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ORIGINAL REPORT

Programmed Death-Ligand 1 Expression and Response to the Anti–Programmed Death 1 Antibody Pembrolizumab in Melanoma

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ABSTRACT

Purpose

Expression of programmed death-ligand 1 (PD-L1) is a potential predictive marker for response and outcome after treatment with anti–programmed death 1 (PD-1). This study explored the relationship between anti–PD-1 activity and PD-L1 expression in patients with advanced melanoma who were treated with pembrolizumab in the phase Ib KEYNOTE-001 study (clinical trial information: NCT01295827).

Patients and Methods

Six hundred fifty-five patients received pembrolizumab10 mg/kg once every 2 weeks or once every 3 weeks, or 2 mg/kg once every 3 weeks. Tumor response was assessed every 12 weeks per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 by independent central review. Primary outcome was objective response rate. Secondary outcomes included progression-free survival (PFS) and overall survival (OS). Membranous PD-L1 expression in tumor and tumor-associated immune cells was assessed by a clinical trial immunohistochemistry assay (22C3 antibody) and scored on a unique melanoma (MEL) scale of 0 to 5 by one of three pathologists who were blinded to clinical outcome; a score ≥ 2 (membranous staining in ≥ 1% of cells) was considered positive.

Results

Of 451 patients with evaluable PD-L1 expression, 344 (76%) had PD-L1–positive tumors. Demographic and staging variables were equally distributed among PD-L1–positive and –negative patients. An association between higher MEL score and higher response rate and longer PFS (hazard ratio, 0.76; 95% CI, 0.71 to 0.82) and OS (hazard ratio, 0.76; 95% CI, 0.69 to 0.83) was observed ($P < .001$ for each). Objective response rate was 8%, 12%, 22%, 43%, 57%, and 53% for MEL 0, 1, 2, 3, 4, and 5, respectively.

Conclusion

PD-L1 expression in pretreatment tumor biopsy samples was correlated with response rate, PFS, and OS; however, patients with PD-L1–negative tumors may also achieve durable responses.

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INTRODUCTION

Cancer cells can exploit many pathways to evade the immune system.[1] One such mechanism involves the exploitation of endogenous inhibitory checkpoints that serve to terminate the immune response after antigen activation.[2-4] Antibodies against cytotoxic T-lymphocyte–associated protein 4, such as ipilimumab, release one such negative regulatory pathway,[5] which results in a survival benefit in patients with metastatic melanoma.[6,7] Programmed death 1 (PD-1) is another key immune inhibitory checkpoint. The PD-1 pathway is a powerful regulator of peripheral tolerance. Initially described in 1992,[8] PD-1 is preferentially expressed by activated T cells, B cells, and myeloid cells in the setting of chronic antigen exposure.[9] Two ligands for PD-1 have been identified to date: programmed death-ligand 1 (PD-L1) and programmed death-ligand 2. PD-L1 was first described in 2000[10] and is widely expressed by myeloid and lymphoid tissues as well as in nonlymphoid tissues, such as the

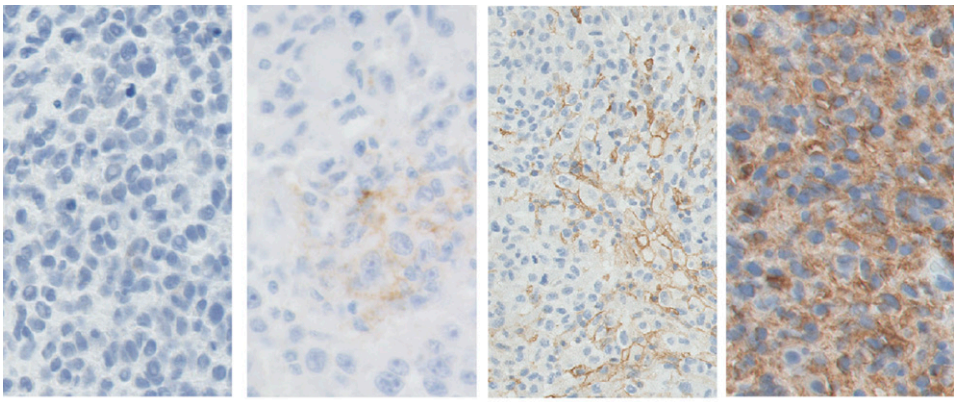

Fig 1. Photomicrographs showing immunohistochemical staining of programmed death-ligand 1 (PD-L1; 22C3 antibody) in melanoma (MEL) samples. MEL score ≥ 2 is considered PD-L1 positive. PD-L1 staining is evidenced by the presence of the brown chromogen, whereas blue is hematoxylin counterstain.

lung, heart, pancreas, and placenta.[11] In the tumor microenvironment, PD-L1 is known to be induced by types I and II interferon. Both tumor cells and tumor-infiltrating cells of multiple cancers have been shown to express PD-L1.[12-15]

