Tumor Biology 43 (2021) 99–113
DOI:10.3233/TUB-200049
IOS Press

Lack of CD44 overexpression and application of concurrent chemoradiotherapy with cisplatin independently indicate excellent prognosis in patients with HPV-positive oropharyngeal cancer

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Received 2 July 2020
Accepted 28 November 2020

Abstract.

BACKGROUND: HPV-16 positivity in patients with squamous cell carcinoma of oropharynx (OPSCC) is associated with better prognosis. However, in more than 40% of HPV infected patients progression of cancer disease is observed, which indicates the presence of cancer cells resistant to therapy. Some studies suggest that there may be a subpopulation of cancer stem cells (CSCs), which simultaneously exhibit unlimited ability to self-renew and differentiate towards neoplastic cells. The relation between HPV16 infection and biomarkers of CSCs is unclear.

OBJECTIVE: The aim of the study was to compare the expression of CD44, CD98, ALDH1/2 and P16 in oropharyngeal cancer patients with or without HPV16 infection, as well as to analyze the prognostic potential of selected CSCs biomarkers in these two subgroups.

METHODS: The study was performed in a group of 63 patients. HPV16 infection status was analyzed by quantitative polymerase chain reaction, while CD44, CD98, ALDH1/2 and P16 expression by immunohistochemistry. In survival analysis, two endpoints were applied: overall survival (OS) and disease-free survival (DFS).

RESULTS: Among 63 cancers, HPV16 infection was found in 25 tumors (39.7%), overexpression of CD44, CD98, ALDH1/2 and P16 in 43 (68.2%), 30 (47.6%), 33 (52.4%) and 27 (42.9%) cancers, respectively. In the HPV16-positive subgroup, DFS rate of 100% was observed in patients with tumors characterized by lack of CD44 overexpression and those treated with

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concurrent chemoradiotherapy with cisplatin (CisPt-CRT). In the HPV16-negative subgroup 100% of DFS was noticed for patients ($n = 6$) with P16 immunopositive tumors. In this subgroup none of the CSCs biomarkers evaluated in the study had any impact on OS or DFS. In patients with HPV16-positive oropharyngeal cancer, lack of CD44 overexpression and application of CisPt-CRT were found to be positive prognostic factors.

Keywords: Oropharynx, cancers, HPV16, cancer stem cells, biomarkers, prognosis

1. Introduction

The 5-year overall survival (OS) for patients with advanced squamous cell carcinomas of head and neck (HNSCC) is approximately 50% [1]. One of the reasons of these disappointing outcomes is a high rate of local recurrence and distant metastases after treatment [1], which indicates the presence of cancer cells resistant to therapy. Some studies suggest that there may be a subpopulation of cancer stem cells (CSCs), which simultaneously exhibit unlimited ability to self-renew and differentiate towards neoplastic cells [2]. However, with regard to prognosis of HNSCC patients, it should be taken into account that, as was shown in many studies (for review see [3]) and meta-analyses [4–7], better prognosis is related to Human Papillomavirus (HPV) infection. This infection (most often with HPV16) is currently considered an important etiological factor in the development of squamous cell carcinoma of the oropharynx (OPSCC) [3–7]. However, the impact of CSCs biomarkers on HPV infection and prognosis of patients with OPSCC is not fully understood. Some studies indicate overexpression of selected CSCs markers (CD44, CD98, and ALDH1) in HPV-positive HNSCC compared to those without infection [8, 9], others did not confirm these findings [10, 11]. Some authors have shown that CD98 overexpression indicated worse prognosis, irrespective of viral infection [11], others have revealed that absent/week CD44 expression was associated with significantly better disease free survival (DFS), but only in the in the subgroup with HPV positivity [12]. In these studies prognostic potential of CSCs biomarkers and HPV infection was not analyzed in relation to treatment type. Therefore, the aim of the present study was to compare the expression and prognostic potential of selected CSCs markers (CD44 CD98, ALDH1/2) between the subgroups of patients with HPV-positive and HPV-negative OPSCC. Additionally, the analysis was subdivided, for the first time, into subgroups of patients treated with different modalities (radiotherapy - RT, cisplatin-based chemotherapy - CisPt-CRT and induction chemotherapy followed by RT).

2. Material and methods

2.1. Patients selection

A series of 63 patients with OPSCC treated between 2007 and 2014, in Maria Sklodowska-Curie National Research Institute of Oncology, Cracow Branch, Poland have been qualified for the study. Inclusion criteria were: SCC of the oropharynx, lack of previously cancer treatment, no distant metastases at the time of diagnosis, follow-up time no shorter than 5 years until the end of 2019.

2.2. Histpathological verification

For 63 patients, haematoxylin/eosin-stained sections were reviewed independently by two pathologists in order to confirm histological diagnosis, grade and degree of keratinization. For further analysis, they also indicated paraffin blocks with at least 50% of tumor neoplasm.

2.3. Assessment of HPV16 infection

HPV16 presence was assessed based on DNA extracted from 4 μm thick FFPE sections and ReliaPrep™FFPE gDNA Miniprep System (Promega, Madison, USA). All details concerning this procedure were described earlier [13]. Briefly, after 1 min incubation with mineral oil at 80 °C, addition of Solution Buffer and centrifugation, samples were incubated with Proteinase K for the whole night at 56 °C and then for 1 h at 80 °C. After cooling, RNAse A treatment for 5 min. and incubation with mixture of BL Buffer and 100% ethanol, the aqueous phase was transferred to the Binding Column. DNA was eluted with 50 μl of Elution Buffer. Quantity and quality (A260/280 and A260/230 ratios) of DNA were assessed spectrophotometrically with Biophotometer Plus (Eppendorf AG, Hamburg, Germany). DNA samples were stored at –20 °C until analysed.

HPV16 presence was detected by quantitative PCR, using primers (F: GAG AAC TGC AAT GTT TCA GGA CC, R:TGT ATA GTT GTT TGC AGC TCT GTG C) and TaqMan probe (6FAM-CAG GAG CGA CCC AGA AAG TTA CCA CAG TT-TAMRA) specific for 81 bp fragment of HPV16 *E6* gene (all synthesized by Thermo FisherScientific, Waltham, USA). All details concerning qPCR were given earlier [13]. CaSki cervical cell line with insert of HPV16 was added to each qPCR series as positive control. The negative control was a sample containing water instead of DNA attached to each qPCR series. Each sample was tested in duplicate.

