Mini review article

PD-1/PD-L1: A NOVEL TARGET FOR IMMUNOTHERAPY IN PATIENTS WITH ADVANCED AND METASTATIC NON-SMALL CELL LUNG CANCER

RECEPTOR PROGRAMIRANE SMRTI/LIGAND PROGRAMIRANE SMRTI: NOVI PRISTUP U IMUNOLOŠKOJ TERAPIJI KOD PACIJENATA SA UZNAPREDOVALIM I METASTAZNIM NESITNOĆELIJSKIM KARCINOMOM PLUĆA

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Abstract

Immune checkpoints are receptor-ligand interactions that either enhance or turn down the activation of T-lymphocytes. Among them, the interaction of Programmed death receptor-1 (PD-1) and Programmed death ligand-1 (PD-L1) shows suppressive effect of T-lymphocytes, preventing over activation of T-lymphocytes in inflammation. However, some tumor cells can also express PD-L1 and use it as a strategy to escape the host's immune response, by binding to PD-1 on T-lymphocytes and inducing their anergy and apoptosis. Hence, a promising approach in cancer therapy would be focused on preventing the tumor cells' PD-L1 from binding to PD-1 of immune cells by application of monoclonal antibodies against either PD-1 or PD-L1. These novel drugs are known as PD-1/PD-L1 inhibitors, and have been tested in patients with non-small cell lung cancer (NSCLC), particularly in advanced stages of the disease. Compared to conventional chemotherapy, PD-1/PD-L1 inhibitors showed fewer adverse effects, and presented encouraging results in terms of improved survival of NSCLC patients, mostly when used as a second line therapy. The therapeutic agents that inhibit PD-1/PD-L1 binding will have a major clinical value if tumor cells express PD-L1 on their membranes. Therefore, reliable interpretation of PD-L1 expression in tumors is needed for optimal selection of patients who would benefit from PD-1/PD-L1 inhibitors. However, PD-L1 as a biomarker needs further improvement, since the estimation of PD-L1 expression is dependent on several factors, such as number of analyzed tumor cells, used antibody, cut-off values.

Sažetak

Imunološke kontrolne tačke (eng. Immune checkpoints) su interakcije receptora liganda koje pojačavaju ili smanjuju aktivaciju T-limfocita. Interakcija receptora programirane smrti (eng. Programmed death receptor-1, PD-1) sa ligandom programirane smrti (engl. Programmed death ligand-1, PD-L1) ima supresivno dejstvo na T-limfocite tako što sprečava njihovu preteranu aktivaciju tokom zapaljenja. Neke tumorske ćelije, međutim, takođe mogu da eksprimiraju PD-L1 i koriste to kao strategiju da izbegnu imunološki odgovor domaćina tako što se vezuju za PD-1 na T-limfocitima i indukuju njihovu anergiju i apoptozu. Obećavajući pristup u terapiji kancera bio bi usmeren na sprečavanje vezivanja tumorskog PD-L1 i limfocitnog PD-1 primenom monoklonskih antitela na PD-1 ili PD-L1. Ovi novi lekovi su poznati kao PD-1/PD-L1 inhibitori i testirani su na pacijentima sa nesitnoćelijskim karcinomom pluća, naročito u uznapredovalim stadijumima bolesti. U odnosu na konvencionalnu hemioterapiju, PD-1/PD-L1 inhibitori su imali manje neželjenih dejstava, a pokazali su ohrabrujuće rezultate u smislu poboljšanog preživljavanja kod pacijenata sa nesitnoćelijskim karcinomom pluća kada su korišćeni uglavnom kao druga linija terapije. Terapija PD-1/PD-L1 inhibitorima ima terapijski efekat ukoliko tumorske ćelije eksprimiraju PD-L1 na svojoj membrani. Zbog toga je pravilna interpretacija PD-L1 ekspresije u tumorima neophodna za optimalni odabir pacijenata koji bi imali koristi od inhibicije PD-1/PD-L1. Analiza PD-L1 kao biomarkera zahteva, međutim, dalje unapređenje budući da procena PD-L1 ekspresije zavisi od nekoliko faktora, kao što su broj analiziranih tumorskih ćelija, upotrebljeno antitelo ili granične vrednosti.