Recent clinical trials that assessed monoclonal antibodies against PD-1 and PD-L1 have shown that these therapies provide antitumor activity against diverse tumor histologies. Pembrolizumab, a humanized immunoglobulin G4 monoclonal antibody against PD-1, has been assessed in multiple clinical trials and has demonstrated antitumor activity and a manageable safety profile in patients with solid tumors and hematologic malignancies.[16-27] Although these data highlight the effectiveness of PD-1 pathway inhibition, it is clear that most patients, even those with prototypically immunogenic tumors, such as melanoma, will not achieve objective responses. Currently, the best marker of response to anti–PD-1 and anti–PD-L1 antibodies is the presence of PD-L1 in tumor cells or tumor-associated stromal cells.[14,15,28] Despite the availability of preliminary evidence that supports the use of PD-L1 as a biomarker, published correlative data remain scarce. Here, we report on the relationship between anti–PD-1 activity and PD-L1 expression in patients with advanced melanoma who were treated with pembrolizumab in the KEYNOTE-001 clinical trial.

PATIENTS AND METHODS

Study Design and Conduct

KEYNOTE-001, an international, multicohort, open-label, phase I study that assessed the efficacy and safety of pembrolizumab in patients with advanced melanoma, was sponsored by Merck & Co (Kenilworth, NJ). The study was conducted in accordance with the protocol, Good Clinical Practice standards, and the Declaration of Helsinki. The protocol and its amendments were approved by the relevant institutional review boards or ethics committees of the participating institutions. All patients provided written informed consent to participate. The study was registered with ClinicalTrials.gov.

As described previously,[24] patients with ipilimumab-naive, ipilimumab-treated, or ipilimumab-refractory melanoma were enrolled in non–randomly assigned and randomly assigned cohorts and were treated with pembrolizumab 2 mg/kg once every 3 weeks, 10 mg/kg once every 3 weeks, or 10 mg/kg once every 2 weeks. Regardless of dose or schedule, pembrolizumab was administered intravenously over a 30-minute period on day one of each cycle and continued until disease progression, intolerable toxicity, withdrawal of consent, or investigator decision.

Patient Eligibility

Detailed eligibility criteria have been published previously.[16,17] In brief, enrolled patients were ≥ 18 years of age with advanced unresectable melanoma, had measurable disease per investigator assessment, an Eastern Cooperative Oncology Group performance status of 0 or 1, and adequate organ function. Previous systemic therapy was limited to two or fewer regimens for patients with ipilimumab-naive disease and was unlimited for patients who were previously treated with ipilimumab. A new tumor biopsy sample collected within 60 days of the first pembrolizumab dose was required. Key exclusion criteria included active autoimmune disease, systemic corticosteroid therapy (except for managing ipilimumab-related adverse events in patients with ipilimumab-refractory disease), and previous treatment with a PD-1 or PD-L1 inhibitor. There was no protocol requirement for baseline magnetic resonance imaging of the brain, but patients with previously treated brain metastases could enroll if they were clinically stable for ≥ 8 weeks. A later protocol amendment permitted patients with brain metastases who were stable for ≥ 4 weeks to enroll.

Assessments

Tumor response was assessed every 12 weeks per RECIST v1.1[29] by independent central review and per immune-related response criteria[30] by investigator review. Pembrolizumab treatment decisions were made on the basis of immune-related response criteria. The primary end point was the

Table 1. Melanoma (MEL) Scoring System for PD-L1 Expression

Definition	MEL Score
No membrane staining	0
Membrane staining in tumor and tumor-associated immune cells, range	
> 0% - < 1%	1
≥ 1% - < 10%	2
≥ 10% - < 33%	3
≥ 33% - < 66%	4
≥ 66%	5

NOTE. PD-L1 expression was assessed by immunohistochemistry using the 22C3 antibody. MEL score ≥ 2 is considered PD-L1 positive.
Abbreviation: PD-L1, programmed death-ligand 1.

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objective response rate (ORR) assessed per RECIST v1.1 by central review. Secondary end points included response duration (time from first documentation of response to first documentation of disease progression), progression-free survival (PFS; time from start of treatment to documentation of disease progression or death as a result of any cause), and overall survival (OS; time from start of treatment to death as a result of any cause).

PD-L1 expression was assessed in pretreatment tumor biopsy samples by using an investigational version of an immunohistochemistry assay, which is now commercially available (PD-L1 IHC 22C3 pharmDx; Dako North America, Carpinteria, CA) and approved by the US Food and Drug Administration for use in non–small-cell lung cancer. All samples were obtained within 60 days of the first pembrolizumab dose and were analyzed either internally at Dako North America (n = 195; 30%) or at Labcorp Clinical Trials (Los Angeles, CA; n = 460; 70%). Each sample was scored by one of three pathologists who were blinded to clinical outcome. Examples of stained samples are shown in Fig 1.

