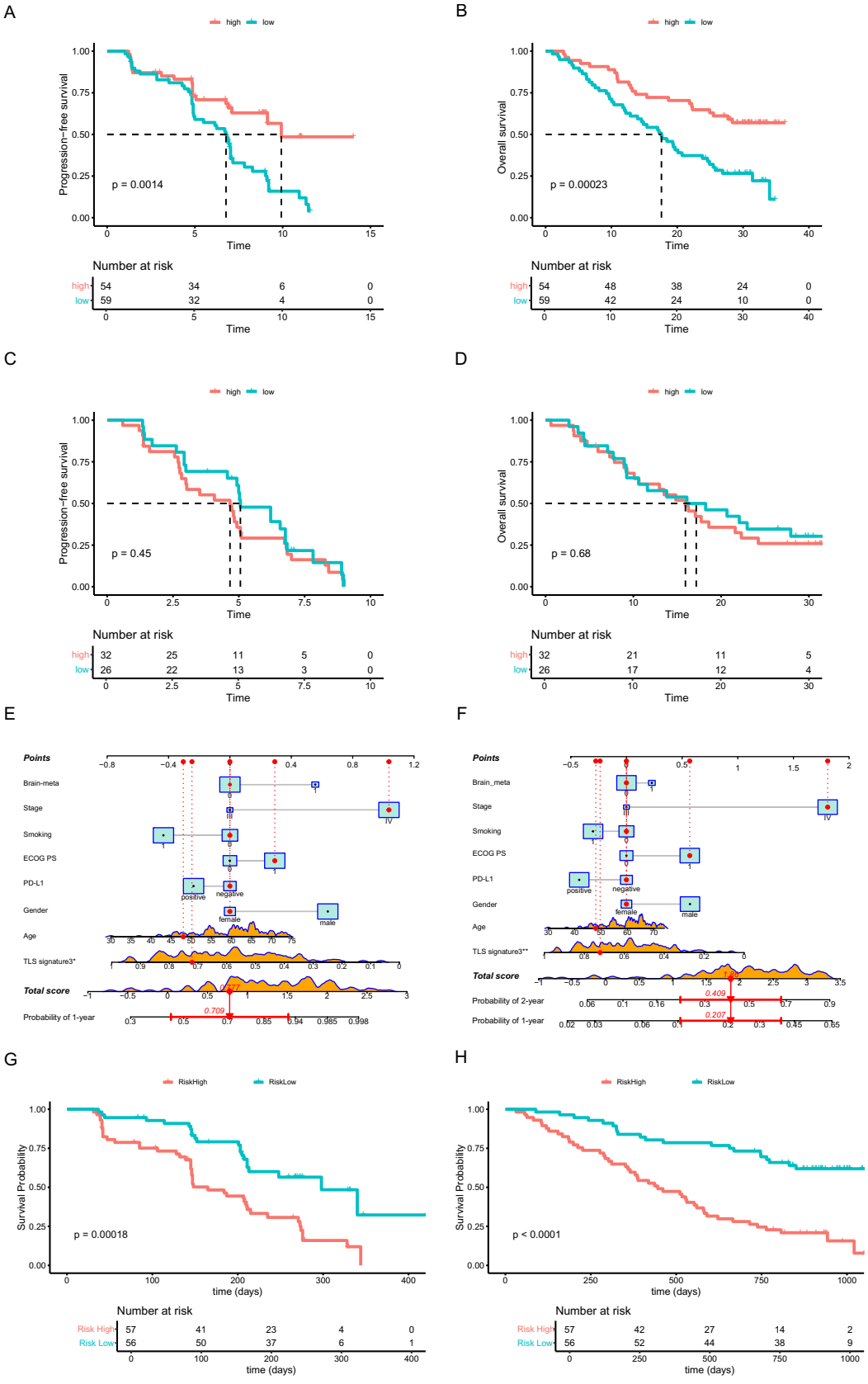
Following intersection analysis of the four groups (OAK-PFS, OAK-OS, POPLAR-PFS, POPLAR-OS), TLS signatures 3 and 4 were found to demonstrate predictive power for immunotherapy monotherapy in both cohorts. TLS signature 4 was subsequently excluded due to its prognostic significance in the chemotherapy cohort of the POPLAR trial.

Finally, after screening (Fig. 1), TLS signatures 3, which exhibited superior predictive capacity, was selected for subsequent analysis.

