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Programmed Death Ligand 1 (PD-L1) Expression and its Association with Driver Mutations among Patients with Non-Small Cell Lung Cancer in a Private Tertiary Care Setting

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ABSTRACT

Objective. The advent of immunotherapy has significantly changed the treatment and management of patients with advanced non-small cell lung cancer (NSCLC). Prior to initiation of immunotherapy, evaluation of programmed death ligand 1 (PD-L1) expression is required. One factor that affects PD-L1 expression in NSCLC is the presence of oncogenic driver mutations; however, little data on its association is available, especially in the Philippine setting. The study aims to determine the prevalence of PD-L1 expression and its association with driver mutations among patients with non-small cell lung cancer in a private tertiary care hospital in the Philippines.

Methodology. The study was undertaken for a period of two years from July 2017-July 2019 at St. Luke's Medical Center and included 446 NSCLC samples. PD-L1 was evaluated by immunohistochemistry using 22C3 anti-PD-L1 antibody clone, EnVision FLEX visualization system on Autostainer Link 48. Patient demographics and data on driver mutation testing were recorded. Statistical analysis was performed using logistic regression.

Results. PD-L1 expression was observed in 273 (61.20%) of 446 cases, 119 (61.20%) of which demonstrated high PD-L1 expression while 154 (34.50%) had low PD-L1 expression. There was no significant association between PD-L1 expression and EGFR mutation, ALK mutation, age, and gender. For histologic type, high PD-L1 expression was significantly associated with adenocarcinoma and non-small cell carcinoma, NOS.

Conclusion. The overall prevalence of PD-L1 expression in non-small cell lung carcinoma is 61.20% based on the cases included. Although we did not find an association between PD-L1 expression and EGFR and ALK mutation, our study observed that ALK-mutated NSCLCs have 4.7 odds of having high PD-L1 expression, however, a higher sample size is warranted to truly determine significant association. The outcome of this study may provide help in the stratification of patients and predict those who will benefit from immunotherapy.

Key words: non-small cell lung cancer, programmed death ligand 1, PD-L1, driver mutation

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INTRODUCTION

Lung cancer is one of the leading causes of cancer-related mortality worldwide. It is the most common cause of cancer death in the US and UK based on recent data from the American Lung Association (2019) and Cancer Research UK (2016), respectively.[1,2] In the Philippines, data from the Department of Health – Philippine Cancer Control Program showed lung to be the most common site of cancer among Filipino men and the third most common among Filipino women.[3] Moreover, the World Health Organization – Cancer Country Profile (Philippines) in 2020 recorded the highest cancer-related mortality rate for lung (17.9%) followed by the liver (11%) and colorectum (10.2%).[4] Lung cancer is generally categorized into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), with NSCLC accounting for 80% to 85% of cases.[5] Subtypes of NSCLCs include adenocarcinoma (40%), squamous cell carcinoma (25%-30%), and large cell carcinoma (10%-15%).

Immunotherapy has provided good clinical outcomes and is now considered as treatment option in patients with advanced NSCLC.[6] The tumor cells may express an

inhibitory cell surface molecule, called the programmed cell death ligand 1 (PD-L1) which combines with the programmed cell death 1 (PD-1) receptor expressed by T-cells, resulting in the inhibition of T-cell proliferation and activation.[7] This pathway has been used by cancer cells to escape immune surveillance. Hence, by blocking the interaction of PD-L1 with PD-1, immune function is restored and the cancer cells are recognized by the T-cells as foreign. This provides the basis of immunotherapy. The expression of PD-L1 on NSCLC cells, currently evaluated by immunohistochemistry (IHC), predicts the responsiveness of the tumor cells to anti-PD-1/PDL1 drugs.[8] Several of these drugs (nivolumab, pembrolizumab, and atezolizumab) have already been approved by the FDA for the treatment of advanced non-small cell lung cancer.[9]

