CHAPTER 1

Translation in Immunology: The Role of Translational Biomarkers to Guide Clinical Use of Immunotherapy for Cancer

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Introduction

For over a century, the role of the immune system in controlling and eradicating tumors has been a subject of intense debate. Since the 1800s, it has been recognized that the immune system also plays an important pathologic protumor role in tumor initiation and progression. Virchow commented on the interaction between inflammation, leukocytes, and cancer in his article from 1863 [1]. More than a hundred years later, we are still extricating the complexities of the interaction between cancers and the host immune system. More recently, Schreiber, Old, and Smyth described the process in which cancer and the immune system interact with each other, termed “cancer immunoediting” [2]. Cancer immunoediting describes a contiguous process that the immune system influences and shapes developing tumors. This process can result in successful rejection of the tumor or generate a tumor through immunologic evasion, the latter of which we now know can occur by multiple mechanisms and more often than not through any one of a number of immune suppressive pathways [3].

Despite the long-standing interest in host antitumor immunity, it was only recently that immunotherapy emerged as one of the effective treatment options for cancer. In the past decade, several new immunotherapies, such as immune checkpoint blockade agents, tumor antigen–targeted monoclonal antibodies, and a cell-based dendritic vaccine, were approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple cancer types. In particular, the immune checkpoint blockade agents, which are treatments that target cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PDL-1), have gained impetus as potent anticancer therapies and have shown promising results across several tumor types, leading to a widespread revolution in cancer treatments and a massive shift in laboratory investigations. Since this form of therapy targets the host’s regulatory components of the immune system rather than specific oncogenic mutations or tumor cells themselves, immune checkpoint blockade has been shown to be effective across multiple cancer types. Furthermore, given that the immune system has the capacity for
long-term memory, patients who respond to this form of immunotherapy frequently have durable responses, which can protect against disease progression for months and years [4–6].

While the early results of immune checkpoint blockade have been quite promising, only about a third of patients benefit from single agent therapy, accounting for both partial and complete responses, defined by the FDA as the objective response rate (ORR). Not all tumor types are equally responsive to immune checkpoint blockade, for reasons that as of yet remain unclear. Emerging studies suggest that combination treatments adding additional immunotherapies or other modalities to immune checkpoint blockade results in ORRs that appear to be higher in many cases. However, in most cases the superiority of combination therapy over monotherapy is still not well proven. Chen and Mellman et al. introduced the concept of the cancer-immunity cycle, which describes the interactions and processes of how the immune system recognizes and eradicates cancer cells [7]. To ensure effective antitumor activities, a series of stepwise events, including release of cancer cell antigens, antigen presentation, priming and activation, trafficking of T cells to tumors, infiltration of T cells into tumors, recognition of cancer cells by T cells, and killing of cancer cells, must be initiated and properly expanded. This cancer-immunity cycle hypothesis provides potential opportunities to intervene, and provides rationale for combination therapy consisting of multiple immunotherapies to improve clinical responses [8]. Additionally, several other combination approaches, including with chemotherapy, anti-angiogenic therapy, and hormonal therapy, are being considered [5, 9, 10]. In this chapter, potential and established biomarkers that can be used as prognostic indicators or as identifiers of patients who will benefit more from these immune checkpoint blockade agents are reviewed. Thus, the impressive therapeutic activity of immune checkpoint blockade, seen in recent years, has solidified the science of translational biomarkers, which enable more rapid, sensible deployment of novel clinical approaches for the select groups of patients who are most likely to benefit.

**Biomarkers for anti-CTLA-4**

Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is an immune checkpoint that down-regulates immune responses. CTLA-4 functions predominantly early in the cancer-immunity cycle during T cell activation and enhances the immunosuppressive activity of regulatory T cells (Treg cells) [11, 12]. In contrast to PD-1 or PDL-1, which is typically thought to modulate antigen-experienced effector cells in inflammatory environments, CTLA-4 engages in the priming phase and regulates the amplitude of early activation of naïve and memory T cells [13]. Ipilimumab was the first immune checkpoint blockade agent approved by FDA, is a humanized monoclonal antibody against CTLA-4, and is indicated for advanced melanoma. However, the response rate for single-agent ipilimumab is merely 10%, and ipilimumab has several concerning mechanistic-based toxicities [14]. Common serious toxicities associated with ipilimumab are dermatitis, enterocolitis, endocrinopathies, liver abnormalities, and uveitis [15]. Therefore, it is critical to identify biomarkers that can be used to select patients who are more likely to benefit from this toxic therapy.

