

Supplementary Material 1. List of antibodies for immunohistochemistry.

Serial tissue sections (5µm thickness) were cut from formalin-fixed and paraffin-embedded blocks containing representative sections and stained using a Roche Ventana Benchmark Ultra automated slide stainer (Ventana Medical Systems, Roche, France) with the Ultraview DAB IHC Detection Kit (Roche, France) according to the manufacturer's instructions. Prediluted primary antibodies were detected using a diaminobenzidine (DAB) staining and nuclei were counterstained with haematoxylin. Internal negative controls were established for each marker by omitting the primary antibody from reaction.

Cell marker	Species Isotype	Company (Clone)
PD-L1	Mouse monoclonal	Roche Diagnostics (SP263)
PD-1	Mouse monoclonal	Roche Diagnostics (NAT105)
CD3	Rabbit Monoclonal	Roche Diagnostics (2GV6)
CD4	Rabbit Monoclonal	Roche Diagnostics (SP35)
CD8	Rabbit Monoclonal	Roche Diagnostics (SP57)

PD-L1: Programmed Cell Death Ligand 1; PD-1: Programmed Cell Death 1; CD: Cluster of Differentiation.

Supplementary Material 2. QuPath settings.

Specificity of staining was checked by pathologists in every case. Technical set-up for image processing and image analysis as well as the quantitative study were performed by GB and SDA. Compared to similar studies, large areas were analysed (range 4–10 mm²), to overcome the intratumor variability. To ensure the reproducibility of our data obtained through digital analysis, specimens were randomly selected and manually checked for plausibility.

Parameter	CD3 DAB	CD4 DAB	CD8 Red	PD-1 DAB
Scan magnification	20x	20x	20x	40x
Stain vectors	Pick manually	QuPath default	QuPath default	QuPath default
Detection image	Hematox OD	Hematox OD	Optical Density Sum	Hematox OD
Requested pixel size	0.5	0.5	0.5	0.5
Background radius	8	8	8	8
Median filter radius	0	0	0	0
Sigma	1.5	1.5	1.5	1
Minimum area	10	10	10	10
Maximum area	100	100	100	100
Intensity threshold	0.1	0.1	0.1	0.1
Max. background intensity	2	2	2	2
Split by shape	on	on	on	On
Exclude DAB	off	off	off	Off
Cell expansion	1.5	1.5	1.5	1.5
Include cell nucleus	on	on	on	on
Smooth boundaries	on	on	on	on
Make measurements	on	on	on	on
Score compartment	Nucleus DAB OD mean	Nucleus DAB OD mean	Nucleus DAB OD mean	Nucleus DAB OD mean
Threshold 1+	0.3	0.6	0.8	0.3
single threshold	on	on	on	on

Supplementary Material 3. List of antibodies for FACS analysis.

Cell marker	Isotype	Company (Clone)
PD-1	mouse IgG1κ	BD Biosciences (MIH4)
CD3	mouse IgG2aκ	Miltenyi Biotec (BW264/56)
CD4	mouse IgG2aκ	Miltenyi Biotec (VIT4)
CD8	recombinant human IgG1	Miltenyi Biotec (REA734)
CD56	mouse IgG1κ	Miltenyi Biotec (AF12-7H3)
CD16	recombinant human IgG1	Miltenyi Biotec (REA423)
CD19	recombinant human IgG1	Miltenyi Biotec (REA675)
Granzyme B	recombinant human IgG1	Miltenyi Biotec (REA226)
Ki67	recombinant human IgG1	Miltenyi Biotec (REA183)
Perforin	recombinant human IgG1	Miltenyi Biotec (REA1061)
CD25	mouse IgG2bκ	Miltenyi Biotec (4E3)
FOXP3	recombinant human IgG1	Miltenyi Biotec (REA1253)

Supplementary Table 1.
Quantitative immunohistochemical parameters.

