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Cancer vaccines and their targets

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Target tumor associated antigens (TAAs) recognized by T cells. Which is the best TAA or combination of?

1. Shared/self/differentiation TAAs (natural *vs.* modified)
2. Shared/self/cancer testis or germinal TAAs
3. Universal TAAs (survivin, hTERT)
4. Mutated, unique TAAs
5. Cancer Stem Cells TAAs

1-2. Shared self TAAs

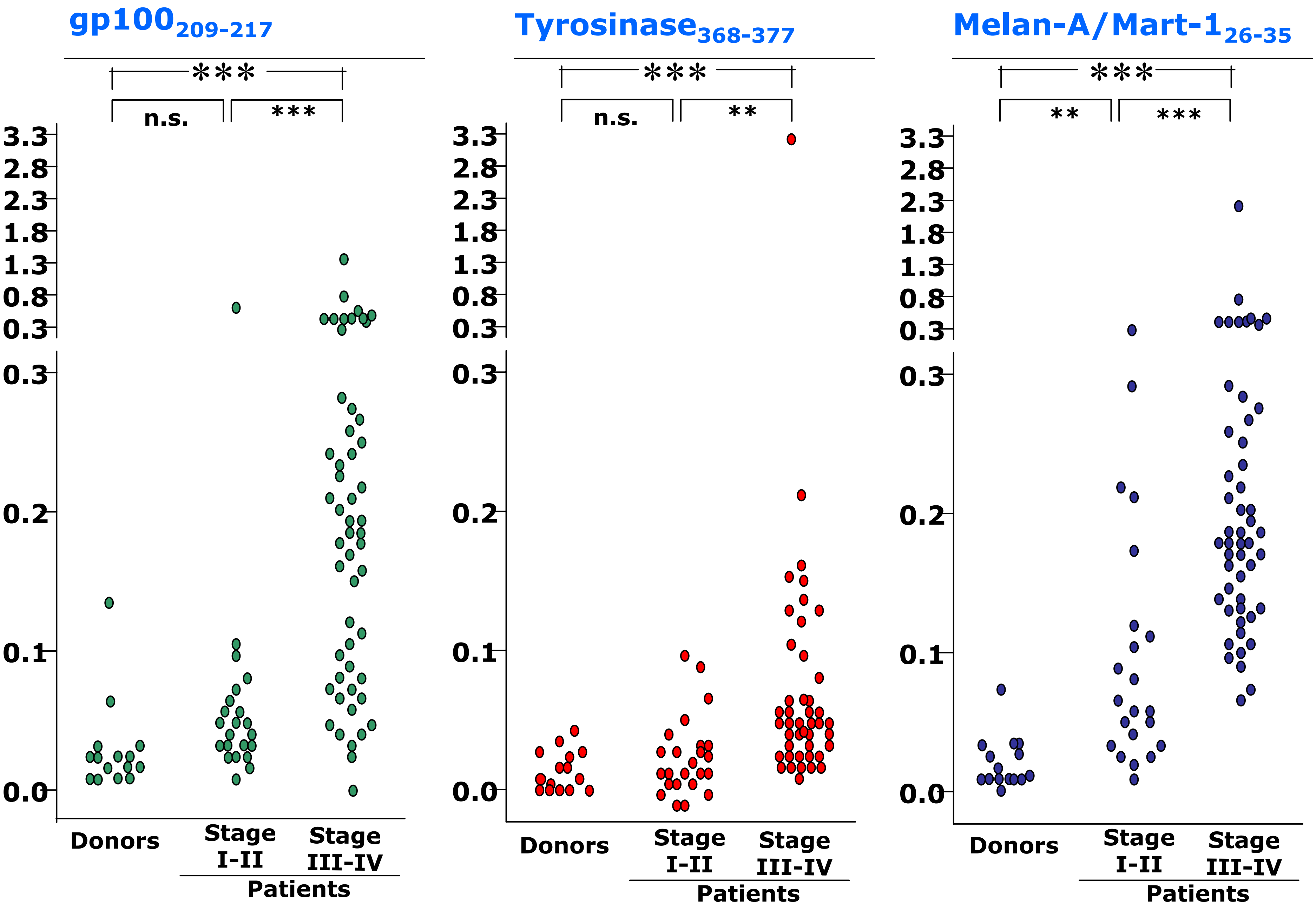
- Normal subjects and cancer patients show some form of tolerance to “self” TAAs (*immune ignorance, peripheral or central tolerance, low frequency of T cell precursors*).

- Tolerance needs to be broken in order to induce a T cell immune response against “self” TAAs.

- Thus, these TAAs are considered to be “weak antigens”

In vivo tolerance break (immunogenicity) of shared self TAAs

FREQUENCY OF ANTI-MAA CD8 T LYMPHOCYTES AND PROGRESSION OF THE DISEASE (*Anichini et al., Cancer Res 2004*)



COA-1, a colorectal cancer TAA whose T cell recognition increases with tumor progression

(Maccalli et al., Cancer Res 2003; Maccalli et al. Clin Cancer Res 2008)

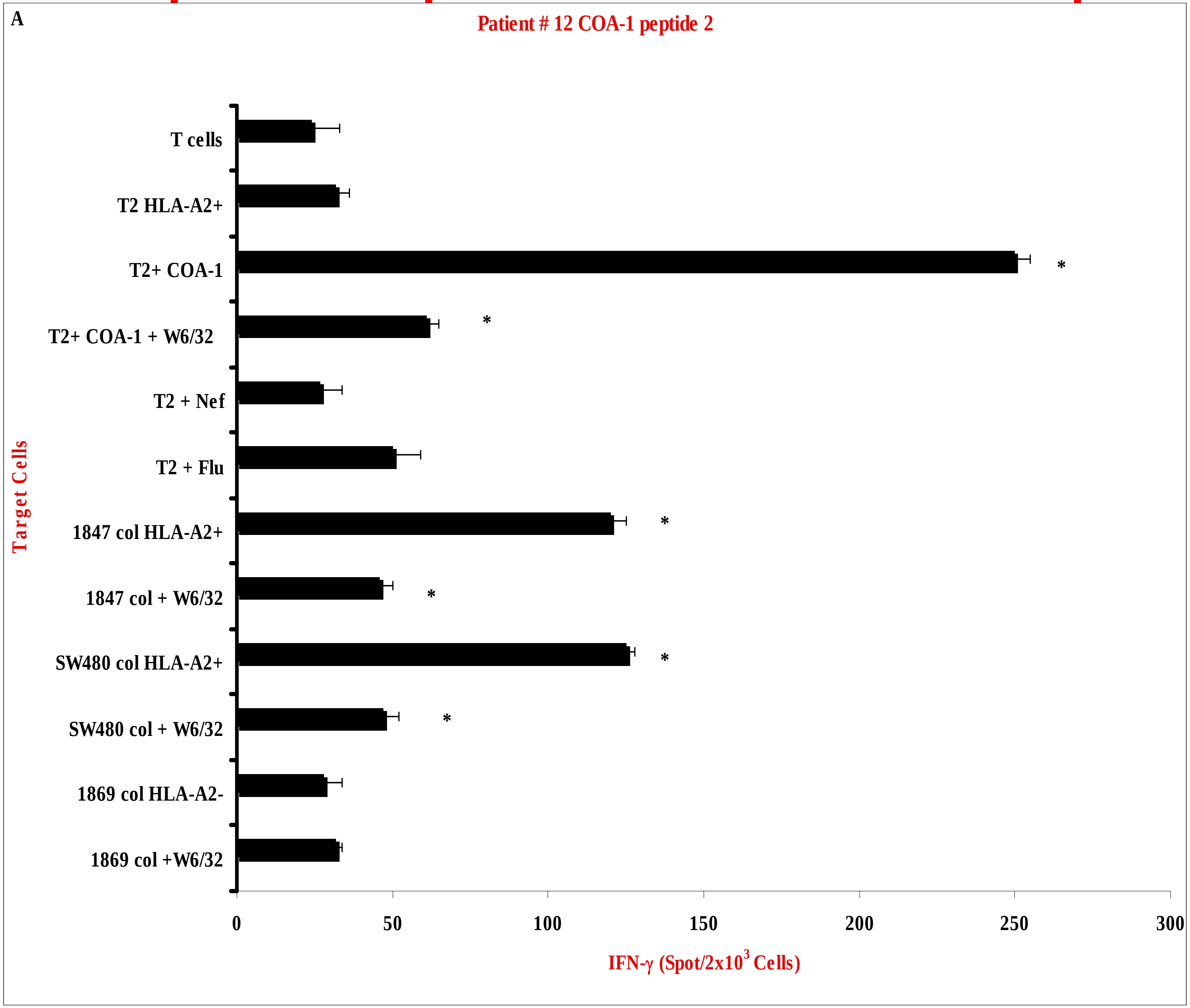
- ***COA-1 is encoded by the UBXD5*** **gene, a regulator of RhoA functions affecting polymerization of actin fibers.**

CD4+ T cell-mediated anti-COA-1 response in metastatic *but not early CRC*

Patient #	Stage	COA-1		
		*N. specific cultures**		
1	I	0/24		
2	II	0/24		
3	II	0/24		
4	II	0/24	**0/4**	
5	IV	**8/11**		
6	IV	**6/24**		
7	IV	**11/24**		
8	IV	**2/24**	**4/4**	

***T cell cultures specifically recognizing COA-1 and tumor cells.**

HLA-A*0201-related COA-1 epitopes elicit $CD8^+$ T cell tumor specific responses in metastatic CRC patients



CONCLUSIONS

- A hierarchy exists in the spontaneous recognition of “self” TAAs.
- Melan-A/MART-1 and CEA (not shown) are the most frequently recognized TAAs in melanoma and CRC, respectively.
- Recognition of “self” TAAs increases with the increased tumor burden (e.g. Melan-A/MART1, COA-1)

Results of first generation (1998-2008) of self peptide-based vaccination of metastatic melanoma patients (Phase I/II studies)

Type of peptide Ag	N. of patients	Clinical response (CR+PR) %	Immune response %
Lineage related (e.g. MelanA)	159	14	20-65
Cancer/Testis (e.g. MAGE)	92	17	30-50
DC peptides	124	16	56
DC lysates	106	18	46

In a recent study *Slingluff et al. (2008)* reported 100% immune response in melanoma patients vaccinated with 12 peptides.

