

## Supplementary Materials: Cell-free HPV-DNA as Biomarker for Oropharyngeal Squamous Cell Carcinoma—A Step towards Personalized Medicine?

Nora Wuerdemann, Rishabh Jain, Anne Adams, Ernst-Jan M. Speel, Steffen Wagner, Simon A. Joosse and Jens P. Klussmann

**Table S1.** Extra information on studies included in the review and meta-analysis.

Study	Year	Target Gene	Volume	Treatment	HPV+ Tissue Status	Concentration of cfHPV-DNA (at first Diagnosis)	Tube	Follow up Time
Cao et al. [22]#**	2012	E6, E7, L1	NR	Chemoradiotherapy	p16 staining PCR/ HPV In-situ hybridization	Median = 222 copies/ml (0–5,500 copies/ml)	NR	12–22 months
Ahn et al. [23]#**	2014	E6, E7	NR	Surgery/Chemoradiotherapy	HPV In-situ hybridization qPCR	NR	Citrate	49 months
Dahlstrom et al. [24]#**	2015	E6/E7	500 µL	Chemoradiotherapy	PCR	<8000 copies/mL E6 Median = 83 copies/mL E7 Median = 43 copies/mL	NR	67 months
Wang et al. [25]#	2015	E7	6 mL	Surgery/Chemoradiotherapy	PCR	NR	NR	12 months
Kuhs et al. [26]#	2017	Antibody: HPV16- L1, E1, E2,E4, E6, E7 HPV6,11,18,31,33,45,58- E6	NR	Surgery/Chemoradiotherapy	PCR (DNA/RNA testing) p16 staining HPV In-situ hybridization	NR	NR	307 days
Lee et al. [27]#**	2017	39-amplicon single pool panel covering 34 distinct regions of HPV16 genome	5 mL	Chemoradiotherapy	p16 staining qPCR	NR	NR	12 weeks
Chera et al. [28]#	2019	E7	2–5 mL	Chemoradiotherapy	p16 staining	HPV16 n = 84 Median = 419 copies/mL(8– 22,579 copies/mL) High risk HPV strain n = 8 Median = 124	BCT tubes (Streck)	16.5 months

						copies/mL (71–15,829 copies/mL)		
Damerla et al. [29]#	2019	E6,E7	4–5 mL	Surgery/Chemoradiotherapy	HPV In-situ hybridization p16 staining	Mean= 1,218 copies/mL (0 to 13,163 copies/mL)	BCT tubes (Streck) or BD vacutainer K2 EDTA tubes	NR
Nguyen et al. [30]#	2020	E7	NR	Chemoradiotherapy/ Immunotherapy	p16 staining	Median= 880 copies/mL (11–161,680 copies/mL)	EDTA	20 months
Reder et al. [31]#	2020	E6,E7	500 µL	Surgery/Chemoradiotherapy	p16 staining PCR	E6 Median= 9,283 copies/mL (0–79,850 copies/mL) E7 Median = 12,699 copies/ml (0–1,50,838 copies/ml)	BCT tubes (Streck)	NR
Mazurek et al. [32]	2016	E6,E7	1 mL	Chemoradiotherapy	NR	OPSCC 9.60 ± 6.23 ng/ml > other HNSCC 7.67 ± 4.44 ng/ml	NR	NR
Jeannot et al. [33]	2016	E7	200µL	NR	NR	4,605 copies/ml	NR	NR
Rutkowski et al. [34]	2017	E6,E7	1 mL	Chemoradiotherapy	NR	NR	K <sub>3</sub> EDTA	Follow up time is given separately for each of the 55 patients
Hanna et al. [35]	2018	E7	NR	Cytotoxic/ Immunotherapy	p16 staining PCR/ HPV In-situ hybridization	Cutoff value = 5–10 copies/ml	NR	133 days
Veyer et al. [36]	2019	E6	200µL	NR	p16 staining/HPV16 molecular detection	Median = 319.4 copies/ml (0–1,47,416.7 copies/ml)	EDTA	2.84 years
Chera et al. [37]**	2020	E6, E7	2–5 mL	Chemoradiotherapy	p16 staining	NR	BCT tubes (Streck)	23 months

**Abbreviations:** NR- not reported, qPCR- Quantitative PCR, BCT- Blood collection tube, OPSCC- Oropharyngeal squamous cell carcinoma. # studies included in meta-analysis at first diagnosis and \*\* studies included in meta-analysis during follow-up.

