Characteristics of patients from University Hospital of Essen - Germany

	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)		Nivolumab +ipilimumab therapy (no, %)		TOTAL (no, %)	
Total	14	26,4	24	45,3	15	28,3	53	100
Median age, years (range 20-89)	72		68		59		63	
Sex								
male	10	18,9	13	24,5	7	13,2	30	56,6
female	4	7,5	11	20,8	8	15,1	23	43,4
Stage								
III	2	3,8	1	1,9	1	1,9	4	7,5
IV	12	22,6	23	43,4	14	26,4	49	92,5
unknown	0		0		0		0	
Serum lactate dehydrogenase								
normal (< ULN)	11	20,8	23	43,4	12	22,6	46	86,8
elevated (≥ ULN)	2	3,8	1	1,9	3	5,7	6	11,3
unkown	1	1,89	0		0		1	1,89
BRAF								
wt	7	13,2	15	28,3	9	17,0	31	58,5
mut	4	7,5	7	13,2	5	9,4	16	30,2
unknown	3	5,66	2	3,77	1	1,89	6	11,3
Brain metastasis								
yes	1	1,9	8	15,1	5	9,4	14	26,4
no	13	24,5	16	30,2	10	18,9	39	73,6
unknown	0		0		0		0	
Bone metastasis								
yes	1	1,9	3	5,7	5	9,4	9	17,0
no	13	24,5	20	37,7	10	18,9	43	81,1
unknown	0		1		0		1	1,89
Line of treatment								
1	7	13,2	11	20,8	8	15,1	26	49,1
2	4	7,5	9	17,0	3	5,7	16	30,2
≥ 3	3	5,7	4	7,5	4	7,5	11	20,8

Characteristics of patients from Hôpital Saint-Louis Policlinique de Dermatologie - Paris, France

	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)		Nivolumab +ipilimumab therapy (no, %)	TOTAL (no, %)	
Total	42	47,7	46	52,3	0	88	100
Median age, years (range 24-92)	60,5		67,5			64,5	
Sex							
male	25	28,4	25	28,4		50	56,8
female	17	19,3	21	23,9		38	43,2
Stage							
III	6	6,8	9	10,2		15	17,0
IV	36	40,9	37	42,0		73	83,0
unknown	0		0			0	
Serum lactate dehydrogenase							
normal (< ULN)	24	27,3	31	35,2		55	62,5
elevated (≥ ULN)	17	19,3	13	14,8		30	34,1
unknown	1	1,1	2	2,3		3	3,4
BRAF							
wt	26	29,5	31	35,2		57	64,8
mut	16	18,2	15	17,0		31	35,2
unknown	0		0			0	
Brain metastasis							
yes	16	18,2	10	11,4		26	29,5
no	26	29,5	36	40,9		62	70,5
unknown	0		0			0	
Bone matastasis							
yes	11	12,5	8	9,1		19	21,6
no	31	35,2	38	43,2		69	78,4
unknown	0		0			0	
Line of treatment							
1	15	17,0	26	29,5		41	46,6
2	22	25,0	17	19,3		39	44,3
≥ 3	5	5,7	3	3,4		8	9,1

Characteristics of patients from Center for Medical Research, University of Tübingen Medical School – Tübingen, Germany

	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)		Nivolumab +ipilimumab therapy (no, %)		TOTAL (no, %)	
Total	0		68	61,3	43	38,7	111	100
Median age, years (range 29-94)			74	35-94	63		69	
Sex				0,0				
male			43	38,7	25	22,5	68	61,3
female			25	22,5	18	16,2	43	38,7
Stage								
III								
IV			68	61,3	43	38,7	111	100
unknown			0		0		0	
Serum lactate dehydrogenase								
normal (< ULN)			39	35,1	17	15,3	56	50,5
elevated (≥ ULN)			29	26,1	25	22,5	54	48,6
unknown			0		1		1	0,9
BRAF								
wt			27	24,3	16	14,4	43	38,7
mut			19	17,1	13	11,7	32	28,8
unknown			22	19,8	14	12,6	36	32,4
Brain metastasis								
yes								
no								
unknown								
Bone metastasis								
yes								
no								
Line of treatment								
1			53	47,7	16	14,4	69	62,2
2			12	10,8	13	11,7	25	22,5
≥ 3			3	2,7	14	12,6	17	15,3

Characteristics of patients from Center for Medical Research, University of Chicago – Illinois, USA

