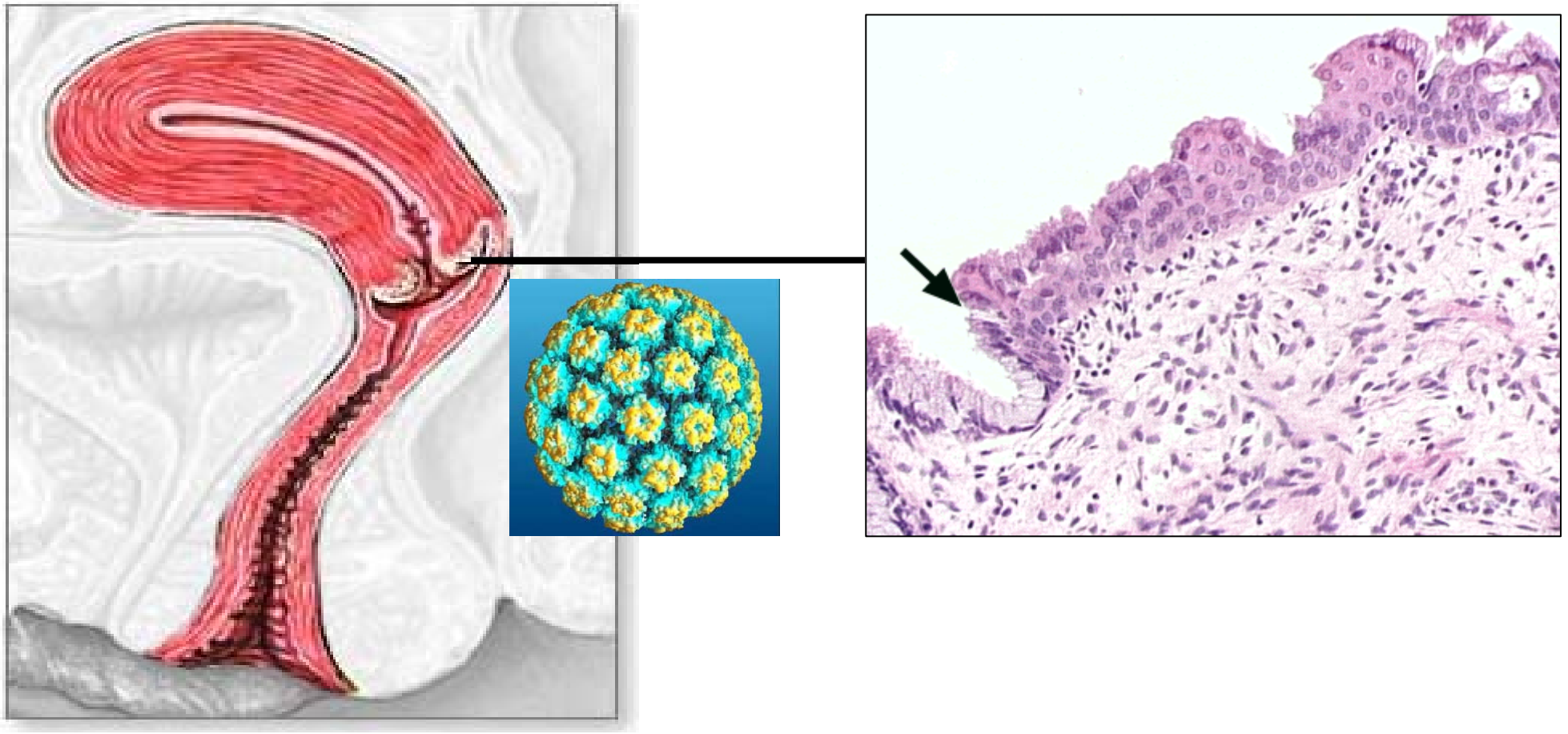
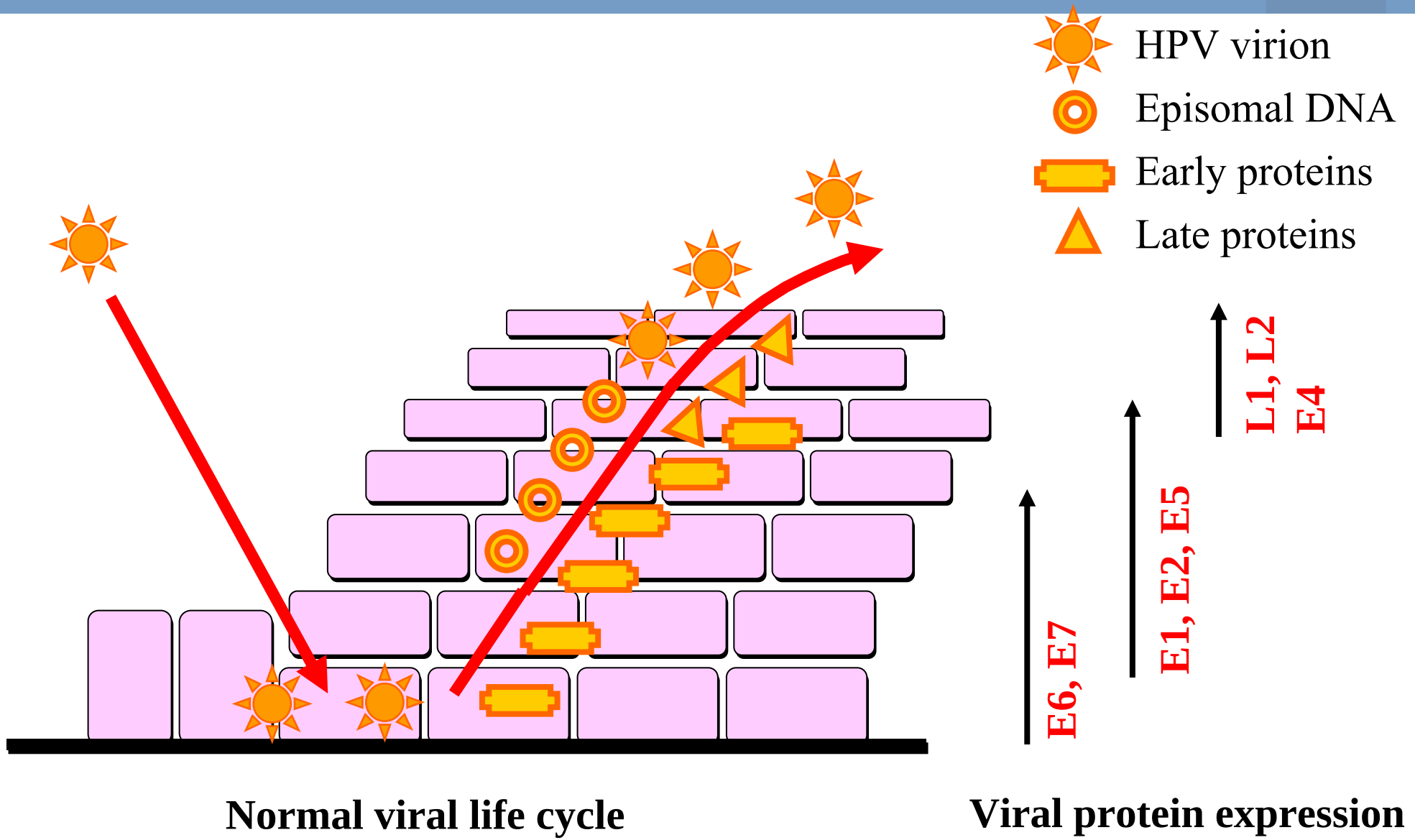
Therapeutic vaccination against HPV16-induced disease

Cornelis J.M.Melief MD, PhD

HPV infection



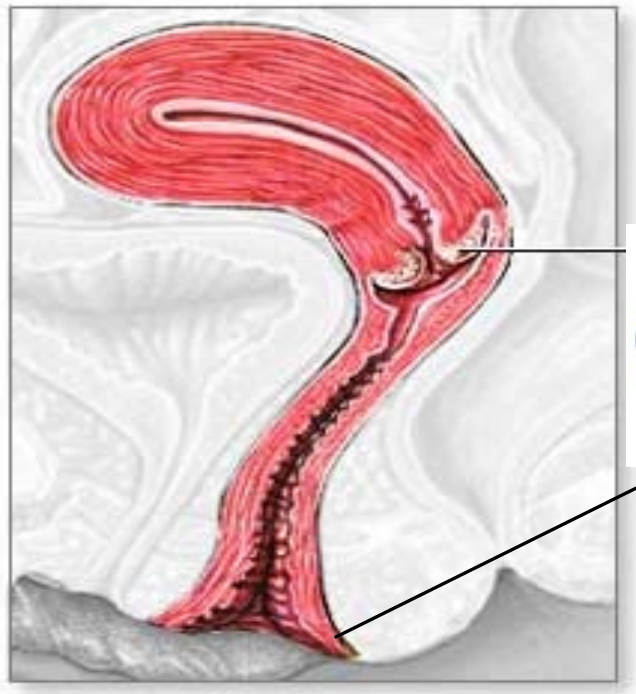
HPV infection cycle is linked to keratinocyte differentiation program



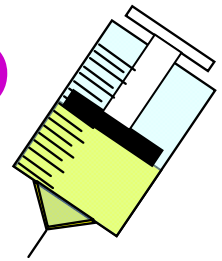
Natural history of cell-mediated adaptive immune response to high risk HPV16

>99%

minority



?



immunity

CD4+ Th1/Th2 immunity to E2, E6, E7 & L1

CD8 immunity to E6 (E7?)

T cells Circulate & Migrate

immune failure

No E6,E7 CD4+ immunity

Impaired CD4+ T-cells

Infrequent CD8+ T-cells

Regulatory T-cells

Overview of different types of potential therapeutic vaccines

- **Viral vector based vaccines: TA-HPV, MVA**
  tremendous problems with antigenic competition by vector sequences
- **DNA vaccines**
  Inefficient way to achieve long-lived antigen expression in DC
- **DC based vaccines**
  laborious and expensive. Direct in vivo DC targeting of antigen more attractive
- **Protein vaccines : TA-CIN, E6E7 Iscomatrix**
  relatively inefficient CD8 CTL induction
- **Peptide vaccines: Minimal HLA class I binding peptides**
  exogenous loading of MHC class I molecules ▸ tolerance. Lack of proper CD8 memory responses due to lack of CD4 help

⟶ Synthetic Long Peptide vaccines

Long-peptide vaccine comprises both a HPV16 E7 CD8+ and a CD4+Th-epitope

Minimal CTL peptide epitope $E7^{49-57}$: RAHYNIVTF

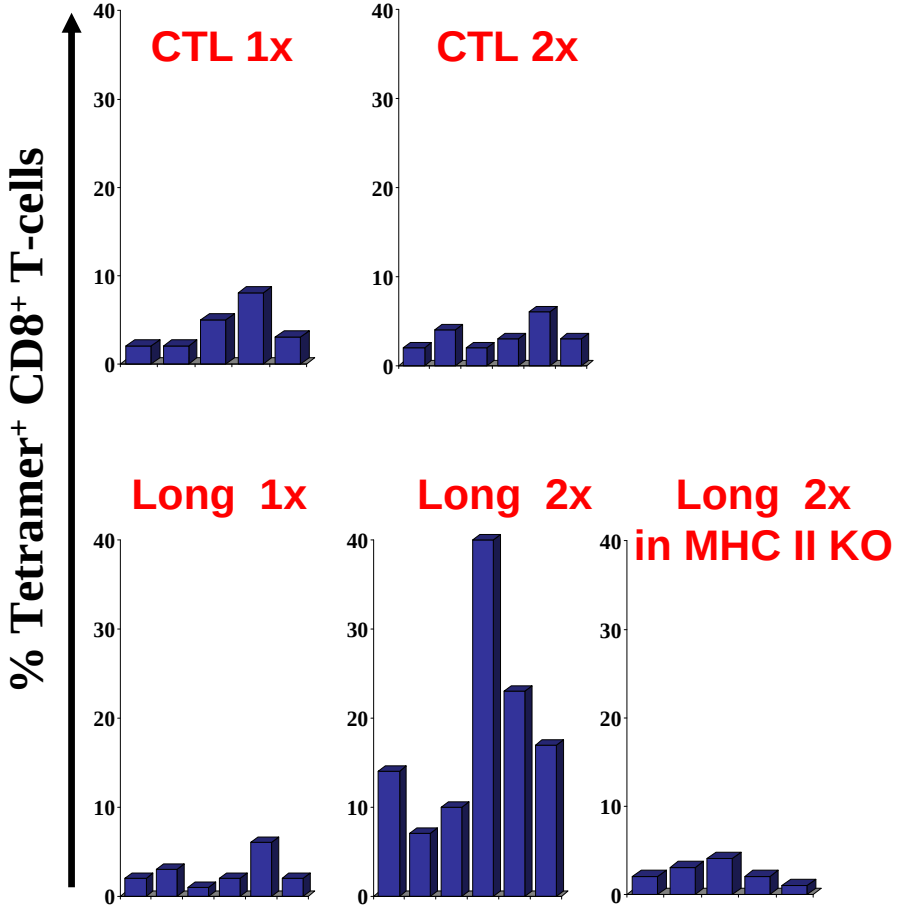
Long peptide $E7^{43-77}$: GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR

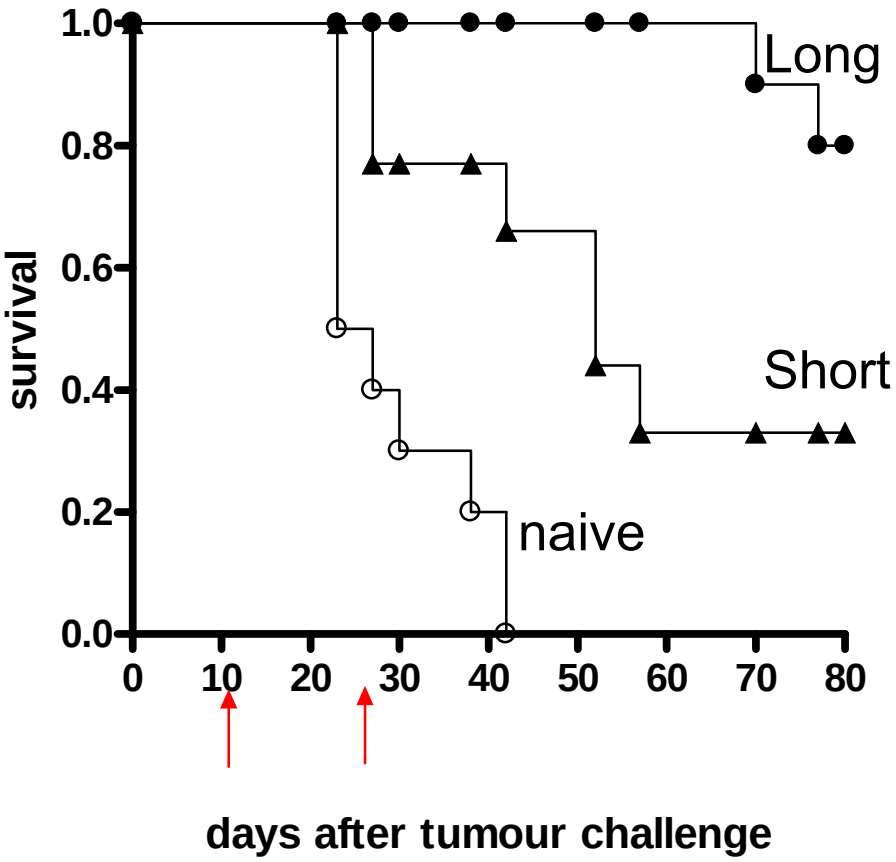
Th epitoop

Zwaveling et al, J. Immunol. ,2002

Long peptide vaccine in HPV16 Mouse Tumour Model

GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR

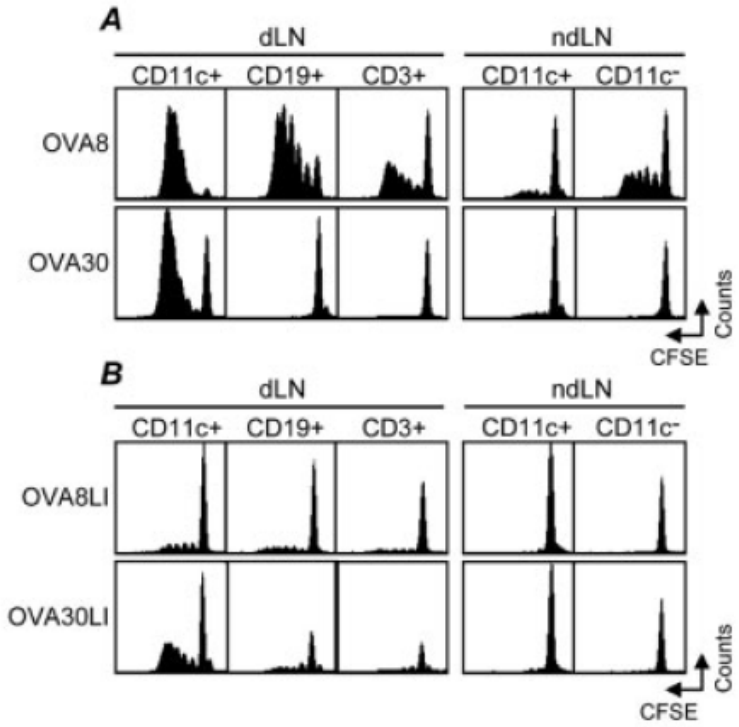




Zwaveling et al, J. Immunol. ,2002

Antigen presentation after vaccination with extended peptides is predominantly focused onto DC in the draining lymph node

Bijker et al EJI,2008



A), B) Cells presenting antigen to CFSE-labeled OT1 T cells 2 days after peptide injection

