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Significant association of PD-L1 expression with human papillomavirus positivity and its prognostic impact in oropharyngeal cancer

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ABSTRACT

Background: The programmed death pathway plays a role in persistent human papillomavirus (HPV) infection as well as in resistance to immune elimination during malignant progression. In this study, we examined PD-L1 expression by immunohistochemistry and tumour infiltrating lymphocytes (TIL) in 214 patients with oropharyngeal squamous cell cancer (OPSCC) to assess its clinical significance.

Results: HPV-positive OPSCC were significantly more likely to express PD-L1 than HPV-negative OPSCC (85.2% vs 57.1%, $p < 0.05$). PD-L1 staining was more likely to be associated with TILs in HPV-positive OPSCC (67.9% vs 49.6%, $p = 0.01$). Relative to those patients with HPV-positive/PD-L1-positive OPSCC, patients with HPV negative/PD-L1 negative OPSCC were 6.4 times more likely to develop a local recurrence, 5.8 times more likely to develop an event and 6.5 times more likely to die. Within the HPV positive cases, PD-L1 expression also significantly impacted on the outcomes with PD-L1 negative cases more likely to develop a locoregional recurrence (HR 4.16), to have an event (HR 2.5) and to die (HR 3.16). Evidence of an interaction between HPV status and PD-L1 expression was found for overall survival ($p < 0.005$).

Conclusion: Our findings suggested that different immune profiles in oropharyngeal cancer by HPV status and the effect of HPV on the outcomes is modified by PD-L1 expression.

Introduction

The oncogenic virus, human papillomavirus (HPV), is the causative agent in the majority (up to 70%) of oropharyngeal squamous cell carcinomas (OPSCC) in the western world [1–3]. HPV-positive OPSCC is clinically and biologically distinct from smoking-related (HPV-negative) OPSCC. HPV-positive OPSCC are usually non-keratinizing, undifferentiated or basaloid in appearance [4]. Patients with HPV-positive OPSCC tend to be younger, have more advanced nodal disease at diagnosis but better outcomes due to improved response to therapy [5–9]. The immune system plays a vital role in the development of cancers, with progression from pre-invasive to invasive disease and, in turn, metastatic disease being strongly dependant on the cancer's ability to escape immune surveillance. It is likely that HPV-positive OPSCC and HPV-negative OPSCC escape the immune surveillance through different mechanisms during their respective development.

Programmed Death Ligand 1 (PD-L1), is a cell-surface protein that can be expressed on a variety of cell types including cancer cells, macrophages, T-cells and other tissues. It has been proposed that the Programmed Death (PD) pathway plays a role in persistent HPV infection as well as in resistance to immune elimination during malignant progression. The better prognosis associated with HPV-positive OPSCC may also be due to a more favourable immune profile and its associated immunogenicity than HPV-negative OPSCC.

In this study, we determined the prognostic significance of PD-L1 expression in a well-defined cohort of OPSCC by their HPV status. In addition, we examined whether the PD-L1 status modified the known prognostic effect of HPV in OPSCC.

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Table 1
Demographic and clinical characteristics of the study population by HPV & PD-L1 status.

