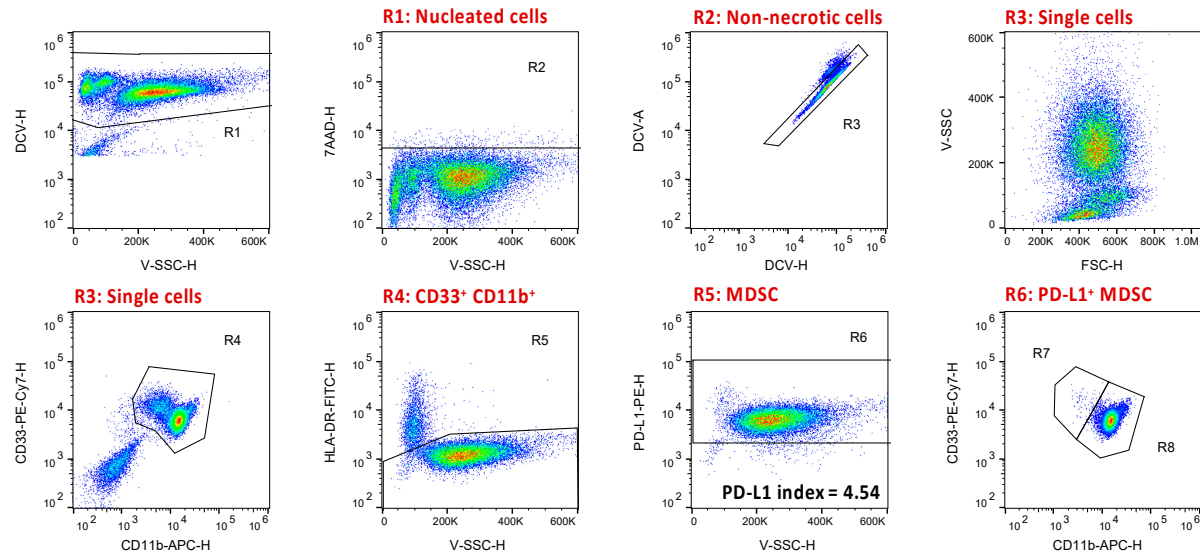
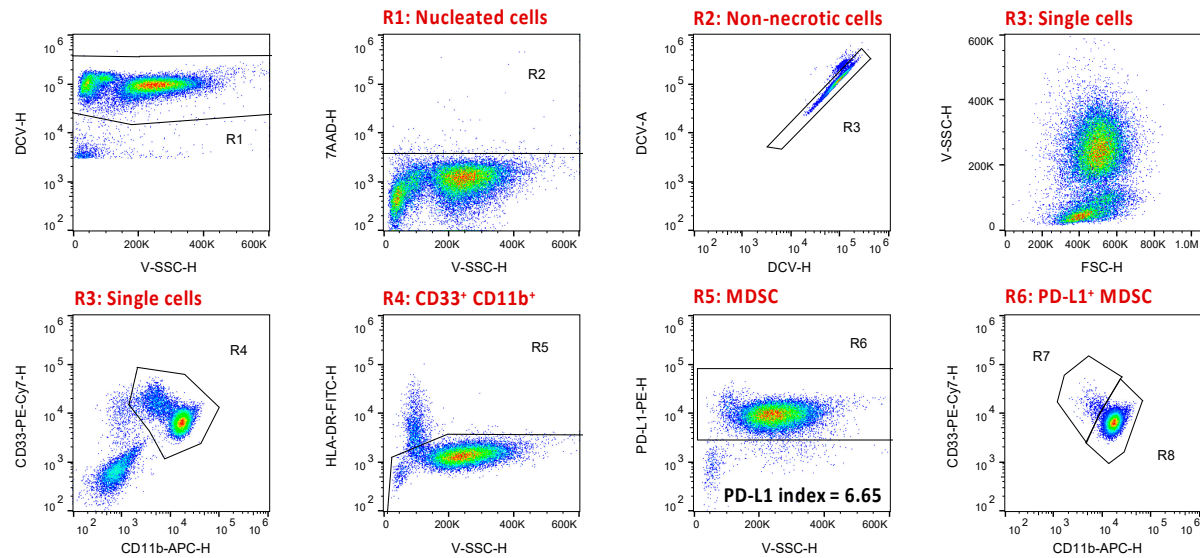


