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Real-world prevalence of PD-L1 expression in non-small cell lung cancer: an Australia-wide multi-centre retrospective observational study

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Summary

An investigator-initiated, Australia-wide multi-centre retrospective observational study was undertaken to investigate the real-world prevalence of programmed death ligand-1 (PD-L1) expression in non-small cell lung carcinoma (NSCLC). Multiple centres around Australia performing PD-L1 immunohistochemistry (IHC) were invited to participate. Histologically confirmed NSCLC of any stage with a PD-L1 IHC test performed for persons aged ≥18 years between 1 January 2018 and 1 January 2020, and eligible for review, were identified at each centre, followed by data extraction and de-identification, after which data were submitted to a central site for collation and analysis. In total data from 6690 eligible PD-L1 IHC tests from histologically (75%) or cytologically (24%) confirmed NSCLC of any stage were reviewed from persons with a median age of 70 years, 43% of which were female. The majority (81%) of tests were performed using the PD-L1 IHC SP263 antibody with the Ventana BenchMark Ultra platform and 19% were performed using Dako PD-L1 IHC 22C3 pharmDx assay. Reported PD-L1 tumour proportion score (TPS) was ≥50% for 30% of all tests, with 62% and 38% scoring PD-L1 ≥1% and <1%, respectively. Relative prevalence of clinicopathological features with PD-L1 scores dichotomised to <50% and ≥50%, or to <1% and ≥1%, were examined. Females scored ≥1% slightly more often than males (64% vs 61%, respectively, p=0.013). However, there was no difference between sexes or age groups (<70 or ≥70 years) where PD-L1 scored ≥50%. Specimens from patients with higher stage (III/IV) scored ≥1% or ≥50% marginally more often compared to specimens from patients with lower stage (I/II) ($p \leq 0.002$). Proportions of primary and metastatic specimens did not differ where PD-L1 TPS was ≥1%, however more metastatic samples scored TPS ≥50% than primary samples (metastatic vs primary; 34% vs 27%, p<0.001). Cytology and biopsy specimens were equally reported, at 63% of specimens, to score TPS ≥1%, whereas cytology samples scored TPS ≥50% slightly more often than biopsy samples (34% vs 30%, respectively, p=0.004). Resection specimens (16% of samples tested) were reported to score TPS ≥50% or ≥1% less often than either biopsy or cytology samples (p<0.001). There was no difference in the proportion of tests with TPS ≥1% between PD-L1 IHC assays used, however the proportion of tests scored at TPS ≥50% was marginally higher for 22C3 compared to SP263 (34% vs 29%, respectively, p<0.001). These real-world Australian data are comparable to some previously published global real-world data, with some differences noted.

Key words: Non-small cell lung carcinoma; prevalence; PD-L1; SP263; 22C3.

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INTRODUCTION

Programmed death-1 (PD-1) is a receptor expressed on the surface of activated T-cells and when bound by one of its ligands, programmed death ligand-1 (PD-L1) and PD-L2, can lead to inhibition of a cytotoxic T-cell response.[1–3] The use of this pathway by tumours assists them to avoid cytotoxic inflammatory responses and to escape immune surveillance.[2,3] The use of immune checkpoint inhibitors (ICIs) in patients with advanced stage or metastatic non-small cell lung carcinoma (NSCLC) has improved patient survival and outcomes, particularly with agents targeting PD-1 and PD-L1.[1,2] However, the most robust results in terms of overall response rate are seen after patient stratification with PD-L1 immunohistochemical (IHC) expression,[2–4] as demonstrated in several pivotal clinical trials over the last decade with different ICIs using specific PD-L1 IHC assays, each with their own scoring algorithm and cut-off level.[3,5–9]

Until recently, most data about the prevalence of PD-L1 expression in NSCLC patients with correlations to patient demographics and clinicopathological characteristics have emerged from clinical trials.[10] In response to this, in 2019, Dietel and co-workers published their findings from a retrospective observational study, called the EXPRESS study, in which they examined the global, regional and country-specific prevalence of PD-L1 expression in 2617 advanced stage or metastatic NSCLC patients who had PD-L1 tests performed on histological specimens using the PD-L1 IHC 22C3 pharmDx kit in 45 centres in 18 countries.[11] These tests were prospectively and consecutively performed in various diagnostic laboratories for the purposes of the study and not as a requirement for entry into a clinical trial, and so the percentage of tumour samples not evaluable for PD-L1 testing could be determined. Limited correlations between PD-L1 expression in patients' tumours with baseline demographic and clinicopathological characteristics were also performed. In addition to the work from Dietel *et al.*, other groups have performed similar real-world investigations[12–27] with some conflicting results, particularly as to reported rates of PD-L1 expression in the various populations examined,[11–16,19,21–24,26,27] the PD-L1 IHC assay system used,[11–13,15–18,21–24,26] and with respect to age, sex, type of specimen, site of specimen and histological subtype.[16,17,19,22–24]

Our objectives were to investigate the prevalence of PD-L1 expression in a real-world Australian setting in a truly retrospective manner, correlating with patient demographics and clinicopathological characteristics, and to compare our results to those of some of the other published real-world studies.

