Supplementary Online Content

Puzanov I, Ribas A, Robert C, et al. Association of *BRAF* V600E/K mutation status and prior BRAF/MEK inhibition with pembrolizumab outcomes in advanced melanoma: pooled analysis of 3 clinical trials. *JAMA Oncol*. Published online July 16, 2020. doi:10.1001/jamaoncol.2020.2288.

eMethods

eTable 1. Univariate Analysis of Factors Associated With Best Overall Response per RECIST v 1.1 per Investigator Review

eTable 2 Multivariate Analysis of Factors Associated With Best Overall Response per RECIST v 1.1 per Investigator Review, Accounting for Patients With Missing Baseline Tumor Size

eTable 3. Multivariate Analysis of Factors Associated With Progression-Free Survival per RECIST v1.1 per Investigator Review With Study as a Covariate

eTable 4. Any-Grade Treatment-Related Adverse Events That Occurred in ≥5% of Patients

eFigure 1. Overall Response Rate in Evaluable Patients (N = 1558) With *BRAF* Wild-Type Versus Mutant Melanoma

eFigure 2. Four-Year Progression-Free Survival (PFS) and Overall Survival (OS) in Patients With *BRAF* Wild-Type Versus Mutant Melanoma

eFigure 3. Kaplan-Meier Estimates of Overall Survival

This supplementary material has been provided by the authors to give readers additional information about their work.

eMETHODS

Study Design and Patients

KEYNOTE-001, an international, open-label, phase 1 study of pembrolizumab, enrolled patients with locally advanced/metastatic melanoma not amenable to local therapy and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 who were either ipilimumab naive (received ≤2 prior systemic treatments for melanoma), ipilimumab treated (<12 weeks from first ipilimumab dose and >6 weeks from last dose with documented progressive disease [PD]), or ipilimumab refractory (documented PD within 24 weeks of last ipilimumab dose). KEYNOTE-002, an international, phase 2, randomized controlled trial to compare pembrolizumab with investigator choice of chemotherapy, enrolled patients with ECOG performance status 0 or 1, who were ipilimumab-refractory (received ≥2 doses of ipilimumab with confirmed PD after last ipilimumab dose), and had unresectable stage III/IV melanoma not amenable to local therapy. KEYNOTE-006, an international, randomized, open-label, phase 3 study to compare pembrolizumab with ipilimumab, enrolled patients with unresectable stage III/IV ipilimumab-naive melanoma not amenable to local therapy, who had received ≤1 prior systemic therapy for advanced/metastatic disease, had received no previous treatment with anti–CTLA-4, anti–programmed death 1 [PD-1], or anti–programmed death ligand 1 [PD-L1] agents, and with known *BRAF*^{V600} mutation status.

Regarding BRAF mutation status, in KEYNOTE-001, ipilimumab-naive patients with *BRAF*$^{V600E/K}$ mutation might have previously received treatment with a BRAFi ± MEKi and ipilimumab-refractory patients with *BRAF*$^{V600E/K}$ mutation were required to have received prior BRAFi ± MEKi therapy. In KEYNOTE-002, patients with *BRAF*$^{V600E/K}$ mutation were required to have previously received BRAFi ± MEKi therapy. In KEYNOTE-006, patients with *BRAF*$^{V600E/K}$ mutation might have previously received prior BRAFi ± MEKi therapy as first-line systemic therapy; however, BRAFi ± MEKi therapy was not required for patients with normal lactate dehydrogenase levels and no clinically significant tumor-related symptoms or evidence of rapid disease progression.

PD-L1 Expression

PD-L1 expression was assessed in tumor biopsy samples by immunohistochemistry using the 22C3 PD-L1 IHC assay (Agilent Technologies, Santa Clara, CA)**.**

Statistical Analysis

To determine baseline risk factors associated with best overall response, univariable analysis of each independent variable was conducted; factors for which the univariable test had a *P* value of less than 0.05 and the factor had clinical relevance were selected for the multivariable logistic regression model. A stepwise selection method was used to select risk factors in the final model. No multiplicity adjustments were made in the univariate analysis.