Several serum biomarkers, such as lactate dehydrogenase (LDH), C-reactive protein (CRP), vascular endothelial growth factor (VEGF), and soluble CD25 (sCD25), have been shown to be associated with ipilimumab treatment in patients with advanced melanoma [16–19]. Higher baseline levels of LDH and VEGF were associated with reduced ipilimumab treatment response in patients with metastatic melanoma [16–19]. Higher baseline levels of LDH and VEGF were associated with reduced ipilimumab treatment response in patients with metastatic melanoma. However, subsequent reductions in LDH, CRP, and Treg as well as an increase in absolute lymphocyte count after ipilimumab treatment were significantly associated with improved overall survival (OS) and disease control rate. sCD25 acts as a decoy receptor for IL-2. While recombinant IL-2 improves efficacy of ipilimumab, sCD25 inhibits the anticancer effects of ipilimumab, and the high level of baseline sCD25 appears to confer resistance to ipilimumab [16]. However, most of these studies were small retrospective database reviews, and at this time, no confirmatory clinical trials have been done to support the routine use of these biomarkers for the selection of patients who should receive ipilimumab.
Given that ipilimumab exerts its antitumor activity through activation and increasing proliferation of T cells, serial measurements of absolute lymphocyte counts (ALC) in the blood after treatment have also been investigated as a pharmacodynamic biomarker of ipilimumab [20, 21]. After ipilimumab therapy, an ALC ≥ 1000/μL at week seven or an increase in ALC from baseline at week twelve was significantly associated with improved OS [18, 22, 23]. Besides a simple absolute count of lymphocytes, which can be heterogeneous, CD4+ICOS+ T cells, an activated T cell subset, have been used to track immune response after ipilimumab therapy as a pharmacodynamics marker. Four independent studies demonstrated that patients who had a sustained increase in CD4+ICOS+ T cells over twelve weeks after ipilimumab therapy had significant improvement in OS [24–28]. This consistent finding is intriguing because ICOS (inducible T cell costimulatory) costimulation is associated with Th2 immune responses, suggesting the possibility that antibodies are involved in the clinical activity of CTLA-4 blockade [29].

Since T cells recognize processed peptides presented by host major histocompatibility complex molecules, mutations in cancers can produce unique peptides that can be recognized by T cells, termed mutated neoantigens [30]. The antigenicity of these neoantigens may affect the function of the protein, and a passenger mutation with no functional role may still generate sufficient immune responses, although the potential for immune escape based on antigen loss is still possible. However, a greater mutational load in the tumors can potentially produce more neoantigens, which will result in a larger repertoire of existing tumor-specific T cells, and less chances of antigen-loss variant escape. Given the fact that immune checkpoint blockade agents exert their activity by unleashing these preexisting tumor-specific T cells, it was initially hypothesized that tumors with higher mutational loads would respond better to this form of therapy [30]. This hypothesis was substantiated based on the early results of studies with ipilimumab, which has activity in the cancer with the highest mutational load, melanoma. In two melanoma studies of ipilimumab, patients who responded to ipilimumab had a statistically significant higher median mutation load in their tumors compared to patients who did not respond. However, there appeared to be no distinct cutoff that can be used to identify patients who would not benefit from ipilimumab therapy [31, 32]. The inability to establish a cutoff may reflect important variations such as HLA allelic variation and immunogenicity of the putative neoantigens, both of which may limit the utility of the mutational load as a response indicator [33].

Despite years of trials and retrospective studies, to date no companion diagnostic test has been approved by the FDA to identify patients who are more likely to benefit from ipilimumab. Thus, additional translational studies of patients undergoing therapy should be designed and implemented to aid in identifying the patients most likely to respond.

**Biomarkers for anti-PD-1/PDL-1 therapies**

Programmed cell death protein 1 or PD-1 (also known as PDCD1) and its ligand PD-1 ligand 1 or PDL-1 (also known as B7-H1) are key immune checkpoints that down-regulate antitumor effects of T cells in the tumor microenvironment [34, 35]. PDL-1 engages PD-1 and inhibits proliferation and cytokine production of T cells [36]. Several preclinical studies demonstrated that inhibition of the PD-1/PDL-1 interaction enhances T cell responses and augments their antitumor activities [34, 37, 38]. The potential translational biomarkers for anti-PD-1/PDL-1 can be categorized into either immune-related or genomic-related biomarkers [39].

