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Review Article

Pathology Section

Programmed Cell Death Ligand 1 Immunoexpression in Head and Neck Squamous Cell Carcinoma: A Narrative Review of Considerations for the Histopathologist

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ABSTRACT

Head and Neck Squamous Cell Carcinoma (HNSCC) is one of the most common malignancies worldwide with most of the patients presenting in advanced stage. The associated high mortality and poor prognosis of the tumour has rendered the search for better therapeutic options than the traditional modalities of surgery, radiotherapy and chemotherapy. Immunotherapy targeting Programmed Cell Death Protein 1/Programmed Cell Death Ligand 1 (PD-1/PD-L1) has been approved in recurrent/ metastatic HNSCC cases. PD-L1 expressing HNSCCs are likely to respond to immunotherapy. PD-L1 expression has been studied in peripheral blood utilising Circulating Tumour Cells (CTC) and soluble exosomes. Despite these non invasive techniques, PD-L1 expression has been most frequently assessed by immunohistochemistry (IHC) application on the Formalin Fixed Paraffin Embedded (FFPE) tissue of the tumour. Currently, many antibody clones and various IHC platforms are available for PD-L1, but only 22c3 has been approved by the Food and Drug Administration (FDA). Although, the availability of multiple options has made PD-L1 assessment possible and affordable at many centres worldwide, but various procedural, pre-analytical, and analytic issues have to be considered prior to the interpretation of immunoexpression of PD-L1. In this review, the authors aim to highlight the problem areas, and to understand the implications of PD-L1 expression in HNSCC. The authors propose recommendations for the optimal assessment of PD-L1 expression in HNSCC on IHC.

Keywords: Oral squamous cell carcinoma, Oropharyngeal squamous cell carcinoma, Pembrolizumab, Prognosis, Programmed cell death ligand-1

INTRODUCTION

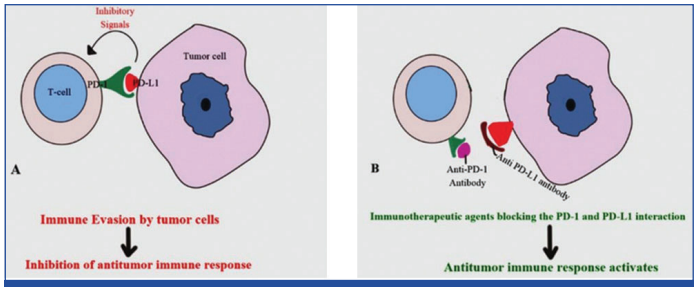
Head and Neck Cancers (HNC) are malignant tumours of the upper aerodigestive tract including oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. Squamous Cell Carcinoma (SCC) accounts for >90% of HNC. HNC are the sixth most common malignancies accounting for approximately 0.93 million cases worldwide in 2020 [1]. HNC account for 26% and 8% of all cancers in India in males and females, respectively [1]. The HNC burden is higher in India as compared to western countries. The HNC incidence rates are higher in north-eastern part of India [2].

Despite the most advanced treatment options available in the current era, the 5-year survival rate of Head and Neck Squamous Cell Carcinoma (HNSCC) is only 50%. More than 50% of HNSCC show recurrence and metastasis within three years [3]. In the recent past, surgery, radiotherapy, chemotherapy or a combination of these, have been used in the treatment of HNSCC cases. The associated morbidity with these therapeutic options has led to a search for safer and more efficient therapeutic modalities [4]. In the past few years, cancer immunotherapy has shown promising results in the management of cancer at various sites. Immune checkpoint proteins, especially the Programmed Cell Death Ligand 1/Programmed Cell Death Protein 1 (PD-L1/PD-1) axis, have been targeted in many clinical trials in many tumours and have shown promising results [4-6]. In the context of HNC, immunotherapeutic options like immune checkpoint inhibitors, tumour vaccines, cell-based therapy and cytokine therapy are being investigated [7,8]. In 2016, two PD-1/PD-L1 inhibitors, nivolumab and pembrolizumab, were approved by both US and EU regulatory agencies for second-line treatment of recurrent or metastatic HNSCC, but evaluation of PD-L1 status has not been mandated for the administration of nivolumab. In 2019, pembrolizumab has been approved for first-line treatment of HNSCC in selected cases [9]. Recently, PD-L1 expression levels have been studied in peripheral blood, in Circulating Tumour Cells (CTCs) and in the form of soluble exosomes [10,11]. Despite these new techniques, Immunohistochemistry (IHC) is still the most widely used technique for assessment of PD-L1 status [12]. Although the PD-L1 expression has been studied in multiple tumours, and multiple antibody clones are available, standardisation of the technique for evaluation of immune-expression is the need of the hour.