Correlations between TLS gene signatures and treatment outcomes

We further explored the correlations between aforementioned TLS gene signature and patient outcomes in ORIENT11 cohort. Stratified by TLS gene signature 3, the TLS-high group exhibited prolonged PFS (median PFS [mPFS] 9.920 [95% CI NR–NR] vs 6.770 [95% CI 5.834–7.706], $p = 0.001$) and OS (mOS NR vs 17.600, $p < 0.001$) compared with the TLS-low group (Fig. 2A, B). Baseline clinicopathologic and genomic characteristics of patients stratified by TLS gene signature 3 are detailed in Table 1. Multivariate cox analysis identified the TLS signature 3 as a significant independent prognostic factor for PFS (HR = 2.14, 95% CI 1.24–3.69, $p = 0.006$, Table 2) and OS (HR = 2.24, 95% CI 1.34–3.74, $p = 0.002$, Table 3).

To establish a prognostic model for patients undergoing CIT, we constructed a multivariate Cox-based nomogram incorporating eight clinicopathological parameters: age, gender, stage, ECOG PS, PD-L1 expression, smoking history, brain metastasis status, and TLS gene signature 3. The nomogram provides quantitative risk stratification for


A
high
low
Progression−free survival
p = 0.0014
Time
Number at risk
high 54 34 6 0
low 59 32 4 0
B
Overall survival
p = 0.00023
Number at risk
high 54 48 38 24 0
low 59 42 24 10 0
C
p = 0.45
Number at risk
high 32 25 11 5 0
low 26 22 13 3 0
D
p = 0.68
Number at risk
high 32 21 11 5
low 26 17 12 4
E
Points
Brain-meta
Stage
Smoking
ECOG PS
PD-L1
Gender
Age
TLS signature3*
Total score
Probability of 1-year
F
Brain_meta
TLS signature3**
Probability of 2-year
G
RiskHigh
RiskLow
Survival Probability
p = 0.00018
time (days)
Number at risk
Risk High 57 41 23 4 0
Risk Low 56 50 37 6 1
H
p < 0.0001
Risk High 57 42 27 14 2
Risk Low 56 52 44 38 9

◂**Fig. 2** Comparative survival analysis and nomogram by TLS signature 3: chemoimmunotherapy versus chemotherapy in ORIENT-11 trial. **A**, **B** Sintilimab-chemotherapy combination outcomes. **C**, **D** Chemotherapy-alone outcomes. A TLS nomogram was constructed to predict the probability of 1-year progression-free survival using Kaplan–Meier (**E**, **G**). A TLS nomogram was established to predict the probability of 1-year and 2-year overall survival using Kaplan–Meier. **F**, **H** ECOG PS, Eastern Cooperative Oncology Group Performance Status; PD-L1, programmed death-ligand 1; TLS, tertiary lymphoid structure

1-/2-year OS and 1-year PFS through a point-scale scoring system, where each variable category is assigned, weighted points reflecting its prognostic contribution (Fig. 2E, F). Total risk scores were calculated by summing individual parameter scores for each patient. Risk stratification revealed that high-risk patients had significantly inferior OS compared to low-risk counterparts (mOS 14.87 vs NR months; $p < 0.001$; Fig. 2H). The ROC analysis demonstrated robust discriminative capacity of the OS nomogram, with AUC values of 0.79 (Supplementary Fig. 1). Similarly, the PFS prediction model showed clinically meaningful stratification, where low-risk patients achieved median PFS of 9.93 months versus 5.50 months in the high-risk group ($p < 0.001$, Fig. 2G). The nomogram maintained satisfactory discrimination power with 1-year PFS (AUC = 0.75, Supplementary Fig. 1).

TLS gene signature 3 and PD-L1 expression

Subsequent analysis evaluated the correlation between PD-L1 expression and TLS gene signature 3 in the CIT cohort. Of 82 patients (72.6%) with PD-L1 TPS ≥ 1%, a higher proportion exhibited a high TLS gene signature score (85.2%) compared to those with a low score (61.0%). A weak but significant positive correlation was observed between TLS gene signature 3 and PD-L1 TPS (Pearson coefficient = 0.271, $p = 0.004$, Supplementary Table 3).