Somatic mutation in the cancer cell genome is classified into two: driver mutations and passenger mutations. Driver mutations confer growth advantage on cancer cells and are positively selected during cancer evolution whereas passenger mutations do not confer growth advantage and thus do not contribute to cancer development.[10] Oncogenic driver mutations that are recommended for testing by the National Cancer Comprehensive Cancer Network (NCCN) for NSCLC include epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1), and BRAF as these are the genotypes with approved targeted therapies that have been shown to improve patient's survival.[11,12] Other mutations that are implicated in the pathogenesis of NSCLC include KRAS, MET, LB1, PIK3CA, and RET.[13] EGFR is dysregulated in a number of NSCLCs either by protein overexpression, gene amplification, or mutation.[14] It is mutated in 10% of cases from North America and Western Europe and to as high as 30-50% of patients in East Asia. In a recent study by Nee-Estuye-Evangelista et al., the frequency of EGFR mutation among Filipinos is found to be at 49.4%.[15] EGFR mutation may be detected in both solid tissue biopsies and liquid biopsies.[16] Like those with EGFR mutation, patients with the ALK rearrangement (EML4-ALK fusion) are also commonly found in younger population who are non-smokers.[17] Detection of the EML4-ALK translocation may be done by immunohistochemistry (IHC), fluorescence in-situ hybridization (FISH), and next generation sequencing (NGS) panels.[17] This translocation is found in 3%-7% of NSCLC patients.[18] ROS1, a receptor tyrosine kinase, is seen in 1-2% of NSCLCs that carry a translocation between ROS1 and other genes, the most common being CD74.[19] Like patients with ALK-mutated tumors, patients with ROS1 mutation are also typically found in younger patients who are never smokers.[20] These mutations may be identified by FISH or NGS panels. Lastly, BRAF mutations, most commonly BRAF V600E, have been observed in 1-3% of NSCLCs and found in patients who are smokers.[21] They are typically detected using polymerase chain reaction (PCR) sequencing or NGS panels.

PD-L1 expression on tumor cells has been shown to be predictor of outcomes of immunotherapy and its association with driver gene mutation status has been a focus of several studies in the recent years.[22] A study by Yang et al., concluded that the use of PD-L1 inhibitors is a promising option in the management of advanced NSCLCs with KRAS driver mutation and not with EGFR and ALK.[23] This is also consistent with the results of the meta-analytic study by Lan et al., where they found that PD-L1 expression in NSCLCs is lower in EGFR-mutated tumors and higher in KRAS-mutated tumors.[24] The association between PD-L1 expression and driver gene mutation in clinical tumor specimens is not well characterized. This study aims to determine the prevalence rate of PD-L1 expression and to determine its association with driver gene mutations among patients in the Philippines diagnosed with non-small cell lung cancer.

METHODOLOGY

A retrospective cross-sectional study was conducted at the Section of Histopathology and Section of Cellular Immunology and Immunogenetics, Institute of Pathology, at St. Luke's Medical Center. The study was undertaken for a period of two (2) years from July 2017 to July 2019.

Sample size

All specimens with a histopathologic diagnosis of non-small cell lung carcinoma submitted for PD-L1 testing with or without driver mutation analysis at St. Luke's Medical Center.

Inclusion criteria

All specimens with a histopathologic diagnosis of non-small cell lung carcinoma submitted for PD-L1 testing at St. Luke's Medical Center from July 2017 to July 2019 with or without driver gene mutation analysis were included.

Exclusion criteria

Specimens submitted for PD-L1 testing with less than 100 tumor cells were labeled suboptimal and excluded from the study. Specimens submitted with driver mutation testing but with indeterminate/equivocal results were also excluded.

PD-L1 testing

Immunohistochemistry was performed on formalin-fixed paraffin embedded (FFPE) tissues using 22C3 anti-PD-L1 antibody clone, EnVision FLEX visualization system (Agilent, USA) on Autostainer Link 48, an FDA-approved method. Tumor cells showing membranous staining for PD-L1 were evaluated as positive cells. The immunostaining results were based on tumor proportion score (TPS): (1) No Expression (Negative) – no staining or detected in $<1\%$ of tumor cells, (2) Low PD-L1 Expression – staining in $\geq 1\%$-49% of tumor cells, and (3) High PD-L1 Expression – staining in $\geq$ 50% of tumor cells. Two molecular pathologists conducted the evaluation.