Table S2. Quality assessment of the studies included in meta-analysis.

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Text	Reference Standard	Flow and Timing	Patient Selection	Index Text	Reference Standard
Cao et al. [22]#	L	L	L	L	L	L	L
Ahn et al. [23]#	L	L	L	L	L	L	L
Dahlstrom et al. [24]#	L	L	L	L	L	L	L
Wang et al. [25]#	L	L	L	L	L	L	L
Kuhs et al. [26]#	L	L	L	L	L	L	L
Lee et al. [27]#	L	L	L	L	L	L	L
Chera et al. [28]#	L	L	L	L	L	L	L
Damerla et al. [29]#	L	L	L	L	L	L	L
Nguyen et al. [30]#	H	L	L	L	H	L	L
Reder et al. [31]#	L	L	L	L	L	L	L

**Abbreviations:** L- Low risk of bias, H- High risk of bias. # studies included in meta-analysis at first diagnosis. # studies included in meta-analysis at first diagnosis.

**Table S3.** Primers and probes used in the different studies of the review and meta-analysis.

Studies	HPV-cfDNA Primers	Probes	Method
Cao et al. [22]###	HPV16 E6/7	HPV16 E6/E7	qPCR
	F- 5'-GAACCGAAACCGGTTAGTATAA-3'	5'-FAM-AGGACCCACAGGAGCGACCC-BHQ1 -3'	
	R- 5'-ATGTATAGTTGTTTGCAGCTCTGT-3'	HPV18 E6/E7	
	HPV18 E6/E7	5'-CO560-	
	F- 5'- GGACCGAAAACGGTGTATATAA-3'	ATGTGAGAAAACACACCACAATACTATGGCGCG-BHQ1	
	R- 5'- CAGTGAAGTGTTTCAGTTCGGT-3'	-3'	
Ahn et al. [23]###	HPV L1		qPCR
	F- 5'- TTTGTTACTGTGGTAGATACATC-3'		
	R- 5'- GAAAAATAAACTGTAATCATATTC-3'		
	HPV E6	HPV E6	
	F- 5'-TCAGGACCCACAGGAGCG-3'	5'-FAM-	
	R- 5'-CCTCACGTGCGCAGTAACTGTTG-3'	CCCAGAAAAGTTACCACAGTTATGCACAGAGCT-TAMRA -3'	
Dahlstrom et al. [24]###	HPV E7	HPV E7	qPCR
	F- 5'-CCGGACAGAGCCCATTACAA-3'		
	R- 5'-CGAATGTCTACGTGTGTGCTTTG-3'	5'-FAM-CGCACAACCGAAGCGTAGAGTCACACT-TAMRA -3'	
	Not provided in the literature	Not provided in the literature	
Wang et al. [25]#	The primers used in this article was referenced to the study- Vogelstein, B., & Kinzler, K. W. (1999). Digital pcr. <i>Proceedings of the National Academy of Sciences</i> , 96(16), 9236-9241.	The probes used in this article was referenced to the study- Vogelstein, B., & Kinzler, K. W. (1999). Digital pcr. <i>Proceedings of the National Academy of Sciences</i> , 96(16), 9236-9241.	ddPCR
Kuhs et al. [26]#	-	-	Multiplex serologic testing
Lee et al. [27]###	-	-	HPV16-detect
Chera et al. [28]#	HPV16	HPV16	ddPCR
	F- 5'- TGA CTCTACGCTTCGGTTG -3'	5'-FAM- CGTACAAAGCACACACGTAGACATTCGTAC-	
	R- 5'- GCCCATTAACAGGTCTTCC -3'	ZEN -3'	
	HPV18	HPV18	

