Society for Immunotherapy of Cancer

2012 Primer in Tumor Immunology and Biological Therapy of Cancer

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Tumor Antigen, Tumor Immunogenicity and Immunization

LUDWIG INSTITUTE FOR CANCER RESEARCH

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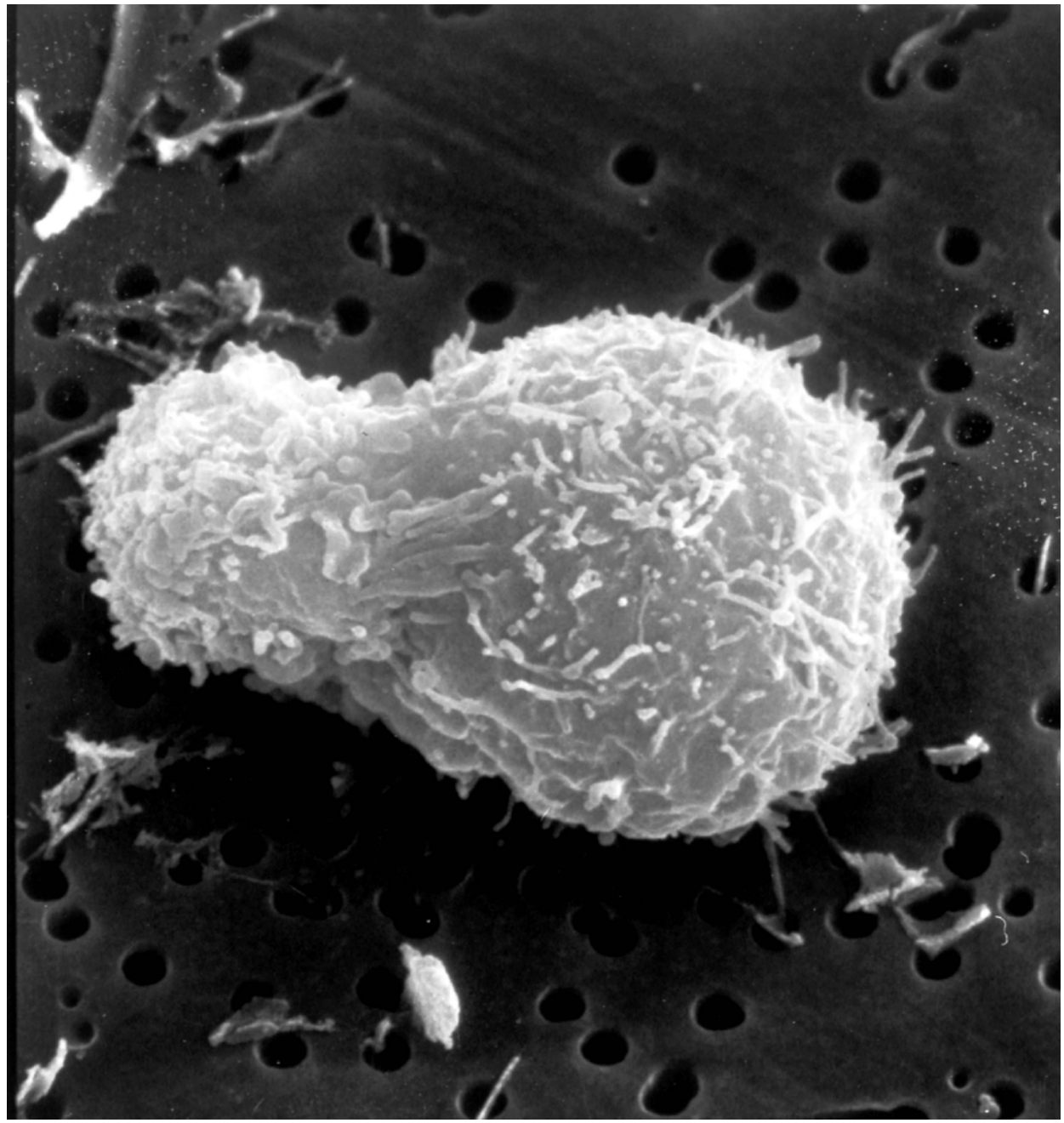
No relevant financial relationships to disclose

