SUPPLEMENTARY TABLES AND FIGURE

Supplementary Table 1. Patient demographic and clinical characteristics [A]

Characteristic	All patients (*N* = 124)	Non-progressors (*N* = 14)	Resistant (*N* = 110)	Original resistance definitions		SITC resistance definitions	
				Primary (N=61)	Secondary (N=49)	Primary (N=76)	Secondary (N=32)
Age							
Mean (SD)	59.5 (12.4)	58.2 (12.7)	59.7 (12.4)	60.1 (12.13)	59.3 (12.93)	60.1 (12.82)	59.5 (11.56)
Gender (binary)							
Female	57 (46.0)	5 (35.7)	52 (47.3)	27 (44.3)	25 (51.0)	39 (51.3)	11 (34.4)
Male	67 (54.0)	9 (64.3)	58 (52.7)	34 (55.7)	24 (49.0)	37 (48.7)	21 (65.6)
Histology							
Acral	76 (61.3)	10 (71.4)	66 (60.0)	35 (57.4)	31 (63.3)	46 (60.5)	19 (59.4)
Mucosal	44 (35.5)	4 (28.6)	40 (36.4)	24 (39.3)	16 (32.7)	27 (35.5)	12 (37.5)
Unknown primary [B]	4 (3.2)	0 (0.0)	4 (3.6)	2 (3.3)	2 (4.1)	3 (3.9)	1 (3.1)
BRAF status							
Patients with data	122	14	108	60	48	75	31
V600	15 (12.3)	4 (28.6)	11 (10.2)	6 (10.0)	5 (10.4)	7 (9.3)	4 (12.9)
WT	105 (86.1)	10 (71.4)	95 (88.0)	54 (90.0)	41 (85.4)	67 (89.3)	26 (83.9)
Other	2 (1.6)	0 (0.0)	2 (1.9)	0 (0.0)	2 (4.2)	1 (1.3)	1 (3.2)
KIT status							
Patients with data	104	12	92	49	43	62	28
Mutant	5 (4.8)	0 (0.0)	5 (5.4)	0 (0.0)	5 (11.6)	3 (4.8)	2 (7.1)

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Characteristic	All patients (*N* = 124)	Non-progressors (*N* = 14)	Resistant (*N* = 110)	Original resistance definitions		SITC resistance definitions	
				Primary (N=61)	Secondary (N=49)	Primary (N=76)	Secondary (N=32)
WT	99 (95.2)	12 (100)	87 (94.6)	49 (100.0)	38 (88.4)	59 (95.2)	26 (92.9)
NRAS status							
Patients with data	104	11	93	49	44	61	30
Mutant	15 (14.4)	1 (9.1)	14 (15.1)	9 (18.4)	5 (11.4)	10 (16.4)	4 (13.3)
WT	89 (85.6)	10 (90.9)	79 (84.9)	40 (81.6)	39 (88.6)	51 (83.6)	26 (86.7)
M stage at baseline biopsy c							
M0	6 (4.8)	3 (21.4)	3 (2.7)	2 (3.3)	1 (2.0)	2 (2.6)	1 (3.1)
M1a	44 (35.5)	2 (14.3)	42 (38.2)	27 (44.3)	15 (30.6)	34 (44.7)	7 (21.9)
M1b	9 (7.3)	1 (7.1)	8 (7.3)	3 (4.9)	5 (10.2)	3 (3.9)	4 (12.5)
M1c	65 (52.4)	8 (57.1)	57 (51.8)	29 (47.5)	28 (57.1)	37 (48.7)	20 (62.5)
Metastasis at baseline biopsy							
Brain	6 (4.8)	1 (7.1)	5 (4.5)	4 (6.6)	1 (2.0)	5 (6.6)	0 (0.0)
Liver	18 (14.5)	1 (7.1)	17 (15.5)	11 (18.0)	6 (12.2)	14 (18.4)	3 (9.4)
ECOG performance status							
1	123 (99.2)	14 (100)	109 (99.1)	60 (98.4)	49 (100.0)	75 (98.7)	32 (100.0)
2	1 (0.8)	0 (0.0)	1 (0.9)	1 (1.6)	0 (0.0)	1 (1.3)	0 (0.0)
Serum lactase dehydrogenase status at initiation of anti-PD-1							