Melanoma samples were scored on the MEL 0 to 5 scale, which was derived from a scheme commonly used for hormone receptors in breast cancer.[31] The MEL score was described using the hormone receptor terminology of the Allred proportion score in the European Union pembrolizumab label. However, we abandoned that terminology herein to avoid confusion, because, other than the 0 to 5 scale, PD-L1 scoring for melanoma differs considerably from scoring hormone receptors. Of note, PD-L1 scoring is distinct from that used for non–small-cell lung cancer. Table 1 lists the correspondence between the MEL 0 to 5 scores and the percentage of stained cells. Cells scored include tumor cells themselves as well as mononuclear inflammatory cells, which are intercalated within or

Table 2. Patient Baseline Characteristics

Characteristic	PD-L1 Positive (n = 344)	PD-L1 Negative (n = 107)	Total (N = 451)	*P**
Age, years				.7
Median	62.0	62.0	62.0	
Range	18-94	23-86	18-94	
Sex, No. (%)				.2
Male	215 (62)	59 (55)	274 (61)	
Female	129 (38)	48 (45)	177 (39)	
Race, No. (%)				.9
White	337 (98)	104 (97)	441 (98)	
Asian	3 (1)	2 (2)	5 (1)	
Multiracial	1 (0.3)	0 (0)	1 (0.2)	
ECOG PS, No. (%)				.9
0	228 (66)	71 (66)	299 (66)	
1	116 (34)	35 (33)	151 (33)	
Missing	0 (0)	1 (1)	1 (0.2)	
BRAF mutation status, No. (%)				.7
Mutant	68 (20)	33 (31)	101 (22)	
Wild-type	273 (79)	73 (68)	346 (77)	
Unknown	3 (1)	1 (1)	4 (1)	
M staging of extent of metastasis,† No. (%)				.6
M0	4 (1)	2 (2)	6 (1)	
M1a	27 (8)	12 (11)	39 (9)	
M1b	51 (15)	13 (12)	64 (14)	
M1c	262 (76)	80 (75)	342 (76)	
Brain metastasis, No. (%)				.7
Yes	25 (7)	6 (6)	31 (7)	
No	318 (92)	101 (94)	419 (93)	
Unknown	1 (0.3)	0 (0)	1 (0.2)	
Previous ipilimumab, No. (%)				.006
Yes	200 (58)	46 (43)	246 (54)	
No	144 (42)	61 (57)	205 (46)	
Previous systemic therapies, No. (%)				.3
0	73 (21)	29 (27)	102 (23)	
1	108 (31)	33 (31)	141 (31)	
2	91 (27)	31 (29)	122 (27)	
≥ 3	72 (21)	14 (13)	86 (19)	
LDH level, No. (%)				.6
Normal	205 (60)	61 (57)	266 (59)	
Elevated	132 (38)	44 (41)	176 (39)	
Unknown	7 (2)	2 (2)	9 (2)	
Baseline tumor size,‡ mm				.2
Mean	134	152	138	
Range	10-535	11-699	10-699	

NOTE. Melanoma score ≥ 2 is considered PD-L1 positive.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PD-L1, programmed death-ligand 1; PS, performance status.
*Comparison between PD-L1 positive and PD-L1 negative within each variable.
†M0, no distant metastasis; M1a, metastasis to skin, subcutaneous tissues, or distant lymph nodes; M1b, metastasis to lung; M1c, metastasis to all other visceral sites or distant metastases at any site associated with elevated levels of serum LDH.
‡Baseline tumor size was calculated as the sum of the longest diameters of all target lesions for patients with measurable disease per RECIST, version 1.1, by independent central review at baseline.

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are contiguous with tumor nests. Inflammatory cells within the stroma, distinct from the tumor nests, are excluded from scoring. MEL scores of 0 or 1, corresponding to $<$ 1% staining, are considered to be negative, whereas scores of 2 to 5, corresponding to $\geq$ 1% staining, are considered positive. The positive and negative interpretation is based on receiver operating characteristic analysis, which favored the true-positive rate—and, thus, negative predictive value—at the expense of the false-positive rate.

Statistical Analyses

ORR was assessed in all patients with evaluable PD-L1 expression who received one or more doses of study treatment and had measurable disease per RECIST v1.1 by central review. ORR and its associated 95% CI was estimated by using the exact binomial method. The Kaplan-Meier method was used to estimate time-to-event outcomes, including PFS, duration of response, and OS, in all patients with evaluable PD-L1 expression who received one or more doses of study treatment. The relationship between tumor PD-L1 positivity and ORR was explored by using the Miettinen and Nurminen method.[32] The relationship between ORR and PD-L1 expression via the MEL score was explored by using logistic regression analysis. The relationships between tumor PD-L1 expression and PFS and OS were explored by Cox proportional hazards regression analysis. All analyses were performed using a data cutoff date of October 18, 2014.