To control for the differences in inclusion and exclusion criteria across clinical studies (KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006), the clinical study was added as a covariate to the model on baseline factors associated with progression-free survival (PFS) (eTable 3). We conducted a sensitivity analysis wherein the model included the clinical study (KEYNOTE-001, KEYNOTE-002, or KEYNOTE-006) as a covariate.

A key assumption in the logistic regression model was that the logits were linearly related to each independent variable; this assumption was examined using the logit plot. In the final model, the logit plot of the continuous variable albumin/albumin upper limit of normal demonstrated that this assumption was valid. The Hosmer-Lemeshow Goodness-of-fit test was used to test model performance. No interaction terms were included in the model. Available case analysis was used to address missing data. The majority of missing data was for baseline tumor size; therefore, a sensitivity analysis was used where all patients with missing baseline tumor size were categorized into a third subgroup. The results of the sensitivity analyses were consistent with the final model.

eTable 1. Univariate Analysis of Factors Associated With Best Overall Response per RECIST v 1.1 per Investigator Review

Risk Factor	Effect	Odds Ratio	95% Confidence Interval	*P*
Age group	<65 vs ≥65	0.732	(0.60-0.90)	.00293
Baseline LDH level	Elevated vs normal	0.406	(0.32-0.51)	<.00001
BRAF mutation	Mutant vs wild type	0.790	(0.63-1.00)	.04597
Brain metastasis	No vs yes	1.055	(0.75-1.48)	.75556
Baseline tumor size	≤93 mm vs >93 mm[a]	2.951	(2.34-3.72)	<.00001
Metastatic staging	M0/M1a/M1b vs M1c	1.788	(1.42-2.25)	<.00001
ECOG performance status at screening	0 vs 1	1.499	(1.20-1.87)	.00029
Ipilimumab exposure	Exposed vs naive	0.606	(0.49-0.75)	<.00001
LDH/(LDH ULN)	—	0.476	(0.39-0.58)	<.00001
No. of metastasis locations	—	0.862	(0.81-0.92)	<.00001
Prior systemic BRAFi therapy	No vs yes	1.724	(1.31-2.27)	.00011
PD-L1 status	Unknown vs PD-L1 negative	2.055	(1.45-2.91)	.04340
	PD-L1 positive vs PD-L1 negative	2.454	(1.81-3.33)	<.00001
No. of prior melanoma systemic therapies	0 vs ≥3	2.236	(1.60-3.12)	<.00001
	1 vs ≥3	1.444	(1.03-2.03)	.97702
	2 vs ≥3	1.333	(0.92-1.92)	.43886
Sex	Female vs male	0.615	(0.50-0.76)	<.00001
Clinical study	KEYNOTE-001 vs KEYNOTE-006	0.921	(0.73-1.16)	.01945
	KEYNOTE-002 vs KEYNOTE-006	0.512	(0.39-0.68)	<.00001
Planned treatment for period KEYNOTE-001	10 mg/kg Q2W vs 2 mg/kg Q3W	1.759	(1.31-2.36)	.00112
	10 mg/kg Q3W vs 2 mg/kg Q3W	1.443	(1.10-1.89)	.42447
Weight, kg	—	1.009	(1.00-1.01)	.00086

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; Q2W, every 2 weeks; Q3W, every 3 weeks; ULN, upper limit of normal.

[a]Cutoff chosen based on the value that showed the most significant difference in response.

eTable 2 Multivariate Analysis[a] of Factors Associated With Best Overall Response per RECIST v 1.1 per Investigator Review, Accounting for Patients With Missing Baseline Tumor Size

Risk Factor	Effect	Odds Ratio	95% Confidence Interval	*P*
Albumin/albumin ULN	units = -1[b]	0.206	(0.07-0.60)	.00356
Baseline LDH level	Elevated vs normal	0.588	(0.46-0.75)	.00003
Baseline tumor size	>93 mm vs ≤93 mm[c]	0.474	(0.36-0.62)	.00034
Ipilimumab exposure	Exposed vs naive	0.744	(0.59-0.93)	.01001
Prior systemic BRAFi therapy	Yes vs no	0.666	(0.50-0.89)	.00697
PD-L1 status	Negative vs Positive	0.476	(0.35-0.66)	<.00001
Sex	Female vs male	0.620	(0.49-0.78)	.00004

Abbreviations: LDH, lactate dehydrogenase; ULN, upper limit of normal.

