research article

PD-L1 expression in squamous-cell carcinoma and adenocarcinoma of the lung

Urska Janzic[1], Izidor Kern[2], Andrej Janzic[3], Luka Cavka[4], Tanja Cufer[1]

[1] Department of Medical Oncology, University Clinic Golnik, Golnik, Slovenia
[2] Department of Pathology, University Clinic Golnik, Golnik, Slovenia
[3] University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia
[4] Department of Medical Oncology, Institute of Oncology, Ljubljana, Slovenia

Radiol Oncol 2017; 51(3): 357-362.

Received 27 April 2017
Accepted 9 August 2017

Correspondence to: Urska Janzic, M.D., Department of Medical Oncology, University Clinic Golnik, Golnik 36, 4204 Golnik, Slovenia. Phone: +386 4 1240 266; Fax: +386 4 256 9174; E-mail: urska.janzic@klinika-golnik.si

Disclosure: No potential conflicts of interest were disclosed.

Background. With introduction of immunotherapy (IT) into the treatment of advanced non-small-cell lung cancer (NSCLC), a need for predictive biomarker became apparent. Programmed death ligand 1 (PD-L1) protein expression is most widely explored predictive marker for response to IT. We assessed PD-L1 expression in tumor cells (TC) and immune cells (IC) of squamous-cell carcinoma (SCC) and adenocarcinoma (AC) patients.

Patients and methods. We obtained 54 surgically resected tumor specimens and assessed PD-L1 expression by immunohistochemistry after staining them with antibody SP142 (Ventana, USA). Clinicopathological characteristics were acquired from the hospital registry database. Results were analyzed according to cut-off values of ≥ 5% and ≥ 10% of PD-L1 expression on either TC or IC.

Results. 29 (54%) samples were AC and 25 (46%) were SCC. PD-L1 expression was significantly higher in TC of SCC compared to AC at both cut-off values (52% *vs.* 17%, $p = 0.016$ and 52% *vs.* 14%, $p = 0.007$, respectively) no difference in PD-L1 expression in IC of SCC and AC was found. In AC alone, PD-L1 expression was significantly higher in IC compared to TC at both cut-off values (72% *vs.* 17%, $p < 0.001$ and 41% *vs.* 14%, $p = 0.008$, respectively), while no significant difference between IC and TC PD-L1 expression was revealed in SCC.

Conclusions. Our results suggest a significantly higher PD-L1 expression in TC of SCC compared to AC, regardless of the cut-off value. PD-L1 expression in IC is high in both histological subtypes of NSCLC, and adds significantly to the overall positivity of AC but not SCC.

Key words: lung cancer; squamous-cell lung cancer; adenocarcinoma; tumor cells; immune cells; PD-L1 expression

Introduction

Immunotherapy with checkpoint inhibitors (CPIs) is becoming a new standard of treatment for metastatic non-small cell lung cancer (NSCLC) patients. Thus far, three agents, two anti-PD-1 inhibitors and one PD-L1 inhibitor, have proven antitumor efficacy in terms of improved response rates and overall survival compared to standard chemotherapy in the second-line setting, namely nivolumab, pembrolizumab and atezolizumab.[1-6] Moreover, pembrolizumab also showed survival advantage over standard chemotherapy in the first-line setting, while nivolumab failed to deliver the same, the main difference probably being patient selection criteria in the clinical trials.[7,8] Patients that benefit from immune checkpoint inhibitors have durable responses with mild toxicities that are mostly immune-related. However, only about 20% of unselected NSCLC patients actually respond to these therapies.[9,10]

