

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Prognostic and Predictive Value of PD-L1 in Patients with Lung Cancer

Mirjana Rajer

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/66757>

Abstract

Improved understanding of the molecular mechanisms has led to identification of checkpoint signalling and development of checkpoint inhibitors in the treatment of many cancers, including lung cancer. To be able to select the patients who benefit most from checkpoint inhibitors, predictive biomarkers are needed. Currently, the only predictive biomarker that has been approved in clinical use is PD-L1, the ligand of the inhibitory T-cell checkpoint PD-1. The use of PD-L1 as a predictive biomarker is confounded by multiple unresolved issues, from testing issues (e.g., cut-off values for positivity) to clinical use (e.g., the response to anti-PD-1 and anti-PD-L1 antibodies in patients without any expression of PD-L1). Even more open questions exist in the evaluation of PD-L1 as a prognostic biomarker. In the future, we expect that an improved understanding of immune system, tumor microenvironment, mechanism of action of immunotherapeutic drugs, and PD-L1 testing methods will elucidate the value of PD-L1 as a prognostic and predictive biomarker in detail.

Keywords: immunotherapy, PD-L1, prognostic factor, predictive factor, lung cancer

1. Introduction

Immunotherapy represents an important step forward in the management of patients with lung cancer. Careful selection of patients, who benefit most from any new treatment including immunotherapy, is essential. To be able to select the patients, prognostic and predictive markers are needed. Prognostic biomarkers provide information about the patient's overall cancer outcome, regardless of the therapy. Predictive biomarkers give information about the effect of a therapeutic intervention [1–3].

PD-1 is a T cell immune checkpoint involved in dampening autoimmunity in the peripheral effector phase of T-cell activation, leading to immune tolerance of cells expressing its ligands PD-L1 and PD-L2. Activation of PD-1–PD-L1 leads to peripheral immunological tolerance in T cells. Multiple solid tumors (melanoma, RCC, and lung cancer) express PD-L1 to generate immunosuppressive tumor microenvironment and avoid destruction of their cells by T lymphocytes. In healthy tissues, PD-1 is thought to limit the activity of antigen-specific T cells to prevent collateral tissue damage during infection. In cancer, the PD-1 pathway can be exploited by some tumor cells to inactivate T cells [4].

The importance of the PD-L1 together with the development of drugs that inhibit its action suggest a candidate for a prognostic and/or predictive biomarker: PD-L1 expression in tumor or inflammatory cells. The (few) trials that evaluated PD-L1 as a prognostic biomarker yielded inconclusive results. Some of them showed that patients with PD-L1 or PD-1 positive expression have significantly shorter overall survival, while in others, no correlation between biomarker expression and outcome was seen. Many questions remain open even when considering PD-L1 as a predictive biomarker, for example, what cut-off percentage of expression can be considered as positive or can PD-L1 testing be performed only on fresh or archival tissues also. Above all, the most important question whether PD-L1 can really be considered as a predictive biomarker still has to be answered. In the lack of definitive answers, currently researchers propose other biomarkers as supplemental (e.g., pre-existing CD8+ T, cytokines, ...) [4].

In this chapter, the value of PD-L1 as a prognostic and predictive biomarker will be presented together with all open questions and possible answers.

2. Change in the insight of lung cancer

Lung cancer was first described a century ago, and since then every year, around 1.6 million of new cases are diagnosed [4]. Nonsmall cell lung cancer (NSCLC) remains the main cause of death due to cancer worldwide. About 60% of lung cancers are diagnosed at Stage IV, and these patients have a very poor prognosis, since 5-year survival is only 1–4% [5]. New treatments, developed in the last years, lead to improved survival in the different groups of NSCLC patients with advanced disease. Patients who benefit most are those with adenocarcinomas and specific mutations, such as EGFR activating mutations and ALK translocations. A median survival of 20 months has been reached with EGFR tyrosine kinase inhibitors (EGFR-TKI), which can be considered a milestone in the treatment of advanced lung cancer. Despite promising results, many patients with advanced lung cancer still have a poor prognosis. Patients with squamous carcinomas, patients with adenocarcinomas not harboring specific mutations, and patients with small cell lung cancers still have a poor prognosis, with survival often less than a year. To be able to improve survival in lung cancer in general, the next step should be to focus on the treatment of those patients. Immunotherapy nowadays seems a step forward in reaching this goal [1].

Changes in lung cancer have become evident in recent years. A strong connection between lung cancer and smoking is supposed to be one of the main reasons [4]. Main changes observed

are the increase of adenocarcinomas at the expense of squamous tumors and a change in the position of cancers. Previously centrally located, they now arise mainly at the periphery of the lung. Possible explanations are changes in the smoking patterns. Nicotine is known to be one of the most addictive substances in the world. To be able to get enough nicotine with new sorts of cigarettes (light and ultra light low-nicotine, and low-tar), smokers have to inhale deeper and for a longer time. This leads to the change of locations of tumors, since the smoke now enters deeply in the lungs and stays there for a longer time [6–8]. The increased incidence of adenocarcinomas is also suspected to be related to new forms of cigarettes and a higher amount of nitrosamines in them. Nitrosamines are known to cause adenocarcinomas in experimental animal models, and it is supposed that the same carcinogenic process occurs also in smokers [4, 9].

In recent years, changes at the “macroscopic level” are supplemented by the insight in the microscopic world of the lung cancer such as discovery of EGFR mutations and more and more important knowledge about complex immunologic interactions between tumors and the host environment [1].

