ORIGINAL ARTICLE

Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

Edward B. Garon, M.D., Naiyer A. Rizvi, M.D., Rina Hui, M.B., B.S., Natasha Leighl, M.D., Ani S. Balmanoukian, M.D., Joseph Paul Eder, M.D., Amita Patnaik, M.D., Charu Aggarwal, M.D., Matthew Gubens, M.D., Leora Horn, M.D., Enric Carcereny, M.D., Myung-Ju Ahn, M.D., Enriqueta Felip, M.D., Jong-Seok Lee, M.D., Matthew D. Hellmann, M.D., Omid Hamid, M.D., Jonathan W. Goldman, M.D., Jean-Charles Soria, M.D., Marisa Dolled-Filhart, Ph.D., Ruth Z. Rutledge, M.B.A., Jin Zhang, Ph.D., Jared K. Lunceford, Ph.D., Reshma Rangwala, M.D., Gregory M. Lubiniecki, M.D., Charlotte Roach, B.S., Kenneth Emancipator, M.D., and Leena Gandhi, M.D., for the KEYNOTE-001 Investigators*

ABSTRACT

BACKGROUND

We assessed the efficacy and safety of programmed cell death 1 (PD-1) inhibition with pembrolizumab in patients with advanced non–small-cell lung cancer enrolled in a phase 1 study. We also sought to define and validate an expression level of the PD-1 ligand 1 (PD-L1) that is associated with the likelihood of clinical benefit.

METHODS

We assigned 495 patients receiving pembrolizumab (at a dose of either 2 mg or 10 mg per kilogram of body weight every 3 weeks or 10 mg per kilogram every 2 weeks) to either a training group (182 patients) or a validation group (313 patients). We assessed PD-L1 expression in tumor samples using immunohistochemical analysis, with results reported as the percentage of neoplastic cells with staining for membranous PD-L1 (proportion score). Response was assessed every 9 weeks by central review.

RESULTS

Common side effects that were attributed to pembrolizumab were fatigue, pruritus, and decreased appetite, with no clear difference according to dose or schedule. Among all the patients, the objective response rate was 19.4%, and the median duration of response was 12.5 months. The median duration of progression-free survival was 3.7 months, and the median duration of overall survival was 12.0 months. PD-L1 expression in at least 50% of tumor cells was selected as the cutoff from the training group. Among patients with a proportion score of at least 50% in the validation group, the response rate was 45.2%. Among all the patients with a proportion score of at least 50%, median progression-free survival was 6.3 months; median overall survival was not reached.

CONCLUSIONS

Pembrolizumab had an acceptable side-effect profile and showed antitumor activity in patients with advanced non–small-cell lung cancer. PD-L1 expression in at least 50% of tumor cells correlated with improved efficacy of pembrolizumab. (Funded by Merck; KEYNOTE-001 ClinicalTrials.gov number, NCT01295827.)

The authors' affiliations are listed in the Appendix. Address reprint requests to Dr. Garon at the Translational Oncology Research Laboratory, David Geffen School of Medicine at UCLA, 2825 Santa Monica Blvd., Suite 200, Santa Monica, CA 90404, or at egaron@mednet.ucla.edu.

*A complete list of investigators who enrolled patients in the KEYNOTE-001 trial is provided in the Supplementary Appendix, available at NEJM.org.

This article was published on April 19, 2015, at NEJM.org.

N Engl J Med 2015;372:2018-28.
DOI: 10.1056/NEJMoa1501824

Lung cancer is the leading cause of cancer-related death worldwide.[1,2] Platinum-based chemotherapy, with or without maintenance therapy and subsequently followed by second-line cytotoxic chemotherapy, is standard treatment for most patients with advanced non–small-cell lung cancer, with a median survival of approximately 1 year.[3,4]

One hallmark of cancer is immune evasion, in which the immune system does not mount an effective antitumor response.[5] Programmed cell death 1 (PD-1) is a negative costimulatory receptor expressed primarily on the surface of activated T cells.[6,7] The binding of PD-1 to one of its ligands, PD-L1 or PD-L2, can inhibit a cytotoxic T-cell response.[8,9] Tumors can co-opt this pathway to escape T-cell–induced antitumor activity.[10-12] Pembrolizumab, a highly selective, humanized monoclonal IgG4 kappa isotype antibody against PD-1, can disrupt the engagement of PD-1 with its ligands and impede inhibitory signals in T cells, with resultant tumor recognition by cytotoxic T cells.

In clinical trials, anti–PD-1 and anti–PD-L1 antibodies produce durable responses in approximately 20% of unselected patients with advanced non–small-cell lung cancer.[13-16] Developing reliable, validated biomarkers that identify patients with an increased probability of response to these antibodies remains a challenge.[16,17] Because the PD-1 pathway may be a key mechanism of immune escape in a subgroup of patients with non–small-cell lung cancer, PD-L1 expression in tumor or inflammatory cells is a candidate biomarker. However, PD-L1 expression has not been formally validated as a biomarker in contemporaneously collected tumor tissue.

As part of the large, international, phase 1 KEYNOTE-001 trial, we evaluated the side effects, safety, and antitumor activity of pembrolizumab in patients with advanced non–small-cell lung cancer. We also sought to define and validate a tumor PD-L1 expression level associated with an enhanced likelihood of benefit from pembrolizumab.

METHODS

PATIENTS

Patients with non–small-cell lung cancer were assigned to multiple expansion cohorts (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). (Details are provided in the protocol, available at NEJM.org.) Eligible patients (age, ≥18 years) had locally advanced or metastatic non–small-cell lung cancer, an Eastern Cooperative Oncology Group performance status of 1 or less (a 5-point scale on which higher numbers reflect greater disability), and adequate organ function. Key exclusion criteria included a history of pneumonitis, systemic immunosuppressive therapy, or active autoimmune disease.

