



Prevalence of PD-L1 Expression in Patients with Advanced Non-Small Cell Lung Cancer Treated in Vajira Hospital

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Abstract

Objectives: Advanced non-small cell lung cancer (NSCLC) is one of the world-wide most common newly diagnosed cancer and cause of cancer deaths. Platinum-based chemotherapy was the backbone of management until an immune checkpoint inhibitor (ICI) as monotherapy or combination with chemotherapy is demonstrated survival benefits over chemotherapy. Program cell death ligand -1 (PD-L1) expression is still the best, even though not perfect biomarker of response to an ICI. This study was intended to determine the prevalence of PD-L1 expression among Thai advanced NSCLC patients.

Methods: The investigators performed a descriptive study of patients with advanced NSCLC who had formalin-fixed paraffin-embedded (FFPE) tissue specimens available, in January 1, 2016 to December 31, 2018, in the Department of Anatomical Pathology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University. The baseline patients' demographics were collected. The PD-L1 expression was determined by the PD-L1 IHC 22C3 pharmDx qualitative immunohistochemical assay. The primary endpoint was to assess the prevalence of PD-L1 expression (TPS \geq 1%) among patients with advanced NSCLC. The secondary endpoints were to find out its correlation with demographics and independent factors of 1-year survival.

Results: There were 79 NSCLC patients who had FFPE tissue specimens available. However, only specimens from 35 patients had adequate quality for PD-L1 analysis. Only three of them (8.6%) had PD-L1 expression. No baseline demographics was associated with its expression. ECOG performance status was the only independent determining factor of 1-year survival. (SR 75.42, p-value < 0.001)

Conclusion: Unexpected low prevalence of PD-L1 expression was demonstrated. The inadequacy of tissue specimen would be the obstacle of PD-L1 analysis. The investigators remind the clinicians that the proper amounts of tumor samplings are suggested for further biomarker analysis.

Keywords: PD-L1 expression, prevalence, advanced NSCLC



ความชุกของการสำแดง PD-L1 ในผู้ป่วยมะเร็งปอดระยะลุกลาม ที่รักษาในเวชพยาบาล

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บทคัดย่อ

วัตถุประสงค์: มะเร็งปอดเป็นมะเร็งที่พบได้บ่อยทั่วโลก และเป็นสาเหตุการตายจากมะเร็งในลำดับต้น ๆ เคมีบำบัดสูตรที่มีแพลตตินั่มเป็นการรักษาหลัก ปัจจุบันมียาที่ออกฤทธิ์กระตุ้นให้ระบบภูมิคุ้มกันจดจำเซลล์มะเร็งและฆ่ามะเร็งได้ (immune checkpoint inhibitor) การใช้ยาดังกล่าวเดี่ยว ๆ หรือร่วมกับเคมีบำบัดให้ผลการรักษาดีกว่าเคมีบำบัด และบางรายอาจมีผลตอบสนองยาวนาน การตรวจการสำแดงโปรตีน Program cell death ligand -1 (PD-L1) บนเซลล์มะเร็งมีส่วนในการทำนายผลการตอบสนองต่อยานี้ การศึกษานี้จึงศึกษาความชุกของการสำแดง PD-L1 ในผู้ป่วยมะเร็งปอดระยะลุกลามของคนไทย

วิธีดำเนินการวิจัย: การศึกษานี้เป็นการศึกษาวิเคราะห์จากการสังเกต ผู้ป่วยที่ศึกษาคือผู้ป่วยมะเร็งปอดชนิดเซลล์ไม่เล็กระยะลุกลามที่มีชิ้นเนื้อทางพยาธิวิทยาเก็บที่แผนกพยาธิวิทยาวิพยาบาลในช่วงวันที่ ๑ มกราคม ๒๕๕๙ ถึง ๓๑ ธันวาคม ๒๕๖๑ ผู้วิจัยเก็บข้อมูลพื้นฐานต่างๆ การตรวจหาการสำแดงโปรตีน PD-L1 ใช้วิธีย้อมด้วยแอนติบอดี 22C3 วัตถุประสงค์หลักของการศึกษาคือหาความชุกของการสำแดงโปรตีน PD-L1 วัตถุประสงค์รองคือหาปัจจัยที่สัมพันธ์กับการสำแดง และปัจจัยอิสระที่บ่งชี้การรอดชีวิตที่หนึ่งปี