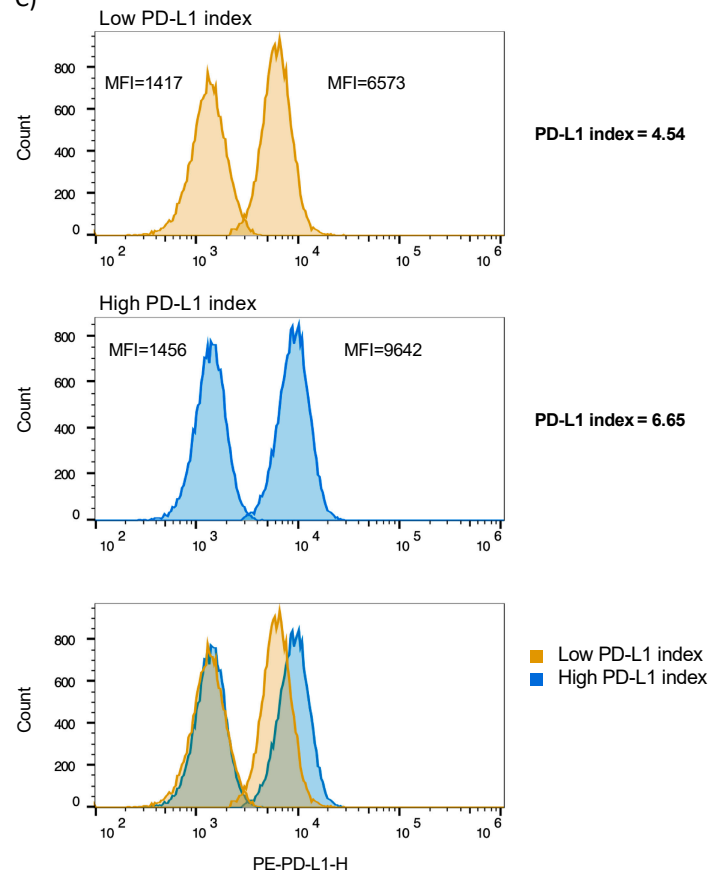
A) Low PD-L1 index patient



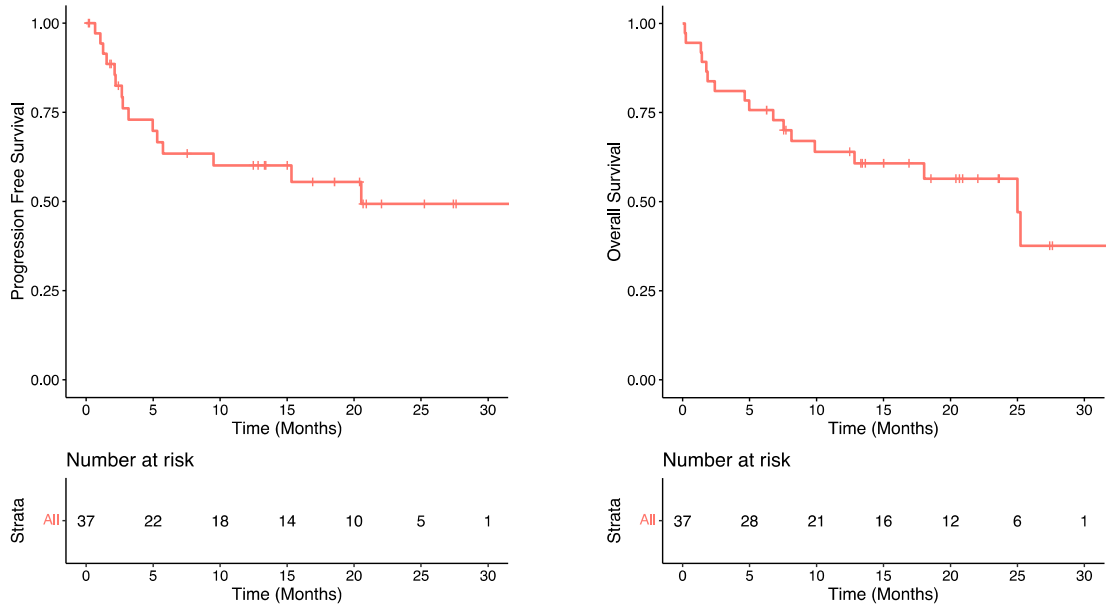
B) High PD-L1 index patient



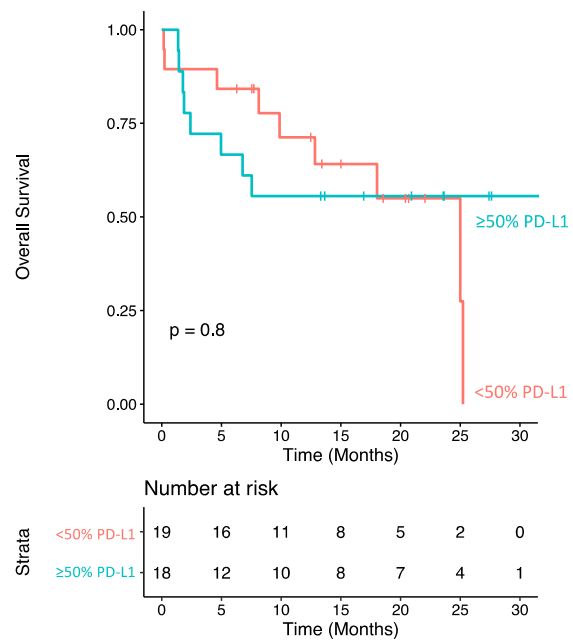
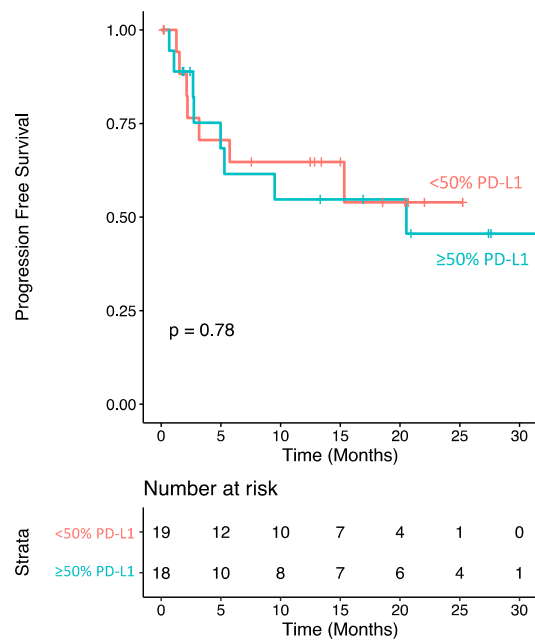
C)



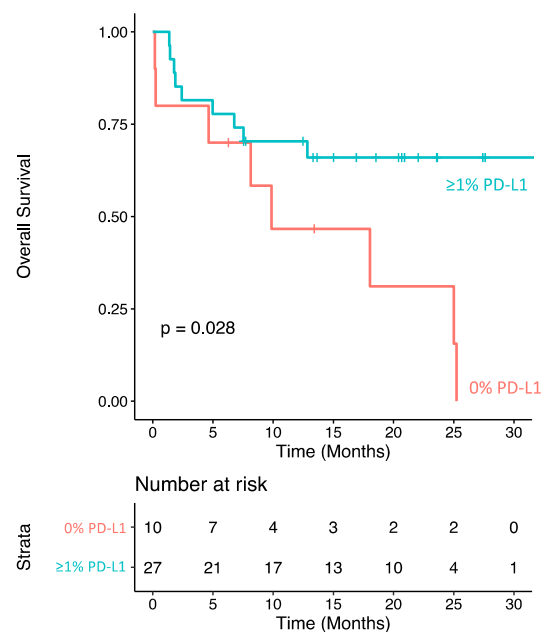
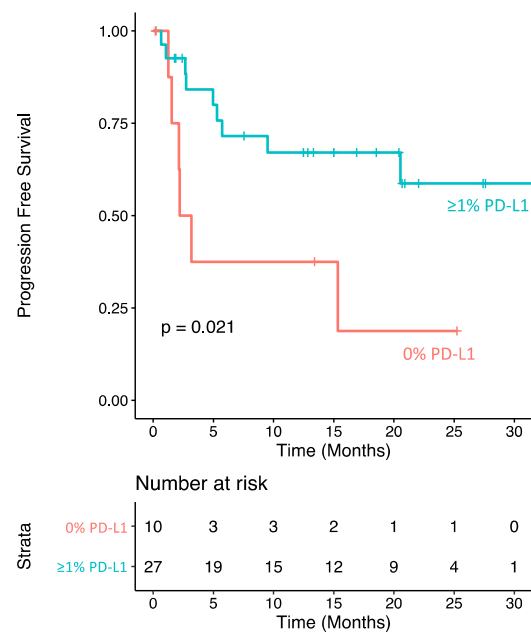
**Figure S1. Comparison of two patients with different PD-L1 index.** Representative analysis of a low PD-L1 index patient with a value of 4.54 (A) and a high PD-L1 index patient with a value of 6.65 (B). The mean fluorescence intensity (MFI) and standard deviation are used to calculate the PD-L1 index, where patients with a high PD-L1 index show a higher reactivity against the PE-PD-L1 antibody.



