Development of a Companion Diagnostic for Pembrolizumab in Non–Small Cell Lung Cancer Using Immunohistochemistry for Programmed Death Ligand-1

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• *Context.*—Programmed death ligand-1 (PD-L1) expression by tumors may enable them to avoid immunosurveillance.

***Objective.*—To develop a PD-L1 immunohistochemical assay using the 22C3 anti–PD-L1 murine monoclonal antibody on the Dako platform as a possible companion diagnostic for pembrolizumab in patients with non–small cell lung cancer.**

***Design.*—Tumor samples from 146 patients with non–small cell lung cancer treated with pembrolizumab in KEYNOTE-001 and for whom response data were available were scored according to their staining intensity by a single pathologist using 4 methods: percentage of tumor cells staining at any intensity (PS1), moderate/strong intensity (PS2), strong intensity (PS3), and H-score (PS1 + PS2 + PS3). The cutoff score for predicting response to pembrolizumab was determined using receiver operating characteristic analysis. Progression-free and overall survival were assessed in patients with measurable disease per Response Evaluation Criteria in Solid Tumors, version 1.1 (n = 146).**

***Results.*—The 4 scoring methods assessed performed similarly; PS1 with a 50% cutoff score is the simplest and easiest method to implement in practice. Response to pembrolizumab was observed in 19 of 44 patients (43%) with a PS1 score of 50% or higher and 8 of 102 patients (8%) with PS1 lower than 50% (odds ratio, 8.93). Median progression-free and overall survival was 4.0 months and not yet reached, respectively, for patients with a PS1 of 50% or higher, and 2.1 and 6.1 months, respectively, for those with PS1 lower than 50%.**

***Conclusion.*—The PD-L1 immunohistochemical assay shows the potential for enrichment of trial populations and as a companion diagnostic tool in non–small cell lung cancer.**

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Programmed death receptor-1 (PD-1) is a regulatory protein that can be expressed on the surface of T cells, B cells, natural killer T cells, activated monocytes, and dendritic cells.[1] The interaction between PD-1 and its ligands, PD-L1 and PD-L2, the expression of which is upregulated in the presence of inflammation,[2] evokes inhibitory signaling that ultimately regulates the balance between T-cell activation, tolerance, and immunopathology.[1] The expression of PD-1 ligand-1 (PD-L1) on tumor cells is thought to be a part of a negative feedback loop that ultimately regulates the function of the PD-1 pathway.[3] PD-1, PD-L1, and PD-L2 are also overexpressed in various types of tumors,[2] including non–small cell lung cancer (NSCLC).[4,5] This adoption of the PD-1, so-called immune checkpoint, pathway by some tumors enables them to evade surveillance by the immune system, allowing them to grow unchecked.[6,7]

The association between tumor PD-L1 expression and prognosis in patients with solid tumors, including NSCLC, has been explored, with findings suggesting a generally negative prognostic effect.[8] With respect to NSCLC, a retrospective analysis of NSCLC specimens using a prototype immunohistochemical (IHC) assay with the 22C3 antibody clone revealed that PD-L1 expression in tumors was not associated with improved prognosis compared with those without PD-L1 expression.[9,10] Furthermore, several recent meta-analyses have suggested a negative prognostic effect of tumor PD-L1 expression in this disease.[11–13]

Pembrolizumab (MK-3475) is an anti–PD-1 monoclonal antibody of the immunoglobulin (Ig) G4-κ isotype that blocks the binding of PD-1 with its ligands[14]; pembrolizumab has demonstrated robust antitumor activity and has an acceptable toxicity profile in multiple tumor types,[2,15–17] including NSCLC.[18,19] Pembrolizumab is currently approved in several countries for the treatment of advanced melanoma, and in the United States it is approved for the treatment of advanced NSCLC that expresses PD-L1 as determined by

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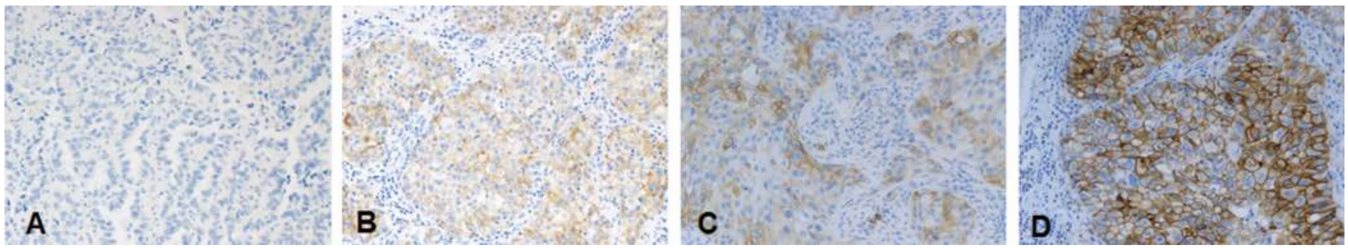

Figure 1. *Examples of different intensities of programmed death ligand-1 (PD-L1) immunostaining in samples of non–small cell lung cancer tumors: (A) none (0), (B) weak (1+), (C) moderate (2+), and (D) strong (3+). Four methods of scoring staining intensity were evaluated: (1) a proportion score (PS) of 1, percentage of cells staining at any intensity (ie, B and C); (2) PS2, percentage of cells staining at moderate/strong intensity (ie, C and D); (3) PS3, percentage of cells staining at strong intensity only (ie, D); and (4) H score = PS1 + PS2 + PS3 (0–300). The bound antibody on the cell membranes was visualized using a horseradish peroxidase–labeled secondary antibody (brown color); the sections were counterstained using hematoxylin (blue color) to reveal cell bodies (original magnification ×20).*

a test approved by the US Food and Drug Administration (FDA), with disease progression on or after platinum-containing chemotherapy.