[a]Analysis included all response-evaluable patients, regardless of *BRAF*-mutation status.
[b]One unit decrease was used to ensure the odds ratio direction for all risk factors was the same.
[c]Cutoff chosen based on the value that showed the most significant difference in response.

eTable 3. Multivariate Analysis of Factors Associated With Progression-Free Survival per RECIST v1.1 per Investigator Review With Study as a Covariate

Risk Factor	Effect	Hazard Ratio	95% CI	*P*
Baseline LDH level	Elevated vs normal	1.432	(1.257-1.632)	<.0001
ECOG performance status at screening	1 vs 0	1.199	(1.059-1.358)	.0043
PD-L1 status	Negative vs positive	1.536	(1.311-1.800)	<.0001
Baseline tumor size	>93 mm vs ≤93 mm[b]	1.477	(1.288-1.694)	<.0001
Prior systemic BRAFi therapy	Yes vs no	1.307	(1.131-1.510)	.0003
Sex	Female vs male	1.223	(1.086-1.379)	.0010
Clinical Study	KEYNOTE-001 vs KEYNOTE006	0.891	(0.756-1.049)	.1666
Clinical Study	KEYNOTE-002 vs KEYNOTE-006	1.178	(1.004-1.382)	.0441
Albumin	≤0.834 vs >0.834	1.233	(1.086-1.400)	.0012

Abbreviations: BRAFi, BRAF inhibitor; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PD-L1, programmed death ligand 1; RECIST, Response Evaluation Criteria in Solid Tumors.
[a]Analysis included all patients regardless of *BRAF*-mutation status. [b]Cutoff chosen based on the value that showed the most significant difference in response.

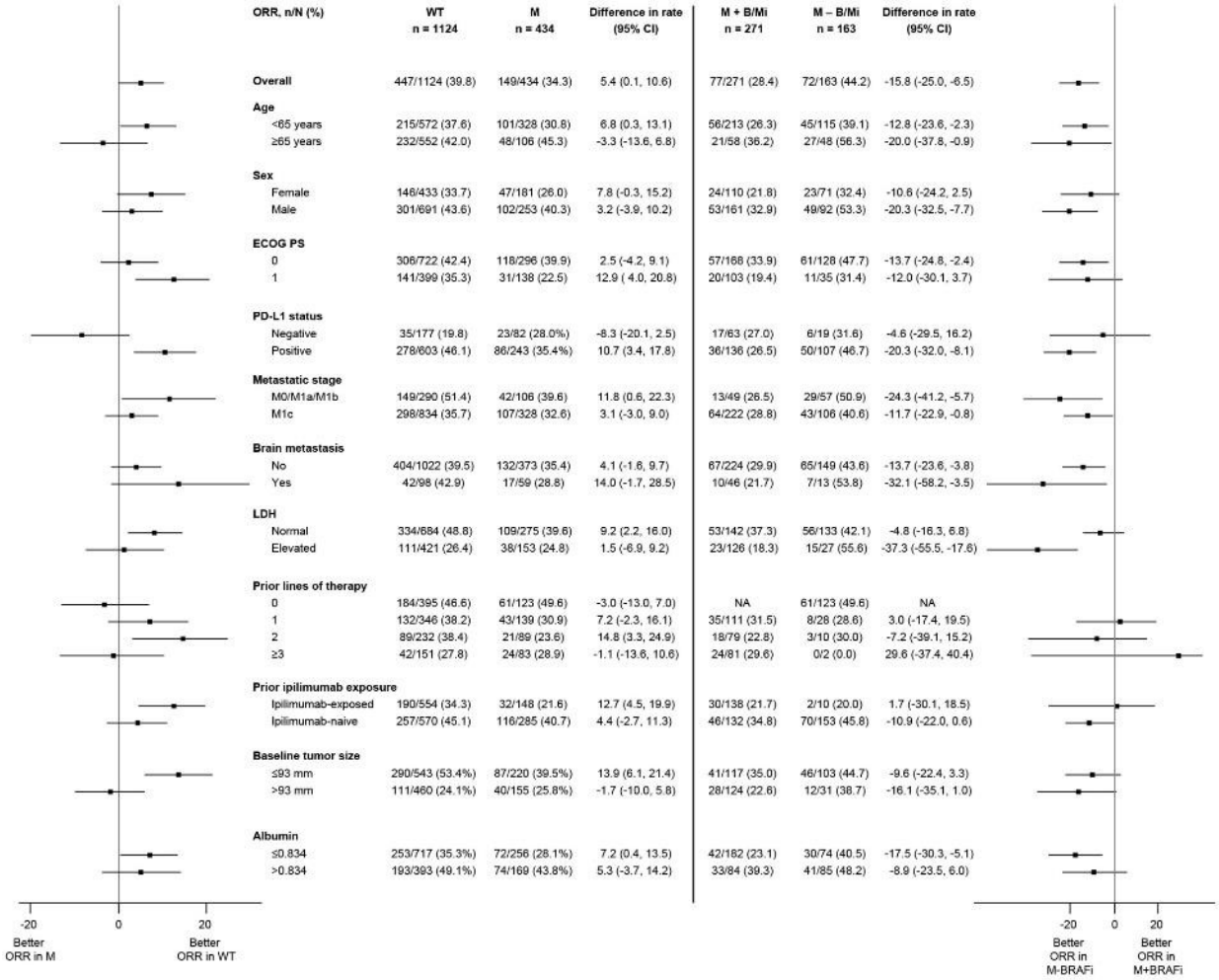
eTable 4. Any-Grade Treatment-Related Adverse Events That Occurred in ≥5% of Patients