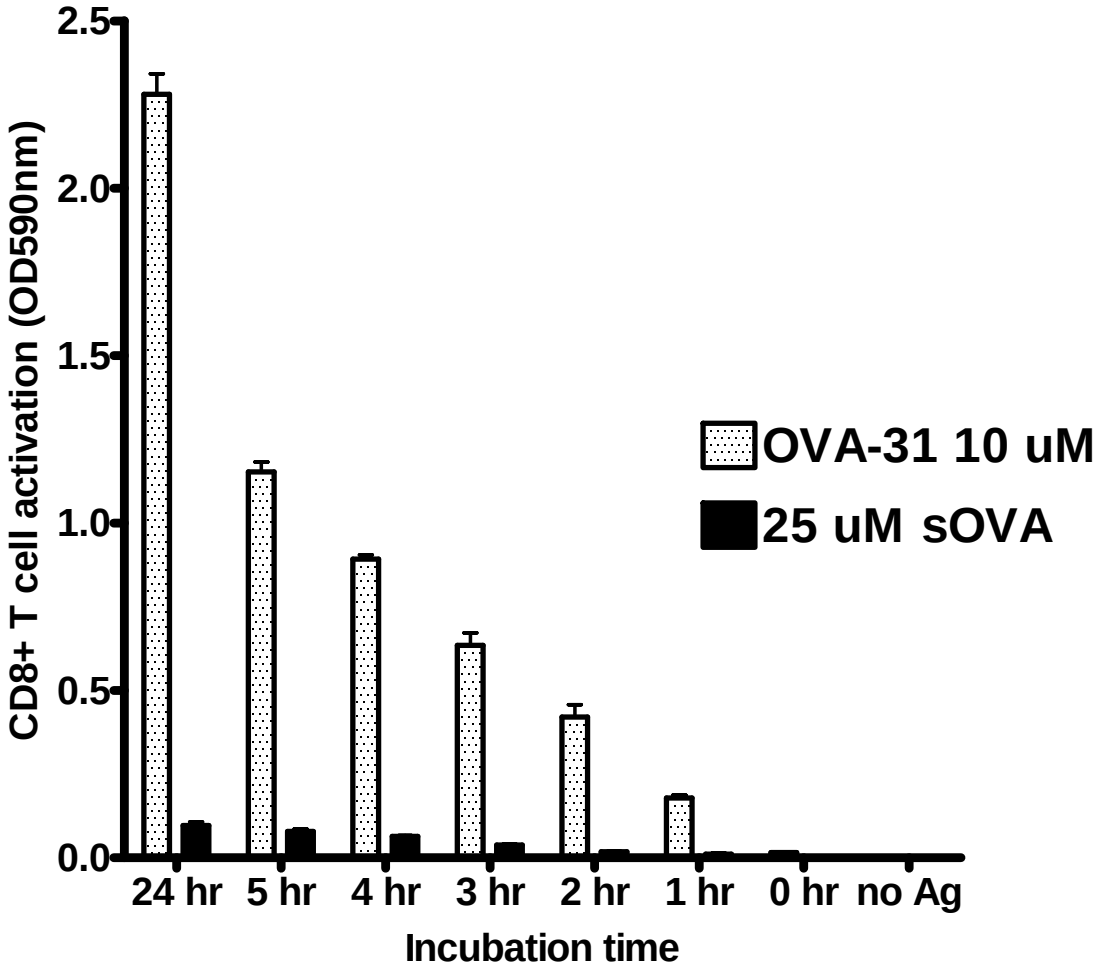
Comparison of SLP with intact protein vaccination for access to cross-presentation pathways for protective CD8+ and CD4+ T cells

(Zhang et al. J. Biol. Chemistry 284, 9184, 2009)

- **HIV-NEF-derived SLP are superior to intact NEF protein, because:**

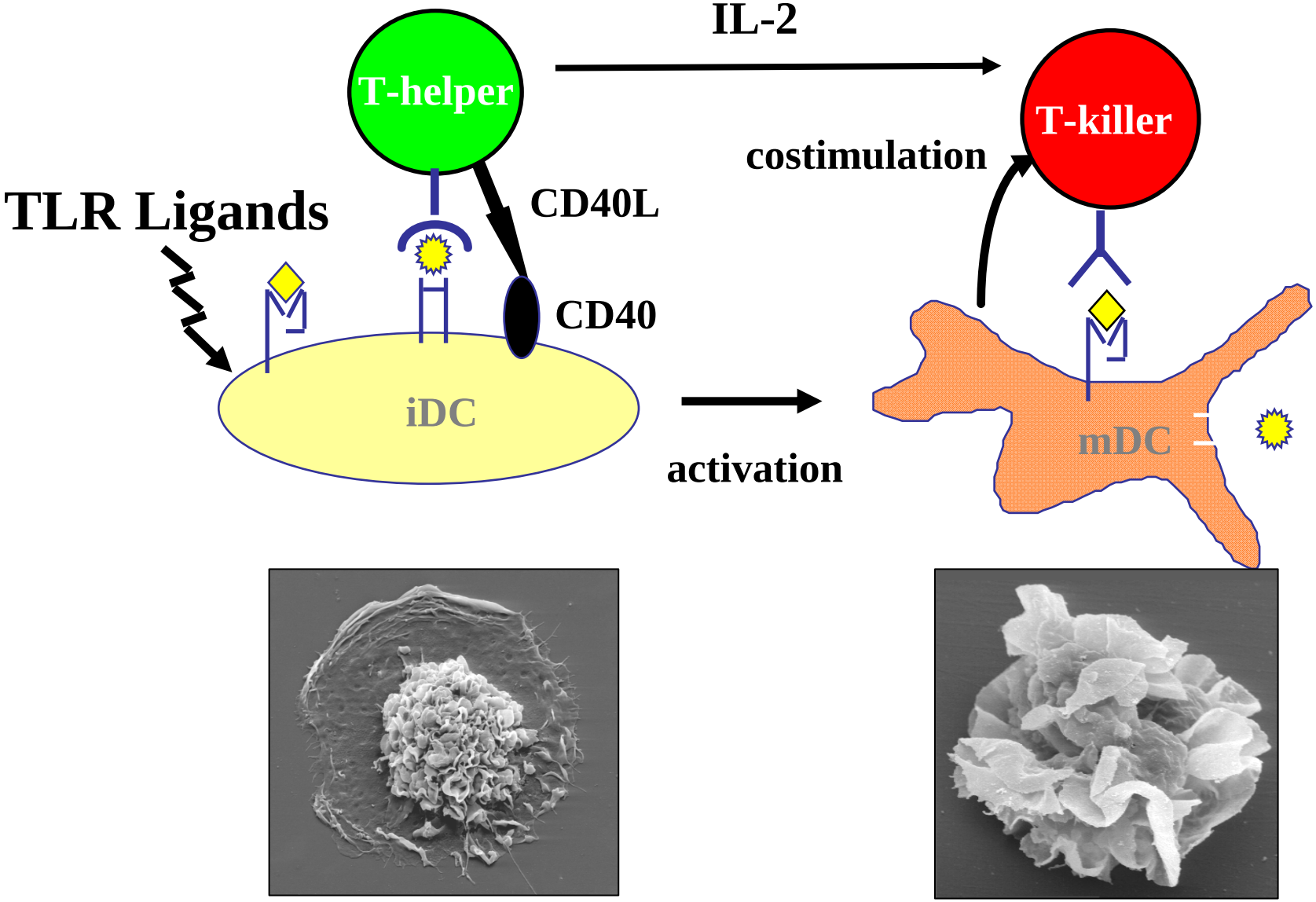
1. SLP traffick not only to endosomes, but also to the cytosol
2. SLP activate CD4+ and CD8+ cells. Intact protein activates mainly CD4+ cells
3. SLP vaccination protects much better than protein against challenge with a lethal does of recombinant –Nef vaccinia virus
4. SLP need to be properly adjuvanted

Efficiency of processing by mouse DC of long peptide versus intact protein



Kinetics of MHC class I antigen presentation. To determine the efficiency of MHC class I presentation of exogenously loaded antigen, cross -presentation, DC were cultured with soluble Ovalbumin protein or the derived synthetic long peptide (OVA -31) encoding the immunodominant MHC class I epitope, SIINFEKL presented in the context of K^b molecules. DC were pulsed for 0,1,2,3,4,5 and 24 h with the antigens followed by extensive washing and mild paraformaldehyde fixation to inhibit further processing beyond above mentioned timepoints. DC were then co -cultured further O/N in the presence of the CD8 T cell hybridoma (B3Z) which produces IL -2 upon recognition of K^b/SIINFEKL.

License to Kill

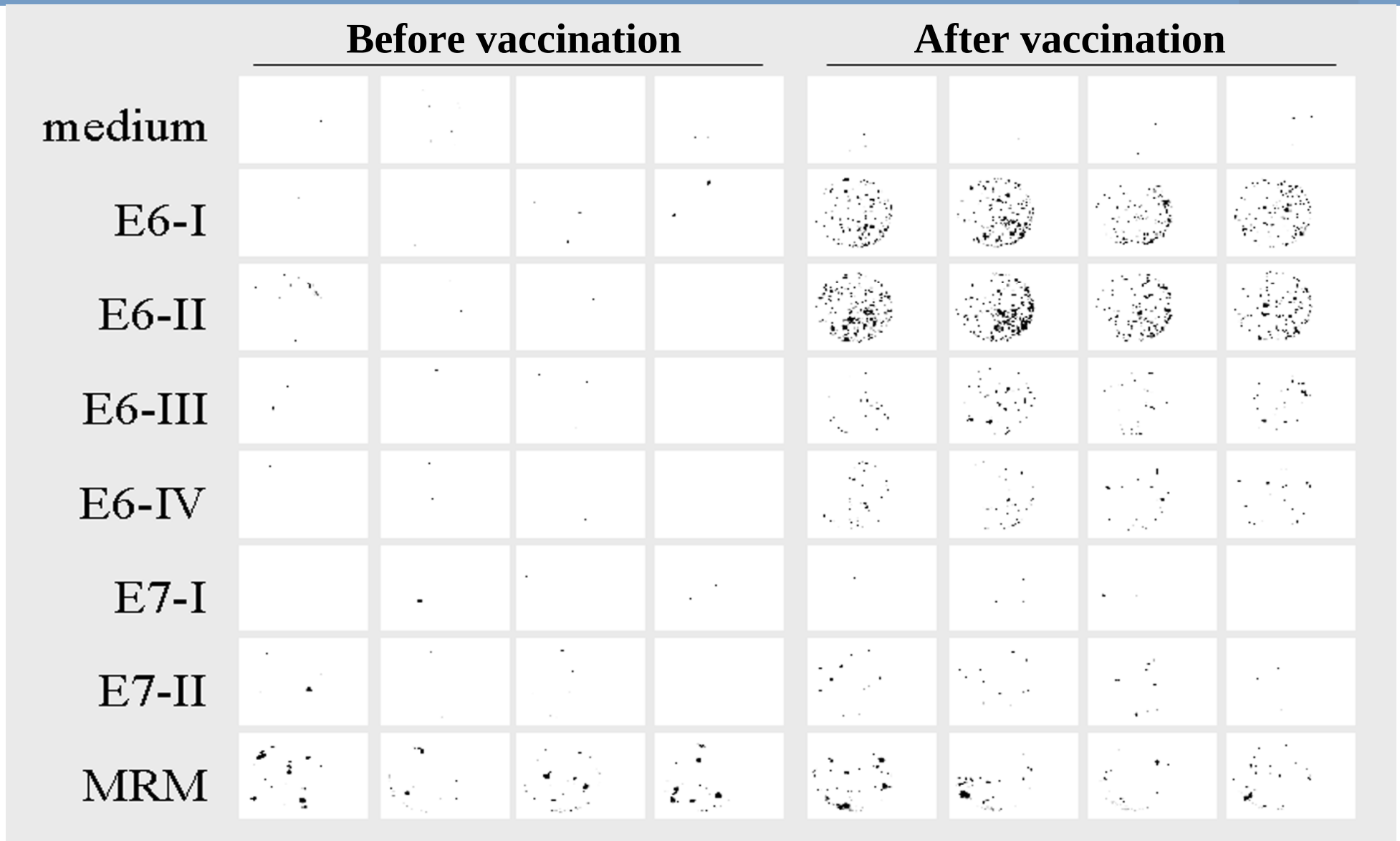


Bennett et al., Schoenberger et al., Nature, 1998

Clinical grade HPV16 therapeutic vaccine consists of synthetic overlapping long peptides comprising all potential CTL and Th epitopes.

Phase I, end stage cervical cancer

Interferon γ Elispot assay



Kenter, Clin Cancer Res, 2008

The NEW ENGLAND JOURNAL of MEDICINE

Volume 361:1838-1847 November 5, 2009 Number 19

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Vaccination against HPV-16 Oncoproteins for Vulvar Intraepithelial Neoplasia

Gemma G. Kenter, M.D., Ph.D., Marij J.P. Welters, Ph.D., A. Rob P.M. Valentijn, Ph.D., Margriet J.G. Lowik, Dorien M.A. Berends-van der Meer, Annelies P.G. Vloon, Farah Essahsah, Lorraine M. Fathers, Rienk Offringa, Ph.D., Jan Wouter Drijfhout, Ph.D., Amon R. Wafelman, Ph.D., Jaap Oostendorp, Ph.D., Gert Jan Fleuren, M.D., Ph.D., Sjoerd H. van der Burg, Ph.D., and Cornelis J.M. Melief, M.D., Ph.D.

Vaccination of 20 HPV16+ VIN3 patients with HPV16 SLP vaccine

Kenter et al. NEJM, 2009

HPV16-induced premalignant lesion of vulva

Non-specific symptoms: pain, itching, burning

Diagnosis: vulvoscopy, biopsies

Non-treated: can progress to cancer

Therapy: surgery, laser vaporization (mutilating)

Chronic disease: recurrence following standard treatment

Chronic disease: Only 1.3% resolves spontaneously

Trial Design, Phase II, HPV16+ Vulvar Intraepithelial Neoplasia (VIN III)

Endpoints

Immunology

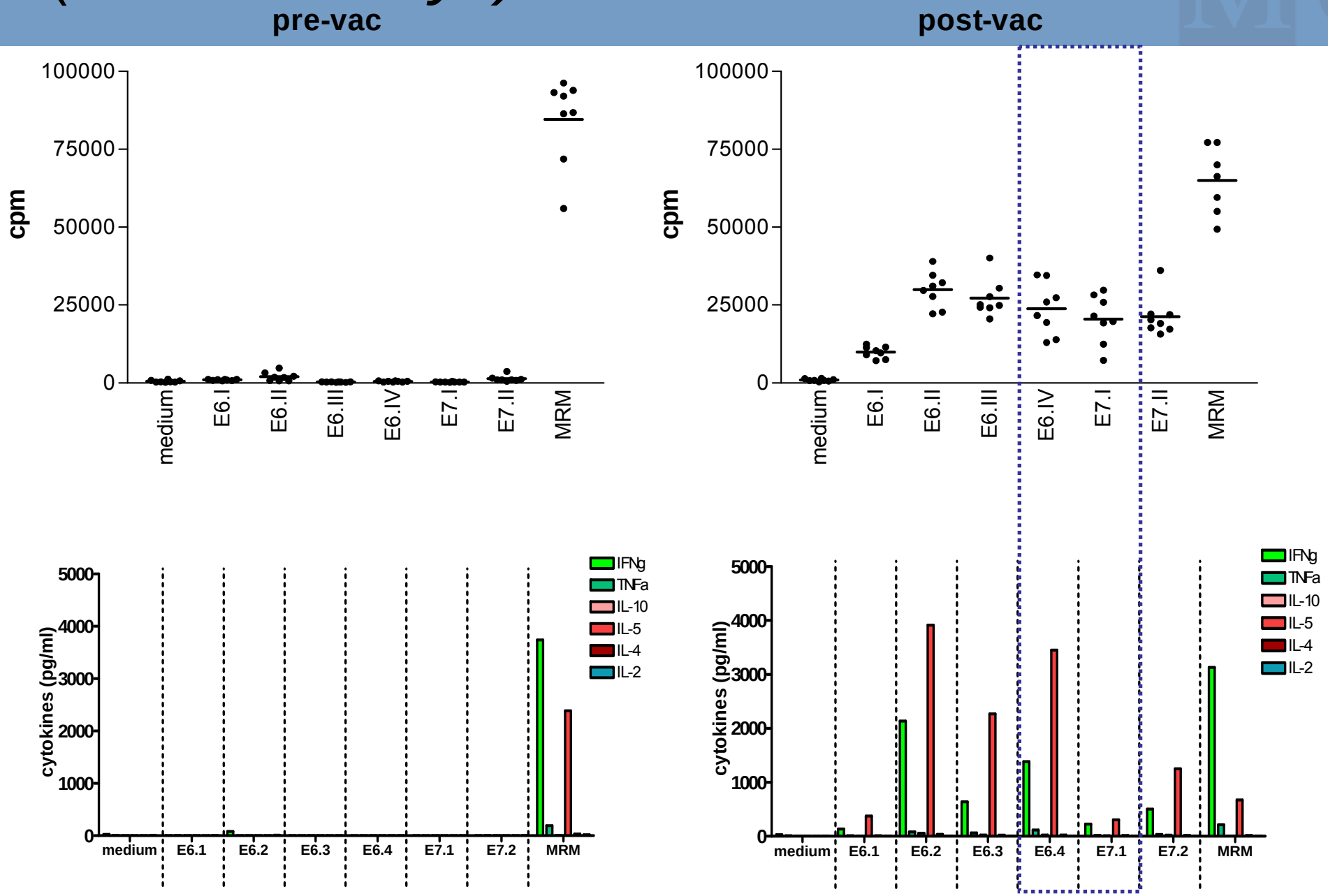
- Proliferation assay
- IFNγ ELISPOT
- Cytokine analysis (CBA, ELISA)
- CD4/CD8 analyses (ICS)

