FCO • 15(1) • 2024 • 36–44
DOI: 10.2478/fco-2023-0036

sciendo

Forum of Clinical **Oncology**

Changes in soluble PD-L1 levels in patients with advanced non-small cell lung cancer

Research Article

Thang Ba Ta[1]*, Nhung Thi Kim Pham[1], Dung Tien Nguyen[1], Tien Viet Tran[2], Cuong Tan Tran[1], Thuc Cong Luong[3], Hoan Ngoc Vu[4], Hien Thi Dieu Le[5], Bang Ngoc Dao[1]

[1]*Respiratory Center, Military Hospital 103, Vietnam Military Medical University*
[2]*Department of Infectious Diseases, Military Hospital 103, Vietnam Military Medical University*
[3]*Cardiovascular Center, Military Hospital 103, Vietnam Medical Military University*
[4]*Department of Military Epidemiology, Vietnam Medical Military University*
[5]*Respiratory Department, Hai Phong University of Medicine and Pharmacy*

Received 19 May 2024; Accepted 2 August 2024

Abstract: Soluble programmed death-1 ligand (sPD-L1) in the serum of non-small cell lung cancer (NSCLC) patients is a crucial factor in disease prognosis and an indicator for immunotherapy response. This study aimed to evaluate changes in sPD-L1 levels in advanced NSCLC patients. Between May 2018 and October 2022, 80 patients with advanced-stage NSCLC and 30 healthy volunteers participated in a prospective study.

The findings revealed a significant rise in sPD-L1 concentration in the lung cancer group compared to the normal control group (1.08 vs. 0.42, $p < 0.001$). No apparent correlation was found between sPD-L1 concentration and membrane-bound programmed death-1 ligand (mPD-L1) expression. The optimal diagnostic threshold for sPD-L1 was set at 0.92 ng/mL, showing a sensitivity of 56.25% and specificity of 77.33%, with an area under the curve (AUC) of 0.743 ($p = 0.001$).

Although increased sPD-L1 levels were prevalent in individuals with advanced age, prolonged disease duration, large tumor size, and finger clubbing, these associations did not reach statistical significance ($p > 0.05$). In conclusion, sPD-L1 levels significantly increased in advanced NSCLC patients compared to controls ($p < 0.05$), with no association observed with mPD-L1 ($p = 0.304$). The study suggests that sPD-L1 concentration can be a specific biomarker for guiding the diagnosis of advanced NSCLC, with a recommended cutoff value of 0.92 ng/mL, demonstrating a sensitivity of 56.25% and specificity of 77.33% ($p = 0.001$). Importantly, no apparent relationship was established between sPD-L1 levels and clinical or paraclinical characteristics in advanced NSCLC patients.

Keywords: *soluble PD-L1 (sPD-L1) • membrane PD-L1 (mPD-L1) • non-small cell lung cancer (NSCLC)*

1. Introduction

Lung cancer, a malignant disease with high incidence and mortality rates in adults, particularly non-small cell lung cancer (NSCLC), is often diagnosed at advanced stages due to silent progression and late-stage detection[1,2]. Advanced-stage patients require a comprehensive treatment approach, combining various interventions. Chemotherapy and radiotherapy, though long-established, pose complications, especially in elderly patients with multiple comorbidities[3].

Immunotherapy has emerged as a primary treatment for advanced-stage NSCLC, demonstrating promising efficacy in clinical practice[4,5]. Various markers, including programmed cell death ligand-1 (PD-L1), have been explored for immunotherapy applications, proving significant in identifying and prognosing NSCLC[6].

PD-L1 expression is commonly observed in human cancer, presenting as two forms: membrane-bound PD-L1 (mPD-L1) and soluble PD-L1 (sPD-L1). sPD-L1, a variant of PD-L1, circulates in the serum of lung cancer patients, autoimmune diseases, and viral infections. Over 20 different mechanisms leading to increased sPD-L1 secretion have been identified[7,8]. While mPD-L1 testing requires tissue samples, sPD-L1 testing can be performed with blood samples. Despite showing proven

* *E-mail: bsdaongocbang@gmail.com*

Open Access. © 2024 Thang Ba Ta *et al.*, published by Sciendo. BY-NC-ND This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License.

diagnostic and therapeutic value, mPD-L1 testing has limitations, such as the need for biopsy specimens containing a minimum number of cancer cells (at least 100 cancer cells per sample), particularly challenging in cases evaluating cancer progression posttreatment[9,10]. In contrast, sPD-L1 testing offers a simple, easily implemented, and minimally invasive sample collection technique. However, ongoing research is essential to validate its diagnostic and prognostic value for NSCLC patients[8,11].

