Beyond the Percentages of PD-L1-Positive Tumor Cells: Induced Versus Constitutive PD-L1 Expression in Primary and Metastatic Head and Neck Squamous Cell Carcinoma

Theresa Scognamiglio¹ · Yao-Tseng Chen²

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Abstract Anti-PD1 antibody has been approved for metastatic squamous cell carcinoma of the head and neck (SCCHN) and objective response rates of approximately 20% have been reported. Defining PD-L1 expression at $\geq 1\%$ tumor cells as positive, PD-L1-positive tumors showed a higher response rate. However, it is unclear whether 1% is the optimal cutoff, and studies on lung cancer suggested 50% cutoff as a stronger predictive biomarker. 96 primary SCCHN from oropharynx and oral cavity and 34 corresponding metastatic lesions were typed for membranous PD-L1 expression. p16 immunohistochemistry was used as a surrogate marker for HPV status in SCCHN from the oropharynx. Fifty-two of 96 (54%) tumors were PD-L1-positive, 72% if PD-L1 expression in tumor-infiltrating immunocytes was also included as positive. Fifteen of 34 primary-metastasis tumor pairs differed in PD-L1 expression, and p16(+) cases more frequently showed PD-L1 expression in immunocytes than p16(–) cases (82 vs. 45%, $p < 0.05$). PD-L1-positive SCCHN showed two distinct patterns of expression. In the induced pattern of expression, PD-L1-positive tumor cells were limited to the periphery of tumor nests at the tumor–immunocyte interface, comprising $< 5\%$ of tumor cells, and were almost always associated with PD-L1-positive immunocytes. In contrast, tumors with constitutive PD-L1

expression had a higher percentage of positive tumor cells, often diffusely distributed throughout the tumor, and often were not accompanied by PD-L1-positive immunocytes. We propose that distinguishing these two biologically distinctive patterns of PD-L1 expression and typing metastatic instead of primary lesions might better predict immunotherapeutic response to anti-PD1/PD-L1 regimens beyond just the percentage of PD-L1-positive tumor cells.

Keywords PD-L1 · Squamous cell carcinoma · HPV · Immunohistochemistry

Background

Cancer immunotherapy, specifically immune checkpoint blockade with immunostimulatory monoclonal antibodies, has been shown to be an effective treatment modality in multiple tumor types, leading to sustained complete or partial remission in a subset of patients. Multiple T cell co-inhibitory or co-stimulatory signal molecules have been and are being explored, and anti-CTLA4, anti-PD1 and anti-PD-L1 have been FDA-approved. In comparison to anti-CTLA4 that has primarily been found to be effective in melanoma, anti-PD-1 and anti-PD-L1 showed treatment response in multiple tumor types, including non-small cell lung cancer (NSCLC), renal cancer, Hodgkin lymphoma, urothelial cancer, head and neck squamous cell carcinoma, etc. One main reason for this broader spectrum of treatment response in anti-PD1 versus anti-CTLA4 stemmed from the expression of PD-L1—one of two receptor ligands of PD1—in a subset of tumor cells. The effectiveness of anti-PD1 (or anti-PD-L1) thus resulted from the blockade of PD1-PD-L1 interaction not only at the priming phase of T cell-dendritic cell interaction, but also at

✉ Theresa Scognamiglio
ths9004@med.cornell.edu

Yao-Tseng Chen
ytchen@med.cornell.edu

¹ Department of Pathology and Laboratory Medicine,
Weill Cornell Medicine, 525 East 68th Street Starr-1005,
New York, NY 10065, USA

² Department of Pathology and Laboratory Medicine,
Weill Cornell Medicine, 525 East 68th Street, New York,
NY 10065, USA

the effector phase of T cell-tumor cell interaction, likely by eliminating an immune escape mechanism utilized by the PD-L1-positive tumor cells [1–4].

