Lung Cancer 89 (2015) 181–188

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma

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ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form 29 April 2015
Accepted 9 May 2015

Keywords:
Lung cancer
NSCLC
Programmed death-ligand 1
Immunohistochemistry
Immunotherapy
Biomarker

ABSTRACT

Objectives: Immune checkpoint blockade using inhibitors of programmed death-1 have shown promise in early phase clinical trials in NSCLC and programmed death-ligand 1 (PD-L1) tumoral expression could potentially be a useful predictive marker. Data reporting the prevalence of PD-L1 expression in NSCLC and clinicopathologic associations is very limited. We sought to determine the frequency of PD-L1 expression in NSCLC and investigate associations with clinicopathologic features and patient outcome.
Materials and methods: PD-L1 expression was analyzed using immunohistochemistry (Merck; clone 22C3) in 678 stages I–III NSCLC and 52 paired nodal metastases using tissue microarrays. Tumors with ≥50% cells showing positive membrane staining were considered to have high expression of PD-L1.
Results: PD-L1 expression of any intensity was identified in 32.8% of cases. High PD-L1 expression was found in 7.4% of NSCLC. Squamous cell carcinomas (8.1%) and large cell carcinomas (12.1%) showed high PD-L1 expression more commonly than adenocarcinomas (5.1%) but this was not statistically significant ($p = 0.072$). High PD-L1 expression was associated with younger patient age and high tumor grade ($p < 0.05$). There was no association with gender, tumor size, stage, nodal status, *EGFR* or *KRAS* mutation status. In multivariate analysis, patients with high PD-L1 expression had significantly longer overall survival ($p < 0.05$).
Conclusions: PD-L1 is expressed at high levels in a significant proportion of NSCLC and appears to be a favorable prognostic factor in early stage disease. As there are potential sampling limitations using tissue microarrays to assess heterogeneously expressed biomarkers, and as the results may differ in advanced stage disease, further studies are recommended.

1. Introduction

Evasion of host immunity has recently been recognized as an emerging hallmark of malignancy [1] with increasing evidence that tumors induce an immunosuppressive milieu in the local microenvironment allowing them to escape host immunity [2]. While tumor cells may be susceptible to adaptive

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http://dx.doi.org/10.1016/j.lungcan.2015.05.007
0169-5002/

immune responses when tumoral genetic and epigenetic alterations produce novel cellular antigens, successful immunologic eradication of tumors is a rare event [2,3]. Tumors develop multiple strategies to evade host immune responses including reduced expression of major histocompatibility complex molecules, loss of tumor antigens, inadequate co-stimulation of T-cells, production of immunosuppressive mediators such as TFG-β, recruitment of immunosuppressive inflammatory cells such as regulatory T cells and myeloid-derived suppressor cells, and expression of immune inhibitory ligands such as programmed death – ligand 1 (PD-L1) [2,4].

Programmed death (PD)-1 (also known as CD279), a member of the immunoglobulin superfamily, is a receptor expressed on the surface of activated T-cells, regulatory T cells, B-cells, NK cells, activated monocytes and dendritic cells, that negatively regulates their proliferation and activation [5,6]. PD-L1 (also known as B7-H1 or CD274) and PD-L2 are the two known ligands for PD-1 with PD-L1 being the main mediator of immunosuppressive effects [6]. PD-L1 is a 290aa type I transmembrane surface glycoprotein encoded by the CD274 gene located on chromosome 9 [6]. In addition to being aberrantly expressed in some tumor cells, PD-L1 is also expressed on antigen presenting cells, activated B- and T-cells and endothelial cells [5–7]. Expression of PD-L1 occurs in many solid tumors including carcinoma of the lung [8–12], pancreas [13], colon [14], stomach [15], breast [16], ovary [17], cervix [18], kidney [19], and urothelium [20], as well as melanomas [21–23].

Interaction of PD1 with its ligand PD-L1 leads to apoptosis or inactivation of activated T-cells. In vivo animal models have shown that anti-tumor T-cell function is inhibited by PD-L1 expression on tumor cells and this effect can be reversed by blockade of PD-L1 with monoclonal antibodies leading to inhibition of tumor growth [24,25]. There is evidence that the immunosuppressive PD-1/PD-L1 axis induces T-cell tolerance in NSCLC [26]. CD8+ tumor infiltrating lymphocytes (TILs) in NSCLC have increased PD-1 expression and demonstrate immune dysfunction including reduced proliferation and cytokine production, that can be partly restored by PD-L1 inhibition [26].

