

Supplementary Tables

Supplementary Table 1. Patients, timepoints, and the sequencing panels used in this study.

Patient	Pre-treatment	End-of-treatment	3MFU	Recurrence	3MFU/RECR	Control
Cervix cancer cohort						
P1	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P2	HPV E6&E7(+)	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P3	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P5	-	HPV16(+)&(-)	HPV16(+)&(-)	HPV16(+)&(-)	-	-
P6	HPV E6&E7(+)	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P7	-	HPV16(+)&(-)	-	-	-	-
P11	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P13	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P15	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P8	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P9	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P17	-	-	HPV16(+)&(-)	-	-	-
P12	-	HPV16(+)&(-)	HPV16(+)&(-)	-	-	-
P4	-	HPV33(+)	-	-	HPV33(+)	-
P19	HPV E6&E7(+)	HPV33(+)	HPV33(+)	-	-	-
P10	HPV E6&E7(+)	HPV45(+)	HPV45(+)	-	-	-
P14	HPV E6&E7(+)	HPV52(+)	-	HPV52(+)	-	-
Control cohort 1 (n=1)	-	-	-	-	-	HPV E6&E7(+), HPV16(+)&(-), HPV33(+), HPV45(+), HPV52(+)
Oropharynx cancer cohort						
13 OPC patients	5HPVs(+)&(-) & E6&E7(+)	-	-	-	-	-
5 SW48 samples	5HPVs(+)&(-) & E6&E7(+)	-	-	-	-	-
Control cohort 2						

21 controls	-	-	-	-	-	HPV16(+) & E6&E7(+)
28 controls						5HPVs(+)&(-) & E6&E7(+)

Abbreviation: 3MFU = 3 month post-treatment follow up; RECR = recurrence; 5HPVs(+)&(-) include HPV-16, HPV-18, HPV-31, HPV-33 and HPV-35

Supplementary Table 2. Accuracy of HPV genotyping

HPV type	# Positive	# Negative	Sensitivity	Specificity	Precision	Accuracy
HPV-16	22	67	95.45%	94.03%	84.00%	94.38%
HPV-18	7	82	85.71%	100%	100%	98.88%

Supplementary Table 3. ctDNA quantification using full-length viral capture HPV-seq for plasma cfDNA samples from 33 cervix cancer and 13 oropharynx cancer patients

Patient	Timepoint	Tissue HPV type	dPCR copies/mL		Plasma HPV-seq attributes		
			E6	E7	# HPV fragments	MAPQ>30	Copies/mL
Cervix cancer: Individual Patient Samples							
HPV dPCR Positive Samples							
P14	RECR	HPV-52	527.27	553.77	62478	99.90%	793.08
P4	end-of-treatment	HPV-33	440.94	387.42	33674	98.20%	384.21
P14	end-of-treatment	HPV-52	189.06	212.26	11958	99.90%	252.30
P7	end-of-treatment	HPV-16	71.60	59.66	11696	99.80%	152.19
P4	3MFU/RECR	HPV-33	69.12	62.21	6342	98.40%	71.93
P15	end-of-treatment	HPV-16	12.80	26.52	324	100%	9.29
P5	RECR	HPV-16	0	2.95	450	99.70%	3.20
P17	3MFU	HPV-16	1.04	2.09	56	98.20%	0.31
P6	3MFU	HPV-16	21.80	0	0	0	0
P11	end-of-treatment	HPV-16	13.12	13.12	0	0	0
P13	end-of-treatment	HPV-16	0	11.65	0	0	0
HPV dPCR Negative Samples							

