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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

The association of clinicopathologic features and peripheral blood parameters with high PD-L1 expression in non-small cell lung cancer

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ABSTRACT

The association of clinicopathologic features and peripheral blood parameters with high PD-L1 expression in non-small cell lung cancer

Introduction: Programmed death ligand 1 (PD-L1) is a marker that widely used for prediction of response to immunotherapy. Dynamic alteration of PD-L1 expression are the major problems for reflection of the actual status of the PD-L1. So, we aimed to investigate the factors that may be associated with PD-L1 expression in lung cancer.

Materials and Methods: The patients diagnosed with non-small cell lung cancer were enrolled, retrospectively. The patients were stratified according to PD-L1 expression level as $\geq 50\%$ and $< 50\%$.

Results: Totally, 217 patients were enrolled. The clinicopathologic features were similar between two groups, except the amount of cigarette consumption. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, and systemic immune-inflammotry index were found significantly lower in PD-L1 $\geq 50\%$ ($p < 0.001$, $p = 0.006$ and $p = 0.003$, respectively) and also negatively correlated with PD-L1 level ($\rho = -0.255$, $p < 0.001$; $\rho = -0.17$, $p = 0.013$; $\rho = -0.185$, $p = 0.006$, respectively).

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Conclusion: According to the results of our study, peripheral blood parameters can be used to the prediction of the high PD-L1 expression and can be used for reflection of current PD-L1 expression.

Key words: PD-L1; NLR; PLR; SII, immune check-point inhibitors

ÖZ

Küçük hücreli dışı akciğer kanserinde yüksek PD-L1 düzeyi ile ilişkili klinikopatolojik faktörlerin ve periferik kan parametrelerinin belirlenmesi

Giriş: “Programmed death ligand 1 (PD-L1)” immünoterapilere yanıtı predikte etmek için yaygın olarak kullanılan bir belirteçtir. Bununla birlikte PD-L1 düzeyinin dinamik değişimi gerçek zamanlı PD-L1 düzeyinin yansıtılması için büyük bir problemdir. Bu sebeple bu çalışmada akciğer kanseri tanısı olan hastalarda PD-L1 düzeyi ile ilişkili olabilecek faktörlerin saptanması amaçlanmıştır.

Materyal ve Metod: Küçük hücreli dışı akciğer kanseri tanısı olan hastalar retrospektif olarak çalışmaya dahil edildi. Hastalar PD-L1 ekspresyon düzeyine göre $< 50\%$ ve $\geq 50\%$ olmak üzere ikiye ayrıldı.

Bulgular: Toplam 217 hasta çalışmaya dahil edildi. Her iki grubun sigara tüketimi dışında klinikopatolojik özellikleri benzerdi. PD-L1 düzeyi $\geq 50\%$ olan grupta nötrofil lenfosit oranı, platelet lenfosit oranı ve sistemik immün inflamatuvar indeks anlamlı olarak daha düşük saptandı (sırasıyla; $p < 0.001$, $p = 0.006$ ve $p = 0.003$). Aynı zamanda bu parametreler PD-L1 ekspresyon düzeyi ile negatif korele olarak saptandı (sırasıyla, $\rho = -0.255$, $p < 0.001$; $\rho = -0.17$, $p = 0.013$; $\rho = -0.185$, $p = 0.006$).

Sonuç: Bizim çalışma sonuçlarımıza göre periferik kan parametreleri PD-L1 düzeyini predikte etmek için kullanılabilir ve böylece güncel PD-L1 ekspresyon düzeyi yansıtılabilir.

Anahtar kelimeler: PD-L1; NLO; PLO; SII; immün kontrol noktası inhibitörleri

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common cancer, worldwide (1). In the past, treatment options for NSCLC were limited with only chemotherapy. However, many therapeutic advances have occurred in the last years. Immune checkpoint blockade is one of the most exciting advances for the treatment of most cancer types including NSCLC. Programmed cell death ligand-1 (PD-L1), programmed cell death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) inhibitors are the agents that used for the treatment of many cancer types. Immune checkpoint inhibitors (ICIs) demonstrating their efficacy by inhibiting to binding of PD-1 and CTLA-4 with their ligands. Ultimately, inhibition of these interactions activate the host immune system and promote immune-mediated elimination of tumor cells (2). Today, pembrolizumab and nivolumab, as PD-1 inhibitors; atezolizumab, durvalumab and avelumab, as PD-L1 inhibitors and ipilimumab as CTLA-4 inhibitor are the agents that showed efficacy with phase 3 clinical trials in NSCLC (3-8). Despite the success of ICIs, only a small fraction of the patients benefit from these agents. Therefore, the search for a biomarker that predicts the response of the ICIs has begun and many biomarkers were detected for prediction of the response like PD-L1, tumor mutational burden (TMB) and effector T cell signature.