On PBMC and Biopsies (VIN lesion, vaccination site)

Clinical responses

- Symptoms
- Change in lesion size
- Change in histology
- Change in HPV detection

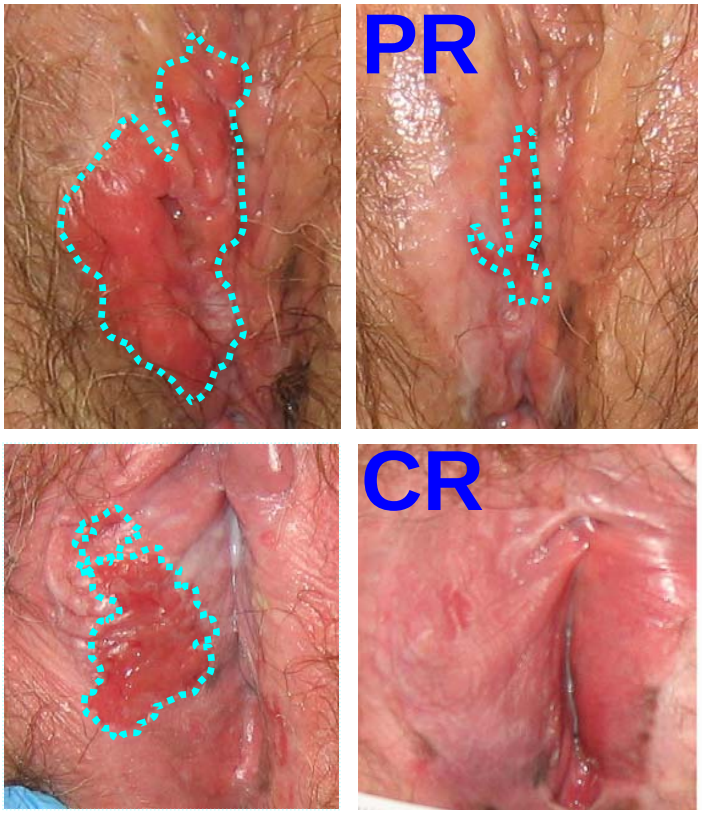
Lymphocyte Proliferation Test (*ex-vivo 6 days)*

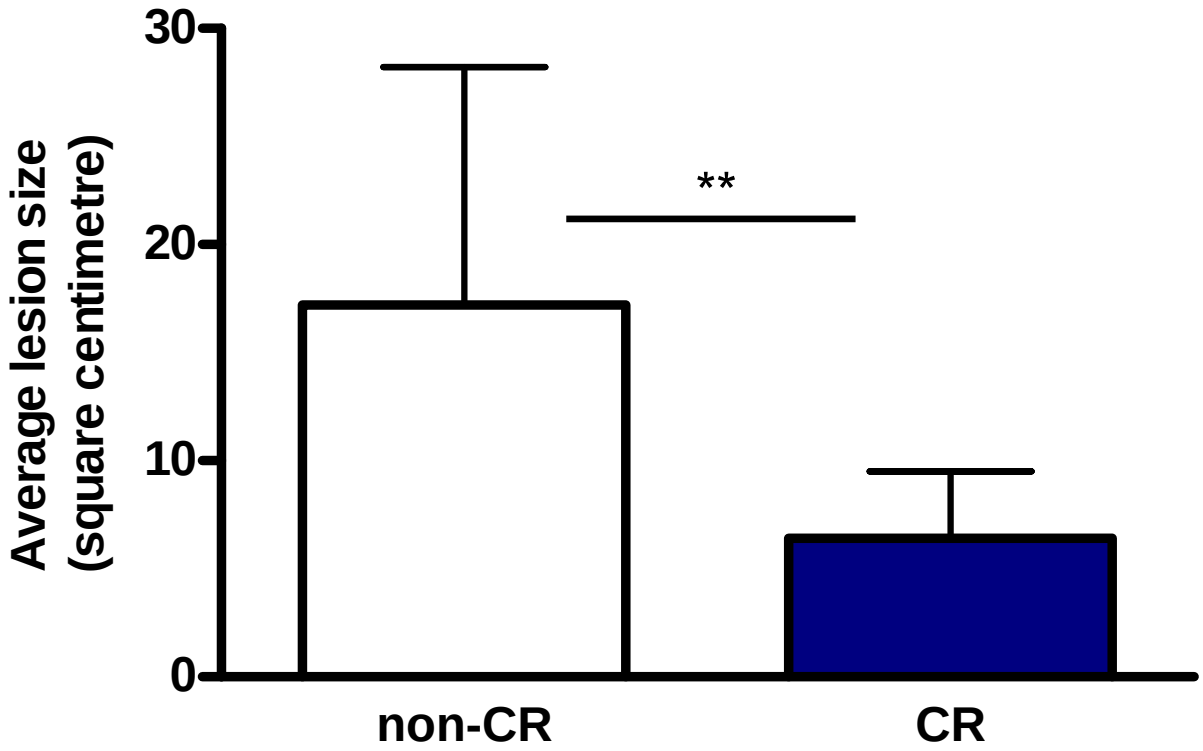


HPV16-SLP vaccination in VIN3
Clinical results at 24 months

Kenter et al., New Engl. J Med. 2009

Pre vax **Post vax**



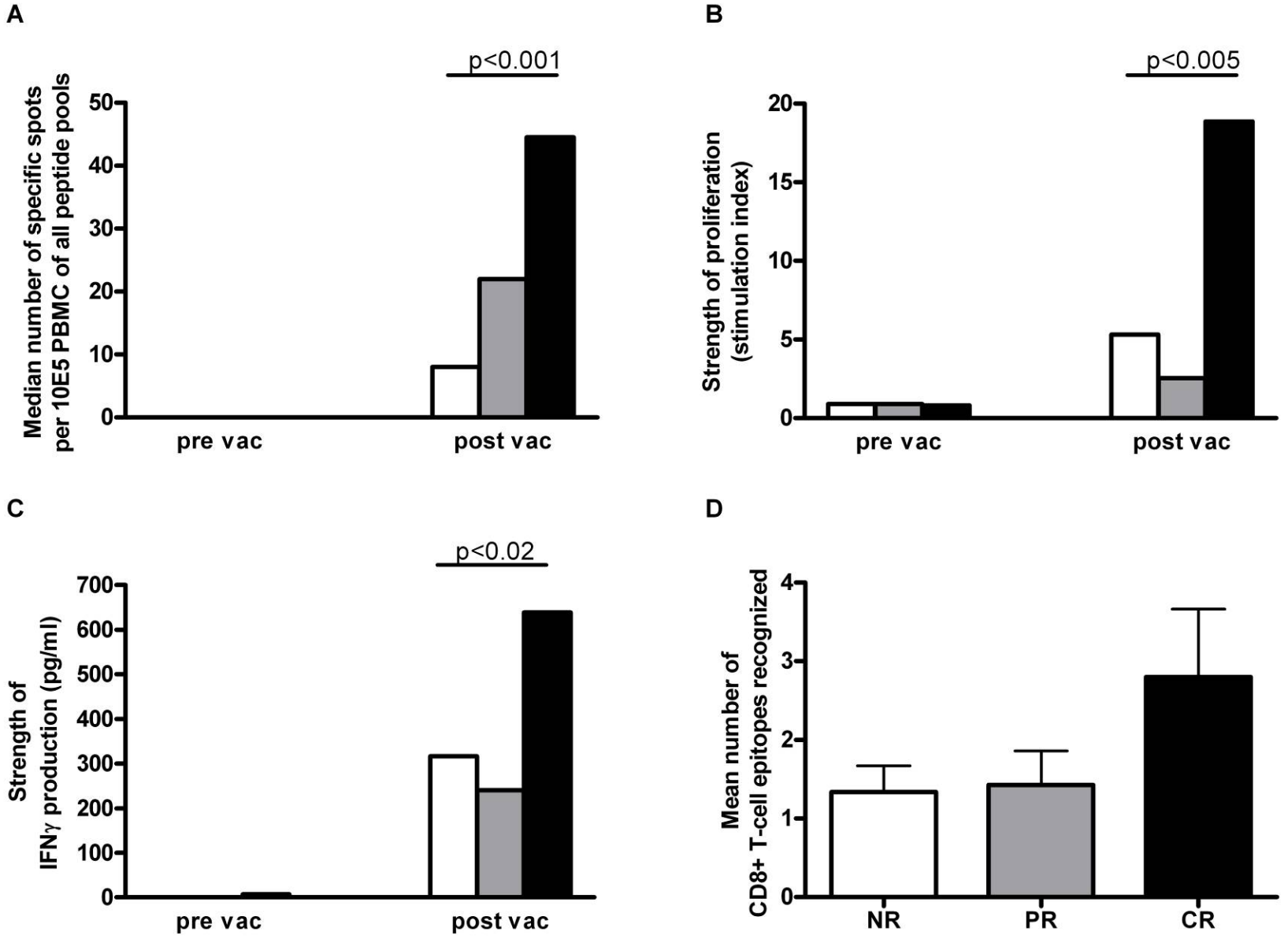


10 NR/PR,

9/9 CR

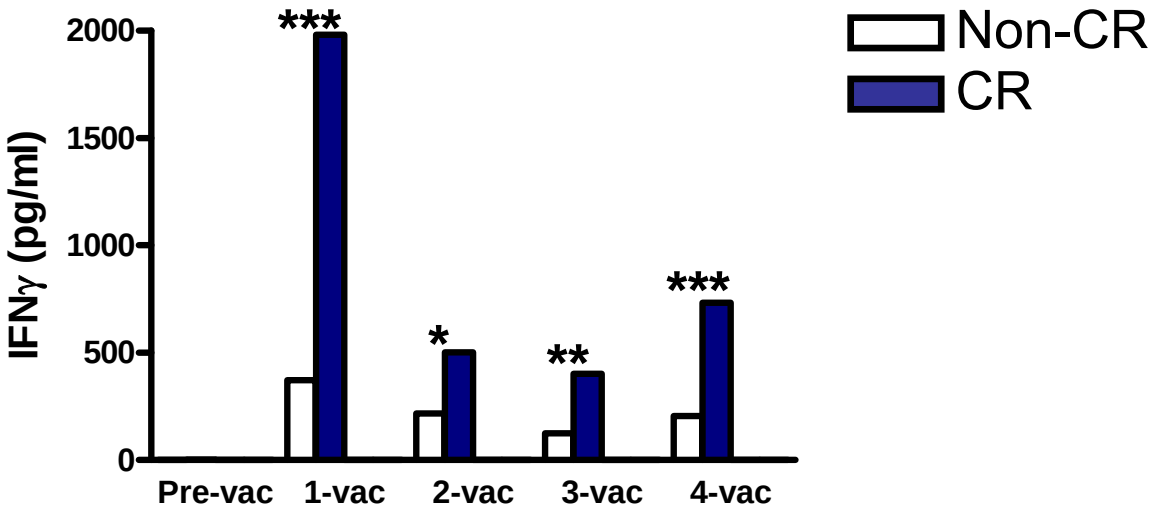
T-cell response after SLP® vaccination of VIN3 patients correlates with clinical outcome

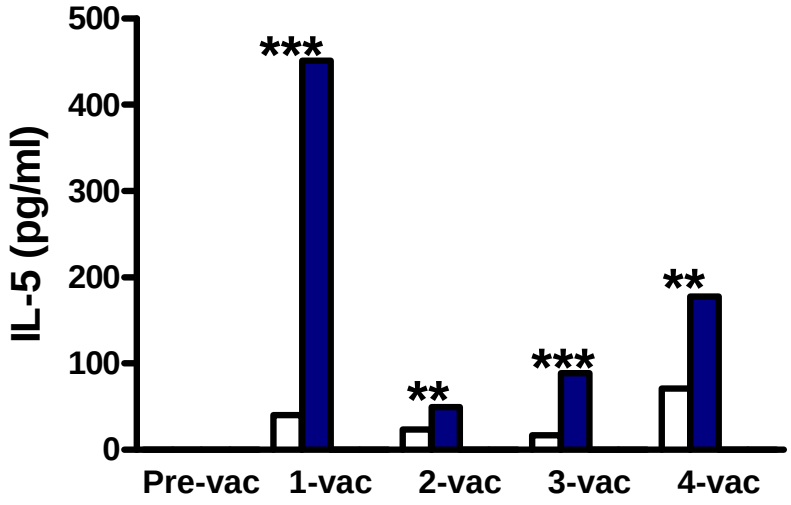
Kenter et al, New England J. of Med, 2009



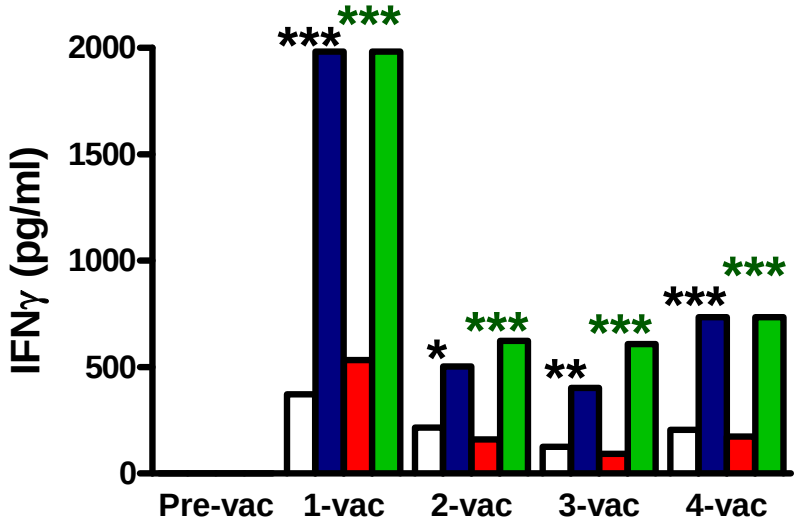
Peak of cytokine production after the first vaccination

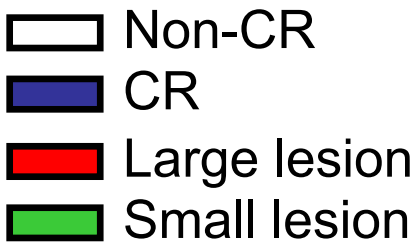
Welters et al. PNAS 2010.

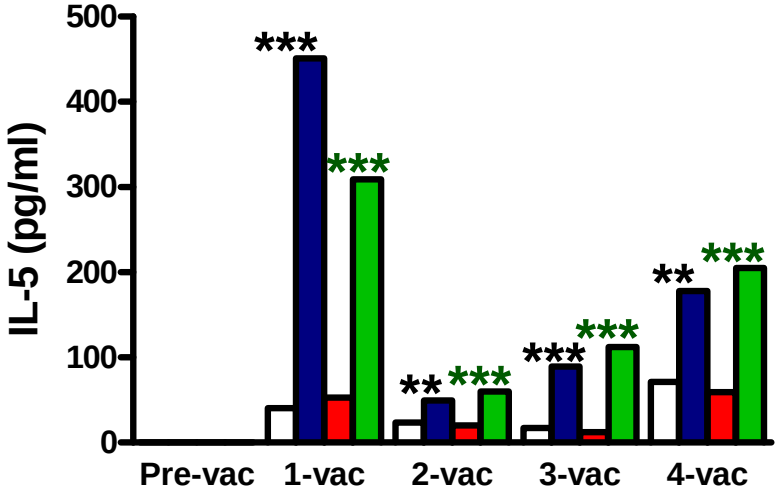




Is the size of lesion of influence on vaccine-induced immunity? Welters et al. PNAS 2010.





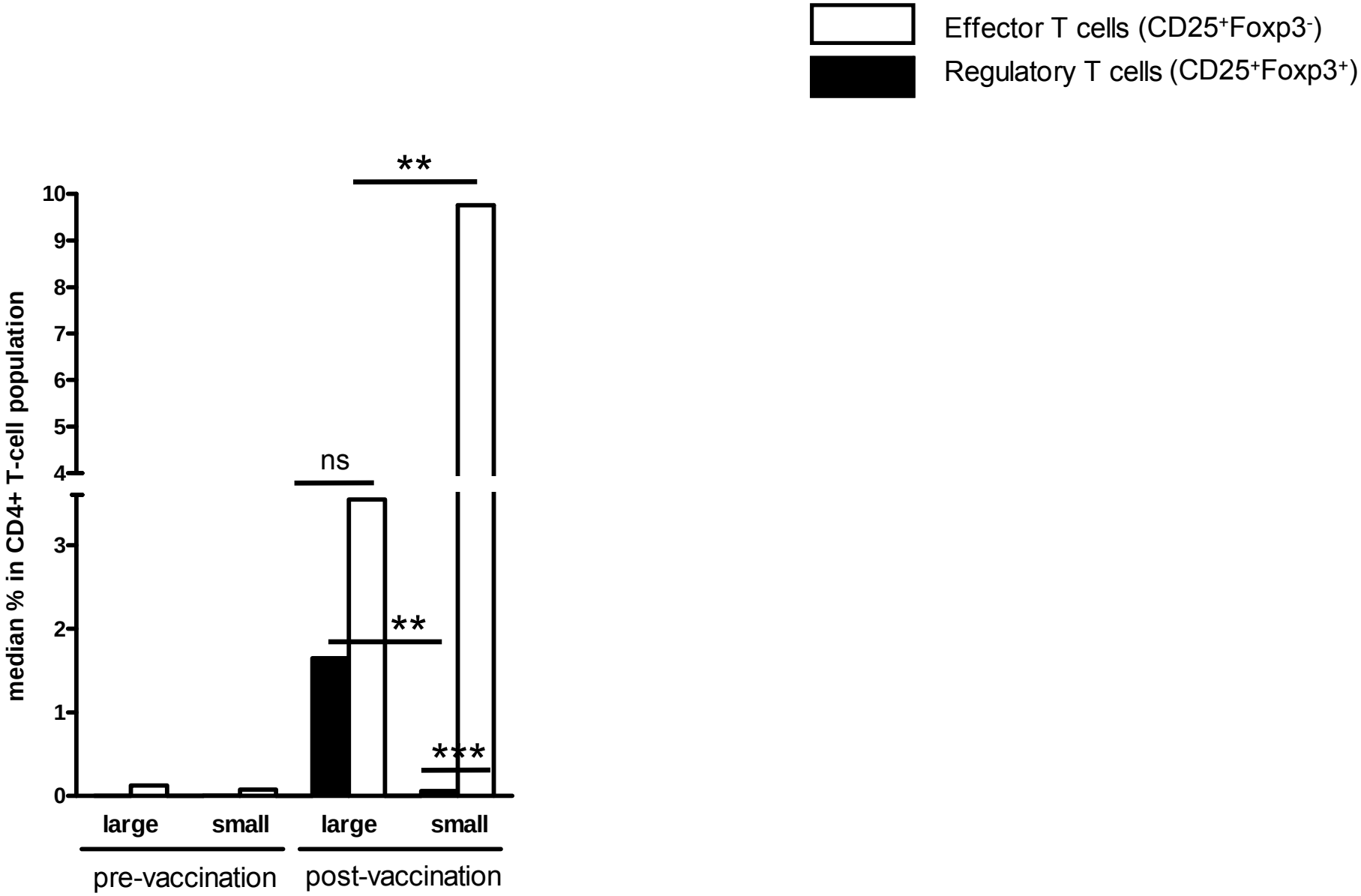


IFNγ / IL-10

Sample	Large	Small	P-value
Pre-vac	0	0	0.52
1-vac	16.53	21.80	0.44
2-vac	9.24	23.53	**0.007**
3-vac	6.04	19.90	**0.009**
4-vac	13.34	28.25	**0.001**

Tregs induced/boosted by vaccination only in patients with large VIN3 lesions

Welters et al. PNAS 2010.