CYTOLYTIC T LYMPHOCYTES (CTL),

the major effectors of anti-tumor adaptive immunity

- **Direct lysis (perforin, GZB)**
- **IFN-γ, TNF-α**
- **Fas L**



Topics

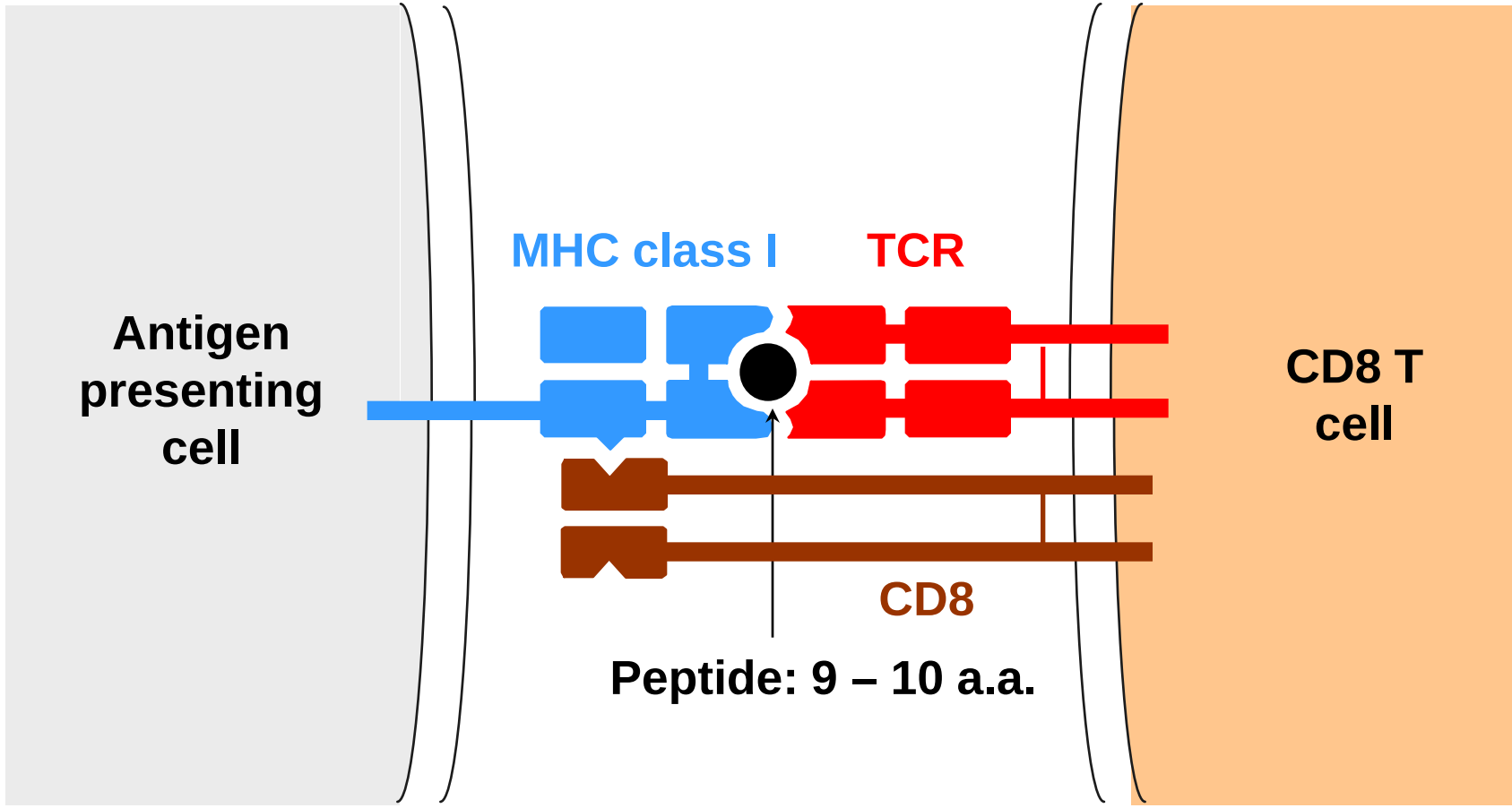
Antigen recognition

Antigen processing

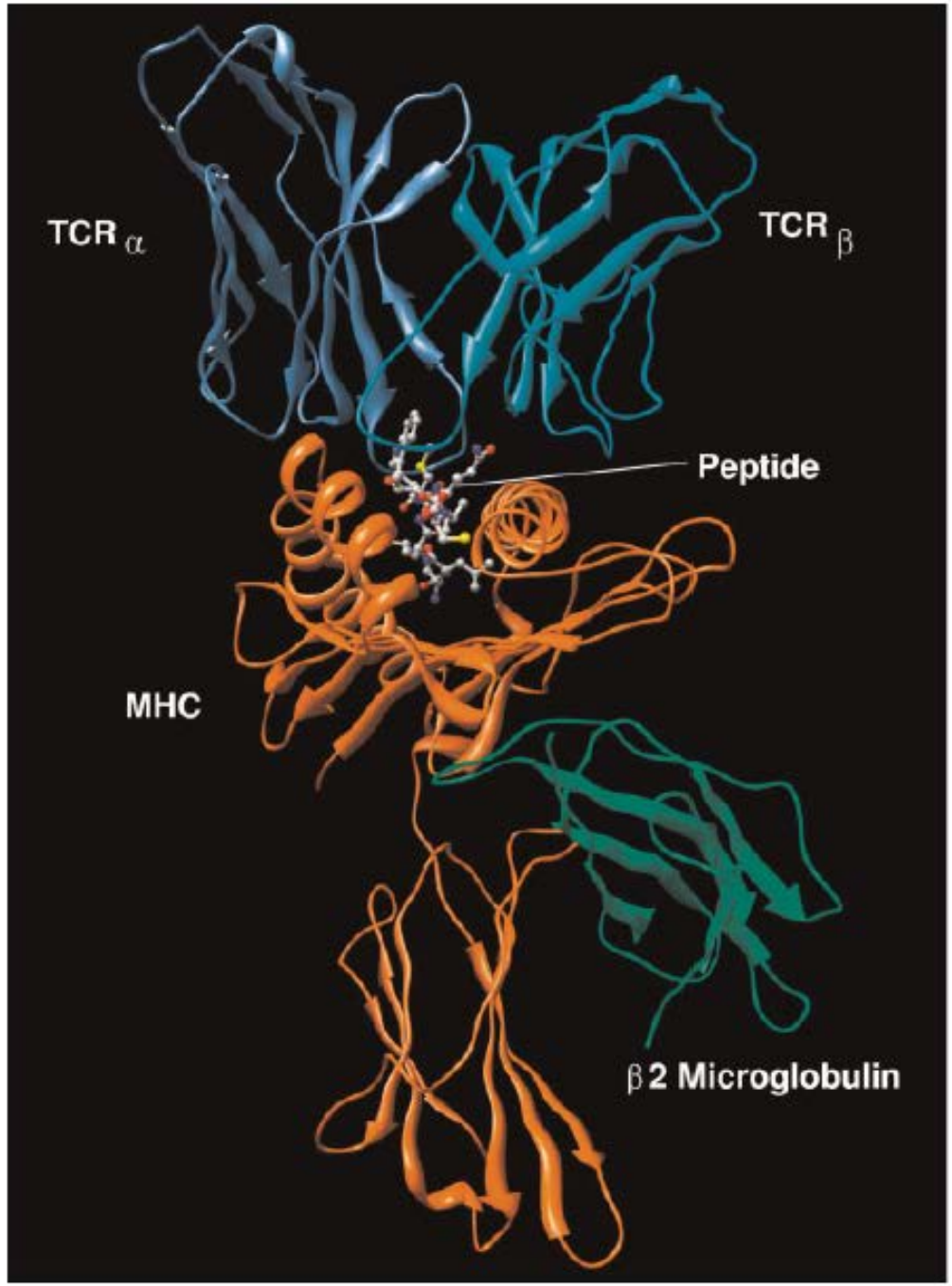
The CD8 T cell response

Eliciting CD8 T cell responses by vaccination

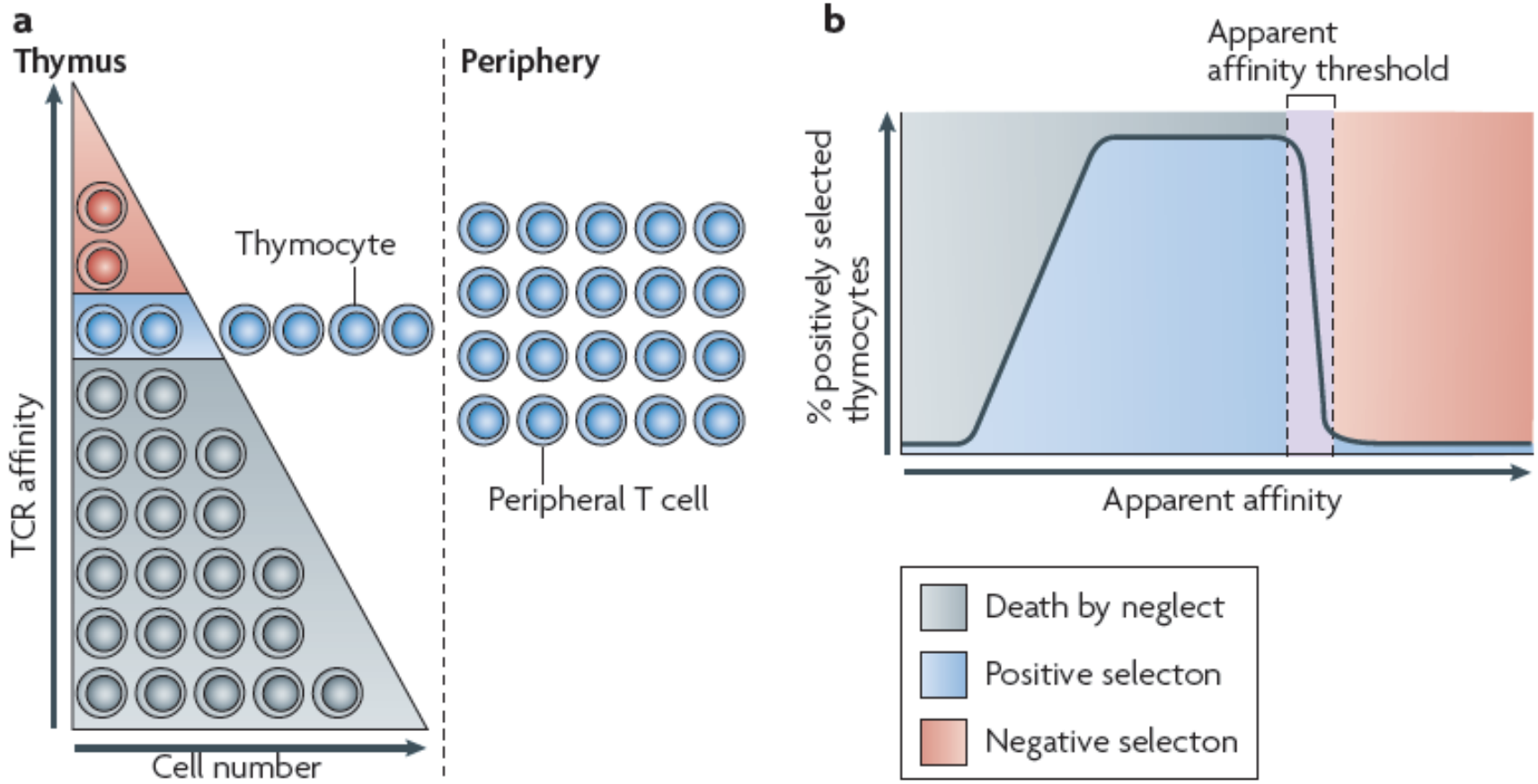
Antigen Recognition by Antigen-Specific CD8 T Cells



$t_{1/2}$

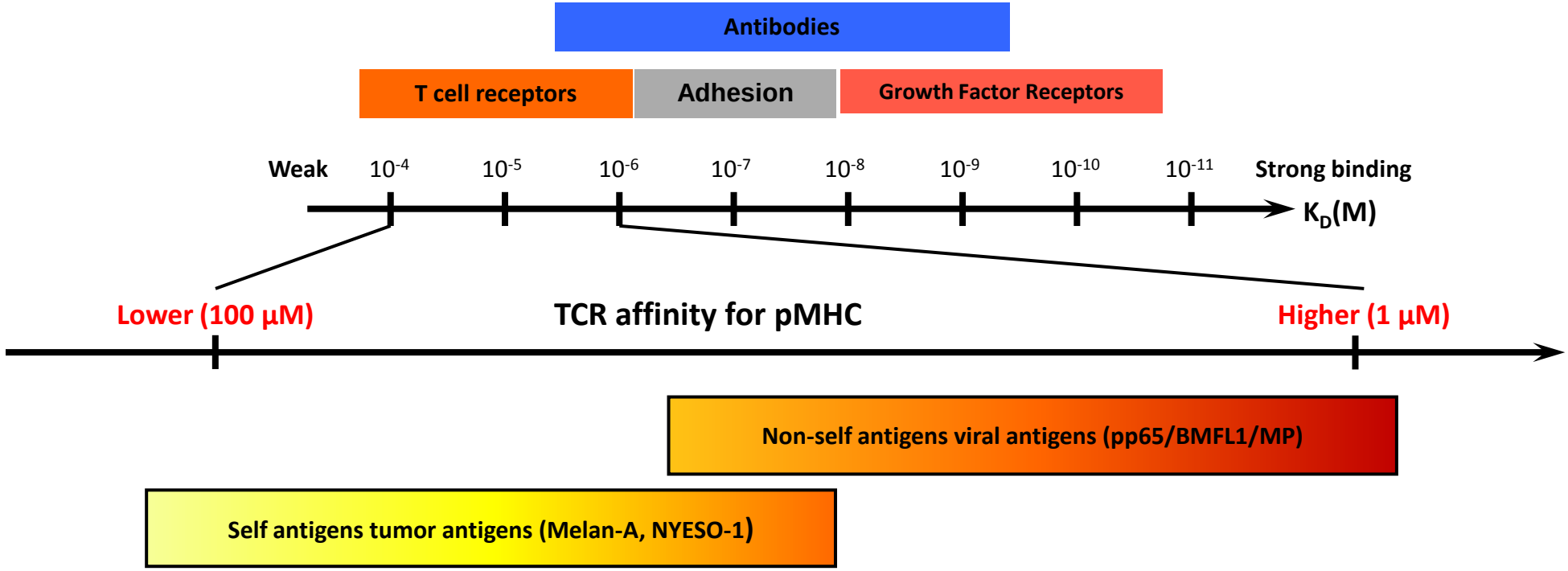


Thymic selection depends on T-cell receptor affinity for self peptide–MHC complexes



Palmer & Naeher, Nat Rev Immunol 2009

Antigen recognition by specific cytolytic T lymphocytes (CTL)



In melanoma, antigen-specific CD8 T cells often develop, but their protective activity is limited by the relative lack of high affinity TCRs against self-(tumor) antigen

Multiple approaches to enhance the avidity of antigen recognition by tumor reactive T cells

Designed by N. Rufer et al.

Approaches to identify T cell-defined tumor antigens

1- Genetic: molecular cloning guided by T cells

2- Biochemical: isolation and sequencing of antigenic peptides

3- Reverse immunology: web-based algorithms

T cell-defined tumor antigens

1- Shared, tumor specific antigens (e.g: MAGE-A3, NY-ESO-1)

2- Differentiation antigens (e.g: gp100, PSA, CEA)

3- Overexpressed antigens (e.g: HER-2/neu, WT-1)

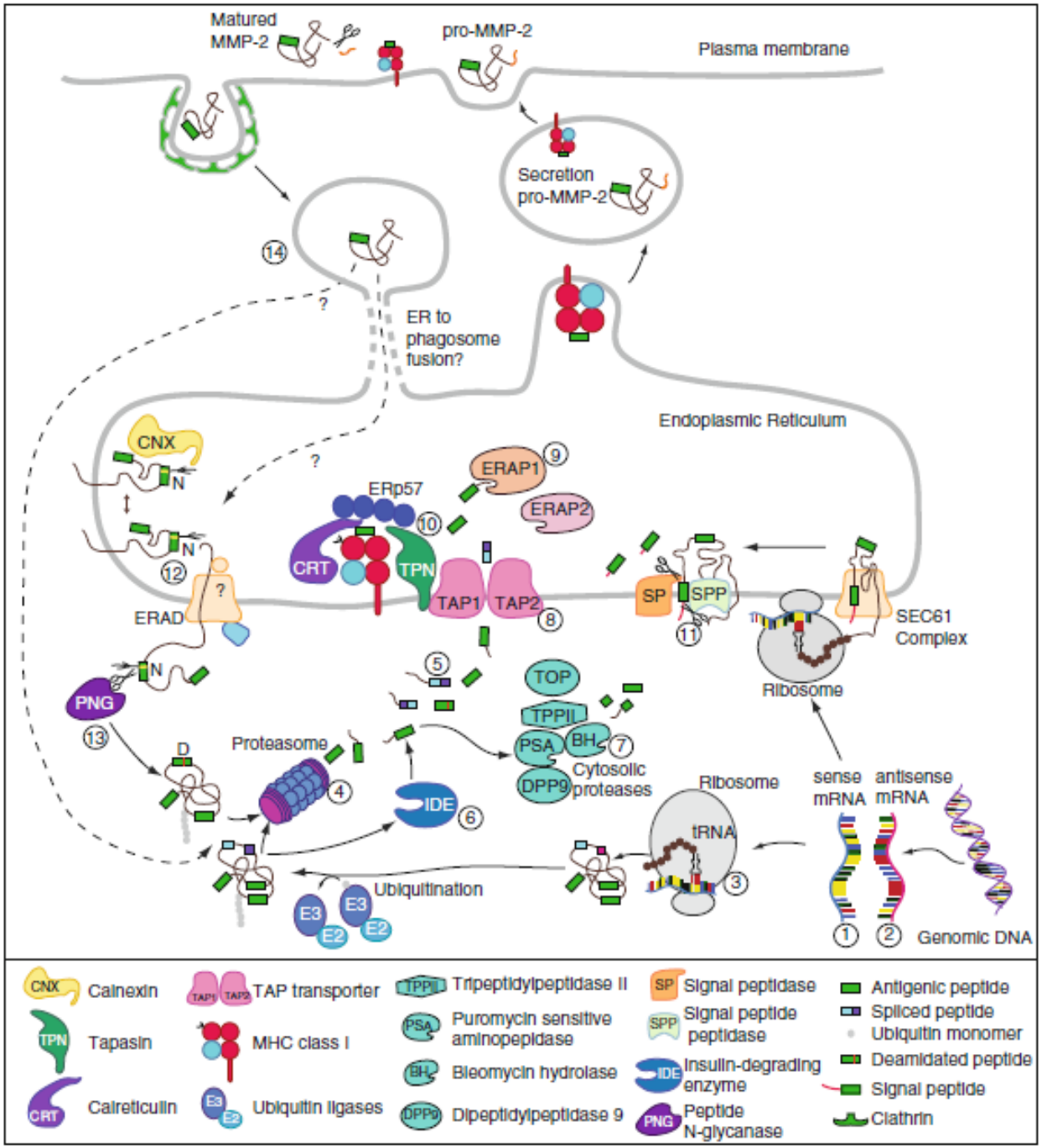
4- Mutated, unique antigens (e.g: MUM-1, idiotypes)