STUDY OVERSIGHT

The protocol and its amendments were approved by the relevant institutional review board or ethics committee at each study center. The study was conducted in accordance with Good Clinical Practice guidelines. All the patients provided written informed consent before any study-related procedures were performed.

Merck Sharp & Dohme, a subsidiary of Merck, sponsored the study, which was designed by representatives of the sponsor and academic advisors. Data were collected by the investigators and their site personnel. The authors and representatives of the sponsor performed the data analysis and interpretation. All the authors had full access to the data. The first author wrote the first draft of the manuscript. Medical writing and editorial assistance were provided by the APO Group and funded by Merck. All the authors participated in the review and editing process, approved the submitted draft of the manuscript, vouch for the accuracy and completeness of the data reported, and attest that the study was conducted in accordance with the protocol.

STUDY DESIGN AND TREATMENT

The primary objectives were to evaluate the safety, side-effect profile, and antitumor activity of pembrolizumab. Patients received intravenous pembrolizumab at a dose of 2 mg or 10 mg per kilogram of body weight every 3 weeks or 10 mg per kilogram every 2 weeks over a 30-minute period (Table S1 in the Supplementary Appendix). The studied doses were selected on the basis of pharmacologic models.[18,19]

STUDY ASSESSMENTS

Toxic effects were graded with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Scheduled

computed tomography or magnetic resonance imaging was performed every 9 weeks. Treatment continued until confirmed disease progression by investigator-assessed immune-related response criteria,[20] a decision by investigators, unacceptable toxicity, or withdrawal of consent. Although immune-related response criteria were evaluated, the primary radiographic assessment was Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1,[21] as assessed by independent central review.

BIOMARKER ANALYSIS FOR PATIENT ELIGIBILITY

A contemporaneous biopsy sample was required (Table S1 in the Supplementary Appendix). We used the anti–PD-L1 antibody clone 22C3 (Merck) and a prototype immunohistochemical assay to determine the PD-L1 status for eligibility (see the Methods section in the Supplementary Appendix). PD-L1 positivity was defined as membranous staining in at least 1% of cells (neoplastic and intercalated mononuclear inflammatory cells) within tumor nests or a distinctive staining pattern caused by the infiltration of mononuclear inflammatory cells in the stroma that formed a banding pattern adjacent to tumor nests.

BIOMARKER CUTOFF SELECTION

After we observed an initial relationship between PD-L1 expression (as assessed using the prototype assay) and the efficacy of pembrolizumab,[22] the protocol was amended to add a coprimary end point to evaluate the efficacy in patients with previously treated non–small-cell lung cancer that expressed a high level of PD-L1. A total of 51 patients had been enrolled at the time of the amendment.

We used receiver-operating-characteristic (ROC) curves to analyze data from the training group in order to define a potential biomarker cutoff (see the Methods section in the Supplementary Appendix). We assessed PD-L1 expression using a clinical-trial assay developed by Dako that used the same 22C3 antibody without knowledge of the results of the prototype assay. During cutoff selection only, we analyzed archival specimens when contemporaneous biopsy specimens were not acceptable for enrollment. Results were reported as the percentage of neoplastic cells showing membranous staining of PD-L1 (proportion score) (Fig. 1). The population that was analyzed for cutoff selection included all treated patients with measurable disease according to investigator-assessed immune-related response criteria at baseline and tissue that could be evaluated by the clinical-trial assay. Patients, investigators, and representatives of the sponsor were unaware of the results of the clinical-trial assay until all the patients had been followed for at least 19 weeks. At that time, the sponsor was made aware of the results, and data regarding the objective response rate were merged with the PD-L1 results. On the basis of limited follow-up duration, we used confirmed and unconfirmed responses, as determined with the use of the investigator-assessed immune-related response criteria, as the radiographic end point for cutoff selection. These results were confirmed in the patients who had centrally measurable disease according to RECIST.

BIOMARKER VALIDATION

An independent population of previously treated and previously untreated patients were included in the validation group. Before we assessed tumor PD-L1 expression using the clinical-trial assay in the validation group, we identified deterioration of the PD-L1 antigen in tumor-bank samples that had been sectioned more than 6 months before staining. Thus, only treated patients in the validation group with measurable disease at baseline whose slides were sectioned within 6 months before staining were evaluated for cutoff validation. Patients, investigators, and the sponsor were unaware of the proportion score until all patients had been followed for at least 5 months. At that time, the sponsor was made aware of the results, and response-rate data were merged with PD-L1 results, as assessed by the clinical-trial assay.

STATISTICAL ANALYSIS

In the validation group, we performed the primary analysis of the antitumor activity of pembrolizumab in previously treated patients whose tumors expressed PD-L1 at levels above the cutoff that was identified in the training group. On the basis of data from the training group, we assumed that half of the samples from patients who would be enrolled in the anticipated validation group would have PD-L1 expression above the cutoff and that previously treated patients whose samples were above the cutoff would have a response rate of at least 30%. On the conservative assumption of a 15% response rate with standard chemotherapy,[23] we determined that enrollment of 75 previously treated patients who were

