


PATIENT CODE	SEX-AGE	STAGE	ARM	PATIENT CODE	SEX-AGE	STAGE	ARM
INT01/001	F61	IIIB	VAX	INT01/023	M52	IIIB	OBS
INT01/002	F64	IIIB	OBS	INT01/024	F56	IIIA	VAX
INT01/003	M57	IIC	VAX	INT01/025	M30	IIB	OBS
INT01/004	M68	IIB	VAX	INT01/026	F36	IIIB	OBS
INT01/005	F69	IIIA	VAX	INT01/027	F41	IIIB	OBS
INT01/006	M49	IIIB	VAX	INT01/028	F38	IIIB	VAX
INT01/007	F28	IIIB	VAX	INT01/029	M43	IIIA	OBS
INT01/008	M67	IIB	VAX	INT01/030	F45	IIB	VAX
INT01/009	M44	IIIB	VAX	INT01/031	F46	IIIA	VAX
INT01/010	M55	IIB	OBS	INT01/032	F55	IIB	OBS
INT01/011	F38	IIIB	VAX	INT01/033	F48	IIIB	VAX
INT01/012	M77	IIB	OBS	INT01/034	F63	IIB	VAX
INT01/013	M68	IIB	VAX	INT01/035	M68	IIB	OBS
INT01/014	M58	IIC	OBS	INT01/036	F36	IIIB	OBS
INT01/015	F40	IIB	OBS	INT01/037	M38	IIIB	OBS
INT01/016	F56	IIB	OBS	SIE01/001	M43	IIIC	OBS
INT01/017	F49	IIIC	OBS	SIE01/002	F51	IIIA	OBS
INT01/018	M51	IIIA	OBS	SIE01/003	F71	IIIC	VAX
INT01/019	F54	IIIB	OBS	SIE01/004	F56	IIIA	VAX
INT01/020	M60	IIB	VAX	PDA01/001	M66	IIB	VAX
INT01/021	M56	IIIC	VAX	PDA01/002	M46	IIB	VAX
INT01/022	M41	IIIB	OBS				

 PBMC of patients available for Treg analysis

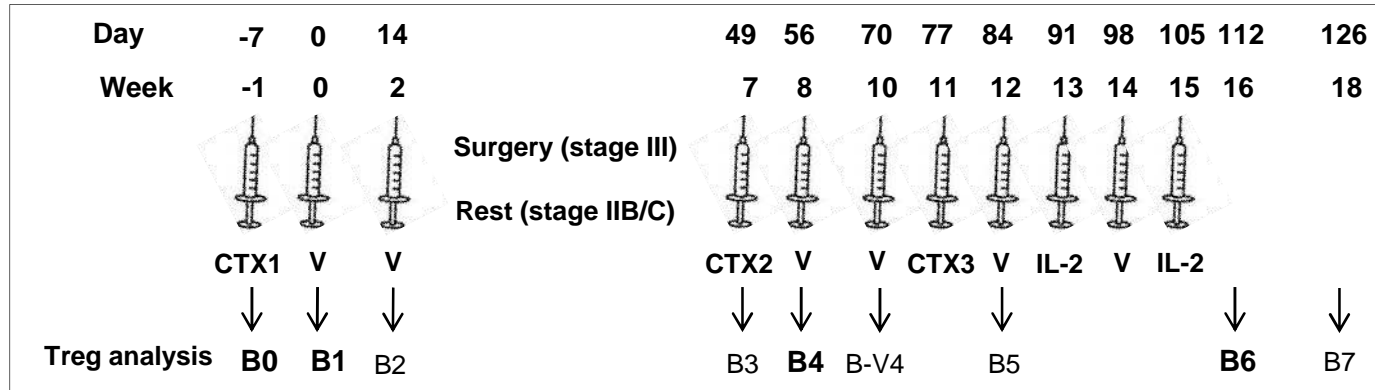
Online table 1. Patients Characteristics

	Foxp3 EXPRESSION		
SCORE	I	II	III
CRL	0	4	5
VAX	2	6	1

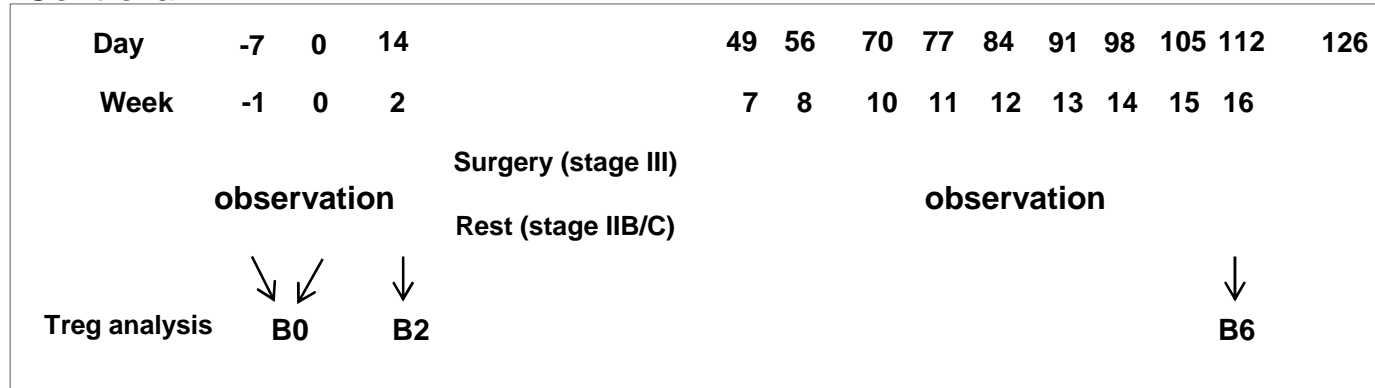
Online table 2. Foxp3 expression in LN tissues