5- Virus encoded antigens (e.g: HPV-16 E6/E7, HBV, HCV)

http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm

> 300 T cell defined tumor antigens, short peptides

Pathways for processing and presentation of MHC class I-restricted tumor antigens



Aberrant transcription or mRNA splicing

Translation of alternative or cryptic ORFs

Post-translational modifications

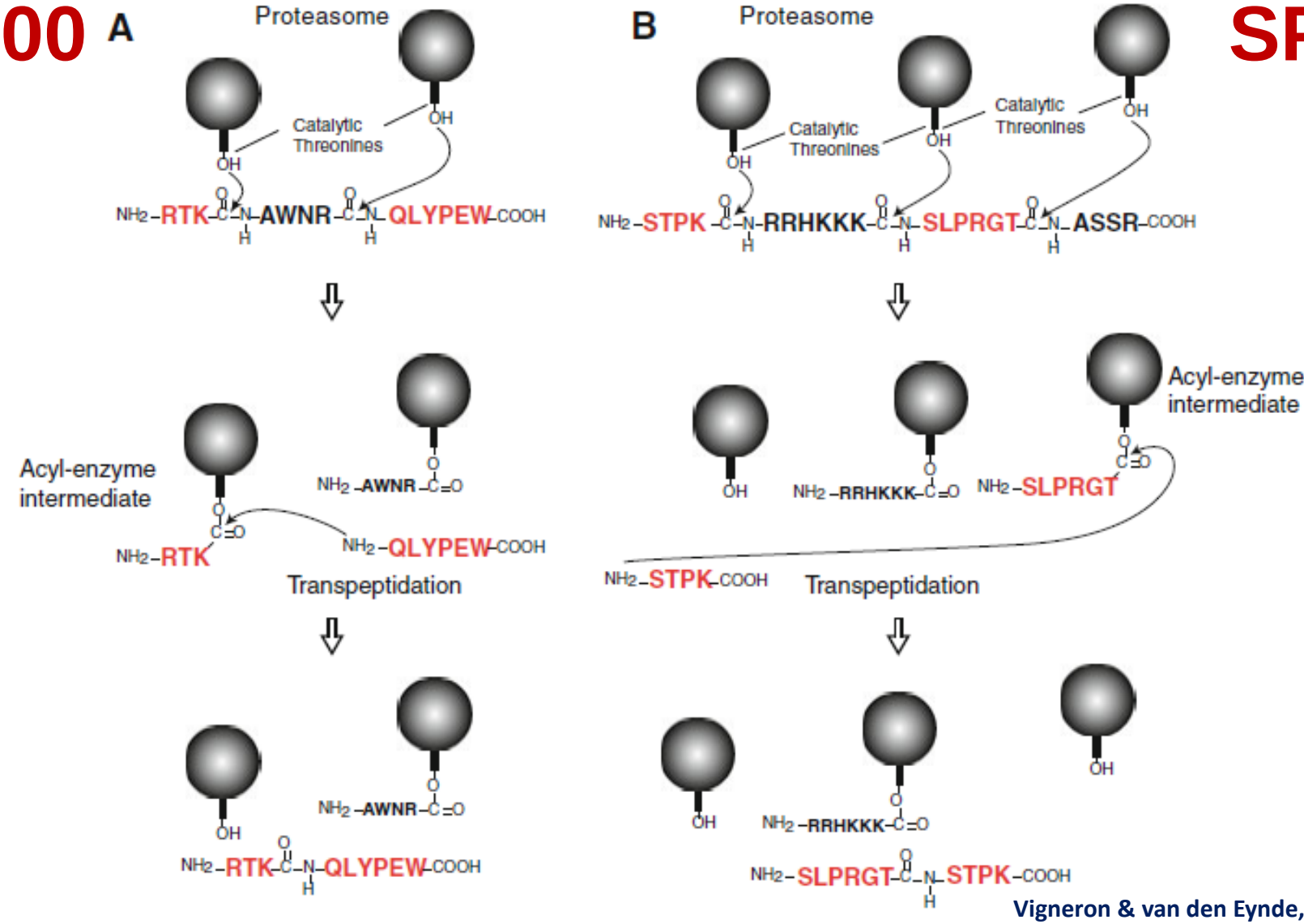
Splicing fragments that are originally non-contiguous in the parental protein

Cytosolic proteases, other than the proteasome, produce or destroy antigenic peptides

Vigneron & van den Eynde, Cell Mol Life Sci 2011

Model of the peptide splicing reaction inside the proteasome

gp100



SP110

Vigneron & van den Eynde, Cell Mol Life Sci 2011

Determinants of peptide antigenicity

TCR contacts

Altered peptide ligands

(APLs)

MHC anchor residues

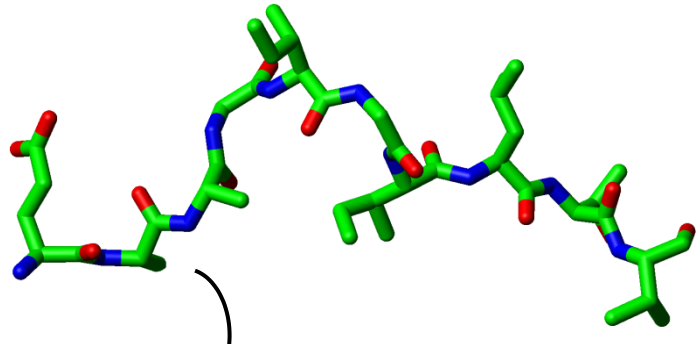
MHC variant peptides

(MVPs)

Edwards & Evavold, Immunol Res 2011

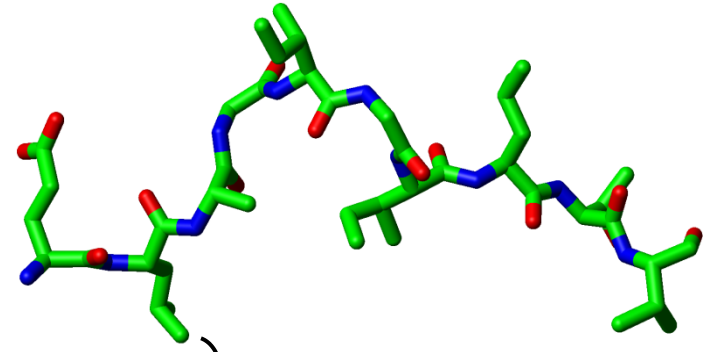
Melan-A/MART-1 MVPs

Natural decapeptide

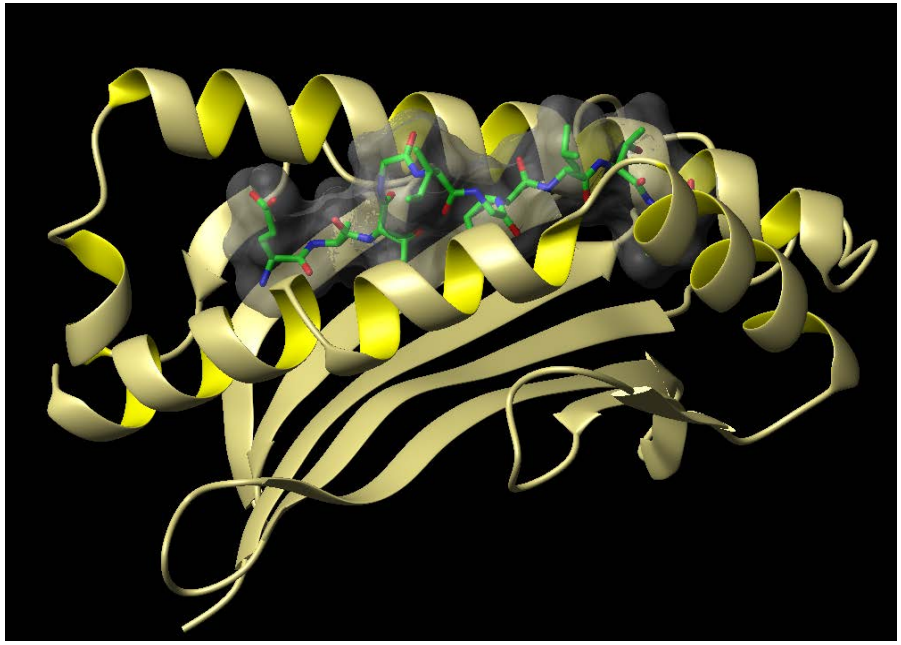


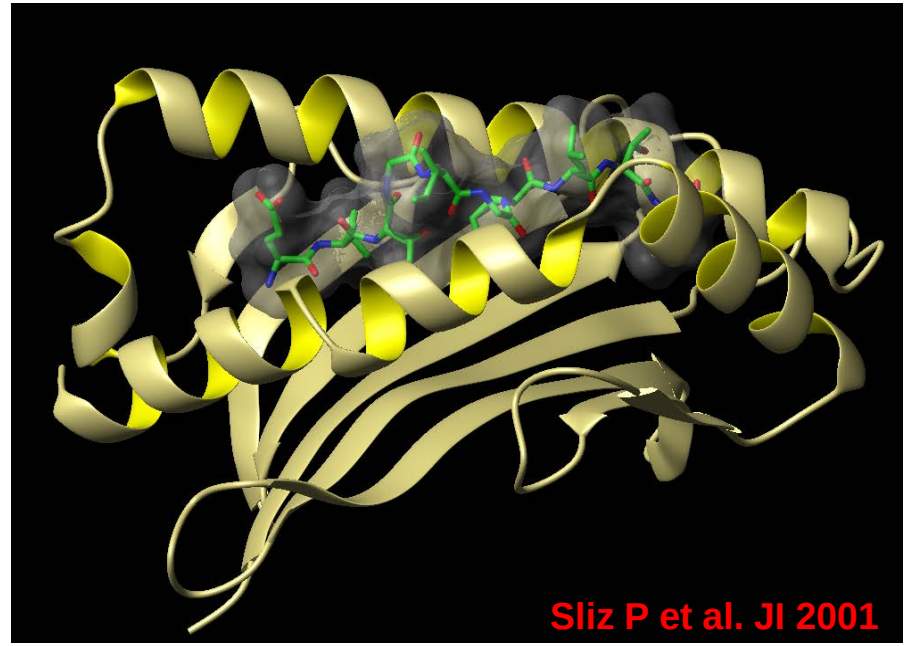
E **A** A G I G I L T V

Decapeptide analog



E **L** A G I G I L T V





Tumor antigenic peptide MVPs in clinical or preclinical testing

- gp 100 (melanocyte/melanoma)

  IMDQVPFSV

  YLEPGPVTV

- CEA (epithelial carcinomas)