Manipulation of the host-tumor relationship in the effort to improve anti-tumor T-cell immunity provides a promising therapeutic opportunity. Blockade of inhibitory immune signalling between PD-1 and its ligands PD-L1 and PD-L2 is a potential mechanism to promote immune eradication of tumor cells and early phase clinical trials of anti-PD-1 and anti-PD-L1 monoclonal antibodies have demonstrated clinical activity in melanoma, renal cell carcinoma as well as advanced stage NSCLC [27–29]. Interestingly, there is some evidence that tumor expression of PD-L1 is associated with tumor response to PD-1/PD-L1 pathway inhibition suggesting PD-L1 expression may be a predictive marker of response to treatment [29].

Data reporting the prevalence and prognostic significance of PD-L1 expression in NSCLC and association with varying histological subtypes, mutation status and patient characteristics is very limited with most studies to date being small and in Asian populations [8–12]. In this study, we present the clinicopathological features associated with PD-L1 expression in the largest cohort of early stage NSCLC reported to date from a Western country.

2. Materials and methods

2.1. Patient cohort

The retrospective cohort consisted of 681 patients who underwent surgical resection of early stage NSCLC between 1990 and 2008 in the Sydney Local Heath District which includes Royal Prince Alfred Hospital and Concord Repatriation General Hospital, Sydney, Australia. H&E stained sections were reviewed by a pulmonary pathologist (WC) and histological subtyping was assessed using the current World Health Organisation 2004 classification [30]. Staging was undertaken according to the 7th edition AJCC tumor, node, metastasis (TNM) classification [31]. Information was collated on patient demographics, pathological features, TNM stage, and overall survival. Preoperative staging using PET scanning commenced on these patients in December 1994 and there was no statistically significant difference in overall survival between patients managed prior to and after this time ($p = 0.31$). Patients with stage II disease who underwent resection from 2004 onwards (45 (6.6%)) may have been offered adjuvant chemotherapy.

Formalin-fixed paraffin-embedded (FFPE) samples of tumor and normal bronchial epithelium were used to construct tissue microarrays (TMAs) using a Manual Tissue Arrayer (MTA-1, Beecher Instruments Tissue Arrayer) with a core size of 1 mm as previously described [32–34]. At least 3–6 tumor cores were selected from each primary tumor in areas previously marked by a pathologist (WC). Regional nodal metastases from 52 cases were also included in the TMAs. Approval for this study was obtained from the Ethics Review Committees of the relevant institutions.

2.2. Immunohistochemistry

Each TMA was sectioned at 4 μm and IHC was performed using a mouse monoclonal anti-PD-L1 primary antibody (Merck; clone 22C3) at 1:500 dilution, incubated for 60 min at room temperature on a Dako autostainer/PTLink using alternative low pH target retrieval buffer (Dako, K8005) and the Envision Flex+ Kit (Dako; K8002). Immunohistochemical expression of PD-L1 was assessed by determining the percentage of cells showing weak (1+), moderate (2+) or strong (3+) membranous staining. For the percentage of positively staining cells, the average was taken from all cores available for each tumor. H-scores were also calculated by multiplying the percentage of positively staining cells for each core by the intensity of staining (giving a score that ranged from 0 to 300) and the average score was taken from all the cores from each tumor. A range of thresholds to determine high PD-L1 expression were investigated and the threshold producing the greatest prognostic significance was determined to be the optimal cut point. Significant p-values ($p < 0.05$) for overall survival were obtained using cut-points ranging from 32% to 77% with the lowest p-value obtained with a cut-point of 57%. This was rounded down to produce an optimal cut-point of 50% as it is a number more readily assessable by pathologists (Supplementary Fig. 1). As such tumors with at least 50% of cells showing membrane staining of any intensity were considered PD-L1 positive. Using the H-score, significant p-values for overall survival were obtained using cut-points ranging from 34 to 125 with the lowest p-value obtained with a cut point of 54 (data not shown). Tumors with an H-score $\geq$50 were therefore considered PD-L1 positive. Two pathologists undertook the IHC scoring (WC and RV) and there was excellent concordance for determining PD-L1 positivity using the percentage of positively staining cells (Cohen's kappa = 0.79), intensity (weighted Cohen's kappa = 0.83) and the H score (Cohen's kappa = 0.84).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2015.05.007

