rapid communications

Pembrolizumab Plus Ipilimumab Following Anti-PD-1/L1 Failure in Melanoma

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abstract

PURPOSE Combination of antiprogrammed cell death protein-1 (PD-1) plus anti–cytotoxic T-cell lymphocyte-4 (anti-CTLA-4) immunotherapy shows greater response rates (RRs) than anti-PD-1 antibody alone in melanoma, but RR after initial anti-PD-1 and programmed death ligand-1 (PD-L1) antibody progression awaits robust investigation. Anti-CTLA-4 antibody alone after anti-PD-1/L1 antibody progression has a historical RR of 13%. We report the results of the first prospective clinical trial evaluating ipilimumab 1 mg/kg plus pembrolizumab following progression on anti-PD-1 immunotherapy.

METHODS Patients with advanced melanoma who had progressed on anti-PD-1/L1 antibody as immediate prior therapy (including non–anti-CTLA-4 antibody combinations) were eligible. Patients received pembrolizumab 200 mg plus ipilimumab 1 mg/kg once every 3 weeks for four doses, followed by pembrolizumab monotherapy. The primary end point was RR by irRECIST. After 35 patients, the trial met the primary end point and was expanded to enroll a total of 70 patients to better estimate the RR.

RESULTS Prior treatments included 60 on anti-PD-1 antibody alone and 10 on anti-PD-1/L1 antibody–based combinations. Thirteen patients had progressed in the adjuvant setting. The median length of prior treatment with anti-PD-1/L1 antibody was 4.8 months. Response assessments included five complete and 15 partial responses, making the irRECIST RR 29% among the entire trial population. The median progression-free survival was 5.0 months, and the median overall survival was 24.7 months. The median duration of response was 16.6 months. There was no difference in median time on prior anti-PD1/L1 or time to PD1 + CTLA4 initiation between responders and nonresponders. Grade 3-4 drug-related adverse events occurred in 27% of patients. Responses occurred in PD-L1–negative, non-T-cell–inflamed, and intermediate tumor phenotypes.

CONCLUSION To our knowledge, this is the first prospective study in melanoma of pembrolizumab plus low-dose ipilimumab after anti-PD-1/L1 immunotherapy failure, demonstrating significant antitumor activity and tolerability.

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ASSOCIATED CONTENT

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Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

For patients with advanced melanoma, the introduction of targeted and immune checkpoint inhibitor therapies has greatly improved systemic therapy outcomes.[1] With antibodies against the immune checkpoints cytotoxic T-lymphocyte–associated antigen 4 (anti–cytotoxic T-cell lymphocyte-4 [CTLA-4]; ipilimumab) and programmed cell death protein-1 (PD-1; pembrolizumab and nivolumab), some patients have achieved long-term disease control, with 5-year survival rates ranging from 34% to 52%.[2,3] Combination immunotherapy using both anti-PD-1 and anti-CTLA-4 antibodies demonstrates a numerically higher response rate (RR), progression-free survival (PFS), and overall survival (OS) relative to anti-PD-1 antibody monotherapy, but with a grade 3-4 toxicity rate of up to 59%.[4] This has prompted many treating oncologists to begin with single-agent anti-PD-1 therapy, with the prospect of using ipilimumab in the second line upon treatment failure.

However, in patients who have experienced tumor progression on anti-PD-1 and programmed death ligand-1 (PD-L1) antibody monotherapy, no prospective trials of ipilimumab or an anti-PD-1 plus anti-CTLA-4

CONTEXT

Key Objective

To assess the efficacy of pembrolizumab plus low-dose ipilimumab in advanced melanoma in patients refractory to an antiprogrammed cell death protein-1 (PD-1) and programmed death ligand-1 (PD-L1) antibody.

Knowledge Generated

Among 70 enrolled patients, 20 confirmed responses were observed (irRECIST response rate 29%) with a median duration of response of 16.6 months. Grade 3-4 adverse events occurred in 27% of patients, and responses occurred primarily among PD-L1–negative, non-T-cell–inflamed, and intermediate tumor phenotypes.

Relevance

To our knowledge, this is the first prospective study in melanoma of pembrolizumab plus low-dose ipilimumab after anti-PD-1/L1 immunotherapy failure, demonstrating significant antitumor activity and tolerability.

antibody combination exist. Post hoc assessment of 97 patients who crossed over to ipilimumab directly after pembrolizumab in the randomized phase III KEYNOTE-006 trial demonstrated a 13% objective response rate (ORR).[5] Similarly, a retrospective review of treatment outcomes in 355 patients with progression on an anti-PD-1/L1 antibody demonstrated that those who subsequently received ipilimumab achieved an RR of 13% versus 32% in those who received ipilimumab of dose 3 mg/kg once every 3 weeks for four doses plus an anti-PD-1/L1 antibody. The OS also appeared to favor the combination group at 20.4 months versus 8.8 months, respectively.[6] These findings support the hypothesis that combined PD-1 and CTLA-4 inhibition can be effective after progression on a prior anti-PD-1/L1. The use of this combination in the second line might also theoretically spare the higher toxicity rate for patients who only required single-agent anti-PD-1 therapy for disease control.

