

# Association of *BRAF* V600E/K Mutation Status and Prior BRAF/MEK Inhibition With Pembrolizumab Outcomes in Advanced Melanoma

## Pooled Analysis of 3 Clinical Trials

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 Supplemental content

**IMPORTANCE** The optimal sequencing of immune checkpoint inhibitors and targeted therapy for *BRAF* V600E/K-mutant melanoma is not well established.

**OBJECTIVE** To assess the association of *BRAF* wild-type (WT) or *BRAF* V600E/K-mutant status and BRAF inhibitor (BRAFi) with or without MEK inhibitor (MEKi) therapy with response to pembrolizumab.

**DESIGN, SETTING, AND PARTICIPANTS** This study is a post hoc subgroup analysis of pooled data from 3 multinational, multisite studies: KEYNOTE-001 (data cutoff September 1, 2017), KEYNOTE-002 (data cutoff May 30, 2018), and KEYNOTE-006 (data cutoff December 4, 2017). Patients included in this analysis were adults with advanced melanoma and known *BRAF* V600E/K tumor status who had received pembrolizumab.

**INTERVENTIONS** Patients received pembrolizumab in dosages of 2 mg/kg every 3 weeks, 10 mg/kg every 2 weeks, or 10 mg/kg every 3 weeks.

**MAIN OUTCOMES AND MEASURES** End points were objective response rate (ORR) and progression-free survival (PFS) assessed by Response Evaluation Criteria in Solid Tumors, version 1.1, and overall survival (OS). Objective response rates, 4-year PFS, and OS rates were compared in the following patient subgroups: *BRAF* WT vs *BRAF* V600E/K-mutant melanoma and *BRAF* V600E/K-mutant melanoma with vs without previous treatment with BRAFi with or without MEKi therapy.

**RESULTS** The overall study population (N = 1558) included 944 men (60.6%) and 614 women (39.4%). The mean (SD) age was 60.0 years (14.0). The ORR was 38.3% (596/1558), 4-year PFS rate was 22.0%, and 4-year OS rate was 36.9%. For patients with *BRAF* WT (n = 1124) and *BRAF* V600E/K-mutant melanoma (n = 434), ORR was 39.8% (n = 447) and 34.3% (n = 149), 4-year PFS rate was 22.9% and 19.8%, and 4-year OS rate was 37.5% and 35.1%, respectively. Patients with *BRAF* V600E/K-mutant melanoma who had (n = 271) vs had not (n = 163) previously received BRAFi with or without MEKi therapy had baseline characteristics with worse prognosis; ORR was 28.4% (n = 77) and 44.2% (n = 72), 4-year PFS rate was 15.2% and 27.8%, and 4-year OS rate was 26.9% and 49.3%, respectively.

**CONCLUSIONS AND RELEVANCE** Results of this subgroup analysis support the use of pembrolizumab for treatment of advanced melanoma regardless of *BRAF* V600E/K mutation status or receipt of prior BRAFi with or without MEKi therapy.

JAMA Oncol. doi:10.1001/jamaoncol.2020.2288  
Published online July 16, 2020.

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The outlook for patients with metastatic melanoma has improved considerably with the availability of targeted agents and immune checkpoint inhibitors. For patients with metastatic *BRAF* wild-type (WT) tumors, current guidelines recommend anti-programmed death 1 (PD-1) monotherapy or anti-PD-1/anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) combination therapy.<sup>1</sup> In approximately 40% of patients with metastatic melanoma, the melanoma contains a *BRAF* mutation, and more than 90% of those have an activating *BRAF* V600E/K mutation.<sup>2</sup> The standard of care for *BRAF* V600-mutant melanoma includes the combination of a BRAF inhibitor and a MEK inhibitor (BRAFi and MEKi) as well as immunotherapy.<sup>1</sup> Because these regimens have distinct mechanisms and toxic effects, the therapy sequence that achieves optimal efficacy and tolerability in patients with *BRAF* V600-mutant melanoma remains unknown.<sup>3</sup>

Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2). Pembrolizumab is approved in many countries for 1 or more advanced cancers, including unresectable or metastatic melanoma.<sup>4,5</sup> The efficacy of pembrolizumab in metastatic melanoma was initially established in the KEYNOTE-001 trial.<sup>6,7</sup> In this study involving treatment-naïve and previously treated patients, a lower objective response rate (ORR) was seen in patients with *BRAF* V600E/K-mutant melanoma ( $n = 133$ ) than in those with WT ( $n = 442$ ) melanoma (26% vs 36%).<sup>8</sup> However, a similar response rate was observed in patients who were treatment naïve regardless of *BRAF* V600E/K mutation status (50% vs 45% in *BRAF* V600E/K-mutant vs WT), suggesting that the reduced response rate seen in the overall population with *BRAF* V600E/K mutation might have been attributed to prior BRAFi with or without MEKi (BRAFi and MEKi or BRAFi alone) therapy rather than mutation status.<sup>8</sup> In line with these observations, several retrospective analyses suggest that treatment with the anti-CTLA-4 antibody ipilimumab after BRAFi with or without MEKi therapy may be associated with poor outcomes, and translational studies suggest that most biopsied mitogen-activated protein kinase (MAPK) inhibitor-resistant melanoma metastases have a poorly immunogenic tumor microenvironment,<sup>9,10</sup> raising questions as to the most effective therapy sequence for patients with *BRAF* V600-mutant melanoma.<sup>11,12</sup> This post hoc subgroup analysis was conducted to assess the effect of *BRAF* V600E/K mutation status and BRAFi with or without MEKi therapy on response to pembrolizumab in patients with ipilimumab-refractory and ipilimumab-naïve advanced melanoma enrolled in the KEYNOTE-001,<sup>6,7</sup> KEYNOTE-002,<sup>13,14</sup> or KEYNOTE-006<sup>15,16</sup> studies.

