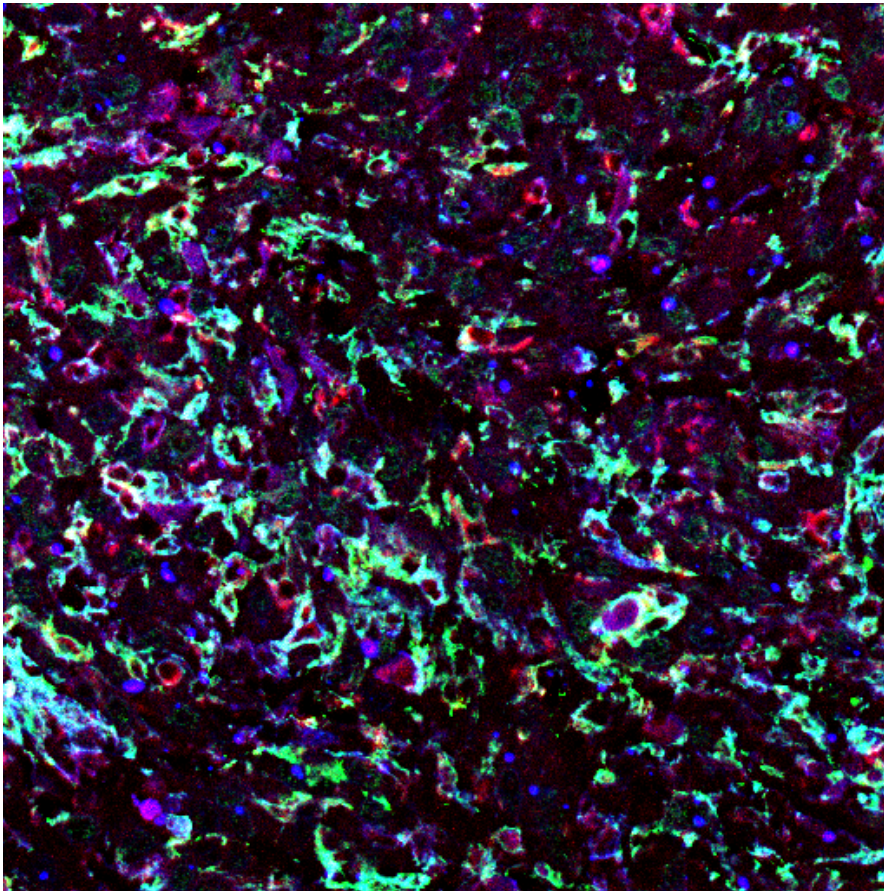


# Key host-tumor interactions for effective immunotherapy of cancer

Thorbald van Hall  
Dept. of Medical Oncology  
Leiden University Medical Center  
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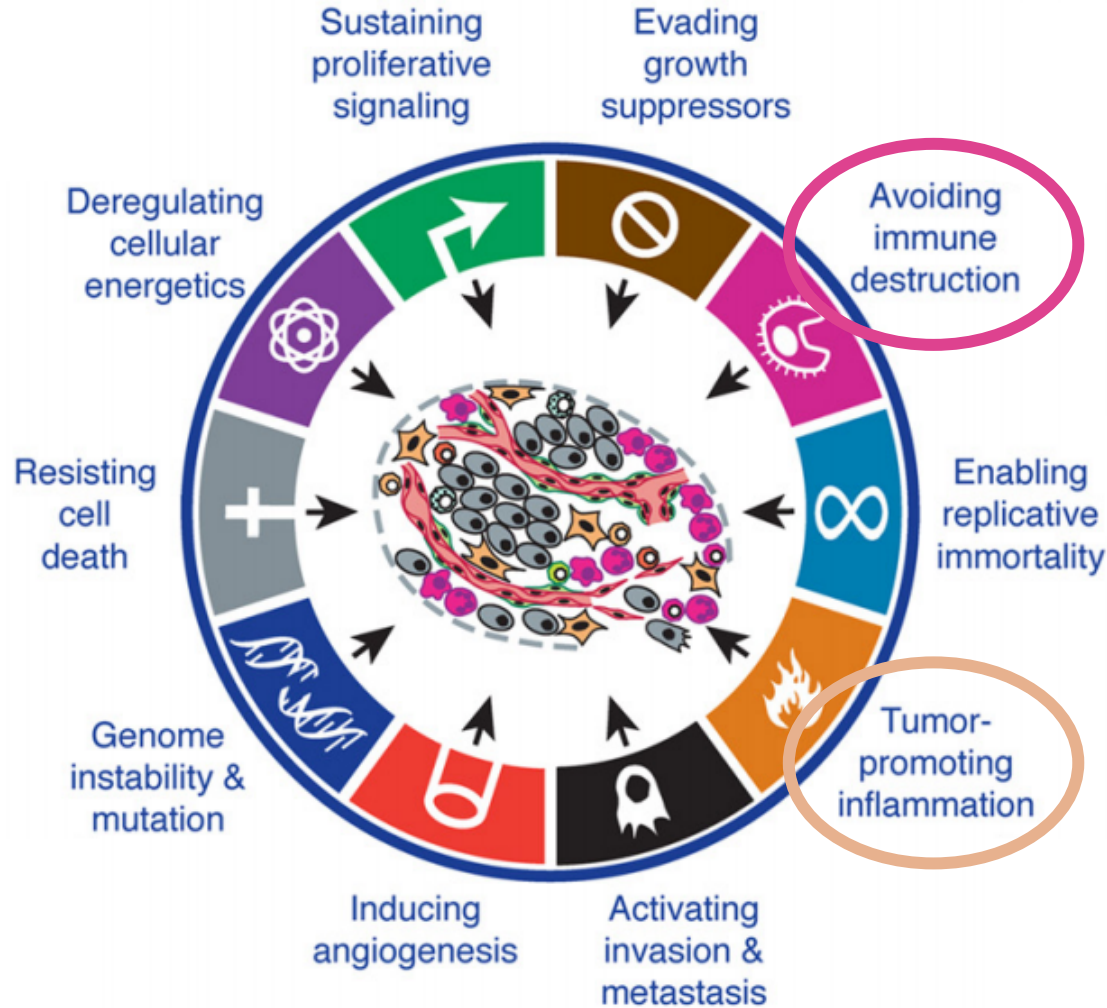
# Cancer lesions, more than cancerous cells...