2.4. Immunohostochemistry - analysis of CD44, CD98, ALDH1/2 and P16 expression

Immunohistochemistry (IHC) was performed on 4 μm paraffin sections mounted on Super Frost Plus slides (Menzel - Gläser, Germany). First, slides were deparaffinised and hydrated through a series of xylens and alcohols. To unmask antigens, 50 min incubation in TRS (pH = 6.1, cat. no. S1699, DakoCytomation, Denmark) preheated to 96 °C was applied. The activity of endogenous peroxidases was blocked with a 0.3% hydrogen peroxide for 30 min. One-hour incubation with an primary antibody (CD44 Monoclonal Antibody, cat. no. MA5-13890, dilution: 1:2000, Thermo, Fisher Scientific, Fremont, CA, USA; CD98 Monoclonal Antibody (E-5), cat. no. sc-376815, dilution 1:100; ALDH1/2 Monoclonal Antibody (H-8); cat. no. sc-166362, dilution: 1:150, both Santa Cruz Biotechnology, Inc., Dallas, USA) was applied. BrightVision system (Immunologic, Duiven, Netherlands) and DAB (Vector Laboratories, Inc., Burlingame, CA, USA) was used for staining visualization. Immunoreactivity of CD44, CD98 and ALDH1/2 was assessed using histological score (H-score) as: H-score = (1 × percentage of weakly positive cells) + (2 × percentage of moderately strong positive cells) + (3 × percentage of strongly positive cells), giving a range of 0 to 300 [14, 15]. We decided to assume H score study (which include the sum of individual H-scores for each intensity level seen) in order to better clarity of immunostaining scoring of all three CSCs biomarkers and the transparency of the results.

Expression of P16 in the group of studied tumors was analyzed by us earlier as a part of study concerning differences in the prognosis of HPV-16 positive patients according to viral load and expression of P16 [16]. P16 immunostaining was assessed using CINtec® Histology Kit (Roche, Heidelberg, Germany) according to the manufacturer's procedure. Immunopositivity was defined according to Lewis et al. [17] as follows: > 75% of positive staining cells or > 50% staining with > 25% confluent areas of positive staining.

2.5. Statistical analysis

Descriptive statistics were used to determine mean values and SE of continuous variables. Relationships between categorized variables were examined by Pearson's $\chi 2$ test. In the survival analysis,

two endpoints were considered: 5-year OS (time from the end of therapy until death from any cause within 5 years after completing the treatment) and 5-year DFS (time from the end of therapy until the first documented evidence of cancer progression - treatment failure, locoregional recurrence, distant metastasis within 5 years after completing the treatment). The probability of survival was estimated using the Kaplan–Meier method. Differences between the course of survival curves were compared by log-rank test. The minimum P-value method of the log-rank test was applied for the selection of cut-off points for overexpression of CD44, CD98 and ALDH1/2. At the beginning of this strategy, the mean, median, and percentiles: 75th, 67th, 33rd, and 25th were we analyzed as cut-off points. Next, other values were tested. To distinguish independent factors affecting survival, multivariate analysis was carried out using the Cox proportional hazards model. Two-sided p values of < 0.05 were considered significant. All statistical analyses were performed using Statistica v.13.3 program.

3. Results

3.1. Clinical characteristics of patients with oropharyngeal squamous cell carcinoma

Detailed characteristics of 63 patients with OPSCC is presented in Table 1. Most patients were subjected to CisPt-CRT ($n = 28$, 44.4%), which was used as definitively ($n = 22$, 78.6%) or after surgery ($n = 6$, 21.4%). As part of CisPt-CRT, total dose of RT ranged from 40 to 70 Gy (mean: 66.1 Gy$\pm$1.0), applied in 14 – 35 fractions of 2.0 – 2.2 Gy. Cisplatin (CisPt) was administered during RT according to two regimens: 100 mg CisPt/m^2 every 3rd week of RT in 2–3 courses or 40 mg CisPt/m2 every week of RT in 3 – 6 courses, depending on patient's condition and early normal tissue response. In 19 patients (30.2%) radiotherapy was used definitively ($n = 6$, 31.6%) or in adjuvant setting after surgery ($n = 13$, 68.4%). Total dose of RT was 20.0 – 74.0 Gy, with mean value of 59.5 Gy, fraction dose of 1.8 – 4.0 Gy, and number of fractions of 5 – 40. Altogether, 19 patients (30.2%) underwent surgery. Meanwhile, 16 patients (25.4%) were treated with induction chemotherapy (CisPt + 5-fluorouracil + taxanes) followed by RT (total dose: 20 – 70 Gy, with mean value of 59.5 Gy, fraction dose: 1.8 – 4 Gy, number of fractions: 5 – 40).

The mean follow-up time was 42.0 months $\pm$ 4.4 and ranged from 0 to 113 months. In 45 patients (71.4%) cancer regression was observed, whereas in 18 (28.6%) progression occurred (in 2 cases treatment failure, in 12 local recurrence and in 4 distant metastases) from 0 to 39 months after completing treatment (mean: 12.0 months $\pm$ 2.5).

3.1.1. HPV infection status, CD44, CD98, ALDH1/2 and P16 expression and their correlation with epidemiological, clinical, and biological parameters

Among 63 OPSCCs, amplification of a fragment of HPV16 *E6* gene was found in 25 tumors (39.7%) (Table 1), with mean Ct value of 28.5 $\pm$ 1.0 (28.5 – 38.9). The mean values of H score for CD44, CD98, and ALDH1/2 immunostaining were 158.2 $\pm$ 88.8, 123.0 $\pm$ 78.5, and 145.5 $\pm$ 90.6. Cut-off points for overexpression of CD44, CD98, and ALDH1/2, chosen by minimal p value of log-rank test, were 110.0, 120.0, and 120.0. Overexpression of CD44, CD98, and ALDH1/2 were found in 43 (68.2%), 30 (47.6%), and 33 (52.4%) cancers (Table 1). The proportion of HPV-negative tumors was significantly higher in younger patients ($p = 0.033$), abusing alcohol ($p = 0.011$), as well as among tumors with lack of P16 immunoreactivity ($p = 0.000$) and CD98 overexpression ($p = 0.01$) as compared to older patients, not abusing alcohol and lacking CD98 overexpression. CD44 overexpression was significantly associated with higher clinical stages ($p = 0.024$) and lack of P16 immunoreactivity ($p = 0.040$). Absence of CD98 overexpression was significantly more common in females ($p = 0.014$), in patients with lower level of smoking ($p = 0.034$) and abusing alcohol ($p = 0.007$), as well as among tumors with

