Supplementary Table 1: Antibodies and staining conditions

Protein	Clone	Supplier	Catalog#	Species	Dilution	HIER*	Protocol ID†
CD3	LN10	Leica	PA0553	Mouse	pre-diluted	ER2/20 min	HUP Refine
CD8	c8/144b	Dako	M7103	Mouse	1:40	ER1/20 min	HUP Refine
CD45 (LCA)	2B11 + PD7/26	Dako	M0701	Mouse	1:200	ER1/10 min	HUP Refine
gH2AX	20E3	Cell Signaling	9718S	Rabbit	1:50	ER2/20 min	HUP 60/20
PDL1	E1J2J	Cell Signaling	15165BF	Rabbit	1:500	ER2/20 min	HUP 60/20
S4/8 pRPA32	(polyclonal)	Abcam	ab87277	Rabbit	1:100	ER2/20 min	HUP Refine

***HIER**: Heat-induced epitope retrieval method (detailed in Supplementary Methods)
†**Protocol ID:** The two different IHC staining protocols are detailed in Supplementary Methods.

Supplementary Table 2: Characteristics of total cohort (n=851)

Variable	Categories	Number	Percent
Overall pathologic stage (8^{th} edition AJCC)	Stage I	645	75.8%
	Stage II	190	22.3%
	Stage III	16	1.9%
Pathologic T-stage	pT0	3	0.4%
	pT1	350	41.1%
	pT2	412	48.4%
	pT3	69	8.1%
	pT4	17	2.0%
Pathologic N-stage	pN0	125	14.7%
	pN1	590	69.3%
	pN2	133	15.6%
	pNx	3	0.4%
Treatment	Surgery only	187	22.0%
	Surgery + radiotherapy	311	36.6%
	Surgery + chemoradiotherapy	250	29.4%
	Unknown	103	12.1%
Smoking history	≤10 pack-years	554	65.1%
	>10 pack-years	255	30.0%
	Unknown	42	4.9%
Overall clinical stage (8^{th} edition AJCC)	Stage I	697	81.9%
	Stage II	55	6.5%
	Stage III	58	6.8%
	Unknown	41	4.8%
Clinical T-stage	cT1	213	25.0%
	cT2	382	44.9%
	cT3	32	3.8%
	cT4	47	5.5%
	cTx	109	12.8%
	cT0	9	1.1%
	Unknown	59	6.9%
Clinical N-stage	cN0	121	14.2%
	cN1	634	74.5%
	cN2	30	3.5%
	cN3	5	0.6%
	Unknown	61	7.2%
Primary tumor site	Tonsil	459	53.9%
	Tongue base	317	37.3%
	Overlap	62	7.3%
	Synchronous	5	0.6%

(continued)

	Unknown primary	8	0.9%
Pathologic level IV/V nodes	No	744	87.4%
	Yes	77	9.1%
	Unknown	30	3.5%
Margin status	Negative	610	71.7%
	In-situ or Close (<2mm)	160	18.8%
	Positive	57	6.7%
	Unknown	24	2.8%
Lymphovascular space Invasion	No	559	65.7%
	Yes	256	30.1%
	Unknown	36	4.2%
Perineural invasion	No	689	81.0%
	Yes	125	14.7%
	Unknown	37	4.4%
Pathologic extranodal extension	No	599	70.4%
	Yes	228	26.8%
	Unknown	24	2.8%
# positive nodes	Mean	2.62	
	Range	0-48	
Age	Median	60	
	Range	32-89	
Sex	Male	741	87.1%
	Female	110	12.9%
Race	White	785	92.2%
	Non-white	62	7.3%
	Unknown	4	0.5%
Charlson comorbidity index	0	562	66.0%
	≥1	149	17.5%
	Unknown	140	16.5%

Supplementary Table 3: Characteristics of matched cases and controls highlighting imperfectly matched traits (red text)