Therefore, this study aims to investigate changes in sPD-L1 concentration in patients with advanced NSCLC.

2. Methods

2.1. Patients

Between May 2018 and October 2022, the cross-sectional study (N = 110) enrolled 80 patients diagnosed with advanced-stage NSCLC and 30 healthy volunteers (control group). The control group consisted of individuals undergoing routine health check-ups at the Clinic of Military Hospital 103. Inclusion criteria for the lung cancer group involved newly diagnosed patients with stage IIIB and IV NSCLC with PS 0–2. Exclusion criteria included concurrent cancers, immune-compromising conditions, and history of use of immune-suppressing drugs including systemic corticosteroids. Similar criteria were applied to the control group, emphasizing regular health check-ups and excluding individuals with cancer or immune-related conditions. Detailed clinical assessments, radiologic examinations, diagnostic biopsies, and immunohistochemical analyses were conducted, alongside the examination of mPD-L1 expression in tissue samples and sPD-L1 concentration in serum. The normal control group underwent blood tests, chest X-rays, and sPD-L1 concentration testing in serum. Lung cancer staging followed the 7th edition TNM classification by the Union for International Cancer Control (UICC), with the histological type determined using the World Health Organization (WHO) classification of lung tumors (2015)[13]. The study received ethical approval from the Institutional Review Board of Military Hospital 103 (No. 99B/15-Aug-2022/VMMU-IRB), and the two groups provided informed consent.

2.2. mPD-L1 Immunohistochemistry

mPD-L1 expression testing utilizes immunohistochemical techniques with a single PD-L1 antibody [rabbit anti-human monoclonal antibody PD-L1 (clone 73-10) from Leica, USA], performed on the Leica BOND-MAX automated staining system. The intensity of PD-L1 staining is assessed on a scale from 0 to 2+ as follows: 0 (negative): no staining or faint staining in less than 1% of tumor cells; 1+ (low): weak staining in 1%–49% of tumor cells; and 2+ (strong): strong staining in more than 50% of tumor cells. The standard requires a minimum of 100 cancer cells[14]. We used the Tumor Proportion Score (TPS) as a critical metric used to quantify PD-L1 expression in tumor cells. It serves as a key determinant in selecting patients for certain immunotherapies, particularly those targeting the PD-1/PD-L1 pathway. Both positive and negative control samples are used to validate the specificity and sensitivity of the PD-L1 antibody.

2.3. sPD-L1 Enzyme-Linked Immunosorbent Assay

Serum was obtained by centrifugation (1300×g for 10 min) and then aliquoted and stored at −80 °C until study analysis. sPD-L1 concentration testing employs a pre-assembled enzyme-linked immunosorbent assay (ELISA) kit with a specific PD-L1 antibody (SEA788Hu) from Cloud-Clone Corporation (USA)[15]. The linearity was validated by testing serial dilutions of known sPD-L1 concentrations, achieving an acceptable R^2 value of greater than 0.99. The assay demonstrated a detection limit of 0.1 ng/mL, validated by spiking known amounts of sPD-L1 into sample matrices and measuring the lowest detectable signal above the background. The assay specificity was confirmed through cross-reactivity and interference testing, ensuring selective detection of sPD-L1. Intra-assay and inter-assay precision were validated, with acceptable coefficients of variation (CVs) of less than 10%. In addition, sPD-L1 concentrations were normalized to total protein concentration measured by a bicinchoninic acid (BCA) assay, allowing the expression of sPD-L1 levels relative to total protein content in the samples.

2.4. Statistical Analysis

In the statistical analysis, data were processed using the medical statistics software SATA 15.0. Categorical variables were delineated by frequencies and proportions, while normally distributed continuous variables were presented using mean and standard deviation and non-normally distributed ones were described by median and interquartile range. The

Mann–Whitney test was used to compare medians for variables lacking a normal distribution. Receiver operating characteristic (ROC) curve analysis was conducted to calculate the area under the curve (AUC) and determine the optimal cutoff point for sPD-L1, optimizing sensitivity and specificity. AUC ≥0.60: poor accuracy; ≥0.70: average accuracy, ≥0.80: good accuracy; and ≥0.90: very good accuracy. The cutoff value is selected at the point with the highest Youden index (J) with J = Se + Sp 1, and the threshold value is determined with the "Cutpt" and "Roctab" commands in Stata 15.0 software. Univariate logistic regression analysis chooses a testing threshold of $p < 0.2$ to select variables, combining characteristics based on clinical experience to include in the multivariable logistic regression model, to predict the likelihood of occurrence, indicates high or low PD-L1 levels, the optimal model is when most of the subjects are accounted for by the model and (in this study, the number of subjects satisfying this model is 67 patients) Pseudo R^2 >20%, $p < 0.05$.