	All Patients (N = 214)	HPV Positive (N = 81) (37.9%)	HPV Negative (N = 133) (62.1%)	*P*-value	PD-L1 Positive (N = 145) (67.8%)	PD-L1 Negative (N = 69) (32.2%)	*P*-value
Median age at diagnosis (range)	59 (31–83)	54 (31–77)	62 (41–83)	0.001	59 (31–77)	59 (41–83)	0.748
Gender							
– Female	48 (22.4%)	18 (22.2%)	30 (22.6%)	1.000	39 (26.9%)	9 (13.0%)	0.024
– Male	166 (77.6%)	63 (77.8%)	103 (77.4%)		106 (73.1%)	60 (87.0%)	
Smoking status							
– Non-smoker	23 (10.7%)	17 (21.0%)	6 (4.5%)	< 0.005	20 (13.8%)	3 (4.3%)	0.056
– Ex-smoker	64 (29.9%)	30 (37.0%)	34 (25.6%)		52 (35.9%)	12 17.4%)	
– Current smoker	127 (59.4%)	34 (42.0%)	93 (70.0%)		73 (50.3%)	54 (78.2%)	
Grade							
– 1	53 (24.8%)	11 (13.6%)	42 (31.6%)	0.003	28 (19.3%)	25 (36.2%)	< 0.005
– 2–3	161 (75.2%)	70 (86.4%)	91 (68.4%)		117 (80.7%)	44 (63.8%)	
T stage							
– 1	38 (17.8%)	24 (29.6%)	14 (10.5%)	< 0.005	30 (20.7%)	8 (11.6%)	0.071
– 2	74 34.6%)	26 (32.1%)	48 (36.1%)		50 (34.5%)	24 (34.8%)	
– 3	69 32.2%)	19 (23.5%)	50 (37.6%)		43 (29.7%)	26 (37.7%)	
– 4	33 (15.4%)	12 (14.8%)	21 (15.8%)		22 (15.2%)	11 (15.9%)	
N stage (Missing = 1)							
– 0	90 (42.3%)	18 (22.2%)	72 (54.5%)	< 0.005	48 (33.1%)	42 (61.8%)	< 0.005
– 1	41 (19.2%)	15 (18.5%)	26 (19.7%)		29 (20.0%)	12 (17.6%)	
– 2	69 (32.4%)	39 (48.2%)	30 (22.7%)		59 (40.7%)	10 (14.7%)	
– 3	13 (6.1%)	9 (11.1%)	4 (3.0%)		9 (6.2%)	4 (5.9%)	
TNM Stage							
– 1	17 (7.9%)	4 (4.9%)	13 (9.8%)	< 0.005	10 (6.9%)	7 (10.1%)	< 0.005
– 2	35 (16.4%)	6 (7.4%)	29 (21.8%)		20 (13.8%)	15 (21.7%)	
– 3	66 (30.8%)	19 (23.5%)	47 (35.3%)		39 (26.9%)	27 (39.1%)	
– 4	96 (44.9%)	52 (64.2%)	44 (33.1%)		76 (52.4%)	20 (29.0%)	
Treatment							
– Radiotherapy + Chemotherapy	50 (23.4%)	32 (39.5%)	18 (13.5%)	0.007	36 (24.8%)	14 (20.3%)	0.004
– Radiotherapy Alone	42 (19.6%)	9 (11.1%)	33 (24.8%)		23 (15.9%)	19 (27.5%)	
– Surgery + Radiotherapy ± Chemo	90 (42.1%)	35 (43.2%)	55 (41.4%)		67 (46.2%)	23 (33.3%)	
– Surgery ± Chemo	32 (15.0%)	5 (6.2%)	27 (20.3%)		19 (13.1%)	13 (18.8%)	
HPV Status							
– Positive	81 (37.9%)				69 (47.6%)	12 (17.4%)	< 0.005
– Negative	133 (62.1%)				76 (52.4%)	57 (82.6%)	
PD-L1 Status							
– Positive	145 (67.8%)	69 (85.2%)	76 (57.1%)	< 0.005			
– Negative	69 (32.2%)	12 (14.8%)	57 (42.9%)				

Methods and materials

Patients

The study cohort consisted of 214 patients with T1-4 N0-3 M0 (American Joint Committee in Cancer Staging System 7th edition) OPSCC treated with curative intent. The study was approved by the ethics committee (Protocol X12-0141). Demographic data, clinicopathological data, treatment details and follow up data were obtained from institutional databases. The histopathological tumour subtype and tumour grade were reviewed in all cases.

Laboratory studies

We tested for HPV status of the OPSCC by DNA status and p16 expression [1]. The presence and type of HPV DNA were determined by E6-based multiplex tandem PCR assay using a modification of the Tandem method of Stanley and Szewezuk [10]. This assay can simultaneously detect and identify 21 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82, 53, 6, 11 and 26). Measured quantities of equine herpesvirus were introduced during the extraction process to monitor the efficiency of DNA extraction and removal of PCR inhibitors. The expression of p16 was determined by semiquantitative immunohistochemistry using the JC2 p16 antibody (Neomarkers, Fremont, USA), as previously reported [11]. Staining was typically strong and diffuse in both the nucleus and cytoplasm of cancer cells and was recorded as positive if seen in more than 50% of cancer cells [12]. An HPV-positive OPSCC was defined as one both testing positive for HPV DNA and demonstrating p16 overexpression by immunohistochemistry [9,13].