MATERIALS AND METHODS

Pathologists in diagnostic pathology laboratories around Australia were contacted and invited to participate in the study, with agreement that all persons aged ≥18 years with NSCLC of any stage (AJCC 8th edition) who had received a PD-L1 IHC test as part of their routine pathologic work-up retrospectively performed between 1 January 2018 and 1 January 2020 be included, with the request that consecutive tests be included. Data in relation to patient outcome or any known mutations or rearrangements were not requested or included. The details of a small cohort of patients included in our database have been previously published.[27] Testing of either the primary tumour or a metastatic site on biopsy, resection or cytology specimens and assessment made using any of the harmonised PD-L1 antibodies or assays[28,29] in a previously validated test in each laboratory within that timeframe were accepted. All PD-L1 IHC tests were previously performed at each laboratory on formalin-fixed paraffin-embedded (FFPE) tumour blocks, with confirmation of the presence of an adequate sample (more than 100 tumour cells specified for Dako PD-L1 IHC 22C3 pharmDx assay), and according to the standard protocols accompanying each IHC assay kit. The PD-L1 tests were then scored by the reporting pathologist in each laboratory according to the established format for scoring and reporting practices at their site. Data collected from pathology reports included: person age and sex; histological subtype of NSCLC including squamous cell carcinoma (SCC), adenocarcinoma, and large cell carcinoma (LCC)/NSCLC not otherwise specified (NOS) but excluding sarcomatoid carcinoma or neuroendocrine carcinoma; specimen type (resection, biopsy or cytology sample); specimen site (primary or metastatic sample); stage (I/II or III/IV); PD-L1 antibody assay system used; and the PD-L1 test result. Due to the coding agreed upon for data collection and used by some laboratories, it was not possible to capture the types of biopsy specimens (i.e., whether bronchial biopsies or core biopsies), types of cytology specimens [i.e., whether endobronchial ultrasound (EBUS) or pleural fluid samples] or location of metastatic sites sampled for all contributing laboratories. PD-L1 test results were accepted in various formats including high (≥50%) or negative/low (<50%), as a tumour proportion score (TPS) or by buckets (PD-L1 0%, 1–49%, ≥50%). Each contributing centre was responsible for the collection and de-identification of data which were submitted to the central site investigators for collation and analysis either as data input to a previously prepared Microsoft Excel spreadsheet or as de-identified reports in electronic or paper format. All de-identified data were prepared using Excel (Microsoft, USA) and analysed using SPSS Statistics version 28.0.1.1 (IBM, USA). Continuous variables were expressed as median (range). Categorical data were expressed as frequencies and/or percentages of the cohort. For comparative purposes, the cohort was dichotomised by: sex; age groups at time of test, <70 years or ≥70 years; PD-L1 IHC expression groups, <1% or ≥1% and <50% or ≥50%. Relative proportion analysis of clinicopathological features with respect to PD-L1 result groups were examined using Chi-square test for independence with Yates continuity correction, with p values <0.05 indicating statistical significance. With our literature review, we elected to focus on studies published either around or after the time of publication of the 2019 study by Dietel *et al.*[11] from as many different countries and continents as possible. Of note, our literature review was not a systematic review and not all-inclusive due to the presence of many published studies.

RESULTS

This investigator-initiated, multi-centre Australia-wide study was conducted at 10 metropolitan pathology centres including one public laboratory from each of South Australia, Queensland, Western Australia and Tasmania, three public laboratories contributing from Victoria, two public laboratories contributing from New South Wales and one private laboratory contributing from Queensland, noting that public laboratories did include private cases in their case mix. In total, de-identified data were collected from 6690 eligible retrospectively performed PD-L1 IHC tests carried out between 1 January 2018 and 1 January 2020. A specific timeframe in which the PD-L1 tests were performed at each laboratory was chosen to ensure consecutive performance of the tests to limit bias. However, it was not possible to capture the proportion of tests that were not able to be evaluated given the current study inclusion criteria. Data in relation to patient outcome or any known mutations or rearrangements were not requested or obtained. Table 1 shows the demographic and clinical characteristics of the tests. Most (n=5396, 81%) of the 6690 tests were performed using the PD-L1 IHC SP263 antibody with the Ventana BenchMark Ultra platform (Ventana Medical Systems, USA) and the remaining 19% (n=1294) were performed using Dako PD-L1 IHC 22C3 pharmDx assay (Agilent, USA) (Table 1).

