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# PD-L1 expression predicts longer disease free survival in high risk head and neck cutaneous squamous cell carcinoma

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**Summary**

Programmed cell death (PD-1) and its ligand (PD-L1) inhibitors have shown clinical response in many tumours. PD-L1 data are limited in head and neck cutaneous squamous cell carcinoma (HNcSCC) and no clinical trials of PD-1/PD-L1 inhibitors are published. We performed PD-L1 immunohistochemistry on 74 cases of high risk HNcSCC with 38 matched metastases and evaluated clinicopathological associations, prognostic significance and heterogeneity in matched metastases. We observed PD-L1 expression in >5% of primary tumour cells in 29 cases (39.2%), primary tumour infiltrating lymphocytes (TILs) in 40 cases (70.2%), metastatic tumour cells in 15 cases (39.5%), and metastatic TILs in 18 cases (47.4%). PD-L1 expression in >5% of primary tumour cells was associated with an inflammatory phenotype ($p = 0.04$), and in primary TILs with clear margins ($p = 0.05$). PD-L1 expression in >5% of primary tumour cells ($p = 0.01$), primary TILs ($p = 0.001$), and metastatic TILs ($p = 0.02$) was associated with improved disease free survival. PD-L1 expression in >5% of tumour cells was heterogeneous between primary and metastatic tumours in 13 cases (34.2%). PD-L1 expression is common in HNcSCC supporting the rationale for a clinical trial of PD-1/PD-L1 inhibitors. PD-L1 expression in tumour cells or TILs predicts longer disease free survival and demonstrates temperospatial heterogeneity.

*Key words:* Skin cancer; squamous cell carcinoma; immunohistochemistry; PD-L1; PD-1; tumour biomarkers; tumour microenvironment; tumour heterogeneity; disease-free survival.

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## INTRODUCTION

Immune checkpoints including programmed cell death ligand 1 (PD-L1) and its receptor (PD-1) are inhibitory pathways that suppress host T cells and moderate the physiological immune response to limit tissue damage and maintain self-tolerance.[1,2] Many of these checkpoints are ligand-receptor interactions and therefore amenable to pharmacological blockade that can reactivate T cells to eliminate tumour cells.[1] Indeed clinical data for PD-1/PD-L1 inhibitors have shown response rates ranging from 10 to 50% in metastatic melanoma, non-small cell lung cancer (NSCLC), metastatic renal cell carcinoma, metastatic urothelial carcinoma, ovarian cancer, haematological malignancies and head and neck squamous cell carcinoma.[3] However, there are no data regarding the prognostic significance of PD-L1 expression and no published clinical trials evaluating PD-1/PD-L1 inhibitors in high risk head and neck cutaneous squamous cell carcinoma (HNcSCC).

Cutaneous squamous cell carcinoma (cSCC) is one of the most common and expensive malignancies and the incidence continues to rise with the ageing population.[4] The vast majority of cases occur on the UV-exposed head and neck region.[5] Although most are localised and curable, advanced disease requires radical surgery and post-operative radiotherapy leading to significant functional morbidity.[6] Given this rising burden of disease and the limited systemic therapeutic options for advanced cSCC, novel treatments such as immune checkpoint inhibitors need to be explored.[7]

A recent meta-analysis demonstrated that immunohistochemical expression of PD-L1 in tumour cells was associated with clinical response to PD-1/PD-L1 inhibitors in metastatic melanoma and non-squamous NSCLC but not in renal cell carcinoma or squamous NSCLC.[8] Similarly, others have shown an association between clinical response to PD-1/PD-L1 blockade and PD-L1 expression in tumour cells and tumour infiltrating lymphocytes (TILs) across a range of tumour types.[9] Data regarding PD-L1 expression in cSCC is just emerging, being limited to two cohort studies, and represents an area of need given the dearth of therapeutic options for advanced, unresectable HNcSCC.[10,11] This study