  YLSGANLDL

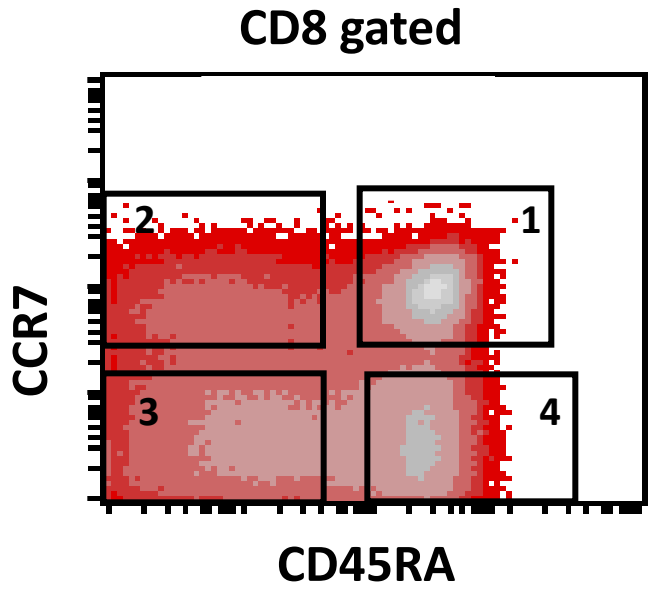
- NY-ESO-1/LAGE-1 (cancer/testis)

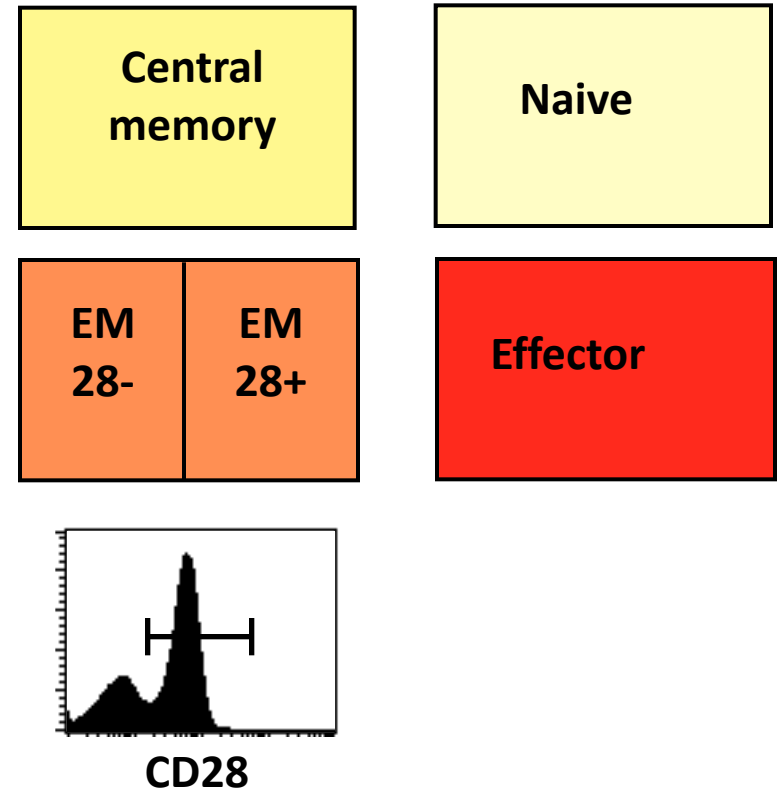
  SLLMWITQA

  SLLMWITQV

Monitoring the tumor antigen specific CD8 T cell response

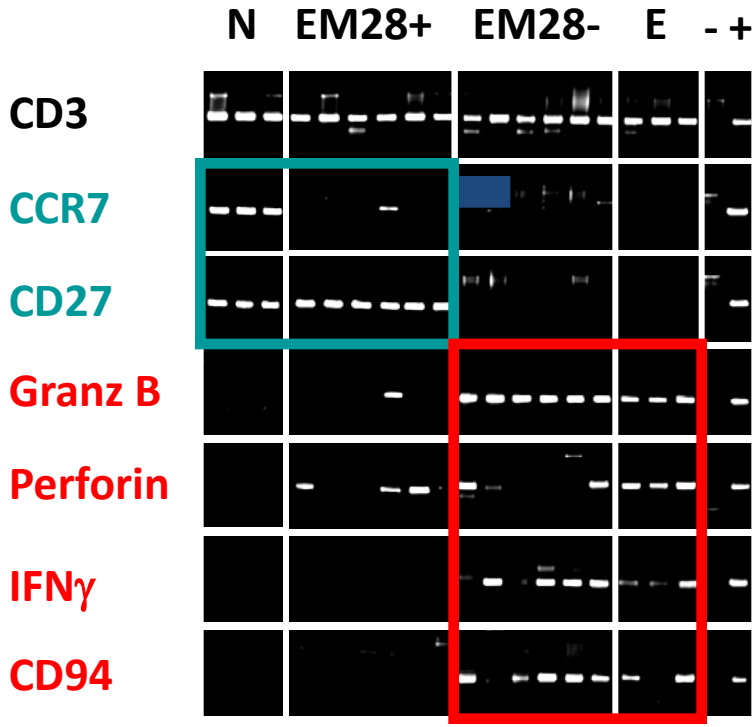
Six major CD8+ T-cell subsets…. in humans



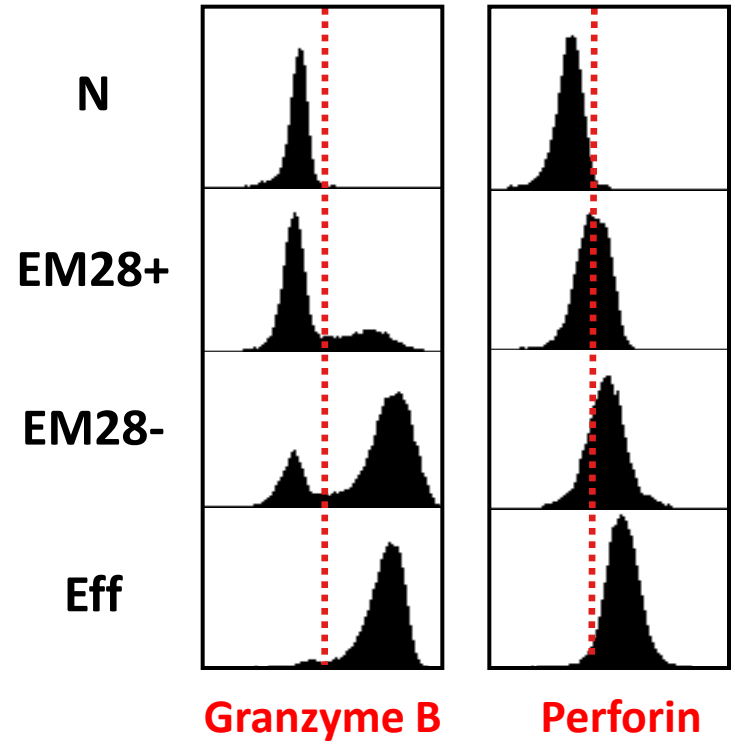


Differential lytic effector function displayed by EM28+ and EM28- T cell subsets

gene expression by 5 cell RT-PCR



protein expression by intracellular staining



Romero et al. JI 2007

Monitoring a CTL response to a well defined tumor antigen

Enumeration and phenotype by flow cytometry: Tetramers^{PE}, CD45RA^{APC}, CCR7^{FITC}

IFN-γ production by flow cytometry (intracellular, secretion)

ELISPOT

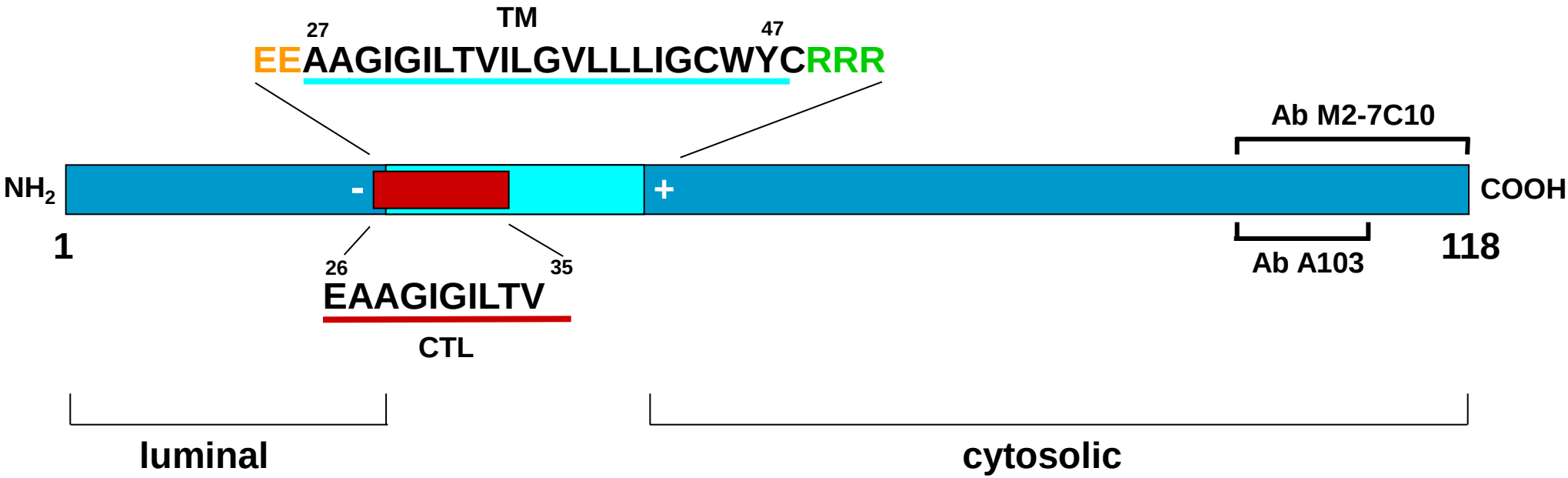
Lytic activity

TCR usage

Functional avidity

Replicative history: telomere length (Flow-FISH); content of TCR rearrangement excision circles (TRECs)

The melanocyte/melanoma differentiation antigen: Melan-A/MART-1



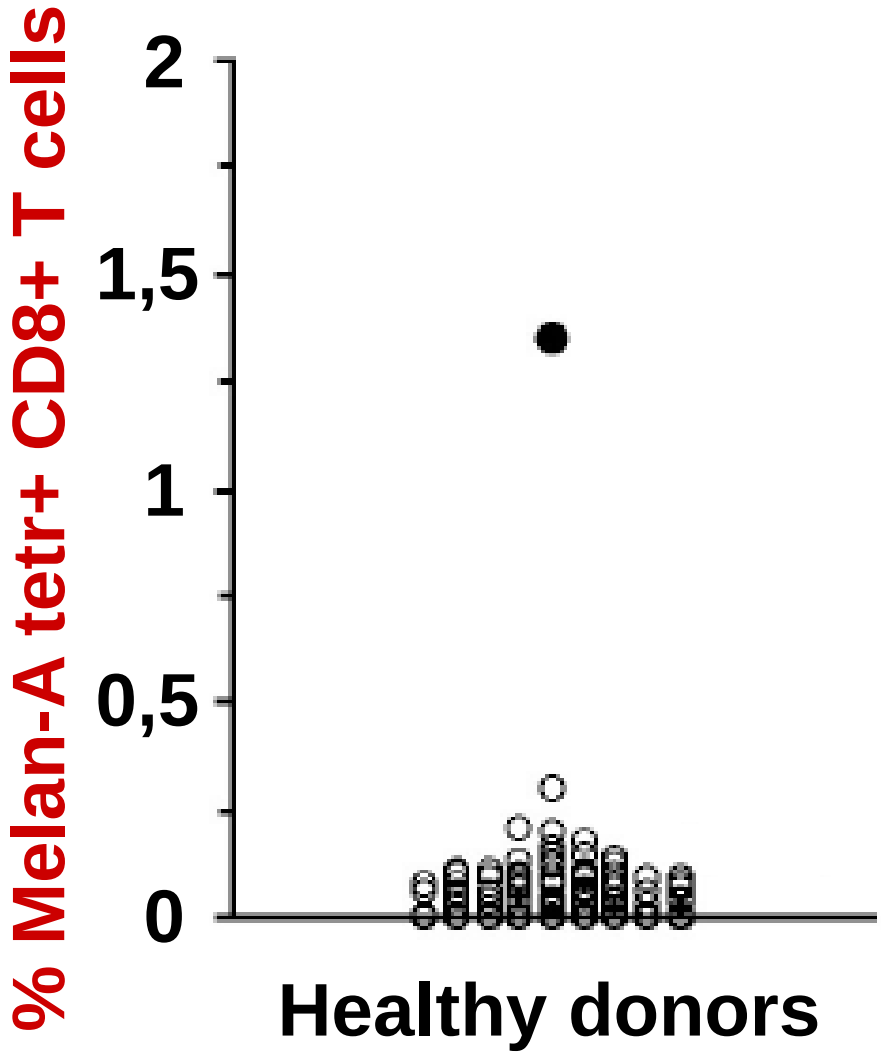
- 118 a.a., apparent MW 22-24 kD
- Melanocyte lineage specific
- Function unknown