In both PD-L1-positive (TPS ≥ 1%) and PD-L1-negative (TPS < 1%) patients, objective response rates (ORR) were higher in the high TLS score group (78.3% and 62.5%, respectively) versus the low TLS score group (66.7% and 47.8%, $p = 0.060$; Supplementary Table 4). For PD-L1-negative patients, median PFS and OS were not reached (95% CI NR–NR) in the high TLS group, compared to 6.24 months (95% CI 4.35–8.13) and 12.17 months (95% CI 4.60–19.75) in the low TLS group. Among PD-L1-positive patients, the high TLS group exhibited a median PFS of 9.92 months (95% CI 6.27–13.57) and unreached OS, whereas the low TLS group had a median PFS of 6.90 months (95% CI 6.59–7.21) and OS of 19.33 months (95% CI 15.80–22.86, Fig. 3A, B).

Similar results were observed for the CD274 mRNA expression. High CD274 mRNA expression was more prevalent in tumors with a high TLS score (72.2%) versus a low score (20.3%). A moderate positive correlation was observed between TLS gene signature 3 and CD274 expression (Pearson coefficient = 0.521, $p < 0.001$; Supplementary Table 3). ORR was higher in the high TLS group for both high (82.1% and 75.0%) and low (60.0% and 55.3%) CD274 expression subgroups ($p = 0.060$, Supplementary Table 4).

Among patients with high CD274 expression, the high TLS group had a median PFS of 9.92 months (95% CI 6.54–13.30) and unreached OS, versus 4.99 months (95%

Table 1 Baseline characteristics on the basis of the TLS signature 3

Characteristics		Total cohort	Combined therapy		*P* value	Chemotherapy		*P* value
			TLS high	TLS low		TLS high	TLS low	
No. of patients		171	54	59	–	32	26	–
Age, years	Median (range)	61 (44)	61 (32)	61 (44)	0.905	62 (33)	58 (33)	0.056
Gender	Male	132 (77.2)	41 (75.9)	46 (78.0)	0.797	27 (84.4)	18 (69.2)	0.169
	Female	39 (22.8)	13 (24.1)	13 (22.0)		5 (15.6)	8 (30.8)	
ECOG PS	0	56 (32.7)	18 (33.3)	17 (28.8)	0.604	13 (40.6)	8 (30.8)	0.437
	1	115 (67.3)	36 (66.7)	42 (71.2)		19 (59.4)	18 (69.2)	
Smoking history	No	60 (35.1)	20 (37.0)	22 (37.3)	0.978	9 (28.1)	9 (34.6)	0.595
	Yes	111 (64.9)	34 (63.0)	37 (62.7)		23 (71.9)	17 (65.4)	
PD-L1 level	Positive	121 (70.8)	46 (85.2)	36 (61.0)	0.004	26 (81.3)	13 (50.0)	0.012
	Negative	50 (29.2)	8 (14.8)	23 (39.0)		6 (18.8)	13 (50.0)	
Stage	III	17 (9.9)	8 (14.8)	1 (1.7)	0.013	4 (12.5)	4 (15.4)	0.752
	IV	154 (90.1)	46 (85.2)	58 (98.3)		28 (87.5)	22 (84.6)	
Brain Metastasis	No	153 (89.5)	50 (92.6)	51 (86.4)	0.289	28 (87.5)	24 (92.3)	0.545
	Yes	18 (10.5)	4 (7.4)	8 (13.6)		4 (12.5)	2 (7.7)	

Combined therapy: sintilimab combined with platinum-based chemotherapy

TLS tertiary lymphoid structures; *ECOG PS* Eastern cooperative oncology group performance status; *PD-L1* programmed death-ligand 1

Table 2 Univariate and multivariate Cox regression analysis regarding progression free survival in chemoimmunotherapy cohort