In this narrative review, the authors address the role of PD-L1, practical challenges in the assessment and interpretation of PD-L1 expression in HNSCC cases.

How does PD-L1 Work in HNSCC?

PD-1 and PD-L1 are two immune checkpoint proteins. PD-1 is also known as CD279 and is encoded by PDCD1 gene located on chromosome 2q37.3. This protein is chiefly expressed by activated T cells [13]. PD-L1 is also known as B7H1 or CD274. It is encoded by a gene located on 9p24.1 and is expressed by tumour cells, T lymphocytes, B lymphocytes and Antigen Presenting Cells (APCs) [14,15]. PD-L1 on binding with its receptor (PD-1), confers protection to tumour cells from cell death [Table/Fig-1]. PD-L1 also reduces activity of tumour-infiltrating effector CD4 and CD8 T cells that express PD-1. Thus, tumour cells can evade host immune response by expressing PD-L1 [16].



[Table/Fig-1]: The mechanism of action of PD-1/PD-L1 and their inhibitors.

Assessment of PD-L1 on Immunohistochemistry

PD-L1 is the most studied predictive and prognostic biomarker in HNSCC [17]. It is most frequently assessed by IHC on the Formalin Fixed Paraffin Embedded (FFPE) tissue obtained from the biopsy or resection of the primary cancer. Different antibody clones have been used by different researchers e.g., 22C3, 5H1 and CAL10 [18]. Automated and manual staining procedures have been used [19,20].

PD-L1 Positivity: The Definition

PD-L1 expression is considered positive if complete or partial membranous staining is present [21]. The differentiation of cytoplasmic or membranous staining in the immune cells can be problematic. This is because of scant cytoplasm of the lymphocytes. Hence, the localisation of the positive staining can be ignored while assessing expression by the immune cells and any positivity in the immune cells should be considered as expression. The localisation of expression, i.e., cytoplasmic or membranous or even nuclear, may vary among the radioresistant and radiosensitive HNSCC cells [22]. Schulz D et al., used flow cytometry to demonstrate that a strong PD-L1 expression is seen in the nuclear and cytoplasmic fractions of radioresistant HNSCC cells, with a reduction of PD-L1 in nuclear fraction on irradiation. On the other hand, the radiosensitive cells did not express PD-L1 [22].

PD-L1 Expression Quantification: The Scoring Systems

Two scoring methods are used for evaluation of PD-L1 expression in HNSCC worldwide. These are Tumour Proportion Score (TPS) and Combined Positive Score (CPS). The enumeration is primarily carried out manually, where a minimum of 100 viable tumour cells are assessed and the number of cells with a positive expression are counted. For TPS, only the tumour cells with a positive expression are counted. For CPS, tumour infiltrating lymphocytes and histiocytes with positive expression are also counted in addition to the positive tumour cells [20,23]. In other malignancies, immune score is also observed, which is the number of only immune cells with a positive expression over 100 tumour cells. But this scoring method has not been used widely in HNSCC.

$$TPS = \frac{\text{Number of PDL1 positive tumour cells}}{\text{Total number of variable tumour cells}} \times 100$$

CPS={the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages)/the total number of viable tumour cells}.* 100

$$CPS = \frac{\text{Number of PDL1 positive tumor cells, lymphocytes, histiocytes}}{\text{Total number of viable tumor cells}} \times 100\%$$

The cut-off for a positive expression of PD-L1 has been kept a low of CPS ≥1% [24]. Some authors have used a cut-off of ≥5% [19]. This implies that PD-L1 expression can be considered positive even if tumour cells are negative for PD-L1. Further, the expression of PD-L1 has been categorised as low or high expression, trying different cut-off levels of ≥50% and ≥20%. The significance of this difference in the level of expression is still unclear. High PD-L1 expression on immune cells has been reported to be a favourable prognostic marker, while the tumour cell expression does not show any such association [19,25].