3. Results

3.1. Clinical Characteristics and Histological Findings

The majority of lung cancer patients were in stage IV (80%), presenting hazy masses in 66.25% and malignant pleural effusion in 47.5%. Adenocarcinoma (ADC) dominated histologically (72.5%), followed by SCC (20%) and ASC (7.5%). *EGFR* mutation occurred in 24.0% of cases. Comparing the lung cancer group to normal groups, the mean serum PD-L1 concentration was significantly higher (1.08 vs. 0.56 ng/mL and 1.08 vs. 0.42 ng/mL, respectively; $p < 0.001$). mPD-L1 expression was observed in 61.2% of patients, with 28.75% having high expression and 32.5% having low expression. Group demographics showed no significant age or body mass index (BMI) differences, but the control group had a lower male ratio ($p < 0.001$), with nonsmokers in the control group. sPD-L1 concentrations did not significantly differ by gender, age group, smoking history, disease stage, tumor size, or histological type ($p > 0.05$). The mean sPD-L1 value in lung cancer patients with mPD-L1 expression was 1.09 ng/mL, which was slightly higher than that in the non-mPD-L1 group (0.93 ng/mL), with no statistically significant difference ($p = 0.3$). No correlation was found between sPD-L1 and mPD-L1 expression levels ($p = 0.726$).

3.2. Diagnostic Threshold and Multivariate Regression Analysis

Comparing the study group to the control group, the diagnostic threshold for sPD-L1 was determined at 0.92 ng/mL, with 56.25% sensitivity and 83.33% specificity [AUC = 0.756; 95% confidence interval (CI): 0.663–0.849; $p = 0.001$]. Multivariate regression analysis indicated associations between increased sPD-L1 and factors like age, disease onset duration, tumor size, and finger clubbing, although statistical significance was not reached for most factors ($p > 0.05$). The model demonstrated statistical significance overall ($p = 0.031$), suggesting a potential role for sPD-L1 in diagnosing advanced NSCLC.

4. Discussion

4.1. Characteristics of the Study Group

Table 1 outlines the common characteristics of the study group. The findings align with previous research and selection criteria for this study. The patients were typically older, predominantly male, and with a smoking history, larger tumor size, ADC histology, and advanced-stage disease. Gender differences between the study group and the control group were influenced by smoking habits in Vietnam, where the majority of smokers are male, a primary risk factor for NSCLC. In addition, gender differences were observed between the UTP group and the normal control group. Despite limited relevance in previous studies, gender differences were maintained in our control group[16]. Previous studies also reported the highest proportion of ADC in NSCLC[17].

4.2. mPD-L1 Expression and Quantification

In this study, 49 patients (61.25%) exhibited mPD-L1 expression, with a median of 10 ng/mL. Among these, 23 patients (46.93%) showed high expression. The positive rate of mPD-L1 varied across studies, subjects, and the type of immunotherapy used [Table 5]. Despite using different PD-L1 antibodies, our study's TPS findings were concordant, with thresholds set at ≥1% (positive) and <1% (negative). The TPS ≥1% subgroup was further divided at cutoffs ≥50% and <50%. Our study, using the PD-L1 73-10 antibody, correlated with the clinical trials JAVELIN Lung 100 and JAVELIN Lung 200, reporting positive mPD-L1 rates of 56.4% and 66%, respectively, while our study found a positive rate of 61.25%[29,30].

Table 1: Characteristics of study subjects.