Despite demonstrating the highest ORR, PFS, and OS, the combination of nivolumab plus ipilimumab at 3 mg/kg treatment is not uniformly used in advanced melanoma because of serious immune-related adverse events.[4] A strategy for mitigating toxicity with this combination immunotherapy is administration of low-dose ipilimumab, 1 mg/kg, in combination with an anti-PD-1 antibody. The randomized Checkmate-511 study compared nivolumab with ipilimumab at 1 mg/kg or 3 mg/kg, demonstrating that high-grade adverse events (grades 3-5) occurred in 34% versus 48% of patients, respectively, whereas exploratory efficacy outcomes were similar.[7] No prospective studies have yet described the utility of anti-PD-1 plus anti-CTLA-4 antibody therapy following progression on anti-PD-1/L1 monotherapy.

Multiple biomarkers of anti-PD-1 treatment response, such as PD-L1 immunohistochemistry and gene expression profiling centered on interferon-associated transcripts, have been identified to stratify immunotherapy treatment outcomes. T-cell–inflamed gene expression, which more broadly describes the tumor microenvironment than PD-L1 staining alone, perhaps has the strongest predictive association with response in melanoma.[8] Immunotherapy biomarkers have been predominately explored in the treatment-naïve setting, and it remains unclear what relationship these biomarkers have with response after failure on an anti-PD-1/L1 antibody.

Herein, we report outcomes from a phase II clinical trial for the combination of pembrolizumab plus low-dose ipilimumab following progression on an anti-PD-1/L1 antibody in advanced melanoma. We also report baseline tumor biomarkers of clinical response to this combination immunotherapy regimen.

METHODS

Study Design and Participants

In this open-label, single-arm phase II trial, we recruited patients from seven medical centers across the United States. Eligible adult patients had unresectable or metastatic melanoma with known *BRAF* mutation status, uveal melanoma excluded, measurable disease according to immune-related RECIST (irRECIST) version 1.1,[9] and adequate organ function and performance status. Patients with previous grade 3-4 toxicity from anti-PD-1/L1 antibody therapy leading to treatment discontinuation were excluded. All patients must have experienced disease progression during treatment with an anti-PD-1/L1 antibody immediately before accrual to this study or disease progression within 6 months of adjuvant anti-PD-1 antibody without intercurrent therapy. For patients without clear clinical progression on the prior anti-PD-1/L1 antibody, a confirmatory scan was recommended but not required. Patients with active CNS metastases were excluded (previously treated, stable CNS disease allowed); no prior anti-CTLA-4 antibody use in the metastatic setting was permitted. Full inclusion and exclusion criteria are listed in the study Protocol (online only [pp 12-15]). The study Protocol was reviewed and approved by the appropriate institutional review boards at each participating site. We conducted the study in accordance with the Protocol with subsequent

amendments and with the Declaration of Helsinki. All patients provided written informed consent.

Procedures

Patients received pembrolizumab (200 mg) intravenously along with low-dose ipilimumab (1 mg/kg) intravenously once every 3 weeks for four doses, followed by pembrolizumab intravenously 200 mg once every 3 weeks for up to 2 years. Treatment continued until confirmed radiographic disease progression, intolerable toxicity, patient withdrawal of consent, investigator decision, or completion of therapy. Per irRECIST, patients with initial evidence of radiographic progression were allowed to continue through to a second confirmatory scan to account for tumor flare; however, patients with evidence of clinical deterioration or clear objective disease progression, as assessed by the treating physician, were not required to have repeat imaging for confirmation of progressive disease. Study treatments continued for up to 2 years, although patients who attained a complete response confirmed on at least two scans after a minimum of 24 weeks of study treatment could discontinue treatment early. Full discontinuation criteria are included in the study Protocol (pp 25-26). No dose reductions of pembrolizumab or ipilimumab were permitted.

Immunohistochemistry for tumor PD-L1 expression and gene expression profiling were performed on archival formalin-fixed paraffin-embedded (FFPE). Full methods for PD-L1 scoring, as well as procedures for RNA extraction and gene expression profiling by RNAseq, are included in the Appendix (online only).