Until now, PD-L1 (programmed death ligand 1) protein expression determined by immunohistochemistry (IHC) has been most widely explored as a putative predictive biomarker for response to CPIs in cancer, also in NSCLC. The PD-L1 expression can be explored on tumor cells (TC) and or tu-

mor-infiltrating immune cells (IC).[10-12] In NSCLC, PD-L1 expression was mainly evaluated on TC, while data on PD-L1 expression on IC is scarce and its importance is yet to be validated.[10,13-16] In general, higher PD-L1 expression correlated with higher overall response rates (ORRs) and consequently better overall survival (OS) across majority of clinical trials. This was demonstrated in clinical trials assessing activity of pembrolizumab and atezolizumab in histologically unselected NSCLC and nivolumab in non-squamous lung carcinoma (non-SCC).[2-6] On the contrary, in the trial studying nivolumab activity exclusively in squamous-cell lung carcinoma (SCC) no major differences in efficacy were observed regarding PD-L1 expression.[1] So there seems to be a vital difference between PD-L1 expressions in major subtypes of NSCLC, which makes them react differently to CPIs. One possible reason is that mutational burden is probably higher in patients with SCC, which might be related to their smoking status.[17,18]

PD-L1 expression determination is also subjected to antibody clone, assay platform and cut-off values used in a particular study. In drug development programs, specific diagnostic tests, including antibody clone and staining platform have been used and validated for each particular PD-L1/PD-1 inhibitor.[10-12] Substitutability of these tests and antibodies is still uncertain. Harmonization trials addressing the question of interchangeability between them showed no major differences considering certain antibodies with only outlying of SP142 antibody being less sensitive for TC, but not for IC staining, compared to other antibodies.[13-16] The importance of tissue specimen selection seems to be vital, since data suggest that PD-L1 expression in tumor tissue is indeed heterogeneous and small biopsy specimens showed lower PD-L1 positivity compared to surgical resection specimens.[19]

Within this research, we studied PD-L1 expression in tumor resection specimens, in TC and IC, of two most common NSCLC histology subtypes - adenocarcinoma (AC) and SCC.

Patients and methods

Patient selection

This prospective study was conducted on surgical specimens from patients with primary operable NSCLC, diagnosed and treated at the University Clinic Golnik from 2006–2015. The specimens were collected and stored at the Laboratory of pathology at the same clinic. Tumor specimens of consecutive patients diagnosed with squamous-cell carcinoma and adenocarcinoma were included.

Patient characteristics

Baseline clinicopathological characteristics, such as age at diagnosis, sex and smoking status were obtained from the University Clinic Golnik hospital lung cancer registry database.

Smoking status categories were divided into current smoker, never smoker and former smoker, the latter defined as a person that quit smoking more than a year before the initial diagnosis of lung cancer.

This study was conducted according to the Declaration of Helsinki and was approved by National Ethics Committee (approval number 40/04/12).

Tumor tissue

The specimens were formalin-fixed, paraffin embedded (FFPE), sliced into 4 μm sections and stained for PD-L1 with a rabbit monoclonal antibody SP142 (Ventana/Roche, USA) on an automated platform (Benchmark, Ventana/Roche, USA). Three independent investigators (I.K., U.J. and L.C.) examined whole slices without prior knowledge of the clinicopathological features of the patients. The presence of IC (yes/no) was evaluated for each individual specimen. Percentage of PD-L1 positive immunohistochemical reaction was evaluated in TC and IC, ranging from 0–100%, regardless of staining intensity. The staining of TC on the cell membrane was regarded as positive, whereas IC showed PD-L1 positive reaction in the cytoplasm. Human placenta was also immunostained as a control tissue for PD-L1 expression. The results were then statistically analyzed for two preplanned cut-off values, namely 5% or higher and 10% or higher PD-L1 expression in either TC or IC.

Statistical considerations

Statistical analyses were performed using SPSS v22 software. Inter-rater agreement of PD-L1 expression in TC and IC was evaluated using Fleiss kappa. PD-L1 expression in either TC or IC was evaluated for unconditional association with the dependent variables using a Chi-square test for categorical data and t-test for continuous data. Association between expression in TC and IC was evaluated with McNemar test. In all analyses the p value of < 0.05 was considered statistically significant.