PD-L1 immunohistochemistry/Tumour-Infiltrating Lymphocytes

Immunohistochemical staining for PD-L1 (Cell Signaling clone E1L3N run at 1:200 concentration) was conducted on an Autostainer Plus (Dako-Agilent Technologies) using 4 µm-thick tissue sections. Sections were dehydrated for 1 h at 60 °C and heat-induced epitope retrieval was performed using EnVision FLEX target retrieval solution for 20 min at 97 °C. The sections were then cooled to room temperature in TBST wash buffer for 5 min. PD-L1 staining underwent signal amplification using the Envision flex Mouse linker (K8022) followed by Envision FLEX kit (K8023) with a DAB chromagen (Dako–Agilent technologies) prior to counterstaining with haematoxylin. Positive PD-L1 status was by the presence of membranous tumour cell staining and the percentage of positive tumour cells (≥1% positivity) in a representative section of each tumour at a minimum of 10 high power field. The tumour-stroma interface was also *examined in each case and any PD-L1 staining of tumor cells in direct contact with stroma at the tumor-stroma interface was regarded as positive*. Tumour-infiltrating lymphocytes (TIL) were defined as lymphocytes within the tumor and the peritumoral area was not included in this assessment. The percentage of tumour-infiltrating lymphocytes was assessed semi-quantitatively using a four-tier scale: 0 = 0%, 1 ≤ 25%, 2 = 25– < 75%, 3 ≥ 75%. In tumours that showed positive staining for PD-L1, it was noted whether this was associated with TILs or not. The pathologists also scored the PD-L1 positivity in tumour associated macrophages as either present (any positive staining) or absent. All scoring was conducted blinded to HPV status and clinical outcomes.

Statistical analyses

Comparisons between demographic and clinicopathological characteristics were undertaken using t-tests for continuous variables and chi-squared tests for categorical variables. Locoregional failure was defined as clinical, radiological and/or pathological evidence of recurrence at the primary site or in the regional nodal area. An event was defined as recurrence of the OPSCC at any site or death from any cause. Times to locoregional failure, any event, death from OPSCC or death from any cause were calculated from the time of diagnosis. Patients were censored at last follow-up or death where applicable. For the analysis of time to death from any cause, patients were censored at last follow-up if they were alive. Univariate and multivariable time-to-event analyses were performed using Cox proportional hazards regression analysis. The Kaplan-Meier method was used to construct time-to-event curves.

Results

The baseline characteristics by HPV status and PD-L1 expression of the study cohort are shown in Table 1. Eighty-one of 214 patients (37.9%) had HPV-positive OPSCC (HPV DNA-positive, p16-positive). Patients with HPV-positive OPSCC were significantly younger at diagnosis (median age 54), more likely to be never smokers (21.0%), and had higher tumour grade (86.4% grade 2–3), higher N-stage (59.3% N2/3) and higher overall tumour stage compared to patients with HPV-negative OPSCC.

HPV-positive tumours were strongly associated with PD-L1 positivity (85.2% vs 57.1%, $p < 0.05$) (Fig. 1). Within the HPV-positive group, there was no significant difference in PD-L1 positivity by smoking status (never smoker 88.2%, ex and current smoker 84.4%). The number of never smokers within the HPV-negative group was too small for meaningful statistical analysis. The PD-L1 staining was more likely to be associated with TILs in HPV-positive OPSCC (67.9% vs 49.6%, $p = 0.01$, Table 2). However, there was no difference for PD-L1 staining at the tumour/stroma interface by HPV status.

Patients with PD-L1 positive OPSCC were more likely to have higher grade and higher nodal stage disease. They were also more likely to have TIL within the tumours.

Characteristics of TIL infiltration within tumours

In terms of the presence of TILs within the primary tumours, HPV-positive tumours were significantly more likely to have > 25% TIL distribution within the tumour than HPV-negative cancers (85.2% vs 51.1%, $p = 0.0003$, Table 2). The intensity of TILs was also greater in HPV-positive OPSCC (moderate to dense, 81.4% vs 47.4%, $p < 0.005$). The PD-L1 staining was higher in tumour associated macrophages in HPV positive OPSCC than HPV-negative OPSCC (85.2% vs 64.7%, $p < 0.005$).

