

Supplementary Information

Tumour-Infiltrating Lymphocytes predict for outcome in HPV-positive Oropharyngeal

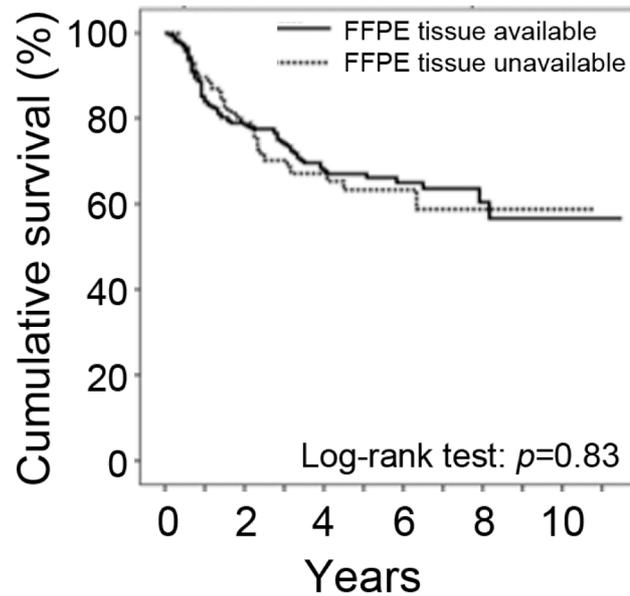
Cancer

Ward MJ *et al*

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Figure S1. Kaplan-Meier survival curves of patients with and without archival pathology material available. The latter were excluded from the study. Demographics of the patients are shown in supplementary Table S1

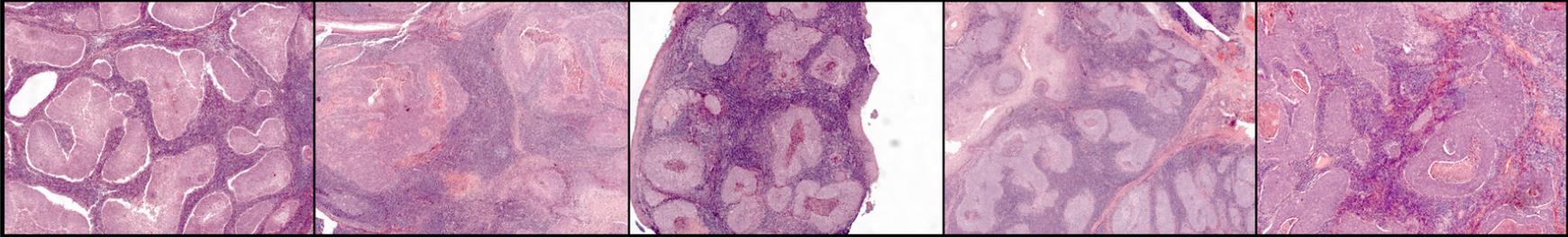


No. at risk		0	2	4	6	8	10
FFPE available	269	175	106	52	19	4	
FFPE unavailable	132	66	39	18	4	1	

Figure S2. TIL-levels

H+E stained sections showing individual examples of five TIL_{high} (A) and five TIL_{low} (B) tumors. All x50 magnification.