Antigens recognized by T cells:

1) Shared, self differentiation proteins expressed also on normal cells (e.g. MART-1, CEA, PSA)

2) Shared self Cancer/Testis expressed by different tumors and by normal testis or placenta (e.g. MAGE, NY-ESO-1)

3) **Shared, universal TAAs predominantly expressed by tumor cells (e.g. *survivin*, hTERT)**

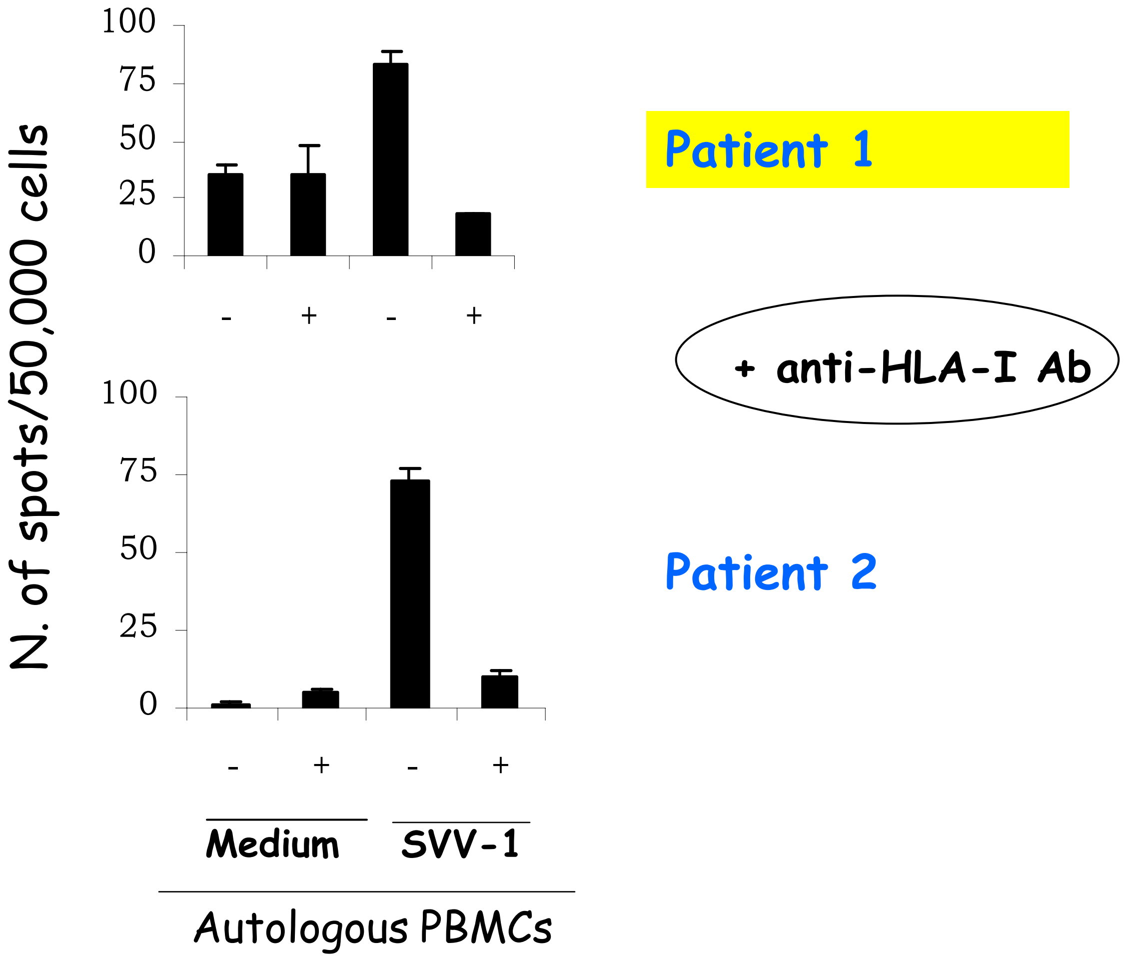
4) Unique, expressed only by a single tumor (e.g. CDK4/m, α-actin-m)

SURVIVIN (SVV)

Member of Inhibitor of Apoptosis Proteins

- **Expression pattern:**
  - **abundant during fetal development**
  - **silenced in normal adult tissues**
  - **over-expressed in most common human cancers**

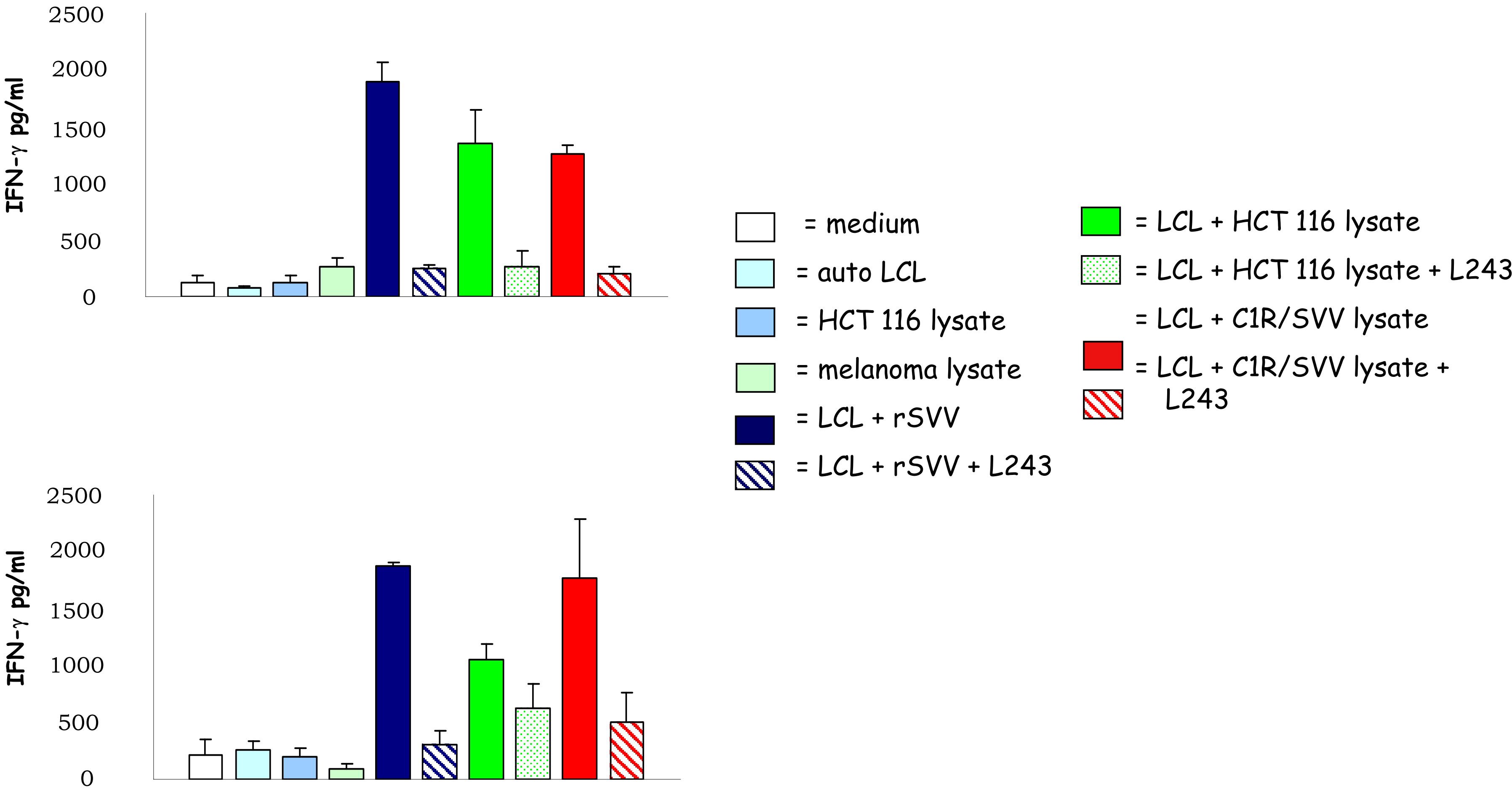
IN VITRO INDUCTION OF HLA CLASS I-RESTRICTED ANTI-SVV-1_{95-104} T CELLS IN PBMCs OF RECTAL CANCER PATIENTS



(Casati *et al*., Cancer Res 2003)

MHC-II RESTRICTED RESPONSE OF COLORECTAL CANCER PATIENTS TO rSVV PROTEIN OR SVV+CANCER LYSATE

IFN-γ ELISA



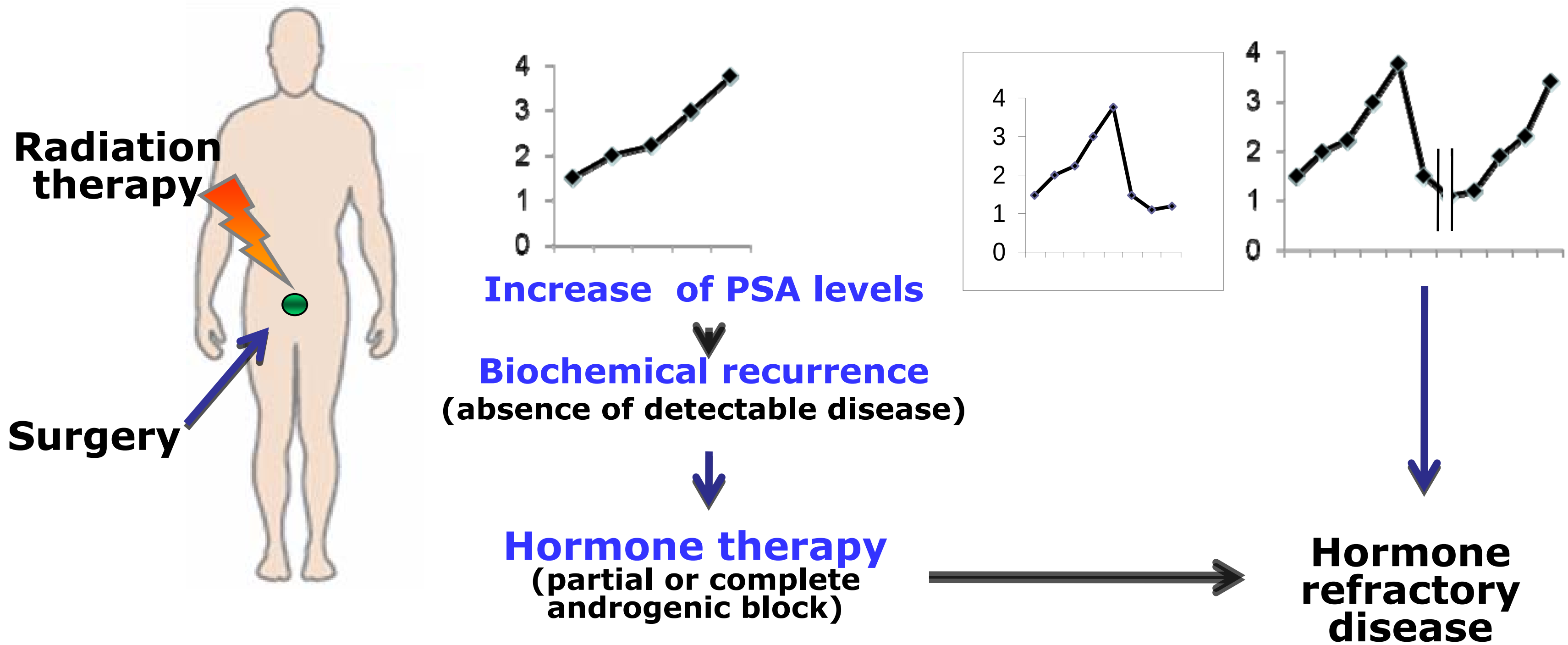
(Casati C *et al*., Cancer Res, 2003)

Conclusion:

SVV is an universal TAA that can elicit both HLA-I and HLA-II-restricted T cell responses

