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ORIGINAL ARTICLE



# Prognostic impact of PD-L1 in oropharyngeal cancer after primary curative radiotherapy and relation to HPV and tobacco smoking

Jacob K. Lilja-Fischer[a], Jesper G. Eriksen[a], Jeanette B. Georgsen[b], Thao T. Vo[c], Stine R. Larsen[d], Jonathan Cheng[c], Michael Busch-Sørensen[e], Deepti Aurora-Garg[c], Torben Steiniche[b] and Jens Overgaard[a]

[a]Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [c]Merck & Co. Inc, Kenilworth, NJ, USA; [d]Department of Pathology, Odense University Hospital, Odense, Denmark; [e]Busch-Sørensen Consulting ApS, Virum, Denmark

**ABSTRACT**

**Background:** Incidence of oropharyngeal squamous cell carcinoma (OPSCC) is rising rapidly in many western countries due to *Human papillomavirus* (HPV) and tobacco smoking, with a considerable overlap. Immunotherapy directed at the PD1/PD-L1 axis have shown promise in head and neck cancer and other cancer types. PD-L1 expression may indicate a poorer prognosis, and at the same time indicate a possible benefit of anti-PD-L1 immunotherapeutic agents. The primary aim of this study was to establish the prognostic effect of PD-L1 expression after primary curative radiotherapy alone.

**Material and methods:** A cohort of 303 OPSCC patients treated with primary, curative intended radiotherapy was established. PD-L1 expression was evaluated by immunohistochemistry on formalin fixed, paraffin embedded tissue sections. PD-L1 positivity was defined as a Combined Positive Score (CPS) $\geq$1, indicating staining of either tumor cells, lymphocytes or macrophages.

**Results:** Median follow-up was 5.3 years. With 199 deaths, there was no difference in overall survival between patients with PD-L1+ and PD-L1− tumors (adjusted hazard ratio [aHR] and 95% confidence interval [CI]: 1.0 [0.71–1.4]). Also, locoregional failure was similar between the two groups (aHR 1.1 [CI: 0.68 − 1.7]). Tumors were PD-L1+ in 76% of cases, significantly more among HPV p16+ tumors (82% vs. 70%, $p = .01$). Interestingly, higher prevalence of PD-L1+ expression was seen in HPV p16+ patients with <10 pack-years of tobacco-smoking (93%) compared to HPV p16+ smokers (76%) or HPV p16-negative patients (70%) ($p = .003$).

**Conclusion:** PD-L1 expression had no prognostic significance in OPSCC patients treated with primary radiotherapy alone. A substantial proportion of OPSCC tumors show PD-L1 overexpression, especially in HPV p16+ tumors in patients with little or no smoking history.

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## Introduction

Oropharyngeal squamous cell carcinoma (OPSCC) is a rapidly growing health concern, and in many western countries now the most common type of head and neck cancer, due to infection with *Human Papillomavirus* (HPV), and to a lesser extent tobacco smoking [1,2]. HPV + OPSCC is established as a separate disease entity, distinct from HPV− OPSCC and squamous cell carcinomas of other head and neck tumor sites [3,4].

Prognosis after primary radiotherapy or chemo-radiotherapy is markedly better in patients with HPV-associated disease compared to those with tobacco-related OPSCC [5]. However, studies have shown that patients with HPV + OPSCC and a significant history of tobacco smoking still have a poor prognosis [6,7]. Current challenges in the treatment of OPSCC is how to personalize treatment in order to improve cure rates for those with a poor prognosis, as well as reduce treatment related morbidity without compromising treatment outcome in those with a more favorable prognosis.

The role of the immune system in cancer biology and treatment has seen renewed interest in recent years, and the ability to avoid immune destruction of tumor cells have been acknowledged as a hallmark of cancer [8]. Large-scale efforts to characterize human cancer has described distinct immune subclasses across multiple cancer types [9]. In head and neck cancer, multiple gene expression subtypes have been described, which also correlate with HPV, immune response and possibly tobacco smoking [4,10].