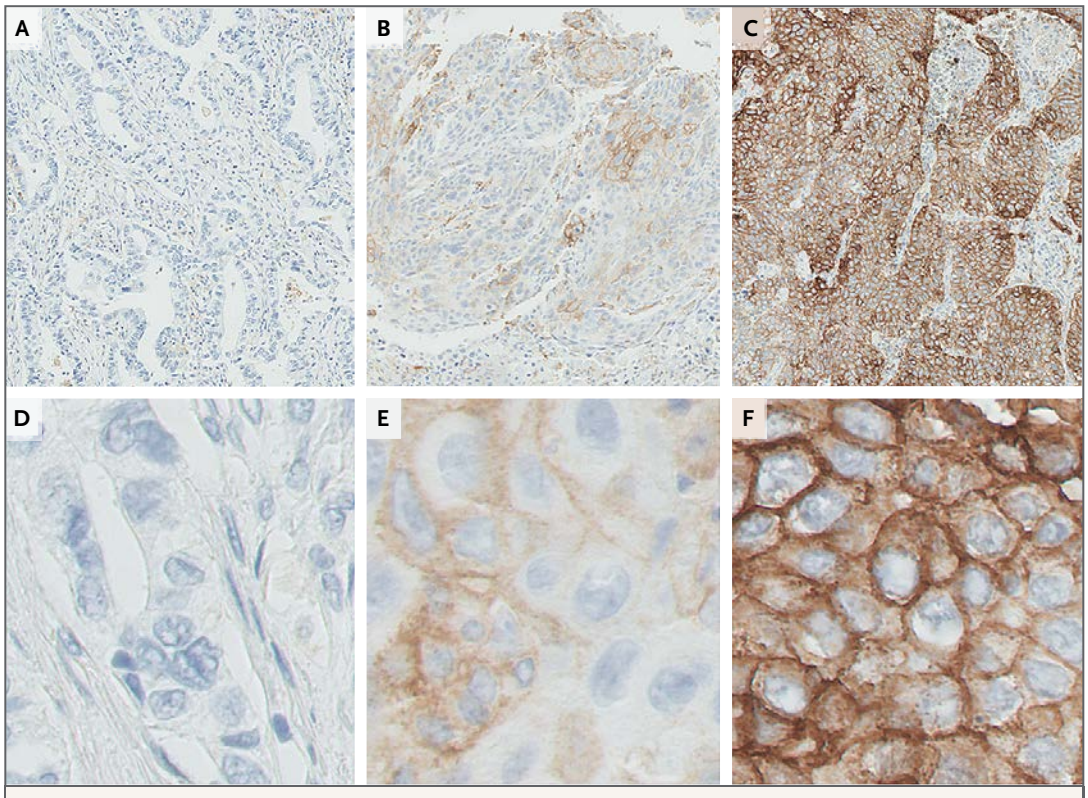


Figure 1. PD-L1 Expression in Non–Small-Cell Lung Cancers.

Results were reported as the percentage of neoplastic cells showing membranous staining of programmed cell death ligand 1 (PD-L1) (proportion score). Shown are tumor samples obtained from patients with a proportion score of less than 1% (Panel A), a score of 1 to 49% (Panel B), and a score of at least 50% (Panel C) (all at low magnification). Tumor samples with the corresponding proportion scores are shown at a higher magnification in Panels D through F. PD-L1 staining is shown by the presence of the brown chromogen. The blue color is the hematoxylin counterstain.

receiving pembrolizumab at a dose of 10 mg per kilogram every 3 weeks would provide a power of 85% to exclude a response rate of 15% or less in patients with samples that were above the cutoff (with a one-sided P value of 0.025). According to the protocol, we could combine data for previously treated patients who received pembrolizumab at a dose of 10 mg per kilogram every 3 weeks with data for previously treated patients who received 10 mg per kilogram every 2 weeks and previously untreated patients if response rates were similar. (Details are provided in the full statistical analysis plan in the protocol at NEJM.org.)

We used the clinical-trial assay to evaluate all screened samples that had sufficient tissue to estimate prevalence in unselected patients. Data are reported as the percentage of samples that could be assessed. To avoid ascertainment bias, we included in the analysis slides that were sectioned more than 6 months before staining for PD-L1.

Response rates with 95% confidence intervals were estimated by means of the binomial exact method. The response duration was defined as the time from first documented evidence of response until progression, according to RECIST. Progression-free and overall survival were defined as the time from the first dose of pembrolizumab to progression, according to RECIST, or death (for progression-free survival) or death alone (for overall survival). We used the Kaplan–Meier method to calculate median values for the duration of response, progression-free survival, and overall survival. The Cochran–Armitage trend test was performed to evaluate the equality of the response rate in groups with a proportion score of at least 50%, a score of 1 to 49%, and a score of less than 1%. The analysis cutoff was August 29, 2014.

