Real-world programmed death-ligand 1 prevalence rates in non-small cell lung cancer: correlation with clinicopathological features and tumour mutation status

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ABSTRACT

Aims The detection of programmed death-ligand 1 (PD-L1) protein expression on tumour cells by immunohistochemistry (IHC) is a predictor of response to immune checkpoint inhibitors. New immunotherapeutic options are changing the treatment paradigm for patients with advanced non-small cell lung cancer (NSCLC). The aim of this retrospective study was to investigate real-world prevalence of PD-L1 expression in NSCLC and any correlations with clinicopathological features.

Methods We reviewed 425 NSCLC cases at a Sydney metropolitan hospital that had PD-L1 IHC (SP263 clone) expression estimated as part of routine diagnostic assessment during a 30-month period.

Results Overall, 32.2% of cases were negative for PD-L1 expression (<1%), 40.3% demonstrated low expression (1%–49%) and 27.5% exhibited high protein expression (≥50%). High PD-L1 expression rates were more likely in non-lung resection cases and in *KRAS* mutant NSCLC as opposed to *KRAS* wildtype, while lower expression rates were more commonly found in *EGFR* mutant NSCLC compared with *EGFR* wildtype tumours.

Conclusions Ongoing observation and comparison of PD-L1 expression rates is an important practice for ensuring its validity as a predictive biomarker. The results from our study align with and contribute to the growing field of published real-world PD-L1 prevalence rates in NSCLC.

INTRODUCTION

Lung cancer is the most common type of cancer worldwide, impacting approximately 2.1 million people and causing an estimated 1.7 million deaths each year.[1] In Australia, an estimated 13 000 new cases of lung cancer are expected to be diagnosed in 2019 and around 9000 Australians will succumb to the disease, making it the leading cause of mortality from cancer.[2]

Approximately 85%–90% of lung cancers are classified as non-small cell lung cancer (NSCLC), and the majority of these patients are diagnosed with advanced stage disease where complete surgical resection is no longer a treatment option and systemic approaches are required. In this setting, new immune-based approaches such as immune checkpoint inhibitors are an important therapeutic option for patients with NSCLC.

First-line treatment of advanced lung cancer has traditionally been dictated by histological subtype, consisting of platinum-based combination chemotherapy regimens for NSCLC. Treatment options have since become more directed with the identification of targetable oncogenic driver mutations such as *epidermal growth factor receptor* (*EGFR*), *anaplastic lymphoma kinase* (*ALK*) and *c-ros oncogene 1* (*ROS1*).[3] However, for the majority of patients without therapy-sensitive mutations, chemotherapy is still the primary treatment option. The recent emergence of immune checkpoint inhibitors is providing an additional option for patients with advanced NSCLC.

One of the immune signalling complexes currently targeted in the development of immunomodulating therapies is the programmed cell death 1 (PD-1) pathway. PD-1 receptors are present on activated CD4 and CD8 T cells, B cells, monocytes, natural killer cells and dendritic cells, while its ligands PD-L1 and PD-L2 are expressed on the surface of dendritic cells and macrophages.[4] Under normal circumstances, the PD-1/PD-L1 interaction ensures only appropriate immune responses are initiated, thus safeguarding against autoimmunity. PD-1/PD-L1 ligation moderates T cell receptor-mediated responses and disrupts the activity of T cell activation.[4] T cells primed with mutated antigens exhibited by dendritic cells are stimulated into becoming cytotoxic T cells. These activated lymphocytes then recognise and bind to specific target cells, releasing apoptosis-inducing cytotoxins.[5]

It is now understood that many solid tumours including NSCLC have the ability to co-opt specific adaptive immune pathways.[6] PD-L1 is frequently overexpressed on the surface of malignant and inflammatory cells in the tumour microenvironment and will bind to PD-1 receptors on circulating cytotoxic lymphocytes. This interaction deactivates the cytotoxic function of T cells, allowing tumour cells to evade immune surveillance.[7] For many tumours, PD-L1 overexpression confers worse prognosis, thus making immunotherapeutic intervention a promising treatment avenue.[8–10]

