

# Concordance, Correlation, and Clinical Impact of Standardized PD-L1 and TIL Scoring in SCCHN

Stijn Jeroen De Keukeleire <sup>1,2,\*</sup>, Tijl Vermassen <sup>1,3,4</sup>, Philippe Deron <sup>5</sup>, Wouter Huvenne <sup>3,5</sup>, Frédéric Duprez <sup>6</sup>, David Creytens <sup>4,7</sup>, Jo Van Dorpe <sup>7</sup>, Liesbeth Ferdinande <sup>7</sup> and Sylvie Rottey <sup>1,3,4</sup>

<sup>1</sup> Department of Medical Oncology, University Hospital Ghent, 9000 Ghent, Belgium; tijl.vermassen@uzgent.be (T.V.); sylvie.rottey@ugent.be (S.R.)

<sup>2</sup> Department of Internal Medicine, University Hospital Brussels, 1090 Jette, Belgium

<sup>3</sup> Drug Research Unit Ghent, Ghent University Hospital, 9000 Ghent, Belgium

<sup>4</sup> Cancer Research Institute Ghent (CRIG), 9000 Ghent, Belgium

<sup>5</sup> Department of Head and Neck Surgery, Ghent University Hospital, 9000 Ghent, Belgium; philippe.deron@uzgent.be (P.D.); wouter.huvenne@uzgent.be (W.H.)

<sup>6</sup> Department of Radiation Oncology, Ghent University Hospital, 9000 Ghent, Belgium; frederic.duprez@uzgent.be

<sup>7</sup> Department of Pathology, Ghent University Hospital, 9000 Ghent, Belgium; david.creytens@uzgent.be (D.C.); jo.vandorpe@uzgent.be (J.V.D.); liesbeth.ferdinande@ugent.be (L.F.)

\* Correspondence: stijn.dekeukeleire@uzbrussel.be; Tel.: +32-2-477-41-11

**Table S1. Paired analysis for CPS**

Tissue	N	Median (range)	P
Biopsy ↔ resection	44	2 (0–60) ↔ 5 (0–70)	0.0085
Resection ↔ lymph node	27	5 (0–100) ↔ 8 (0–100)	0.0495
Biopsy ↔ lymph node	18	1 (0–15) ↔ 8 (0–45)	0.0076
Primary tumor ↔ metastasis	36	5 (0–100) ↔ 8 (0–100)	0.3669
Biopsy ↔ resection ↔ lymph node	11	1 (0–10) ↔ 5 (1–30) ↔ 5 (0–20)	0.0017 *

\* significant difference for biopsy versus resection and for biopsy versus lymph node. CPS, combined positive score.

**Table S2. Correlation for CPS between paired tissue material**

Correlation	n	r	P
Biopsy versus resection	44	0.3537	0.0185
Resection versus lymph node	27	0.8592	<0.0001
Biopsy versus lymph node	18	−0.0281	0.9119
Primary tumor versus metastasis	36	0.7512	<0.0001

CPS, combined positive score.

**Table S3. Association for CPS between paired tissue material***A. Biopsy versus resection*

		Biopsy			<i>P</i>
		<1	1–19	≥20	
<b>Resection</b>	<1	2 (14)	5 (19)	0 (0)	0.1986
	1–19	11 (79)	16 (59)	1 (33)	
	≥20	1 (7)	6 (22)	2 (67)	

*B. Biopsy versus lymph node*

		Biopsy			<i>P</i>
		<1	1–19	≥20	
<b>Lymph node</b>	<1	3 (38)	0 (0)	0 (0)	0.1054
	1–19	4 (50)	8 (80)	0 (0)	
	≥20	1 (13)	2 (20)	0 (0)	

*C. Resection versus lymph node*

		Resection			<i>P</i>
		<1	1–19	≥20	
<b>Lymph node</b>	<1	1 (100)	3 (18)	0 (0)	0.0016
	1–19	0 (0)	13 (77)	3 (33)	
	≥20	0 (0)	1 (6)	6 (67)	

*D. Primary tumor versus metastasis*

		Primary tumors			<i>P</i>
		<1	1–19	≥20	
<b>Metastasis</b>	<1	2 (40)	0 (0)	4 (18)	0.0082
	1–19	1 (20)	6 (67)	2 (9)	
	≥20	2 (40)	3 (33)	16 (73)	

Data denote numbers (% per column). *P* value for bicategorical variables calculated using Fisher Exact test, for tricategorical variables using Chi Square test. CPS, combined positive score.

**Table S4. Association of CPS between primary tumor-based biopsy, primary tumor-based resection and lymph node material**

Case	Biopsy		Resection		Lymph node	
	Continuous	Categorical.	Continuous	Categorical.	Continuous	Categorical.
1	0	<1	5	1–19	0	<1
2	0	<1	5	1–19	0	<1
3	0	<1	1	1–19	2	1–19
4	0	<1	4	1–19	5	1–19
5	0	<1	5	1–19	8	1–19
6	1	1–19	10	1–19	8	1–19
7	2	1–19	2	1–19	5	1–19
8	2	1–19	25	≥20	8	1–19
9	3	1–19	3	1–19	2	1–19
10	4	1–19	30	≥20	20	≥20
11	10	1–19	28	≥20	15	1–19

Samples with perfect concordance according to categorical CPS are indicated in green. Perfect concordance was found in 3/11 (27%) cases. CPS, combined positive score.