RESULTS

Study Design and Patient Characteristics

Between December 2011 and September 2013, 655 patients with advanced melanoma were enrolled in KEYNOTE-001 (Appendix Fig A1, online only). Among these 655 patients, biopsy samples from 451 patients that were collected in the 60 days before the first dose of pembrolizumab was administered were evaluable for PD-L1 expression. Of 451 evaluable patients, 344 (76%) had PD-L1–positive tumors and 107 (24%) had PD-L1–negative

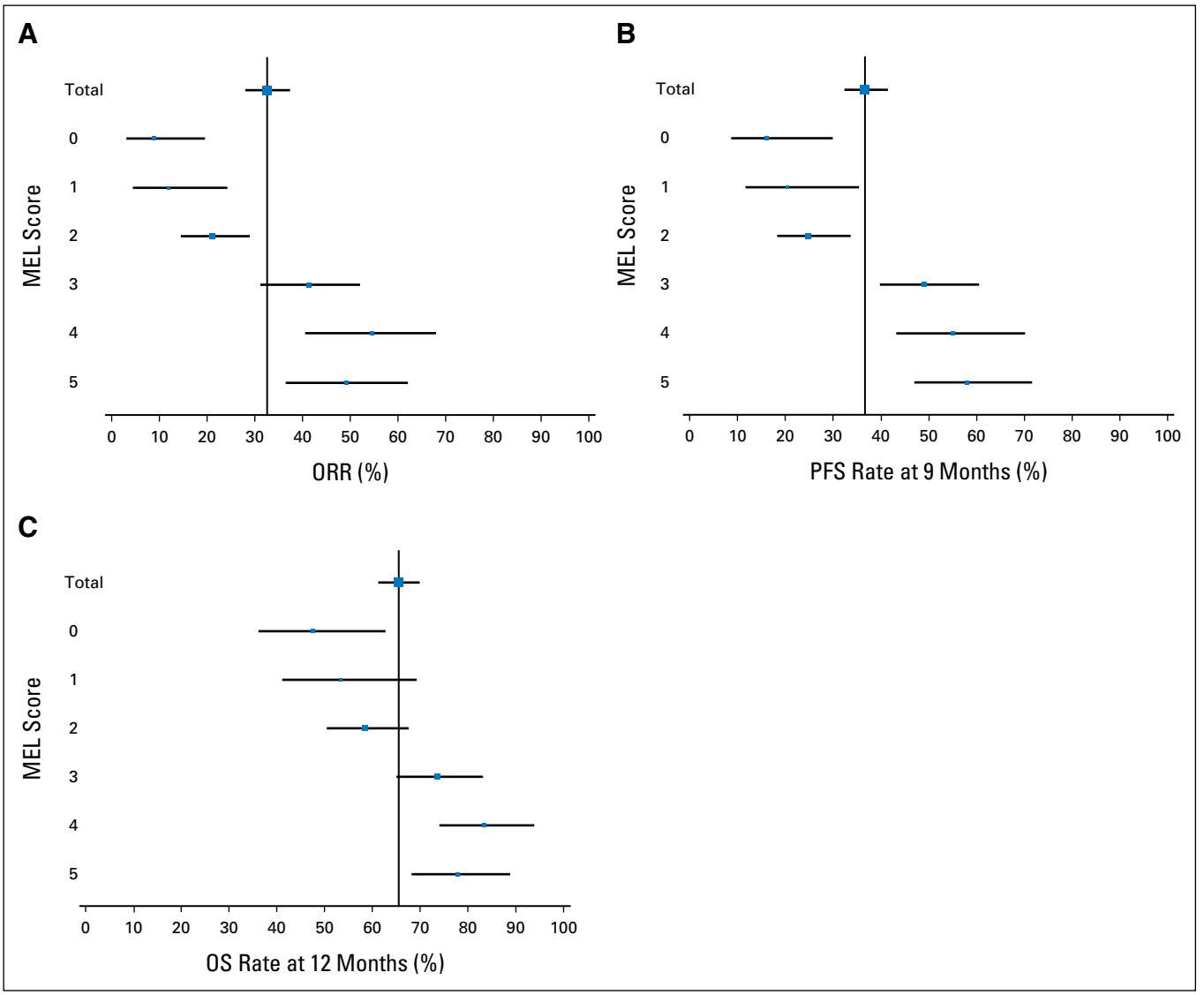

Fig 2. Efficacy according to melanoma (MEL) score for programmed death-ligand 1 (PD-L1) expression. (A) Objective response rate (ORR) and associated 95% CIs. (B) Kapan-Meier estimates of progression-free survival (PFS) and associated 95% CIs at 9 months. (C) Kaplan-Meier estimates of overall survival (OS) and associated 95% CIs at 12 months. Response was assessed per RECIST, version 1.1, by independent central review. MEL score $\geq$ 2 is considered PD-L1 positive.

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tumors. Patient baseline demographics and disease characteristics were mostly similar between patients with PD-L1–positive and those with PD-L1–negative tumors, with the exception of higher percentages of *BRAF* wild-type tumors and ipilimumab treatment in patients with PD-L1–positive tumors (Table 2). A breakdown of key baseline characteristics by MEL score is presented in Appendix Table A1 (online only).