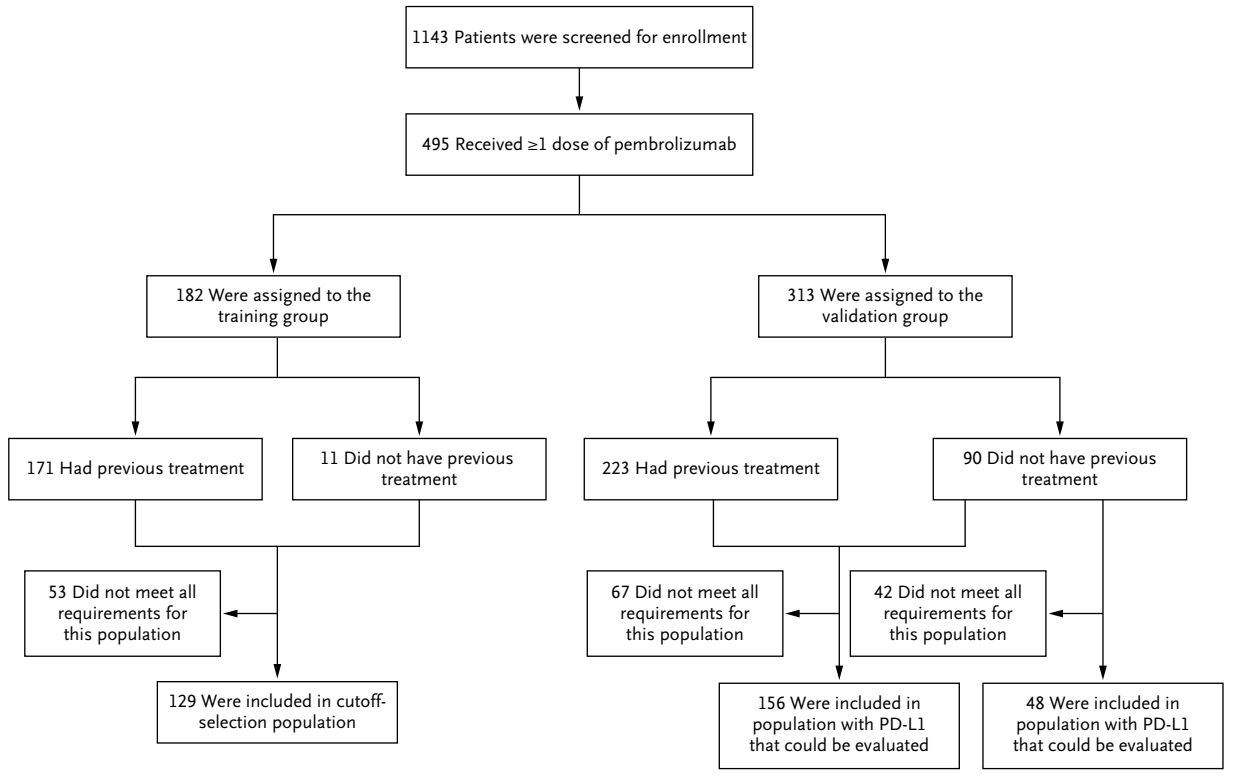


Figure 2. Enrollment and Assignment to Training Group and Validation Group.

PD-L1 status for eligibility was determined with the use of the anti–PD-L1 antibody clone 22C3 and a prototype immunohistochemical assay. Among the 1143 patients who underwent screening, regardless of the interval between slide sectioning and staining, 824 patients had tumor samples that could be evaluated by the clinical-trial assay, which used the same 22C3 antibody. For the training group, the PD-L1 cutoff was selected from a population that included patients with measurable disease according to immune-related response criteria by investigator review and whose tumors could be evaluated by the clinical-trial assay; 146 patients were included in this cutoff-selection population. When response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) by central review, 129 patients were included. One patient who did not meet all the requirements at the time of the cutoff selection did meet all eligibility requirements at the time of data lock on August 29, 2014. In the validation group, the biomarker-evaluable population included patients in whom disease could be evaluated by central review at baseline and who had slides that were sectioned within 6 months before staining and for whom a proportion score could be assigned. The numbers of patients with slides that were sectioned within 6 months before staining and had tumor tissue evaluated by the clinical-trial assay were 136 in the training group and 220 in the validation group.

RESULTS

PATIENTS

From May 2012 through February 2014, a total of 495 patients received at least 1 dose of pembrolizumab (Fig. 2). The clinical characteristics of the patients were typical of those with advanced non–small-cell lung cancer (Table S2 in the Supplementary Appendix). At the time of the data cutoff, the median duration of follow-up was 10.9 months (range, 5.2 to 27.5), and 115 patients (23.2%) continued to receive treatment.

ADVERSE EVENTS

Treatment-related adverse events occurred in 351 patients (70.9%), with no clear difference according to dose or schedule (Table S3 in the Supplementary Appendix). The most common treatment-related adverse events were fatigue, pruritus, and decreased appetite (Table 1). Adverse events of grade 3 or higher were reported in 47 of 495 patients (9.5%). The only treatment-related adverse events of an inflammatory or immune-mediated nature that occurred in more than 2% of patients were infusion-related reactions (in 15 patients

[3.0%]), hypothyroidism (in 34 patients [6.9%]), and pneumonitis (in 18 patients [3.6%]). One infusion reaction led to treatment discontinuation. All the patients with hypothyroidism were successfully treated with medical therapy. Pneumonitis of grade 3 or greater was observed in 9 patients (1.8%), including 1 (0.2%) who died. At the time of this analysis, 2 cases of pneumonitis (both grade 1 or 2) were ongoing.

OVERALL EFFICACY

The overall response rate was 19.4% (95% confidence interval [CI], 16.0 to 23.2), which included a response rate of 18.0% (95% CI, 14.4 to 22.2) in the 394 previously treated patients and 24.8% (95% CI, 16.7 to 34.3) in the 101 previously untreated patients. The best overall response was stable disease in 21.8% of patients (Table S4 in the Supplementary Appendix). The response rate was similar regardless of dose, schedule, and histologic analysis (Table S5 in the Supplementary Appendix). Current or former smokers had a response rate of 22.5%, as compared with 10.3% among patients who had never smoked cigarettes (Table S5 in the Supplementary Appendix).

At the time of this analysis, 84.4% of patients with a response had no disease progression, and the median duration of response was 12.5 months (range, 1.0 to 23.3) in all patients, 10.4 months (range, 1.0 to 10.4) in previously treated patients, and 23.3 months (range, 1.0 to 23.3) in previously untreated patients. Median progression-free survival was 3.7 months (95% CI, 2.9 to 4.1) for all the patients, 3.0 months (95% CI, 2.2 to 4.0) for previously treated patients, and 6.0 months (95% CI, 4.1 to 8.6) for previously untreated patients (Fig. S1 in the Supplementary Appendix). Median overall survival was 12.0 months (95% CI, 9.3 to 14.7) for all the patients, 9.3 months (95% CI, 8.4 to 12.4) for previously treated patients, and 16.2 months (95% CI, 16.2 to not reached) for previously untreated patients (Fig. S2 in the Supplementary Appendix).

BIOMARKER SELECTION

Overall, 182 patients were assigned to the training group to define a PD-L1 cutoff (Fig. 2). At the time of cutoff selection, 129 patients had measurable disease, according to RECIST, by central review and samples that could be evaluated by the clinical-trial assay; 25 samples were archival.