Characteristic	All patients (*N* = 124)	Non-progressors (*N* = 14)	Resistant (*N* = 110)	Original resistance definitions		SITC resistance definitions	
				Primary (N=61)	Secondary (N=49)	Primary (N=76)	Secondary (N=32)
therapy							
Elevated	16 (12.9)	1 (7.1)	15 (13.6)	11 (18.0)	4 (8.2)	12 (15.8)	3 (9.4)
Normal	93 (75.0)	12 (85.7)	81 (73.6)	42 (68.9)	39 (79.6)	54 (71.1)	26 (81.3)
Unknown	15 (12.1)	1 (7.1)	14 (12.7)	8 (13.1)	6 (12.2)	10 (13.2)	3 (9.4)
Neutrophil to lymphocyte ratio							
Mean (SD)	2.1 (0.1)	1.8 (0.5)	2.1 (1.0)	2.3 (1.0)	2.0 (1.0)	2.14 (0.981)	2.13 (1.119)
Median (range)	1.9 (0.7–6.2)	1.6 (1.2–3.0)	1.9 (0.7–6.2)	2.0 (0.9–5.4)	1.8 (0.7–6.2)	1.87 (0.7–5.4)	1.90 (0.9–6.2)
Rounds of previous systemic therapies							
0	108 (87.1)	14 (100)	94 (85.5)	51 (83.6)	43 (87.8)	63 (82.9)	29 (90.6)
1	15 (12.1)	0 (0.0)	15 (13.6)	9 (14.8)	6 (12.2)	12 (15.8)	3 (9.4)
2	1 (0.8)	0 (0.0)	1 (0.9)	1 (1.6)	0 (0.0)	1 (1.3)	0 (0.0)
Type of previous systemic therapy							
Patients with data	16	0	16	10	6	13	3
BRAF and/or MEK/MAP2K inhibitor	2 (12.5)	NA	2 (12.5)	2 (20.0)	0 (0.0)	2 (15.4)	0 (0.0)
CTLA-4 inhibitor	1 (6.3)	NA	1 (6.3)	1 (10.0)	0 (0.0)	1 (7.7)	0 (0.0)
Dacarbazine or CVD	12 (75.0)	NA	12 (75.0)	7 (70.0)	5 (83.3)	9 (69.2)	3 (100.0)

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Characteristic	All patients (*N* = 124)	Non-progressors (*N* = 14)	Resistant (*N* = 110)	Original resistance definitions		SITC resistance definitions	
				Primary (N=61)	Secondary (N=49)	Primary (N=76)	Secondary (N=32)
Paclitaxel + carboplatin	1 (6.3)	NA	1 (6.3)	0 (0.0)	1 (16.7)	1 (7.7)	0 (0.0)
Site of baseline biopsy							
Patients with data	124	14	110	61	49	76	32
Skin	56 (45.2)	2 (14.3)	54 (49.1)	28 (45.9)	26 (53.1)	36 (47.4)	16 (50.0)
Lymph node	24 (19.4)	5 (35.7)	19 (17.3)	10 (16.4)	9 (18.4)	14 (18.4)	5 (15.6)
Mucosal surface	21 (16.9)	3 (21.4)	18 (16.4)	12 (19.7)	6 (12.2)	13 (17.1)	5 (15.6)
Other	23 (18.6)	4 (28.6)	19 (17.3)	11 (18.0)	8 (16.3)	13 (17.1)	6 (18.8)
Site of Post-treatment biopsy							
Patients with data	NA	NA	90	53	37	68	21
Skin	NA	NA	30 (33.3)	15 (28.3)	15 (40.5)	20 (29.4)	9 (42.9)
Lymph node	NA	NA	19 (21.1)	12 (22.6)	7 (18.9)	16 (23.5)	3 (14.3)
Mucosal surface	NA	NA	10 (11.1)	5 (9.4)	5 (13.5)	7 (10.3)	3 (14.3)
Other	NA	NA	31 (34.4)	21 (39.6)	10 (27.0)	25 (36.8)	6 (28.6)

CVD, cisplatin, vinblastine and dacarbazine; ECOG, Eastern Cooperative Oncology Group; NA, not applicable; SD, standard deviation; SITC, Society for Immunotherapy of Cancer; WT, wild-type.

