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PD-L1 Testing in Guiding Patient Selection for PD-1/PD-L1 Inhibitor Therapy in Lung Cancer

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Abstract

Immunotherapy with programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) targeted monoclonal antibodies has dramatically changed the therapeutic and prognostic landscape for several types of malignancy. PD-1 and PD-L1 are immune checkpoint proteins whose binding ultimately result in T cell exhaustion and self-tolerance. Blocking this pathway “releases the breaks” on the immune system and allows for attack of tumor cells that express PD-L1. The clinical trials that led to The Food and Drug Administration (FDA) approval of these agents used different immunohistochemical (IHC) platforms with various PD-L1 antibodies to assess for PD-L1 expression on either tumor cells or tumor-infiltrating immune cells. There are four PD-L1 IHC assays registered with the FDA, using four different PD-L1 antibodies (22C3, 28–8, SP263, SP142), on two different IHC platforms (Dako and Ventana), each with their own scoring systems. Attempts at harmonization of PD-L1 IHC antibodies and staining platforms are underway. While PD-L1 IHC can be used to predict likelihood of response to anti-PD-1 or anti-PD-L1 therapy, a proportion of patients that are negative can have response and identification of alternative biomarkers is critical to further refine selection of patients most likely to respond to these therapies.

1. Introduction

Immunotherapy with programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) targeted monoclonal antibodies has dramatically changed the therapeutic and prognostic landscape for several types of malignancy. PD-1 is a receptor present on the surface of activated T and B cells and binds to its ligands PD-L1 and programmed death-ligand 2 (PD-L2). PD-L1 is found on many normal tissues, including placenta, vascular endothelium, epithelium, muscle, pancreatic islet cells, as well as on B cells, T cells, and macrophages among other cell types [1]. The binding of PD-1 to PD-L1 induces a pathway which acts to inhibit the cytotoxic/cytolytic effector functions of T lymphocytes, a process which is also termed T cell exhaustion. This is an important auto-regulatory response to local inflammation such that local tissues do not get damaged as bystanders in the immune

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response [2]. PD-L1 is also expressed on the surface of tumor cells, some of which have found ways to upregulate PD-L1 expression leading to suppression of the host immune response and tolerance to tumor. It thus follows theoretically that suppressing the PD-1/PD-L1 pathway would “release the breaks” and induce an immune system attack on tumor cells. The ultimate goal is improved overall survival, which has been demonstrated in multiple clinical trials across multiple disease sites.

Immune checkpoint inhibitors were first approved in melanoma, specifically ipilimumab (cytoxic T-lymphocyte antigen 4 inhibitor, Bristol-Myers Squibb) which received FDA approval in March 2011. In September 2014 the first anti-PD-1 antibody, pembrolizumab (Merck) was approved by the US FDA for use in metastatic melanoma. Since then, therapeutic monoclonal antibodies that target either PD-1 or PD-L1 have been FDA approved for use in non-small cell lung cancer (NSCLC), renal cell carcinoma, bladder cancer, head and neck cancer, Merkel cell carcinoma, Hodgkin lymphoma, gastric cancer, hepatocellular carcinoma, and microsatellite instability-high cancer regardless of histology, with approval pending in other diseases. Interestingly, the only setting where PD-L1 positivity is specified in the FDA approval as a precondition to therapy with a PD-1/PD-L1 antibody is pembrolizumab in the treatment of NSCLC.

Early studies in multiple cancer types have shown improved outcomes in patients treated with anti-PD-1 antibodies whose tumors are found to have PD-L1 expression, prompting further investigation of PD-L1 as a predictive biomarker for PD-1 response despite PD-1 having multiple other ligands. In the phase 1 study of nivolumab (anti PD-1 antibody, Bristol-Myers Squibb) in multiple cancer types, the murine antihuman PD-L1 monoclonal antibody 5H1 was used to evaluate pretreatment tumor specimens from 42 patients. In this study, PD-L1 positivity was defined by 5% or more of tumor cells. None of the 17 patients that had PD-L1 negative tumors had an objective response, while 9 of 25 (35%) patients with PD-L1 positive tumors had a response (P-0.006) [3]. A group evaluating immunohistochemical (IHC) features from patients with melanoma, NSCLC, renal cell carcinoma, colorectal carcinoma, or prostate cancer on the phase I nivolumab trial, including PD-1, PD-L1 and PD-L2 expression, as well as patterns of immune cell infiltration and lymphocyte subpopulations, assessed 41 pretreatment tumor specimens and found that of the evaluated features, it was tumor PD-L1 expression that correlated the most with objective response to anti-PD-1 therapy [4]. The PD-L1 monoclonal antibody 5H1 was again used in this study [4] but was later abandoned in favor of a commercial assay developed by Dako using rabbit anti-human clone 28-8.

There are four PD-L1 IHC assays registered with the FDA, using four different PD-L1 antibodies (22C3, 28-8, SP263, SP142), on two different IHC platforms (Dako and Ventana), each with their own scoring systems. Varying antibody clones and platforms have been approved for each available PD-1 and PD-L1 inhibitor, making comparison amongst trials difficult. This review will focus on the trials leading to the approval of PD-1 and PD-L1 inhibitors in NSCLC with a specific focus on how PD-L1 expression correlates with response and review issues related to determination of PD-L1 status and refinement of patient selection for PD-1/PD-L1 directed therapy. We will also discuss harmonization

studies evaluating the interchangeability of the assays as well as potential alternative biomarkers of response to immunotherapy.