Table 1. Adverse Events in 495 Patients in the Treated Population.*

Adverse Event	Any Grade	Grade 3–5
	no. of patients (%)	
Fatigue	96 (19.4)	4 (0.8)
Pruritus	53 (10.7)	0
Decreased appetite	52 (10.5)	5 (1.0)
Rash	48 (9.7)	1 (0.2)
Arthralgia	45 (9.1)	2 (0.4)
Diarrhea	40 (8.1)	3 (0.6)
Nausea	37 (7.5)	4 (0.8)
Hypothyroidism	34 (6.9)	1 (0.2)
Asthenia	24 (4.8)	5 (1.0)
Anemia	21 (4.2)	0
Dyspnea	21 (4.2)	19 (3.8)
Pyrexia	21 (4.2)	3 (0.6)
Decreased weight	19 (3.8)	2 (0.4)
Dry skin	18 (3.6)	0
Pneumonitis†	18 (3.6)	9 (1.8)
Elevation in aspartate aminotransferase	15 (3.0)	3 (0.6)
Vomiting	14 (2.8)	3 (0.6)
Dermatitis acneiform	13 (2.6)	0
Myalgia	13 (2.6)	0
Cough	12 (2.4)	0
Elevation in alanine aminotransferase	11 (2.2)	2 (0.4)
Chills	10 (2.0)	0
Constipation	10 (2.0)	2 (0.4)
Infusion-related reaction	15 (3.0)	1 (0.2)

* Listed are events that were considered to be related to treatment by the investigator and were reported in at least 2% of patients.
† Included among patients with pneumonitis is one patient with grade 5 interstitial lung disease.

After evaluation of several methods for pathological assessment (see the Methods section in the Supplementary Appendix), membranous PD-L1 expression in at least 50% of tumor cells (proportion score, ≥50%) was selected as the cutoff on the basis of the ease of use and ROC analysis (Fig. S3 in the Supplementary Appendix). The response rate according to RECIST by central review at this cutoff was 36.6% (95% CI, 22.1 to 53.1) at the time that the data were merged.

BIOMARKER VALIDATION

The validation group included 313 patients: 223 previously treated patients and 90 previously un-

treated patients (Fig. 2). Of the patients in the validation group who were initially classified as PD-L1–positive by the prototype assay, 23 patients (21 of whom had disease that could be evaluated at baseline) were PD-L1–negative by the clinical-trial assay. PD-L1 status could not be assessed by the clinical-trial assay in 83 patients, including 61 whose samples were sectioned more than 6 months before staining.

The response rate was 45.2% (95% CI, 33.5 to 57.3) in the 73 patients with a proportion score of at least 50%, including 43.9% (95% CI, 30.7 to 57.6) in previously treated patients and 50.0% (95% CI, 24.7 to 75.3) in previously untreated patients, values that numerically exceeded the response rate in the training group (Table S6 in the Supplementary Appendix). The response rate for patients with a proportion score of at least 50% exceeded both the group with a score of 1 to 49% and the group with a score of less than 1% both for previously treated patients ($P<0.001$) and for previously untreated patients ($P=0.01$) (Table S7 in the Supplementary Appendix). The response rate for patients with a proportion score of at least 50% was 42.3% when those without disease that could be measured at baseline were included. Little difference in response rate was observed according to dose, schedule, or smoking status (Table S8 in the Supplementary Appendix). After the pooling of data from the training and validation groups post hoc, evaluation according to quartile suggested that a higher proportion score was associated with a greater response rate within the group with a proportion score of 1 to 49% and the group with a score of at least 50% (Fig. S4 in the Supplementary Appendix).

ESTIMATED PREVALENCE OF PD-L1

Among the 1143 screened patients, 824 had samples that could be evaluated by the clinical-trial assay, with a prevalence of 23.2% of patients with a proportion score of at least 50%, 37.6% with a score of 1 to 49%, and 39.2% with a score of less than 1% by the clinical-trial assay. The prevalence of a proportion score of at least 50% was 24.9% among previously untreated patients and 22.7% among previously treated patients (Table S9 in the Supplementary Appendix). Among treated patients, there was no clear difference in PD-L1 staining according to mutational status of the gene encoding epidermal growth factor receptor (*EGFR*), whereas a numerically higher percentage of patients with *KRAS* mutations had increased PD-L1 staining (Table S10 in the Supplementary Appendix). There were too few patients with *ALK* rearrangements to draw conclusions.