Adverse Event, No. (%)	WT n = 1124	M n = 434	M + B/Mi n = 271	M – B/Mi n = 164
Any	922 (82.0)	333 (76.7)	194 (71.6)	139 (84.7)
Fatigue	388 (34.5)	120 (27.6)	66 (24.4)	54 (33.1)
Pruritus	294 (26.2)	90 (20.7)	47 (17.3)	43 (26.4)
Diarrhea	207 (18.4)	74 (17.1)	39 (14.4)	35 (21.5)
Rash	232 (20.6)	56 (12.9)	29 (10.7)	27 (16.6)
Arthralgia	172 (15.3)	53 (12.2)	34 (12.5)	19 (11.7)
Vitiligo	131 (11.7)	56 (12.9)	26 (9.6)	30 (18.4)
Nausea	144 (12.8)	62 (14.3)	34 (12.5)	28 (17.2)
Hypothyroidism	93 (8.3)	50 (11.5)	25 (9.2)	25 (15.3)
Asthenia	107 (9.5)	48 (11.1)	28 (10.3)	20 (12.3)
Myalgia	93 (8.3)	33 (7.6)	16 (5.9)	17 (10.4)
Headache	69 (6.1)	29 (6.7)	12 (4.4)	17 (10.4)
Decreased appetite	111 (9.9)	21 (4.8)	15 (5.5)	6 (3.7)
Cough	77 (6.9)	24 (5.5)	12 (4.4)	12 (7.4)
Pyrexia	66 (5.9)	17 (3.9)	12 (4.4)	5 (3.1)
Dyspnea	53 (4.7)	23 (5.3)	13 (4.8)	10 (6.1)
Vomiting	49 (4.4)	26 (6.0)	15 (5.5)	11 (6.7)
AST increased	48 (4.3)	23 (5.3)	13 (4.8)	10 (6.1)
ALT increased	45 (4.0)	24 (5.5)	13 (4.8)	11 (6.7)
Abdominal pain	38 (3.4)	22 (5.1)	11 (4.1)	11 (6.7)
Hyperthyroidism	28 (2.5)	20 (4.6)	10 (3.7)	10 (6.1)
Dry mouth	41 (3.6)	24 (5.5)	12 (4.4)	12 (7.4)
Dry skin	47 (4.2)	18 (4.1)	9 (3.3)	9 (5.5)
Chills	57 (5.1)	10 (2.3)	5 (1.8)	5 (3.1)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; + B/Mi, prior treatment with BRAFi and/or MEK inhibitor; – B/Mi, no prior treatment with BRAFi and/or MEK inhibitor; M, mutant *BRAF*$^{V600E/K}$; WT, wild type *BRAF*^{V600}.