Formal clinical assessments of response were completed per irRECIST version 1.1 before initiation of study therapy and every 12 weeks thereafter, with intervals of at least every 3 weeks required for confirmation of disease progression. At all treatment and follow-up visits, we assessed physical examination status, vital signs, and laboratory values. Adverse events were documented according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Outcome Analysis

The primary objective of this study was to determine the ORR of pembrolizumab with low-dose ipilimumab following initial progression on an anti-PD-1/L1 antibody in advanced melanoma. Secondary objectives included summarizing PFS (defined as time on study treatment until immune-related progressive disease, clear clinical progression, or death) and safety. Exploratory objectives included associations of baseline tumor gene expression patterns with clinical outcomes. Although not prespecified end points in the study Protocol, OS (defined as the time from enrollment to death from any cause) and duration of response (DOR; defined as the time from first evidence of response until disease progression or death) were also assessed.

Statistical Analysis

The initial design of this study sought to test a null hypothesis of a 10% RR (based on known historical controls for ipilimumab) versus a 30% alternative.[5] Simon's optimal two-stage design was initially employed to enroll up to a total of 35 patients. In the first stage, 12 patients were enrolled with two responses required to initiate the second stage, where a total of 35 patients would be enrolled (one-sided alpha .10, 90% power). Given the investigator-initiated and open-label nature of the study, no formal accrual hold was planned for interim analysis. It was observed that two patients responded within the first five patients accrued and there were seven responders in the first 12 patients of the Simon design. The primary end point for the total study was met when eight responses were observed in the first 17 patients, after which the trial was expanded to enroll a total of 70 patients in an exploratory manner to better describe the ORR in this population.[10] We assessed ORR and, using the Kaplan-Meier method, estimated PFS and OS in all enrolled patients as well as DOR in patients achieving a partial or complete response. We provided descriptive statistics for baseline patient characteristics and for adverse events. Frequency of adverse events for each grade level was recorded among all patients receiving at least one dose of study therapy.

Available tumor specimens were analyzed for PD-L1 expression and gene expression profiling; these biomarkers were then compared with clinical response outcomes. We used a previously described T-cell inflammation score,[11] which correlates with other validated interferon-γ gene signatures (Appendix).[12] This trial is registered with ClinicalTrials.gov identifier: NCT02743819. The trial is closed to enrollment, but patients remain on treatment and in observation for safety and ongoing response assessments.

RESULTS

From December 9, 2016 to November 4, 2019, 70 patients were enrolled and treated with at least one dose of pembrolizumab plus low-dose ipilimumab. Before enrollment, the median time on prior anti-PD-1/L1 antibody was 4.8 months (Appendix Fig A1, online only). Thirteen study patients (19%) progressed previously in the adjuvant setting. Two patients received adjuvant ipilimumab and completed this treatment 15 and 22 months before receiving an anti-PD-1/L1 antibody. Progression to prior anti-PD-1/L1 therapy was determined by confirmatory scans in 55 (79%) patients and by assessment of clinical progression by the treating physician in 15 (21%) patients. Most patients were male and had cutaneous melanoma; 20 patients (29%) had *BRAF*^{V600} mutations. Additionally, 34 patients (49%) had M1c or M1d disease, and 22 patients (31%) had elevated serum lactate dehydrogenase (LDH) concentrations (Table 1).

TABLE 1. Baseline Characteristics

Characteristic	Study Patients Receiving ≥ 1 Dose (N = 70)
Age, years	
Median	64
Range	27-87
Sex, No. (%)	
Male	47 (67)
Female	23 (33)
BRAF status, No. (%)	
Mutant (V600)	20 (29)
Wild type	50 (71)
AJCC stage, No. (%)	
IIIc (unresectable)	12 (17)
IV	58 (83)
M1a	15 (21)
M1b	9 (13)
M1c	27 (39)
M1d	7 (10)
Melanoma subtypes, No. (%)	
Cutaneous (non-acral)	62 (89)
Acral	7 (10)
Mucosal	1 (1)
Adjuvant anti-PD-1 Ab progression	13 (19)
Baseline LDH, No. (%)	
≤ ULN	48 (69)
> ULN	17 (24)
≥ 2× ULN	5 (7)
History of brain metastases (treated), No. (%)	
Yes	7 (10)
No	53 (90)
Liver metastases, No. (%)	
Yes	17 (24)
No	53 (76)
Prior lines of systemic therapy (mean = 1), No. (%)	
Anti-PD-1 Ab alone	60 (86)
Anti-PD-1/L1 Ab combination (non–anti-CTLA-4 Ab)	8 (11)
Anti-PD-L1 Ab combination (non–CTLA-4 Ab)	2 (3)
Prior adjuvant ipilimumab (pre–anti-PD-1/L1 Ab)	2 (3)
Adjuvant interferon (pre–anti-PD-1/L1 Ab)	3 (4)
Prior *BRAF*-directed therapy (pre–anti-PD-1/L1 Ab)	5 (7)

Abbreviations: Ab, antibody; AJCC, American Joint Committee on Cancer; CTLA-4, cytotoxic T-cell lymphocyte-4; LDH, lactate dehydrogenase; M, distant metastasis; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; ULN, upper limit of normal.