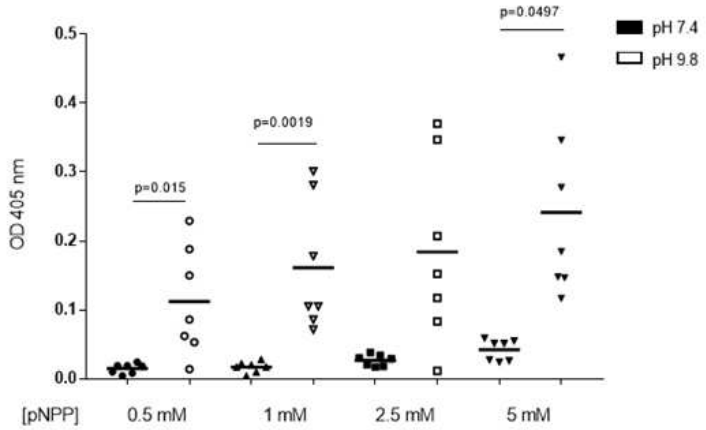
	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)		Nivolumab +Ipilimumab therapy (no, %)		TOTAL (no, %)	
Total	5	10	39	78	6	12	50	100
Median age, years (range 19-85)	61		62		52			
Sex								
male	4	8	19	38	3	6	26	52
female	1	2	20	40	3	6	24	48
Stage								
III	1	2	6	12			7	14
IV	4	8	33	66	6	12	43	86
unknown	0		0		0		0	
Serum lactate dehydrogenase								
normal (< ULN)	3	6	31	62	4	8	38	76
elevated (≥ ULN)	2	4	8	16	2	4	12	24
unknown	0		0		0		0	
BRAF								
wt	2	4	25	50	1	2	28	56
mut	3	6	10	20	5	10	18	36
unknown	0		4	8	0		4	8
Brain metastasis								
yes	1	2	3	6	0		4	8
no	4	8	36	72	6	12	46	92
unknown	0		0		0		0	
Bone metastasis								
yes	1	2	8	16	1	2	10	20
no	4	8	31	62	5	10	40	80
unknown	0		0		0		0	
Line of treatment								
1	5	10	28	56	3	6	36	72
2			8	16	3	6	11	22
≥ 3			3	6			3	6

Characteristics of patients from University of Zürich Hospital-Schlieren - Switzerland

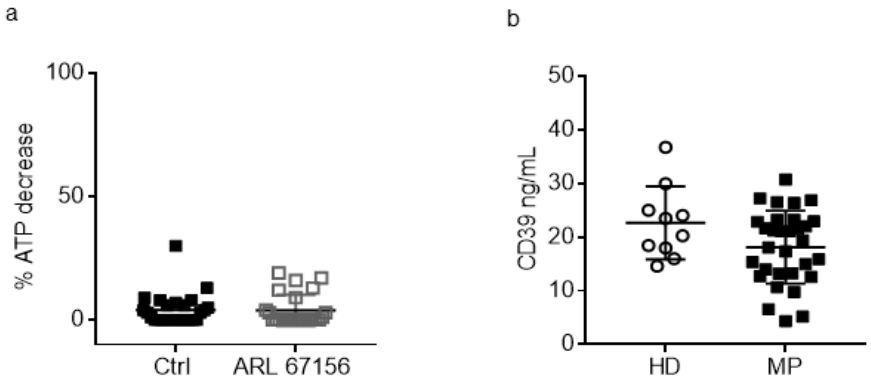
	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)		Nivolumab +ipilimumab therapy (no, %)		TOTAL (n, %)	
Total	21	14,6	94	65,3	29	20,1	144	100
Median age, years (range 27-93)	60		67		61		65	
Sex								
male	16	11,1	63	43,8	18	12,5	97	67,4
female	5	3,5	31	21,5	11	7,6	47	32,6
Stage								
III	9	6,5	10	6,9	3	2,1	22	15,3
IV	12	8,3	83	57,6	26	18,1	121	84,0
unknown	0		1	0,7	0		1	0,7
Serum lactate dehydrogenase								
normal (< ULN)	19	13,2	77	53,5	18	12,5	114	79,2
elevated (≥ ULN)	2	1,4	17	11,8	11	7,6	30	20,8
unknown	0		0		0		0	
BRAF								
wt	13	9,0	64	44,4	19	13,2	96	66,7
mut	6	4,2	29	20,1	10	6,9	45	31,3
unknown	2	1,4	1	0,7	0		3	2,0
Brain metastasis								
yes	2	1,4	11	7,6	4	2,8	17	11,8
no	19	13,2	83	57,6	25	17,4	127	88,2
unknown	0		0		0		0	
Bone metastasis								
yes	2	1,4	30	20,8	7	4,9	39	27,1
no	18	12,5	63	43,8	22	15,3	103	71,5
unknown	1	0,7	1	0,7	0		2	1,4
Line of treatment								
1	9	6,3	18	12,5	11	7,6	38	26,4
2	4	2,8	23	16,0	8	5,6	35	24,3
≥ 3	8	5,6	53	36,8	10	6,9	71	49,3