3. Immune system and cancer

When cells transform to be malignant, activation of innate and adaptive immune responses occurs. The purpose of this activation is control of early cancer growth by elimination of cancer cells. Cancer cells have different genetic and epigenetic alterations leading to an expression of different antigens that can be recognized and eliminated by the immune system [10].

The process starts at the cancer site, where tumor cells disintegrate and tumor antigens become available to the immune system. Antigen presenting cells (APC) uptake these antigens and under maturation, signals, activate, and migrate to the lymph nodes or tertiary lymphoid structures [11]. Maturation signals are necessary, since without them immune tolerance rather than activation occurs. Examples of maturation signals are intracellular proteins, heat-shock proteins, DNA, ATP, uric acid, etc. Activated APCs migrate to lymph nodes, where they present as antigens in the context of major histocompatibility complex Classes I and II molecules to the T lymphocytes. Antigen-specific CD 4 and CD 8 T lymphocytes recognize the antigens and become activated. Costimulatory and coinhibitory signals are essential for this activation to regulate and balance a proper immunological response. CD 28 complex represents a costimulatory signal and acts together with other stimulatory and inhibitory signals on T lymphocytes. They regulate T-cell activation, differentiation, survival, and effector function. Examples of costimulatory receptors are GITR, OX 40, CD 30, and CD 40, while coinhibitory signals, beside LAG, TIM, BTLA, VISTA, etc., include also CTLA-4 and PD-1 with its ligand PD-L1 currently implemented as important targets of cancer immunotherapy. After activation, T lymphocytes migrate to the tumors through and the systemic vasculature by following a chemokine gradient. T cells then go through the process of extravasation, migrate into the tumor, and recognize the tumor targets that lead finally to tumor cell destruction [11]. Lymphocytic infiltration of tumors is frequently observed in a variety of human cancers and in numerous trials tumor-infiltrating lymphocytes have been correlated with a more favorable prognosis [10].

When tumors develop, tumor cells acquire several mutations that lead to tumor “immortality.” The results of these mutations are abnormal proteins on the cell surface that can be recognized by the immune system. A higher number of mutations, commonly called mutation burden, is connected to higher immunogenicity of tumors. NSCLC and melanomas are cancers with the highest burden among several solid tumors and because of that they are believed to be good targets for immunotherapy [1]. The problem of immune recognition and cancer is that cancers have the ability to evade the immune system, and this is one of the hallmarks of cancer [5]. Several mechanisms of immune evasion exist, but one of the most important is that tumor-infiltrating lymphocytes become inactivated by the effect of PD-L1 expression on tumor cells [10, 12].

4. Why do we need prognostic and predictive biomarkers

Prognostic and predictive biomarkers have become important in recent years with the development of new highly selective therapies. A prognostic biomarker offers insight into the possible natural evolution of the disease and most likely outcome like duration of survival of the patients. It is not related to treatment, but rather to tumor biology. It helps physicians and patients to predict the course of the disease [1, 13–15]. Before a marker is labelled predictive, the effect of a marker as a prognostic marker must be taken into account [16].

A predictive biomarker, on the other hand, predicts the effect of the treatment that should be different in patients with the biomarker compared to those without it [1, 7]. In lung cancer, examples of predictive biomarkers include the presence of EGFR-activating mutations and response to EGFR-TKIs. Predictive biomarkers are important in treatment decisions because they can improve the treatment effectiveness and at the same time, reduce costs and potential harm to the patients by avoiding treatment when the biomarker is not present [1]. In a trial presented by Lopes *et al.*, the use of biomarkers to select proper patients in clinical trials resulted in a sixfold increase in clinical trial success [17].

Regarding immunotherapy, the median duration of response, once the response is achieved, is often longer than response to classical chemotherapy and even some targeted agents. However, response rates in nonselected populations are still low; they are achieved in only 15–20% of the patients. This fact, together with the high expenses of immunotherapy, leads to the necessity of finding reliable predictive biomarkers to identify which patients are most likely to benefit from it [16].

5. PD-L1 as a prognostic and predictive biomarker

Programmed cell death protein 1 (PD-1) is a cell surface protein that has two ligands PD-L1 and PD-L2. PD-1 and PD-L1 negatively regulate immune responses. The PD-1/PD-L1 pathway mediates one of the mechanisms of cancer “escape” from the immune system. Cancer microenvironment induces PD-L1 expression on tumor cells that results in inhibition of the immune response, permitting cancer growth, progression, and metastases [5, 18].

PD-L1 expression has been studied widely in different trials as a prognostic and predictive biomarker. Poor responses and high expression of PD-L1 have been found in several cancers including melanoma, breast, bladder, ovary, pancreas, kidney, esophagus, and hematologic malignancies [1, 18, 19]. Results are not constant; some authors report no correlation or even improved survival of patients with tumors highly expressing PD-L1 [18, 20].

Regarding expression of PD-L1 as a prognostic biomarker in lung cancer, several contradictory data have been published. Many authors suggest that high expression is connected to better prognosis, while others found just the opposite [19]. Cha *et al.* evaluated the prognostic significance of PD-L1 expression in 323 surgically resected lung adenocarcinomas Stages I–III. PD-L1 expression in tumor cells was positive in 60 of the cases (18.6%). PD-L1 positivity was more frequent in male patients ($p = 0.001$), tumors greater than 3 cm ($p = 0.03$), higher-stage tumors ($p < 0.001$), solid, predominant tumors ($p < 0.001$), and EGFR wild-type tumors ($p = 0.022$). Higher expression (over 50%) was more prevalent in former or current smokers compared to nonsmokers ($p = 0.026$) and was associated with more pack-years of smoking ($p = 0.016$) [19]. Survival analysis was performed on 316 patients who underwent complete surgical resection (Stages I–IIIA disease). Poor, recurrence-free, and overall survival in patients with high PD-L1 expression assessed with univariate analysis were reported (both $p < 0.001$), exact median PFS and overall survival (OS) were not reported in numbers (e.g., months). Authors conclude that high PD-L1 expression is associated with poor prognosis of patients [19].