This understanding of PD1-PD-L1 biology would suggest PD-L1 expression in tumor cells as a potential predictive marker in the treatment response to anti-PD1 and particularly anti-PD-L1 antibodies, and this subject has been examined in almost all anti-PD1-PD-L1 clinical trials, but the results have been highly variable [5–9]. One factor that complicated the evaluation of PD-L1 in cancer was whether PD-L1 positivity should be defined solely by its expression in tumor cells, or should PD-L1 expression in the immune cells in the tumor microenvironment also be considered positive. PD-L1 is normally expressed in a subset of immune cells, including T cells and mononuclear cells, but not in normal epithelial cells except the reticular epithelium at the crypt of the tonsil [10]. Various types of cancer cells, however, have been found to express PD-L1, most often as induced expression by IFN- γ secreted by adjacent immune cells. Most of the earlier studies evaluated only PD-L1 expression in the tumor cells, but the expression in immunocytes in the tumor microenvironment has since been recognized as being just as important or even more important [6, 11]. This notion is illustrated in the study of Chow et al. (KEYNOTE-012) in SCCHN [6] in which no difference in overall response rate (ORR) was seen in the PD-L1-positive ($\geq 1\%$) versus negative groups if only tumor cells were evaluated, whereas the PD-L1-positive group did better if immune cells were included in the immunohistochemical scoring (22 vs. 4%, $p=0.021$).

When comparing PD-1/PD-L1 expression in SCCHN versus other epithelial cancers, one unique feature is its association with HPV, which has been shown to be the driver in carcinogenesis in 40–80% of oropharyngeal carcinoma, but not in SCCHN arising from the oral cavity or other head and neck sites. The HPV oncoproteins, including E6 and E7, are exogenous immunogenic tumor antigens in the human host and would be expected to elicit CD4 and CD8 T cell responses. The studies to address whether this subset of SCCHN would be more likely to be PD-L1 positive (and may in turn be more likely to respond to anti-PD1/PD-L1), however, have yielded variable results [7, 9, 10, 12–14].

Given the observation that only ~20% of metastatic/recurrent SCCHN would respond to immune checkpoint blockade, further evaluation of potential predictive markers such as HPV status and PD-L1 expression in these tumors is warranted for better patient selection and management. In the present study, we evaluated squamous cell carcinomas from the oropharynx and oral cavity for PD-L1 expression by immunohistochemistry (IHC) and two distinct patterns of PD-L1 expression were observed. The PD-L1 expression in p16(+) versus p16(–) tumors and in primary versus metastatic foci of carcinoma was also evaluated.

Methods

Tissue Samples

Normal and tumor tissues used for this study were obtained from the Department of Pathology at New York Presbyterian Hospital-Weill Cornell Medical Center. 96 SCCHN were evaluated in total, including 46 from the oral cavity and 50 from the oropharynx. Of these, 34 had metastatic disease to cervical lymph nodes in the resection specimens, and both the primary and metastatic tumors were examined.

Immunohistochemical Analysis

The immunohistochemical staining was accomplished using the Bond III Autostainer (Leica Microsystems, Illinois, USA). Three rabbit anti-PD-L1 monoclonal antibodies—SP263 (Ventana), SP142 (Spring Bioscience) and E1L3N (Cell Signaling)—were tested using normal tonsil as the positive control. All three showed the expected positive staining at the base of the crypt and SP263 was selected for its stronger staining and better signal/background ratio, a finding also observed in the recently published data of the Blueprint project [15]. Immunohistochemical staining for p16 (clone E6H4, MTM Laboratories) was performed as the surrogate marker for HPV infection in the oropharynx, and strong nuclear and cytoplasmic staining in $> 70\%$ of the tumor cells was interpreted as p16(+).

Formalin fixed and paraffin-embedded tissue sections were baked and deparaffinized. Antigen retrieval was achieved by heating the slides in Bond Epitope Retrieval Solution 1 (ER1) (Leica Microsystems) at 99–100 °C for 30 min. Sections were then incubated with the pre-diluted SP263 for 15 min, post-primary (equivalent to secondary antibody) for 8 min, polymer for 8 min, endogenous peroxidase block for 5 min, diaminobenzidine (DAB) for 10 min and hematoxylin for 5 min (Leica Microsystems). The sections were then dehydrated in 100% ethanol and mounted.

