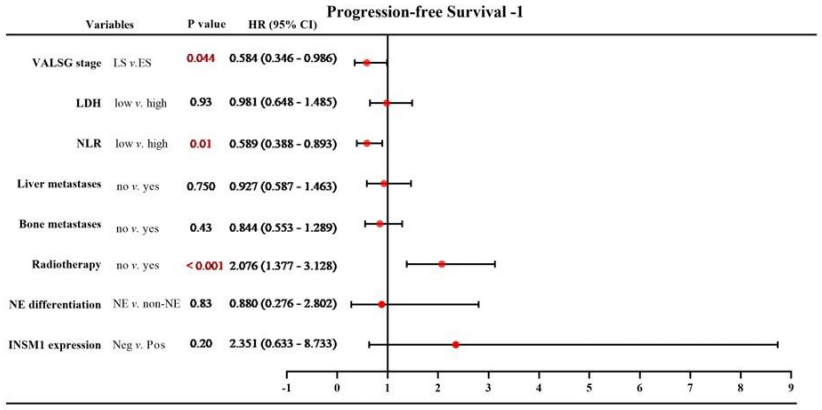
Figure S1

A



B

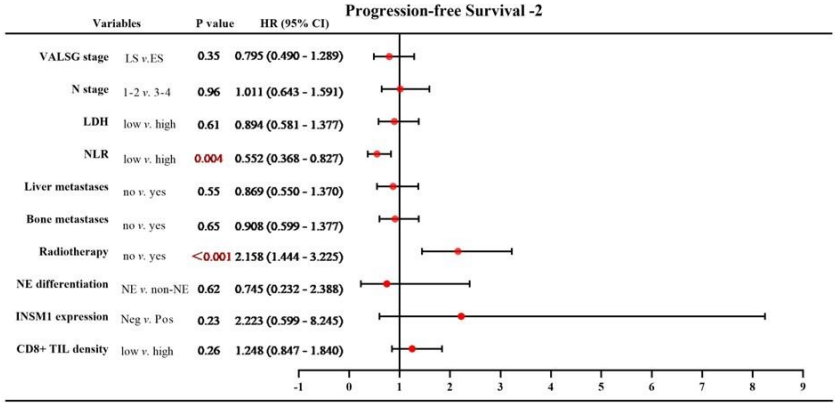


Figure S1. Multivariate analyses for PFS1 and PFS2 of clinicopathologic parameters in the entire cohort.
The multivariable Cox analysis confirmed the independent prognostic roles of VALSG stage (P=0.02; HR =0.502; 95% CI: 0.276, 0.913), and elevated NLR and radiotherapy administration independently predicted PFS1 and PFS2 outcomes.