The number of cores with viable tumor available for PD-L1 assessment ranged from 1 to 6 and there was no statistically significant difference in PD-L1 score versus number of cores for assessment (Supplementary Table 1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2015.05.007

2.3. Mutation testing

All adenocarcinomas underwent *EGFR* and *KRAS* mutation testing using OncoCarta v1.0 Panel and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) technology on the MassArray platform, as previously described [35]. ALK assessment was also undertaken using immunohistochemistry (Novocastra mouse monoclonal antibody p80 ALK antibody, Clone 5A4, NCL-ALK, Leica, Germany, and the rabbit monoclonal ALK XP antibody, clone D5F3, 3633P from Cell Signalling Technology, USA), as previously described [36], with all positively staining cases subsequently undergoing FISH analysis for confirmation of *ALK* rearrangement using the Vysis LSI *ALK* Dual Colour, Break Apart Rearrangement Probe (Abbott Molecular, USA).

2.4. Statistical Analyses

Statistical analyzes were undertaken using R version 2.15.2 (The R Foundation for Statistical Computing). Clinicopathologic variables were tested using Pearson's Chi squared with Fisher's Exact test (categorical variables) and two-sample *t*-test (continuous variables) or Cramer's V and Wilcoxon–Mann–Whitney tests for parametric and non-parametric testing, respectively. Overall survival was assessed using the Kaplan–Meier method and log-rank tests. Statistical significance was set at $p < 0.05$ for two sides. Multivariate analysis was performed using a forward step-wise method of the Cox proportional hazard regression model.

3. Results

3.1. Expression of PD-L1

Amongst all NSCLC, 28.2% of primary tumors showed staining for PD-L1 of any intensity in at least 1% of tumor cells and 20.1% of cases demonstrated staining in at least 5% of cells. The mean percentage of tumor cells showing PD-L1 staining of any intensity was 7.7% (range 0–100%). Using a threshold of at least 50% of cells showing staining to determine positivity, 7.4% of all primary tumors were found to be PD-L1 positive (Fig. 1). Most positively staining tumors showed weak intensity membrane staining (75%) with 25% showing moderate to strong staining (Fig. 2) Among the 52 primary tumors with nodal metastases available for assessment, only 2 (3.8%) of the primary tumors were PD-L1 positive. Using an H-score threshold of ≥50 to determine positivity, 8.4% of primary tumors were PD-L1 positive. Staining for PD-L1 was also seen in

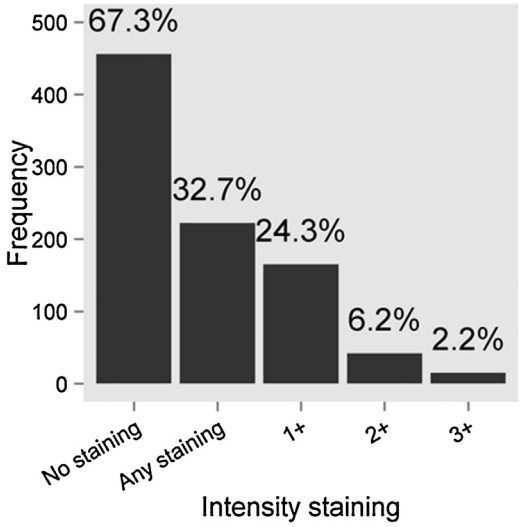

Fig. 2. Distribution plot of PD-L1 intensity of staining.