TIME Parameters (n= 115)	Median	Mean $\pm$ SD	Range
TILs, n/mm²			
CD3+	725.00	865.91 $\pm$ 629.13	45.89 – 3488.89
<i>i</i>CD3+	475.26	593.73 $\pm$ 477.68	34.46 – 2507.50
sCD3+	1034.12	179.83 $\pm$ 187.04	96.00 – 5896.96
CD4+	522.20	638.59 $\pm$ 516.73	38.00 – 2626.20
<i>i</i>CD4+	341.25	471.58 $\pm$ 45.56	17.19 – 2430.22
sCD4+	791.94	920.88 $\pm$ 727.80	29.78 – 3454.55
CD8+	297.62	393.14 $\pm$ 323.52	7.50 – 1593.25
<i>i</i>CD8+	175.13	295.84 $\pm$ 287.09	6.30 – 1647.69
sCD8+	415.70	512.72 $\pm$ 417.95	5.83 – 2230.00
PD-1 +	131.25	179.83 $\pm$ 187.04	8.67 – 1101.75
<i>i</i>PD-1 +	84.50	158.20 $\pm$ 212.83	1.05 – 1520.00
sPD-1 +	151.57	241.33 $\pm$ 287.09	8.00 – 1721.43

TILs: Tumor Infiltrating Lymphocytes; *i*: intratumoral; s: stromal; SD: standard Deviation

Supplementary Table 2.
FACS parameters.

PB (<i>n</i>= 115), n/mm³	Median	Mean ± SD	Range
CD3+	1226.36	1255.52 ± 476.22	223.20 – 2516.64
CD3+CD4+	718.59	749.57 ± 327.98	130.12 – 1876.38
CD3+CD8+	377.56	410.78 ± 242.47	86.82 – 1482.81
CD14+	395.25	462.21 ± 313.67	73.86 – 2359.07
CD19+	358.87	438.19 ± 313.38	1.90 – 1392.79
CD3-CD56+CD16+ NKs			
bright	14.87	17.67 ± 11.58	4.12 – 77.24
dim	264.66	312.19 ± 224.73	26.07 – 1905.12
CD4+CD25+FOXP3+ Tregs	81.90	81.90 ± 62.51	5.29 – 281.10
CD4+PD-1+	93.61	102.72 ± 51.03	27.66 – 321.35
CD4+GnZB+	32.73	51.73 ± 53.18	0 – 276.75
CD4+Perf+	18.75	27.23 ± 25.12	2.91 – 144.94
CD4+Ki67+	22.35	26.17 ± 15.54	3.6 – 84.33
CD8+PD-1+	56.21	80.55 ± 71.64	10.76 – 487.02
CD8+GnZB+	191.03	220.47 ± 191.12	14.58 – 1033.43
CD8+Perf+	138.84	182.05 ± 161.85	12.85 – 981.46
CD8+Ki67+	9.34	10.78 ± 7.19	2.49 – 45.12

Supplementary Table 3.
Univariate Cox regression on clinicopathological and TIME parameters.

Clinicopathological Parameters	Univariate Analysis		
	HR	95% CI	<i>p</i>
Gender , female vs. male	1.409	0.748 – 2. 654	0.288
Age , < 65 vs. ≥ 65 years	0.880	0.440 - 1.763	0.719
Histology ADC vs. SCC	0.704	0.317 – 1.563	0.388
Smoking Status (never vs current/former)	2.199	0.677 – 7.145	0.190
TNM Stage (I vs. II vs. III)	2.481	1.726 – 3.566	<0.001
Lymphovascular Invasion (no vs. yes)	2.678	1.307 – 5.485	0.007
Pleural Invasion (no vs. yes)	2.053	1.089 – 3.868	0.026