Figure S2

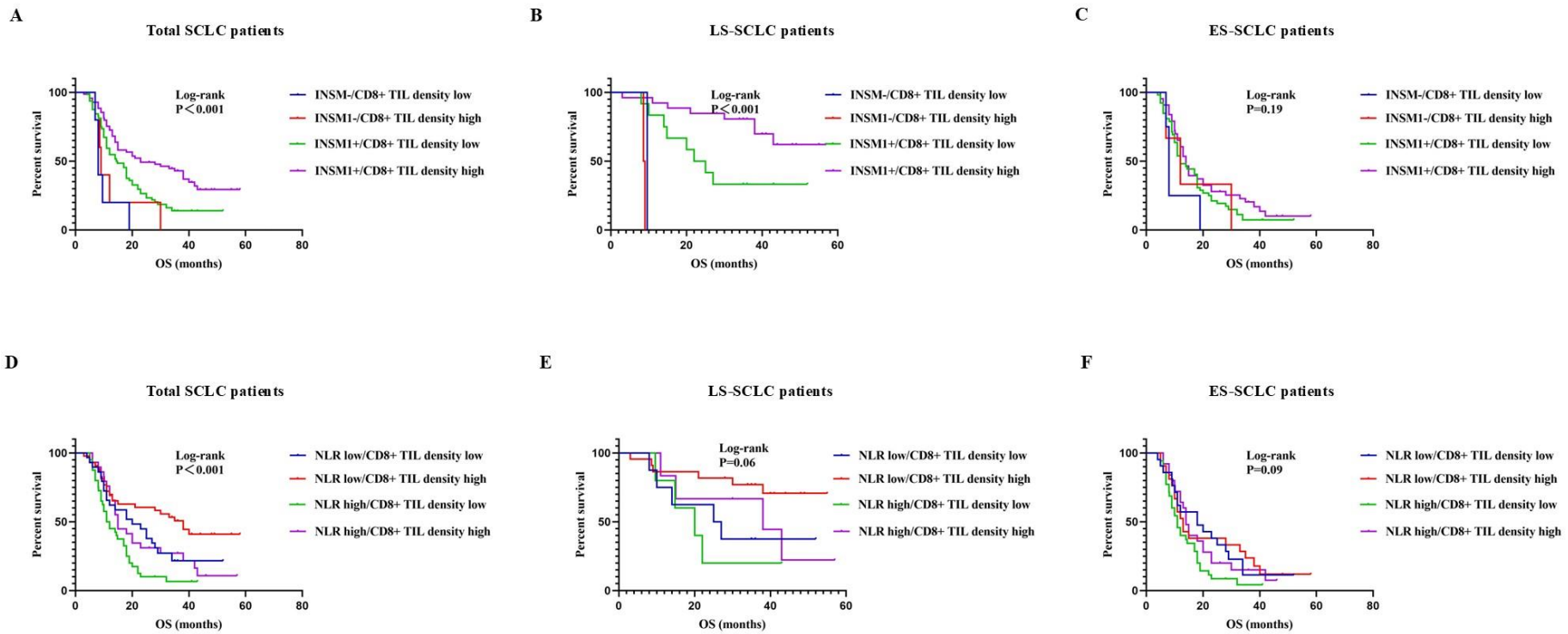


Figure S2. Kaplan – Meier curves of OS according to four groups of the INSM1/NLR-CD8+ TIL density composite biomarker in the overall cohort, LS-SCLC, and ES-SCLC.

No combination demonstrated statistically significant survival differentials across all three cohorts.

Table S1. Antibodies used for immunohistochemistry

Marker	Clone	Catalog number	Company	Dilution	Positive reactivity location
ASCL1	24B72D11.1	#556604	BD Biosciences, San José, CA, USA	1:50	Nucleus
NEUROD1	EPR17084	#ab205300	Abcam, Cambridge, U.K.	1:200	Nucleus
POU2F3	E5N2D	#36135	Cell Signaling Technology, Leiden, The Netherlands	1:300	Nucleus
YAP1	EP1674Y	#ab52771	Abcam, Cambridge, U.K.	1:300	Nucleus, cytoplasm
INSM1	EPR23199-37	#ab305104	Abcam, Cambridge, UK	1:200	Nucleus
MHC I	EMR8-5	#ab70328	Abcam, Cambridge, U.K.	1:500	Cell membrane
PD-L1	22C3	M3653	Agilent DaKo, Denmark	1:50	Partial or complete membrane
Rb	EPR17512	#ab181616	Abcam, Cambridge, U.K.	1:1000	Nucleus
CD8	SP16	ZA-0508	ZSGB-BIO	Working solution	Cell membrane

Abbreviations: ASCL1, achaete-scute family BHLH transcription factor 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes1-associated transcriptional regulator; MHC I, major histocompatibility complex class I; PD-L1, programmed death-ligand 1.

Table S2. Demographics of the included patients and samples

Parameter	**No. (%)**
Age (year),	62 (36–79)
Median (range)	
⩽65	93 (65.0)
>65	50 (35.0)
Sex	
Male	116 (81.1)
Female	27 (18.9)
Smoking history	
No	31 (21.7)
Yes	112 (78.3)
ECOG PS	
0-1	135 (94.4)
⩾2	8 (5.6)
Liver metastases	
No	102 (71.3)
Yes	41 (28.7)
Brain metastases	
No	124 (86.7)
Yes	19 (13.3)
Bone metastases	
No	96 (67.1)
Yes	47 (32.9)
VALSG stage	
LS	41 (28.7)
ES	102 (71.3)
TNM stage, case number (%)	
II	5 (3.5)
III	36 (25.2)

IV	102 (71.3)
T stage	
1-2	45 (31.5)
3-4	98 (68.5)
N stage	
0-2	60 (42.0)
3	83 (58.0)
Radiation therapy	
No	65 (45.5)
Yes	78 (54.5)
ICIs inhibitors	
PD-L1 inhibitors	107 (74.8)
PD-1 inhibitors	36 (25.2)
anti-TIGIT antibody	
No	135 (94.4)
Yes	8 (5.6)
≥second-line immunotherapy	
No	37 (25.9)
Yes	33 (23.1)
Unknow	73 (51.0)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; VALSG, Veterans Administration Lung Cancer Study Group; TNM, tumor-node-metastasis; ICIs, immune checkpoint inhibitors; TIGIT, T cell immunoglobulin and the immunoreceptor tyrosine-based inhibitory motif domain.

