Peripheral blood Th9 cells are a possible pharmacodynamic biomarker of nivolumab treatment efficacy in metastatic melanoma patients

Supplemental information

Material and methods

Tumor proliferation assay

B16 melanoma cells were cultured in complete DMEM supplemented with 10% FCS/10 mM Hepes/100 units/ml penicillin/100 mg/ml streptomycin/2 mM L-glutamine (Invitrogen) at 5% CO_2 for 24 h with or without 10 ng/mL of recombinant murine IL-9 (BioLegend). Finally, the number of harvested cells was counted using a hematocytometer.

IL-9 stimulation assay of human melanoma cell lines

Human melanoma cell lines, A375 and SK-MEK-28, were commercially purchased from ECACC or ATCC. They were cultured in complete DMEM supplemented with 10% FCS/10 mM Hepes/100 units/ml penicillin/100 mg/ml streptomycin/2 mM L-glutamine (Invitrogen) at 5% CO_2 for 24 h with or without 10 ng/mL of recombinant murine IL-9 (BioLegend). The number of harvested cells was counted using a hematocytometer. The following fluorescent-labeled monoclonal antibodies were used for surface staining of these melanoma cells: HLA-ABC-PE (BioLegend), IL-9R-PE (BioLegend), PD-L1-PE/Cy7 (BioLegend), HLA-DR-APC (eBioscience). After surface staining, the cells were permeabilized and then fixed

according to the manufacturer's instructions using the Cytofix/Cytoperm kit (BD Biosciences). Acquisition was performed by eight-color flow cytometry using FACS Fortessa with FACS Diva software (both from BD Biosciences). The compensation control was performed with BD CompBeads (BD Biosciences). FlowJo software (Tree Star) was used for analysis.

Supplemental Table 1

Laboratory data of the patients with nivolumab treatment.

Res: responders, Non: non-responders, SD: standard deviation, WBC: white blood cells, LDH: lactate dehydrogenase

	Unit	**Before**					**After**				
		Res	**SD**	**Non**	**SD**	***P* value**	**Res**	**SD**	**Non**	**SD**	***P* value**
Age		69.5	10.2	65	12.2	0.129	-	-	-	-	-
WBC	/μL	4579	930.7	5206	1937	0.309	5326	2068	6990	4349	0.23
Neutrophils	/μL	2950	835.4	3549	1805	0.296	3542	1987	5231	4388	0.227
Lymphocytes	/μL	1207	309.7	1114	431.6	0.512	1294	470.4	1045	506.5	0.158
Monocytes	/μL	288	62.31	355.7	135.7	0.119	355.1	164.4	388.1	218.3	0.648
Eosinophils	/μL	101.9	75.32	132.6	126	0.451	108.5	80.33	220.4	207.6	0.09
Basophils	/μL	32.44	23.21	27.53	21.66	0.524	29.27	24.62	31.35	24.18	0.806
LDH	IU/L	283.8	163	343.6	391.8	0.627	297.7	265.9	427.1	513.8	0.43

Supplemental Table 2

The size of melanoma lesion (length × width, mm^2) of Braf/Pten mutation mice.

		Day 0	Day 5	Day 10	Day 15
Control	#1	10	12	14	16
	#2	25	30	36	40
	#3	6	8	10	12
αIL-9	#1	10	24	26	32
	#2	12	24	36	72
	#3	49	104	120	182
	#4	45	72	100	131

Supplemental Table 3

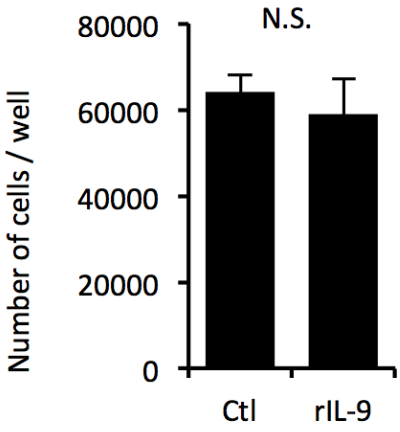
Clinical information of the patients enrolled in the immunohistochemistry of IL-9 and CD8.