**Table S5. Paired analysis for TIL<sub>str</sub>**

Tissue	N	Median (range)	P
Biopsy ↔ resection	41	15 (1–90) ↔ 20 (0–80)	0.2363
Resection ↔ lymph node	18	20 (1–80) ↔ 20 (0–65)	0.8871
Biopsy ↔ lymph node	14	30 (5–90) ↔ 20 (0–50)	0.1167
Primary tumor ↔ metastasis	25	20 (0–80) ↔ 20 (1–65)	0.6263
Biopsy ↔ resection ↔ lymph node	8	30 (5–90) ↔ 20 (5–80) ↔ 18 (0–50)	0.4630

str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S6. Correlation for TIL<sub>str</sub> between paired tissue material**

Correlation	n	r	P
Biopsy versus resection	41	0.4807	0.0015
Resection versus lymph node	18	0.1394	0.5811
Biopsy versus lymph node	14	0.0713	0.8087
Primary tumor versus metastasis	25	0.1671	0.4247

str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S7. Association of TIL<sub>str</sub> between primary tumor-based biopsy, primary tumor-based resection and lymph node material**

Case	Biopsy		Resection		Lymph node	
	Continuous	Categorical.	Continuous	Categorical.	Continuous	Categorical.
1	15	<20	40	≥20	20	≥20
2	20	≥20	20	≥20	10	<20
3	10	<20	5	<20	4	<20
4	–	–	20	≥20	65	≥20
5	5	<20	20	≥20	20	≥20
6	18	<20	50	≥20	–	–
7	40	≥20	20	≥20	50	≥20
8	60	≥20	50	≥20	15	<20
9	20	≥20	–	–	20	≥20
10	15	<20	70	≥20	–	–
11	60	≥20	8	<20	50	≥20

Samples with perfect concordance according categorical TIL<sub>str</sub> are indicated in green. Perfect concordance was found in 4/11 (36%) cases. Of note, TILs could not be determined for 4 cases (one biopsy, one resection and two lymph nodes). str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S8. Correlation between pathological parameters and time**

Samples	n	r	P value
<b>A. <math>\Delta</math>CPS – <math>\Delta</math>time</b>			
All samples	56	–0.0784	0.5658
Biopt versus resection	44	0.1820	0.2370
Biopt versus lymph node	18	0.1217	0.6305
Resection versus lymph node	9	0.0687	0.8605
<b>B. <math>\Delta</math>CPS – <math>\log(\Delta</math>time)</b>			
All samples	56	–0.0758	0.5789
Biopt versus resection	44	0.1492	0.3339
Biopt versus lymph node	18	0.1778	0.4804
Resection versus lymph node	9	–0.0492	0.9000
<b>C. <math>\Delta</math>TIL<sub>str</sub> – <math>\Delta</math>time</b>			
All samples	53	–0.1646	0.2389
Biopt versus resection	41	0.1505	0.3476
Biopt versus lymph node	13	–0.1479	0.6296
Resection versus lymph node	8	–0.0919	0.8287
<b>D. <math>\Delta</math>TIL<sub>str</sub> – <math>\log(\Delta</math>time)</b>			
All samples	53	–0.1428	0.3076
Biopt versus resection	41	0.1167	0.4676
Biopt versus lymph node	13	–0.1463	0.6335
Resection versus lymph node	8	–0.0444	0.9169

$\Delta$ CPS,  $\Delta$ TILs and  $\Delta$ time were calculated as the difference in CPS, in TILs and in time, respectively, between the second and the first specimen type. For ‘all samples’, the specimens with the longest  $\Delta$ time were selected. CPS, combined positive score; str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S9. Correlation between CPS and TIL<sub>str</sub>**

<b>Correlation</b>	<b>n</b>	<b>r</b>	<b>P</b>
<i>A. All samples</i>			
CPS versus TIL <sub>str</sub>	146	0.2067	0.0123
CPS versus log (TIL <sub>str</sub> )	144	0.2151	0.0096
Log (CPS) versus TIL <sub>str</sub>	116	0.2274	0.0141
Log (CPS) versus log (TIL <sub>str</sub> )	116	0.2692	0.0035
<i>B. Biopsy</i>			
CPS versus TIL <sub>str</sub>	53	0.0550	0.6959
CPS versus log (TIL <sub>str</sub> )	53	0.0853	0.5438
Log (CPS) versus TIL <sub>str</sub>	39	0.1799	0.2732
Log (CPS) versus log (TIL <sub>str</sub> )	39	0.1835	0.2634
<i>C. Resection</i>			
CPS versus TIL <sub>str</sub>	62	0.2680	0.0352
CPS versus log (TIL <sub>str</sub> )	61	0.2331	0.0707
Log (CPS) versus TIL <sub>str</sub>	53	0.2508	0.0701
Log (CPS) versus log (TIL <sub>str</sub> )	53	0.2698	0.0507
<i>D. Lymph node</i>			
CPS versus TIL <sub>str</sub>	25	0.1584	0.4494
CPS versus log (TIL <sub>str</sub> )	25	0.2338	0.2607
Log (CPS) versus TIL <sub>str</sub>	21	0.2291	0.3177
Log (CPS) versus log (TIL <sub>str</sub> )	21	0.3983	0.0737
<i>E. Distant metastasis</i>			
CPS versus TIL <sub>str</sub>	6	0.9757	0.0009
CPS versus log (TIL <sub>str</sub> )	5	0.8293	0.0825
Log (CPS) versus TIL <sub>str</sub>	3	0.9737	0.1464
Log (CPS) versus log (TIL <sub>str</sub> )	3	0.9081	0.2750
<i>F. Samples selected for survival analysis</i>			
CPS versus TIL <sub>str</sub>	80	0.2332	0.0373
CPS versus log (TIL <sub>str</sub> )	77	0.2134	0.0623
Log (CPS) versus TIL <sub>str</sub>	68	0.2396	0.0491
Log (CPS) versus log (TIL <sub>str</sub> )	68	0.2870	0.0177

For log (TIL<sub>str</sub>), all cases with TILs = 0% were excluded. For log (CPS), all cases with CPS = 0 were excluded. For log (TIL<sub>str</sub>) and log (CPS), all cases with no TIL<sub>str</sub> = 0% and / or CPS = 0 were excluded. CPS, combined positive score; str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S10. DFS analysis since time of tissue acquirement**