The Melan-A/MART-1 specific CD8 T cell repertoire in healthy HLA-A2 individuals

- One in 1,400 blood CD8 T lymphocytes are specifically labeled with fluorescent multimers
- Phenotypically and functionally they are naive T cells
- High level of positive selection in human thymus
- They undergo limited expansion in the peripheral immune system
- Unique example of « immunological ignorance » in humans

Pittet et al. 1999; Zippelius et al. 2004

Ex vivo detectable HLA-A2/Melan-A specific CD8 T cells in humans



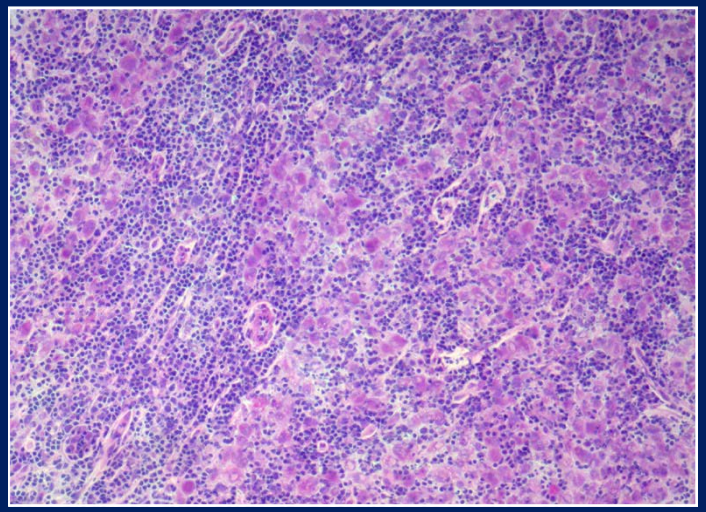
Mean = 0.08 %
(n = 76)

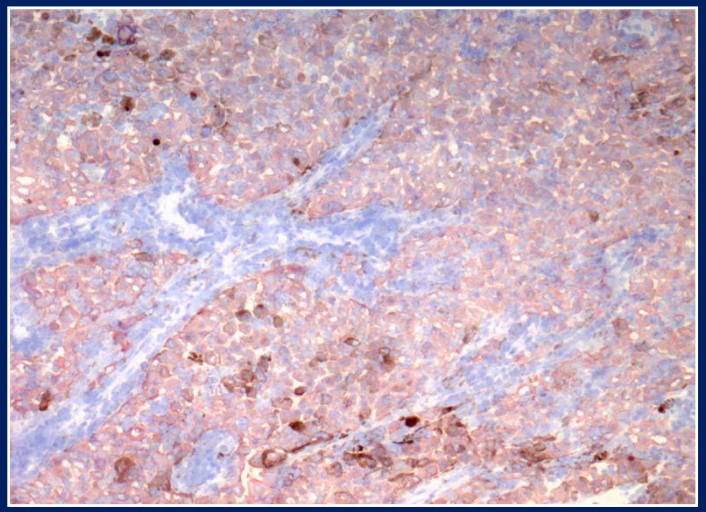
Voelter V. … Appay V, 2008

Metastatic (stages III and IV) melanoma patients (HLA-A2)

LAU 465 – Lymph node metastasis

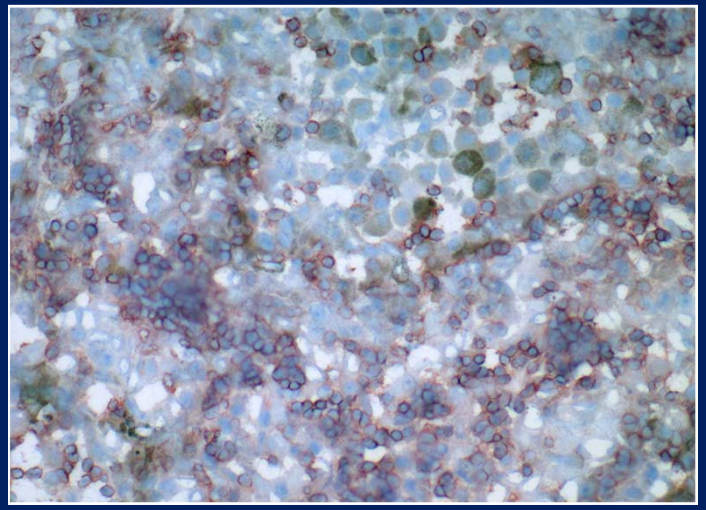
H&E

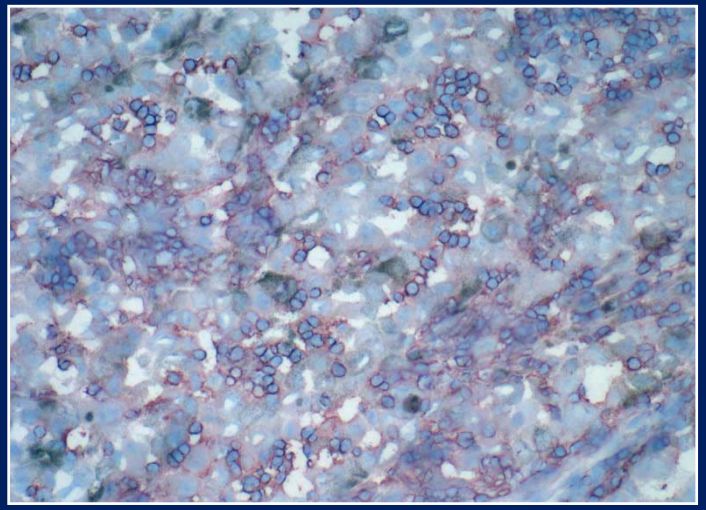




Melan-A/
MART-1

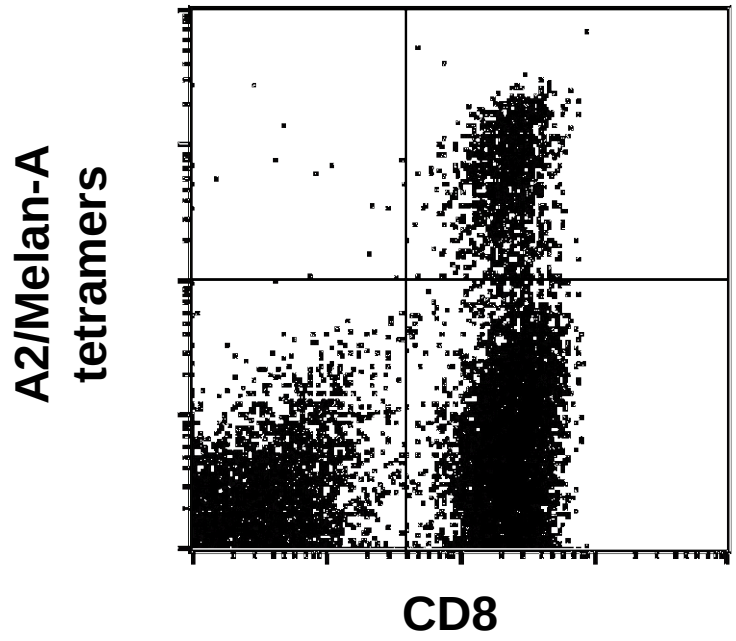
CD3





CD8

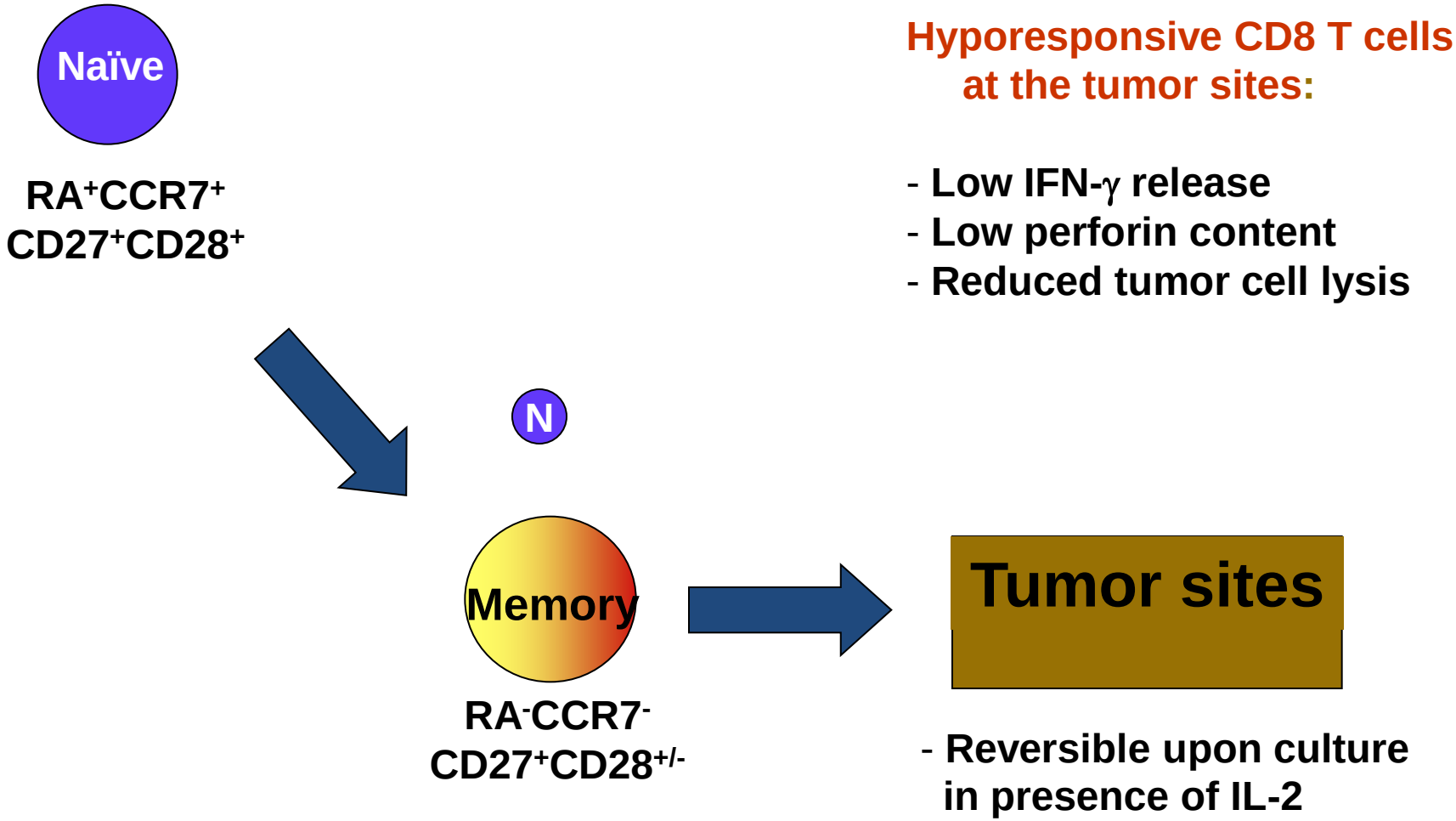
A2/Melan-A tetramer^{+} cells in TILNs ex vivo