Pair	Recurrence						Control					
number	Smoking	pT	pN	pOverall	Treatment	Systemic agent	Smoking	pT	pN	pOverall	Treatment	Systemic agent
1	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin
2	≤10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin
3	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
4	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin
5	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
6	≤10pk-yr	pT1	pN1	1	Surgery + RT		≤10pk-yr	pT1	pN1	1	Surgery + RT	
7	≤10pk-yr	pT1	pN1	1	Surgery + RT		≤10pk-yr	pT1	pN1	1	Surgery + RT	
8	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin
9	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Carboplatin/paclitaxel	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Carboplatin/paclitaxel
10	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
11	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cetuximab	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cetuximab
12*	≤10pk-yr	pT3	pN0	2	Surgery + RT		≤10pk-yr	pT3	pN0	2	Surgery + RT	
14	>10pk-yr	pT2	pN1	1	Surgery Only		>10pk-yr	pT2	pN1	1	Surgery Only	
15	>10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin	>10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin
16	>10pk-yr	pT0	pN2	2	Surgery + CRT	Cetuximab	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
18	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cetuximab	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin
19	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
20	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
21	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
22	≤10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin
23	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin
24	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	>10pk-yr	pT1	pN2	2	Surgery + CRT	Cisplatin
25	>10pk-yr	pT1	pN2	2	Surgery + CRT	Cetuximab	>10pk-yr	pT1	pN2	2	Surgery + CRT	Cetuximab
26*	≤10pk-yr	pT3	pN0	2	Surgery + RT		≤10pk-yr	pT3	pN0	2	Surgery + RT	
27	≤10pk-yr	pT1	pN2	2	Surgery + RT		≤10pk-yr	pT1	pN2	2	Surgery + RT	
28	≤10pk-yr	pT2	pN2	2	Surgery Only		≤10pk-yr	pT3	pN1	2	Surgery Only	
29†	>10pk-yr	pT2	pN2	2	Surgery + RT		>10pk-yr	pT1	pN2	2	Surgery + RT	
30	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin
31	≤10pk-yr	pT1	pN1	1	Surgery + CRT	Cisplatin	≤10pk-yr	pT1	pN1	1	Surgery + CRT	Cisplatin
32	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cetuximab	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cetuximab
33	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin
34	>10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	>10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin

(continued)

35	>10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin	>10pk-yr	pT2	pN1	1	Surgery + CRT	Cisplatin
36	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
37	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT1	pN1	1	Surgery + RT	
38	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
39	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
40	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT1	pN1	1	Surgery + RT	
41*	≤10pk-yr	pT2	pN0	1	Surgery + RT		≤10pk-yr	pT2	pN0	1	Surgery + RT	
42	≤10pk-yr	pT1	pN1	1	Surgery + RT		≤10pk-yr	pT1	pN1	1	Surgery + RT	
43	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
44	≤10pk-yr	pT2	pN1	1	Surgery + RT		≤10pk-yr	pT2	pN1	1	Surgery + RT	
45	>10pk-yr	pT2	pN1	1	Surgery + RT		>10pk-yr	pT2	pN1	1	Surgery + RT	
46	>10pk-yr	pT2	pN0	1	Surgery Only		>10pk-yr	pT2	pN0	1	Surgery Only	
48	>10pk-yr	pT1	pN1	1	Surgery Only		>10pk-yr	pT1	pN1	1	Surgery Only	
49†	>10pk-yr	pT1	pN2	2	Surgery + RT		>10pk-yr	pT1	pN2	2	Surgery + RT	
50	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	>10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin
51	>10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin	>10pk-yr	pT3	pN1	2	Surgery + CRT	Cisplatin
52*	≤10pk-yr	pT2	pN0	1	Surgery + RT		>10pk-yr	pT2	pN0	1	Surgery + RT	
54	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin	≤10pk-yr	pT2	pN2	2	Surgery + CRT	Cisplatin

*Cases and controls were sequenced from primary tumor and not metastatic lymph nodes.
†Same control was used for two cases
pk-yr = pack years, RT = radiation therapy, CRT = chemoradiation

Supplementary Table 4: Characteristics of HPV+ OPSCC patients in three validation cohorts

Cohort	Total patients	Patients receiving nonsurgical therapy	Patients with recurrence	Months to last recurrence event	Potential controls
UNC	89	81	15	58.6	53
JHU	47	16	4	48.7	19
TCGA	53	29	6	41.6	12

Supplementary Methods

Matching strategy in absence of a perfect control match

Six recurrent cases for which optimal pT and/or pN control matches were not identified without disrupting matching by the other criteria (R16, R24, R28, R29, R37, R40) were paired with controls with different pT and/or pN but the same pathologic overall stage. One of these six cases (R16), which received adjuvant cetuximab, was matched with a control that received cisplatin. These differences are highlighted in Supplementary Table 3.