Table 1
Relation between HPV16 status, CD44, CD98, ALDH1/2 expression and epidemiological and clinical features of 63 patients with squamous cell carcinoma of oropharynx

	All (%)[a]	HPV16 infection			CD44			CD98			ALDH1/2		
		Yes N (%)[b]	No N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)
All	63 (100.0)	25 (39.7)	38 (60.3)		43 (68.2)	20 (31.8)		30 (47.6)	33 (52.4)		33 (52.4)	30 (47.6)	
Age													
≤58 years[c]	28 (44.4)	7 (25.0)	21 (75.0)		20 (71.4)	8 (28.6)		16 (57.1)	12 (42.9)		16 (57.1)	12 (42.9)	
>58 years	35 (55.6)	18 (51.4)	17 (48.6)	**0.033**	23 (65.7)	12 (34.3)	0.628	14 (40.0)	21 (60.0)	0.176	17 (48.6)	18 (51.4)	0.498
Gender													
Female	15 (23.8)	9 (60.0)	6 (40.0)		8 (53.3)	7 (46.7)		3 (20.0)	12 (80.0)		7 (46.7)	8 (53.3)	0.612
Male	48 (76.2)	16 (33.3)	32 (66.7)	0.066	35 (72.9)	13 (27.1)	0.155	27 (56.3)	21 (43.7)	**0.014**	26 (54.2)	22 (45.8)	
Status in the Karnofsky scale													
<80%	26 (41.3)	11 (42.3)	15 (57.7)		19 (73.1)	7 (26.9)		15 (57.7)	11 (42.3)		15 (57.7)	11 (42.3)	
≥80%	37 (58.7)	14 (37.8)	23 (62.2)	0.721	24 (64.9)	13 (35.1)	0.491	15 (40.5)	22 (59.5)	0.180	18 (48.6)	19 (51.4)	0.479
The level of smoking-Brinkman index[d]													
≤520[c]	34 (54.0)	14 (41.2)	20 (58.8)		21 (61.8)	13 (38.2)		12 (35.3)	22 (64.7)		18 (52.9)	16 (47.1)	
>520	29 (46.0)	11 (37.9)	18 (62.1)	0.793	22 (75.9)	7 (24.1)	0.231	18 (62.1)	11 (37.9)	**0.034**	15 (51.7)	14 (48.3)	0.923
The level of drinking[e]													
Low	28 (44.4)	16 (57.1)	12 (42.9)		16 (57.1)	12 (42.9)		8 (28.6)	20 (71.4)		14 (50.0)	14 (50.0)	
High	35 (55.6)	9 (25.7)	26 (74.3)	**0.011**	27 (77.1)	8 (22.9)	0.090	22 (62.9)	13 (37.1)	**0.007**	19 (54.3)	16 (45.7)	0.735
T stage													
2	15 (23.8)	6 (40.0)	9 (60.0)		6 (40.0)	9 (60.0)		8 (53.3)	7 (46.7)		7 (46.7)	8 (53.3)	
3	32 (50.8)	15 (46.9)	17 (53.1)		24 (75.0)	8 (25.0)		11 (34.4)	21 (65.6)		14 (43.7)	18 (56.3)	
4	16 (25.4)	4 (25.0)	12 (75.0)	0.344	13 (81.2)	3 (18.8)	**0.024**	11 (68.7)	5 (31.3)	0.070	12 (75.0)	4 (25.0)	0.110
N stage													
0	10 (15.9)	2 (20.0)	8 (80.0)		9 (90.0)	1 (10.0)		8 (80.0)	2 (20.0)		4 (40.0)	6 (60.0)	
1	13 (20.6)	5 (38.5)	8 (61.5)		10 (76.9)	3 (23.1)		3 (23.1)	10 (76.9)		7 (53.8)	6 (46.2)	
2	35 (55.6)	16 (45.7)	19 (54.3)		22 (62.9)	13 (37.1)		17 (48.6)	18 (51.4)		20 (57.1)	15 (42.9)	
3	5 (7.9)	2 (40.0)	3 (60.0)	0.540	2 (40.0)	3 (60.0)	0.176	2 (40.0)	3 (60.0)	0.058	2 (40.0)	3 (60.0)	0.741
Grade													
1	25 (39.7)	10 (40.0)	15 (60.0)		19 (76.0)	6 (24.0)		11 (44.0)	14 (56.0)		15 (60.0)	10 (40.0)	
2	33 (52.4)	14 (42.4)	19 (57.6)		19 (57.6)	14 (42.4)		15 (45.5)	18 (54.5)		16 (48.5)	17 (51.5)	
3	5 (7.9)	1 (20.0)	4 (80.0)	0.633	5 (100.0)	0 (0.0)	0.093	4 (80.0)	1 (20.0)	0.317	2 (40.0)	3 (60.0)	0.580

(Continued)

Table 1
(Continued)