Treating patients with anti-PD-1/PD-L1 monoclonal antibodies such as pembrolizumab, a humanised IgG4 antibody directed against PD-1,

upregulates the T cell-mediated immune response of the adaptive immune pathway.[11] The KEYNOTE-001 clinical trial of pembrolizumab treatment in a range of solid tumours found a positive association between PD-L1 expression and treatment response in NSCLC.[7] KEYNOTE-010 followed on with a validation of these treatment results, demonstrating prolonged overall survival for patients positive for PD-L1, defined as PD-L1 expression on at least 1% of tumour cells, compared with docetaxel chemotherapy in the second-line setting.[12] In the subgroup analysis of patients with PD-L1 high tumours, defined as PD-L1 expression on at least 50% of tumour cells, the efficacy for pembrolizumab appeared more pronounced.[12] Similarly, first-line studies have shown prolonged survival in patients with PD-L1 high tumours with no targetable *EGFR* or *ALK* alterations, treated with pembrolizumab compared with those treated with platinum doublet chemotherapy,[13] cementing its use as monotherapy in patients with PD-L1 high NSCLC. Current guidelines recommend first-line treatment with pembrolizumab monotherapy for patients with PD-L1 Tumour Proportion Score (TPS) ≥50% and a combination of chemotherapy and pembrolizumab regardless of the PD-L1 TPS.[14–16]

Evaluating and comparing PD-L1 expression rates in real-world diagnostic settings is therefore an important process to ensure patients are accessing the most effective treatments available. The aims of this retrospective study were to review the local prevalence of PD-L1 expression in NSCLC and correlate findings with clinicopathological features including specimen types, histological tumour subtypes and tumour mutation status.

MATERIALS AND METHODS

Patient cohort

Patients diagnosed with NSCLC who had PD-L1 immunohistochemistry (IHC) evaluation (SP263 clone) as part of routine diagnostic assessment between January 2017 and June 2019 at Royal Prince Alfred Hospital department of Tissue Pathology and Diagnostic Oncology (Sydney, Australia) were included in the study.

PD-L1 assessment by IHC

Immunohistochemical assessment was carried out using PD-L1 SP263 clone (rabbit monoclonal anti-PD-L1 antibody, Roche Diagnostics, Rotkreuz, Switzerland). Sections for staining were sourced from representative tumour formalin-fixed paraffin-embedded (FFPE) tissue blocks routinely processed following fixation in 10% buffered neutral formalin. For cytology specimens, fine needle aspirate material was placed in Hank's solution or saline, while small clots and tissue fragments were preserved in formalin before being centrifuged into cell pellets. Cell blocks were then produced using agar cytology preparation prior to fixation in 10% neutral buffered formalin and processed as FFPE blocks. Sections from both histology blocks and cell blocks were cut at 3 µm, mounted onto Trajan Series 3 adhesive slides (Trajan Scientific, Victoria, Australia) and oven dried for 60 min at 65°C.

IHC was performed via heat induced epitope retrieval using OptiView DAB IHC Detection Kit (Ventana Medical Systems, Arizona, USA) on a Ventana BenchMark ULTRA IHC/ISH System (Ventana Medical Systems). A section of tonsil tissue was included on each test slide as an external control to verify performance of PD-L1 assay.

Evaluation of PD-L1 expression

PD-L1 expression was evaluated as a Tumour Proportion Score (TPS) by one of two appropriately trained pathologists (WAC or AM). The visual assessment categorises representative samples as negative (<1% positive tumour cells), low (1%–49% cells) and high (≥50% cells). TPS was reported as a percentage of tumour cells showing partial or complete membrane staining, regardless of intensity, relative to all viable tumour cells. Any immune cells present positive for PD-L1 staining were not included in scoring.