Variables			Univariate HR (95% CI, *P* value)	Multivariate HR (95% CI, *P* value)
TLS signature 3	High	54 (47.8)	–	–
	Low	59 (52.2)	2.30 (1.36–3.91, $p=0.002$)	2.14 (1.24–3.69, $p=0.006$)
TLS subtype 1	$TLS^{low}TME^{cold}$	53 (46.9)	–	–
	$TLS^{low}TME^{hot}$	6 (5.3)	0.67 (0.24–1.88, $p=0.444$)	
	$TLS^{high}TME^{cold}$	6 (5.3)	0.86 (0.31–2.42, $p=0.778$)	
	$TLS^{high}TME^{hot}$	48 (42.5)	0.37 (0.21–0.66, $p=0.001$)	
TLS subtype 2	$TLS^{low}PD\text{-}L1^{negative}$	23 (20.4)	–	
	$TLS^{low}PD\text{-}L1^{positive}$	36 (31.9)	0.84 (0.46–1.52, $p=0.554$)	
	$TLS^{high}PD\text{-}L1^{negative}$	8 (7.1)	0.20 (0.05–0.86, $p=0.030$)	
	$TLS^{high}PD\text{-}L1^{positive}$	46 (40.7)	0.44 (0.23–0.84, $p=0.013$)	
TLS subtype 3	$TLS^{low}CD274^{low}$	47 (41.6)	–	
	$TLS^{low}CD274^{high}$	12 (10.6)	0.99 (0.49–2.00, $p=0.973$)	
	$TLS^{high}CD274^{low}$	15 (13.3)	0.47 (0.20–1.11, $p=0.085$)	
	$TLS^{high}CD274^{high}$	39 (34.5)	0.42 (0.23–0.78, $p=0.006$)	
Age	Median (Range)	61 (44.0)	1.03 (1.00–1.06, $p=0.043$)	1.03 (1.00–1.06, $p=0.039$)
Gender	Female	26 (23.0)	–	
	Male	87 (77.0)	1.67 (0.89–3.09, $p=0.110$)	
PD-L1 level	Negative	31 (27.4)	–	
	Positive	82 (72.6)	0.85 (0.50–1.45, $p=0.562$)	
ECOG PS	1	78 (69.0)	–	
	0	35 (31.0)	0.66 (0.38–1.15, $p=0.146$)	
Smoking History	No	42 (37.2)	–	
	Yes	71 (62.8)	1.10 (0.66–1.84, $p=0.720$)	
Stage	III	9 (8.0)	–	
	IV	104 (92.0)	4.38 (1.07–17.93, $p=0.040$)	2.76 (0.65–11.69, $p=0.168$)
Brain-meta	No	101 (89.4)	–	
	Yes	12 (10.6)	2.14 (1.05–4.35, $p=0.036$)	1.80 (0.88–3.67, $p=0.109$)

TLS tertiary lymphoid structures; *ECOG PS* Eastern cooperative oncology group performance status; *HR* hazard ratio; *PD-L1* programmed death-ligand 1; *TME* tumor microenvironment

CI 1.87–8.11) and 19.40 months (95% CI 10.23–28.57) in the low TLS group. For low CD274 expression, median PFS and OS were not reached in the high TLS group, compared to 6.83 months (95% CI 5.77–7.89) and 14.87 months (95% CI 8.52–21.22) in the low TLS group (Fig. 3C, D).

TLS gene signature 3 and tumor immune infiltration

Correlation analysis between tumor immune infiltration (assessed by ImmuneScore) and TLS gene signature 3 revealed that hot tumors (high ImmuneScore) were more frequent in the high TLS group (88.9%) than in the low TLS group (10.2%).

TLS gene signature 3 correlated strongly with tumor microenvironment (TME) in ORIENT 11 (Pearson coefficient = 0.787, $p<0.001$; Supplementary Table 3) and in TCGA lung adenocarcinoma (odds ratio = 49.22, $p<0.001$; Supplementary Fig. 2). In hot tumors, ORR was higher in the high TLS group (81.3% vs 50.0%), whereas in cold tumors, it was 33.3% versus 60.4% ($p=0.060$, Supplementary Table 4).

For hot tumor patients, median PFS and OS were not reached in high TLS group, versus 7.00 months (95% CI 5.71–8.29) and 24.90 months (95% CI NR–NR) in low group. In cold tumor patients, the median PFS and OS were, 3.09 (95% CI 0.00–10.532) and 12.67 (95% CI 3.51–21.83) months in high TLS gene signature score group versus 6.70 (95% CI 4.56–8.84) and 17.23 (95% CI 13.30–21.16) months in low TLS gene signature score group (Fig. 3E, F).

Discussion

In this study, we screened and validated a TLS-derived gene signature (TLS signature 3) as a predictive biomarker for response to first-line CIT in advanced NSCLC. Our findings

Table 3 Univariate and multivariate Cox regression analysis regarding overall survival in chemoimmunotherapy cohort