Patient outcome analysis

The median follow up time was 48.5 months (range 1–318 months). A total of 75 patients (35.0%) developed a recurrence. Locoregional recurrence occurred in 57 patients as the first site of relapse. Of these, 39 patients developed a local recurrence only and 18 patients developed a regional recurrence. Eighteen patients developed distant metastases as the first site of relapse, none of whom had loco-regional failure at the time of the diagnosis of distant metastases. There were 142 events and 129 deaths from any cause, of which 68 patients died from OPSCC while 61 died from unrelated causes.

Univariate associations of patient and disease characteristics with outcomes are shown in Table S1. Both HPV status and PD-L1 expression predicted for locoregional recurrence, development of an event and death. Age, smoking status and T stage were predictors for event and overall survival.

In the multivariate analysis (Table 3), age was a significant prognostic factor for overall survival (HR 1.47, 95% 1.01–2.15, $p = 0.046$). T stage was a significant prognostic factor for both event-free survival and overall survival. HPV status was a significant prognostic factor for locoregional recurrence, event and death. Patients with HPV-negative OPSCC were more likely to develop a locoregional recurrence (HR 6.63, 95% CI 2.86–15.37, $p < 0.005$), an event (HR 5.45, 95% CI 3.22–9.22, $p < 0.005$) and to die (HR 5.79, 95% CI 3.32–10.10, $p < 0.005$). Patients with PD-L1 positive OPSCC had better event-free survival (HR 0.66, 95% 0.44–0.99, $p = 0.043$) but there was no significant difference in locoregional recurrence (HR 0.57, 95% CI 0.30–1.07, $p = 0.08$) or overall survival (HR 0.70, 95% 0.46–1.07, $p = 0.097$) when compared to patients with PD-L1 negative OPSCC. TIL was not a significant prognostic factor for locoregional recurrence, event and death after adjusting for the other variables.

The effects of a combination of HPV status and PD-L1 expression on outcomes are shown in multivariate Kaplan-Meier models (Fig. 2a–c). There was a significant difference in the risk of locoregional recurrence, event-free survival and overall survival by HPV/PD-L1 combination. The best outcomes were seen in those patients with HPV-positive/PD-L1 positive OPSCC and the worst outcomes were seen in HPV-negative/PD-L1-negative OPSCC (Table 4). Patients with HPV negative/PD-L1 negative OPSCC were 6.4 times more likely to develop a local recurrence, 5.8 times more likely to develop an event and 6.5 times more likely to die than patients with HPV positive/PD-L1 positive OPSCC. Within the HPV positive cases, PD-L1 expression also significantly impacted on the outcomes with PD-L1 negative cases more likely to develop a locoregional recurrence (HR 4.16, 95% CI 1.17–14.77, $p = 0.027$), to have an event (HR 2.5, 95% CI 1.09–9.97, $p < 0.05$) and to die (HR 3.16, 95% CI 1.33–7.50, $p = 0.008$). Interaction terms were tested for HPV status and PD-1 expression. Evidence of an interaction between HPV status and PD-L1 expression was found for overall survival ($p < 0.005$, supplementary data).

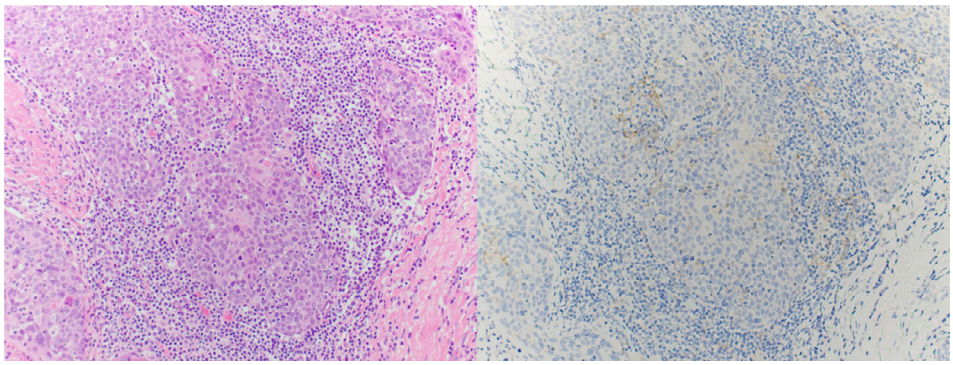

Fig. 1a. The H&E (left) and PD-L1 (right) immunohistochemistry staining of a HPV-positive/PDL1 positive OPSCC.