Driver mutation testing

EGFR mutation

Detection of the most common EGFR mutations was performed using the Cobas Z 480 Analyzer (Roche Diagnostics, USA). DNA extraction from formalin-fixed paraffin embedded tissues was done followed by PCR amplification and detection of target DNA using complementary primer pairs and oligonucleotide probes labeled fluorescent dyes using Real time PCR analysis. The amplification and detection reagents were provided in the Cobas Roche EGFR Mutation Test v1 kit (Roche

Diagnostics, USA). The most common EGFR mutations detected by the Cobas Z 480 Analyzer includes:

- Exon 18: G719X (G719A, G719C, and G719S)
- Exon 19: deletions and complex mutations
- Exon 20: S768I, T790M, and insertions
- Exon 21: L858R and L861Q

ALK rearrangement

Detection of ALK rearrangement was performed either by immunohistochemistry (IHC) or fluorescent in-situ hybridization (FISH). By IHC assay, the anti-ALK (clone D5F3) rabbit monoclonal primary antibody was used, with the OptiView™-diaminobenzidine IHC detection kit and OptiView™ amplification kit, on a fully automated IHC-staining platform. By FISH assay, a total of 50 cells were analyzed using US-FDA approved ALK 2p23dual color break apart Vysis probes from Abbott Molecular. This test was validated on formalin-fixed paraffin-embedded tissues.

ROS1 rearrangement

Detection of ROS1 gene rearrangement was performed using fluorescent in-situ hybridization assay. A total of 100 cells were analyzed using laboratory validated ROS1 6q22.1(genomic location: 117,288,300-117,425,855) dual colorbreak apart Vysis probes from Abbott Molecular. This test was validated on formalin-fixed paraffin-embedded tissues.

BRAF mutation

Detection of the most common BRAF exon 15 (codon 600) was performed on Cobas z 480 analyzer using the Cobas BRAF V600 Mutation Test. This is a rtPCR based assay for targeted, qualitative, non-discriminatory detection of the most common mutations in BRAF, including V600E, and some non-V600E mutations (V600D, V600E2 and V600K), which represents approximately 90% of all BRAF mutations. DNA extraction from formalin-fixed paraffin-embedded tissues was followed by rtPCR amplification and detection of target DNA by primers and probes. Mutant and negative quality controls were included in each run.

KRAS mutation

Detection of the most common KRAS exons 2 and 3 (codons 12, 13 and 61) was performed on Cobas z 480 analyzer using the Cobas KRAS mutation test. This is a rtPCR based assay for targeted, qualitative, non-discriminatory detection of the most common mutations in KRAS. DNA extraction from formalin-fixed paraffin-embedded tissues was followed by rtPCR amplification and detection of target DNA by primers and probes. Mutant and negative quality controls were included in each run.

Statistical analysis

Data was encoded and processed using Microsoft Excel and SPSS. The association between PD-L1 expression and driver gene mutations were analyzed using logistic regression. A p-value of <0.05 is considered statistically significant.

Ethical clearance

Ethical clearance was obtained from the Institutional Ethics Review Board.

RESULTS

Demographic data

A total of 446 subjects were included in the study. The mean age (± standard deviation) 65.8 ± 11.3 years old with a minimum age of 21 years old and a maximum age of 92 years old. Three hundred fifteen (70.6%) subjects were aged 60 years old and above and 131 (29.4%) were less than 60 years of age. There were 263 (59.0%) males and 183 (41.0%) females. Most of the specimens (68.2%) were obtained through biopsy. The most common histopathologic diagnosis was adenocarcinoma (69.7%) followed by non-small cell carcinoma, NOS (19.1%) (Table 1).

PD-L1 expression in non-small cell lung carcinoma

PD-L1 expression was determined among subjects with non-small cell lung carcinoma. There were 173 (38.8%) of 446 subjects with no PD-L1 expression and 273 (61.20%) subjects with PD-L1 expression. Among the 273 subjects, 154 (34.5%) had low PD-L1 expression and 119 (26.7%) had high PD-L1 expression.

PD-L1 expression and driver mutations

EGFR mutation testing was only performed on 356 out of 446 subjects. One hundred forty-nine (41.9%) was EGFR-mutated and 207 (58.1%) was EGFR-negative (Figure 1). Among those with EGFR mutation, 140 (39.4%) had single mutation and only 9 (2.5%) had dual mutations. Of the 149 EGFR mutations, 44.30% had mutation of Exon 19 followed by mutation of Exon 21 at 39.6%. The most common dual mutation was the combination of Exon 19 and Exon 20 at 3.36%. There were 283 subjects who underwent ALK mutation determination. Among these 283 subjects, 16 (5.7%) were ALK-mutated. Only 68 of 446 subjects had ROS1 mutation testing and 67 (98.5%) were negative for ROS1 mutation. Of the 26 of 446 subjects who underwent BRAF testing, 25 (96.2%) were negative for BRAF mutation. Two subjects who had KRAS testing were both negative for KRAS mutation.