Relationship Between PD-L1 Expression and Efficacy

The confirmed ORR per RECIST v1.1 by independent central review was 33% (95% CI, 28% to 37%) in 405 patients who were evaluable for both PD-L1 expression and tumor response. An association between a higher MEL score for PD-L1 expression and ORR was observed ($P < .001$; Fig 2; panel A). The highest and lowest ORRs were observed in patients with tumors with a MEL score of 4 (ORR, 57%) and 0 (ORR, 8%). The pattern of change in tumor size compared with baseline seemed to vary by MEL score (Fig 3). Among evaluable patients at week 12, 35%, 38%, 57%, 78%, 84%, and 86% showed reductions from baseline tumor measurements for MEL scores 0 to 5, respectively. Of note, patients with MEL scores of 4 or 5, 33% and 22%, respectively, showed a reduction of ≥ 50% at week 12. Qualitatively, patients with MEL scores of 0 or 1 were less likely to have a decrease from baseline tumor size overall, whereas more patients with MEL scores of 2 to 5 had a 100% decrease from baseline.

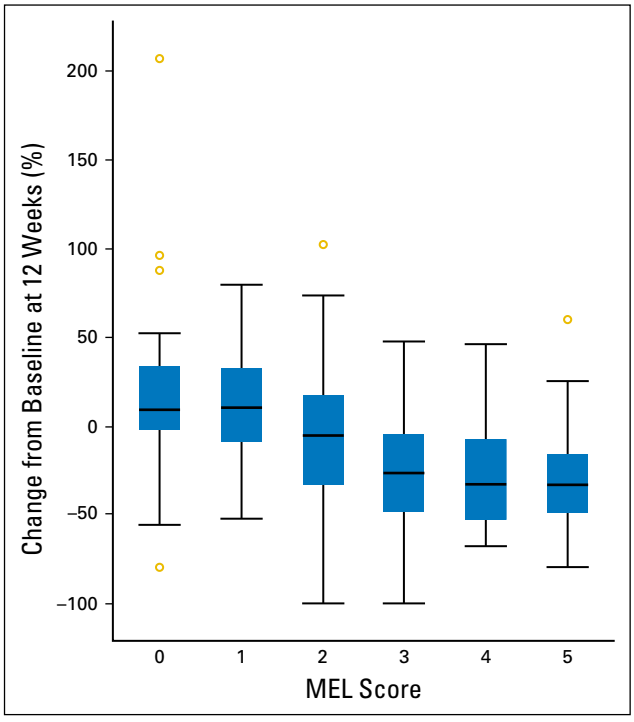

Fig 3. Box plots of observed percentage change from baseline in tumor size at week 12 by melanoma (MEL) score. The analysis population was patients with evaluable programmed death-ligand 1 (PD-L1) expression who had tumor size data at week 12 (n = 292). MEL score, 0 (0% staining); MEL score, 1 (> 0% to < 1% staining); MEL score, 2 (≥ 1% to < 10% staining); MEL score, 3 (≥ 10% to < 33% staining); MEL score, 4 (≥ 33% to < 66% staining); and MEL score, 5 (≥ 66% to 100% staining). MEL score ≥ 2 is considered PD-L1 positive. Outer boundaries of boxes represent the 1st and 3rd quartiles, and lines in between represent the median.

In 451 patients who were evaluable for PD-L1 expression, associations between a higher MEL score and the 9-month PFS and 12-month OS rates were observed (Fig 2; panels B and C). Similar to ORR, patients with tumors with a MEL score of 4 had the best outcomes in terms of survival, whereas a similar proportion of patients with tumors with MEL scores of 4 and 5 were alive and free of progression at 9 months. Associations between higher PD-L1 MEL score and PFS (hazard ratio [HR], 0.76; 95% CI, 0.71 to 0.82; $P < .001$) and OS (HR, 0.76; 95% CI, 0.69 to 0.83; $P < .001$) were observed. Similarly, significant associations were also observed when PD-L1 expression was categorized as positive (ie, MEL score 2 to 5) or negative (ie, MEL score 0 or 1; Fig 4), with a HR of 0.51 (95% CI, 0.40 to 0.65) and 0.50 (95% CI, 0.37 to 0.67) for PFS and OS, respectively.