In the phase 1b KEYNOTE-001 study, tumor PD-L1 expression in at least 50% of tumor cells was correlated with improved response to pembrolizumab treatment in patients with NSCLC.[18] This finding raises the possibility that tumor PD-L1 expression (or the degree thereof) could be employed as a diagnostic biomarker to identify those patients who are most likely to respond to pembrolizumab, not only optimizing patient benefit and reducing the number who are treated unnecessarily, but also as a method of trial population enrichment. To this end, a variety of assays have been developed to evaluate tumor PD-1/PD-L1 status. Unfortunately, as noted by Carbognin et al[20] in a recent sensitivity analysis, the parameters used to determine tumor positivity—including PD-1/PD-L1 antibodies, source of tumor sample (ie, primary tumor versus metastatic tissue), the number of samples assayed for each patient, whether immune cells were considered when defining positivity, whether cell surface or cytoplasmic staining was considered positive, and cutpoints for positivity—have varied across different drug development programs. This lack of a precise and extrapolative definition of tumor PD-1/PD-L1 positivity restricts the utility of the current findings pertaining to responders versus nonresponders and highlights the need for a definitive method of evaluating tumor PD-1/PD-L1 status. In an effort to compare the performance of the various assays, several pharmaceutical and diagnostics companies, including Merck and Dako, are collaborating with the FDA and leaders from the American Society of Clinical Oncology and American Association for Cancer Research to develop a blueprint for assay analysis.[21]

The main objectives of the present study, which used a cohort of patients with NSCLC enrolled in the KEYNOTE-001 trial, were to develop an IHC assay for PD-L1 and to determine the optimal scoring method for PD-L1 staining that predicts response to pembrolizumab in NSCLC. This IHC assay has been approved in the United States as the companion diagnostic for pembrolizumab in advanced NSCLC (IHC 22C3 pharmDx test, Dako, Carpinteria, California).

MATERIALS AND METHODS

Patient Population in the KEYNOTE-001 Study

A detailed account of the NSCLC cohorts in the KEYNOTE-001 trial is published elsewhere.[18] The patients fulfilled the following key eligibility criteria: advanced NSCLC, Eastern Cooperative Oncology Group performance status 0 or 1, 1 or more measurable lesions, provision of a newly obtained tumor sample for PD-L1 IHC assessment, and adequate organ function as determined by baseline laboratory testing. The pembrolizumab dosing schedule was 2 or 10 mg/kg every 3 weeks, or 10 mg/kg every 2 weeks, for 24 months or less or until disease progression, intolerable toxicity, or investigator or patient choice. Response was assessed every 9 weeks. Objective response rates were based on immune-related response criteria by investigator review. Progression-free survival was based on Response Evaluation Criteria in Solid Tumors,

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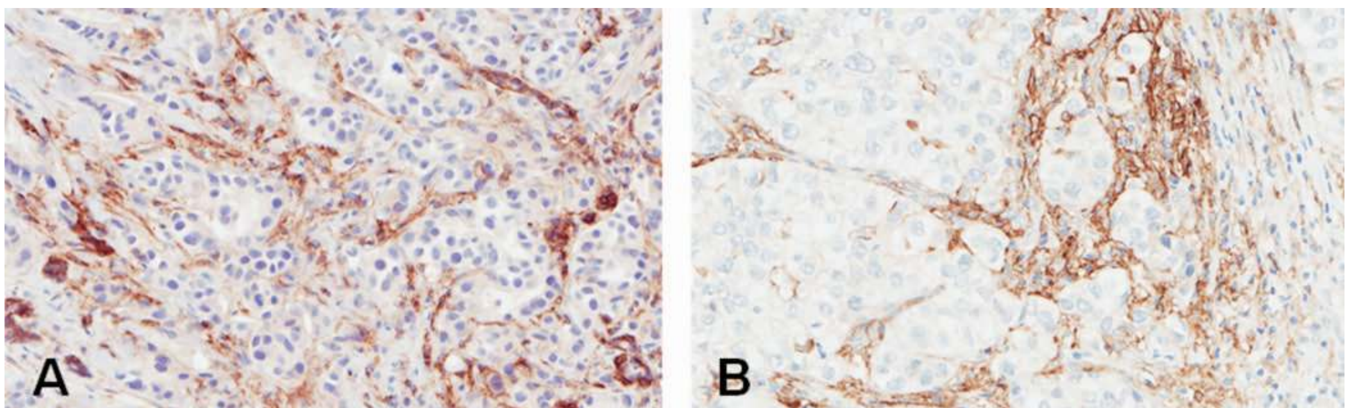

Figure 2. *Two examples (A and B) of interface patterns in non–small cell lung cancer, obtained using the prototype programmed death ligand-1 (PD-L1) immunohistochemical assay. PD-L1 immunostaining is colored brown; the sections were counterstained with hematoxylin (blue; original magnification ×20).*

Table 1. Comparison of the Scoring Methods Evaluated for the Prototype and Clinical Trial Assays		
	Prototype Assay	**Clinical Trial (Dako) Assay**
Standard scores (PS1, PS2, PS3, HS)[a]	Includes both tumor and immune cells located within the tumor nests, demonstrating partial or complete membrane staining	Includes exclusively tumor cells demonstrating partial or complete membrane staining
Interface pattern	Present or absent	Not included
Adequate sample	≥50 viable tumor cells or ≥5 viable PD-L1–positive cells	≥100 viable tumor cells

Abbreviations: HS, histology or H-score; PD-L1, programmed death ligand-1; PS, proportion score.