The median age of persons at the time of PD-L1 IHC testing was 70 years (range 19–98 years), with 3183 (48%) aged <70 years (Table 1). Tests for females were less frequent than for males across both age groups (41–45% females vs 55–59%

males, p=0.002) (Supplementary Table 1, Appendix A). Stage was unknown for around half of the tests reported (56%, n=3774). Where stage information was known (n=2916), 74% were stage III/IV. Specimen site was reported for all but 12 test samples (<1%). Around two-thirds of tests were performed using a primary site specimen (n=4114, 62%) and 2564 (38%) were performed using a metastatic site specimen. Specimens tested for PD-L1 were predominantly biopsy samples (n=3942, 59%), followed by cytology samples (n=1615, 24%), with 16% of tests carried out on surgical resection specimens (n=1075) and 1% of unknown type (n=58). As noted above, details as to biopsy specimen type, cytology specimen type and location of metastatic sites sampled were not able to be collected from all contributing laboratories. At 69% of tests, adenocarcinoma was the most frequent histological subtype tested (n=4592) followed by SCC (n=1499, 22%), with LCC/NSCLC NOS assigned to 9% of tests (n=591). Nearly all PD-L1 IHC test results were reported and provided as a TPS (n=6640, 99%). PD-L1 percentage scores were ≥50% for 30% (n=1998) of all tests, with a third scoring between 1–49% (n=2162, 32%), and 38% scoring <1% (n=2530). In total, 62% of tests scored ≥1% (Table 1).

Prevalence of clinicopathological features was examined between PD-L1 tests, dichotomised by PD-L1 IHC result <50% or ≥50%, and <1% or ≥1% (Table 2), by age group dichotomised at the median age of 70 years at time of testing (<70 or ≥70 years), and sex (Table 2; Supplementary Table 1, Appendix A). There was no difference in the relative proportions of tests with a PD-L1 result ≥50% by age group or sex (p>0.05) (Table 2). More broadly for tests with a TPS ≥1%, there was no differences by age group; however, females scored PD-L1 ≥1% slightly more often than males (64% vs 61%, respectively, p=0.013) (Table 2).

In general, there were differences by histological subtype, specimen site and type, stage and assay used between the dichotomised PD-L1 result groups (Table 2; Supplementary Table 1, Appendix A). Comparisons of histological subtypes with respect to PD-L1 result showed that LCC/NSCLC NOS specimens scored ≥50% more often than those with SCC or adenocarcinoma (38% vs 26% or 30%, respectively, p<0.001); however, the prevalence of adenocarcinoma scoring ≥50% was higher compared to SCC (30% vs 26%, p=0.002). For tests dichotomised at 1%, relative proportions were the same for SCC compared to adenocarcinoma or LCC/

Table 1 Demographics and characteristics of PD-L1 test cohort (N=6690)

Characteristic	*n* or median (range)			
	All tests	PD-L1 ≥50%	PD-L1 ≥1%	PD-L1 <1%
Total tests	6690	1998	4160	2530
Age				
Median, years (IQR)	70 (63–76)	70 (62–76)	70 (63–76)	70 (63–76)
Range, years	19–98	30–93	19–98	20–95
<70	3183	979	1984	1199
≥70	3507	1019	2176	1331
Gender				
Female	2881	867	1841	1040
Male	3809	1131	2319	1490
Histological type				
Squamous cell carcinoma	1499	387	955	544
Adenocarcinoma	4592	1384	2804	1788
NSCLC NOS/LCC	591	224	398	193
Missing/unknown	8	3	3	5
Specimen type				
Surgical resection	1075	251	598	477
Biopsy	3942	1179	2499	1443
Cytology	1615	547	1025	590
Missing/unknown	58	21	38	20
Specimen site				
Primary	4114	1125	2539	1575
Metastatic	2564	869	1615	949
Missing/unknown	12	4	6	6
Stage				
I/II	744	175	423	321
III/IV	2172	699	1373	799
Unknown	3774	1124	2364	1410
PD-L1 antibody				
SP263	5396	1562	3325	2071
22C3	1294	436	835	459
Unknown	0	0	0	0
PD-L1 result				
TPS (%)	6640	1983	4111	2529
High[a]	3	3	3	0
Negative/low[b]	30	0	30	0
Bucket categories[c]	17	12	16	1

IQR, interquartile range; LCC, large cell carcinoma; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; TPS, tumour percent score.
[a] High ≥50%, thus assigned TPS 51% for analysis.
[b] Negative/low <50%, thus assigned 49% for analysis.
[c] Assigned TPS bucket categories (PD-L1 0%, 1–49%, ≥50%).

