Supplementary data
Supplementary Table 1. Univariate and multivariate analysis on PFS

HR (95% C.I.) GENDER (M vs F) 1.01 (0.61-1.69) p=0.96 0.81 (0.45-1.47) p=0.49 AGE (>=64 vs <64) 1.39 (0.83-2.32) p=0.21 1.72 (0.89-3.34) p=0.11 BRAF (mutated vs wild-type) 1.31 (0.75-2.28) p=0.35 1.28 (0.66-2.51) p=0.46 BRAIN metastasis (yes vs no) Metastasis at nivolumab start (lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 0.74 (0.29-1.90) p=0.53 (visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY (first-line vs not first-line) 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH (elevated vs normal) 1.14 (0.64-2.04) p=0.65 many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration (>=32 vs <32) 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because dichotomized concentration was used		Univariate	Multivariate
AGE (>=64 vs <64) 1.39 (0.83-2.32) p=0.21 1.72 (0.89-3.34) p=0.11 BRAF (mutated vs wild-type) 1.31 (0.75-2.28) p=0.35 1.28 (0.66-2.51) p=0.46 BRAIN metastasis (yes vs no) Metastasis at nivolumab start (lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 0.74 (0.29-1.90) p=0.53 (visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY (first-line vs not first-line) 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration (>=32 vs <32) 0.61 (0.36-1.01) P=0.058 Not considered because dichotomized concentration		HR (95% C.I.)	HR (95% C.I.)
BRAF (mutated vs wild-type) 1.31 (0.75-2.28) p=0.35 1.28 (0.66-2.51) p=0.46 BRAIN metastasis (yes vs no) Metastasis at nivolumab start (lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY (first-line vs not first-line) 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.61 (0.36-1.01) P=0.058 Not considered because 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration (>=32 vs <32) 0.67 (0.95-0.99) p=0.01	GENDER (M vs F)	1.01 (0.61-1.69) p=0.96	0.81 (0.45-1.47) p=0.49
BRAIN metastasis (yes vs no) Metastasis at nivolumab start (lung vs soft tissue) (visceral vs soft tissue) LINE OF THERAPY (first-line vs not first-line) Deliver (elevated vs normal) Nivolumab regimen (240 mg vs 3 mg/kg) Nivolumab concentration Nivolumab concentration Not considered because 0.73 (0.31-1.74) p=0.48 0.73 (0.31-1.74) p=0.48 0.73 (0.31-1.74) p=0.48 0.73 (0.31-1.74) p=0.73 Not considered because 0.74 (0.29-1.90) p=0.53 1.16 (0.50-2.68) p=0.74 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 1.14 (0.64-2.04) p=0.12 0.58 (0.32-1.05) p=0.074 Not considered because 0.73 (0.31-1.74) p=0.48 0.87 (0.41-1.86) p=0.73 Not considered because Not considered because dichotomized concentration	AGE (>=64 vs <64)	1.39 (0.83-2.32) p=0.21	1.72 (0.89-3.34) p=0.11
(yes vs no) 1.70 (0.99-2.92) p=0.057 1.67 (0.81-3.46) p=0.16 Metastasis at nivolumab start (lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 0.74 (0.29-1.90) p=0.53 (visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too many missing value Nivolumab regimen 0.240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	BRAF (mutated vs wild-type)	1.31 (0.75-2.28) p=0.35	1.28 (0.66-2.51) p=0.46
(yes vs no) Metastasis at nivolumab start (lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 0.74 (0.29-1.90) p=0.53 (visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too (elevated vs normal) 1.14 (0.64-2.04) p=0.65 many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	BRAIN metastasis	1 70 (0 00 2 02) n=0 057	1.67 (0.91.2.46) n=0.16
(lung vs soft tissue) 0.72 (0.31-1.71) p=0.46 0.74 (0.29-1.90) p=0.53 (visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too delevated vs normal Not considered because of too many missing value Nivolumab regimen 0.73 (0.31-1.74) p=0.48 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.54 (0.30-0.98) p=0.043 Not considered because Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	(yes vs no)	1.70 (0.33-2.32) p-0.037	1.07 (0.81-3.40) β-0.10
(visceral vs soft tissue) 1.14 (0.63-2.09) p=0.66 1.16 (0.50-2.68) p=0.74 LINE OF THERAPY 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too delevated vs normal Nivolumab regimen 0.40 mg vs 3 mg/kg 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	Metastasis at nivolumab start		
LINE OF THERAPY (first-line vs not first-line) 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 Not considered because of too many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration (>=32 vs <32) 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	(lung vs soft tissue)	0.72 (0.31-1.71) p=0.46	0.74 (0.29-1.90) p=0.53
(first-line vs not first-line) 0.67 (0.40-1.11) p=0.12 0.58 (0.32-1.05) p=0.074 LDH Not considered because of too (elevated vs normal) 1.14 (0.64-2.04) p=0.65 many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	(visceral vs soft tissue)	1.14 (0.63-2.09) p=0.66	1.16 (0.50-2.68) p=0.74
LDH Not considered because of too (elevated vs normal) 1.14 (0.64-2.04) p=0.65 many missing value Nivolumab regimen 240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	LINE OF THERAPY		
(elevated vs normal) 1.14 (0.64-2.04) p=0.65 many missing value Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration 0.61 (0.36-1.01) P=0.058 0.54 (0.30-0.98) p=0.043 Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	(first-line vs not first-line)	0.67 (0.40-1.11) p=0.12	0.58 (0.32-1.05) p=0.074
Nivolumab regimen (240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration (>=32 vs <32)	LDH		Not considered because of too
(240 mg vs 3 mg/kg) 1.08 (0.51-2.32) p=0.84 0.73 (0.31-1.74) p=0.48 (480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration (>=32 vs <32)	(elevated vs normal)	1.14 (0.64-2.04) p=0.65	many missing value
(480 mg vs 3 mg/kg) 0.90 (0.47-1.71) p=0.74 0.87 (0.41-1.86) p=0.73 Nivolumab concentration (>=32 vs <32)	Nivolumab regimen		
Nivolumab concentration (>=32 vs <32)	(240 mg vs 3 mg/kg)	1.08 (0.51-2.32) p=0.84	0.73 (0.31-1.74) p=0.48
(>=32 vs <32)	(480 mg vs 3 mg/kg)	0.90 (0.47-1.71) p=0.74	0.87 (0.41-1.86) p=0.73
Not considered because Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	Nivolumab concentration		
Nivolumab concentration 0.97 (0.95-0.99) p=0.01 dichotomized concentration	(>=32 vs <32)	0.61 (0.36-1.01) P=0.058	0.54 (0.30-0.98) p=0.043
			Not considered because
was used	Nivolumab concentration	0.97 (0.95-0.99) p=0.01	dichotomized concentration
			was used