#	Age	Sex	TNM	Stage	Type
1	52	F	pT2bN1aM0	IIIB	SSM
2	68	M	pT2N0M0	IB	ALM
3	48	M	pT4aN1aM0	IIIA	SSM
4	78	M	T4bN3M0	IIIC	ALM
5	71	M	pT2aNX	IB	SSM
6	73	F	pT1aN0M0	IA	ALM
7	90	M	pT4bNxM0	IIC	ALM
8	69	F	pT4bN0M0	IIC	Mucosal
9	79	M	T4bN3M0	IIIC	ALM
10	89	M	pT4bN3M0	IIIC	LMM

M: male, F: female, ALM: acral lentiginous melanoma, SSM: superficial spreading melanoma, LMM: lentigo maligna melanoma

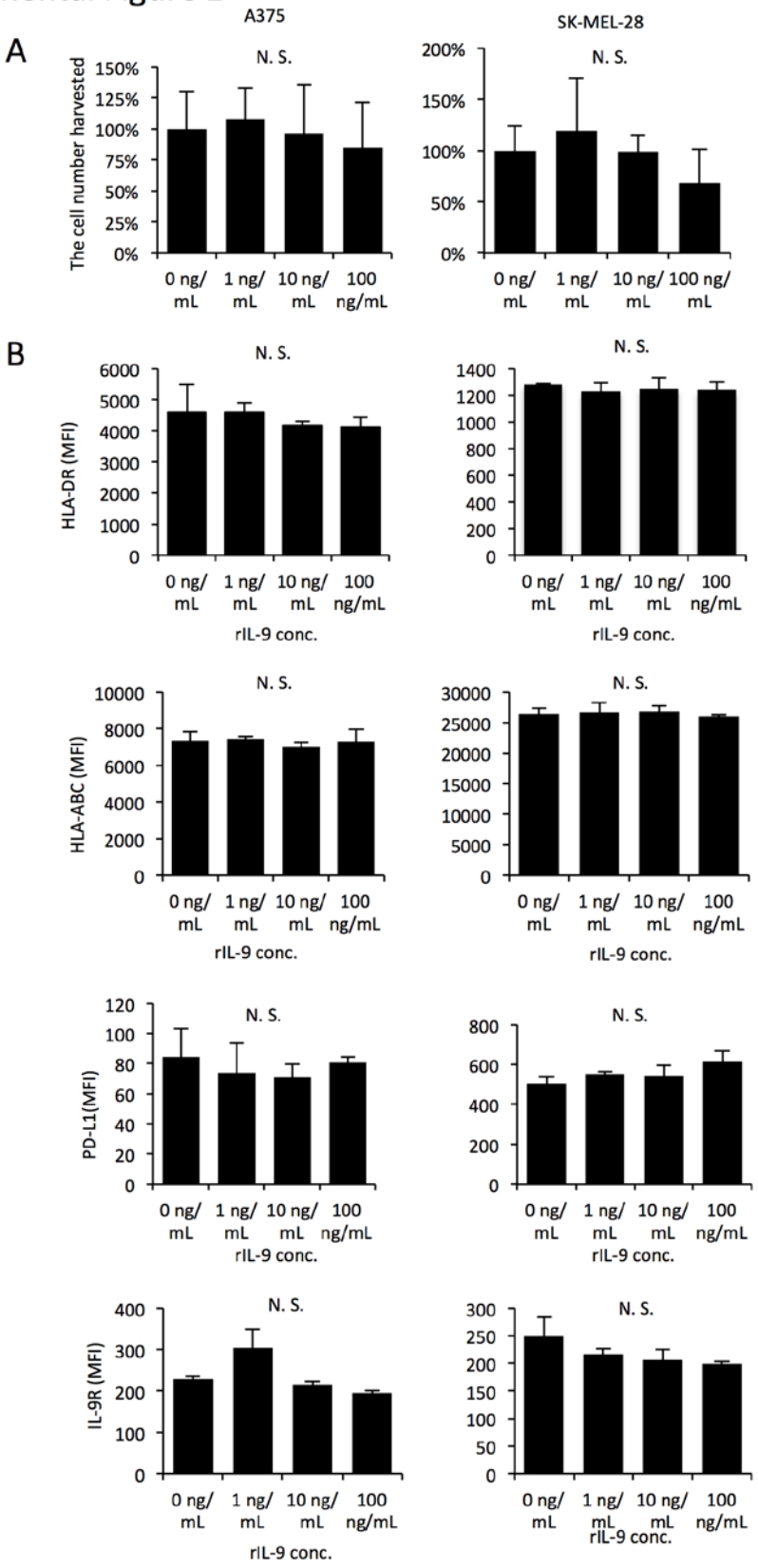
Supplemental Figure 1

A



B16 melanoma cells were cultured in the presence or absence of rIL-9. No significant difference was observed in the proliferation of the cells, irrespective of the presence of rIL-9.