Table 2 Comparative categorical analysis of known clinical parameters dichotomised by PD-L1 results <50% versus ≥50% and <1% versus ≥1%

Variable	Total (N)	Comparative PD-L1 result groups (*n*, %)					
		Score <50%	Score ≥50%	*p* value	Score <1%	Score ≥1%	*p* value
Sex							
Male	3809	2678 (70%)	1131 (30%)	0.743	1490 (39%)	2319 (61%)	0.013
Female	2881	2014 (70%)	867 (30%)		1040 (36%)	1841 (64%)	
Age, years							
<70	3183	2204 (69%)	979 (31%)	0.136	1199 (38%)	1984 (62%)	0.831
≥70	3507	2488 (71%)	1019 (29%)		1331 (38%)	2176 (62%)	
Stage							
I/II	744	569 (76%)	175 (24%)	<0.001	321 (43%)	423 (57%)	0.002
III/IV	2172	1473 (68%)	699 (32%)		799 (37%)	1373 (63%)	
Specimen site							
Primary	4114	2989 (73%)	1125 (27%)	<0.001	1575 (38%)	2539 (62%)	0.310
Metastasis	2564	1695 (66%)	869 (34%)		949 (37%)	1615 (63%)	
Histologic type							
SCC	1499	1112 (74%)	387 (26%)	0.002	544 (36%)	955 (64%)	0.072
Adenocarcinoma	4592	3208 (70%)	1384 (30%)		1788 (39%)	2804 (61%)	
SCC	1499	1112 (74%)	387 (26%)	<0.001	544 (36%)	955 (64%)	0.130
LCC/NSCLC NOS	591	367 (62%)	224 (38%)		193 (33%)	398 (67%)	
Adenocarcinoma	4592	3208 (70%)	1384 (30%)	<0.001	1788 (39%)	2804 (61%)	0.004
LCC/NSCLC NOS	591	367 (62%)	224 (38%)		193 (33%)	398 (67%)	
Specimen type							
Resection	1075	824 (77%)	251 (23%)	<0.001	477 (44%)	598 (56%)	<0.001
Cytology	1615	1068 (66%)	547 (34%)		590 (37%)	1025 (63%)	
Biopsy	3942	2763 (70%)	1179 (30%)	0.004	1443 (37%)	2499 (63%)	0.983
Cytology	1615	1068 (66%)	547 (34%)		590 (37%)	1025 (63%)	
Resection	1075	824 (77%)	251 (23%)	<0.001	477 (44%)	598 (56%)	<0.001
Biopsy	3942	2763 (70%)	1179 (30%)		1443 (37%)	2499 (63%)	
Assay							
22C3	1294	858 (66%)	436 (34%)	0.001	459 (35%)	835 (65%)	0.057
SP263	5396	3834 (71%)	1562 (29%)		2071 (38%)	3325 (62%)	

N, total number of tests in the cohort; SCC, squamous cell carcinoma; LCC, large cell carcinoma; NSCLC, non-small-cell lung cancer; NOS, not otherwise specified. Percent values have been rounded and may not equal 100.
p value determined using 2×2 Chi-square test for independence with Yates continuity correction, $p<0.05$ significant.

NSCLC NOS specimens. LCC/NSCLC NOS scored ≥1% slightly more often than adenocarcinoma specimens (67% vs 61%, respectively, $p=0.004$) (Table 2). Specific examination of the differences between adenocarcinoma vs SCC ($n=6091$) by sex, showed that more females had adenocarcinoma tested for PD-L1 than SCC (48% vs 30%, respectively, $p<0.001$), whereas more males had SCC tested for PD-L1 than adenocarcinoma (70% vs 52%, respectively, $p<0.001$) (Supplementary Table 1, Appendix A). Similarly, there was a difference between these two histological subtypes by age group, although it was only marginal (<70 years vs ≥70 years: SCC 59% vs adenocarcinoma 51%, $p<0.001$) (Supplementary Table 1, Appendix A).

The prevalence of primary site specimens with a PD-L1 result ≥50% was slightly lower than that for metastatic specimens (27% vs 34%, respectively, $p<0.001$). However, primary and metastatic specimens equally scored ≥1% (62–63% from each site) (Table 2). For persons aged 70 years or older, NSCLC at metastatic sites was tested less often compared to primary site tumour samples, however the difference was marginal (49% vs 54%, respectively, $p<0.001$) (Supplementary Table 1, Appendix A). Around half of metastatic site specimens were cytology samples (53%). There was no difference in relative proportions when comparing specimen type pairs by age group. There was no difference between the use of biopsy and cytology specimens by sex (Supplementary Table 1, Appendix A). Cytology and biopsy specimens with a TPS ≥1% were equally reported, at 63% of specimens, whereas cytology samples scored TPS ≥50% slightly more often than biopsy samples (34% vs 30%, respectively, $p=0.004$). Resection specimens, representing 16% of all tested specimens (Table 1), were reported to have TPS ≥50% (23%) or ≥1% (56%) less often than either biopsy (30%) or cytology (34%) samples ($p<0.001$ each). Cytology specimens scored a TPS ≥1% more often compared to resection specimens (56% vs 63%, respectively, $p<0.001$) (Table 2).

For tests with known stage information, higher stage (III/IV) was associated with a higher proportion scoring PD-L1 ≥50% (stage III/IV vs I/II: 32% vs 24%, $p<0.001$), and more broadly where PD-L1 ≥1% (stage III/IV vs I/II: 63% vs 57%, $p=0.002$).

There were no differences in the proportion of tests with a TPS ≥1% between PD-L1 IHC assay systems; however the proportion of tests with a TPS ≥50% was marginally higher for 22C3 compared to SP263 (34% vs 29%, respectively, $p<0.001$) (Table 2).