Results

A total of 54 tumor samples were examined, 29 of them (54%) were AC and 25 (46%) were SCC. The majority of samples were retrieved from male patients 34 (63%) and 20 (37%) from female. The mean (SD) age at diagnosis was 62.4 (8.6) years. Most of the patients were smokers (46%) or ex-smokers (39%). Patient characteristics are listed in Table 1.

PD-L1 positive reaction in either TC or IC is shown in Figure 1. In TC, the expression of PD-L1 was equal or higher than 10% in 17 samples, between 5% and 10% in only 1 sample, lower than 5% in 14 samples and completely absent in 22 samples. The inter-rater agreement in this case was almost perfect ($\kappa = 0.89$; $p < 0.001$ at 5% cut-off value). Significantly higher rates of TC PD-L1 positivity were determined in SCC than in AC, at both 5% and 10% cut-off values (52% *vs.* 17%; $p = 0.016$ and 52% *vs.* 14%; $p = 0.007$, respectively). The proportion of PD-L1 positivity in TC of AC and SCC is depicted in Figure 2.

Compared to the expression in TC, PD-L1 was more often present in IC. PD-L1 expression in IC was equal or higher than 10% in 28 samples, between 5% and 10% in 12 samples, lower than 5% in 6 samples and completely absent in 8 samples. In this case the inter-rater agreement was lower as in the determination of TC ($\kappa = 0.12$; $p = 0.119$ at 5% or higher cut-off value). There were no significant differences observed in IC PD-L1 positivity rates be-

TABLE 1. Clinicopathological patient characteristics

Total	N = 54
Histology	
Squamous-cell carcinoma	25 (46%)
Adenocarcinoma	29 (54%)
Sex	
Male	34 (63%)
Female	20 (37%)
Age (years)	
Mean (SD)	62.4 (8.6)
Smoking status	
Current smoker	25 (46%)
Former-smoker (>1 year)	21 (39%)
Non-smoker	0 (0%)
Unknown	8 (15%)

