
Supplementary information

$\gamma\delta$ T cells are effectors of immunotherapy in cancers with HLA class I defects

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Supplementary information

Supplemental Table 1. Characteristics of clinical samples from 71 patients with MMR-d cancers from the DRUP study.

See separate - Excel table

Supplemental Table 2. Immune marker gene sets for bulk RNA-seq analyses.

See separate - Excel table

Supplemental Table 3. Characteristics of clinical samples from 2,256 patients with MMR-p cancers from the Hartwig database.

See separate - Excel table

Supplemental Table 4. Characteristics of clinical samples from 17 patients with ICB-naïve MMR-d colon cancer.

Patient ID	Tumor location	HLA class I status	$\beta 2m$ status	<i>B2M</i> status	Subgroup
CRC 1	Flexura lienalis	Positive	Positive	Wildtype	$\beta 2m^+$
CRC 2	Coecum	Defect	Mut pattern	Wildtype	$\beta 2m^+$
CRC 3	Flexura hepatica	Positive	Positive	Wildtype	$\beta 2m^+$
CRC 6	Descendens	Defect	Defect	Mutated	$\beta 2m^-$
CRC 19	Ascendens	Defect	Defect	Mutated	$\beta 2m^-$
CRC 52	Ascendens	Defect	Mut pattern	Wildtype	$\beta 2m^+$
CRC 67	Transversum	Positive	Positive	Wildtype	$\beta 2m^+$
CRC 84	Sigmoid	Defect	Defect	Wildtype	$\beta 2m^-$
CRC 94 #	Sigmoid	Defect	Positive	Wildtype	$\beta 2m^+$
CRC 96* #	Transversum	Defect	Defect	Mutated	$\beta 2m^-$
CRC 102	Ascendens	Defect	Positive	Wildtype	$\beta 2m^+$
CRC 134* #	Ascendens	Defect	Defect	Mutated	$\beta 2m^-$
CRC 154* #	Flexura hepatica	Positive	Positive	Wildtype	$\beta 2m^+$
CRC 159*	Coecum	Defect	Mut pattern	Wildtype	$\beta 2m^+$
CRC 167* #	Ascendens	Defect	Mut pattern	Wildtype	$\beta 2m^+$
CRC 177	Flexura hepatica	Defect	Positive	Wildtype	$\beta 2m^+$
CRC 195	Flexura hepatica	Defect	Positive	Wildtype	$\beta 2m^+$

* Used for single-cell RNA-sequencing experiments, # Used for cell culturing experiments. *B2M*, $\beta 2$ -microglobulin; MMR, mismatch repair.

Supplemental Table 5. Characteristics of patient-derived organoids from MMR-d colorectal cancer.

Sample	PDTO-1 WT	PDTO-1 <i>B2M</i> ko	PDTO-2 WT	PDTO-2 <i>B2M</i> ko
Tumor	CRC	CRC	CRC	CRC
MMR status	deficient	deficient	deficient	deficient
Biopsy / Resection	Resection	Resection	Biopsy	Biopsy
Biopsied lesion	Colon	Colon	Peritoneal	Peritoneal
Primary / Metastasis	Primary	Primary	Metastasis	Metastasis
Authenticated	Confirmed by SNP	Confirmed by STR	Confirmed by SNP	Confirmed by SNP
Mycoplasma	Negative	Negative	Negative	Negative
Characteristics	Luciferase transduced; parental line of PDTO-1 <i>B2M</i> -KO	<i>B2M</i> -knockout of PDTO-1 WT	Parental line of PDTO-2 <i>B2M</i> -KO	<i>B2M</i> -knockout of PDTO-2 WT
<i>B2M</i> knockout	-	sgRNA targeting <i>B2M</i> (GGCCGAGATGTCTCGCTCC G) cloned into LentiCRISPR v2 plasmid	-	sgRNA targeting <i>B2M</i> (GGCCGAGATGTCTCGCTCC G) cloned into LentiCRISPR v2 plasmid

Supplemental Table 6. Antibodies used for imaging mass cytometry of colon cancers.