Parameter	Univariate analysis		
	Median DFS (95% CI)	HR (95% CI)	<i>P</i>
<b>T stage</b>			
1–2	3.7 (1.5–4.0)	1	0.2520
3–4	1.6 (0.9–4.9)	1.42 (0.78–2.59)	
<b>N stage</b>			
0	2.4 (1.4–4.0)	1	0.4212
1+	3.7 (1.2–4.9)	0.78 (0.43–1.42)	
<b>AJCC stage</b>			
I–II	2.8 (1.0–3.4)	1	0.6562
III–IV	2.7 (1.4–4.9)	1.15 (0.62–2.12)	
<b>Alcohol abuse (≥30 U / week)</b>			
No	2.7 (1.0–4.9)	1	0.5335
Yes	2.4 (1.3–3.7)	1.21 (0.66–2.24)	
<b>Smoking history</b>			
<20 PY	3.4 (0.8–3.4)	1	0.4141
≥20 PY	2.6 (1.3–4.0)	1.34 (0.66–2.70)	
<b>TIL<sub>str</sub></b>			
<20%	1.4 (0.8–2.3)	1	0.0126
≥20%	4.0 (2.6–4.9)	0.45 (0.24–0.84)	
<b>CPS</b>			
<1	1.5 (0.1–3.4)	1	0.4070
≥1	2.7 (1.5–4.9)	0.68 (0.78–1.70)	

Parameters that reached significance on univariate analysis, were included in the multivariate analysis. CI, confidence interval; CPS, combined positive score; DFS, disease-free survival; HR, hazard ratio; PY, pack-years; str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S11. OS analysis since time of diagnosis**

Parameter	Univariate analysis			Multivariate analysis	
	Median OS (95% CI)	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
<b>T stage</b>					
1–2	NR	1		1	
3–4	3.8 (1.9–5.0)	2.12 (1.07–4.18)	0.0310	2.06 (1.05–4.03)	0.0351
<b>N stage</b>					
0	NR	1			
1+	4.4 (2.4–5.0)	1.09 (0.55–2.14)	0.8055		
<b>AJCC stage</b>					
I–II	NR	1			
III–IV	4.2 (2.6–5.0)	1.73 (0.88–3.43)	0.1148		
<b>Alcohol abuse (≥30 U / week)</b>					
No	5.0 (3.9–5.0)	1			
Yes	3.8 (2.4–4.4)	1.82 (0.92–3.61)	0.0867		
<b>Smoking history</b>					
<20 PY	NR	1			
≥20 PY	4.2 (2.6–5.0)	2.00 (0.92–4.35)	0.0815		
<b>TIL<sub>str</sub></b>					
<20%	2.4 (1.6–4.4)	1		1	
≥20%	5.0 (3.9–5.0)	0.41 (0.21–0.80)	0.0098	0.42 (0.21–0.83)	0.0123
<b>CPS</b>					
<1	1.4 (0.4–1.6)	1			
≥1	5.0 (3.8–5.0)	0.33 (0.11–1.01)	0.0516		

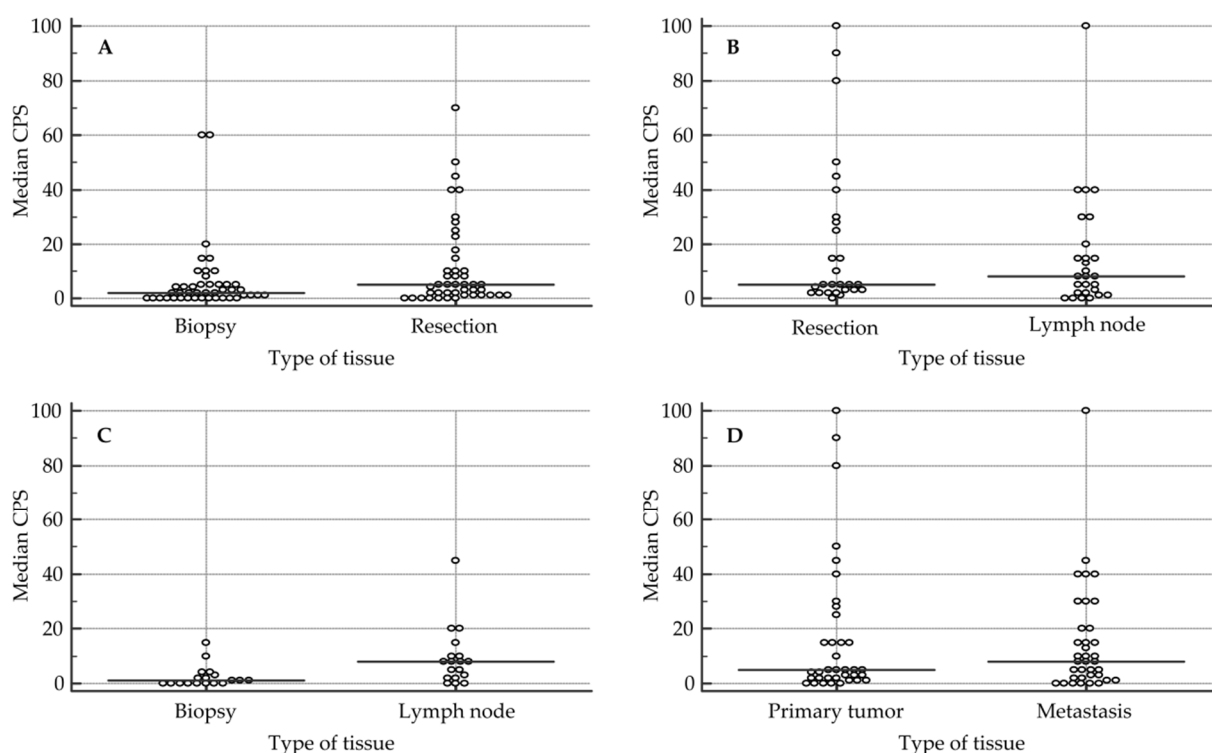
Parameters that reached significance on univariate analysis, were included in the multivariate analysis. CI, confidence interval; CPS, combined positive score; HR, hazard ratio; OS, overall survival; PY, pack-years; str, stromal; TIL, tumor infiltrating lymphocyte.