DISCUSSION

PD-1 and PD-L1 inhibitors are rapidly emerging as a central therapeutic modality for patients with advanced melanoma. Results of recent clinical trials have shown the superiority of pembrolizumab over chemotherapy for patients with ipilimumab-refractory melanoma and, if $BRAF^{V600}$ mutant, a BRAF inhibitor;[25] of pembrolizumab over ipilimumab for patients with ipilimumab-naive melanoma;[26] of nivolumab over chemotherapy as first-line therapy;[33] and of nivolumab alone or combined with ipilimumab over ipilimumab as first-line therapy.[34] Anti–PD-1 and anti–PD-L1 antibodies are also widely active across diverse nonmelanoma cancers.[16-27] Given the targeted nature of these antibodies, it seems logical that PD-L1 expression should be correlated with outcome; however, the data that have emerged so far are complex and ambiguous. Whereas PD-L1 expression seems to correlate with response, the thresholds required for response vary widely by both tumor histology and immunohistochemistry method used.[14,15] PD-L1 assays that incorporate different cut points to define PD-L1 positivity also pose a challenge for interpretation.[35] The variability of tumor PD-L1 status according to assay and cut point used is illustrated by the PD-L1 positivity rates observed in similar advanced melanoma populations in the current study (76.3%) and in a recent randomized trial of ipilimumab plus nivolumab versus each agent alone in patients with treatment-naive melanoma (23.6%).[34] Along with differences in assays and antibodies, use of newly collected versus archival tumor samples may impact the rate of PD-L1 positivity.[31] Furthermore, PD-L1 expression is dynamic and is affected by many factors, including prior therapy and presence of tumor-infiltrating immune cells,[34] which may limit its ability to discriminate between patients who will and will not respond to therapy. Physicians should be made aware of the various antibodies and assays in use, including their differences and their limitations. Of note, the clinical use of the assay described here may be less uniform in practice than readings done by more experienced pathologists. Nonetheless, tumor PD-L1 expression remains of predictive value.[36,37] One of the critical unanswered questions is whether PD-L1 expression is correlated with survival, which remains the gold standard for therapeutic intervention. Other correlative markers, such as $CD8^+$ T-cell infiltration of the tumor or of the tumor margin, have also been studied.[36] Whereas these are promising biomarker candidates,

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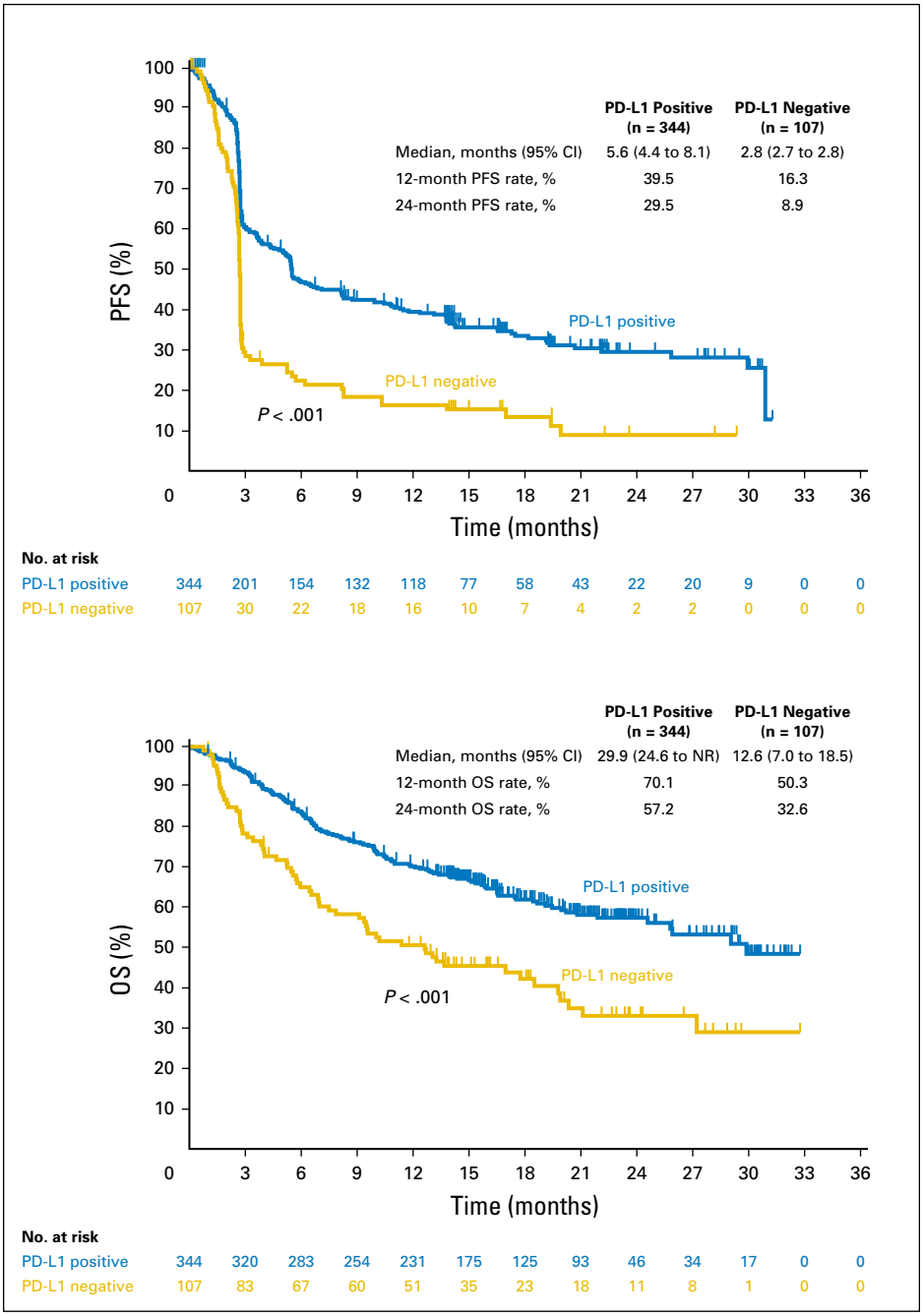

