Programmed Death Ligand-1 Immunohistochemistry—A New Challenge for Pathologists

A Perspective From Members of the Pulmonary Pathology Society

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• The binding of programmed death ligand-1 and ligand-2 (PD-L1 and PD-L2) to PD-1 blocks T-cell–mediated immune response to tumor. Antibodies that target programmed death receptor-1 (PD-1) will block the ligand-receptor interface, thereby allowing T cells to attack the tumor and increase antitumor immune response. In clinical trials, PD-1 inhibitors have been associated with an approximately 20% overall response rate in unselected patients with non–small cell lung cancer, with sustained tumor response in a subset of patients treated by these immune checkpoint inhibitors. Facing a proliferation of PD-L1 immunohistochemistry clones, staining platforms, and scoring criteria, the pathologist must decide on the feasibility of introducing a newly approved companion diagnostic assay that may require purchase not only of a specific antibody kit but of a particular staining platform. Given the likely reality that clinical practice may, in the near future, demand access to 4 different PD-L1 antibodies coupled with different immunohistochemistry platforms, laboratories will be challenged with deciding among this variety of testing methods, each with its own potential benefits. Another immediate challenge to PD-L1 testing in lung cancer patients is that of access to adequate tumor tissue, given that non–small cell lung cancer samples are often extremely limited in size. With PD-L1 testing it has become clear that the historically used US regulatory approach of one assay–one drug will not be sustainable. One evolving concept is that of complementary diagnostics, a novel regulatory pathway initiated by the US Food and Drug Administration, which is distinct from companion diagnostics in that it may present additional flexibility. Although pathologists need to face the practical reality that oncologists will be asking regularly for the PD-L1 immunohistochemistry status of their patients' tumors, we should also keep in mind that there may be room for improvement of biomarkers for immunotherapy response. The field is rich with opportunities for investigation into biomarkers of immunotherapy response, particularly in the form of collaborative, multidisciplinary studies that incorporate oncologists, pathologists, and basic scientists. Pathologists must take the lead in the rational incorporation of these biomarkers into clinical practice.

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Programmed death receptor-1 (PD-1) is a type 1 membrane protein of the immunoglobulin superfamily[1] that has an important role in restricting immune-mediated tissue damage secondary to inflammation and/or infection. This immunomodulatory receptor is expressed on the surface of T and B cells, natural killer cells, natural killer–T cells, dendritic cells, and macrophages[2] and is overexpressed on the surface of exhausted T cells. Based on the hypothesis that its blockade can restore the function of exhausted T cells,[3] PD-1 is considered a key immune barrier receptor expressed by activated T cells.[4]

The binding of programmed death ligand-1 and ligand-2 (PD-L1 and PD-L2) to PD-1 blocks T-cell–mediated immune response to tumor.[5,6] Among the ligands belonging to the B7 family (PD-L1, PD-L2, B7-H3, and B7-H4), PD-L1 is one of the most important membrane inhibitory ligands and the most studied in lung cancer clinical trials.[7] Antibodies that target either PD-1 or PD-L1 will block this ligand-receptor interface, thereby allowing T cells to attack the tumor and increase antitumor immune response. The clinical benefit of this approach to cancer killing became clear when trials using antagonists against the T-cell regulator, CTLA4, such as ipilimumab, and subsequently PD-1, showed a survival benefit in patients with metastatic melanoma and led to remarkable response in a variety of solid tumors refractory to other therapies.[8] In lung cancer, a series of high-profile clinical trials have demonstrated the benefit of PD-1 inhibitors pembrolizumab (Keytruda, Merck, Kenilworth, New Jersey) in advanced non–small cell lung cancer (NSCLC) and nivolumab (Opdivo, Bristol-Myers Squibb, New York, New York) in advanced squamous and nonsquamous NSCLC; both have been approved as second-line therapies by the US Food and Drug Administration (FDA).[9–11] PD-L1 inhibitors atezolizumab (Roche, Basel, Switzerland) and durvalumab (AstraZeneca, London, United Kingdom) have demonstrated efficacy in a number of tumor types[12,13]; although only preliminary clinical data are available on these PD-L1 inhibitors, it is possible they will be approved for clinical use in 2016.

In clinical trials, PD-1 inhibitors are associated with an approximately 20% overall response rate in unselected patients with NSCLC, with sustained tumor response in a subset of patients treated by these immune checkpoint inhibitors. Of particular relevance to pathologists, Garon et al[9] showed that patients whose tumors had PD-L1 expression in 50% or greater malignant cells by immunohistochemistry (IHC) were significantly more likely to respond to pembrolizumab than those with less than 50% malignant cell expression. Their study used the Dako PD-L1 IHC 22C3 pharmDx test on the Autostainer Link 48 (Dako, Carpinteria, California), and the FDA approved this combination of antibody clone and detection system as a companion diagnostic for selecting lung cancer patients for pembrolizumab therapy. In contrast, response rates to nivolumab are significantly greater in patients with nonsquamous NSCLC, showing 1% or greater tumor cell positivity using the Dako detection system but a different antibody clone (28-8, Abcam, Cambridge, Massachusetts).[11] Response rates in PD-L1–positive patients in these trials were 31% to 52%, but notably up to 16% of PD-L1–negative patients also showed treatment response, indicating that PD-L1 expression enriches for responders but the absence of expression is not an absolute indicator of the lack of benefit. PD-L1 expression did not predict differential response to nivolumab in lung squamous cell carcinoma as compared with docetaxel.[10] Published abstracts from trials of the PD-L1 inhibitors from atezolizumab (POPLAR trial [NCT01903993])[14] and durvalumab[15] describe the use of different PD-L1 IHC testing platforms (Roche and Ventana) and clones (SP142 and SP263, respectively). Furthermore, the POPLAR trial adds yet another complexity to the biomarker scoring approach by suggesting that PD-L1 expression on tumor-infiltrating immune cells may also predict response (Table).