**Table S12. OS analysis since time of tissue acquirement**

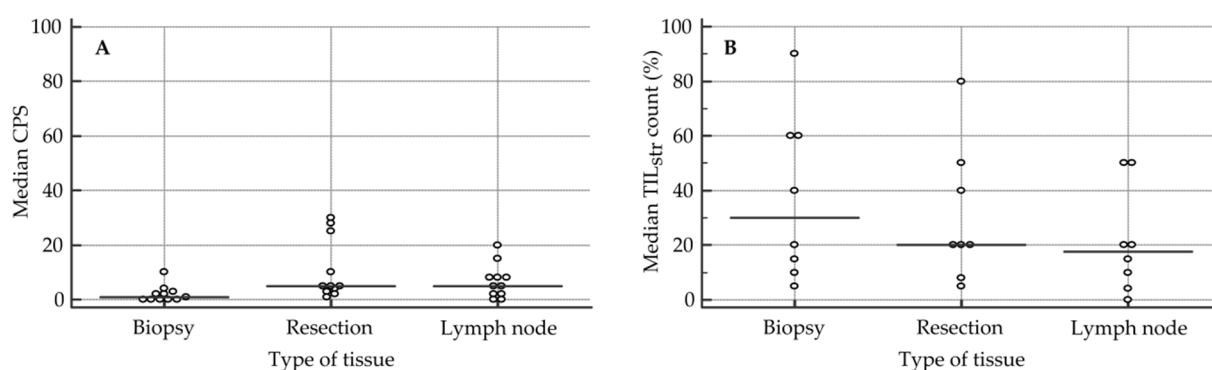
Parameter	Univariate analysis			Multivariate analysis	
	Median OS (95% CI)	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
<b>T stage</b>					
1–2	NR	1		1	
3–4	3.3 (1.5–4.9)	2.07 (1.05–4.09)	0.0363	2.17 (1.11–4.27)	0.0241
<b>N stage</b>					
0	4.1 (2.6–4.1)	1			
1+	4.9 (1.8–4.9)	1.06 (0.54–2.09)	0.8589		
<b>AJCC stage</b>					
I–II	NR	1			
III–IV	3.7 (1.9–4.9)	1.63(0.82–3.28)	0.1664		
<b>Alcohol abuse (≥30 U / week)</b>					
No	4.9 (3.7–4.9)	1			
Yes	3.3 (1.8–3.3)	1.92 (0.97–3.80)	0.0603		
<b>Smoking history</b>					
<20 PY	NR	1			
≥20 PY	3.7 (2.4–4.9)	2.02 (0.93–4.42)	0.0766		
<b>TIL<sub>str</sub></b>					
<20%	1.7 (1.3–2.4)	1		1	
≥20%	4.9 (3.7–4.9)	0.36 (0.20–0.72)	0.0038	0.36 (0.18–0.71)	0.0033
<b>CPS</b>					
<1	1.4 (0.1–1.5)	1		1	
≥1	4.9 (3.6–4.9)	0.31 (0.10–0.95)	0.0406	0.49 (0.21–1.15)	0.1000

Parameters that reached significance on univariate analysis, were included in the multivariate analysis. CI, confidence interval; CPS, combined positive score; HR, hazard ratio; OS, overall survival; PY, pack-years; str, stromal; TIL, tumor infiltrating lymphocyte.