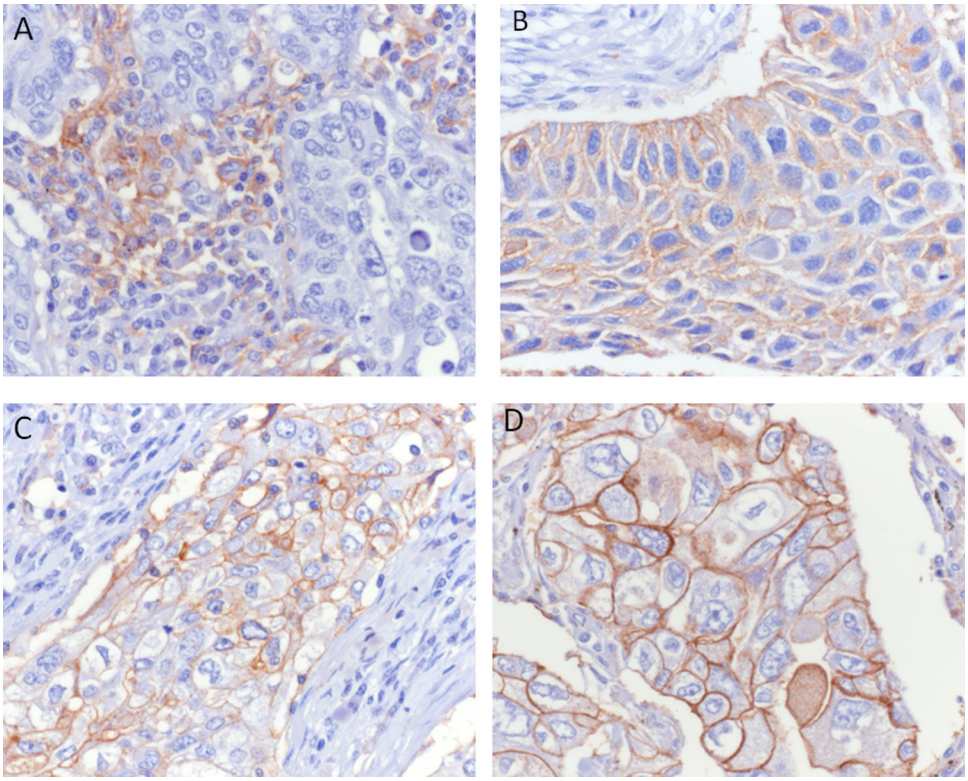

Fig. 1. PD-L1 immunohistochemical staining in NSCLC. (A) LCC with no staining for PD-L1 (interstitial macrophages provide a positive internal control). (B) Positive membranous PD-L1 staining in an SCC and (C, D) a poorly differentiated adenocarcinoma. (Original magnification 200× for A–C, 400× for D).

Table 1
PD-L1 expression in NSCLC and associations with clinicopathologic features.

Clinical features	PD-L1 staining percentage			PD-L1 H-Score		
	<50%	≥50%	*p*-value	< 50	≥50	*p*-value
n	628 (92.6%)	50 (7.4%)		621 (91.6%)	57 (8.4%)	
Age (years)						
Median	69	65.5	<0.05	69	67	0.07
Gender, *n* (%)						
Male	444 (93.1%)	33 (6.9%)	0.52	181 (90%)	20 (10%)	0.36
Female	184 (91.5%)	17 (8.5%)		440 (92.2%)	37 (7.8%)	
Tumor size (mm)						
Median	40	42	0.08	40	45	0.02
Stage group, *n* (%)						
I	316 (93.2%)	23 (6.8%)	0.66	313 (92.3%)	26 (7.7%)	0.58
II–III	312 (92%)	27 (8%)		308 (90.9%)	31 (9.1%)	
Lymph node, *n* (%)						
Negative	552 (92.2%)	47 (7.8%)	0.25	545 (91%)	54 (9%)	0.13
Positive	76 (96.2%)	3 (3.8%)		76 (96.2%)	3 (3.8%)	
Histologic tumor subype, *n* (%)						
ADC s	262 (94.9%)	14 (5.1%)	0.14	261 (94.6%)	15 (5.4%)	0.13
SCCs	249 (91.9%)	22 (8.1%)		244 (90%)	27 (10%)	
LCCs	102 (87.9%)	14 (12.1%)		102 (87.9%)	14 (12.1%)	
Mixed	3 (100%)	0 (0%)		3 (100%)	0 (0%)	
Others	12 (100%)	0 (0%)		11 (91.7%)	1 (8.3%)	
Grade, *n* (%)						
I	49 (100%)	0 (0%)	<0.01	52 (100%)	0 (0%)	<0.01
II	258 (95.2%)	13 (4.8%)		277 (94.2%)	17 (5.8%)	
III	280 (88.9%)	35 (11.1%)		292 (88%)	40 (12%)	
Vessel invasion, *n* (%)						
Absent	337 (92.1%)	29 (7.9%)	1	333 (91%)	33 (9%)	1
Present	73 (92.4%)	6 (7.6%)		72 (91.1%)	7 (8.9%)	
EGFR, *n* (%)						
Wild-type	222 (93.7%)	15 (6.3%)	0.23	222 (93.7%)	15 (6.3%)	0.23
Mutant	33 (100%)	0 (0%)		33 (100%)	0 (0%)	
KRAS, *n* (%)						
Wild-type	174 (95.6%)	8 (4.4%)	0.26	174 (95.6%)	8 (4.4%)	0.26
Mutant	81 (92%)	7 (8%)		81 (92%)	7 (8%)	
ALK, *n* (%)						
Wild-type	252 (94.4%)	15 (5.6%)	1	252 (94.4%)	15 (5.6%)	1
Mutant	3 (100%)	0 (0%)		3 (100%)	0 (0%)	