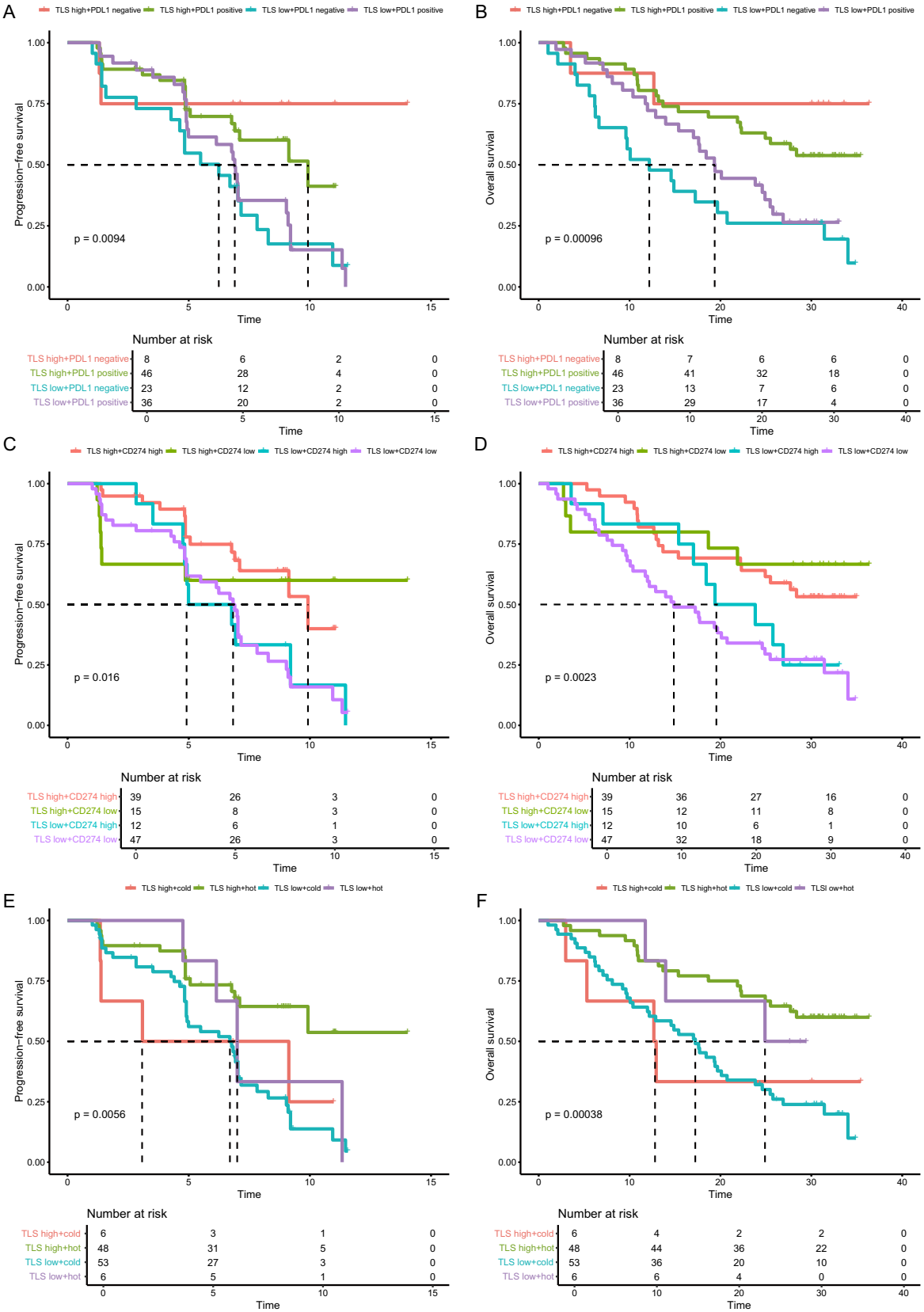
Variables			Univariate HR (95% CI, *P* value)	Multivariate HR (95% CI, *P* value)
TLS signature 3	High	54 (47.8)	–	–
	Low	59 (52.2)	2.51 (1.51–4.17, $p<0.001$)	2.24 (1.34–3.74, $p=0.002$)
TLS subtype 1	$TLS^{low}TME^{cold}$	53 (46.9)	–	
	$TLS^{low}TME^{hot}$	6 (5.3)	0.45 (0.14–1.47, $p=0.187$)	
	$TLS^{high}TME^{cold}$	6 (5.3)	0.87 (0.31–2.44, $p=0.791$)	
	$TLS^{high}TME^{hot}$	48 (42.5)	0.33 (0.19–0.57, $p<0.001$)	
TLS subtype 2	$TLS^{low}PD\text{-}L1^{negative}$	23 (20.4)	–	
	$TLS^{low}PD\text{-}L1^{positive}$	36 (31.9)	0.69 (0.38–1.26, $p=0.226$)	
	$TLS^{high}PD\text{-}L1^{negative}$	8 (7.1)	0.17 (0.04–0.75, $p=0.019$)	
	$TLS^{high}PD\text{-}L1^{positive}$	46 (40.7)	0.34 (0.18–0.64, $p=0.001$)	
TLS subtype 3	$TLS^{low}CD274^{low}$	47 (41.6)	–	
	$TLS^{low}CD274^{high}$	12 (10.6)	0.78 (0.38–1.63, $p=0.511$)	
	$TLS^{high}CD274^{low}$	15 (13.3)	0.29 (0.11–0.74, $p=0.010$)	
	$TLS^{high}CD274^{high}$	39 (34.5)	0.41 (0.23–0.73, $p=0.002$)	
Age	Median (Range)	61 (44.0)	1.03 (1.00–1.06, $p=0.031$)	1.03 (1.00–1.06, $p=0.059$)
Gender	Female	26 (23.0)	–	
	Male	87 (77.0)	1.48 (0.81–2.67, $p=0.200$)	
PD-L1 level	Negative	31 (27.4)	–	
	Positive	82 (72.6)	0.69 (0.41–1.16, $p=0.161$)	
ECOG PS	1	78 (69.0)	–	–
	0	35 (31.0)	0.46 (0.26–0.83, $p=0.010$)	0.59 (0.32–1.076, $p=0.082$)
Smoking history	No	42 (37.2)	–	
	Yes	71 (62.8)	1.07 (0.65–1.75, $p=0.791$)	
Stage	III	9 (8.0)	–	–
	IV	104 (92.0)	8.92 (1.24–64.36, $p=0.030$)	5.45 (0.74–40.18, $p=0.097$)
Brain-meta	No	101 (89.4)	–	
	Yes	12 (10.6)	1.67 (0.85–3.28, $p=0.137$)	