Facing this proliferation of PD-L1 IHC clones, staining platforms, and scoring criteria, the pathologist must decide on the feasibility of introducing a newly approved companion diagnostic assay that may require purchase not only of a specific antibody kit but of a particular staining platform. Given that the FDA-approved Dako 22C3 companion diagnostic became available only in October of 2015 and that the 22C3 clone cannot be purchased apart from the approved kit, many laboratories have already technically validated other readily available and far less costly commercial antibodies as laboratory-developed tests (LDTs). Efforts to compare the performance of 2 commonly used antibody clones, E1L3N from Cell Signaling Technology and Ventana-SP142 (anticipated to serve as part of the companion diagnostic for atezolizumab), have demonstrated fair to poor concordance[16]; however, these studies are limited by outdated antibody optimization techniques and the lack of a clear clinical gold standard in the form of response data. The importance of this latter issue cannot be understated. Overall, the performance of these LDTs as biomarkers for response to immunotherapies has not been clinically validated. Retrospective comparison of LDTs versus approved diagnostics may be possible at the individual institutions that participated in published trials, but these institutional efforts may ultimately be underpowered to demonstrate equivalence.

Given the likely reality that clinical practice may, in the near future, demand access to 4 different PD-L1 antibodies coupled with different IHC platforms, some laboratories may find it more practical and economical to use an institutional LDT to screen potential patients for treatment with an anti–PD-1/PD-L1 agent. When necessary, positive confirmation may be performed at a commercial laboratory that offers a PD-L1 IHC assay specific for the agent of use. This approach, however, introduces an inevitable delay to start of therapy. Alternatively, individual laboratories may choose to offer a single companion diagnostic assay tailored to the practices and preferences of their requesting oncologists while retaining the option of sending out samples to other laboratories that offer different assays.

To reduce the chance of false-negative results, more concerted efforts to cross compare performance of available antibodies and protocols are needed. As a result of the FDA-American Association for Cancer Research-American Society of Clinical Oncology–sponsored workshop "Complexities in Personalized Medicine: Harmonizing Companion Diagnostics Across a Class of Targeted Therapies" that took place on March 24, 2015, a blueprint project to evaluate the comparability of the various PD-L1 assays being developed is ongoing.[17] In a separate effort, the National Comprehensive Cancer Network is collaborating with Bristol-Myers Squibb to assess variability across assays, heterogeneity within individual samples, and concordance of pathologist interpretation.[18] Although these efforts may move us toward greater standardization, decades of experience with assays such as HER2 and ER IHC have shown that even with

Programmed Death Ligand-1 Inhibitors					
Drug	**Company**	**FDA Approval**	**mAb/Platform**	**Scoring Criteria**	**Comment**
Pembrolizumab (Keytruda)	Merck (Kenilworth, New Jersey)	FDA approved for NSCLC	22C3 (DAKO pharmDx)/ Link 48 Autostainer (Dako, Carpenteria, California)	≥50% tumor cells	Companion diagnostic[a] (as of October 2015)
Nivolumab (Opdivo)	Bristol-Myers Squibb (New York, New York)	FDA approved for squamous and nonsquamous NSCLC	28-8 (DAKO pharmDx)/ Link 48 Autostainer	≥1% tumor cells	Complementary diagnostic[b] (as of October 2015); predictive only in nonsquamous carcinomas
Atezolizumab (MPDL3280)	Roche (Basel, Switzerland)	Expected in 2016	SP142 (Ventana, Tucson, Arizona)	Tumor cells and/ or tumor-infiltrating immune cells	In development
Durvalumab (MEDI4736)	Astra-Zeneca (London, United Kingdom)	Expected in 2016	SP263 (Ventana)	≥25% tumor cells	In development

Abbreviations: FDA, US Food and Drug Administration; mAb, monoclonal antibody; NSCLC, non–small cell lung cancer.