	All (%)[a]	HPV16 infection			CD44			CD98			ALDH1/2		
		Yes N (%)[b]	No N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)	Over expression N (%)[b]	Lack of over expression N (%)	*p* value (test χ^2)
Keratinization													
Yes	35 (44.4)	11 (31.4)	24 (68.6)		27 (77.1)	8 (22.9)		18 (51.4)	17 (48.6)		17 (48.6)	18 (51.4)	
No	28 (55.6)	14 (50.0)	14 (50.0)	0.134	16 (51.1)	12 (42.9)	0.090	12 (42.9)	16 (57.1)	0.498	16 (57.1)	12 (42.9)	0.498
HPV16 infection (qPCR)													
Yes	25 (39.7)				14 (56.0)	11 (44.0)		7 (28.0)	18 (72.0)		11 (44.0)	14 (56.0)	
Not	38 (60.3)				29 (76.3)	9 (23.7)	0.090	23 (60.5)	15 (39.5)	**0.011**	22 (57.9)	16 (42.1)	0.280
P16 immunopositivity													
Yes	27 (42.9)	21 (77.8)	6 (22.2)		8 (29.6)	19 (70.4)		6 (22.2)	21 (77.8)		12 (44.4)	15 (55.6)	
Not	36 (57.1)	4 (11.1)	32 (88.9)	**0.000**	20 (55.6)	16 (44.4)	**0.040**	24 (66.7)	12 (33.3)	**0.000**	21 (58.3)	15 (44.4)	0.275
CD44 expression													
Overexpression	43 (68.3)	14 (32.6)	29 (67.4)					20 (46.5)	23 (53.5)		23 (53.5)	20 (46.5)	
Lack of overexpression	20 (31.7)	11 (55.0)	9 (45.0)	0.090				10 (50.0)	10 (50.0)	0.796	10 (50.0)	10 (50.0)	0.796
CD98 expression													
Overexpression	30 (47.6)	7 (23.3)	23 (76.7)		20 (66.7)	10 (33.3)					17 (43.3)	13 (56.7)	
Lack of overexpression	33 (52.4)	18 (54.5)	15 (45.5)	**0.011**	23 (69.7)	10 (30.3)	0.796				16 (48.5)	17 (51.5)	0.516
ALDH1/2 expression													
Overexpression	33 (47.6)	11 (33.3)	22 (66.7)		23 (69.7)	10 (30.3)		17 (51.5)	16 (48.4)				
Lack of overexpression	30 (52.4)	14 (46.7)	16 (53.3)	0.280	20 (66.7)	10 (33.3)	0.796	13 (43.3)	17 (57.7)	0.516			
Treatment													
Definitive CisPt-CRT or surgery + CisPt-CRT	28 (44.4)	13 (46.4)	15 (53.6)		18 (64.3)	10 (35.7)		9 (32.1)	19 (57.9)		16 (57.1)	12 (42.9)	
Definitive RT or surgery + RT	19 (30.2)	7 (36.8)	12 (63.2)		14 (73.7)	5 (26.3)		10 (52.6)	9 (47.4)		8 (42.1)	11 (57.9)	
Induction CT + definitive RT	16 (25.4)	5 (31.2)	11 (68.8)	0.585	11 (68.7)	5 (31.3)	0.793	11 (68.7)	5 (31.3)	0.056	9 (56.3)	7 (43.7)	0.561
Treatment outcome													
Regression of cancer disease	45 (71.4)	21 (46.7)	24 (53.3)		28 (62.2)	17 (37.8)		18 (40.0)	27 (60.0)		23 (51.1)	22 (48.9)	
Treatment failure	2 (3.2)	1 (50.0)	1 (50.0)		2 (100.0)	0 (0.0)		1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)	
Local recurrence	12 (19.1)	2 (16.7)	10 (83.3)		10 (83.3)	2 (16.7)		9 (75.0)	3 (25.0)		8 (66.7)	4 (33.3)	
Distant metastases	4 (6.3)	1 (25.0)	3 (75.0)	0.259	3 (75.0)	1 (25.0)	0.387	2 (50.0)	2 (50.0)	0.198	1 (25.0)	3 (75.0)	0.528
Survival													
Alive at the last follow-up	34 (54.0)	17 (50.0)	17 (50.0)		21 (61.8)	13 (38.2)		13 (38.2)	21 (61.8)		15 (44.1)	19 (55.9)	
Death from cancer disease	15 (20.0)	3 (29.4)	12 (80.0)		13 (86.7)	2 (13.3)		11 (73.3)	4 (26.7)		8 (53.3)	7 (46.7)	
Death from others reasons	14 (35.7)	5 (35.7)	9 (64.3)	0.133	9 (64.3)	5 (35.7)	0.211	6 (42.9)	8 (57.1)	0.070	10 (71.4)	4 (28.6)	0.226

CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]Colunm percentage. [b]Row percentage. [b]Median value. [d]Number of cigarettes per day × years of smoking. [e]Low level of drinking—no alcohol and occasional drinkers (at most two drinks a day, especially with a meal) high level of drinking—more than 15 drinks high percentage alcohol in a week and alcoholics.

P16 immunoreactivity ($p = 0.000$). The distribution of tumors with or without ALDH1/2 overexpression was not significantly dependent on assessed epidemiological, clinical and biological parameters.

3.2. Survival analysis in the group of 63 patients with OPSCC

Because of low number of treatment failures ($n = 2$) and distant metastasis ($n = 4$), in the survival analysis two endpoints were applied: OS and DFS. In the series of 63 patients with OPSCC, 5-year OS and DFS were: 50.7% and 66.3%. In univariate analysis, significantly higher OS was found in females ($p = 0.008$), patients with: lower level of smoking ($p = 0.024$), alcohol abuse ($p = 0.023$), lower T ($p = 0.001$), N ($p = 0.046$) stages, non-keratinizing tumors ($p = 0.032$), with P16 positivity ($p = 0.030$) and those treated with CisPt-CRT ($p = 0.009$) (Table 2). Among features indicating significantly better DFS were: lower level of smoking ($p = 0.026$), lower T stage ($p = 0.000$), lack of keratinization ($p = 0.045$), HPV16 presence (($p = 0.048$), P16 positivity ($p = 0.001$), CD98 overexpression ($p = 0.041$) and application of CisPt-CRT ($p = 0.000$).

All parameters showing statistically significant influence on survival in univariate analysis were included in multivariate analysis. For OS they were: gender, level of smoking, alcohol abuse, T and N stages, keratinization status, P16 immunoreactivity and treatment type. In the case of DFS, level of smoking, T stage, keratinization status, HPV16 infection, P16 immunoreactivity, CD98 overexpression and treatment type were included. For both endpoints (OS and DFS), lower T stage and P16 immunoreactivity were independent favorable prognostic factors (Table 3).

3.3. The influence of CSCs biomarkers on survival in the subgroups of patients with different HPV16 status

Separate analysis was carried out for the subgroups of HPV16 positive and negative patients. In HPV16-positive cases, lower smoking level ($p = 0.037$) and lower N stage ($p = 0.028$) showed significant impact on OS (Table 4). In this subgroup, DFS of 100% was found for patients with tumors lacking CD44 overexpression and patients treated with concurrent CisPt-CRT. Significantly better DFS was also related to lower T stages.