A



B

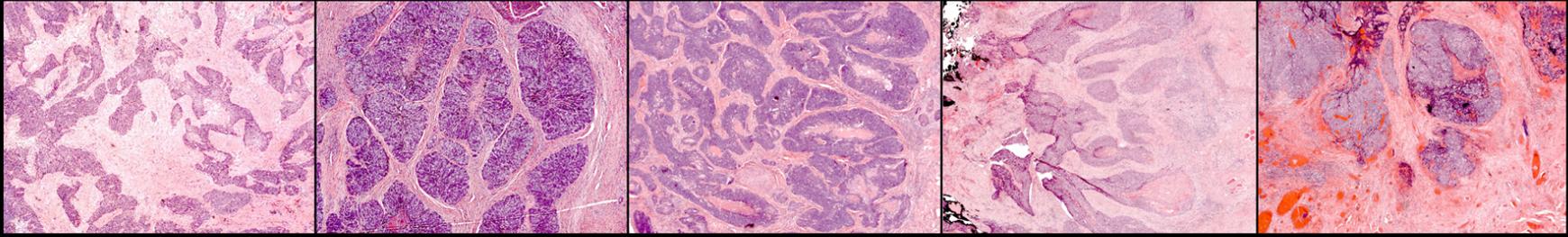
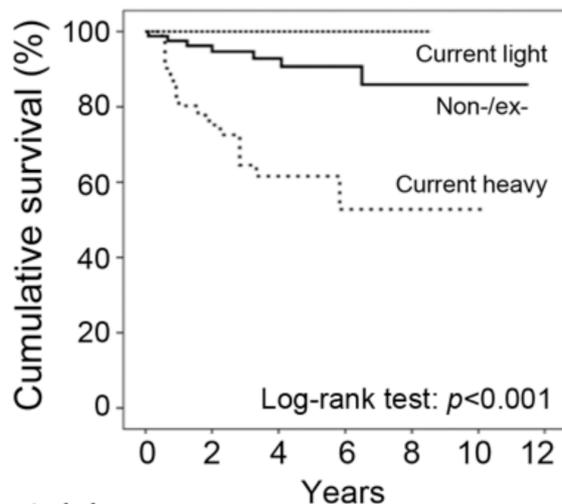


Figure S3. Kaplan-Meier survival curves of HPV-positive patients according to smoking history.

Patients who are non-, ex- or current light smokers (<10 pack years) have improved survival compared to those who are current heavy smokers (>10 pack years) in HPV-positive OPSCC (cohorts combined).



	No. at risk					
	0	2	4	6	8	10
Non-/ex smoker	82	62	43	20	8	1
Current light-smoker	10	8	7	4	1	
Current heavy-smoker	41	29	17	6	2	1

Table S1: Demographics of patients with and without archival pathology samples available

Characteristic		Entire Cohort n=442 Number (%)	FFPE Tissue Available n=290 Number (%)	FFPE Tissue Unavailable n=152 Number (%)
Gender	<i>Male</i>	325 (73.5)	211 (72.8)	114 (75)
	<i>Female</i>	117 (26.5)	79 (27.2)	38 (25)
Age	<i><50</i>	86 (19.5)	58 (20)	28 (18.4)
	<i>50-69</i>	268 (60.6)	176 (60.7)	92 (60.5)
	<i>70+</i>	88 (19.9)	56 (19.3)	32 (21.1)
	<i>Mean (SD)</i>	59.2 (11.3)	59.6 (11.6)	58.9 (10.7)
Smoking	<i>Non Smoker</i>	81 (18.3)	55 (19.0)	26 (17.1)
	<i>Current Smoker <10/day</i>	23 (5.2)	18 (6.2)	5 (3.3)
	<i>Current Smoker >10/day</i>	184 (41.6)	119 (41.0)	65 (42.8)
	<i>Ex-Smoker</i>	95 (21.5)	60 (20.7)	35 (23.0)
	<i>Not Known</i>	59 (13.3)	38 (13.1)	21 (13.8)
Alcohol	<i>Non/Ex Drinker</i>	61 (13.8)	37 (12.8)	24 (15.8)
	<i>Current Drinker</i>	271 (61.3)	176 (60.7)	95 (62.5)
	<i>Not Known</i>	110 (24.9)	77 (26.6)	33 (21.7)
Tumor Site	<i>Tonsil</i>	236 (53.4)	162 (55.9)	74 (48.7)
	<i>Tongue Base</i>	118 (26.7)	78 (26.9)	40 (26.3)
	<i>Other Oropharynx</i>	88 (19.9)	50 (17.2)	38 (25)
Median Length of Follow Up in Months (Range)		49 (2-137)	58 (8-137)	33 (2-137)
Disease Stage	<i>I</i>	30 (6.8)	21 (7.2)	9 (5.9)
	<i>II</i>	42 (9.5)	31 (10.7)	11 (7.2)
	<i>III</i>	60 (13.6)	37 (12.8)	23 (15.1)
	<i>IV</i>	306 (69.2)	199 (68.6)	107 (70.4)
	<i>Not Known</i>	4 (0.9)	2 (0.7)	2 (1.3)
Tumour Stage	<i>T1</i>	109 (24.6)	76 (26.2)	33 (21.7)
	<i>T2</i>	148 (33.5)	114 (39.3)	34 (22.4)
	<i>T3</i>	56 (12.7)	29 (10.0)	27 (17.8)
	<i>T4</i>	120 (27.1)	67 (23.1)	53 (34.9)
	<i>Tx</i>	4 (0.9)	3 (1.0)	1 (0.7)
	<i>Not Known</i>	5 (1.1)	1 (0.3)	4 (2.6)
Nodal Stage	<i>N0</i>	103 (23.3)	69 (23.8)	34 (22.4)
	<i>N1</i>	61 (13.8)	39 (13.4)	22 (14.5)
	<i>N2</i>	252 (57.0)	166 (57.2)	86 (56.6)
	<i>N3</i>	22 (5.0)	14 (4.8)	8 (5.3)
	<i>Not Known</i>	4 (0.9)	2 (0.7)	2 (1.3)
Grade	<i>Well/Moderately Differentiated</i>	168 (38.0)	110 (37.9)	58 (38.2)
	<i>Poorly Differentiated</i>	250 (56.6)	180 (62.1)	70 (46.1)
	<i>Not Known</i>	24 (5.4)	-	24 (15.8)