Aguiar *et al.* recently published a meta-analysis aiming to answer the question about PD-L1 as a predictive biomarker. The analysis included 13 studies with 1979 patients who were treated with checkpoint inhibitors. Five different checkpoint inhibitors were used: nivolumab in six trials, atezolizumab in three trials, pembrolizumab in one trial, MEDI4736 in two, and avelumab in one trial. The most frequent histology was nonsquamous NSCLC (67%). Majority of the patients were previously treated (84%), male (58%), current or previous smokers (64%), and with ECOG performance status 1 (62%). All included trials reported overall response rate (ORR). The ORR in 652 PD-L1–positive patients was 29%, and 13% among 915 PD-L1–negative patients. Difference was statistically significant (relative risk (RR) 2.08, 95% confidence interval (CI) 1.49–2.91, $p < 0.01$). The ORR increased proportionally with increase in PD-L1 expression, regardless of tumor histology or line of treatment (Pearson’s correlation $r = 0.43$). Regarding PFS, authors evaluated 24 weeks PFS rate. Data were available for 767 patients. In PD-L1–positive patients 24 weeks PFS was 35% (358 patients), while in 409 PD-L1–negative patients, it was 26% (RR 0.79, 95% CI 0.71–0.89, $p < 0.01$). One-year overall survival (OS) was reported in nine trials with 1396 patients. OS did not differ between the PD-L1–positive and PD-L1–negative groups. Among the 617 positive patients the rate was 28%, while among 779 PD-L1–negative, it was 27% (RR 0.96, 95% CI 0.87–1.06, $p = 0.39$). Authors also evaluated the response to immune checkpoint inhibitors compared to docetaxel in PD-L1 negative patients (PD-L1 expression below 1%). Two Phase III and one Phase II trials compared nivolumab or atezolizumab with docetaxel in the second-line setting. Among the 407 PD-L1–negative patients, ORR was 12% in both arms (RR 1, 95% CI 0.59–1.7, $p = 1$). PFS was also similar between the two arms (hazard ratio (HR) 0.98, $p = 0.93$ for PFS). Regarding OS even if not statistically significant, there was even a trend toward better survival among patients receiving checkpoint inhibitors (HR 0.83, $p = 0.12$). Authors conclude that even if tumor PD-L1

expression is related to improved survival and better outcome of patients with advanced NSCLC, PD-L1 currently cannot be considered as a biomarker for selection of patients who benefit from immune checkpoint inhibitors treatment since many PD-L1–negative patients also benefit from them, and the response is not inferior to the response to chemotherapy [1].

Other authors like Schmidt *et al.* also evaluated prognostic and predictive values of PD-L1 expression. Three hundred and twenty-one curatively resected NSCLC patients who had available tumor material and clinical data from the Thoracic Departments in Ostrcapepeln, Germany, were included. Median age of patients was 66 years and all NSCLC histology included, except NSCLC non-other specified. Cut-off for positivity was $\geq 5\%$ of tumor cells PD-L1–positive. Twenty-four percent of cells expressed PD-L1 in NSCLC samples. For the whole group, PD-L1 expression was not the prognostic factor for OS ($p = 0.256$). The better survival in the patients with PD-L1–positive tumors compared to patients with no PD-L1 expression was observed only in some subgroups: squamous histology (HR 0.45, 95% CI 0.25–0.83, $p = 0.005$), patients that received adjuvant therapy (HR 0.35, CI 0.14–0.86, $p = 0.01$), had positive lymph nodes revided by surgery (HR 0.47, CI 0.26–0.85, $p = 0.005$), and had greater tumor size (HR 0.55, CI 0.36–0.84, $p = 0.004$). According to the authors, these findings suggest that PD-L1 expression might be prognostic in these subgroups of patients, but because of the small sample size, firm conclusions cannot be done [21].

Cooper retrospectively analyzed 681 Australian patients who underwent surgical resection for Stages I–III NSCLC between 1990 and 2008 for PD-L1 expression. Tumors with $\geq 50\%$ of the cells showing positive membrane staining were considered to have high expression of PD-L1. Several clinicopathological factors were compared regarding PD-L1 expression. Associations were encountered comparing patient's age, tumor size, and grade. High PD-L1 expression was associated with younger patient's age ($p = 0.07$). Median age in the "younger" group was 67 and "older" group 69 years. High PD-L1 expression was statistically significantly higher in bigger tumors (median size 45 mm vs. 40 mm, $p = 0.02$) and tumors with higher histological grade ($p < 0.01$). Even if not statistically significant, squamous and large cell tumors had more "high" expression compared with adenocarcinomas (8.1 and 12.5 vs. 5.1% of samples, $p = 0.13$). High PD-L1 expression was not associated with factors such as gender, tumor size, stage, nodal involvement, EGFR, k-ras, or ALK mutations. Authors also compared patients' outcome between the two PD-L1 groups. Patients with high expression had longer median survival compared to patients with low (113.2 months vs. 85.5 months, $p = 0.023$). High PD-L1 as the prognostic factor for better survival was confirmed in Cox model (HR 0.59, 95% CI 0.4–0.8, $p > 0.01$). Even if high PD-L1 expression was a prognostic factor in this trial, authors conclude that the evidence was still not firm enough to claim its prognostic value, and they state that any prognostic significance relates not on single marker of immune signals, but to the overall balance of the host-tumor immune response [22].