2. PD-1 Inhibitors

Pembrolizumab (anti-PD-1 antibody, Merck) and nivolumab are FDA approved for use in previously treated metastatic NSCLC, and pembrolizumab is also FDA approved for use in the first-line setting in patients with PD-L1 expression greater than 50% and in combination with carboplatin/pemetrexed in the first-line setting regardless of PD-L1 status.

2.1 Second-line Treatment

2.1.1 Nivolumab—CheckMate 017, the phase 3 trial of nivolumab versus docetaxel in previously treated squamous NSCLC, evaluated PD-L1 with an automated IHC assay [Dako] and a rabbit monoclonal antihuman PD-L1 antibody, clone 28–8 [Epitomics], with samples classified as positive when staining of the tumor cell membrane was observed at prespecified expression levels of 1%, 5%, or 10% [5] (See Table 1). Similar rates of objective responses were seen in patients regardless of PD-L1 status, with PD-L1 expression being neither prognostic nor predictive of any of the efficacy endpoints [5]. In contrast, in Checkmate 057, the phase 3 trial of nivolumab in previously treated nonsquamous NSCLC that used the 28–8 clone and Dako IHC, there was an advantage in the PD-L1-positive patients treated with nivolumab, with nearly double median overall survival (OS) in patients with at least 1% PD-L1 positivity treated with nivolumab as compared with docetaxel [6]. There were no meaningful differences in OS between the nivolumab and docetaxel groups when looking at patients who were PD-L1-negative [6]. The authors concluded that despite the lack of improvement in OS between nivolumab and docetaxel in nonsquamous PD-L1 negative patients compared with docetaxel, nivolumab remains a reasonable treatment option given its improved safety profile, and the durability of responses when they do occur [6]. Indeed nivolumab is FDA approved for use in metastatic NSCLC in the second-line setting, regardless of PD-L1 expression. The 2 year update of CheckMate 017 and CheckMate 057 confirmed a greater magnitude of benefit in non-squamous NSCLC patients that were PD-L1-positive and treated with nivolumab; whereas PD-L1 expression was neither predictive nor prognostic in squamous NSCLC patients [7]. The pembrolizumab KEYNOTE trials did not include subgroup analysis data looking at PD-L1 positivity and response by histology.

2.1.2 Pembrolizumab—The phase 1b study of pembrolizumab in the treatment of advanced NSCLC, KEYNOTE 001, used the anti-PD-L1 antibody clone 22C3 [Merck] to assess PD-L1 expression at various levels, with positivity defined by membranous staining in at least 1% of cells within tumor nests[8]. PD-L1 staining of at least 50% correlated with improved efficacy of pembrolizumab [8]. Based on data from KEYNOTE 001, in October 2015, pembrolizumab was approved by the FDA for use in patients with metastatic NSCLC who have progressed after first-line treatment and with PD-L1 positive tumors as assessed by 1% staining using the companion diagnostic PD-L1 IHC 22C3 pharmDx test. Long term follow up data from KEYNOTE 001 revealed that OS increased with increasing PD-L1 tumor proportion score [9]. In the phase 2/3 trial of pembrolizumab versus docetaxel, KEYNOTE 010, all patients had PD-L1 expression of at least 1% of tumor cells, which was

assessed using the 22C3 antibody and Dako IHC [10]. In the general patient population, OS was significantly longer for pembrolizumab treated patients as compared with docetaxel [10]. The subset of patients with at least 50% staining for PD-L1 had significantly longer OS with pembrolizumab versus docetaxel, as well as significantly longer progression free survival (PFS) [10]. KEYNOTE 010 data was further analyzed by PD-L1 expression of various levels, with staining of 1%–24%, 25%–49%, 50%–74%, and greater than or equal to 75% [11]. In the patients treated with pembrolizumab, OS, PFS, and objective response rate (ORR) generally increased along with increasing PD-L1 expression, with the longest OS and PFS and highest ORR in patients with PD-L1 expression greater than or equal to 75% of tumor cells [11].

2.2 First-line Treatment

2.2.1 Nivolumab—In CheckMate 012, a phase 1 multicohort trial of nivolumab in the first-line setting, PD-L1 expression was assessed using a validated IHC assay [Dako] with the 28–8 clone, with positivity defined as at least 1% of tumor cells [12]. In the cohort of patients treated with combination nivolumab and platinum-based doublet chemotherapy, there was no association between PD-L1 expression and OS or PFS, with equivalent responses seen across PD-L1 expression levels [12]. In the cohort of patients treated with nivolumab monotherapy, clinical activity was observed regardless of PD-L1 expression, with higher ORRs in patients with higher PD-L1 expression [13]. However, CheckMate 026, a phase 3 trial of nivolumab in the first-line setting demonstrated no difference in PFS between the nivolumab and chemotherapy groups even among patients that had greater than 5% PD-L1 expression [14] [15].Nivolumab is not FDA approved in the first-line setting.

2.2.2 Pembrolizumab—In a phase 3 trial in the first-line setting, pembrolizumab was shown to significantly improve OS and PFS as compared to platinum-based chemotherapy in patients with over 50% expression of PD-L1 as assessed by the 22C3 pharmDx IHC assay [Dako] [16]. As a result of this trial, in October 2016 pembrolizumab was approved in the first-line setting for patients with greater than 50% PD-L1 expression. One can postulate that the difference in the first-line pembrolizumab trial meeting its primary endpoint and not the first-line nivolumab trial is related to patient selection.