Fig 4. Efficacy by PD-L1 positivity. (A) Kaplan-Meier estimate of progression-free survival (PFS) assessed per RECIST, version 1.1, by independent central review. (B) Kaplan-Meier estimate of overall survival (OS). Melanoma score ≥ 2 is considered PD-L1 positive. NR, not reached; PD-L1, programmed death-ligand 1.

there are currently few prospective data that assess their potential use.

To our knowledge, the data described here represent one of the most comprehensive analyses of the relationship between PD-L1 expression and treatment outcome presented to date for any tumor. KEYNOTE-001 was a large phase I trial, with 655 patients enrolled in the melanoma arms. Of these 655 patients, 451 patients had tumor samples that were evaluable for PD-L1 expression by using the 22C3 antibody. Clinical outcomes in KEYNOTE-001 were measured rigorously, with radiologic response to therapy evaluated per RECIST v1.1 by independent central review. Using this robust dataset, a relationship was observed between PD-L1 expression assessed by using a unique MEL scoring system and ORR. The highest ORR was observed among patients with an MEL score of 4. Of most importance, survival was also correlated with PD-L1 expression. Reflective of the significance of PD-L1 expression, Kaplan-Meier curves for PFS and OS diverged when PD-L1 expression was categorized as positive (ie, MEL score 2 to 5) and negative (ie, MEL score 0 or 1).

Given the data described here, PD-L1 expression is correlated with clinical outcome in patients with advanced melanoma. Whereas there is some uncertainty regarding the optimal level of PD-L1 expression, a level that corresponds to MEL scores of 3 to 5 seems to best capture the population that is most highly responsive

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to PD-1 blockade. Conversely, MEL scores of 0 or 1 represents the least responsive population, and a MEL score of 2, which represents the largest proportion of patients (29%), seems to be intermediate, with many patients experiencing a response to therapy. Furthermore, tumors with the highest levels of PD-L1 staining exhibited deeper responses (33% and 22% of patients showed reductions of $\geq$ 50% at week 12 with MEL scores of 4 and 5, respectively). Although there was no clear evidence of differences between MEL score and age or sex, there did seem to be some evidence of increased PD-L1 positivity with prior ipilimumab treatment; however, it is not clear that this relationship is reproducible in other studies, as PD-L1 expression—using the same assay and scoring guidelines—was observed in 81% of ipilimumab-naive patients in KEYNOTE-006.[26]

Randomized controlled studies are needed to better understand the clinical benefits of pembrolizumab in patients with PD-L1–positive and PD-L1–negative melanoma, particularly given that a proportion of patients who have PD-L1–negative tumors may experience a durable response with pembrolizumab. Of note, both intertumoral and intratumoral heterogeneity of PD-L1 expression have been observed,[37,38] as have changes in PD-L1 expression over time.[13,39] Therefore, analysis of a single tissue sample, as in this study, may result in the classification of a tumor with some PD-L1–positive areas as PD-L1 negative. Further limitations of this analysis are that the study included non–randomly assigned cohorts, and a sizeable fraction (31%) of the overall study population was not evaluable for PD-L1 expression.

Important questions remain regarding the relationship between PD-L1 expression and the therapeutic effects of anti–PD-1 and anti–PD-L1 antibodies. It is clear that even with a MEL score of 0 in the assay used in this study, durable responses were still observed; response rates were even higher in the other MEL score groups. The high prevalence of PD-L1 positivity observed in this study, along with the durable responses observed in PD-L1–negative tumors, suggest that pembrolizumab treatment should not be limited to patients with PD-L1–positive tumors. Ongoing clinical trials with correlative studies will further delineate the role of PD-L1 expression in melanoma.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Final approval of manuscript: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Programmed Death-Ligand 1 Expression and Response to the Anti–Programmed Death 1 Antibody Pembrolizumab in Melanoma