Table S2: Detailed Treatment Breakdown

	HPV-Positive OPSCC N=149	HPV-Negative OPSCC N=121
	<i>Number (%)</i>	<i>Number (%)</i>
<i>Surgery Alone</i>	5 (3.4)	20 (16.5)
<i>Surgery with Postoperative Radiotherapy</i>	44 (29.5)	32 (26.4)
<i>Surgery with Postoperative Chemoradiotherapy</i>	7 (4.7)	1 (0.8)
<i>Radiotherapy Alone</i>	24 (16.1)	34 (28.1)
<i>Concurrent Chemoradiotherapy</i>	31 (20.8)	14 (11.6)
<i>Neoadjuvant Chemotherapy followed by Chemoradiotherapy</i>	31 (20.8)	13 (10.7)
<i>Neoadjuvant Chemotherapy followed by Radiotherapy</i>	3 (2.0)	5 (4.1)
<i>Neoadjuvant Chemotherapy followed by Surgery with Postoperative Radiotherapy</i>	4 (2.7)	1 (0.8)

Table S3. Demographics of cohorts stratified by HPV-status and TIL-Levels

		UHS Cohort				PFT/BLT Cohort			
		HPV-negative n=82	HPV-positive TIL _{low} n=15	HPV-positive TIL _{mod} n=35	HPV-positive TIL _{high} n=51	HPV-negative n=51	HPV-positive TIL _{low} n=8	HPV-positive TIL _{mod} n=20	HPV-positive TIL _{high} n=22
		Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Gender	<i>Male</i>	56 (68.3)	13 (86.7)	26 (74.3)	38 (74.5)	37 (72.5)	6 (75.0)	15 (75.0)	15 (68.2)
	<i>Female</i>	26 (31.7)	2 (13.3)	9 (25.7)	13 (25.5)	14 (27.5)	2 (25.0)	5 (25.0)	7 (31.8)
Age at Diagnosis	<i><50</i>	11 (13.4)	6 (40.0)	8 (22.9)	14 (27.5)	7 (13.7)	1 (12.5)	5 (25.0)	6 (27.3)
	<i>50-69</i>	49 (59.8)	8 (53.3)	18 (51.4)	33 (64.7)	32 (62.7)	6 (75.0)	11 (55.0)	14 (63.6)
	<i>70+</i>	22 (26.8)	1 (6.7)	9 (25.7)	4 (7.8)	12 (23.5)	1 (12.5)	4 (20.0)	2 (9.1)
	<i>Mean (SD)</i>	61.87 (11.74)	54.93 (12.14)	60.09 (13.68)	55.31 (9.25)	59.98 (12.05)	56.88 (10.29)	57.30 (12.38)	56.64 (10.22)
Smoking	<i>Non-Smoker</i>	7 (8.5)	4 (26.7)	9 (25.7)	13 (25.5)	3 (5.9)	1 (12.5)	5 (25.0)	12 (54.5)
	<i>Current Smoker <10/day</i>	1 (1.2)	-	2 (5.7)	5 (9.8)	7 (13.7)	-	1 (5.0)	2 (9.1)
	<i>Current Smoker >10/day</i>	50 (61.0)	5 (33.3)	9 (25.7)	13 (25.5)	27 (52.9)	4 (50.0)	7 (35.0)	2 (9.1)
	<i>Ex-Smoker</i>	15 (18.3)	6 (40.0)	8 (22.9)	14 (27.5)	5 (9.8)	3 (37.5)	3 (15.0)	5 (22.7)