Table S3. Comparisons of clinicopathologic parameters between LS-SCLC and ES-SCLC

	VALSG stage		
Parameter	**LS**	**ES**	**P value**
Age			0.85
⩽65 years	26 (63.4%)	67 (65.7%)	
> 65 years	15 (36.6%)	35 (34.3%)	
Sex			0.35
Male	31 (75.6%)	85 (83.3%)	
Female	10 (24.4%)	17 (16.7%)	
Smoking story			0.37
No	11 (26.8%)	20 (19.6%)	
Yes	30 (73.2%)	82 (80.4%)	
ECOG PS			0.44
0-1	40 (97.6%)	95 (93.1%)	
⩾2	1 (2.4%)	7 (6.9%)	
LDH			**<0.001**
Low	30 (81.1%)	44 (45.4%)	
High	7 (18.9%)	53 (54.6%)	
NLR			**0.001**
Low	30 (73.2%)	42 (42.0%)	
High	11 (26.8%)	58 (58.0%)	
PLR			0.10
Low	22 (53.7%)	38 (38.0%)	
High	19 (46.3%)	62 (62.0%)	
Radiotherapy			**＜0.001**
No	9 (22.0%)	56 (54.9%)	
Yes	32 (78.0%)	46 (45.1%)	
ICIs inhibitors			0.14
PD-L1 inhibitors	27 (65.9%)	80 (78.4%)	
PD-1 inhibitors	14 (34.1%)	22 (21.6%)	

Anti-TIGIT antibody			＞0.99
No	39 (95.1%)	96 (94.1%)	
Yes	2 (4.9%)	6 (5.9%)	
ASCL1 expression			0.76
Negative	5 (12.2%)	10 (9.8%)	
Positive	36 (87.8%)	92 (90.2%)	
NEUROD1 expression			0.36
Negative	25 (61.0%)	53 (52.0%)	
Positive	16 (39.0%)	49 (48.0%)	
POU2F3 expression			＞0.99
Negative	36 (87.8%)	89 (87.3%)	
Positive	5 (12.2%)	13 (12.7%)	
YAP1 expression			0.15
Negative	36 (87.8%)	97 (95.1%)	
Positive	5 (12.2%)	5 (4.9)	
INSM1 expression			＞0.99
Negative	3 (7.3%)	7 (6.9%)	
Positive	38 (92.7%)	95 (93.1%)	
NE differentiation			＞0.99
NE phenotype	37 (90.2%)	93 (91.2%)	
Non-NE phenotype	4 (9.8%)	9 (8.8%)	
Rb expression			0.47
Negative	32 (78.0%)	86 (84.3%)	
Positive	9 (22.0%)	16 (15.7%)	
Tumor PD-L1 expression			0.49
Negative	40 (97.6%)	101 (99.0%)	
Positive	1 (2.4%)	1 (1.0%)	
Stroma PD-L1 expression			0.64
Negative	32 (78.0%)	84 (82.4%)	

Positive	9 (22.0%)	18 (17.6%)	
MHC I expression			0.44
Negative	29 (70.7%)	64 (62.7%)	
Positive	12 (29.3%)	38 (37.3%)	
CD8+ TIL density			**0.02**
Low	13 (31.7%)	56 (54.9%)	
High	28 (68.3%)	46 (45.1%)	

Abbreviations: SCLC, small cell lung carcinoma; LS-SCLC, limited-stage SCLC; ES-SCLC, extensive-stage SCLC; VALSG, Veterans Administration Lung Cancer Study Group; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ICIs, immune checkpoint inhibitors; TIGIT, T cell immunoglobulin and the immunoreceptor tyrosine-based inhibitory motif domain; ASCL1, achaete-scute family BHLH transcription factor 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes1-associated transcriptional regulator; NE, neuroendocrine; PD-L1, programmed death-ligand 1; MHC I, major histocompatibility complex class I; TIL, tumor-infiltrating lymphocyte.

Note: Significant P values are highlighted in bold.