**Immune-related biomarkers**

PD-1 and PDL-1 immune checkpoint blockade agents are thought to exert their activity mainly by enhancing the antitumor activities of preformed host immune responses [40]. Thus, the amount of preexisting immune infiltrate in the tumor at baseline prior to anti-PD-1/PDL-1 treatment was one of the first translational biomarker candidates to be explored. In melanoma, higher numbers of preexisting CD8+ T cells, particularly at the invasive tumor margin, have been shown to associate with tumor regression in patients treated with anti-PD-1 therapy (pembrolizumab) [40]. Comparing between responders and nonresponders, responding
patients had significantly higher numbers of CD8+, PD-1+, and PDL-1+ cells at the invasive tumor margin and a more clonal T cell antigen receptor repertoire. Furthermore, patients who responded to therapy had significant increases in CD8+ T cells both inside the tumors and at the invasive margins. Similar findings, in which an increase in CD8+ T cell infiltration after anti-PD-1 therapy correlates with tumor regression, were also observed in another study with pembrolizumab in melanoma and nivolumab in solid tumors in a phase I study [41, 42].

Another immune-related biomarker that has received a great deal of attention is tumor cell–associated PDL-1 expression. PDL-1 is widely expressed in the tumor microenvironment not only on the tumor cells but also in subsets of immune cells, particularly macrophages, dendritic cells, and activated T, B, and NK cells as well as other nonmalignant cells, including endothelial cells as part of a physiological process to down-regulate host immune responses in inflammatory microenvironment [43–45]. The distribution of PDL-1 expression differs among tumor types. In certain type of cancers, PDL-1 is expressed on both tumor cells and immune infiltrating cells. These types of cancers include squamous cell carcinoma of the head and neck (SCCHN), melanoma, breast cancer, and renal cell carcinoma [46–50]. However, in other forms of cancers such as colorectal (CRC) and gastric cancer, PDL-1 is expressed almost exclusively on the immune-infiltrating cells but rarely on the tumor cells [51, 52].

In the initial phase I trial of nivolumab, an anti-PD-1 antibody, in 39 patients with advanced solid malignancies, 9 biopsied samples were available for PDL-1 assessment by immunohistochemistry. Among these 9 patients, 3 out of 4 patients with membranous expression of PDL-1 responded to nivolumab. Objective responses were not observed in the other 5 patients without PDL-1 expression [42]. Similar findings were subsequently observed in a larger trial of nivolumab, which demonstrated no objective response in patients with PDL-1-negative tumors. In contrast, patients with PDL-1 expression of ≥5% of tumor cells were twice as likely to respond compared to the overall study population [39, 53]. While PDL-1 expression can be used to identify patients who are more likely to respond to anti-PD-1 therapy, subsequent studies have shown that objective responses could still be observed in some patients with PDL-1-negative tumors [54]. In an analysis of multiple anti-PD-1 trials, the average ORR of anti-PD-1 therapy was approximately 29% across 15 trials in various solid malignancies. Among patients with PDL-1-positive tumors, the ORR was 48% compared to 15% in PDL-1-negative tumors [55]. These findings exemplified that PDL-1 negativity cannot be used to exclude patients from anti-PD-1 therapies but rather to enrich patients who are more likely to benefit from this therapy.

Of note, while PD-1 is the actual target of anti-PD-1 therapy, expression of PD-1 does not appear to provide any additional predictive value [50]. Tumeh et al. demonstrated that more complex parameters, such as close proximity of PD-1+ cells to PDL-1+ cells, proliferation of CD8+ T cells measured by Ki67 and CD8 costaining, and markers of IFNγ signaling, provided superior predictive value compared to a single marker [40].