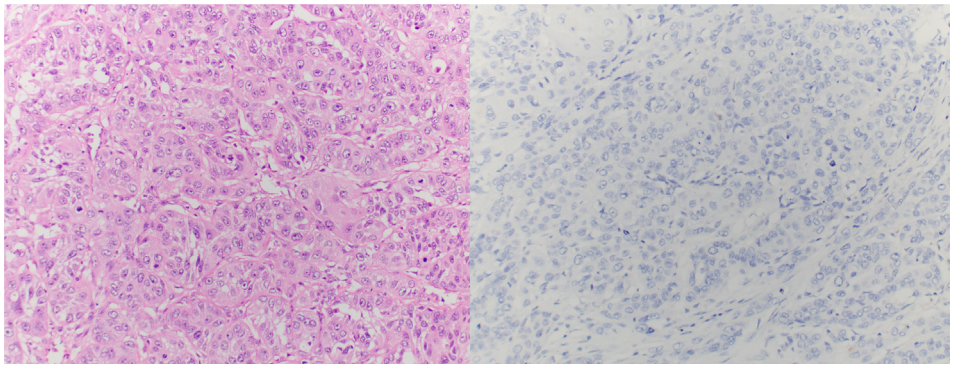

Fig. 1b. The H&E (left) and PD-L1 (right) immunohistochemistry staining of a HPV-positive/ PDL1 negative OPSCC.

Discussion

The current study of 214 patients with OPSCC confirmed a strong correlation between HPV positivity and PD-L1 expression; 85.2% of HPV-positive OPSCC expressed PD-L1 compared with only 57.1% of HPV-negative OPSCC. OPSCCs with PD-L1 expression were also more likely to have TILs within the tumour. The clinical outcomes varied significantly by HPV status and PD-L1 expression with the best outcome seen in patients with HPV positive/PD-L1 positive OPSCC.

In other studies, the prognostic significance of PD-L1 expression in head and neck cancer were less clear cut and could be related to detection method and inclusion of all subsites of head and neck cancer. For example, in a study of 305 patients with oral squamous cell carcinoma, high levels of PD-L1 expression were associated with a poorer overall survival in males and smokers [14]. In that study, Lin et al used immunohistochemistry on tissue microarrays and in the majority of the cases, only one core was used in the arrays. Therefore, the tissue cores used might not be representative of the whole tumour. This is particularly important in evaluating PD-L1 expression since in some tumours, only a minority of tumour cells may be positive [15]. On the other hand, Oliveira-Costa et al. showed that low expression of PD-L1 in oral squamous cell carcinoma was an independent prognostic factor for worse disease-free survival [16].

In terms of the PD-L1 expression by HPV status, a large study including all subsites of head and neck from the German Cancer Consortium Radiation Oncology Group showed a similar correlation between HPV status and PD-L1 expression to that demonstrated in our study [17]. In 161 patients treated with surgery and adjuvant chemoradiation, PD-L1 expression was present in 53.7% of the 67 cases of HPV-positive cancers and 28.7% of the HPV-negative cases. The authors also reported that local progression-free survival, distant metastasis-free survival and overall survival were significantly better in those patients with a high level of PD-L1 expression. They also found that high PD-L1 expression was a strong prognostic factor in patients with HPV-negative cancer. However, they did not show an effect of PD-L1 status on clinical outcome in patients with HPV-positive head and neck SCC. The lack of prognostic significance in HPV-positive cancer observed in that study might be due to great variation in the HPV positivity rate and the prognosis among the cancers involving different subsites of the head and neck region. A recent meta-analysis of 12,263 patients with head and neck squamous cell carcinoma demonstrated a significant variation of HPV positivity within the head and neck subsites: 45.8% for oropharynx, 24.2% for oral cavity and 22.1% for larynx [18]. We have previously shown in 99 cases of tonsillar cancer that PD-L1 expression was associated with better outcomes in patients treated with conventional surgery, chemotherapy and radiation therapy [19].