TLS Tertiary lymphoid structures; *ECOG PS* Eastern cooperative oncology group; *HR* hazard ratio; *PD-L1* programmed death-ligand 1; *TME* tumor microenvironment

are consistent with prior evidence highlighting TLS as dynamic hubs of tumor-immune interactions, where organized T/B cell responses enhance anti-tumor immunity. In the ORIENT-11 cohort, TLS-high patients (defined by signature 3) exhibited significantly longer median PFS and OS compared to TLS-low counterparts, underscoring its clinical relevance in CIT regimens.

In the initial screening phase, we evaluated the predictive power of 17 TLS gene signatures derived from different cancer types within the OAK and Poplar cohort, identifying one specific gene set: TLS signatures 3. The rigorous screening process—requiring signatures to predict immunotherapy response while lacking prognostic value in chemotherapy cohorts—validated the specificity of signature 3 for immune-mediated efficacy, distinguishing it from generic tumor biology markers.

TLS signature 3, composed of 19 genes related to T helper 1 (TH1) cells and B cells, aligns with growing evidence that TLS serve as functional hubs for antitumor immunity. TLS facilitate localized immune activation by fostering interactions among B cells, T cells, and dendritic cells, potentially enhancing immunotherapy efficacy [26]. Tumors with high TLS density exhibit elevated levels of activated T cells ($CD38^+$ and $CD69^+$) and effector memory $CD8^+$ T cells, along with upregulated genes associated with T cell activation, TH1 polarization, chemotaxis, and cytotoxicity [12, 13, 27–29]. In NSCLC, microdissected TLS analysis confirmed that TLSs drive a TH1-polarized and $CD8^+$ T cell-mediated antitumor immune response [27]. B cells in TLSs directly target tumor antigens by producing high-affinity antibodies (such as IgG and IgA). Additionally, B cells can function as antigen-presenting cells (APCs), activating $CD4^+$ T cells via major histocompatibility complex (MHC) class II molecules and