Table S4. Univariate analyses for prognostic significance of clinicopathologic parameters in LS-SCLC patients

Clinicopathologic parameters	PFS1		PFS2		OS	
	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)
Age, ≤65 *v.* > 65 years	0.36	0.693 (0.318-1.511)	0.36	0.719 (0.356-1.451)	0.43	0.693 (0.278-1.725)
Sex, male *v.* female	0.89	1.069 (0.429-2.665)	0.30	1.585 (0.673-3.733)	0.44	1.550 (0.510-4.712)
Smoking story, no *v.* yes	0.84	0.910 (0.365-2.270)	0.47	0.739 (0.327-1.672)	0.28	0.539 (0.177-1.644)
T stage, 1-2 *v.* 3−4	0.79	0.895 (0.397-2.015)	0.54	0.804 (0.399-1.622)	0.56	0.752 (0.285-1.983)
N stage, 0-2 *v.* 3	0.65	0.834 (0.378-1.841)	0.40	0.741 (0.369-1.487)	0.20	0.549 (0.219-1.376)
ECOG PS, 0-1 *v.* ≥2	0.73	0.700 (0.094-5.231)	0.75	0.726 (0.097-5.406)	0.57	2.149 (0.313-9.801)
LDH, low *v.* high	0.27	0.582 (0.224-1.509)	0.14	0.514 (0.211-1.255)	0.12	0.443 (0.157-1.249)
NLR, low *v.* high	**0.03**	0.409 (0.182-0.921)	0.16	0.580 (0.273-1.234)	0.09	0.449 (0.180-1.122)
PLR, low *v.* high	0.34	0.685 (0.317-1.480)	0.71	0.876 (0.436-1.760)	0.40	0.671 (0.266-1.692)
Radiotherapy, no *v.* yes	**0.005**	3.335 (1.426-7.802)	**0.004**	3.126 (1.425-6.858)	**0.02**	3.063 (1.191-7.879)
ICIs inhibitors, PD-L1 inhibitors *v.* PD-1 inhibitors	0.11	2.043 (0.854-4.892)	0.22	1.582 (0.765-3.269)	0.42	1.530 (0.548-4.270)
Anti-TIGIT antibody, no *v.* yes	0.94	1.083 (0.146-8.015)	0.62	0.694 (0.164-2.926)	0.84	1.229 (0.163-9.284)
≥second line immunotherapy, no v. yes	0.11	3.169 (0.782-12.845)	0.30	1.979 (0.540-7.252)	0.94	0.948 (0.211-4.266)
ASCL1 expression, Neg *v.* Pos	0.31	1.738 (0.593-5.095)	0.46	1.492 (0.517-4.303)	**0.009**	4.726 (1.484-15.046)
NEUROD1 expression, Neg *v.* Pos	0.06	2.315 (0.968-5.540)	**0.02**	2.368 (1.119-5.010)	0.09	2.436 (0.871-6.816)
POU2F3 expression, Neg *v.* Pos	0.31	0.576 (0.196-1.687)	0.15	0.493 (0.187-1.302)	**0.02**	0.245 (0.079-0.761)
YAP1 expression, Neg *v.* Pos	0.59	1.396 (0.418-4.655)	0.99	1.005 (0.388-2.607)	0.76	0.826 (0.240-2.843)
NE differentiation, NE phenotype *v.* non-NE phenotype	**0.04**	0.308 (0.100-0.947)	**0.04**	0.305 (0.099-0.941)	**＜0.001**	0.093 (0.026-0.332)
INSM1 expression, Neg *v.* Pos	0.09	2.990 (0.857-10.430)	0.09	2.999 (0.860-10.462)	**0.001**	24.994 (4.015-155.591)
Rb expression, Neg *v.* Pos	0.51	1.428 (0.491-4.149)	0.51	1.327 (0.568-3.101)	0.48	1.560 (0.453-5.373)

Tumor PD-L1 expression, Neg *v.* Pos	0.46	0.469 (0.062-3.553)	0.45	0.455 (0.060-3.448)	**0.03**	0.073 (0.008-0.750)
Stromal PD-L1 expression, Neg *v.* Pos	0.77	1.157 (0.436-3.071)	0.66	0.837 (0.377-1.858)	0.68	1.266 (0.420-3.822)
MHC I expression, Neg *v.* Pos	0.39	0.699 (0.311-1.572)	0.87	0.938 (0.449-1.962)	0.66	0.807 (0.306-2.128)
CD8+ TIL density, Low *v.* High	0.053	2.180 (0.991-4.797)	0.056	2.048 (0.980-4.280)	**0.03**	2.821 (1.127-7.060)

Abbreviations: SCLC, small cell lung carcinoma; LS-SCLC, limited-stage SCLC; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ICIs, immune checkpoint inhibitors; TIGIT, T cell immunoglobulin and the immunoreceptor tyrosine-based inhibitory motif domain; ASCL1, achaete-scute family BHLH transcription factor 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes1-associated transcriptional regulator; NE, neuroendocrine; PD-L1, programmed death-ligand 1; MHC I, major histocompatibility complex class I; TIL, tumor-infiltrating lymphocyte.