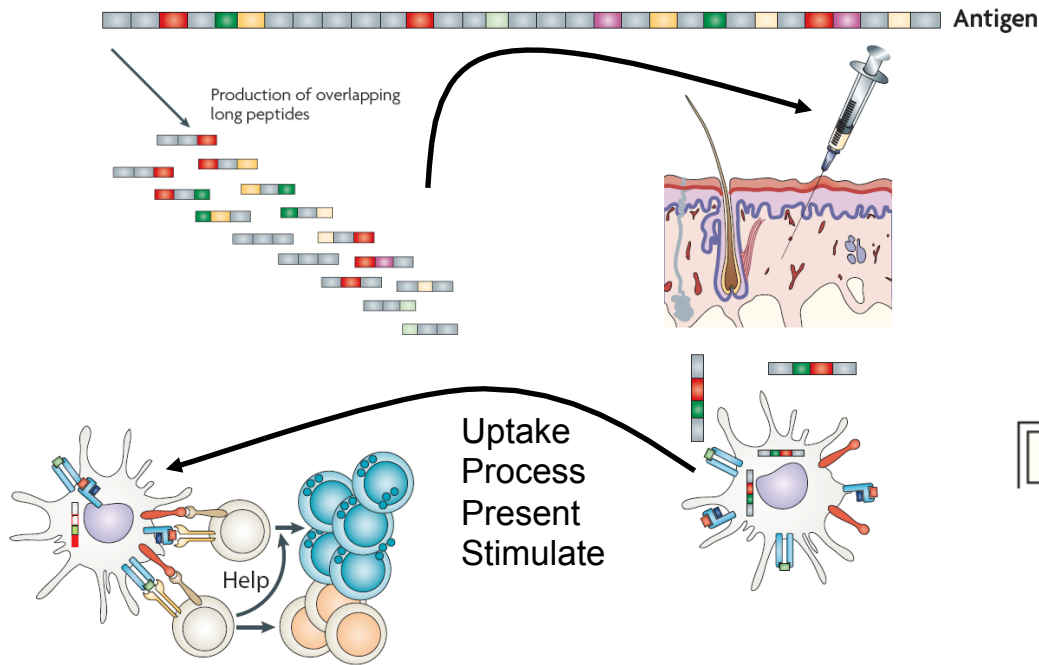
Gastrointestinal stromal tumor, stained for CD33, CD14 and HLA-DR

- ❖ Altered extracellular matrix
- ❖ Cancer Associated Fibroblasts
- ❖ Infiltrating immune cells:
  - TAM
  - MDSC
  - DC
  - T reg
  - T helper
  - CTL
  - NK cells
  - B cells
  - Eosinophils
  - Neutrophils
  - ....

# Immune system: a hallmark of cancer



# Therapeutic SLP (Synthetic Long Peptide) vaccine



THE NEW ENGLAND JOURNAL of MEDICINE

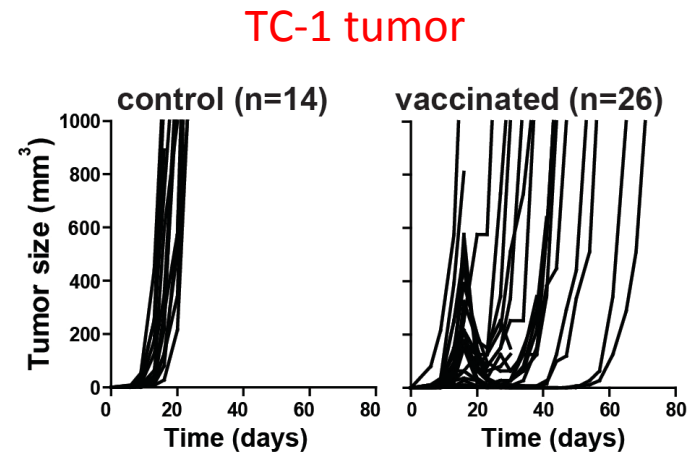
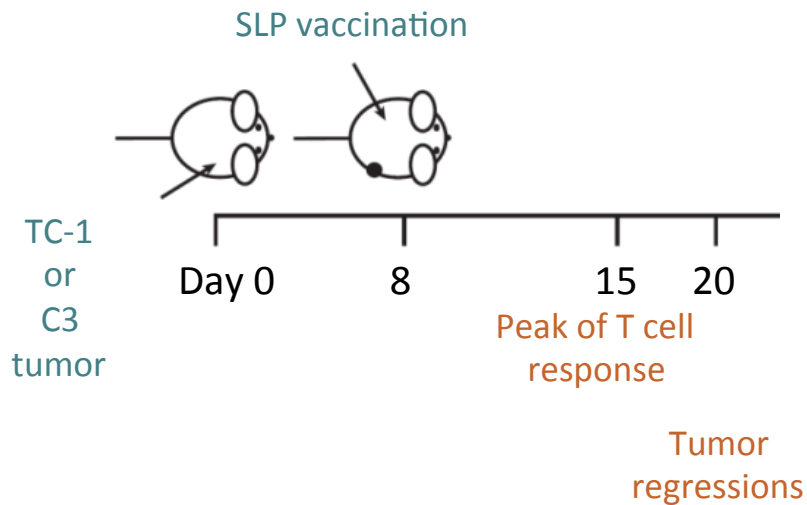
ORIGINAL ARTICLE