Statistical analysis

Correlation of PD-L1 expression with demographic and clinicopathological variables was performed using IBM SPSS V.25.0. Calculated probability values of <0.05 were considered statistically significant. For the purposes of Pearson's χ^2 analysis, PD-L1 status was assessed as either negative (<50%) or positive (≥50%).

RESULTS

PD-L1 prevalence and clinicopathological features

We assessed NSCLC cases for PD-L1 prevalence and found 67.8% (288/425) demonstrating any level of staining, with 40.3% (171/425) exhibiting low expression of TPS 1%–49% and 27.5% (117/425) with high expression of TPS ≥50% (figure 1, table 1). The remaining 32.2% (137/425) displayed negative PD-L1 TPS.

Tumour specimens were predominantly from sites of primary origin, typically lung resections, lung biopsies or bronchial

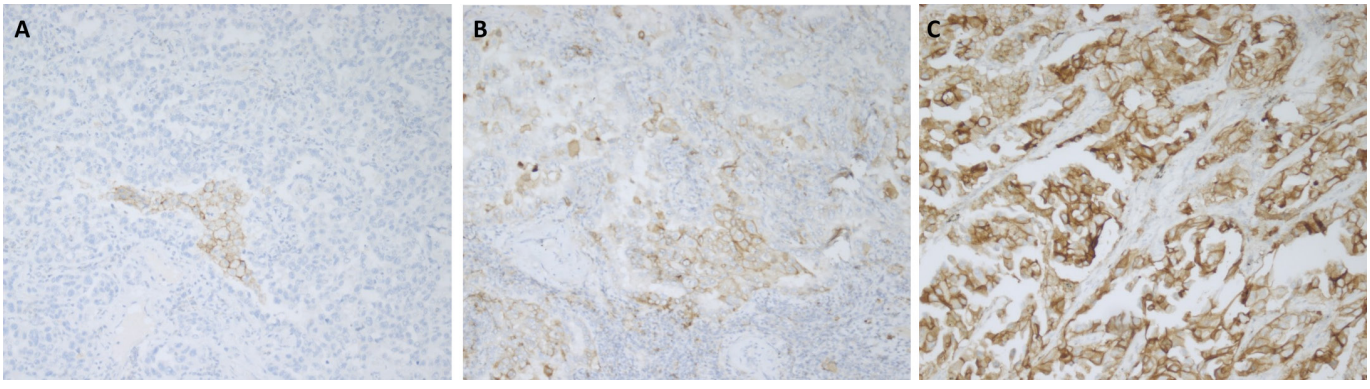

Figure 1 PD-L1 immunohistochemical staining of NSCLC cases. (A) PD-L1 tumour proportion score 0 (staining in macrophages only). (B) PD-L1 tumour proportion score 10%. (C) PD-L1 tumour proportion score 100%. 100× magnification. NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1.

Table 1 Patient characteristics and clinicopathological features correlated with PD-L1 tumour proportion score