~ 15 %

A2/gp100, A2/tyrosinase, A2/MAGE-10, A2/LAGE and A2/NY-ESO-1 tetramer^{+} cells were non detectable (<0.0 4%)

Evidence of Melan-A specific response in two thirds of metastatic melanoma patients, accumulating in tumor lesions

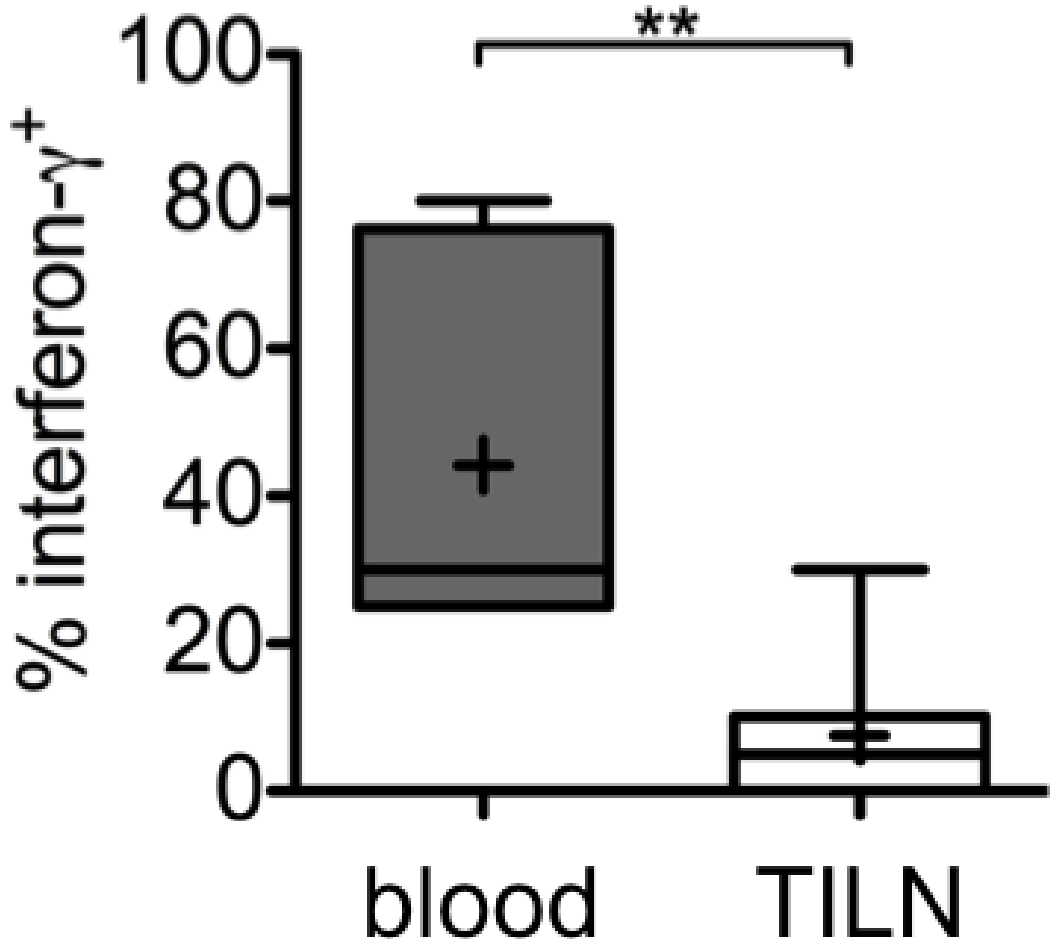


Hyporesponsive CD8 T cells at the tumor sites:

- **Low IFN-γ release**
- **Low perforin content**
- **Reduced tumor cell lysis**

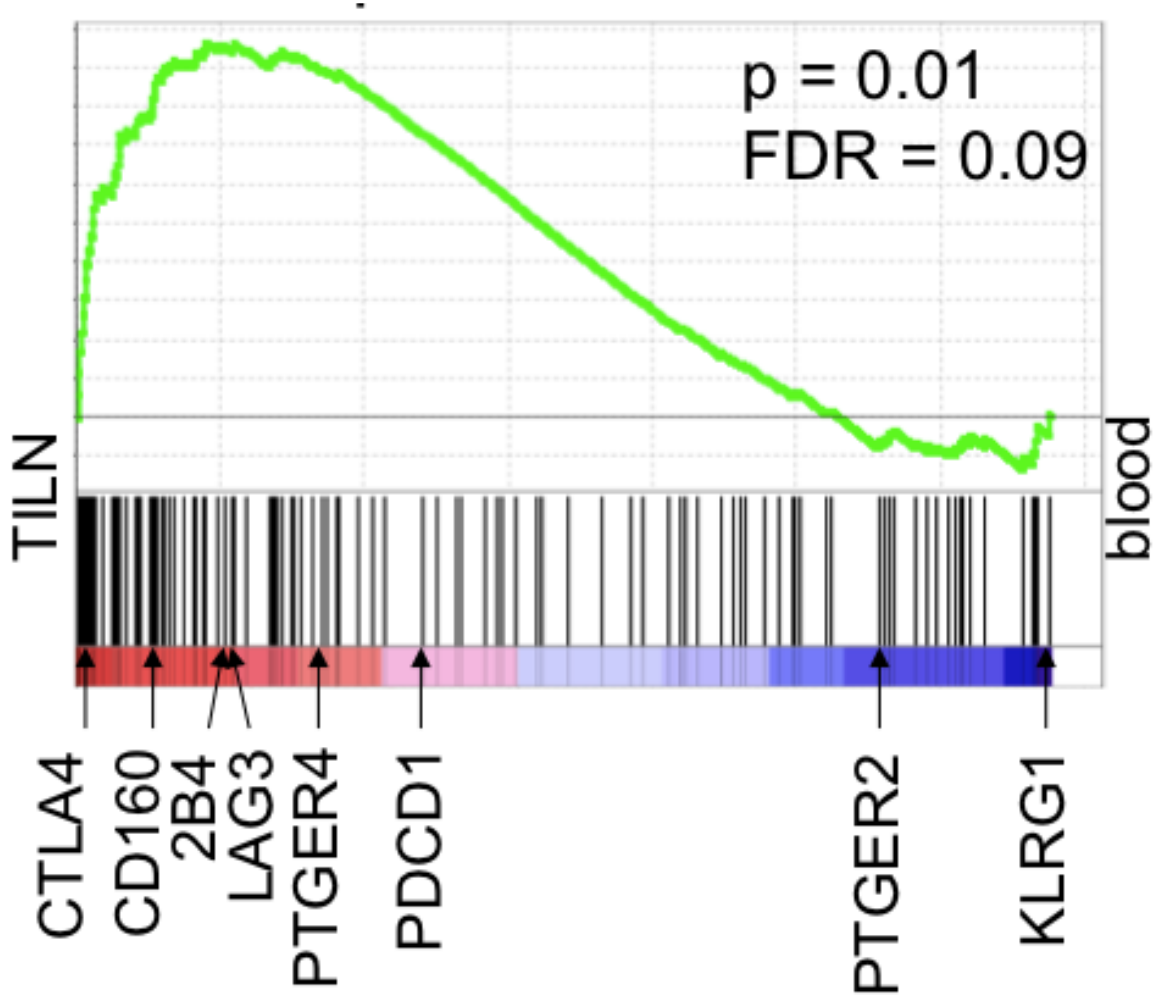
- **Reversible upon culture in presence of IL-2**

Tumor antigen specific IFN-γ release in specifically blunted in tumor infiltrating lymphocytes



Zippelius et al. 2004; Baitsch, Speiser et al. 2011

Exhausted gene set enrichment in Melan-A-specific CD8 T cells from metastatic lymph nodes



Baitsch, Speiser et al, 2011

Can we rekindle tumor antigen specific T cells by vaccination?

Subunit vaccines: defined molecular composition

Minimal number of molecularly defined vaccine modules

Target antigen (signal 1)

Immunological adjuvant (at least signal 2?, >)

Delivery vehicle

Cancer vaccines, the many ways to deliver Ags

Synthetic peptide: short, exact epitopes; or long peptides, multiple epitopes

Recombinant protein

Recombinant viral like particles (VLPs)

Naked, stabilized nucleic acids:

- **DNA plasmids**
- **mRNA**

Recombinant viruses (many candidates):

- **Pox**
- **Adeno**
- **Retro, lenti**
- **Sindbis**

Recombinant bacteria:

- **Salmonella**
- **Listeria**

Dendritic cells

NOBEL PRIZE 2011 PHYS & MED

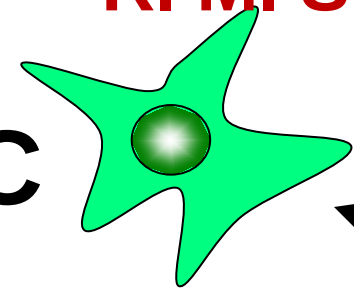
Nature's adjuvant: mature dendritic cells

Activated T helper cell

CD40 ligand

The late
R. M. Steinman

NK cells



DC

J. Hoffmann
&
B. Beutler

TLR agonists

Endogenous innate R. ligands (DAMPs)

Pathogenic microbes!

Cytokines

TLR agonists as vaccine adjuvants

TLR-4: LPS, MPLA present in various formulations (e.g. GSKBio adjuvants)

TLR-3: proven in preclinical models, ongoing in clinical trials

TLR-7: Aldara™ (Imiquimod) being tested in cancer patients

TLR-9: synthetic oligos bearing CpG motifs; the most potent in terms of adjuvanticity

Results of sequential phase I clinical trials in patients with stage III/IV melanoma in Lausanne

Adjuvant	TLR agonist	Immune response
None	-	0/6
AS02B (GSK Bio)	TLR4	1/12
K. pneum. outer membrane P40 (P. Fabre)	TLR2	0/9
Montanide (IFA, Seppic)	-	12/17
rtLAG-3 (Immutep)	DC activator	?