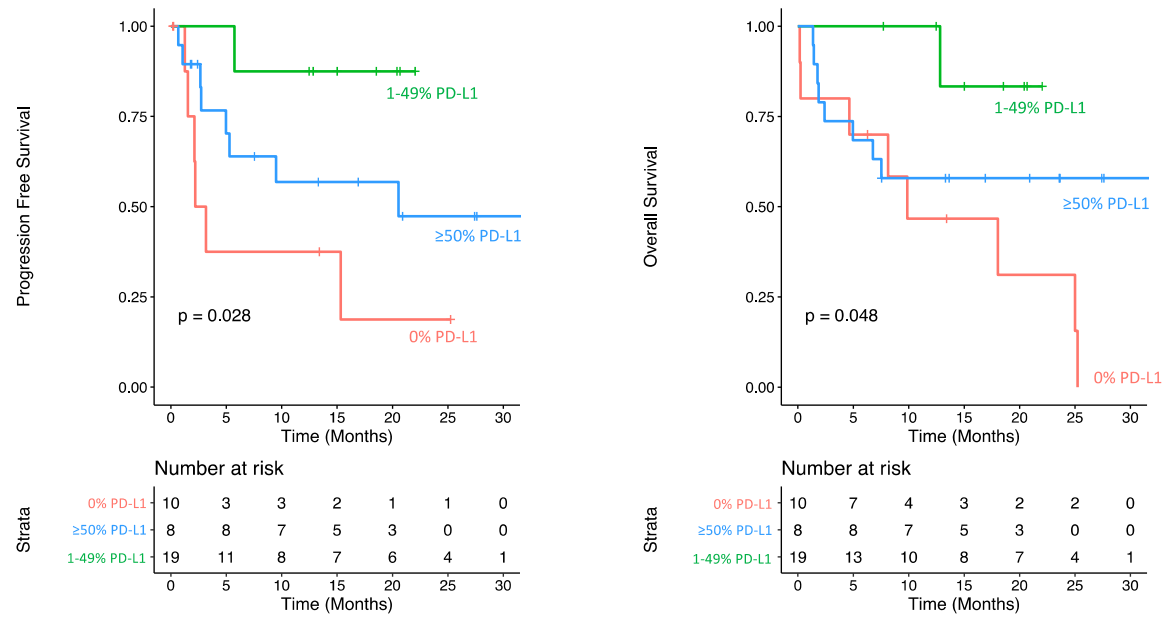
**Figure S2. Kaplan-Meier plot of progression-free survival and overall survival from stage III-IV NSCLC patients.**



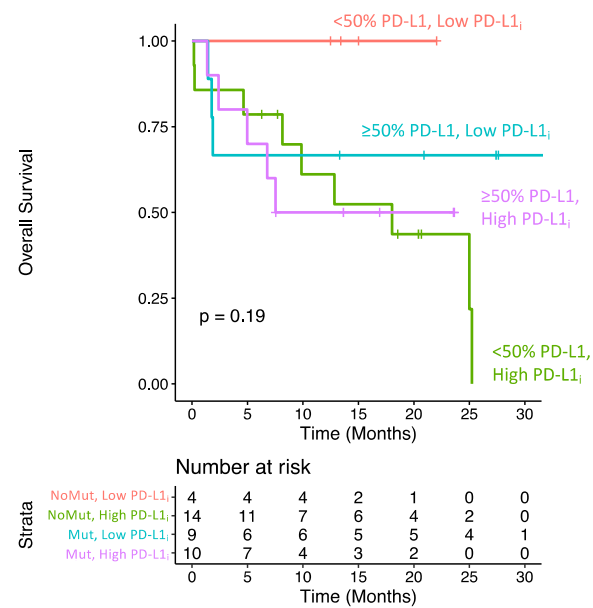
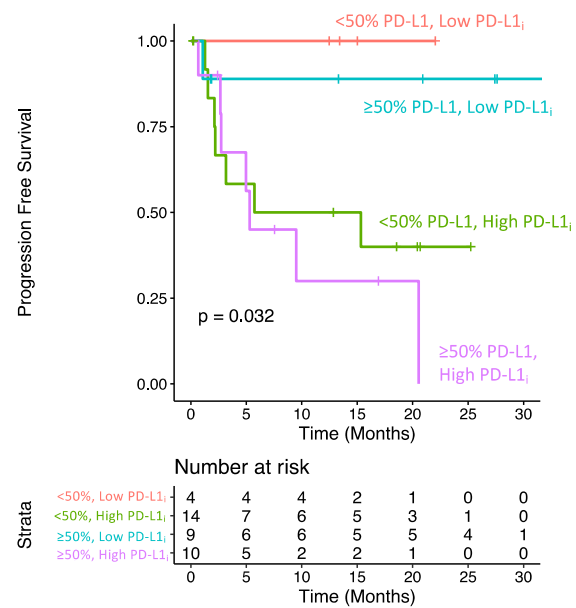
**Figure S3. PD-L1 expression of tumor tissue, assessed by immunohistochemistry, did not predict PFS nor OS at baseline.**



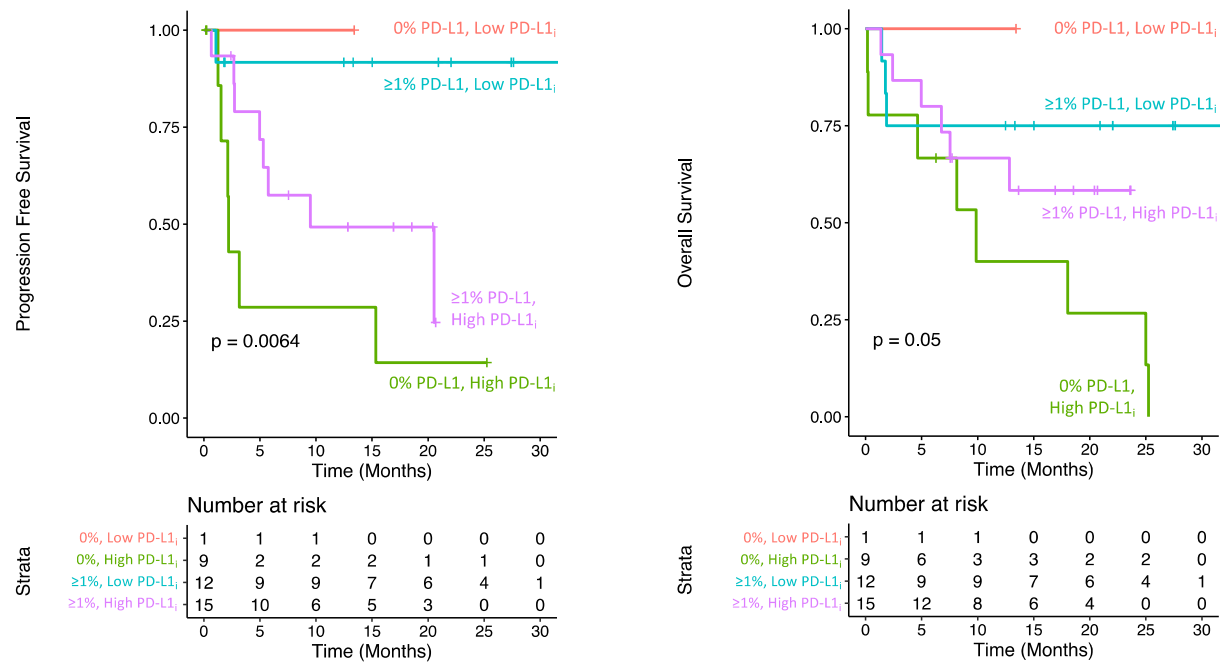
**Figure S4. PD-L1 expression of tumor tissue stratified by expression and no expression predicted PFS and OS at baseline.**