Characteristics of patients from Melanoma, Cancer Immunotherapy and Development Therapeutics Unit, Istituto Nazionale Tumori-IRCCS Fondazione "G. Pascale" - Naples, Italy

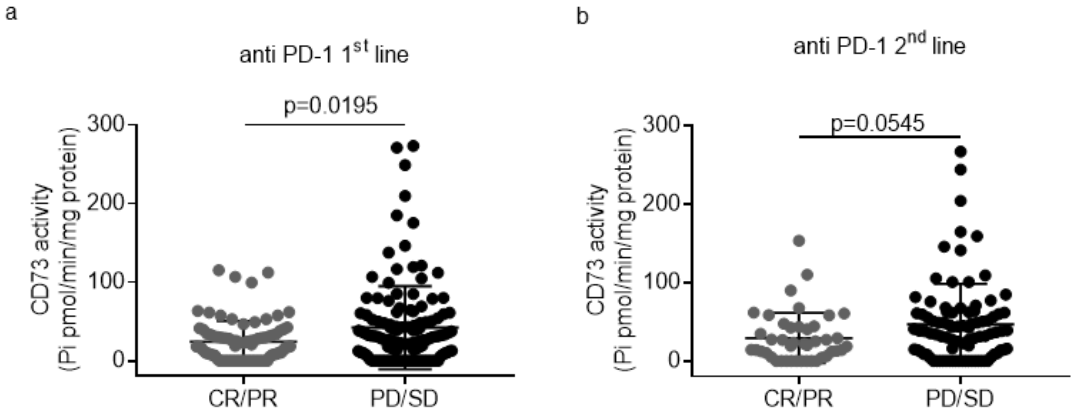
	Nivolumab therapy (no, %)		Pembrolizumab therapy (no, %)	Nivolumab +ipilimumab therapy (no, %)	TOTAL (n, %)	
Total	100	100	0	0	100	100
Median age, years (range 21-91)	61,5				61,5	21-91
Sex						
male	53	53			53	53
female	47	47			47	47
Stage						
III	4	4			4	4
IV	94	94			94	94
unknown	2	2			2	2
Serum lactate dehydrogenase						
normal (< ULN)	50	50			50	50
elevated (≥ ULN)	25	25			25	25
unknown	25	25			25	25
BRAF						
wt	60	60			60	60
mut	38	38			38	38
unknown	2	2			2	2
Brain metastasis						
yes	24	24			24	24
no	74	74			74	74
unknown	2	2			2	2
Bone metastasis						
yes	16	16			16	16
no	84	84			84	84
unknown	0	0			0	0
Line of treatment						
1	46	46			46	46
2	35	35			35	35
≥ 3	19	19			19	19