By the data cutoff date of April 28, 2020, the median follow-up was 12.0 months (interquartile range [IQR], 6.0-22.6 months); among patients still alive, the median follow-up time was 13.3 months (IQR, 6.2-23.1 months). At the time of the analysis, five patients (7%) were continuing to receive pembrolizumab monotherapy and one patient had completed 2 years of study therapy. All other patients have discontinued because of clinical or radiographic disease progression in 47 patients (67%), adverse events in nine patients (13%), elective discontinuation of therapy after complete response in four patients (6%), death unrelated to study therapy in two patients (3%), or withdrawal of consent in two patients (3%). Fifty-three patients (76%) received four or more doses of pembrolizumab plus low-dose ipilimumab. Of those who did not complete four cycles of study therapy, the median number of cycles was 2 (IQR, 1.75-2.25) and reasons for discontinuation among these patients were progressive disease (n = 11), adverse events (n = 4), and death (n = 2). For patients continuing pembrolizumab monotherapy, the median number of cycles was 9 (IQR, 5.0-15.8).

Twenty of 70 enrolled patients (29%) achieved a confirmed response (95% CI, 18.4 to 40.6), including five complete (7.2%) and 15 partial responses (21.4%) (Fig 1). Three additional patients had unconfirmed responses, all with subsequent disease progression. Eight patients were nonevaluable for radiographic assessment of the primary end point but are included in the overall analysis for ORR (as nonresponders) and secondary end points. Of the eight nonevaluable patients, five had clear clinical progression before first response assessment, two patients died of complications related to disease progression before restaging, and one patient withdrew consent after experiencing grade three immune-related hepatitis after one dose of study therapy. The median PFS was 5 months (95% CI, 2.8 to 8.3). In responding patients, the median DOR was 16.6 months (95% CI, 7.9 to not reached). The median OS was 24.7 months (95% CI, 15.2 to not reached) (Appendix Fig A2, online only). At the time of data lock, 14 of 20 responses (70%) were ongoing.

Responses were observed across clinical and biomarker subgroups (Table 2), including patients who progressed on an anti-PD-1/L1 antibody in the adjuvant setting (two of 13 [15%]), patients with liver metastases and previously treated CNS metastases (six of 25 [17%]), and patients with an elevated LDH at study entry (six of 22 [27%]). No association between time on prior anti-PD-1/L1 antibody therapy and subsequent response to study therapy was observed. The median time between prior anti-PD-1/L1 treatments and initiating pembrolizumab plus low-dose ipilimumab was 1.5 months in responders vs 1.4 months

in nonresponders. More responses were observed in PD-L1–negative archival tumors (15 of 39 [38%]) as compared with PD-L1–positive archival tumors (four of 27 [15%]). T-cell–inflamed gene expression scores were calculated across all patients with archival tissue adequate for RNA extraction (n = 58). Two T-cell–inflamed tumors were observed among the 20 responders, with the remainder being either non-T-cell–inflamed or intermediate (Table 3).

Treatment-related adverse events occurred in 62 of 70 patients (87%), with the most frequent events being pruritis, rash, diarrhea, fatigue, nausea, and transaminase elevations (Table 4). Grade 3-4 treatment-related adverse events occurred in 19 of 70 patients (27%), with the most common being colitis or diarrhea, rash, and transaminase elevations. Only one patient experienced a grade four treatment-related adverse event, which was a concurrent lipase elevation in a patient with grade three pancreatitis.