In the subgroup with HPV16-negative tumors, female gender ($p = 0.008$), lower T stages ($p = 0.000$), lack of keratinization ($p = 0.041$), and treatment with definitive CisPt-CRT or surgery + CisPt-CRT or definitive RT or surgery + RT ($p = 0.040$) indicated significantly higher OS in univariate analysis (Table 5). For DFS, these parameters were: lower T stages ($p = 0.003$), lower N stages ($p = 0.008$), P16 immunopositivity ($p = 0.029$) and treatment with definitive CisPt-CRT or surgery + CisPt-CRT or definitive RT or surgery + RT ($p = 0.013$).

In the subgroup of HPV16-positive patients, level of smoking and N stage were included in multivariate Cox regression analysis concerning OS. These two parameters were identified as independent prognostic factors (Table 6). In case of DFS, T stage, CD44 expression and treatment type were evaluated. CD44 expression and treatment type were independent prognostic factors. In the subgroup of patients with HPV16-negative tumors, in multivariate analysis gender, T stage, keratinization status, and treatment type for OS and T stage, N stage, P16 immunoreactivity and treatment type for DFS were included. This analysis revealed T stage for OS and T and N stages for DFS to be independent prognostic factors.

4. Discussion

In this retrospective study, we have shown, according to our knowledge for the first time, DFS of 100% in OPSCC patients with tumors lacking CD44 overexpression (Table 4). DFS for HPV16-positive

Table 2
Univariate Cox proportional hazard model for 5-year overall and 5-year disease free survival of 63 patients with with squamous cell carcinoma of oropharynx

	Overall survival				Disease free survival			
	Response N (%)[a]	HR	95% CI	Log-rank *p*	Response N (%)[a]	HR	95% CI	Log-rank *p*
Age:								
≤58 years[b]	11/28 (39.3)	1.034			19/28 (62.0)	1.183		
> 58 years	23/35 (65.7)	1.000	0.663 – 1.613	0.110	26/35 (74.3)	1.000	0.684 – 2.045	0.562
Gender								
Female	13/15 (86.7)	1.000			12/15 (80.0)	1.000		
Male	21/48 (43.7)	3.679	1.485 – 9.112	**0.008**	33/48 (68.7)	3.002	1.082 – 8.331	0.254
Status in the Karnofsky scale								
≤80%	1/9 (11.1)	2.411			5/9 (55.5)	1.707		
> 80%	33/54 (38.9)	1.000	0.408 – 1.464	**0.194**	40/54 (74.1)	1.000	0.831 – 3.506	0.128
The level of smoking-Brinkman index[c]								
≤520[b]	23/34 (67.6)	1.000			28/34 (82.3)	1.000		
> 520[b]	11/29 (37.9)	2.327	1.096 – 4.941	**0.024**	17/29 (58.6)	3.375	1.097 – 7.848	**0.026**
The level of drinking[d]								
Low	20/28 (71.4)	1.000			23/28 (82.1)	1.000		
High	14/40 (77.1)	2.468	1.090 – 5.584	**0.023**	22/35 (62.9)	2.538	0.902 – 7.145	0.065
T stage								
2	10/15 (66.7)	1.000			13/15 (86.7)	1.000		
3	21/32 (65.6)	2.143	1.271 – 3.614		26/32 (81.2)	1.593	1.280 – 8.853	
4	2/16 (12.5)	2.692	1.293 – 5.603	**0.001**	6/16 (37.5)	3.551	1.346 – 6.294	**0.000**
N stage								
0	5/10 (50.0)	1.000	0.755 – 4.127		8/10 (80.0)	1.282	0.728 – 2.256	
1	12/13 (92.3)	1.000			12/13 (92.3)			
2	15/35 (42.8)	10.564	1.413 – 8.858		22/35 (62.8)	7.089	0.920 – 4.590	
3	2/5 (40.0)	12.904	0.932 – 9.053	**0.046**	3/5 (60.0)	9.427	0.725 – 8.117	0.168
Grade								
1	13/25 (52.0)	1.861	0.539 – 2.501		17/25 (68.0)	1.558	0.744 - 4.279	
2	9/33 (57.6)	1.000			26/33 (78.8)	1.000		
3	2/5 (40.0)	2.564	0.449 – 5.457	0.796	2/5 (40.0)	3.068	0.789 – 11.078	0.258
Keratinization								
Yes	15/35 (42.9)	1.339			22/35 (62.9)	1.518		
No	19/28 (67.9)	1.000	0.845 – 2.065	**0.032**	23/28 (82.1)	1.000	0.857 – 2.689	**0.045**
HPV16 infection (qPCR)								
Present	17/25 (68.0)	1.000			21/25 (84.0)	1.000		
Absent	17/38 (44.7)	1.844	0.290 – 1.015	0.086	24/38 (63.1)	2.808	1.990 – 6.578	**0.048**
P16 immunoreactivity								
Yes	21/27 (77.8)	1.000			25/27 (92.6)	1.000		
No	23/36 (63.9)	2.514	1.3450 –5.818	**0.030**	20/36 (55.6)	6.416	2.718 – 9.411	**0.001**
CD44 expression								
Overexpression	21/43 (48.8)	1.289			28/43 (65.1)	2.045		
Lack of overexpression	13/20 (65.0)	1.000	0.801 – 2.075	0.191	17/20 (85.0)	1.000	1.072 – 3.901	0.075
CD98 expression								
Overexpression	13/30 (43.3)	1.376			18/30 (60.0)	2.426		
Lack of overexpression	21/33 (63.6)	1.000	0.863 – 2.194	0.157	29/33 (87.9)	1.000	1.272 – 4.627	**0.041**
ALDH1/2 expression								
Overexpression	15/33 (45.5)	1.261			23/33 (69.7)	1.354		
Lack of overexpression	19/30 (63.3)	1.000	0.804 – 1.978	0.090	22/30 (73.3)	1.000	0.777 – 2.358	0.468

(Continued)

Table 2
(Continued)

	Overall survival				Disease free survival			
	Response N (%)[a]	HR	95% CI	Log-rank *p*	Response N (%)[a]	HR	95% CI	Log-rank *p*
Treatment								
Definitive CisPt-CRT or surgery + CisPt-CRT	19/28 (67.9)	1.000			25/28 (89.3)	1.000		
Definitive RT or surgery + RT	12/19 (63.2)	1.892	1.027 – 3.486		14/19 (73.7)	1.742	0.829 – 3.660	
Induction CT + definitive RT	3/16 (18.8)	2.734	1.194 – 3.519	**0.009**	6/16 (37.5)	2.850	1.198 – 3.860	**0.000**

HR: hazard ratio; CI: confidence interval; CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]Row percentage. [b]Median values. [c]Number of cigarettes per day x years of smoking. [d]Low level of drinking - no alcohol and occasional drinkers (at most two drinks a day, especially with a meal) high level of drinking - more than 15 drinks of high percentage alcohol in a week and alcoholics.