[a] PS1, staining at any intensity; PS2, staining at moderate/strong intensity; PS3, staining at strong intensity only; HS = PS1 + PS2 + PS3.

version 1.1 (RECIST v1.1), by central review. Outcome analysis took into account patients who may have stopped therapy but experienced delayed response as long as the patient had continued to have imaging submitted to the independent imaging vendor per the protocol; imaging was permitted to stop once progression of disease by immune-related response criteria was determined by the investigator.

PD-L1 Immunohistochemical Prototype Assay

The prototype PD-L1 IHC assay was used to determine enrollment of patients in KEYNOTE-001 and was performed at a single laboratory site at QualTek (Goleta, California), which is a College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA)–accredited laboratory. Commercially available reagents from the Dako EnVision FLEX+ HRP-Polymer kit were used (Dako No. K8012) with the anti–PD-L1 (clone 22C3) antibody. Additional details of the 22C3 PD-L1 antibody clone are described elsewhere.[22]

The prototype assay was developed prior to the development of the clinical trial assay. A full description of the prototype IHC assay is provided in the supplementary methods of Garon et al.[18] In brief, the unstained slides of NSCLC samples sectioned at 4 to 5 microns were first baked at 60°C, deparaffinized in xylene, and rehydrated through a series of graded ethanols into distilled water. Antigens in the tissue were unmasked by placing the slides into a steamer with the Dako EnVision FLEX low-pH Target Retrieval Solution, and staining was achieved using the TechMate IHC platform, which included an additional proteinase K antigen-retrieval step (Dako No. S3020, diluted 1:160 in Dako No. K8012 EnVision FLEX+ wash buffer). The slides were incubated overnight in the dark in a humidified chamber off platform with the 22C3 antibody diluted in Dako Primary Antibody Diluent (2 μg/mL) or a mouse IgG11κ as a negative control for each sample. Detection of the primary antibody or mouse IgG11κ was performed using EnVision FLEX+ Polymer reagents (horseradish peroxidase polymer, diaminobenzidine chromogen, and diaminobenzidine enhancer), washing with EnVision FLEX+ wash buffer between the incubation steps. The slides were counterstained with hematoxylin, coverslipped, and viewed with the aid of a light microscope.

PD-L1 22C3 Immunohistochemical Clinical Trial Assay

This clinical trial assay was developed after the prototype assay; it was developed in a distributable format with the intent of serving as a possible companion diagnostic for pembrolizumab. The PD-L1 IHC staining procedure was performed using the Dako Autostainer Link 48 platform. Slides were baked at 60°C, followed by deparaffinization, rehydration, and target retrieval on the Dako PT Link Pre-Treatment Module (Dako No. PT100) using the Dako EnVision FLEX Target Retrieval Solution, low pH (citrate-based buffered solution at pH 6.1). Staining was performed on the Dako Automated Link 48 staining platform. Following incubation with the monoclonal mouse anti-human PD-L1 antibody, clone 22C3, or the negative control reagent, the Dako EnVision FLEX+ Polymer

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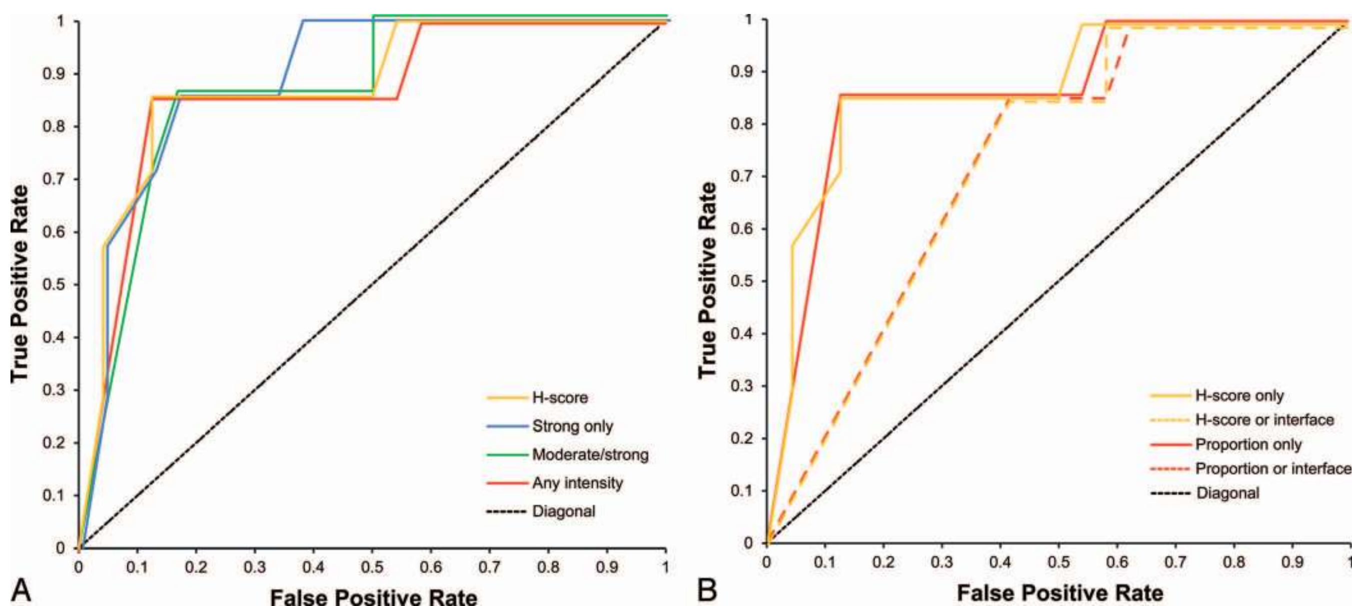