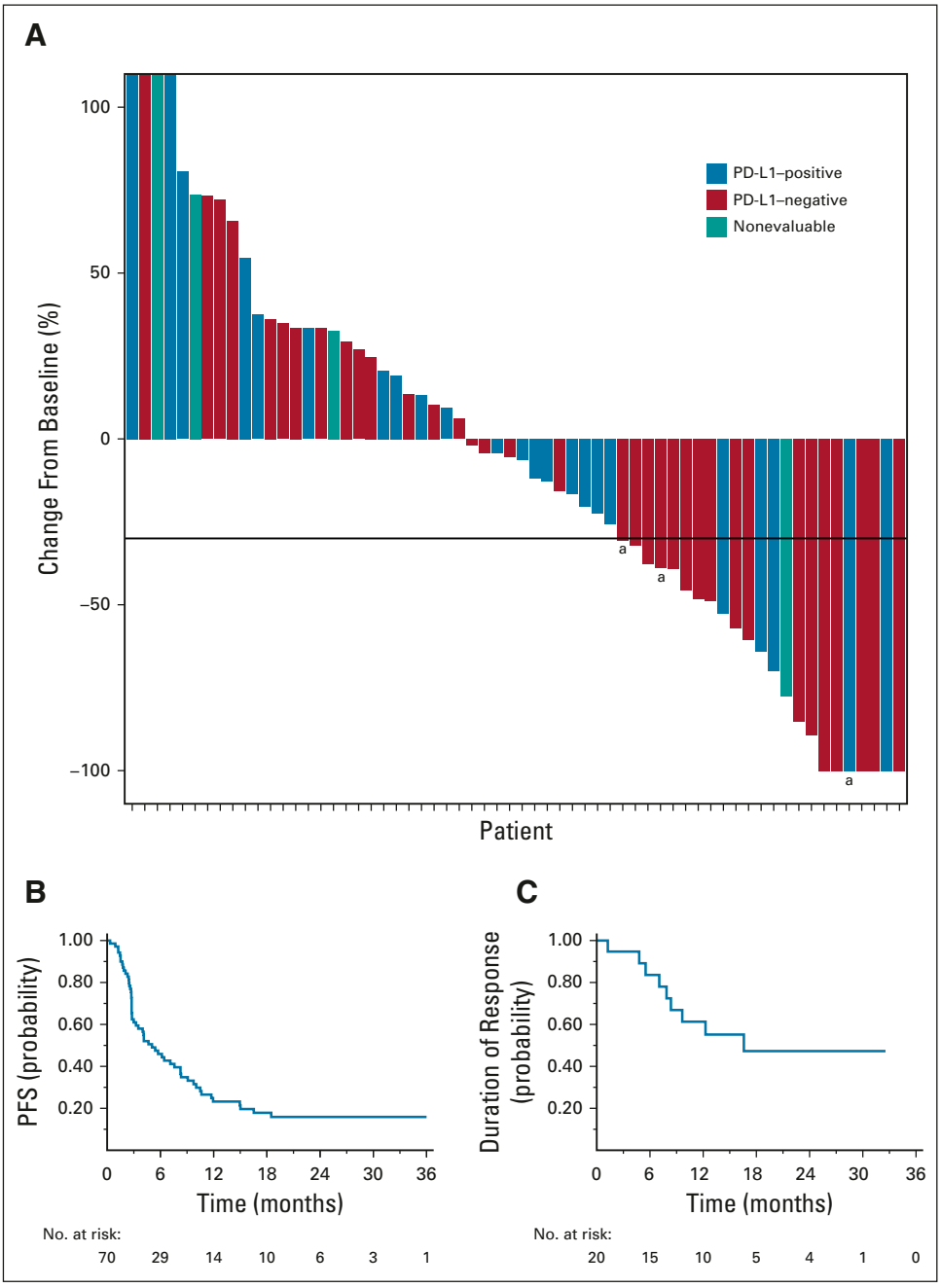

FIG 1. Antitumor activity. (A) Best percentage change from baseline in the sum of the longest diameters of target lesions in all patients with at least one on-treatment radiographic assessment. Kaplan-Meier estimates of (B) PFS and (C) duration of response. [a]Unconfirmed response. PD-L1, programmed death ligand-1; PFS, progression-free survival.

TABLE 2. RRs in Overall Study Population and by Clinical and Biomarker Subgroups

Response subgroups	Overall irRECIST RR 20/70 (29%), No. (%)
After adjuvant anti-PD-1 Ab progression	2/13 (15)
Liver or CNS disease	6/24 (17)
Acral or mucosal melanoma	1/8 (14)
Elevated LDH	6/22 (27)
LDH in normal range	14/48 (29)
BRAF status	
Mutant	5/20 (25)
Wild type	15/50 (30)
Time on prior anti-PD-1/L1 treatment	
> 6 months	4/25 (16)
< 6 months	16/45 (36)
> 12 months	0/8 (0)
Assessment of progression to prior anti-PD-1/L1 treatment	
Confirmed progressive disease (irPD)	14/55 (25)
Clinical progressive disease	6/15 (40)
PD-L1 status[a]	
PD-L1–positive	4/27 (15)
PD-L1–negative	15/39 (38)

Abbreviations: Ab, antibody; irPD, immune-related progressive disease; LDH, lactate dehydrogenase; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; RR, response rate.

[a]One responder with PD-L1 stain inadequate for scoring.

Four other patients also experienced concurrent grade three treatment-related adverse events. There were no deaths related to study treatment. The median time to onset of grade 3-4 adverse events was 55 days.

DISCUSSION

We performed this prospective study of patients with advanced melanoma refractory to an anti-PD-1/L1 antibody and demonstrate that the combination of pembrolizumab plus low-dose ipilimumab has substantial antitumor activity and a manageable toxicity profile. Responses were observed in 29% of all study patients. Responses are ongoing in 70% of responding patients, with a median response duration of 16.6 months. Responses were also observed across all clinical subgroups, including patients with elevated LDH and brain and liver metastases and in patients who progressed on an anti-PD-1/L1 antibody in the adjuvant setting. Most responses were observed among patients who progressed on a prior anti-PD-1/L1 antibody within 6 months, although responses were also observed among those with longer prior treatment. Additionally, these responses occurred predominantly among patients with intermediate to non-T-cell–inflamed and PD-L1–negative tumors. The study regimen showed a relatively low rate of toxicity, with grade 3-4 adverse events occurring in 27% of all study patients.