There are several technical difficulties and limitations of using PDL-1 expression as a biomarker for anti-PD-1/PDL-1 therapies. First, the expression of PDL-1 is variable in multiple tumor biopsies collected over time and/or from different anatomical sites in each individual patient [39]. This variable expression represents a potential pitfall of developing PDL-1 IHC as an absolute biomarker based on a single biopsied tumor specimen. Moreover, the tumors used to evaluate PDL-1 expression were collected after varied duration of treatment among multiple clinical trials. Some of the trials used tumors collected right before the initiation of therapy, and some trials used the tumors from the initial diagnosis. The tumors that were collected after the initial diagnosis, which could have been months or years before the initiation of therapy, may not have reflected the PDL-1 status at the time of therapy. Furthermore, the expression of PDL-1 is not uniform within the tumors. Focal expression of PDL-1 could be missed in small core needle biopsy specimens, resulting in false negative results [56].

**Genomic-related biomarkers**

To date, no specific oncogenic mutations have been shown to associate with outcome in patients treated with anti-PD-1/PDL-1 therapy as an independent
variable. However, several aberrant oncogenic drivers and signaling pathways have been shown to associate with PDL-1 expression. PTEN mutations resulting in constitutive activation of the PI3K-AKT pathway have been shown to associate with higher PDL-1 expression in glioma cells [57]. Similar findings were observed with constitutive ALK signaling activation, which was found to associate with increased PDL-1 expression via activation of STAT3 in certain lymphomas and lung cancers [58]. Additionally, in a subset of lung adenocarcinomas, KRAS mutations were associated with increased PDL-1 expression and denser inflammation compared to wild-type tumors [59]. Nevertheless, there appeared to be no significant difference in PDL-1 expression in non-small-cell lung cancer (NSCLC) tumors with mutant EGFR and those with wild-type EGFR [60]. Furthermore, in melanoma, a previous study also demonstrated no significant difference in PDL-1 expression between BRAF-V600E mutated vs. wild-type tumors [61]. Consistent with this finding, the response to anti-PD-1 therapy appeared to be similar in patients with BRAF-V600E mutation and BRAF wild-type tumors [6, 62].

Given that genes encoding for both PDL-1 and another PD-1 ligand, PDL-2, are located on the 9p24.1 locus, translocations or amplifications of 9p24.1 locus also have been shown to increase PDL-1 and PDL-2 expression on the surface of tumors. Amplification of 9p24.1 has been observed in several tumor types, including Hodgkin lymphomas [63, 64], mantle cell lymphomas [65], gastric cancers [66], and breast cancer [67]. Up to 97% of classical Hodgkin’s lymphomas have alterations of the PDL-1 and PDL-2 loci: either polysomy, copy number gain, or amplification resulting in PDL-1 overexpression. Furthermore, consistent with the known capability of virus-caused up-regulation of the PD-1/PDL-1 pathway, Epstein-Barr virus infection, which is common in Hodgkin’s lymphoma, also contributes to overexpression of PDL-1. As a result of these two mechanisms, a large proportion of classical Hodgkin’s lymphoma have increased PDL-1 expression [68]. Corresponding to these findings, the initial phase I study of nivolumab in 23 patients with relapsed or refractory Hodgkin's lymphoma, with the majority progressing after autologous stem-cell transplantation and brentuximab vedotin, showed a remarkable ORR of 87%, including 17% with a complete response, 70% partial response, and 13% with stable disease [63, 64]. Similar findings were observed in a subsequent multi-center, single arm phase II trial of nivolumab in 80 patients with classical Hodgkin’s lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin. However, the expression of PDL-1 on Reed-Sternberg cells was not required and patients were enrolled regardless of their PDL-1 expression status. ORR was observed in 66.3% of patients, with 9% complete response, 58% partial response, and 23% stable disease [69]. Based on these promising results, the FDA granted the accelerated approval of nivolumab for the treatment of patients with Hodgkin's disease in this setting.

Similar to that reported with ipilimumab, mutational burden is another key factor that has been found to be associated with clinical response to anti-PD-1/PDL-1 therapies. Early studies of anti-PD-1 indicated that these agents appear to have activity across all cancers with the highest median mutation loads, namely, melanoma, NSCLC, SCCHN, bladder cancer, and gastric cancer. The ORR for anti-PD-1 in these cancer types was more than 15% across the board [53, 70, 71]. In contrast, the ORR is relatively low in cancers with low mutational loads, such as prostate and pancreatic cancers. In a small study of patients with lung cancer receiving pembrolizumab, higher mutational burden was associated with improved response to this agent [72]. Nevertheless, and much like that observed for ipilimumab, there is no clear cutoff for the number of actual mutations that can be used for the purpose of patient selection. Currently, beyond the number of mutations, there are several computational algorithms that can be used to predict the numbers of potential neoantigens. However, to date, these algorithms are still highly imperfect and at present not suitable for use for routine clinical management.