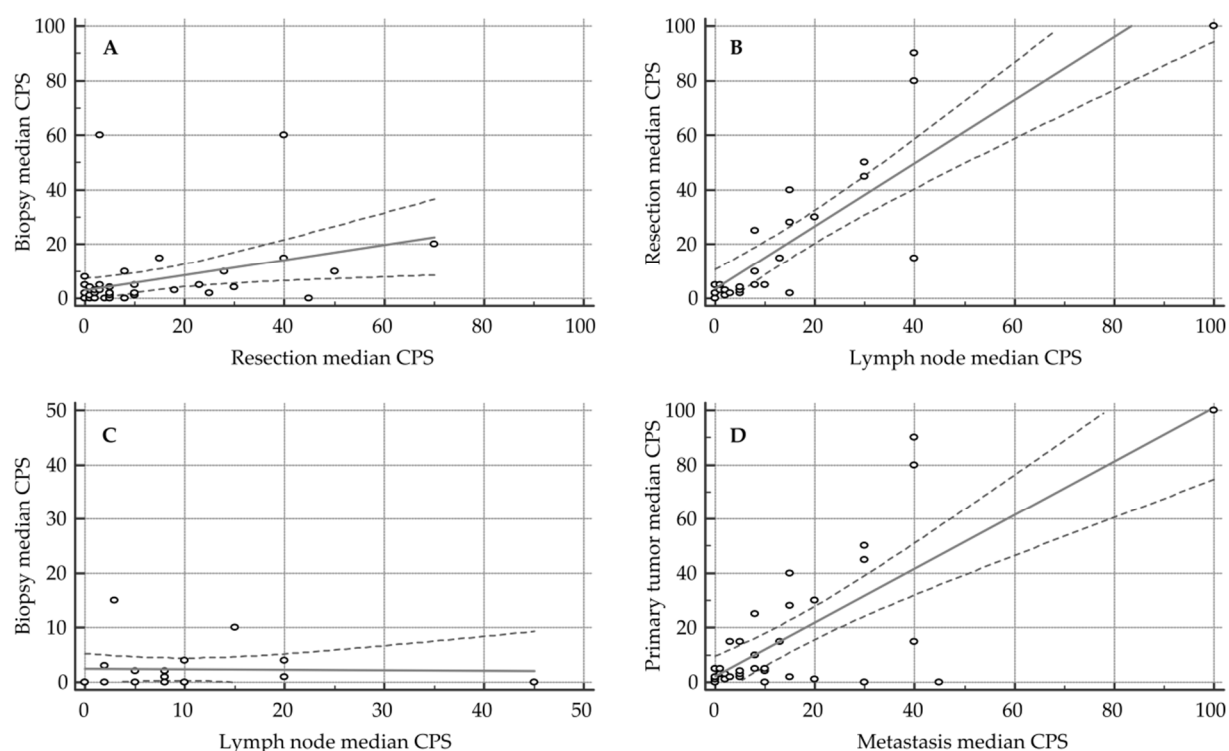




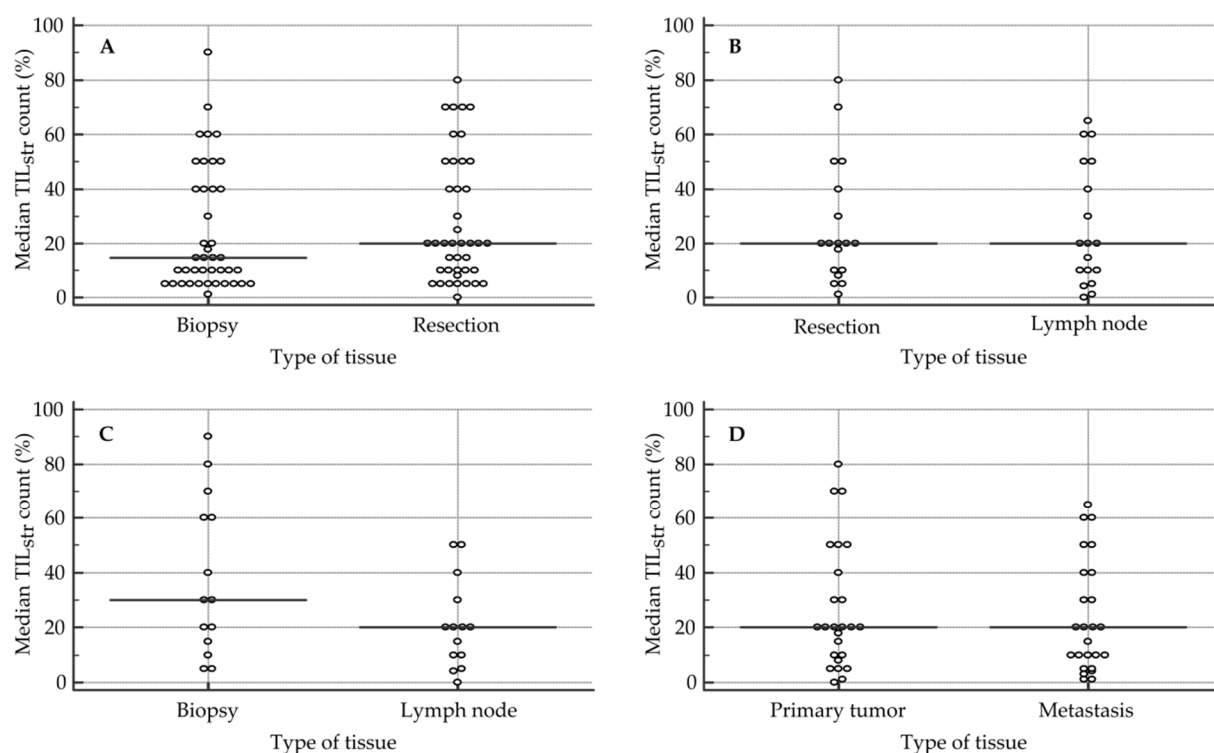
**Figure S1.** Comparison for CPS between paired tissue specimens. Median CPS is indicated in the Y-axis, type of tissue in the X-axis. Comparisons are illustrated for (A) biopsy versus resection material ( $P = 0.0085$ ), (B) resection versus lymph node material ( $P = 0.0495$ ), (C) biopsy versus lymph node material ( $P = 0.0076$ ), and (D) primary tumor versus metastatic material ( $P = 0.3669$ ). CPS, combined positive score.



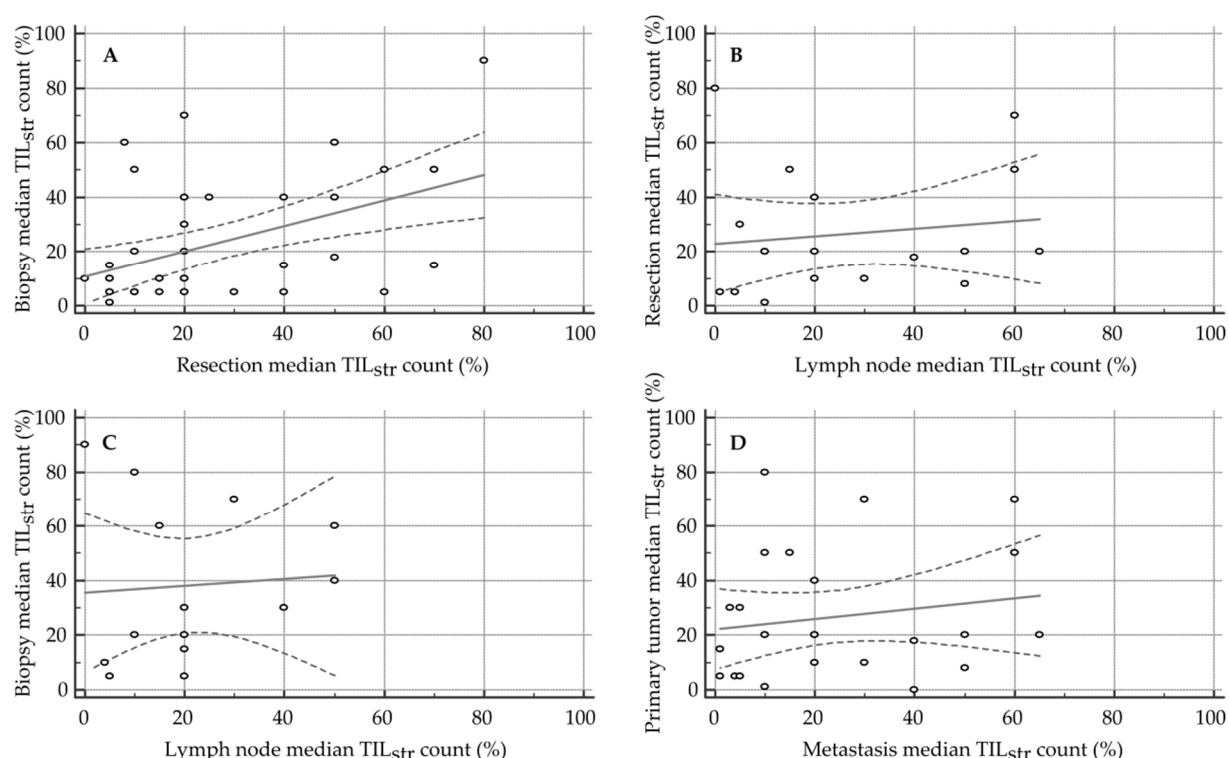
**Figure S2.** Comparison for CPS and TIL<sub>str</sub> between patients for who biopsy, resection and lymph node material is present. Median CPS or TIL<sub>str</sub> count is indicated in the Y-axis, type of tissue in the X-axis. Comparisons are illustrated for (A) median CPS ( $P = 0.0017$ ), (B) TIL<sub>str</sub> count ( $P = 0.4630$ ). CPS, combined positive score; str, stromal, TIL, tumor infiltrating lymphocyte.