## Methods

### Study Design and Patients

Results of the KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006 trials have been published.<sup>6,7,13-16</sup> There were important differences in inclusion criteria regarding prior treatments and

## Key Points

**Question** Does *BRAF* V600E/K mutation status or previous BRAF inhibitor (BRAFi) with or without MEK inhibitor (MEKi) therapy affect response to pembrolizumab in patients with advanced melanoma?

**Findings** This post hoc analysis of 3 randomized clinical trials (KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006) involved 1558 patients with advanced melanoma and known *BRAF* tumor status (*BRAF* wild-type or *BRAF* V600E/K-mutant melanoma) who had all been treated with pembrolizumab and some of whom had undergone prior treatment with BRAF inhibitors with or without MEK inhibitors. Patients with *BRAF* wild-type and *BRAF* V600E/K-mutant melanoma had objective response rates (ORRs) of 39.8% and 34.3%, respectively, and similar respective rates of 4-year progression-free survival (PFS; 22.9% and 19.8%) and overall survival (OS; 37.5% and 35.1%); patients with *BRAF* V600E/K-mutant melanoma who had vs had not received previous BRAFi with or without MEKi had baseline characteristics with worse prognosis: lower ORR (28.4% vs 44.2%), 4-year PFS (15.2% vs 27.8%), and OS (26.9% vs 49.3%).

**Meaning** The results of this study support the use of pembrolizumab for the treatment of advanced melanoma regardless of *BRAF* V600E/K mutation status or prior BRAF inhibitor with or without MEK inhibitor therapy.

baseline lactate dehydrogenase (LDH) levels in these 3 studies (eMethods in the Supplement).

Briefly, in the open-label, phase 1 KEYNOTE-001 study of pembrolizumab, eligible patients were ipilimumab naïve, ipilimumab treated, or ipilimumab refractory. Ipilimumab-naïve patients with *BRAF* V600E/K mutation could have previously received BRAFi with or without MEKi therapy, and ipilimumab-refractory patients with *BRAF* V600E/K mutation were required to have received prior BRAFi with or without MEKi therapy.

The randomized, double-blind, phase 2 KEYNOTE-002 study compared pembrolizumab with chemotherapy in patients with ipilimumab-refractory advanced melanoma. Patients with *BRAF* V600E/K mutation were required to have previously received BRAFi with or without MEKi therapy.

The randomized, open-label, phase 3 KEYNOTE-006 study compared pembrolizumab with ipilimumab in patients with ipilimumab-naïve melanoma who had received no or 1 prior systemic therapy for advanced/metastatic disease. Patients with *BRAF* V600E/K mutation might have previously received prior BRAFi with or without MEKi therapy as first-line systemic therapy; however, BRAFi with or without MEKi therapy was not required for patients with normal LDH levels and no clinically significant tumor-related symptoms or evidence of rapid disease progression.

All studies were sponsored by Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey, and were conducted in accordance with the Declaration of Helsinki<sup>17</sup> and the International Council for Harmonisation and Good Clinical Practice guidelines. All protocols and amendments were approved by the appropriate institutional review boards or ethics committees. All patients provided written informed consent.

### Treatments, Assessments, and End Points

Patients received pembrolizumab in 1 of the following regimens: 2 mg/kg every 3 weeks, 10 mg/kg every 2 weeks, or 10 mg/kg every 3 weeks. As previously described, efficacy and safety profiles were similar across pembrolizumab regimens<sup>6,7,13,15,16,18</sup>; therefore, pembrolizumab dose groups were pooled across studies for this analysis.

Best overall response in all studies was assessed per Response Evaluation Criteria in Solid Tumors, version 1.1, by investigator review.<sup>6,13,15</sup> In the KEYNOTE-001 study, response was assessed every 12 weeks, and in KEYNOTE-002 and KEYNOTE-006, response was assessed at week 12, every 6 weeks until week 48, and then every 12 weeks thereafter.<sup>13-16</sup> End points examined in this pooled analysis were ORR, progression-free survival (PFS), and overall survival (OS).

### Statistical Analysis

Data from the intention-to-treat (ITT) populations were pooled from all the studies. Data cutoffs were as follows: September 1, 2017 (KEYNOTE-001), May 30, 2018 (KEYNOTE-002), and December 4, 2017 (KEYNOTE-006). Summary statistics for ORR are provided as percentages. For PFS and OS, 4-year rates are provided. Comparisons between the following patient subgroups were performed: *BRAF* V600 WT vs *BRAF* V600E/K-mutant and, among those with mutant disease, previous BRAFi with or without MEKi vs no previous BRAFi with or without MEKi treatment. The Miettinen and Nurminen method was used to determine 95% CIs and *P* values. The Kaplan-Meier method was used to estimate median PFS and OS. For patients lost to follow-up, data from the date of last contact were used. To determine baseline risk factors associated with best overall response, 1-tailed univariable analysis of each independent variable was conducted; factors for which the univariable test had a *P* value of less than 0.05 and those factors that had clinical relevance were selected for the multivariable logistic regression model (eMethods in the Supplement). A *P* value less than 0.05 was considered as significant.