PD-L1 is the most researched and widely used biomarker for prediction of the response to PD-1 and PD-L1 monoclonal antibodies. PD-L1 is expressed on the surface of the several types of the tumor as well as on tumor-infiltrating immune cells in the tumor microenvironment. PD-L1 expression is measured by using the tumor proportion score (TPS). In the clinical trials that investigated the efficacy of ICIs, anti PD-1 and PD-L1 therapy were found that effective in patients with both PD-L1 positive (TPS $\geq 1\%$) or negative (TPS $< 1\%$). However, the efficacy was found statistically superior in the PD-L1 positive group than negative (9). Especially, the results of the clinical trials were also suggested that the high expression level of PD-L1 (TPS $\geq 50\%$) can strongly predict the response of ICIs in NSCLC (3,10). Intratumoral heterogeneity and dynamic alteration of PD-L1 expression are the major problems for reflection of the actual status of the PD-L1 expression level (11). In the literature, there are many trials that evaluated the correlation between PD-L1 level and various markers including cytokines, transcription factors, tumor-infiltrating lymphocytes density or clinicopathological features in various malignancy (12-14). However, the methods for analyses of these factors are expensive and difficult to access for a daily routine investigation. Because of that, we also aimed to investigate the association between high PD-L1 expression and clinicopathologic features and also peripheral blood

parameter which is a non-invasive, easily accessible-assessable and also can be reflected host inflammatory status.

MATERIALS and METHODS

The patients who diagnosed with advanced NSCLC and admitted to Ankara Atatürk Chest Disease and Chest Surgery Training and Research Hospital and Ankara Yildirim Beyazit University Hospital in 2018 and 2019 were enrolled. The patient's records were obtained by using the electronic database of the hospital, retrospectively. Inclusion criteria were follow; (1) the patients with more than 18 years old, (2) the patients who had metastatic or local advance that non-eligible for curative treatment and had measurable disease as assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1., (3) the patients who was known PD-L1 expression level at the time of diagnosis. Exclusion criteria of our study were also followed; (1) The patients who had active infection, (2) the patients with received drugs that can be affect blood parameters, (3) the patients who had blood product transfusion within 1 month before enrolment, (4) the patients who had not complete data.

Before PD-L1 analyses, all pathologic samples were routinely assessed with the histo-immunochemistry examination. If the tumor sample was adequate for advance examination, PD-L1 analyses were performed in the experienced laboratory. PD-L1 expression was assessed in formalin-fixed tumor samples obtained at the diagnosis with the use of the available PD-L1 IHC 22C3 pharmDx assay (Dako North America). The PD-L1 expression level was measured by TPS which described as the percentage of viable tumor cells showing partial or complete membrane staining, relative to all viable tumor cells. The level of the PD-L1 TPS $\geq 50\%$ was identified as a high PD-L1 expression level. In addition, the patients were stratified according to PD-L1 expression as PD-L1 TPS of 50% or greater and less than 50%.

Complete blood count (CBC) parameters, albumin, globulin, LDH, CRP levels were used for the analysis of the relationship between PD-L1 and peripheral blood parameters. Neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR) were calculated as followed: neutrophil/lymphocytes and platelet/lymphocyte, respectively. The systemic immune-inflammation index (SII) was also calculated by using the formula as follow: (Neutrophil x

Platelets)/Lymphocyte. All of the blood samples were obtained at the diagnosis and before the initiation of any treatment.

Statistical analyses were performed using the SPSS software version 23. Categorical variables were compared using the Chi-square or Fisher's exact test, where appropriate. The variables were investigated using visual (histogram, probability plots) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Mann-Whitney U test was used to compare non-normal disturbed and ordinal variables with the groups with PD-L1 $< 50\%$ and $\geq 50\%$. While investigating the association between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A 5% Type-I error level was used to infer statistical significance. A p value of less than 0.05 was considered to show a statistically significant result.

Local Clinical Research Ethics Committee's approval was obtained.

