Supplementary Table S1

Case[†]	Gender[a]	Smoking status	Alcohol status[b]	Type	Site	Sub-site[c]	T[d]	N[e]	M[f]	R[g]	P16[h]
1	M	Previous	X	Primary	Hypopharynx	Pyriform fossa	3	2b	0	Y	-
2	M	Current	Current	Primary	Oral	Floor of mouth	2	2c	0	N	-
3	F	Current	Current	Primary	Larynx	N/A	3	0	0	N	N/A
4	M	Previous	Current	Primary	Oropharynx	Tonsil	3	2b	0	N	+
5	F	Never	Current	Primary	Hypopharynx	X	3	1	1	N	N/A
6	M	Never	Never	Primary	Oral	Tongue	4	2a	0	N	N/A
7	M	Previous	Current	Primary	Oral	Tongue	1	1	0	Y	N/A
8	M	Current	Current	Primary	Oral	Floor of mouth	2	2b	0	N	N/A
9	F	Previous	Never	Primary	Oral	Floor of mouth	2	2c	0	N	N/A
10	M	Current	Current	Primary	Oral	Tongue	3	0	0	N	N/A
11	M	Current	Current	Primary	Oral	Tongue	1	0	0	N	N/A
12	F	Never	Current	Primary	Oral	Mandible	4a	0	0	Y	N/A
13	F	Current	Current	Primary	Oral	Mandible	1	0	0	N	N/A
14	F	Current	Current	Primary	Oral	Tongue	4	0	0	N	+
15	M	Previous	Previous	Primary	Oral	Mandible	4a	2b	0	N	-
16	M	Previous	Previous	Primary	Larynx	X	4a	0	0	N	N/A
17	M	Current	Previous	Recurrent	Oropharynx	Tonsil	2	0	0	Y	-
18	M	Never	Previous	Primary	Larynx	X	4a	1	0	N	N/A
19	F	Never	Current	Primary	Oral	Tongue	2	0	0	Y	X
20	M	Current	Current	Primary	Oral	Tongue	4a	2b	0	N	-
21	M	Current	Current	Primary	Oral	Floor of mouth	2	2c	0	N	X
22	M	Current	Current	Primary	Oropharynx	Tonsil	X	X	X	N	X
23	F	Current	Current	Primary	Oropharynx	Tongue base	4a	0	0	N	-
24	M	Never	Current	Primary	Oropharynx	Tonsil	2	2c	0	N	+

[a] Abbreviations: F, female; M, male
[b] Abbreviations: X, no data available
[c] Abbreviations: X, no data available, N/A, not applicable
[d] T stage. Abbreviations: X, no data available; 1, tumour ≤ 2 cm; 2, tumour > 2 cm; 3, tumour > 4 cm; 4a, moderately advanced local disease; 4b, very advanced local disease
[e] N stage. Abbreviations: X, no data available; 0, No regional lymph node metastasis; 1, Metastasis in a single ipsilateral lymph node ≤ 3 cm in greatest dimension; 2a, Metastasis in a single ipsilateral lymph node > 3 cm but not more than 6 cm in greatest dimension; 2b, Metastasis in a multiple ipsilateral lymph nodes - none > 6 cm in greatest dimension; 2c, Metastasis in bilateral or contralateral lymph nodes - none > 6 cm in greatest dimension; 3, Metastasis in a lymph node > 6 cm in greatest dimension
[f] M stage. Abbreviations: X, no data available; 0, no distant metastasis; 1, distant metastasis
[g] Tumour recurrence. Abbreviations: 0, no; 1, yes
[h] p16 status determined by immunohistochemistry. Abbreviations: X, no data available; N/A, not applicable; -, negative; +, positive

[†]Normal specimens were taken at least 3 cm from the tumour margin and histologically examined to confirm tissue was non-cancerous