Of particular interest, it has materialized that programmed death-ligand 1 (PD-L1) expressed on the surface of tumor cells or macrophages interact with programmed cell death protein 1 (PD1) receptors on the surface of cytotoxic T lymphocytes and help suppress the antitumor activity of these. On this basis, PD-L1 overexpressing (PD-L1+) tumors may

CONTACT Jacob K. Lilja-Fischer jacob@oncology.au.dk Department of Experimental Clinical Oncology, Aarhus University Hospital, Nørrebrogade 44, Aarhus C, DK-8000, Denmark

Supplemental data for this article can be accessed here.

have a poorer prognosis than PD-L1-negative (PD-L1−) tumors, and may at the same time be more sensitive to treatment with anti-PD-L1 immunotherapeutic agents [11,12]. Indeed, there is evidence for the prognostic value of PD-L1 expression in several cancer types, and PD-L1+ status is used to indicate treatment with these agents [12].

Recently, two large randomized phase III trials of anti-PD1 immunotherapeutic agents in recurrent or metastatic head and neck cancer have been reported, establishing benefit compared to standard chemotherapeutic regimes [13,14]. This suggests a clinical benefit of immunotherapy in head and neck cancer, but a possible role as adjuvant to primary treatment and interaction with HPV and other clinical factors in OPSCC is still unresolved [15–17].

Here we detail the prognostic effect of PD-L1 expression in OPSCC after primary radiotherapy alone, in the largest study to date. Furthermore, we explore the association to tobacco smoking, which is a key prognostic factor in HPV + OPSCC.

## Material and methods

### Study population

A cohort of 400 patients was selected to reflect a nationally representative sample of patients with primary OPSCC from 2000 to 2012 in Denmark. Denmark has an almost 100% public health care system with uniform standard of care for all patients. Patient and tumor characteristics as well as follow-up and outcome data were collected from the Danish Head and Neck Cancer database, and supplemented by medical records. Tobacco smoking history was available in all but one patient. From the 400 identified patients, we included patients that completed radiotherapy with curative intent as monotherapy, primarily with 66–68Gy, 2 Gy/fraction, six fractions/week and who had available tumor tissue, leaving 303 patients for analysis. Patients were staged according to UICC TNM 7th edition. This was a retrospective, observational study with no direct patient contact. Permissions were obtained from the regional ethical committee and the Danish Data Protection Agency.

### Immunohistochemistry

Tumor tissue samples were collected during routine clinical practice. For immunohistochemical staining (IHC) formalin-fixed, paraffin-embedded tumor sections were cut at 4–5 μm and placed onto positively charged slides.

PD-L1 staining (Agilent's investigational PD-L1 PharmDx IHC Assay (clone 22C3)) was performed on the Autostainer Link 48 (Agilent), per the manufacturer's instructions. Briefly, a negative isotype-matched antibody control was run for each sample, along with positive and negative controls in each staining run. All stained slides were evaluated by a board-certified pathologist for PD-L1 membrane staining. All the PD-L1 IHC assays were done at Q2 Solutions (Edinburg, UK) as the central laboratory.

Primarily, PDL1+ expression was defined as a Combined Positive Score (CPS) ≥1, calculated as the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by total number of tumor cells evaluated, multiplied by 100. In sensitivity analyses, we also considered Tumor Proportion Score (TPS), defined as % of neoplastic cells expressing PD-L1 at any intensity, and Mononuclear Inflammatory Density Score (MIDS), defined as the estimate of PD-L1 expressing mononuclear inflammatory cells associated with neoplastic cells. A minimum of 100 viable tumor cells were evaluated [18].

HPV status was determined using p16 IHC clone E6H4 (Roche) as a surrogate marker. Staining was done on VENTANA BenchMark ULTRA, a fully automated staining instrument. HPV oncogene expression was assessed using a >70% cut-point of tumor cells stained for p16 IHC.

### Statistical analyses

The primary outcome measures were locoregional failure and overall survival. Overall survival was defined as time to death from any cause, and estimated by Kaplan–Meier method. Locoregional failure was defined as persistence or recurrence of squamous cell carcinoma at the T- or N-site. Additionally, disease-specific mortality was defined as death from or with the disease. Cumulative incidence estimates were corrected for competing risks (for locoregional failure: distant failure and death; for disease-specific mortality: death from other causes). Cumulative incidence curves were estimated by Aalen-Johansen method. Cumulative incidence and confidence intervals at 5 years were estimated by pseudo values method [19]. Time to event of interest was measured starting from end of radiotherapy.

Post-radiotherapy neck dissection was not routinely performed and was not considered part of the primary treatment.