SVV-based vaccines in prostate cancer

Standard treatment for prostate carcinoma



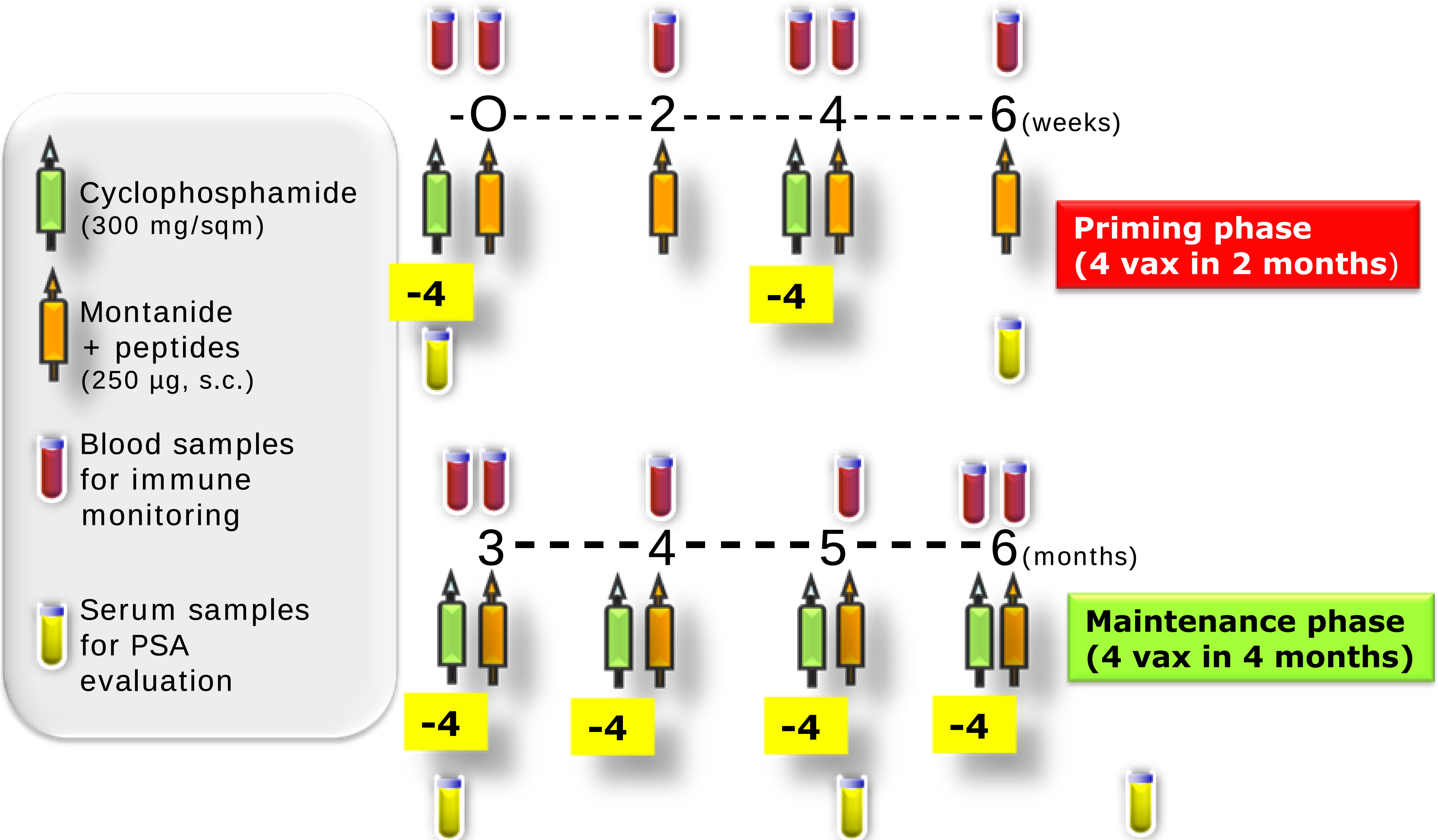
Phase II trial of multiple peptide vaccination in HLA-A0201+ prostate carcinoma patients with biochemical recurrence after conventional therapies

The vaccine

PSMA1	**LLHETDSAV**
PSMA2	**ALFDIESKV**
Survivin (SVV-1)	**LMLGEFLKL**

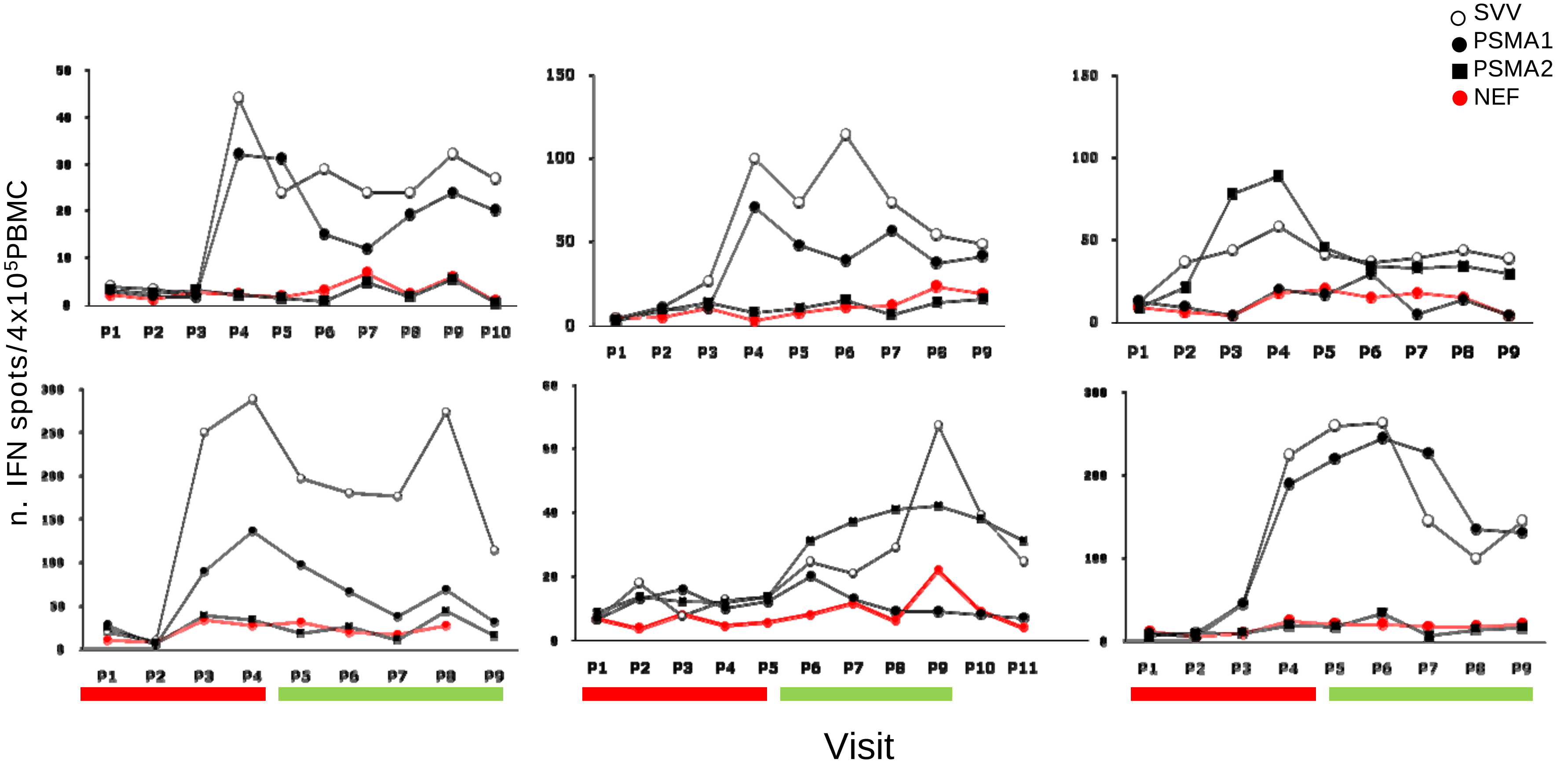
- **Low-dose cyclophosphamide (300 mg/m²)**
  (for T reg down modulation)

Vaccine schedule



Anti-peptide reactivity

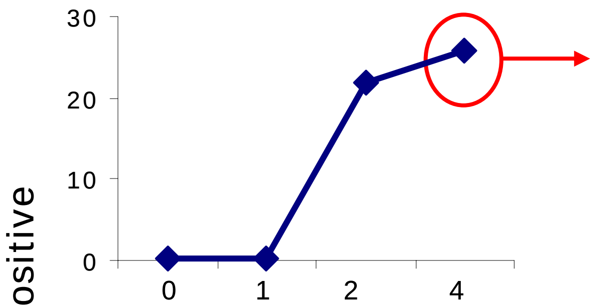
IFNγ Elispot , uncultured PBMCs

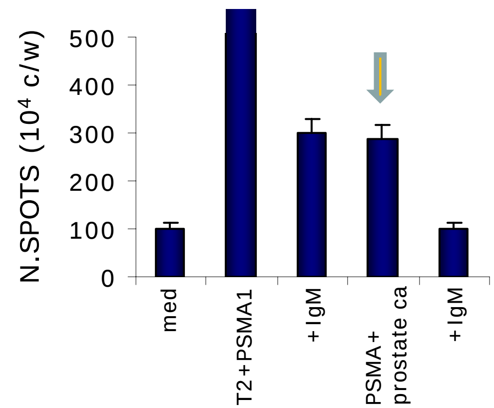


T cells activated *in vivo* with natural peptides efficiently recognize tumor cells

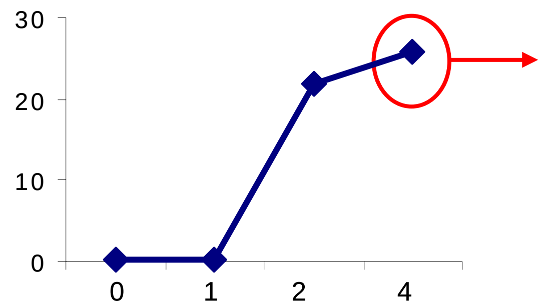
(upon *in vitro* sensitization)

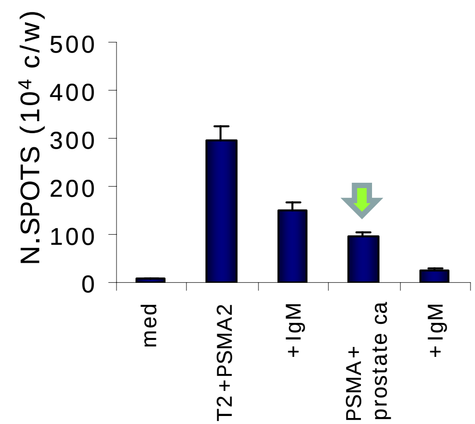
Anti-PSMA1
(tumor recognition in 7/7 cases)



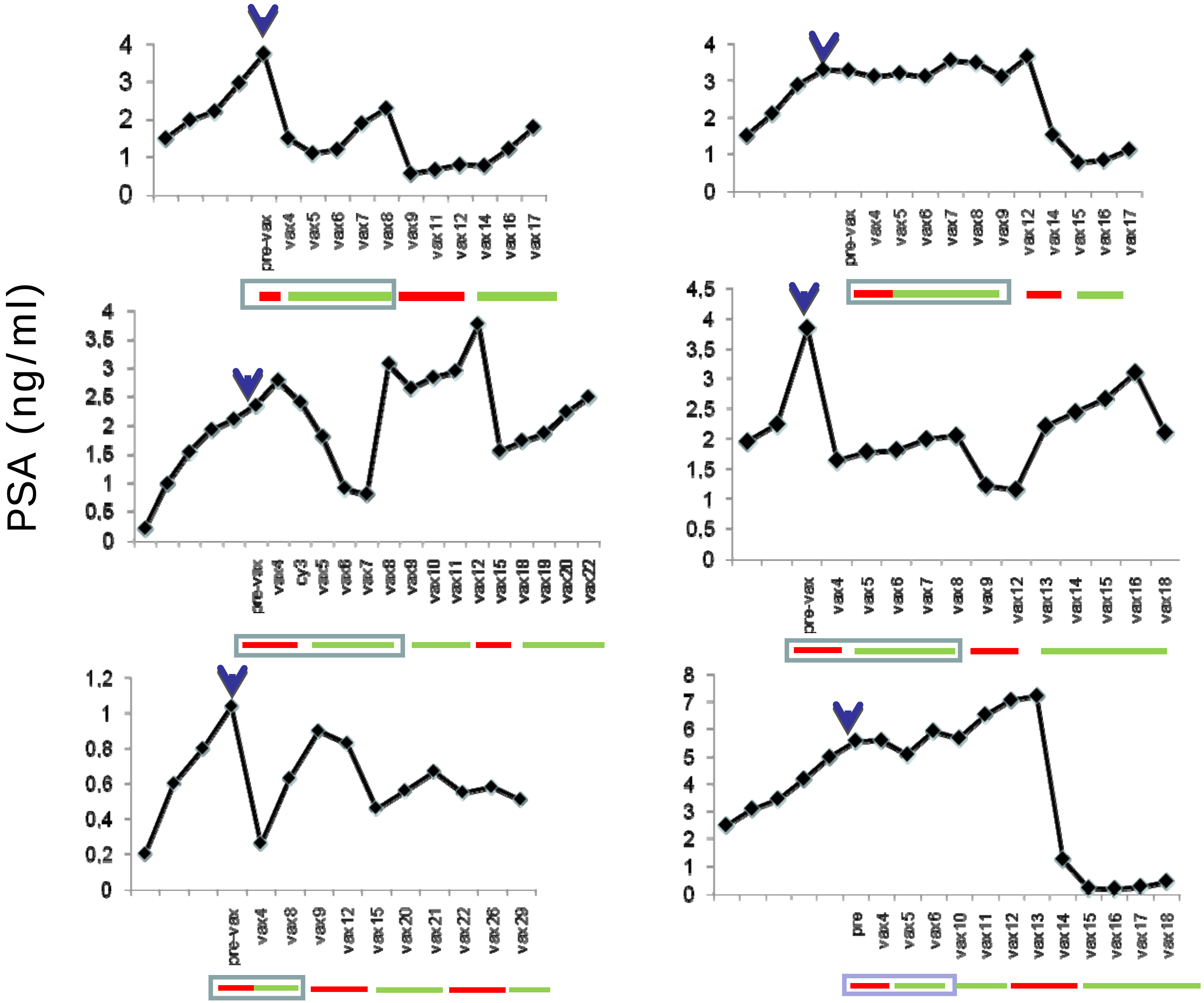


Anti-SVV
(tumor recognition in 1/7 cases)

