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Predictive biomarkers of response to PD-1/PD-L1 immune checkpoint inhibitors in non–small cell lung cancer

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Abstract

Inhibitors of the programmed cell death 1 (PD-1) pathway show the potential to substantially increase the efficacy of therapy for various malignancies, including non–small cell lung cancer (NSCLC). At the same time, substantial effort has been invested in finding biomarkers predicting which patients will respond best to this immune checkpoint inhibition. PD-L1 expression in tumor cells and the tumor microenvironment, genetic alterations and mutational load in tumor cells, and pre-existing immunity and its enhancement during treatment through tumor-infiltrating immune cells have been associated with outcomes of immune checkpoint inhibition. Here, we review the reported predictive biomarkers of response to PD-1 pathway immune checkpoint inhibitors in NSCLC, mainly focusing on results obtained with clinical trials.

Keywords

Immune checkpoint inhibition; PD-1; PD-L1; Biomarker; NSCLC

1. Introduction

Various chemotherapeutic agents were developed to treat patients with advanced-stage non–small cell lung cancer (NSCLC) patients. However, standard platinum doublet therapy seems to have reached a plateau in terms of efficacy [1,2]. The development of agents targeting vascular endothelial growth factor, use of histology to guide chemotherapy decisions, and addition of maintenance therapy have led to improvements of several months in overall survival (OS) over platinum doublet therapy alone, particularly for non-squamous NSCLC [3]. To extend survival further, novel types of drugs that target specific molecular pathways have been developed over the past decade [4]. For example, tyrosine kinase inhibitors (TKIs) specific for the epidermal growth factor receptor (EGFR) have changed clinical practice

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dramatically in a specific subset of NSCLC patients. Despite dramatic responses, however, acquired drug resistance to EGFR-TKIs develops in almost all patients who receive these treatments, usually within 1 year, limiting improvement in patient outcomes [5,6]. Novel treatment options are clearly warranted to improve the prognosis of NSCLC patients.

Since 2010, clinical trials of antibodies directed against the immunosuppressive molecules programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) have shown both encouraging safety profiles and remarkable antitumor activity in various malignancies, including NSCLC [7–16]. PD-1 is expressed in activated T-cells, and inhibits their function upon binding to the PD-1 ligands PD-L1 (also called B7-H1 or CD274) and programmed cell death ligand 2 (PD-L2, also called B7-DC or CD273), which is expressed on several types of immune cells and tumor cells [17,18]. Thus, PD-1 and PD-1–ligand interactions are a major mechanism of immunosuppression within the tumor microenvironment, and inhibiting these interactions can significantly enhance T-cell function, resulting in antitumor activity [8,19].

To date, various therapeutic antibodies inhibiting either PD-1 or PD-L1 have been developed for clinical application in the treatment of NSCLC and other malignancies. Substantial effort also has been invested in finding mechanism-based predictive biomarkers to identify the patients who will respond best to PD-1 pathway inhibition. These predictive biomarkers are important to consider when we select patients likely to respond favorably to PD-1 pathway inhibition, and when we evaluate treatment options that are available for individual patients with NSCLC and other cancers that may respond to these inhibitors. Here, we review the reported predictive biomarkers of response to PD-1 pathway immune checkpoint inhibitors in patients with NSCLC, mainly focusing on results of clinical trials.

2. Impact of PD-1 pathway inhibition in NSCLC

2.1. Rationale for PD-1 pathway inhibition

In NSCLC patients, the overexpression of PD-1 on CD8⁺ T-cells has been observed, and tumor-infiltrating CD8⁺ T-cells have high PD-1 expression and impaired immune function, including reducing cytokine production capability and impairing capacity to proliferate [20]. At the same time, the overexpression of PD-L1 in tumor cells can escape the immune response to the cancer cells and consequently facilitate tumor progression and metastasis [21,22]. Indeed, high PD-L1 expression in tumor cells was significantly associated with poor differentiation of the tumor cells and poor prognosis in many malignancies, including NSCLC [23,24]. Thus, inhibiting the PD-1 pathway may facilitate restoration of tumor-specific T-cell function and diminish tumor-induced immunosuppression [25].