Immunohistochemistry IHC was performed on paraffin embedded LN tissues using anti-Foxp3 mAb. Anti-Foxp3 Ab immunoreactivity was scored as grade I (0%-5%), II (5%-10%) and III (> 10%) according to the number of Foxp3 positive nuclei found in lymphocyte T area; number of positive cases for control patients (CRL, n=9) and vaccinated patients (VAX, n=9) are reported.

Vaccination arm

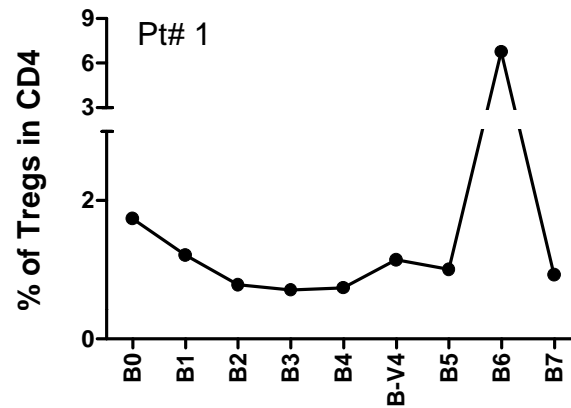


Control arm



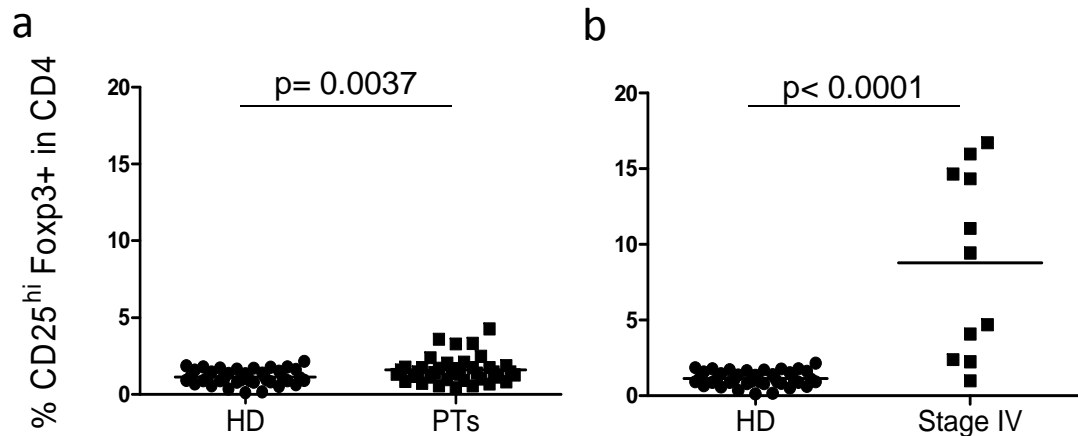
Schematic representation of the vaccination schedule Vaccination arm: V= peptide vaccine comprised of HLA-A*0201-restricted (Melan-A/MART-1[27L], gp100[210M], NY-ESO-1[165V], and Survivin[97M]) 250 µg each emulsified in Montanide ISA-51. CTX= Cyclophosphamide i.v. 300 mg/m². IL-2 s.c. 3x10⁶ IU/day for 3 days. B= blood samples collected; bold font: samples available for all the patients. Control arm: observation only.

Online figure 1



IL-2-mediated Treg increases are transient Graph shows the frequency profile of CD4⁺CD25^{hi}Foxp3⁺ Tregs in gated CD4⁺ lymphocytes in patient PBMC. A representative case is reported (Pt# 1).

Online figure 2



Treg frequency in melanoma patients Difference in the frequencies of Tregs, CD25^{hi}Foxp3⁺ cells in CD4 lymphocytes, between healthy donors (HD, n= 42) and patients enrolled in the study (PTs, n= 32) is reported in panel (a). Panel (b) shows the frequency of Tregs in stage IV melanoma patients with high tumor burden (Stage IV, n= 11) compared to that in healthy donors (HD) (p value calculated with Student's t-Test).

Online figure 3