Feature	NSCLC (n_1 = 80)	Control (n_2 = 30)	p
General Characteristics			
Age (X ± SD) (years)	65.58 ± 12.15	61.23 ± 15.84	0.096*
Male	59 (73.75%)	14 (46.67%)	0.007[a]
Female	21 (26.25%)	16 (53.33%)	
Smoking	38 (47.5%)	8 (26.67%)	< 0.049[a]
BMI	20,85 ± 2,41	21,1 ± 2,06	0.624*
Clinical Features:			
Fatigue	36 (45.0%)	0	
Weight loss	32 (40.0%)	0	
Fever	11 (13.75%)	0	
Clubbing	7 (8.75%)	0	
Peripheral Lymphadenopathy	15 (18.75%)	0	
Cough	66 (82.5%)	0	
Sputum	22 (27.5%)	0	
Hemoptysis	9 (11.25%)	0	
Chest pain	52 (65.0%)	0	
Dyspnea	29 (36.25%)	0	
Bronchial Obstruction	6 (7.5%)	NA	
Consolidation syndrome	11 (13.92%)	NA	
Pleural effusion	36 (45.0%)	NA	
Mediastinal Syndrome	4 (5.0%)	NA	
CT Chest Imaging Characteristics:			
Nodules	27 (23.75%)	NA	
Mass	53 (66.25%)	NA	
Largest Tumor Size (X±SD) (mm)	47.73 ± 21.46	NA	
Pleural effusion	38 (47.5%)	NA	
Mediastinal lymph nodes	21 (26.25%)	NA	
Atelectasis	15 (18.75%)	NA	
Staging		NA	
IIIB	16 (20.0%)	NA	
IV	64 (80.0%)	NA	
Pathology and Gene mutations:			
ADC	58 (72.5%)	NA	
SCC	16 (20.0%)	NA	
ASC	6 (7.5%)	NA	
EGFR mutation	24 (30.0%)	NA	
sPD-L1 in PBMC:			
Median (Min – Max)	1.08 (0.01 – 5.56)	0.42 (0.01 – 1.66)	< 0.001**
mPD-L1 molecular expression:			
Negative	31 (38.75%)	NA	
Positive	49 (61.25%)	NA	
Expression level			
- Low (1 – 49%)	26 (32.5%)	NA	
- High (≥ 50%)	23 (28.75)	NA	
Median (min – max)	10 (0 – 50)	NA	
X ± SD (%)	27.82 **± 33.61**	NA	

*Kruskal–Wallis test a: Chi^2 test **: Mann–Whitney test

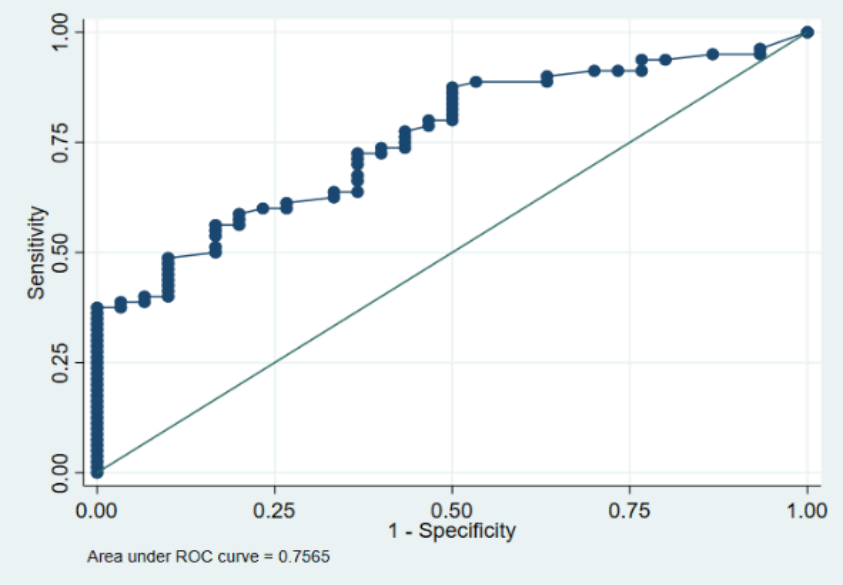
Table 2: Values of sPD-L1 concentration according to clinical and paraclinical characteristics.

Feature	Subgroup	Median (IQR)	p**
Gender	Male (n = 59)	1.09 (0.5 - 2.56)	0.67
	Female (n = 21)	0.93 (0.6 - 1.49)	
Smoking	Yes (n = 38)	1.08 (0.56 - 2.43	0.965
	No (n = 42)	1.03 (0.59 - 2.28)	
Staging	IIIB (n = 16)	1.06 (0.68 - 2.16)	0.764
	IV (n = 64)	1.08 (0.52 - 2.36)	
Age	≤ 65 (n = 40)	1.04 (0.55 - 2.49)	0.577
	> 65 (n = 40)	1.1 (0.67 - 2.27)	
Tumor size	< 30 mm (n = 27)	1.49 (0.65 - 2.29)	0.981
	≥ 30 mm (n = 53)	1.07 (0.56 - 2.28)	
Phenotype	ADC (n = 58)	0.96 (0.59 - 2.25)	0.763
	SCC (n = 16)	1.17 (0.46 - 2.24)	
	ASC (n = 6)	2.0 (0.57 - 3.59)	
EGFR mutation	Positive (n = 24)	1.125 (0.69 - 2.2125)	0.187
	Negative (n = 56)	2.56 (0.865 - 4.80)	