2.2. Clinical trials of PD-1 pathway inhibitors for NSCLC patients

PD-1 or PD-L1 inhibitors for NSCLC currently in clinical use or in development are shown in Table 1. The PD-1 inhibitor, nivolumab (Opdivo) is a fully human IgG4 antibody, and the PD-1 inhibitors pembrolizumab (Keytruda) and PDR001 are engineered humanized IgG4 antibodies. In 2015, nivolumab received U.S. Food and Drug Administration (FDA) approval for the treatment of advanced (metastatic) NSCLC that has progressed during or

after platinum-based chemotherapy. Pembrolizumab also received FDA approval in 2015 for the treatment of advanced (metastatic) NSCLC that has progressed after other treatments and whose tumors express PD-L1.

No PD-L1 inhibitors have received FDA approval for the treatment of NSCLC to date. Atezolizumab (MPDL3280A) and durvalumab (MEDI4736) are humanized IgG1 antibodies. BMS-936559 (MDX-1105) is a fully human IgG4 antibody, and avelumab (MSB0010718C) is a fully human IgG1 antibody. To date, BMS-936559 has not been developed further for the treatment of NSCLC, and additional trials using atezolizumab, durvalumab, and avelumab are in progress.

3. Candidate predictive biomarkers of response to PD-1 pathway inhibitors

Candidate predictive biomarkers of response to PD-1 pathway inhibitors have been investigated in subanalyses in clinical trials. PD-L1 expression, pre-existing immune status thorough tumor-infiltrating CD8+ T-cells and its enhancement during treatment, and genetic alterations and mutational load in tumor cells have been significantly associated with outcomes in patients treated with these inhibitors. Some of these factors are currently under intense study individually and in combination to find more sensitive and specific predictors of the clinical efficacy of PD-1 pathway inhibitors for the treatment of NSCLC.

3.1. PD-L1 expression in tumor cells

Early studies revealed a significant correlation between pretreatment PD-L1 expression in tumor cells on immunohistochemical analysis (IHC) and the likelihood of response to PD-1 inhibitors in several types of malignancies [7,9]. In contrast, some recent studies have shown that baseline PD-L1 status is independent of treatment response [10,12].

Fig. 1 summarizes PD-L1 positivity rates according to histologic subtype and the associations between PD-L1 expression and clinical benefit in patients with NSCLC. All of the analyzed trials used nivolumab, and the PD-L1 positivity of tumor cells on IHC was defined by a 5% threshold [11,12,14,15,19,26–30]. In general, there was a higher response rate in patients with PD-L1–positive disease than in patients with PD-L1–negative disease. In the CheckMate-057 trial, targeting non-squamous NSCLC, PD-L1 expression was associated with improved efficacy across all endpoints (OS, PFS, and duration of response) at all expression levels [11]. The most improvement was seen in patients with PD-L1 expression 5% and 10%, but was evident at PD-L1 expression levels as low as 1%. On the other hand, PD-L1 expression on squamous NSCLC did not significantly predict response across the prespecified expression levels (1%, 5%, and 10%) in the CheckMate-017 trial [12]. Thus, the relationship between PD-L1 expression and outcomes after treatment with nivolumab appears to depend on tumor type and histologic class.

Regarding pembrolizumab, high PD-L1 expression on tumors was associated with better outcome than low PD-L1 expression in KEYNOTE-001 trial [13]. This trial enrolled 495 patients, of whom 182 patients were assigned to a training group and 313 patients to a validation group. The data from the training group were used to define the clinical cutoff for the PD-L1 assay. On the basis of receiver operating characteristic analysis, a proportion

score of $\geq 50\%$ was selected, which corresponds to PD-L1 membrane expression in at least 50% of the tumor cells. In the validation group, patients with a proportion score of $\geq 50\%$ had a higher response rate and longer OS and PFS than patients with a proportion score of $<50\%$.