*Notes*Wilcoxon–Mann–Whitney test for age and tumor size, Fisher's exact testest used for other parameters.
Abbreviations: IHC, immunohistochemistry; ADC, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma.

macrophages and lymphocytes which provided a positive internal control (Fig. 1A). Prominent tumor infiltrating lymphocytes were seen in association with PD-L1 expressing tumor cells in some cases.

3.2. Correlation of PD-L1 expression with clinicopathologic features

PD-L1 expression according to patient characteristics and tumor pathological features are presented in Table 1. Squamous cell carcinomas (8.1%) and large cell carcinomas (12.1%) tended to show high PD-L1 expression more commonly than adenocarcinomas (5.1%) but this was not statistically significant ($p = 0.072$). There was no significant difference in terms of PD-L1 expression between ADCs and non-ADC subtypes (14/276 (5.1%) versus 36/402 (9%), $p = 0.072$) or between SCCs and non-SCCs subtypes (22/271 (8.1%) versus 28/407 (6.9%), $p = 0.55$). High PD-L1 expression was associated with younger patient age (using ≥50% staining to determine positivity) and high tumor grade ($p < 0.05$) (using % or *H*-score to determine positivity). There was no association between PD-L1 expression and gender, tumor stage, nodal status, or *ALK*, *EGFR* or *KRAS* mutation status.

3.3. Correlation of PD-L1 expression with overall survival

Amongst all NSCLC cases, patients with high PD-L1 expression (≥50% positively staining cells) had significantly longer overall survival compared to those with low PD-L1 expression in univariate analysis (113.2 months vs 85.5 months, $p = 0.023$) (Fig. 3a). This prognostic association was also observed in squamous cell carcinomas ($p = 0.023$) and non-adenocarcinomas ($p < 0.01$) (Fig. 3b and c) but not in adenocarcinomas ($p = 0.91$ (Fig. 3d). In the multivariate Cox model controlling for stage and patient age, high PD-L1 expression (≥50% positively staining cells) was confirmed as an independent significant predictor of improved overall survival (HR 0.65, 95% CI 0.45–0.85, $p < 0.05$) (Table 2). Using the same model, high PD-L1 expression determined by H-score ≥50 was also an independent significant predictor of greater overall survival (HR 0.59, 95% CI 0.4–0.8, $p < 0.01$).

When different thresholds were used to determine high PD-L1 expression, cut points ranging from 32% to 77% also produced prognostically significant *p*-values in univariate analysis and 33–63% in multivariate analysis (data not shown).

There was no difference in overall survival between patients who had no expression of PD-L1 versus those with any staining (≥1%) (data not shown).

4. Discussion

Immune reactions are highly regulated through a complex and dynamic arrangement of stimulatory and inhibitory signals with negative regulatory pathways playing a critical role in tolerance. Expression of PD-L1 in tumors assists in immune tolerance and evasion of host immunity by down regulating anti-tumor T-cell responses and providing protection from immune destruction [3,5–7]. While there is early data suggesting that PD-L1 expression