Table 2
Characteristics of PD-L1 expression and TIL infiltration by HPV status.

	All Patients (N = 214)	HPV Positive (N = 81)	HPV Negative (N = 133)	P-value for heterogeneity
Tumour PD-L1 staining				
– Positive	145 (67.8%)	69 (85.2%)	76 (57.1%)	< 0.005
– Negative	69 (32.2%)	12 (14.8%)	57 (42.9%)	
Presence of any staining associated with TILs				
– Yes	121 (56.5%)	55 (67.9%)	66 (49.6%)	0.011
– No	93 (43.5%)	26 (32.1%)	67 (50.4%)	
Staining at the tumour/stroma interface (for those with positive PD-L1 staining)				
– Yes	120 (82.8%)	58 (84.1%)	62 (81.5%)	0.693
– No	25 (17.2%)	11 (15.9%)	14 (18.4%)	
TILs (n, %)				
– Yes	206 (96.3%)	80 (98.8%)	126 (94.7%)	0.132
– No	8 (3.7%)	1 (1.2%)	7 (5.3%)	
TILs Distribution				
– 0 (no TIL)	8 (3.7%)	1 (1.2%)	7 (5.3%)	< 0.005
– 1 (< 25%)	69 (32.2%)	11 (13.6%)	58 (43.6%)	
– 2 (25– < 75%)	81 (37.9%)	28 (34.6%)	53 (39.8%)	
– 3 (> 75%)	56 (26.2%)	41 (50.6%)	15 (11.3%)	
Density of TILs				
– 0 (no TIL)	8 (3.7%)	1 (1.2%)	7 (5.3%)	< 0.005
– 1 (sparse)	77 (36.0%)	14 (17.3%)	63 (47.4%)	
– 2 (moderate)	86 (40.2%)	36 (44.4%)	50 (37.6%)	
– 3 (intense)	43 (20.1%)	30 (37.0%)	13 (9.8%)	
Macrophage Staining				
– Yes	155 (72.4%)	69 (85.2%)	86 (64.7%)	< 0.005
– No	59 (27.6%)	12 (14.8%)	47 (35.3%)	

Table 3
Multivariate associations of patient and disease characteristics with outcomes.

Characteristic	Locoregional Failure (n = 57)		Any event (n = 142)		Death from any cause (n = 129)	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age at diagnosis						
< 60	1		1		1	
≥ 60	1.04 (0.60, 1.80)	0.889	1.43 (1.00, 2.05)	0.052	1.47 (1.01, 2.15)	0.046
Gender						
– Female	1		1		1	
– Male	0.86 (0.44, 1.69)	0.667	1.05 (0.67, 1.65)	0.822	1.20 (0.74, 1.94)	0.470
Smoking status						
– Non-smoker	1		1		1	
– Current/Ex-smoker	1.13 (0.37, 3.43)	0.825	1.61 (0.73, 3.52)	0.235	1.58 (0.68, 3.66)	0.286
T stage						
– 1	1		1		1	
– 2	1.20 (0.46, 3.11)	0.705	1.08 (0.60, 1.96)	0.790	1.13 (0.59, 2.16)	0.719
– 3	1.54 (0.60, 4.00)	0.370	1.53 (0.86, 2.75)	0.151	1.75 (0.93, 3.29)	0.085
– 4	1.58 (0.53, 4.74)	0.416	2.02 (1.05, 3.89)	0.036	2.34 (0.16, 4.74)	0.018
N stage (Missing = 1)						
– 0	1		1		1	
– 1	1.80 (0.87, 3.71)	0.113	1.48 (0.92, 2.36)	0.103	1.47 (0.90, 2.40)	0.127
– 2	4.20 (1.90, 9.28)	< 0.005	2.95 (1.77, 4.93)	< 0.005	2.86 (1.68, 4.85)	< 0.005
– 3	1.84 (0.41, 8.33)	0.431	2.26 (0.93, 5.47)	0.070	2.41 (0.99, 5.86)	0.051
Grade						
– 1	1		1		1	
– 2–3	0.99 (0.54, 1.83)	0.978	0.93 (0.63, 1.37)	0.700	0.94 (0.63, 1.41)	0.769
HPV status						
– Positive	1		1		1	
– Negative	6.63 (2.86, 15.37)	< 0.005	5.45 (3.22, 9.22)	< 0.005	5.79 (3.32, 10.10)	< 0.005
PD-L1 Status						
– Positive	0.57 (0.30, 1.07)	0.081	0.66 (0.44, 0.99)	0.044	0.70 (0.46, 1.07)	0.097
– Negative	1		1		1	