ผลการวิจัย: ผู้ป่วยที่ตรงตามคุณลักษณะเกณฑ์เข้าของงานวิจัย มีทั้งหมด ๗๙ คน แต่มีผู้ป่วยเพียง ๓๕ คนที่มีชิ้นเนื้อเพียงพอต่อการวิเคราะห์ ผลพบว่าผู้ป่วยเพียงสามราย (ร้อยละ ๘.๖) ที่ตรวจพบการสำแดง ผู้วิจัยไม่พบว่ามีลักษณะพื้นฐานใดที่มีความสัมพันธ์กับการสำแดง ผู้ป่วยที่มีสภาพร่างกายดีเท่านั้นที่เป็นปัจจัยอิสระสัมพันธ์กับการรอดชีวิตที่หนึ่งปี

สรุปผลการวิจัย: ความชุกของการสำแดงโปรตีน PD-L1 ต่ำกว่าที่คาดมาก สาเหตุเป็นเพราะชิ้นเนื้อไม่เพียงพอต่อการวิเคราะห์ ผู้วิจัยแนะนำให้เห็นความสำคัญของการเก็บชิ้นเนื้อผู้ป่วยรายใหม่ให้มากเพียงพอต่อการวิเคราะห์หาตัวตอบสนองทางชีวภาพ

คำสำคัญ: การสำแดงพีดีแอล ความชุก มะเร็งปอดชนิดเซลล์ไม่เล็กระยะลุกลาม

Introduction

Lung cancer is the world-wide second most common newly diagnosed cancer, closely behind female breast cancer; however, it is the most common cause of cancer death according to Global Cancer Statistics 2020¹. In Thailand, lung cancer is the second most commonly newly diagnosed cancer and the second most common cause of cancer deaths behind liver cancers². The major breakthroughs in lung cancer treatment are the inventions of molecular-targeted therapies³. Lung cancer is right now divided into those with and without targetable mutations. Around half of the Asian-Pacific patients with pulmonary adenocarcinoma harbored an Epidermal growth factor receptor (EGFR) mutation⁴. An EGFR tyrosine kinase inhibitor led to nearly one-year progression free survival (PFS) and up to three-year overall survival (OS)⁵. The rest of the targetable mutations such as *ALK* rearrangements, *ROS1* rearrangements, *BRAF* mutations, *NTRK* fusions have specific efficacious drugs, as well³. On the other hand, among those without targetable mutations, there was a limited numbers of options beyond platinum-based chemotherapy. An immune checkpoint inhibitor (ICI) alone or in combination with platinum-based chemotherapy has been the new paradigm of management for a patient without targetable mutation. Compared to platinum-based chemotherapy, pembrolizumab, an anti-PD-1 antibody, alone or combined with platinum-based chemotherapy consistently led to longer OS. Moreover, it would result in uncommon but possible durable response⁶. However, the magnitude of survival benefit seemed to be most among those with higher Program cell death ligand -1 (PD-L1) expression. Many studies reported that PD-L1 expression, regardless of using pembrolizumab as monotherapy or combination therapy, was associated with an increased efficacy⁷⁻⁹. Furthermore, the efficacy of the ICIs besides pembrolizumab are also associated with PD-L1 expression as well¹⁰⁻¹¹. This study was intended to determine the prevalence of PD-L1 expression (as defined as TPS \geq 1%) among

Thai advanced non-small cell lung cancer (NSCLC) patients.