LONGITUDINAL OUTCOMES ON THE BASIS OF PD-L1 STAINING

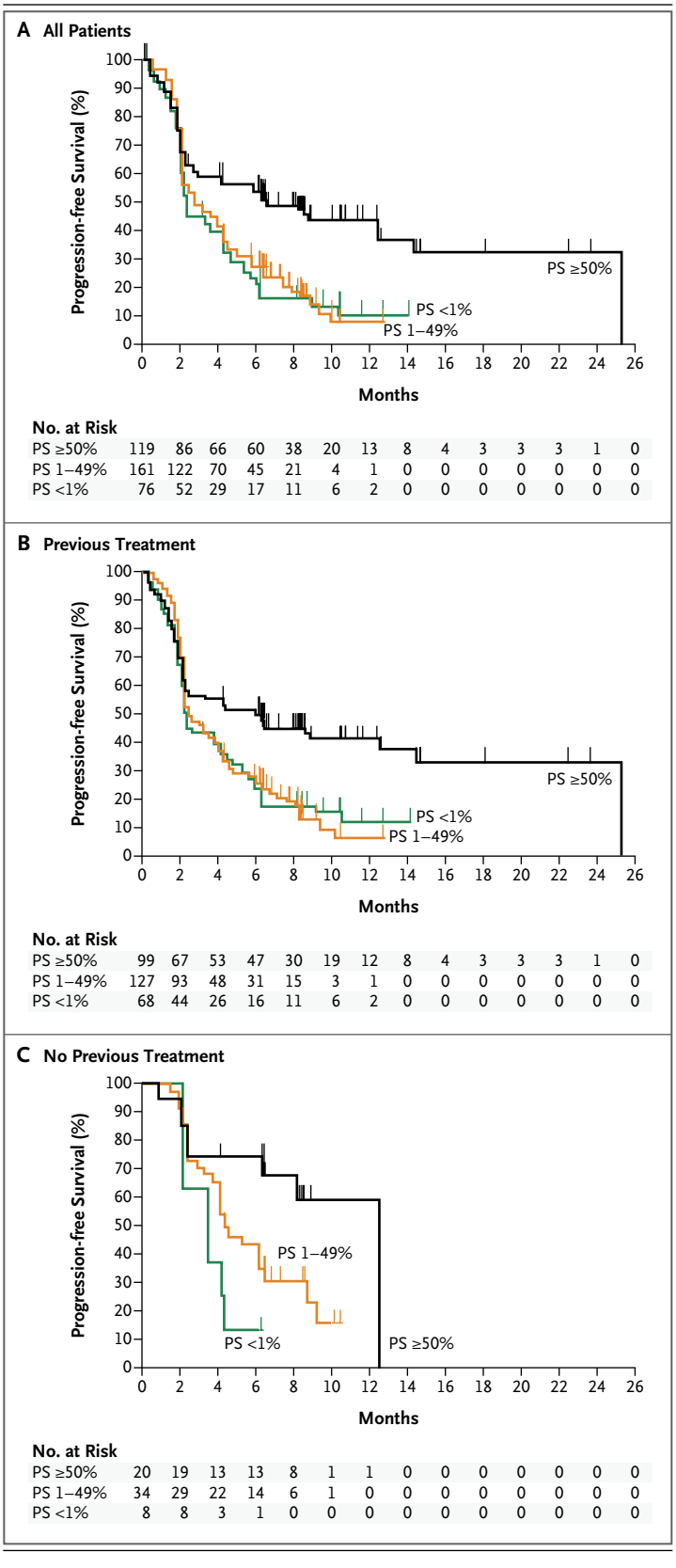
Median progression-free survival among patients with a proportion score of at least 50% was 6.3 months (95% CI, 2.9 to 12.5) for all patients, 6.1 months (95% CI, 2.1 to 12.5) for previously treated patients, and 12.5 months (95% CI, 2.4 to 12.5) for previously untreated patients (Fig. 3). Median overall survival among patients with a proportion score of at least 50% was not reached in the total population (95% CI, 13.7 months to not reached), in previously treated patients (95% CI, 9.3 to not reached), and in previously untreated patients (95% CI, not reached to not reached) (Fig. 4). Progression-free and overall survival were shorter among patients with a proportion score of 1 to 49% or a score of less than 1% than among those with a score of at least 50% (Fig. 3 and 4). The median duration of response was similar regardless of proportion score: 12.5 months (range, 2.1 to 23.3) for a proportion score of at least 50%, 7.2 months (range, 1.4 to 8.3) for a proportion score of 1 to 49%, and not reached (range, 1.0 to 10.8) for a proportion score of less than 1% (Fig. S5 in the Supplementary Appendix).

DISCUSSION

In this study, we evaluated a large series of patients with non–small-cell lung cancer (some of whom had received previous therapy and some who had not) who were treated with various doses and regimens of the anti–PD-1 inhibitor pembrolizumab. At a dose of 10 mg per kilogram every 2 or 3 weeks or 2 mg per kilogram every 3 weeks, pembrolizumab showed an acceptable side-effect profile and produced durable responses. We found no significant differences in efficacy or side-effect profile between patients receiving the dose of 10 mg per kilogram every 2 weeks and those receiving the same dose every 3 weeks, which echoed the findings in the melanoma cohorts of KEYNOTE-001.[24-26] The interpretation of response among patients receiving 2 mg per kilogram is limited by the lack of data for that dose. The 2-mg dose is being evaluated in a recently enrolled cohort of KEYNOTE-001, as well as in the phase

Figure 3. Progression-free Survival.

Shown are Kaplan–Meier estimates of progression-free survival according to the proportion score (PS) — the percentage of neoplastic cells with membranous PD-L1 staining — for 356 patients in the training and validation groups who had slides that were sectioned within 6 months before staining (Panel A), including 294 previously treated patients (Panel B) and 62 previously untreated patients (Panel C).



2–3 KEYNOTE-010 study (ClinicalTrials.gov number, NCT01905657). Among doses with similar efficacy, the lowest dose is usually recommended.

The analysis of PD-L1 expression permitted the identification of patients with an enhanced likelihood of a response to pembrolizumab. A proportion score of at least 50% was associated with a higher response rate and longer progression-free and overall survival than was a proportion score of less than 50% in both previously untreated patients and previously treated patients, which indicates that this is a subgroup of patients in whom the PD-L1 pathway can be successfully targeted. Furthermore, the magnitude of benefit that was observed in previously treated patients clearly exceeds that anticipated with standard therapy, with median overall survival not reached among patients with a proportion score of at least 50%, regardless of previous treatment, at the time of data cutoff. This finding suggests that a proportion score of at least 50% may represent a new biomarker for the treatment of non–small-cell lung cancer, although interpretation in the context of other immune checkpoint inhibitors and their respective biomarkers[15,16] may require cross-compound comparisons.

The study design, which does not include a non-pembrolizumab comparator, prevents the assessment of the prognostic implications of PD-L1 expression. Although the results of studies are inconsistent regarding the association between PD-L1 expression and prognosis among patients with non–small-cell lung cancer, retrospective analysis of specimens with the use of the 22C3 clone suggests that PD-L1 expression does not have a positive prognostic effect,[27,28] a finding that is consistent with results of a recent meta-analysis of outcome on the basis of PD-L1 expression.[29] Although the effect of pooling data from patients with different treatment histories who were receiving different doses of pembrolizumab is unclear, the modest differences in