Table 3
Multivariate Cox proportional hazard model carried out in whole group of 63 patients with squamous cell carcinoma of oropharynx and separately in the subgroups with HPV16 positivity and HPV16 negativity

	Overall survival			Disease free survival		
	HR	95% CI	*p*-value[a]	HR	95% CI	*p*-value[a]
T stage						
2 + 3	1.000			1.000		
4	3.921	1.847 – 8.323	0.000	5.423	2.039 – 14.425	0.000
P16 immunoreactivity						
Yes	1.000			1.000		
No	3.015	1.214 – 7.491	0.017	6.347	1.429 – 18.191	0.015

HR: hazard ratio; CI: confidence interval; CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]*p* -value was examined by the Cox proportional hazard model for multivariate survival analysis.

patients with tumors characterized by CD44 overexpression was significantly lower (71.4%). In the HPV16-negative subgroup, CD44 expression did not correlate with survival (Table 5). Näsman et al. [12] have obtained similar results in a group of 225 patients with HPV-positive OPSCC, majority of which were treated with conventional or accelerated RT. In HPV-positive patients, they have shown that absent/weak CD44 expression indicated significantly better 3-year DFS (96%) as compared to cases showing medium/strong CD44 staining intensity (86%). In HPV-negative patients, CD44 expression did not influence survival. However, some authors have shown opposite results, i.e. shorter survival time or shorter recurrence-free interval for patients with decreased CD44 expression [18, 19], or no association between expression of CD44 and prognosis in OCSCC [20]. It should be also noticed that prognostic potential of CD44 expression in relation to HPV infection is not entirely clear. Contrary to us, Linge et al. [21], in the group of 195 patients with HNSCC treated with postoperative chemotherapy have noticed that low CD44 expression was associated with better prognosis in HPV negative subgroup. In turn, Motegi et al. [22], in the group of 58 OPSCC, which were treated with intensity modulated radiation therapy and most of which (79%) received induction and/or concurrent chemotherapy, have found 100% of locoregional control for patients with lack of CD44 overexpression and P16 immunopositivity. This finding is confirm by the results of Cohen et al. [23], who in the

Table 4

Univariate Cox proportional hazard model for 5-year overall and 5-year disease free survival in the subgroups of patients with with squamous cell carcinoma of oropharynx with HPV16 positivity assessed by qPCR ($n = 25$)

	Overall survival				Disease free survival			
	Response N (%)[a]	HR	95% CI	Log-rank p	Response N (%)[a]	HR	95% CI	Log-rank p
Age								
≤58 years[b]	4/7 (57.1)	1.430			7/7 (100.0)	1.000		
>58 years	13/18 (72.2)	1.000	0.167 – 2.931	0.613	14/18 (77.8)	1.114	0.284 – 4.521	0.188
Gender								
Female	7/9 (77.8)	1.000			8/9 (88.9)	1.000		
Male	10/16 (62.5)	1.676	0.338 – 8.308	0.518	13/16 (81.2)	1.725	0.179 –6.615	0.630
Status in the Karnofsky scale								
<80%	7/11 (63.6)	1.510			8/11 (72.7)	4.536		
≥80%	10/14 (71.4)	1.000	0.379 – 6.010	0.560	13/14 (92.9)	1.000	0.476 – 8.190	0.155
The level of smoking-Brinkman index[c]								
Low	12/14 (85.7)	1.000			13/14 (92.9)	1.000		
High	5/11 (45.4)	4.787	0.960 – 13.874	**0.037**	8/11 (72.7)	4.898	0.505 – 7.487	0.137
The level of drinking[d]								
Low	13/16 (81.2)	1.000			15/16 (93.7)	1.000		
High	4/9 (44.4)	3.224	0.768 – 10.545	0.092	6/9 (63.7)	6.410	0.476 – 12.222	0.073
T stage								
2 + 3	15/21 (71.4)	1.000			19/21 (90.5)	1.000		
4	2/4 (50.0)	2.098	0.421 – 8.462	0.381	2/4 (50.0)	6.654	0.932 – 17.504	**0.043**
N stage								
0 + 1	7/7 (100.0)	1.000			6/7 (85.7)	1.000		
2 + 3	10/18 (55.5)	5.291	0.335 – 15.875	**0.028**	15/18 (83.3)	1.648	0.169 – 6.106	0.645
Grade								
1	6/10 (60.0)	1.845			7/10 (70.0)	1.964		
2 + 3	10/14 (71.4)	1.000	0.398 – 4.572	0.482	13/14 (92.8)	1.000	0.4952 – 4. 961	0.222
Keratinization								
Yes	7/11 (63.6)	1.454			9/11 (81.8)	1.415		
No	10/14 (71.4)	1.000	0.363 – 5.827	0.595	12/14 (85.7)	1.000	0.199 – 5.064	0.729
P16 immunoreactivity								
Yes	16/21 (64.3)	1.741			19/21 (90.5)	1.000		
No	1/4 (25.0)	3.390	0.608 – 14.228	0.080	2/2 (50.0)	5.433	0.761 – 18.785	0.091
CD44 expression								
Overexpression	9/14 (64.3)	1.741			10/14 (71.4)	8.006		
Lack of overexpression	8/11 (72.7)	1.000	0.335 – 5.875	0.637	11/11 (100.0)	1.000	0.813 – 18.830	**0.049**
CD98 expression								
Overexpression	5/7 (71.4)	1.000			5/7 (71.4)	2.104		
Lack of overexpression	12/18 (66.7)	1.403	0.149 – 3.684	0.703	16/18 (88.9)	1.000	0.294 – 15.032	0.428
ALDH1/2 expression								
Overexpression	7/11 (63.6)	1.478			9/11 (81.8)	1.442		
Lack of overexpression	10/14 (71.4)	1.000	0.368 – 5.931	0.580	12/14 (85.7)	1.000	0.202 – 10.276	0.714
Treatment								
Definitive CisPt-CRT or surgery + CisPt-CRT	10/13 (76.9)	1.000			13/13 (100.0)	1.000		
Definitive RT or surgery + RT	5/7 (71.4)	1.170	0.195 – 7.010	0.363	5/7 (71.4)	4.503	1.831 – 8.721	**0.049**
Induction CT + definitive RT	2/5 (40.0)	1.762	0.784 – 3.958		3/5 (60.0)	5.698	1.942 – 12.411	

HR: hazard ratio; CI: confidence interval; CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]Row percentage. [b]Median values. [c]Number of cigarettes per day x years of smoking. [d]Low level of drinking - no alcohol and occasional drinkers (at most two drinks a day, especially with a meal) high level of drinking - more than 15 drinks of high percentage alcohol in a week and alcoholics.