Methods

A descriptive study was performed in patients with advanced (stage IV according to American Joint Committee on Cancer (AJCC) 8th Edition¹² NSCLC, regardless of cell types with available documented clinical data and formalin-fixed, paraffin-embedded (FFPE) tumor tissues in the archive of Department of Anatomical Pathology, Faculty of Medicine Vajira Hospital. Primary objective of this study was to determine the prevalence of PD-L1 expression among Thai advanced NSCLC patients. The secondary one was to evaluate what clinical factors were associated with PD-L1 expression and independent factors of survival.

The PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in FFPE NSCLC tissue. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation. The Tumor Proportion Score (TPS) is the percentage of viable tumor cells showing partial or complete membrane staining (\geq 1+) relative to all viable tumor cells present in the sample (positive and negative). The specimen should be considered to have PD-L1 expression, if TPS \geq 1%¹³. The cost of the PD-L1 analysis were compassionately funded by the MSD Pharmaceutical Thailand. The study protocol had been approved by Navamindradhiraj University Ethical Committee. (COA 068/63)

The inclusion criteria are advanced NSCLC patients with age more than eighteen with available documented clinical data and FFPE tumor tissues in the archive of Department of Anatomical Pathology, Faculty of Medicine Vajira Hospital. The exclusion criteria is patients had other malignancy and/or was taken tissue to PD-L1 stained before.

Statistical analysis

In order to determine the prevalence, the calculated sample size, from the formular¹⁴,

$$n = \frac{Z_{\alpha/2}^2 p(1 - p)}{d^2}$$

n = sample size

$Z_{\alpha/2}$ = level of confidence according to the standard normal distribution (For a level of confidence of 95%, $\alpha = 0.05$ and $Z_{\alpha/2} = 1.96$)

d = tolerated margin of error (d = 0.07)

p = estimated proportion of the population based on the study by Sorensen et al.¹⁵

The prevalence of PD-L1 expression (p) = 0.75 based on the study by Sorensen et al.¹⁵ was 150 patients. The investigators collected demographic data including age, sex, stages at diagnosis (AJCC stage M1a vs M1b and M1c), histopathology, smoking status and ECOG performance status from the written and electronic medical records. The time since cancer diagnosis to death from any causes was also recorded. One-year survival was assessed. The descriptive statistics were reported as numbers and per cent or median and IQR as appropriate by Chi-square test. Fisher exact test was used to compare the demographic data between those with and without PD-L1 expression. Kaplan-Meier method was used to estimate the overall survival (OS) outcome. The 1-year survival rates among different groups of interest were calculated using log rank test and reported as per cent and 95% confidence interval (95% C.I.). Hazard ratio (HR) of 1-year survival between different groups of interest were calculated using Cox proportional hazard model.

Results

There were 79 NSCLC patients who had FFPE tissue specimens available in the Department of Anatomical Pathology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University. However, only specimens from 35 patients had sufficient quality for PD-L1 analysis. Sixteen of them were male, the rest (19 of 35) were female. Most of them (21, 60%) aged more than 60 years at NSCLC diagnosis. Due to Thailand's health care policy, all of the participants with fair to good ECOG performance status (from 0 to 2) were treated with platinum-based chemotherapy. A molecular targeted therapy, if eligible and reimbursable was prescribed as the later-line of treatment. The majority (30, 85.7%) had non-squamous NSCLC histology and had either M1b or M1c (32, 91.4%) stage at diagnosis. Around two-thirds (23, 65.7%) of them were never smokers. Nearly all of them (33, 94.3%) had ECOG performance status of 0-2. And nearly three-fourths (25, 71.4%) had 1-year survival. Those who had poor ECOG performance status (3-4) could not live beyond a year. The investigators found that only 3 (8.6%) had PD-L1 expression (TPS >1%). No association between PD-L expression and any baseline patients' demographics including sex, age, histology and smoking status was demonstrated (table 1).

Table 2 showed comparisons of the one-year survival rate in different patients' demographics and PD-L1 expression. The investigators found that only poor ECOG performance status (3-4) that correlated with significantly worse 1-year survival.

Regarding the predictive factor of survival, only ECOG performance status of 0-2 was associated with better 1-year survival. PD-L1 expression, sex, age, histology, stages, and smoking status were not correlated (table 3).