Supplementary Table S2

Case[†]	Age at Diagnosis (yr)	Gender[a]	Smoking status[b]	Alcohol status[c]	Site	Sub-site[d]	T[e]	N[f]	M[g]	R[h]	HPV status[i]
1	62	F	Previous	Previous	Oropharynx	Tonsil	2	0	0	N	-
2	57	F	N/A	N/A	Oropharynx	Tonsil	2	2a	0	N	+
3	50	F	Current	Current	Oropharynx	Base of tongue	4	2a	0	N	-
4	56	M	Previous	N/A	Oropharynx	X	1	1	0	Y	-
5	57	M	Current	Previous	Oropharynx	Tonsil	3	2a	0	X	-
6	49	F	Never	Previous	Oropharynx	Tonsil	2	1	0	Y	+
7	57	M	Never	Previous	Oropharynx	Base of tongue	4	0	0	N	+
8	42	M	Never	Previous	Oropharynx	Tonsil	2	2b	0	N	+
9	48	M	Current	Current	Oropharynx	Base of tongue	4	2b	0	N	-
10	72	M	Current	Never	Oropharynx	Soft palate	2	0	0	N	-
11	51	F	Previous	Previous	Oropharynx	X	4	0	0	N	-
12	51	M	Current	Current	Oropharynx	Soft palate	4	0	0	Y	-
13	52	M	Never	Current	Oropharynx	Tonsil	4	0	0	N	+
14	54	M	Never	Previous	Oropharynx	Tonsil	2	0	0	Y	+
15	47	F	Current	Previous	Oropharynx	Tonsil	1	0	0	Y	-
16	51	M	Previous	Previous	Oropharynx	X	4	0	0	N	-
17	51	M	Never	Previous	Oropharynx	Tonsil	2	2c	0	N	+
18	70	M	Previous	Previous	Oropharynx	Base of tongue	4	2a	0	N	-
19	45	M	Current	Current	Oropharynx	Soft palate	2	2c	0	N	-
20	67	F	Current	Previous	Oropharynx	Tonsil	2	2b	0	N	-
21	46	F	Never	Previous	Oropharynx	Tonsil	2	1	0	N	-
22	56	F	Current	Previous	Oropharynx	Base of tongue	4	0	0	N	-
23	59	M	Previous	Previous	Oropharynx	Tonsil	2	0	0	N	+
24	51	M	Never	Previous	Oropharynx	Base of tongue	2	2a	0	N	+
25	48	M	Never	Previous	Oropharynx	Tonsil	1	2a	0	N	-
26	40	M	Previous	Never	Oropharynx	Base of tongue	2	2b	0	N	-
27	66	M	Previous	Current	Oropharynx	Base of tongue	2	2b	0	Y	+
28	66	M	Previous	Previous	Oropharynx	Base of tongue	3	0	0	N	-
29	45	M	Never	Previous	Oropharynx	Tonsil	X	3	0	N	+
30	57	F	Current	Previous	Oropharynx	Tonsil	1	1	0	N	+
31	62	M	Current	Previous	Oropharynx	Base of tongue	4	2	X	N	-
32	58	M	Current	N/A	Oropharynx	Tonsil	3	0	X	N	-
33	57	F	Never	Previous	Oropharynx	Base of tongue	3	2b	0	N	-
34	46	M	Previous	Previous	Oropharynx	Tonsil	2	2a	X	N	+
35	41	M	Previous	Previous	Oropharynx	Tonsil	3	2	0	N	-
36	75	F	Previous	Previous	Oropharynx	Soft palate	2	0	0	Y	-
37	67	M	Current	Previous	Oropharynx	Tonsil	3	1	0	N	-
38	72	M	N/A	Previous	Oropharynx	Base of tongue	2	3	X	Y	-
39	56	M	Previous	Previous	Oropharynx	Tonsil	2	3	X	Y	-
40	55	M	Never	Previous	Oropharynx	Base of tongue	2	2	0	Y	-
41	50	M	Never	Previous	Oropharynx	Tonsil	3	2b	0	N	-
42	51	M	Current	Current	Oropharynx	Tonsil	3	2b	X	N	-
43	53	M	Current	Previous	Oropharynx	Base of tongue	3	1	X	Y	-
44	45	F	Current	Current	Oropharynx	Soft palate	3	2c	X	N	-
45	51	M	Current	Current	Oropharynx	Soft palate	1	0	0	Y	-
46	66	M	Never	Current	Oropharynx	Base of tongue	1	3	X	Y	-
47	56	M	Current	Previous	Oropharynx	Tonsil	2	0	0	N	NA
48	58	F	Previous	Previous	Oropharynx	Tonsil	1	0	0	N	NA
49	62	M	Current	Previous	Oropharynx	Soft palate	2	0	0	N	-
50	53	M	Current	Previous	Oropharynx	Piriform	2	1	X	N	-
51	74	M	Current	Never	Oropharynx	Tonsil	3	2c	X	N	-
52	64	M	Current	Previous	Oropharynx	Tonsil	X	X	X	N	-
53	46	M	Previous	X	Oropharynx	Base of tongue	X	X	X	Y	-