Problem Areas in Signing out the PD-L1 Expression

With appropriate positive and negative controls and a standardised protocol, the interpretation of results of PD-L1 expression is not difficult. But, certainly there are problem areas in the evaluation. Following points should be considered before signing out the report for PD-L1 expression in HNSCC.

Preanalytical Conditions

FFPE tissue is the most frequently used source of tissue for IHC. But, cell-blocks and tissue microarray have also been used. For FFPE, cold ischaemia time is an important consideration. The cold ischaemia time >60 minutes should be prohibited. The biopsy tissue should be immediately immersed in 10% Neutral Buffered Formalin (NBF) for at least six hours and up to 48 hours. For cell-blocks, the cytological sample should be prefixed in ethanol and is then followed by cell-block preparation and subsequent transfer to the 10% NBF. The sections should be 3 or 4 µm thick [26].

Variability of Expression with Different Antibody Clones and Interobserver Variability

The FDA has approved the clone 22C3 on the CDx autostainer platform [27]. But currently, many antibody clones for PD-L1 IHC are available besides 22C3. These are 28-8, SP263, SP142, 73-19, and EIL3N. Of these, clones SP142 and SP263, respectively have a 100% specificity and 96% sensitivity on Ventana Benchmark platform in classifying HNSCC as PD-L1 positive or negative [28]. Clone 73-19 shows higher staining rates of tumour and immune cells. Staining results of other clones were comparable [29,30]. Interobserver variability is usually not an important consideration in PD-L1 expression/scoring as almost perfect to moderate interobserver agreement is seen in HNSCC and other cancers [28,30,31]. However, it is recommended that at least two pathologists should independently evaluate PD-L1 expression, which is not a new practice in oncopathology. Also, a brief training for PD-L1 scoring methodology should be considered for a new authorised signatory. An appropriate positive and a negative control should be run with each test and should be endorsed on the report.

Temporal Heterogeneity in PD-L1 Expression

Temporal heterogeneity is the time-dependent variation in the PD-L1 expression during the course of the disease and has been studied in HNSCC. Significant difference in the expression has been reported at the time of initial diagnosis and on recurrence [32,33]. The expression may even change after neoadjuvant therapy. The PD-L1 expression can change after irradiation, especially in radioresistant HNSCC cells [22]. In HNSCC patients with relapse who had received radiotherapy, Delafoy A et al., reported that PD-L1 expression may switch from positive to negative and vice versa [33]. This can be because the neoadjuvant radiotherapy might modify the immunogenicity of the tumour and can even alter the mechanisms involved in immune tolerance [33]. This can have implications in the decision about which neoadjuvant therapy to start along with immunotherapy to harvest maximum response. But, this longitudinal assessment of PD-L1 expression in a case may imply repeated biopsies during the course of the disease. A baseline PD-L1 assessment should be done at the time of diagnosis and prior to commencement of any neoadjuvant therapy. If the decision about the commencement of immunotherapy is to be made later at any point of time during the course of the disease, it is advisable to consider the PD-L1 expression interpretation on the recent-most biopsy specimen, as 'representative' [34].

Intratumoural Heterogeneity of PD-L1 Expression

The expression of PD-L1 may vary in a tumour in different areas [35,36]. The variable distribution of PD-L1 expression in a tumour may lead to discrepancy between the immunoexpression levels in a biopsy and the corresponding resection specimen. A discordance of 30-50% has been reported between the paired biopsy and resection samples [34,35,37]. This variation can be attributed to spatial variation of heterogenous clones of cancer cells with invasiveness, tumour differentiation and heterogeneity of inflammatory cell infiltrate [38].

Intertumoural heterogeneity of PD-L1 expression, i.e., difference in the expression between primary and paired metastatic site has also been identified. The metastatic deposits of HNSCC have been found to have a higher rate of PD-L1 expression [34,39]. Hence, assessment of PD-L1 should be performed on the resected primary tumour instead of biopsy or metastatic tissue. A minimum of two blocks should be tested, to overcome the intratumoural heterogeneity of the immunoexpression of PD-L1.