## 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.

# Mouse models for HPV16-induced cancer

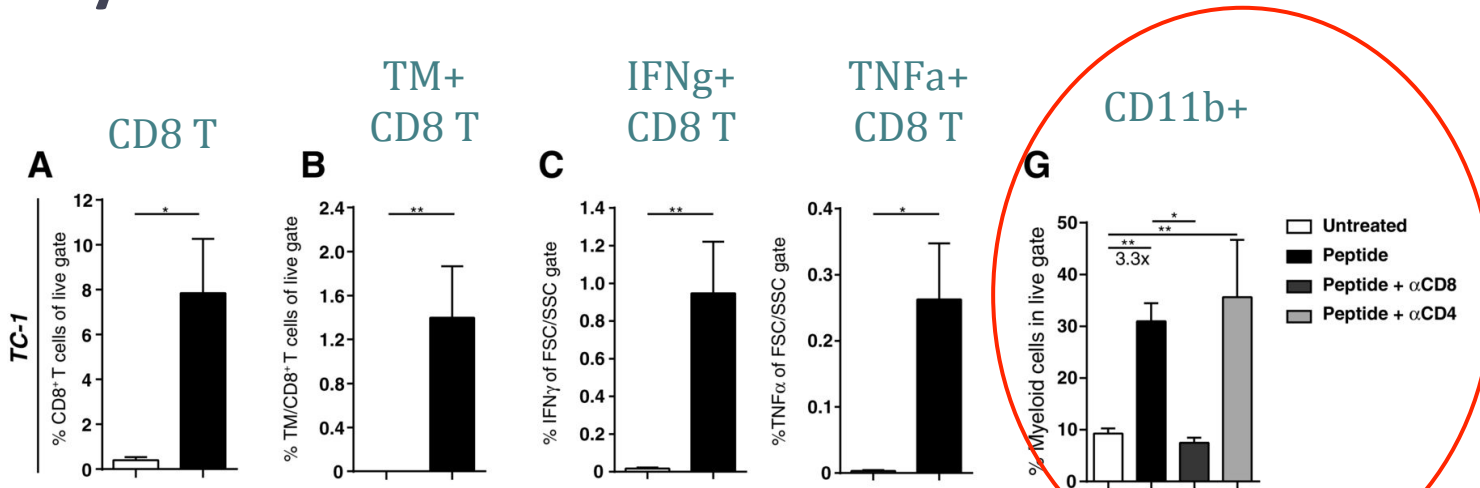
CTL epitope  
**Long peptide E7<sup>43-77</sup> : GQAEPDRAHYNIVTFCKCDSTLRLCVQSTHVDIR**  
Th epitope