**Figure S5. PD-L1 expression of tumor tissue stratified in three levels of expression.**

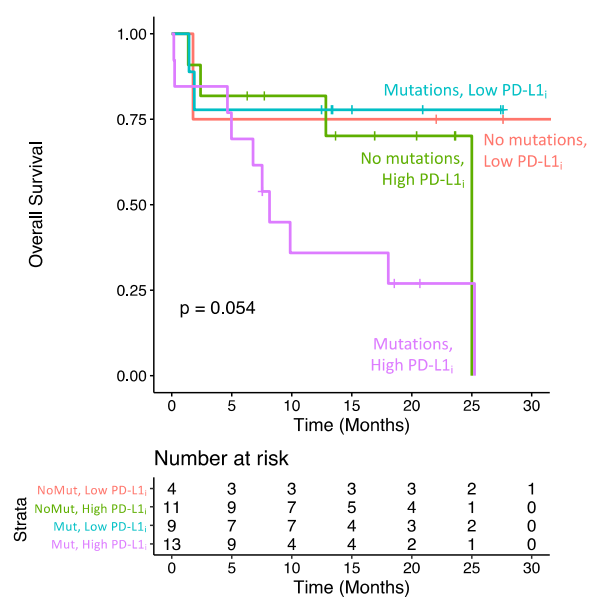
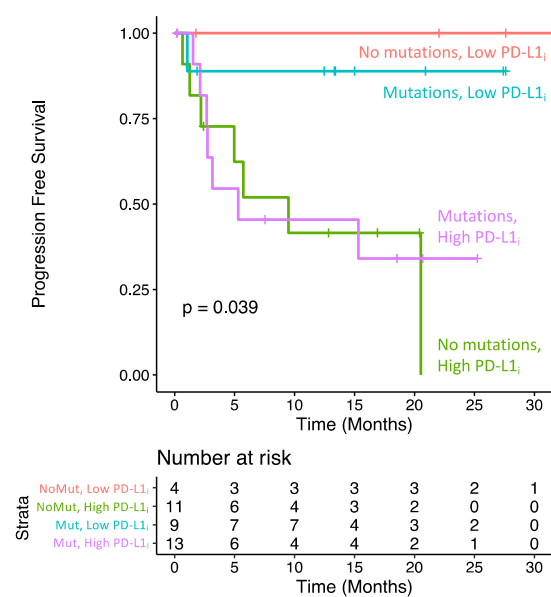


**Figure S6. The combination of tumor PD-L1 expression and PD-L1 index did not improve the PFS nor OS prediction at baseline.**

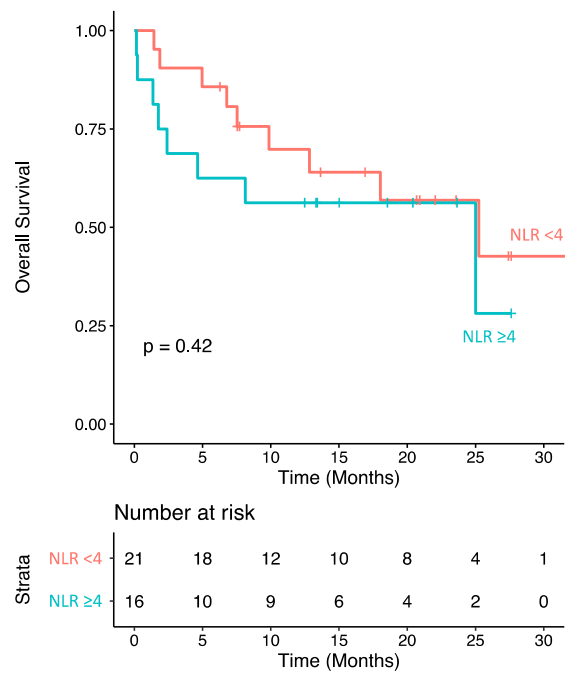
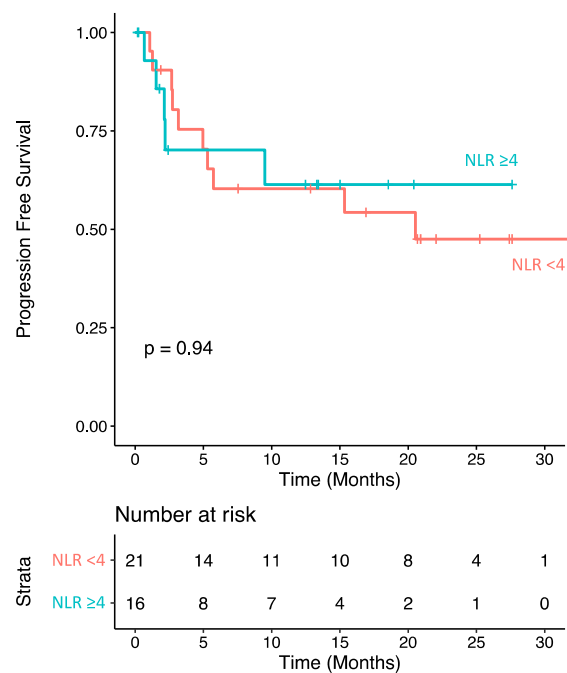


**Figure S7. The combination of tumor PD-L1 expression and PD-L1 index did not improve the PFS nor OS prediction at baseline.**