Table 1:

showed base-line patients' demographics and correlation with the PD-L1 expression

Variables	All Patients		PD-L1 Expression				p-value*
	n	(%)	Positive		Negative		
			n	(%)	n	(%)	
Overall	35	(100.0)	3	(8.6)	32	(91.4)	-
Sex							
Male	16	(45.7)	2	(66.7)	14	(43.8)	0.582
Female	19	(54.3)	1	(33.3)	18	(56.3)	
Age (years)							
<60 years	9	(25.7)	1	(33.3)	8	(25.0)	1.000
≥60 years	26	(74.3)	2	(66.7)	24	(75.0)	
Histology							
Non squamous cell cancer	30	(85.7)	3	(100)	27	(84.4)	0.668
Squamous cell cancer	5	(14.3)	0	(0.0)	5	(15.6)	
Stage							
M1a	3	(8.6)	0	(0.0)	3	(9.4)	1.000
M1b and M1c	32	(91.4)	3	(100)	29	(90.6)	
Smoking status							
Non-smoking	23	(65.7)	2	(66.7)	21	(65.6)	0.952
<20 packs-year	1	(2.9)	0	(0.0)	1	(3.1)	
≥20 packs-year	11	(31.4)	1	(33.3)	10	(31.3)	
Performance status							
0-2	33	(94.3)	3	(100)	30	(93.8)	1.000
3-4	2	(5.7)	0	(0.0)	2	(6.3)	
1-year survival							
No	10	(28.6)	1	(33.3)	9	(28.1)	1.000
Yes	25	(71.4)	2	(66.7)	23	(71.9)	

*Fisher exact test.

Table 2:

showed comparisons of the one-year survival rate among different patients' demographics and PD-L1 expression

Variables	1-year Survival		p-value*
	SR	95%CI	
Overall	71.11	(52.93 - 83.30)	-
PD-L1 Expression			
Negative	71.50	(52.34 - 84.04)	0.401
Positive	66.67	(5.41 - 94.52)	
Sex			
Male	68.75	(40.46 - 85.63)	0.243
Female	73.31	(47.23 - 87.94)	
Age at diagnosis (years)			
<60 years	63.49	(33.12 - 82.97)	0.617
≥60 years	76.19	(51.94 - 89.33)	
Histology			
Non squamous cell cancer	40.00	(5.20 - 75.28)	0.468
Squamous cell CA	76.39	(56.73 - 87.99)	
Stage			
M1a	66.67	(5.41 - 94.52)	0.513
M1b and M1c	71.50	(52.34 - 84.04)	
Smoking status			
Non-smoker	78.02	(54.97 - 90.22)	0.210
Smoker	58.33	(27.01 - 80.09)	
Performance status			
0-2	75.42	(56.78 - 86.88)	<0.001
3-4	0	(NR)	

Abbreviations: NR, data not report.

*Log-rank test.

Table 3:

Univariate and multi-variate analysis of overall survival.

Factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	p-value	HR _{adj}	95%CI	p-value
PD-L1 Expression						
Negative	1.00	Reference		1.00	Reference	
Positive	0.43	(0.06 - 3.24)	0.415	0.35	(0.04 - 3.01)	0.341
Sex						
Male	1.00	Reference		1.00	Reference	
Female	0.61	(0.26 - 1.41)	0.248	0.52	(0.12 - 2.18)	0.370
Age at diagnosis (years)						
<60 years	1.00	Reference		1.00	Reference	
≥60 years	0.80	(0.34 - 1.9)	0.618	0.85	(0.29 - 2.44)	0.759
Histology						
Squamous cell cancer	1.00	Reference		1.00	Reference	
Non squamous cell cancer	0.64	(0.18 - 2.18)	0.472	0.55	(0.14 - 2.12)	0.382
Stage						
M1a	1.00	Reference		1.00	Reference	
M1b and M1c	1.93	(0.26 - 14.46)	0.520	1.92	(0.22 - 16.68)	0.553
Smoking status						
Non-smoker	1.00	Reference		1.00	Reference	
Smoker	1.72	(0.73 - 4.07)	0.215	0.96	(0.21 - 4.45)	0.958
Performance status						
0-2	1.00	Reference		1.00	Reference	
3-4	41.38	(3.64 - 469.81)	0.003	34.08	(2.55 - 456.34)	0.008

Note: HR, Hazard Ratio; HR_{adj}, Adjusted Hazard Ratio; CI, confident interval.