Antibody	Metal	Clone	Supplier	Catalog number	Lot number	Incubation time (temperature)	Dilution
β -catenin	89Y	D10A8	CST	8480BF	8	Overnight (4°C)	1:100
CD103	168 Er	EPR4166(2)	Abcam	ab221210	GR3355784-7	5h (RT)	1:50
CD11b	144 Nd	D6X1N	CST	49420BF	4	5h (RT)	1:100
CD11c	176 Yb	EP1347Y	Abcam	ab216655	GR3357092-9	5h (RT)	1:100
CD14	163 Dy	D7A2T	CST	56082BF	2	5h (RT)	1:100
CD15	171 Yb	MC480	CST	4744BF	5	Overnight (4°C)	1:100
CD163	173 Yb	EPR14643-36	Abcam	93498BF	Not available	5h (RT)	1:50
CD20	142 Nd	E7B7T	CST	48750BF	9179056	Overnight (4°C)	1:100
CD204	164 Dy	J5HTR3	Thermo Fisher Scientific	14-9054-95	4338161	5h (RT)	1:50
CD3	153 Eu	EP449E	Abcam	ab271850	GR3341846-3	Overnight (4°C)	1:50
CD31	147 Sm	89C2	CST	3528BF	Not available	Overnight (4°C)	1:100
CD38	169 Tm	EPR4106	Abcam	ab226034	GR3378690-1	Overnight (4°C)	1:100
CD39	157 Gd	EPR20627	Abcam	ab236038	GR3274485-6	5h (RT)	1:100
CD4*	145 Nd	EPR6855	Abcam	ab181724	GR3285644-10	Overnight (RT)	1:100
CD45	149 Sm	D9M8I	CST	13917BF	11	Overnight (4°C)	1:50
CD45RO	165 Ho	UCHL1	CST	55618BF	2	Overnight (4°C)	1:100
CD56	167 Er	E7X9M	CST	99746BF	2	5h (RT)	1:100
CD57	151 Eu	HNK-1 / Leu-7	Abcam	ab269781	GR3373313-3	Overnight (4°C)	1:100
CD68	143 Nd	D4B9C	CST	76437BF	2	Overnight (4°C)	1:100
CD7	174 Yb	EPR4242	Abcam	ab230834	Not available	5h (RT)	1:100
CD8 α	146 Nd	D8A8Y	CST	85336BF	Not available	5h (RT)	1:50
Cleaved caspase-3	172 Yb	5A1E	CST	9664BF	24	5h (RT)	1:100
D2-40	166 Er	D2-40	BioLegend	916606	B316467	Overnight (4°C)	1:100
FOXP3	159 Tb	D608R	CST	12653BF	8	Overnight (4°C)	1:50
Granzyme B	150 Nd	D6E9W	CST	46890BF	3	5h (RT)	1:100
Histone H3	209	D1H2	CST	4499BF	Not available	Overnight (4°C)	1:50
HLA-DR	141 Pr	TAL 1B5	Abcam	ab176408	GR3384096-1	5h (RT)	1:100
ICOS	161 Dy	D1K2T™	CST	89601BF	4	5h (RT)	1:50
IDO	162 Dy	D5J4E™	CST	86630BF	7	Overnight (4°C)	1:100
Ki-67	152 Sm	8D5	CST	9449BF	11	Overnight (4°C)	1:100

LAG-3	155 Gd	D2G40™	CST	15372BF	Not available	5h (RT)	1:50
p16ink4a	175 Lu	D3W8G	CST	92803BF	2	Overnight (4°C)	1:100
Pan-keratin	198 Pt	C11 and AE1/AE3	CST / BioLegend	4545BF / 914204	12 / B302316	Overnight (4°C)	1:50
PD-1	160 Gd	D4W2J	CST	86163BF	7	5h (RT)	1:50
PD-L1	156 Gd	E1L3N ^R	CST	13684BF	17	Overnight (4°C)	1:50
T-bet	170 Er	4B10	BioLegend	644825	B298378	5h (RT)	1:50
TCR δ^*	148 Nd	H41	Santa Cruz	sc-100289	D3021	Overnight (RT)	1:50
TGF β	115 In	TB21	Thermo Fisher Scientific	MA5- 16949	151471	5h (RT)	1:100
TIM-3	154 Sm	D5D5R™	CST	45208BF	9	5h (RT)	1:100
Vimentin	194 Pt	D21H3	CST	5741BF	9	Overnight (4°C)	1:50
VISTA	158 Gd	D1L2G™	CST	64953BF	7	5h (RT)	1:100

* Detection with metal-conjugated secondary antibodies. CST, Cell Signaling Technology.

Supplemental Table 7. Antibodies used for immunophenotyping of $\gamma\delta$ T cells by flow cytometry.