Assessment of the prognostic effect of PD-L1 overexpression, defined as CPS ≥1, was by crude and adjusted Cox proportional hazards regression, with the following variables selected *a priori* due to their established independent prognostic significance in OPSCC: Locoregional failure was adjusted for p16 status, T classification (T1–2 vs. T3–4), N classification (N0 vs. N1 vs. N2–3), disease stage (I–II vs. III–IV), tobacco smoking status (current vs. former/never) and pack-years of smoking (continuous variable). Disease-specific mortality and overall survival was furthermore adjusted for age (continuous variable), gender and WHO performance status.

Analyses were performed for the entire cohort as well as stratified by HPV p16 status by dividing the cohort into two subsets. An analysis of interaction was performed by adding an interaction term to the Cox regression analysis, to determine evidence of differential effect by HPV p16 status.

Association between clinical parameters and PD-L1 status was tested by rank-sum test for continuous data, and chi-squared test for categorical data. Correlation between TPS and MIDS was assessed with Kendall's tau. Estimates are presented with 95% confidence interval (CI) and *p*-values less than 5% were considered significant.

## Results

### Patient characteristics

A total of 303 patients with OPSCC treated with curative radiotherapy were included, of which 156 (51%) were HPV p16+. The majority of patients had locoregionally advanced disease, with 81% classified as stage III or IV, and 79% had a tobacco smoking history of ten or more pack-years. Patients were treated with 66–68 Gy of radiotherapy in 33 − 34 fractions. Radiotherapy was delivered as 2 D radiotherapy, 3 D conformal radiotherapy or intensity-modulated radiotherapy. The hypoxic radiosensitizer nimorazole were administered in 93% of patients, while only 3% received concomitant cisplatin. Further details are given in Table 1.

### PD-L1 expression

Measured as CPS ≥1, 76% of tumors were PD-L1 positive. This was significantly higher among HPV p16+ patients (82% vs. 70%, $p = .01$), and remained so when evaluating other cutoffs, although absolute proportions of PD-L1+ tumors obviously changed. MIDS and TPS were also higher among HPV p16+ patients (Table 2). TPS distribution was severely skewed, with the majority being negative or staining only weakly, while a minority had a high proportion of positive tumor cells (median TPS: 1, interquartile range: 0–5). TPS was weakly associated with MIDS (Kendall's tau = 0.25).

Among HPV p16− patients, there were no differences between PD-L1+ and PD-L1− patients with regards to clinical and tumor characteristics. Among HPV p16+ patients, patients with PD-L1− tumors were more likely to be former or current smokers, and had accumulated significantly more tobacco pack-years (Table 1).

### Survival outcomes

Median follow-up was 5.3 years (Interquartile range: 5.0−12 years). There were 199 deaths, 107 deaths from or with the disease, and 112 locoregional failures during follow-up.

Locoregional failure was 34% (CI: 28–39%) at five years for the entire cohort, significantly worse in patients with HPV p16- tumors. There was no effect of PD-L1 status on locoregional failure, neither in the entire cohort or when stratified by HPV p16 status. Adjusted hazard ratio was 1.1 (CI: 0.7−1.7) for the entire cohort, and there was no evidence of a prognostic effect of PD-L1+ status in either HPV p16+ or HPV p16− subgroups (Figure 1 and Table 3).