Table 1. Demographic data of patients with non-small cell lung carcinoma (n=446)

Age (years)	
Mean	65.8 ± 11.3
Median	67.0
Minimum	21
Maximum	92
	Frequency (%)
Age Group	
<60 years	131 (29.4)
≥60 years	315 (70.6)
Sex	
Female	183 (41.0)
Male	263 (59.0)
Specimen Type	
Biopsy	304 (68.2)
Fluid cytology	51 (11.4)
Lobectomy/Resection	29 (6.5)
Not available	62 (13.9)
Histopathologic diagnosis	
Adenocarcinoma	311 (69.7)
Adeno squamous carcinoma	7 (1.6)
Non-small cell carcinoma, NOS	85 (19.1)
Pleomorphic carcinoma	2 (0.4)
Squamous cell carcinoma	41 (9.2)

Table 2. Association of PD-L1 expression with EGFR and ALK mutation

Driver mutation	High PD-L1 expression				Low PD-L1 expression			
	N (%)	OR	CI	*p* value	N (%)	OR	CI	*p* value
EGFR-mutated	36 (24.2)	0.687	0.402 – 1.174	0.17	53 (35.6)	0.848	0.518 – 1.387	0.51
EGFR negative	62 (30.0)				74 (35.7)			
ALK-mutated	8 (50.0)	4.7	0.973 – 22.873	0.05	6 (37.5)	2.8	0.560 – 14.458	0.21
ALK negative	78 (29.2)				97 (36.3)			

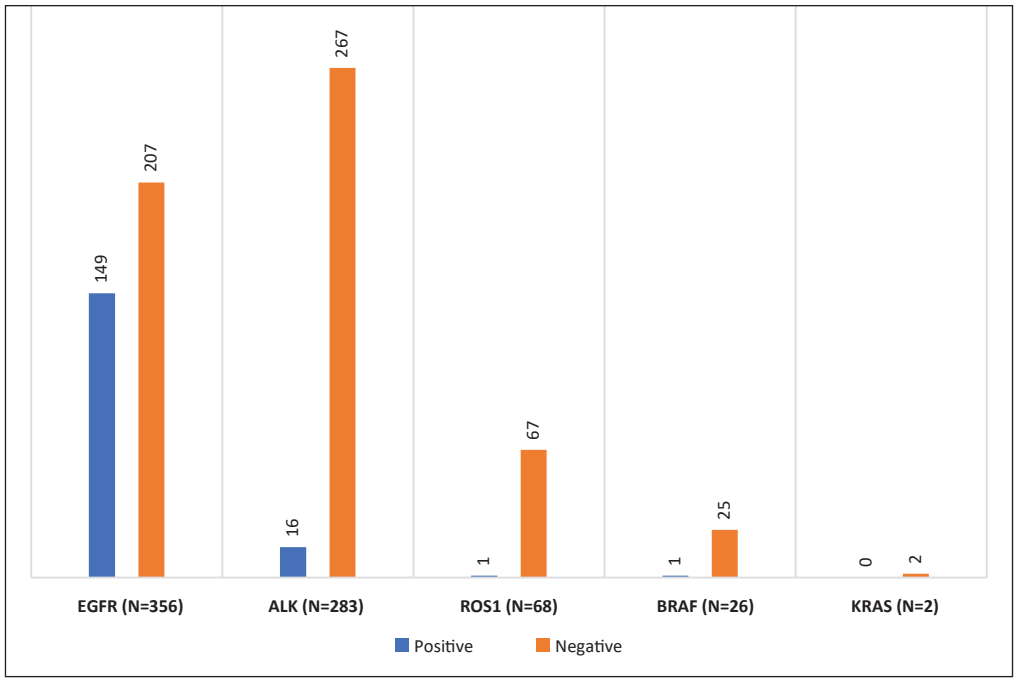

Figure 1. Frequency of driver mutations in non-small cell lung carcinoma.

EGFR

Low PD-L1 expression (35.7%) is more frequent among EGFR-negative NSCLCs while majority of EGFR-mutated NSCLCs did not express PD-L1 (40.3%) (Figure 2). In this study, however, no significant association is found between EGFR mutation and high PD-L1 expression (OR=0.687, 95% CI: 0.402-1.174, p=0.17) and low PD-L1 expression (OR=0.848, 95% CI: 0.518-1.387, p=0.51) (Table 2).