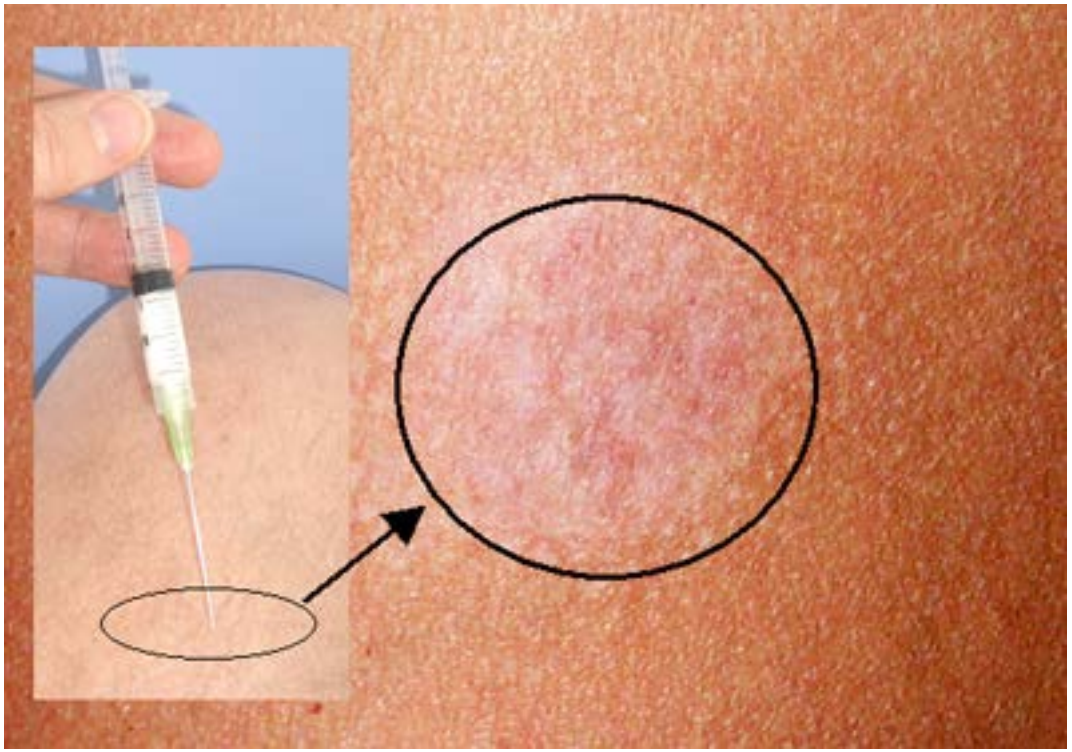
Molecularly defined therapeutic vaccines: synergy between IFA and TLR9 agonist

100 μg peptide (analog)

500 μg CpG-ODN (type B) [PF 676]

Montanide ISA 51, 1 ml

Monthly s.c. injections

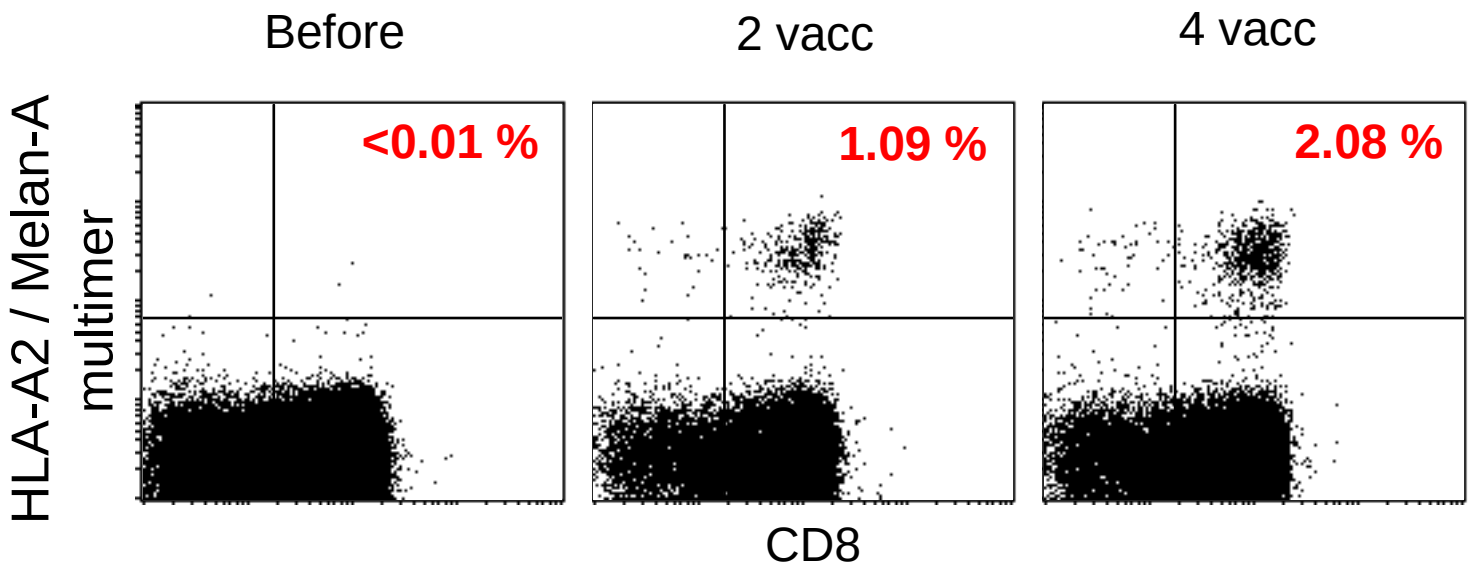


Strongly increased T cell frequency

after 4 vaccinations with

Melan-A peptide + CpG 7909 + IFA

(PF-3512676)



ex vivo flow cytometry analysis of PBMC

Speiser et al. JCI 2005 115:739

Vaccination with CpG 7909 (PF 676) + peptide analogue + IFA :

- rapid and efficient T cell responses, frequency 10-fold higher than without CpG

- peak response 7 to11 days after booster injection

-generation of (central and) effector memory T cells expressing perforin, granzyme B and IFNγ

- progressive generation of CD28- effector T cells

- killing of melanoma cells, IFNγ secretion

Other peptide based vaccines extensively tested in melanoma:

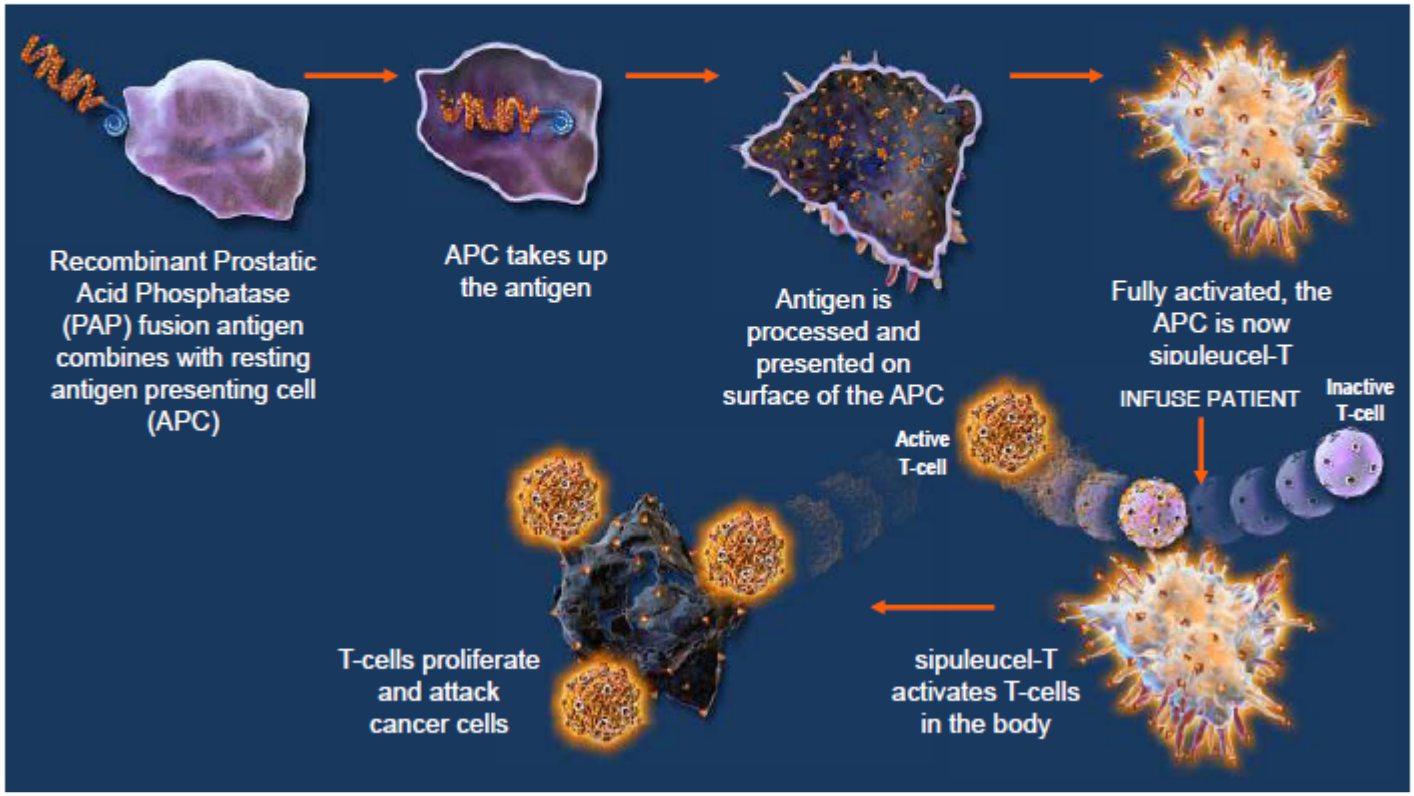
MAGE-A3$_{168 – 176}$, HLA-A1

NY-ESO-1$_{157 – 165}$, HLA-A2

gp100$_{209 – 217}$ analog +/- high dose IL-2

(extended survival reported by Schwartzentruber 2009)

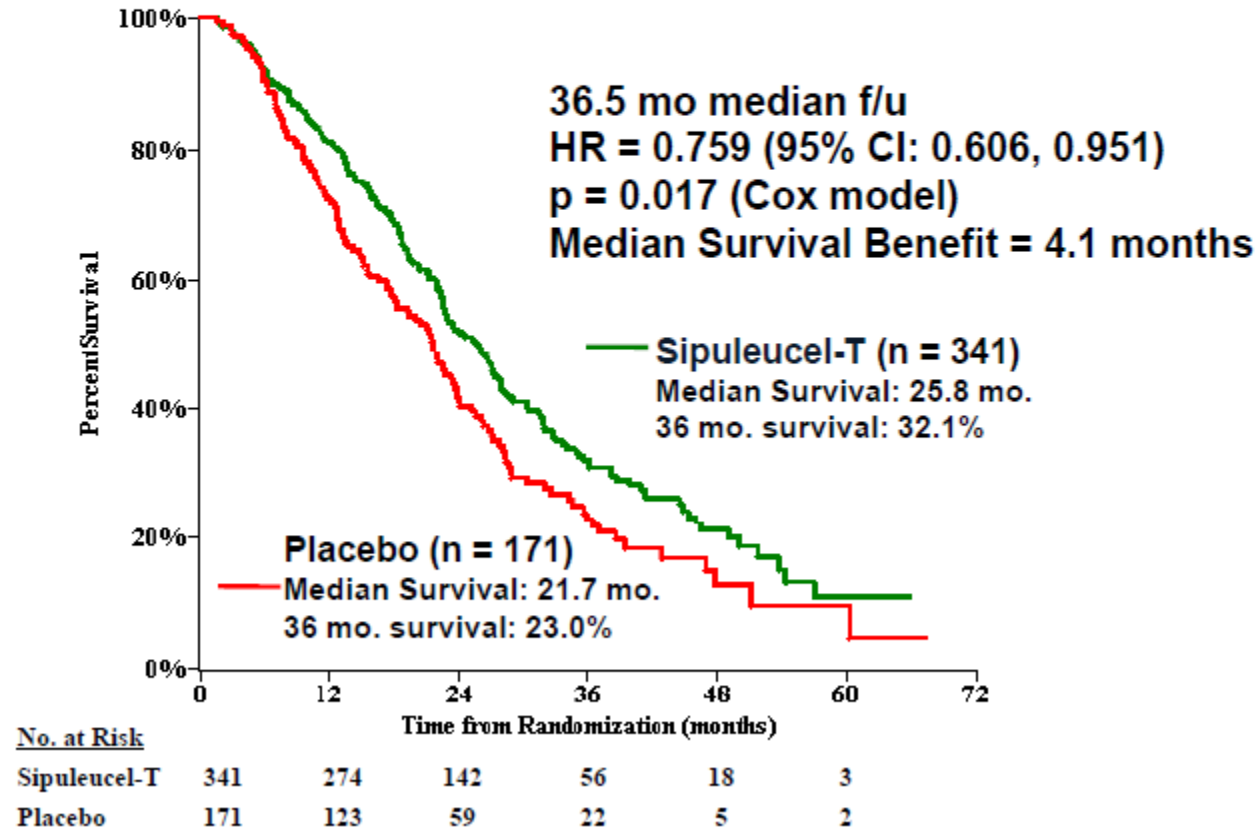
Sipuleucel-T (Provenge): autologous “APCs” cultured with antigen-cytokine fusion protein