P8	end-of-treatment	HPV-16	0	0	94	98.9%	1.28
P1	end-of-treatment	HPV-16	0	0	44	100%	0.66
P3	end-of-treatment	HPV-16	0	0	24	100%	0.34
P5	end-of-treatment	HPV-16	0	0	10	100%	0.12
P12	end-of-treatment	HPV-16	0	0	2	100%	0.03
P1	3MFU	HPV-16	0	0	0	-	0
P2	end-of-treatment	HPV-16	0	0	0	-	0
P2	3MFU	HPV-16	0	0	0	-	0
P3	3MFU	HPV-16	0	0	0	-	0
P5	3MFU	HPV-16	0	0	0	-	0
P6	end-of-treatment	HPV-16	0	0	0	-	0
P8	3MFU	HPV-16	0	0	0	-	0
P9	end-of-treatment	HPV-16	0	0	0	-	0
P9	3MFU	HPV-16	0	0	0	-	0
P11	3MFU	HPV-16	0	0	0	-	0
P12	3MFU	HPV-16	0	0	0	-	0
P13	3MFU	HPV-16	0	0	0	-	0
P15	3MFU	HPV-16	0	0	0	-	0
P19	end-of-treatment	HPV-33	0	0	0	-	0
P19	3MFU	HPV-33	0	0	0	-	0
P10	end-of-treatment	HPV-45	0	0	0	-	0
P10	3MFU	HPV-45	0	0	0	-	0

Oropharynx cancer: Individual Patient Samples

HPV dPCR Positive Samples

OPC8	pre-treatment	HPV-16	12925.02	13048.90	75772	99.90%	35119.21
OPC1	pre-treatment	HPV-16	9491.00	7968.38	211654	99.98%	28611.55
OPC10	pre-treatment	HPV-16	6052.22	6026.24	432532	99.98%	25960.12
OPC5	pre-treatment	HPV-16	5021.90	4432.08	569668	99.98%	23535.47
OPC12	pre-treatment	HPV-16	5605.84	6269.95	319978	99.98%	16848.72

OPC13	pre-treatment	HPV-16	1592.93	1847.60	121640	99.97%	8120.31
OPC9	pre-treatment	HPV-16	1860.36	3199.94	14388	99.94%	6077.61
OPC7	pre-treatment	HPV-16	1317.51	1361.13	60342	99.98%	4315.83
OPC6	pre-treatment	HPV-16	1227.26	1104.35	69340	99.98%	4024.84
OPC4	pre-treatment	HPV-16	385.86	372.46	37466	99.98%	1167.01
OPC2	pre-treatment	HPV-16	83.93	46.08	3880	99.97%	208.48
OPC11	pre-treatment	HPV-16	23.92	16.64	242	99.59%	119.37
OPC3	pre-treatment	HPV-16	4.19	2.49	174	100%	6.74

Abbreviation: 3MFU = 3 month post-treatment follow up; RECR = recurrence

Supplementary Table 4. Fragment length distributions for sequenced human and HPV DNAs

Patient	Timepoint	HPV type	# Fragments		Median fragment length		Δ fragment length	p-value
			Human	HPV	Human	HPV		
Cervix cancer: Individual Patient Samples								
HPV dPCR Positive Samples								
P14	RECR	HPV-52	123651	17052	170	148	22	3×10^{-180}
P4	end-of-treatment	HPV-33	138270	10035	166	143	23	5.7×10^{-321}
P7	end-of-treatment	HPV-16	197669	3710	166	153	13	1.4×10^{-218}
P14	end-of-treatment	HPV-52	123912	3583	171	138	33	$< 1 \times 10^{-320}$
P4	3MFU/RECR	HPV-33	197744	1969	171	150	21	5.9×10^{-125}
P5	RECR	HPV-16	153455	125	167	150	17	3.1×10^{-07}
P15	end-of-treatment	HPV-16	209455	102	165	162	3	0.12
P17	3MFU	HPV-16	158689	15	168	125	43	2.8×10^{-06}
HPV dPCR Negative Samples								
P8	end-of-treatment	HPV-16	122680	33	168	180	-12	0.01
P1	end-of-treatment	HPV-16	110289	13	171	155	16	0.2
P3	end-of-treatment	HPV-16	113824	9	175	135	40	0.04
P5	end-of-treatment	HPV-16	144870	3	174	133	41	$< 1 \times 10^{-320}$
P12	end-of-treatment	HPV-16	125281	1	169	121	48	-
Cervix cancer: Groups								