## Results

### Patients and Efficacy

Data from 1558 pembrolizumab-treated patients with known *BRAF* V600E/K mutation status across studies (647 patients from KEYNOTE-001, 361 from KEYNOTE-002, and 550 from KEYNOTE-006) were pooled for this subgroup analysis. Overall, 944 (60.6%) were male and 614 (39.4%) were female; the mean (SD) age of participants was 60.0 (14.0) years. Of these, 1124 (72.1%) had *BRAF* WT and 434 (27.9%) had *BRAF* V600E/K-mutant melanoma; 271 (62.4%) were treated previously with BRAFi with or without MEKi and 163 (37.6%) were BRAFi with or without MEKi therapy naive. A total of 29 patients (1.9%) were lost to follow-up. For the overall analysis population (*N* = 1558), the ORR was 38.3% (596/1558), the 4-year PFS rate was 22.0%, and the 4-year OS rate was 36.9%.

### *BRAF* Wild-Type vs *BRAF* V600E/K-Mutant Melanoma

Although baseline characteristics were generally similar between the *BRAF* WT and *BRAF* V600E/K-mutant subgroups, because of differences in inclusion criteria across the 3 studies, there were some key distinctions among patients included. Most patients (271 of 434 [62.4%]) in the mutant subgroup had previously received BRAFi with or without MEKi therapy (Table 1). Additionally, more patients with *BRAF* WT than mutant disease were older ( $\geq 65$  years, 552/1125 [49.1%] vs 106/434 [24.4%]) and had previously received ipilimumab treatment (554/1124 [49.3%] vs 148/434 [34.1%]). Univariate analysis revealed that patients with *BRAF* WT melanoma had a higher ORR than those with *BRAF* V600E/K-mutant melanoma (447/1124 [39.8%] vs 149/434 [34.3%]; 5.4% difference [95% CI, 0.1-10.6]) (eFigure 1 in the Supplement) and a similar 4-year PFS rate (22.9% vs 19.8% of patients) (eFigure 2A in the Supplement). Median PFS was numerically longer (hazard ratio, 0.84; 95% CI, 0.74-0.96) for patients with *BRAF* WT (7.9 months [95% CI, 6.4-9.7]) than for patients with *BRAF* V600E/K-mutant melanoma (5.6 months [95% CI, 4.2-6.7]) (Figure, A), as was the 4-year OS rate (37.5% vs 35.1%) (eFigure 2B in the Supplement) and median OS (23.0 months [95% CI, 20.2-26.7] vs 21.8 months [95% CI, 17.1-27.3]) (eFigure 3A in the Supplement). The ORR in patients with *BRAF* WT melanoma (447/1124 [39.8%]) was similar to that in patients with *BRAF* V600E/K-mutant disease (72/163 [44.2%]) not previously treated with BRAFi with or without MEKi.

### Patients With *BRAF* V600E/K-Mutant Melanoma Previously Treated With a BRAFi With or Without MEKi vs Patients With BRAFi With or Without MEKi Therapy-Naive Melanoma

Baseline characteristics were different between patients with *BRAF* V600E/K-mutant disease previously treated with a BRAFi with or without MEKi and those who were BRAFi with or without MEKi therapy naive (Table 1). A greater proportion of patients previously treated with BRAFi with or without MEKi had a higher Eastern Cooperative Oncology Group performance status (103/271 [38.0%] vs 35/163 [21.5%]), PD-L1-negative tumors (63/271 [23.2%] vs 19/163 [11.7%]), M1c disease (222/271 [81.9%] vs 106/163 [65.0%]), stable brain metastases (46/271 [17.0%] vs 13/163 [8.0%]), elevated serum LDH levels (126/271 [46.5%] vs 27/163 [16.6%]), undergone more prior lines of therapy ( $\geq 3$  lines, 81/271 [29.9%] vs 2/163 [1.2%]), undergone prior ipilimumab treatment (138/271 [50.9%] vs 10/163 [6.1%]), larger tumors ( $> 93$  mm, 124/271 [45.8%] vs 31/163 [19.0%]), and lower albumin levels (area under the receiver operating curve  $\leq 0.834$ , 182/271 [67.2%] vs 74/163 [45.4%]).

BRAFi with or without MEKi-treated patients had a lower ORR with pembrolizumab than those who were BRAFi with or without MEKi therapy naive (77/271 [28.4%] vs 72/163 [44.2%]; -15.8% difference [95% CI, -25.0 to -6.5]) (eFigure 1 in the Supplement). In subgroup analysis by baseline characteristics, lower ORR to pembrolizumab was observed in BRAFi with or without MEKi-treated patients vs BRAFi with or without MEKi therapy-naive patients across subgroups. The greatest differences were observed for the following factors: age 65 years and older (21/58 [36.2%] vs 27/48 [56.3%]; difference, -20.0%), PD-L1 positive status (36/136 [26.5%] vs 50/107