RESULTS

Totally, 217 patients were enrolled to study. The median age of patients was 63 years (min-max; 24-89). The rate of male and female patients were 82.9% and 17.1%, respectively. The vast majority of patients had adenocarcinoma histology (64.2%). Subsequently, 26.5% and 9.3% of patients had squamous cell carcinoma (SCC) and non-otherwise specified (NOS) histology. At the time of diagnosis, 68.7% of patients were metastatic and 31.3% of patients were also local advance (Stage 3A-3C). The rate of the active smoker, former smoker, and non-smoker patients were 33.6%, 54.2%, and 12.1%, respectively. The median amount of cigarette consumption was 40 packages/year in patients who were active smokers and former smokers. A hundred-eleven of 217 (51.2%) patients were PD-L1 TPS $< 1\%$. The forty-five of 217 patients (20.7%) were PD-L1 TPS 1-49% and 61 of whom (28.1%) were also PD-L1 TPS $\geq 50\%$.

When the whole group separated into two groups according to PD-L1 TPS as $\geq 50\%$ and $< 50\%$; 28.1% of patients were in TPS of 50% or greater and 71.9% of patients were also in TPS of less than 50%. The baseline clinicopathologic feature was statistically similar between the two groups, except the amount of smoking consumption. The median amount of

smoking consumption was statistically higher in TPS of 50% or greater compared with the TPS of less than 50%. Detailed data were shown in Table 1.

In univariate analyses for the association between PD-L1 expression and peripheral blood parameters; NLR, PLR, and SII were found significantly different between TPS $\geq$ 50% and $<$ 50%. NLR, PLR and SII were significantly lower in patients with TPS $\geq$ 50% than $<$ 50%. Detailed data of analyses were shown in

Table 2. When evaluated the correlation between PD-L1 TPS and the parameter which were found significantly related to PD-L1 TPS expression in univariate analyses. NLR, PLR and SII were found negatively correlated with PD-L1 TPS expression ($\rho = -0.255$, $p < 0.001$ for NLR and PD-L1 TPS; $\rho = -0.17$, $p = 0.013$ for PLR and PD-L1 TPS, $\rho = -0.185$, $p = 0.006$ for SII and PD-L1 TPS). The graphical view of the correlation of NLR, PLR, and SII with PD-L1 TPS were shown in Figure 1,2 and 3, respectively.

Table 1. The association of PD-L1 TPS expression and clinicopathologic feature

Parameter	PD-L1 TPS < 50%	PD-L1 TPS $\geq$ 50%	p
Age (median, min-max)	63 (24-85)	64 (31-89)	0.32
Sex (M/F)	126/30	53/8	0.13
Histologic subtype			0.32
Adenocarcinoma	103	36	
SCC	40	18	
NOS	12	8	
Smoking status			0.17
Active smoker	47	27	
Former smoker	87	30	
Non-smoker	21	5	
Blood group			
A	60	23	
B	15	7	0.30
AB	11	2	
O	42	8	
The cigarette consumption (median, package/year)	40	50	< 0.001
Current stage			0.30
Metastatic	105	44	
Local advance	51	17	

M: Male, F: Female, SCC: Squamous cell carcinoma, NOS: Non-otherwise specified, TPS: Tumor proportional score.

Table 2. The association of PD-L1 TPS expression and peripheral blood parameters

Parameter	PD-L1 TPS < 50% (median)	PD-L1 TPS $\geq$ 50% (median)	p
Hemoglobin (g/dL)	13.2	12.90	0.66
RDW	14.6	14.7	0.41
LDH (mg/dL)	214	215.5	0.84
CRP (mg/dL)	10.5	7.06	0.28
Albumin (mg/dL)	3.80	3.83	0.69
NLR	3.93	2.73	< 0.001
PLR	181.6	151.1	0.006
SII	1239.5	1009.2	0.003

RDW: Red cell distribution width, CRP: C-reactive protein, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, SII: Systemic immune-inflammation index, TPS: Tumor proportional score.

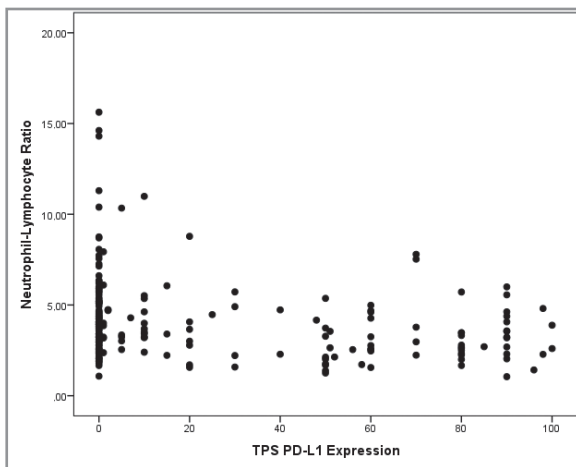


Figure 1. The correlation between NLR and PD-L1 TPS expression ($\rho = -0.255$, $p < 0.001$).