Expression Patterns of PD-L1

Two patterns of PD-L1 expression have been identified- induced and constitutive expression. The constitutive expression had a higher proportion of tumour cells with positive expression and a diffuse pattern. The immune score in this type of expression is low. On the other hand, in the induced pattern of expression, the proportion of PD-L1 positive tumour cells is low, expression is limited to the peripheral part of the tumour nests and sheets, and is accompanied by a higher immune score. The identification of the two types of expression pattern may predict the immunotherapeutic response in a better way [40]. The pattern of expression by tumour can also be identified as diffuse or patchy. The patchy pattern of PD-L1 expression has been found to be an independent risk factor for overall survival [36]. Hence, the pattern(s) of PD-L1 expression as constitutive/induced or patchy/diffuse may also be reported.

Variability in Expression in Archived Material

Significant reduction in PD-L1 expression has been reported in both TPS and CPS, between the initial sections and the sections from the blocks stored for 20-48 months [41]. However, additional studies are required to prove the fact. In this context, the International Association for the Study of Lung Cancer (IASLC) atlas of PD-L1 testing has suggested that FFPE tissue blocks older than three years should not be used for IHC staining [42].

Expected Results

HNSCC is a highly immunogenic malignancy [43]. These tumours show a significant upregulation of tumour infiltrating lymphocytes and PD-L1 expression, as compared to normal oral mucosa [44]. Hence, 40-70% of HNSCC have relatively high expression of PD-L1 [45,46]. This high immunogenicity of HNSCC is because of a high number of somatic mutations producing many neoantigens [47]. Also, these tumours, especially oropharyngeal HNSCC, are associated with Human Papillomavirus (HPV) infection. HPV association may trigger T cell immune response against the viral antigen expression [48]. But, this high level of immunogenicity may fade later during disease progression [49]. This can be due to overall immune exhaustion of the tumour cells and immune cells, declaring a clear victory of the tumour.

Implications of the Positive PD-L1 Expression

Assessment of PD-L1 was first introduced with the aim of selecting the candidates for PD-1/PD-L1 immunotherapy. The two approved immune checkpoint inhibitors in HNSCC are pembrolizumab and nivolumab. These drugs have been approved for recurrent/metastatic HNSCC and for palliative treatment. For administration of pembrolizumab, baseline assessment of PD-L1 expression is required. On the other hand, assessment of PD-L1 expression is not mandated for nivolumab monotherapy in recurrent cases of HNSCC [23,50]. As is expected a positive expression should indicate a good response to PD-L1 inhibitors. But, the data from the clinical trials show only moderate overall response rate of 20% as compared to much higher response rate in PD-L1 expressing tumours of other sites [51].

The temporal and intratumoural heterogeneity of PD-L1 expression in HNSCC may be responsible for the differential response. The function, expression and localisation of PD-L1 depends on the differentiation status, cell cycle phase as well as the tumour microenvironment. Increased membranous expression is seen during S phase of the cell cycle [41].

PD-L1 Expression versus Human Papillomavirus Status

PD-1/PD-L 1 plays a role in viral replication and adaptive immune resistance in HPV-positive Oropharyngeal Squamous Cell Carcinoma (OPSCC). Worldwide, p16 has been accepted as a surrogate IHC marker for HPV infection.

In OPSCC, a significant association has been reported between PD-L1 and p16 expression [52-54]. Patients with p16 and PD-L1 expression on immune cells in OPSCC had a favourable overall survival, whereas patients negative for p16 and PD-L1 expression on immune cells showed worse outcome [25]. Contradictory studies have reported no association between p16 and PD-L1 expression in oral cavity [19,51] and OPSCC [55].

A New Role for PD-L1: Prognosticator

Recently there have been a significant rise in the number of publications evaluating the prognostic impact of PD-L1 expression in HNSCC [19,20,25,35,36,38,55-77]. However, the results are inconstant and often contradictory [36,55,59,61,64,65,76,77]. The impact of PD-L1 expression on the outcome and prognosis is discussed in the subsequent section.

Survival and Outcome

Some authors have reported that PD-L1 expression in HNSCC is a poor prognostic marker, resulting into a reduced overall survival [57,59]. Studies with neutral results report that PD-L1 expression has no significant impact on overall survival in HNSCC cases [55,58,60,61]. While some other studies have reported PD-L1 expression as a favourable prognostic factor in HNSCC, and such cases have a longer overall survival [33,56]. This is most likely because HNSCC are actually a heterogeneous group of tumours with respect to different anatomical sites and differing aetiological factors.