6. Immune checkpoint inhibitors in lung cancer and PD-L1 as predictive biomarker

The aim of cancer immunotherapies is to change the adoptive immune system toward tumor rejection. One of the possible approaches is immune checkpoint blockade, which aims to

relieve inhibition of immune checkpoints [5, 23]. NSCLC was considered to be an immunotherapy nonresponsive tumor type, when the earliest clinical trials with interleukins, vaccines, and interferon failed to show clinical benefit. More recently, good treatment responses and improved overall survival have been observed with the use of immune checkpoint inhibitors. Patients that respond to these treatments have durable responses that are usually longer than responses to chemotherapy or targeted therapies. However, some drawbacks exist in nonselected patients' response and can be seen only in 15–20% of the patients, and the cost of these drugs is extremely high [12, 16].

Currently, two checkpoint inhibitors (PD-L1 inhibitors) pembrolizumab and nivolumab are being used in everyday clinical practice, but it is supposed that soon others such as atezolizumab, durvalumab, and avelumab will enter into everyday clinical use [24]. Quick development of new immunotherapeutic drugs is not followed by proper development in optimal biomarkers for patient selection. In clinical trials, anti-PD-1 and anti-PD-L1 antibodies produce 20% of response in nonselected population [25]. This can be well seen in the interpretation of clinical trials with nivolumab and pembrolizumab. General conclusions from nivolumab trials is that patient selections should not be done on the PD-L1 expression, while pembrolizumab trials suggest the opposite [16].

6.1. Nivolumab

CheckMate 017, a Phase III trial, compared treatment with docetaxel in one arm and nivolumab in second arm in patients with Stage IIIB or Stage IV squamous NSCLC that progressed on the first-line therapy with platinum-containing regimen. From October 2012–December 2013, 272 eligible patients were included. Patients were randomized to receive either nivolumab at dose 3 mg/kg or docetaxel 75 mg/m² every 3 weeks. The primary endpoint was OS. Median age was 63, most were men (76%), had ECOG performance status score 1 (76%), and were current or former smokers (92%). The nivolumab group had longer overall survival (9.2 vs. 6 months, HR 0.59, $p < 0.001$) and longer progression-free survival (3.5 vs. 2.8 months, HR = 0.62, $p < 0.001$). Response was 20% in nivolumab and 9% in docetaxel arm ($p = 0.008$). PD-L1 expression was assessed retrospectively in 83% (225 of 272) of the patients who underwent randomization. Patients were categorized as positive if the expression was 1, 5, or 10%. No differences in overall survival was found between different expression groups in patients that received nivolumab or docetaxel regardless of the percentage (%) of positivity (>1% HR 0.96, CI 0.45–1.05; >5% HR 0.53, CI 0.3–0.89; >10% HR 0.70, CI 0.47–1.01). Authors concluded that the expression of PD-L1 was not a prognostic or a predictive factor for treatment with nivolumab [16, 26].

CheckMate 057 was a second Phase III trial comparing nivolumab with docetaxel for patients with previously treated nonsquamous NSCLC Stage IIIB or Stage IV that progressed on previous therapy. From November 2012 to December 2013, 582 patients were randomized to receive either 3 mg/kg of nivolumab every 3 weeks or docetaxel 75 mg/m² every 3 weeks. Patients treated with nivolumab had longer overall survival (12.2 vs. 9.4 months, HR 0.73, $p = 0.002$) and a higher response rate (19% vs. 12%, $p = 0.02$) compared to patients receiving docetaxel. Differences in PFS were not established (2.3 months for nivolumab vs. 4.2 for docetaxel, HR 0.92, CI 0.77–1.11, $p = 0.39$). PD-L1 expression was evaluated in the same way

as in CheckMate 017 with cut-offs at 1, 5, and 10%. PD-L1 testing was performed in 78% (455 of 582) of the patients. Objective response rate was 9% (CI 5–16) in patients with less than 1% PD-L1 expression, 36% (CI 26–46) in patients with >5%, and 37 (CI 7–48) in patients with more than 10% expression. Similarly, differences were seen in OS, with patients who have expression more than 5% (median OS 19.4 months), having longer survival with nivolumab treatment compared to patients with expression less than 1% (median OS 10.5 months) ($p < 0.01$). The study showed a predictive association between PD-L1 expression and benefit from anti-PD-1 treatment. Although the benefit of nivolumab was observed in the overall population, the magnitude of benefit across all the efficacy endpoints appeared to be greater among patients whose tumors expressed PD-L1 than among those whose tumors did not express PD-L1 [16, 27].