eFigure 1. Overall Response Rate in Evaluable Patients (N = 1558) With *BRAF* Wild-Type Versus Mutant Melanoma

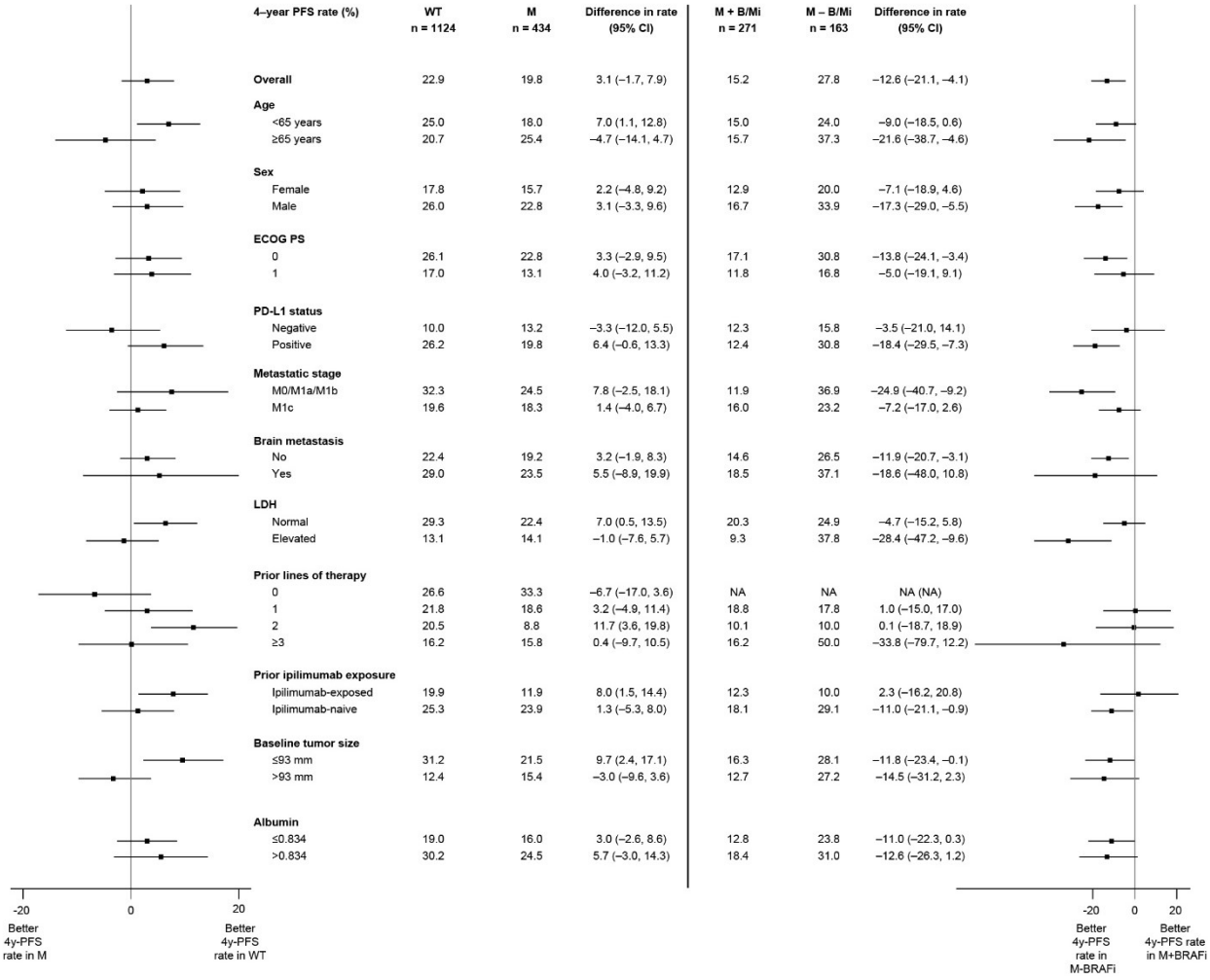
Overall response rate in patients with WT versus mutant melanoma (left) and patients who were BRAFi ± MEKi treated versus BRAFi ± MEKi therapy naive (right). Abbreviations: +, B/Mi, prior treatment with BRAFi and/or MEK inhibitor; –, B/Mi, no prior treatment with BRAFi and/or MEK inhibitor; diff, difference; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; M, mutant $BRAF^{V600E/K}$; NA, not applicable; ORR, objective response rate; WT, wild-type $BRAF^{V600}$.