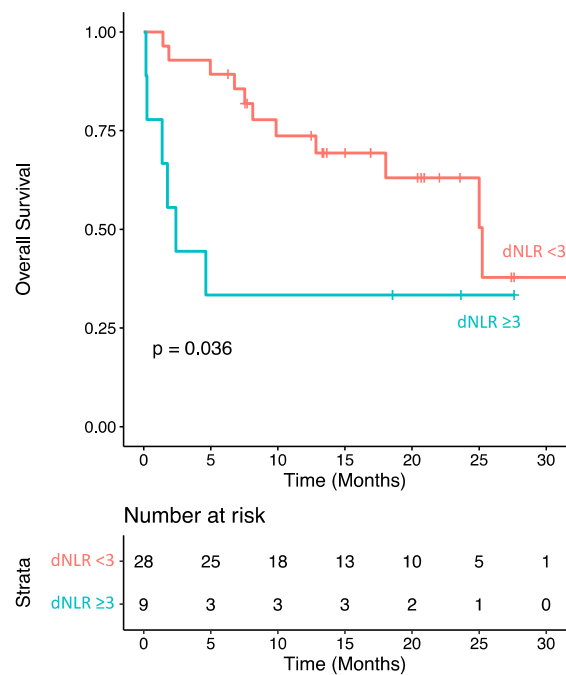
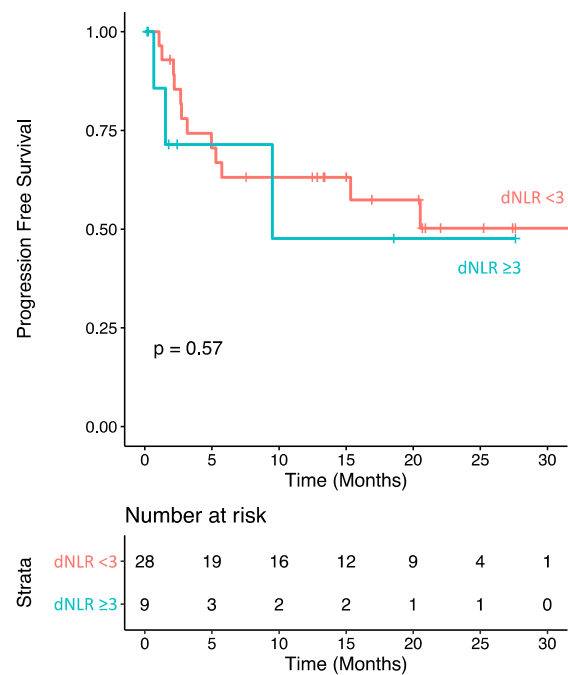




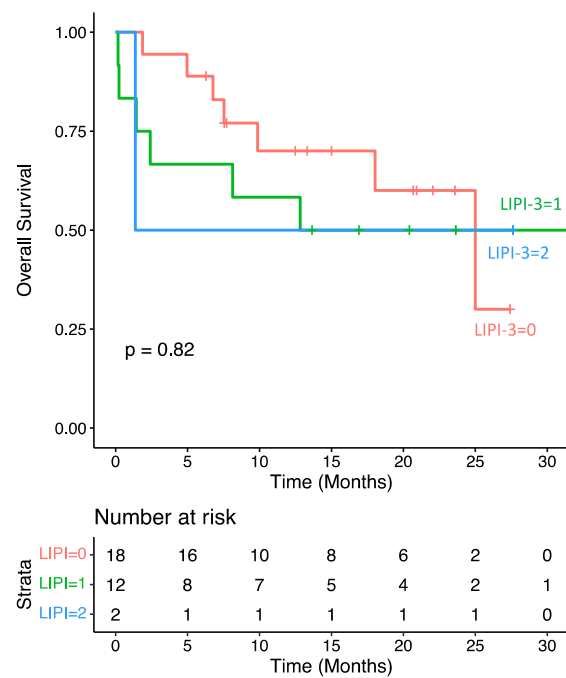
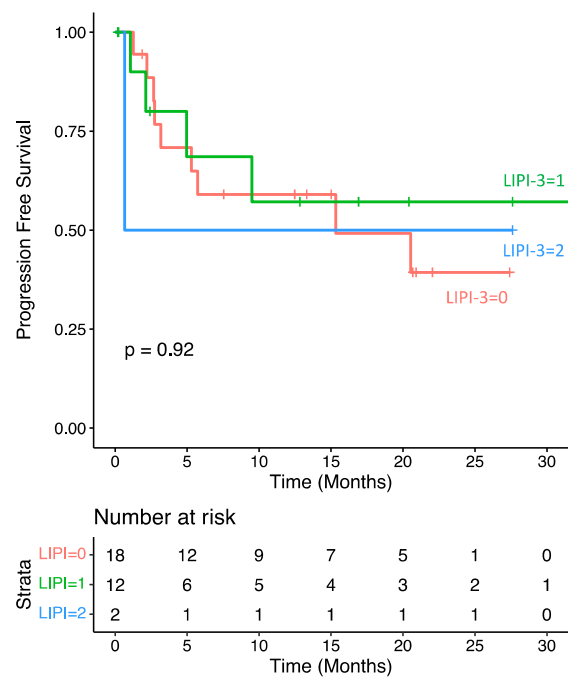
**Figure S8. The presence of driver mutations combined with PD-L1 index did not improve the PFS nor OS prediction at baseline.**



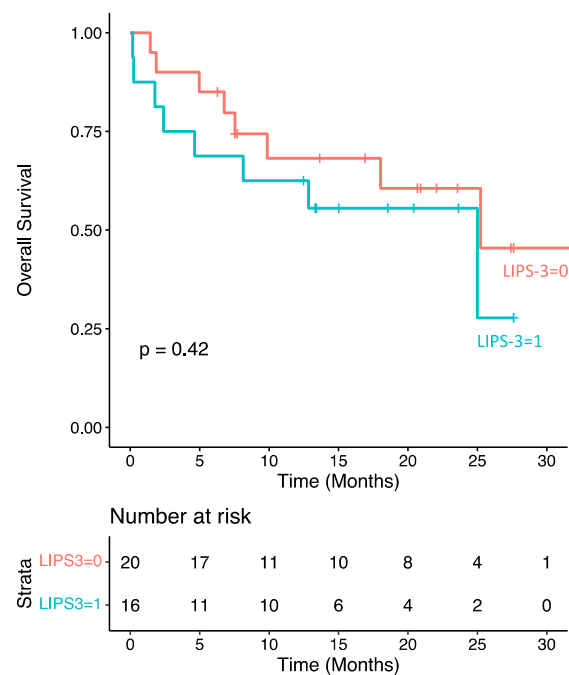
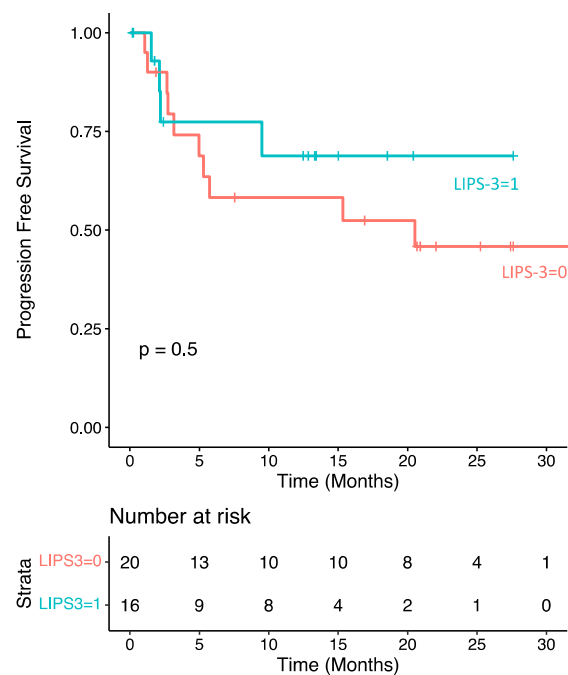
**Figure S9. Neutrophil-to-lymphocyte ratio (NLR) did not predict PFS nor OS at baseline.**