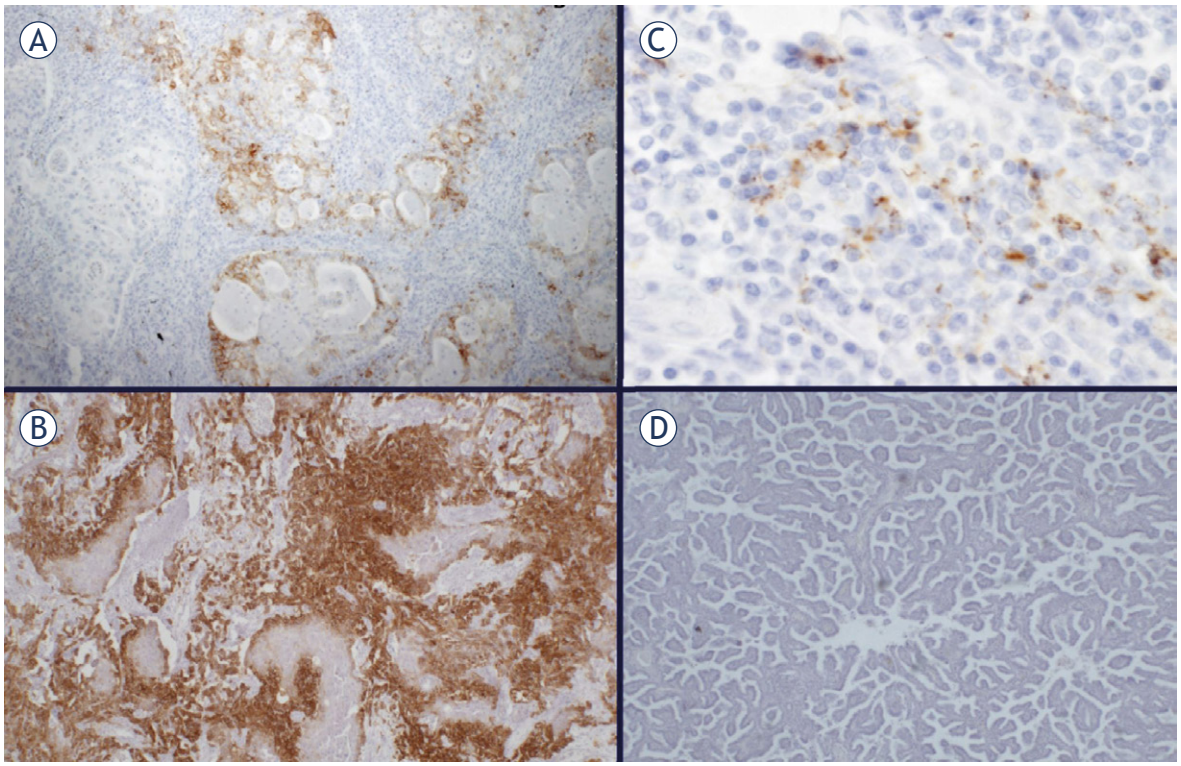

FIGURE 1. PD-L1 expression in NSCLC. Positive membranous reaction in tumor cells in adenocarcinoma **(A)**, and squamous-cell carcinoma **(B)**, positive cytoplasmic reaction in immune cells **(C)**, negative reaction in adenocarcinoma **(D)**.

TABLE 2. PD-L1 positivity according to histology for cut-off value of 5% and higher and 10% and higher

	Tumor specimens N	PD-L1 positivity (cut-off ≥ 5%)				PD-L1 positivity (cut-off ≥ 10%)			
		TC or IC	TC	IC	*p value*	TC or IC	TC	IC	*p value*
		N (%)	N (%)	N (%)	*TC vs. IC*	N (%)	N (%)	N (%)	*TC vs. IC*
Total	54	43 (80)	18 (33)	40 (74)		31 (57)	17 (31)	28 (52)	
Adenocarcinoma	29	21 (72)	5 (17)	21 (72)	***< 0.001***	12 (41)	4 (14)	12 (41)	***0.008***
Squamous-cell carcinoma	25	22 (88)	13 (52)	19 (76)	*0.146*	19 (76)	13 (52)	16 (64)	*0.508*

IC = immune cells; TC = tumor cells. Statistically significant results are in bold.

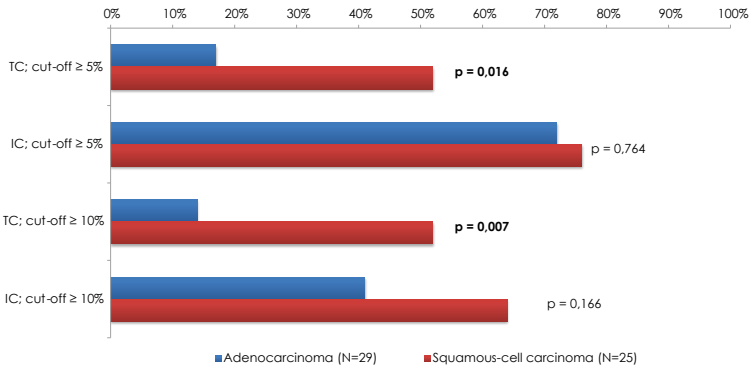

FIGURE 2. Proportions of PD-L1 positive samples of adenocarcinoma and squamous-cell carcinoma in tumor cells (TC) and immune cells (IC) at pre-defined cut-off values (≥ 5% and ≥ 10%) with corresponding p values.

tween SCC in AC samples at both 5% and 10% cut-off values (76% *vs.* 72%; p = 0.764 and 64% *vs.* 41%; p = 0.166, respectively) (Figure 2). However, while at the lower cut-off level of 5% the PD-L1 expression in IC was similar in AC and SCC samples, the numerically higher, but not statistically significant, higher PD-L1 positivity rate was observed in SCC. At the same time, this PD-L1 expression in IC was the only noticed difference in PD-L1 expression by predefined cut-off values. (Figure 2)

Higher levels of expression of PD-L1 were observed in IC than in TC. Approximately one third of samples were PD-L1 positive in TC irrespective of the cut off level, compared to more than half of the samples that were positive in IC at 10% cut-off and three quarters at 5% cut-off. The difference in expression between IC and TC reached the level of significance in AC, but not in SCC at both cut-of values (Table 2).