Discussion

Even though this study could not recruit enough patients as determined by sample calculations due to limited budgets. The investigated demonstrated that most FFPE specimens for quantitating PD-L1 expression were less than adequate. This finding would remind the clinicians that large amount of tumor tissues is required for full investigation of biomarkers. Oncogene

alterations can be assessed from a microscopic tissue specimen, if adequate amount of undamaged tumor DNA is guaranteed. Moreover, detection of circulating cell-free DNA from a patient's blood has been widely available in routine clinical practice with comparable sensitivity and specificity to tissue testing¹⁶. However, an FFPE specimen for PD-L1 testing requires at least 100 viable tumor cells in a stained slide¹³.

Regarding the prevalence of PD-L1 expression, based on Keynote-189 study conducted in previously-untreated advanced non-squamous NSCLC, 63.4% of enrolled patients had PD-L1 TPS $\geq 1\%$ ⁷. According to Keynote-407 conducted in previously-untreated advanced squamous NSCLC, 63.3% of enrolled patients had PD-L1 TPS $\geq 1\%$ ⁸. Sensitizing *EGFR* and *ALK* mutations had to be excluded in all of the patients enrolled in clinical trials of immune checkpoint inhibitors, either monotherapy or combination with chemotherapy. Pooled analysis from Keynote-001, -010 and -024 found that around 67% of enrolled patients had PD-L1 TPS $\geq 1\%$. The prevalence of PD-L1 expression was similar in treatment-naïve and previously treated patients with advanced NSCLC. Despite missing data, the prevalence seemed to be similar across other demographic and disease characteristics examined¹⁷. Dietel, et al. reported the real-world prevalence of PD-L1 expression in locally advanced or metastatic NSCLC from centers across the world. Of 2617 patients who met inclusion criteria, 2368 (90%) had PD-L1 data; 1232 (52%) had PD-L1 TPS $\geq 1\%$, and 1136 (48%) had PD-L1 TPS $< 1\%$. The most common reason for not having PD-L1 data (n = 249) was insufficient tumor cells (< 100) on the slide (n = 170 [6%]). Prevalence of *EGFR* mutations (19%) and *ALK* alterations (3%) was consistent with prior reports from metastatic NSCLC studies. Again, the prevalence of both PD-L1 TPS $\geq 50\%$ and TPS $\geq 1\%$ among patients with advanced NSCLC was similar across geographic regions¹⁸. The investigators deduced that around two-thirds of advanced NSCLC patients without targetable mutations, especially *EGFR* and *ALK* had PD-L1 expression. On the contrary, the investigators showed only 8.6% of this cohort of patients had PD-L1 expression. The investigators could not explain this unexpected low prevalence. The high prevalence of *EGFR* mutations among Asian patients was presumably not an explanation. Azuma et al. revealed that the presence of *EGFR* mutations and

adenocarcinoma histology were significantly associated with increased PD-L1 expression¹⁹.

In terms of the PD-L1 expression as a prognostic survival, the investigators found no association in accordance with the report by Sorensen et al¹⁵. They did not demonstrate association between PD-L1 expression and OS when PD-L1 expression levels were stratified by either median or tertiles. The investigators noted that ECOG performance was still the strong independent prognostic factor of survival.

Limitations

Due to limited budgets of this study, the investigators could not recruit enough patients as planned. Moreover, insufficient amount of tumor cells in FFPE specimens was the major obstacle of PD-L1 analysis. All of the participants were treated with platinum-based chemotherapy as the first-line therapy. An *EGFR* TKI or other molecular-targeted therapies had not yet approved as the first-line treatment for every Thai patient at the time of cancer diagnosis. Further biomarker studies in NSCLC patients conducted in Thai patients is warranted.