Figure 3. *Comparison of scoring methods by receiver operating characteristic analysis using the prototype immunohistochemical assay in 31 non–small cell lung cancer samples. A, Comparison of the different scoring methods: percentage of cells stained at any intensity (PS1), moderate/strong intensity (PS2), strong intensity only (PS3), or H-score (PS1 + PS2 + PS3). B, Comparison of H-score, interface, and proportion of all tumor cells stained.*

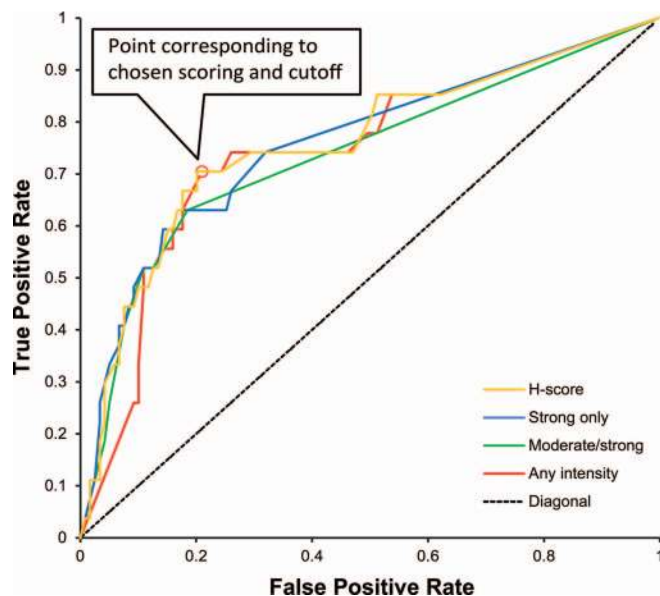

Figure 4. *Comparison of scoring methods (PS1, PS2, PS3, and H-score) by receiver operating characteristic analysis using the clinical trial assay in 146 non–small cell lung cancer samples.*

reagents, including a mouse linker, horseradish peroxidase polymer, diaminobenzidine chromogen, and diaminobenzidine enhancer, were used for primary antibody detection. Slides were washed between incubation steps with EnVision FLEX+ wash buffer and were counterstained with hematoxylin at the end of the procedure. Slides were rinsed in distilled water and subjected to an ethanol dehydration series and xylene changes before being coverslipped. Staining was interpreted with the aid of a light microscope.

Scoring Methods

A single pathologist examined and scored the prototype PD-L1 IHC staining in all of the samples. A different pathologist scored the clinical trial assay. Four standard methods of scoring PD-L1 immunohistochemical staining were evaluated to allow investigation of the potential correlation between PD-L1 expression and clinical response to pembrolizumab: (1) proportion score (PS1), the percentage of tumor cells staining at any intensity; (2) PS2, the percentage of tumor cells staining with moderate or strong intensity; (3) PS3, the percentage of tumor cells staining with a strong intensity only; and (4) the H-score (HS; 0–300), the sum of PS1 + PS2 + PS3. Figure 1 shows examples of PD-L1 membrane staining at different intensities, as assessed using the clinical trial assay, with Figure 1, A, showing no PD-L1 staining; Figure 1, B, weak PD-L1 staining (1+ intensity); Figure 1, C, moderate staining (2+ intensity); and Figure 1, D, strong staining (3+ intensity). In addition, the "interface" or "stroma" pattern (Figure 2) was scored as either present or absent.

The application of the scoring differed slightly between the prototype and clinical trial assay (Table 1). As described in Table 1, for both the prototype and clinical trial assay, partial or complete membrane staining was assessed for PD-L1 IHC scoring. It is noted that the minimum tumor cell requirement differed between the prototype assay and the clinical trial assay. This was because the early use of the prototype assay was as an enrollment assay for KEYNOTE-001,[18] and it was determined that using a minimum of 50 total tumor cells, having 1 positive tumor cell would deem a sample positive for enrollment in the trial, because it would have 1% or more of tumor cells positive for PD-L1. Therefore, it was determined that a reasonable approach would be to consider the presence of either 5 positive cells or 50 total tumor cells (if negative) as adequate for evaluation. When developing the assay as a companion diagnostic, it was determined that based on previous approved IHC tests and minimum thresholds for tumor content, it was more appropriate to focus only on samples with at least 100 viable tumor cells.