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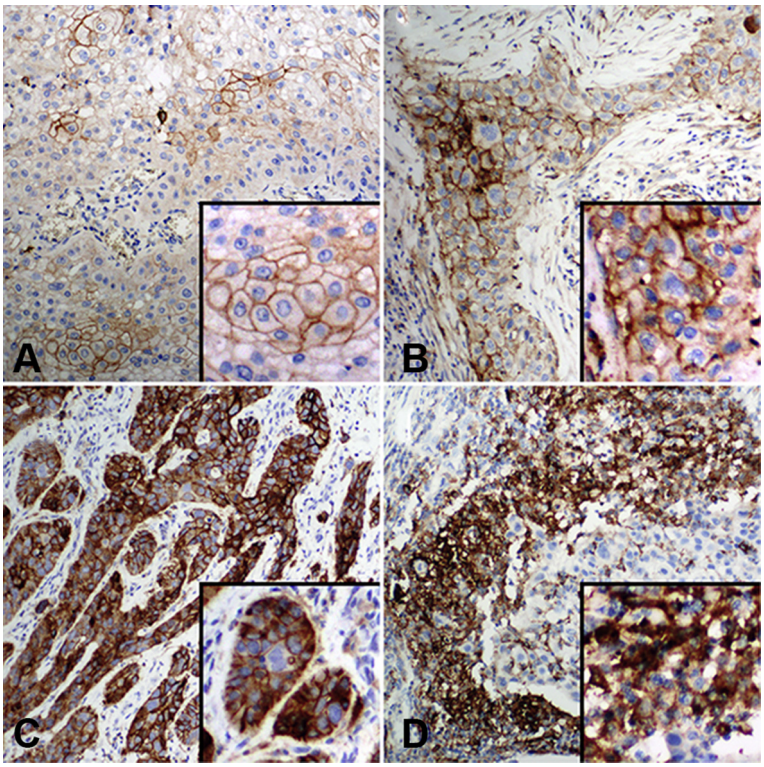


**Fig. 1** Immunohistochemistry for programmed cell death ligand 1 (PD-L1) by intensity of staining in head and neck cutaneous squamous cell carcinoma (HNcSCC). (A) PD-L1 staining intensity 1+ in tumour cells; (B) PD-L1 staining intensity 2+ in tumour cells; (C) PD-L1 staining intensity 3+ in tumour cells; (D) PD-L1 staining intensity 3+ in tumour infiltrating lymphocytes.

evaluates PD-L1 expression in primary and metastatic tumour cells and TILs. The association of PD-L1 expression with conventional prognostic factors and survival has been evaluated. Heterogeneity in the expression of PD-L1 between primary carcinomas and their metastases has been investigated.

## MATERIALS AND METHODS

A total of 74 patients with high risk HNcSCC[12] and detailed clinicopathological information were identified from the Sydney Head and Neck Cancer Institute database (1997–2015) after approval from the institution's Human Research Ethics Committee. Of these 74 patients, 35 had high risk primary HNcSCC without nodal metastases and the remaining 39 had metastases to cervical or intraparotid lymph nodes. Tumour-containing archival, formalin fixed, paraffin embedded tissue blocks were retrieved for all 74 primary specimens and 38 of 39 metastatic specimens.

### Clinical and follow-up data

Data on patient demographics, post-operative adjuvant therapy, local failure, regional failure, distant metastases and survival were obtained.

### Histopathological review

The archival slides from all 74 primary tumours and 38 metastases were reviewed for tumour size, depth of invasion, tumour differentiation, margins of resection, lymphovascular and perineural invasion, and for evaluation of tumour infiltrating lymphocytes. All tumours were staged using the 7th edition of the American Joint Committee on Cancer Cancer (AJCC) Staging Manual pTNM staging system for primary cSCC.[12]

### Quantification of tumour infiltrating lymphocytes (TILs)

TILs at the infiltrative front of the tumour were quantified as 0–3 as per Busam *et al.* for primary cutaneous melanoma as well as Klintrup *et al.* and Huh *et al.* for colorectal carcinoma as follows: no lymphocytes invading the

**Table 1** Cohort characteristics ($n$ = 74)

| Characteristic | $n$ (%) |
|---|---|
| Mean age, years (range) | 69.9 (34–100) |
| Sex | |
| Male | 64 (86.5%) |
| Female | 10 (13.5%) |
| Mean tumour diameter (range) | 32.7mm (3–160mm) |
| Mean tumour thickness (range) | 14.4mm (0.3–70mm) |
| Perineural invasion | 37 (50%) |
| Lymphovascular invasion | 13 (17.6%) |
| Tumour infiltrating lymphocytes | |
| Present | 57 (77%) |
| 1. Patchy | 32 (43.2%) |
| 2. Moderate | 19 (25.7%) |
| 3. Diffuse | 6 (8.1%) |
| T category | |
| T1 | 11 (14.9%) |
| T2 | 46 (62.2%) |
| T3 | 4 (5.4%) |
| T4 | 13 (17.6%) |
| Node status | |
| N0 | 35 (47.3%) |
| N1 | 12 (16.2%) |
| N2a | 3 (4.1%) |
| N2b | 22 (29.7%) |
| N2c | 2 (2.7%) |
| Differentiation | |
| Well | 5 (6.8%) |
| Moderate | 47 (63.5%) |
| Poor | 22 (29.7%) |
| Margins | |
| Clear | 21 (28.4%) |
| Close | 25 (37.8%) |
| Involved | 28 (37.8%) |
| Metastases, present | 39 (52.7%)[a] |
| Follow up, mean years (range) | 2.2 (0.1–7.9) |

[a] One metastatic specimen did not undergo PD-L1 assessment

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