TIME Parameters n/mm ²	Univariate Analysis		
	HR	95% CI	<i>P</i>
CD3+ ≥ 725.00 high vs. low	0.290	0.140 – 0.601	<0.001
<i>i</i>CD3+ ≥ 475.26	0.366	0.181 – 0.741	0.005
<i>s</i>CD3+ ≥ 1034.12	0.335	0.166 – 0.679	0.002
CD4+ ≥ 516.73	0.246	0.115 – 0.524	<0.001
<i>i</i>CD4+ ≥ 481.20	0.417	0.208 – 0.836	0.014
<i>s</i>CD4+ ≥ 727.80	0.248	0.116 – 0.528	<0.001
CD8+ ≥ 323.52	0.197	0.086 – 0.450	<0.001
<i>i</i>CD8+ ≥ 175.13	0.243	0.111 – 0.534	<0.001
<i>s</i>CD8+ ≥ 417.95	0.233	0.106 – 0.511	<0.001
PD-1 + ≥ 131.25	2.604	1.278 – 5.307	0.008
<i>i</i>PD-1+, ≥ 84.50	1.719	0.878 – 3.365	0.114
<i>s</i>PD-1+, ≥ 151.57	1.760	0.899 – 3.44	0.099
PD-1-to-CD8 ratio ≥ 0.5	3.623	1.771 – 7.410	<0.001
<i>i</i>-PD-1-to-CD8 ratio ≥ 0.5	3.343	1.631 – 6.849	<0.001
<i>s</i>-PD-1-to-CD8 ratio ≥ 0.5	5.397	2.446 – 11.910	<0.001
PD-L1 expression (neg vs. pos vs. high)	1.366	0.854 – 2.186	0.193

Supplementary Table 4.
Univariate Cox regression on peripheral blood parameters.

PB Parameters n/mm ³	Univariate Analysis		
	HR	95% CI	<i>p</i>
CD3+, high vs low	0.545	0.280 – 1.060	0.074
CD3+CD4+ high	0.514	0.422 – 1.540	0.806
CD3+CD8+ high	0.570	0.293 – 1.109	0.098
CD14+ high	1.181	0.619 – 2.250	0.614
CD19+ high	0.756	0.396 – 1.444	0.397
CD3-CD56+CD16+ NKs	0.845	0.442 – 1.614	0.609
bright high	0.939	0.492 – 1.790	0.848
dim high	0.832	0.436 – 1.590	0.578
CD4+CD25+FOXP3^{high} Tregs high	1.571	0.811 – 3.042	0.181
CD4+PD-1+ high	1.145	0.599 – 2.188	0.681
CD4+GnZB+ high	0.784	0.411 – 1.497	0.461
CD4+Perf+ high	0.943	0.493 – 1.805	0.860
CD4+Ki67+ high	1.108	0.580 – 2.118	0.756
CD8+PD-1+ high	0.677	0.352 – 1.303	0.243
CD8+GnZB+ high	0.540	0.278 – 1.051	0.070
CD8+Ki-67+ high	0.597	0.309 – 1.152	0.124
CD8+Perf+ high	0.721	0.376 – 1.382	0.324