A
TLS high+PDL1 negative
TLS high+PDL1 positive
TLS low+PDL1 negative
TLS low+PDL1 positive
Progression−free survival
p = 0.0094
Time
Number at risk
TLS high+PDL1 negative 8 6 2 0
TLS high+PDL1 positive 46 28 4 0
TLS low+PDL1 negative 23 12 2 0
TLS low+PDL1 positive 36 20 2 0
B
Overall survival
p = 0.00096
Number at risk
TLS high+PDL1 negative 8 7 6 6 0
TLS high+PDL1 positive 46 41 32 18 0
TLS low+PDL1 negative 23 13 7 6 0
TLS low+PDL1 positive 36 29 17 4 0
C
TLS high+CD274 high
TLS high+CD274 low
TLS low+CD274 high
TLS low+CD274 low
p = 0.016
Number at risk
TLS high+CD274 high 39 26 3 0
TLS high+CD274 low 15 8 3 0
TLS low+CD274 high 12 6 1 0
TLS low+CD274 low 47 26 3 0
D
p = 0.0023
Number at risk
TLS high+CD274 high 39 36 27 16 0
TLS high+CD274 low 15 12 11 8 0
TLS low+CD274 high 12 10 6 1 0
TLS low+CD274 low 47 32 18 9 0
E
TLS high+cold
TLS high+hot
TLS low+cold
TLS low+hot
p = 0.0056
Number at risk
TLS high+cold 6 3 1 0
TLS high+hot 48 31 5 0
TLS low+cold 53 27 3 0
TLS low+hot 6 5 1 0
F
TLS high+cold
TLS high+hot
TLS low+cold
TLSl ow+hot
p = 0.00038
Number at risk
TLS high+cold 6 4 2 2 0
TLS high+hot 48 44 36 22 0
TLS low+cold 53 36 20 10 0
TLS low+hot 6 6 4 0 0
Time

◂**Fig. 3** Comparative survival analysis stratified by the TLS classification model. **A** PFS of patients receiving combination therapy stratified by TLS gene signature 3 and PD-L1 level **B** OS of patients receiving combination therapy stratified by TLS gene signature 3 and PD-L1 level **C** PFS of patients receiving combination therapy stratified by TLS gene signature 3 and CD274 mRNA expression level **D** OS of patients receiving combination therapy stratified by TLS gene signature 3 and CD274 mRNA expression level **E** PFS of patients receiving combination therapy stratified by TLS gene signature 3 and ImmuneScore **F** OS of patients receiving combination therapy stratified by TLS gene signature 3 and ImmuneScore. PFS, progression-free survival;OS, overall survival; PD-L1, programmed death-ligand 1

stimulating $CD8^+$ T cells through cross-presentation [30]. TH1 and B cells drive anti-tumor immunity in TLS through synergistic interactions (with TH1 cells dominating cellular immune responses while B cells mediate humoral immunity). Their presence and functional status serve as critical determinants of TLS, and may provide potential biomarkers for cancer immunotherapy.

The multivariable Cox nomogram incorporating TLS signature 3 and clinicopathological factors (age, gender, stage, etc.) demonstrated robust discriminative power (AUC: 0.75–0.79), providing a personalized tool for risk stratification in CIT candidates. Such models may facilitate precision oncology by guiding CIT eligibility, particularly in PD-L1-equivocal cases.

While PD-L1 is an established biomarker for immunotherapy, TLS signature 3 adds incremental value, particularly in PD-L1-negative subsets. In PD-L1-negative patients, high TLS scores were associated with numerically higher ORR and prolonged survival, which aligns with the hypothesis that TLS fosters an immunogenic TME, potentially augmenting PD-L1-independent immune activation [22]. Combined evaluation of TLS and PD-L1 may thus improve patient stratification beyond PD-L1 alone. Although TLS signature 3 demonstrated potential to stratify outcomes in PD-L1-negative patients (n = 31), the limited sample size reduces statistical power and increases the risk of type II error. The observed survival benefits should be considered exploratory, requiring validation in larger cohorts. Regarding TME, TLS signature 3 strongly correlated with hot tumor phenotypes, supporting the paradigm that TLS serves as a functional hub for adaptive immunity, facilitating lymphocyte recruitment and activation. The superior outcomes in TLS-high patients may reflect pre-existing antitumor immunity, which is further amplified by CIT.

While TLS signature 3 demonstrates robust predictive value, its current reliance on RNA-seq may limit immediate clinical adoption. To address this, we are developing targeted assays (e.g., qRT-PCR, NanoString) and immunohistochemistry (IHC) surrogates (e.g., CXCL13) to simplify detection. These approaches could integrate with existing PD-L1 testing, offering a practical workflow for patient stratification. Future studies will validate these surrogates clinically and analytically, with the goal of enabling precision immunotherapy in routine practice.

Several limitations warrant consideration. First, the TLS signature was derived from transcriptomic data, which may not fully capture spatial heterogeneity in TLS distribution. Future studies incorporating multiplex IHC are needed to validate these findings. Second, the biological mechanisms linking TLS signature 3 to CIT response remain speculative; functional studies are required to elucidate whether TLS actively potentiate therapy or merely reflect a pre-existing immune-permissive niche. Third, the small sample size of PD-L1-negative subgroups limits definitive conclusions about TLS efficacy in this population. Lastly, the heterogeneity in TLS composition (e.g., mature vs immature) was not assessed, and future work should explore whether specific TLS subtypes confer differential benefits.