**Table 1.** Patient, tumor and treatment characteristics. PD-L1 status according to combined positive score ≥1.

| | All patients ($n = 303$) | HPV p16+ ($n = 156$) | | | HPV p16− ($n = 147$) | | |
|---|---|---|---|---|---|---|---|
| | | PD-L1+ ($n = 128$) | PD-L1− ($n = 28$) | $p$ Value | PD-L1+ ($n = 103$) | PD-L1− ($n = 44$) | $p$ Value |
| Age, years | | | | | | | |
| Median, IQR | 59 (53–65) | 57 (52–64) | 59 (51–66) | .9 | 60 (55–66) | 60 (55–66) | .9 |
| Gender | | | | | | | |
| Male | 217 (72%) | 94 (73%) | 20 (71%) | .8 | 70 (68%) | 33 (75%) | .4 |
| Female | 86 (28%) | 34 (27%) | 8 (29%) | | 33 (32%) | 11 (25%) | |
| Performance status | | | | | | | |
| 0–1 | 277 (91%) | 123 (96%) | 27 (96%) | .9 | 87 (84%) | 40 (91%) | .3 |
| 2–3 | 26 (9%) | 5 (4%) | 1 (4%) | | 16 (16%) | 4 (9%) | |
| Tumor subsite | | | | | | | |
| Base of tongue and vallecula | 68 (22%) | 26 (20%) | 1 (3%) | .02 | 33 (32%) | 8 (18%) | .1 |
| Tonsil and tonsillary fossa | 205 (68%) | 99 (77%) | 24 (86%) | | 56 (54%) | 26 (59%) | |
| Other oropharynx | 30 (10%) | 3 (2%) | 3 (11%) | | 14 (14%) | 10 (23%) | |
| T category | | | | | | | |
| T1–2 | 182 (60%) | 84 (66%) | 17 (61%) | .6 | 60 (58%) | 21 (48%) | .2 |
| T3–4 | 121 (40%) | 44 (34%) | 11 (39%) | | 43 (42%) | 23 (52%) | |
| N category | | | | | | | |
| N0 | 81 (27%) | 21 (16%) | 6 (21%) | .1 | 37 (36%) | 17 (39%) | .9 |
| N1 | 59 (19%) | 26 (20%) | 10 (36%) | | 17 (17%) | 6 (14%) | |
| N2–3 | 163 (54%) | 81 (63%) | 12 (43%) | | 49 (48%) | 21 (48%) | |
| M category | | | | | | | |
| M0 | 303 (100%) | 128 (100%) | 28 (100%) | | 103 (100%) | 44 (100%) | |
| Stage | | | | | | | |
| I–II | 58 (19%) | 16 (12%) | 4 (14%) | .8 | 30 (29%) | 8 (18%) | .2 |
| III–IV | 245 (81%) | 112 (88%) | 24 (86%) | | 73 (71%) | 36 (82%) | |
| Differentiation | | | | | | | |
| Low | 167 (55%) | 83 (65%) | 18 (64%) | 1 | 45 (44%) | 21 (48%) | .7 |
| Moderate and high | 136 (45%) | 45 (35%) | 10 (36%) | | 58 (56%) | 23 (52%) | |
| Tobacco smoking category | | | | | | | |
| Never | 48 (16%) | 40 (31%) | 2 (7%) | .007 | 5 (5%) | 1 (2%) | .3 |
| Former | 86 (28%) | 53 (41%) | 10 (36%) | | 19 (18%) | 4 (9%) | |
| Current | 168 (55%) | 34 (27%) | 16 (57%) | | 79 (77%) | 39 (89%) | |
| N/A | 1 (<1%) | 1 (1%) | 0 (0%) | | 0 (0%) | | |
| Tobacco pack-years | | | | | | | |
| Median, IQR | 31 (12–47) | 15 (0–34) | 40 (25–53) | .0001 | 40 (27–53) | 38 (25–54) | .7 |
| ≥10 pack-years | 240 (79%) | 76 (59%) | 24 (86%) | .008 | 98 (95%) | 42 (95%) | .9 |
| <10 pack-years | 63 (21%) | 52 (41%) | 4 (14%) | | 5 (5%) | 2 (5%) | |
| Treatment | | | | | | | |
| ≥66 Gy | 303 (100%) | 129 (100%) | 28 (100%) | | 102 (100%) | 44 (100%) | |
| Nimorazole | 283 (93%) | 122 (95%) | 28 (100%) | .2 | 94 (91%) | 39 (89%) | .6 |
| Chemotherapy | 10 (3%) | 7 (5%) | 0 (0%) | .05 | 2 (2%) | 1 (2%) | .9 |

$p$ Values are from chi-squared test except age and pack-years which are from ranksum test.