In the phase 2 study of carboplatin and pemetrexed with or without pembrolizumab for non-squamous NSCLC in the first-line setting, there was a difference in the primary endpoint of ORR, 55% in the pembrolizumab and chemotherapy group vs 29% in the chemotherapy group as well as a difference in PFS, 13.0 months vs 8.9 months [17]. Exploratory analyses found similar rates of ORR regardless of PD-L1 expression [17]. These data suggest increased efficacy of combining cytotoxic therapy with PD1/PD-L1 inhibitors above that of chemotherapy alone. As a result of this trial, pembrolizumab in combination with carboplatin/pemetrexed was approved by the United States FDA in May 2017 for use in metastatic non-squamous NSCLC in the first-line setting regardless of tumor PD-L1 expression. A confirmatory phase III clinical trial is underway.

3. PD-L1 Inhibitors

Atezolizumab is a humanized IgG1 monoclonal anti-PD-L1 antibody [F Hoffmann-La Roche/Genentech] that was FDA approved for use in previously treated metastatic NSCLC regardless of PD-L1 status, based on the results of two international, randomized trials, POPLAR and OAK. In POPLAR, the phase 2 study of atezolizumab versus docetaxel, PD-L1 expression on both tumor and tumor-infiltrating immune cells was assessed using the Ventana SP142 IHC assay [Ventana Medical Systems, Tucson, AZ, USA] [18]. Tumor cells expressing PD-L1 were scored as a percentage of total tumor cells (TC3≥50%, TC2≥5% and <50%, TC1≥1% and <5%, and TC0<1%) and tumor-infiltrating immune cells were scored as a percentage of tumor area (IC3≥10%, IC2≥5% and <10%, IC1≥1% and <5%, and IC0<1%) [18]. There was an OS benefit in patients treated with atezolizumab and increasing improvement in OS correlated with PD-L1 IHC expression on tumor cells and tumor-infiltrating immune cells [18]. OS in patients with TC0 and IC0 PD-L1 status in the atezolizumab group was similar to the docetaxel group [18]. Long-term follow up of POPLAR reveals an improvement in median OS in the atezolizumab group versus docetaxel in almost every subgroup of PD-L1 expression, except in the TC0 and IC0 group where median OS was the same [19]. In OAK, the phase 3 trial of atezolizumab versus docetaxel, there was an OS benefit among patients treated with atezolizumab, with median OS of 13.8 vs 9.6 months [20]. This benefit was seen regardless of PD-L1 expression and even in patients with less than 1% PD-L1 expression, who had a 25% improvement in overall survival with atezolizumab as compared to docetaxel [21]. PD-L1 expression was assessed on both tumor cells and tumor-infiltrating cells, with the Ventana SP142 assay [20].

Avelumab [Pfizer] is a human IgG1 monoclonal antibody PD-L1 inhibitor. In the phase 1b trial (JAVELIN Solid Tumor) investigating avelumab in previously treated patients with metastatic or recurrent NSCLC, PD-L1 expression on tumor and immune cells was assessed using a Dako assay with a rabbit monoclonal antibody clone 73–10 [22]. Tumor cell staining was assessed at prespecified levels of ≥ 1%, ≥ 5%, ≥ 25%. Immune cells were considered to stain positive for PD-L1 at ≥ 10% and were assessed in hotspots [22]. Neither the proportion of patients with objective response nor OS outcomes differed between PD-L1 positive and negative patients at any prespecified expression level [22].

Durvalumab [Astra Zeneca], formerly MEDI4736, is a human IgG1 monoclonal antibody PD-L1 inhibitor. Phase 1/2 data in the third-line setting for patients with metastatic NSCLC revealed ORR of 14% in the general population, and 23% in PD-L1 positive patients [23]. PD-L1 was evaluated using the SP263 assay, with positivity defined as staining of 25% or more of tumor cells. Interestingly, ORR was higher in squamous (21%) than non-squamous (10%) patients [23].

In most of the completed combination immunotherapy studies of PD-1/PD-L1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) antibodies, PD-L1 expression does not seem to correlate with outcomes in advanced NSCLC patients. In a phase 1/2 study of pembrolizumab plus ipilimumab (KEYNOTE 021, cohorts D and H), there was no link between PD-L1 status as determined by the 22C3 antibody and ORR or median OS [24]. In a phase 1b study of durvalumab plus tremelimumab (anti-CTLA-4 antibody, AstraZeneca),

which assessed PD-L1 status with the Ventana SP263 assay, with samples considered positive if ≥25% of tumor cells showed membrane staining, evidence of clinical activity was noted in both patients with PD-L1 positive tumors and PD-L1 negative tumors [25]. However, in the phase 1 multicohort study of nivolumab plus ipilimumab (CheckMate 012), the combination had increased clinical activity in patients whose tumors express PD-L1, as determined by the 28–8 antibody [26]. The proportion of patients that achieved a response was near 90% in patients whose tumors had greater than 50% PD-L1 expression [26]. The phase 3 trial (ARCTIC Study) of durvalumab with or without tremelimumab for previously treated patients with advanced NSCLC is assessing PD-L1 status with the SP263 assay, and is currently ongoing [27].