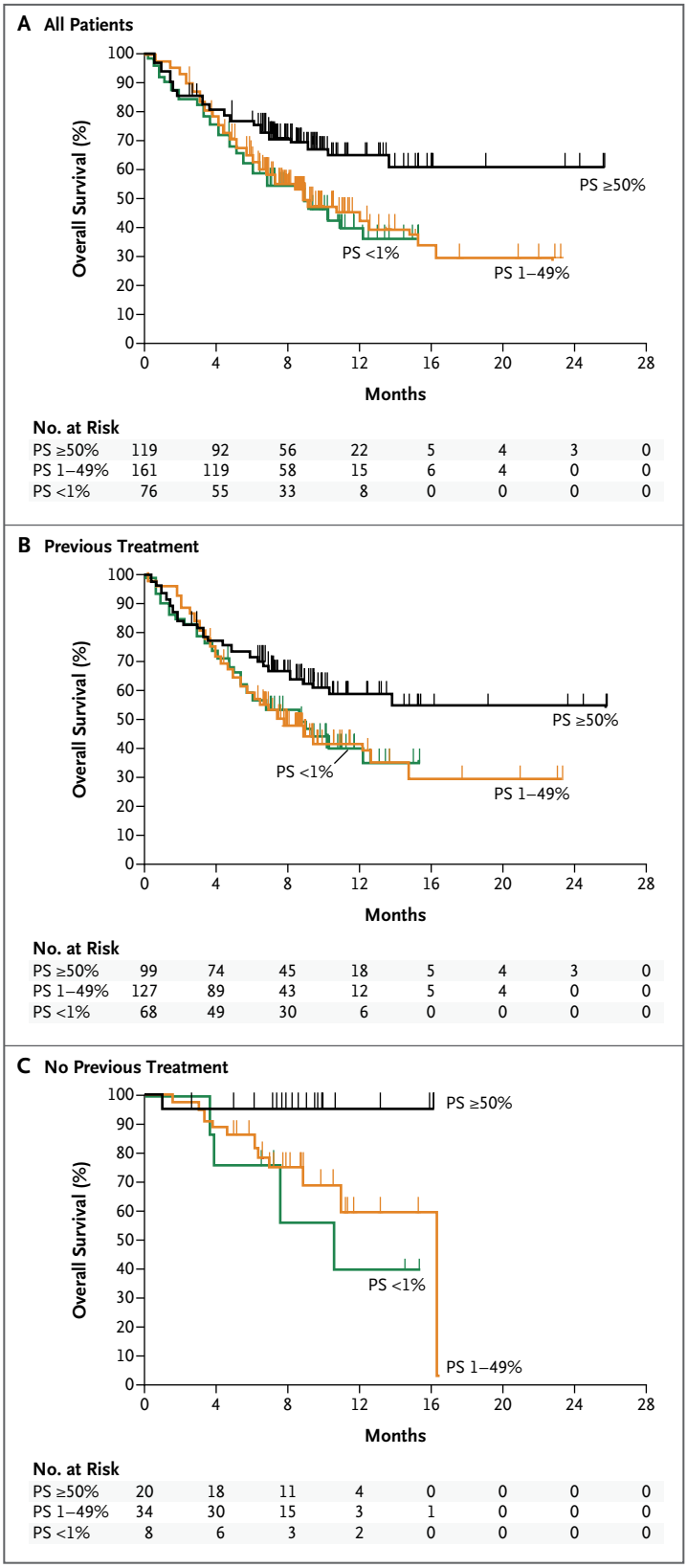


Figure 4. Overall Survival.

Shown are Kaplan–Meier estimates of overall survival according to the proportion score for 356 patients in the training and validation groups who had slides that were sectioned within 6 months before staining (Panel A), including 294 previously treated patients (Panel B) and 62 previously untreated patients (Panel C).

outcome on the basis of these factors support the analysis as conducted.

Current or former smoking status was associated with an increased response to treatment, an association that was also observed by other investigators and is hypothesized to be based on a higher mutational burden in these patients.[30,31] However, when assessed according to PD-L1 subgroup, the response rate was similar according to smoking status. This finding, along with the much higher response rate among patients with a proportion score of at least 50%, as compared with that of current or former smokers, suggests that although smoking history may provide insight into a patient profile associated with a greater benefit for pembrolizumab, PD-L1 expression is a better predictor of response.

Evaluation according to the quartile of proportion score suggests a positive correlation between the response rate and PD-L1 expression, although the analysis was limited by the small sample size in some groups and large confidence intervals for response. These data in combination with responses among patients with a proportion score of less than 1% suggest that tumor PD-L1 expression is not associated with the ideal test characteristics of approved genetically based biomarkers.[32-34] We did not seek to identify a PD-L1 cutoff that would capture all patients with a possible response but rather one that would identify patients with a greater likelihood of response. Although responses that were observed in patients for whom responses would not have been predicted by PD-L1 staining could result merely from tumor heterogeneity, it is more likely that tumor PD-L1 expression alone does not accurately assess the dynamic immune microenvironment. Additional diagnostic approaches, including assessment of the genomic landscape and the presence of preexisting CD8+ T cells and cytokines in tumor samples, could supplement PD-L1 expression as a means of identifying patients who might have a response to pembrolizumab.[17,35-37]

In the screened population, a proportion score of at least 50% was seen in approximately one quarter of patients with advanced or metastatic non–small-cell lung cancer. However, prevalence should be interpreted with caution, given that slides that were sectioned more than 6 months before staining were included in the estimates and PD-L1 expression could be affected by previous treatment or by disease stage. This potentially dynamic expression of the PD-L1 protein[38-40] led us to focus our analysis on contemporaneously collected tissue samples. Determination of whether archival samples can be substituted for those collected contemporaneously, the true prevalence of the proportion-score subgroups, and the degree of clinical benefit in patients with non–small-cell lung cancer with a proportion score of less than 50% are being assessed in ongoing randomized trials enrolling both previously treated and previously untreated patients.

Pembrolizumab showed modest toxicity. Treatment-related serious adverse events of grade 3 or greater severity were observed in less than 10% of patients, a proportion that is lower than that anticipated with chemotherapy. Pneumonitis is an immune-mediated adverse event of particular relevance to patients with non–small-cell lung cancer. Despite the presence of coexisting conditions that could precipitate or exacerbate this inflammatory process, the overall incidence of pneumonitis was less than 4%, with a severity of grade 3 or less in half the patients.