DISCUSSION

In this retrospective Australia-wide study, results of 6990 PD-L1 tests from persons tested via the pathology services of the 10 metropolitan contributing centres are reported. It was agreed by all participating institutions that PD-L1 test results were to be extracted for the specific timeframe of 1 January 2018 to 1 January 2020 to ensure that consecutively reported tests were captured, to limit bias. However, because results of only reported PD-L1 tests were extracted, it was not possible to determine the number of tests which were not evaluable. Looking at this metric in other published real-world studies we examined,[11,13,14,16–18,20,22] there was a broad range of patients with specimens not evaluable, ranging from 2.5% ($n=22$) to

53%,[14] with the most common reported reason being insufficient tumour cells in the specimen.[11,14,16–18,20,22] The Dako 22C3 IHC pharmDx assay specifically requires at least 100 viable tumour cells for adequate assessment, a requirement which in real-life practice is translated across to use of the other PD-L1 immunoassays. The broad range and its upper limit of just over 50% of patients in one study underscores the reality of clinical practice in which more testing is required from increasingly small specimens, with the majority of locally advanced or metastatic NSCLC patients only ever having a small biopsy or cytology specimen, from which a diagnosis and further testing is required. Data in relation to patient outcome or any known mutations or rearrangements were not requested or obtained.

Of the 6690 PD-L1 tests reviewed here, the reported prevalence of PD-L1 TPS scores ≥50% was 30%, TPS ≥1% was 62%, TPS between 1–49% was 32%, and TPS <1% was 38%. These results are roughly equivalent to a handful of previously published real-world studies,[17,18,20,25] but not the majority.[11–16,19,21–24,26,27] The reasons for this are not apparent, though may include differences in PD-L1 immunoassay systems used, different patient populations including ethnicity, stage, type and site of specimens and histological subtypes of tumours and different interpretations by the various pathologists, with exploration of some of these factors in the following discussion. Interestingly our results are reasonably equivalent to the KEYNOTE clinical trial results reported by Aggarwal *et al.*[10] Comparison of patient demographics and clinicopathological features of studies with equivalent results to ours did not reveal many similarities with the majority of studies reporting exclusive use of the Dako 22C3 IHC pharmDx assay,[10,17,18] and only two studies[20,25] reporting use of a range of IHC assays like us. In a similar vein, most studies with equivalent results included predominantly stages III–IV NSCLC patients[10,17,20,25] whereas we included a range of stages, although in one study stage was mostly unknown.[18]

Indeed, the majority of real-world studies we examined used the Dako 22C3 IHC pharmDx assay[11,12,15–18,21,22,24,26] with three other studies[14,19,27] exclusively using the Ventana SP263 IHC assay, another exclusively using the Ventana SP142 IHC assay,[13] another using the Dako 28-8 IHC assay at one centre,[23] and a few[20,25] using a range of IHC assays like us. This emphasises a point highlighted in recent literature[28,29] which is that the majority of these studies used a PD-L1 diagnostic assay, rather than using a laboratory-developed test, which was only reported by one study in one centre in Sweden.[23]

Prevalent use of the Ventana SP263 IHC assay in Australia is explained by most laboratories having Ventana staining platforms, with only a minority having the Dako AutoStainer Link 48 platform with consequent use of the Dako 22C3 pharmDx IHC assay.[26] In comparison, widespread use of the Dako 22C3 pharmDx IHC assay overseas perhaps indicates a preference for laboratories in Europe and Asia for Dako staining platforms. Of note, we found no difference in the proportion of tests with a TPS ≥1% between the different PD-L1 IHC assay systems used; however, the proportion of tests with a TPS ≥50% was marginally higher for the Dako 22C3 pharmDx IHC assay compared to the PD-L1 SP263 IHC assay (34% vs 29%, respectively, $p<0.001$) (Table 2). The reason for this is not clear but may relate to differences in the patient cohorts in the different laboratories.

Turning to our results in more detail, with respect to PD-L1 results and age and sex, we found no difference in the relative proportions of tests with a PD-L1 result ≥50% by age group or sex and no difference by age group for TPS ≥1%. However, we found that females scored PD-L1 ≥1% slightly more often than males (64% vs 61%, respectively, $p=0.013$) (Table 2). Other investigators have also reported no overall differences between age and PD-L1 expression,[11,16,17,20,21,26] though not all, with Yang *et al.*[22] reporting higher PD-L1 expression in patients <75 years, and Evans *et al.*[16] finding on detailed subgroup analysis a statistically significant 'spike' in PD-L1 expression in patients ≥90 years, which they could not explain. In contrast to our marginal correlation between female sex at PD-L1 TPS ≥1%, the majority of studies reported no differences between sex and PD-L1 expression,[11,16,17,20,21,26] whilst three groups found that male sex predicted higher PD-L1 expression.[15,22,24]