4. PD-L1 Assays

Assessing for PD-L1 expression in an attempt to predict response to PD-1 or PD-L1 inhibitor therapy is not as straightforward as one would imagine. There is no uniformity in PD-L1 assessment among the clinical trials that were performed to approve these agents. A different companion diagnostic antibody clone with associated IHC platform was used for each approved anti-PD-1 or anti-PD-L1 inhibitor, and it is not clear how interchangeable these assays are. This is problematic when considering that hospital laboratories are then faced with the decision of which PD-L1 diagnostic IHC platform and clone to use given cost constraints. It may make the most clinical sense to carry the 22C3 assay that was approved for use with pembrolizumab, given this is the only agent that was approved in the setting of PD-L1 positivity in NSCLC only, whereas the other approved agents can be used regardless of PD-L1 expression. There is variability in the definition of “PD-L1 positivity” in various trials and some studies included analysis of unevaluable samples with the PD-L1-negative tumors. There was also a difference in which cells were evaluated for PD-L1 expression, with some studies finding a correlation with response and tumor cell PD-L1 staining and some finding an association with response and a combination of tumor and tumor-infiltrating immune cell staining. Additionally, some trials required biopsy prior to enrollment, whereas others relied on archival tissue, which is problematic since PD-L1 expression is thought to change over time. For instance, PD-L1 expression was shown to be dynamically induced by IFN-γ in a mouse model in the melanoma tumor microenvironment [28]. There is also intratumor heterogeneity [29], so a biopsy of one site at one point in time may not be the most appropriate biomarker.

Besides the variation in PD-L1 antibody and platform, there are other technical issues to consider with regards to PD-L1 testing. Assessing formalin-fixed tissue as compared to freshly frozen tissue can underestimate PD-L1 expression [30]. Gadiot et al. found a range of PD-L1 expression when assessing samples with multiple differing anti-PD-L1 antibodies in both formalin fixed and freshly frozen tissue [30]. There is a paucity of data on PD-L1 testing in cytology preparations, which can be clinically problematic as this is the predominant sample type in some institutions [31]. Because there are only two small hydrophilic regions on PD-L1 which would be amenable for IHC detection, IHC antibodies typically bind PD-L1 at sites that are structurally unique compared to those of therapeutic PD-L1 antibodies [1].

In a meta-analysis comparing PD-1/PD-L1 inhibitors to docetaxel in the second-line setting for treatment of advanced NSCLC, the benefit from PD-1/PD-L1 inhibitors was limited to the PD-L1 > 1% subgroup [32]. For PD-L1 > 1% patients versus PD-L1 < 1% patients treated with PD-1/PD-L1 inhibitors, the odds ratio of ORR was 2.18 (95% CI 1.45–3.29; p = 0.0002) [32]. In a separate meta-analysis including 1,612 patients from 13 trials, the overall response rate was statistically significantly higher in the PD-L1 positive group (RR 2.06 [95% CI 1.50 – 2.83]) [33]. The authors concluded that PD-L1 overexpression can be considered a predictive biomarker for response to immune checkpoint inhibitors in NSCLC, independent of previous treatments or tumor histology [33]. A third meta-analysis including 6,800 patients from 51 trials of PD-1/PD-L1 antibodies in various cancer types found that as compared with tumors with negative PD-L1 expression, tumors with positive PD-L1 expression had a significantly higher clinical response rate (41.4% versus 26.5%) with RR = 1.92 (95% CI: 1.53–2.41, P < 0.001) [34].

Attempts to standardize IHC assays are necessary to help formulate guidelines. In a public workshop in March 2015, the FDA, the American Society of Clinical Oncology (ASCO), and the American Association for Clinical Research (AACR), announced efforts aimed at harmonizing companion diagnostics across PD-1/PD-L1 directed therapies, involving collaboration between pharmaceutical and diagnostic companies [35]. The Blueprint PD-L1 IHC Comparison Project is a collaboration between the International Association for the Study of Lung Cancer, the AACR, and four pharmaceutical companies (Bristol-Myers Squibb, Merck & Co. Inc, AstraZeneca, and Genentech/Roche) [36]. The phase 1 feasibility study of Blueprint assessed 39 NSCLC tumors with four PD-L1 IHC assays (Dako 22C3, Dako 28–8, Ventana SP142, and Ventana SP263), with results showing comparable staining of the 22C3, 28–8, and SP263 assays [36]. The SP142 assay had fewer stained tumor cells and did not correlate as well [36]. Blueprint phase 1 also indicated that immune cell staining had greater variability than tumor cell staining [36]. In 14 of 38 samples (37%), a different classification would be made depending on which assay was used [36], which would have obvious impacts on treatment selection. Nineteen of 38 samples (50%) were classified above (or as PD-L1 positive) the selected cutoffs of all assays [36]. Five of 38 samples (13%) were classified below the selected cutoffs of all IHC assays [36].

A study evaluating 493 commercially available samples from NSCLC patients indicated good concordance between the Ventana SP263 assay, Dako 28–8 assay, and Dako 22C3 assay [37]. Specifically, there was an overall percentage agreement of greater than 90% across multiple expression cut-offs [37]. The National Comprehensive Cancer Network (NCCN) is collaborating with Bristol-Myers Squibb, in a separate effort in lung cancer, to evaluate variability across assays, heterogeneity within samples, and concordance of pathologist interpretation [31] [38]. In France the national health system is carrying out a validation study of PD-L1 expression using different antibodies and platforms in solid and hematologic tumors [31]. An early German effort at harmonization of PD-L1 IHC in pulmonary squamous cell and adenocarcinoma evaluated interobserver concordance in two sets of 15 resection specimens [39]. Four clinical trial assays including 28–8, 22C3, SP142, and SP263 as well as two laboratory developed assays were interpreted independently by nine pathologists [39]. Proportion scoring of PD-L1 positive carcinoma cells showed moderate interobserver concordance coefficients for the six step scoring system that was