Ključne reči:
imunološka terapija,
nesitnoćelijski karcinom pluća,
PD-1,
PD-L1

Among malignant tumors, lung cancer is the leading cause of death worldwide. According to the annual report of the Institute of National Health of Serbia from 2016, lung cancer was the most common malignant tumor in men, and the second most common malignancy in women in Serbia (1). Two major types of lung cancer are non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC); however, NSCLC encompasses about 85% of all lung cancers (2). Depending on the stage of the disease, as well as the overall health of a patient, the therapy of advanced stages of NSCLC involves radiotherapy, chemotherapy or their combination (3). However, the clinical success of such therapeutic approach is limited, as NSCLC is sometimes unresponsive to both the conventional (such as platinum based) and targeted chemotherapeutical agents (tyrosine kinase inhibitors – TKI, such as erlotinib) (4). Furthermore, the responses are often of short duration, and there are significant adverse effects of chemotherapy (5). In addition, the patients with a mutation in EGFR gene receive targeted TKI chemotherapy (erlotinib or gefitinib) and they are expected to have a better response; however, the acquired resistance to those agents is a common problem nowadays (6). Therefore, alternative therapeutic approaches, such as immunotherapy, are necessary to improve the survival in patients with advanced and metastatic NSCLC.

Considering that the initial immunotherapy studies with application of BCG vaccine, IL-2, cytokines, or antitumor vaccination did not show satisfactory results in NSCLC patients, it appeared that NSCLC is a non-immunogenic tumor and, hence, not a suitable target for immunotherapy (7). However, immune checkpoint inhibitors emerged recently as promising agents for the treatment of advanced stages of NSCLC (8).

Immune checkpoints are receptor-ligand interactions that either enhance or turn down the activation of T-lymphocytes. Programmed death receptor-1 (PD-1) is one of the immune checkpoint proteins expressed by T-, B- and NK-cells (9). The ligand binding to the PD-1 is marked as Programmed death ligand-1 (PD-L1, also known as B7-H1 or CD274). The expression of PD-L1 is induced by proinflammatory cytokines (IFN γ) in immune cells with the purpose of preventing over activation of T cells and extensive destruction of tissue (10). However, some tumor cells can also express PD-L1 and use it as a strategy to escape host immune response. Namely, when a tumor cell expresses PD-L1, it binds to PD-1 and decreases proliferation of the T-cells, reduces production of cytokines, and induces T-cell anergy or apoptosis (11). In that way, tumor cells can avoid immune surveillance, which leads to progression and spreading of the cancer. Therefore, therapeutic approach based on preventing the tumor cells' PD-L1 from binding to PD-1 of immune cells (**Figure 1**) would allow the immune system to recognize the tumor cells and attack them (12).

PD-1/PD-L1 inhibitors

So far, several monoclonal antibodies to PD-1 and PD-L1 were tested in NSCLC patients (**Table 1**) and showed favorable therapeutic response in some patients (13).