Ag: PAP

Cytok: GM-CSF

IMPACT Overall survival
Final analysis (n = 349 events)



Hormone refractory metastatic prostate carcinoma

Other therapeutic cancer vaccines in use

Oncophage, Russia from 2008

Provenge, USA from 2010

Prostvac, USA on fast track

Two vaccines in randomized phase III clinical trials in patients with lung cancer

Rt MAGE-A3 protein (GSK Bio)

MUC-1 peptide (Merck – Biomira)

HPV16 E7 + E6 long synthetic peptide cocktail vaccine

Approx. 15 peptides (~25 – 20 amino acids)

N = 20 patients with VIN

10+ complete tumor regression (> 50%)

Correlation with overall specific immune response

Cancer vaccine development:

Frequent induction of vaccine specific T cell responses (40- 80 %)

Sporadic tumor regression (3 – 10 %)

Favourable clinical outcome in even higher proportions of patients (up to 25 %)

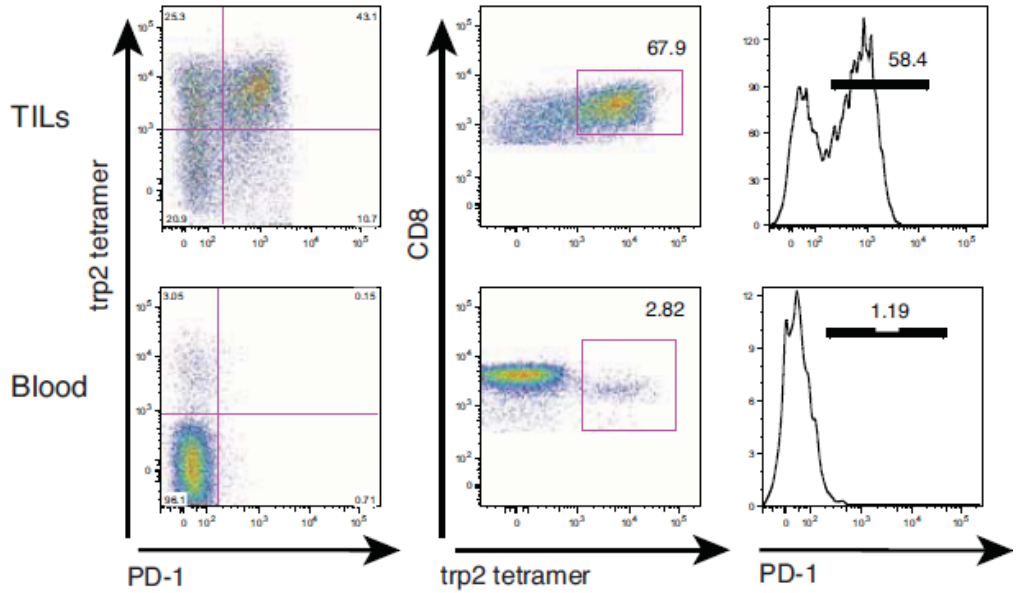
Correlation with immune response

Two major weaknesses in current therapeutic cancer vaccines

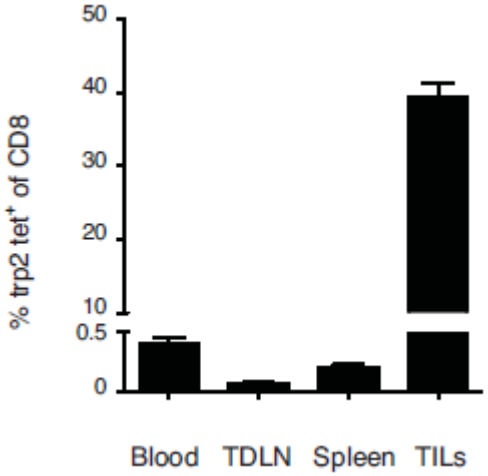
Low potency – need for optimization

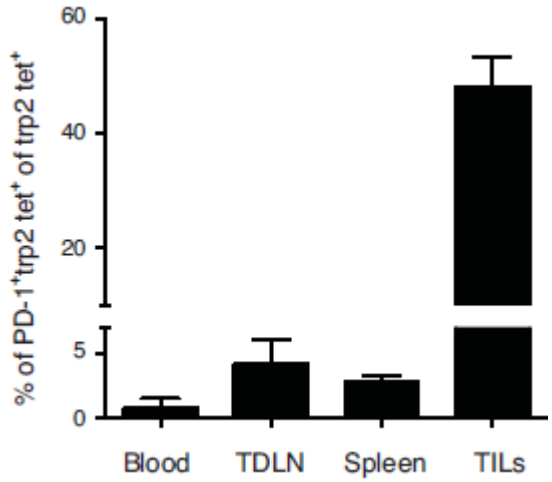
Short lived responses – memory formation, negative modulation at the tumor sites

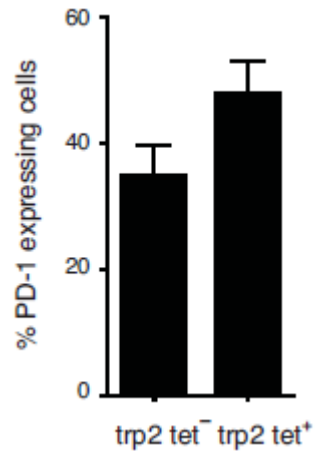
PD-1 expression of trp2-specific CD8+ T cells after therapeutic rLV vaccination

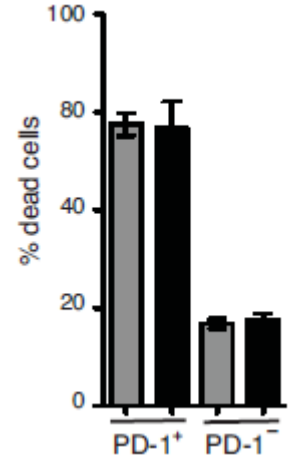


Sierro et al. EJI 2011

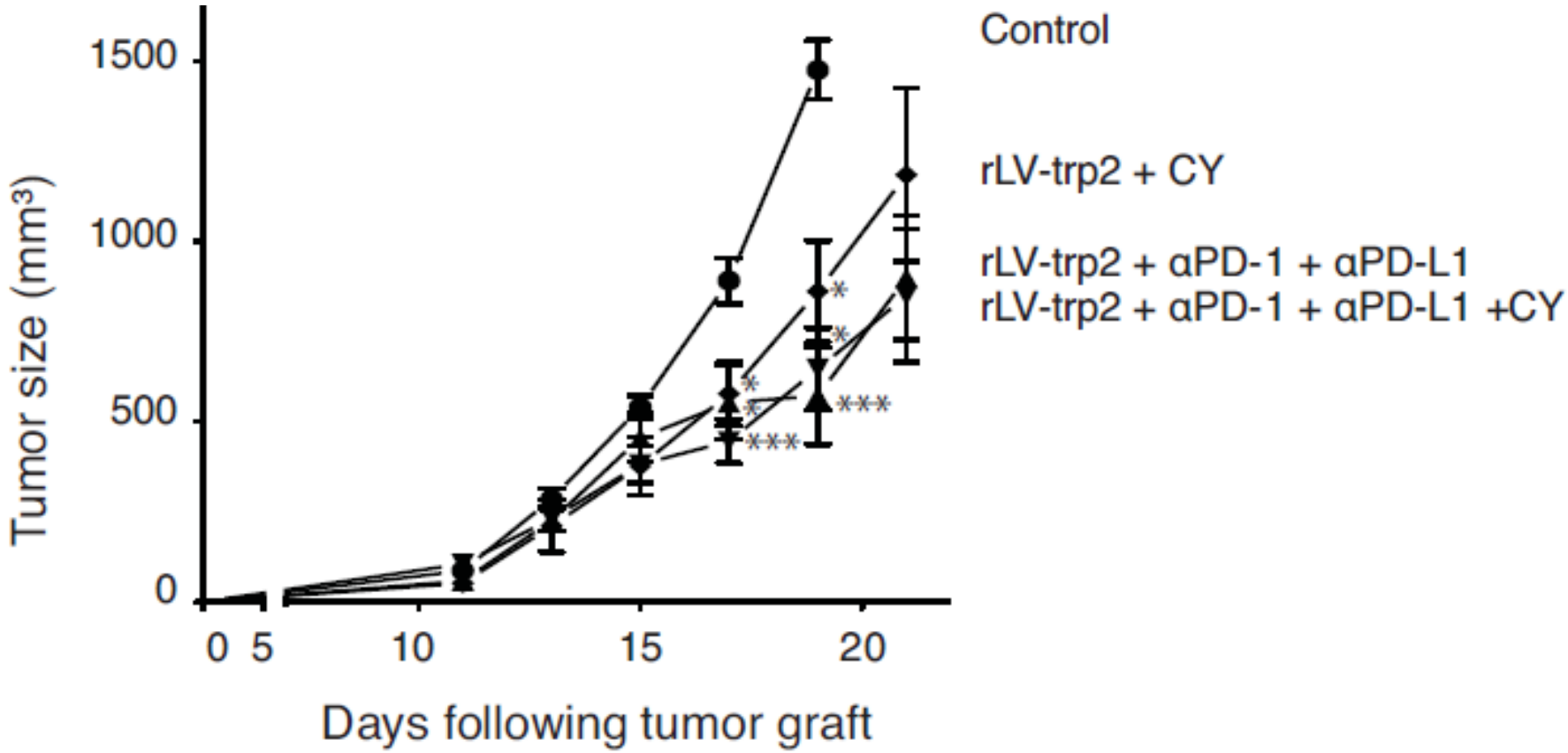








Blockade of PD-L1/PD-L1 pathway and rLV-trp2: Combination therapy ameliorates tumor control



Cancer vaccine development:

Comprehensive immunomonitoring

Clinical endpoints, modified response criteria

Predictive biomarkers, in development:

e.g. ICAM-1 levels in infused cells – Provenge
Gene signatures, immune response genes

Vaccines combined with immunomodulators or standard tts
Anti-CTLA-4 mAb approved for melanoma in 2011

Thanks!