In conclusion, we have shown the efficacy and safety of pembrolizumab for previously treated and previously untreated patients with non–small-cell lung cancer. Prospective testing of PD-L1 expression is feasible and retrospectively identified patients with an enhanced likelihood of having a clinical benefit from treatment with pembrolizumab.

Supported by Merck Sharp & Dohme, a subsidiary of Merck.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and their families and caregivers for participating in the study; Tricia Brown, M.S., and Melanie Leiby, Ph.D. (of the APO Group, Yardley, PA) for their assistance in the preparation of the manuscript; and Roger Dansey, Eric Rubin, Alise Reicin, Marty Huber, Cong Chen, and Andrea Perrone (all of Merck) for their critical review of the manuscript or study support.

APPENDIX

The authors' affiliations are as follows: the David Geffen School of Medicine at the University of California, Los Angeles, Santa Monica (E.B.G., J.W.G.), the Angeles Clinic and Research Institute, Los Angeles (A.S.B., O.H.), the University of California, San Francisco, San Francisco (M.G.), and Dako, Carpinteria (C.R.) — all in California; Columbia University (N.A.R.) and Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College (M.D.H.) — all in New York; Westmead Hospital, University of Sydney, Westmead, NSW, Australia (R.H.); Princess Margaret Cancer Centre, Toronto (N.L.); Yale University, New Haven, CT (J.P.E.); South Texas Accelerated Research Therapeutics, San Antonio (A.P.); Abramson Cancer Center of the University of Pennsylvania, Philadelphia (C.A.); Vanderbilt Ingram Cancer Center, Nashville (L.H.); Catalan Institute of Oncology Badalona, Badalona (E.C.), and Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology, Barcelona (E.F.) — both in Spain; Samsung Medical Center (M.-J.A.) and Seoul National University (J.-S.L.) — both in Seoul, South Korea; Institut Gustave Roussy and Université Paris-Sud, Villejuif, France (J.-C.S.); Merck, Kenilworth, NJ (M.D.-F., R.Z.R., J.Z., J.K.L., R.R., G.M.L., K.E.); and the Dana–Farber Cancer Institute, Boston (L.G.).

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012, version 1.0. Cancer incidence and mortality worldwide: IARC Cancer Base No. 11. Lyon, France: International Agency for Research on Cancer, 2013 (http://globocan.iarc.fr).
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5-29.
3. Leighl NB. Treatment paradigms for patients with metastatic non-small-cell lung cancer: first-, second-, and third-line. Curr Oncol 2012;19:Suppl 1:S52-S58.
4. Gerber DE, Schiller JH. Maintenance chemotherapy for advanced non-small-cell lung cancer: new life for an old idea. J Clin Oncol 2013;31:1009-20.
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
6. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol 2002;2:116-26.
7. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677-704.
8. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:1027-34.
9. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8:793-800.
10. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565-70.
11. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol 2011;29:235-71.
12. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
13. Gettinger S, Herbst RS. B7-H1/PD-1 blockade therapy in non-small cell lung cancer: current status and future direction. Cancer J 2014;20:281-9.
14. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.
15. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.
16. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563-7.

17. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568-71.
18. Lindauer A, Valiathan C, Mehta K, et al. Translational pharmacokinetic/pharmacodynamic model of tumor growth inhibition by the new anti-PD1 monoclonal antibody MK-3475. Presented at the 23rd Meeting of the Population Approach Group in Europe, Alicante, Spain, June 10–13, 2014. abstract.
19. Ahamadi M, Prohn M, Rossenu S, et al. Population pharmacokinetics of MK-3475, a human anti-PD-1 monoclonal antibody in patients with progressive locally advanced or metastatic carcinoma, melanoma, and non-small cell lung cancer. Presented at the 23rd Meeting of the Population Approach Group in Europe, Alicante, Spain, June 10–13, 2014. abstract.
20. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20.
21. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
22. Gandhi L, Balmanoukian A, Hui R, et al. MK-3475 (anti-PD-1 monoclonal antibody) for non-small cell lung cancer: antitumor activity and association with tumor PD-L1 expression. Cancer Res 2014;74:CT105. abstract.
23. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589-97.
24. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti–PD-1) in melanoma. N Engl J Med 2013;369:134-44.
25. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014;384:1109-17.
26. Robert C, Joshua AM, Weber JS, et al. Pembrolizumab (pembro; MK-3475) for advanced melanoma: randomized comparison of two dosing schedules. Ann Oncol 2014;25:Suppl 5:LBA34. abstract.
27. Sun JM, Zhou W, Choi Y-L, et al. PD-L1 expression and survival in patients with non-small cell lung cancer (NSCLC) in Korea. J Clin Oncol 2014;32:Suppl 15:8066. abstract.
28. 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. abstract.
29. 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;41:450-6).
30. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
31. D'Incecco A, Andreozzi M, Ludovini V, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer 2015;112:95-102.
32. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
33. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced *ALK*-positive lung cancer. N Engl J Med 2013;368:2385-94.
34. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in *ALK*-positive lung cancer. N Engl J Med 2014;371:2167-77.
35. 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;13:84-8.
36. Bald T, Landsberg J, Lopez-Ramos D, et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. Cancer Discov 2014;4:674-87.
37. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189-99.
38. Liu J, Hamrouni A, Wolowiec D, et al. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-gamma and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. Blood 2007;110:296-304.
39. Kondo A, Yamashita T, Tamura H, et al. Interferon-gamma and tumor necrosis factor-alpha induce an immunoinhibitory molecule, B7-H1, via nuclear factor-kappaB activation in blasts in myelodysplastic syndromes. Blood 2010;116:1124-31.
40. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 2012;4:127ra37.