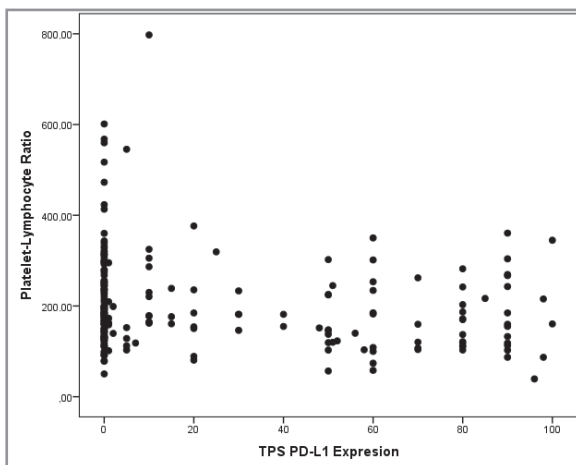


Figure 2. The correlation between PLR and PD-L1 TPS expression ($\rho = -0.17$, $p = 0.013$).

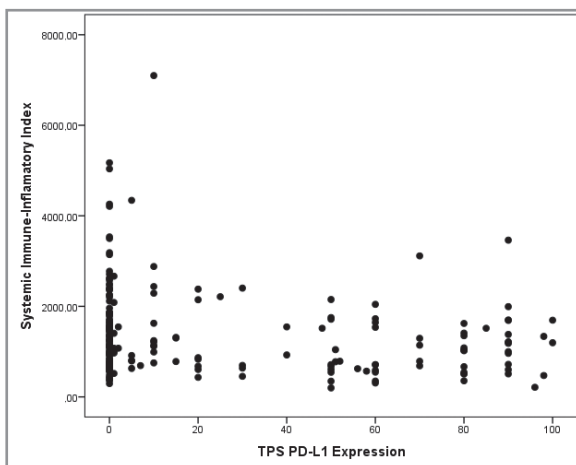


Figure 3. The correlation between SII and PD-L1 TPS expression ($\rho = -0.185$, $p = 0.006$).

DISCUSSION

In our study that evaluated the association between PD-L1 expression and clinicopathologic features and peripheral blood parameters in NSCLC, 28.1% of the patients had PD-L1 TPS expression $\geq 50\%$. When compare clinicopathologic features between the patients with PD-L1 TPS $< 50\%$ and $\geq 50\%$, the only amount of cigarette consumption was found significantly different. In patients with TPS $\geq 50\%$, the median cigarette consumption was significantly higher than patients with TPS $< 50\%$. The other clinicopathologic features were similar between the two groups. In addition, NLR, PLR, and SII were found significantly low in patients with PD-L1 TPS $\geq 50\%$. Also, NLR, PLR, and SII were found significantly negatively correlated with PD-L1 TPS.

In previous trials, superior outcomes were found with ICIs that targeted PD-1 and PD-L1 in the NSCLC patients with PD-L1 TPS $\geq 50\%$ (3,7,10). In addition, current guidelines suggested immune checkpoint inhibitor as the first treatment option for the NSCLC with PD-L1 TPS $\geq 50\%$. The prevalence of the patients with PD-L1 expression of 50% or greater were found between 16%-40% in the previous phase 3 trials (15). In addition, according to preliminary results of the global, multicentre EXPRESS trial that investigated the prevalence of PD-L1 expression in the real world, 540 of 2435 patients (22%) were found that PD-L1 TPS $\geq 50\%$ (16). In our trial, we found that the prevalence of the PD-L1 TPS $\geq 50\%$ was 28.1%. When compared with the result of the EXPRESS trial, the rate of TPS $\geq 50\%$ is higher in our trial. This may be the result of the geographic/ethnic difference of PD-L1 expression or the low number of patients in our study than the EXPRESS trial.

