

Antibody	Provider	Concentration used	Clone
gp100	DBS	1/25	HMB45
Melan-A	Dako	1/25	A103
Tyrosinase	Novocastra	4 µg/mL	T311
pan-MAGEs	Santa Cruz	8 µg/mL	-
NY-ESO-1	Santa Cruz	10 µg/mL	E978
CD8	Dako	5 µg/mL	C8/144B
CD4	Dako	2.5 µg/mL	MT310
Foxp3	ebioscience	5 µg/mL	236A/E7
IL-10	Diaclone	2 µg/mL	B-S10
TGF-β	Serotec	2 µg/mL	TB21
IFN-γ	Santa Cruz	20 µg/mL	G23
TNF-α	DBS	1/50	28401.111
IDO1	Serotec	1/100	10,1
STAT3	Lifespan	1/100	-
PD-1	ebioscience	5 µg/mL	J105
PD-L1	ebioscience	5 µg/mL	MIH1
CTLA-4	ebioscience	5 µg/mL	14D3

Supplementary Table 1. Monoclonal antibodies used to assess the expression of predictive markers: T-cell infiltrate (CD4 and CD8), pro-inflammatory cytokines (IFN-γ and TNF-α), transcription factor STAT3, immunosuppressive cytokines (IL-10 and TGF-β), IDO1 and Foxp3, negative checkpoint molecules (PD-1, PD-L1 and CTLA-4) and melanoma-associated antigens (Melan-A, gp100, tyrosinase, NY-ESO-1 and pan-MAGEs)

BRAF V600	forward	AGG TGA TTT TGG TCT AGC TAC AGT
	reverse	ATG GAT CCA GAC AAC TGT TCA AAC
BRAF V600E	forward	AGG TGA TTT TGG TCT AGC TAC AGA
	reverse	ATG GAT CCA GAC AAC TGT TCA AAC
BRAF 15F	forward	5'-TCA TAA TGC TTG CTC TGA TAG GA-3'
BRAF 15R	reverse	5'-GGC CAA AAA TTT AAT CAG TGG A-3'
NRAS 2F	forward	5'-CCC CCA GGA TTC TTA CAG AA-3'
NRAS 2R	reverse	5'-ATA CAC AGA GGA AGC CTT CG-3'

Supplementary Table 2. Primers used for amplification and sequencing to detect BRAF and NRAS mutations

Markers	Overall clinical response			Local cutaneous response		
	RC or RP or SD (Mean [Min-Max])	P (Mean [Min-Max])	p-value	RC or RP or SD (Mean [Min-Max])	P (Mean [Min-Max])	p-value
CD8	0.8[0-1]	1[0-2]	0.76	1[0-2]	0.7[0-2]	0.498
CD4	1.6[1-2]	1.83[1-3]	0.68	1.88[1-3]	1.33[1-2]	0.252
Foxp3	0.4[0-1]	0.167[0-1]	0.48	0.25[0-1]	0.33[0-1]	0.895
GP100	1.8[0-3]	2.5[0-4]	0.40	2.25[0-4]	2[0-3]	0.833
Melan-A	2.8[1-4]	2[0-4]	0.51	2.375[0-4]	2.33[0-4]	1
Tyrosinase	1.8[0-4]	1[0-4]	1.00	1.125[0-4]	2[1-4]	0.229
MAGEs	1.4[0-4]	1.33[0-4]	1.00	1.375[0-4]	1.33[0-4]	0.515
NY-ESO-1	0.8[0-4]	0.5[0-1]	0.59	0.75[0-4]	0.33[0-1]	0.904
IL-10	1[0-4]	0.33[0-2]	0.49	0.63[0-4]	0.67[0-2]	0.896
TGF- β 1	1.8[1-2]	2.33[0-4]	0.56	1.75[0-4]	3[2-4]	0.132
IFN- γ	2[0-4]	1.33[0-4]	0.39	1.5[0-4]	1[0-2]	0.670
TNF- α	0.4[0-2]	0.33[0-2]	1.00	0.25[0-2]	0.667[0-2]	0.542
IDO1	1.6[0-4]	0.67[0-2]	0.21	1.25[0-4]	0.67[0-2]	0.812
STAT3	0[0-0]	0[0-0]	1.00	0[0-0]	0[0-0]	NA
PD-1	1.8[0-4]	1.33[0-4]	0.61	2.13[0-4]	0[0-0]	0.11
PD-L1	0[0-0]	1[0-4]	0.22	0.5[0-4]	0.667[0-2]	0.649
CTLA-4	1[0-4]	1.16[0-4]	0.62	0.75[0-4]	0.667[0-2]	0.123
BRAF status	2/6	3/7	1	3/9	2/2	1

Supplementary Table 3. Correlation of tissue immunity markers before treatment with the overall clinical response and local cutaneous response. *CR*, complete regression; *PR*, partial regression; *SD*, stable disease; *P*, progression; *IL*, interleukin; *IFN- γ* , interferon-gamma; *TNF- α* , tumor necrosis factor-alpha; *IDO*, indoleamine dioxygenase; *CD*, cluster of differentiation; *CTLA-4*, Cytotoxic T-Lymphocyte Antigen 4; *TGF- β* , Transforming growth factor beta; *MAGE*, Melanoma-associated antigen; *NY-ESO-1*, New-York Esophageal Squamous Cell Carcinoma.

Patient code	Check point	CD8	CD4	Foxp3	gp100	Melan-A	Tyrosinase	MAGE	NY-ESO-1	IL-10	TGF-b	IFN-γ	TNF-a	IDO1	STAT3	PD-1	PD-L1	CTLA-4	Overall clinical response	Local cutaneous response
5 A-C	T0	1	1	0	3	1	1	1	0	0	2	2	2	0	0	4	0	4	CR	CR
	M3	2	1	0	4	1	0	0	0	0	3	2	2	0	0	4	0	1		
6 O-A	T0	2	3	0	1	1	0	1	1	0	1	1	0	0	0	4	0	1	P	SD
	M3	2	1	0	0	0	0	0	0	1	0	2	0	0	0	2	0	4		
7 G-M	T0	1	1	0	2	3	0	0	0	1	2	0	0	4	0	4	0	0	PR	CR
	M3	2	1	1	1	4	0	1	0	0	3	2	0	0	1	0	0	3		
15 T-J	T0	1	2	0	4	0	0	2	1	0	4	1	0	2	0	4	4	0	P	PR
	M3	2	3	0	4	4	4	4	4	0	0	4	0	1	0	1	0	4		

Supplementary Table 4. Immunohistochemistry of cutaneous immunity markers and melanoma antigens performed on 4 cutaneous biopsies taken from 4 patients before and 3 months after treatment initiation in injected lesions.