ALK

Low PD-L1 expression is more frequent among ALK-negative NSCLCs while high PD-L1 expression is more frequent among ALK-mutated NSCLCs (Figure 2). ALK-mutated NSCLCs are 4.7x more likely to have high PD-L1 expression (OR=4.7, 95% CI: 0.973-22.873, p=0.05) and 2.8x more likely to have low PD-L1 expression (OR=2.8, 95% CI: 0.560-14.458, p=0.21) but the differences are not significant (Table 2).

ROS1, BRAF, KRAS

Most of the subjects with ROS1 negative NSCLCs have high PD-L1 expression while those with BRAF-negative NSCLCs have low PD-L1 expression (Figure 2). Only two subjects underwent KRAS mutation testing and both showed positive PD-L1 expression. The relationship between ROS1, BRAF, and KRAS mutation with PD-L1 expression cannot be determined due to the limited number of participants who underwent testing for these driver mutations.

PD-L1 expression and clinicopathologic factors

Age

The majority of the subjects belong to the elderly age group (≥60 years old). Most of the subjects in both groups did not express PD-L1. There is no significant association between age and high PD-L1 expression (OR=1.05, 95% CI: 0.63-1.76, p=0.84) and low PD-L1 expression (OR=0.91, 95% CI: 0.56-1.48, p=0.70) (Table 3).

Gender

The study population comprised of males (59%) more than females (41%). Most of the male subjects did not express PD-L1 (40.7%). Females have 1.6 odds of having low PD-L1 expression but the difference is not significant (OR=1.6, 95% CI: 1.00-2.49, p=0.05) (Table 3).

Histologic Type

Adenocarcinoma was the predominant histologic type. Subjects diagnosed with adenocarcinoma are 2.9 times more likely to have high PD-L1 expression than subjects diagnosed with squamous cell carcinoma. In addition, subjects diagnosed with non-small cell carcinoma, NOS are 4 times more likely to have high PD-L1 expression than subjects diagnosed with squamous cell carcinoma. The high PD-L1 expression in adenocarcinoma (OR=2.91, 95% CI: 1.04-8.09, p=0.04) and non-small cell carcinoma, NOS (OR=4.4, 95% CI: 1.44-13.40, p=0.01) histologic group are both statistically significant (Table 3).