Table 3: Relationship between mPD-L1 and sPD-L1 expression.

mPD-L1 expression		sPD-L1 Median (IQR)	p**
Not expressed (n = 31)		0.93 (0.48 - 2.06)	0.304
Expressed (n = 49)		1.09 (0.64 - 2.56)	
Expression level	- Low (n = 26)	1.21 (0.65 - 2.7)	0.726
	- High (n = 23)	1.04 (0.49 - 2.56)	

**: Mann-Whitney test



Logistic regression	UTP=1.455 x sPDL1 – 0.335
Cutoff	0.92 ng/mL *(Youden (J)=0.4)*
Se	56.25%
Sp	83.33%
AUC (CI95%)	0.756 (0.663 - 0.849)
p	0.001

Figure 1: ROC curve of serum PD-L

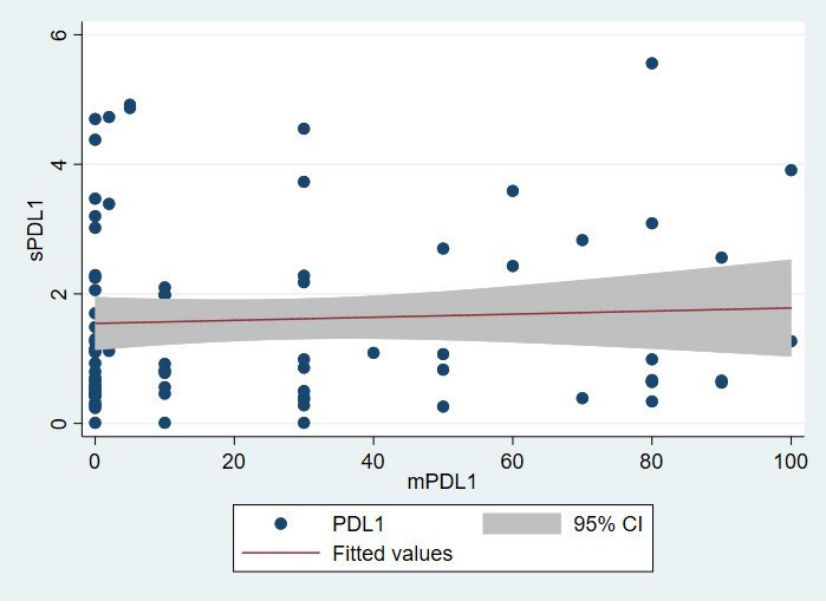


Figure 2: Distribution characteristics of mPD-L1 and sPD-L1.

Table 4: Logistic regression model between increased sPD-L1 and mPD-L1 and clinical and paraclinical characteristics of NSCLC.

sPDL1 expression low – high	OR	SE	z	p	95% CI	
Age	1.06	0.0317	1.85	0.065	0.996	1.121
Gender	0.34	0.2732	-1.34	0.179	0.072	1.635
BMI	0.86	0.1049	-1.27	0.204	0.673	1.088
Disease duration	1.003	0.0068	0.52	0.604	0.990	1.017
Clubbing	9.49	14.413	1.48	0.139	0.483	186.36
Bronchial Obstruction	0.06	0.0753	-2.19	0.029	0.004	0.7417
Tumor size	1.01	0.0152	0.77	0.442	0.982	1.042
Metastases	0.21	0.2167	-1.51	0.131	0.027	1.595
ADC	0.79	0.5333	-0.35	0.726	0.209	2.968
_cons	1.56	5.2885	0.13	0.896	0.002	1202.81
p = 0.031; R2 = 0.20						

Generally, mPD-L1 expression rates were consistently above 60% across studies [Table 5].