	<i>Not Known</i>	9 (11.0)	-	7 (20.0)	6 (11.8)	9 (17.6)	-	4 (20.0)	1 (4.5)
Alcohol	<i>Non/Ex Drinker</i>	12 (14.6)	3 (20.0)	4 (11.4)	7 (13.7)	5 (9.8)	1 (12.5)	2 (10.0)	3 (13.6)
	<i>Current Drinker</i>	57 (69.5)	8 (53.3)	21 (60.0)	35 (68.6)	22 (43.1)	5 (62.5)	11 (55.0)	15 (68.2)
	<i>Not Known</i>	13 (15.9)	4 (26.7)	10 (28.6)	9 (17.6)	24 (47.1)	2 (25.0)	7 (35.0)	4 (18.2)
Tumour Site	<i>Tonsil</i>	35 (42.7)	12 (80.0)	20 (57.1)	35 (68.6)	24 (47.1)	4 (50.0)	11 (55.0)	17 (77.3)
	<i>Tongue Base</i>	24 (29.3)	2 (13.3)	12 (34.3)	13 (25.5)	11 (21.6)	3 (37.5)	6 (30.0)	5 (22.7)
	<i>Other Oropharynx</i>	23 (28.0)	1 (6.7)	3 (8.6)	3 (5.9)	16 (31.4)	1 (12.5)	3 (15.0)	0 (0.0)
Disease Stage	<i>I</i>	9 (11.0)	0 (0.0)	0 (0.0)	1 (2.0)	9 (17.6)	0 (0.0)	1 (5.0)	1 (4.5)
	<i>II</i>	12 (14.6)	1 (6.7)	2 (5.7)	3 (5.9)	9 (17.6)	2 (25.0)	0 (0.0)	1 (4.5)
	<i>III</i>	7 (8.5)	2 (13.3)	5 (14.3)	3 (5.9)	10 (19.6)	0 (0.0)	4 (20.0)	6 (27.3)
	<i>IV</i>	54 (65.9)	11 (73.3)	28 (80.0)	44 (86.3)	22 (43.1)	6 (75.0)	15 (75.0)	14 (63.6)
	<i>Not Known</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)
Grade	<i>Well/Moderately Differentiated</i>	44 (53.7)	3 (20.0)	10 (28.6)	6 (11.8)	31 (60.8)	4 (50.0)	7 (35.0)	3 (13.6)
	<i>Poorly Differentiated</i>	38 (46.3)	12 (80.0)	25 (71.4)	45 (88.2)	20 (39.2)	4 (50.0)	13 (65.0)	19 (86.4)
Treatment	<i>Surgery+/- Radiotherapy</i>	31 (37.8)	7 (46.7)	13 (37.1)	23 (45.1)	25 (49.1)	4 (50.0)	5 (25.0)	7 (31.8)
	<i>Radiotherapy</i>	25 (30.5)	3 (20.0)	8 (22.9)	7 (13.7)	9 (17.6)	1 (12.5)	2 (10.0)	2 (9.1)
	<i>Chemoradiotherapy</i>	20 (24.4)	4 (26.7)	13 (37.1)	21 (41.2)	12 (23.5)	3 (37.5)	12 (60.0)	12 (54.5)
	<i>None/Palliative</i>	6 (7.3)	1 (6.7)	1 (2.9)	0 (0.0)	5 (9.8)	0 (0.0)	1 (5.0)	1 (4.5)
EGFR	<i>Negative</i>	26 (31.7)	6 (40.0)	14 (40.0)	36 (70.6)	8 (15.7)	3 (37.5)	9 (45.0)	10 (45.5)
	<i>Positive</i>	49 (59.8)	6 (40.0)	20 (57.1)	10 (19.6)	42 (82.4)	5 (62.5)	11 (55.0)	12 (54.5)
	<i>Not Known</i>	7 (8.5)	3 (20.0)	1 (2.9)	5 (9.8)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table S4. T-cell subset counts and ratios