The first one that obtained the approval from US Food and Drug Administration (FDA) was Nivolumab (4). Nivolumab is a monoclonal IgG4 antibody which targets PD-1. It was shown that Nivolumab as second-line therapy had a positive effect in 18% of NSCLC patients (with ≥5% tumor cells expressing PD-L1 by immunohistochemistry) who had previously not responded to platinum-based chemotherapy or TKI (14). In most cases, the
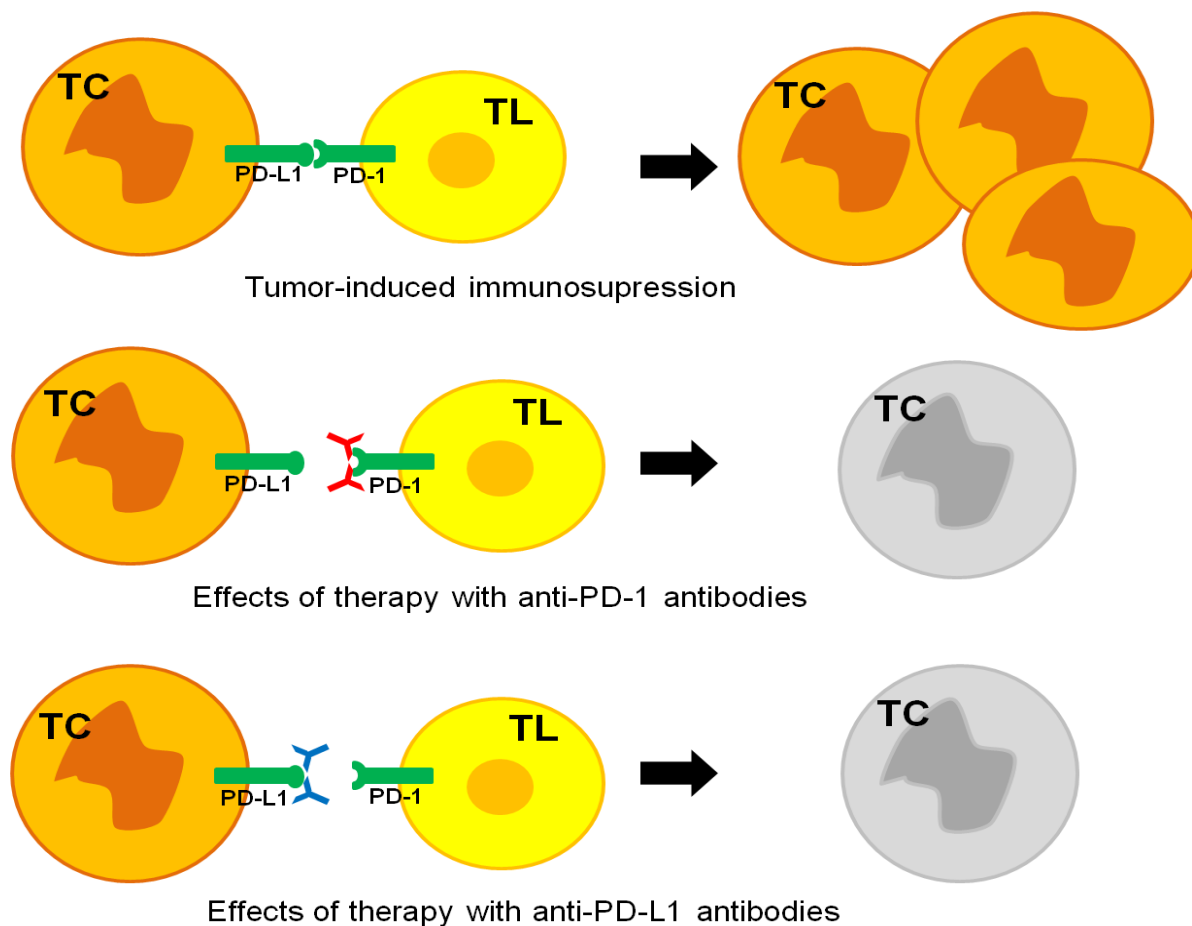
Figure 1. The PD-1/PD-L1 as a new target for immunotherapy of non-small cell lung cancer: PD-L1 expressed on tumor cell binds to PD-1 on T-lymphocyte leading to its anergy and apoptosis. Hence, the tumor cell avoids the host immunity and cancer can grow and progress. The therapeutic approach with PD-1/PD-L1 inhibitors encompasses application of monoclonal antibodies directed either against PD-1 or PD-L1, in both cases preventing binding of tumor cell PD-L1 with the PD-1 on T-lymphocyte. In that way, T-lymphocyte is functional and can attack tumor cells. Abbreviations: TL – T-lymphocyte, TC – tumor cell

Table 1. Immune checkpoint inhibitors for treatment of NSCLC and monoclonal antibodies used for detection of PD-L1 expression in NSCLC

Agent	Company	Target	Monoclonal antibody	Staining platform
Nivolumab	Bristol-Myers Squibb	PD-1	Clone 28-8	Dako Link 48
Pembrolizumab	Merck	PD-1	Clone 22C3	Dako Link 48
Atezolizumab	Roche	PD-L1	Clone SP142	Ventana
Durvalumab	AstraZeneca	PD-L1	Clone SP263	Ventana
Avelumab	Pfizer/Merck Serono	PD-L1	Clone 73-10	Dako Link 48

therapeutic effect of Nivolumab lasted more than one year (14). However, it was shown in the clinical trial CheckMate 026 that as a first-line therapy, Nivolumab was not superior to platinum-based doublet chemotherapy in the patients with stage IV NSCLC and ≥5% tumor cells expressing PD-L1, considering that it did not improve progression free survival in those patients (15). Nevertheless, the adverse effects were notably less frequent with Nivolumab, especially serious or life threatening side effects that were recorded in 18%, compared to 51% of patients receiving platinum-based doublet chemotherapy (15).

Pembrolizumab is another monoclonal antibody to PD-1 and it was approved by the FDA for advanced and metastatic NSCLC (16). In a clinical trial (KEYNOTE-001) on 495 patients with NSCLC, it was shown that Pembroli-zumab as a second-line therapy had an overall response rate of 19.4%, median progression-free survival of nearly 4 months and overall survival of 12 months (17). Overall response rate and survival was higher in the patients where more than 50% of the tumor cells were PD-L1 positive by immunohistochemistry (17). Recent data suggest that this drug can be used even as a first line of therapy in NSCLC patients, showing improved response rate and survival compared to the chemotherapy (18).

Atezolizumab, the first monoclonal antibody targeting PD-L1, improved overall survival compared to Docetaxel in previously treated NSCLC, even in the patients with low or undetectable PD-L1 expression (19).