Scoring of PD-L1 Expression

The expression of PD-L1 in tumor cells and in the immune cells was evaluated separately. A 4-tier scoring system was used to score PD-L1 staining in tumor cells: 0 (no staining), 1+ (any tumor cell staining, $< 5\%$ of tumor cells), 2+ (5–50% of tumor cells), or 3+ ($> 50\%$ of tumor cells). Only membranous staining was interpreted as positive, and staining intensity, being heterogeneous in most cases, was not included in the scoring.

PD-L1 expression in immunocytes, unlike in tumor cells, was only classified as positive or negative and not further enumerated. This is in recognition of the fact that the number of tumor-associated immunocytes varied

widely among individual cases and also within individual tumor, and a significant interobserver and intraobserver variation would be expected in any attempts to quantify these PD-L1 infiltrates in percentages, as was evident from the Blueprint comparison project on lung cancer [15]. Only lymphocytes and histiocytes in the tumor microenvironments were included in the evaluation, and lymphoid tissues outside of the tumor–stroma interface, e.g. uninvolved tonsillar lymphoid tissue and lymph nodes distant from the metastatic carcinoma, were excluded.

Statistical Analysis

The frequencies of PD-L1 expression between various subgroups, e.g. p16(+) versus p16(–), were compared using chi-squared test. $P < 0.05$ was considered statistically significant.

Results

The frequencies of PD-L1 expression in tumor cells and in immunocytes are summarized in Table 1.

PD-L1 Expression in Immunocytes at the Tumor–Stroma Interface

Fifty-eight of 96 cases (60%) showed PD-L1 expression in the immunocytes in the tumor microenvironments, whereas 38 (40%) of the cases were negative. The PD-L1-positive immunocytes were morphologically small lymphocytes and histiocytes. They were identified either within the tumor nests as intratumoral tumor-infiltrating immunocytes, or at the interface of tumor nests and the adjacent stroma, forming a characteristic lace-like pattern (Fig. 1a, b). In 17 of the 58 cases, these PD-L1-positive immunocytes were the only PD-L1 positivity detected, and all tumor cells were PD-L1-negative. The remaining 41

cases showed variable PD-L1 positivity in the tumor cells (see below).

Induced Versus Constitutive Expression of PD-L1 Expression in Tumor Cells

Fifty-two of 96 (54%) cases showed PD-L1 expression in tumor cells, including $< 5\%$ (1+) tumor cells in 22, 5–50% (2+) in 18, and $> 50\%$ (3+) in 12 cases. In all 22 cases with $< 5\%$ PD-L1-positive tumor cells, the PD-L1 expressing tumor cells were located at the periphery of the tumor nests adjacent to the tumor–stroma interface, and 21 of the 22 cases had concomitant expression of PD-L1 in the adjacent immunocytes, indicating induced PD-L1 expression in these cells as a result of tumor–immunocyte interactions (Fig. 1c, d).

In contrast to this peripheral distribution pattern, in the 30 cases with 2–3+ PD-L1 positivity, i.e. in $> 5\%$ tumor cells, PD-L1 positive tumor cells were located beyond the tumor–stroma interface (Fig. 2). Eighteen showed 5–50% PD-L1-positive tumor cells (Fig. 2a), and the remaining 12 (13% of all cases in the cohort) had $> 50\%$ positivity, many of them showing diffuse staining in $> 90\%$ of tumor cells (Fig. 2b). The finding that the PD-L1-positive tumor cells were not limited to the tumor–stroma interface in these cases, and PD-L1-positive tumor-infiltrating lymphocytes were indeed absent in 10 of the 30 cases, indicated that these tumor cells expressed PD-L1 constitutively, in contrast to the induced expression in PD-L1-1+ tumors.

Mixed Pattern of PD-L1 Expression

The lace-like pattern of induced expression and the diffuse pattern of constitutive expression were not mutually exclusive, and this mixed pattern of PD-L1 expression was best exemplified in two cases (Fig. 3a, b). In these two cases, the tumor–stroma interface pattern was seen diffusely throughout the tumor, but the constitutive expression pattern was also observed in a well-demarcated area of the tumor, presumably representing a subclone of the tumor cells (Fig. 3a, b).

p16 Status and PD-L1 Expression

40 of 96 cases were p16(+), including 39 of 50 (78%) oropharynx cases and only 1 of 46 cases from the oral cavity. After excluding the single oral cavity p16(+) case (since p16-positivity is not a reliable HPV surrogate marker at this site), 32 of the 39 p16(+) cases (82%), all from oropharynx, demonstrated PD-L1-positive immunocytes, significantly higher than the frequency seen in p16(–) cases (25/56, 45%, $p < 0.05$).