used as well as good concordance coefficients of the dichotomous proportion cut offs, ≥1, ≥5, ≥10, ≥50% [39]. Scoring of immune cells yielded lower interobserver concordance coefficients [39]. The 28–8 and 22C3 assays stained similar proportions of carcinoma cells in 12 of 15 cases [39]. SP142 stained fewer carcinoma cells than the other three assays in 4 of 15 cases [39]. Rimm et al. evaluated serial histologic sections of 90 archival NSCLC specimens using the Dako 28–8 assay, Dako 22C3 assay, Ventana SP142 assay, and the E1L3N antibody on the Leica Bond platform [40]. The SP142 assay was found to be an outlier, detecting significantly less PD-L1 expression in tumor and immune cells [40]. The 22C3 assay also showed statistically significant lower staining than the 28–8 or E1L3N assays, but this was only significant when using the mean of the pathologists' scores [40].

5. Alternative Biomarkers

Other biomarkers that have been shown to correlate with PD-1 inhibitor efficacy include the molecular smoking signature, higher neoantigen burden, and DNA repair pathway mutations [41]. Whole exome sequencing was used to examine nonsynonymous mutation burden from two cohorts of patients treated with pembrolizumab and higher nonsynonymous mutation burden was associated with clinical activity of pembrolizumab [41]. In the discovery cohort of sixteen patients, the median number of nonsynonymous mutations per sample was 302 in patients with durable clinical benefit versus 148 in patients without durable benefit of treatment with pembrolizumab [41]. Confirmed ORR and PFS were both higher in patients with high nonsynonymous mutation burden [41]. In the validation cohort of 18 patients, the rates of patients with durable clinical benefit and PFS were also significantly greater in patients with high nonsynonymous mutation burden [41]. The ORR in tumors with the molecular smoking signature was 56% versus 17% in tumors with never-smoking signatures [41]. Interestingly, while the molecular smoking signature did correlate with efficacy of pembrolizumab, self-reported smoking status did not [41]. Mutations in DNA repair and replication were found in responders with the highest mutation burden, including mutations in POLD1, POLE, and MSH2 [41]. An exploratory analysis of the phase 3 study of nivolumab versus platinum based chemotherapy in the first-line setting (CheckMate 026) found that patients with high tumor mutation burden had a benefit in PFS with nivolumab treatment as compared with platinum based chemotherapy [42]. In the neoadjuvant setting, mutation burden and neoantigen density were associated with deeper pathologic response to treatment with nivolumab in patients with early-stage resectable NSCLC [43].

Interferon gamma (IFN-γ) mRNA expression has been shown to correlate with response to PD-L1 inhibitors, specifically durvalumab in the setting of advanced NSCLC as assessed in the phase 1/2 study revealing response rate 33% (14 of 43) and 8% (6 of 79) in IFN-γ positive and IFN-γ negative patients respectively [44] [45]. The numerically highest rates of response were seen in the combined IFN-γ positive and PD-L1 positive patients [44]. PD-L1 was assessed using the Ventana SP263 assay and samples were considered positive if 25% or more of cells were stained at any intensity [44].

Gopalakrishnan et al. have studied oral and intestinal microbiome samples via 16S rRNA gene sequencing in patients with metastatic melanoma treated with anti-PD-1 therapy [46]. The authors found significant differences in the diversity and composition of the gut

microbiome in patients that had a response according to RECIST criteria versus non-responders [46]. There were no clear differences in the oral microbiomes [46]. Immune profiling by an IHC panel demonstrated significantly increased immune infiltrates in baseline tumor samples of responders [46].

The Society for Immunotherapy of Cancer recently reconvened the Immune Biomarkers Task Force, comprised of an international multidisciplinary panel of experts, with the ultimate goals of identifying biomarkers predictive of clinical outcomes and elucidating why some patients do not respond to immunotherapy [47]. The most recent meeting, Working Group 4, focused on the complexity of the tumor microenvironment as well as novel tools to aid in such broad analyses [47].

6. Conclusion

Clinicians should be aware that while PD-L1 IHC can be used to predict likelihood of response to anti-PD-1 or anti-PD-L1 therapy in certain patient populations, the association between PD-L1 expression and response is not straightforward, and a proportion of patients with PD-L1 negative tumors can derive benefit from treatment. There are four PD-L1 IHC assays registered with the FDA, using 4 different PD-L1 antibodies (22C3, 28–8, SP263, SP142), on two different IHC platforms (Dako and Ventana), each with their own scoring systems. Attempts to standardize IHC assays to further explore the clinical utility of PD-L1 testing are underway. While harmonization studies have given early indication that the 22C3, 28–8, and SP263 assays are comparable, data are needed regarding the interchangeability of the assays as it pertains to response. To improve patient selection, alternative biomarkers are needed.