AstraZeneca reported that PD-L1 inhibitor named Durvalumab in combination with another immune check-

point inhibitor (tremelimumab) did not improve progression-free survival compared to standard chemotherapy in patients whose more than 25% of tumor cells expressed PD-L1, as determined by the Ventana PD-L1 (SP263) assay (20).

Avelumab showed an acceptable safety profile and antitumour activity in patients with progressive or treatment-resistant NSCLC (21), especially in patients with PD-L1-positive tumors (22).

It is expected that the therapeutic agents that inhibit PD-1/PD-L1 binding will have clinical value only if tumor cells express PD-L1. Therefore, before introducing PD-1/PD-L1 inhibitors to the therapy of NSCLC, immunohistochemical assays have to be performed to evaluate the degree of expression of PD-L1 in tumor cells (**Figure 2**). So far, five immunohistochemical markers were developed that correspond to particular epitopes and monoclonal antibodies designed to bind to PD-L1 (**Table 1**).
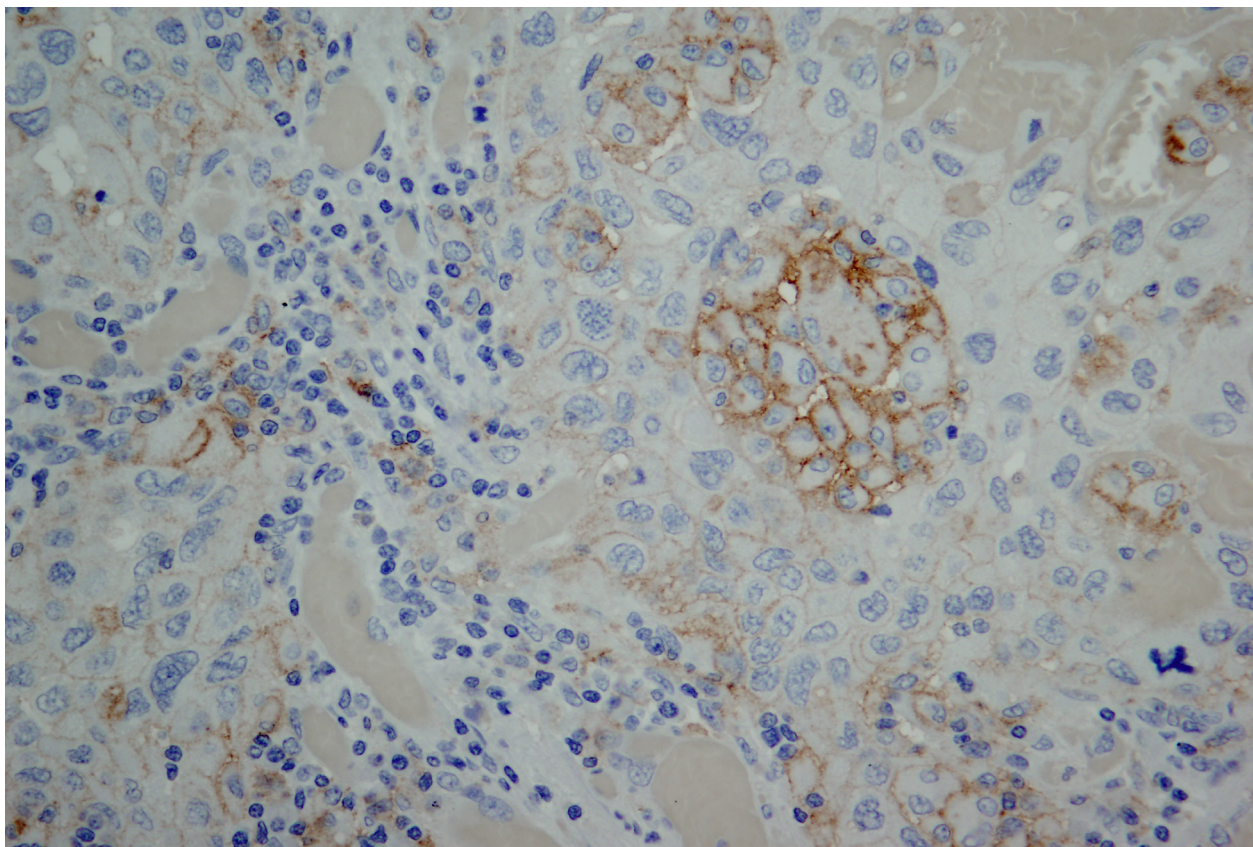
Figure 2. Squamous cell lung carcinoma stained with monoclonal anti-PD-L1 antibody (clone 22C3) showing membranous positivity in tumor cells.