Comparing NSCLC histological subtypes to PD-L1 expression, we found that LCC/NSCLC NOS scored ≥50% more often than SCC or adenocarcinoma (38% vs 26% or 30%, respectively, $p<0.001$); however, more adenocarcinoma cases scored PD-L1 TPS ≥50% in comparison to SCC cases (30% vs 26%, $p=0.002$). For PD-L1 tests dichotomised at 1%, the relative proportions were the same for all three NSCLC histological subtypes, although, LCC/NSCLC NOS scored PD-L1 TPS ≥1% slightly more often than adenocarcinoma specimens (67% vs 61%, respectively, $p=0.004$) (Table 2). In the studies we examined, there was a range of different findings, with some studies reporting higher PD-L1 expression in SCC compared to adenocarcinoma,[12,18,20–24,26] others reporting higher PD-L1 expression in adenocarcinoma and NSCLC, NOS in comparison to SCC,[17] and others reporting no difference, statistical or otherwise[11,16,19,27] at any expression level. One interesting finding in some studies we reviewed was the correlation between higher PD-L1 expression and certain adenocarcinoma subtypes, most notably those associated with poorer prognosis such as solid and micropapillary predominant adenocarcinomas.[12,16,24] In addition, despite finding no significant difference in rates of PD-L1 expression between adenocarcinoma and SCC, Evans *et al.*[16] found that rarer NSCLC subtypes showed distinct staining patterns with lower PD-L1 expression in neuroendocrine carcinomas and higher PD-L1 expression in sarcomatoid carcinomas. We were unable to examine either of these correlations because our data acquisition did not capture adenocarcinoma subtypes or other NSCLC subtypes.

Examining specimen site with respect to PD-L1 expression in our cohort showed that the prevalence of specimens from primary sites with a PD-L1 score ≥50% was slightly lower than that for metastatic specimens (27% vs 34%, respectively, $p<0.001$). However, there were fairly equal numbers of specimens from both primary and metastatic sites which scored PD-L1 ≥1% (62–63% from each site). Other studies have reported that metastatic site specimens showed higher PD-L1 expression than primary site specimens,[12,16,18,19,30] although other groups found no difference between PD-L1 expression and site of tumour sampled.[17,20–24,27] Hwang *et al.*[18] examined concordance of PD-L1 expression in 27 patients with paired primary and metastatic tumour samples and found only weak concordance (kappa coefficient = 0.48). However, they found moderate concordance (kappa coefficient = 0.67) in 103 patients with paired biopsy and resection specimens. Further work is needed to resolve this issue of differing PD-L1

expression levels between primary and metastatic sites as it potentially may have a bearing on selection of patients for treatment with ICIs, with the risk of artificial over-inflation of PD-L1 expression in a metastatic site sample in comparison to a primary site sample, with the attendant risk of adverse side effects with no treatment benefit. Despite these concerns, in real-world clinical practice, such issues are not always taken into consideration with the necessity to confirm metastatic disease and ease of access to a particular tumour site more often driving choice of tumour site sampled.

Turning to the type of specimen examined, we found that cytology and biopsy specimens were scored at PD-L1 TPS $\geq$1% in equal numbers (63% of specimens each); whereas we found that cytology samples were scored at PD-L1 TPS $\geq$50% slightly more often than biopsy samples (34% vs 30%, respectively, p=0.004). In contrast to the finding with biopsy specimens at TPS $\geq$1%, cytology specimens were scored at a TPS $\geq$1% more often in comparison to resection specimens (63% vs 56%, respectively, p<0.001) (Table 2). Additionally, we found that resection specimens, representing 16% of all tested specimens (Table 1), were scored at PD-L1 TPS $\geq$50% (23%) or TPS $\geq$1% (56%) less often than either biopsy (30%) or cytology (34%) samples (p<0.001 each). In the studies we examined, Skov *et al.* found that cytology samples expressed PD-L1 TPS $\geq$50% more often than histology samples (36% vs 27%), which their multivariate analysis suggested was because cytology specimens were more often from higher stage patients. Of note in the clinical trials which led to approval of different ICIs, only histological specimens were allowed, which led to the diagnostic IHC assays only being approved for histology specimens. Similar to Skov *et al.*,[17] whose patient cohort included approximately 28% with only a cytology sample for diagnosis and PD-L1 testing, our patient cohort included 24% with only a cytology sample. There is still some debate in the literature as to whether PD-L1 results are accurate in cytology samples. However, in practice cytology cell blocks and smears are widely used for testing, because if not used, a sizeable proportion of NSCLC patients would be excluded from ICI therapy if they were unable to undergo histological sampling. Furthermore, there are several recent studies demonstrating that treatment outcome with ICI therapy in NSCLC patients was comparable between those who had PD-L1 expression assessed on a cytology or on a histology specimen, suggesting that PD-L1 results obtained via assessment of cytology specimens can also be used to guide selection of therapy for ICI therapy.[31,32] Because we did not collect patient outcome data, we were not able to examine this. Jin *et al.*[12] found that surgical resections had much lower rates of PD-L1 expression than small biopsy samples, without specific mention of cytology samples. Other studies found no correlation between specimen type, mostly comparing biopsy versus resection specimens, and PD-L1 expression levels.[16,20–23,27]