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References

1. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Molecular Cancer Therapeutics. 2015; 14(4):847–856. [PubMed: 25695955]
2. Swaika A, Hammond WA, Joseph RW. Current state of anti-PD-L1 and anti-PD-1 agents in cancer therapy. Molecular Immunology. 2015; 67(2, Part A):4–17. [PubMed: 25749122]
3. Topalian SL, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. New England Journal of Medicine. 2012; 366(26):2443–2454. [PubMed: 22658127]
4. Taube JM, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti–PD-1 Therapy. Clinical Cancer Research. 2014; 20(19): 5064–5074. [PubMed: 24714771]
5. Brahmer J, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015; 373(2):123–135. [PubMed: 26028407]
6. Borghaei H, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015; 373(17):1627–1639. [PubMed: 26412456]
7. Borghaei H, et al. Nivolumab (nivo) vs docetaxel (doc) in patients (pts) with advanced NSCLC: CheckMate 017/057 2-y update and exploratory cytokine profile analyses. ASCO Meeting Abstracts. 2016; 34(15_suppl):9025.
8. Garon EB, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015; 372(21):2018–2028. [PubMed: 25891174]

9. Hui R, et al. Long-term OS for patients with advanced NSCLC enrolled in the KEYNOTE-001 study of pembrolizumab (pembro). ASCO Meeting Abstracts. 2016; 34(15_suppl):9026.
10. Herbst RS, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. The Lancet. 2016; 387(10027):1540–1550.
11. Baas P, et al. Relationship between level of PD-L1 expression and outcomes in the KEYNOTE-010 study of pembrolizumab vs docetaxel for previously treated, PD-L1-Positive NSCLC. ASCO Meeting Abstracts. 2016; 34(15_suppl):9015.
12. Rizvi NA, et al. Nivolumab in Combination With Platinum-Based Doublet Chemotherapy for First-Line Treatment of Advanced Non–Small-Cell Lung Cancer. Journal of Clinical Oncology. 2016; 34(25):2969–2979. [PubMed: 27354481]
13. Gettinger S, et al. Nivolumab Monotherapy for First-Line Treatment of Advanced Non–Small-Cell Lung Cancer. Journal of Clinical Oncology. 2016; 34(25):2980–2987. [PubMed: 27354485]
14. Socinski M, et al. NSCLC, metastaticCheckMate 026: A phase 3 trial of nivolumab vs investigator's choice (IC) of platinum-based doublet chemotherapy (PT-DC) as first-line therapy for stage iv/recurrent programmed death ligand 1 (PD-L1)−positive NSCLC. Annals of Oncology. 2016; 27(suppl_6):LBA7_PR-LBA7_PR.
15. Carbone DP, et al. First-Line Nivolumab in Stage IV or Recurrent Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2017; 376(25):2415–2426. [PubMed: 28636851]
16. Reck M, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2016; 375:1823–1833. [PubMed: 27718847]
17. Langer CJ, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. The Lancet Oncology. 2016; 17(11):1497–1508. [PubMed: 27745820]
18. 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. The Lancet. 2016; 387(10030):1837–1846.
19. Smith DA, et al. Updated survival and biomarker analyses of a randomized phase II study of atezolizumab vs docetaxel in 2L/3L NSCLC (POPLAR). ASCO Meeting Abstracts. 2016; 34(15_suppl):9028.
20. Rittmeyer A, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. The Lancet. 2016
21. Barlesi F, et al. Primary analysis from OAK, a randomized phase III study comparing atezolizumab with docetaxel in 2L/3L NSCLC. ESMO Meeting Abstract. 2016; LBA44_PR
22. Gulley JL, et al. Avelumab for patients with previously treated metastatic or recurrent non-small-cell lung cancer (JAVELIN Solid Tumor): dose-expansion cohort of a multicentre, open-label, phase 1b trial. The Lancet Oncology. 2017
23. Rizvi N, et al. Safety and clinical activity of MEDI4736, an anti-programmed cell death-ligand 1 (PD-L1) antibody, in patients with non-small cell lung cancer (NSCLC). J Clin Oncol. 2015; 33(Suppl) abstr 8032.
24. Gubens MA, et al. Phase I/II study of pembrolizumab (pembro) plus ipilimumab (ipi) as second-line therapy for NSCLC: KEYNOTE-021 cohorts D and H. ASCO Meeting Abstracts. 2016; 34(15_suppl):9027.
25. Antonia S, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. The Lancet Oncology. 2016; 17(3):299–308. [PubMed: 26858122]
26. Hellmann MD, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. The Lancet Oncology. 2017; 18(1):31–41. [PubMed: 27932067]
27. Planchard D, et al. A Phase III Study of Durvalumab (MEDI4736) With or Without Tremelimumab for Previously Treated Patients With Advanced NSCLC: Rationale and Protocol Design of the ARCTIC Study. Clinical Lung Cancer. 2016; 17(3):232–236.e1. [PubMed: 27265743]