	HPV-positive N=149	HPV-negative N=121	p-value
<i>Total T-Cell (CD4+CD8)</i>	111.7	53.7	<0.001
<i>CD8⁺</i>	57.1	24.0	<0.001
<i>CD4⁺</i>	54.0	29.9	<0.001
<i>Foxp3⁺</i>	32.1	16.7	<0.001
<i>Proportion CD8⁺</i>	51.1%	44.7%	<0.001
<i>Proportion CD4⁺</i>	48.9%	54.0%	<0.001
<i>Proportion Foxp3⁺</i>	31.2%	36.8%	0.08
<i>CD4:CD8 Ratio</i>	0.96:1	1.21:1	<0.001
<i>Foxp3:CD8 Ratio</i>	0.78:1	0.69:1	<0.001

Table S5. Treatment received by HPV-positive/TIL_{low} patients

	HPV-positive/High-Risk
	Cohorts Combined n=28
	<i>Number (%)</i>
<i>Surgery with Postoperative Radiotherapy</i>	6 (20.7)
<i>Surgery with Postoperative Chemoradiotherapy</i>	4 (13.8)
<i>Radiotherapy Alone</i>	8 (27.6)
<i>Concurrent Chemoradiotherapy</i>	3 (10.3)
<i>Neoadjuvant Chemotherapy followed by Chemoradiotherapy</i>	7 (24.1)

Table S6. Position of genes describing effector CD8⁺ T cells (*Best et al, 2013*) in a rank-ordered list of genes (1-15,031) differentially expressed between HNSCC survivors (survival >3 years, mean 49 months) and non-survivors (survival < 3 years, mean 16 months), (*Thurlow et al, 2010*); with genes enriched in survivors at the top of the list.

Gene Symbol	Gene Title	Rank
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9 (4-1BB)	126
CCR2	Chemokine (C-C motif) receptor 2	177
KLRC3	Killer cell lectin-like receptor subfamily C, member 3	265
ZDHHC2	Zinc finger, DHHC-type containing 2	325
PLEK	Pleckstrin	473
CXCR3	Chemokine (C-X-C motif) receptor 3	546
ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	584
CCL4	Chemokine (C-C motif) ligand 4	590
KLRG1	Killer cell lectin-like receptor subfamily G, member 1	915
LIF	Leukemia inhibitory factor (cholinergic differentiation factor)	1028
GZMB	Granzyme B (granzyme 2, CTL-associated serine esterase 1)	1256
IFNG	Interferon, gamma	1288
IRF8	Interferon regulatory factor 8	1345
CTLA4	Cytotoxic T-lymphocyte-associated protein 4	1407
IL18RAP	Interleukin 18 receptor accessory protein	1411
KLRK1	Killer cell lectin-like receptor subfamily K, member 1	1424
CX3CR1	Chemokine (C-X3-C motif) receptor 1	1490
S100A4	S100 calcium binding protein A4	1559
PIK3AP1	Phosphoinositide-3-kinase adaptor protein 1	1562
CD200	CD200 molecule	1632
ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1677
ID2	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	1729
ITGAM	Integrin, alpha M (complement component 3 receptor 3 subunit)	1763
GZMA	Granzyme A (granzyme 1, CTL-associated serine esterase 3)	1836
IL3	Interleukin 3 (colony-stimulating factor, multiple)	1921
IL2	Interleukin 2	2001
MYO1F	Myosin IF	2009
XCL1	Chemokine (C motif) ligand 1	2262
GZMK	Granzyme K (granzyme 3; tryptase II)	2353
PTPRJ	Protein tyrosine phosphatase, receptor type, J	2603
IL21	Interleukin 21	2674
DOCK5	Dedicator of cytokinesis 5	2710
IRF4	Interferon regulatory factor 4	3157
NR4A1	Nuclear receptor subfamily 4, group A, member 1	3280
ITGAX	Integrin, alpha X (complement component 3 receptor 4 subunit)	3524