[a] Abbreviations: F, female; M, male

[b] Abbreviations: N/A, not applicable

[c] Abbreviations: X, no data available, N/A, not applicable

[d] Abbreviations: X, no data available

[e] T stage. Abbreviations: X, no data available; 1, tumour $\leq$ 2 cm; 2, tumour > 2 cm; 3, tumour > 4 cm; 4a, moderately advanced local disease; 4b, very advanced local disease

[f] N stage. Abbreviations: X, no data available; 0, No regional lymph node metastasis; 1, Metastasis in a single ipsilateral lymph node $\leq$ 3 cm in greatest dimension; 2a, Metastasis in a single ipsilateral lymph node > 3 cm but not more than 6 cm in greatest dimension; 2b, Metastasis in a multiple ipsilateral lymph nodes - none > 6 cm in greatest dimension; 2c, Metastasis in bilateral or contralateral lymph nodes - none > 6 cm in greatest dimension; 3, Metastasis in a lymph node > 6 cm in greatest dimension

[g] M stage. Abbreviations: X, no data available; 0, no distant metastasis; 1, distant metastasis

[h] Tumour recurrence. Abbreviations: N, no; Y, yes

[i] HPV status determined by in-situ hybridisation histochemistry. Abbreviations: X, no data available; N/A, not applicable; -, negative; +, positive

[†] Tissue microarrays (TMA) were prepared with HNSCC specimens from 53 patients who underwent surgery at the University Hospitals Coventry and Warwickshire NHS Trust, UK or the Royal Liverpool and Broadgreen University Hospitals NHS Trust, UK

Supplementary Table S3. Characteristics of HNSCC cell lines

Cell line	Patient details				Clinical features			
	Age	Gender[a]	Smoking	Alcohol	Origin[b]	Site	Pathological stage	TP53 codon
92-VU-040T	65	F	-	-	NP	Tongue	T3N0	WT
93-VU-147T	58	M	+	+	NP	Floor of mouth	T4N2	c.770T>G

[a] Abbreviations: F, female; M, male.

[b] Abbreviations: NP, new primary.

[c] 92-VU-040T cells are a moderately differentiated HPV-negative HNSCC line derived from biopsies of a primary squamous cell carcinoma of the oral cavity (tongue) of a 65 year-old female patient and expresses wild-type p53 (1-4). Synonym: VU-SCC-040. https://web.expasy.org/cellosaurus/CVCL_JL62 (5)

[d] 93-VU-147T cells are a moderately differentiated HNSCC line, which was derived from biopsies of a primary squamous cell carcinoma of the oral cavity (floor of mouth) of a 58 year-old male patient (1-4). The cells contain a heterozygous mutation in the TP53 gene (c.770T>G, p.L257R) predicted to render the translated p53 protein non-functional (6). The cells also contain integrated HPV-16 DNA (1-4). Synonym: VU-SCC-047. https://web.expasy.org/cellosaurus/CVCL_L895 (5).

References

1. Hermsen M A, Joenje H, Arwert F, Welters M J, Braakhuis B J, Bagnay M, et al. Centromeric breakage as a major cause of cytogenetic abnormalities in oral squamous cell carcinoma. Genes Chromosomes Cancer 1996;15:1-9
2. White J S, Weissfeld J L, Ragin C C, Rossie K M, Martin C L, Shuster M, et al. The influence of clinical and demographic risk factors on the establishment of head and neck squamous cell carcinoma cell lines. Oral Oncol 2007;43:701-12
3. Steenbergen R D, Hermsen M A, Walboomers J M, Joenje H, Arwent F, Meijer C J, et al. Integrated human papillomavirus type 16 and loss of heterozygosity at 11q22 and 18q21 in an oral carcinoma and its derivative cell line. Cancer Res 1995;55:5465-71
4. Rampias T, Sasaki C, Weinberger P, and Psyrri A. E6 and E7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. J Natl Cancer Inst 2009;101:412-23
5. Bairoch A. The Cellosaurus: a cell line knowledge resource. https://web.expasy.org/cellosaurus/
6. Kimple R J, Smith M A, Blitzer G C, Torres A D, Martin J A, Yang R Z, et al. Enhanced radiation sensitivity in HPV-positive head and neck cancer. Cancer Res 2013;73:4791-800