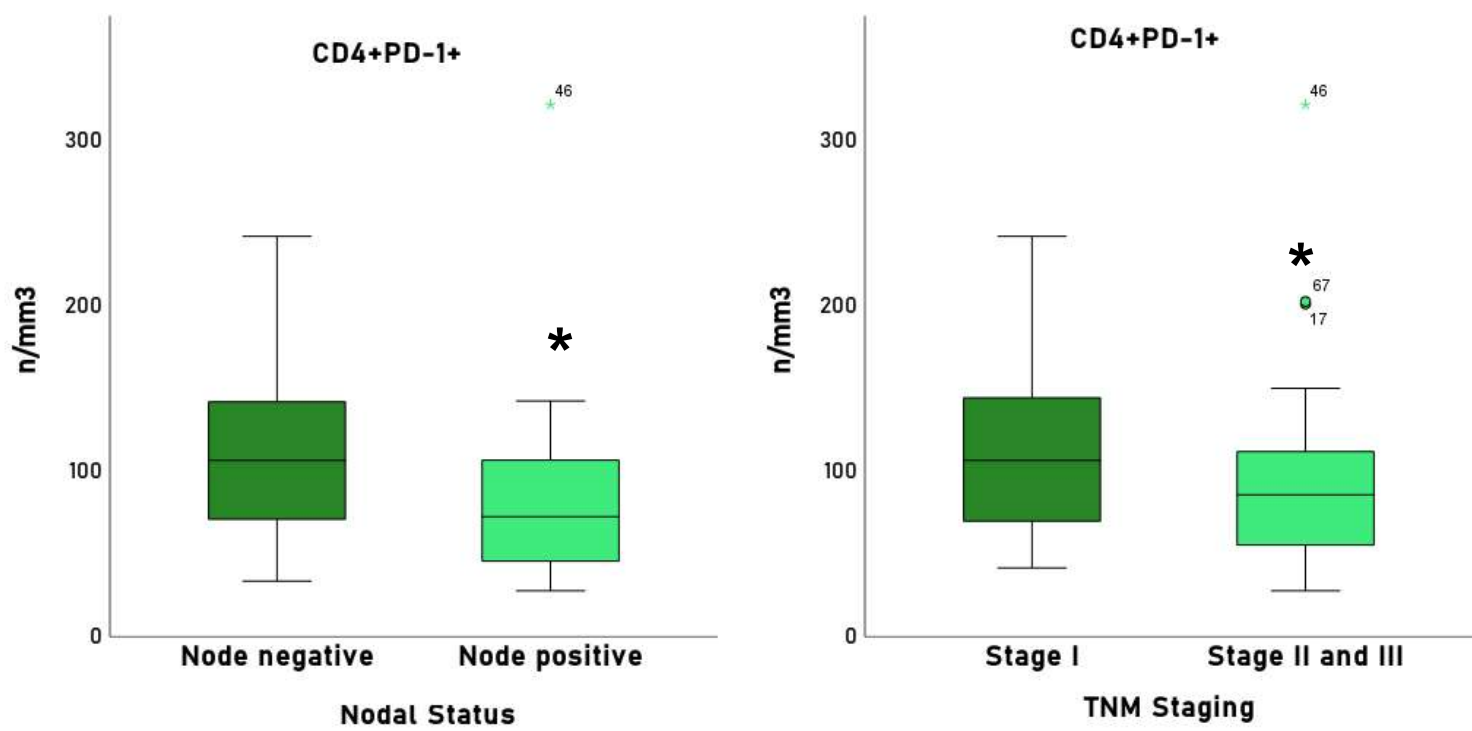
GnZB: granzyme B; Perf: perforin

Supplementary Table 5.
Multivariate Cox regression.

Parameters	Multivariate Analysis		
	HR	95%CI	<i>P</i>
TNM Stage (<i>I vs. II vs. III</i>)	1.551	0.900 – 2.671	0.114
Histology (<i>ADC vs. SCC</i>)	0.232	0.079 – 0.680	0.008
Lymphovascular Invasion (<i>yes vs. no</i>)	1.634	0.694 – 3.846	0.261
CD3+ TILs (<i>high vs. low</i>)	0.508	0.204 – 1.262	0.145
s-CD4+ TILs (<i>high vs. low</i>)	0.603	0.227–1.601	0.310
<i>i</i>-CD8+ TILs (<i>high vs. low</i>)	0.333	0.120–0.920	0.034
PD-1-to-CD8 TILs ratio ≥ 0.5	3.296	1.426–7.621	0.005
PB CD3+ (<i>high vs. low</i>)	0.779	0.304–1.997	0.604
PB CD8+ (<i>high vs. low</i>)	0.523	0.133–2.054	0.353
PB CD8+GnZB+ (<i>high vs. low</i>)	0.785	0.227–2.722	0.703

TILs: tumor infiltrating lymphocytes; s: stromal; *i*: intratumoral; PB: peripheral blood; GnZB: granzyme B

Supplementary Figure 1.
Peripheral Blood Analysis on Circulating Lymphocytes.



Supplementary Figure 2.

Kaplan-Meier curves survival curves considering *stromal*-PD1-to-CD8 ratio.