Characteristics	N (%)	TPS <1%	TPS ≥1%	TPS <25%	TPS ≥25%	TPS <50%	TPS ≥50%	P value
All patients	425	137 (32.2)	288 (67.8)	281 (66.1)	144 (33.9)	308 (72.5)	117 (27.5)	
Age (years)								
<70	204	60 (29.4)	143 (70.1)	138 (67.6)	66 (32.4)	151 (74)	53 (26)	0.49
≥70	221	77 (34.8)	144 (65.2)	144 (65.2)	77 (34.8)	157 (71)	64 (29)	
Sex								
Female	174	56 (32.2)	118 (67.8)	114 (65.5)	60 (34.5)	126 (72.4)	48 (27.6)	0.98
Male	251	81 (32.3)	170 (67.7)	167 (66.5)	84 (33.5)	182 (72.5)	69 (27.5)	
Specimen								
Lung resection	138	62 (44.9)	76 (55.1)	103 (74.6)	35 (25.4)	111 (80.4)	27 (19.6)	<0.01
Other*	287	75 (26.1)	212 (73.9)	178 (62)	109 (38)	197 (68.6)	90 (31.4)	
Specimen type								
Histology	347	120 (34.6)	227 (65.4)	231 (66.6)	116 (33.4)	252 (72.6)	95 (27.4)	0.88
Cytology	78	17 (21.8)	61 (78.2)	50 (64.1)	28 (35.9)	56 (71.8)	22 (28.2)	
Specimen origin								
Primary	269	95 (35.3)	174 (64.7)	181 (67.3)	88 (32.7)	201 (74.7)	68 (25.3)	0.17
Metastasis	156	42 (26.9)	114 (73.1)	100 (64.1)	56 (35.9)	107 (68.6)	49 (31.4)	
Histological subtype								
ADC	328	111 (33.8)	217 (66.2)	220 (67.1)	108 (32.9)	238 (72.6)	90 (27.4)	0.94
Non-ADC	97	26 (26.8)	71 (73.2)	61 (62.9)	36 (37.1)	70 (72.2)	27 (27.8)	
SCC	66	21 (31.8)	45 (68.2)	45 (68.2)	21 (31.8)	52 (78.8)	14 (21.2)	0.21
Non-SCC	359	116 (32.3)	243 (67.7)	236 (65.7)	123 (34.3)	256 (71.3)	103 (28.7)	
NSCLC-NOS	31	5 (16.1)	26 (83.9)	16 (51.6)	15 (48.4)	18 (58.1)	13 (41.9)	0.06
Non-NOS	394	132 (33.5)	262 (66.5)	265 (67.3)	129 (32.7)	290 (73.6)	104 (26.4)	

*'Other' represents lung core biopsies, fine needle aspirates, cytological collections, metastatic resections or metastatic core biopsies.
ADC, adenocarcinoma; NOS, not otherwise specified; NSCLC, non-smallcell lung cancer; PD-L1, programmed death-ligand 1; SCC, squamous cell carcinoma; TPS, Tumour Proportion Score.

biopsies (63.3% (269/425)). Specimens collected from metastatic sites included lymph node biopsies, brain resections, bone or other sites (36.7% (156/425)). The majority of cases were assessed for PD-L1 status using histology specimens (81.6% (347/425)) rather than cytology specimens (18.4% (78/425)).

Tumour samples obtained from lung resections were significantly more likely to demonstrate high PD-L1 expression when compared with all 'other' specimens including lung core biopsies, fine needle aspirates, cytological collections, metastatic resections or metastatic core biopsies (p value <0.05). No significant difference was identified in PD-L1 expression between histology and cytology NSCLC cases (p value 0.88) or between primary and metastatic cases (p value 0.17).

Among our NSCLC cohort, 77.2% (328/425) were adenocarcinomas, 15.5% (66/425) squamous cell carcinoma and 7.3% (31/425) were NSCLC, not-otherwise specified. The PD-L1 prevalence rates found in adenocarcinoma cases were 33.8% (111/328) negative, 38.7% (127/328) low and the 27.4% (90/328) high. Cases of squamous cell carcinoma displayed a similar PD-L1 stratification of 31.8% (21/66) negative, 47% (31/66) low TPS and 21.2% (14/66) high TPS. Not otherwise specified tumours were found to have slightly higher rates of PD-L1 expression, with 16.1% (5/31) negative, 41.9% (13/31) low TPS and 41.9% (13/66) high TPS, although this was not statistically significant.

A notable variation in PD-L1 expression rates was found between tumours with *Kirsten rat sarcoma viral oncogene homologue gene* (*KRAS)* mutations and *EGFR* mutations, with 40.2% (39/97) of *KRAS*-positive tumours demonstrating high PD-L1 TPS as opposed to 11.1% (6/54) of *EGFR* positive tumours (table 2). We did not observe significant variations in PD-L1 expression for tumours with *v-Raf murine sarcoma viral oncogene homologue* (*BRAF)* mutations or *ALK* translocations.