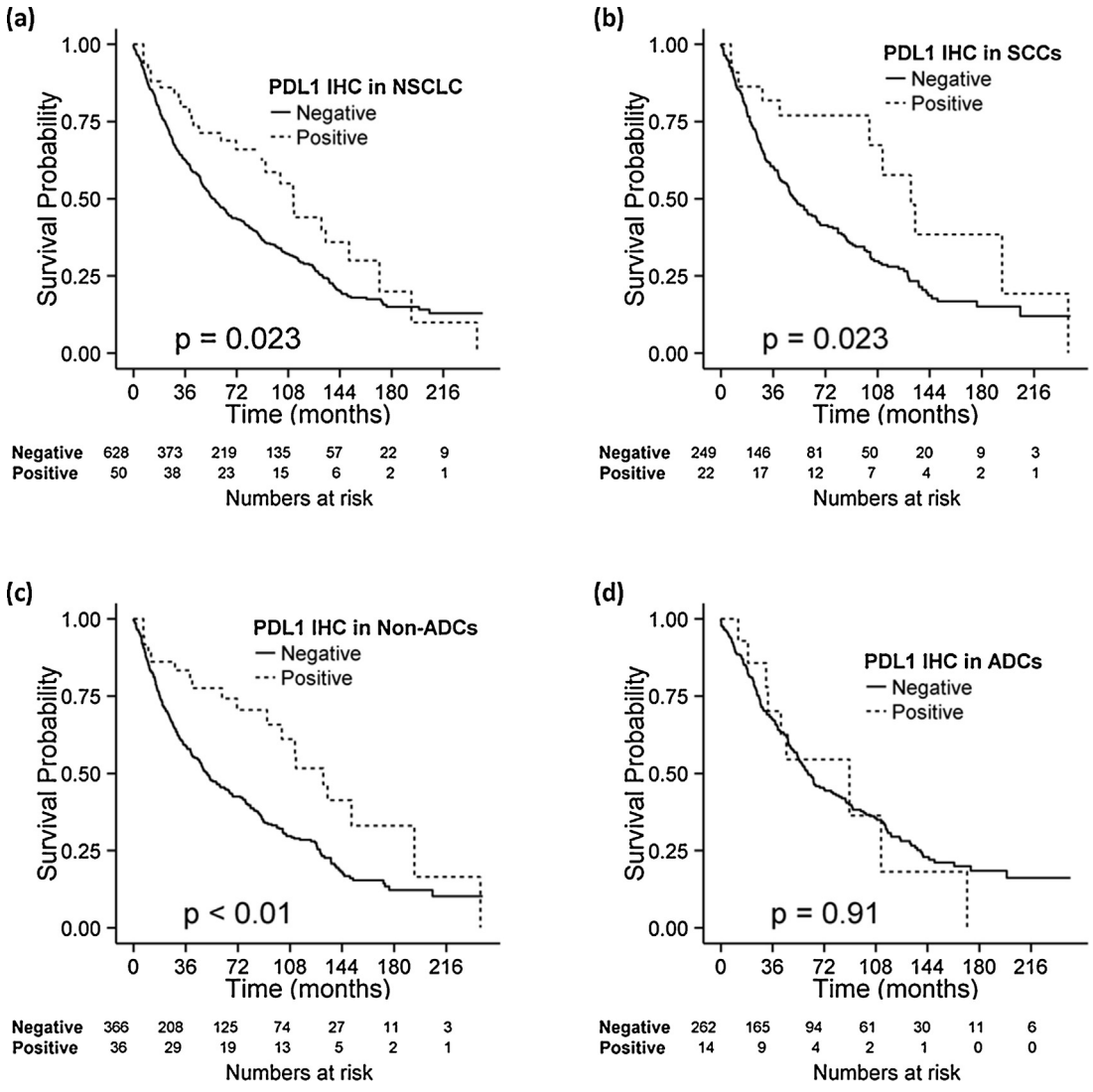

Fig. 3. Kaplan meier curves (a) all NSCLC, (b) SCCs, (C) non-ADCs, and (d) ADCs.

is predictive of tumor response to blockade of the PD-1/PD-L1 pathway in a variety of solid tumors including NSCLC [27], very little is known about the prevalence of PD-L1 expression in NSCLC and if there is any association with clinicopathological variables.

In the largest published cohort to date of surgically resected early stage NSCLC, we found high PD-L1 expression in a minority of tumors (7.4%). Only 7 other peer reviewed non-clinical trial studies have reported prevalence rates of PD-L1 expression in NSCLC [8–12,37,38] and only 5 of these utilized IHC in FFPE tissue [8–12]. Of the IHC studies in FFPE tissue, most have found a higher proportion of cases with high PD-L1 expression ranging from 19.6% to 57.5% [9–12] although they mostly used lower thresholds to determine high expression than in our study. Using a threshold of 5% of cells staining to determine positivity, Yang et al. [12] found PD-L1 in 39.9% of stage I lung adenocarcinomas and Boland et al. [9] in 19.6% of surgically treated SCCs. This is similar to our finding of 20.1% of early stage NSCLC showing staining in at least 5% of tumor cells. In contrast Chen et al. [10] reported that in a Chinese population, 57.5% of stage I-III NSCLC had high PD-L1 expression as determined by a complex scoring method based on intensity and percentage of positively staining cells (effectively a modified H score equivalent to 50% 1+ or 10% 3+). The latter is more in line with early data from clinical trials suggesting about 45–50% of advanced stage NSCLC express PD-L1 [39], although differing thresholds have been used to determine positive expression. We also attempted to compare PD-L1 expression in primary tumors and corresponding nodal metastases, which has not previously been reported in NSCLC. However, the small number of positive cases in this group

Table 2
Multivariate analysis of prognostic significance of PD-L1 expression in NSCLC.