6.2. Pembrolizumab

Keynote 001 was a Phase I trial in which patients received three different schedules of treatment with pembrolizumab (2 mg/kg every 2 weeks, 10 mg/kg every two or every 3 weeks). From May 2012 to February 2014, 495 patients with Stage IIIB or Stage IV of NSCLC were included. ORR with pembrolizumab treatment was 19.4%, and PFS and OS were 3.7 and 12.5 months, respectively. PD-L1 was assessed and cut-off defined at 50% of tumor cells expression. In defining PD-L1 positivity, authors used receiver-data-characteristic curves (ROC). Prevalence of PD-L1 positivity (>50%) was 23.2, 1–49, 37.6, and 39.2% less than 1%. Among patients with the score of at least 50%, the progression-free survival was 6.3 (95% CI 2.9–12.5) months, and overall survival was not reached. Progression-free and overall survival were shorter among patients with a proportion score of 1–49% or a score of less than 1% than among those with a score of at least 50%. Median PFS was 6.1 months (CI 4.2–not reached) in positive group and 4 months (CI 2.1–2.4) in <1% group. Median OS was not reached in a positive group and 10.4 (CI 5.8–not reached) in <1% group. Authors conclude that a proportion score of at least 50% may represent a new biomarker for the treatment of nonsmall-cell lung cancer [16, 25].

Keynote 010 was a Phase II/III trial comparing two schedules of pembrolizumab (2 mg/kg every 3 weeks, 10 mg/kg every 3 weeks) with docetaxel 75 mg/m² for patients with PD-L1 expression (>1% of tumor cells) that progressed on previous chemotherapy. Between August 2013 and February 2015, 1034 patients were included. Median overall survival was 10.4 months with pembrolizumab 2 mg/kg, 12.7 months with pembrolizumab 10 mg/kg, and 8.5 months with docetaxel. Overall survival was significantly longer for both doses of pembrolizumab vs. docetaxel (pembrolizumab 2 mg/kg vs. docetaxel: HR 0.71, 95% CI 0.58–0.88, $p = 0.0008$), and (pembrolizumab 10 mg/kg vs. docetaxel: HR 0.61, 95% CI 0.49–0.75, $p < 0.0001$). Median progression-free survival was 3.9 months with pembrolizumab 2 mg/kg, 4.0 months with pembrolizumab 10 mg/kg, and 4.0 months with docetaxel, with no significant difference for pembrolizumab 2 mg/kg or for pembrolizumab 10 mg/kg. As expected, patients with at least 50% of tumor cells expressing PD-L1 had overall survival significantly longer with pembrolizumab 2 mg/kg than with docetaxel (median 14.9 months vs. 8.2 months; HR 0.54, 95% CI 0.38–0.77, $p = 0.0002$), and with pembrolizumab 10 mg/kg than with docetaxel (17.3 months vs. 8.2 months; HR 0.50, 95% CI 0.36–0.70; $p < 0.0001$). For this patient population, progression-free survival

was also significantly longer with pembrolizumab 2 mg/kg than with docetaxel (median 5.0 months vs. 4.1 months, HR 0.59, 95% CI 0.44–0.78, $p=0.0001$), and with pembrolizumab 10 mg/kg than with docetaxel (5.2 months vs. 4.1 months, 95%, HR 0.59, 95% CI 0.45–0.78, $p < 0.0001$). This trial confirmed the results of Keynote 001 and added additional data to the assumed predictive value of PD-L1 positivity [16, 28].

6.3. Atezolizumab

PD-L1 expression in trials with atezolizumab is reported somehow differently. First expression is reported in tumor cells (TC) and immune cells infiltrating tumor (IC) separately. Tumors are categorized into four different groups:

- TC3/IC3: at least 10% of cells expressing PD-L1.
- TC2/IC2: at least 5% of cells expressing PD-L1.
- TC1/IC1: at least 1% of cells expressing PD-L1.
- TC0/IC0: less than 1% of cells expressing PD-L1.

The association between the response to atezolizumab and PD-L1 expression was first assessed in a Phase I trial of Herbst *et al.* Two hundred and seventy-seven patients with advanced incurable cancer received MPDL-3280A (atezolizumab) intravenously every 3 weeks. Responses (complete and partial responses) were observed in 32 of 175 (18%) with all tumor types including NSCLC, melanoma, renal cell carcinoma, and other tumors (including colorectal cancer, gastric cancer, and head and neck squamous cell carcinoma). Authors found the association between response and the expression of PD-L1. The association of response to atezolizumab treatment and tumor-infiltrating immune cell PD-L1 expression was statistically significant ($p = 0.007$), while the association with tumor cell PD-L1 expression was not ($p = 0.920$) [29].

POPLAR was a Phase II trial comparing treatment with atezolizumab with docetaxel in patients progressing on previous chemotherapy treatments. One hundred forty-four patients between August 2013 and March 2014 randomly received either atezolizumab 1200 mg or docetaxel 75 mg/m² once every 3 weeks. Patients on atezolizumab had longer overall survival (12.6 vs. 9.7 months, HR = 0.73, $p = 0.04$), although no differences in response rate or progression-free survival were encountered. Higher PD-L1 expression in TC/IC was associated with improved overall survival (TC3/IC3 HR 0.49, 95% CI 0.22–1.07, $p = 0.068$; TC2/3 or IC2/3 HR 0.54, 95% CI 0.33–0.89. $p = 0.014$, TC1/2/3 or IC1/2/3 HR 0.59, 95% CI 0.40–0.85, $p = 0.005$; TC0 and IC0 HR 1.04. 95% CI 0.62–1.75, $p = 0.871$). Authors believe PD-L1 expression is predictive for atezolizumab's benefits [16, 30].