4.3. sPD-L1 Concentration and Diagnostic Value

sPD-L1, a soluble form generated by cleavage or splicing of PD-L1 mRNA, circulates in the serum of lung cancer, autoimmune, and viral infection patients[33,34]. Our study demonstrated significantly higher sPD-L1 concentrations compared to both control groups ($p < 0.01$) [Table 3]. This aligns with previous research showing elevated sPD-L1 levels in cancer patients[11]. The diagnostic ROC curve analysis revealed an optimal AUC of 0.817 when compared to the normal control group. The diagnostic cutoff for UTP was 0.92 ng/mL, providing 56.25% sensitivity and 77.33% specificity. Thus, sPD-L1 could serve as a promising prognostic indicator for lung cancer alongside other biomarkers[34,40]. However, the prognostic value of sPD-L1 in immune therapy response was not evaluated in our study. Multivariate regression analysis indicated associations between elevated sPD-L1 levels and older age, prolonged disease duration, larger tumor size, and finger clubbing, though statistical significance was not reached ($p > 0.05$). Further exploration with a larger sample size is warranted to validate these findings and improve model utility[14,37].

Table 5. mPD-L1 expression ratio in some clinical trials on NSCLC.

Clinical trials	Positive mPD-L1 expression ratio	PD-L1 antibody type
CheckMate 057[18]	455/582 (78%)	28-8
CheckMate 017[19]	225/272 (83%)	28-8
CheckMate 012[20]	32/46 (70%)	28-8
CheckMate 227[21]	1189/1739 (68%)	28-8
Keynote 189[22]	63%	22C3
Keynote 001[23]	60,8%	22C3
Keynote 010[24]	1475/2222 (66%)	22C3
Keynote 021[25]	56,8%	22C3
Keynote 042[26]	1978/3019 (66%)	22C3
Keynote 024[27]	500/1653 (30,2%)	22C3
POPLAR[28]	109/287 (37,9%)	SP142
JAVELIN Lung 100[29]	88/156 (56,4%)	73-10
JAVELIN Lung 200[30]	527/792 (66%)	73-10

5. Conclusions

The concentration of sPD-L1 is significantly higher in patients with advanced NSCLC when compared to the control group ($p < 0.05$). No correlation is observed between sPD-L1 concentration and mPD-L1 expression. The concentration of sPD-L1 could be applied as a prognosis indicator in advanced NSCLC, with a threshold of 0.92 ng/mL, achieving a sensitivity of 56.25% and specificity of 77.33%. There is no significant correlation found between sPD-L1 concentration and clinical or paraclinical characteristics in advanced NSCLC patients ($p > 0.05$).

Limitations

The study only selected patients with advanced NSCLC, and therefore, the changes in sPD-L1 concentration in early-stage NSCLC patients have not been assessed. In addition, the limited number of patients resulted in only three types of NSCLC being included in the new tissue type. The study has also not evaluated the role of sPD-L1 in monitoring immune treatment response in NSCLC patients.

Clinical implications and future direction

The study results indicate a significant increase in sPD-L1 concentration in the advanced NSCLC patient group ($p < 0.05$), and there is no correlation with mPD-L1 ($p = 0.304$). The sPD-L1 concentration serves as a diagnostic marker for advanced NSCLC with a sensitivity of 56.25% and specificity of 77.33%, making it applicable as an orienting diagnostic marker. There is no substantial correlation between sPD-L1 concentration and clinical or paraclinical characteristics in advanced NSCLC patients. Due to the limited number of samples obtained, we plan to study the association between sPD-L1 and *EGFR* and *ALK* mutations when more data becomes available.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Institutional Review Board Statement

The protocol was approved by the Ethics Committee of Military Hospital 103 (No. 99B/15-Aug-2022/VMMU-IRB). The study was conducted using good clinical practice guidelines and it adhered to the principles of the Declaration of Helsinki.

Informed Consent Statement

All participants provided written informed consent. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data supporting this research are available from the authors on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; CT, computed tomography; mPD-L1, membrane-bound programmed cell death ligand-1; MRI, magnetic resonance imaging; OS, overall survival; PET-CT, positron emission tomography and computed tomography; PFS, progression-free survival; sPD-L1, soluble programmed cell death ligand-1; UICC, Union for International Cancer Control; VMMU, Vietnam Military Medical University.

References

[1] WHO (2023). Lung cancer. https://www.who.int/news-room/fact-sheets/detail/lung-cancer

[2] Sung H., Ferlay J., Siegel R.L., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 71(3): 209-249. doi: 10.3322/caac.21660.

[3] Society A.C. (2019). Key Statistics for Lung Cancer, https://www.cancer.org/content/cancer/en/cancer/non-small-cell-lung-cancer/about /key-statistics/.

[4] Borghaei H., Paz-Ares L., et al. (2015). Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med; 373(17): 1627-1639. doi: 10.1056/NEJMoa1507643.

