

Response to pembrolizumab in metastatic colorectal cancer with microsatellite instability and markedly reduced HLA-I expression suggests NK cell involvement

Authors:

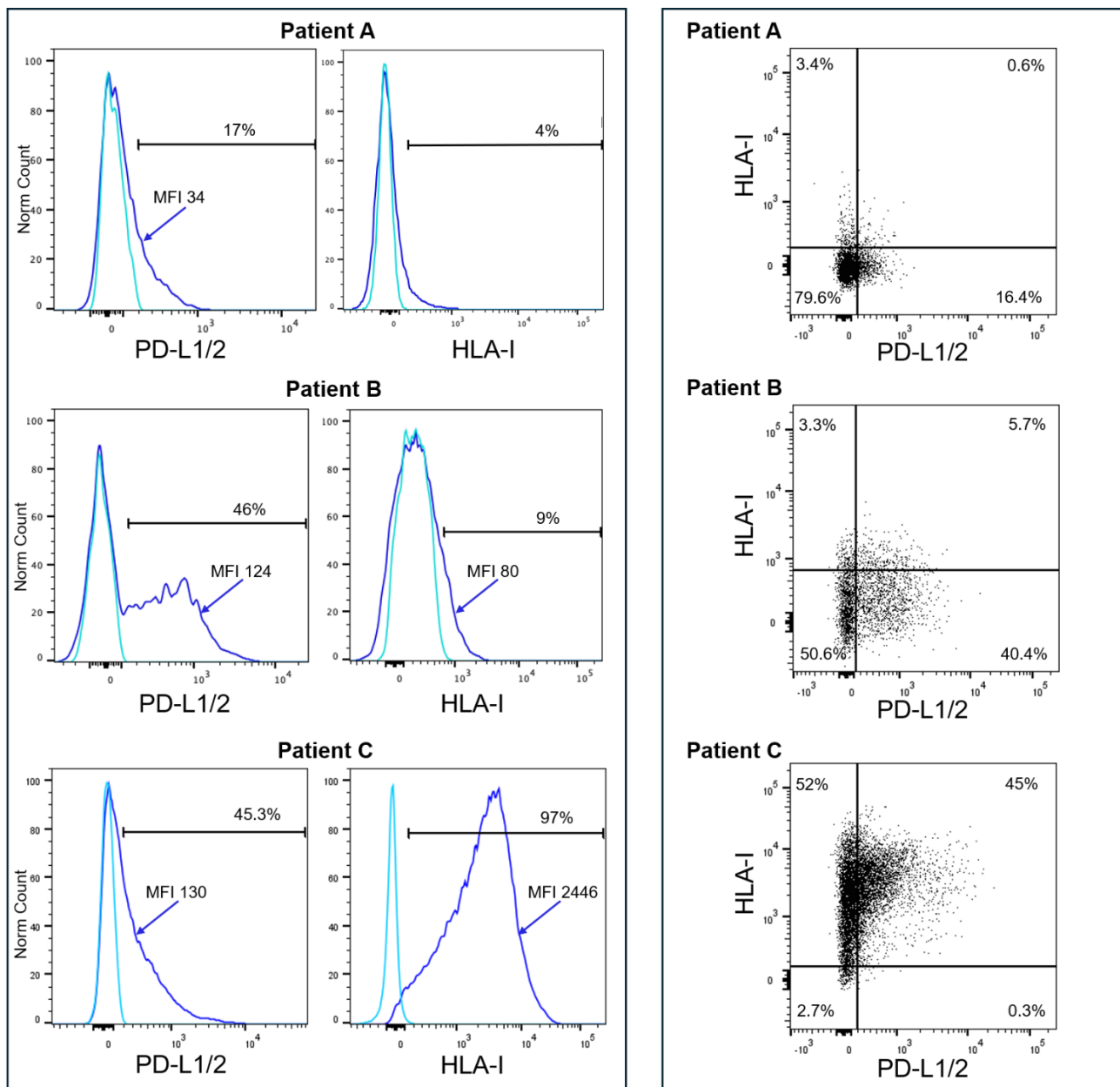
Nicoletta Provinciali^{1,2^}, Chiara Giordano^{1^}, Valentina Obino^{1^}, Matteo Bozzo³, Mariangela Rutigliani⁴, Simona Candiani^{3,5}, Simona Carlomagno⁶, Silvia Pesce^{1,5}, Marco Greppi¹, Enrico Ciferri⁷, Tania Buttiron Webber², Laura Paleari⁸, Andrea Barberis⁷, Marco Filauro⁷, Matteo Clavarezza², Emanuela Marcenaro^{1,5}, Mariella Della Chiesa^{1,5*}, Andrea De Censi^{2,9#}, Simona Sivori^{1,5*#}