BIRCH trial was a Phase II trial in which patients with locally advanced or metastatic NSCLC were divided into three cohorts. All the patients received atezolizumab at the dose of 1200 mg every 3 weeks. Patients were included if they had TC2–3/IC 2-3 PD-L1 expression. One hundred thirty-nine patients in Cohort I received atezolizumab as first-line treatment, 267 patients in Cohort II as second, and 253 in Cohort III as third or more line of treatment. Higher PD-L1 expression was associated with higher response rate in all cohorts.

Name of the trial (phase)	Compound	PD-L1 expression and OS (HR compared to control arm or months)	PD-L1 expression and PFS (HR compared to control arm or months)	PD-L1 expression and RR (%)
Checkmate 017 (Phase 3)	Nivolumab versus docetaxel	<1% (0.58)	<1% (0.66)	<1% (17)
		≥1% (0.69)	≥1% (0.67)	≥1% (17)
		<5% (0.70)	<5% (0.75)	<5% (15)
		≥5% (0.53)	≥5% (0.54)	≥5% (21)
		<10% (0.70)	<10% (0.70)	<10% (16)
		≥10% (0.50)	≥10% (0.58)	≥10% (19)
Checkmate 057 (Phase III)	Nivolumab versus docetaxel	<1% (0.90)	<1% (1.19)	<1% (9)
		≥1% (0.59)	≥1% (0.70)	≥1% (31)
		<5% (1.01)	<5% (1.31)	<5% (10)
		≥5% (0.43)	≥5% (0.54)	≥5% (36)
		<10% (1.00)	<10% (1.24)	<10% (11)
		≥10% (0.40)	≥10% (0.52)	≥10% (37)
Keynote 001 (Phase I)	Pembrolizumab	<1% 10.4 months	<1% 4.0 months	<1% (10.7)
		1–49% 10.6 months	1–49% 4.1 months	1–49% (16.5)
		≥50% not reported	≥50% 6.4 months	≥50% (45.2)
Keynote 010 (Phase III)	Pembrolizumab 2 mg/kg versus docetaxel	>1% (0.71)	>1% (0.88)	>1% (18)
		≥50% (0.54)	≥50% (0.59)	≥50% (30)
Keynote 010 (Phase III)	Pembrolizumab 10 mg/kg versus docetaxel	>1% (0.61)	>1% (0.79)	>1% (18)
		≥50% (0.50)	≥50% (0.59)	≥50% (29)
Atezolizumab (Phase I)	Atezolizumab	TC0–2/IC0–2 16 months TC3/IC3 18 months	TC0–2/IC0–2 4 months TC3/IC3 4 months	TC0–2/IC0–2 (16) TC3/IC3 (48)
POPLAR (Phase 2)	Atezolizumab versus docetaxel	TC0/IC (1.04)	TC0/IC (1.12)	TC0/IC (8)
		TC0–2/IC 0–2 (0.59)	TC0–2/IC 0–2 (0.85)	TC0–2/IC 0–2 (18)
		TC2–3/IC 2–3 (0.54)	TC2–3/IC 2–3 (0.72)	TC2–3/IC 2–3 (22)
		TC3/IC3 (0.49)	TC3/IC3 (0.60)	TC3/IC3 (38)
BIRCH (Phase II, Cohort I)	Atezolizumab	TC2–3/IC2–3 (82%)*	TC2–3/IC2–3 5.5 months	TC2–3/IC2–3 (19)
		TC3/IC3 (79%)*	TC3/IC3 5.5 months	TC3/IC3 (26)
BIRCH (Phase II, Cohort II)	Atezolizumab	TC2–3/IC2–3 (76%)*	TC2–3/IC2–3 2.8 months	TC2–3/IC2–3 (17)
		TC3/IC3 (80%)*	TC3/IC3 4.1 months	TC3/IC3 (24)
BIRCH (Phase II, Cohort III)	Atezolizumab	TC2–3/IC2–3 (71%)*	TC2–3/IC2–3 2.8 months	TC2–3/IC2–3 (17)
		TC3/IC3 (75%)*	TC3/IC3 4.2 months	TC3/IC3 (27)
Avelumab (Phase Ib)	Avelumab	>1% (4.6 months)	>1% (5.9 weeks)	>1% (10)
		≤1% (8.9 months)	≤1% (12.0 weeks)	≤1% (15)
Durvalumab (Phase I/II)	Durvalumab	<25% not reported	<25% not reported	<25% (5)
		≥25% not reported	≥25% not reported	≥25% (27)

*Six-month overall survival.

Table 1. Response to checkpoint inhibitors according to PD-L1 expression.

In Cohort I, ORR was 26% (95% CI 16–39) in patients with TC3/IC2 vs. 19% (95% CI 13–27) in patients with TC2–3/IC 2–3. In Cohort II, ORR was 24% (95% CI 17–32) in patients with TC3/IC2 vs. 17% (95% CI 13–22) in patients with TC2–3/IC 2–3. In Cohort III ORR was 27% (95% CI 19–36) in patients with TC3/IC2 vs. 17% (95% CI 13–23) in patients with TC2–3/IC 2–3. These results also suggest that PD-L1 is a possible biomarker for treatment with atezolizumab [31].

6.4. Avelumab and durvalumab

Patients received avelumab in a Phase Ib trial. The response rate, median progression-free survival, and median overall survival were 13.6%, 11.6 weeks and 8.4 months. PD-L1 cut-off for positivity was 1% of the expression and was associated with higher response rate, longer progression-free, and overall survival [16]. Response rate of 16% was achieved in Phase I/II trial of durvalumab treatment. PD-L1 positivity was defined as an expression of more than 25% of cells and was correlated with higher response rate [16].