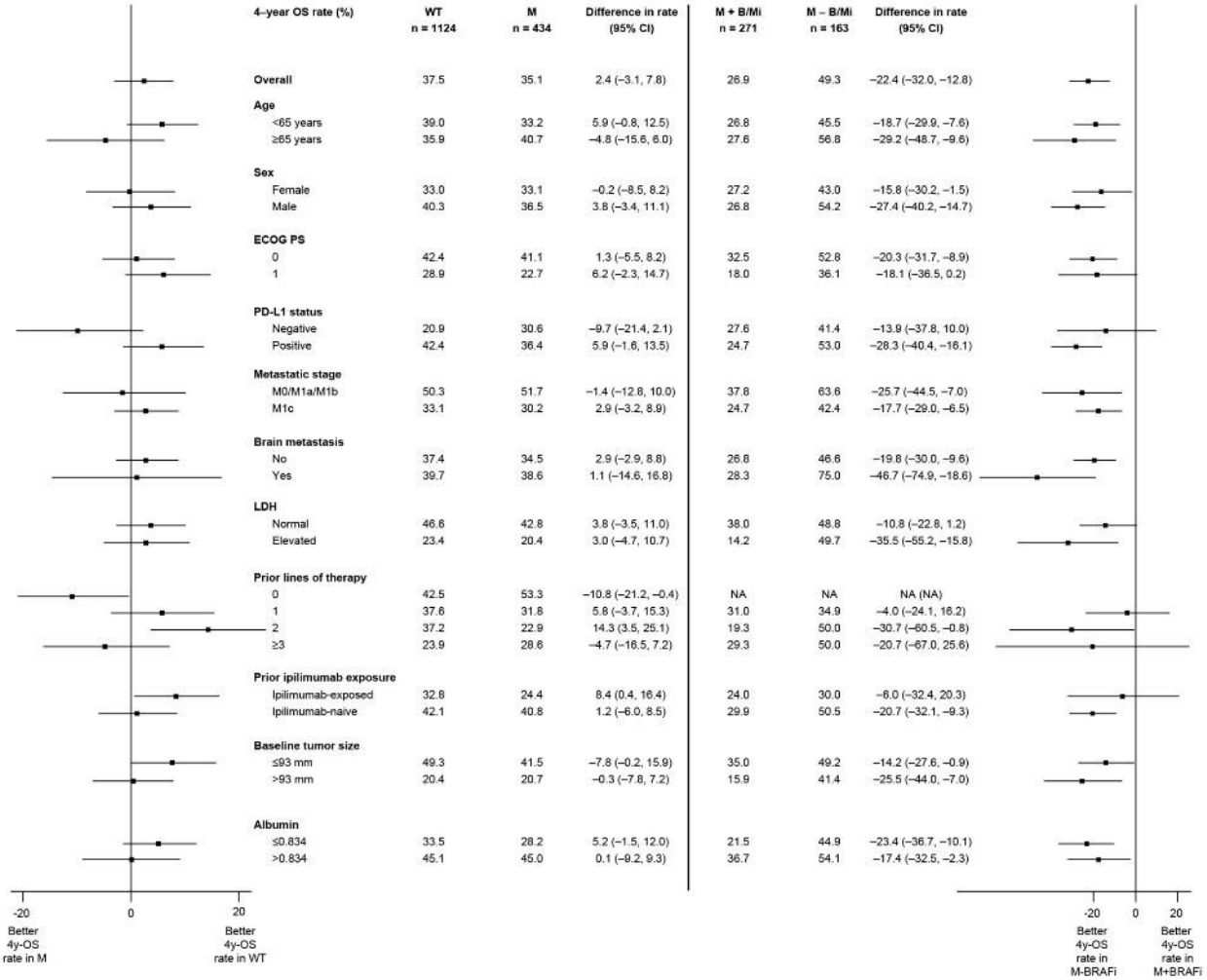
eFigure 2. Four-Year Progression-Free Survival (PFS) and Overall Survival (OS) in Patients With *BRAF* Wild-Type Versus Mutant Melanoma