Conclusions (1)
HPV16-SLP vaccine in VIN3 patients

Kenter et al. New Engl. J. Med. 361: 1838-1847, 2009

HPV16-SLP vaccine induces :

- ✓ HPV16 specific T-cell proliferation in 20/20 VIN III patients.
- ✓ HPV16 specific IFNγ-producing CD4+ T-cells in 19/20 patients.
- ✓ IFNγ-producing CD8+ T-cells in 19/20 patients.
- ✓ Migration of HPV16 spec.T-cells to vaccination site in 7/18 patients.
- ✓ Complete clearance of the VIN grade III lesion in:

  5/20 patients, 3 months after the last vaccination,

  9/19 patients, 12 months after the last vaccination.

- ✓ Partial clearance of VIN grade III lesion in 5/20 patients, 12 months after the last vaccination. Overall clinical benefit in 14 of 20 patients
- ✓ Complete clearance of HPV 16 infection in 4/20 patients, 3 months after the last vaccination.

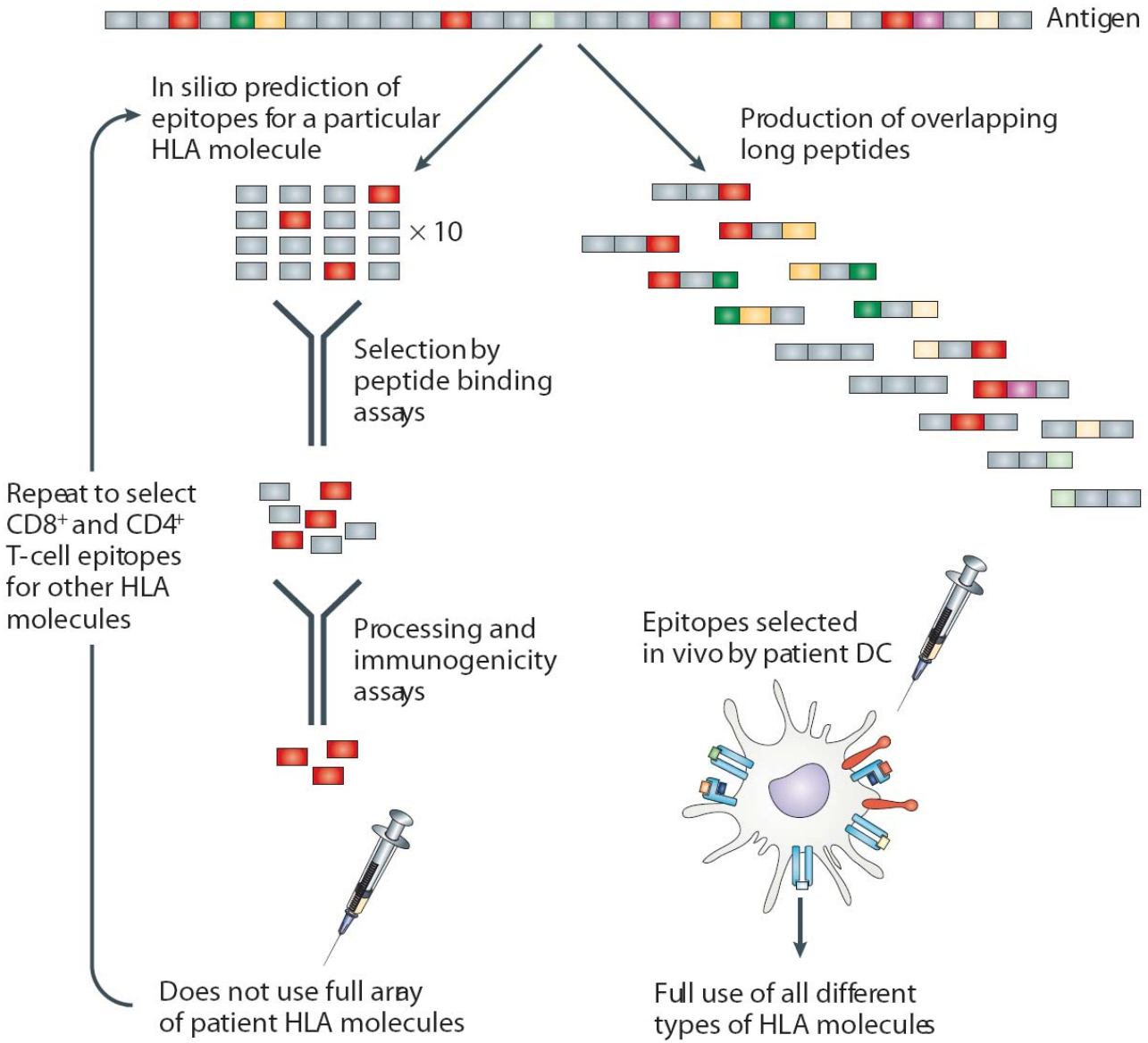
Conclusions (2)
HPV16-SLP vaccine in VIN3 patients

- Follow-up 24 months after the last vaccination:
- All 9 patients with a CR still have a CR
- 4/10 patients with NR or PR have developed micro-invasive vulvar carcinoma. All of these patients had VIN3 lesions of more than 10 years duration

Conclusions (3)
HPV16-SLP vaccine in VIN3 patients

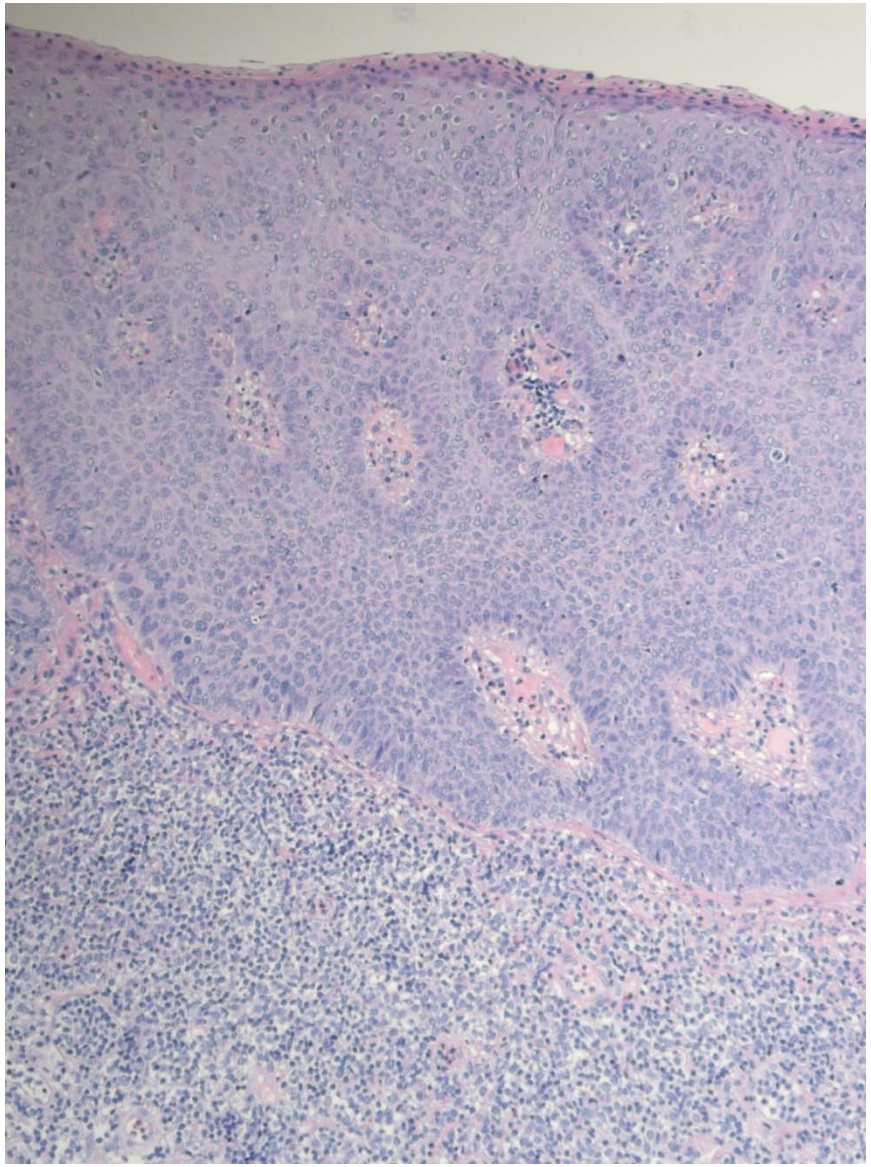
- HPV16 SLP vaccine is able to restore the $CD4^+$ and $CD8^+$ T-cell response to HPV16 E6 and E7 in VIN3 patients.
- HPV16 SLP vaccine is able to induce clinical responses in 79% of vaccinated subjects (32% PR, 47% CR).
- The strength of the HPV16 SLP vaccine-induced $CD4^+$ T-cell response as measured by a combination of proliferation and IFNγ production (LST, CBA, ELISPOT) correlates with clinical responses.
- The success of immunotherapy is determined by the ratio of vaccine-prompted effector T cells over CD4+CD25+ Foxp3+ regulatory T cells
- Robust and durable response to synthetic long peptides is ascribed to simultaneous presentation of many class I and class II epitopes by Dendritic Cells in the absence of antigenic competition.