Trials with checkpoint inhibitors are summarized in **Table 1**.

7. The problems of PD-L1 as a biomarker

The assessment of PD-L1 tumor expression is currently a controversial issue with more open questions than known facts. PD-L1 expression is a dynamic rather than static process that varies according to different tumor and host factors (15–30). Several data show that anti-PD-L1 drugs are more effective in patients whose tumors express PD-L1, but responses in the population of patients without the expression lead to the conclusion that these patients also benefit from this treatment. How to select right patients for anti-PD-L1 treatment remains an open question in these circumstances [5].

7.1. Tests

Currently, several different monoclonal antibodies for testing PD-L1 positivity exist. In a meta-analysis of Aguiar *et al.* among 13 included trials, 5 different antibodies were used (DAKO 28-8, DAKO 22C3, VENTANA SP 142, and two nonspecified), and 3 different threshold values for immunohistochemical positivity selected (1, 5, and 50%). Authors conclude that standardized approach to PD-L1 status assessment is lacking [1]. For the details of Aguiar's meta-analysis, please see the Section 5 of this chapter.

Today several differences exist between tests:

1. Every test uses its own antibody.
2. Some tests evaluate the percentage of tumor cells stained, while others evaluate not only tumor cells but also tumor infiltrating immune cells.
3. Cut-off points and scoring systems differ between tests.
4. Different staining techniques (manual vs. automated).

All these differences make the comparison between results of different tests difficult if not impossible [16, 23].

7.2. Positivity and response to treatment

The threshold of immunohistochemical PD-L1 expression positivity is not well established today. Thresholds of 1, 5, or 50% of positive cells are being used in different trials. It is estimated that any expression of PD-L1 can be found in 45–50% of NSCLC biopsies [18]. In a trial of Cha *et al.*, the threshold of 5% showed the most significant *p* value regarding overall survival predictions [19]. For details of Cha *et al.* trial, please see the Section 5 of this chapter. Even if considered positive (regardless of the positivity cut-off), not all patients respond to the treatment with immunotherapy. Response is seen only in 15–45% of “positive” patients and on the other end, many “negative” patients also respond [16].

7.3. Concordance

Checkpoint inhibitors recently demonstrated efficacy in the treatments of metastatic NSCLC that are being used more and more in clinical trials and everyday clinical practice. Despite their benefits, the association of responses with a predictive biomarker PD-L1 remains uncertain. Today, several PD-L1 IHC tests exist, and concordance between them is not very clear. Sheffield *et al.* compared multiple methods of testing. Tissue microarrays of matched primary and metastatic NSCLCs were used to compare four different PD-L1 IHC techniques. Tissues from 80 patients were included. Multiple IHC methodologies showed a high rate of agreement (Kappa = 0.67). Concordance between PD-L1 positivity among different tests (antibodies) was from 73.4 to 76%. Determination of which test is the best one is challenging due to the lack of a reference standard [32].

Significant discordance between the PD-L1 status of primary tumors and metastases was observed. PD-L1 status of primary and metastatic tumors was discordant in 17 (22%) cases. Because of the variability of the biomarker, also changes in positivity during immunotherapy treatment have been studied. Discordance between primary tumor and metastatic sites is supposed to be because of the intratumoral heterogeneity and sampling bias [32]. Variability seems to be even more substantial, since immunohistochemical status changes during treatment in 12–35% of the patients [16].

7.4. Tissue

Keynote 001 trial revealed another unanswered issue in the PD-L1 expression testing. Deterioration in the PD-L1 expression in archival tissue samples that had been sectioned 6 months or more before testing was encountered. Researches decided to evaluate only the samples taken in the 6-months period before testing. Of the 1143 screened patients, only 824 had eligible tumor samples. Why this deterioration in expression occurs, and how to treat patients with pembrolizumab that do not have tissue available for testing are another of the two questions to be solved [25].

7.5. Open questions

To be able to continue the story of the PD-L1 as a predictive biomarker, these open questions will have to be answered [33]:

1. Is a predictive biomarker in immunotherapy necessary?
2. Is localization of the biomarker important (expression of PD-L1 in tumor and/or tumor-infiltrating lymphocytes)?
3. What is/are the optimal detection test/tests?
4. Does PD-L1 expression change over time (after different treatments such as chemotherapy and irradiation) and space (primary tumor vs. metastases)?

8. Conclusion

Immune checkpoint inhibitors are new and very promising treatment options in patients with several solid malignancies including lung cancer. For an optimal selection of patients who benefit most, predictive biomarkers are needed. PD-L1 has been suggested as a potential biomarker, but due to many open questions, today, it is not considered the only reliable immunotherapy biomarker since responses can be encountered even in patients considered PD-L1 negative. A possible solution to this issue is finding new and maybe more reliable predictive biomarkers that will for sure evolve in the next years of immunotherapy treatment development.

Author details

Mirjana Rajer

Address all correspondence to: mrajer@onko-i.si

Institute of Oncology Ljubljana, Ljubljana, Slovenia

References

- [1] Aguiar, P.N., Jr., et al., The role of PD-L1 expression as a predictive biomarker in advanced non-small-cell lung cancer: a network meta-analysis. *Immunotherapy*, 2016. 8(4): pp. 479–488.
- [2] Homet Moreno, B. and Ribas, A., Anti-programmed cell death protein-1/ligand-1 therapy in different cancers. *Br J Cancer*, 2015. 112(9): pp. 1421–1427.
- [3] McDermott, D.F. and Atkins, M.B., PD-1 as a potential target in cancer therapy. *Cancer Med*, 2013. 2(5): pp. 662–673.