Finally looking at stage, acknowledging that it was unknown in 56% of our cohort, we found that samples from stages III/IV patients more often had a PD-L1 test $\geq$50% than from stages I/II patients (32% and 24%, respectively, p<0.001) and more broadly where PD-L1 TPS was $\geq$1% (stage III/IV vs I/II: 63% vs 57%, p=0.002), which is in accordance with previous reports,[17,24,33,34] though not all,[21,22] with some studies reporting no correlation between PD-L1 expression and stage. Skov *et al.*[17] reported that at PD-L1 TPS $\geq$50%, tumours from people with lower stages were associated with a lower prevalence of PD-L1 expression with an odds ratio of 0.31 for stage I versus stage IV. Wang *et al.*[34] reported that for a TPS of $\geq$50% there was a statistically significant higher prevalence rate of PD-L1 expression seen in patients with stage IV metastatic NSCLC than those with lower stage and locally advanced NSCLC.

Limitations of our study include its retrospective nature, not obtaining data in relation to patient outcome, any known mutations or rearrangements, other NSCLC histologies or neuroendocrine tumours, location of metastatic sites sampled, and the types of biopsy and cytology specimens from all contributing laboratories. Due to these limitations, we were unable to examine any correlations between these factors and PD-L1 expression in our patient population. Additionally, we were also only able to obtain stage information in 44% of the patient population.

CONCLUSION

In conclusion, we report the results of an investigator-initiated, Australia-wide retrospective observational study in which the PD-L1 expression rates of 6690 stages I–IV NSCLC tests are correlated with various clinicopathological features. In this real-world retrospective cohort, TPS was $\geq$50% in 1998 (30%), $\geq$1% in 4160 (62%), between 1–49% in 2162 (32%), and <1% in 2530 (38%), which is equivalent to some of the other published real-world studies. We found significant relationships between PD-L1 expression scores $\geq$50% and histological type, specimen types and sites, and stage, but not by age group or sex.

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Dedication: This work is dedicated to the memory of our colleague and friend, Dr Annabelle Mahar.

Ethical approval: The conduct of this study was carried out in Australia as approved by the lead Central Adelaide Health Network (CALHN) HREC (study number: 13659), and the Tasmania Health and Medical HREC (study number: 24054), and in accordance with site governance approvals from contributing centres. Patient consent was waived at each institution.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2023.08.008.