On the basis of these results, in 2015 the FDA approved two assays to detect PD-L1 protein by IHC along with the PD-1 inhibitors nivolumab and pembrolizumab to treat NSCLC. The clinical uses of these two assays are rather different. For nivolumab, the FDA approved the PD-L1 IHC 28-8 pharmDx assay (Dako, Carpinteria, CA) to detect PD-L1 protein expression levels and help physicians determine which patients will benefit most from treatment with nivolumab. The suggested cutoff for positive staining is $\geq 1\%$ at any intensity based on the clinical trial result [11]. FDA approved this assay as a complementary diagnostic, meaning its use is not mandated prior to nivolumab administration. Unlike nivolumab, pembrolizumab is approved only for patients with PD-L1-positive tumors as determined by the PD-L1 IHC 22C3 pharmDx kit (Dako) companion diagnostic that was approved at the same time as the drug. The PD-L1 IHC 22C3 pharmDx kit requires the EnVision FLEX visualization system on the Autostainer Link 48 from Dako. Scoring of the stained slides is based on a tumor proportion score, or the percentage of viable tumor cells displaying partial or complete membrane staining. A tumor proportion score of $\geq 50\%$ in the 22C3 pharmDx kit is considered positive according to the KEYNOTE-001 trial results. For consistent interpretation of the tumor proportion score by pathologists, Dako provides an interpretation manual and Web-based instruction (<http://www.dako.com/>). These companion diagnostic tests require a notable investment by the histology laboratory and the pathologist.

When using PD-L1 expression on IHC as a predictive biomarker, we need to take into consideration that PD-L1 assessment on IHC has been performed in different ways, using different antibodies, different technical staining platforms, and different definitions of PD-L1 positivity in different clinical trials. Furthermore, PD-1 expression within tumors may be heterogeneous and dynamic. Indeed, several trials assessing PD-L1 expression used archived samples, and intervening treatments might have affected PD-L1 status. Regarding the definitions of PD-L1 positivity, more stringent cutoffs for PD-L1 expression would better enrich for responders but fail to identify a larger absolute number of potential responders, while a less stringent PD-L1 expression cutoff might capture more of the responders but reduce the predictive power of the assay. In addition, it is important to remember that PD-L1 is an inducible and dynamic biomarker and needs to be considered differently from other well-known oncologic predictive biomarkers such as actionable *EGFR* mutations and *ALK* fusion in NSCLC. To elucidate some of the issues on existing assays to assess PD-L1 IHC expression as predictive marker in NSCLC, there are two ongoing projects comparing the performance of several diagnostics and research commercially available antibodies: the Blue Print study, coordinated by the International Association for the Study of Lung Cancer (IASLC), and a study sponsored by the US National Cancer Center Network (NCCN). The results of these studies will help to determine how better assess PD-L1 IHC expression in lung cancer tissue specimens.

3.2. Tumor microenvironment/immune effector cells

In improving patient selection and treatment outcomes, it is critical to understand why many PD-L1-positive tumors fail to respond to PD-1 pathway inhibitors while some PD-L1-negative tumors respond to these inhibitors. Several explanations independent of baseline PD-L1 in tumor cells involving the immune microenvironment surrounding the tumor have been suggested.

First, as we mentioned above, PD-L1 is an inducible and dynamic biomarker subject to changes due to the tumor microenvironment. There is a question that if archived specimens are analyzed, PD-L1 expression status could differ temporally and anatomically between analyzed specimens and the actual treated tumor. In the analysis of 95 paired archival and fresh NSCLC tumor samples, the rate of concordance of PD-L1 expression status in tumor cells or tumor-infiltrating immune cells between fresh and archival tissue was high when the same type of tissue procurement method was used (resection or biopsy), whereas the rate of concordance decreased when different methods of procurement were used [31]. Indeed, pembrolizumab provided benefit compared with docetaxel irrespective of whether fresh or archival tumor samples were used to assess PD-L1 expression in KEYNOTE-010 trial [16]. Second, not only tumor cells but also tumor-infiltrating immune cells express PD-L1, and PD-L1 expression in immune cells could be more relevant to inhibitor response than its expression in tumor cells. Third, PD-L1 expression is constitutive in some cases, caused by activation of a pathway such as PI3 K/AKT within the tumor, rather than induced by an adaptive response to antigen-specific T-cells [32]; if PD-L1 is overexpressed on a tumor lacking an appropriate immune infiltrate, PD-1 pathway inhibition may have no effect because the tumor is lacking the effector cells that attack cancer cells. The response to immune checkpoint inhibitors also will largely depend on the individual's immune status. In Table 2, we summarize reported molecular predictive biomarkers for PD-1 pathway inhibitors in NSCLC.