Supplementary Table S4. shRNA sequences used in lentiviral vectors.

shRNA sequences[1]	
ID	**Sequence**
PBF shRNA#1	5' GAT CAT CAC CAT GTC GGT A 3'
PBF shRNA#2	5' AAC GCT AAC TGC ACA CGA A 3'
PBF shRNA#3	5' GGT TGT GAA ATA CAG CGT A 3'
PTTG shRNA#1	5' GGT CTG GAC CTT CAA TCA AAG 3'
PTTG shRNA#2	5' GCC TTA CCT AAA GCT ACT AGA 3'

[1]Preliminary screening showed that PTTG shRNA#1 and PBF shRNA#1 gave the greatest knockdown in HNSCC cells (PTTG shRNA#1: ~90%; PBF shRNA#1: ~80%). Based on these results cells transduced with either PTTG or PBF shRNA#1 were expanded and antibiotic-resistant colonies selected to provide a genetically homogenous and clonal cell population. In addition, cells lentivirally transduced with Scrambled (Scr) shRNA, which contains a scrambled sequence with no known homology to human sequences, were also expanded.

Supplementary Table S5. Primary antibodies used in study.

Commercial Taqman Assays		
Antibody	**Clone**	**Supplier**
PBF	Rabbit polyclonal; Custom	Eurogentec
PBF-pY174	Rabbit monoclonal; Custom	Covalab
PTTG	Mouse monoclonal 1gG2 [DCS-280]	Abcam
PTTG-pT60[1,2]	Rabbit monoclonal; Custom	Covalab
H2A.X S139P	Mouse monoclonal [JBW301] [16-193]	Millipore
HA[3]	Mouse monoclonal 1gG1 [16B12]	BioLegend
HA[4]	Rabbit polyclonal [Y-11] [sc-805]	Santa Cruz Biotechnology
p53	Mouse monoclonal IgG2 [DO-1] [sc-126]	Santa Cruz Biotechnology
p53[5]	Rabbit polyclonal [FL-393] [sc-6243]	Santa Cruz Biotechnology
p53 pS15	Rabbit polyclonal [9284]	Cell Signaling Technology
β-actin	Mouse monoclonal [AC-15]	Sigma-Aldrich

[1]T60 phospho-specific PTTG antibody was produced by CovalAb using three peptides; CUK-1323A long phospho-peptide: FDAPPALPKAT**pR**KAL, CUK-1323B short phospho-peptide: LPKAT**pR**KA, and CUK-1323C control peptide: FDAPPALPKATRKAL. Both CUK-1323A and B were used for immunisations to obtain antibodies specific to the phospho-modification. The serum was then purified against the control peptide to remove non-specific antibodies and against CUK-1323B to retain only specific antibodies. The antibody was then immunopurified from serum.

[2]For peptide blocking experiments, the phospho-specific PTTG-T60 antibody was incubated with a two-fold excess of neutralising peptide (supplied by CovalAb) for 2 hours with gentle shaking prior to incubation with Western blot membrane or HNSCC tissue section. Densitometry was performed for Western blot analysis as previously described (Read ML *et al.,* 2014).

[3]Anti-HA antibody used to detect exogenous expression of HA-tagged PTTG (e.g. Fig. 5A and C; Supplementary Fig. S10B) and PBF (e.g. Fig. 5B and D) in whole cell lysates.

[4,5]Anti-HA and p53 antibodies used to validate successful immunoprecipitation in co-IP assays (e.g. Supplementary Fig. S10A and S10C).