	F- 5'- TGAAGCCAGAATTGAGCTAG -3' R- 5'- AGGACAGGGTGTTCAGAA -3' HPV31	5'-LNA- CAGACGACCTTCG-HEX -3'  HPV31	
	F- 5'- AGCACACAAGTAGATATTCGC -3' R- 5'- TAGTAGAACAGTTGGGGCA -3' HPV33	5'-LNA- TAACAGCTCTTGC -HEX-3'  HPV33	
	F- 5'- TAACACCACAGTTCGTTTATGT -3' R- 5'- ACAATATTCAGTGTGCCATA -3' HPV35	5'-LNA- TGACCTACG AACCC-HEX -3'  HPV35	
	F- 5'- TGAGGCGACACTACGTC -3' R- 5'- GTGCCATTAATAAATCTTCCAA -3'	5'-LNA- AGAGCACACACAT-HEX -3'	
<b>Damerla et al.</b> <b>[29]#</b>	HPV16 F- 5'- TATGCACAGAGCTGCAAACA -3' R- 5'- GCAAAGTCATATACCTCACGTC -3' HPV33	HPV16 5'-FAM- TGTGTGTACTGCAAGCAACAGTTACTG – lowaBlack -3' HPV33	ddPCR qPCR
	F- 5'- CCACAGTTCGTTTATGTGTC -3' R- 5'- TGCCATAAGTAGTTGCTGT -3'	5'-FAM- AGTACAGCAAGTGACCTACGAACCA – lowaBlack -3'	
<b>Nguyen et al.</b> <b>[30]#</b>	Same as that of Jeannot et al. HPV16 F- 5'- TCCAGCTGGACAAGCAGAAC -3' R- 5'- CACAACCGAAGCGTAGAGTC -3'	Same as that of Jeannot et al. HPV16 5'-FAM- ACAGAGCCCATTACAAT –Taqman probe -3'	ddPCR
<b>Reder et al.</b> <b>[31]#</b>	HPV E6 F- 5'- GCACCAAAGAGAAGTGAATG -3' R- 5'- GTTGCAGCTCTGTGCATAACTG -3' HPV E7 F- 5'- CAGCTCAGAGGAGGAGGATG -3' R- 5'- GTAATGGCTCTGTCCGGT -3'	HPV E6 5'-JOE- AGGACCCACAGGAGCGACCCAGAAAGTTAC- BHQ1 -3' HPV E7 5'-FAM- AGATGGTCCAGCTGGACAAGCAG AACCGG-BHQ1 -3'	qPCR
<b>Mazurek et al.</b> <b>[32]</b>	HPV16 F- 5'- TTGCAGATCATCAAGAACACGTAGA -3' R- 5'- CAGTAGAGATCAGTTGTCTCTGGTTGC -3'  HPV18 F- 5'- AGAGGCCAGTGCCATTCGT -3' R- 5'- GTTCTCTGCGTCGTGGAGT -3'	HPV16 5'-FAM- AATCATGCATGGAGATACACCTACATTGCATGA- TAMRA -3'  HPV18 5'-FAM- TCCTGTCGTGCTCGGTTGCAGC -TAMRA-3'	TaqMan based TERT amplification