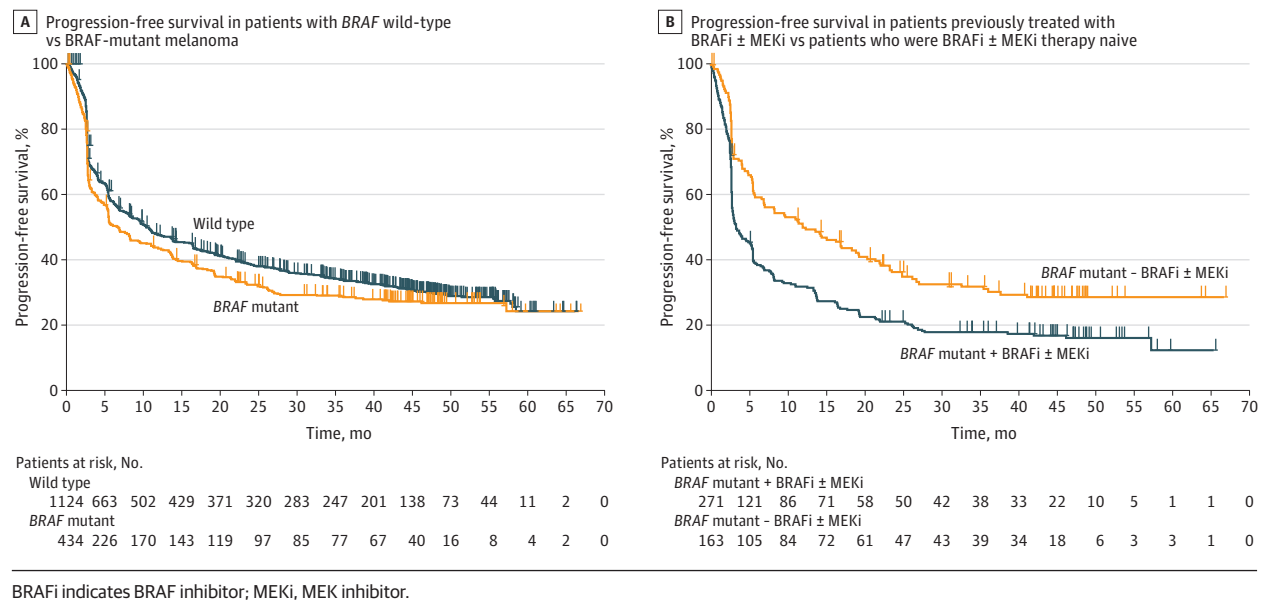
Table 1. Patient Baseline Demographics and Disease Characteristics

Characteristic	No. (%)			
	<i>BRAF</i> WT (n = 1124)	<i>BRAF</i> V600E/K- mutant (n = 434)	<i>BRAF</i> V600E/K-mutant with prior BRAFi with or without MEKi treatment (n = 271)	<i>BRAF</i> V600E/K-mutant with no prior BRAFi with or without MEKi treatment (n = 163)
Age, mean (SD), y	62.4 (13.2)	53.8 (14.2)	53.4 (13.6)	54.5 (15.1)
<65	572 (50.9)	328 (75.6)	213 (78.6)	115 (70.6)
≥65	552 (49.1)	106 (24.4)	58 (21.4)	48 (29.4)
Male	691 (61.5)	253 (58.3)	161 (59.4)	92 (56.4)
ECOG PS				
0	721 (64.1)	296 (68.2)	168 (62.0)	128 (78.5)
1	400 (35.6)	138 (31.8)	103 (38.0)	35 (21.5)
Missing	3 (0.3)	0	0	0
PD-L1 status				
Positive	603 (53.6)	243 (56.0)	136 (50.2)	107 (65.6)
Negative	177 (15.7)	82 (18.9)	63 (23.2)	19 (11.7)
Unknown	151 (13.4)	46 (10.6)	31 (11.4)	15 (9.2)
Missing	193 (17.2)	63 (14.5)	41 (15.1)	22 (13.5)
Metastatic stage				
M0/M1a/M1b	290 (25.8)	106 (24.4)	49 (18.1)	57 (35.0)
M1c	834 (74.2)	328 (75.6)	222 (81.9)	106 (65.0)
Brain metastasis				
No	1022 (90.9)	373 (85.9)	224 (82.7)	149 (91.4)
Yes	98 (8.7)	59 (13.6)	46 (17.0)	13 (8.0)
Missing	4 (0.4)	2 (0.5)	1 (0.4)	1 (0.6)
LDH level				
Elevated	421 (37.5)	153 (35.3)	126 (46.5)	27 (16.6)
Normal	684 (60.9)	275 (63.4)	142 (52.4)	133 (81.6)
Missing	19 (1.7)	6 (1.4)	3 (1.1)	3 (1.8)
Prior lines of therapy				
0	154 (13.7)	41 (9.4)	0	41 (25.2)
1	346 (30.8)	139 (32.0)	111 (41.0)	28 (17.2)
2	232 (20.6)	89 (20.5)	79 (29.2)	10 (6.1)
≥3	151 (13.4)	83 (19.1)	81 (29.9)	2 (1.2)
Missing	241 (21.4)	82 (18.9)	0 (0)	82 (50.3)
Prior ipilimumab exposure				
Exposed	554 (49.3)	148 (34.1)	138 (50.9)	10 (6.1)
Naive	570 (50.7)	285 (65.7)	132 (48.7)	153 (93.9)
Missing	0	1 (0.2)	1 (0.4)	0
Baseline tumor size, mm				
≤93	543 (48.3)	220 (50.7)	117 (43.2)	103 (63.2)
>93	460 (40.9)	155 (35.7)	124 (45.8)	31 (19.0)
Missing	121 (10.8)	59 (13.6)	30 (11.1)	29 (17.8)
Albumin				
AUC ≤0.834	717 (63.8)	256 (59.0)	182 (67.2)	74 (45.4)
AUC >0.834	393 (35.0)	169 (38.9)	84 (31.0)	85 (52.1)
Missing	14 (1.2)	9 (2.1)	5 (1.8)	4 (2.5)
Prior systemic BRAFi therapy				
No	1101 (98.0)	163 (37.6)	0	163 (100)
Yes	23 (2.0)	271 (62.4)	271 (100)	0

Abbreviations: AUC, area under the receiver operating characteristics curve; BRAFi, BRAF inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; MEKi, MEK inhibitor; PD-L1, programmed death ligand 1; WT, wild type.