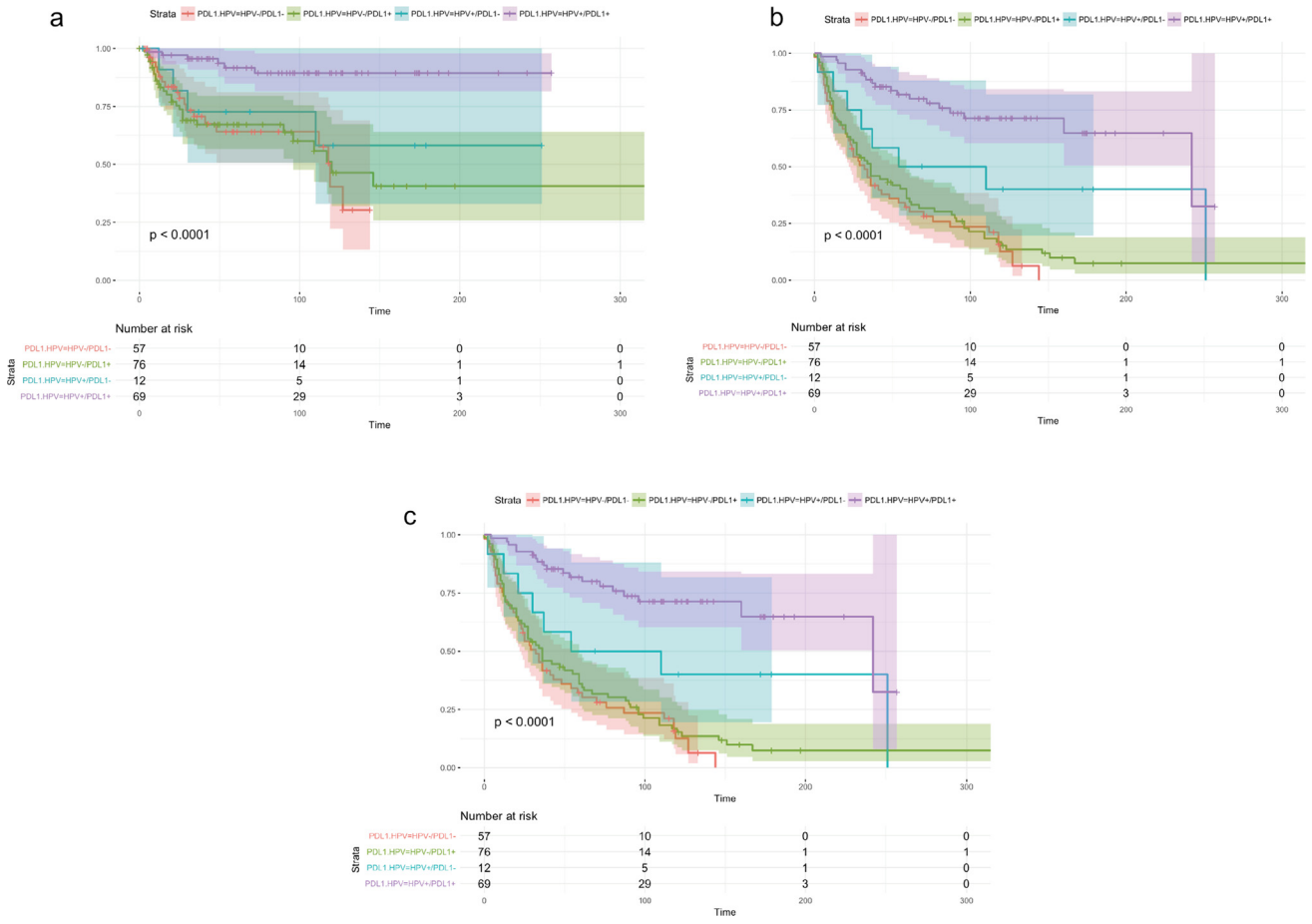

Fig. 2. (a–c) Kaplan-Meier curves by HPV and PD-L1 status. (a) Locoregional recurrence. (b) Event free survival. (c) Overall survival.