Table 1 PD-L1 expression in SCCHN tumor cells and in immunocytes at tumor–stroma interface

	PD-L1 in tumor cells				Total cases
	0	1+	2+	3+	
PD-L1 in immunocytes					
Positive	17	21	13	7	58 (60%)
Negative	27	1	5	5	38 (40%)
Total cases	44 (46%)	22 (23%)	18 (19%)	12 (13%)	96

Fig. 1 Representative images of PD-L1 expression in immunocytes and induced expression in tumor cells. **a** and **c** PD-L1 positive immunocytes formed a distinctive laced pattern surrounding individual tumor nests in both cases (magnification $\times 40$). **b** High power image of **a**, showing PD-L1-positive cells to be lymphocytes and histiocytes

only, and not tumor cells (magnification $\times 200$). **d** High power image of **c**, showing PD-L1-positive lymphocytes and histiocytes, as well as a small subset ($< 5\%$) of the tumor cells at the tumor–stroma interface (arrow) (magnification $\times 200$)

Fig. 2 Representative images of constitutive expression of PD-L1 in SCCHN. Membranous PD-L1 staining was observed in neoplastic squamous cells in both **a** and **b** with no spatial relationship to the tumor–stroma interface. **a** $< 50\%$ (but $> 5\%$) of the tumor cells were

positive in this case, scored as 2+. **b** $> 90\%$ of the tumor cells were positive, including isolated tumor cells on the right of the field, scoring this case as 3+ (magnification $\times 200$)

Fig. 3 Mixed induced and constitutive expression pattern of PD-L1 in SCCHN. The two expression patterns of PD-L1 expression were not mutually exclusive, as seen in these two cases. **a** The induced pattern is seen in the lower left aspect of the image, whereas tumor clusters in the center and upper parts were diffusely positive. Benign

squamous mucosa on the surface is negative. **b** Most of the section in this case showed induced pattern with only immunocyte staining. Tumor cells are negative except for one single cluster of tumor cells with strong diffuse staining (at top center) (magnification $\times 20$)

Table 2 PD-L1 expression in metastatic SCCHN in comparison to primary tumors

	PD-L1 staining		# of cases
	Primary	Metastasis	
PD-L1 unchanged	0	0	7
	1+	1+	4
	2+	2+	2
	3+	3+	6
	Total		19
PD-L1 increased	0	1+	4
	0	2+	1
	0	3+	1
	1+	3+	1
	2+	3+	1
	1+	2+	1
	Total		9
PD-L1 decreased	1+	0	2
	2+	0	1
	2+	1+	2
	3+	2+	1
	Total		6

In comparison, p16(+) and p16(−) SCCHN showed almost identical frequencies of constitutive PD-L1 expression (13/40 vs. 17/56, or 33 vs. 30%).

PD-L1 Expression in Primary and Metastatic Tumors

Thirty-four primary-metastasis tumor pairs were tested for PD-L1 expression, including 21 pairs from oral cavity and 13 pairs from oropharynx. PD-L1 expression in tumor cells

was concordant in 19 of 34 pairs (56%). Of the 15 discordant cases, 9 showed an increase in PD-L1 expression in the metastasis, including 6 from negative to positive. On the other hand, 6 showed decreased PD-L1 expression, with 3 from positive to negative. These changes are detailed in Table 2 and an example is shown in Fig. 4.

In regard to PD-L1 expression in immunocytes, 26 of 34 cases showed identical PD-L1 status in the paired samples, whereas 6 changed from negative to positive in the metastatic lesions, and 2 changed from positive to negative.