Another specific genetic subset that has been shown to associate with higher mutation burden and better response to anti-PD-1/PDL-1 is tumors with DNA mismatch repair (MMR) defects. Genes in the MMR complex are often found to be mutated, deleted, or epigenetically silenced in several
cancers, including CRC, gastric, endometrial, ampullary, duodenal, and prostate cancers. MMR-deficient genotypes account for approximately 4% of all solid tumors and can be identified by detecting microsatellite instability (MSI) or by immunohistochemical staining of MMR proteins [39]. These tumors with MMR defect have a 10- to 100-fold increase in mutational burden compared to MMR-proficient tumors. Furthermore, colon cancers with MSI exhibit several other features that predict sensitivity to anti-PD-1/PDL-1 therapy. These features include high CD8+ T cell infiltration, CD4+ T cells with the Th1 phenotype, high levels of PD-1, PDL-1, CTLA-4, lymphocyte activation gene (LAG3), and IFNγ-inducible immune inhibitory metabolic enzyme (IDO1) [51, 73]. Despite a generally low rate of response in CRC patients, there was a patient with CRC who had a durable complete response in the initial phase I trial of nivolumab [42]. Subsequent analysis of this patient's tumor demonstrated an MSIh phenotype [74]. This finding was confirmed in a larger phase II trial of pembrolizumab in patients with tumors harboring MMR defects. In this particular trial, patients with MMR-deficient and -proficient CRC were enrolled. The ORR was 40% in MMR-deficient CRC compared to 0% in MMR-proficient CRC. Similar high response rates were also observed in another cohort of patients with MMR-deficient non-CRC with an ORR of 71% [60].

Besides somatic mutations, integration of onco-genic viruses in cancer genomes represents another form of genetic alterations that can produce neo-antigens. There are several human cancers that are driven by viruses, namely, Epstein-Barr virus, human papillomavirus, Merkel cell polyomavirus (MCPyV), human T-lymphotropic virus 1 (HTLV-1), Kaposi sarcoma-associated herpes virus (KSHV), hepatitis B, and hepatitis C viruses. Early studies demonstrated that these viral-associated cancers might have high response rates to anti-PD-1/PDL-1 therapies. Approximately 80% of Merkel cell carcinomas are associated with MCPyV infection, and patients with Merkel cell carcinoma often produce MCPyV T-antigen-specific T cells and antibodies [75, 76]. A high ORR of 56% was observed in a phase II trial of pembrolizumab in this group of patients, which might be indicative of activation of latent MCPyV-specific immune effectors [77]. Similar findings were also observed in hepatocellular carcinoma, in which the ORR was 36% among hepatitis C infected patients compared to 15% in noninfected patients [78].

Approved anti-PD-1/PDL-1 blockade agents and biomarkers in clinical use

Since 2014, several agents targeting this particular pathway have been approved or are under consideration by the FDA. Presently, three PD-1-PDL-1 targeting agents have been approved by the FDA, namely, pembrolizumab, nivolumab, and atezolizumab. Multiple other agents targeting this particular pathway are currently under clinical development. The agents targeting PD-1 currently in clinical development include pidilizumab, AMP-224, and AMP-514, as well as agents targeting its ligand, PDL-1, including BMS-936559, durvalumab, and avelumab [79].

Pembrolizumab, a humanized monoclonal IgG4 antibody against PD-1, was the first PD-1/PDL-1 targeting agent approved by the FDA. It was approved in September 2014. Pembrolizumab is currently indicated for the treatment of unresectable or metastatic melanoma patients, whose tumors express PDL-1, either as an initial treatment or subsequent treatment after progressing on ipilimumab and/or a BRAF inhibitor, the first or later line treatment of patients with metastatic NSCLC, and the treatment of patients with recurrent or metastatic SCCHN after progressing on platinum-containing chemotherapy [80–83].

