

Supplemental tables

Parameter	CD4 High No. (%)	CD4 Low No. (%)	P value	CD8 High No. (%)	CD8 Low No. (%)	P value	CD20 High No. (%)	CD20 Low No. (%)	P value
Age, y									
<65	37 (51.4%)	35 (48.6%)	0.99	36 (50.0%)	36 (50.0%)	0.74	37 (51.4%)	35 (48.6%)	0.99
≥65	38 (52.1%)	35 (47.9%)		39 (53.4%)	34 (46.6%)		38 (52.1%)	35 (47.9%)	
Sex									
Male	33 (42.9%)	44 (57.1%)	0.04	38 (46.8%)	41 (53.2%)	0.43	39 (50.7%)	38 (49.3%)	0.87
Female	49 (60.5%)	32 (39.5%)		44 (54.3%)	37 (45.7%)		43 (53.1%)	38 (46.9%)	
Smoker									
No	13 (43.3%)	17 (56.7%)	0.42	14 (46.7%)	16 (53.3%)	0.84	15 (50.0%)	15 (50.0%)	0.84
Yes	66 (52.4%)	60 (47.6%)		64 (50.8%)	62 (49.2%)		66 (52.4%)	60 (47.6%)	
Histology									
ADC	59 (55.7%)	47 (44.3%)	0.46	51 (48.1%)	55 (51.9%)	0.99	60 (56.6%)	46 (43.4%)	0.09
SCC	19 (47.5%)	21 (52.5%)		19 (47.5%)	21 (52.5%)		16 (40.0%)	24 (60.0%)	
Stage									
I-II	18 (56.3%)	14 (43.7%)	0.69	17 (53.1%)	15 (46.9%)	0.84	17 (53.1%)	15 (46.9%)	0.85
III-IV	63 (50.8%)	61 (49.2%)		62 (50.0%)	62 (50.0%)		63 (50.8%)	61 (49.2%)	
PD-L1 Status									
PD-L1<1%	23 (39.7%)	35 (60.3%)	0.05	20 (34.5%)	38 (65.5%)	0.0026	28 (48.3%)	30 (51.7%)	0.86
PD-L1≥1%	42 (58.3%)	30 (41.7%)		45 (62.5%)	27 (37.5%)		37 (51.4%)	35 (48.6%)	

Table S1. Association between TILs density and clinicopathological variables.

Variable	Stratification	#Cases TCR Burden Cohort
Age	<65	99 (51.6%)
	≥65	93 (48.4%)
Gender	Male	87 (45.3%)
	Female	105 (54.7%)
Smoking	Smoker	167 (87.0%)
	Non-Smoker	25 (13.0%)
Stage	I-II	23 (13.1%)
	III-IV	152 (86.9%)
Histology	Adenocarcinoma	149 (77.6%)
	Squamous	30 (15.6%)
	Other	13 (6.8%)
PD-L1 Status	PD-L1<1%	79 (41.1%)
	PD-L1≥1%	113 (58.9%)
Treatment	Nivolumab	95 (49.5%)
	Pembrolizumab	91 (47.4%)
	Atezolizumab	6 (3.1%)

Table S2. Clinicopathological description of TCR burden cohort

Parameter	TIM-3		P value	PD-1		P value	LAG-3		P value
	High No. (%)	TIM-3 Low No. (%)		High No. (%)	Low No. (%)		High No. (%)	Low No. (%)	
Age, y									
<65	43 (53.1%)	38 (46.9%)	0.53	45 (55.6%)	36 (44.4%)	0.76	42 (51.8%)	39 (48.2%)	0.88
≥65	40 (47.6%)	44 (52.4%)		44 (52.4%)	40 (47.6%)		42 (50.0%)	42 (50.0%)	
Sex									
Male	47 (56.0%)	37 (44.0%)	0.10	42 (50.0%)	42 (50.0%)	0.45	42 (50.0%)	42 (50.0%)	0.88
Female	39 (57.1%)	52 (42.9%)		51 (56.0%)	40 (44.0%)		44 (48.4%)	47 (51.6%)	
Smoker									
No	18 (50.0%)	18 (50.0%)	0.99	18 (50.0%)	18 (50.0%)	0.71	23 (63.9%)	13 (36.1%)	0.06
Yes	68 (49.3%)	70 (50.7%)		74 (53.6%)	64 (46.4%)		62 (44.9%)	76 (55.1%)	
Histology									
ADC	59 (49.2%)	61 (50.8%)	0.59	68 (56.7%)	52 (43.3%)	0.21	60 (50.0%)	60 (50.0%)	0.21
SCC	23 (54.8%)	19 (45.2%)		19 (45.2%)	23 (54.8%)		16 (38.1%)	26 (61.9%)	
Stage									
I-II	16 (54.3%)	19 (45.7%)	0.71	17 (48.6%)	18 (51.4%)	0.45	14 (40.0%)	21 (60.0%)	0.70
III-IV	69 (50.0%)	69 (50.0%)		78 (56.5%)	60 (43.5%)		62 (44.9%)	76 (55.1%)	
PD-L1 Status									
PD-L1<1%	25 (34.7%)	47 (65.3%)	0.0007	34 (47.2%)	38 (52.8%)	0.64	30 (42.9%)	40 (57.1%)	0.15
PD-L1≥1%	54 (62.8%)	32 (37.2%)		44 (51.2%)	42 (48.8%)		47 (54.7%)	39 (45.3%)	

Table S3. Association between expression of exhaustion markers and clinicopathological variables.

Parameter	RQI High No. (%)	RQI Low No. (%)	P value
Age, y			
<65	41 (56.9%)	31 (43.1%)	0.19
≥65	34 (46.0%)	40 (54.0%)	
Sex			
Male	41 (53.3%)	36 (46.7%)	0.75
Female	41 (50.6%)	40 (49.4%)	
Smoker			
No	19 (63.3%)	11 (36.7%)	0.16
Yes	62 (48.8%)	65 (51.2%)	
Histology			
ADC	58 (54.7%)	48 (45.3%)	0.46
SCC	19 (47.5%)	21 (52.5%)	
Stage			
I-II	15 (46.9%)	17 (53.1%)	0.56
III-IV	66 (53.2%)	58 (46.8%)	
PD-L1 Status			
PD-L1<1%	26 (45.6%)	31 (54.4%)	0.11
PD-L1>1%	44 (61.1%)	28 (38.9%)	

Table S4. Association between RQI and clinicopathological variables.

Supplementary figures legend

Figure S1. Antibody validation and batch correlation.

Figure S2. Cox Continuous Univariate Analysis for TIL density (**A and B**) and for exhaustion markers in the whole cohort (**C and D**) in PD-L1 positive patients (**E and F**) and in negative patients (**G and H**).

Figure S3. TLS representation in the cohort and association with number of FOV. Kaplan-Meier survival analysis for TIL density based on PD- L1 status.

Figure S4. A RQI for CD4, CD8, CD20, tumor cells, stromal cells, immune cells and integrated. **B-G** Results show the association between density of individual cells and integrated RQI.

Figure S5. Survival analysis for RQI of individual cells.