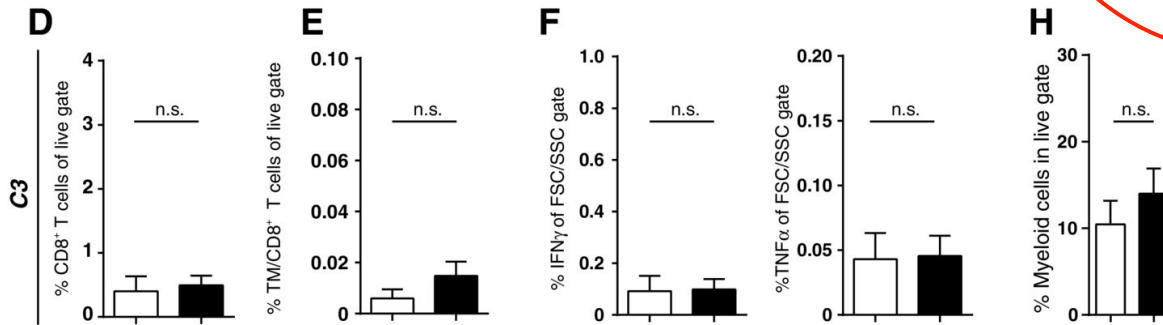
C3 tumor is not responsive

# Vaccine-induced CD8 T-cells infiltrate TC-1 tumors and recruit myeloid cells

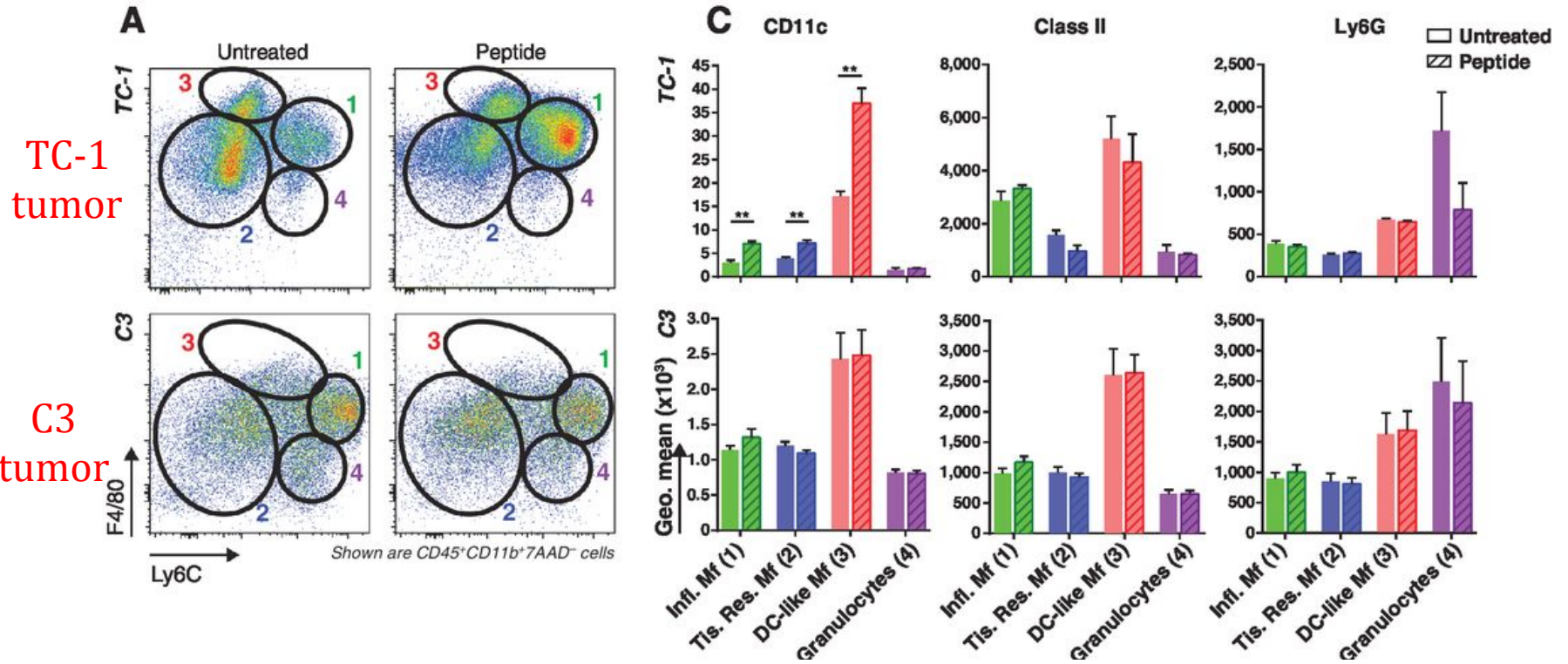
TC-1 tumor



C3 tumor



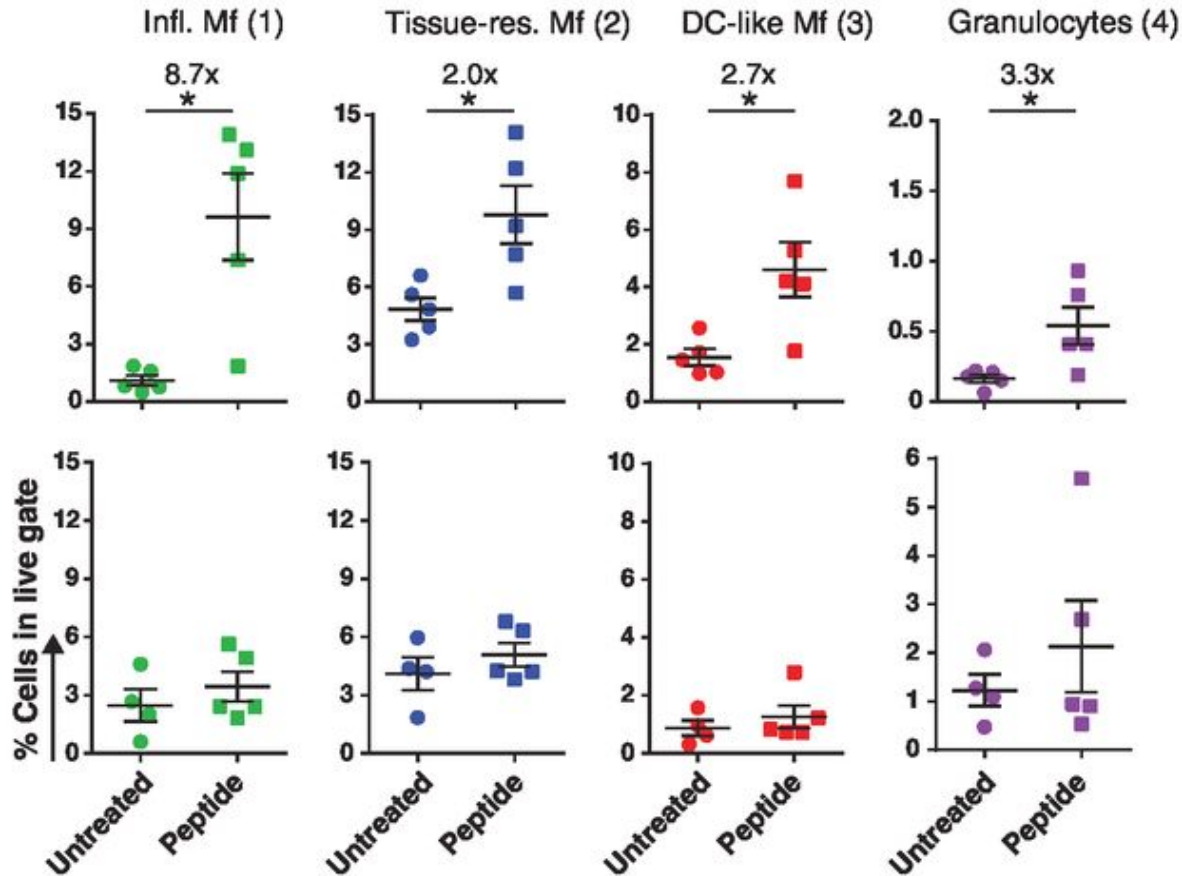
# Vaccination with SLP changes the macrophage subsets