Conclusion

Among Thai patients with advanced NSCLC, the PD-L1 expression was unexpected low (8.6%). Most of FFPE specimens were less than adequacy for determination of PD-L1 expression. In the era of the biomarker-directed management of lung cancer, obtaining the proper amounts of tumor samplings is the pre-requisite of cancer management, not only for cancer diagnosis, but also for guiding more efficacious treatments.

Conflict of interest

The authors declare no conflict of interest.

References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021;71(3):209-49.
- World Health Organization International Agency for Research on Cancer. GLOBOCAN 2020: Estimated Thailand's cancer incidence, mortality and prevalence in 2020 [Internet]. 2021 [cited 2021 May 9]. Available from: <http://gco.iarc.fr>
- Stinchcombe TE. Targeted Therapies for Lung Cancer. In: Reckamp K, editor. *Lung Cancer: treatment and Research*. Switzerland: Springer; 2016. p165-82.
- Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* 2014 ;9(2):154-62.
- Kim C, Liu SV. First-line EGFR TKI therapy in non-small-cell lung cancer: looking back before leaping forward. *Ann Oncol* 2019;30(12): 1852-5.
- Onoi K, Chihara Y, Uchino J, Shimamoto T, Morimoto Y, Iwasaku M, et al. Immune Checkpoint Inhibitors for Lung Cancer Treatment: A Review. *J Clin Med* 2020 6;9(5):1362.
- Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* 2018 ;378(22):2078-92.
- Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, et al. Pembrolizumab plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2018 22;379(21):2040-51.
- Mok TS, Wu Y-L, Kudaba I, Kowalski DM, Cho BC, Turna HZ et al. 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* 2019;393: 1819–30.
- Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* 2018; 378(24):2288-301.
- Park S, Choi YD, Kim J, Kho BG, Park CK, Oh IJ, Kim Y-C. Efficacy of immune checkpoint inhibitors according to PD-L1 tumor proportion scores in non-small cell lung cancer. *Thorac Cancer* 2019;11:408–14.
- Detterbec FC. The eighth edition TNM stage classification for lung cancer: What does it mean on main street? *J Thorac Cardiovasc Surg* 2018;155:356-9.
- Agilent Dako. PD-L1 IHC 22C3 pharmDx Interpretation Manual – NSCLC. For in-vitro diagnostic use [Internet] 2018 [cited 2021 May 9]. Available from: <http://www.agilent.com>
- Daniel WW. *Biostatistics : a foundation for analysis in the health sciences*. 6th ed. New York: Wiley, 1995.
- Sorensen SF, Zhou W, Dolled-Filhart M, Georgsen JB, Wang Z, Emancipator K, et al. PD-L1 Expression and Survival among Patients with Advanced Non-Small Cell Lung Cancer Treated with Chemotherapy. *Transl Oncol* 2016; 9:64-9.
- Scilla KA, Rolfo C. The Role of Circulating Tumor DNA in Lung Cancer: Mutational Analysis, Diagnosis, and Surveillance Now and into the Future. *Curr Treat Options Oncol* 2019;20(7):61.

17. Aggarwal C, Abreu DR, Felip E, Carcereny E, Gottfried M, Wehler T, et al. Prevalence of PD-L1 expression in patients with non-small cell lung cancer screened for enrollment in KEYNOTE-001,-010, and-024(1060p). *Ann oncol* 2016;27 Suppl 6:vi359-78.
18. Dietel M, Savelov N, Salanova R, Micke P, Biigras G, Hida T, et al. Real-world prevalence of programmed death ligand 1 expression in locally advanced or metastatic non-small-cell lung cancer: The global, multicenter EXPRESS study. *Lung Cancer* 2019;134:174-9.
19. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann Oncol* 2014;25(10):1935-40.