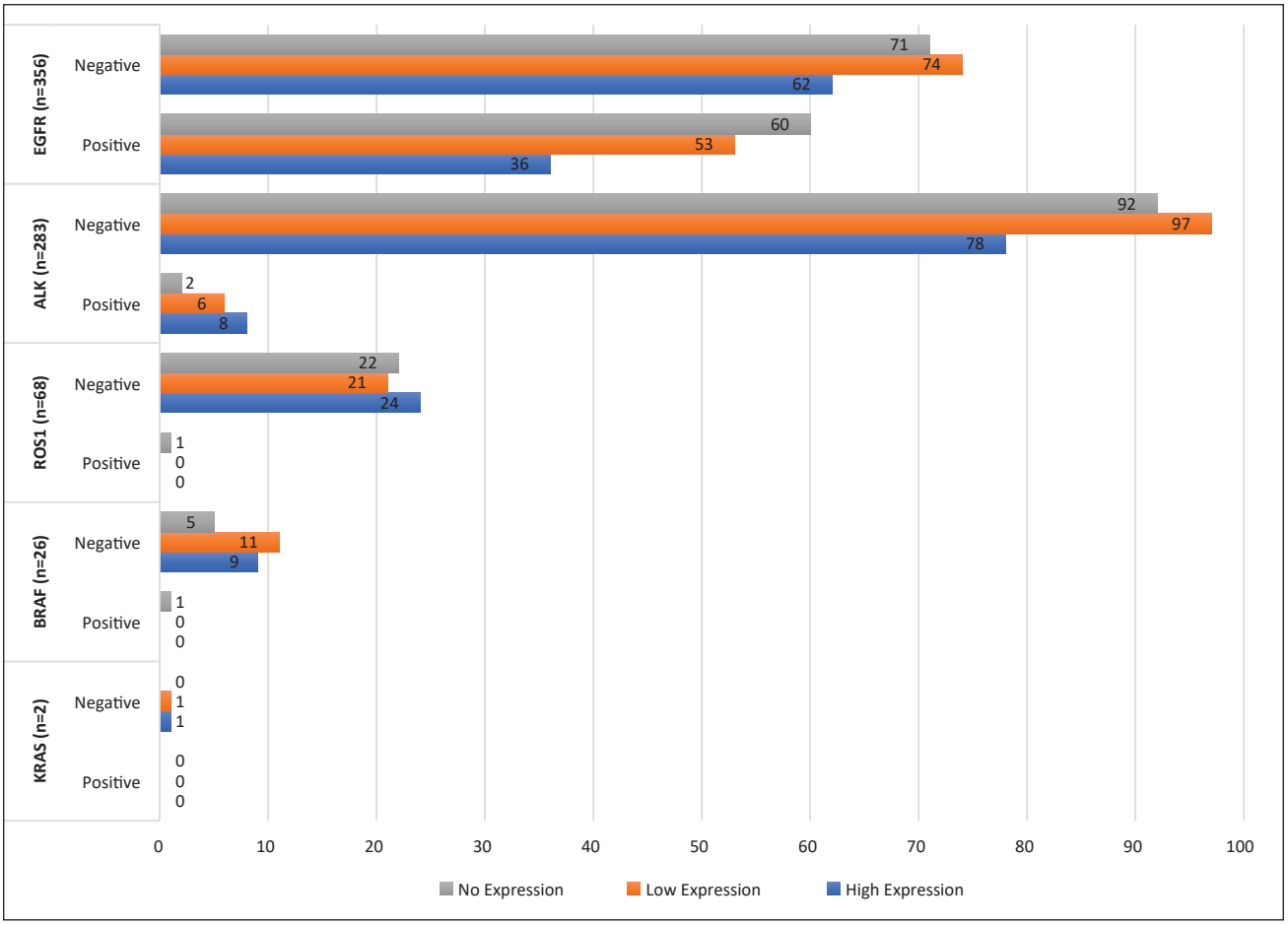

Figure 2. PD-L1 expression and driver mutations.

Table 3. Association of PD-L1 expression with age, gender and histologic type

Characteristic	High PD-L1 expression				Low PD-L1 expression			
	N (%)	OR	CI	*p* value	N (%)	OR	CI	*p* value
Age group								
<60 years	37 (28.2)	1.05	0.63 – 1.76	0.84	43 (32.8)	0.91	0.56 – 1.48	0.70
≥60 years	82 (26.0)				111 (35.2)			
Sex								
Female	43 (23.5)	0.89	0.54 – 1.46	0.64	74 (40.4)	1.60	1.00 – 2.49	0.05
Male	76 (28.9)				80 (30.4)			
Histopathologic diagnosis								
Adenocarcinoma	84 (27.0)	2.91	1.04 – 8.09	**0.04**	103 (33.1)	0.91	0.56 – 1.48	0.70
Adenosquamous carcinoma	2 (28.6)	4.09	0.46 – 36.59	0.21	3 (42.9)	1.04	0.50 – 2.16	0.91
Non-small cell carcinoma NOS	27 (31.8)	4.40	1.44 – 13.40	**0.01**	32 (30.6)	2.36	0.35 – 15.98	0.38
Squamous cell carcinoma	5 (12.2)				15 (36.6)			

DISCUSSION

Targeted immunotherapy has been used successfully in the United States since 2014 for the treatment of various advanced cancers such as NSCLCs. In the Philippines, recent approval by the Philippine Food and Drug Administration was given for pembrolizumab, a landmark drug that may be used as first line treatment in advanced NSCLC making immunotherapy a reality for the Filipinos.[25] The drug works by blocking the interaction of PD-L1, present in the cancer cells, with the PD-1 receptor, present in T-cells. Currently, the 2019 National Comprehensive Cancer Network (NCCN) immunotherapy guidelines in the treatment and management of advanced NSCLC require the evaluation of PD-L1 expression by immunohistochemistry prior to initiation of immunotherapy.[11] This set of guidelines is also followed by many international and local societies in Oncology including the American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), and the Philippine Society of Medical Oncology (PSMO). The NCCN strongly recommends single-agent pembrolizumab as first line treatment for patients with high PD-L1 expression (≥50%) because use of single agent pembrolizumab as first line treatment improves overall survival by twofold in patients with high PD-L1 expression. For patients with low PD-L1 expression (≥1%-49%), the treatment varies for non-squamous NSCLC and squamous NSCLC. For patients with low PD-

L1 expression and non-squamous NSCLC, single-agent pembrolizumab is not recommended as first line treatment. Instead, the preferred approach is combination therapy with pembrolizumab/permetrexed with either cisplatin or carboplatin. This combination therapy has resulted in reduced risk of death by 51% vs chemotherapy alone based on the KEYNOTE-189 trial.[26] For patients with low PD-L1 expression and squamous NSCLC, NCCN recommends treatment with pembrolizumab/carboplatin in addition to either paclitaxel or nab-paclitaxel.