CI: confidence interval; HR: hazard ratio; LDH: lactate dehydrogenase.

Supplementary Table 2. Univariate and multivariate analysis on OS

	Univariate	Multivariate
	HR (95% C.I.)	HR (95% C.I.)
GENDER (M vs F)	0.87 (0.50-1.51) p=0.62	0.64 (0.34-1.21) p=0.17
AGE (>=64 vs <64)	1.47 (0.84-2.58) p=0.18	1.59 (0.78-3.24) p=0.20
BRAF (mutated vs wild-type)	1.08 (0.58-2.00) p=0.80	0.96 (0.46-1.99) p=0.91
BRAIN metastasis (yes vs no)	1.71 (0.95-3.09) p=0.07	1.50 (0.69-3.25) p=0.30

Metastasis at nivolumab start		
(lung vs soft tissue)	0.67 (0.25-1.79) p=0.42	0.65 (0.22-1.95) p=0.44
(visceral vs soft tissue)	1.21 (0.62-2.34) p=0.58	1.37 (0.54-3.49) p=0.51
LINE OF THERAPY		
(first-line vs not first-line)	0.67 (0.39-1.18) p=0.16	0.52 (0.27-1.00) p=0.051
LDH		Not considered because of too
(elevated vs normal)	1.31 (0.70-2.46) p=0.40	many missing value
Nivolumab regimen		
(240 mg vs 3 mg/kg)	1.33 (0.61-2.88) p=0.47	1.05 (0.43-2.57) p=0.91
(480 mg vs 3 mg/kg)	0.77 (0.37-1.61) p=0.49	0.79 (0.34-1.83) p=0.59
Nivolumab concentration		
(>=32 vs <32)	0.54 (0.31-0.95) P=0.03	0.42 (0.22-0.82) p=0.01
		Not considered because
Nivolumab concentration	0.97 (0.94-0.99) p=0.007	dichotomized concentration
		was used

CI: confidence interval; HR: hazard ratio; LDH: lactate dehydrogenase.

Supplementary Table 3. Renal and hepatic function in patients with higher and lower concentrations of nivolumab.

Patient with higher	Average (range)	Normal Range
concentration of nivolumab		
Creatinine	0.84 (0.4-2)	0.51- 0.95 mg/dl
eGFR		
female	99.9 (14.8-209)	> 85 mL/min
male	115.8 (56.4-190.5)	> 90 mL/min
Albumin	4.1 (3.4-4.7)	3.5-5.3 g/dl
ALT	24 (6-119)	4-32 U/L
AST	18.78 (9-53)	4-32 U/L
GGT	42.2 (7-243)	< 40 U/L
Patients wits lower		
concentration of nivolumab		
Creatinine	0.87 (0.4-1.6)	0.51- 0.95 mg/dl

eGFR		
female	100,4 (25.4-209.12)	> 85 mL/min
male	110.9 (56.41-190.5)	> 90 mL/min
Albumin	3.8 (3.4-4.5)	3.5-5.3 g/dl
ALT	20.7 (7-51)	4-32 U/L
AST	18.7 (9-29)	4-32 U/L
GGT	43.1 (8-198)	< 40 U/L

ALT: alanine aminotransferase; AST: aspartate transaminase; GGT: gamma-glutamyl transferase; eGFR: estimated glomerular filtration rate

Supplementary Figure 1. Relationship between nivolumab serum concentration and patients' response.