Synthetic Long Peptide vaccine concept

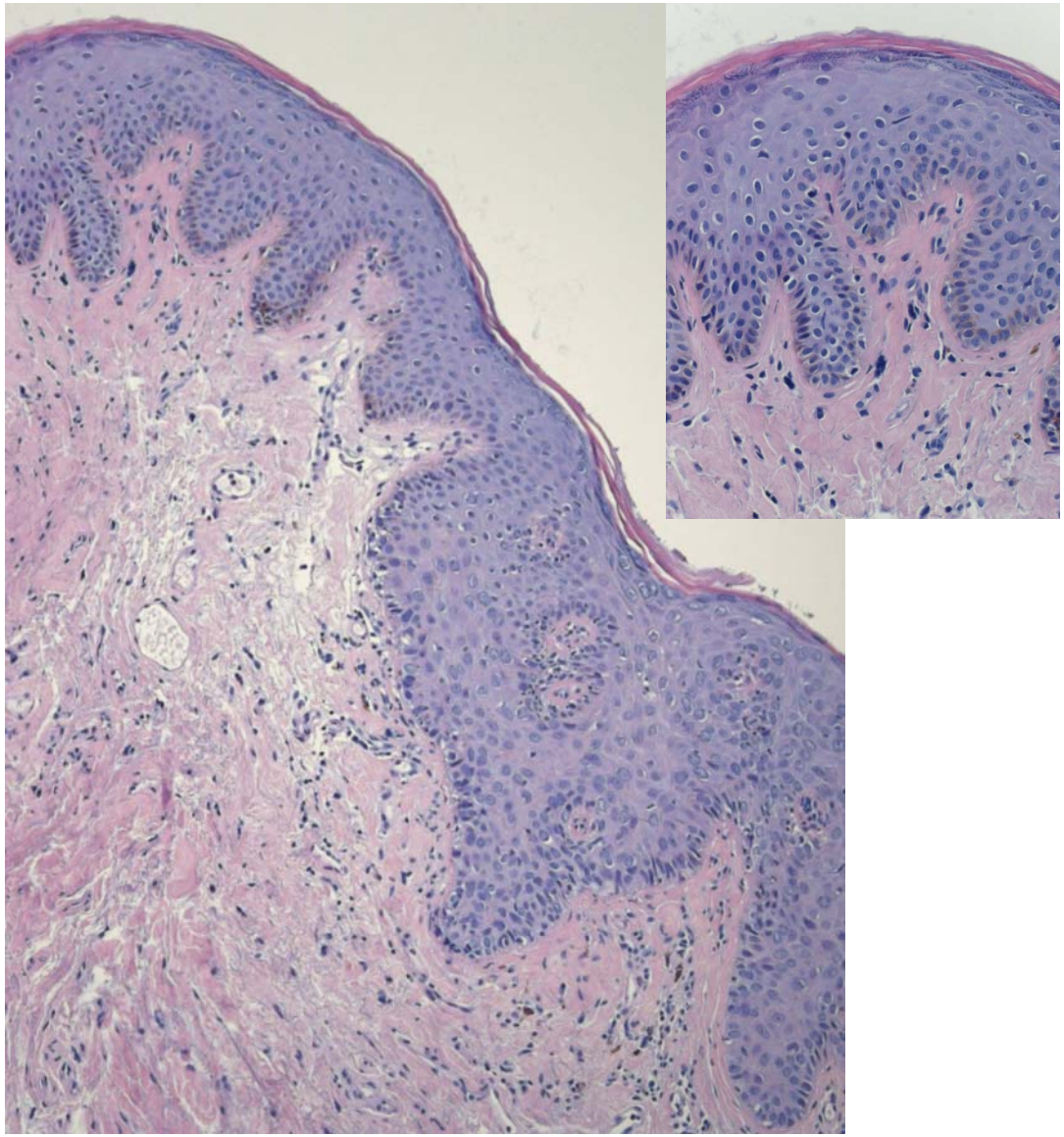


Melief & Van der Burg,Nature Reviews Cancer 2008

Histology of completely cleared VIN lesion

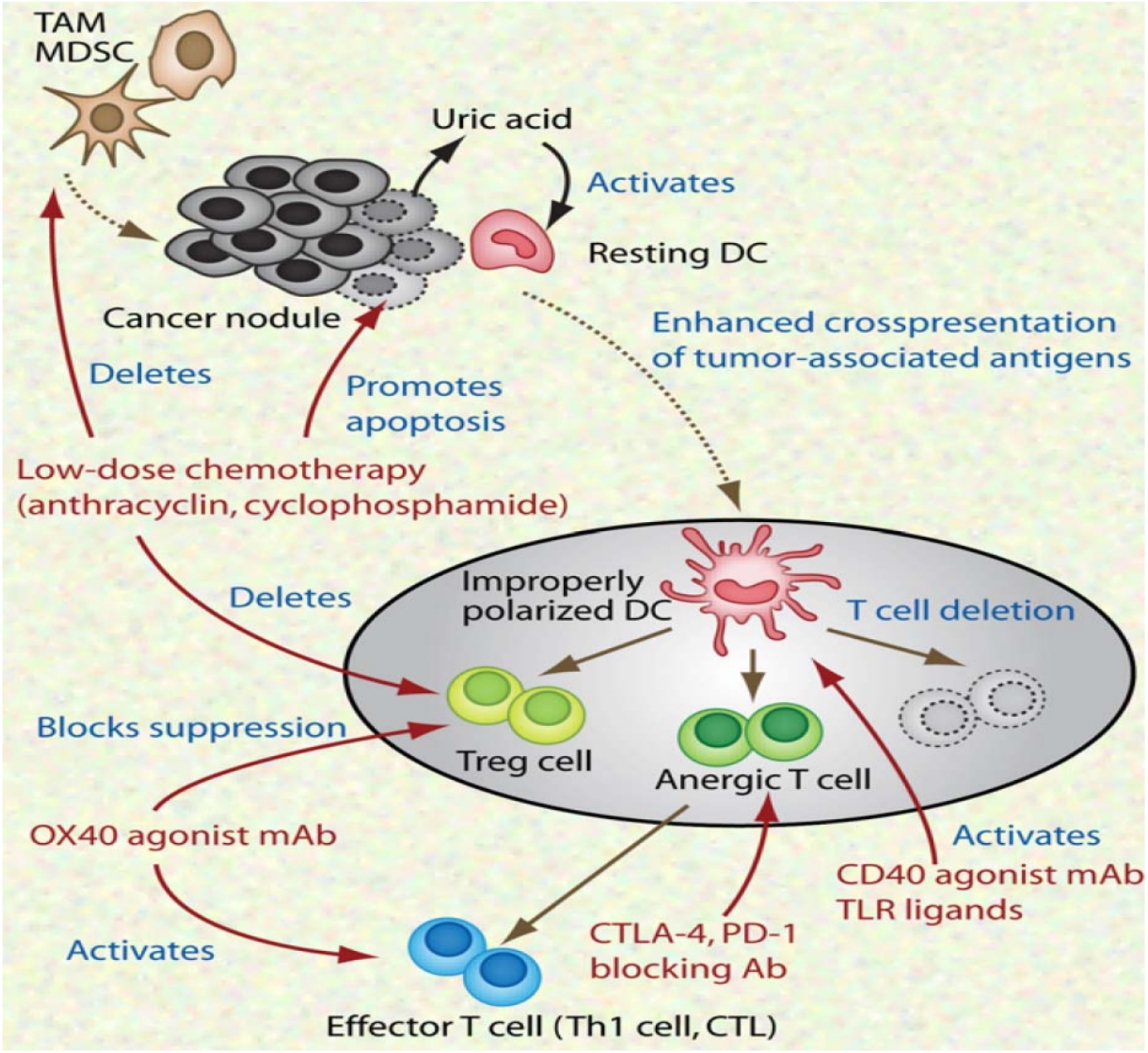


Pre-vaccination



Post-vaccination

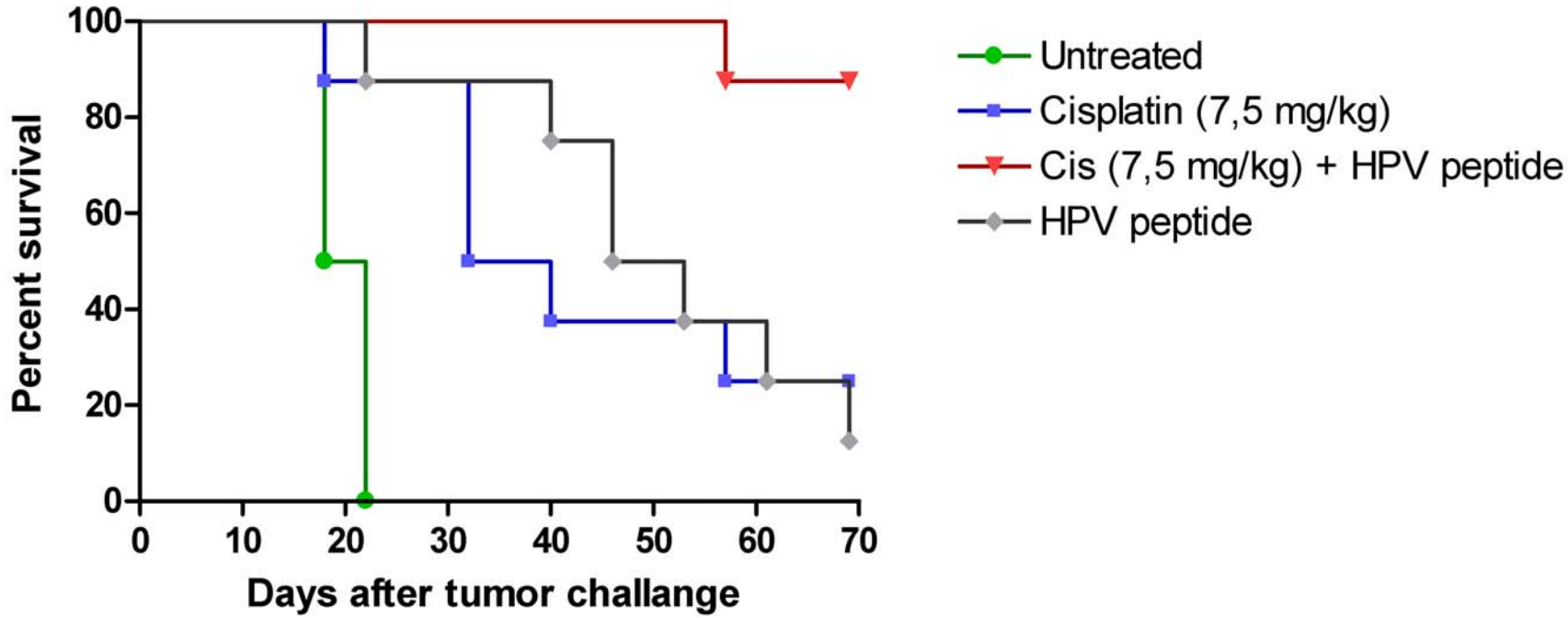
Sites of action of Immunotherapy of Cancer



Melief, Immunity, 2008

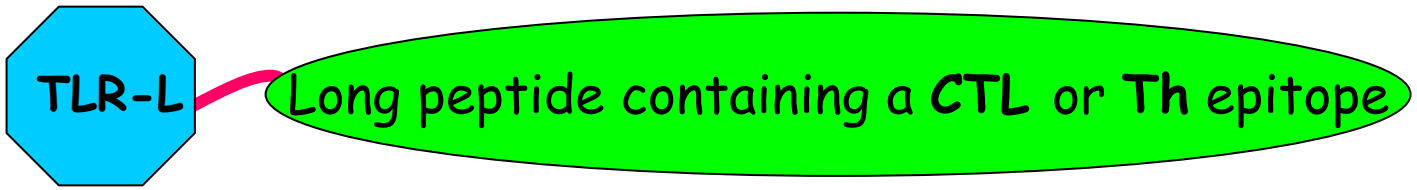
Chemo-immunotherapy of HPV16-positive established tumor TC-1 in mice

Treatment of tumor-bearing (TC-1) B6 mice with peptide vaccination in combination with cisplatin (Exp: Chemo-4)



Next generation of synthetic vaccines

Khan et al. J. Biol. Chem`2008



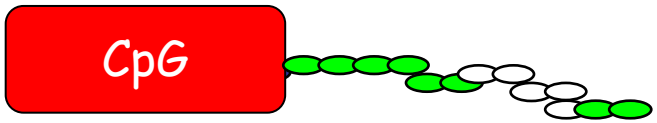
Fundamental study:

* Cell biology of TLR-L conjugates in DCs
(Uptake, routing, antigen presentation)

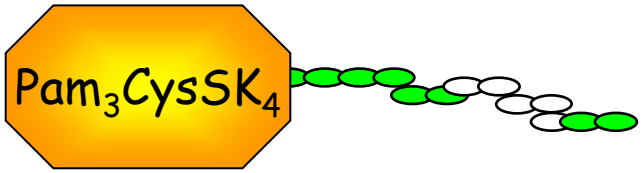
* Immunological response (T-cell induction and Tumor protection)

The constructs...

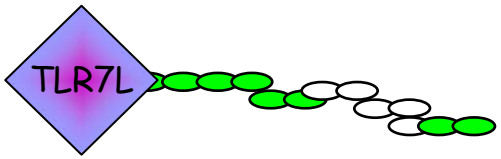
* Longer peptides containing the Ovalbumin CTL or Th epitope.
* Longer peptides containing the MuLV ENV 119-137 Th epitope.



endosomes (TLR9)



Cell surface (TLR2)

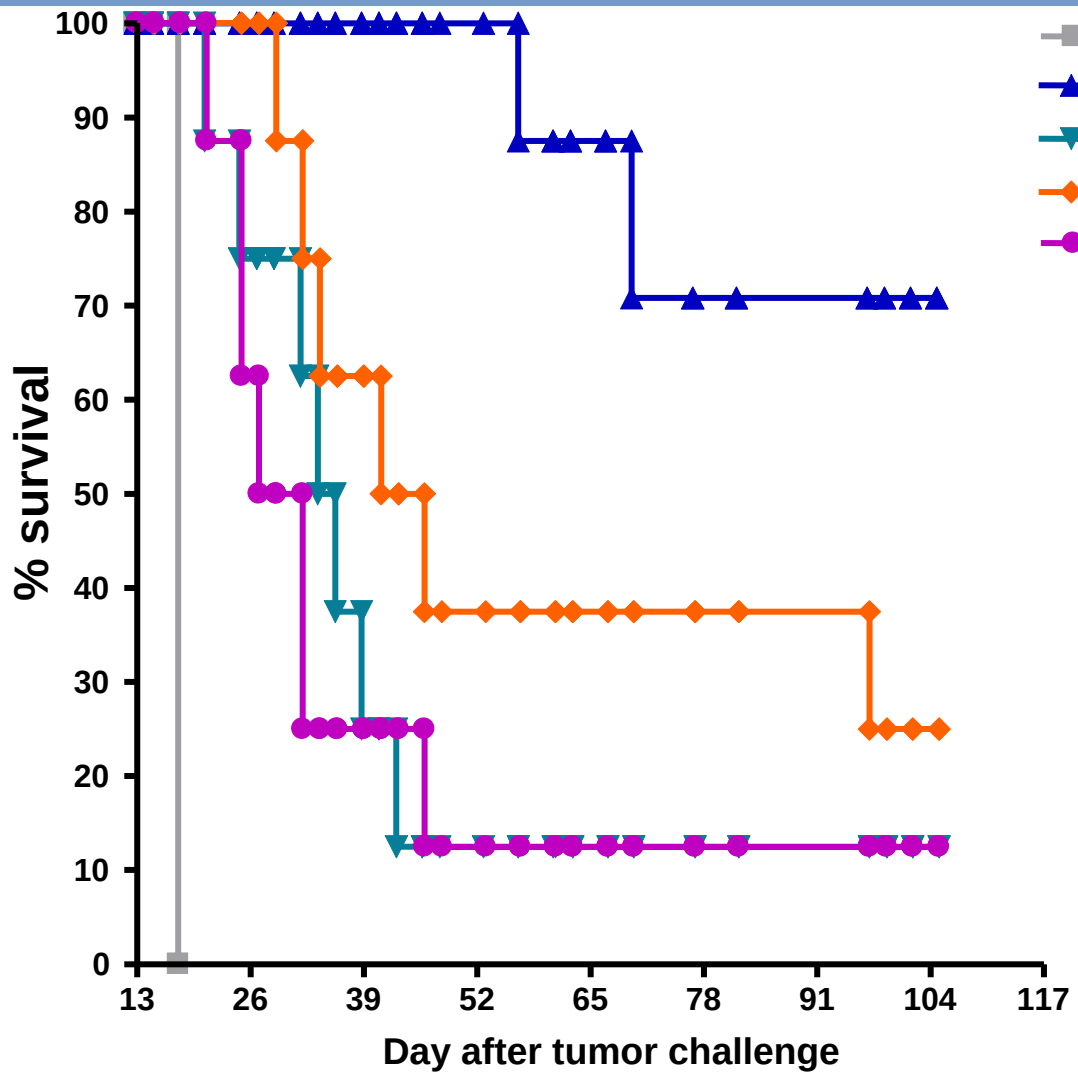


endosomes (TLR7)

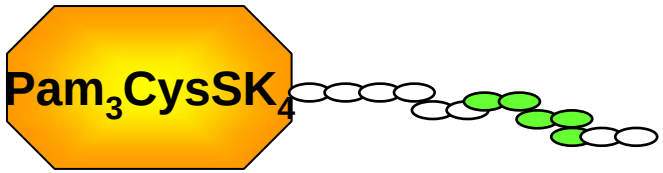
Adjuvant **Antigen**

Tumor protection by CTL and T helper vaccination with combined long peptide-TLR ligand conjugates

EG7 (OVA) tumor



naive (IFA + Pam)
Pam-conj(CTL)/Pam-conj(Th)
Pam-conj(CTL)
Pam-conj(Thelp)
Th pep+ CTL pep + Pam

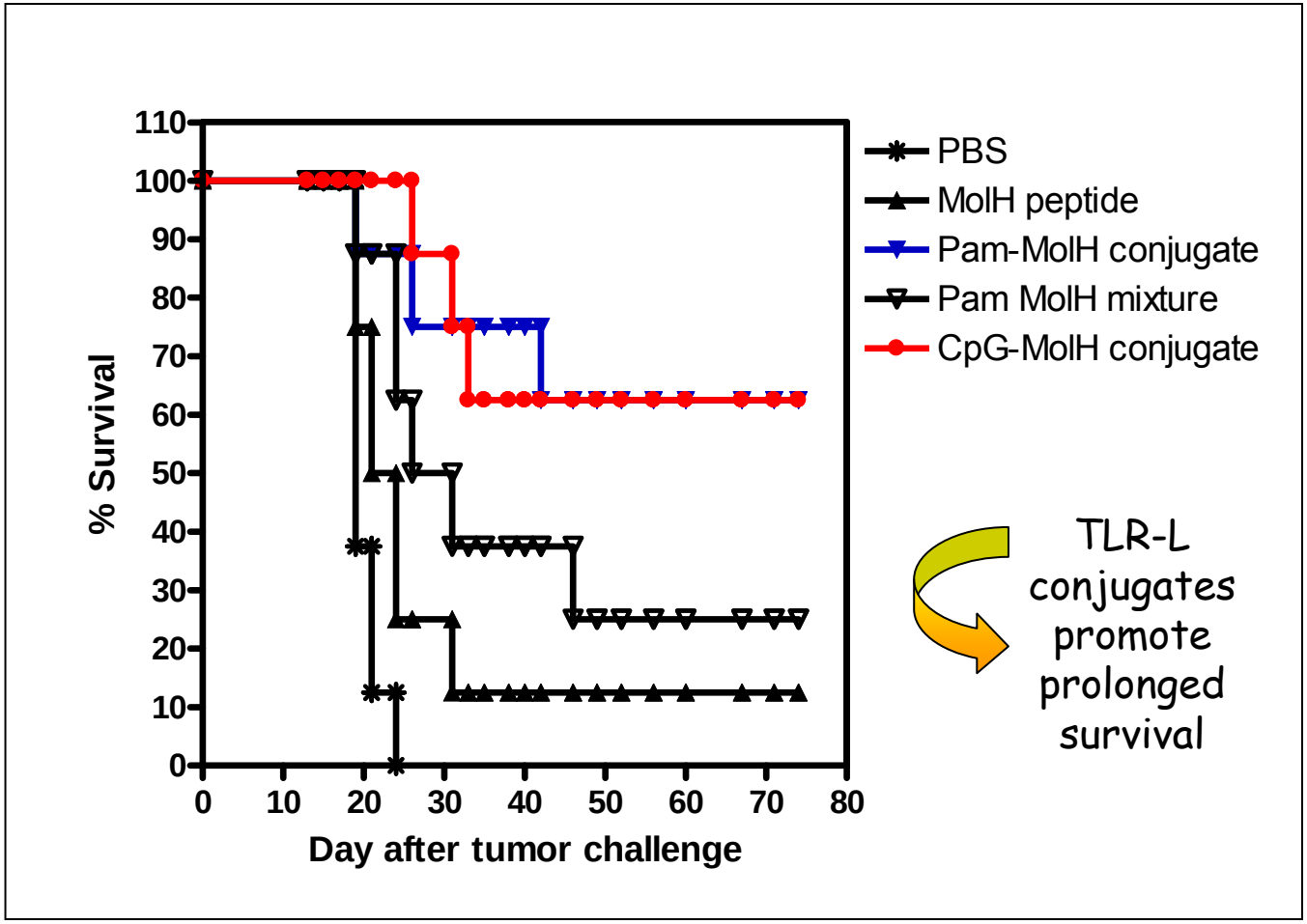


TLR2 ligand Long peptide

Tumor protection experiment

RMA MuLV Leukemia (dependent on CD4+ T-cells)

Unpublished S. Khan, C. Britten



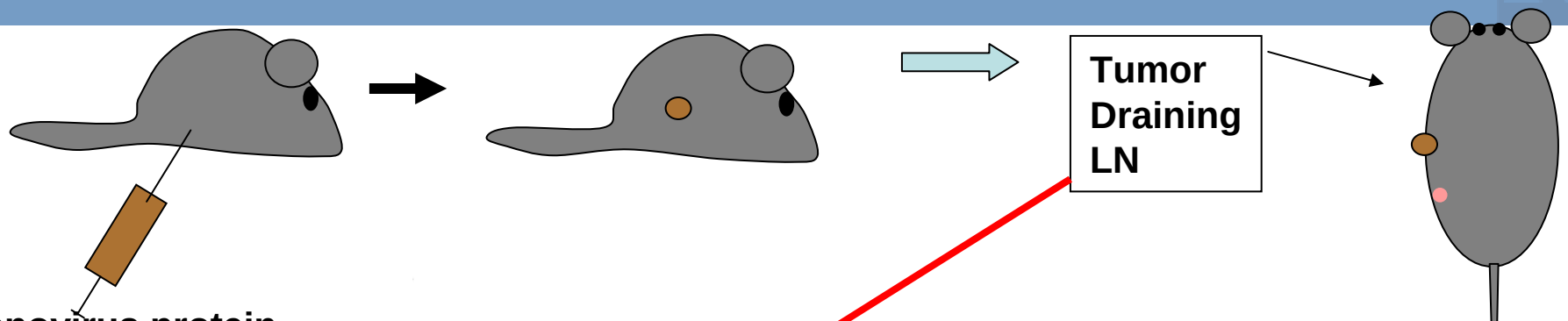
Reduction of toxicity of immunotherapy with anti-mouse CD40 agonist monoclonal antibody

Local re-programming of tumor-specific T-cells by anti-CD40 agonistic antibodies allows systemic anti-tumor immunity with low toxicity