Immunohistohemical assessment of PD-L1 expression

Considering that most antibodies used for PD-L1 immunohistochemistry bind to extracellular domains of PD-L1, linear membrane staining is expected as a marker of expression (23). Indeed, PD-L1 staining is defined as "complete circumferential or partial linear plasma membrane staining of tumor cells at any intensity" (23). In this context, cytoplasmic staining cannot be considered for scoring purposes, except with use of the SP263 antibody clone. In addition, SP142 assay shows both membranous and granular cytoplasmic staining in tumor cells (24), but this is dependent on the Ventana detection system (25).

It is important to note that viable tumor cells are not the only cells that can be stained positively for PD-L1. As a matter of fact, necrotic tumor cells, nonmalignant cells and immune cells (e.g., infiltrating lymphocytes or macrophages) may also stain positively for PD-L1 (23). However, positivity of these cells is not considered except in the case of SP142 assay, where the IHC report takes into account positive immune cells together with viable tumor cells (25).

Scoring and reporting PD-L1 positivity

Evaluation of PD-L1 expression is done through the scoring schemes specific for each of the IHC assays. A minimum of 100 viable tumor cells are required to determine the percentage of PD-L1 stained tumor cells (26), except in SP142 assay where a minimum of 50 cells with associated stroma are needed.

In case of 28-8 antibody the following scoring is employed: <1% of positive tumor cells (TC), 1-5% TC, 5-10% TC and ≥10% positive TC (27). It was shown that Nivolumab showed better effect than Docetaxel, if PD-L1 positivity was at least 1%, even more if higher than 5% or 10% (28).

For the clone 22C3, the tumor proportion score (TPS) is used for scoring and classifies the PD-L1 expression into three groups: no PD-L1 expression (TPS<1%), PD-L1 expression (TPS 1 – 49%) and high PD-L1 expression (TPS ≥ 50%) (29). To use Pembrolizumab as the first-

line therapy, the TPS must be higher than 50%, while TPS in the range between 1% and 49% makes the patient eligible for this drug as the second- or third-line (26).

For the SP142 assay, different scoring systems have been reported so far, but they all rely not only on counting positive tumor cells but also positive immune cells (25). Specifically, according to Ventana PD-L1 (SP142) Assay guide, if any membrane staining of any intensity is present in at least 50% of tumor cells, the sample is reported as "TC ≥ 50%". Otherwise, positivity of immune cells (IC) settled in tumor stroma has to be assessed, so that two categories are defined: "TC<50% and IC>10%" and "TC<50% and IC<10%" (25). However, in the clinical trials, the positivity of tumor cells was scored as TC0 (<1% of positive TC), TC1 (1-5%), TC2 (5-50%) and TC3 (≥50%), whereas positivity of immune cells was scored as IC0 (<1% of positive IC), IC1 (1-5%), IC2 (5-10%) or IC3 (≥10% or positive IC) (25, 30).

In SP263 assay, PD-L1 positivity means "membranous and/or cytoplasmic expression at any intensity greater than background staining" in at least 25% of tumor cells if the Durvalumab therapy is being considered (31). If Nivolumab therapy is being considered, SP263 can still be used, but the scoring scheme as used for 28-8 assay is recommended: less than 1%, 1% to 5%, 5% to 10%, and 10% or greater (31).

In 73-10 assay, early clinical trials reported expression in less than 1% of TC or expression in 1% or more TC (32). Yet, this assay is still under development.

Finally, the **Figure 3** shows a diagnostic algorithm highlighting which tumor types should be stained for PD-L1.

Pitfalls in interpretation

The expression of PD-L1 depends significantly on the used antibody (34), and the interpretation of positive staining depends on cut-off values of stained tumor cells, as described above. For example, in the study of Janzic et al. (35) it was shown that 17% of patients with NSCLC were classified as PD-L1 positive, at a cut-off value of 5%, while setting a higher cut-off value (10%) resulted in fewer positive patients (14%).

There are several possible issues in interpretation, such as non-specific background staining (due to improper drying, improper deparaffinization, or incomplete rinsing of the slides), edge artifacts (due to pre-fixation tissue drying or drying during staining), crush artifacts, necrosis or poor fixation (23). Moreover, non-malignant cells can also express PD-L1 (e.g. alveolar macrophages or inflammatory cells) and it is essential to distinguish them from tumor cells and not count them in the PD-L1 expression report (23). Only in the case of SP142 assay, stained immune cells located in the tumor stroma are counted if less than 50% tumor cells are PD-L1-positive (25). Except in the case of SP263 assay, cytoplasmatic staining is excluded (31). For example, necrotic tumor cells show cytoplasmatic staining and should be excluded from scoring of PD-L1 expression (23).