Among the non-squamous lung cancer cohort expressing high PD-L1 with mutation results available, 42.9% (39/91) were *KRAS* positive and 6.6% (6/91) were *EGFR* positive. Known *EGFR/ALK* wildtype tumours demonstrated a negative, low and high PD-L1 prevalence of 25.5% (60/235), 39.6% (93/235) and 34.9% (82/235), respectively. Wildtype *EGFR/ALK/ROS1* tumours were more likely to exhibit high PD-L1 compared with tumours with an *EGFR* mutation, *ALK* rearrangement or *ROS1* translocation (p value<0.05). Rates of PD-L1 expression among the subset of non-squamous tumours not exhibiting clinically actionable genetic mutations were 28.1% (36/128) negative, 39.1% (50/128) low and 32.8% (42/128) high.

DISCUSSION

To date, this is the largest retrospective study to analyse PD-L1 expression in NSCLC using Australian data and adds to mounting international research surrounding the role of PD-L1 status in lung cancer. The published rates of PD-L1 prevalence are extremely variable, complicating the utilisation of biomarkers for predicting patient responses to immune-checkpoint inhibitors such as pembrolizumab.

The PD-L1 prevalence rates found in our cohort are in keeping with those reported in KEYNOTE clinical trials assessing the efficacy of pembrolizumab in treating a range of solid tumours including NSCLC, with rates ranging from 30.7% to 39.2% negative, 37.6%–39.1% low TPS and 23.2%–30.2% high TPS published.[7 12 13 17] Further consensus of our prevalence rates come from a more recent study investigating PD-L1 expression in the UK, with rates of 44.4% negative TPS, 25.0% low TPS

Table 2 Mutation status of non-squamous carcinoma cases correlated with PD-L1 tumour proportion score

Mutation status	N (%)	TPS <1%	TPS ≥1%	TPS <25%	TPS ≥25%	TPS <50%	TPS ≥50%	P value
KRAS								
Positive	97	20 (20.6)	77 (79.4)	53 (54.6)	44 (45.4)	58 (59.8)	39 (40.2)	<0.01
Negative	202	63 (31.2)	139 (68.8)	136 (67.3)	66 (32.7)	150 (74.3)	52 (25.7)	
Unknown	60	31 (51.7)	29 (48.3)	46 (76.7)	14 (23.3)	47 (78.3)	13 (21.7)	
EGFR								
Positive	54	22 (40.7)	32 (59.3)	45 (83.3)	9 (16.7)	48 (88.9)	6 (11.1)	<0.001
Negative	246	62 (25.2)	184 (74.8)	144 (58.5)	102 (41.5)	161 (65.4)	85 (34.6)	
Unknown	59	32 (54.2)	27 (45.8)	46 (78)	13 (22)	47 (79.7)	12 (20.3)	
BRAF								
Positive	11	4 (36.4)	7 (63.6)	8 (72.7)	3 (27.3)	9 (81.8)	2 (18.2)	0.38
Negative	287	80 (27.9)	207 (72.1)	181 (63.1)	106 (36.9)	199 (69.3)	88 (30.7)	
Unknown	61	32 (52.5)	29 (47.5)	47 (77)	14 (23)	48 (78.7)	13 (21.3)	
ALK								
Positive	6	1 (16.7)	5 (83.3)	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	0.48
Negative	306	94 (30.7)	212 (69.3)	196 (64.1)	110 (35.9)	214 (69.9)	92 (30.1)	
Unknown	47	21 (44.7)	26 (55.3)	36 (76.6)	11 (23.4)	37 (78.7)	10 (21.3)	
ROS1								
Positive	3	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)	*
Negative	309	94 (30.4)	215 (69.6)	198 (64.1)	111 (35.9)	217 (70.2)	92 (29.8)	
Unknown	47	21 (44.7)	26 (55.3)	36 (76.6)	11 (23.4)	37 (78.7)	10 (21.3)	