Local treatment induces a systemic CD8+ CTL response

Marieke F. Fransen, Ramon Arens

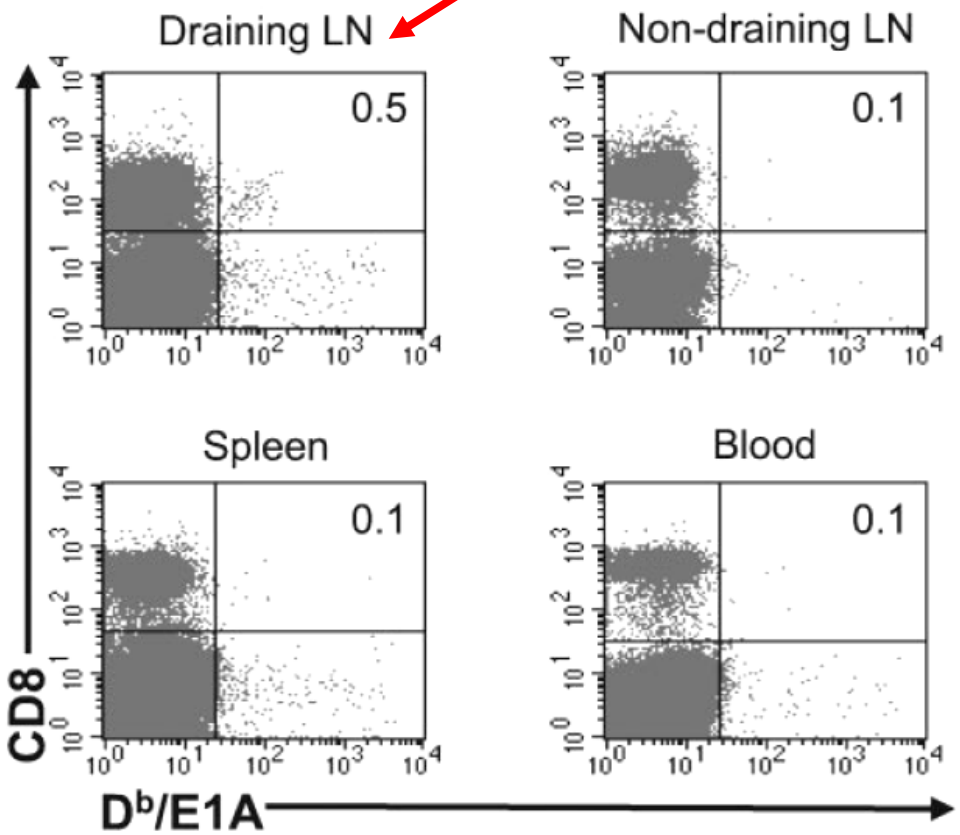
Experimental model



Tumor Draining LN

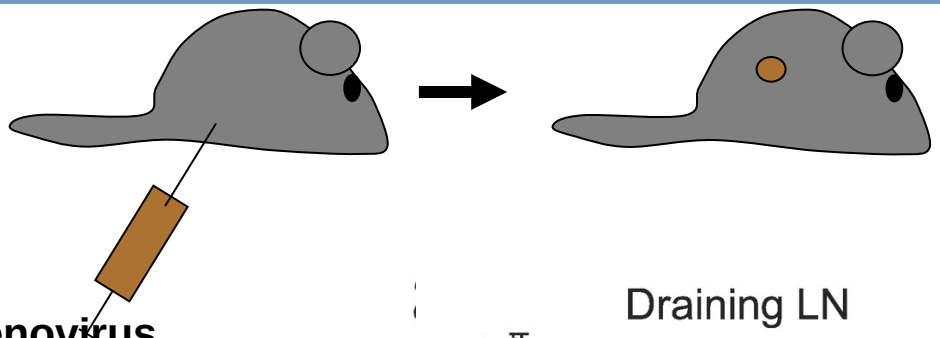
Adenovirus protein E1A-expressing -tumor cells

Endogenous response



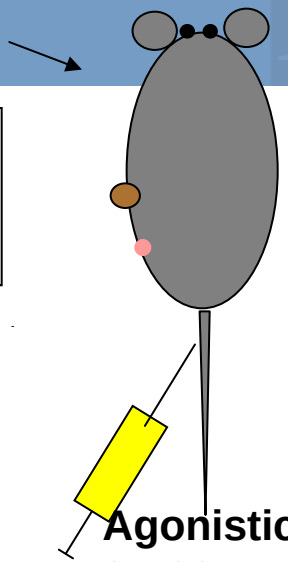
Van Mierlo et al. PNAS, 2002

Systemic anti-CD40 induces systemic anti-tumor CD8 CTL response



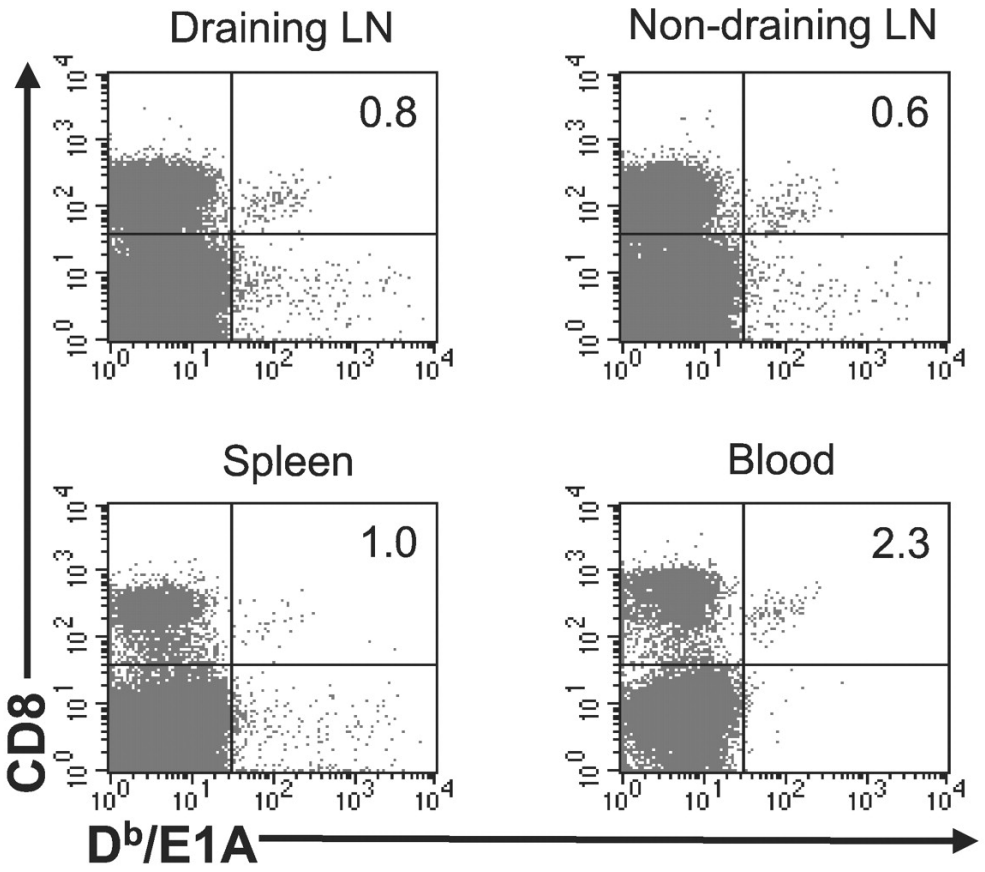
Adenovirus protein E1A-expressing tumor cells

Tumor Draining LN



Agonistic anti-CD40 3*100 µg, systemic =High dose, IV

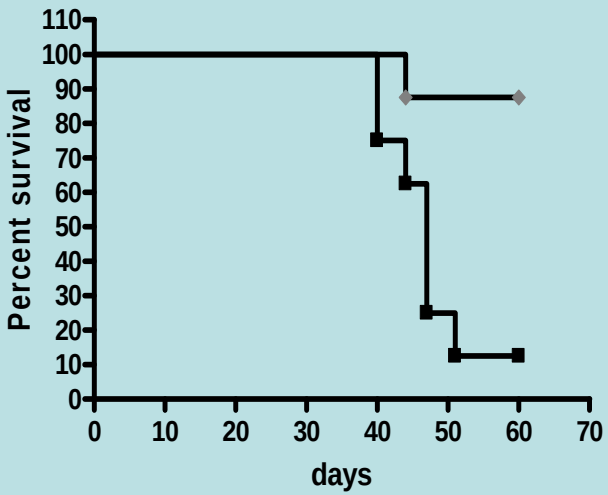
Endogenous response



Effector T-cells

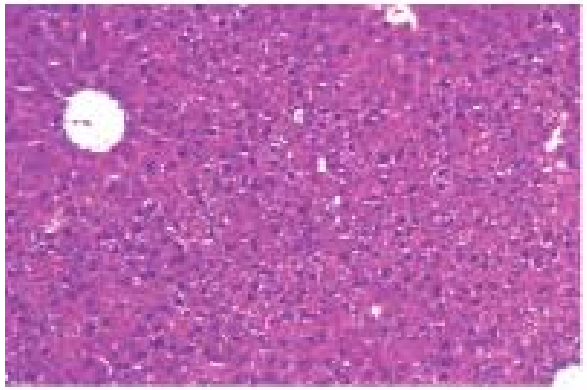
Van Mierlo et al. PNAS, 2002

Systemic anti-CD40 treatment of tumor bearing mice

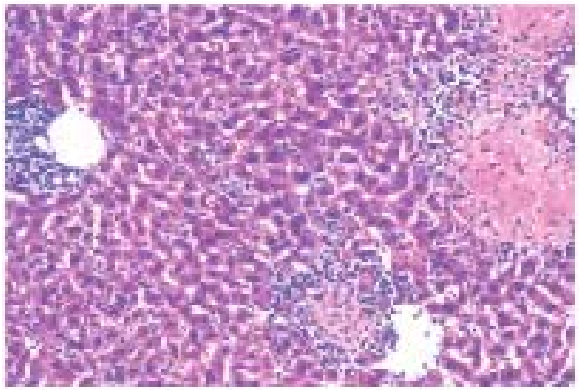


Strong anti-tumor CD8 T-cell response

Effective tumor clearing



Liver
Naïve



Liver
Anti-CD40
3*100 µg IV

Strong systemic toxicity

Splenomegaly

Hypothesis:

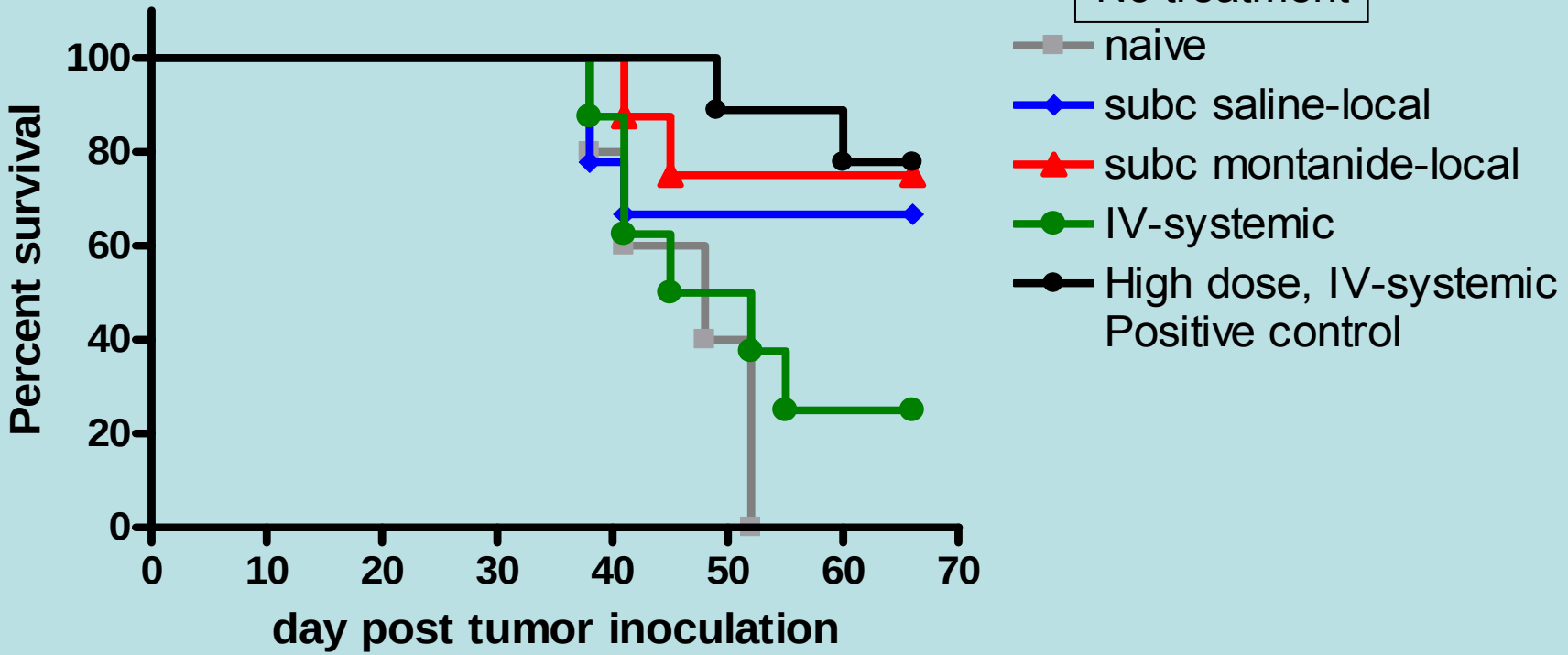
Can we activate the anti-tumor CD8 T-cells without causing systemic toxicity?

-Lower dose

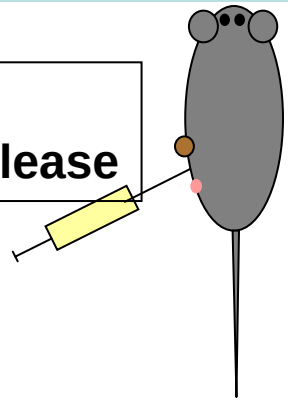
-Local injection

-Slow-release formulation

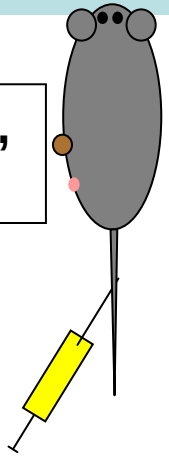
Lower dose: 30 microgram anti-CD40, different administrations