**Table 2.** Distribution of PD-L1 expression.

| | All (n = 303) | HPV p16+ (n = 156) | HPV p16− (n = 147) | *p* Value |
|---|---|---|---|---|
| Combined positive score (CPS) | | | | |
| PD-L1 positive (CPS ≥ 1) | 231 (76%) | 128 (82%) | 103 (70%) | .01[a] |
| PD-L1 negative (CPS = 0) | 72 (24%) | 28 (18%) | 44 (30%) | |
| CPS ≥ 10 | 80 (26%) | 59 (38%) | 21 (14%) | <.001[a] |
| CPS < 10 | 223 (74%) | 97 (62%) | 126 (86%) | |
| CPS ≥ 20 | 42 (14%) | 34 (22%) | 8 (5%) | <.001[a] |
| CPS < 20 | 261 (86%) | 122 (78%) | 139 (95%) | |
| Tumor proportion score (TPS) | | | | |
| Mean (95% CI) | 5.2 (3.8-6.5) | 6.8 (4.6-9.0) | 3.4 (1.8-5.0) | .003[b] |
| TPS > 1% | 105 (35%) | 67 (43%) | 38 (26%) | .002[c] |
| TPS ≤ 1% | 198 (65%) | 89 (57%) | 109 (74%) | |
| TPS > 10% | 35 (12%) | 24 (15%) | 11 (7%) | .03[c] |
| TPS ≤ 10% | 268 (88%) | 132 (85%) | 136 (93%) | |
| Mononuclear inflammatory densitory score (MIDS) | | | | |
| 0 | 44 (15%) | 17 (11%) | 27 (18%) | <.001[c] |
| 1 | 165 (54%) | 73 (47%) | 92 (63%) | |
| 2 | 81 (27%) | 56 (36%) | 25 (17%) | |
| 3 | 12 (4%) | 9 (6%) | 3 (2%) | |
| 4 | 1 (<1%) | 1 (1%) | 0 (0%) | |

[a]Chi-squared test.
[b]Ranksum test.
[c]Chi-squared test for trend.

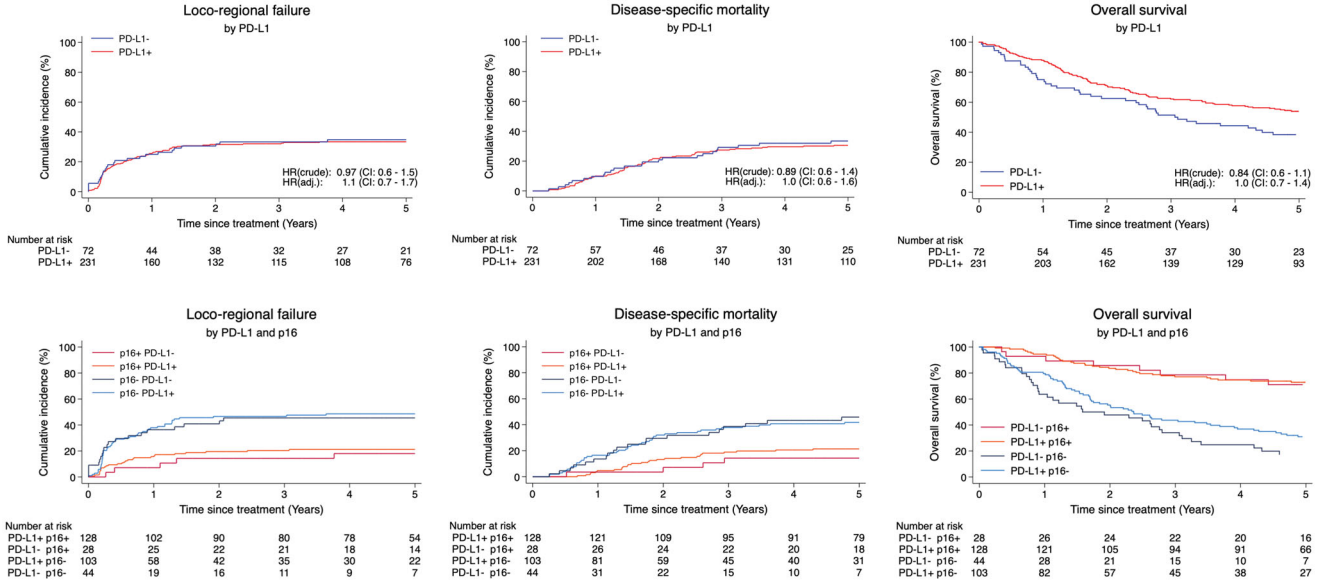


**Figure 1.** Loco-regional failure, disease-specific mortality and overall survival stratified by PD-L1 and PD-L1/p16. PD-L1 was evaluated as Combined Positive Score ≥1. Cumulative incidence estimates corrected for competing risks (loco-regional failure: distant failure and death; disease-specific mortality: death from other causes). Adjusted hazard ratio (HR) adjusted as follows: Locoregional failure adjusted for p16, T classification, N classification, disease stage, tobacco smoking status and pack-years of smoking. Disease-specific mortality and overall survival furthermore adjusted for age, gender and performance status. CI: 95% confidence interval.

