Supplementary Online Content

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BRAF/MEK inhibition with pembrolizumab outcomes in advanced melanoma: pooled analysis of

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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.

Risk Factor	Effect	Odds	95% Confidence	P	
		Ratio	Interval		
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	\leq 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	0 vs 1	1.499	(1.20-1.87)	.00029	
screening					
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	2.055	(1.45-2.91)	.04340	
	negative				
	PD-L1 positive vs PD-L1	2.454	(1.81-3.33)	<.00001	
	negative				
No. of prior melanoma systemic	0 vs≥3	2.236	(1.60-3.12)	<.00001	
therapies	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	0.921	(0.73-1.16)	.01945	
	KEYNOTE-006				
	KEYNOTE-002 vs	0.512	(0.39-0.68)	<.00001	
	KEYNOTE-006				
Planned treatment for period	10 mg/kg Q2W vs 2 mg/kg	1.759	(1.31-2.36)	.00112	
KEYNOTE-001	Q3W				
	10 mg/kg Q3W vs 2 mg/kg	1.443	(1.10-1.89)	.42447	
	Q3W				
Weight, kg	<u> </u>	1.009	(1.00-1.01)	.00086	

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

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

^aCutoff 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	P
			Interval	
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.

^aAnalysis included all response-evaluable patients, regardless of *BRAF*-mutation status.

^bOne unit decrease was used to ensure the odds ratio direction for all risk factors was the same.

°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	Р
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. ^aAnalysis included all patients regardless of *BRAF*-mutation status. ^bCutoff chosen based on the value that showed the most significant difference in response.

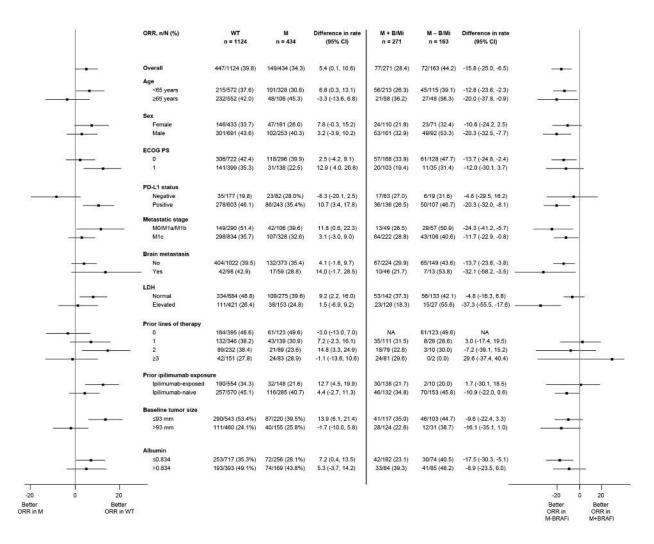
Adverse French No. (9/)	WT	Μ	M + B/Mi	M – B/Mi
Adverse Event, No. (%)	n = 1124	n = 434	n = 271	n = 164
Any	922 (82.0)	333 (76.7)	194 (71.6)	139 (84.7)
Fatigue	388 (34.5)	120 (27.6)	66 (24.4)	54 (33.1)
Pruritus	294 (26.2)	90 (20.7)	47 (17.3)	43 (26.4)
Diarrhea	207 (18.4)	74 (17.1)	39 (14.4)	35 (21.5)
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)

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

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 \pm MEKi treated versus BRAFi \pm 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 \pm MEKi treated versus BRAFi \pm 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 \pm MEKi treated versus BRAFi \pm 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}.