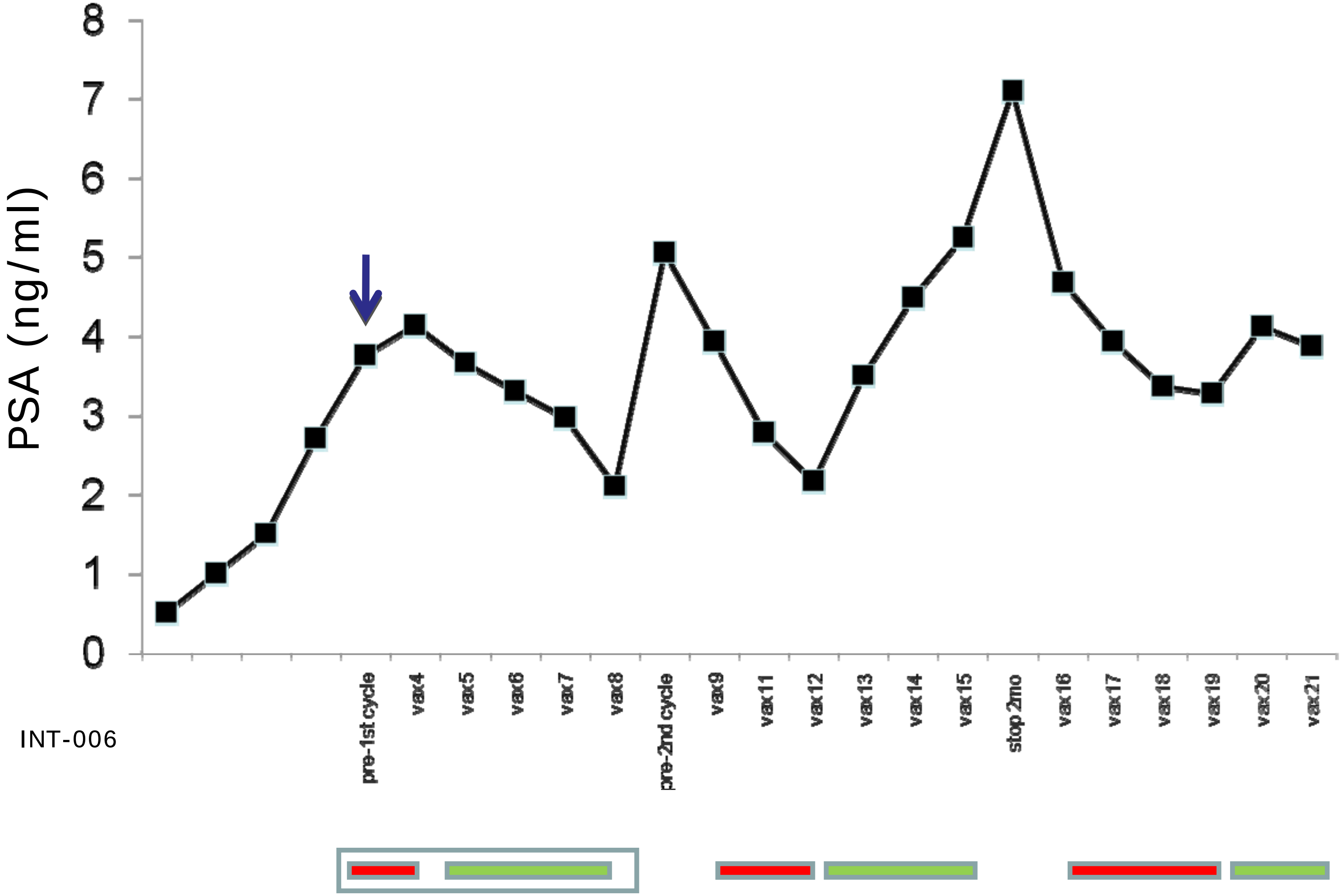




Effects of vaccination on PSA levels

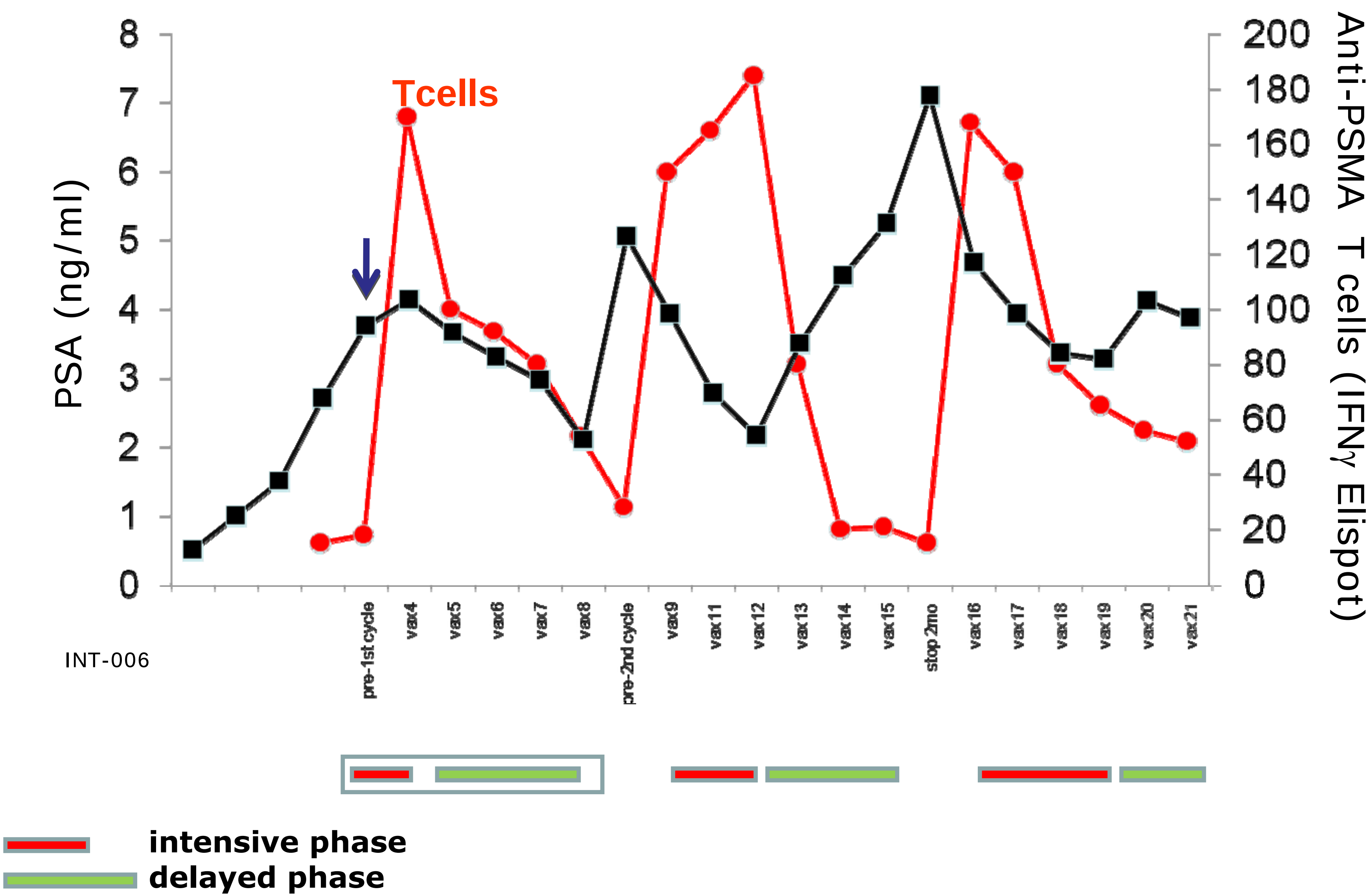


Effects of priming vs maintenance vaccination on PSA levels



intensive phase
delayed phase

Effects of priming vs maintenance vaccination on PSA levels



Conclusions: Unique TAAs

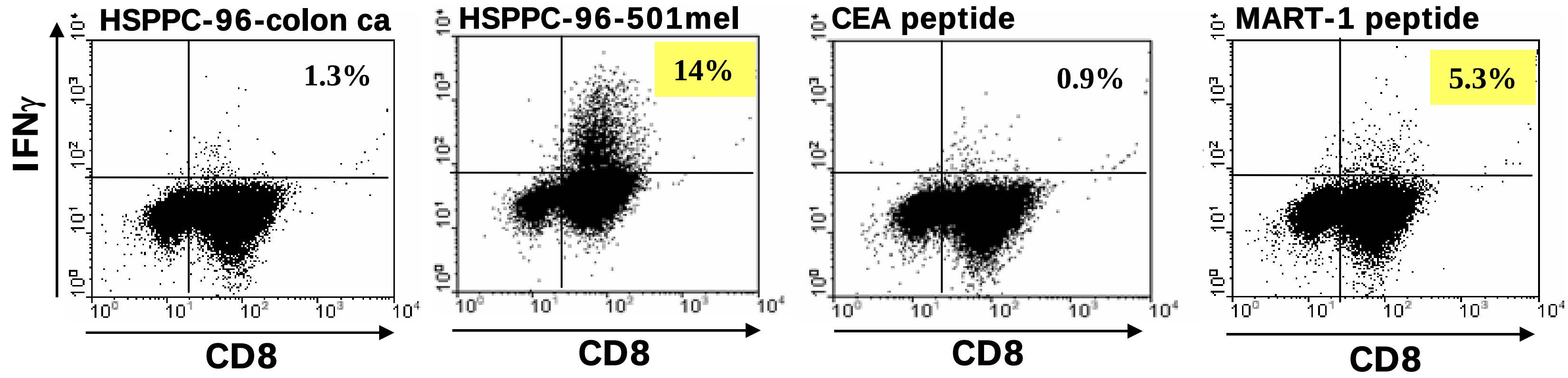
- Derive from mutated genes often involved in maintaining the neoplastic state (e.g.CDK4m)
- Expressed by a variety of human tumors
- Immunogenic *in vivo*
- Immunodominant over self TAAs