Discussion

Review of the literature revealed that various criteria have been employed in the scoring of PD-L1 expression in SCCHN, and thresholds as low as 1% or as high as > 20% have been used [6, 7, 9, 10, 12–14, 16–18], resulting in the reported frequency of PD-L1 expression ranging from 17 [16] to 68% [13] in this tumor type. Excluding those using tissue microarray and which had the potential problem of sampling artifact, most studies demonstrated PD-L1 positivity in at least 40–50% of cases [6, 7, 9, 10, 13, 18]. Examining PD-L1 expression in tumor cells only, we found PD-L1 expression in 54% (52/96) of SCCHN, in line with previous studies. If PD-L1 expression in immune cells (but not tumor cells) at the tumor–stroma interface was also included, the positive rate would be 72% (69/96), similar to the 78% reported by Seiwart et al. [9] or 81% reported by Chow et al. [6] However, if $\geq 5\%$ or $\geq 50\%$ had been used as the cutoff, the PD-L1 expression rate would have dropped to 31 or 13%, respectively, in our study. Ferris et al. [7] also stratified their data based on different cut-offs, and a similarly significant

Fig. 4 Discordant expression of PD-L1 between primary and metastatic SCCHN. Primary tumor in the tonsil (**a**, H&E stain) showed PD-L1 expression in immunocytes within the tumor nests and at the tumor–immunocyte interface, but not in the tumor cells (**b**). In meta-

static carcinoma involving a cervical lymph node (**c**, H&E stain), **d** >50% of the tumor cells were PD-L1-positive. Occasional PD-L1-positive immunocytes were also noted (magnification $\times 200$)

drop in PD-L1-positive rate was observed, from 57% ($\geq 1\%$) to 37% ($\geq 5\%$) and 27% ($\geq 10\%$).

This substantial drop-off in the frequency of PD-L1-positive tumors following a minor change of cutoff threshold from 1 to 5% (or 10%) has important implications in the evaluation of PD-L1 as a prognostic or predictive biomarker. Moreover, the variability in defining PD-L1 positivity could profoundly affect the results of clinical trials, and this was best illustrated by the anti-PD1 trials in non-small cell lung cancer (NSCLC). In the nivolumab trials in NSCLC, PD-L1 positivity was stratified as membranous staining in 1, 5, or 10% of tumor cells (e.g. in CheckMate-017), and it was concluded that PD-L1 expression was “neither prognostic nor predictive of benefit” [5], leading to the approval of nivolumab as second-line therapy in NSCLC irrespective of PD-L1 expression status in tumor. In contrast, in the KEYNOTE-024 trial for pembrolizumab, 1, 1–49, and $\geq 50\%$ was used to define a “proportion score” of PD-L1 expression, and the ORR was found to be significantly higher in cases with a score of $\geq 50\%$ [8], leading to the approval of pembrolizumab only in this highly selected PD-L1-positive

subset of NSCLC patients, in conjunction with a pairing companion diagnostics (22C3 PhamDx). The decision of the pharmaceutical sponsor to use a low PD-L1 cutoff in its nivolumab trials, however, likely has caused the subsequent failure in the phase III nivolumab trial as a first-line therapy in advanced NSCLC (CheckMate-026). In contrast, in the pembrolizumab trial, by setting a $\geq 50\%$ PD-L1 expression in tumor as an eligibility criterion, it was found to be effective both as a second-line and a first-line therapeutic agent and has been approved for both clinical settings.

In SCCHN, in the study of Chow et al. [6] (KEYNOTE-012) that led to the approval of pembrolizumab in the treatment of SCCHN, $\geq 1\%$ of PD-L1 expression in either the tumor cells or the stromal immunocytes was defined as positive, and the ORR was found to be significantly higher in the PD-L1-positive cases (22 vs. 4%, $p=0.021$). In comparison, the nivolumab trial on SCCHN (CheckMate-141) evaluated only PD-L1 in tumor cells, and expression in immune cells was excluded [7]. Using ≥ 1 , ≥ 5 and $\geq 10\%$ in tumor cells as cutoff values, ORR of 17.0, 22.2 and 27.9% were reported, respectively, in contrast to 12.3% in the $< 1\%$

Table 3 Correlation between overall response rate (ORR) and PD-L1 positivity in anti-PD-1 SCCHN clinical trials