Note: Significant P values are highlighted in bold.

Table S5. Univariate analyses for prognostic significance of clinicopathologic parameters in ES-SCLC patients

Clinicopathologic parameters	PFS1		PFS2		OS	
	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)
Age, ≤65 *v.* > 65 years	0.48	0.858 (0.560-1.315)	0.65	0.908 (0.597-1.379)	0.16	0.733 (0.475-1.132)
Sex, male *v.* female	0.48	0.821 (0.478-1.411)	0.53	1.187 (0.692-2.037)	0.57	0.853 (0.489-1.487)
Smoking story, no *v.* yes	0.22	1.372 (0.825-2.281)	0.74	1.086 (0.663-1.777)	0.62	1.142 (0.678-1.922)
T stage, 1-2 *v.* 3−4	0.41	0.828 (0.528-1.299)	0.40	0.828 (0.533-1.286)	0.80	0.943 (0.600-1.480)
N stage, 0-2 *v.* 3	0.60	0.889 (0.572-1.384)	0.18	0.743 (0.481-1.146)	0.70	0.915 (0.585-1.430)
ECOG PS, 0-1 *v.* ≥2	0.39	0.671 (0.270-1.667)	0.81	1.117 (0.453-2.757)	0.95	0.971 (0.393-2.398)
LDH, low *v.* high	0.25	0.779 (0.510-1.189)	0.10	0.709 (0.469-1.071)	0.28	0.791 (0.517-1.211)
NLR, low *v.* high	**0.04**	0.629 (0.409-0.968)	**0.007**	0.566 (0.373-0.859)	0.10	0.692 (0.449-1.068)
PLR, low *v.* high	0.20	0.755 (0.491-1.161)	0.23	0.775 (0.512-1.174)	0.52	0.869 (0.564-1.337)
Liver metastases, no *v.* yes	0.10	0.699 (0.456-1.070)	**0.03**	0.637 (0.420-0.966)	0.10	0.694 (0.453-1.064)
Brain metastases, no *v.* yes	0.07	1.659 (0.947-2.908)	**0.04**	1.780 (1.029-3.078)	0.12	1.580 (0.884-2.826)
Bone metastases, no *v.* yes	0.08	0.691 (0.457-1.047)	0.08	0.694 (0.462-1.043)	**0.01**	0.571 (0.372-0.878)
Radiotherapy, no *v.* yes	**0.03**	1.569 (1.039-2.371)	**0.01**	1.653 (1.105-2.473)	0.19	1.325 (0.872-2.013)
ICIs inhibitors, PD-L1 inhibitors *v.* PD-1 inhibitors	0.86	0.956 (0.576-1.584)	0.83	1.055 (0.645-1.726)	0.30	1.326 (0.781-2.249)
Anti-TIGIT antibody, no *v.* yes	0.59	1.258 (0.543-2.914)	0.72	1.163 (0.507-2.665)	0.61	1.244 (0.542-2.856)
⩾ second line immunotherapy, no v. yes	0.86	0.947 (0.519-1.729)	0.99	0.998 (0.551-1.806)	0.49	0.806 (0.439-1.481)
ASCL1 expression, Neg *v.* Pos	**0.04**	2.013 (1.028-3.939)	**0.01**	2.391 (1.215-4.707)	**0.04**	1.997 (1.027-3.884)
NEUROD1 expression, Neg *v.* Pos	0.77	1.064 (0.706-1.605)	0.82	1.048 (0.704-1.560)	0.88	1.033 (0.682-1.564)
POU2F3 expression, Neg *v.* Pos	0.71	0.893 (0.496-1.608)	0.52	0.823 (0.458-1.480)	0.32	0.733 (0.398-1.349)
YAP1 expression, Neg *v.* Pos	0.93	1.044 (0.422-2.582)	0.97	1.015 (0.411-2.509)	0.96	1.029 (0.375-2.823)