Supplemental Material

Table S1 Flow cytometry and IHC reagents				
Monoclonal antibody	Fluorochrome	Source	Cat. No.	RRID
Goat Anti-Mouse IgG2a	FITC	Southern Biotech	1080-02	AB_2794476
Goat Anti-Mouse IgG2b	PE	Southern Biotech	1010-09	AB_2794129
CD3	PE-CF594	BD Horizon™	562280	AB_11153674
CD14	PE-CF594	BD Horizon™	562335	AB_11153663
CD19	PE-CF594	BD Horizon™	562294	AB_11154408
CD33	PE-CF594	BD Horizon™	562492	AB_2713912
CD45	APC/BUV496	BD Horizon™	569101	AB_3684778
CD56	BV480	BD Horizon™	566124	AB_2739525
CD56	PE-Cy7	Beckman Coulter	A21692	AB_2892144
CD49a	BV786	BD OptiBuild™	742362	AB_2740720
CD90	BV480	BD OptiBuild™	746286	AB_2743616
CD103	BV711	BD Horizon™	563162	AB_2738039
CD127	BV650/PE-CF594	BD Horizon™	563225	AB_2738081
EpCAM	BV786	BD Horizon™	565685	AB_2744364
FixableStain620		BD Horizon™	564996	AB_2869636
HLA-ABC	BV650	BD OptiBuild™	740581	AB_2740282
KIR2DL1/S1	FITC	Miltenyi Biotec	130-118-973	AB_2733621
KIR2DL2/L3/S2	FITC	BD Pharmingen™	559784	AB_397325
KIR3DL1/L2/S1-IgG2a	Non-conjugated	Produced in our laboratory		
NKG2A	PE-Cy7	Beckman Coulter	B10246	AB_2687887
PD-1-IgG2b	PE or non-conjugated	Miltenyi Biotec	130-120-382	AB_2752069
PD-L1	APC-R700	BD Horizon™	565188	AB_2739101
PD-L2	APC-R700	BD Horizon™	557926	AB_647162
Isotype IgG1	BV650	BD Horizon™	563231	AB_2869470

Isotype IgG1	APC-R700	BD Horizon™	564974	AB_2869632
HLA Class 1 ABC		Abcam	ab225636	
MLH1		VENTANA®	790-5091	AB_2336022
MSH2		VENTANA®	790-5093	AB_2936886
MSH6		VENTANA®	790-5092	AB_2936885
PD-L1		VENTANA®	40-49077	
PMS2		VENTANA®	790-5094	AB_3669003

Supplementary Figure 1



Surface expression of PD-L1/2 and HLA-I on tumor cells in patients A, B and C

Left panels: histogram representation of PD-L1/2 and HLA-I surface expression on tumor cells (blue lines) of Patients A, B and, as positive control, of Patient C, compared to their corresponding isotype controls (light blue lines). The bar gates were set based on the isotype controls and the percentage of positive cells refers to PD-L1/2⁺ or to HLA-I⁺ tumor cells. Isotype-corrected Median Fluorescence Intensity (MFI) for the whole tumor population is indicated for both markers.

Right panels: dotplots showing the reciprocal surface expression of HLA-I and PD-L1/2 on tumor cells of Patients A, B and C. The percentages of cells are indicated in each quadrant.

Tumor cells were gated as viable Lin-CD45-EpCAM⁺CD90⁻ cells in all panels.