Sample description, mRNA Library preparation and sequencing

Two tumor tissue sample were macrodissected from FFPE blocks of each patient. RNA sample quality was assessed by RNA TapeStation (Agilent Technologies) and quantified by AccuBlue® Broad Range RNA Quantitation assay (Biotium). Total RNA libraries were created using Illumina Stranded with RiboZero plus kit. Final library quantity was estimated by Qubit 2.0 (ThermoFisher) and quality was assessed by TapeStation HSD1000 ScreenTape (Agilent Technologies). Final library size was ~350bp with an insert size of about 200bp based on Illumina® 8-nt unique dual-indices. Libraries were pooled in equimolar proportions and sequenced on an Illumina® Novaseq platform with a read length configuration of 150 for 80M paired end reads per sample (40M in each direction).

Processing of RNASeq data

Low-quality bases and adapter sequences from raw RNA sequencing FASTQ files were trimmed using Trimmomatic-0.32 [1]. Filtered high quality sequencing reads were then aligned to the GRCh38 genome [2] with STAR v2.7.1a [3] with –twopassMode, --outFilterIntronMotifs and RemoveNoncanonical parameters. Quantification of reads aligned to exonic regions was conducted using STAR's –quant mode and HTSeq2.0 [4]. DeSeq2 collapse replicate was used for combining replicates and normalization [5].

Viral transcript alignment

Revised HPV16 reference genome and genomic annotations were obtained from the papillomavirus episteme (PaVE) [6]. Star genome index was generated using genomeSAindexNbases 8 as per size of HPV16 genome (7904 bases). Unaligned reads after human reference genome alignment were used to perform alignment with revised HPV16 viral genome with using STAR v2.7.1a –twopassMode. Quantification of reads aligned to exonic regions was conducted using STAR's –quant mode and HTSeq2.0[4].

GSEA, GSVA, and calculation of combined score from TPS and ISS

Gene set enrichment analysis (GSEA) [7] was performed on the Hallmark gene set (H subset, n=50 pathways) [8] and non-disease-associated KEGG Legacy pathways (subset of CP pathways, n=147) [9] using the fgsea R package [10] wi4th 1000 permutations. Gene set variation analysis (GSVA) scores were generated for unique genes within pathways associated with immune suppression (n=921) and tumor progression (n=1569) using the ssGSEA R package [11] with options set to kcdf =" gaussian," maximum group size= "2000", and mx.diff parameter= "true". GSVA scores were generated for external cohorts using gene lists obtained from case control cohort for tumor progression and immune suppression with same parameters.

Multivariate logistic regression analysis was used to construct a combined score from GSVA-derived ISS and TPS scores. Considering significant correlation between these scores,

interaction term was also incorporated in the calculation of combined scores by the following equation: *Combined Score= (TPS x 2.826) + (ISS x 3.521) + (ISS x TPS x -1.894) – 2.66*.

Details of R v4.2 usage

Youden-index was calculated using the cutpointr R package [12]. R package pROC was used for ROC analysis [13]. For survival analysis, the Survminer [14] R package was used.

Immunohistochemistry details

Heat-induced epitope retrieval was performed per manufacture protocol using combinations of the following two buffers: Epitope Retrieval 1 (ER1) (Leica, Cat#AR9961) and Epitope Retrieval 2 (ER2) (Leica, Cat#AR9640). IHC staining was performed by one of two protocols, HUP 60/20 and HUP Refine, which differ in primary antibody incubation times. The HUP Refine protocol uses an incubation time of 15 minutes with the primary antibody and 8 minutes with the secondary antibody. The HUP 60/20 protocol uses an incubation time of 60 minutes for primary antibody and 20 minutes for secondary antibody. The anti-mouse or anti-rabbit secondary antibodies used in both protocols are part of the Refine Detection Kit (Cat#DS9800).

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