# Vaccination with SLP massively enhances numbers of macrophages

TC-1  
tumor

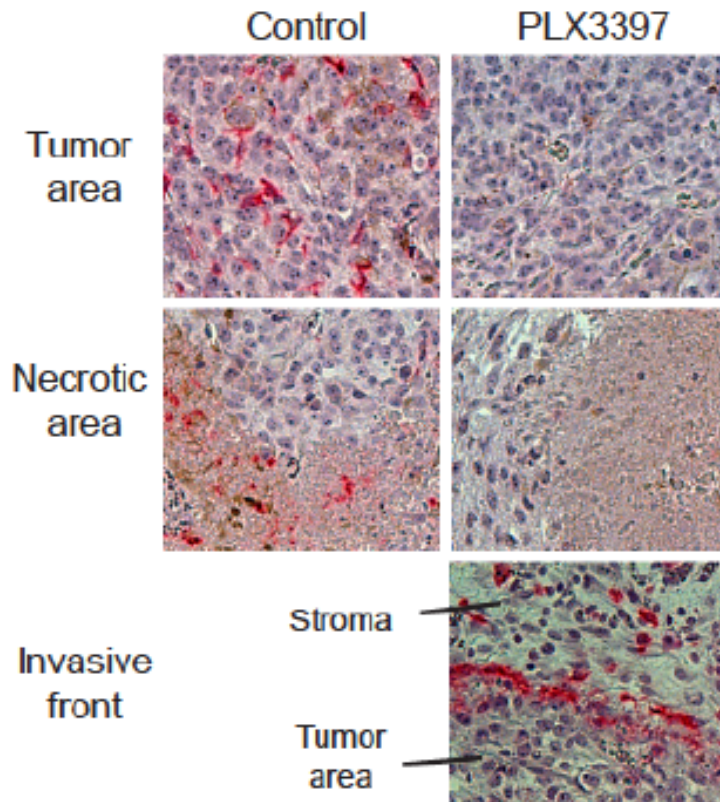
C3  
tumor



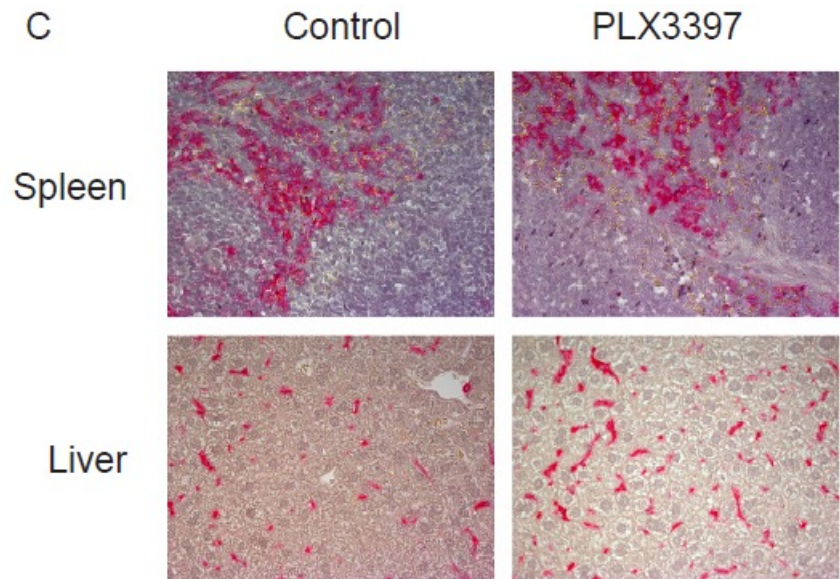


# Application of M-CSF receptor inhibitor to deplete F4/80<sup>+</sup> macrophages

F4/80 in tumor



F4/80 in other tissues