*Number of positive cases too small for analysis.
ALK, anaplastic lymphoma kinase gene; *BRAF*, v-Raf murine sarcoma viral oncogene homologue B; *EGFR*, epidermal growth factor receptor gene; *KRAS*, Kirsten rat sarcoma viral oncogene homologue gene; PD-L1, programmed death-ligand 1; *ROS1*, ROS proto-oncogene 1; TPS, Tumour Proportion Score.

and 30.6% high TPS established.[18] Our results also closely align with those from a similar Australian study evaluating PD-L1 prevalence in NSCLC conducted by Ye *et al*, where rates of 27% negative, 41% low and 32% high were demonstrated.[19] This is an encouraging finding owing to the anticipated similarities between cohort demographics.

Differences are observed when comparing our findings with those from the global, multicentre EXPRESS study. Dietel *et al*[20] found comparatively lower PD-L1 expression, with negative, low and high rates of 48.4%, 29.4% and 22.2% in all NSCLC patients included in the study. A greater discordance is seen between our population and a recent Polish study, where 0 point (<1%), 1 point (1%–49%) and 2 points (≥50%) rates of 67.4%, 22.3% and 10.3% were established.[21] However, variations between these studies and our investigation should be noted. Researchers from the larger, multicentre EXPRESS study used an alternate assay (PD-L1 IHC 22C3), excluded cytology cases and notably included patients from Asia-Pacific regions, accounting for 28% of the cohort. This study found tumour samples with PD-L1 TPS ≥50% were less common among patients with sensitising *EGFR* mutations and *ALK* translocations.[20] The Polish study by Pawelczyk *et al*[21] involved an Eastern European population and did not assess any correlation between mutation status and PD-L1 expression.

When NSCLC cases with known *EGFR* mutations or *ALK* translocations were excluded from our cohort, the rate of cases demonstrating high TPS increased from 28.8% (49/170) to 34.9% (82/235). The prevalence of high PD-L1 similarly rose among *EGFR/ALK* negative cases in the EXPRESS study, increasing from 22% to 27% high TPS.[20] This association between *EGFR/ALK* wildtype tumours and lower PD-L1 expression is further demonstrated in a large-scale meta-analysis of PD-L1 expression in NSCLC specimens by Lan *et al*.[22]

An interesting finding from our study is the association between specimen type and PD-L1 status, with samples from lung resections less likely to exhibit high PD-L1 expression compared with 'other' specimens. This discordance may relate to the recognised intratumoural and intertumoural heterogeneity inherent within lung cancers. The polyclonal evolution of cancer generates cellular diversity both within individual tumours and between primary lung tumours and corresponding metastatic lesions.[23] This diversity is apparent at the genetic, epigenetic, transcriptomic and proteomic levels and manifests as variability in immunohistochemical staining patterns within tumours.[24 25] The resection specimens also represent early-stage disease possibly suggesting tumour stage may influence PD-L1 expression levels, although we did not have complete staging data for our cohort to substantiate this hypothesis. Published literature is currently inconclusive regarding an association between PD-L1 expression and tumour stage. Some studies indicate no decisive link,[7 26 27] while others demonstrate an association between advanced stage III/IV NSCLC and high PD-L1 positivity.[28–31] A large meta-analysis by Zhang *et al* involving 11 444 patients across 47 studies supports this correlation, where higher rates of PD-L1 expression positively correlated with a variety of clinicopathological features including higher tumour stage and grade.[32]

It has been well established that the immunoreactivity of antibodies may be affected by clinicopathological features such as stage of disease, prior chemotherapy or radiotherapy and presence of tumour-infiltrating lymphocytes.[23 33–36] While important factors that could effect PD-L1 expression, assessment of these was outside the scope of our study, as was evaluating paired resection and biopsy samples.