<b>Jeannot et al.</b> [33]	HPV16	HPV16	ddPCR
	F- 5'- TCCAGCTGGACAAGCAGAAC -3' R- 5'- CACAACCGAAGCGTAGAGTC -3'	5'-FAM- ACAGAGCCCATTACAAT –Taqman probe -3'	qPCR
	HPV18	HPV18	
	F- 5'- AACATTACCAGCCCGACGA -3' R- 5'- TCGTCTGCTGAGCTTTCTAC -3'	5'-FAM- AACACAACGTCACACAA –Taqman probe -3'	
<b>Rutkowski et al.</b> [34]	Same as that of Mazurek et al.	Same as that of Mazurek et al.	TaqMan based TERT amplification
	HPV16	HPV16	
	F- 5'- TTGCAGATCATCAAGAACACGTAGA -3' R- 5'- CAGTAGAGATCAGTTGTCTCTGGTTGC -3'	5'-FAM- AATCATGCATGGAGATACACCTACATTGCATGA- TAMRA -3'	
	HPV18	HPV18	
	F- 5'- AGAGGCCAGTGCCATTCGT -3' R- 5'- GTTCTCTGCGTCGTTGGAGT -3'	5'-FAM- TCCTGTCGTGCTCGGTTGCAGC -TAMRA-3'	
	<b>Hanna et al.</b> [35]	HPV16	ddPCR
	F- 5'- GCTCAGAGGAGGAGGATGAAATAGA -3' R- 5'- GAGTCACACTTGCAACAAAAGGTT -3'	5'-FAM- CCGGACAGAGCCCATTACAAT –NFQ -3'	
	HPV18	HPV18	
	F- 5'- AGGAAGAAAACGATGAAATAGATGGAGTT -3' R- 5'- GGCTTCACACTTACAACACATAC -3'	5'-FAM- ACCAGCCCGACGAGCCGA –NFQ -3'	
	HPV31	HPV31	
	F- 5'- CAGCTCAGATGAGGAGGATGTC -3' R- 5'- ACTTACACTGACAACAAAAGGTAACGA -3'	5'-FAM- AACCGGACACATCCAATTAC -NFQ-3'	
	HPV33	HPV33	
	F- 5'- CGGCCAGATGGACAAGCA -3' R- 5'- TGCTGTACTGTTGACACATAAACGA -3'	5'-FAM- CCGACGAGCCGAACCACAG -NFQ -3'	
	HPV45	HPV45	
	F- 5'- CAGATGGAGTTAGTCATGCACAAC -3' R- 5'- CCGTCACACTTACAACATACACACA -3'		
	<b>Veyer et al.</b> [36]	Not provided in the literature	ddPCR
<b>Chera et al.</b> [37]	Same as that of Chera et al.	Same as that of Chera et al.	ddPCR
	** HPV16	HPV16	

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F- 5'- TGA	5'-FAM-
CTCTACGCTTCGGTTG -3'	CGTACAAAGCACACACG
R- 5'- GCCC	TAGACATTCGTAC-
ATTAAACAGGTCTTCC -3'	ZEN -3'
HPV18	HPV18
F- 5'- TGA	5'-LNA-
AAGCCAGAATTGAGCTAG -3'	CAGACGACCTTCG-HEX -3'
R- 5'- AGG	HPV31
ACAGGGTGTTCAGAA -3'	5'-LNA- TAACAGCTCTTGC -HEX-3'
HPV31	HPV33
F- 5'- AGC	5'-LNA- TGACCTACG AACCC-HEX -3'
CACACAAGTAGATATTCGC -3'	HPV35
R- 5'- TAG	5'-LNA- AGAGCACACACAT-HEX -3'
TAGAACAGTTGGGGCA -3'	
HPV33	
F- 5'- TAA	
CACCACAGTTCGTTTATGT -3'	
R- 5'- ACA	
ATATTCAGTGTGCCATA -3'	
HPV35	
F- 5'- TG	
AGGCGACACTACGTC -3'	
R- 5'- GT	
GCCCATTAATAAATCTTCAA -3'	

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**Abbreviations:** NR- not reported, qPCR- Quantitative PCR, ddPCR- Droplet digital PCR. # studies included in meta-analysis at first diagnosis and \*\* studies included in meta-analysis during follow-up.



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