**Table 3.** Hazard ratio and 95% confidence intervals for PD-L1 positive versus PD-L1 negative from Cox regression.

| | All patients (*n* = 303) | HPV p16+, *n* = 157 | HPV p16−, *n* = 146 | *p* Value (interaction) |
|---|---|---|---|---|
| Locoregional failure | (112 events) | (38 events) | (74 events) | |
| Crude HR | 0.97 (0.63–1.5) | 1.4 (0.56–3.3) | 1.0 (0.62–1.7) | .7 |
| Adjusted HR[a] | 1.1 (0.68–1.7) | 1.4 (0.54–3.7) | 0.90 (0.53–1.5) | .7 |
| Disease-specific mortality | (107 events) | (38 events) | (69 events) | |
| Crude HR | 0.89 (0.57–1.4) | 1.5 (0.62–3.8) | 0.85 (0.51–1.4) | .4 |
| Adjusted HR[b] | 1.0 (0.62–1.6) | 1.2 (0.44–3.1) | 0.87 (0.50–1.5) | .8 |
| Overall survival | (199 events) | (73 events) | (126 events) | |
| Crude HR | 0.84 (0.61–1.1) | 1.3 (0.71–2.4) | 0.80 (0.55–1.2) | .4 |
| Adjusted HR[b] | 1.0 (0.71–1.4) | 1.1 (0.53–2.1) | 0.89 (0.59–1.4) | .9 |

[a]Adjusted for p16, T and N classification, disease stage, tobacco smoking status and pack-years of tobacco smoking.
[b]Also adjusted for age, gender and performance status. PD-L1 status according to CPS ≥ 1.

Disease-specific mortality was 31% (CI: 26–37%) at 5 years, worse with HPV p16-negative tumors, and there was no effect of PD-L1 status, with an adjusted hazard ratio of 1.0 (CI: 0.6–1.6).

Five-year overall survival for the entire cohort was 50% (CI: 44–56%), again significantly worse for patients with HPV p16− tumors. PD-L1 status had no effect on overall survival (adjusted hazard ratio 1.0 [CI: 0.71−1.4]; Table 4).

### *Survival outcomes with other measures of PD-L1 expression*

To explore whether other measures or cut-points might have an influence on survival outcomes, we explored overall survival and locoregional failure using CPS ≥10, CPS ≥20, TPS >1%, TPS >10%, MIDS ≥1 or MIDS ≥2 as definition of PD-L1 positivity, again evaluating crude and adjusted hazard ratios for the entire cohort and stratified by HPV p16-status.

**Table 4.** Absolute 5-year estimates of overall survival and locoregional failure (with 95% CI).

| | All patients ($n = 303$) | HPV p16+, $n = 157$ | HPV p16−, $n = 146$ |
|---|---|---|---|
| Locoregional failure | | | |
| All | 34% (28–39%) | 21% (14–27%) | 48% (40–56%) |
| PD-L1+ | 33% (27–39%) | 21% (14–28%) | 49% (40–57%) |
| PD-L1− | 35% (24–46%) | 18% (6–30%) | 45% (34–57%) |
| Disease-specific mortality | | | |
| All | 31% (26–37%) | 20% (14–26%) | 43% (35–51%) |
| PD-L1+ | 31% (25–37%) | 20% (14–27%) | 43% (34–52%) |
| PD-L1− | 34% (23–45%) | 20% (8–31%) | 42% (31–54%) |
| Overall survival | | | |
| All | 50% (44–56%) | 73% (65–79%) | 27% (20–34%) |
| PD-L1+ | 54% (47–69%) | 73% (64–80%) | 17% (6–30%) |
| PD-L1− | 38% (27–50%) | 71% (50–84%) | 31% (22–40%) |

Estimates by Kaplan–Meier method for OS and by pseudo-values method for LRF and DSM. PD-L1 status according to Combined Positive Score ≥1.