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References

1. Reck M. Pembrolizumab as first-line therapy for metastatic non-small-cell lung cancer. *Immunotherapy* 2018; 10: 93–105.
2. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Transl Med* 2016; 8: 32rv4.
3. Garon EB, Rizvi NA, Hui R, *et al*. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; 372: 2018–28.
4. Topalian SL, Hodi FS, Brahmer JR, *et al*. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; 366: 2443–54.
5. Reck M, Rodriguez-Abreu D, Robinson AG, *et al*. Pembrolizumab versus chemotherapy for PD-L1-positive non-small cell lung cancer. *N Engl J Med* 2016; 375: 1823–33.
6. Borghaei H, Paz-Ares L, Horn L, *et al*. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; 373: 1627–39.
7. Fehrenbacher L, Spira A, Ballinger M, *et al*. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multi-centre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; 387: 1837–46.
8. Rebelatto MC, Midha A, Mistry A, *et al*. Development of a programmed cell death ligand-1 immunohistochemical assay validated for analysis of non-small cell lung cancer and head and neck squamous cell carcinoma. *Diagn Pathol* 2016; 11: 95.
9. Antonia SJ, Balmanoukian A, Brahmer J, *et al*. Clinical activity, tolerability, long-term follow-up of durvalumab in patients with advanced NSCLC. *J Thorac Oncol* 2019; 14: 1794–806.
10. Aggarwal C, Rodriguez-Abreu D, Felip E, *et al*. Prevalence of PD-L1 expression in patients with non-small cell lung cancer screened for enrolment in KEYNOTE-001, -010, and -024. *Ann Oncol* 2016; 27 (Suppl 6): vi359–78.
11. Dietel M, Savelov N, Salanova R, *et al*. Real-world prevalence of programmed death ligand 1 expression in locally advanced or metastatic non-small-cell lung cancer: the global, multi-center EXPRESS study. *Lung Cancer* 2019; 134: 174–9.
12. Jin Y, Shen X, Pan Y, *et al*. Correlation between PD-L1 expression and clinicopathological characteristics of non-small cell lung cancer: a real-world study of a large Chinese cohort. *J Thorac Dis* 2019; 11: 4591–601.
13. Li C, Liu J, Xie Z, *et al*. PD-L1 expression with respect to driver mutations in non-small cell lung cancer in an Asian population: a large study of 1370 cases in China. *Ther Adv Med Oncol* 2020; 12: 1758835920965840.
14. Vrankar M, Kern I, Stanic K. Prognostic value of PD-L1 expression in patients with unresectable stage III non-small cell lung cancer treated with chemoradiotherapy. *Radiat Oncol* 2020; 15: 247.
15. Jazieh AR, Bounedjar A, Bamefleh H, *et al*. Expression of immune response markers in Arab patients with lung cancer. *JCO Glob Oncol* 2020; 6: 1218–24.
16. Evans M, O'Sullivan B, Hughes F, *et al*. The clinicopathological and molecular characteristics of PD-L1 expression in non-small cell lung cancer: analysis of a series of 10,005 cases tested with the 22C3 assay. *Pathol Oncol Res* 2020; 26: 79–89.
17. Skov BG, Rorvig SB, Jensen THL, Skov T. The prevalence of programmed death ligand-1 (PD-L1) expression in non-small cell lung cancer in an unselected, consecutive population. *Mod Pathol* 2020; 33: 109–17.
18. Hwang DM, Albaqer T, Santiago RC, *et al*. Prevalence and heterogeneity of PD-L1 expression by 22C3 assay in routine population-based and reflexive clinical testing in lung cancer. *J Thorac Oncol* 2021; 16: 1490–500.
19. Garg G, Prasad KT, Singh N, *et al*. Programmed death-ligand 1 expression and tumor-infiltrating lymphocytes in non-small cell lung cancer: association with clinicopathologic parameters. *J Pathol Trans Med* 2021; 55: 398–405.
20. Rubio-Viqueria B, Tarruella MM, Lazaro M, *et al*. PD-L1 testing and clinical management of newly diagnosed metastatic non-small cell lung cancer in Spain: MOREL study. *Lung Cancer Manag* 2021; 10: LMT53.
21. Zhu Y, Lin S, Wang Y, *et al*. Study on PD-L1 expression in NSCLC patients and related influencing factors in the real world. *Comput Math Methods Med* 2021; 2021: 3273347.
22. Yang X, Jiang L, Jin Y, *et al*. PD-L1 expression in Chinese patients with advanced non-small cell lung cancer (NSCLC): a multi-center retrospective observational study. *J Cancer* 2021; 12: 7390–8.
23. Mansour MSI, Malmros K, Mager U, *et al*. PD-L1 expression in non-small cell lung cancer specimens: association with clinicopathological factors and molecular alterations. *Int J Mol Sci* 2022; 23: 4517.
24. Fu F, Deng C, Sun W, *et al*. Distribution and concordance of PD-L1 expression by routine 22C3 assays in East-Asian patients with non-small cell lung cancer. *Respir Res* 2022; 23: 302.
25. How SH, Tho LM, Liam CK, *et al*. Programmed death-ligand 1 expression and use of immune checkpoint inhibitors among patients with advanced non-small-cell lung cancer in a resource-limited country. *Thorac Cancer* 2022; 13: 1676–83.
26. Ye L, Leslie C, Jacques A, *et al*. Programmed death ligand-1 expression in non-small cell lung cancer in a Western population and correlation with clinicopathologic features. *Mod Pathol* 2019; 32: 524–31.
27. Holmes M, Mahar A, Lum T, *et al*. Real-world programmed death-ligand 1 prevalence rates in non-small cell lung cancer: correlation with clinicopathologic features and tumour mutation status. *J Clin Pathol* 2021; 74: 123–8.
28. Hirsch FR, McElhinny A, Stanforth D, *et al*. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol* 2016; 12: 208–22.
29. Tsao MS, Kerr KM, Kockx M, *et al*. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of the Blueprint phase 2 project. *J Thorac Oncol* 2018; 13: 1302–11.
30. Uruga H, Bozkurtlar E, Huynh TG, *et al*. Programmed cell death ligand (PD-L1) expression in stage II and III adenocarcinomas and nodal metastases. *J Thorac Oncol* 2017; 12: 458–66.
31. Tajarernmuang P, Ofiara L, Beaudoin S, *et al*. Real-world outcomes of patients with advanced non-small cell lung cancer treated with anti-PD1 therapy on the basis of PD-L1 results in EBUS-TBNA vs histological specimens. *Chest* 2021; 160: 743–53.
32. Lau SCM, Rabindranath M, Weiss J, *et al*. PD-L1 assessment in cytology samples predicts treatment response to checkpoint inhibitors in NSCLC. *Lung Cancer* 2022; 171: 42–6.
33. Kerr KM, Thunnissen E, Dafni U, *et al*. A retrospective cohort study of PD-L1 prevalence, molecular associations and clinical outcomes in patients with NSCLC: results from the European Thoracic Oncology Platform (ETOP) Lungscape Project. *Lung Cancer* 2019; 13: 95–103.
34. Wang H, Agulnik J, Kasymjanova G, *et al*. Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. *Ann Oncol* 2018; 29: 1417–22.