3.2.1. PD-L1 expression in the tumor microenvironment—Several reports show a positive correlation between PD-L1 expression in the tumor microenvironment and outcomes of treatment with PD-1 pathway inhibitors. Herbst et al. reported the association of PD-L1 expression in tumor-infiltrating immune cells with treatment response to atezolizumab in several solid tumor types, including NSCLC [10]. However, PD-L1 expression in tumor-infiltrating immune cells has been reported to vary according to tumor type, and there are still few data on PD-L1 expression in these immune cells in NSCLC [33]. The role of PD-L1 expression in the NSCLC tumor environment as a predictive biomarker for PD-1 pathway inhibition requires further evaluation in the context of clinical trials.

3.2.2. PD-L2 expression in tumor cells and infiltrating immune cells—PD-L2 expression by tumors as a mechanism of immune evasion has been described [34]. Few reports analyze the prognostic values of PD-L2 expression in PD-1 pathway inhibitors, and the predictive value of PD-L2 expression in NSCLC has yet to be defined.

3.2.3. CD8 and T-cell and effector functional markers—The levels of certain immune cells predict response to immune checkpoint inhibitors. CD8 expression can be

found on natural killer and dendritic cells but is predominantly expressed on the surface on cytotoxic T-cells. CD8+ T-cells are a crucial component of the cellular immune system and cell-mediated antitumor immune responses [35,36]. Strong lymphocytic infiltration has been associated with good clinical outcomes in many types of malignancies, including NSCLC [37,38]. High densities of CD3+ T-cells, CD8+ cytotoxic T-cells, and CD45RO+ memory T-cells were associated with longer disease-free survival and/or OS in patients with many types of malignancies who underwent surgical resection of the primary tumor [35]. In NSCLC, CD8+ T-cell density has been reported as a significant prognostic factor alone or in combination with other markers [39]. Donnem et al. thoroughly analyzed the impact of stromal CD8+ tumor-infiltrating lymphocytes (TILs) as an immunoscore in NSCLC, reported that stromal CD8+ TILs density has independent prognostic value in resected NSCLC, and suggested that CD8 positivity is a good candidate marker for establishing a TNM immunoscore [40].

Several investigators found that the presence of CD8+ T-cells was also a potent predictor of response to PD-1 pathway inhibitors, suggesting that these cells drive the response to PD-1 pathway immune checkpoint inhibitors. In NSCLC, a subanalysis using the KEYNOTE-001 trial sample showed a significantly higher density of pre-existing CD8+ cells in the tumors of responders than in non-responders (percentage CD8+ nucleated cells: 17.7% vs. 5.6%, respectively) [41]. Although further investigations in larger samples are needed to determine the predictive impact of these biomarkers in PD-1 pathway inhibitors, these findings suggest that a patient's intrinsic immune system substantially affects the effectiveness of PD-1 pathway inhibitors.

3.2.4. Gene expression analysis—Several other candidate predictive biomarkers associated with immunosuppressive cells were reported in a gene expression analysis of clinical trial samples for solid tumors treated with atezolizumab [10]. Higher gene expression of *CTLA4* and lower expression of *CX3CL1* (fractalkine) before treatment correlated with response to atezolizumab. In melanoma, pretreatment tumors in the response group demonstrated elevated expression of *IFN γ* as well as IFN γ -inducible genes, including *IDO1* (indoleamine 2,3-dioxygenase 1) and *CXCL9*. These associations were, however, weak in patients with NSCLC. Further investigation is required to apply comprehensive gene expression analysis as a potential predictive biomarker to guide selection of patients who benefit most from immune checkpoint inhibitors in patients with NSCLC.

3.3. Gene alterations and phenotypic alterations in tumor cells

3.3.1. Tumor mutational load—Preliminary data suggest that tumors harboring high levels of somatic mutations may be highly sensitive to immune checkpoint inhibitors [42,43]. Somatic mutations in tumor cells could produce neoantigens, and the recognition of neoantigens by T-cells seems important for the activity of PD-1 pathway inhibitors.