In summary, none of these measures had any significant impact on outcome (Supplementary Table 1).

### *PD-L1 expression and relation to HPV and smoking*

To further explore the connection between PD-L1 expression and tobacco-smoking seen in patients with HPV p16+ tumors, we stratified this group into HPV + smokers (≥10 pack-years) and HPV + nonsmokers (<10 pack-years). This revealed, that PD-L1 expression was significantly higher among HPV + nonsmokers by TPS, MIDS or CPS, and that HPV + smokers had PD-L1 expression similar to HPV p16− tumors (Figure 2, Supplementary Table 2).

## Discussion

In summary, we found no prognostic value of PD-L1 expression in OPSCC treated with primary curative radiotherapy with regards to locoregional failure, disease-specific mortality or overall survival. This persisted even after considering other PD-L1 measures and cutoffs. PD-L1 expression was consistently higher in HPV p16+ tumors, almost exclusively limited to patients with little or no tobacco smoking history.

Few other studies have examined the distribution and prognostic effect of PD-L1 expression after primary radiotherapy in OPSCC specifically: In a cohort of 190 patients with HPV + OPSCC primarily treated with chemoradiotherapy, Solomon *et al* found no effect of PD-L1 expression in tumor cells, but did note a positive prognostic effect in a subgroup of patients with strong staining of immune cells [20]. In a smaller study of 92 OPSCC patients treated with primary radiotherapy, Fukushima et al. evaluated staining of ≥1% of either immune cells or tumor cells. A prognostic effect on progression-free survival and overall survival was not confirmed in multivariate analyses [21]. Similarly, other studies of

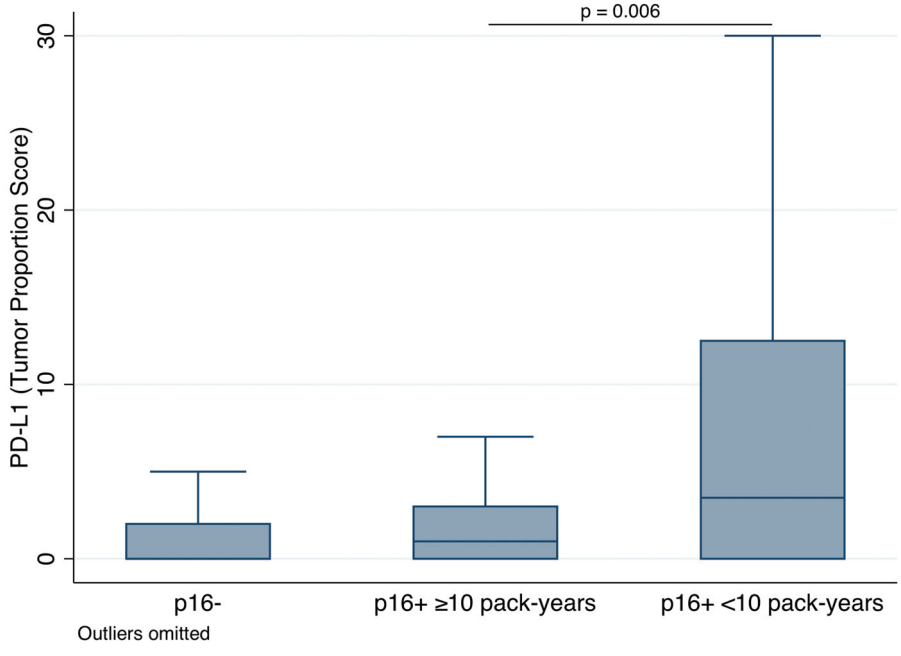


**Figure 2.** PD-L1 expression in relation to HPV and tobacco smoking.

patients in which surgery played a larger role has yielded equally conflicting results [22–24].

A recent meta-analysis found no prognostic effect of tumor PD-L1 expression in head and neck squamous cell carcinoma. However, PD-L1 expression was evaluated in multiple ways, and treatment was not accounted for [25]. Higher PD-L1 expression in HPV + tumors were also found in other studies [21,22,24,26], and one other study similarly found higher PD-L1 expression among nonsmokers [22].