PLX3397 = kinase inhibitor for M-CSF receptor

# Which macrophage subsets are depleted by M-CSF receptor inhibitor?



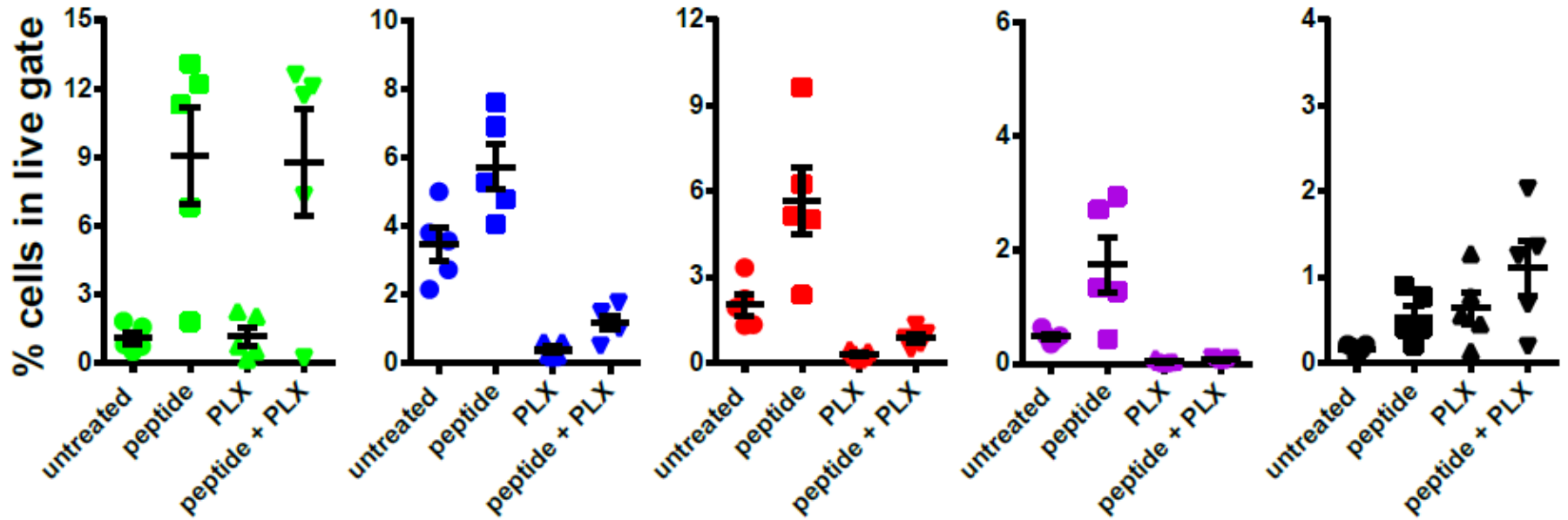
Infl Mf

Tissue res Mf

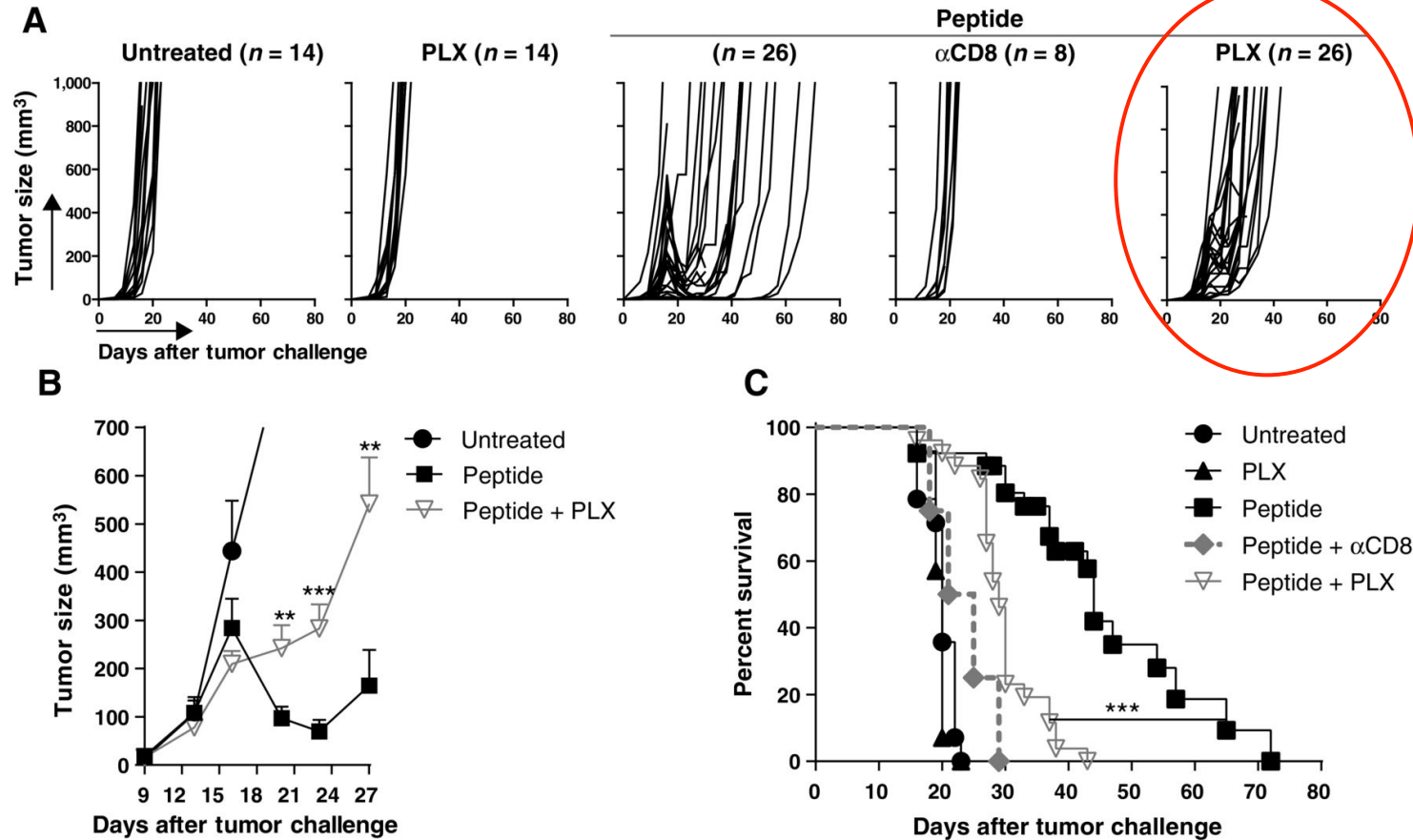
DC-like

Tissue Mf

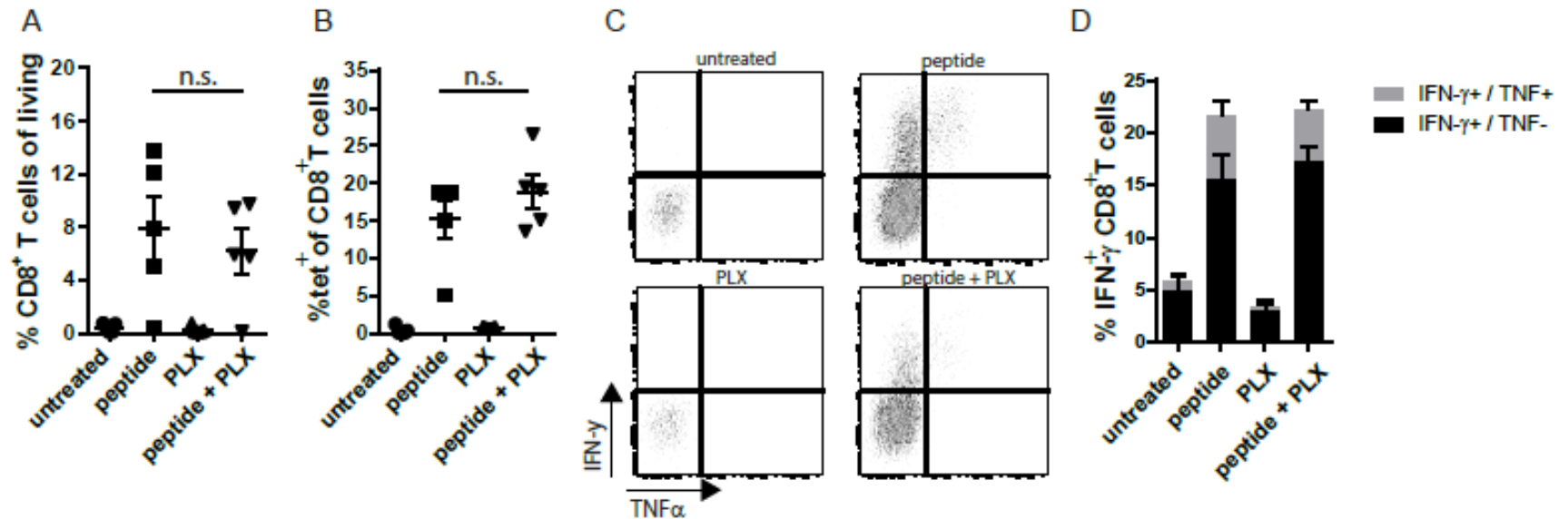
granulocytes