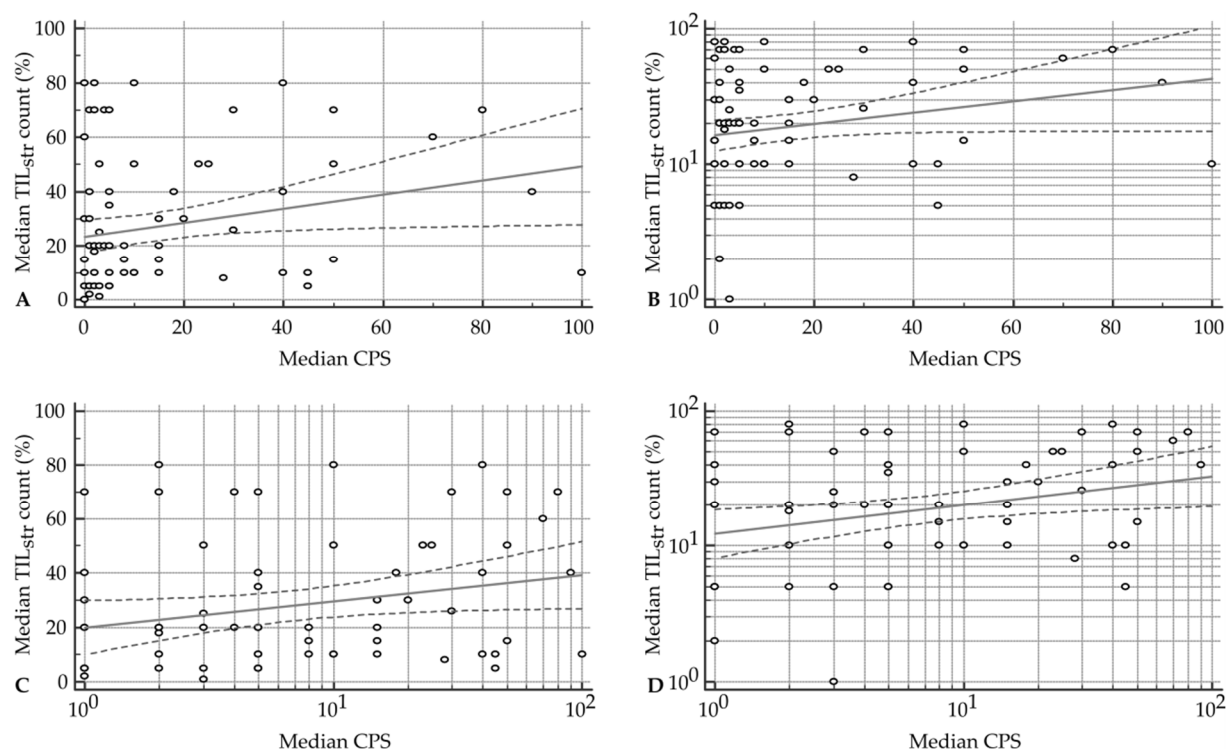
**Figure S3.** Correlation for CPS between paired tissue specimens. Correlations are illustrated for (A) biopsy versus resection material ( $r = 0.3537$ ;  $P = 0.0185$ ), (B) resection versus lymph node material ( $r = 0.8592$ ;  $P < 0.0001$ ), (C) biopsy versus lymph node material ( $r = -0.0281$ ;  $P = 0.9119$ ), and (D) primary tumor versus metastatic material ( $r = 0.7512$ ;  $P < 0.0001$ ). CPS, combined positive score.



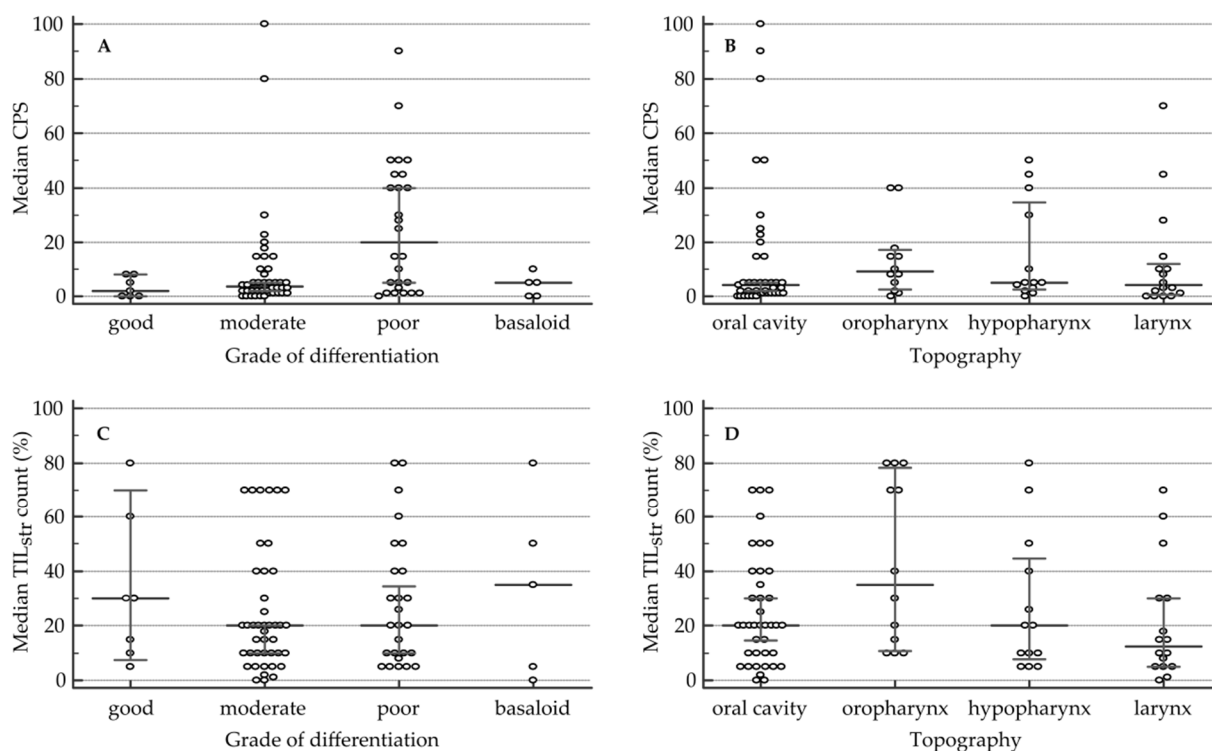
**Figure S4.** Comparison for TIL<sub>str</sub> count between paired tissue specimens. Median TIL<sub>str</sub> count is indicated in the Y-axis, type of tissue in the X-axis. Comparisons are illustrated for (A) biopsy versus resection material ( $P = 0.2363$ ), (B) resection versus lymph node material ( $P = 0.8871$ ), (C) biopsy versus lymph node material ( $P = 0.1167$ ), and (D) primary tumor versus metastatic material ( $P = 0.6263$ ). str, stromal, TIL, tumor infiltrating lymphocyte.