Antibody	Fluorochrome	Clone	Supplier	Catalog number	Lot number	Dilution
CD16	PE	B73.1	BD Biosciences	332779	9045985	1:60
CD103	FITC	Ber-ACT8	BD Biosciences	550259	2332847	1:10
CD122/IL-2R β	BV421	TU27	BioLegend	339010	B313155	1:20
CD132/IL-2R γ	APC	TUGh4	BioLegend	338608	B293032	1:80
CD161	BV605	DX12	BD Biosciences	563863	7030586	1:20
CD25/IL-2R α	PE-Cy7	M-A251	BD Biosciences	557741	9301660	1:25
CD215/IL-15R α	PE	JM7A4	BioLegend	330208	B265801	1:80
CD226/DNA M-1	BV510	DX11	BD Biosciences	742494	9203072	1:150
CD3	Am Cyan	SK7	BD Biosciences	339186	9161745	1:20
CD32	APC	FLI8.26	BD Biosciences	559769	184743	1:20
CD38	PE-Cy7	HIT2	eBioscience	25-0389-42	4319912	1:200
CD39	APC	A1	BioLegend	328210	B249211	1:60
CD45RA	FITC	L48	BD Biosciences	335039	8227525	1:30
CD45RA	PE-Dazzle594	HI100	Sony	2120730	126470	1:20
CD45RO	PerCP-Cy5.5	UCHL1	Sony	2121110	138351	1:20
CD56	APC-R700	NCAM16.2	BD Biosciences	565139	5251693	1:150
CD64	FITC	10.1	BD Biosciences	555527	58058	1:20
CD69	PerCP-Cy5.5	FN50	BioLegend	310925	B266970	1:200
CD8 α	BV605	SK1	BD Biosciences	564115	7092	1:100
CD94	BV605	HP-3D9	BD Biosciences	743950	7138571	1:200
GITR	PE	108-17	BioLegend	371204	B244963	1:50
Granzyme B*	PE	GB11	eBiosciences	12-8899-41	1928380	1:50
KIR2DL1	PE	HP3-E4	BD Biosciences	556063	86798	1:20
KIR2DL1/S1	PE	EB6	Beckman Coulter	A09778	12	1:50
KIR2DL2/L3/S2	PE	GL183	Beckman Coulter	IM2278U	200051	1:50
KIR2DL4	PE	181703	R&D Systems	FAB2238P	AAHO0209081	1:10
KIR2DS4	PE	FES172	Beckman Coulter	IM3337	200037	1:40
KIR3DL1	PE	DX9	BD Biosciences	555967	121769	1:80
KIR3DL1/S1	PE	Z27	Beckman Coulter	IM3292	200044	1:20
KIR3DL2	PE	#539304	R&D Systems	FAB2878P	ADBO0217051	1:10
LAG3	PE-Cy7	11C3C65	BioLegend	369309	B289009	1:100
NKG2A	APC	z199	Beckman Coulter	A60797	200046	1:30
NKG2C	PE	134591	Beckman Coulter	FAB138P	LCN0818011	1:20
NKG2D	PE-Cy7	1D11	BD Biosciences	562365	9045733	1:300

NKp44	APC	P44-8	BioLegend	325109	B160899	1:20
NKp46	PE	9E2	BioLegend	331907	B150121	1:20
PD-1	PE	MIH4	eBioscience	12-9969-42	1952441	1:30
Perforin*	PE-Cy7	dG9	BioLegend	308125	B215704	1:20
TCR $\gamma\delta$	BV421	11F2	BD Biosciences	744870	9340519	1:80
TCR $\gamma\delta$	BV650	11F2	BD Biosciences	745359	7222894	1:40
TCR V δ 1	FITC	TS8.2	Invitrogen	TCR2730	UH286015	1:50
TCR V δ 2	PerCP-Cy5.5	B6	BioLegend	331424	B279957	1:200
TIGIT	APC	1D11	BD Biosciences	562365	9045733	1:300
Live/dead	nIR	n.a.	Life Technologies	L10119	1808830	1:1000

* Detected intracellularly

Videos (supplied separately):

Video 1. Killing of CRC cells upon co-culture with $\gamma\delta$ T cells. Killing of HCT-15 cells (red) by $\gamma\delta$ T cells (V δ 1⁺) from a MMR-d colon cancer in the presence of caspase-3/7 (green). $\gamma\delta$ T cells were added at T=6h, and images were taken until 12 hours after (T=18h). Cancer cell apoptosis is visualized in yellow.

Video 2. Pronounced cancer cell apoptosis upon co-culture with PD-1⁺ (V δ 1⁺) as compared to PD-1⁻ $\gamma\delta$ T cells. Killing of HCT-15 cells (red) by PD-1⁺ (V δ 1⁺, left panel) as compared to PD-1⁻ (V δ 2⁺, right panel) $\gamma\delta$ T cells from a MMR-d colon cancer in the presence of caspase-3/7 (green). $\gamma\delta$ T cells were added at T=5h, and images were taken until 12 hours after (T=17h). Cancer cell apoptosis is visualized in yellow.