Supplementary data

Supplementary Table 1. Univariate and multivariate analysis on PFS

	Univariate HR (95% C.I.)	Multivariate 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)	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) (visceral vs soft tissue)	0.72 (0.31-1.71) p=0.46 1.14 (0.63-2.09) p=0.66	0.74 (0.29-1.90) p=0.53 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	Not considered because of too many missing value
Nivolumab regimen (240 mg vs 3 mg/kg) (480 mg vs 3 mg/kg)	1.08 (0.51-2.32) p=0.84 0.90 (0.47-1.71) p=0.74	0.73 (0.31-1.74) p=0.48 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
Nivolumab concentration	0.97 (0.95-0.99) p=0.01	Not considered because dichotomized concentration was used

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

Supplementary Table 2. Univariate and multivariate analysis on OS

	Univariate HR (95% C.I.)	Multivariate 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) (visceral vs soft tissue)	0.67 (0.25-1.79) p=0.42 1.21 (0.62-2.34) p=0.58	0.65 (0.22-1.95) p=0.44 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 (elevated vs normal)	1.31 (0.70-2.46) p=0.40	Not considered because of too many missing value
Nivolumab regimen (240 mg vs 3 mg/kg) (480 mg vs 3 mg/kg)	1.33 (0.61-2.88) p=0.47 0.77 (0.37-1.61) p=0.49	1.05 (0.43-2.57) p=0.91 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
Nivolumab concentration	0.97 (0.94-0.99) p=0.007	Not considered because 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 concentration of nivolumab	Average (range)	Normal Range
Creatinine	0.84 (0.4-2)	0.51- 0.95 mg/dl
eGFR female male	99.9 (14.8-209) 115.8 (56.4-190.5)	> 85 mL/min > 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.