
Supplementary information

**PLA2G2D in tumour-draining lymph nodes
regulates anti-tumour immunity**

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PLA2G2D in tumour-draining lymph nodes regulates anti-tumour immunity

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SUPPLEMENTARY DATA

Supplementary Tables 1 & 3-7 (Supplementary Table 2: separate .xlsx file)

Supplementary Table 1 – Melanoma IMC patient characteristics

Supplementary Table 1 - Characteristics of melanoma patients selected for Imaging Mass Cytometry. P-values for categorical variables were calculated using a Chi-squared test. For continuous variables, P-values were calculated using an independent sample T test.

	ALL PATIENTS N=21		STAGE 2		STAGE 3		P VALUE
			Recurrence >96 months	Recurrence <48 months	Recurrence > 60 months	Recurrence < 24 months	
PATIENT CHARACTERISTICS							
AGE (MEAN, 95%CI)		52.9 (48.2- 57.6)	54 (44.9- 63.1)	52.6 (40.0- 65.3)	50.5 (37.5- 63.6)	54.8 (38.8- 70.8)	0.920
SEX							0.613
	Female	9	2	1	3	3	
	Male	12	3	4	3	2	
TUMOR CHARACTERISTICS							
BRESLOW (MM) MEAN (95%CI)		3.3 (2.5-4.1)	3.8 (0.6-7.0)	3.6 (1.4-5.9)	2.8 (1.1-4.4)	3.2 (1.9-4.5)	0.791
ULCERATION							
	Absent	10	5	1	3	1	0.036
	Present	11	0	4	3	4	
HISTOLOGY							
	SSM	12	3	3	2	4	
	NM	6	0	2	3	1	
	Unknown	3	2	0	1	0	
LOCATION							0.637
	Arm	5	2	2	0	1	
	Leg	4	0	1	2	1	
	Trunk	10	2	2	4	2	
	Head & neck	2	1	0	0	1	
SLN SURGICAL REMOVAL							
SN REGION							0.682
	Axillary	13	3	4	3	3	
	Iguinal- iliac	6	1	1	3	1	
	cervical	2	1	0	0	1	
OUTCOME							
TIME TO RECURRENCE (MONTHS)				14.6 ± 11.3		11.8 ± 5.3	

Supplementary Table 3 - Melanoma validation cohort

Supplementary Table 3 - Characteristics of melanoma patients selected as a validation cohort. P-values for categorical variables were calculated using a Chi-squared test. For continuous variables, P-values were calculated using an independent sample T test.

		NO RECURRENCE > 60 MONTHS N=10	RECURRENCE < 24 MONTHS N=14	P-VALUE
PATIENT CHARACTERISTICS				
AGE (MEAN, 95%CI)		57.5 (51.2-63.8)	55.9 (50.9-60.8)	0.652
SEX				0.408
	Female	6	5	
	Male	4	9	
PRIMARY TUMOR CHARACTERISTICS				
BRESLOW (MM) MEAN (95%CI)		3.9 (2.7-5.1)	5.2 (3.4-7.0)	0.195
ULCERATION				0.134
	Absent	6	3	
	Present	4	10	
	unknown	0	1	
HISTOLOGY				0.257
	SSM	6	3	
	NM	3	5	
	SSM/NM	0	1	
	ALM	1	2	
	Unknown	0	3	
LOCATION				0.804
	Upper limb	2	2	
	Lower limb	5	6	
	Trunk	3	6	
OUTCOME				
TIME TO RECURRENCE (MONTHS) MEAN ± SD		11.8 ± 6.3		
RELAPSE SITE				
	Lymphnodes		2	
	Lung		5	
	Liver		2	
	Bone		1	
	intransit		3	
	Multiple organs		1	

Supplementary Table 4 – NSCLC patient characteristics

Supplementary Table 4 - Characteristics of non-small cell lung cancer patients. P-values for categorical variables were calculated using a Chi-squared test. For continuous variables, P-values was calculated using an independent sample T test.

		NO RECURRENCE > 60 MONTHS N= 9	RECURRENCE < 60 MONTHS N= 9	P-VALUE
PATIENT CHARACTERISTICS				
AGE (MEAN, SD)		62.2 years (6.0)	69.3 years (12.8)	0.152
SEX				0.165
	Female	7	4	
	Male	2	5	
PRIMARY TUMOR CHARACTERISTICS				
T-STAGE				0.228
	T1	2	1	
	T2	4	3	
	T3	2	3	
	T4	1	2	
N-STAGE				1.000
	N1	6	6	
	N2	3	3	
HISTOLOGY				0.332
	Squamous cell carcinoma	4	2	
	Adenocarcinoma	4	6	
	NSCLC NOS	1	1	
TDLN LOCATION				0.999
	N1 station (hilar, 9,10,11,12)	8	8	
	N2 station (4)	-	1	
PD-L1				0.089
	NEG	3	3	
	POS	2	5	
LOCATION				0.564
	Right upper lobe	5	5	
	Middle lobe	-	1	
	Right lower lobe	1	2	
	Left upper lobe	1	-	
	Left lower lobe	2	1	
OUTCOME				
TIME TO RECURRENCE (MONTHS) MEAN ± SD			23 ± 14	
RELAPSE SITE				
	Lymph nodes		2	
	Lung		2	
	Pleura		1	
	Bone		1	
	Cerebral		2	
	Multiple organs		1	

Supplementary Table 5 – IMC Antibodies

Supplementary Table 5 - Imaging Mass Cytometry panel.