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Appendix

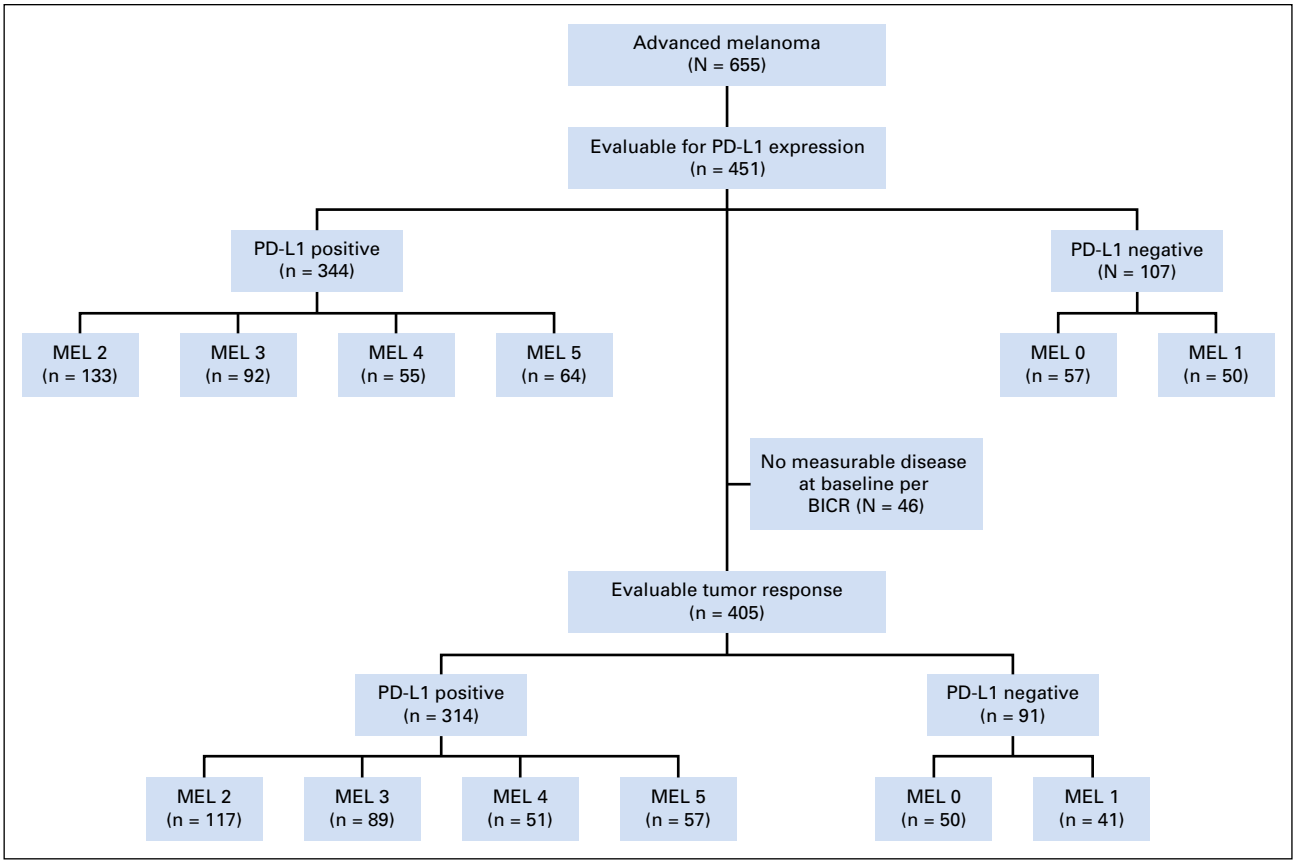

Fig A1. CONSORT diagram. BICR, blinded independent central review; MEL, melanoma; PD-L1, programmed death-ligand 1.

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Table A1. Key Patient Baseline Characteristics by MEL Score for PD-L1 Expression

Characteristic	MEL Score						*P**
	0	1	2	3	4	5	
Age, years							.40
< 65	37 (14)	28 (10)	86 (32)	55 (21)	29 (11)	33 (12)	
≥ 65	20 (11)	22 (12)	47 (26)	37 (20)	26 (14)	31 (17)	
Sex							.10
Male	25 (9)	34 (12)	80 (29)	58 (21)	37 (14)	40 (15)	
Female	32 (18)	16 (9)	53 (30)	34 (19)	18 (10)	24 (14)	
Prior ipilimumab							.03
Yes	24 (10)	22 (9)	82 (33)	47 (19)	37 (15)	34 (14)	
No	33 (16)	28 (14)	51 (25)	45 (22)	18 (9)	30 (15)	
M stage†							.41
M0	1 (20)	1 (20)	1 (20)	2 (40)	0 (0)	1 (20)	
M1a	4 (10)	8 (21)	8 (21)	5 (13)	6 (15)	8 (21)	
M1b	9 (14)	4 (6)	21 (33)	8 (12)	10 (16)	12 (19)	
M1c	43 (13)	37 (11)	103 (30)	77 (22)	39 (11)	43 (13)	

NOTE. Data are given as No. (%). MEL score ≥ 2 is considered PD-L1 positive.
Abbreviations: MEL, melanoma; PD-L1, programmed death-ligand 1.
*Comparison across subgroups within each variable.
†M0, no distant metastasis; M1a, metastasis to skin, subcutaneous tissues, or distant lymph nodes; M1b, metastasis to lung; M1c, metastasis to all other visceral sites or distant metastases at any site associated with elevated levels of serum LDH.

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