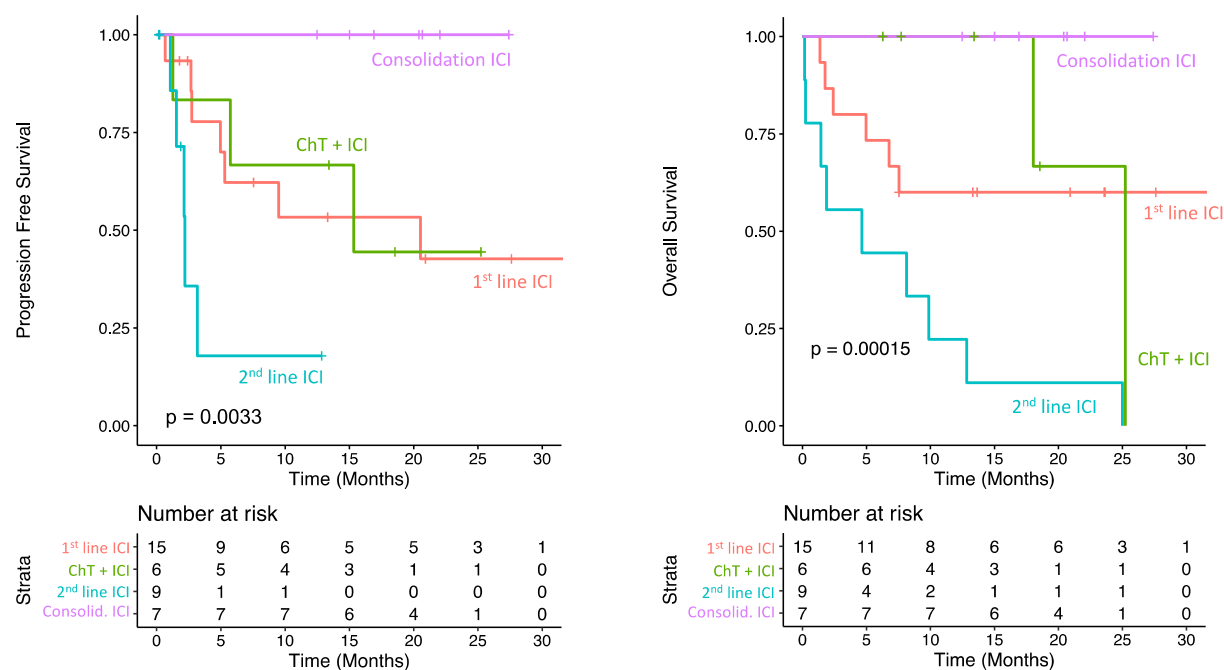
**Figure S10. Derived neutrophil-to-lymphocyte ratio (dNLR) did not predict PFS nor OS at baseline.**



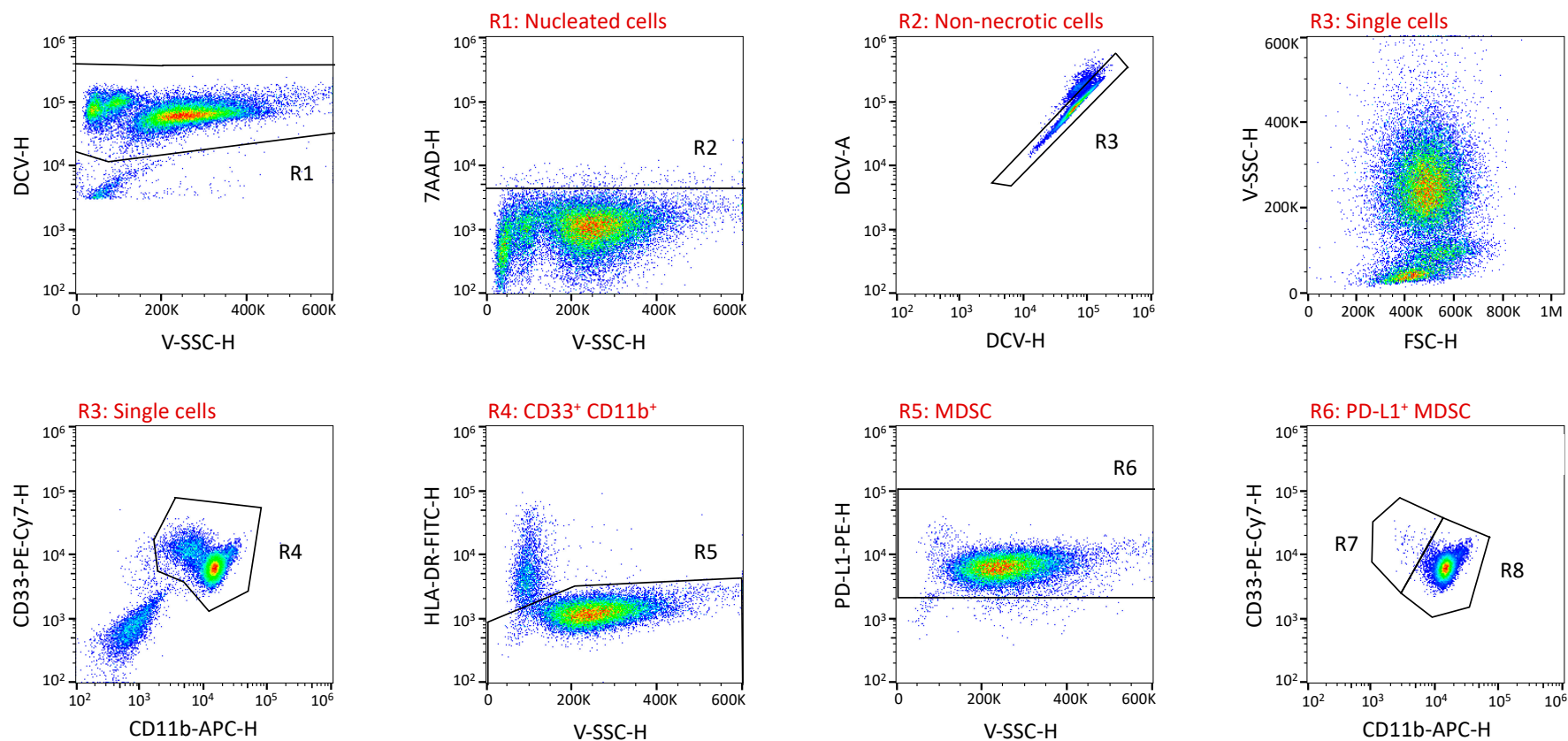
**Figure S11. Lung immune prognostic index (LIPI) did not predict PFS nor OS at baseline.**



**Figure S12. Lung immuno-oncology prognostic score-3 (LIPS-3) did not predict PFS nor OS at baseline.**



**Figure S13. Treatment-strategy influence on PFS and OS.** In first-line ICI and first-line chemotherapy plus ICI (ChT + ICI), pembrolizumab is administered. In second-line ICI the therapy indication is pembrolizumab, nivolumab and atezolizumab, and in consolidation ICI after chemoradiation, durvalumab is administered.