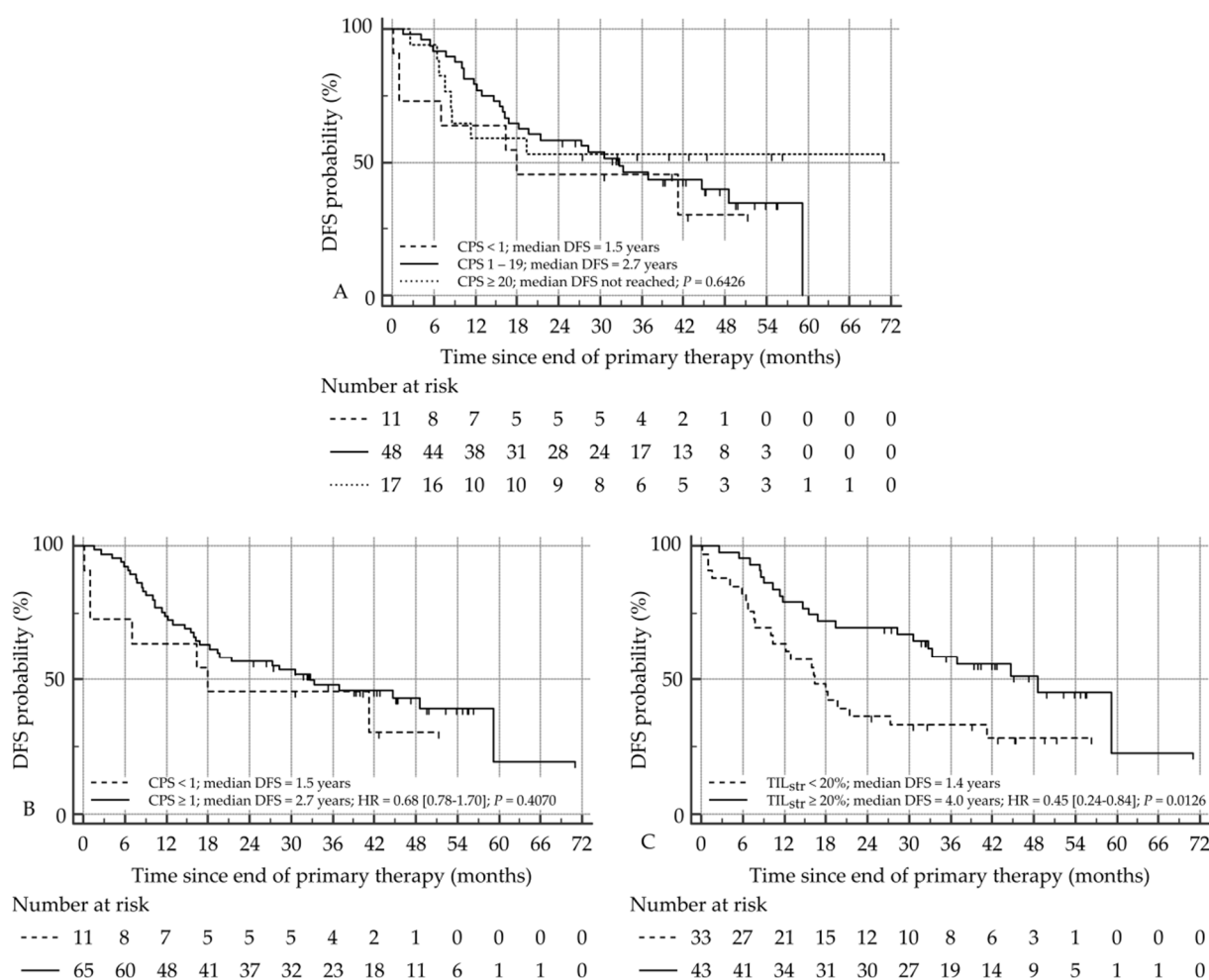
**Figure S5.** Correlation for TIL<sub>str</sub> between paired tissue specimens. Correlations are illustrated for (A) biopsy versus resection material ( $r = 0.4807$ ;  $P = 0.0015$ ), (B) resection versus lymph node material ( $r = 0.1394$ ;  $P = 0.5811$ ), (C) biopsy versus lymph node material ( $r = 0.0713$ ;  $P = 0.8087$ ), and (D) primary tumor versus metastatic material ( $r = 0.1671$ ;  $P = 0.4247$ ). str, stromal; TIL, tumor infiltrating lymphocyte.