Commercial Taqman Assays	
Gene target	**Catalogue Number**
PBF	Hs01036322_m1
PTTG	Hs00851754_u1
TP53	Hs01034249_m1
CDKN1A	Hs00355782_m1
BCL2	Hs00248075_m1
RAD51	Hs00947967_m1
BRCA1	Hs01556193_m1
PPIA	Hs04194521_s1

Supplementary Table S6. List of Taqman® assays (ThermoFisher Scientific) used in study. PPIA was used as internal control. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Supplementary Table S7. p53 mutational status of tumour samples in HNSCC TCGA with profiled HPV status.

Subsite	HPV status	TCGA samples per subgroup	Mutation data available[1]	MUT p53[2]	WT p53[3]
Larynx	-ve	18	17	17(100%)	0(0)
	+ve	3	2	2(100%)	0(0)
Oral Cavity	-ve	45	44	35(79.5%)	9(20.5%)
	+ve	2	1	1(100%)	0(0)
Oropharynx	-ve	8	8	8(100%)	0(0)
	+ve	32	28	1(3.6%)	27(96.4%)

[1]Total number of HNSCC samples with profiled HPV status and corresponding p53 mutation data available in TCGA. Data corresponds to Supplementary Fig. S2D-S2E.
[2]Number of HNSCC tumours with mutant p53. Alterations include missense mutations, truncating mutation and deep deletions. Percentages in brackets.
[3]Number of HNSCC tumours with wild-type p53. Percentage in brackets.

Supplementary Table S8

Clinical and pathological features		PTTG			Fisher's exact test
		Mann-Whitney *U* test			
		No. of patients	H-score (mean±SEM)	p-value	p-value
Age (yr)	<50	13	37.9±11.0	0.21	1.00
	>50	38	61.0±9.4		
Gender[a]	F	14	34.9±7.7	0.23	0.20
	M	37	62.7±9.9		
Smoking status	Never	14	76.8±14.8	0.07	0.12
	Previous/Current	35	47.0±9.1		
Alcohol status	Never	3	76.7±43.2	0.49	0.55
	Previous/Current	44	52.9±7.9		
Sub-site	Tonsil	25	59.3±10.2	0.67	0.75
	Base of tongue	15	59.7±17.2		
T stage	pT1-2	29	54.4±8.7	0.99	0.37
	pT3-4	19	62.7±15.3		
N stage	pN0-1	23	70.0±14.2	0.39	0.78
	pN2-3	26	46.1±7.2		
Recurrence	No	36	57.8±8.0	0.34	0.76
	Yes	14	51.7±18.9		
HPV status[b]	-	36	44.2±8.7	**0.05**	**0.05**
	+	13	82.0±15.8		

[a] Abbreviations: F, female; M, male
[b] Abbreviations: -, negative; +, positive

Supplementary Table S9[1]

Gene	MUT p53 Subgroup v WT p53 (*n* = 157)	N	P_L	P_B	P_T	Median Survival v WT p53 (68.43 months)
PBF	HIGH (Q3Q4)	180	5.39×10^{-4}	1.50×10^{-4}	1.17×10^{-4}	32.19
	LOW (Q1Q2)	180	0.016	0.046	0.021	46.98
	HIGH (Q4)	90	0.0012	5.48×10^{-4}	4.19×10^{-4}	32.19
	LOW (Q1)	90	0.017	NS	0.029	49.41
PTTG	HIGH (Q3Q4)	180	4.88×10^{-4}	8.36×10^{-4}	3.26×10^{-4}	32.62
	LOW (Q1Q2)	180	0.017	0.011	0.0053	47.93
	HIGH (Q4)	90	0.0032	0.0147	0.0053	35.51
	LOW (Q1)	90	NS	NS	NS	52.27

[1]Expression groups Q3Q4 and Q1Q2, as well as Q1 and Q4, compared for both PTTG and PBF in MUT p53 versus WT p53 HNSCC tumours. In all cases the significant difference in overall survival was greater with high expression groups (i.e. Q3Q4 or Q4) for PBF and PTTG than corresponding low expression groups (i.e. Q1Q2 and Q1) in MUT p53 versus WT p53 tumours. *P*-values were determined using the indicated test (L=Log Rank, B=Breslow, T=Tarone-Ware). Median survival times were also shorter in all high expression groups (i.e. Q3Q4 or Q4) compared to corresponding low expression groups.