Anti-PD1 mAb	Clinical trial	PD-L1 scoring*	PD-L1 positivity cut-off (%)	Overall response rate		Reference
				PD-L1-positive (%)	PD-L1-negative (%)	
Pembrolizumab	KEYNOTE 012	Tumor cells only	> 1	19	16	[6]
		Tumor cells + immunocytes	> 1	22	4**	
	KEYNOTE 055	Tumor cells + immunocytes	> 1	18	12	[19]
		Tumor cells + immunocytes	> 50	27	13	
Nivolumab	CheckMate141	Tumor cells only	> 1	17.0	12.3	[7]
		Tumor cells only	> 5	22.2	11.2	
		Tumor cells only	> 10	27.9	10.2	

*PD-L1 antibody 22C3 (Dako) used for KEYNOTE 012 and KEYNOTE 055; Clone 28-8 (Epitomics) used for CheckMate141

**Statistically significant, $p=0.021$

group. This trend of higher response rate in PD-L1-positive SCCHN was also observed in the more recently published KEYNOTE-055 trial [19], and these clinical trial results are summarized in Table 3. However, despite these positive correlations between PD-L1 expression and ORR in both nivolumab and pembrolizumab trials, the sponsors of both studies successfully claimed that there was a significant, albeit lower, survival benefit in patients with PD-L1-negative tumors. As a result, both were approved for all metastatic SCCHN, and PD-L1 immunotyping was not required. However, if we consider the relatively low ~20% response rate with this “all-eligible” approach and the high cost of this treatment, one might argue that anti-PD1 is probably best reserved for SCCHN patients with tumors that express PD-L1 in a substantial proportion of tumor cells, and patients with tumors that show no or low PD-L1 expression might benefit from being advised to seek other treatments first if other options exist.

The question that follows is: how should PD-L1 positivity be best defined? At least three factors would need to be considered in order to answer this complex question: (a) the anti-PD-L1 antibody clone used, (b) the inclusion (or exclusion) of PD-L1 expression in immune cells as PD-L1-positive, and (c) the cutoff percentage for PD-L1 positivity in tumor cells. The question of different diagnostic PD-L1 antibodies has been discussed in several recent studies [15, 20–22]. We have also compared SP263 (Ventana), SP142 (Spring Bioscience) and E1L3N (Cell Signaling) in our study, and we found SP263 to show the strongest staining intensity in the positive (tonsil) control and have used this antibody in this study. Blueprint PD-L1 IHC Assay Comparison Project, an industrial-academic collaborative project, has recently compared 22C3, 28-8, SP142 and SP263, and comparable results were found between 22C3, 28-8 and SP263, whereas fewer positive cells were detected by SP142 [15]. It thus appears that SP263, in addition to the FDA-approved 22C3, can be reliably used for PD-L1 typing. SP-142, however,

gave more variable and often weaker staining results and should be avoided.

The second issue that should be discussed is whether and how immunocyte expression of PD-L1 should be included in the PD-L1 scoring of tumors, and how this should be recorded. Review of the literature showed that this had been performed inconsistently, potentially leading to confusions in comparing results from different studies and clinical trials. The nivolumab trial on SCCHN, for instance, specified that PD-L1 expression was defined based on “tumor PD-L1 membrane expression...in a minimum of 100 tumor cells” [7], and immunocyte expression was not evaluated. In contrast, in the phase 1b trial (KEYNOTE-012) of pembrolizumab, the PD-L1 positivity was defined as “detected in at least 1% of tumor or inflammatory cells or stroma” [9], and the expression between tumor cells and immunocytes was combined into a single score, rather than evaluated separately. It was only in its subsequent expansion cohort that the authors separately evaluated “tumor cells only” and “tumor and immune cells”, and in fact concluded that the latter correlated to a significant increase in ORR [6]. This finding was further supported by the study of Kim et al. [11] in which the tumor expression and immunocyte expression were separately evaluated and the latter shown to be a favorable prognostic factor in SCCHN patients. Given these data, we believe that the tumor expression and immunocyte expression should both be evaluated and recorded separately, as we have done in this study.