It is recommended to obtain serial tissue sections, where the first one would be used for H/E staining (to assess preservation and staining quality), one for PD-L1 staining, and one would serve as a negative control. For external positive control, human tonsils can be used, depending on the manufacturer's recommendations (23).

In clinical trials, immunohistochemistry is mostly performed on biopsies; hence, it is unknown whether cell block cytology specimens would be a reliable source for evaluation of PD-L1 expression (36), especially considering that certainly many of cytology specimens do not provide a minimum of 100 cells for assessment of PD-L1 positivity and that none of the PD-L1 assays have been validated for cytology (23, 36). Future studies are needed to evaluate the applicability of PD-L1 assays in cytology specimens.

Is PD-L1 a prognostic and/or predictive marker?

It is still unknown whether the immunohistochemical expression of PD-L1 reflects the prognosis of the cancer (9, 37). Various studies showed contradicting results. In a study by Wang et al., expression of PD-L1 was shown to be a negative prognostic marker in patients with NSCLC, which could be explained by poorer differentiation of PD-L1 positive tumors (38). In contrast, Brahmer et al. (39) did not observe significant correlation between PD-L1 expression level and prognosis. The differences among the studies might originate from the use of different antibodies and various cut-off values used to interpret positivity (24).

Borghaei et al. (28) showed a positive predictive value of PD-L1 expression in non-squamous NSCLC patients treated with Nivolumab (with cut-off values of 1%, 5% and 10% of stained tumor cells), in comparison to those treated with Docetaxel. Likewise, Garon et al. (26) reported that expression of PD-L1 was a positive predictive marker in both squamous and non-squamous NSCLC treated with Pembrolizumab (with cut-off value of 50% of stained tumor cells). In contrast, PD-L1 staining did not sufficiently discriminate between responders and non-responders for all immune checkpoint inhibitors (18). Therefore, there is still a need for a better predictive biomarker. Atezolizumab improved overall survival compared to chemotherapy (Docetaxel) in previously treated NSCLC, regardless of immunohistochemical PD-L1 expression (OAK trial) (19, 40). Although there was a 3.7 months longer overall survival in patients with low or undetectable PD-L1 expression, compared to the group on Docetaxel, a high PD-L1 expression (≥50% of tumor cells) was associated with more than twofold increase in overall survival compared to Docetaxel (40). That suggests that the PD-L1 expression level is not a reliable biomarker for selecting the patients that should receive Atezolizumab treatment, but also that it can be used to identify the patients that would gain more benefit from Atezolizumab.
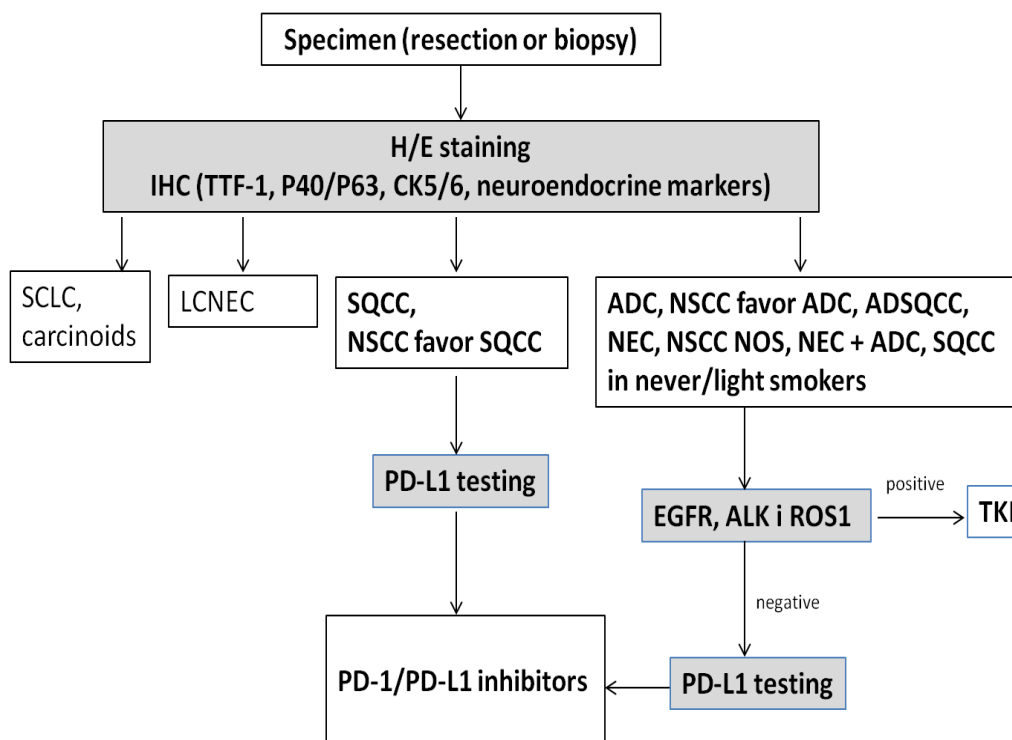
Figure 3. When should PD-L1 IHC tests be performed? - Diagnostic algorithm based on the recommendations from Tsao et al. (33) (Abbreviations: IHC - immunohistochemistry, SCLC - small cell lung cancer, LCNEC - large cell neuroendocrine cancer, SQCC - squamous cell carcinoma, NSCC – non-small cell cancer, ADC - adenocarcinoma, ADSQCC - adenosquamous carcinoma, NSCC NOS - NSCC not other specified, NEC - neuroendocrine carcinoma, TKI - thyrosine kinase inhibitors.)