TABLE 3. Responses by T-Cell–Inflamed Gene Expression Signature

	Best Overall Response				
T Cell Inflammation Score	CR	PR	SD	PD	Total, No. (%)
T-cell–inflamed	1	1	0	3	5 (9)
Intermediate	1	9	8	18	34 (59)
Non-T-cell–inflamed	2	6	3	8	19 (33)

NOTE. Response categories by T-cell–inflamed GEPs in all patients with available tumor specimens passing RNA quality control (n = 58).

Abbreviations: CR, complete response; GEP, gene expression profile; PD, progressive disease; PR, partial response; SD, stable disease.

Prolonged response, even after cessation of therapy, is a hallmark feature of immune checkpoint inhibitors.[3,13] The durable responses observed with pembrolizumab plus low-dose ipilimumab suggest that this benefit may carry forward when using an anti-PD-1 plus anti-CTLA-4 antibody combination after anti-PD-1 antibody failure. These findings may be particularly informative for future drug development after failure of an anti-PD-1/L1 antibody by setting an important benchmark for therapies in this space.

Although high rates of toxicity have previously limited safe delivery of anti-PD-1 plus anti-CTLA-4 combinations, the 27% grade 3-4 adverse event rate observed with pembrolizumab plus low-dose ipilimumab reflects a comparatively manageable side effect profile. Currently, there is no prospective comparison of toxicity for anti-PD-1 and anti-CTLA-4 antibody combinations in the anti-PD-1/L1 antibody–refractory setting, although high-grade toxicity rates in the anti-PD-1/L1 antibody–naïve setting surpass 50%.[4] In this study, we used a 1 mg/kg dose of ipilimumab to mitigate such high rates of toxicity, while seeking to maintain antitumor activity, as this strategy has shown promise in the anti-PD-1/L1–naïve setting.[7,14] The 1 mg/kg ipilimumab dose may, to an extent, explain the relatively favorable rates of toxicity observed with pembrolizumab plus low-dose ipilimumab. We would, however, make only cautious comparisons between toxicity rates between the anti-PD-1/L1 antibody–refractory and antibody–naïve settings. As anti-PD-1/L1 antibody refractory patients who experienced high-grade toxicity were ineligible for our study, toxicity rates observed with ipilimumab combination in the anti-PD-1/L1 antibody–refractory setting may be subject to this selection bias.

Responses to pembrolizumab plus low-dose ipilimumab were observed predominantly among PD-L1–negative and intermediate to non-T-cell–inflamed tumors. This is contrary to what has been observed in the anti-PD-1/L1 antibody–naïve setting, where biomarkers of T-cell inflammation and PD-L1 expression enrich for response.[8,15,16] Previously, MHC class II expression has been shown to predict

TABLE 4. TRAEs

Variable	Grade 1-2	Grade 3-4[a]
Any TRAE, No. (%)	62/70 (87)	19/70 (27)
TRAEs in > 10 of patients		
Pruritis	28 (40)	
Rash (maculopapular, acneiform, and papulopustular)	25 (36)	4 (6)
Colitis or diarrhea	28 (40)	6 (9)
Fatigue	23 (33)	0
Nausea	17 (24)	1 (1)
ALT/AST increased	14 (20)	4 (6)
Anorexia	14 (20)	0
Arthralgia	7 (10)	0
TRAEs leading to treatment discontinuation, No. (%)		
Rash maculopapular	0	1 (1)
Skin and subcutaneous tissue disorders—others (vasculitis)	0	1 (1)
AST and/or ALT elevation	0	2 (3)
Colitis or diarrhea	0	1 (1)
No. of patients with ≥ grade 3 toxicity at least possibly related to study drug, No. (%)[b]		
Colitis or diarrhea	NA	6 (9)
Rash (acneiform or maculopapular)	NA	4 (6)
AST and/or ALT elevation	NA	4 (6)
Lipase elevation[a]	NA	3 (4)
Acute kidney injury	NA	2 (3)
Hyperglycemia	NA	2 (3)
Pancreatitis	NA	1 (1)
Skin and subcutaneous tissue disorders—others, specify (vasculitis)	NA	1 (1)
Anemia	NA	1 (1)
Nausea	NA	1 (1)
Lymphocyte count decreased	NA	1 (1)
Lymphocyte count increased	NA	1 (1)
Lung infection	NA	1 (1)
Alkaline phosphatase elevation	NA	1 (1)

Abbreviations: NA, not available; TRAE, treatment-related adverse event.

[a]A single grade 4 lipase elevation TRAE occurred concurrently in a patient with grade 3 pancreatitis.