Region-wise, in OPSCC, PD-L1 expression alone did not affect the overall survival in most of the studies [19,55]. HPV positive PD-L1 expressing OPSCC had a favourable outcome as compared to HPV-/PD-L1- [25,63].

In oral cavity SCC, PD-L1 expression had no impact on the overall survival [36,64]. Cases with higher PD-L1 expression and presence of tumour infiltrating lymphocytes have an improved survival and lower recurrence rates [65]. Another study reported a poorer overall survival rate associated with PD-L1 expression by tumour cells and immune cells in oral SCC [66].

In hypopharyngeal SCC, PD-L1 expression was associated with poorer overall survival [67]. Other studies have reported no association between PD-L1 expression and survival [68].

In laryngeal SCC, PD-L1 expression by immune cells had an improved disease free survival [69,70]. Another study reported no association between PD-L1 expression and overall survival in laryngeal SCC [71].

The overall variability in results from different studies can be because of the overall heterogeneity of HNSCCs. Also, the antibody clone used for evaluation of PD-L1 can also affect the prediction of the outcome [35,54].

Lymph Node Metastasis, Stage and Recurrence

PD-L1 expression in HNSCC has been found to be associated with lymph node metastasis [72-74]. Many other studies have reported no impact of PD-L1 expression on the stage of the disease [19,20]. The increased propensity for metastasis in PD-L1 expressing HNSCC can be attributed to its role in favouring cell motility, including cell spreading, migration and invasion [75].

PD-L1 expression has been reported to be associated with a lower recurrence rate in laryngeal SCC [76]. On the contrary, recurrent tongue SCC showed higher PD-L1 expression [77].

With the above discussed outcomes of HNSCC, it can be concluded that the expression of PD-L1 alone cannot predict the outcome of the disease. But, if combined with other factors e.g., HPV status and tumour infiltrating lymphocytes, the prognosis can be predicted for specific sites. Still, larger multi-institutional studies with a large sample size can actually lead to some significant conclusions.

Future Directions

Fine Needle Aspiration Cytology (FNAC) material can be used to make cell-blocks and perform PD-L1 IHC. Paintal AS and Brockstein BE reported that PD-L1 interpretation in cell-blocks or core biopsy had a positive predictive and negative predictive value of 100% and 28% at a cut-off of CPS ≥1% [37]. In the coming decade, oncology is likely to undergo a metamorphosis with utilisation of Artificial Intelligence (AI). With reference to PD L1 expression, automated PD-L1 scoring using AI has been attempted in HNSCC. Puladi B et al., reported a higher inter-rater correlation between human-machine compared to human-human correlation for CPS and immune cell score [78]. However, additional studies are required to prove the utility of cell-block IHC as well as AI in PD-L1 assessment.

Proposed Guidelines for Interpretation of PD-L1 Expression on Immunohistochemistry

For correct PD-L1 interpretation on IHC, following guidelines are proposed:

1. PD-L1 assessment should preferably be performed on a treatment naïve biopsy or resection specimens. If by any chance, neoadjuvant therapy was given to the patient, a disclaimer should be made on the final report.
2. Resection specimens should be preferred over biopsy specimens. IHC staining and interpretation should be done on a minimum of two sections.
3. The cold ischaemia time should be taken into account and fresh FFPE tissue should be used. Blocks older than three years should not be used for IHC, instead, a fresh biopsy should be advised, if possible.
4. At least two independent and trained observers should interpret the IHC and an average should be reported as the final result.
5. The quality check of the IHC set up and the clone should be done time-to-time and with every new batch of antibody. Appropriate controls should be run with each test.

CONCLUSION(S)

PD-L1, an immune checkpoint protein, is frequently upregulated in HNSCC. PD-L1 expression is frequently assessed by IHC. Presently, multiple antibody clones and platforms are available for PD-L1 IHC. Because of non-standardisation of the pre-analytical, analytical and post-analytical phases involved, the predictive and prognostic impact of PD-L1 immunoexpression remains questionable. The guidelines proposed in the review will help standardise the procedure and interpretation of PD-L1 immunoexpression.

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