**Figure S14. Flow cytometry panel and gating strategy for identification and classification of PD-L1<sup>+</sup> MDSCs.** Representative analysis of a peripheral blood sample from an NSCLC patient. Nucleated cells are selected based on DCV<sup>+</sup> events (R1) and both necrotic cells (R2) and doublets (R3) are excluded from the analysis. CD33<sup>+</sup>CD11b<sup>+</sup>HLA-DR<sup>-/lo</sup> events are then selected as MDSCs (R4-5). PMA-stimulated cells are highly reactive to the anti-PD-L1 monoclonal antibody and are selected as PD-L1<sup>+</sup> MDSCs (R6). MDSCs can be further classified into monocytic (R7) and polymorphonuclear MDSCs (R8).

**Table S1. Univariate PFS analysis**



Variables			Stratified Kaplan-Meier model				Cox regression			
	Stratum value	N	PD events (%)	Censored (%)	Median (95% CI)	Log-rank (p-value)	N	Contrast	Pr > ChiSq	Hazard Ratio (95% CI)
PD-L1 index groups	High PD-L1 index	24	14 (58.33%)	10 (41.67%)	5.73 (2.67,20.53)	0.0042	37	PD-L1 index: High vs Low PD-L1 index	0.0212	11.01 (1.43,84.67)
	Low PD-L1 index	13	1 (7.69%)	12 (92.31%)	NR (NR,NR)					
Sex	Female	10	1 (10.00%)	9 (90.00%)	NR (20.53,NR)	0.0025	37	Sex: Male vs Female	0.0143	13.44 (1.68,107.53)
	Male	27	14 (51.85%)	13 (48.15%)	5.73 (2.67,NR)					
Age	≤68 years	19	8 (42.11%)	11 (57.89%)	20.53 (3.17,NR)	0.9048	37	Age: >68 vs ≤68 years	0.9046	1.06 (0.38,2.95)
	>68 years	18	7 (38.89%)	11 (61.11%)	15.33 (2.73,NR)					
Smoker	No	2	1 (50.00%)	1 (50.00%)	NR (3.17,NR)	0.9975	37	Smoker: Yes vs No	0.9975	1.00 (0.13,7.62)
	Yes	35	14 (40.00%)	21 (60.00%)	20.53 (5.30,NR)					
Former smokers	No	13	6 (46.15%)	7 (53.85%)	20.53 (3.17,NR)	0.9142	37	Former smokers: Yes vs No	0.9137	0.94 (0.34,2.66)
	Yes	24	9 (37.50%)	15 (62.50%)	NR (2.73,NR)					
Current smokers	No	26	10 (38.46%)	16 (61.54%)	NR (2.73,NR)	0.9119	37	Current smokers: Yes vs No	0.9119	1.06 (0.36,3.11)
	Yes	11	5 (45.45%)	6 (54.55%)	20.53 (1.27,NR)					
PS (ECOG)	0	11	4 (36.36%)	7 (63.64%)	NR (3.17,NR)	0.4236	37	Performance status (ECOG): 1,2 vs 0	0.4279	1.59 (0.50,5.01)
	1,2	26	11 (42.31%)	15 (57.69%)	15.33 (2.73,NR)					
	IV	25	13 (52.00%)	12 (48.00%)	15.33 (2.73,NR)					
Cancer staging	III	12	2 (16.67%)	10 (83.33%)	NR (9.50,NR)	0.0558	37	Cancer staging: IV vs III	0.0757	3.87 (0.87,17.21)
	IV	25	13 (52.00%)	12 (48.00%)	15.33 (2.73,NR)					
Histology	Adenocarcinoma	24	10 (41.67%)	14 (58.33%)	NR (5.30,NR)	0.4614	37	Histology: Adenocarcinoma vs Squamous cell carcinoma	0.4641	0.67 (0.23,1.97)
	Squamous cell carcinoma	13	5 (38.46%)	8 (61.54%)	NR (1.53,NR)					
Number of metastases	1 metastasis	17	9 (52.94%)	8 (47.06%)	15.33 (2.73,NR)	0.6687	25	Number of metastases: ≥2 vs 1 metastasis	0.6695	1.30 (0.39,4.26)
	≥2 metastases	8	4 (50.00%)	4 (50.00%)	NR (0.67,NR)					

Variables			Stratified Kaplan-Meier model				Cox regression			
	Stratum value	N	PD events (%)	Censored (%)	Median (95% CI)	Log-rank (p-value)	N	Contrast	Pr > ChiSq	Hazard Ratio (95% CI)
KRAS mutation	No	28	11 (39.29%)	17 (60.71%)	NR (3.17,NR)	0.8396	37	KRAS mutation: Yes vs No	0.8397	1.13 (0.36,3.54)
	Yes	9	4 (44.44%)	5 (55.56%)	20.53 (0.67,NR)					
BRAF mutation	No	34	14 (41.18%)	20 (58.82%)	20.53 (4.97,NR)	0.5744	37	BRAF mutation: Yes vs No	0.5796	0.56 (0.07,4.30)
	Yes	3	1 (33.33%)	2 (66.67%)	NR (9.50,NR)					
TP53 mutation	No	33	13 (39.39%)	20 (60.61%)	NR (5.30,NR)	0.4095	37	TP53 mutation: Yes vs No	0.4169	1.87 (0.41,8.43)
	Yes	4	2 (50.00%)	2 (50.00%)	NR (1.27,NR)					
ICI duration	≤4.6 months	19	11 (57.89%)	8 (42.11%)	2.73 (1.53,5.30)	0.0001	37	ICI duration: >4.6 vs ≤4.6 months	<b>0.0009</b>	0.13 (0.04,0.44)
	>4.6 months	18	4 (22.22%)	14 (77.78%)	NR (20.53,NR)					
LDH	≤247 U/L	21	10 (47.62%)	11 (52.38%)	15.33 (3.17,NR)	0.5002	32	LDH: ≤247 U/L vs >247 U/L	0.5031	1.49 (0.47,4.76)
	>247 U/L	11	4 (36.36%)	7 (63.64%)	NR (1.07,NR)					
Tumoral PD-L1	<50%	18	7 (38.89%)	11 (61.11%)	NR (2.20,NR)	0.9614	37	Tumoral PD-L1: ≥50% vs <50%	0.9615	1.03 (0.37,2.84)
	≥50%	19	8 (42.11%)	11 (57.89%)	20.53 (2.73,NR)					

PD, progressive disease; CI, confidence interval; Pr>ChiSq, Chi-Squared test; NR, not reached; PS, Performance status. Significant p-values are highlighted in bold.