[a] According to the FDA, a *companion diagnostic* is one whose use is "essential to the safe and effective use of a corresponding therapeutic product" and is typically developed contemporaneously with the clinical development of that particular therapeutic. See "In Vitro Comparison Diagnostic Devices." [21]

[b] The definition of *complementary diagnostic* has not been formally established by the FDA at the time of this writing. One proposed definition is that of a test that is not required for use of a therapeutic product, but that may be used to identify patients most likely to derive benefit. See Ray's[22] article on the FDA's recent approval of Opdivo.

highly standardized scoring criteria using FDA-approved assays, there remains substantial discordance when testing is performed in a widely distributed manner. This issue is likely to only be amplified by the diversity of antibody clones targeting the intracellular domain (E1L3N and SP142) versus the extracellular domain (SP263, 22C3, and 28-8) of PD-L1 and a plethora of detection systems. Most laboratories cannot bear the burden of high-volume send-out testing; as a result, it is almost inevitable that predictive diagnostics for immunotherapeutics will become a distributed practice, and interlaboratory reproducibility will necessarily become a key element of quality assurance.

Another immediate challenge is that of access to adequate tumor tissue, given that NSCLC samples are often extremely limited in size. The demand for genomic testing to dictate first-line therapy in patients with advanced NSCLC may compete with requests for multiple immunotherapy IHC assays. For many patients with NSCLC, a diagnostic specimen obtained through minimally invasive means is often scant and may be readily depleted by the key standard-of-care assays evaluating for targets such as *EGFR* and *ALK*. A further complication is the lack of data on PD-L1 testing in cytology preparations, the dominant sample type in some institutions. As indicated below, the PD-L1 expression is dynamic and may change following targeted therapy and/or chemotherapy/radiation therapy. Thus, it may be less than ideal to determine PD-L1 status using archival tissue. The practical feasibility and risks of rebiopsy must then be carefully weighed against the benefit of knowing the tumor PD-L1 status in making future immunotherapy treatment decisions.

In light of the issues presented here, it is clear that the historically used US regulatory approach of one assay–one drug will not be sustainable. One evolving concept is that of complementary diagnostics, a novel regulatory pathway initiated by the FDA, which is distinct from companion diagnostics in that it may present additional flexibility. Indeed, the Dako PD-L1 IHC 28-8 assay has been given this designation in association with nivolumab therapy in nonsquamous NSCLC, although its use is not required in this setting. Although uncertainty remains about the complementary diagnostic pathway, an alternative approach looking at the use of a class of antibody for selection for a class of drug may best facilitate implementation of a selection approach for immunotherapy agents. This would combat many of the detrimental effects of the multiplicity of antibodies, conditions, and interpretive approaches; however, this would need additional study to demonstrate its clinical validity. Of note, these challenges are being addressed in a more systematic fashion in other countries, for instance in France, where the national health systems aim to standardize PD-L1 IHC for selecting patients for immunotherapies. In France, the national cancer institute is currently supporting a national validation study of PD-L1 expression using different antibodies and platforms in solid and hematologic tumors, including melanoma, lung cancers and mesothelioma, lymphoma, and head and neck carcinoma, with the aim of providing national guidelines and recommendations regarding antibodies, protocols, and scoring systems. It should be noted, however, that these studies can only report on technical equivalence; there is no guarantee that the same predictive performance will be delivered by an LDT.

Although we need to face the practical reality that oncologists will be asking regularly for the PD-L1 IHC status of their patients' tumors, we should also keep in mind that there may be room for improvement of biomarkers for immunotherapy response. Some percentage of patients whose tumors are considered negative for PD-L1 expression demonstrate response to PD-1/PD-L1 inhibitors; indeed, PD-L1 expression analysis is not indicated at all when considering nivolumab therapy for patients with squamous cell carcinoma. The performance of PD-L1 tests is also influenced by cutoffs for positivity; very low cutoffs may fail to maximize the differences in response between positive and negative groups. Sampling errors, tumoral heterogeneity, or testing of tissue obtained at diagnosis rather than at time of progression or relapse may underestimate or

overestimate the percentage of neoplastic cells showing PD-L1 expression.[16,19] There is evidence across different tumor types that mutational signatures, neoantigen burden, and expression of other checkpoint inhibitors may predict response to immunotherapies.[20] It is quite possible that in the future another assay may supersede PD-L1 IHC as the biomarker of choice, assuming these markers can be validated in samples of past or future trials involving these immunotherapy drugs.

The available immunotherapeutics are associated with better response rates and fewer adverse events compared with cytotoxic chemotherapy for patients with NSCLC, and offer a new hope for many patients who have failed to benefit from the genomic revolution that has reshaped the treatment of lung adenocarcinoma in the last decade. As a result, there is a high level of clinical, scientific, and public interest in this new approach to oncologic therapy. The field is rich with opportunities for investigation into biomarkers of immunotherapy response, particularly in the form of collaborative, multidisciplinary studies that incorporate oncologists, pathologists, and basic scientists. Concerted action is needed in the implementation phase, involving all stakeholders including patient advocacy groups, government authorities, and other payers in order to establish the clinical and cost efficacy of immunotherapy in relation to available biomarkers. Pathologists must take the lead in the rational incorporation of these biomarkers into clinical practice. It is imperative that concerned pathology societies gather their experts, consider feasible approaches to addressing these growing logistical and economic challenges, and begin to develop guidelines to inform pathology practice and, ultimately, influence trends in oncology.

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