How about the percentage cut-off in defining PD-L1 “positive” versus “negative” tumors? In earlier studies, a low cut-off value—either 1 or 5%—was often arbitrarily selected and used to correlate to other clinical or pathological parameters, as most “PD-L1-positive” tumors were found to have low percentages of tumor cells expressing PD-L1. It became obvious during our analysis that there were two distinct patterns of PD-L1 expression in SCCHN tumors, and the tumors with low percentages (<5%) of PD-L1-positive

tumor cells were almost always (> 90%) associated with PD-L1-positive immunocytes. The PD-L1-positive tumor cells in such cases were limited to the periphery of the tumor nests, distinctive from the less common pattern in which PD-L1 positive cells are distributed more diffusely in the tumor nests beyond the tumor–stroma interface, often in > 50% of the tumor cells (Fig. 2). We have termed these two patterns “induced pattern” versus “constitutive pattern” of PD-L1 expression, as we believe the first pattern to represent induced PD-L1 expression by cytokines, e.g. interferon- γ , as a result of tumor–immunocyte interactions, representing “adaptive immune resistance” [23]. This is in contrast to the second pattern that represents constitutive, or innate, expression of PD-L1 in tumor cells.

Review of the literature showed that the induced pattern of PD-L1 expression had been observed in most tumor types and would be expected to correlate with the immunogenicity, mutational load, and the presence of tumor-infiltrating lymphocytes in the tumor. In our study, we have found HPV-positive tumors to more frequently show this pattern than the HPV-negative tumors, presumably reflecting the higher immunogenicity of these virally induced tumors. In contrast, the constitutive PD-L1 expression is less common and has only been described in few tumor types in the literature, including in squamous cell carcinoma [10], rare cases of melanoma [23], etc. In SCCHN, Lyford-Pike et al. [10] reported this diffuse pattern in 1 of 14 PD-L1 positive cases, and similar findings were subsequently confirmed [24, 25]. The distinction of these two patterns was not made in the lung cancer trials, but the cases with > 50% positivity in the pembrolizumab trials clearly also represented such constitutive expression.

Despite having been documented in the literature, these two distinctive patterns have not been separately evaluated in any of the clinical trials to date. It is highly likely that tumors with these two different patterns of PD-L1 expression might respond very differently to immunotherapy, particularly to anti-PD-L1 antibodies, and we would propose that these two subtypes of PD-L1-positive tumors be evaluated separately in future PD-1/PD-L1 trials and in aftermarket studies, at least for SCCHN. The binding and internalization of anti-PD-L1 antibody onto PD-L1-positive tumor cells, for instance, could potentially have profound anti-tumor effects analogous to the targeting of HER2-positive tumors with anti-HER2 antibody, and the mechanism of action could be far beyond immune checkpoint blockade. The distinction of these two different PD-L1-positive tumors hence has significant implications in immunotherapy beyond just the percentages of PD-L1-positive cells and this possibility should be carefully explored.

Our finding that PD-L1 expression was often different in the primary tumor and its metastatic counterpart could also be readily explained by recognizing these two different

expression patterns. As tumor cells migrate from primary tumor sites to metastatic foci, it is not surprising that the tumor microenvironments would be altered with secondary effects on the induced PD-L1 expression. On the other hand, the changes in the constitutive expression of tumor cells could easily be explained by tumor heterogeneity, as was clearly exemplified by the two cases that showed well-defined foci of PD-L1 expression in otherwise PD-L1 negative tumors (Fig. 3). Nonetheless, this frequent discordance of PD-L1 expression between primary and metastatic tumors would indicate that the tissue typing for PD-L1 expression should ideally be performed on the metastatic tumor if PD-L1 positivity is included as an eligibility criterion in anti-PD-1/PD-L1 clinical trials.

Conclusions

SCCHN shows two distinctive patterns of PD-L1 expression. In induced expression, only immunocytes and tumor cells at the tumor–stroma interface were positive for PD-L1, and this pattern of expression is more commonly seen in p16(+) tumors. In contrast, a subset of SCCHN shows constitutive expression of PD-L1 in tumor cells, often diffusely in > 50% of tumor cells. Distinguishing these two biologically distinctive patterns of PD-L1 expression and typing metastatic instead of primary lesions might better predict immunotherapeutic response to anti-PD1/PD-L1 regimens beyond just the percentage of PD-L1-positive tumor cells.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest to disclose.

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