- [4] Domagala-Kulawik, J., The role of the immune system in non-small cell lung carcinoma and potential for therapeutic intervention. *Transl Lung Cancer Res*, 2015. **4**(2): pp. 177–190.
- [5] Aguiar, P.N., Jr., et al., A pooled analysis of nivolumab for the treatment of advanced non-small-cell lung cancer and the role of PD-L1 as a predictive biomarker. *Immunotherapy*, 2016. **8**(9): pp. 1011–1019.
- [6] Janssen-Heijnen, M.L. and Coebergh, J.W., Trends in incidence and prognosis of the histological subtypes of lung cancer in North America, Australia, New Zealand and Europe. *Lung Cancer*, 2001. **31**(2–3): pp. 123–137.
- [7] Janssen-Heijnen, M.L. and Coebergh, J.W., The changing epidemiology of lung cancer in Europe. *Lung Cancer*, 2003. **41**(3): pp. 245–258.
- [8] Charloux, A., et al., The increasing incidence of lung adenocarcinoma: reality or artefact? A review of the epidemiology of lung adenocarcinoma. *Int J Epidemiol*, 1997. **26**(1): pp. 14–23.
- [9] Burns, D.M., Changing rates of adenocarcinoma of the lung. *Chem Res Toxicol*, 2014. **27**(8): pp. 1330–1335.
- [10] Santarpia, M., et al., Programmed cell death protein-1/programmed cell death ligand-1 pathway inhibition and predictive biomarkers: understanding transforming growth factor-beta role. *Transl Lung Cancer Res*, 2015. **4**(6): pp. 728–742.
- [11] Zamarin, D. and Postow, M.A., Immune checkpoint modulation: rational design of combination strategies. *Pharmacol Ther*, 2015. **150**: pp. 23–32.
- [12] Chakravarti, N. and Prieto, V.G., Predictive factors of activity of anti-programmed death-1/programmed death ligand-1 drugs: immunohistochemistry analysis. *Transl Lung Cancer Res*, 2015. **4**(6): pp. 743–751.
- [13] Ballman, K.V., Biomarker: predictive or prognostic?. *J Clin Oncol*, 2015. **33**(33): pp. 3968–3971.
- [14] Chan, J.Y., Choudhury, Y., and Tan, M.H., Predictive molecular biomarkers to guide clinical decision making in kidney cancer: current progress and future challenges. *Expert Rev Mol Diagn*, 2015. **15**(5): pp. 631–646.
- [15] Burotto, M., et al., Biomarkers in early-stage non-small-cell lung cancer: current concepts and future directions. *J Thorac Oncol*, 2014. **9**(11): pp. 1609–1617.
- [16] Shukuya, T. and Carbone, D.P., Predictive markers for the efficacy of Anti-PD-1/PD-L1 antibodies in lung cancer. *J Thorac Oncol*, 2016. **11**(7): pp. 976–988.
- [17] Falconi, A., Lopes, G., and Parker, J.L., Biomarkers and receptor targeted therapies reduce clinical trial risk in non-small-cell lung cancer. *J Thorac Oncol*, 2014. **9**(2): pp. 163–169.
- [18] D’Incecco, A., et al., PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer*, 2015. **112**(1): pp. 95–102.

- [19] Cha, Y.J., et al., Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status. *Lung Cancer*, 2016. **97**: pp. 73–80.
- [20] Baxevanis, C.N., et al., Immune biomarkers: how well do they serve prognosis in human cancers?. *Expert Rev Mol Diagn*, 2015. **15**(1): pp. 49–59.
- [21] Schmidt, L.H., et al., PD-1 and PD-L1 expression in NSCLC indicate a favorable prognosis in defined subgroups. *PLoS One*, 2015. **10**(8): p. e0136023.
- [22] Cooper, W.A., et al., PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. *Lung Cancer*, 2015. **89**(2): pp. 181–188.
- [23] Chae, Y.K., et al., Biomarkers for PD-1/PD-L1 blockade therapy in non-small-cell lung cancer: is PD-L1 expression a good marker for patient selection?. *Clin Lung Cancer*, 2016. **17**(5): pp. 350–361.
- [24] Grigg, C. and Rizvi, N.A., PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction?. *J Immunother Cancer*, 2016. **4**: p. 48.
- [25] Garon, E.B., et al., Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*, 2015. **372**(21): pp. 2018–2028.
- [26] Brahmer, J., et al., Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*, 2015. **373**(2): pp. 123–135.
- [27] Borghaei, H., et al., Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*, 2015. **373**(17): pp. 1627–1639.
- [28] Herbst, R.S., 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**(10027): pp. 1540–1550.
- [29] Herbst, R.S., et al., Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*, 2014. **515**(7528): pp. 563–567.
- [30] Fehrenbacher, L., 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): pp. 1837–1846.
- [31] Besse, B., et al., 16LBA 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). *Eur J Cancer*, 2015. **51**: pp. S717–S718.
- [32] Sheffield, B.S., et al., Investigation of PD-L1 biomarker testing methods for PD-1 axis inhibition in non-squamous non-small cell lung cancer. *J Histochem Cytochem*, 2016. **64**(10): 587–600.
- [33] Sundar, R., et al., Immunotherapy in the treatment of non-small cell lung cancer. *Lung Cancer*, 2014. **85**(2): pp. 101–109.