The mismatch repair deficiency was reported to be a potential biomarker in progressive metastatic carcinoma treated with pembrolizumab [44]. Response rates of up to 40% were achieved in mismatch repair-deficient tumors such as colorectal cancer with microsatellite instability (n = 10) in contrast to 0% in mismatch repair-proficient colorectal cancer (n = 18)

after pembrolizumab treatment. Mismatch repair–deficient tumors are associated with a much higher neoantigen load than tumors with proficient mismatch repair. Furthermore, a recent study showed that the mismatch repair–deficient tumor microenvironment strongly express several immune–checkpoint ligands, including PD-1, PD-L1, CTLA-4, LAG-3, and IDO1, suggesting that their active immune microenvironment is counterbalanced by immune inhibitory signals that resist tumor elimination [45].

In NSCLC, a higher non-synonymous mutation burden in tumors was associated with improved objective response, durable clinical benefit, and PFS with pembrolizumab treatment [43]. The efficacy of pembrolizumab also correlated with the molecular smoking signature, higher neoantigen burden, and the presence of DNA repair pathway mutations; each of these factors was also associated with higher mutation burden. A median of 112 candidate neoantigens were identified per tumor (range: 8–610 neoantigens), and the number of neoantigens per tumor correlated with mutation burden. Tumors in patients who had durable clinical benefit from treatment with pembrolizumab had significantly higher candidate neoantigen burdens than those in patients with no durable benefit from treatment, and high candidate neoantigen burden was associated with improved PFS. In addition, NSCLC tumors with both a high clonal (present in all tumor cells) neoantigen burden and low neoantigen intra-tumor heterogeneity were associated with significantly longer PFS with pembrolizumab treatment [46]. Greater PD-L1 expression was observed in tumors harboring a large clonal neoantigen burden and low neoantigen heterogeneity compared to the remaining tumors. These data suggest that neoantigen heterogeneity may influence immune surveillance.

Further intensive investigation of these recognizable neoantigens may reveal an important predictive biomarker model of response to PD-1 pathway inhibitors in NSCLC.

3.3.2. Oncogene mutations—In general, no clear evidence has associated specific oncogenic mutations within tumors with sensitivity to immune checkpoint inhibitors. It has been reported that constitutive oncogene pathway activation could upregulate PD-L1 in tumor cells. In NSCLC, PD-L1 expression can be upregulated by *EGFR* mutation or *ALK* fusion [47–49]. Loss of *PTEN* and the activation of the PI3 K/AKT pathway elevated PD-L1 expression in various malignancies, including NSCLC [50,51]. Both *EGFR* mutation and *ALK* fusion activate the downstream PI3 K/AKT signaling pathway, and constitutive activation of this pathway can upregulate PD-L1. Furthermore, *EGFR* mutation-positive NSCLC may preferentially use PD-1/PD-L1–mediated mechanisms to evade immune surveillance [47]. In contrast, *STK11/LKB1*-inactivating mutations were associated with reduced expression of PD-L1 in *KRAS*-mutant lung adenocarcinomas compared to *STK11/LKB1* wild-type *KRAS*-mutant tumors [52,53]. *LKB1*-mutant lung adenocarcinomas with *KRAS* mutation were largely immune-inert and expressed low level of immune markers [53]. Furthermore, PD-1 targeting antibody was less effective against *LKB1*-mutant lung adenocarcinomas with *KRAS* mutation in preclinical model [52]. These findings suggest potentially distinctive effects on the immune microenvironment in genetic subsets of NSCLC. Although it is still unclear how individual genetic background affects immune checkpoint inhibitor outcomes, further investigation of how oncogene alteration influences

the immune microenvironment in NSCLC would help refine the use of active specific immunotherapy in NSCLC.