Supplemental Figure 2



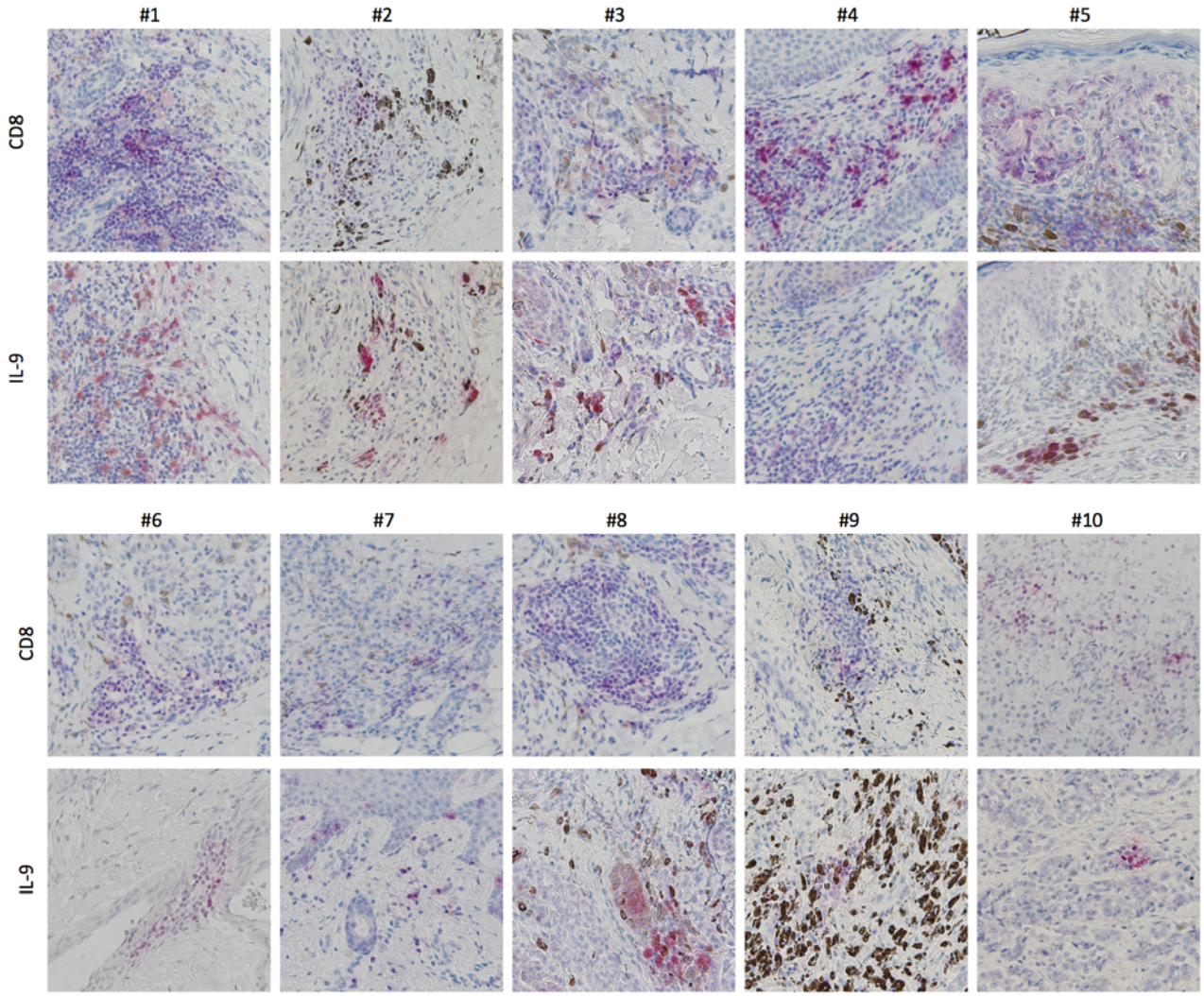
Human melanoma cell lines (A375 and SK-MEL-28) were cultured in the presence or absence of rIL-9.

(A) The cell number after 24 h culture was shown as % of that of control group.

(B) Using flowcytometry, the expression levels of HLA-DR, HLA-ABC, PD-L1, IL-9R were quantified by measurement of each mean fluorescent intensity. No significant difference was observed, irrespective of the presence of rIL-9.

The bar graphs show the representative data.

Supplemental Figure 3



CD8 (upper) and IL-9 (lower) expression in human melanoma tissues from 10 melanoma patients (immunohistochemistry).