Conclusion

In conclusion, our study demonstrates that TLS signature 3 is a promising biomarker for predicting CIT response in advanced NSCLC, complementing PD-L1. Its integration into clinical practice may optimize patient selection and improve outcomes. Further research exploring the mechanistic basis of TLS-immune interactions and validating its utility in diverse therapeutic settings is warranted.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate Individual consent for this retrospective analysis was waived.

Consent for publication Individual consent for this retrospective analysis was waived.

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References

1. Bray F et al (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74(3):229–263
2. Meyer ML et al (2024) New promises and challenges in the treatment of advanced non-small-cell lung cancer. Lancet 404(10454):803–822
3. Desai A, Peters S (2023) Immunotherapy-based combinations in metastatic NSCLC. Cancer Treat Rev 116:102545
4. Grant MJ, Herbst RS, Goldberg SB (2021) Selecting the optimal immunotherapy regimen in driver-negative metastatic NSCLC. Nat Rev Clin Oncol 18(10):625–644
5. Teillaud JL et al (2024) Tertiary lymphoid structures in anticancer immunity. Nat Rev Cancer 24(9):629–646
6. Cui X et al (2025) Tertiary lymphoid structures as a biomarker in immunotherapy and beyond: advancing towards clinical application. Cancer Lett 613:217491
7. Sautès-Fridman C et al (2019) Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer 19(6):307–325
8. Yang Y et al (2020) Efficacy and safety of sintilimab plus pemetrexed and platinum as first-line treatment for locally advanced or metastatic nonsquamous NSCLC: a randomized, double-blind, phase 3 study (Oncology pRogram by InnovENT anti-PD-1-11). J Thorac Oncol 15(10):1636–1646
9. Rittmeyer A et al (2017) Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 389(10066):255–265
10. Fehrenbacher L et al (2016) Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 387(10030):1837–1846
11. Coppola D et al (2011) Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. Am J Pathol 179(1):37–45
12. Gu-Trantien C et al (2013) CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 123(7):2873–2892
13. Hennequin A et al (2016) Tumor infiltration by Tbet+ effector T cells and CD20+ B cells is associated with survival in gastric cancer patients. Oncoimmunology 5(2):e1054598
14. Kroeger DR, Milne K, Nelson BH (2016) Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. Clin Cancer Res 22(12):3005–3015
15. Becht E et al (2016) Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. Clin Cancer Res 22(16):4057–4066
16. Cabrita R et al (2020) Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature 577(7791):561–565
17. Dieu-Nosjean MC et al (2014) Tertiary lymphoid structures in cancer and beyond. Trends Immunol 35(11):571–580
18. Bindea G et al (2013) Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 39(4):782–795
19. Noël G et al (2021) Functional Th1-oriented t follicular helper cells that infiltrate human breast cancer promote effective adaptive immunity. J Clin Invest. https://doi.org/10.1172/JCI139905
20. Meylan M et al (2022) Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. Immunity 55(3):527-541.e5
21. Shang T et al (2023) Tertiary lymphoid structures predict the prognosis and immunotherapy response of cholangiocarcinoma. Front Immunol 14:1166497
22. Vanhersecke L et al (2021) Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nat Cancer 2(8):794–802
23. Kinker GS et al (2023) Mature tertiary lymphoid structures are key niches of tumour-specific immune responses in pancreatic ductal adenocarcinomas. Gut 72(10):1927–1941
24. Patil NS et al (2022) Intratumoral plasma cells predict outcomes to PD-L1 blockade in non-small cell lung cancer. Cancer Cell 40(3):289-300.e4
25. Tang Z et al (2025) Spatial transcriptomics reveals tryptophan metabolism restricting maturation of intratumoral tertiary lymphoid structures. Cancer Cell 43(6):1025–1044
26. Peyraud F et al (2025) Tertiary lymphoid structures and cancer immunotherapy: from bench to bedside. Med 6(1):100546
27. Goc J et al (2014) Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res 74(3):705–715
28. Hiraoka N et al (2015) Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer 112(11):1782–1790
29. Truxova I et al (2018) Mature dendritic cells correlate with favorable immune infiltrate and improved prognosis in ovarian carcinoma patients. J Immunother Cancer 6(1):139
30. Fridman WH et al (2022) B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat Rev Clin Oncol 19(7):441–457