There are a limited number of trials that investigated the clinicopathologic factors related to PD-L1 expression in NSCLC. According to a recently published trial by Pawelczyk et al., sex, smoking status, lymph node metastasis, Ki-67 proliferation index and tumor grade were found significantly different between the group with PD-L1 TPS $< 1\%$, 1-49%, and $\geq 50\%$. The presence of lymph node metastasis, higher tumor grade, active or former smoking were found that related to TPS of 50% or greater. Additionally, the Ki-67 proliferation index was found low positively correlated with PD-L1 expression (17). In another trial that accepted threshold for PD-L1 expression as 5%; smoking status, male gender, adenocarcinoma

histology and absence of driver mutation were found related to PD-L1 expression in NSCLC (18). In our study, we found that the amount of cigarette consumption was significantly higher in a group with PD-L1 TPS $\geq 50\%$. However, there was no difference in smoking status, histologic subtypes, and tumor stage between the patients with PD-L1 TPS $<50\%$ and $\geq 50\%$. In contrast to previous trials, despite the active or former smoker patients were numerically higher in the group with PD-L1 TPS $\geq 50\%$, smoking status was not found significantly related with higher PD-L1 expression. This finding may be related to the low number of non-smoker patients in our trial, especially in the group with PD-L1 TPS $\geq 50\%$. On the other hand, the higher amount of cigarette consumption was found significantly related to high PD-L1 expression. It is known that smoking is linked to the expression of neoantigens and increased numbers of somatic mutations in NSCLC (19). Therefore, the higher amount of cigarette consumption may be related to high PD-L1 expression. But, the exact mechanism of these findings is not clear.

In our knowledge, this is the first study that evaluated the relationship between high PD-L1 expression level and peripheral blood parameters. We found that NLR, PLR, and SII were significantly lower in the group with PD-L1 TPS $\geq 50\%$ and also NLR, PLR and SII were found negatively correlated with PD-L1 expression. It is known that the two main mechanisms are contemplated which may be associated with PD-L1 upregulation; oncogene-mediated PD-L1 expression and immunity-dependent PD-L1 expression. It is a hypothesis that immunity-dependent PD-L1 upregulation plays a more critical role in the suppression of host immunity (9). The cytokines like interferon-gama (IFN- γ) and interleukin-6 (IL-6) play a critical role for PD-L1 upregulation through the JAK-STAT pathway (20,21). IFN- γ is produced by tumor-infiltrating lymphocyte (TIL), and it can up-regulate PD-L1 expression via induced JAK1/JAK2-STAT1/STAT2/STAT3-IRF1 axis, subsequently (21). Another function of IFN- γ is that induce clonal expansion and activation of CD4 and also CD8 T lymphocytes (22). IL-6 is another cytokine that contributes to the up-regulation of PD-L1 expression. Like IFN- γ , IL-6 can promote proliferation and differentiation of lymphocytes through the various mechanisms (23). Increased production of cytokines like IFN- γ , and IL-6 due to cancer development may be induced both of PD-L1 expression and lymphocyte

production, probably. Therefore, the low level of NLR, PLR, and SII due to increased lymphocyte count may be related to high PD-L1 expression. However, the exact mechanism of this relationship is not clearly known.

In previous clinical studies with a limited number, NLR was found as a prognostic marker for NSCLC which treated with PD-1 monoclonal antibodies. According to the results of retrospective trials that evaluated the prognostic value of NLR in NSCLC patients with not known PD-L1 expression status and treated with nivolumab, the lower level of NLR was found that related to high PFS, OS, and ORR (24,25). The results of these studies may be compatible with our study that was found the lower level of NLR was related to high PD-L1 expression. Another study that evaluated the prognostic effect of dynamic change of NLR in advance cancer patients that treated with PD-1/PD-L1 inhibitors, the decreasing of NLR after two courses of ICIs was found that related to the better outcome (26). In the study that investigated relationships between the dynamic change of PD-L1 expression and response to PD-1 blockade in melanoma, tumoral and macrophage PD-L1 expression was found higher in responders than non-responders in the biopsy that performed within 2 months of commencing treatment (27). According to the results of these trials, NLR and PLR that were shown a correlation with PD-L1 expression in our study may be used as a marker to predict the response.

The retrospective design and relatively low number of patient enrollment were the major limitation of our study. However, in our knowledge, this is the first study that evaluated the relationship between high PD-L1 expression and peripheral blood parameters. Based on the results of our study; NLR, PLR, and SII can be used to the prediction of the high PD-L1 expression level. In addition, NLR, PLR and SII, easily accessible-assessable and also cheap markers, can be used for reflection of current PD-L1 expression status in during the treatment with immune checkpoint inhibitors that targeted to PD-1/PD-L1. However, the results of our study must be validated with advanced studies with a large patient population.