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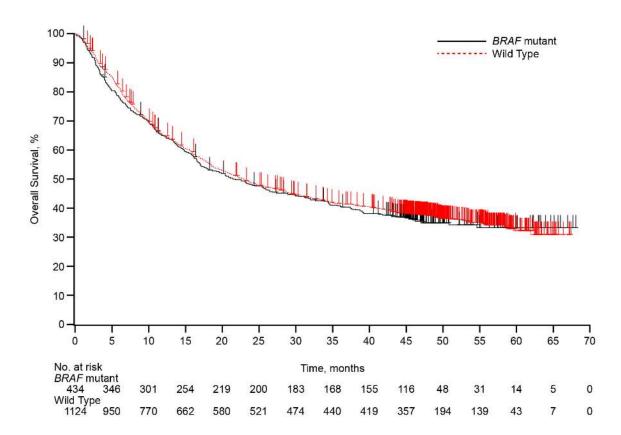
		4–year PFS rate (%)	WT n = 1124	M n = 434	Difference in rate (95% CI)	M + B/Mi n = 271	M – B/Mi n = 163	Difference in rate (95% CI)	
	_ -	Overall	22.9	19.8	3.1 (-1.7, 7.9)	15.2	27.8	-12.6 (-21.1, -4.1)	-
		Age							
	· · · · · · · · · · · · · · · · · · ·	<65 years	25.0	18.0	7.0 (1.1, 12.8)	15.0	24.0	-9.0 (-18.5, 0.6)	
	•	≥65 years	20.7	25.4	-4.7 (-14.1, 4.7)	15.7	37.3	-21.6 (-38.7, -4.6)	
		Sex							
	· · · · · · · · · · · · · · · · · · ·	Female	17.8	15.7	2.2 (-4.8, 9.2)	12.9	20.0	-7.1 (-18.9, 4.6)	
		Male	26.0	22.8	3.1 (-3.3, 9.6)	16.7	33.9	-17.3 (-29.0, -5.5)	
		ECOG PS							
		0	26.1	22.8	3.3 (-2.9, 9.5)	17.1	30.8	-13.8 (-24.1, -3.4)	
		1	17.0	13.1	4.0 (-3.2, 11.2)	11.8	16.8	-5.0 (-19.1, 9.1)	
		PD-L1 status							
12		Negative	10.0	13.2	-3.3 (-12.0, 5.5)	12.3	15.8	-3.5 (-21.0, 14.1)	
		Positive	26.2	19.8	6.4 (-0.6, 13.3)	12.4	30.8	-18.4 (-29.5, -7.3)	
		Metastatic stage							
		- M0/M1a/M1b	32.3	24.5	7.8 (-2.5, 18.1)	11.9	36.9	-24.9 (-40.7, -9.2)	I
		M1c	19.6	18.3	1.4 (-4.0, 6.7)	16.0	23.2	-7.2 (-17.0, 2.6)	
		Brain metastasis							
		No	22.4	19.2	3.2 (-1.9, 8.3)	14.6	26.5	-11.9 (-20.7, -3.1)	
_	•	Yes	29.0	23.5	5.5 (-8.9, 19.9)	18.5	37.1	-18.6 (-48.0, 10.8)	
		LDH Normal	29.3	22.4	7.0 (0.5, 13.5)	20.3	24.9	-4.7 (-15.2, 5.8)	
	-	Elevated	13.1	14.1	-1.0 (-7.6, 5.7)	9.3	37.8	-28.4 (-47.2, -9.6)	
	•	Elevaled	13.1	14.1	-1.0 (-7.0, 5.7)	9.5	37.0	-28.4 (-47.2, -9.0)	_
		Prior lines of therapy							
	•	0	26.6	33.3	-6.7 (-17.0, 3.6)	NA	NA	NA (NA)	
		1	21.8	18.6	3.2 (-4.9, 11.4)	18.8	17.8	1.0 (-15.0, 17.0)	
		_ 2	20.5	8.8	11.7 (3.6, 19.8)	10.1	10.0	0.1 (-18.7, 18.9)	
	-	≥3	16.2	15.8	0.4 (-9.7, 10.5)	16.2	50.0	-33.8 (-79.7, 12.2)	
		Prior ipilimumab exposu			00/15/14/0	10.0	40.0		
		Ipilimumab-exposed	19.9 25.3	11.9 23.9	8.0 (1.5, 14.4)	12.3 18.1	10.0 29.1	2.3 (-16.2, 20.8)	
		Ipilimumab-naive	20.3	23.9	1.3 (-5.3, 8.0)	18.1	29.1	-11.0 (-21.1, -0.9)	
		Baseline tumor size							
		≤93 mm	31.2	21.5	9.7 (2.4, 17.1)	16.3	28.1	-11.8 (-23.4, -0.1)	
		>93 mm	12.4	15.4	-3.0 (-9.6, 3.6)	12.7	27.2	-14.5 (-31.2, 2.3)	
		Albumin							
		≤0.834	19.0	16.0	3.0 (-2.6, 8.6)	12.8	23.8	-11.0 (-22.3, 0.3)	_
		>0.834	30.2	24.5	5.7 (-3.0, 14.3)	18.4	31.0	-12.6 (-26.3, 1.2)	
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	o	20							-20 0 2
r		Better							Better Be
S M		∔y-PFS te in WT							4y-PFS 4y-PF rate in in M+ M-BRAFi