Several studies have shown PD-L1 expression to be associated with the presence or absence of driver gene mutations.[12,24,27-37] In patients with driver mutations, the NCCN guidelines recommend targeted therapy as first line treatment since they typically do not respond well to single-agent immunotherapy. Targeted therapy includes (1) tyrosine kinase inhibitors such as osimertinib. erlotinib, gefitinib, afatinib, and dacomitinib for EGFR-mutated NSCLC; crizotinib, ceritinib, and brigatinib for ALK-mutated NSCLC; crizotinib and ceritinib for ROS1-mutated NSCLC; and (2) kinase inhibitors such as dabrafenib + trametinib for BRAF-mutated NSCLC. Since it has been reported that driver mutations are more common in patients of East Asian origin,[14] including those of Filipino descent,[15] the evaluation of PD-L1 expression becomes an important parameter to assess in the treatment planning of patients with NSCLC.

In our study, we retrospectively assessed PD-L1 expression by immunohistochemistry in NSCLC (n=446) for a period of two years. Overall, 61.20% of subjects had PD-L1 expression while 38.80% had no PD-L1 expression. Among those with positive PD-L1 expression, low PD-L1 expression (≥1%-49%) was detected in 34.50% while high PD-L1 expression (≥50%) was detected in 26.70%. This data is comparable with the studies done by Aggarwal et al. (n=3,880/5,879, 66%), Holmes et al. (n=194/264, 73.4%), and Chang et al. (n=334/500, 66.8%), wherein the prevalence of PD-L1 expression in NSCLC was seen in more than 60% of the study population.[38-40]

Among the 446 subjects, only 356 had EGFR mutation testing. Of the 356, 41.9% (n=149) had EGFR mutation and 58.1% (n=207) had negative EGFR mutation. This high EGFR mutation rate is comparable with several studies done in Asian populations which showed EGFR mutation rate ranging from 30% to as high as 76%.[15,41,42] Correlating PD-L1 expression with EGFR mutation, our study found no significant association. This is comparable with previous studies conducted by Cooper et al., Schmidt et al., and Tang et al.[43-45] Other studies, however, demonstrated high PD-L1 expression in EGFR-mutated NSCLCs,[12,27,28] while some showed that it is higher in EGFR-wildtype NSCLCs.[29-31] Moreover, there are also researches that showed lower PD-L1 expression in EGFR-mutated NSCLCs.[24,32] The variation in the results of these studies may probably be explained by differences in study population, antibody clones used for PD-L1 testing, and variable cut offs for PD-L1 expression.

Two hundred eighty-three (283) of the 446 subjects underwent ALK mutation testing. Of the 283, 5.7% (n=16) were positive for ALK rearrangement while 94.3% (n=267) were negative. In our study, ALK-mutated NSCLCs are 4.7 times more likely to have high PD-L1 expression but this is not statistically significant (OR=4.7, 95% CI: 0.973-22.873, p=0.05). Despite a p-value of 0.05, the confidence interval included the value 1.0 and is very wide which means that we were not able to find a significant difference due to the small sample size (n=283). Hence, we recommend higher sample size for ALK in future studies to truly determine if high PD-L1 expression is correlated with the presence of ALK mutation in NSCLCs. The results of several studies regarding PD-L1 expression and ALK mutation are also conflicting. Some studies have found no association between PD-L1 expression and ALK mutation[24,32,40,46,47] while other studies demonstrated significantly higher PD-L1 expression in ALK-mutated NSCLCs.[32-34] The proposed mechanism behind high PD-L1 expression in ALK-mutated NSCLCs is believed to be due to the upregulation of the MEK/ERK and PI3K/AKT pathway signaling in the tumor cells.[35-37]