ANTIBODY	DILUTION	LABEL	COMPANY	CLONE	#CAT
BCL6	300	172Yb	CST	E5I8I	56263
CD11c	200	142Nd	CST	D3V1E	#45581
CD127	400	168Er	SBT	EPR2955(2)	3168026D
CD14	1000	144Nd	SBT	EPR3653	3144025D
CD141	200	141Pr	CST	E7Y9P	#43514
CD15	100	149Sm	CST	MC480	74180
CD163	500	147Sm	SBT	EDHu1	3147021D
CD169	200	163Dy	Abcam	SP213	ab245735
CD1c	1000	148Nd	Abcam	EPR23189-196	ab270797
CD20	400	161Dy	SBT	H1	3161029D
CD25	100	175Lu	Abcam	SP176	ab238272
CD27	100	171Yb	Abcam	EPR8569	ab256583
CD3	200	170Er	Abcam	SP162	ab245731
CD31	800	151Eu	CST	89C2	85873
CD4	500	156Gd	SBT	EPR6855	3156033D
CD45	1000	152Sm	SBT	D9M8I	3152018D
CD45RA	400	166Er	SBT	HI100	3166031D
CD68	400	159Tb	SBT	KP1	3159035D
CD69	1000	164Dy	Abcam	EPR21814	ab234512
CD8A	400	162Dy	CST	D8A8Y	81575
COLLAGEN	600	169Tm	SBT	Polyclonal	3169023D
CSK-I	1000	195Pt	SBT	-	TIS-00001
CSK-II	1000	196Pt	SBT	-	TIS-00001
CSK-III	1000	198Pt	SBT	-	TIS-00001
CTLA-4	400	160Gd	Abcam	CAL49	ab251599
D2-40	200	153Eu	CST	LpMab-12	#26981
DNAI	800	191Ir	SBT	-	201192A
DNAII	800	193Ir	SBT	-	201192A
FoxP3	1600	155Gd	Abcam	221D	ab255942
GATA3	1600	176Yb	CST	D13C9	#5852
GRANZYME B	400	167Er	SBT	EPR20129-217	3167021D
HLA-DR	1000	174Yb	SBT	LN3	3174025D
IDO	100	145Nd	CST	D5J4E	#86630
Ki-67	200	146Nd	CST	8D5	#9449
PD-1	200	165Ho	CST	EH33	60847
PD-L1	300	150Nd	CST	E1L3N	85164
SOX10	200	158Gd	CST	E6B6I	69661
TCF1	200	173Yb	CST	C63D9	#2203
TIM-3	800	154Sm	CST	D5D5R	81229
TOX	400	143Nd	CST	E6I3Q	#73758

Supplementary Table 6 – Histology antibodies

Supplementary Table 6 - Antibodies used for IHC and multiplex immunofluorescence

ANTIBODY	DILUTION	SPECIES	COMPANY	CLONE	<u>AB INCUBATION</u> time at 37°C
CD11C	2.15 ug/ml	rabbit	Cell Marque	SP157	12 minutes
CD8	1.04 ug/ml	mouse	DAKO	C8/144B	32 minutes
CD68	3.49 ug/ml	mouse	Ventana	KP1	12 minutes
LAMP3	1/100	rabbit	Atlas Antibodies	Polyclonal	60 minutes
CXCL13	1/1200	rabbit	Invitrogen	polyclonal	20 minutes
PLA2G2D	1/800	rabbit	Invitrogen	polyclonal	32 minutes

Supplementary Table 7 – Flowcytometry & culture reagents

Supplementary Table 7 – Flowcytometry Human panel A

PRODUCT	FLUOCHROME	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
CD19	FITC	H1B-19	BD	555412	1:20
CD20	FITC	2H7	Invitrogen / eBioscience	11-0209-42	1:40
CD11C	FITC	B-ly6	BD	564490	1:20
CD68	FITC	Y1/82A	Biolegend	333806	1:10
CD56	FITC	HCD56	Biolegend	318304	1:10
CD14	FITC	61D3	Invitrogen / eBioscience	11-0149-42	1:10
CD16	FITC	3G8	BD	555406	1:10
CD123	FITC	6H6	Biolegend	306014	1:10
HELIX NP BLUE	BV421		Biolegend	425305	