Interobserver reproducibility studies have been published in the PD-L1 IHC 22C3 pharmDx package insert. Scoring of 62 NSCLC specimens (30 PD-L1 negative and 32 PD-L1 positive) with a range of PD-L1 IHC expression, which were stained with PD-L1 IHC 22C3 pharmDx, was performed by 3 pathologists, 1 at each of 3 study sites, on 3 nonconsecutive days. Interobserver analysis was performed between 3 sites on a total of 1674 pairwise comparisons. Average agreements were calculated because no natural reference exists for such reproducibility parameters as site and observer. Confidence intervals for the average agreements were computed using a percentile bootstrap method. At a cutoff of 50% or higher, the average negative agreement was 92.6% (87.8%–96.7%), the average positive agreement was 92.8% (88.1%–96.8%), and the overall agreement was 92.7% (88.1%–96.8%).[23]

The optimal scoring method and the cutoff score for predicting response to pembrolizumab with the clinical trial assay were identified by comparison using receiver operating characteristic curve analysis and were evaluated using the Youden index.[24] Survival analysis was performed using Kaplan-Meier statistics.

RESULTS

Analysis of PD-L1 Immunohistochemical Expression in Normal Tissue

PD-L1 expression in normal tissues was observed as expected in tonsil (crypt epithelium) and placenta (syncytiotrophoblasts) samples, as well as in liver macrophages (Kupffer cells) and lymphoid tissues (data not shown). The expression of PD-L1 on normal tissue macrophages indicates that PD-L1 staining of immune cells is not tumor specific; the focus for the NSCLC cohort was tumor-specific staining.

Preliminary Evaluation of the Predictive Value of PD-L1 Expression Using the Prototype Assay

Preliminary evaluation of the prototype assay in an initial set of 31 NSCLC samples suggested that PD-L1 expression had predictive value. Receiver operating characteristic analysis yielded similar findings for all 4 proposed scoring methods (Figure 3, A). Although incorporation of the interface pattern increased the false-positive rate, it did

Table 2. Summary Results of the 4 Scoring Methods Used for the KEYNOTE-001 Training Set: The 4 Methods Performed Similarly

Scoring Method	Area Under the ROC Curve	Youden Index	Cutoff Score[a]	False-Positive Rate	True-Positive Rate
PS1	0.743	0.494	50	0.210	0.704
PS2	0.758	0.462	11	0.168	0.630
PS3	0.736	0.445	1	0.185	0.630
HS	0.758	0.502	63	0.202	0.704

Abbreviations: HS, histology or H-score; PD-L1, programmed death ligand-1; PS, proportion score; ROC, receiver operating characteristic.

[a] Percentage of tumor cells staining for PD-L1 based on the specific scoring method.

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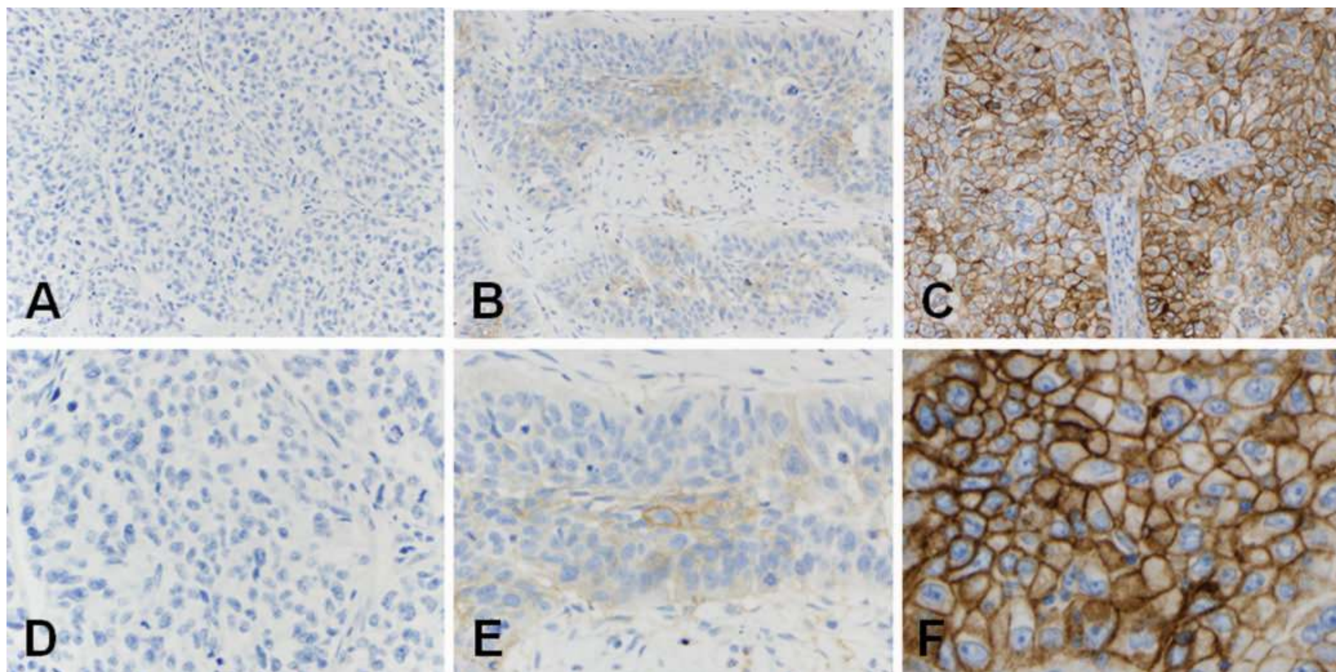

Figure 5. *Programmed death ligand-1 (PD-L1) expression in non–small cell lung cancer tumor samples using the clinical trial assay with tumor proportion score (TPS) of less than 1% (A and D), TPS of 1% to 49% (B and E), and TPS of at least 50% (C and F). PD-L1 immunostaining of cell membranes is colored brown; the sections were counterstained with hematoxylin (blue; original magnifications ×20 [A through C] and ×40 [D through F]).*

not improve the true-positive rate (Figure 3, B) within this initial sample set of 31 samples. Ultimately, the interface pattern was not considered for PD-L1 companion diagnostic development using the 22C3 antibody for NSCLC because: (1) it did not improve the assay performance; (2) it was an extreme pattern of PD-L1–positive inflammatory cell staining, driven predominantly by macrophages; (3) PD-L1 is expressed in macrophages of normal tissue; and (4) the presence of true tumor-infiltrating lymphocytes is low.