28. Spranger S, et al. Up-Regulation of PD-L1, IDO, and T(regs) in the Melanoma Tumor Microenvironment Is Driven by CD8(+) T Cells. Science translational medicine. 2013; 5(200): 200ra116–200ra116.
29. McLaughlin J, et al. Quantitative assessment of the heterogeneity of pd-l1 expression in non–small-cell lung cancer. JAMA Oncology. 2016; 2(1):46–54. [PubMed: 26562159]
30. Gadiot J, et al. Overall survival and PD-L1 expression in metastasized malignant melanoma. Cancer. 2011; 117(10):2192–2201. [PubMed: 21523733]
31. Sholl LM, et al. Programmed Death Ligand-1 Immunohistochemistry—A New Challenge for Pathologists: A Perspective From Members of the Pulmonary Pathology Society. Archives of Pathology & Laboratory Medicine. 2016; 140(4):341–344. [PubMed: 26780537]
32. Abdel-Rahman O. Correlation between PD-L1 expression and outcome of NSCLC patients treated with anti-PD-1/PD-L1 agents: A meta-analysis. Critical Reviews in Oncology/Hematology. 2016; 101:75–85. [PubMed: 26969107]
33. Aguiar P Jr, et al. P2.47 (also presented as PD1.02): The Role of PD-L1 Expression as a Predictive Biomarker in Advanced NSCLC: An Update of a Network Meta-Analysis. Journal of Thoracic Oncology. 2016; 11(10):S247–S248.
34. Zhang T, et al. The efficacy and safety of anti-PD-1/PD-L1 antibodies for treatment of advanced or refractory cancers: a meta-analysis. Oncotarget. 2016; 7(45):73068–79. [PubMed: 27683031]
35. Villaruz LC, Socinski MA. The clinical utility of PD-L1 testing in selecting non-small cell lung cancer patients for PD1/PD-L1-directed therapy. Clinical Pharmacology & Therapeutics. 2016; 100(3):212–214. [PubMed: 27090296]
36. Hirsch FR, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. Journal of Thoracic Oncology. 2017; 12(2): 208–222. [PubMed: 27913228]
37. Ratcliffe MJ, et al. Agreement between Programmed Cell Death Ligand-1 Diagnostic Assays across Multiple Protein Expression Cut-Offs in Non-Small Cell Lung Cancer. Clinical Cancer Research. 2017
38. NCCN NCCN collaborates with Bristol-Myers Squibb to study PD-L1 expression and test interpretation in lung cancer. 2016
39. Scheel AH, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Mod Pathol. 2016; 29(10):1165–1172. [PubMed: 27389313]
40. Rimm DL, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for pd-l1 expression in non–small cell lung cancer. JAMA Oncology. 2017 Mar 9. [Epub ahead of print]. doi: 10.1001/jamaoncol.2017.0013
41. Rizvi NA, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science. 2015; 348(6230):124–128. [PubMed: 25765070]
42. Peters S. Impact of tumor mutation burden on the efficacy of first-line nivolumab in stage IV or recurrent non-small cell lung cancer: an exploratory analysis of CheckMate-026. AACR Abstract # CT082. 2017
43. Chaft JE, et al. Neoadjuvant nivolumab in early-stage, resectable non-small cell lung cancers. Journal of Clinical Oncology. 2017; 35(15_suppl):8508–8508.
44. Higgs BW, et al. 15LBA High tumoral IFN mRNA, PD-L1 protein, and combined IFN mRNA/PD-L1 protein expression associates with response to durvalumab (anti-PD-L1) monotherapy in NSCLC patients. European Journal of Cancer. 2015; 51:S717.
45. Shukuya T, Carbone DP. Predictive Markers for the Efficacy of Anti PD-1/PD-L1 Antibodies in Lung Cancer. Journal of Thoracic Oncology. 2016; 11(7):976–988. [PubMed: 26944305]
46. Gopalakrishnan V, S C, Reuben A, Karpinets T, Hutchinson D, Hoffman K, Prieto P, Tetzlaff M, Lazar A, Davies M, Gershenwald J, Jenq R, Hwu P, Sharma P, Patrick Allison J, Futreal A, Ajami N, Petrosino J, Daniel-MacDougall C, Wargo J. Association of diversity and composition of the gut microbiome with differential responses to PD-1 based therapy in patients with metastatic melanoma. J clin Oncol. 2017; 35(suppl 75) abstract 2.
47. Gnjatic S, et al. Identifying baseline immune-related biomarkers to predict clinical outcome of immunotherapy. Journal for ImmunoTherapy of Cancer. 2017; 5(1):44. [PubMed: 28515944]

48. Roche. Two positive studies of Roche's investigational cancer immunotherapy atezolizumab in specific type of lung cancer presented at 2015 European Cancer Congress. F. Hoffmann-La Roche Ltd; 2015. Media Release, Basel, 27 September 2015. http://www.roche.com/media/store/releases/med-cor-2015-09-27.htm

Key Points

1. In non-squamous non-small cell lung cancer PD-L1 positivity correlates with response to PD-1 inhibitor treatment, but a significant portion of patients that are PD-L1 negative can have a response.
2. Each FDA approved PD-1/PD-L1 antibody was approved in the setting of its own unique PD-L1 assay and harmonization studies are underway, with early studies generally indicating good concordance.

Table 1

Selected PD-1 and PD-L1 inhibitor clinical trials in advanced NSCLC

Study	Description of Study	Type of Biopsy Evaluated	Study Arms	N	Primary Endpoints	PD-L1 assay	Correlation between PD-L1 status and endpoints?	Ref
PD1 inhibitors								
CheckMate 017	Phase III, previously treated squamous NSCLC, any PD-L1 status	Mix of archival and fresh	nivolumab vs docetaxel	272	OS (mos), 9.2 vs 6.0	IHC 28–8 antibody (Dako). PD-L1 positive: staining of tumor cell membrane at any intensity at 1%, 5%, 10% of cells	No	[5]
CheckMate 057	Phase III, previously treated nonsquamo us NSCLC, any PD-L1 status	Mix of archival and fresh	nivolumab vs docetaxel	582	OS (mos), 12.2 vs 9.4	IHC 28–8 antibody (Dako). PD-L1 positive: staining of tumor cell membrane at any intensity at 1%, 5%, 10% of cells	Yes, nivolumab nearly doubled OS as compared to docetaxel in patients whose tumors expressed any PD-L1. No difference in OS between groups in patients whose tumors were negative for PD-L1	[6]
CheckMate 026	Phase III, first-line NSCLC, PD-L1 ≥ 5%	Fresh or archival obtained within 6 mos before enrollment	nivolumab vs investigator's choice chemotherapy	541	PFS (mos), 4.2 vs 5.9	IHC 28–8 (Dako), PD-L1 positive: tumor cell staining at ≥ 5%	No PFS benefit in nivolumab group	[14] [15]
KEYNOTE 001	Phase I, advanced (both previously treated and untreated) NSCLC, any PD-L1 status	Fresh	Pembroizumab	495	efficacy and safety, ORR 19.4%	IHC 22C3 antibody (Dako), PD-L1 positive: membrano us staining in at least 1% of cells within tumor nests or distinctive staining pattern of mononuclear inflammatory cells in the stroma	Yes, response rate nearly double in patients with PD-L1 staining ≥ 50%	[8]
KEYNOTE 010	Phase II/III, previously treated NSCLC, PD-L1 positive	Mix of archival and fresh	pembolizumab vs docetaxel	1034	OS (mos): pembro 2 mg/kg 10.4, pembro 10 mg/kg 12.7, docetaxel 8.5. No difference in PFS.	IHC 22C3 antibody (Dako). PD-L1 positive: membrano us staining in at least 1% of cells within tumor nests or distinctive staining pattern of mononuclear inflammatory cells in the stroma	Yes, OS significantly longer in subgroup of patients with PD-L1 ≥ 50%. OS (mos): pembro 2 mg/kg 14.9, pembro 10 mg/kg 17.3, docetaxel 8.2	[10]