[b]A given patient might be counted in more than one toxicity grade category.

response to anti-PD-1 but not anti-CTLA-4 antibody therapy, suggesting that distinct mechanisms of antigen presentation may drive the differential benefit between the two immune checkpoint inhibitors.[17-19] These findings would also suggest that patients with highest pre-existing IFN-γ–associated immune activation are most likely to benefit from upfront anti-PD-1/L1 antibody therapy and hence may be less represented in the anti-PD-1/L1 antibody–refractory setting. This could explain in part why patients who benefited from pembrolizumab plus low-dose ipilimumab were found to have non-T-cell–inflamed tumor phenotypes. It remains unclear, however, how the addition of ipilimumab drives antitumor responses after a failure of an anti-PD-1/L1 antibody. One possibility is that ipilimumab may enhance the development of de novo antitumor responses in the peripheral immune compartment and/or relieve independent mechanisms of resistance associated with PD-1/L1 expression in the tumor microenvironment. These hypotheses are supported by neoadjuvant ipilimumab studies in which an influx of $CD8^+$ T cells into the tumor microenvironment was observed after treatment.[20] The exact immunologic mechanisms by which ipilimumab may facilitate these antitumor responses require further investigation.

We recognize that our study has certain limitations. Given the constraints of an uncontrolled study, we would await the randomized results of anti-PD-1 plus anti-CTLA-4 antibody combination after anti-PD-1 antibody failure to definitively make a claim of superiority for the combination regimen.[21] Additionally, at the time this study began, there was not a formal consensus to define progression on an anti-PD-1/L1 antibody as it exists currently.[22] In reviewing our study, 79% of patients had two scans to confirm progression on a prior anti-PD-1/L1 antibody, and for patients without confirmatory scans, treating physicians used their clinical judgment to ascertain a lack of benefit from the prior anti-PD-1/L1 antibody. Although RECIST 1.1 remains a predominant treatment response method, we chose irRECIST to more completely describe the clinical outcomes as the primary end point analysis for an immunotherapy trial. Regarding our observations surrounding lack of a T-cell–inflamed tumor phenotype with response, these findings are predicated on baseline, archival FFPE tumor samples. Future studies incorporating pre- and on-treatment biopsies after anti-PD-1/L1 antibody failure will be helpful to fully characterize mechanisms of tumor microenvironment modulation by ipilimumab. We also recognize that in clinical practice, some patients may receive intercurrent BRAF-/MEK-directed therapy after progression on an anti-PD-1/L1 antibody. Outcomes for this specific subgroup await further study.

The combination of pembrolizumab plus low-dose ipilimumab demonstrated significant antitumor activity and tolerability in a multicenter clinical trial. This study demonstrated long-term responses, suggesting that durable survival—a hallmark of immunotherapy activity—may be possible even after failure of an anti-PD-1/L1 antibody. These findings warrant further investigation and support the ongoing effort to directly compare combination of anti-PD-1 and anti-CTLA-4 antibodies with ipilimumab alone in the anti-PD-1/L1 antibody–refractory setting.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

EQUAL CONTRIBUTION

T.F.G., N.I.K., and J.J.L. are co-senior authors.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Pembrolizumab Plus Ipilimumab Following Anti-PD-1/L1 Failure in Melanoma

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APPENDIX

Tumor Biomarker Procedures

PD-L1 scoring. The Cell Signaling Technology EIL3N programmed death ligand-1 (PD-L1) monoclonal antibody was used for PD-L1 scoring. This antibody has been previously validated for PD-L1 scoring and showed high concordance with commercially available PD-L1 antibodies, including DAKO 22C35.[23] Tumor cells were scored by University of Chicago pathologists using the DAKO 22C3 method as taught in the interactive primer course on the DAKO 22C3 website; this method is employed as standard practice for PD-L1 scoring at the University of Chicago. A cutoff of ≥ 1% was used to define PD-L1 positivity as consistent with the melanoma KEYNOTE trials.