Conclusions

Immune checkpoint inhibitors (specifically PD-1/PD-L1 inhibitors) are novel therapeutic agents that can be used for treating non-small cell lung cancer, particularly in advanced stages of the disease. Compared to conventional chemotherapy they show fewer adverse effects, and present encouraging results in terms of improved survival of NSCLC patients, mostly when used as a second line therapy. Reliable interpretation of PD-L1 expression in tumor is needed for optimal selection of patients who would benefit from PD-1/PD-L1 inhibitors.

References

1. Health Statistical Yearbook of Republic of Serbia 2016. Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", Belgrade, 2016, available at: http://www.batut.org.rs/download/publikacije/pub201620171103.pdf (last accessed on May 10th 2018)
2. Sher T, Dy GK, Adjei AA. Small Cell Lung Cancer. Mayo Clin Proc. 2008;83(3):355-67.
3. Besse B, Adjei A, Baas P, et al. 2nd ESMO Consensus Conference on Lung Cancer: non-small-cell lung cancer first-line/second and further lines of treatment in advanced disease. Ann Oncol. 2014;25(8):1475-1484.
4. Antoniu S, Ulmeanu R. Nivolumab for advanced non-small cell lung cancer: an immunologicallymediated tumor checkout. Ann Transl Med 2016;4(10):201.
5. Valecha GK, Vennepureddy A, Ibrahim U, Safa F, Samra B, Atallah JP. Anti–PD-1/PD-L1 antibodies in non-small cell lung cancer: the era of immunotherapy. Expert Rev Anticancer Ther. 2017;17(1):47-59.
6. Shien K, Yamamoto H, Soh J, Miyoshi S, Toyooka S. Drug resistance to EGFR tyrosine kinase inhibitors for non-small cell lung cancer. Acta Med Okayama. 2014;68(4):191-200.
7. Villaruz LC, Kalyan A, Zarour H, et al. Immunotherapy in lung cancer. Transl Lung Cancer Res. 2014;3(1):2-14.
8. Pennock GK, Chow LQM. The Evolving Role of Immune Checkpoint Inhibitors in Cancer Treatment. Oncologist 2015;20(7):812-22.
9. Guan J, Lim KS, Mekhail T, Chang C-C. Programmed Death Ligand-1 (PD-L1) Expression in the Programmed Death Receptor-1 (PD-1)/PD-L1 Blockade: A Key Player Against Various Cancers. Arch Pathol Lab Med. 2017;141(6):851-61.
10. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Dual Faces of IFNgamma in Cancer Progression: A Role of PD-L1 Induction in the Determination of Pro- and Antitumor Immunity. Clin Cancer Res. 2016;22:2329-34.
11. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192(7):1027-1034.
12. Miller RA, Miller TN, Cagle PT. PD-1/PD-L1, Only a Piece of the Puzzle. Arch Pathol Lab Med. 2016;140(11):1187-1188.
13. Anagnostou VK, Brahmer JR. Cancer immunotherapy: a future paradigm shift in the treatment of non-small cell lung cancer. Clin Cancer Res. 2015;21:976-84.
14. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. New Engl J Med.

2012;366(26):2443-54.

15. Socinski M, Creelan B, Horn L, Reck M, Paz-Ares L, Steins M, et al. NSCLC, metastatic 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. Ann Oncol. 2016;27(Suppl.6):L-BA7_PR.

16. Pai-Scherf L, Blumenthal GM, Li H, Subramaniam S, Mishra-Kalyani PS, He K, Zhao H, Yu J, Paciga M, Goldberg KB, et al. FDA Approval Summary: Pembrolizumab for Treatment of Metastatic Non-Small Cell Lung Cancer: First-Line Therapy and Beyond. Oncologist 2017;22(11):1392-1399.