Evaluation of the Predictive Value of PD-L1 Expression Using the Clinical Trial Assay

In total, 182 patients from the KEYNOTE-001 trial were prospectively allocated to a training set to evaluate the response-predictive value of PD-L1 expression.[18] Of the specimens used to determine trial eligibility with the prototype assay, a tumor sample for which a quantitative result could be obtained using the clinical trial assay was available for 146 patients. As with the prototype assay, receiver operating characteristic analysis yielded similar results for all 4 standard tumor scores (Figure 4; Table 2). Although slightly better results were obtained for HS, as assessed by the area under the curve and the Youden index, this system is more difficult to implement in clinical practice. Thus, the simplest scoring method—percentage of tumor cells with staining at any intensity (PS1), now simply referred to as the "proportion score" (PS) or as the "tumor proportion score" (TPS)—was used for further assay development, with a chosen cutoff score of 50% based on the receiver operating characteristic analysis. Examples of tumor samples with different TPSs (ie, 0, <50%, and ≥50%) are shown in Figure 5, with PD-L1–negative staining shown at different magnifications in Figure 5, A and D; 1% to 49% staining shown in Figure 5, B and E; and 50% or more staining shown in Figure 5, C and F.

PD-L1 immunostaining with a TPS of 50% or higher was associated with significantly longer progression-free survival (per RECIST v1.1, central review; Figure 6, A) and overall survival (Figure 6, B) than a TPS lower than 50%. The number of responders (per immune-related response criteria, investigator review) was enriched in the TPS 50% or higher population, with an odds ratio of 8.93 (Table 3).

DISCUSSION

Tumor PD-L1 expression represents a potential companion diagnostic to identify patients who are most likely to respond to pembrolizumab. Such a diagnostic would not only enable more accurate targeting of this therapeutic for the greatest benefit, thus reducing the number of people treated unnecessarily, but also allow for the enrichment of clinical trial populations. To date, there have been no comparisons performed across assays or cutoff points, and as such there is no standardized method of evaluating tumor PD-1/PD-L1 status. This issue was addressed for the 22C3 antibody in the present study by comparing the utility of 4 different methods of scoring tumor staining and establishing the optimum cutoff for the proportion of cells stained so that pembrolizumab responders can be identified. Furthermore, the potential role of different staining patterns (tumor versus inflammatory) was assessed in the contexts of both normal and tumor tissue expression of PD-L1 and clinical outcome in patients with NSCLC treated with pembrolizumab.

The 4 scoring methods yielded similar results. Of the 4 methods, it was determined that the easiest method to implement in clinical practice would be to define tumors as PD-L1 positive if 50% or more of the sample was PD-L1 immunopositive at any staining intensity; incorporation of inflammatory cells did not improve the predictive value.

The existence of multiple tests for PD-L1 expression renders it difficult to compare the findings of clinical studies.

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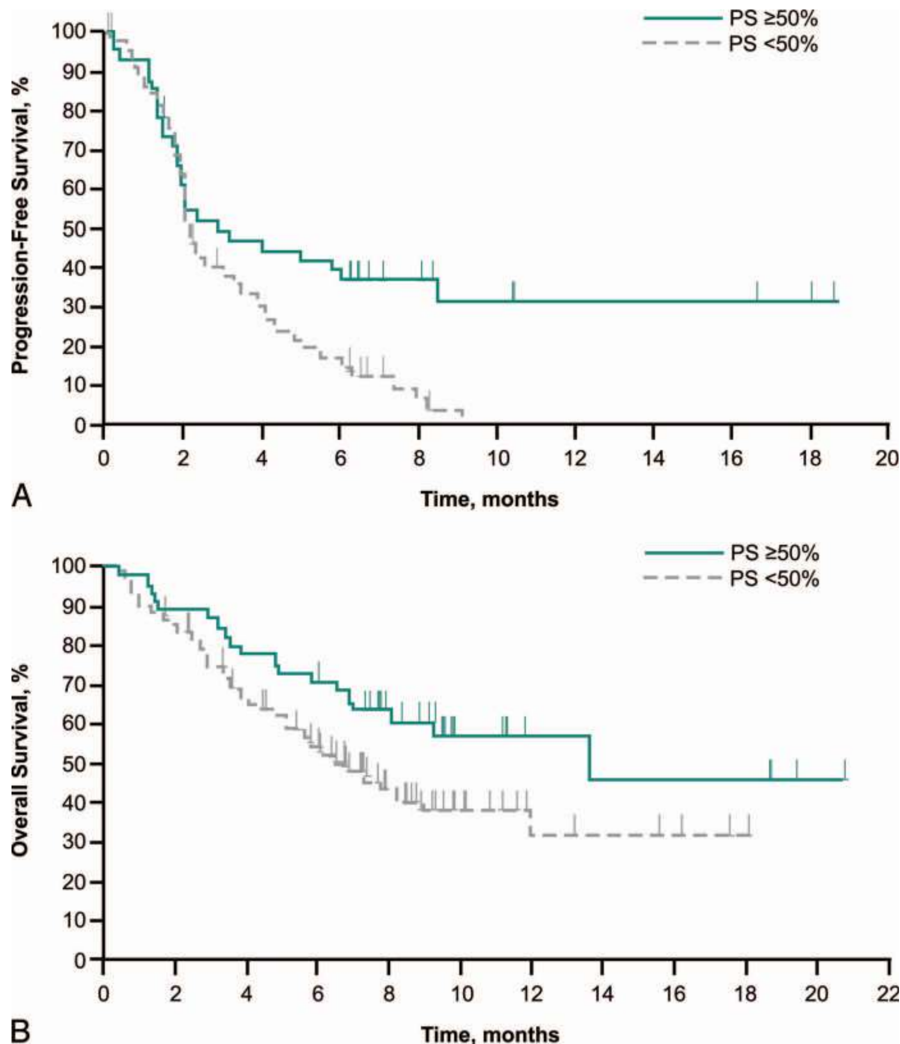