Vaccination with unique TAAs

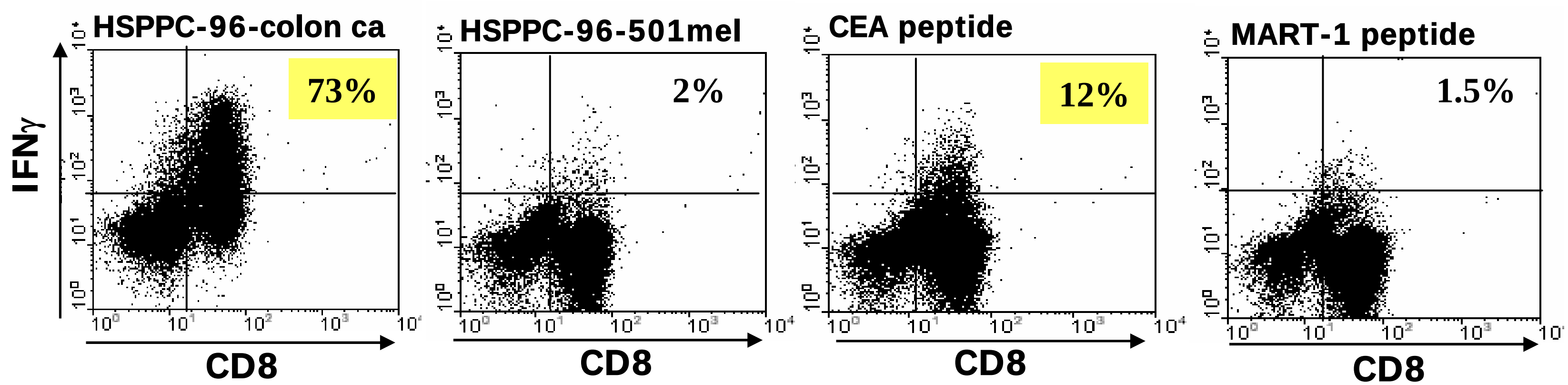
1. Autologous (gene modified?) tumor cells (G-Vax)
2. Autologous HSPs
3. Mutated peptides (wide transcriptome sequencing)

Tumor-derived HSPPC-96 mediate cross-presentation by HLA-A*0201+ monocytes of peptides from know tumor antigens to specific T cell clones

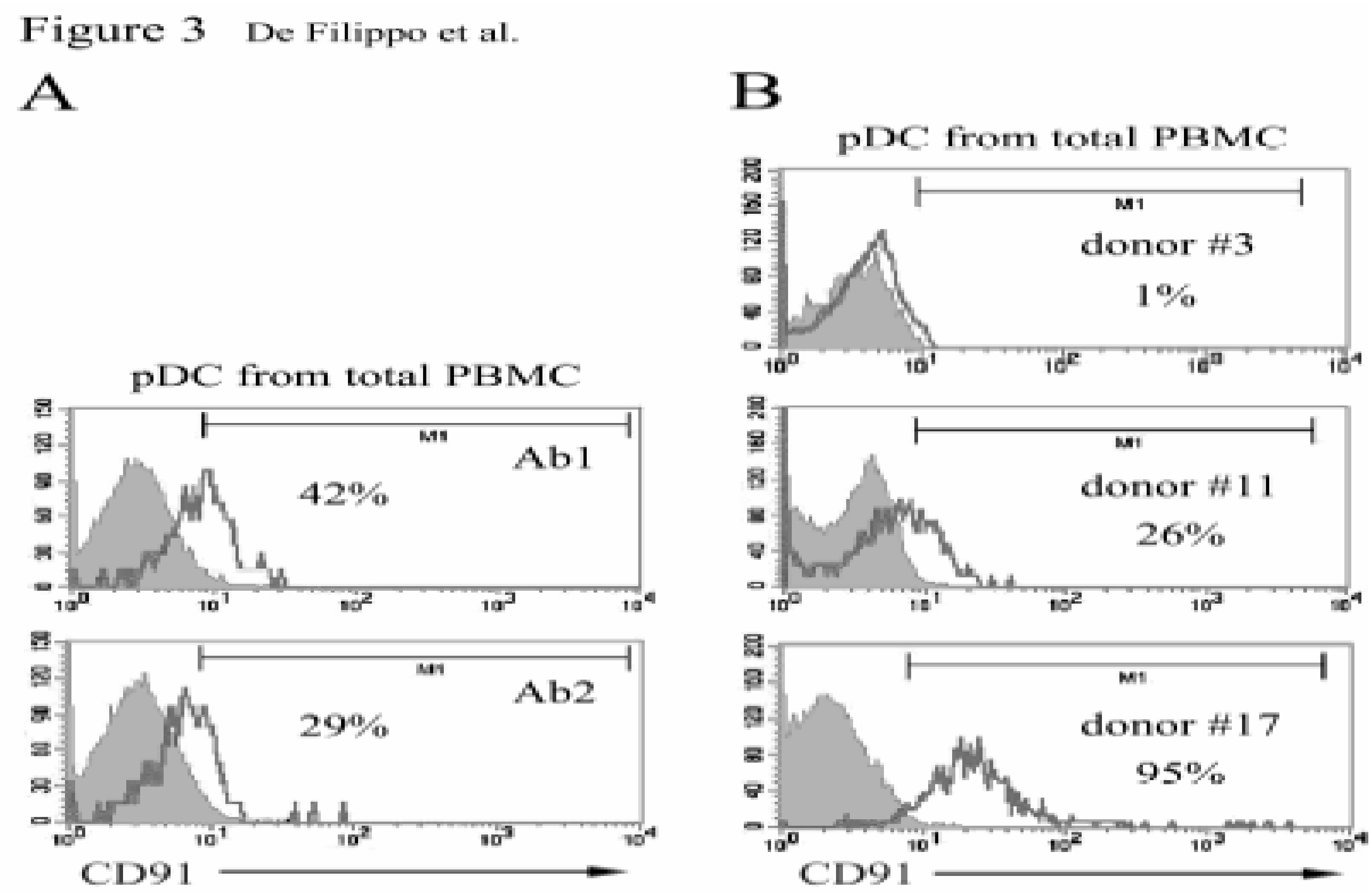
Anti-MART-1 HLA-A2 restricted CD8+ T cell clone



Anti-CEA HLA-A2 restricted CD8+ T cell clone

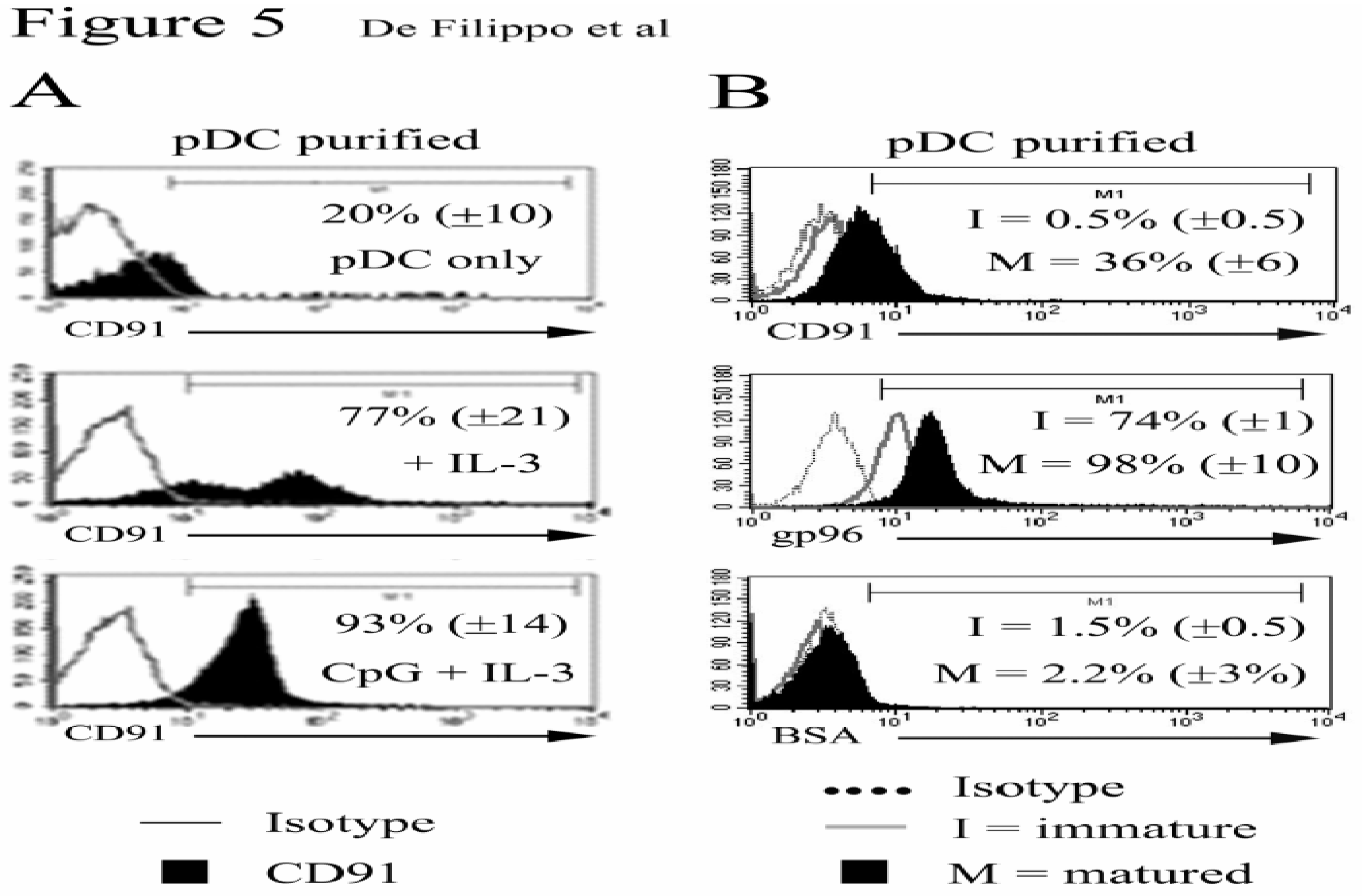


HSP receptor CD91 is heterogeneously expressed on normal donors pDCs



(De Filippo et al. J Immunol 2008)

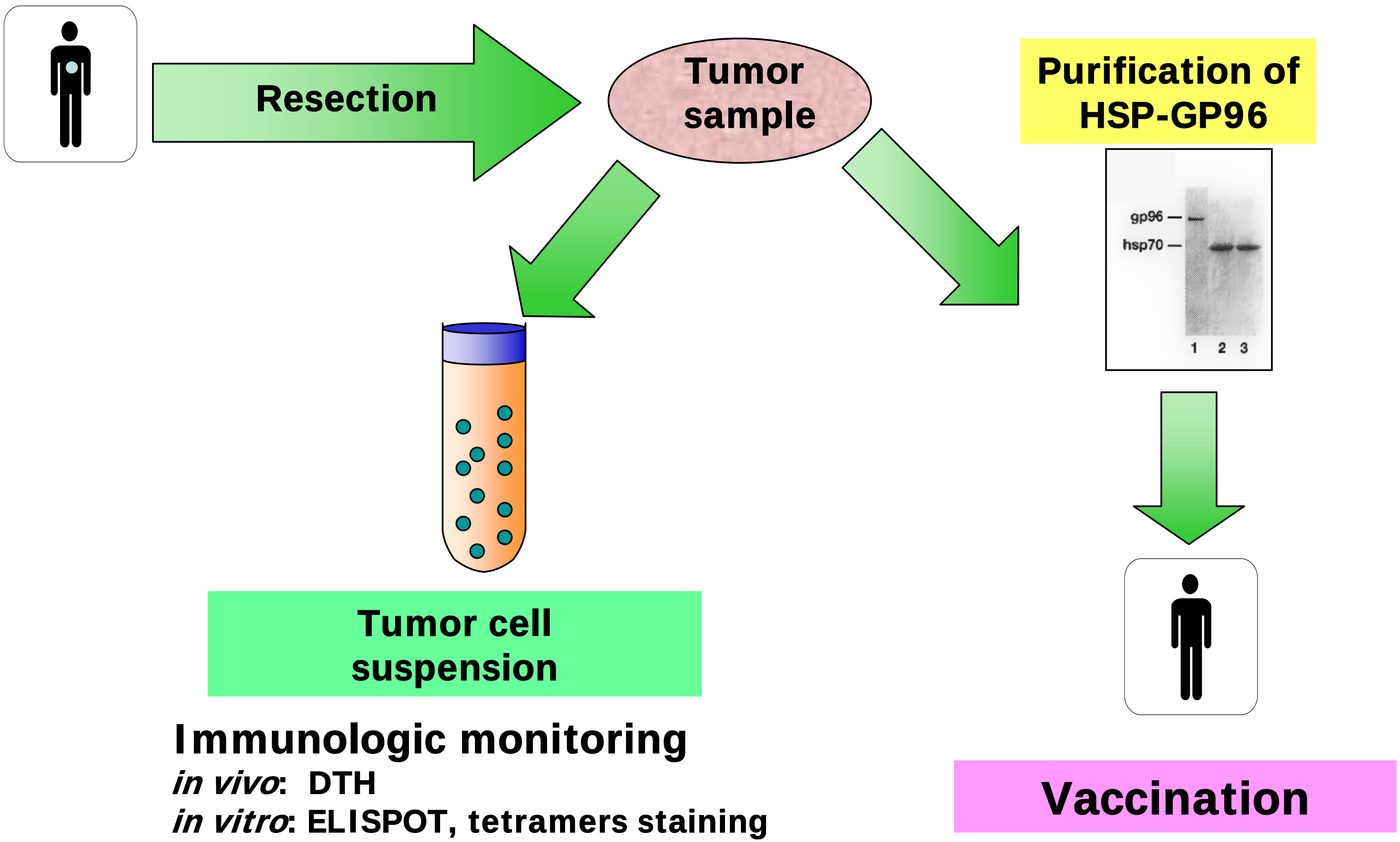
Increased CD91 expression after cell maturation parallels the increase in gp96 binding