[A] All values given as *n* (%) unless otherwise stated.

[B] Only those unknown primary tumors with the clinical appearance of mucosal melanoma were included in the study.

[C] M0, no distant metastases; M1a, non-visceral metastases to skin, soft tissue including muscles, and/or nonregional lymph nodes; M1b, distant metastases to lung; M1c, all other visceral metastases and any distant metastases with elevated lactase dehydrogenase levels.

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Supplementary Table 2. Patient treatment and clinical outcomes [A]

	All patients (*N* = 124)	Non-progressors (*N* = 14)	Resistant (*N* = 110)	Original resistance definitions		SITC resistance definitions	
				Primary	Secondary	Primary	Secondary
Type of anti-PD-1 treatment							
Nivolumab	20 (16.1)	4 (28.6)	16 (14.5)	6 (9.8)	10 (20.4)	8 (10.5)	8 (25.0)
Pembrolizumab	104 (83.9)	10 (71.4)	94 (85.5)	55 (90.2)	39 (79.6)	68 (89.5)	24 (75.0)
Number of cycles received							
Median (range)	10 (2–67)	33 (14–67)	8 (2–64)	5.0 (2–27)	19.0 (5–64)	6.0 (2–27)	23.0 (10–64)
Duration of anti-PD-1 treatment (months)							
Median (range)	6.3 (1.3–45.0)	23.2 (9.4–38.4) [B]	5.4 (1.3–45.0)	2.79 (1.3–18.4)	12.45 (3.7–45.0)	3.45 (1.3–18.4)	13.86 (6.7–45.0)
Duration of response (months) [C]							
Patients with data	94	14	80	31	49	46	32
Median (range)	6.0 (0.0–44.5)	27.2 (15.6–44.5) [B]	4.7 (0.0–40.7)	1.64 (0.0–2.9)	7.87 (3.1–40.7)	1.93 (0.0–5.6)	11.25 (5.9–40.7)
Best overall response							
Complete response	11 (8.9)	7 (50.0)	4 (3.6)	1 (1.6)	3 (6.1)	1 (1.3)	2 (6.3)
Partial response	35 (28.2)	7 (50.0)	28 (25.5)	6 (9.8)	22 (44.9)	8 (10.5)	19 (59.4)
Stable disease	48 (38.7)	0 (0.0)	48 (43.6)	24 (39.3)	24 (49.0)	37 (48.7)	11 (34.4)

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	All patients (N = 124)	Non-progressors (N = 14)	Resistant (N = 110)	Original resistance definitions		SITC resistance definitions	
				Primary	Secondary	Primary	Secondary
Progressive disease	30 (24.2)	0 (0.0)	30 (27.3)	30 (49.2)	0 (0.0)	30 (39.5)	0 (0.0)
Vital status at last follow-up							
Alive	64 (51.6)	14 (100)	50 (45.5)	22 (36.1)	28 (57.1)	27 (35.5)	21 (65.6)
Dead	60 (48.4)	0 (0.0)	60 (54.5)	39 (63.9)	21 (42.9)	49 (64.5)	11 (34.4)

SD, standard deviation; SITC, Society for Immunotherapy of Cancer.

[A] All values given as *n* (%) unless otherwise stated.

[B] Date of last follow-up was used to calculate duration of therapy and response for 9 non-responders whose treatment was ongoing at the end of the study period.

[C] Duration of response is reported for patients with a best overall response of complete response, partial response, and stable disease. Patients with BOR of CR, PR, or SD <90 days (original definition) and <180 days (SITC definition) before progression are considered primary resistance. Patients with a BOR of ≥90 days (original definition) and ≥180 days (SITC definition) before progression are considered secondary resistance.