[46.7%]; –20.3%), metastatic stage (M0/M1a/M1b) (13/49 (23/126 [18.3%] vs 15/27 [55.6%]; –37.3%) (eFigure 1 in the Supplement) [26.5%] vs 29/57 [50.7%]; –24.3%), and elevated LDH level

Figure. Kaplan-Meier Estimates of Progression-Free Survival



Patients who were previously treated with BRAFi with or without MEKi therapy had lower 4-year PFS rates (15.2% vs 27.8%) (eFigure 2A in the Supplement) and shorter median PFS (3.4 months [95% CI, 2.9-5.3] vs 12.0 months [95% CI, 7.0-18.4]) (hazard ratio 1.64 [95% CI, 1.31-2.06]) (Figure, B) than those who were BRAFi with or without MEKi therapy naive. Patients who were previously treated with BRAFi with or without MEKi therapy had lower 4-year OS (26.9% vs 49.3%) (eFigure 2B in the Supplement) and shorter median OS (13.8 months [95% CI, 11.0-17.1] vs 45.4 months [95% CI, 33.9 to not reached]) (eFigure 3B in the Supplement) than those who were BRAFi with or without MEKi therapy naive. Similar to the trend with ORR, lower 4-year PFS rates and 4-year OS rates were observed with pembrolizumab treatment in BRAFi with or without MEKi-treated patients vs BRAFi with or without MEKi therapy-naïve patients across subgroups. Patients who were previously treated with BRAFi with or without MEKi therapy and were 65 years and older (4-year PFS rate, 15.7% vs 37.3%; difference, -21.6%), had disease with metastatic stage M0/M1a/M1b (11.9% vs 36.9%; difference, -24.9%), were PD-L1 positive (12.4% vs 30.8%; difference -18.4%), or had elevated LDH level (9.3% vs 37.8%; difference, -28.4%) had lower 4-year PFS rates than those who were not previously treated with BRAFi with or without MEKi therapy (eFigure 2A in the Supplement). Patients who were previously treated with BRAFi with or without MEKi therapy and 65 years and older (4-year OS rate, 27.6% vs 56.8%; difference, -29.2%), PD-L1-positive (24.7% vs 53.0%, difference, -28.3%), had disease with metastatic stage (M0/M1a/M1b) (37.8% vs 63.6%, difference, -25.7%), or had elevated LDH (14.2% vs 49.7%; difference, -35.5%) had lower 4-year OS rates than those who were BRAFi with or without MEKi therapy naive.

### Factors Associated With Response

Although many baseline characteristics were associated with worse overall response in subgroup analysis (eTable 1 in the Supplement), multivariate analysis confirmed that PD-L1-negative tumors, elevated LDH levels, lower albumin levels, larger baseline tumors, and prior ipilimumab exposure were associated with worse overall response (Table 2). Multivariate analysis accounting for patients with missing baseline tumor size (n = 178) further corroborated these findings (Table 2; eTable 2 in the Supplement). Multivariate analysis of baseline factors associated with worse PFS identified PD-L1-negative tumors, elevated LDH levels, prior ipilimumab exposure, larger baseline tumors, and prior exposure to BRAFi therapy (Table 3). Multivariate analysis with study as a covariate supported these findings (eTable 3 in the Supplement).

### Safety

Any-grade treatment-related adverse events were similar across all subgroups and ranged from 194 of 271 patients (71.6%) to 139 of 164 patients (84.7%) (eTable 4 in the Supplement). The safety profile of pembrolizumab was generally similar regardless of *BRAF* V600E/K mutation status or receipt of prior BRAFi with or without MEKi therapy.

### Discussion

Nearly half of melanomas harbor activating *BRAF* mutations,<sup>19,20</sup> which lead to constitutive activation of the MAPK pathway<sup>21</sup> and provide a target for BRAFi and MEKi therapy.<sup>22</sup> Limited clinical data are available on the association of *BRAF* mutation status or exposure to prior BRAFi with or without MEKi therapy with response to

Table 2. Multivariate Analysis of Factors Associated With Best Overall Response<sup>a</sup>

Risk factor at baseline	Effect	OR (95% CI)	P value
Baseline albumin/albumin ULN	Units = -1 <sup>b</sup>	0.16 (0.05-0.49)	.001
LDH level	Elevated vs normal	0.61 (0.47-0.79)	<.001
Tumor size	>93 mm vs ≤93 mm <sup>c</sup>	0.47 (0.36-0.61)	<.001
Ipilimumab exposure	Ipilimumab exposed vs ipilimumab naive	0.71 (0.56-0.91)	.006
Prior systemic BRAFi therapy	Yes vs no	0.71 (0.52-0.97)	.030
PD-L1 status	Negative vs positive	0.52 (0.37-0.73)	<.001
Sex	Female vs male	0.64 (0.50-0.81)	<.001

Abbreviations: BRAFi, BRAF inhibitor; LDH, lactate dehydrogenase; OR, odds ratio; PD-L1, programmed death ligand 1; ULN, upper limit of normal.

<sup>a</sup> Analysis included all response-evaluable patients, regardless of *BRAF* V600E/K mutation status. Overall response was determined by Response Evaluation Criteria in Solid Tumors, version 1.1, per investigator review

<sup>b</sup> A 1-unit decrease was used to ensure the OR direction for all risk factors was the same.

<sup>c</sup> Cutoff chosen based on the value that showed the most significant difference in response.