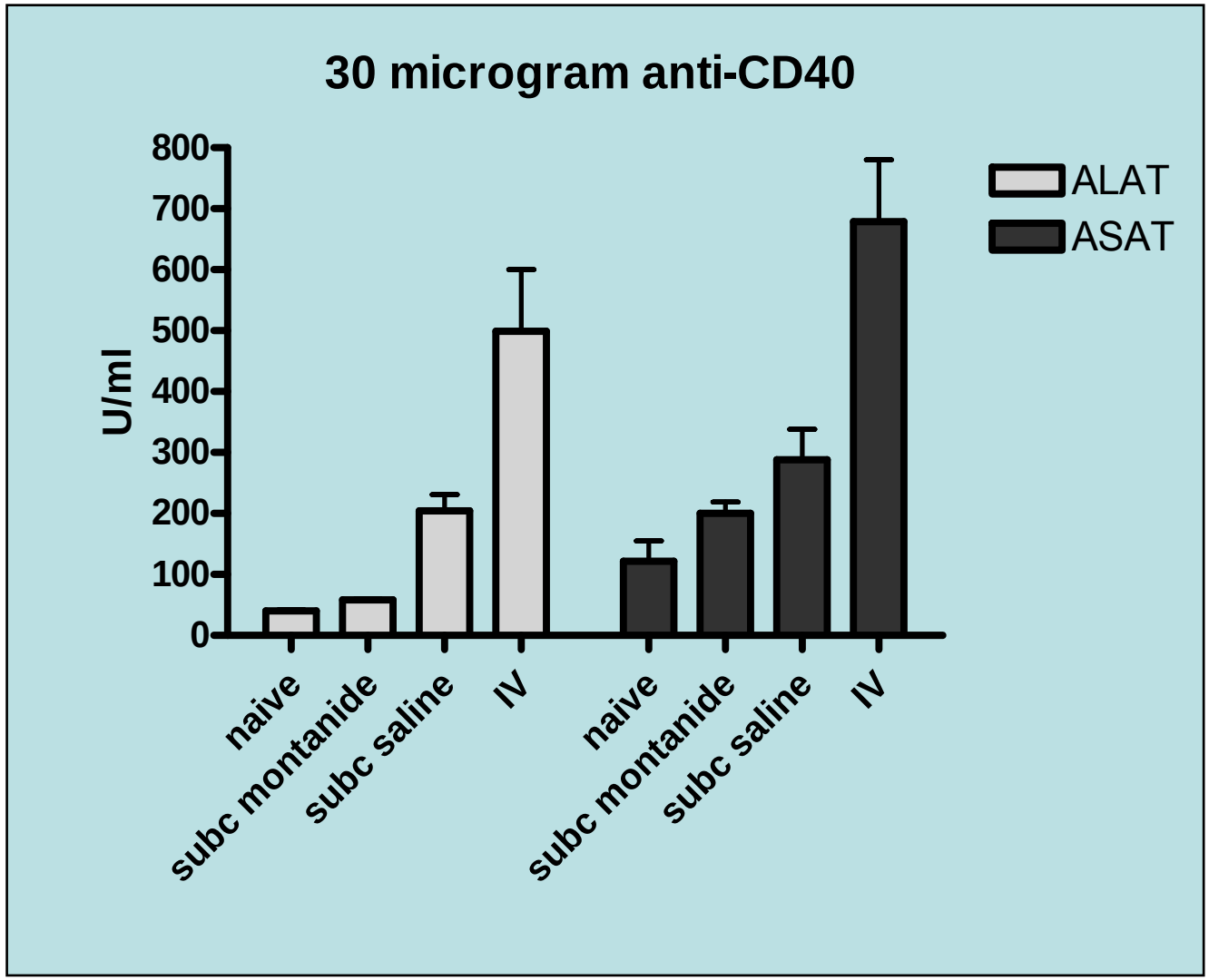
Local injection,
30 µg sc, slow release



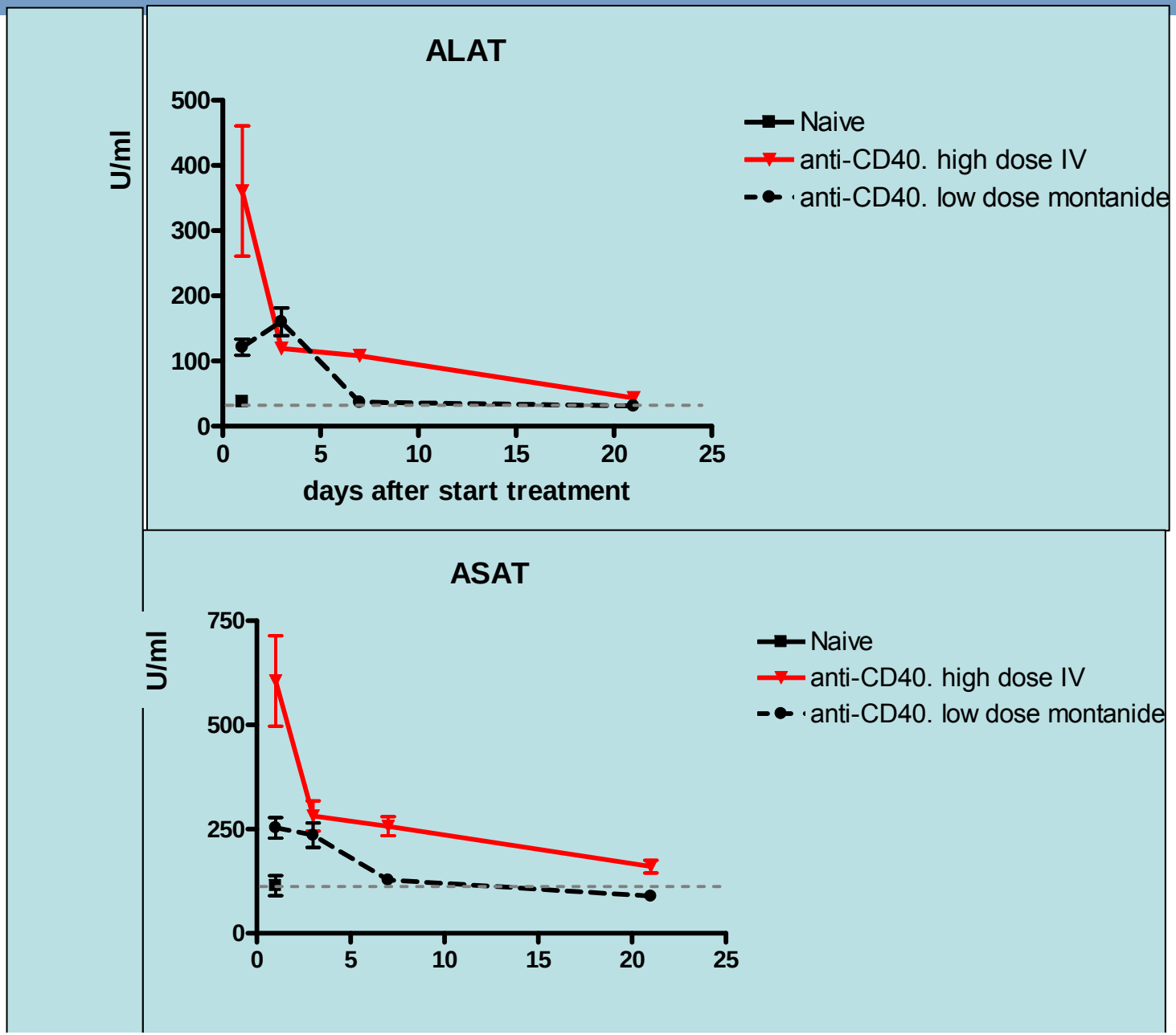
Systemic injection,
3 times 100 µg iv



Toxicity: liver enzymes 24 hours after treatment with anti-CD40 agonist antibody

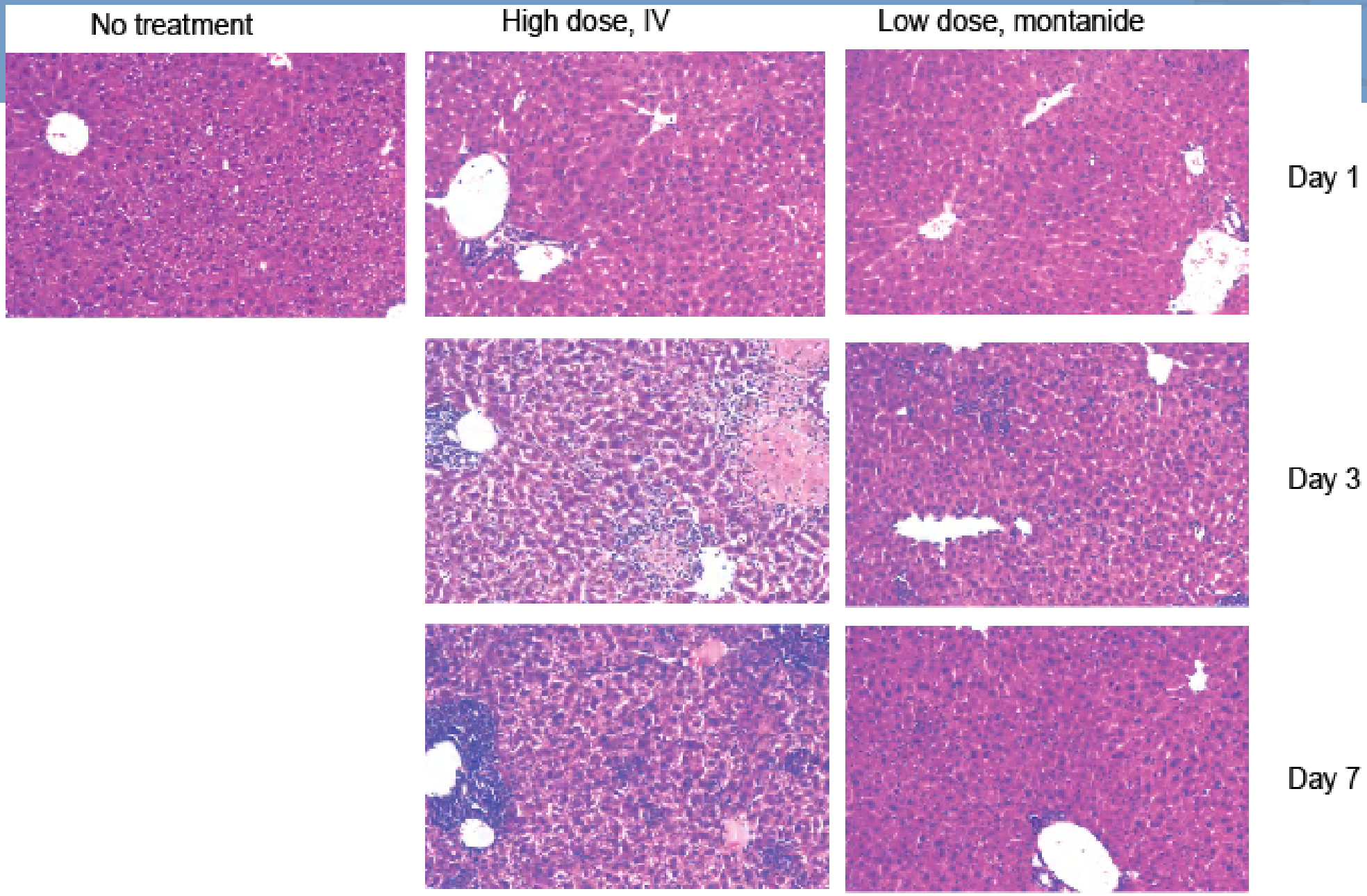


Kinetics of toxicity

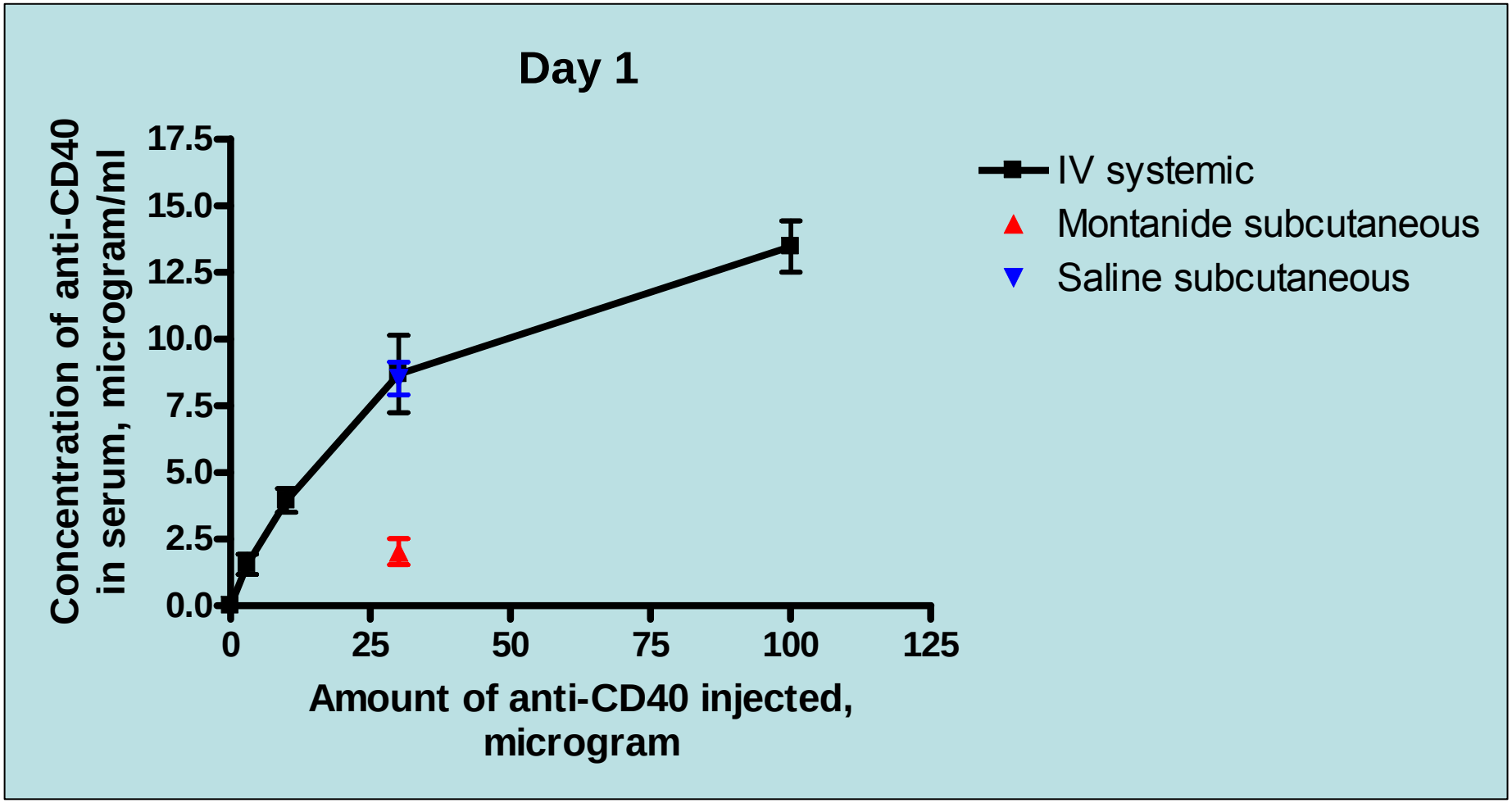


Histology

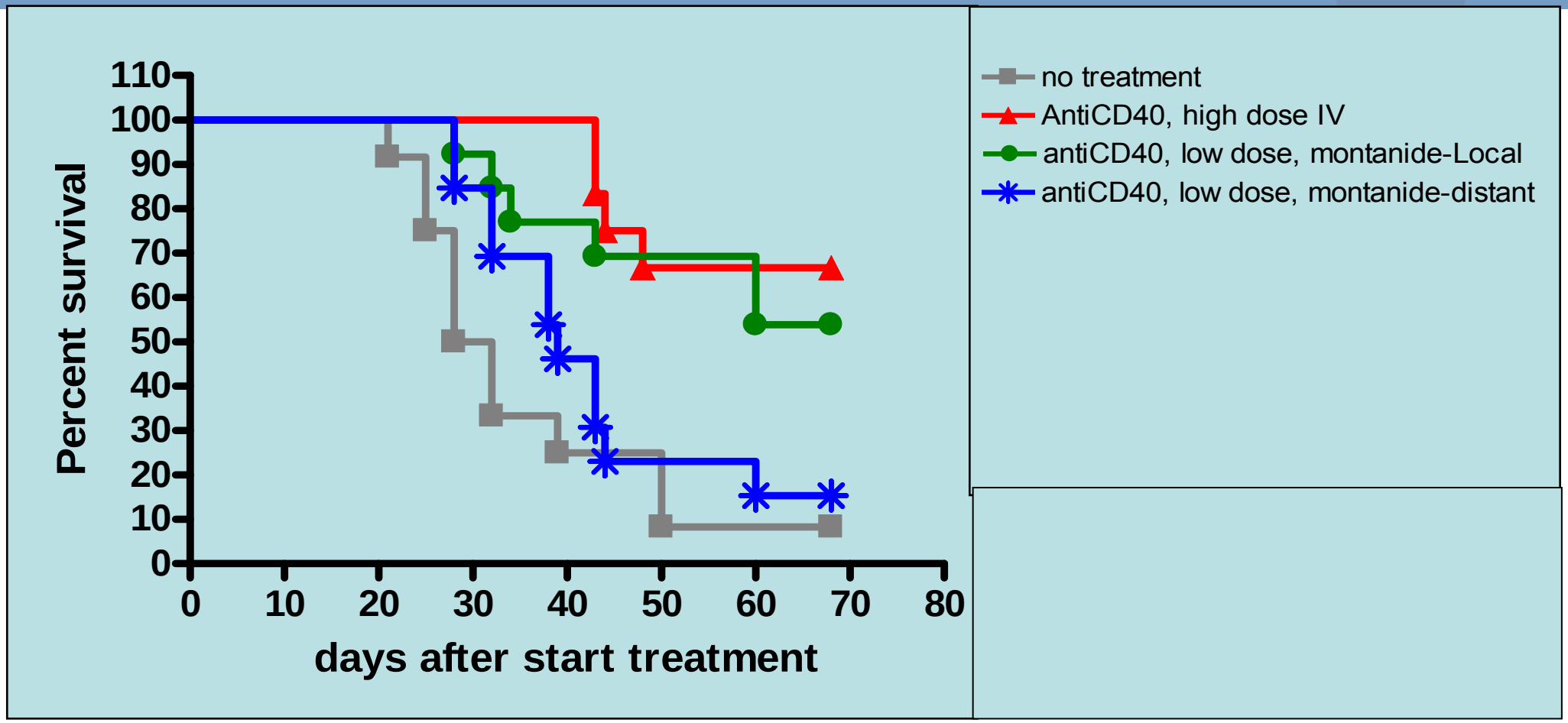
Liver



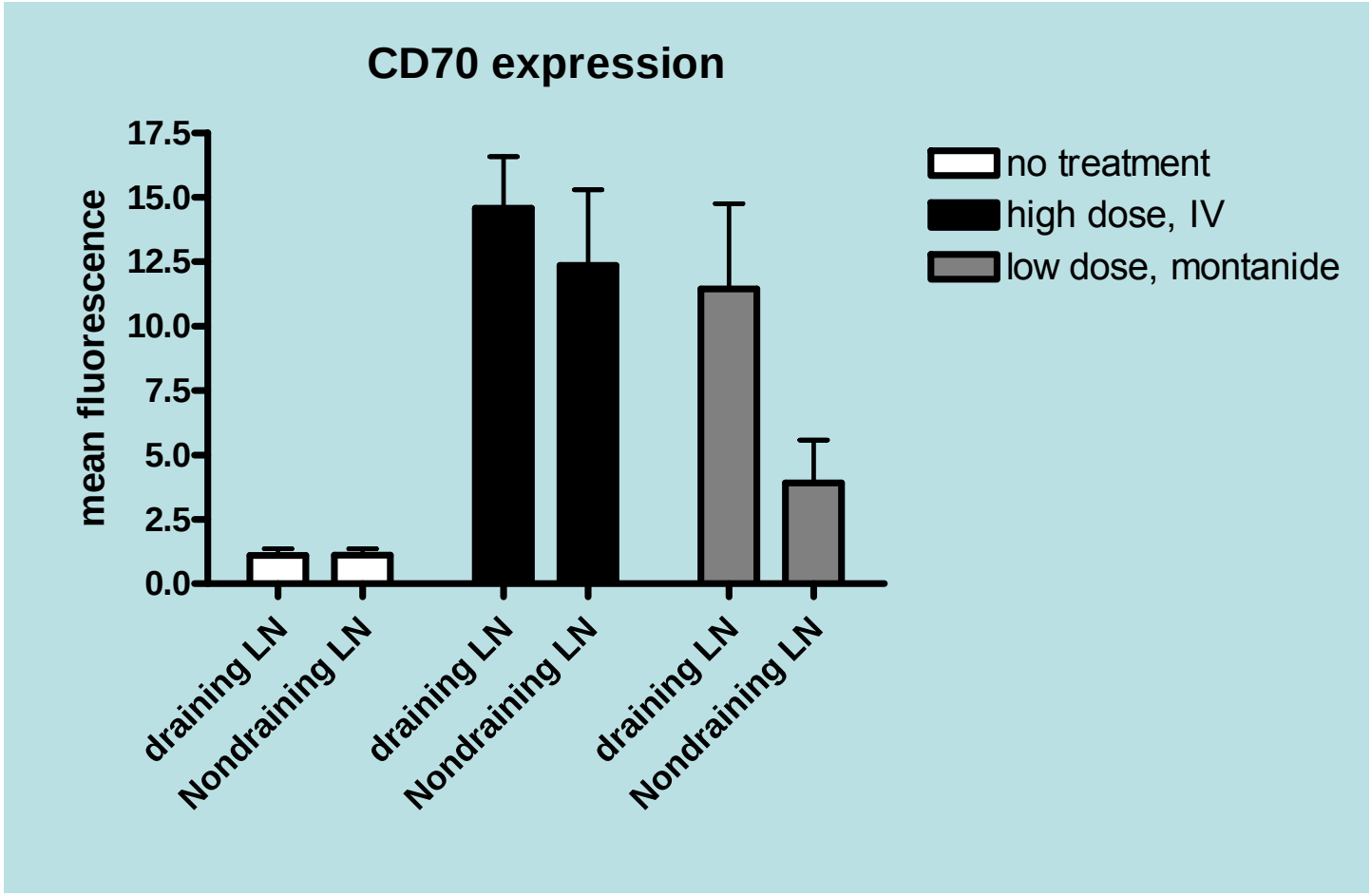
Anti-CD40 in serum



Does the low dose treatment have to be local in the vicinity of a tumor?