The total PD-L1 positivity rate (either in TC or IC) was the same as determined for IC in AC, irrespective of the cut-off value, since all of the positive TC samples were also IC positive. In SCC the total positivity rate was slightly higher than the rates observed in IC, since three samples that were TC positive, were IC negative, irrespective of the cut-off value (Table 2).

Histology was associated with different PD-L1 expression in our 54 samples. In total, the samples obtained from SCC patients were more frequently determined as PD-L1 positive compared to the samples from AC patients, mainly due to a significantly higher rate of PD-L1 positivity of TC in SCC compared to AC histology (Table 2 and Figure 2).

Discussion

In the present study, we showed that PD-L1 expression in lung cancer might differ according to histology and that the selection of TC and/or IC for the evaluation influences the PD-L1 positivity rate in adenocarcinomas. Based on our results, we observed a significantly higher proportion of PD-L1 positivity among SCC than AC, when considering staining in the TC, whereas PD-L1 positivity in IC is quite high in both histological subtypes of NSCLC.

According to our knowledge, only few studies reported PD-L1 expression separately in SCC and AC. Among them, two studies by Yang *et al.*[20,21] mirror our results in terms of higher positivity in SCC. In these studies, the PD-L1 positivity in TC was 56.2% and 39.9% in SCC and AC, respectively. They used another PD-L1 antibody to stain the tissue (Proteintech Group Inc., USA), but similar cut-off point of 5%.

There are also studies with different results in the literature. For example, D'Incecco *et al.*[22] analyzed PD-L1 expression in NSCLC tumor specimens separately for SCC and non-SCC, and reported on TC positivity rate of 30% and 63%, respectively. The reason for different results compared to ours could be the use of different antibody (Ab58810 by Ventana) in this particular study and the fact that they used both whole tissue specimens and small biopsies, which may not reflect the actual PD-L1

expression of the tumor. Since NSCLC tumor specimens are obviously quite heterogeneous, we firmly believe that it is important to have a large tissue specimen for PD-L1 determination, as small specimens are unreliable and might not represent the whole image of PD-L1 positivity in the tumor.[19]

The clinical trials with immune checkpoint inhibitors put the clinical outcomes in perspective according to PD-L1 expression. Most of these trials used only biopsy specimens, while whole tissue specimens were in minority, since the population included patients with advanced NSCLC.[1-6] Two phase 3 trials with NSCLC patients evaluating nivolumab, namely CheckMate 017 and CheckMate 057, were the only two that performed separate clinical trials on SCC and non-SCC subpopulations of advanced NSCLC patients. Results showed similar response rates in the overall population of around 20%, but differences emerged when responses were examined according to PD-L1 expression. While SCC population has a stable response to therapy no matter the cut-off value, ranging from 17–21% in the PD-L1 positive and negative population of patients, responses of non-SCC population are higher with increasing PD-L1 expression, ranging from 31–37% for PD-L1 positive patients and less than 10% in PD-L1 negative population of patients.[1,2] The same applies to undivided advanced NSCLC patient population, treated with pembrolizumab and atezolizumab. It should be noted that these trials recruited substantially more non-SCC than SCC patients and showed that the higher the positivity rate of PD-L1 expression, the better the clinical outcomes.[3-6]