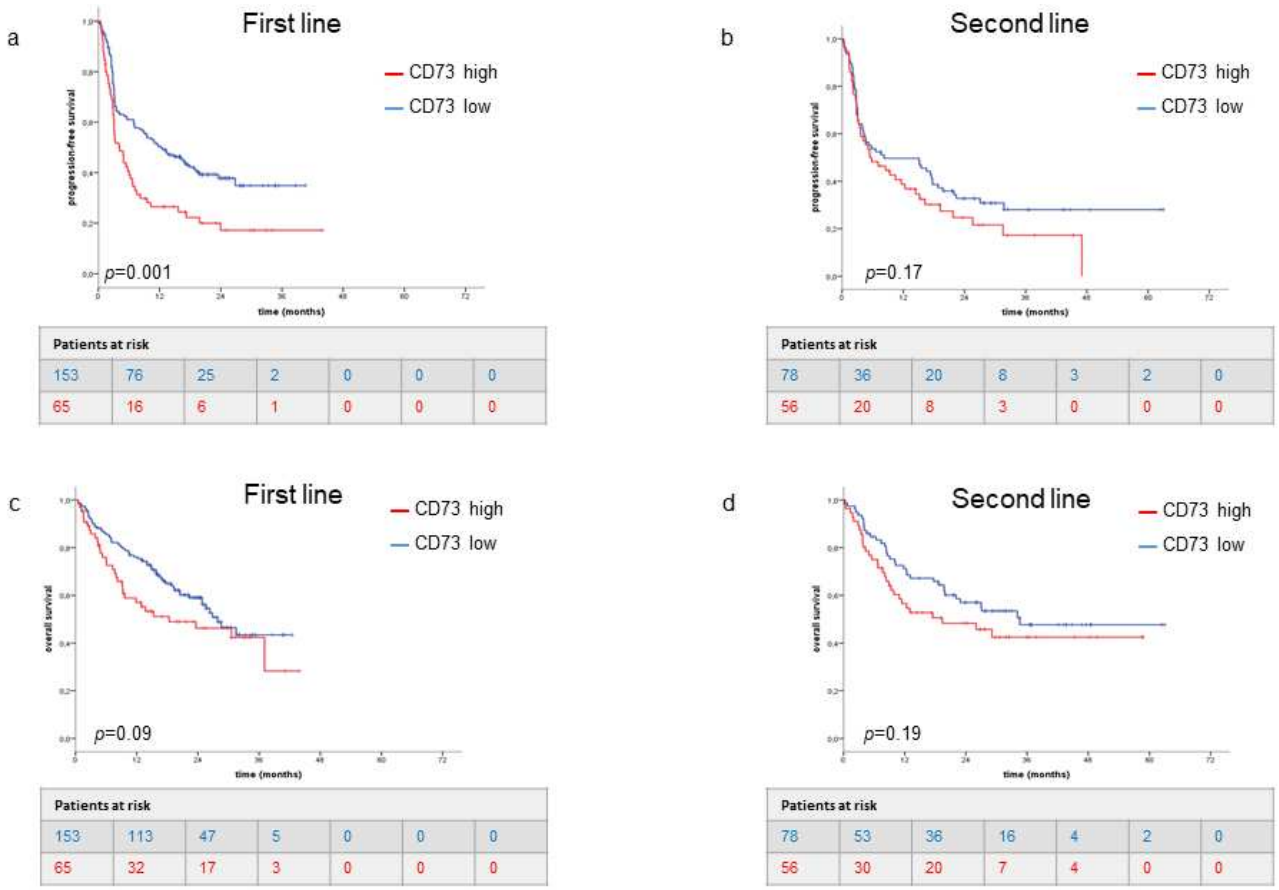
Online Supplementary Figure S1 TNAP activity in serum of melanoma patients. The activity of tissue non-specific alkaline phosphatases (TNAP) was evaluated by colorimetric assay in serum samples of melanoma patients (n=7) at pH 7.4 or pH 9.8, in presence of the substrate p-nytrophenylphosphate (pNPP; 0.5 mM, 1 mM, 2.5 mM, 5 mM). Then, the plate was incubated again at 37°C. The hydrolysis of pNPP mediated by alkaline phosphateses leads to the formation of a yellow product, measured by spectrophotometer at 405 nm. Lane represents mean. Significance was evaluated with Kruskal-Wallis test.



Online Supplementary Figure S2. ATP decrease and CD39 expression in serum samples. a. The ATPase activity was evaluated in presence of ATP (2mM) in serum samples from melanoma patients (n=26). Reaction was stopped with TCA (5%) after 40 min of incubation. The ATP levels were determined in the samples supernatants using the ATP assay kit (ab83355, Abcam). The percentage of ATP hydrolysis was evaluated in presence of the selective CD39 inhibitor, ARL67156 (100µM, Sigma-Aldrich) or vehicle (Ctrl). **b**. CD39 was determined in serum of 31 melanoma patients and 10 healthy subjects, by means of commercially available ELISA kit (Human CD39 ELISA kit, LS-F25268, LifeSpan BioSciences, Inc.). Data are mean ± s.d.



Online Supplementary Figure S3. Baseline levels of serum CD73 activity based on clinical benefit to anti-PD-1 agents as first line or second line treatment. **a.** serum CD73 activity in patients who respond or failed to anti-PD-1 agents in 1st line treatment (CR/PR=82 patients; PD/SD=131 patients) or **b.** in 2nd line treatment (CR/PR=43 patients; PD/SD=90 patients). Mean ± s.d. is shown. Statistical analysis was performed with Mann-Whitney test.



Online Supplementary Figure S4. Associations of baseline CD73 activity with progression-free (top) and overall survival (bottom) in melanoma patients receiving anti-PD-1 agent as first line (a, c) or second line (b, d) treatment. P values were calculated using log-rank test.