17. Lim SH, Sun J-M, Lee S-H, Ahn JS, Park K, Ahn M-J. Pembrolizumab for the treatment of non-small cell lung cancer. Exp Opin Biol Ther. 2016;16(3):397-406.

18. Muller M, Schouten RD, De Gooijer CJ, Baas P. Pembrolizumab for the treatment of non-small cell lung cancer. Expert Rev Anticancer Ther. 2017;17(5):399-409.

19. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, 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(10066):255-65.

20. AstraZeneca reports initial results from the ongoing MYSTIC trial in Stage IV lung cancer, 2017, available at: https://www.astrazeneca.com/media-centre/press-releases/2017/astrazeneca-reports-initial-results-from-the-ongoing-mystic-trial-in-stage-iv-lung-cancer-27072017.html (accessed on May 10th 2018)

21. Gulley JL, Rajan A, Spigel DR, Iannotti N, Chandler J, Wong DJL, Leach J, Edenfield WJ, Wang D, Grote HJ, et al. Avelumab for patients with previously treated metastatic or recurrent non-small-cell lung cancer (JAVELIN Solid Tumor): dose-expansion cohort of a multicentre, open-label, phase 1b trial. Lancet Oncol. 2017;18(5):599-610.

22. Gulley JL, Spigel D, Kelly K, Chandler JC, Rajan A, Hassan R, Wong DJL, Leach J, Edenfield WJ, Wang D, et al. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in advanced NSCLC patients: A phase 1b, open-label expansion trial in patients progressing after platinum-based chemotherapy. J Clin Oncol. 2015; 33(15_suppl):8034-8034.

23. Thunnissen E, Yatabe Y, Lantuéjoul S, Bubendorf L. Immunohistochemistry for PD-L1. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.35-47.

24. Scheel AH, Dietel M, Heukamp LC, Johrens K, Kirchner T, Reu S, Ruschoff J, Schildhaus HU, Schirmacher P, Tiemann M, Warth A, Weichert W, Fischer RN, Wolf J, Buettner R. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Mod Pathol 2016;29(10):1165-1172.

25. Soo RA, Asuncion BR, Buettner R. PD-L1 SP142 Assay. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.63-72.

26. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New Engl J Med. 2015;372(21):2018-28.

27. Lantuéjoul S, Thunnissen E. PD-L1 28-8 pharmDx Assay. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.49-54.

28. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. New Engl J Med 2015;373(17):1627-39.

29. Chou T-Y, Cooper WA, Kerr KM. PD-L1 22C3 pharmDx Assay. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.55-62.

30. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, 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(10030):1837-1846.

31. Dacic S, Warth A. PD-L1 SP263 Assay. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.73-77.

32. Mino-Kenudson M, Warth A, Yatabe Y. PD-L1 73-10 Assay. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.79-82.

33. Tsao MS, Nicholson AG, Hirsch FR. Implementation of PD-L1 Testing for Personalized Therapy for Lung Cancer. In: Tsao MS, Kerr K, Dacic S, Yatabe Y, Hirsch FR (eds). IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Aurora, Colorado: International Association for the Study of Lung Cancer; 2017. p.109-113.

34. Santini FC, Rudin CM. Atezolizumab for the treatment of non-small cell lung cancer. Exp Rev Clin Pharmacol. 2017;10(9):935-45.

35. Janzic U, Kern I, Janzic A, Cavka L, Cufer T. PD-L1 expression in squamous-cell carcinoma and adenocarcinoma of the lung. Radiol Oncol. 2017;51(3):357-62.

36. Kerr KM, Tsao M-S, Nicholson AG, Yatabe Y, Wistuba II, Hirsch FR. Programmed Death-Ligand 1 Immunohistochemistry in Lung Cancer: In what state is this art? J Thor Oncol. 2015;10(7):985-9.

37. Zhang M, Li G, Wang Y, Wang Y, Zhao S, Haihong P, Zhao H. PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis. Sci Rep. 2017;7(1):10255.

38. Wang A, Wang HY, Liu Y, Zhao MC, Zhang HJ, Lu ZY, Fang YC, Chen XF, Liu GT. The prognostic value of PD-L1 expression for non-small cell lung cancer patients: A meta-analysis. Eur J Surg Oncol. 2015;41(4):450-6.

39. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. New Engl J Med. 2015;373(2):123-35.

40. Barlesi F, Park K, Ciardiello F, von Pawel J, Gadgeel S, Hida T, et al. Primary analysis from OAK, a randomized phase III study comparing atezolizumab with Docetaxel in 2L/3L NSCLC. Ann Oncol. 2016;27(Suppl.6):LBA44_PR.