Table 5

Univariate Cox proportional hazard model for 5-year overall and 5-year disease free survival in the subgroups of patients with with squamous cell carcinoma of oropharynx with HPV16 negativity assessed by qPCR ($n = 38$)

	Overall survival				Disease free survival			
	Response N (%)[a]	HR	95% CI	Log-rank p	Response N (%)[a]	HR	95% CI	Log-rank p
Age								
≤58 years[b]	7/21 (33.3)	1.704			12/21 (57.1)	1.606		
>58 years	10/17 (58.8)	1.000	0.690 – 4.208	0.237	12/17 (70.6)	1.000	0.208 – 1.858	0.385
Gender								
Female	6/6 (100.0)	1.000			4/6 (66.7)	1.000		
Male	11/32 (34.4)	5.094	0.951 – 15.875	**0.008**	20/32 (62.5)	1.657	0.368 – 7.448	0.451
Status in the Karnofsky scale								
<80%	5/15 (33.3)	1.754			8/15 (53.3)	2.052		
≥80%	12/23 (52.2)	1.000	0.741 – 4.149	0.207	16/23 (69.6)	1.000	0.169 – 1.403	0.190
The level of smoking-Brinkman index[c]								
Low	11/20 (55.0)	1.000			15/20 (75.0)	1.000		
High	6/18 (33.3)	1.613	0.679 – 3.831	0.269	9/18 (50.0)	2.356	0.787 – 7.056	0.115
The level of drinking[d]								
Low	7/12 (58.3)	1.000			8/12 (66.7)	1.000		
High	10/26 (38.5)	1.630	0.595 – 4.5461	0.323	16/26 (61.5)	1.364	0.426 – 4.363	0.587
T stage								
2 + 3	17/26 (64.3)	1.000			20/26 (76.9)	1.000		
4	0/12 (0.0)	5.955	2.368 – 14.973	**0.000**	4/12 (33.3)	6.189	1.971 – 19.438	**0.003**
N stage								
0 + 1	10/16 (62.5)	1.000			14/16 (87.5)	1.000		
2 + 3	7/22 (31.8)	2.425	0.914 – 6.314	0.057	10/22 (45.4)	5.848	1.301 – 16.281	**0.008**
Grade								
1	16/34 (47.1)	1.741			23/34 (67.5)	1.000		
2 + 3	1/4 (25.0)	1.000	0.335 – 5.875	0.400	1/4 (25.0)	3.948	1.002 – 6.745	0.116
Keratinization								
Yes	8/24 (33.3)	1.741			13/24 (54.2)	3.042		
No	9/14 (64.3)	1.000	0.335 – 5.875	**0.041**	11/14 (78.6)	1.000	0.840 – 11.015	0.067
P16 immunoreactivity								
Yes	5/6 (83.3)	1.000			6/6 (100.0)	1.000		
No	12/32 (37.5)	4.944	0.662 – 16.719	0.056	18/32 (56.3)	5.065	1.311 – 15.420	**0.029**
CD44 expression								
Overexpression	12/29 (41.4)	4.689			18/29 (62.1)	4.271		
Lack of overexpression	5/9 (55.6)	1.000	0.798 – 16.098	0.321	6/9 (66.7)	1.000	0.470 – 18.790	0.546
CD98 expression								
Overexpression	8/23 (34.8)	1.124			13/23 (56.5)	1.482		
Lack of overexpression	9/15 (60.0)	1.000	0.251 – 5.033	0.140	11/15 (73.3)	1.000	0.247 – 8.887	0.196
ALDH1/2 expression								
Overexpression	8/22 (36.4)	2.682			14/22 (63.6)	1.000		
Lack of overexpression	9/16 (56.2)	1.000	0.595 – 12.089	0.132	10/16 (62.5)	1.181	0.195 – 7.138	0.652
Treatment								
Definitive CisPt-CRT or surgery + CisPt-CRT	9/15 (60.0)	1.000			12/15 (80.0)	1.000		
Definitive RT or surgery + RT	7/12 (58.3)	1.915	1.091 – 3.358	**0.040**	9/12 (75.0)	1.254	0.418 – 3.380	**0.013**
Induction CT + definitive RT	1/11 (9.1)	7.416	1.383 – 14.612		3/11 (27.3)	5.867	0.550 – 10.713	

HR: hazard ratio; CI: confidence interval; CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]Row percentage. [b]Median values. [c]Number of cigarettes per day x years of smoking. [d]Low level of drinking - no alcohol and occasional drinkers (at most two drinks a day, especially with a meal) high level of drinking - more than 15 drinks of high percentage alcohol in a week and alcoholics.

Table 6
Multivariate Cox proportional hazard model carried out in the subgroups of patients with squamous cell carcinoma of oropharynx with HPV16 positivity and HPV16 negativity

	Overall survival			Disease free survival		
	HR	95% CI	*p*-value[a]	HR	95% CI	*p*-value[a]
Subgroup of HPV16 positive OPSCC patients ($n = 25$)						
N stage						
0 + 1	1.000					
2 + 3	2.857	1.155 – 7.072	0.023			
The level of smoking-Brinkman index[b]						
Low	1.000					
High	2.237	1.050 – 4.764	0.036			
CD44 expression						
Overexpression				1.000		
Lack of overexpression				3.789	1.387 – 10.352	0.009
Treatment						
Definitive CisPt-CRT or surgery + CisPt-CRT and definitive RT or surgery + RT				1.000		
Induction CT + definitive RT				7.232	2.669 - 13.518	0.000
Subgroup of HPV16 negative OPSCC patients ($n = 38$)						
T stage						
2 + 3	1.000			1.000		
4	5.955	2.368 – 14.973	0.000	4.223	1.310 – 13.616	0.016
N stage						
0 + 1				1.000		
2 + 3				3.925	0.826 – 8.658	0.045