Table 3. Multivariate Analysis of Factors Associated With Progression-Free Survival<sup>a</sup>

Risk factor	Effect	HR (95% CI)	P value
Baseline LDH level	Elevated vs normal	1.45 (1.27-1.65)	<.001
ECOG PS at screening	1 vs 0	1.20 (1.06-1.36)	.004
Ipilimumab exposure <sup>b</sup>	Ipilimumab exposed vs ipilimumab naive	1.13 (1.00-1.28)	.042
PD-L1 status	Negative vs positive	1.58 (1.35-1.84)	<.001
Baseline tumor size	>93 mm vs ≤93 mm <sup>c</sup>	1.44 (1.26-1.65)	<.001
Prior systemic BRAFi therapy	Yes vs no	1.31 (1.14-1.52)	<.001
Sex	Female vs male	1.23 (1.09-1.38)	<.001
Albumin	≤0.834 vs >0.834	1.23 (1.08-1.40)	<.001

Abbreviations: BRAFi, BRAF inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; LDH, lactate dehydrogenase; PD-L1, programmed death ligand 1.

<sup>a</sup> Analysis included all patients, regardless of *BRAF* mutation status. Progression-free survival was determined by Response Evaluation Criteria in Solid Tumors, version 1.1, per investigator review.

<sup>b</sup> The clinical study and prior ipilimumab exposure were highly correlated, and only 1 could be included in the model. See eTable 3 in the Supplement for multivariate analysis with study included as a covariate instead of ipilimumab exposure.

<sup>c</sup> Cutoff chosen based on the value that showed the most significant difference in response.

immunotherapies.<sup>12,20</sup> The results of this of this post hoc pooled subgroup analysis of data from 3 clinical trials show that pembrolizumab may provide clinical benefit in patients with *BRAF* WT and *BRAF* V600E/K-mutant advanced melanoma and in patients who were or were not previously treated with BRAFi with or without MEKi therapy. There were no meaningful differences in the safety profile among pembrolizumab-treated patients in the 4 subgroups. The 1558 patients pooled from 3 pivotal trials form, to our knowledge, the largest data set reported for this critical analysis of the association of *BRAF* V600E/K mutation and prior *BRAF*-directed therapy with therapeutic outcomes of single-agent PD-1 blockade. Similar to our findings, in a retrospective post hoc analysis of pooled data from 4 nivolumab clinical trials, patients with *BRAF* WT melanoma had a response to treatment and a safety profile similar to those of patients with mutant disease; however, that report did not evaluate prior exposure to combined BRAFi and MEKi therapy.<sup>23</sup>

As mentioned previously, because of differences in inclusion criteria regarding patients with *BRAF* V600E/K-mutant disease, a greater proportion of BRAFi with or without MEKi-treated vs BRAFi with or without MEKi therapy-naive patients in the current pooled analysis had baseline characteristics known to be associated with worse outcomes in patients

with melanoma, such as PD-L1-negative tumors (63/271 [23.2%] vs 19/163 [11.7%]), elevated LDH levels (126/271 [46.5%] vs 27/163 [16.6%]), higher Eastern Cooperative Oncology Group performance status (103/271 [38.0%] vs 35/163 [21.5%]), larger baseline tumors (>93 mm, 124/271 [45.8%] vs 31/163 [19.0%]), and lower albumin levels (area under receiver operating curve ≤0.834, 182/271 [67.2%] vs 74/163 [45.4%]) (Table 1). For example, low serum LDH level has been associated with favorable OS in patients with melanoma<sup>24</sup> and PD-L1 expression correlates with pembrolizumab response.<sup>25</sup> Worse outcomes—namely, lower ORR and 4-year PFS and OS rates in patients with *BRAF* V600E/K-mutant melanoma treated with BRAFi with or without MEKi therapy than those who were BRAFi with or without MEKi therapy naive—are likely to have resulted because of the aforementioned imbalance in baseline characteristics.

Another explanation for the lower ORR in patients previously treated with BRAFi with or without MEKi therapy vs those who were naive to BRAFi with or without MEKi therapy is that prior BRAFi with or without MEKi treatment may have led to long-term response in patients with favorable baseline characteristics<sup>26</sup> (normal LDH levels, fewer than 3 sites with metastatic disease) and therefore selection of patients with less favorable characteristics or pembrolizumab resistance.

Inhibition of the MAPK pathway in advanced melanoma has been associated with transcriptional induction of the innate anti-PD-1 resistance signature, involving mesenchymal transition, angiogenesis, hypoxia, and wound-healing genes.<sup>27</sup>

Lower response rates were observed in patients with melanoma and brain metastases treated with nivolumab and ipilimumab after progression on BRAFi and MEKi therapy compared with patients who were treatment naive.<sup>28</sup> Simeone et al<sup>29</sup> reported similar findings from a retrospective study of patients with metastatic melanoma who had previously received ipilimumab when response to pembrolizumab was affected by prior BRAFi treatment; patients with *BRAF* V600-mutant melanoma previously treated with a BRAFi had lower median PFS and disease control rate with pembrolizumab than patients with WT disease.<sup>29</sup> This observation may have important ramifications for therapy sequencing for *BRAF* V600-mutant melanoma and is consistent with observations of earlier retrospective reports that outcomes with ipilimumab were inferior in patients with *BRAF* V600-mutant melanoma previously treated with BRAFi compared with outcomes with ipilimumab as first-line therapy.<sup>12,20</sup>