CONFLICT of INTEREST

The authors indicated no potential conflicts of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: BB, ŞY, ÜY

Analysis/Interpretation: BB, MAŞ

Data Acquisition: BB, MH, MBA, ŞY, ÜY

Writting: BB, ŞY

Critical Revision: MAŞ, BY

Final Approval: BB

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019;69:7-34.
2. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. *Exp Mol Med* 2018;50:165.
3. 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;378:2288-301.
4. 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;378:2078-92.
5. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015;373:123-35.
6. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015;373:1627-39.
7. Barlesi F, Vansteenkiste J, Spigel D, Ishii H, Garassino M, de Marinis F, et al. Avelumab versus docetaxel in patients with platinum-treated advanced non-small-cell lung cancer (JAVELIN Lung 200): an open-label, randomised, phase 3 study. *Lancet Oncol* 2018;19:1468-79.
8. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018;379:2342-50.
9. Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. *BMJ* 2018;362:k3529.
10. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375:1823-33.
11. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Mol Cancer* 2018;17:129.
12. Kitano A, Ono M, Yoshida M, Noguchi E, Shimomura A, Shimoi T, et al. Tumour-infiltrating lymphocytes are correlated with higher expression levels of PD-1 and PD-L1 in early breast cancer. *ESMO Open* 2017;2:e000150.
13. Chang LC, Chen TP, Kuo WK, Hua CC. The protein expression of PDL1 is highly correlated with those of eIF2alpha and ATF4 in lung cancer. *Dis Markers* 2018;2018:5068701.
14. Chen Y, Zhang Y, Chai X, Gao J, Chen G, Zhang W, et al. Correlation between the expression of PD-L1 and clinicopathological features in patients with thymic epithelial tumors. *Biomed Res Int* 2018;2018:5830547.
15. Teixeira C, Vilarino N, Reyes R, Reguart N. PD-L1 expression testing in non-small cell lung cancer. *Ther Adv Med Oncol* 2018;10:1758835918763493.
16. Dietel M, Savelov N, Salanova R, Mücke P, Bigras G, Hida T, et al. 130O Real-world prevalence of PD-L1 expression in locally advanced or metastatic non-small cell lung cancer (NSCLC): the global, multicentre EXPRESS study. *J Thorac Oncol* 2018;13:S74-S75.
17. Pawelczyk K, Piotrowska A, Ciesielska U, Jablonska K, Gletzel-Plucinska N, Grzegorzolka J, et al. Role of PD-L1 expression in non-small cell lung cancer and their prognostic significance according to clinicopathological factors and diagnostic markers. *Int J Mol Sci* 2019;20.
18. Petrelli F, Maltese M, Tomasello G, Conti B, Borgonovo K, Cabiddu M, et al. Clinical and molecular predictors of PD-L1 expression in non-small-cell lung cancer: systematic review and meta-analysis. *Clin Lung Cancer* 2018;19:315-22.
19. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124-8.
20. Mimura K, Teh JL, Okayama H, Shiraishi K, Kua LF, Koh V, et al. PD-L1 expression is mainly regulated by interferon gamma associated with JAK-STAT pathway in gastric cancer. *Cancer Sci* 2018;109:43-53.
21. Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Rep* 2017;19:1189-201.
22. Reed JM, Branigan PJ, Bamezai A. Interferon gamma enhances clonal expansion and survival of CD4+ T cells. *J Interferon Cytokine Res* 2008;28:611-22.
23. Yang R, Masters AR, Fortner KA, Champagne DP, Yanguas-Casas N, Silberger DJ, et al. IL-6 promotes the differentiation of a subset of naive CD8+ T cells into IL-21-producing B helper CD8+ T cells. *J Exp Med* 2016;213:2281-91.
24. Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, et al. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. *Lung Cancer* 2017;111:176-81.

25. Bagley SJ, Kothari S, Aggarwal C, Bauml JM, Alley EW, Evans TL, et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. *Lung Cancer* 2017;106:1-7.
26. Moschetta M, Uccello M, Kasenda B, Mak G, McClelland A, Boussios S, et al. Dynamics of neutrophils-to-lymphocyte ratio predict outcomes of PD-1/PD-L1 blockade. *Biomed Res Int* 2017;2017:1506824.
27. Vilain RE, Menzies AM, Wilmott JS, Kakavand H, Madore J, Guminski A, et al. Dynamic changes in PD-L1 expression and immune infiltrates early during treatment predict response to PD-1 blockade in melanoma. *Clin Cancer Res* 2017;23:5024-33.