Supplementary Table 3. Patient characteristics by acral and mucosal melanoma [A]

Characteristic	Acral (*N* = 76)	Mucosal/Unknown Primary (*N* = 48)
Age		
Mean (SD)	58.9 (12.38)	60.5 (12.6)
Gender		
Female	34 (44.7)	23 (47.9)
Male	42 (55.3)	25 (52.1)
BRAF status		
Subjects with data	74	48
V600	11 (14.9)	4 (8.3)
WT	62 (83.8)	43 (89.6)
Other	1 (1.4)	1 (2.1)
M stage at biopsy [B]		
M0	1 (1.3)	5 (10.4)
M1a	32 (42.1)	12 (25.0)
M1b	5 (6.6)	4 (8.3)
M1c	38 (50.0)	27 (56.3)
ECOG performance status		
1	76 (100.0)	44 (97.9)
2	0 (0.0)	1 (2.1)
Lines of previous systemic therapy		
0	64 (84.2)	4 (90.9)
1	11 (14.5)	4 (9.1)
2	1 (1.3)	0 (0.0)
Type of previous systemic therapy		
Subjects with data	12	4
BRAF and/or MEK inhibitor	1 (8.3)	1 (25.0)
CTLA-4 inhibitor	0 (0.0)	1 (25.0)
Dacarbazine	10 (83.3)	2 (50.0)
Paclitaxel + carboplatin	1 (8.3)	0 (0.0)
Type of anti-PD-1 treatment		
Nivolumab	12 (15.8)	8 (16.7)

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Pembrolizumab	64 (84.2)	40 (83.3)
Cycles received		
Median (range)	10 (2. 67)	11.5 (2, 50)
Duration of anti-PD-1 treatment (months)		
Median (range)	6.29 (1.4, 45.0)	6.67 (1.3, 26.2)

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; WT, wild-type.

[A] All values given as *n* (%) unless otherwise stated.
[B] M0, no distant metastases; M1a, non-visceral metastases to skin, soft tissue including muscles, and/or nonregional lymph nodes; M1b, distant metastases to lung; M1c, all other visceral metastases and any distant metastases with elevated lactase dehydrogenase levels.

Supplementary Table 4. Baseline and post-treatment comparisons of mRNA signature expression levels in the non-progressor and resistant groups [A]

mRNA signature (number of genes in consensus gene set)	Baseline expression, non-progressors versus resistant AUROC (95% CI)	Post-treatment change from baseline, resistant Effect size (95% CI)	Post-treatment change from baseline, primary versus secondary resistance AUROC (95% CI)	
			Original study definition	SITC definition
GEP (18)	0.78 (0.62, 0.94)	0.34 (−0.05, 0.72)	0.53 (0.37, 0.68)	0.51 (0.33, 0.68)
Angiogenesis (16)	0.62 (0.46, 0.78)	0.07 (−0.31, 0.45)	0.48 (0.32, 0.64)	0.59 (0.41, 0.77)
Glycolysis (30)	0.59 (0.37, 0.81)	−0.12 (−0.50, 0.26)	0.51 (0.35, 0.67)	0.53 (0.35, 0.72)
Hypoxia (20)	0.61 (0.39, 0.83)	−0.07 (−0.45, 0.31)	0.51 (0.35, 0.67)	0.47 (0.29, 0.65)
gMDSC (43)	0.51 (0.31, 0.70)	−0.03 (−0.41, 0.35)	0.63 (0.48, 0.79)	0.60 (0.42, 0.78)
mMDSC (218)	0.61 (0.44, 0.77)	0.23 (−0.15, 0.61)	0.60 (0.45, 0.75)	0.61 (0.46, 0.76)
MYC (32)	0.56 (0.35, 0.77)	0.23 (−0.15, 0.61)	0.61 (0.46, 0.77)	0.60 (0.42, 0.77)
Proliferation (227)	0.55 (0.34, 0.77)	0.03 (−0.35, 0.41)	0.61 (0.46, 0.76)	0.55 (0.36, 0.75)
RAS (11)	0.49 (0.27, 0.71)	−0.09 (−0.46, 0.29)	0.57 (0.42, 0.73)	0.67 (0.51, 0.84)
Stroma/EMT/*TGFB1* (51)	0.63 (0.45, 0.81)	−0.12 (−0.50, 0.26)	0.50 (0.34, 0.66)	0.50 (0.27, 0.63)
WNT (13)	0.47 (0.30, 0.64)	−0.42 (−0.81, −0.04)	0.57 (0.42, 0.73)	0.76 (0.62, 0.90)
IFNA1 (3)	0.48 (0.32, 0.65)	−0.50 (−0.89, −0.11)	0.57 (0.42, 0.73)	0.54 (0.36, 0.71)
Cytokine (12)	0.58 (0.38, 0.77)	−0.01 (−0.38, 0.37)	0.57 (0.41, 0.73)	0.62 (0.47, 0.77)