Based on our data as well as data published and described above, SCC seems to be distinct from non-SCC. That reflects both in high PD-L1 positivity and in steady responses to immune checkpoint therapy across SCC subgroup of patients. One of the possible explanations could be high levels of acquired somatic mutations in SCC patients caused with carcinogens such as cigarette smoke, especially because most of the patients with SCC are smokers. Rizvi *et al.* analyzed the responses to pembrolizumab with respect to the mutational burden of NSCLC patients and discovered that patients with a high rate of somatic mutational burden had a much higher rate of responses to pembrolizumab and that those responses were durable.[17,18]

So far, data on the importance of IC in tumor microenvironment are scarce and even less data exist on PD-L1 positivity of these cells and what is their clinical significance. Our study showed high levels of PD-L1 positive IC across all histological subtypes of NSCLC no matter the cut-off value applied. Only one paper reported PD-L1 expression on IC separately, but used different methodology for their determination, so these data are hardly comparable with ours.[23] Most of the clinical trials with immune checkpoint inhibitors used PD-L1 expression on TC as enrichment predictive biomarker.[1-3] The trials with atezolizumab were the only ones that considered PD-L1 expression on both, TC and IC.[6-8] The antibody used in these trials was the same as in our study (Ventana SP142), but their cut-off values were determined a bit different. Since they reported the results of PD-L1 expression on TC and IC together, it cannot be established if one type of cells are more prominent than the other or which cells prevail concerning PD-L1 expression.[4-6]

PD-L1 assays poses major challenges and barriers in comparing results obtained by different IHC assays. When dealing with different antibodies, cut-off values, platforms, tissue specimens, tumor heterogeneity and different types of cells being evaluated, a uniform way of determining PD-L1 expression comes to mind.[11,12] Three trials of harmonization and standardization for quantitative assessment of PD-L1 positivity were already published[13-15] and one was presented in form of an abstract.[16] The major finding of The Blueprint project[13] and of the German study[14], which compared four assays (with corresponding platforms and antibodies Ventana SP142, Ventana SP263, Dako 22C3 and Dako 28-8) was that the Ventana SP142 assay stains less TC than the other three. Staining of the IC was observed across different assays, but with greater variability compared to TC staining, which they clarify by the lack of criteria for scoring of the PD-L1 positive IC component in tumors. This is a viable explanation for our results as well, since we showed IC to be highly positive across both histological subtypes. Whether using Ventana SP142 assay influenced the differences in TC staining between AC and SCC samples observed in our study cannot be ruled out completely.

In addition to the use of specific Ventana SP142 assay, which obviously stains less TC, the limitation of our study is also a relatively small number of patients, which makes results barely comparable to other trials.

In conclusion, we have shown significantly higher levels of PD-L1 expression in TC of SCC compared to AC samples, while no difference in PD-L1 expression on IC was observed. Even though PD-L1 positivity is far from being an optimal predictive marker, higher PD-L1 expression in SCC might reflect a high mutational load in this

smoking related lung cancer. Ongoing research is already oriented at mutational load and smoking gene signatures in addition to other immune markers that might offer a more accurate prediction of response to CPIs in future. Until then, we might feel comfortable to use some of the CPIs in lung cancer without PD-L1 determination, at least in patients with squamous-cell carcinoma.

Acknowledgement

The research was publicly funded by Slovenian Research Agency (ARRS), grant number J3-4076.