A, 4-year PFS rate in evaluable patients (N = 1558) with *BRAF* WT versus mutant melanoma (left), and patients with mutant disease who were BRAFi ± MEKi treated versus BRAFi ± MEKi therapy naive (right). B, 4-year OS rate in evaluable patients (N = 1558) with *BRAF* WT versus mutant melanoma (left), and patients with mutant disease who were BRAFi ± MEKi treated versus BRAFi ± MEKi therapy naive (right). Abbreviations: +, B/Mi, prior treatment with BRAFi and/or MEK inhibitor; – B/Mi, no prior treatment with BRAFi and/or MEK inhibitor; diff, difference; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; M, mutant $BRAF^{V600E/K}$; OS, overall survival; PFS, progression-free survival; PM, person-month; WT, wild-type $BRAF^{V600}$.

A

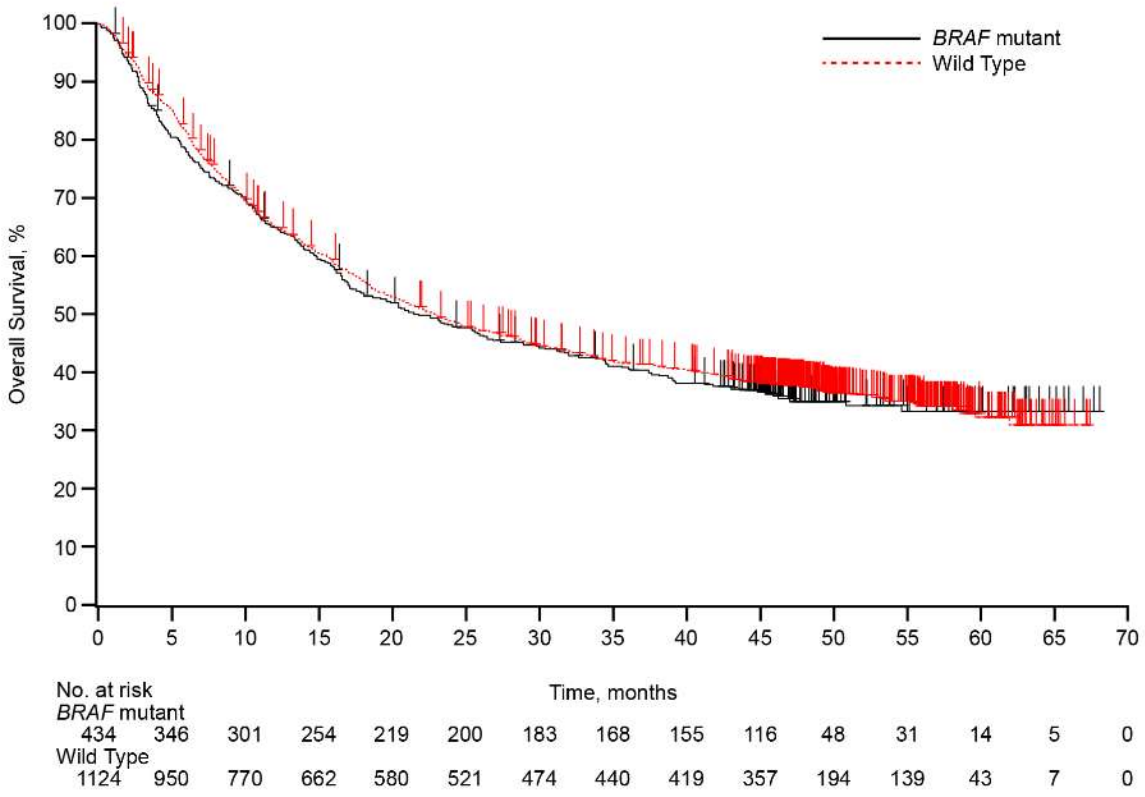


B



eFigure 3. Kaplan-Meier Estimates of Overall Survival
A, Patients with *BRAF* wild-type versus mutant melanoma. B, Patients with mutant disease, and patients who were BRAFi ± MEKi treated versus BRAFi ± MEKi naive

A



B