Ongoing randomized clinical trials will allow these outcomes to be better understood and analyzed for correlative, prognostic, and predictive factors. Results of a 2017 retrospective study<sup>3</sup> to investigate the sequencing of BRAFi with or without MEKi and anti-PD-1 therapy for the treatment of *BRAF* V600-mutant melanoma indicated that BRAFi with or without MEKi and anti-PD-1 therapies were effective when used as front-line therapy, whereas anti-PD-1 therapy was only moderately effective after progression with BRAFi with or without MEKi therapy, and BRAFi therapy resulted in a poor response when used as salvage therapy after anti-PD-1 therapy.<sup>3</sup> Perhaps a subgroup of patients is likely to benefit from both classes of therapies, whereas those with aggressive disease may exhibit poor response to both classes of therapies.<sup>30</sup> The definitive answer to the question regarding the optimal sequence of immunotherapy and targeted therapy for patients with *BRAF* V600E/K-mutated melanoma will come from randomized trials including EA6134,<sup>31</sup> SECOMBIT,<sup>32</sup> EBIN,<sup>33</sup> COWBOY,<sup>34</sup> NeoTrio,<sup>35</sup> and ImmunoCobiVem.<sup>36</sup>

An alternative approach involving a combination of PD-1 inhibitors with BRAFi and MEKi is currently being tested in clinical trials for patients with *BRAF* V600-mutant melanoma.<sup>37,38</sup> This combination strategy is based on the immune-modulating effects of BRAF and MEK inhibition. The phase 2 KEYNOTE-022 trial of pembrolizumab plus dabrafenib and trametinib (triplet therapy) in patients with treatment-naive *BRAF* V600E/K-mutant advanced melanoma showed numerically longer PFS and duration of response with triplet therapy compared with dabrafenib and trametinib

alone.<sup>38</sup> Initial results from the phase 3 COMBI-i trial of spartalizumab plus dabrafenib and trametinib, and the phase 2 TRI-DeNT trial of nivolumab with dabrafenib and trametinib, reported promising preliminary efficacy and manageable safety in *BRAF* V600-mutant melanoma.<sup>37,39</sup> However, the optimal regimen (PD-1 inhibitor and BRAFi and MEKi) and sequence of these therapies remains to be established.

### Limitations

The primary limitations of this analysis were its retrospective nature and the pooling of data from 3 trials with different study designs and patient populations. Of particular note were the different inclusion criteria, particularly criteria pertaining to patients with *BRAF* V600E/K-mutant melanoma, across the 3 studies. Patients with *BRAF* V600E/K-mutant melanoma had to be previously treated with BRAFi with or without MEKi therapy in KEYNOTE-002 and in KEYNOTE-001 if they were ipilimumab refractory. In contrast, in KEYNOTE-006, patients with *BRAF* V600E/K-mutant melanoma who were BRAFi naive were eligible if they had a normal LDH level and no clinically significant tumor-related symptoms or evidence of rapidly progressing melanoma (thereby excluding BRAFi-naive patients with high LDH level and poorer prognosis), whereas those who had received prior BRAFi were eligible regardless of LDH level. Thus, patients with *BRAF* V600E/K-mutant melanoma who received pembrolizumab followed by BRAFi with or without MEKi might have had a better prognosis than those who received the reverse sequence. As in most immunotherapy clinical trials, patients with Eastern Cooperative Oncology Group performance status (0 or 1) were enrolled, which may not reflect a real-world population.<sup>40</sup> Furthermore, in countries where targeted therapies are available for *BRAF* V600E/K-mutant melanoma, patients with *BRAF* WT tumors may be more likely to enroll in clinical studies than patients with *BRAF* V600E/K-mutant melanoma due to a lack of alternative treatment options. Owing to the retrospective nature of the current analysis, definitive conclusions cannot be made regarding sequencing. In addition, the small sample size for some of the subgroups also limits the interpretation.

### Conclusions

In conclusion, results of this post hoc pooled subgroup analysis support the use of pembrolizumab for the treatment of patients with advanced melanoma regardless of *BRAF* V600E/K mutation status or use of BRAFi with or without MEKi therapy. Results of well-designed randomized studies that evaluate potential determinants of outcome will help to determine optimal first-line agents for defined subgroups of patients with advanced melanoma.

#### ARTICLE INFORMATION

Accepted for Publication: April 21, 2020.

Published Online: July 16, 2020.  
doi:10.1001/jamaoncol.2020.2288

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**Obtained funding:** Puzanov.

**Administrative, technical, or material support:** Puzanov, Daud, Carlino, Long, Schadendorf, Homet Moreno, Hamid.

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**Conflict of Interest Disclosures:** Dr Puzanov reports personal fees from Amgen, Bristol-Myers Squibb, Oncorus, and Synthorx outside the submitted work. Dr Ribas reports personal fees from Merck during the conduct of the study; personal fees from 4C Biomed, Advaxis, Amgen, Apricity, Arcus, Chugai, Compugen, CytomX, Five Prime, Genentech, Jounce, Highlight, ImaginAb, Isoplexis, Kite-Gilead, MapKure, Merck, Merus, Novartis, Nurix, RAPT, Rgenix, Lutris, PACT Pharma, Sanofi, Tango, and Vedanta outside the submitted work; and research funding from Agilent and Bristol-Myers Squibb. In addition, Dr Ribas serves on the scientific advisory board and holds stock in 4C Biomed, Apricity, Arcus, Compugen, Highlight, ImaginAb, MapKure, Merus, Rgenix, Lutris, PACT Pharma, and Tango Therapeutics. Dr Ribas served on the scientific advisory board and holds stocks in Advaxis, CytomX, Five Prime, RAPT, Isoplexis, and Kite-Gilead. Dr Ribas reports honoraria for consulting from Amgen, Chugai, Genentech, Jounce, Merck, Novartis, Nurix, Sanofi, and Vedanta. Dr Robert reports personal fees and advisory board compensation from Biothera, Bristol-Myers Squibb, CureVac, Merck Sharp &