Supplementary Table 7 - Flowcytometry Human panel B

PRODUCT	FLUOCHROME	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
CD4	APC-H7	RPA-T4	BD	560158	1:20
CD223 (LAG-3)	AF700	11C3C65	Biolegend	369344	1:20
CD197 (CCR7)	BV510	G043H7	Biolegend	353232	1:20
CD366 (TIM-3)	BV750	F38-2E2	Biolegend	345055	1:20
CD152 (CTLA-4)	BV421	BNI3	BD	562743	1:20
CD39	BV711	TU66	BD	563680	1:20
CD279 (PD-1)	PE	MIH4	Invitrogen / eBioscience	12-9969-42	1:10
CD8A	PerCp-Cy5.5	RPA-T8	Invitrogen / eBioscience	45-0088-42	1:20
CD45RA	PE-TxR	MEM-56	Invitrogen / eBioscience	MHCD45RA17	1:20
CD5	PE-Cy7	UCHT2	Biolegend	300622	1:30
FIXABLE VIABILITY STAIN 575V	BV575		BD	565694	1:500
TOX	APC	REA473	Miltenyi Biotec	130-118-335	1:20
KI67	BV605	Ki67	Biolegend	350522	1:100
TIGIT	BV711	741182	BD	747839	1:20
CD69	FITC	FN50	BD	555530	1:20
CD279 (PD-1)	BV786	EH12.2H7	Biolegend	329939	1:20

Supplementary Table 7 – Flowcytometry Human panel C

PRODUCT	FLUOCHROME	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
CD4	APC-Cy7	RPA-T4	BD	560158	1:25
CD3	AF700	UCHT1	eBioscience	56-0038-42	1:25
CD8	PE-TXR	RPA-T8	BD	562282	1:50
IL-2	BV650	Mq1-17H12	BD	564166	1:25
IFNY	BV711	B27	BD	564039	1:25

Supplementary Table 7 – Flowcytometry Mouse antibodies

PRODUCT	FLUOCHROME	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
CD62L	PE-Dazzle	MEL-14	Biolegend	104448	1:200
CD8	PE	53-6.7	Invitrogen / eBioscience	12-0081-83	1:800
CD44	BV650	IM7	BD	740455	1:400
CD4	APC-H7	GK1.5	BD	560181	1:50
CD45	BV786	30-F11	BD	564225	1:1600
CD3	Biotin	145-2C11	BD	553060	1:100
FIXABLE VIABILITY DYE	eFluor506		Invitrogen / eBioscience	65-0866-14	1:200
CD45	PerCP-Cy5.5	1600	30-F11	eBioscience	45-0451-82
CD11B	PE-Cy7	1000	M1/70	BD	552850
CD44	APC-Cy7	800	IMF7	BD	560568
CD3	FITC	100	145-2C11	BD	553062
CD11C	APC	200	N418	Invitrogen / eBioscience	17-0114-81
CD44	BV650	IM7	Biolegend	103049	1:400
PD1	BV421	J43	BD	562584	1:100
CD8	BV605	53-6.7	Biolegend	100744	1:800
CD4	APC-H7	GK1.5	BD	560181	1:50
CD45	BV786	30-F11	BD	564225	1:1600
CD3	Biotin	145-2C11	BD	553060	1:100
FOXP3	AF700	FJK-165	Invitrogen / eBioscience	56-5773-82	1:100
KI67	FITC	SolA15	Invitrogen / eBioscience	11-5698-82	1:400
CD8	APC	53-6.7	Invitrogen / eBioscience	17-0081-82	1:400
CD3	PE-Cy7	145-2C11	Biolegend	100320	1:100
CD44	PerCP-Cy5.5	IM7	Invitrogen / eBioscience	45-0441-82	1:800
CD45	AF700	30-F11	Biolegend	103128	1:100
CD8	BV786	53-6.7	Biolegend	100750	1:400
IL-2	APC	JES6-5H4	BD	554429	1:100
H-2KB TRP-2 TETRAMER- SVYDFFVWL	PE		Dr. Thorbald van Hall (LUMC)		1:200

Supplementary Table 7 – Flow cytometry materials

PRODUCT	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
Human TruStain FcX		Biolegend	422302	
Brilliant Stain Buffer		BD	563794	
Permeabilization Buffer (10X)		Invitrogen / eBioscience	00-8333-56	
Fixation/Permeabilization Concentrate		Invitrogen / eBioscience	00-5123-43	

Fixation/Permeabilization Diluent	Invitrogen /	00-5223-56
Saponin from Quillaja Bark	eBioscience	S7900-100G
Paraformaldehyde	Merck	16005

Supplementary Table 7 – Cell culture materials

PRODUCT	CLONE	SUPPLIER	CATALOGUE #	CONCENTRATION
RPMI 1640 Medium, GlutaMAX Supplement		ThermoFisher	61870010	
Fetal Bovine Serum		BODINCO BV	BDC-S00O12	1:10
Penicillin-Streptomycin (10,000 U/mL)		ThermoFisher	15140122	1:100
Primocin		InvivoGen	ant-pm-1	100 µg/mL
Purified anti-human CD3	OKT-3	Biolegend	317302	0,5 µg/mL
Purified anti-human CD28	CD28.2	Biolegend	302902	0,2 µg/mL
DMEM glutamax		Gibco	10566016	
Gentamicin		Gibco	15710-049	50 µg/mL
<u>FACS and MACS buffer</u>				
Dulbecco's Phosphate Buffered Saline		Gibco	14190-094	
BSA		Sigma-Aldrich	A-8327	
EDTA		Sigma-Aldrich	03690	