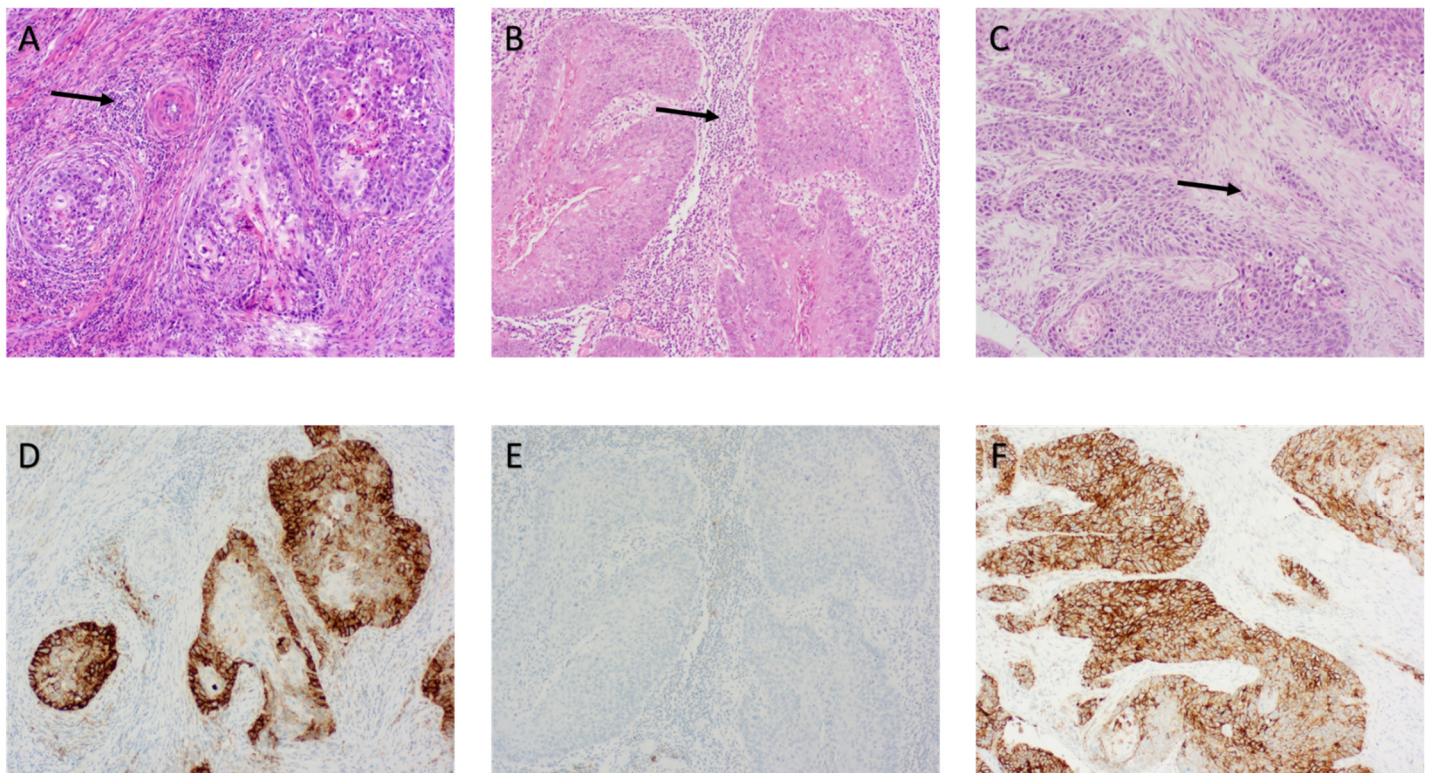
**Figure S6.** Correlation between CPS and TIL<sub>str</sub>, irrespective of tissue origin. Correlations are illustrated for (A) CPS versus TIL<sub>str</sub> ( $r = 0.4807$ ;  $P = 0.0015$ ), (B) CPS versus log (TIL<sub>str</sub>) ( $r = 0.1394$ ;  $P = 0.5811$ ), (C) log (CPS) versus TIL<sub>str</sub> ( $r = 0.0713$ ;  $P = 0.8087$ ), and (D) log (CPS) versus log (TIL<sub>str</sub>) ( $r = 0.1671$ ;  $P = 0.4247$ ). CPS, combined positive score; str, stromal; TIL, tumor infiltrating lymphocyte.



**Figure S7.** Comparison for CPS and TIL<sub>str</sub> count according to tumor differentiation and topography. Median CPS / TIL<sub>str</sub> count is indicated in the Y-axis, tumor differentiation / topography in the X-axis. Comparison are illustrated for (A) CPS according to tumor differentiation ( $P = 0.0101$ ), (B) CPS according to topography ( $P = 0.5130$ ), (C) TIL<sub>str</sub> count according to tumor differentiation ( $P = 0.8128$ ), and (D) TIL<sub>str</sub> count according to topography ( $P = 0.1234$ ). CPS, combined positive score; str, stromal, TIL, tumor infiltrating lymphocyte.



**Figure S8.** Effect of CPS and TIL<sub>str</sub> count on DFS in SCCHN patients. X-axis depicts survival time (in months), Y-axis depicts the cumulative DFS probability. DFS has been depicted for (A) trichotomous CPS, (B) dichotomous CPS, and (C) TIL<sub>str</sub>. Number at risk for each group has been indicated beneath each DFS curve. CPS, combined positive score; DFS, disease-free survival; HR, hazard ratio; SCCHN, squamous cell carcinoma of the head and neck; str, stromal; TIL, tumor infiltrating lymphocyte.



**Figure S9.** Histological sections of SCCHN depicting the heterogeneous profiles of PD-L1 staining and immune infiltration. The upper and lower row indicate paired HE and PD-L1 IHC slides, respectively. Profiles are shown for (1) high TIL<sub>str</sub> (A) and high PD-L1 (D), (2) high TIL<sub>str</sub> (B) and low PD-L1 (E), (3) low TIL<sub>str</sub> (C) and high PD-L1 (F). Arrows: compartment with tumoral stroma. HE, haematoxylin-eosin; IHC, immunohistochemistry; PD-L1, programmed cell death-ligand 1; SCCHN, squamous cell cancer in head and neck; str, stromal; TIL, tumor infiltrating lymphocyte.