Conclusions

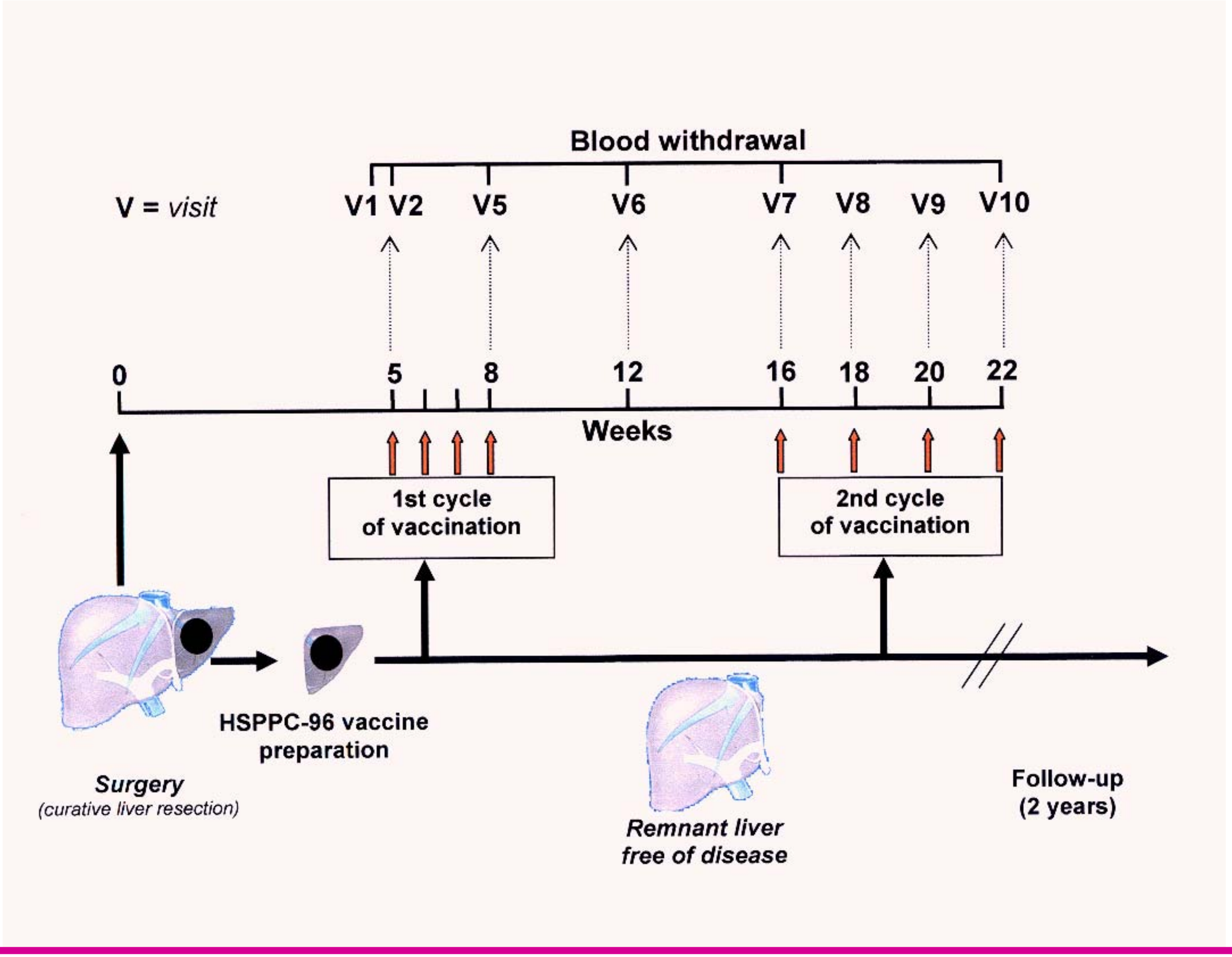
- Gp96 interact with human blood pDCs directly, specifically and functionally.
- In absence of pDC maturation gp96 may down-modulate the immune response.
- CpG up-regulate CD91 thus promoting TLR9 and CD91 synergy.
- Thus, gp96 may bridge innate and adaptive immunity and explain their immunogenicity in vaccinated patients

Vaccination of metastatic patients with HSPPC-96 derived from the autologous tumor



Vaccination of patients with liver metastases of CRC with autologous HSPPC-96

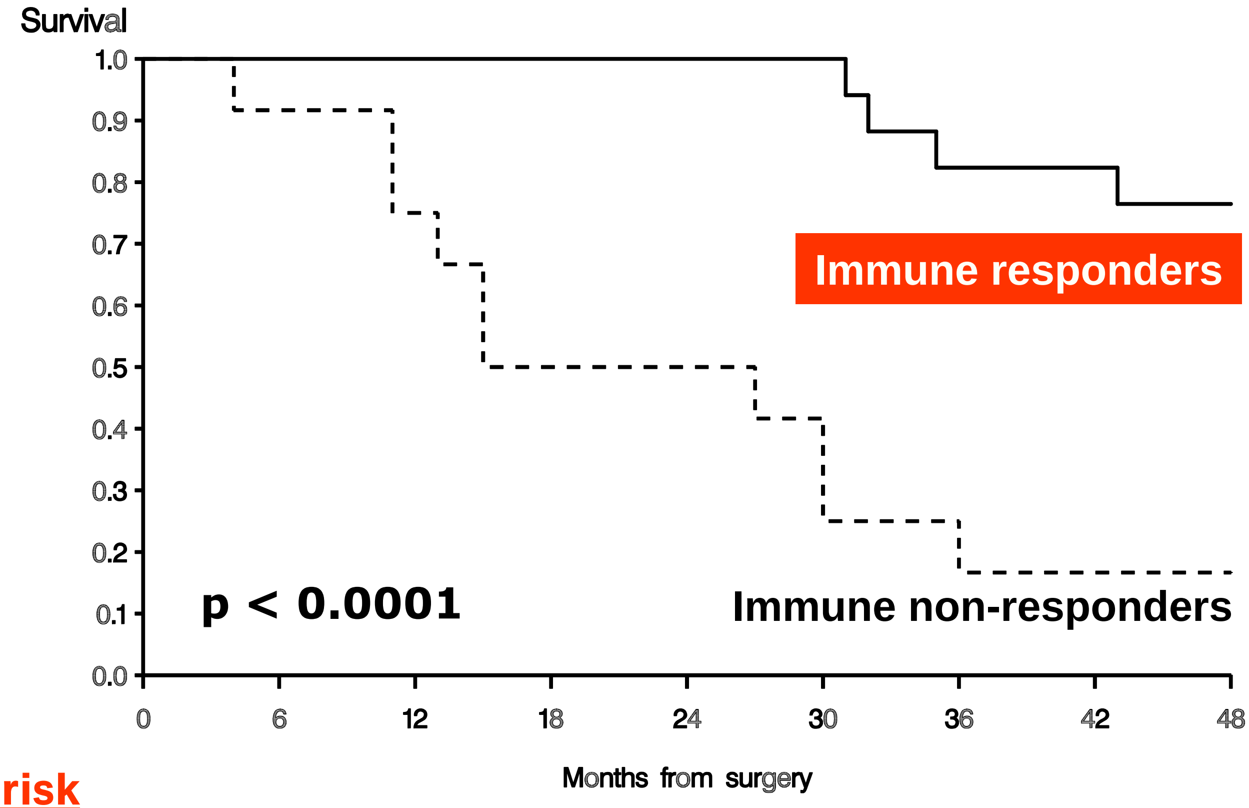
- TREATMENT SCHEME -



Major endpoints

- Feasibility
- Safety
- Induction of T and NK cellular response against CRC cells or peptides (HLA-A2 patients)

OVERALL SURVIVAL AND T CELL RESPONSE INDUCED BY VACCINATION IN PATIENTS WITH LIVER METASTASES FROM CRC

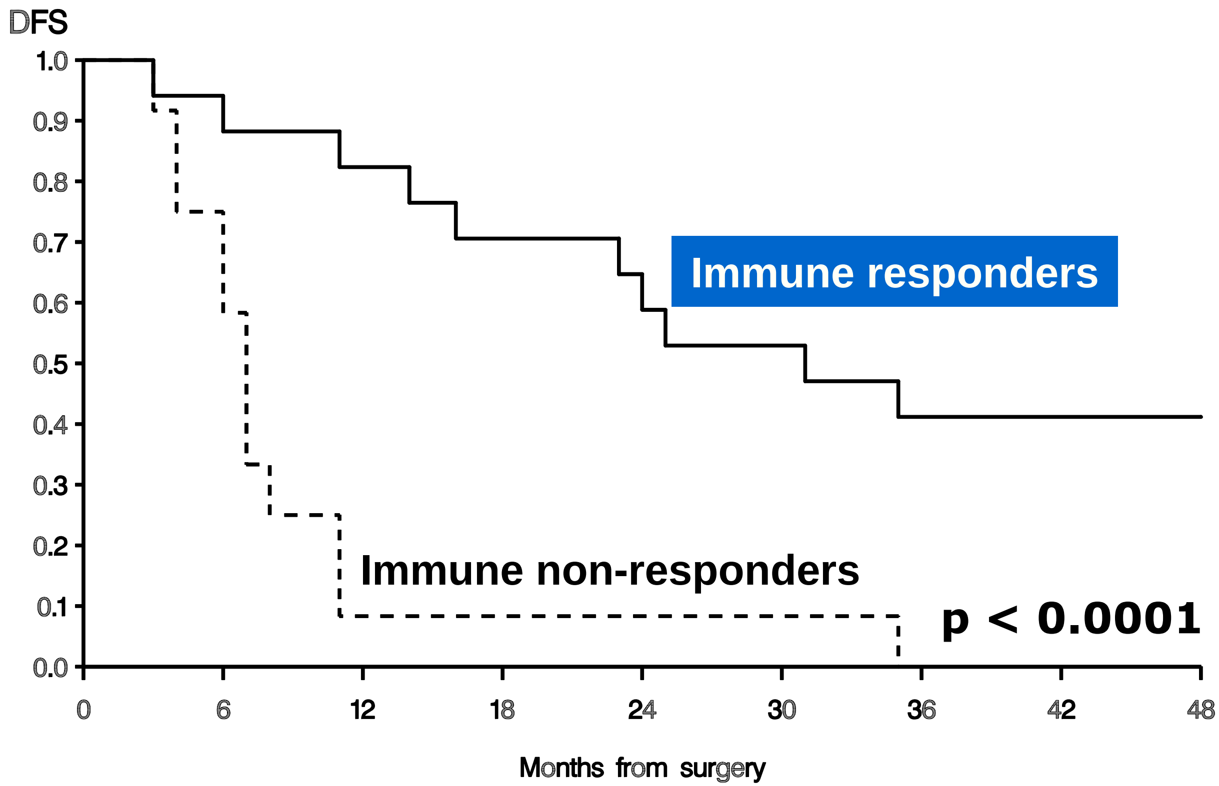


Patients at risk

Immune responders	17	17	17	17	17	17	14	14	11
Immune non-responders	12	11	9	6	6	5	3	2	2

September 2004

DISEASE-FREE SURVIVAL AND T CELL RESPONSE IN PATIENTS WITH LIVER METASTASES FROM CRC



Patients at risk

Immune responders	17	16	14	12	11	9	7	7	6
Immune non-responders	12	9	1	1	1	1			

September 2004

CONCLUSIONS

Vaccination of patients with liver metastases of CRC with autologous HSPPC-96 is feasible and safe.