No.	Category (n = 678; events = 473)	Univariate			Multivariate		
		HR	95% CI	p-value	HR	95% CI	p-value
1	*AJCC 7 TNM Stage II–III vs. Stage I*	1.31	1.22–1.4	<0.01	1.36	1.27–1.45	<0.01
2	*Age ≥60 vs. <60 years*	1.8	1.66–1.94	<0.01	1.82	1.68–1. 96	<0.01
3	*PD-L1 (Positive vs. negative)*	0.64	0.45–0.83	<0.05	0.65	0.45–0.85	<0.05

Factors to be tested on the univariate Cox regression model with p-value > 0.05 include: Tumor size (≥ 7.5 vs. < 7.5 cm); Histologic grade (I vs. II vs. III)

(2 of the primary tumors and 1 metastasis) prevents any meaningful assessment.

Comparison of different studies reporting PD-L1 expression in NSCLC is hindered by discrepant methodologies, different thresholds to determine positivity/high expression and differences in cohorts, particularly racial differences, and variations in the proportions of histological subtypes. The studies analysing PD-L1 IHC expression in NSCLC have each used different antibodies and most were undertaken in Asian populations [8,10–12,37,40] with only 2 including Western populations [9,38]. In one Taiwanese study, more than 2/3 of patients were female and only a minority were smokers [12] while another had a young median age of 66 years [8] which is markedly different to the demographics in our cohort from a Western country. All of the peer reviewed studies included fewer patients (range 52–340) than our study and the studies included patients with a range of disease stages from I-IV, although they were predominantly early stage disease and all were undertaken on surgically obtained samples [8–12,37,38]. As a consequence, the findings could potentially differ considerably from those of patients with advanced stage disease, the commonest stage at presentation, where the majority of tumor specimens are small biopsies rather than surgical excision specimens. Most studies undertook PD-L1 IHC on whole tissue sections [9,11,12], with only one other study utilizing TMAs [38]. When using low thresholds to determine positivity, the use of whole tissue sections could lead to a higher prevalence of positive cases compared to the more limited sampling of a TMA approach.

The relatively high frequency of high PD-L1 expression in LCCs, as was also reported in two other studies [10,12], is not surprising given the association they also found with high grade tumors. We also found an association between PD-L1 expression and high tumor grade but not LCCs. We did not find any significant association, between PD-L1 expression and histological subtypes of NSCLC in keeping with other studies [10,37], but we did find a non-significant trend towards lower expression in adenocarcinomas. Other studies have reported higher expression of PD-L1 in SCCs in a Western cohort [38] and a Korean cohort [40], and in ADC compared to SCCs in both a Chinese population [11] and a Japanese population [8]. While we found high PD-L1 was more frequent in younger patients, perhaps reflecting an adaptive response to greater host immunity in younger patients, others have found an association with older patients [40], while most studies have found no association with age [8,10,12,37,38]. In keeping with other studies, we did not find any association between PD-L1 expression and any other clinicopathologic features including gender [10,12,37,38]. In contrast Azuma et al. [8] reported an association between PD-L1 expression and female never smokers in a Japanese study.

The ability of tumors to suppress host immunity relates at least to some extent to tumor disease burden in several tumor models [41] and in 2 retrospective cohorts high PD-L1 expression was more common in high stage disease [10,40]. However, most studies, including our own, did not find any association between PD-L1 expression and tumor stage [8,11,12,37,38]. We found no association between PD-L1 expression and *EGFR* or *KRAS* mutational status amongst the 276 adenocarcinomas. None of the 33 adenocarcinomas with an *EGFR* mutation showed high expression of PD-L1. By contrast, others have shown *EGFR* mutant tumors are more likely to express PD-L1 [8,42]. Interestingly, there is evidence that oncogenic *EGFR* activation drives immune evasion as PD-L1 expression is upregulated in bronchial epithelial cells expressing mutant EGFR in vitro [42]. In addition, expression of PD-L1 in NSCLC cell lines harboring *EGFR* mutations is down-regulated by EGFR-TKI treatment [42].