Similar to pembrolizumab, nivolumab is a humanized monoclonal IgG4 antibody against PD-1. Currently, nivolumab is indicated as a single agent for the first-line treatment of patients with BRAFV600 wild-type unresectable or metastatic melanoma, metastatic NSCLC progressing after platinum-based chemotherapy, advanced renal cell carcinoma with prior antiangiogenic therapy [84], relapsed Hodgkin lymphoma after autologous hematopoietic stem cell transplantation and posttransplantation brentuximab vedotin, and recurrent or metastatic SCCHN progressing after platinum-based therapy [64, 85–89]. In addition, nivolumab is also indicated in combination with ipilimumab in unresectable or metastatic melanoma patients with BRAF wild-type [90, 91].
In contrast to pembrolizumab and nivolumab, atezolizumab is a humanized monoclonal IgG1 antibody against PDL-1. Atezolizumab is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma progressing after platinum-based chemotherapy [92] and patients with metastatic NSCLC progressing after platinum-based chemotherapy [93].

At present, two established biomarkers are currently in routine clinical use. They are the PDL-1 IHC 22C3 pharmDx assay for pembrolizumab in NSCLC and the PDL-1 IHC 28-8 pharmDx assay for nivolumab in nonsquamous NSCLC and melanoma. Upon the approval of pembrolizumab in NSCLC, the FDA also simultaneously approved the companion diagnostic test, PDL-1 IHC 22C3 pharmDx assay, to guide patient selection. PDL-1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using mouse monoclonal anti-PDL-1 clone 22C3 in formalin-fixed, paraffin-embedded samples. Tumor proportion score (TPS) is used to determine the expression level of PDL-1. PDL-1 is considered positive if TPS ≥ 1% and high PDL-1 expression is defined as TPS ≥ 50%. Currently, pembrolizumab has two indications in metastatic NSCLC, including the first-line therapy for NSCLC patients whose tumors have high PDL-1 expression (TPS ≥ 50%) and no EGFR or ALK genomic aberrations [82]. This approval was based on a large phase II trial of pembrolizumab in patients with squamous and nonsquamous NSCLC, which demonstrated significantly higher ORR, improved PFS, and OS in patients with tumors expressed PDL-1 ≥ 50% [60]. The second indication includes the second or later line of therapy in NSCLC patients progressing on platinum-based chemotherapy. In this indication, the cutoff for TPS is lower than the first indication at ≥ 1% rather than ≥ 50%. This lower cutoff may be due to enhanced sensitivity to immune checkpoint blockade agents among patients with platinum resistance. In contrast, PDL-1 IHC 28-8 pharmDx for nivolumab in NSCLC and melanoma was approved as a complementary companion diagnostic test rather than a required test for patient selection. In two phase III trials of nivolumab, NSCLC patients whose tumors expressed PDL-1 ≥ 1% using PDL-1 IHC 28-8 pharmDx assay had improved OS, but only in the nonsquamous NSCLC group [88, 89]. These assays in current clinical use are summarized in Table 1.1.

**Conclusion**

Immunotherapy, particularly with immune checkpoint blockade, represents a revolutionary paradigm shift in cancer treatment. By enhancing endogenous host immune responses, rather than specifically targeting particular aberrant signaling pathways intrinsic to the tumor cell, this form of treatment has proven to be effective across multiple tumor types. Nonetheless, the response to immunotherapy is not universal and specific translational biomarkers are needed to identify patients who are more likely to benefit from this therapy. To date, there are only two PDL-1 immunohistochemistry assays that are approved by the FDA and are currently in clinical use. However, as our understanding of the interplay between immune system and tumor microenvironment grows, novel mechanistic-based biomarkers and combination therapy will emerge to improve patient selection for this form of therapy.

**Table 1.1 Summary of approved biomarkers for anti-PD-1/PDL-1 blockade agents in clinical use.**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Agent</th>
<th>Disease Setting</th>
<th>Cutoff</th>
<th>Reference</th>
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<tr>
<td>PDL-1 IHC 22C3 PharmDx assay</td>
<td>PDL-1 IHC 22C3 PharmDx assay</td>
<td>1st-line NSCLC without EGFR or ALK mutation For patient selection ≥ 2nd-line NSCLC For patient selection</td>
<td>TPS ≥ 50%</td>
<td>82</td>
</tr>
<tr>
<td>PDL-1 IHC 28-8 PharmDx assay</td>
<td>Nivolumab</td>
<td>Nonsquamous NSCLC For prognostic purpose</td>
<td>TPS ≥ 1%</td>
<td>89</td>
</tr>
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*Note: TPS = tumor proportion score.*
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