# Intratumoral macrophages are required for SLP vaccine–induced tumor regression



# M-CSF receptor kinase inhibitor does not affect CD8 T cell responses

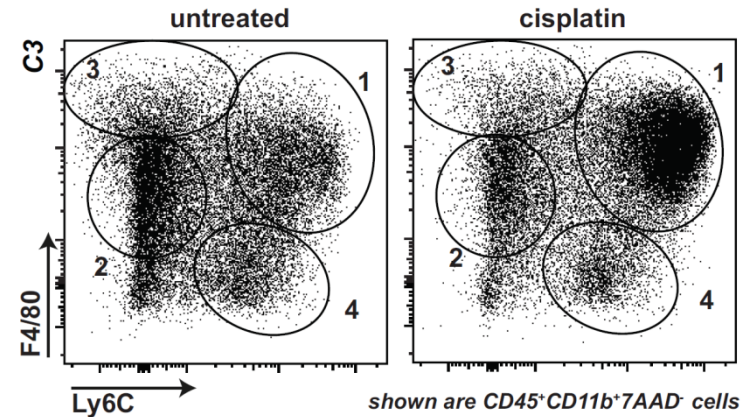
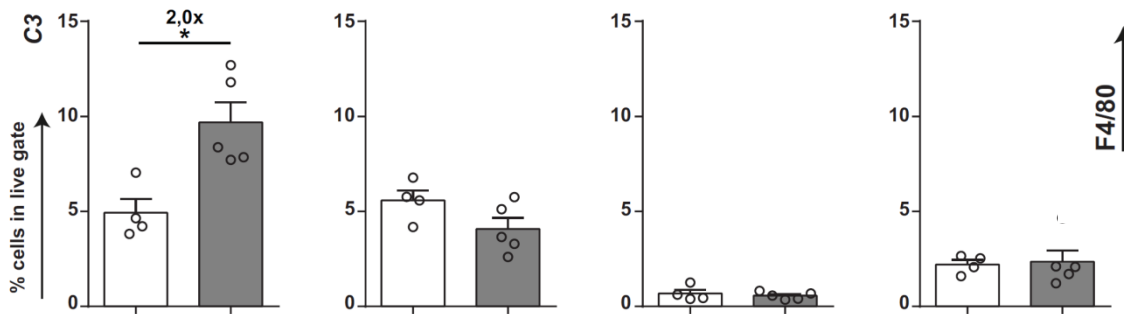
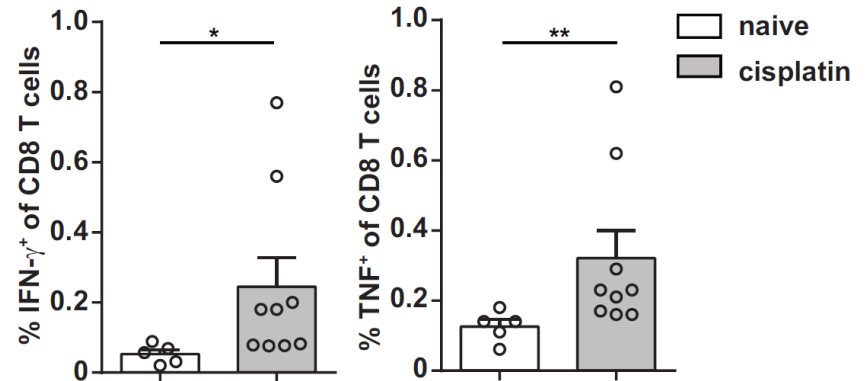
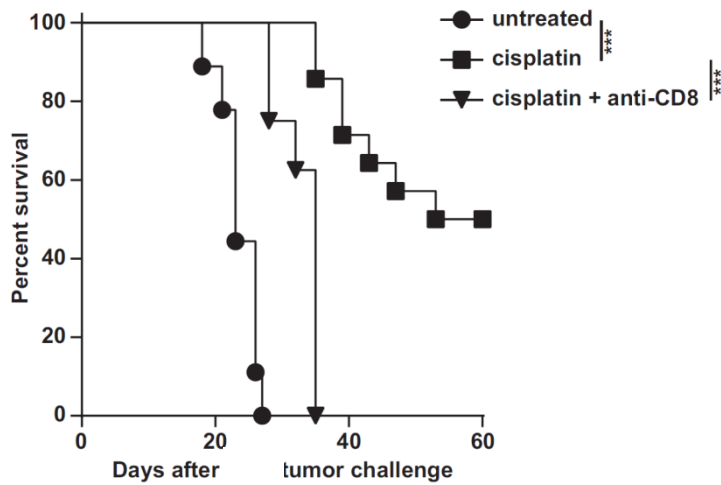