Figure 6. *Progression-free (A) and overall (B) survival in patients with a programmed death ligand-1 (PD-L1) proportion score (PS) of 50% or higher versus less than 50%, as assessed using the clinical trial assay. Response was based on centrally reviewed Response Evaluation Criteria in Solid Tumors (RECIST).*

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Tumor positivity rates reported in the literature range from 13% to 83% depending on the biomarker-defined cohort and the administered therapy.[25] As previously discussed, the differences in rates are likely affected by several factors, including but not limited to the definition of PD-L1 positivity used, the heterogeneity of tumor PD-L1 expression, the presence of immune cells within the tumor, and the tumor type sampled. These factors may also be responsible for the conflicting findings regarding the prognostic value of PD-L1 expression.

Although PD-1/PD-L1 expression has demonstrated value as a predictive biomarker of response to pembrolizumab, reports of its utility as a prognostic indicator have yielded conflicting findings, with some finding better outcomes in patients with PD-L1–expressing NSCLC and others finding poorer outcomes or no correlation.[11] However, 3 recent meta-analyses found that PD-L1 overexpression was associated with a poor prognosis.[11–13] There is some indication that the method of establishing PD-L1 positivity influences the findings on disease outcome; in a subgroup analysis, Pan et al[13] noted that identification of PD-L1 positivity by IHC methods yielded an association with poorer outcome, whereas use of quantitative immunofluorescence yielded no prognostic value. The present study had a single-arm design and thus lacked the comparator arm that would permit assessment of the prognostic value of PD-L1 expression. However, the findings of an epidemiologic study using the prototype IHC assay suggest that it does not possess prognostic value in NSCLC.[9,10]

Taken together, these findings emphasize the clear need for standardization of methodology, including immunohistochemistry and scoring techniques for establishing tumor PD-1/PD-L1 positivity to enable a more accurate determination of the utility of PD-1/PD-L1 status as a biomarker of treatment response.

The responses of patients with NSCLC to pembrolizumab tend to be durable; however, they can vary widely between individuals.[18] Although PD-L1 is useful for predicting which

Table 3. Enrichment for Pembrolizumab Response in Non–Small Cell Lung Cancer Using the Clinical Trial Assay and a Proportion Score 1 Cutoff Score of 50% Programmed Death Ligand-1 Expression in Tumor Cells (as Assessed per Immune-Related Response Criteria by Investigator Review)

Percentage of Tumor Cells Stained	Responders, No.	Nonresponders, No.
<50%	8	94
≥50%	19	25

tumors are most likely to respond to pembrolizumab, PD-L1 is far from a perfect biomarker. Some tumors with TPS less than 50% respond to pembrolizumab, whereas other tumors with TPS of 50% or more do not. One obvious explanation is sampling error: the amount of tissue examined for PD-L1 expression represents a very small fraction of the total body burden of tumor and may not be representative of the overall PD-L1 status. However, a personalized approach to immunotherapy of cancer may prove more challenging than for other targeted therapies. Therapies that target EGFR and KRAS attempt to counteract oncogenic driver mutations, and their mechanism of action is easily understood in terms of the cell biology of cancer. Immunotherapies, on the other hand, are more indirect and complex. Consider, for example, that the target for pembrolizumab is not even on tumor cells, but instead is on the host's immune cells.

The rationale for using PD-L1 as a predictive biomarker is that high expression represents an adaptive modulatory counterresponse by tumor cells to a preceding immune response against the tumor. The purported sequence of events is as follows: neoplastic growth → immune response → adaptive PD-L1 expression (triggered by release of cytokines, most notably interferon-γ) → attenuation of immune response (mediated by the PD-1 receptor). One variation on this theme is that some tumors may effect immune modulation through expression of PD-L2[26] in the absence of concomitant PD-L1 expression; the presence of different molecular alterations could also potentially impact response to specific therapies, representing other possible explanations for pembrolizumab efficacy in tumors with TPS lower than 50%.

The tumor microenvironment most likely encompasses a very complex milieu of factors that may augment or attenuate the host immune response, beyond the PD-1/PD-L1/PD-L2 pathway. One interesting observation is that the probability of response to immune checkpoint inhibitors is proportional to the absolute number of mutations identified by whole-exome sequencing (the "mutational burden"); one hypothesis is that tumors with greater mutational burden evoke a brisker immune response.[27]

It is becoming increasingly evident that the future of targeted therapies in cancer lies at least in part in the ability to focus on specific tumor genetic characteristics. This is likely to involve establishment of genomic, immunohistochemical, and clinical features in a combined diagnostic array, toward individualized targeted treatments.