**Table S2. Multivariate PFS analysis**

Variable		Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
<b>PD-L1 index groups</b>	High vs low PD-L1 index	1.47016	1.11181	1.7485	0.1861	4.350	0.492	38.447
<b>Sex</b>	Male vs Female	2.04976	1.24156	2.7256	0.0987	7.766	0.681	88.515
<b>ICI duration</b>	>4.6 vs ≤4.6 months	-1.05690	0.70383	2.2549	0.1332	0.348	0.087	1.381
<b>Cancer staging</b>	IV vs III stage	1.66037	0.94361	3.0962	0.0785	5.261	0.828	33.442

Pr>ChiSqr, Chi-Squared test. Significant p-values are highlighted in bold.

**Table S3. Univariate analysis for overall survival**

Variable		Stratified Kaplan-Meier model					Cox regression			
	Stratum value	N	PD events N(%)	Censored N(%)	Median (CI 95%)	Log-rank (p- value)	N	Contrast	Pr > ChiSq	Hazard Ratio (CI 95%)
PD-L1 index groups	High PD-L1 Index	24	14 (58.33%)	10 (41.67%)	18.03 (6.77,25.23)	0.0348	37	PD-L1 index: High vs Low PD-L1 Index	0.0462	3.72 (1.02, 13.56)
	Low PD-L1 Index	13	3 (23.08%)	10 (76.92%)	NR (1.87,NR)					
Sex	Female	10	1 (10.00%)	9 (90.00%)	NR (25.23,NR)	0.0007	37	Sex: Male vs Female	0.0273	30.86 (1.47, 648.28)
	Male	27	16 (59.26%)	11 (40.74%)	9.87 (4.63,25.00)					
Age	≤68 years	19	8 (42.11%)	11 (57.89%)	25.00 (7.53,NR)	0.5156	37	Age: >68 vs ≤68 years	0.5173	1.37 (0.53, 3.57)
	>68 years	18	9 (50.00%)	9 (50.00%)	18.03 (4.63,NR)					
Smoker	No	2	2 (100.00%)	0 (0.00%)	17.55 (9.87,25.23)	0.5673	37	Smoker: Yes vs No	0.5702	0.65 (0.15, 2.89)
	Yes	35	15 (42.86%)	20 (57.14%)	25.00 (8.13,NR)					
Former smoker	No	13	5 (38.46%)	8 (61.54%)	25.23 (6.77,NR)	0.5150	37	Former smoker: Yes vs No	0.5171	1.41 (0.50, 4.01)
	Yes	24	12 (50.00%)	12 (50.00%)	25.00 (4.97,NR)					
Current smoker	No	26	14 (53.85%)	12 (46.15%)	25.00 (7.53,NR)	0.3003	37	Current smoker: Yes vs No	0.3087	0.52 (0.15, 1.82)
	Yes	11	3 (27.27%)	8 (72.73%)	NR (2.40,NR)					
Performance status (ECOG)	0	11	5 (45.45%)	6 (54.55%)	25.00 (6.77,NR)	0.5607	37	Performance status (ECOG): 1,2 vs 0	0.5623	1.36 (0.48, 3.89)
	1,2	26	12 (46.15%)	14 (53.85%)	18.03 (4.97,NR)					
Cancer staging	III	12	4 (33.33%)	8 (66.67%)	25.23 (1.87,NR)	0.3298	37	Cancer staging: IV vs III	0.3359	1.74 (0.56, 5.33)
	IV	25	13 (52.00%)	12 (48.00%)	18.03 (6.77,NR)					
Histology	Adenocarcinoma	24	9 (37.50%)	15 (62.50%)	25.23 (12.83,NR)	0.1066	37	Histology: Adenocarcinoma vs Squamous	0.1151	0.46 (0.18, 1.21)
	Squamous	13	8 (61.54%)	5 (38.46%)	9.87 (1.43,NR)					

Variable		Stratified Kaplan-Meier model					Cox regression			
	Stratum value	N	PD events N(%)	Censored N(%)	Median (CI 95%)	Log-rank (p- value)	N	Contrast	Pr > ChiSq	Hazard Ratio (CI 95%)
Number of metastases	1 Metastasis	17	9 (52.94%)	8 (47.06%)	12.83 (6.77,NR)	0.8857	25	Number of metastases: ≥2 vs 1 metastases	0.8857	0.91 (0.27, 3.11)
	≥2 Metastases	8	4 (50.00%)	4 (50.00%)	25.00 (1.37,NR)					
KRAS mutation	No	28	14 (50.00%)	14 (50.00%)	25.00 (8.13,NR)	0.6378	37	KRAS mutation: Yes vs No	0.6392	0.74 (0.21, 2.59)
	Yes	9	3 (33.33%)	6 (66.67%)	NR (1.37,NR)					
BRAF mutation	No	34	16 (47.06%)	18 (52.94%)	25.00 (7.53,NR)	0.6103	37	BRAF mutation: Yes vs No	0.6143	0.59 (0.08, 4.52)
	Yes	3	1 (33.33%)	2 (66.67%)	NR (12.83,NR)					
TP53 mutation	No	33	15 (45.45%)	18 (54.55%)	25.23 (8.13,NR)	0.9201	37	TP53 mutation: Yes vs No	0.9201	0.93 (0.21, 4.14)
	Yes	4	2 (50.00%)	2 (50.00%)	25.00 (2.40,NR)					
ICI duration	≤4.6 months	19	15 (78.95%)	4 (21.05%)	6.77 (1.77,12.83)	<b>&lt;.0001</b>	37	ICI duration: >4.6 vs ≤4.6 months	<b>0.0012</b>	0.03 (0.00, 0.26)
	>4.6 months	18	2 (11.11%)	16 (88.89%)	NR (25.23,NR)					
LDH	≤247 U/L	21	10 (47.62%)	11 (52.38%)	25.00 (6.77,NR)	0.4208	32	LDH: ≤247 U/L vs >247 U/L	0.4251	1.61 (0.50, 5.20)
	>247 U/L	11	4 (36.36%)	7 (63.64%)	NR (1.43,NR)					
Tumoral PD-L1	<50%	18	9 (50.00%)	9 (50.00%)	25.00 (8.13,25.23)	0.6714	37	Tumoral PD-L1: ≥50% vs <50%	0.6720	0.81 (0.31, 2.14)
	≥50%	19	8 (42.11%)	11 (57.89%)	NR (2.40,NR)					

PD, progressive disease; CI, confidence interval; Pr>ChiSq, Chi-Squared test; NR, not reached; PS, Performance status. Significant p-values are highlighted in bold.

**Table S4. Multivariate analysis for overall survival**

Variable		Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
PD-L1 index groups	High vs Low PD-L1 index	0.33590	0.70740	0.2255	0.6349	1.399	0.350	5.598
Sex	Male vs Female	15.63440	1731	0.0001	0.9928	6165031	0.000	.
ICI duration	>4.6 vs ≤4.6 months	-2.15709	1.05814	4.1558	0.0415	0.116	0.015	0.920

Pr>ChiSq, Chi-Squared test. Significant p-values are highlighted in bold.