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 NESITNOČELIJSKIM 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.

Keywords: immunotherapy, non-small cell lung cancer, 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 malignan

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).

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

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

The 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 Pembrolizumab 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.

**Immunohistochemical 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-
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.
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