HPV type							
HPV-16			1336212	4011	168	153	15 1×10^{-320}
HPV-33			336014	12004	168	144	22 1×10^{-320}
HPV-52			247563	20635	171	146	25 1×10^{-320}
Timepoint							
end-of-treatment			1286250	17489	168	144	24 1.0×10^{-52}
3MFU			158689	15	168	125	43 2.8×10^{-06}
RECR			474850	19146	169	148	21 1.0×10^{-106}
Panel							
Single-strand			583577	32639	169	145	24 1×10^{-320}
Dual-strand			1336212	4011	168	153	15 1×10^{-320}
Oropharynx cancer: Individual Patient Samples							
HPV dPCR Positive Samples							
OPC1	pre-treatment	HPV-16	99982	67204	166	142	24 1×10^{-320}
OPC2	pre-treatment	HPV-16	82148	1245	167	141	26 9.7×10^{-223}
OPC3	pre-treatment	HPV-16	80969	55	167	144	23 6.2×10^{-13}
OPC4	pre-treatment	HPV-16	98758	10435	167	152	15 1×10^{-320}
OPC5	pre-treatment	HPV-16	102790	173687	164	140	24 1×10^{-320}
OPC6	pre-treatment	HPV-16	85621	21442	168	144	24 7.5×10^{-186}
OPC7	pre-treatment	HPV-16	90934	14989	166	162	4 8.4×10^{-148}
OPC8	pre-treatment	HPV-16	16334	19337	167	155	12 4.8×10^{-252}
OPC9	pre-treatment	HPV-16	13039	3511	167	158	9 0.25
OPC10	pre-treatment	HPV-16	109318	129727	164	140	24 1×10^{-320}
OPC11	pre-treatment	HPV-16	13499	75	168	151	17 2.5×10^{-11}
OPC12	pre-treatment	HPV-16	93698	94408	164	143	21 1×10^{-320}
OPC13	pre-treatment	HPV-16	95050	33547	166	141	25 1×10^{-320}
Oropharynx cancer: Groups							
HPV type							
HPV16			982140	569662	166	142	24 1×10^{-320}

Abbreviation: 3MFU = 3 month post-treatment follow up; RECR = recurrence

Supplementary Table 5. 38 HPV genomes interrogated by the HPV genotyping panel

HPV genome	HPV name
gi 333071	Human papillomavirus 5 (HPV-5)
gi 60955	Human papillomavirus 6 (HPV-6)
gi 333026	Human papillomavirus 11 (HPV-11)
gi 396910	Human papillomavirus 12 (HPV-12)
gi 333031	Human papillomavirus 16 (HPV-16)
gi 60975	Human papillomavirus 18 (HPV-18)
gi 9627305	Human papillomavirus 26 (HPV-26)
gi 333048	Human papillomavirus 31 (HPV-31)
gi 333049	Human papillomavirus 33 (HPV-33)
gi 396997	Human papillomavirus 35 (HPV-35)
gi 1020234	Human papillomavirus 38 (HPV-38)
gi 333245	Human papillomavirus 39 (HPV-39)
gi 397014	Human papillomavirus 40 (HPV-40)
gi 333211	Human papillomavirus 42 (HPV-42)
gi 397022	Human papillomavirus 45 (HPV-45)
gi 333087	Human papillomavirus 51 (HPV-51)
gi 397038	Human papillomavirus 52 (HPV-52)
gi 9627377	Human papillomavirus 53 (HPV-53)
gi 9628437	Human papillomavirus 54 (HPV-54)
gi 396937660	Human papillomavirus 55 (HPV-55)
gi 397053	Human papillomavirus 56 (HPV-56)
gi 222386	Human papillomavirus 58 (HPV-58)
gi 557236	Human papillomavirus 59 (HPV-59)
gi 9628574	Human papillomavirus 61 (HPV-61)
gi 39932599	Human papillomavirus 62 (HPV-62)