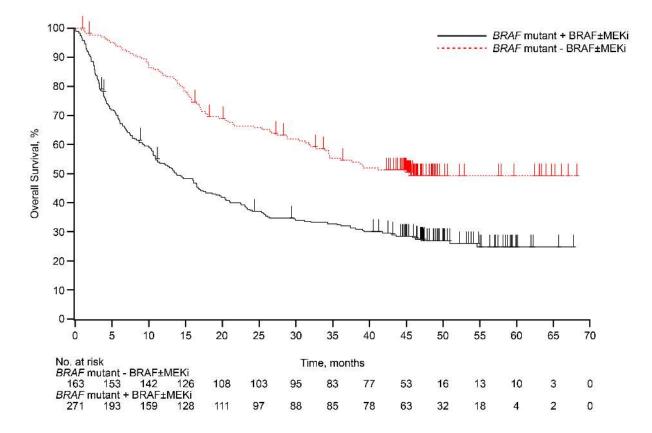
	4-year OS rate (%)	WT n = 1124	M n = 434	Difference in rate (95% CI)	M + B/Mi n = 271	M – B/Mi n = 163	Difference in rate (95% CI)	
·	Overall	37.5	35.1	2.4 (-3.1, 7.8)	26.9	49.3	-22.4 (-32.0, -12.8)	
	Age							
	<65 years	39.0	33.2	5.9 (-0.8, 12.5)	26.8	45.5	-18.7 (-29.9, -7.6)	S
	≥65 years	35.9	40.7	-4.8 (-15.6, 6.0)	27.6	56.8	-29.2 (-48.7, -9.6)	· · · · · · · · · · · · · · · · · · ·
	Sex							
	Female	33.0	33.1	-0.2 (-8.5, 8.2)	27.2	43.0	-15.8 (-30.2, -1.5)	
	Male	40.3	36.5	3.8 (-3.4, 11.1)	26.8	54.2	-27.4 (-40.2, -14.7)	
	ECOG PS							
	0	42.4	41.1	1.3 (-5.5, 8.2)	32.5	52.8	-20.3 (-31.7, -8.9)	
	- 1	28.9	22.7	6.2 (-2.3, 14.7)	18.0	36.1	-18.1 (-36.5, 0.2)	
	PD-L1 status							
	Negative	20.9	30.6	-9.7 (-21.4, 2.1)	27.6	41.4	-13.9 (-37.8, 10.0)	
-	- Positive	42.4	36.4	5.9 (-1.6, 13.5)	24.7	53.0	-28.3 (-40.4, -16.1)	
	Metastatic stage							
	M0/M1a/M1b	50.3	51.7	-1.4 (-12.8, 10.0)	37.8	63.6	-25.7 (-44.5, -7.0)	
	M1c	33.1	30.2	2.9 (-3.2, 8.9)	24.7	42.4	-17.7 (-29.0, -6.5)	
	Brain metastasis			100710-0000				
	No	37.4	34.5	2.9 (-2.9, 8.8)	26.8	46.6	-19.8 (-30.0, -9.6)	8 . 1. 1 .
•	Yes	39.7	38.6	1.1 (-14.6, 16.8)	28.3	75.0	-46.7 (-74.9, -18.6)	•
	LDH							
	Normal Elevated	46.6 23.4	42.8 20.4	3.8 (-3.5, 11.0) 3.0 (-4.7, 10.7)	38.0 14.2	48.8 49.7	-10.8 (-22.8, 1.2) -35.5 (-55.2, -15.8)	
	Elevated	23.4	20.4	3.0 (-4.7, 10.7)	14.2	49.7	-35.5 (-05.2, -15.6)	•
	Prior lines of therapy							
	0	42.5	53.3	-10.8 (-21.2, -0.4)	NA	NA	NA (NA)	
	1 2	37.6 37.2	31.8 22.9	5.8 (-3.7, 15.3)	31.0 19.3	34.9 50.0	-4.0 (-24.1, 16.2)	
	≥3	23.9	28.6	14.3 (3.5, 25.1) -4.7 (-16.5, 7.2)	29.3	50.0	-30.7 (-60.5, -0.8) -20.7 (-67.0, 25.6) -	· · ·
	Prior ipilimumab expos							
		32.8	24.4	8.4 (0.4, 16.4)	24.0	30.0	-6.0 (-32.4, 20.3)	12 12 2 2
	Ipilimumab-naive	42.1	40.8	1.2 (-6.0, 8.5)	29.9	50.5	-20.7 (-32.1, -9.3)	_ _
	Baseline tumor size			301 III 1 688				
	≤93 mm	49.3	41.5	-7.8 (-0.2, 15.9)	35.0	49.2	-14.2 (-27.6, -0.9)	
	>93 mm	20.4	20.7	-0.3 (-7.8, 7.2)	15.9	41.4	-25.5 (-44.0, -7.0)	
	Albumin							
1000	≤0.834	33.5	28.2	5.2 (-1.5, 12.0)	21.5	44.9	-23.4 (-36.7, -10.1)	100 million (100 million)
	>0.834	45.1	45.0	0.1 (-9.2, 9.3)	36.7	54.1	-17.4 (-32.5, -2.3)	
				l,			<u></u>	
0	20							-20 0
	Better							Better
	4y-OS rate in WT							4y-OS rate in

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 \pm MEKi treated versus BRAFi \pm MEKi naive

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