Dohme, Novartis, Pierre Fabre, Roche, and Sanofi, during the conduct of the study. Dr Nyakas reports personal fees for advisory boards and lectures from Novartis, Bristol-Myers Squibb, and Pierre Fabre outside of the submitted work. Dr Daud reports research grants from Bristol-Myers Squibb, CheckMate, Genentech, Incyte, Merck, Pfizer, and Regeneron during the conduct of the study, and grants from OncoSec, personal fees from Regeneron, and stock in Trex outside the submitted work. Dr Arance reports personal fees and travel compensation from Amgen, Bristol-Myers Squibb, Merck, Merck Sharp & Dohme, Novartis, Pierre Fabre, Roche, and Sanofi outside the submitted work. Dr Carlino reports personal fees for advisory boards from Amgen, Bristol-Myers Squibb, Ideaya, Merck Sharp & Dohme, Nektar, Novartis, Pierre Fabre, Merck Serono, Roche, and Sanofi outside the submitted work. Dr O'Day reports research grants and advisory board support from Merck, Biothera, Agenus, Bristol-Myers Squibb, and ImaginAB; research grants from Amgen, Genocoea, Incyte, Viralytics, Oncotherapeutics, Ultimovacs, and Exicure; and consultancy fees from RadImmune and personal fees for advisory boards from Immunsys. Dr Long reports personal fees from Aduro, Amgen, Array, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Oncosec, Pierre Fabre, and Roche outside the submitted work. Dr Dummer reports consultancy fees from Amgen, Bristol-Myers Squibb, Catalym, Merck Sharp & Dohme, Novartis, Pierre Fabre, Roche, Sanofi, Sun Pharma, Second Genome, Regeneron, Alligator, and Takeda outside the submitted work. Dr Schadendorf reports grants, personal fees, nonfinancial support, and other support from Merck Sharp & Dohme during the conduct of the study. Dr Schadendorf also reports grants, personal fees, nonfinancial support, and other from Bristol-Myers Squibb, Novartis, Roche, and Array; personal fees from Immunocore, Eisai, InflaRx, ultimovacs, Neracare, Sun Pharma, and Sandoz; personal fees and other from 4SC and Philogen; personal fees, nonfinancial support, and other from Merck Serono, Sanofi Regeneron, and Pierre Fabre; and personal fees and nonfinancial support from Nektar outside the submitted work. Dr Lutzky reports receiving advisory board fees from Array, Regeneron, Castle, and Kimera, speaker fees from Regeneron and Array, and research funding from Regeneron, Immunocore, Vyriad, Bristol-Myers Squibb, Merck, Viralytics, Pfizer, Idera, and Incyte outside the submitted work. Dr Ascierto reports research funds and fees for advisory boards for Array Biopharma, Bristol-Myers Squibb, and Roche-Genentech; fees for advisory boards and travel from Merck Sharp & Dohme; and fees for advisory boards for 4SC, AstraZeneca, Genmab, Idera, Immunocore, Incyte, MedImmune, Merck Serono, Pierre Fabre, NewLink Genetics, Novartis, Sandoz, Sanofi, Sun Pharma, Syndax, and Ultimovacs outside the submitted work. Dr Tahini reports research funds from Merck during the conduct of the study and personal fees from Bristol-Myers Squibb, Novartis, Genentech-Roche, Array, Sanofi-Genzyme/Regeneron, Immunocore, and BIONTech outside the submitted work. Drs Lin, Mogg, Homet Moreno, and Ibrahim report employment with Merck Sharp & Dohme. Dr Lin reports stock ownership with Merck. Dr Mogg reports personal fees from Merck & Co, Inc, during the conduct of the study; and personal fees from Merck & Co, Inc, outside the submitted work. Dr

Ibrahim reports stock ownership with GlaxoSmithKline. Dr Hamid reports consulting and personal fees from Aduro, Akeso, Amgen, Array, Beigene, Bristol-Myers Squibb, Genentech, GlaxoSmithKline, Immunocore, Incyte, Janssen, Merck, Nextcure, Novartis, Sanofi Regeneron, Seattle Genetics, Tempus, and Zelluna during the conduct of the study; speaker and personal fees from Array, Bristol-Myers Squibb, Novartis, and Sanofi Regeneron during the conduct of the study; and research funding from Arcus, Aduro, Akeso, Amgen, Array, Bristol-Myers Squibb, CytomX, Exelixis, Genentech, GlaxoSmithKline, Immunocore, Incyte, Iovance, Merck, Merck Serono, Moderna, Nextcure, Novartis, Sanofi, Regeneron, Seattle Genetics, Torque, and Zelluna outside the submitted work. No other disclosures were reported.

**Funding/Support:** Funding for this research was provided by Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey.

**Role of the Funder/Sponsor:** Merck Sharp & Dohme had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Additional Contributions:** The authors thank the patients and their families and all investigators and site personnel. The authors also thank the following employees of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey, for critical manuscript review: Scot Ebbinghaus, MD; Scott J. Diede, MD, PhD; and James R. Anderson, PhD. Medical writing and/or editorial assistance was provided by Doyel Mitra, PhD, CMPP, of the ApotheCom pembrolizumab team (Yardley, Pennsylvania). This medical writing and/or editorial assistance was funded by Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey.

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