3.3.3. Epithelial-to-mesenchymal phenotype—Epithelial-to-mesenchymal transition (EMT) is a phenomenon in which cells with epithelial phenotypes acquire mesenchymal characteristics, and EMT plays an important role in cancer metastasis and drug resistance [54]. The transcription factor ZEB1, a known EMT activator, regulates the microRNA-200-dependent expression of PD-L1 on tumor cells and enhances the tumor response to IFN γ [55]. EMT is associated with an inflammatory tumor microenvironment with expression of multiple immune checkpoint molecules and immune activation in lung adenocarcinoma, independent of tumor mutational burden [56]. The expression levels of PD-L1 and PD-1, and IFN and its inducible enzyme IDO, were significantly elevated in tumors with mesenchymal phenotype compared to tumors with epithelial phenotype in lung adenocarcinoma. In addition, higher levels of tumor infiltration by CD3+ T-cell and CD4 + Foxp3+ T-cells were observed in tumors with mesenchymal phenotype compared to tumors with epithelial phenotype. These findings suggest an ongoing immune response in the tumor microenvironment of lung adenocarcinoma with mesenchymal phenotype. Further investigation is required to apply EMT phenotype as a potential predictive biomarker.

3.4. Clinicopathologic biomarkers

3.4.1. Demographic and histologic characteristics—Several clinical factors have been analyzed in relation to response to PD-1 pathway inhibitors in NSCLC. No clear evidence has associated PD-1 pathway inhibitor response with patient age, sex, or race.

Some histologic markers may be associated with response to PD-1 pathway inhibitors. These inhibitors were effective in squamous NSCLC, as mentioned above [12–15,57], and squamous NSCLC consistently expresses molecules such as p63, CK5/6, and 34bE12 and without expression of TTF-1 [58]. In contrast, adenocarcinoma shows more heterogeneity in immune elements. Indeed, squamous NSCLC seems to express known tumor antigens, including melanoma-associated antigen (MAGE)-A3, MAGE-A4, and NY-ESO-1, more frequently than non-squamous NSCLC [59,60].

With regard to the number of prior systemic regimens, a recent clinical trial (BIRCH) showed that the objective response rate to the PD-L1 inhibitor atezolizumab did not significantly vary by treatment line in NSCLC patients with tumors expressing PD-L1 (26% [95% CI: 16–9%] for first line vs. 24% [17–32%] for second line vs. 27% [19%–36%] for third line) [61].

3.4.2. Smoking history—A history of smoking was significantly associated with higher response rate to PD-1 pathway inhibitors [10,13,14]. In general, human tumors associated with environmental mutagens, including ultraviolet light and smoking, have a higher mutational load [62,63], which, as we noted above, has been associated with sensitivity to immune checkpoint inhibitors. Indeed, whole-genome and transcriptome sequencing revealed more mutations per Mbp in NSCLC patients with a history of smoking than in never smokers, with a possible dose-response relationship between the amount and duration of smoking and the frequency of mutations [42]. In addition, smoking can reduce immune

recognition by producing several immunosuppressive factors, including IL-10, TGF β 1, and IDO1, in tumor cells and/or immune cells [64,65]. Thus, smoking may increase the mutational load, leading to neoantigen expression in tumor cells and helping maintain an inflammatory environment, resulting in IFN γ -driven expression of PD-L1, which may be related to immunotherapy responses [32].

3.4.3. Blood-based biomarkers—Blood-based immune biomarkers have also been examined as a less invasive immune monitoring tool in several clinical trials with immune checkpoint inhibitors. No blood-based predictive biomarker has been reported for PD-1 pathway inhibitors in NSCLC so far.

3.5. Tregs and myeloid-derived suppressor cells

Intensive effort has been invested in finding predictive biomarkers for immune checkpoint inhibition using preclinical models, supported by clinical observations. Among these potential markers, immunosuppressive cells have emerged as an important predictor of immune checkpoint inhibitor efficacy.

Several types of cells in the tumor microenvironment have immunosuppressive properties, including Tregs, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages and neutrophils [66–68]. CD4 + CD25+ TILs, the activated phenotype of Tregs, mediate inhibition of autologous T-cell proliferation and prevent the host from mounting an immune response to tumor antigens [68,69]. In NSCLC patients, CD4 + CD25+ Tregs with marked immunosuppressive activity are elevated in peripheral blood [70]. Further investigation revealed that Tregs express and functionally depend on the transcription factor Foxp3 [71]. In addition, the presence of CD4 + CD25 + Foxp3+ Treg and absence of CD8+ T-cells in TILs is associated with poor prognosis in several solid malignancies, suggesting that tumor-reactive CD8+ cytotoxic T-cells are suppressed by Foxp3+ Tregs in tumors [72]. In contrast, high infiltration of Foxp3+ Tregs has been associated with better prognosis in several malignancies [73]. This discrepancy may be due to CD25+ or Foxp3+ T-cell subpopulations in tumor tissues with heterogeneous properties that can be discerned by the use of additional markers [73]. Indeed, CD25 and Foxp3 are also expressed by activated effector T-cells, and there are also Foxp3– suppressor cells [74].