CONCLUSIONS

The newly developed clinical trial assay used in a training-set analysis of KEYNOTE-001 demonstrated that a PD-L1 PS1 score of 50% or higher identifies a subset of patients with NSCLC who are more likely to respond to pembrolizumab. The predictive value of the assay does not appear to be improved by incorporation of inflammatory cells into the scoring system. Assessment of the prognostic value of PD-L1 expression was not possible in this study given its single-arm design. Given the demonstrated antitumor activity of pembrolizumab in NSCLC, the establishment of a standardized method of determining tumor PD-1/PD-L1 positivity, as in the present study, represents a step toward the goal of individualized therapy.

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References

1. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677–704.

2. McDermott J, Jimeno A. Pembrolizumab: PD-1 inhibition as a therapeutic strategy in cancer. *Drugs Today*. 2015;51(1):7–20.

3. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med*. 2007;13(1):84–88.

4. Yamane H, Isozaki H, Takeyama M, et al. Programmed cell death protein 1 and programmed death-ligand 1 are expressed on the surface of some small-cell lung cancer lines. *Am J Cancer Res*. 2015;5(4):1553–1557.

5. Ji M, Liu Y, Li Q, et al. PD-1/PD-L1 pathway in non-small-cell lung cancer and its relation with EGFR mutation. *J Transl Med*. 2015;13:5.

6. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252–264.

7. Mahoney KM, Atkins MB. Prognostic and predictive markers for the new immunotherapies. *Oncology*. 2014;28(suppl 3):39–48.

8. Wu P, Wu D, Li L, Chai Y, Huang J. PD-L1 and survival in solid tumors: a meta-analysis. *PLoS One*. 2015;10(6):e0131403.

9. Sun JM, Zhou W, Choi SJ, et al. PD-L1 expression and survival in patients with non-small cell lung cancer (NSCLC) in Korea. *J Clin Oncol*. 2014;32(suppl 5):8066.

10. Sorensen S, Zhou W, Dolled-Filhart M, et al. Antitumor activity of pembrolizumab (pembro; MK-3475) and correlation with programmed death ligand 1 (PD-L1) expression in a pooled analysis of patients (pts) with advanced non-small cell lung carcinoma (NSCLC). *Ann Oncol*. 2014;25(suppl 4):1328P.

11. Wang A, Wang HY, Liu Y, et al. The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis. *Eur J Surg Oncol*. 2015; 41(4):450–456.

12. Zhou ZJ, Zhan P, Song Y. PD-L1 over-expression and survival in patients with non-small cell lung cancer: a meta-analysis. *Transl Lung Cancer Res*. 2015; 4(2):203–208.

13. Pan ZK, Ye F, Wu X, An HX, Wu JX. Clinicopathological and prognostic significance of programmed cell death ligand1 (PD-L1) expression in patients with non-small cell lung cancer: a meta-analysis. *J Thorac Dis*. 2015;7(3):462–470.

14. Keytruda (pembrolizumab) for injection, for intravenous use [package insert]. Whitehouse Station, NJ: Merck & Co, Inc; 2015.

15. Daud A, Ribas A, Robert C, et al. Long-term efficacy of pembrolizumab (pembro; MK-3475) in a pooled analysis of 655 patients (pts) with advanced melanoma (MEL) enrolled in KEYNOTE-001. *J Clin Oncol*. 2015;33(suppl):9005.

16. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. 2013;369(2):134–144.

17. Patnaik A, Kang SP, Rasco D, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin Cancer Res*. 2015;21(19):4286–4293.

18. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–2028.

19. Rizvi NA, Garon EB, Patnaik A, et al. Safety and clinical activity of MK-3475 as initial therapy in patients with advanced non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2014;32(5 suppl):8007.

20. Carbognin L, Pilotto S, Milella M, et al. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PLoS One*. 2015;10(6):e0130142.

21. Averbuch S, Emancipator K, McCaffery I, et al. Complexities in personalized medicine: harmonizing companion diagnostics across a class of targeted therapies. American Association for Cancer Research Web site. http://www.aacr.org/AdvocacyPolicy/GovernmentAffairs/Documents/FDA-AACR-ASCO-Complexities-in-Personalized-Medicine-Blueprint-Proposal.pdf. Accessed February 8, 2016.

22. Tumeh PC, Harview CL, Yearly JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515(7528):568–571.

23. Clinical and Laboratory Standards Institute. *Clinical and Laboratory Standards Institute* (Formerly NCCCLS) *for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline*. 2nd ed. Wayne, PA: Clinical and Laboratory Standards Insitutute; 2011. Report No. 35. CLSI document I/LA28-A2.

24. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32–35.

25. Kerr KM, Tsao MS, Nicholson AG, Yatabe Y, Wistuba II, Hirsch FR. Programmed death-ligand 1 immunohistochemistry in lung cancer: in what state is this art? *J Thorac Oncol*. 2015;10(7):985–989.

26. Yearly J, Gibson C, Yu N, Moon C, Murphy E, McClanahan T. PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. Paper presented at: 18th ECCO European Cancer Congress; September 25–29, 2015; Vienna, Austria. Abstract 18LBA.

27. Rizvi NA, Hellmann MD, Snyder A et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015; 348(6230):124–128.

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