Approximately 60% of patients developed a T cell response to CRC antigens and a NK response to CRC cells.

Both OS and DFS were longer in T cell responding than in non-responding subjects,independently from other prognostic factors.

Early phase III trials with melanoma and RCC concluded

PHASE III RANDOMIZED TRIAL OF AUTOLOGOUS TUMOR-DERIVED HSPPC-96 *vs*. PHYSICIAN CHOICE IN METASTATIC MELANOMA (*Testori et al. JCO 2008*)

- Trial features: Randomization 2:1 favoring vaccination (215 vs. 107 patients). Physician choice included IL-2 and/or dacarbazine/temozolomide-based therapy and/or surgery

- Results. Overall, patient in ITT vaccination arm *fared similarly* to those in the physician choice arm in terms of survival.

. Subset of patients who received *at least 10 doses* of vaccine showed an extension in median survival of 29% compared with those receiving physician choice treatment.

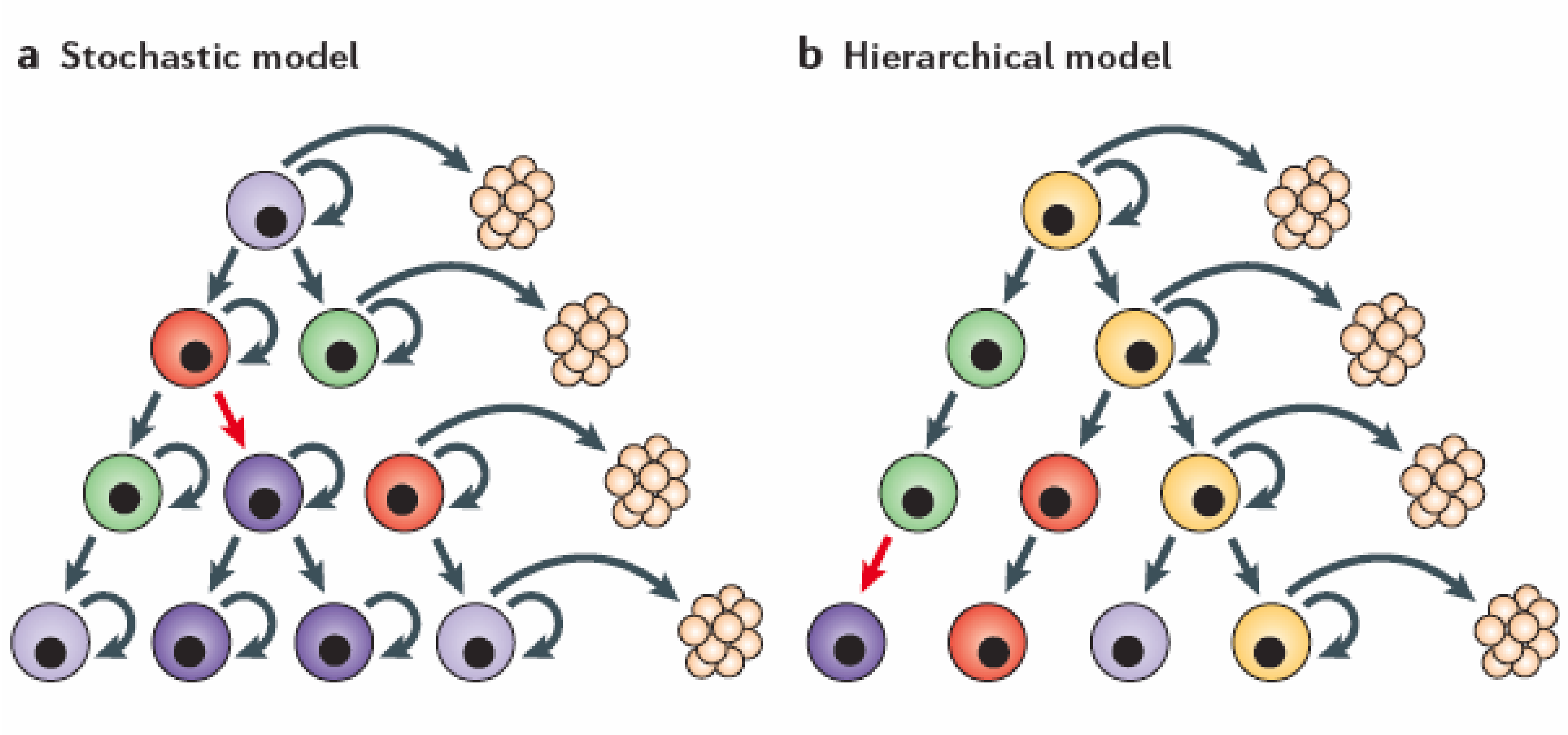
Oncophage® was associated with clinical benefit (P= 0.017) in stage M1a and M1b patients who received at least 10 doses.

- Conclusion: Signs of potential survival benefit in M1a/b patients.

Vaccination with unique TAAs. A new genomic strategy

- Cancer cells contain many somatic mutations detectable by new sequencing technology. These mutations can generate candidate new T cell epitopes.
- Breast and colorectal cancers may accumulate 7-10 new HLA-A*0201 epitopes that may define an individual tailored polyvalent vaccine.
- *(Segal et al., Cancer Res 2008)*

Cancer stem cells (CSC): A new target of immunotherapy?

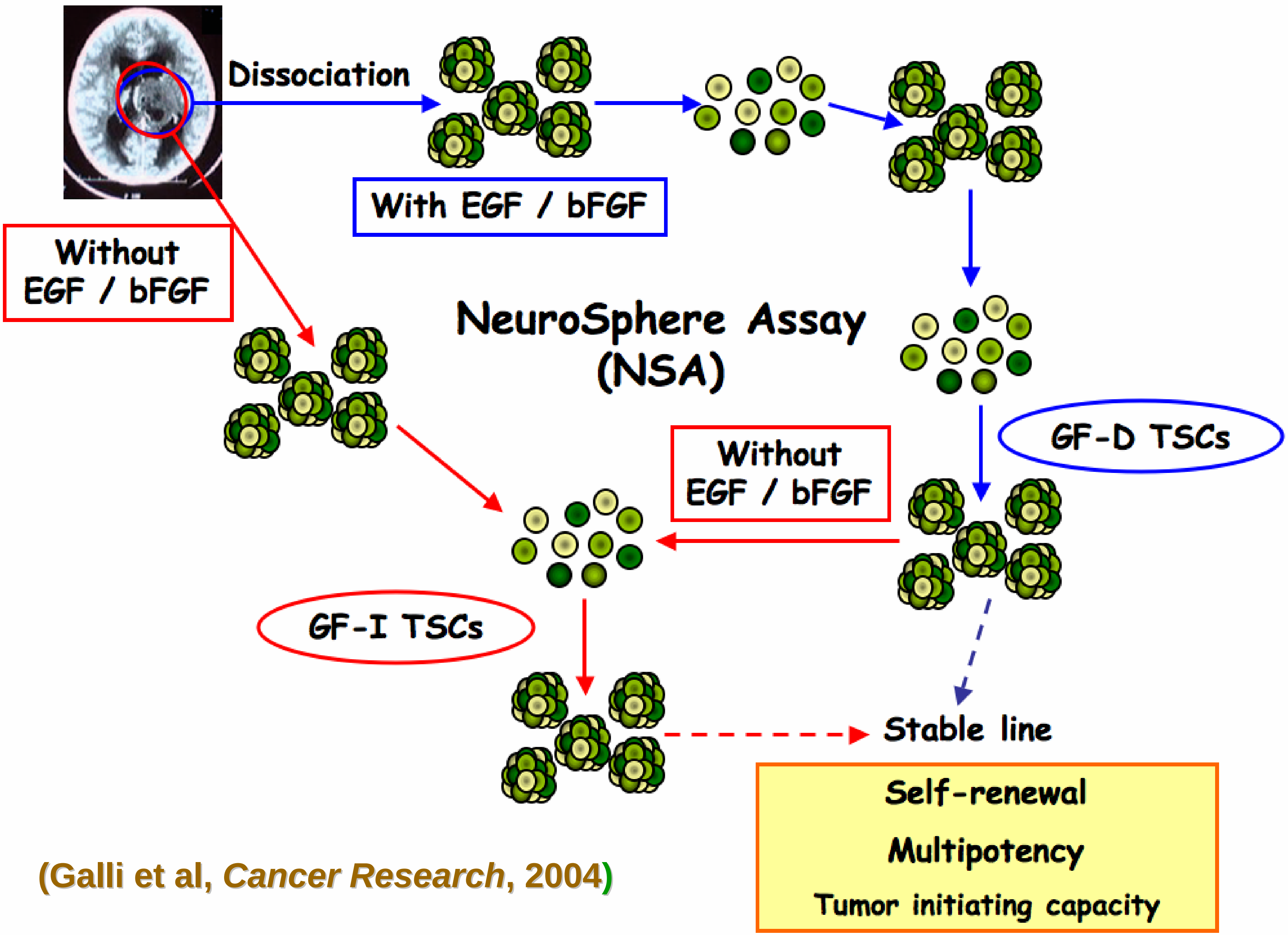


Tumor cells are heterogeneous, but many of them can proliferate extensively, although with a low probability.

Tumor cells are heterogeneous, but only the cancer stem cell subpopulation has the ability to proliferate extensively.

Vescovi, Galli and Reynolds, *Nature Rev Cancer*, 2006

- If the CSC hypothesis is correct then CSCs will represent the most important target of cancer therapy including immunotherapy.



(Galli et al, *Cancer Research*, 2004)

In vitro and *in vivo* expression of MHC, NKG2D ligands and TAAs by GBM-CSCs.