AUROC, area under the receiver operating characteristic; CI, confidence interval; EMT, epithelial-to-mesenchymal transition; GEP, 18-gene T cell-inflamed gene expression profile (Tcell$_{inf}$GEP); gMDSC, granulocytic myeloid-derived suppressor cells; mMDSC, monocytic myeloid-derived suppressor cells; SITC, Society for Immunotherapy of Cancer.
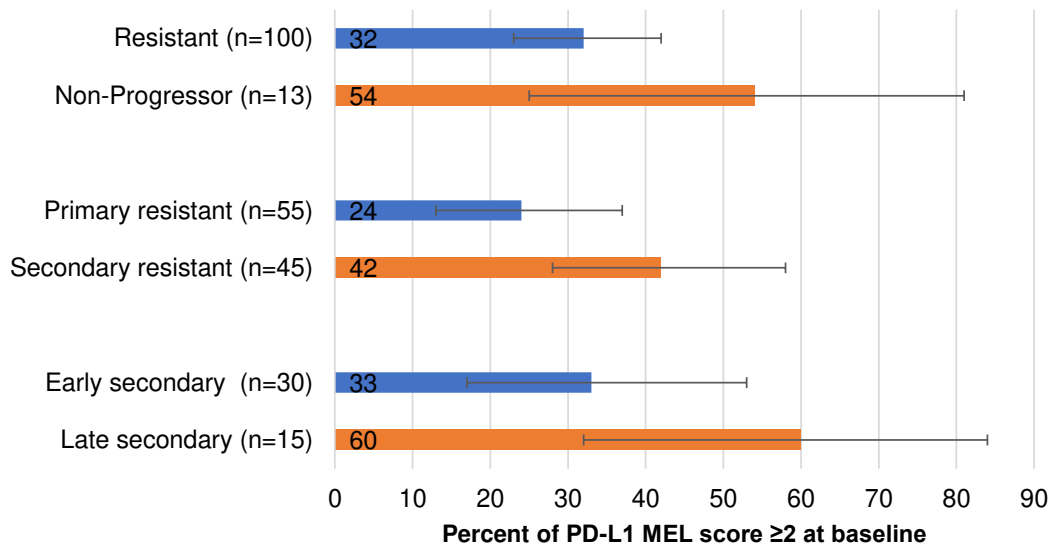[A] All mRNA signatures other than GEP were adjusted for GEP values.

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Supplementary Table 5. Baseline and post-treatment comparisons of infiltrating immune cell densities in the non-progressor and resistant groups [A]

Cell type	Baseline expression, non-progressors versus resistant	Post-treatment change from baseline, primary versus secondary resistance	
		Original study definition	SITC definition
CD8^{+}	0.78 (0.65, 0.90)	0.53 (0.39, 0.67)	0.58 (0.43, 0.73)
CD11c^{+}	0.70 (0.54, 0.87)	0.60 (0.46, 0.75)	0.62 (0.45, 0.78)
FOXP3^{+}	0.73 (0.54, 0.87)	0.58 (0.44, 0.71)	0.69 (0.55, 0.83)

SITC, Society for Immunotherapy of Cancer.

[A] All values given as area under the receiver operating characteristic (95% confidence interval)

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Supplementary Figure 1. Percent PD-L1 MEL score ≥2 at baseline

*Based on primary definitions of resistance.