[5] Gettinger S., Rizvi N.A., et al. (2016). Nivolumab Monotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. J Clin Oncol; 34(25): 2980-2987. doi: 10.1200/JCO.2016.66.9929.

[6] Han Y., Liu D. and Li L. (2020). PD-1/PD-L1 pathway: current researches in cancer. Am J Cancer Res. 2020; 10(3): 727–742. PMCID: PMC7136921.

[7] Sun C, Mezzadra R, Schumacher TN. (2018). Regulation and Function of the PD-L1 Checkpoint. Immunity (2018) 48:434–52. doi: 10.1016/j.immuni.2018. 03.014.

[8] Guixiang Liao, Zhihong Zhao, Yuting Qian, et al. (2021). Prognostic Role of Soluble Programmed Death Ligand 1 in Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis. Front Oncol. 2021; 11: 774131. doi: 10.3389/fonc.2021.774131

[9] Cui S, Su X, Dong L, Qian J, et al. (2017). Programmed Cell Death Ligand 1 Protein Levels Predicted Survival of Non-Small Cell Lung Cancer. J Cancer (2017) 8:4075–82. doi: 10.7150/jca.21415.

[10] Ishii H., Azuma K., Kawahara A., et al. (2015). Significance of Programmed Cell Death - Ligand 1 Expression and its Association With Survival in Patients With Small Cell Lung Cancer. J Thorac Oncol (2015) 10:426–30. doi: 10.1097/JTO.0000000000000414

[11] SoYeon Oh, Soyeon Kim, Bhumsuk Keam, et al. (2021). Soluble PD-L1 is a predictive and prognostic biomarker in advanced cancer patients who receive immune checkpoint blockade treatment. Sci Rep. 2021 Oct 5;11(1):19712. doi: 10.1038/s41598-021-99311-y

[12] Rami-Porta R., Bolejack V., Crowley J., et al. (2015). The IASLC Lung Cancer Staging Project: Proposals for the Revisions of the T Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Lung Cancer. J Thorac Oncol, 10(7): 990-1003. doi: 10.1097/JTO.0000000000000559.

[13] Travis W.D., Brambilla E., Nicholson A.G., et al. (2015). The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. J Thorac Oncol, 10(9): 1243-1260. doi: 10.1097/JTO.0000000000000630.

[14] Teixido C., Vilarino N., Reyes R., et al. (2018). PD-L1 expression testing in non-small cell lung cancer. Ther Adv Med Oncol, 10: 1758835918763493.

[15] Corp. C.-C., Instruction manual, SEA788Hu 96 Tests Enzyme-linked Immunosorbent Assay Kit For Programmed Cell Death Protein 1 Ligand 1 (PDCD1LG1) Organism Species: Homo sapiens (Human).

[16] Chuling Li, Chuwei Li, Chunchun Zhi, et al. (2019). Clinical significance of PD-L1 expression in serum-derived exosomes in NSCLC patients. Journal of Translational Medicine Vol. 17, 355 (2019).

[17] Yumin Wu, Hui Guo, Jiawei Yue (2022).Serum sPD1 and sPDL1 as Biomarkers for Evaluating the Immune State of Lung Adenocarcinoma Patients. Journal of Immunology Research. Vol 2022. doi: 10.1155/2022/9101912

[18] Borghaei H., Paz-Ares L., Horn L., et al. (2015). Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med, 373(17): 1627-1639. doi: 10.1056/NEJMoa1507643.

[19] Brahmer J., Reckamp K.L., Baas P., et al. (2015). Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med, 373(2): 123-135. doi: 10.1056/NEJMoa1507643.

[20] Gettinger S., Rizvi N.A., Chow L.Q., et al. (2016). Nivolumab Monotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. J Clin Oncol, 34(25): 2980-2987. doi: 10.1200/JCO.2016.66.9929.

[21] Hellmann M.D., Ciuleanu T.E., Pluzanski A., et al. (2018). Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med, 378(22): 2093-2104. doi: 10.1056/NEJMoa1801946.

[22] Gandhi L., Rodriguez-Abreu D., Gadgeel S., et al. (2018). Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med, 378(22): 2078-2092. doi: 10.1056/NEJMoa1801005.

[23] Garon E.B., Rizvi N.A., Hui R., et al. (2015). Pembrolizumab for the treatment of non-small-cell

lung cancer. N Engl J Med, 372(21): 2018-2028. doi: 10.1056/NEJMoa1501824.