MDSCs are another heterogeneous population of cells of myeloid origin that are characterized by their immature state and ability to suppress T-cell responses [75]. MDSC levels are often elevated in tumor-bearing hosts and have potent immunosuppressive activities [75,76]. In lung cancer, antibody-mediated MDSC depletion increased antigen-presenting cell activity and augmented the activity of effector T-cells, reducing tumor growth and enhancing therapeutic vaccination responses [77].

The predictive ability of these biomarkers in NSCLC patients treated with PD-1 pathway inhibitors requires evaluation in the context of clinical trials.

4. Conclusion

PD-L1 expression in tumor cells and level of and markers for immune cells in the tumor microenvironment is associated with, but not a definitive predictive biomarker of, response to PD-1 pathway inhibitors in patients with NSCLC and other cancers. CD8+ T-cell activation through treatment and tumor mutational loads also may be important in assessing the potential for PD-1 pathway inhibition. Further understanding of the immune profiles of responders and non-responders, through IHC-based expression and distribution analysis in combination with comprehensive gene expression profiling, will likely elucidate antitumor immune mechanisms and thus inform the use of immune checkpoint inhibitors and potentially lead to novel treatment approaches using immune checkpoint inhibition.

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Fig. 1.

PD-L1 expression and response rate to nivolumab in NSCLC patients.

PD-L1 expression rates in tumor cells on IHC (A) and relationships between PD-L1 expression and overall response rate (B) are summarized. The data are from clinical trials with nivolumab for NSCLC [11,12,14,15], non-squamous NSCLC [11], and squamous NSCLC [12,15]. PD-L1 positivity was defined by a 5% threshold. Mean values for each group are shown. Error bars show ranges of response rates. The chi-square test was used to determine the significance of differences between response rates. ORR: Overall response rate.

Table 1

PD-1 or PD-L1 inhibitors for NSCLC in clinical use or in development.

Target	Drug name	Other names	Molecular Properties	Manufacturer
PD-1	nivolumab	Opdivo, MDX-1106, BMS-936558, ONO-4538	Fully Human IgG4	Bristol-Myers Squibb, Ono Pharmaceutical
	pembrolizumab	Keytruda, lambrolizumab, MK-3475	Humanized IgG4	Merck
	PDR001	None	Humanized IgG4	Novartis Pharmaceuticals
PD-L1	BMS-936559	MDX-1105	Fully Human IgG4	Bristol-Myers Squibb
	atezolizumab	MPDL3280A	Humanized IgG1	Genentech/Roche
	durvalumab	MEDI4736	Humanized IgG1	MedImmune/AstraZeneca
	avelumab	MSB0010718C	Fully Human IgG1	Merck

Table 2
Molecular predictive biomarkers of favorable response to PD-1/PD-L1 inhibitors in NSCLC.

Candidate biomarker	Location of biomarker	Sample collection time	Tumor type (number of NSCLC patients)	Drugs	References
Increased PD-L1	Tumor cells	Pre-treatment	NSCLC (691)	Nivolumab, pembrolizumab	11,13,15,33
Increased PD-L1	Immune cells	Pre-treatment	Solid tumors, including NSCLC (58)	Nivolumab, atezolizumab	10,33
Increased CD8+ T-cell density	Immune cells	Pre-treatment	NSCLC (27)	Pembrolizumab	41
Increased <i>CTLA4</i>	Tumor cells	Pre-treatment	Solid tumors, including NSCLC (unknown)	Atezolizumab	10
Decreased fractalkine (<i>CX3CL1</i>)	Tumor cells	Pre-treatment	Solid tumors, including NSCLC (unknown)	Atezolizumab	10
Increased somatic mutation burden	Tumor cells	Pre-treatment	NSCLC (34)	Pembrolizumab	44
Increased mutation-associated neoantigen burden	Tumor cells	Pre-treatment	NSCLC (31)	Pembrolizumab	44