Study	Description of Study	Type of Biopsy Evaluated	Study Arms	N	Primary Endpoints	PD-L1 assay	Correlation between PD-L1 status and endpoints?	Ref
KEYNOTE 024	Phase III, first-line NSCLC, PD-L1 ≥ 50%	Collected at time of diagnosis of metastatic disease	pembrolizumab vs platinum-based chemo	305	PFS (mos): 10.3 vs 6.0. Secondary endpoint OS, also longer in pembro group	IHC 22C3 antibody (Dako). PD-L1 positive: membrano us staining in at least 50% of tumor cells	Yes, PFS and OS improved in patients with ≥ 50% PD-L1 staining treated with pembrolizumab	[16]
KEYNOTE 021	Phase II, first-line, non-squamous NSCLC, any PD-L1 status	Collected at time of diagnosis of metastatic disease	carboplatin and pemetrexed with pembrolizumab vs carboplatin and pemetrexed	123	ORR, 55% vs 29%	IHC 22C3 antibody (Dako). Patients were stratified by staining of <1% and ≥ 1% tumor cells	No apparent relationship between tumor PD-L1 expression and response	[17]
PD-L1 inhibitors								
POPLAR	Phase II, previously treated NSCLC, any PD-L1 status	Fresh	atezolizumab vs docetaxel	287	OS (mos): 12.6 vs 9.7	IHC SP142 antibody (Ventana). PD-L1 expression on tumor-infiltrating immune cells (IC) and tumor cells (TC) scored as TC0, 1, 2, or 3 and IC0, 1, 2, or 3.	Yes, OS by PD-L1 expression was assessed as a co-primary endpoint. OS benefit increased with increasing PD-L1 expression on TC, IC, or both	[18]
BIRCH	Phase II, first-line or subsequent treatment for NSCLC, PD-L1 positive	Not reported	atezolizumab	667	ORR: between 17% and 27% for the various groups	IHC SP142 antibody (Ventana). PD-L1 expression on tumor-infiltrating immune cells (IC) and tumor cells (TC) scored as TC0, 1, 2, or 3 and IC0, 1, 2, or 3.	Yes, ORR higher in groups with high (TC3 or IC3) PD-L1 expression as compared to combination of medium and high (TC 2/3 or IC 2/3) expression	[48]
OAK	Phase III, previously treated NSCLC, any PD-L1 status	Mix of archival and fresh	atezolizumab vs docetaxel	850 of 1225 analyzed	Median OS (mos): 13.8 vs 9.6	IHC SP142 antibody (Ventana). PD-L1 expression on tumor-infiltrating immune cells (IC) and tumor cells (TC) scored as TC0, 1, 2, or 3 and IC0, 1, 2, or 3.	No. OS benefit was seen regardless of PD-L1 expression. Patients in TC0 and IC0 subgroup also had improved OS. However, more pronounced benefit was seen in pts with high PD-L1.	[20]
ATLANTIC	Phase II, 3rd line setting NSCLC, any PD-L1 status (later restricted to PD-L1 positive)	Not reported	durvalumab	149	ORR 14% in general population, 23% in PD-L1 positive patients. ORR higher in squamous vs non-squamous	IHC SP263 (Ventana). 25% or greater of tumor cells staining considered positive.	Yes, higher response rate in PD-L1 positive patients.	[23]
JAVELIN	Phase 1b, previously treated NSCLC, any PD-L1 status	Mix of archival and fresh	avelumab	184	12% with OR, 50% with stable disease (OR + SD)	IHC 73–10 (Dako). Tumor cell staining assessed at levels ≥ 1%, ≥ 5%, ≥ 25%. Immune	No. Proportion of patients with OR or OS outcome did not differ between PD-L1 positive and negative	[22]

Study	Description of Study	Type of Biopsy Evaluated	Study Arms	N	Primary Endpoints	PD-L1 assay	Correlation between PD-L1 status and endpoints?	Ref
						cells assessed at ≥ 10% in hotspots	patients at any prespecified expression level.	

Abbreviations. PD-1- programmed death 1; PD-L1- programmed death-ligand 1; Ref- reference; NSCLC- non-small cell lung cancer; OS- overall survival; mos- months; IHC- immunohistochemistry; PFS- progression free survival; ORR- objective response rate; OR- objective response; SD- stable disease