This study is a retrospective review of longitudinally-collected clinical data and is the largest to date to specifically assess the prognostic value of PD-L1 expression after primary radiotherapy in oropharyngeal cancer patients. The cohort is a representative sample of oropharyngeal cancer patients who received uniform standard of care. Although most of the included patients would have been treated with concomitant cisplatin by today's standards, the cohort does allow to study the impact of PD-L1 expression on radiotherapy response alone. We also took association with tobacco smoking into account, which is an important prognostic factor in HPV + OPSCC [7]. Patients were staged using UICC TNM 7th edition to allow for comparison between HPV + and HPV− disease.

Comparison across studies is hampered by study differences in the PD-L1 clones used, the PD-L1 stained cells that are evaluated (tumor cells or immune cells or both), and the cut-points used for defining PD-L1 positivity. Nevertheless, recent studies did show high concordance in scoring for PD-L1 expression across 3 of 4 PD-L1 IHC clones/assays [27–29]. Furthermore, our sensitivity analyses confirmed that our conclusions did not differ with other PD-L1 definitions, such as MIDS or TPS, or with other cutoffs used to define PD-L1+ status.

Although this paper only discusses PD-L1 expression, there is a widening understanding of the diverse interplay between tumor and immune system. In fact, multiple pathways are involved from the release of the cancer antigens to the activation of the T-cell response, the infiltration of T-cells in the tumor and finally the killing of the cancer cell. Several other immune markers have thus shown prognostic potential and relation to PD-L1, e.g., CD8+ tumor infiltrating lymphocytes in OPSCC [9,30,31].

Our results support findings from other studies, that the prognostic value of PD-L1 expression after radiotherapy-based treatment is non-existent or weak at best. Our finding of an association between PD-L1 expression to tumor HPV p16 status support those of other studies, but PD-L1 status does not seem to be of prognostic importance in either HPV groups, although a lack of statistical power does make these analyses less valid. The role which lower expression of PD-L1 plays in patients with a significant smoking history is unclear, although other studies in OPSCC or other head and neck cancer patients have shown either no significant difference in the distribution of smoking by PD-L1 or shown higher PD-L1 overexpression among those with higher levels of smoking [32,33]. The results do, however, point to differences in tumor biology or immune system interaction between smokers and nonsmokers with HPV + OPSCC. Whether this is implicated in the better prognosis in nonsmokers seen in multiple studies is yet to be investigated [6,7].

Current challenges in the treatment of OPSCC is how to tailor therapy, with the aim of improving prognosis while minimizing morbidity. Much attention has been given to the concept of de-escalation of therapy in HPV + patients, particularly in those with little or no smoking history [34]. However, the future direction of treatment in HPV + OPSCC is likely to be influenced by the recent failure of two large de-escalation trials, substituting cisplatin for cetuximab concurrent with radiotherapy. This resulted in increased locoregional failures and additional mortality [35,36]. Whether immunotherapy could play a role in the primary treatment of OPSCC remains a matter of research and intense debate [37]. Our finding of high PD-L1 expression in HPV + tumors among patients with no or minor smoking history could point to this as a candidate group for treatment with anti-PD1 immunotherapeutic agents. However, caution must be exercised not to compromise cure rates, as exemplified by the aforementioned trials. Results from the present study should not change current clinical practice, but it does provide biologically interesting observations, and could hint to the importance of the immune system to radiotherapy response in these patients. HPV status remain the strongest prognostic factor for these patients, as is reflected in the recent update of the TNM classification.

To conclude, in this large study of OPSCC patients treated with primary curative radiotherapy alone, we found no prognostic effect of PD-L1 expression on locoregional failure, disease-specific mortality or overall survival. We found higher PD-L1 expression in HPV + tumors, especially in patients with no or minor smoking history.

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## Disclosure statement

T.T.V, J.C. and D.A.G. is employed at Merck & Co., Inc.; T.T.V. and J.C. owns stock in Merck & Co., Inc.; T.S received research funding from Merck & Co., Inc.

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## ORCID

Jacob K. Lilja-Fischer http://orcid.org/0000-0002-6400-6650
Jesper G. Eriksen http://orcid.org/0000-0002-1145-6033
Jeanette B. Georgsen http://orcid.org/0000-0002-9452-2920
Michael Busch-Sørensen http://orcid.org/0000-0002-7326-4686
Torben Steiniche http://orcid.org/0000-0001-5689-3555
Jens Overgaard http://orcid.org/0000-0002-0814-8179

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