HR: hazard ratio; CI: confidence interval; OPSCC- squamous cell carcinoma of oropharynx; CisPt-CRT: concurrent chemoradiotherapy with cisplatin; CT: chemotherapy. [a]*p*-value was examined by the Cox proportional hazard model for multivariate survival analysis. [b]Number of cigarettes per day x years of smoking.

group of 24 lip/oral cavity and 40 OPSCC have noticed the best survival for peripheral/mixed group alone or when combined with universal P16 immunopositivity. However, these results are difficult to compare to those presented by us, because we decided to perform separate analysis in HPV16 positive and HPV16 negative patients and to use qPCR method to assess virus prevalence. P16 is known surrogate marker of HPV presence, however is characterized by relative low specificity, which generates risk of false positive results. Therefore, in the present study prognostic significance of P16 immunoreactivity was analysed separately in HPV16 positive and HPV16 negative patients (identified by qPCR method). We have found 100% of DFS for the small subgroup of patients ($n = 6$) having tumors with HPV negativity (assessed by qPCR) and P16 immunopositivity, whereas in the group of HPV16 positive patients P16 immunoreactivity did not significantly influence patients' survival. These results are in line with the results of meta-analysis of Albers et al. [24], in which 11 studies were included. These authors have shown the highest survival outcomes for patients characterized by $HPV^+/P16^+$ subgroup, intermediate for $HPV^-/P16^+$ subgroup and the shortest for $HPV^+/p16^-$ and $HPV^-/P16^-$, wherein survival for patients with $HPV^-/p16^+$ HNSCC was clearly distinct from survival of patients with other subtypes of tumors. All these observations suggest that $HPV16^-/P16^+$ OPSCC maybe a new relevant HPV-independent subtype, which requires further studies to define its biological characteristics and the same prognosis of patients with this subtype of tumors. Considering prognostic potential of CD44 expression, it should be also noticed that IHC methodology used to assess CD44

expression is not well established. Discrepancies are connected to microscopic analysis of immunostaining, scoring system and cut-off point indicating CD44 overexpression. Cohen et al. [23] during microscopic analysis of CD44 immunostaining had compared prognostic potential of universal gross staining and peripherial/mixed staining. They have found that universal gross staining demonstrated poorer OS as compared to peripherial/mixed staining. In regard to scoring system, it should be noticed that some authors, similarly to us, used semi-quantitative scale, in which number of positively-stained cells and intensity of staining are included [12, 21], while others ignored staining intensity [19]. The differences concern also the cut-off for the number positively stained cells, which is defined at the level of 50% [19], or 26% of positively-stained cells [12]. Taking all these facts into account, it should be stated that although CD44 immunoreactivity has the potential to be a reliable prognostic biomarker for patients with HPV-positive OPSCC, it requires validation of IHC staining method in adequately large and homogeneous group of patients.

In the present study we have shown, according to our best knowledge for the first time, that all HPV16-positive patients treated with CisPt-CRT (definitive or combined with surgery) survived 5 years without cancer progression. DFS for patients treated with RT or induction CT + definitive RT was significantly lower (Table 4). The underlying mechanisms of this observation are unknown. Some experimental researches suggest greater sensitivity of HPV16-positive cancer cells to CisPt combined with irradiation than HPV16-negative cell lines [25–27]. Ziemann et al. [27] have also shown that in HPV-infected cells CisPt induced S phase blockage, whereas after irradiation prolonged G2/M arrest was observed. In their study, addition of CisPt significantly enhanced apoptosis, particularly in HPV-positive cell lines, although no changes in expression of endogenous P53 were noticed. The results of the above-mentioned experimental studies and those presented by us are also in line with the results of two phase III studies evaluating RT-cetuximab vs. CisPt-CRT in HPV-positive OPSCC (De-ESCALaTE and RTOG 1016). These analyses have shown inferior OS and progression-free survival for RT-cetuximab combination [28, 29], what may suggest that HPV-positive cancer cells are exceptionally sensitive to CisPt-CRT. However, the hypothesis about greater sensitivity of HPV-positive cells to CisPt and irradiation should be treated cautiously, particularly in the light of results obtained by some authors showing lack of significant differences in response to CisPt of HPV-positive and HPV-negative HNSCC cell lines [30] or resistance to drug of HPV-positive HNSCCs as compared to HPV-negative cells [31]. It is also worth to notice, that in all meta-analyses regarding relation between HPV infection and prognosis, this relation was tested irrespectively of treatment type [4–7]. Therefore, other factors, including stimulation of immune response through viral infection and/or beneficial epidemiological and clinical features (younger age, white race, better performance status, no addiction to nicotine and alcohol) of HPV positive patients may be responsible for better prognosis [3]. Summarizing this part of discussion, it seems that OPSCC patients with HPV positivity respond better to CisPt- CRT, however, the results of research aimed at explaining mechanisms related to this observation are inconclusive.

The important limitation of the current study is small number of patients included into the analysis and their heterogeneity regarding to treatment type. The analyzed patients' group was treated with chemoradiotherapy, radiation therapy and induction chemotherapy followed by radiotherapy and even in these three subgroups the heterogeneity particularly in radiation dose (range: 20.0 – 74.0 Gy) was noticed. Therefore, in the present analysis we examine the influence of treatment type on survival among HPV16 positive and HPv16 negative patients. We have shown DFS of 100% in HPV16-positive OPSCC patients treated with concurrent CisPt-CRT and those with tumors lacking CD44 overexpression, what is important suggestion that further studies concerning prognostic potential of HPV16 infection and CD44 expression in OPSCC should be performed in homogeneous according to treatment type subgroups of patients (radiation *vs* concurrent chemoradiotherapy). Experimental studies that examine biological basis of different prognosis of HPV16 positive or HPV16 negative and CD44 overexperssoped or no overexpressed OPSCC subtypes are also expected.

Acknowledgments

The study was financially supported partly by Foundation Jakub count. Potocki, Warsaw, Poland (grant number 263/18). This funding did not include any other role of the Foundation in completion of this manuscript.

Conflict of interest

The authors report no conflicts of interest.

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