Table 4
Multivariate associations of patient and disease characteristics with outcomes.

HPV and PD-L1 status	Time to LR failure HR (95% CI)	Time to event HR (95% CI)	Time to death HR (95% CI)
HPV + /PD-L1 +	1	1	1
HPV + /PD-L1-	4.16 (1.17–14.77) p < 0.027	2.50 (1.09–9.97), p < 0.05	3.16 (1.33–7.50), p = 0.008
HPV-/PD-L1 +	6.35 (2.62–15.37), p < 0.005	5.00 (2.99–8.34), p = 0.030	5.86 (3.33–10.32), p < 0.005
HPV-/PD-L1-	6.42 (2.55–16.15), p < 0.005	5.84 (3.42–9.98), p < 0.005	6.51 (3.60–11.78), p ≤ 0.005

In the current study with a larger cohort including all subsites of OPSCC, we once again demonstrated the strong correlation between HPV status and PD-L1 expression. In addition, there was a strong interaction between HPV status and PD-L1 expression and the effect of HPV status on overall survival was modified by PD-L1 expression.

Several mechanisms that may contribute to the better prognosis seen in HPV-positive OSCC have been proposed and these include up-regulation of p53, altered apoptosis, and impaired repair of DNA damage in response to chemotherapy and radiation therapy. Accumulating evidence also suggests that the enhanced immunogenicity observed in HPV-positive tumours might contribute to the improved survival. The Head and Neck SPORE Program data suggested that higher TIL levels were associated with better relapse-free survival and overall survival [20]. Our data showing the different pattern of TIL infiltration and PD-L1 staining in tumour associated macrophages support that HPV-positive OPSCC are more immunogenic. These data build on previous reports that HPV infection rendered the tumours more immunogenic [17,19,21]. Similarly, cutaneous Merkel cell carcinomas caused by Merkel cell polyoma virus infection are more immunogenic than their virus negative counterparts.[22] Our data are consistent with studies in other malignancies showing association of PD-L1 expression with increased tumour infiltrating lymphocytes and survival [23–25]. The higher TIL infiltration rate seen in HPV-positive OPSCC compared to HPV-negative OPSCC could contribute to enhanced response to radiation therapy. In rectal cancer, the baseline TIL, in particular FoxP3 + TIL, correlated with an increased response to pre-operative radiotherapy and better overall survival [26].

The positive correlation between PD-L1 expression and response to immunotherapy in head and neck cancer was first confirmed in the Keynote 012 study.[27] In this study of 132 patients with recurrent or metastatic head and neck SCC treated with pembrolizumab, the overall response rate was significantly higher in patients with PD-L1 positive disease (22% v 4%; P = 0.021). The Checkmate 141 was a phase 3, randomized study of 361 patients with platinum refractory advanced head and neck SCC. Patients were randomized in a 2:1 ratio to receive nivolumab or investigators' choice of single agent systemic therapy (methotrexate, docetaxel, or cetuximab). Nivolumab was superior to standard systemic therapy with median overall survival of 7.5 versus 5.1 months, (hazard ratio for death was 0.70; P = 0.01). In the exploratory biomarker analyses, survival benefit from nivolumab was seen regardless of tumor PD-L1 status or p16 status. However, it appeared that the benefit may be greater when PD-L1 expression was ≥1% or in HPV positive OPSCC.

One weakness of the study is the use of AJCC 7th edition criteria. The study population was staging prior to the establishment of the AJCC 8th edition staging in 2017 which includes a separate staging algorithm for HPV positive OPSCC.

In conclusion, we have demonstrated different immune profiles of HPV-positive and HPV-negative OPSCC and that the effect of HPV on the outcomes is modified by PD-L1 expression. PD-L1 expression and TILs infiltration were associated with better prognosis in this cohort of patients treated with conventional therapy. In future studies evaluating immunotherapy in OPSCC, it will be important to stratify patients based upon their HPV tumour status in the study design and outcome analysis.

Conflicts of interest statement

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2019.03.012.

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