RNA sequencing and gene expression profiling. Tumor RNA was isolated from available formalin-fixed paraffin-embedded (FFPE) tumor samples using the QIAGEN AllPrep DNA/RNA FFPE kit (Qiagen, Hilden, Germany) at the Human Immunologic Monitoring Facility at the University of Chicago according to the manufacturer's instruction. The quality and quantity of RNA were measured on an Agilent 2100 Bio-analyzer using Agilent reagents and protocols (Agilent Technologies, Santa Clara, CA). RNAseq libraries were generated using Illumina TruSEQ Total RNA stranded library–making kits using Illumina protocols (Illumina, San Diego, CA). The quality and quantity of the library were determined using an Agilent 2100 Bio-analyzer using Agilent reagents and protocols. Sequencing data were collected using an Illumina NovaSEQ sequencer and demultiplexed using Illumina bcl2fastq software. The quality of raw reads was assessed by FastQC.[24] Reads were aligned to human reference transcriptome with Gencode gene annotation (GRCh38) by Kallisto.[25] Transcript abundance was quantified at the transcript level using the strand-specific protocol, summarized into gene level using tximport,[26] normalized by the trimmed mean of M values (TMM) method, and $\log_2$-transformed for further analysis. T-cell–inflamed gene expression scores were determined using a 160-gene signature of T-cell inflammation,[11] and the expression for each gene was normalized to its mean expression level by subtracting the average value and dividing by the standard deviation, such that the average value of the normalized data was 0 and the standard deviation was 1. T-cell–inflamed gene expression scores were calculated by taking the median of the normalized values for each gene in the signature. Of the 58 tumor specimens with RNA adequate for analysis, 50 were obtained before initial anti-PD-1/L1 antibody treatment and eight were obtained after anti-PD-1/L1 antibody treatment, but before initiating study treatment with pembrolizumab plus low-dose ipilimumab.

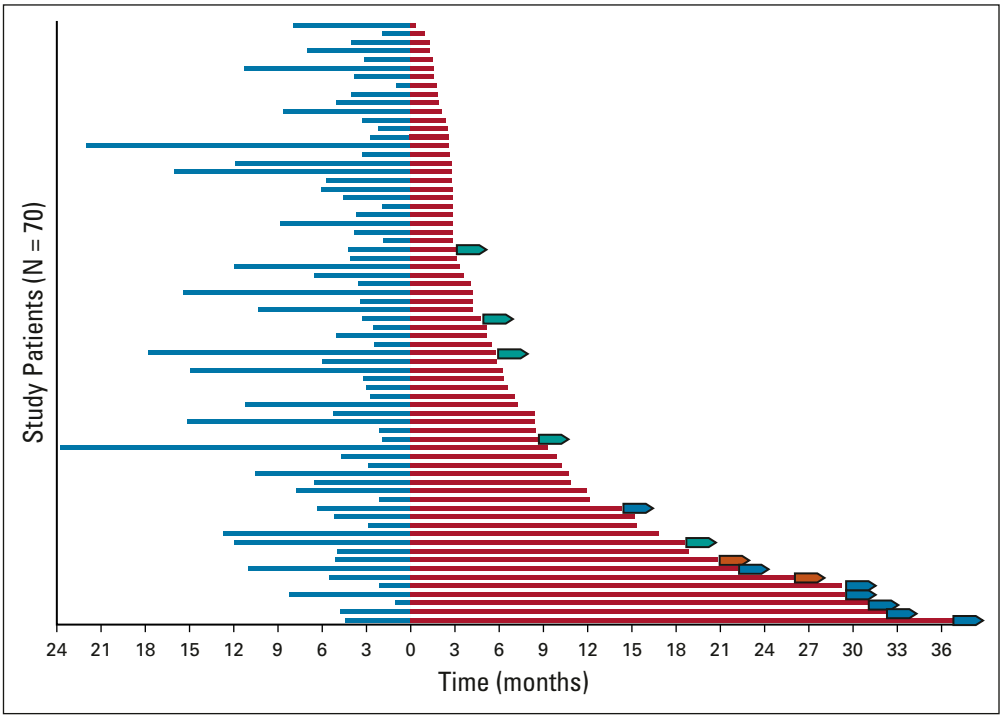

FIG A1. Time on prior anti-PD-1/L1 antibody (blue) versus progression-free time on pembrolizumab plus low-dose ipilimumab (red). Red bars indicate progression-free time after initiating study treatment, blue bars indicate time on prior anti-PD-1/L1 antibody, green arrows indicate patients currently on treatment, blue arrows represent those who either completed treatment or stopped early, and orange arrows represent patients who came off for an adverse event and remain progression-free. PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1.

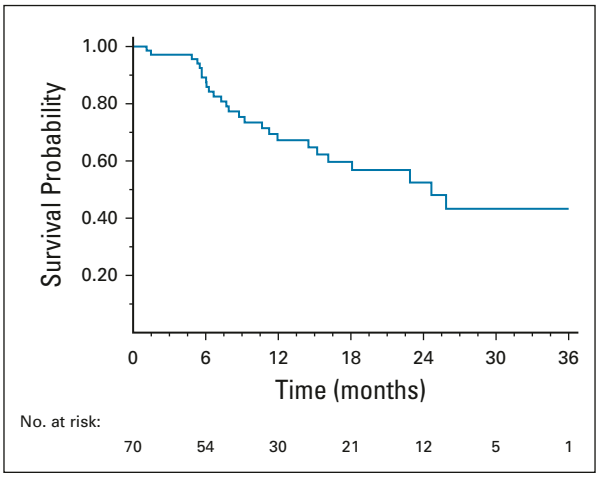

FIG A2. Overall survival in the total population.