[24] Herbst R.S., Baas P., Kim D.W., et al. (2016). Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet, 387(10027): 1540-1550. doi: 10.1016/S0140-6736(15)01281-7.

[25] Gubens M.A., Sequist L.V., Stevenson J.P., et al. (2019). Pembrolizumab in combination with ipilimumab as second-line or later therapy for advanced non-small-cell lung cancer: KEYNOTE-021 cohorts D and H. Lung Cancer, 130: 59-66. doi: 10.1016/j.lungcan.2018.12.015.

[26] Mok T.S.K., Wu Y.L., Kudaba I., et al. (2019). Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet, 393(10183): 1819-1830. doi: 10.1016/S0140-6736(18)32409-7.

[27] Reck M., Rodriguez-Abreu D., Robinson A.G., et al. (2016). Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med, 375(19): 1823-1833. doi: 10.1056/NEJMoa1606774.

[28] Fehrenbacher L., Spira A., Ballinger M., et al. (2016). Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet, 387(10030): 1837-1846. doi: 10.1016/S0140-6736(16)00587-0.

[29] Verschraegen C.F., Jerusalem G., McClay E.F., et al. (2020). Efficacy and safety of first-line avelumab in patients with advanced non-small cell lung cancer: results from a phase Ib cohort of the JAVELIN Solid Tumor study. J Immunother Cancer, 8(2): e001064. doi: 10.1136/jitc-2020-001064.

[30] Barlesi F., Vansteenkiste J., Spigel D., et al. (2018). Avelumab versus docetaxel in patients with platinum-treated advanced non-small-cell lung cancer (JAVELIN Lung 200): an open-label, randomised, phase 3 study. The Lancet. Oncology, 19(11): 1468-1479. doi: 10.1016/S1470-2045(18)30673-9.

[31] Giuseppe Lamberti, Monia Sisi, Elisa Andrini, et al. (2020). The mechanisms of PD-L1 regulation in non-small-cell lung cancer (NSCLC): Which are the involved players?. Cancers (Basel). 2020 Nov; 12(11): 3129. doi: 10.3390/cancers12113129.

[32] P Song, S Wu, L Zhang, X Zeng, et al. (2019). Correlation between PD-L1 expression and clinicopathologic features in 404 patients with lung adenocarcinoma. Interdisciplinary Sciences: Computational Life Sciences, Vol. 11, 258-265. doi: 10.1007/s12539-019-00329-8.

[33] Zhu X., Lang J. Soluble PD-1 and PD-L1: Predictive and prognostic significance in cancer. Oncotarget. 2017;8:97671–97682. doi: 10.18632/oncotarget.18311.

[34] Christian Bailly, Xavier Thuru, and Bruno Quesnel (2021). Soluble Programmed Death Ligand-1 (sPD-L1): A Pool of Circulating Proteins Implicated in Health and Diseases. Cancers (Basel). 2021 Jun; 13(12): 3034. doi: 10.3390/cancers13123034.

[35] Khan M., Zhao Z., Arooj S., et al. (2020). Soluble PD-1: Predictive, prognostic, and therapeutic value for cancer immunotherapy. Front. Immunol. 2020;11:587460. doi: 10.3389/fimmu.2020.587460.

[36] Zhang J., Gao J., Li Y., et al. (2015). Circulating PD-L1 in NSCLC patients and the correlation between the level of PD-L1 expression and the clinical characteristics. Thorac Cancer, 6(4): 534-538. doi: 10.1111/1759-7714.12247.

[37] Okuma Y., Hosomi Y., Nakahara Y., et al. (2017). High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. Lung Cancer, 104: 1-6. doi: 10.1016/j.lungcan.2016.11.023.

[38] Costantini A., Julie C., Dumenil C., et al. (2018). Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. Oncoimmunology, 7(8): e1452581. doi: 10.1080/2162402X.2018.1452581.

[39] Murakami S., Shibaki R., Matsumoto Y., et al. (2020). Association between serum level soluble programmed cell death ligand 1 and prognosis in patients with non-small cell lung cancer treated with anti-PD-1 antibody. Thorac Cancer, 11(12): 3585-3595. doi: 10.1111/1759-7714.13721.

[40] Yumin Wu, Hui Guo, Jiawei Yue, et al. (2022). Serum sPD1 and sPDL1 as Biomarkers for Evaluating the Immune State of Lung Adenocarcinoma Patients. Journal of Immunology Research, Volume 2022, Article ID 9101912, doi.org/10.1155/2022/9101912.