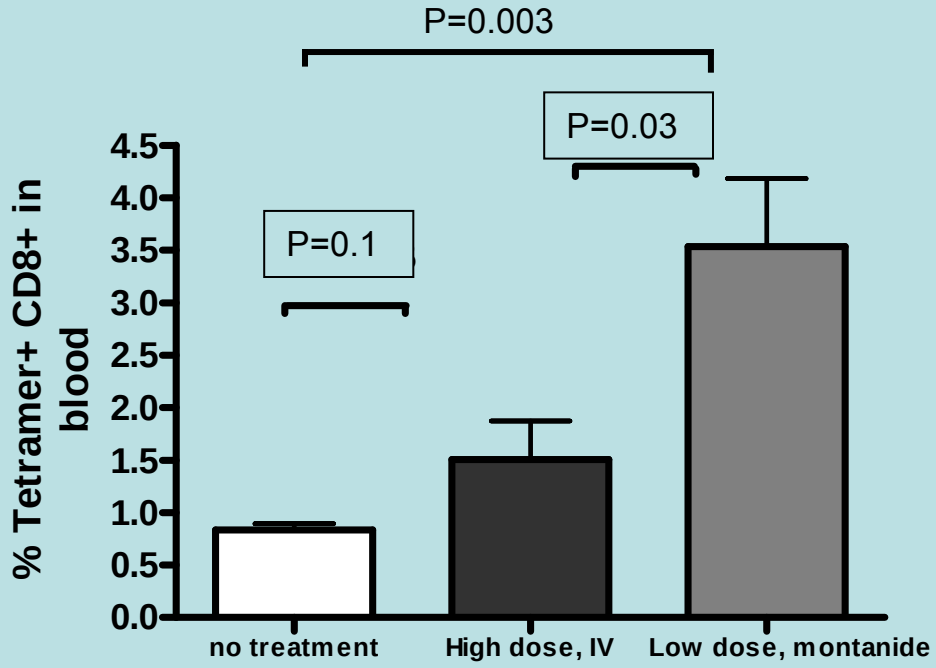


DC-activation in draining lymph node

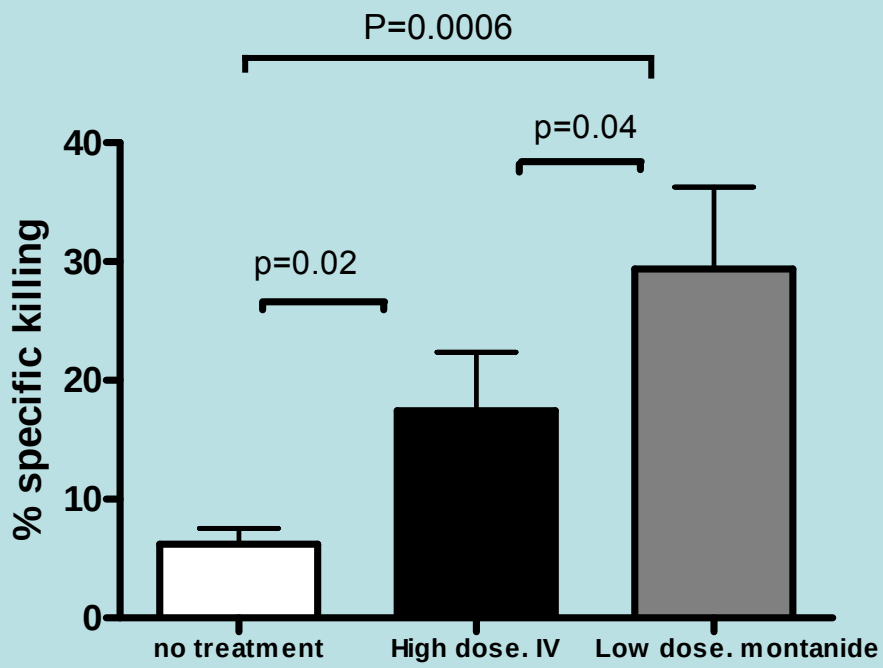


High dose iv versus versus local treatment with anti-CD40

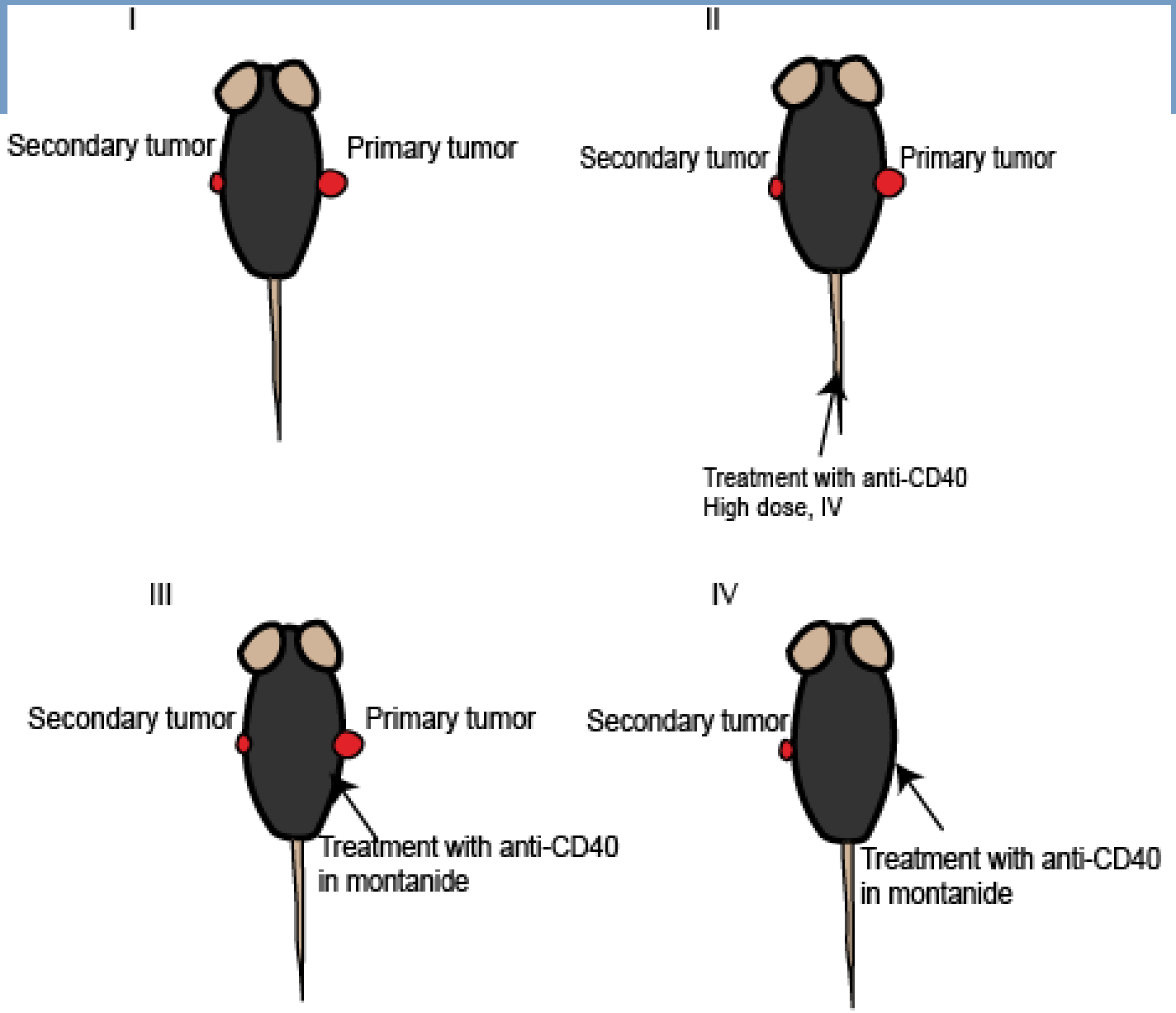
CTL response in blood



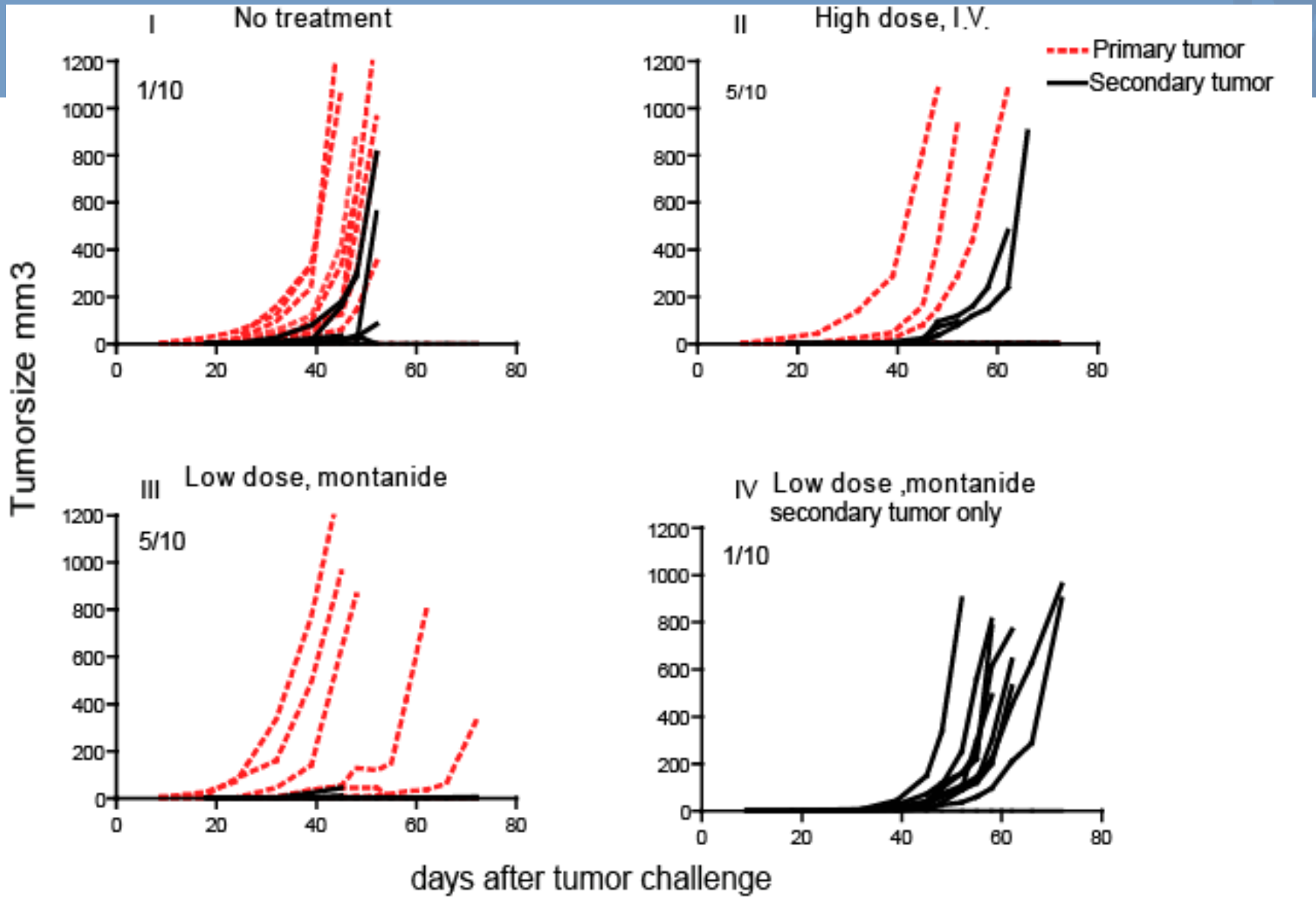
In vivo cytotoxicity



Eradication of secondary tumor:

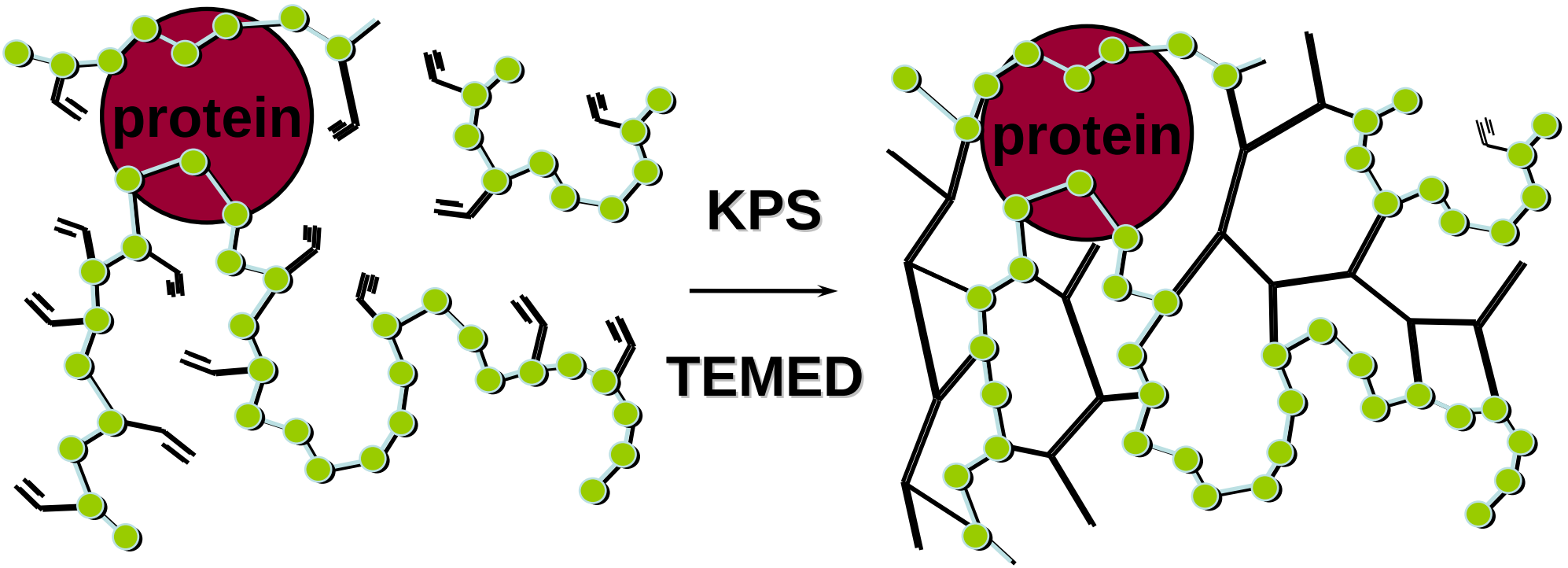


Eradication of secondary tumor:



-Dextran release can be better regulated than that of Montanide.

Dextran-based structures capture proteins, and can be emulsified into gel-like microspheres that are biodegradable and slowly release the proteins. With possibility of regulated release by changing water content.



Entrapped protein in 3-D network

Overall conclusions

- Short peptide vaccines do not work
- Long peptide vaccines harboring both CD4 and CD8 T cell epitopes and requiring DC processing are efficient
- Further improvements possible by adding TLR ligands or especially by conjugating TLR ligands to the long peptides
- For maximally effective cancer treatment develop combination treatment of long peptide vaccination with immunogenic chemotherapy and inhibitors of checkpoint control monoclonal antibodies (CTLA-4 blocker, PD-1, PD-L1 blockers)
- Reduce toxicity of the monoclonal antibody treatments by local delivery in slow release formulation close to tumor-draining lymph nodes

Acknowledgements

Dept of Clinical Oncology

Sytse Piersma
Moniek Heusinkveld
Renske Goedemans
Jeanette van den Hulst
Tamara Ramwadhdoebe
Lien van der Minne
Marij Welters
Sjoerd van der Burg

Alumni

Sander Zwaveling
Annemieke de Jong
Martijn Bijker
Kitty Kwappenberg
Annelies Vloon
Rienk Offringa

Dept of IHB

Selina Khan
Cedric Britten
Linda Stijnenbosch
Marieke Fransen
Farah Essahsah
Kees Franken
Ramon Arens
Ferry Ossendorp

Dept. of Pathology

Gert Jan Fleuren
Katja Jordanova
Hans Morreau
Sandra Uljee

Dept. of Gynaecology

Gemma Kenter
Peggy de Vos-v. Steenwijk
Muriel van den Hende
Margriet Löwik
Dorien Berends-van der Meer
Mariette van Poelgeest

Pharmacy

Jan Wouter Drijfhout
Jaap Oostendorp
Rob Valentijn
Lorraine Fathers

NWO
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