gi 1020290	Human papillomavirus 66 (HPV-66)
gi 3228267	Human papillomavirus 67 (HPV-67)
gi 71726685	Human papillomavirus 68 (HPV-68)
gi 6970418	Human papillomavirus 69 (HPV-69)
gi 1173493	Human papillomavirus 70 (HPV-70)
gi 12084981	Human papillomavirus 71 (HPV-71)
gi 1491683	Human papillomavirus 72 (HPV-72)
gi 1491692	Human papillomavirus 73 (HPV-73)
gi 40804509	Human papillomavirus 81 (HPV-81)
gi 6970427	Human papillomavirus 82 (HPV-82)
gi 5059324	Human papillomavirus 83 (HPV-83)
gi 12958167	Human papillomavirus 84 (HPV-84)
gi 50253426	Human papillomavirus 96 (HPV-96)

Supplementary Table 6. Summary of dPCR results and plasma cfDNA yields

Patient	Timepoint	HPV type	Plasma cfDNA conc (ng/mL)	E6 (copies/mL)	E7 (copies/mL)	E6 (copies/10ng)	E7 (copies/10ng)	E6 (positive droplets)	E7 (positive droplets)
Cervix cancer									
P14	pre-treatment	HPV52	10.048	901.91	851.07	897.6	847	616	582
P14	RECR	HPV-52	11.776	527.27	553.77	447.75	470.25	265	278
P4	end-of-treatment	HPV-33	11.328	440.94	387.42	389.25	342	257	227
P14	end-of-treatment	HPV-52	19.04	189.06	212.26	99.3	111.48	49	57
P19	pre-treatment	HPV33	13.632	0	113.49	0	83.25	0	55
P4	3MFU/RECR	HPV-33	15.36	69.12	62.21	45	40.5	31	28
P7	end-of-treatment	HPV-16	18.08	71.6	59.66	39.6	33	29	24
P6	pre-treatment	HPV16	14.784	9.11	32.52	6.16	22	4	14
P10	pre-treatment	HPV45	5.856	2.83	5.67	4.84	9.68	3	6

P15	end-of-treatment	HPV-16	41.568	12.8	26.52	3.08	6.38	2	4
P17	3MFU	HPV-16	6.624	1.04	2.09	1.575	3.15	1	2
P5	RECR	HPV-16	8.736	0	2.95	0	3.375	0	2
P2	pre-treatment	HPV16	6.56	1.01	1.01	1.54	1.54	1	1
P11	end-of-treatment	HPV-16	99.392	13.12	13.12	1.32	1.32	1	1
P13	end-of-treatment	HPV-16	75.648	0	11.65	0	1.54	0	1
P6	3MFU	HPV-16	121.136	21.8	0	1.8	0	1	0
Oropharynx cancer									
OPC8	pre-treatment	HPV16	18.77	12925.02	13048.90	6886	6952	4376	4422
OPC12	pre-treatment	HPV16	8.88	5605.84	6269.95	6314	7062	3211	3542
OPC5	pre-treatment	HPV16	7.66	5021.90	4432.08	6556	5786	3484	3114
OPC10	pre-treatment	HPV16	11.81	6052.22	6026.24	5126	5104	2964	2954
OPC1	pre-treatment	HPV16	23.07	9491.00	7968.38	4114	3454	2388	2029
OPC9	pre-treatment	HPV16	14.26	1860.36	3199.94	1304.6	2244	832	1399
OPC13	pre-treatment	HPV16	11.81	1592.93	1847.60	1348.6	1564.2	870	1005
OPC6	pre-treatment	HPV16	8.73	1227.26	1104.35	1405.8	1265	999	901
OPC7	pre-treatment	HPV16	13.22	1317.51	1361.13	996.6	1029.6	581	599
OPC4	pre-treatment	HPV16	6.09	385.86	372.46	633.6	611.6	469	452
OPC2	pre-treatment	HPV16	7.48	83.93	46.08	112.2	61.6	72	40
OPC11	pre-treatment	HPV16	15.76	23.92	16.64	15.18	10.56	10	7
OPC3	pre-treatment	HPV16	5.95	4.19	2.49	7.04	4.18	5	3