CSC GB LINES: CELL SURFACE ANTIGENIC PROFILE

CSC Line*	Antigens: MHC-I % (MIF)	MHC-II	MICA	MICB	CD133	EGFR	Survivin	Tumorigenicity
GB0627 I	**70 (138)**	5 (79)	**47 (106)**	**83 (68)**	5 (63)	94 (130)	85%	High
GB0627	1 (23)	4 (23)	ND	2 (25)	20 (99)	55 (123)	60%	High
GB050615	1 (10)	1 (20)	1 (30)	1 (10)	8 (206)	18 (185)	88%	High
GB030616	13 (42)	3 (21)	4 (22)	1 (20)	1 (20)	24 (60)	90%	High

MIF = Mean intensity of fluorescence
*As obtained from neurospheres

TAAs expression by CSC and primary tumor cell lines derived from GBM patients

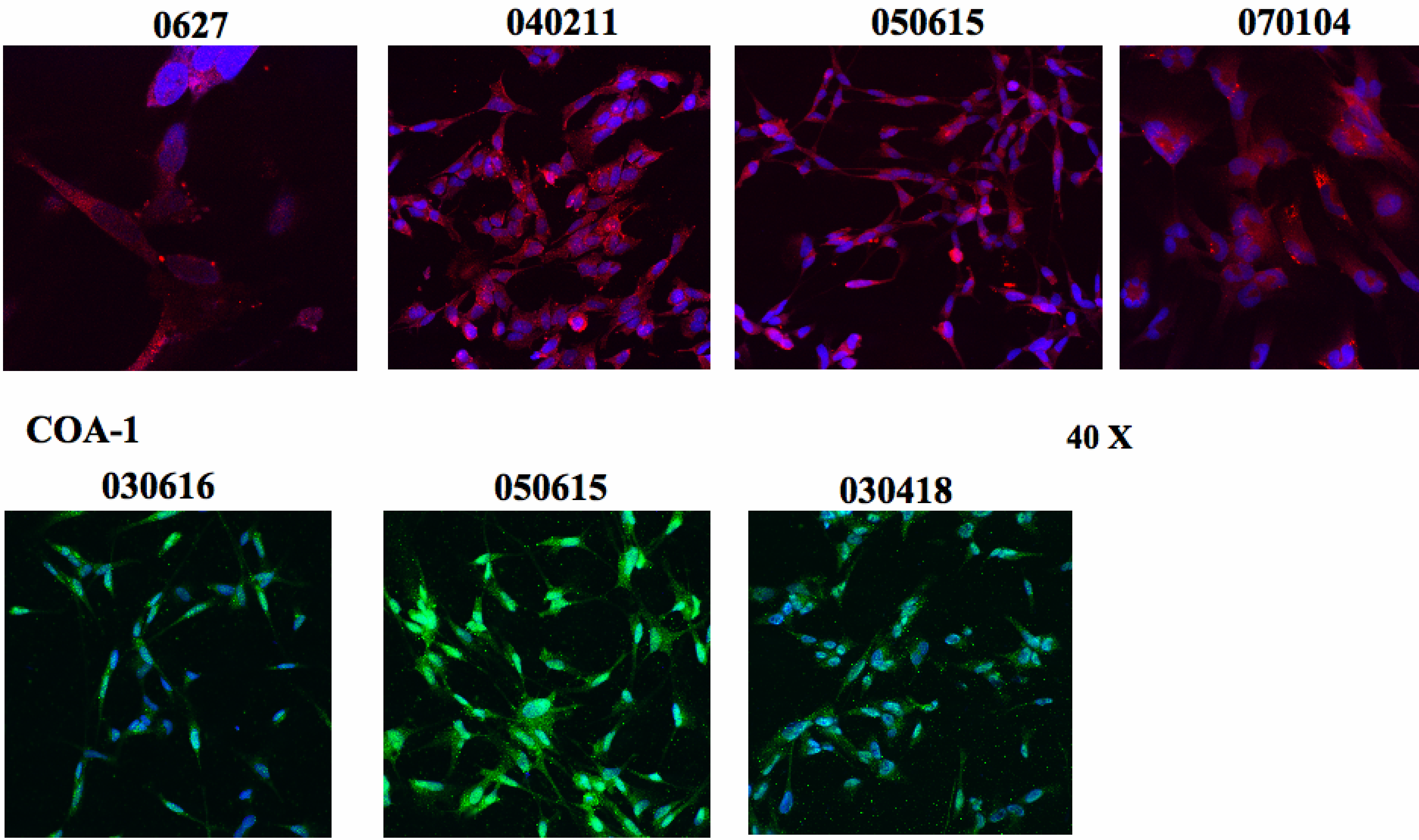
Cell Line		0627 FBS	0627	070112	070112	080125 FBS	080125	080418 FBS	080418
Antigen	IL-13 Rα2	1 [b]	1	1	1	1	1	1	1
	Sox2	7	28	21	106	3	7	20	38

b MRI: ratio between the mean of fluorescence intensity of cells stained with the selected mAb and that of the negative control.

All cell lines (9 CSC and 4 FBS tumor lines) expressed high level of SOX2, while a failure in the expression of IL-13Rα2 was observed. Survivin and COA-1 were also expressed commonly by CSCs. NY-ESO was expressed weakly by few CSC lines; no detection of MAGE and Gp100 occurred.

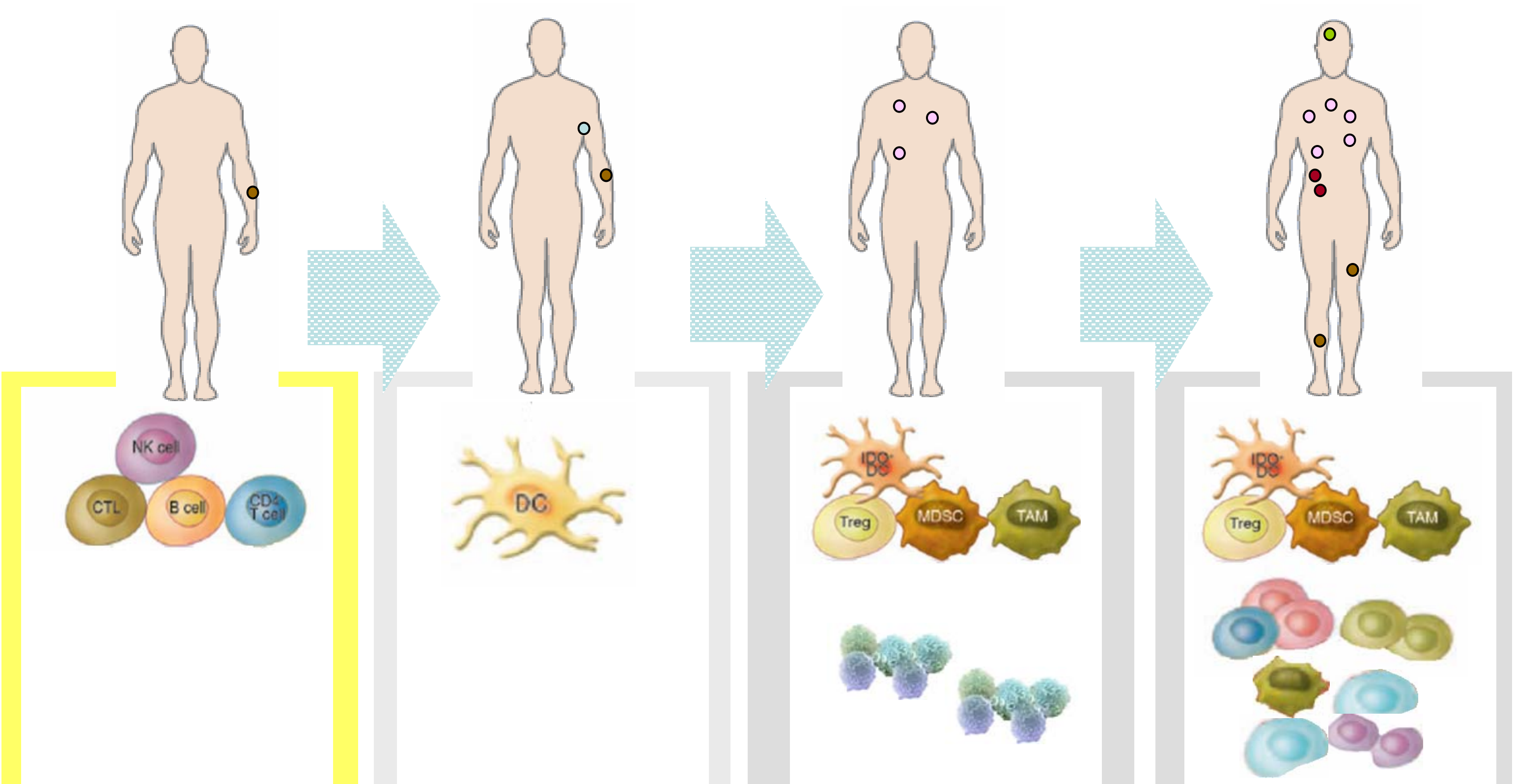
Expression of TAAs by GBM CSCs

Survivin



Results: Efficient expression of Survivin and COA-1 was commonly detected in GBM CSCs. Weak or negative expression of MAGE or NY-ESO-1 by CSC lines.

Immune functions during cancer progression



Effective anti-tumor immune responses

Functional defects in regional lymph nodes

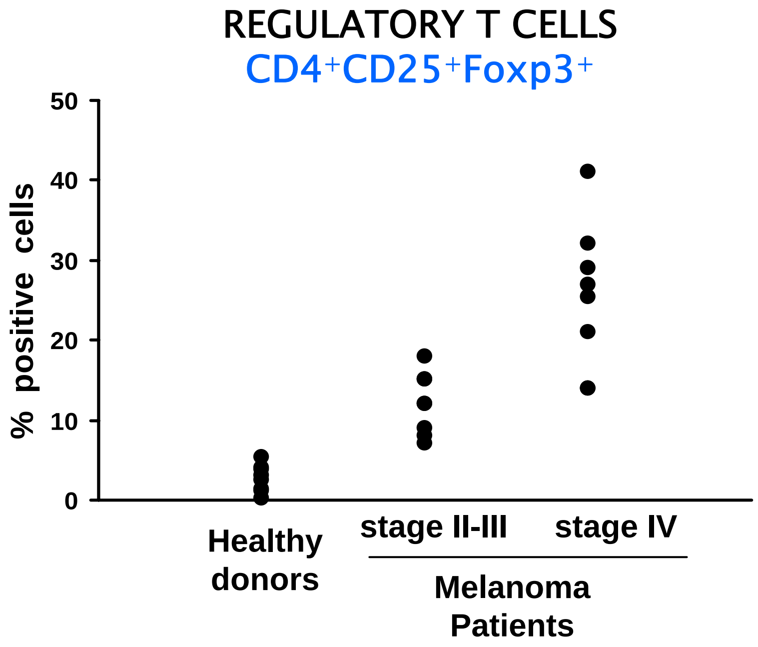
Suppressive immune cells (Treg, MSC)

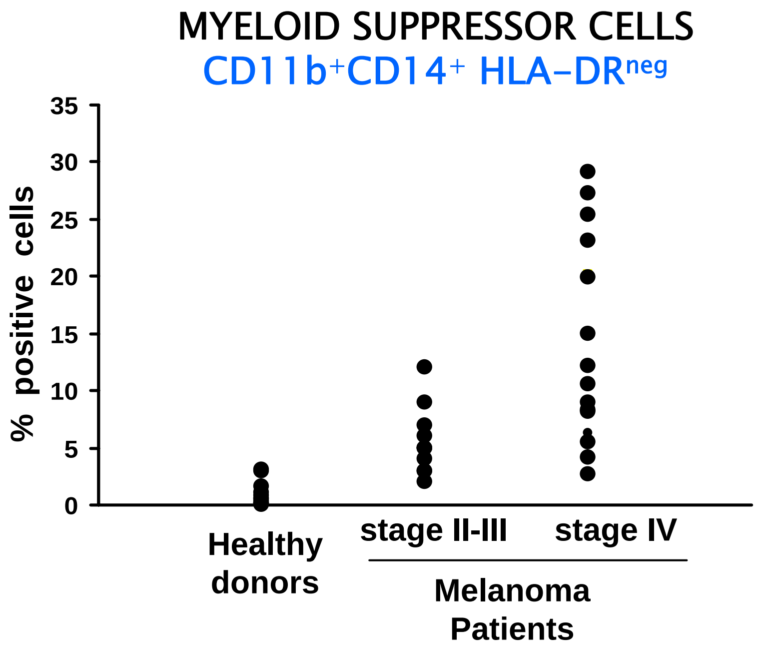
T cell defects or apoptosis

"Ignorant" immune system

Filipazzi et al., J Clin Oncol 2007
Andreola et al. J Exp Med 2002

Immune dysfunctions are less pronounced in early stage melanoma





Rationale for a new generation of cancer vaccines

- **Early disease**
- **<u>Multiple antigens including natural self, unique, CSC and universal TAAs</u>**
- New TLR targeting adjuvants (CpG, HSPs)
- **Down-regulation of Tregs and/or Myeloid Derived Suppressor Cells**
- Immune-monitoring in blood, LNs and tumor tissue.
- **Assessment of patient polymorphisms and tumor gene signatures**

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