NE differentiation, NE phenotype *v.* non-NE phenotype	0.08	0.532 (0.265-1.069)	**0.02**	0.446 (0.221-0.901)	0.30	0.691 (0.346-1.380)
INSM1 expression, Neg *v.* Pos	**0.01**	2.848 (1.289-6.289)	**0.003**	3.385 (1.524-7.520)	0.16	1.750 (0.805-3.804)
Rb expression, Neg *v.* Pos	0.62	0.868 (0.498-1.513)	0.24	0.724 (0.421-1.243)	0.08	0.607 (0.347-1.064)
Tumor PD-L1 expression, Neg *v.* Pos	0.85	1.206 (0.167-8.691)	0.98	1.024 (0.142-7.385)	0.88	0.853 (0.118-6.162)
Stromal PD-L1 expression, Neg *v.* Pos	0.45	0.816 (0.480-1.387)	0.76	1.085 (0.642-1.833)	0.87	1.047 (0.608-1.803)
MHC I expression, Neg *v.* Pos	0.21	1.317 (0.859-2.020)	0.10	1.417 (0.935-2.147)	0.14	1.395 (0.902-2.159)
CD8+ TIL density, Low *v.* High	0.82	0.952 (0.632-1.435)	0.62	1.108 (0.743-1.652)	0.21	1.308 (0.857-1.995)

Abbreviations: SCLC, small cell lung carcinoma; ES-SCLC, extensive-stage SCLC; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ICIs, immune checkpoint inhibitors; TIGIT, T cell immunoglobulin and the immunoreceptor tyrosine-based inhibitory motif domain; ASCL1, achaete-scute family BHLH transcription factor 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes1-associated transcriptional regulator; NE, neuroendocrine; PD-L1, programmed death-ligand 1; MHC I, major histocompatibility complex class I; TIL, tumor-infiltrating lymphocyte.

Note: Significant P values are highlighted in bold.

Table S6. Multivariate analyses for prognostic significance of clinicopathologic parameters in LS- and ES-SCLC

Clinicopathologic parameters	PFS1		PFS2		OS	
	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)
LS-SCLC						
NLR, low *v.* high	0.20	0.562 (0.234-1.349)				
Radiotherapy, no *v.* yes	**0.004**	3.884 (1.550-9.729)			**0.01**	3.960 (1.373-11.419)
ASCL1 expression, Neg *v.* Pos			0.94	0.000 (0.000-4.571E+112)	0.94	0.000 (0.000-3.497E+79)
NEUROD1 expression, Neg *v.* Pos			0.08	1.998 (0.927-4.309)		
POU2F3 expression, Neg *v.* Pos					0.93	2272.571 (0.000-4.267E+77)
NE differentiation, NE *v.* non-NE	**0.03**	0.246 (0.071-0.848)	0.94	0.000 (0.000-1.847E+112)	0.90	0.000 (0.000-8.444E+103)
INSM1 expression, Neg *v.* Pos					0.145	7.590 (0.492-117.041)
Tumor PD-L1 expression, Neg *v.* Pos					0.70	0.597 (0.045-7.868)
CD8+ TIL density, Low *v.* High					0.22	1.888 (0.683-5.215)
ES-SCLC						
NLR, low *v.* high	**0.02**	0.581 (0.370-0.911)	**0.005**	0.515 (0.324-0.818)		
Liver metastases, no *v.* yes			0.37	0.817 (0.523-1.274)		
Brain metastases, no *v.* yes			0.15	1.524 (0.855-2.717)		
Bone metastases, no *v.* yes					**0.01**	0.583 (0.379-0.898)
Radiotherapy, no *v.* yes	**0.01**	1.757 (1.142-2.703)	**0.007**	1.834 (1.182-2.844)		
ASCL1 expression, Neg *v.* Pos	0.96	1.029 (0.322-3.292)	0.84	1.142 (0.302-4.316)	0.056	1.918 (0.983-3.740)
NE differentiation, NE *v.* non-NE			0.91	1.096 (0.218-5.508)		
INSM1 expression, Neg *v.* Pos	0.17	2.610 (0.668-10.208)	0.24	2.709 (0.507-14.476)		

Abbreviations: SCLC, small cell lung carcinoma; LS-SCLC, limited-stage SCLC; ES-SCLC, extensive-stage SCLC; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; Pos, positive; Neg, negative; VALSG, Veterans Administration Lung Cancer Study Group; NLR, neutrophil-to-lymphocyte ratio; ASCL1, achaete-scute family BHLH transcription factor 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; NE, neuroendocrine; PD-L1, programmed death-ligand 1; TIL, tumor-infiltrating lymphocyte.

Note: Significant P values are highlighted in bold.