References

1. 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. doi:10.1056/NEJMoa1504627
2. 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. doi:10.1056/NEJMoa1507643
3. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387:** 1540-50. doi:10.1016/S0140-6736(15)01281-7
4. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; **387:** 1837-46. doi:10.1016/S0140-6736(16)00587-0
5. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; **389:** 255-65. doi:10.1016/S0140-6736(16)32517-X
6. Besse B, Johnson M, Janne PA, Garassino M, Eberhardt WEE, Peters S, et al. Phase II, single-arm trial (BIRCH) of atezolizumab as first-line or subsequent therapy for locally advanced or metastatic PD-L1-selected non-small cell lung cancer (NSCLC). [abstract]. *Eur J Cancer* 2015; **51(Suppl 3):** abstr 16LBA, S717-18. doi:10.1016/S0959-8049(16)31938-4
7. Reck M, Rodriguez-Abreu D, Robinson AG, Hui RN, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *New Engl J Med* 2016; **375:** 1823-33. doi:10.1056/Nejmoa1606774
8. Socinski M, Creelan B, Horn L, Reck M, Paz-Ares L, Steins M, et al. CheckMate 026: A phase 3 trial of nivolumab vs investigator's choice (IC) of platinum-based doublet chemotherapy (PT-DC) as first-line therapy for stage IV/recurrent programmed death ligand 1 (PD-L1)-positive NSCLC. [abstract]. *Ann Oncol* 2016; **27(Suppl):** abstr LBA7. doi:10.1093/annonc/mdw435.39
9. Kumar R, Collins D, Dolly S, McDonald F, O'Brien ME, Yap TA. Targeting the PD-1/PD-L1 axis in non-small cell lung cancer. *Curr Probl Cancer* 2017; **41:** 111-24. doi:10.1016/j.currproblcancer.2016.12.002
10. Califano R, Kerr K, Morgan RD, Lo Russo G, Garassino M, Morgillo F, et al. Immune checkpoint blockade: a new era for non-small cell lung cancer. *Curr Oncol Rep* 2016; **18:** 59. doi:10.1007/s11912-016-0544-7
11. Kerr KM, Tsao MS, Nicholson AG, Yatabe Y, Wistuba, II, Hirsch FR. Programmed death-ligand 1 immunohistochemistry in lung cancer: In what state is this art? *J Thorac Oncol* 2015; **10:** 985-9. doi:10.1097/JTO.0000000000000526
12. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515:** 563-7. doi:10.1038/nature14011
13. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. *J Thoracic Oncol* 2017; **12:** 208-22. doi:10.1016/j.jtho.2016.11.2228
14. Scheel AH, Dietel M, Heukamp LC, Johrens K, Kirchner T, Reu S, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. *Modern Pathol* 2016; **29:** 1165-72. doi:10.1038/modpathol.2016.117
15. McLaughlin J, Han G, Schalper K, Carvajal-Hausdorf D, Pelekanou V, Rehman J et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *Jama Oncol* 2016; **2:** 46-54. doi:10.1001/jamaoncol.2015.3638
16. Ratcliffe MJ, Sharpe A, Midha A, Barker C, Scorer P, Walker J. A comparative study of PD-L1 diagnostic assays and the classification of patients as PD-L1 positive and PD-L1 negative. [abstract]. *Cancer Res* 2016; **76(Suppl):** abstr LB-094. doi:10.1158/1538-7445.AM2016-LB-094
17. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348:** 124-8. doi:10.1126/science.aaa1348
18. Hellmann M, Rizvi N, Wolchok JD, Chan TA. Genomic profile, smoking, and response to anti-PD-1 therapy in non-small cell lung carcinoma. *Mol Cell Oncol* 2016; **3:** e1048929. doi:10.1080/23723556.2015.1048929
19. Ilie M, Long-Mira E, Bence C, Butori C, Lassalle S, Bouhlel L, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. *Ann Oncol* 2016; **27:** 147-53. doi:10.1093/annonc/mdv489
20. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. *Eur J Cancer* 2014; **50:** 1361-9. doi:10.1016/j.ejca.2014.01.018
21. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed cell death-ligand 1 expression is associated with a favourable immune microenvironment and better overall survival in stage I pulmonary squamous cell carcinoma. *Eur J Cancer* 2016; **57:** 91-103. doi:10.1016/j.ejca.2015.12.033
22. D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer* 2015; **112:** 95-102. doi:10.1038/bjc.2014.555
23. Schmidt LH, Kummel A, Gorlich D, Mohr M, Brockling S, Mikesch JH, et al. PD-1 and PD-L1 expression in NSCLC indicate a favorable prognosis in defined subgroups. *PLoS One* 2015; **10:** e0136023. doi: 10.1371/journal.pone.0136023