The association of PD-L1 expression among clinico-pathologic variables (age, gender, and histologic type) was also determined. In this study, no association is found between low and high PD-L1 expression and age group. This finding is consistent with several studies.[32,41,47-49] For gender, many studies have found PD-L1 expression to be positively associated with male gender[32,44,46,47,49,50] while some studies did not observe any significant association.[40,48,51] The positive association with male gender could be due to the higher incidence of cigarette smoking among males which is explained by the proinflammatory effects of smoking and that smoking-induced carcinomas also have high mutational tumor burden in which they express neoantigens that trigger anti-tumor immune responses.[32,52] In our study, however, no significant association is observed between PD-L1 expression and gender. For the histologic type, a study by Skov et al., showed significant difference in PD-L1 expression between adenocarcinoma and squamous cell carcinoma with adenocarcinoma having an odds ratio of 1.8.[53] This is also supported by the study of Mu et al., where PD-L1 expression in adenocarcinoma was significantly higher than in squamous cell carcinoma.[54] Their findings are comparable with the result of our study where we observed high PD-L1 expression in adenocarcinoma (p=0.04) and non-small cell carcinoma, NOS (p=0.01). The clinical significance of this is that patients with advanced lung cancer with a histopathologic diagnosis of adenocarcinoma are more likely to receive and benefit from immunotherapy compared to other histologic subtypes. Other studies, however, demonstrated higher PD-L1 expression in squamous cell carcinoma than adenocarcinoma[24,32,46,50,55] while some studies showed no significant association.[48,56,57] The variation in the results of the studies may again be explained by differences in study population and methodology. The significant association between PD-L1 expression and NSCC, NOS could be because these tumors are more likely adenocarcinoma when immunophenotyping is performed.[58] In this study, however, the cases that were classified as NSCC, NOS, included those wherein the immunohistochemistry results were not conclusive (i.e., TTF-1 and p40 negative cases), as well as those wherein immunohistochemical staining was not performed. Moreover, a formal slide review to confirm the histologic subtype of the tumors was not done.

The histologic subtype was based solely on the final diagnosis in the accompanying histopathology reports.

There are several limitations of the study that should be acknowledged. First, the sample size for the analysis of correlation between PD-L1 expression and driver mutations (EGFR [n=356] and ALK [n=283]) was small; hence, a higher sample size is warranted. Also, we were not able to check for the association of PD-L1 expression with other driver mutations due to the small number of patients who underwent testing for these mutations (ROS1, n=68; BRAF, n=26; and KRAS, n=2). Second, most of the samples from this study are from small biopsies (n=305), from fine needle aspiration and core needle biopsies, which may affect PD-L1 expression due to smaller number of tumor cells available for analysis compared to lobectomy/resection specimens. The tissue samples obtained from small biopsies may not be representative of the whole tumor and may show divergent results due to possible heterogenous expression of PD-L1.[59] It is widely recognized that PD-L1 expression in tumors is heterogenous, which may impact the interpretation on small biopsies versus resections. In a study by Hwang et al., the discordance rates between biopsy and resection specimens were as high as 33.3% (for biopsies greater than or equal to 8 mm^2 area) and 71.4% (for biopsies less than 8 mm^2 area).[60] Lastly, some cases from other hospital institutions that were sent for PD-L1 testing have incomplete data causing them to be excluded from this study and further limiting the sample size.

CONCLUSION

Our study provided baseline data in the Philippines regarding expression of PD-L1 in NSCLC and its association with driver mutations. The prevalence of PD-L1 in NSCLC was 61.20%, with 34.5% of subjects having low expression (≥1-49%) and 26.71% of subjects having high expression (≥50%). No significant association was observed between PD-L1 expression and EGFR and ALK mutations. For EGFR mutation, determining if there is a significant difference in PD-L1 expression among the specific EGFR exon mutations may also be worth looking into. For ALK mutation, although our study observed ALK-mutated NSCLCs to have 4.7 odds of having high PD-L1 expression, a higher sample size is warranted to truly determine if there is significant association (95% CI: 0.973-22.873, *p*-value=0.05). High PD-L1 expression was significantly associated with adenocarcinoma and non-small cell carcinoma, NOS histology. No significant association was observed between PD-L1 expression and age and gender. The outcome of this study may provide help in the stratification of patients and predict those who will benefit from immunotherapy. Overall, since the evaluation of PD-L1 expression is required prior to initiation of immunotherapy, there is a need to explore factors that may affect its expression. Some of these factors, including the presence of oncogenic driver mutations, age, gender, and histologic type, were already explored in this study; however, a higher sample size is still recommended. Correlation with other clinicopathologic parameters such as smoking status, presence of tumor infiltrating lymphocytes, tumor differentiation, tumor size, lymph node metastasis, and TNM stage, is also recommended for future studies as these factors have been found to affect PD-L1 expression as well.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest

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