A significant finding from our study were the equivalent rates of high PD-L1 expression between histology and cytology samples, suggesting that both specimen types are equally valid in assessing PD-L1 status. This is an important finding foremost, as patients with late-stage lung cancer are often not surgical candidates, and second, as 30%–50% of NSCLC cases are diagnosed

based on cytology samples alone.[34] Although our study did not investigate paired histology and cytology samples, these findings are in line with similar studies proposing that cytological sampling is an appropriate technique for assessing PD-L1 expression.[34–37]

Our retrospective study also highlighted an association between higher PD-L1 expression in patients with *KRAS* positive tumours and lower PD-L1 expression in patients with *EGFR* positive tumours. Among this study population, 40.2% of *KRAS*-positive tumours demonstrated PD-L1 TPS ≥50% compared with the wildtype. An inverse relationship was seen in tumours positive for an *EGFR* mutation, with just 11.1% displaying high expression. The mutation results and correlated PD-L1 expression from our study support published research indicating, first, an association between NSCLC harbouring *KRAS* mutations and higher rates of concurrent PD-L1 expression and second, a relationship between *EGFR* positive tumours and lower levels of PD-L1 expression.[35 38–41] However, it must be noted that there is significant contradiction surrounding the relationship between PD-L1 expression and genetic mutations in NSCLC reported in the literature, highlighting the need for further large-scale studies that incorporate varied, international populations.

The emergence of PD-1/PD-L1 as a clinical biomarker for predicting response to immune-checkpoint inhibitors has been an inconsistent process. Issues surrounding establishing optimal PD-L1 cutpoints for accessing immunotherapy, PD-L1 tumourous heterogeneity, the significance of tumour-infiltrating lymphocytes and associated immune cell scoring and the relationship between oncogenic driver mutations and PD-L1 status are yet to reach consensus. The dynamic nature of PD-L1 utilisation is evident through the number of antibodies, assays, positivity cutpoints and evaluation schemes available. Persistent efforts are being made to continually standardise its application as a clinical biomarker.

The retrospective nature of our study lends itself to several inherent limitations. First, our cohort was predominantly from a single institution, and PD-L1 expression was assessed by only two pathologists. In addition, this study did not take into consideration patients' smoking status, tumour stage or treatment history at time of PD-L1 sampling, all of which are factors potentially contributing to PD-L1 elevation or inhibition.[10 42 43]

To date, the prevalence of PD-L1 expression has largely been evaluated in clinical trial populations. Our real-world study supports literature indicating an association between clinicopathological features such as *KRAS* mutant and *EGFR* wildtype NSCLC tumours, and higher rates of PD-L1 expression. Ongoing observation and comparison of PD-L1 expression rates is an important practice for ensuring its validity as a predictive biomarker. The results from our study contribute to the growing field of published real-world PD-L1 prevalence rates in NSCLC.

Take home messages

- Almost 30% of non-small cell lung cancer cases show high programmed death-ligand 1 (PD-L1) expression (tumour proportion score ≥50%) in real-world specimens.
- High PD-L1 expression is associated with *KRAS* mutations.
- Low or absent PD-L1 expression is associated with *EGFR* wildtype status.

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Contributors MH and WAC identified retrospective cases, obtained clinicopathological data and wrote the initial draft of the manuscript. TL optimised and performed immunohistochemical staining. AM and WAC interpreted immunohistochemistry slides. All authors contributed to manuscript revision.

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Competing interests MH, AM, TL and WAC declare no conflict of interests. SK declares compensated advisory board (institutional) from AstraZeneca, Boerhinger, Roche, Merck Sharp & Dohme and Pfizer; honoraria from AstraZeneca, Bristol-Myers-Squibb, Merck Sharp & Dohme, Pfizer and Roche; and travel from AstraZeneca, Boerhinger, Bristol-Myers-Squibb and Roche.

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