While activation of EGFR signalling or other oncogenic signalling pathways may lead to PD-L1 expression, there is also evidence that expression of PD-L1 may more frequently be an adaptive/inducible mechanism in tumors as expression can be induced by interferons in the tumor microenvironment [4,7]. Expression of PD-L1 in tumor cells has been associated with activation of several other oncogenic pathways including the PI3 K/Akt and P13 K/mTOR pathways as well as the STAT3 pathway from ALK activation in NSCLC and ALCL [7].

Interestingly, although PD-L1 mediates immune inhibitory signals, we found patients whose tumors exhibited high expression of PD-L1 were more likely to have a longer overall survival, independent of patient age and tumor stage. This independent prognostic significance was maintained using a range of thresholds to determine high PD-L1 expression (33–63%). The prognostic significance of PD-L1 is not straightforward, however, with several studies producing conflicting results. High PD-L1 has been associated with improved outcome in previous studies of NSCLC using immunofluorescent [38] and IHC [12] techniques. Interestingly, in the latter study, a threshold of 5% was used to determine high expression, a cut point which was not prognostically significant in our study. High PD-L1 has also been reported to be a favorable prognostic factor in small cell lung carcinomas [43] as well as breast carcinoma [16], colorectal adenocarcinoma [14], gastric cancer [44] and melanoma [23]. These results are at odds, however, with two recently presented abstracts on NSCLC [40,45] using the same Merck 22C3 PD-L1 antibody as in our study. One study found high PD-L1 expression was associated with poor overall survival in a Korean population of early and advanced stage NSCLC, particularly in ADCs [40]. In the other study looking at a European population of patients with advanced stage NSCLC treated with chemotherapy, there was no association between PD-L1 expression and overall survival [45]. A number of earlier studies found high PD-L1 was associated with a poorer prognosis in NSCLC [8,10,11,40] as well as several other solid human tumors including carcinoma of the pancreas [13], oesophagus [46], liver [47], ovary [17], kidney [19], urothelium [20], and melanoma [21]. There is evidence that expression of PD-L1 is an adaptive mechanism [23], and may be a marker of tumor response to pressure from host immunity rather than intrinsically dominant tumor immune evasion. In fact, PD-L1 expression has been associated with endogenous immune responses such as TILs in NSCLC [38] as well as other solid tumors [16,23]. It is likely that any prognostic significance relates not to single markers of immune signals but to the overall balance of the host anti-tumor immune response and tumor-mediated immunosuppression, whether adaptive or related to constitutively upregulated immunosuppressive signals mediated by oncogenic driver mutations.

We have assessed PD-L1 expression in a large cohort of early stage NSCLC with the main limitations relating to the retrospective design and the use of tissue microarrays, resulting in only small areas of a tumor being assessed and potentially introducing a sampling error, although the TMAs were designed with at least 3 cores taken from each tumor to help mitigate this possibility. We have shown PD-L1 expression is high in a relatively low proportion of early stage NSCLC and is more common in poorly differentiated tumors and in younger patients but may not relate to mutational status. High PD-L1 expression was paradoxically associated with improved patient outcome, possibly reflecting an adaptive tumor response to an immune pressure from the host in early stage disease. Further data from clinical trials of monoclonal antibodies targeting PD-1 or PD-L1 and utilizing PD-L1 immunohistochemistry as a biomarker in patients with advanced as well as early stage disease will help address the prognostic significance of this ligand. While it remains to be determined if PD-L1 expression in individual primary resected tumors remains stable with disease progression, our study provides a baseline for comparison with future studies.

Conflicts of interest statement

MB has received honoraria from Merck for an advisory board. WC and MB are on advisory boards for Bristol Myers Squibb and Merck, Sharp and Dohme for which they received honoraria. JHY is a Merck employee. For the remaining authors there are no disclosures.

Funding sources

Support from the National Foundation for Medical Research and Innovation, Cancer Institute New South Wales, Chris O'Brien Lifehouse at RPA Grant, The Sydney Breast Cancer Foundation, the National Health and Medical Research Council, Melanoma Institute Australia as well as philanthropic support from ICAP, Mr David Paradice, the Tag family trust, the O'Sullivan Family and the Cameron Family is gratefully acknowledged. Those providing funding support for the research were not involved in the study design, collection, analysis and interpretation of data, or writing of the report.

Acknowledgments

The authors also gratefully acknowledge Merck for the innovation and donation of the anti-PD-L1 MAb clone-22C3.

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