Ag-specific CD8 T cells, analyzed in blood and tumor for activity and location

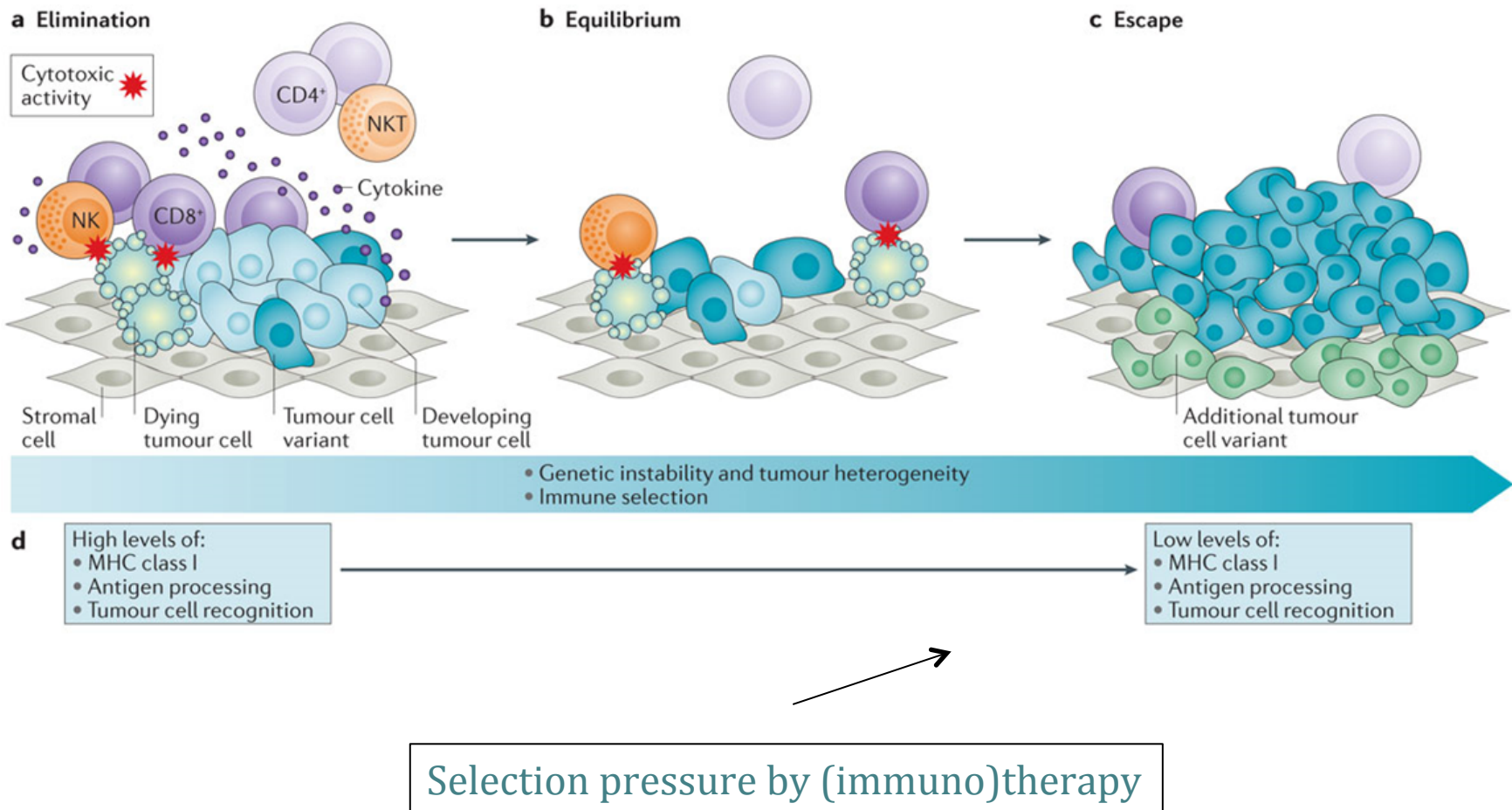
# Conclusion I

- SLP peptide vaccine is strong enough to induce tumor regression responses in the TC-1 model, but not in the C3 model.
- This vaccine elicits tumor-specific CD8 T cells that infiltrate the tumor and recruit different subsets of macrophages.
- The 'TAM' and DC-like macrophages are crucial for tumor regressions (cleaning debris, local T cell support?).
- In general, therapeutic cancer vaccines are not sufficient to induce an tumor regression response, like in the C3 model.
  - Need for active T cell homing and relief of local immuno-suppression

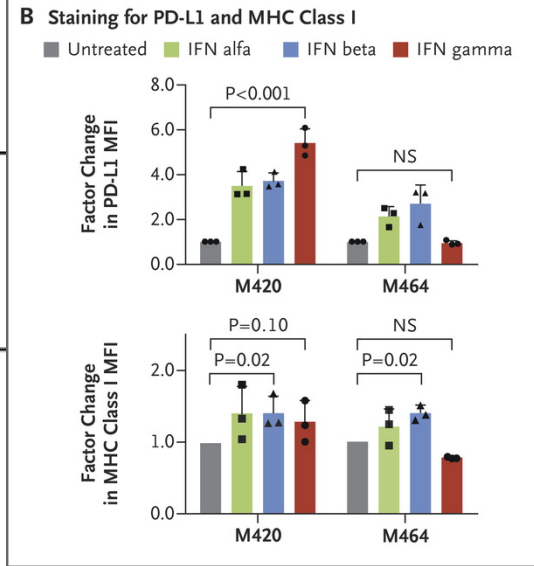
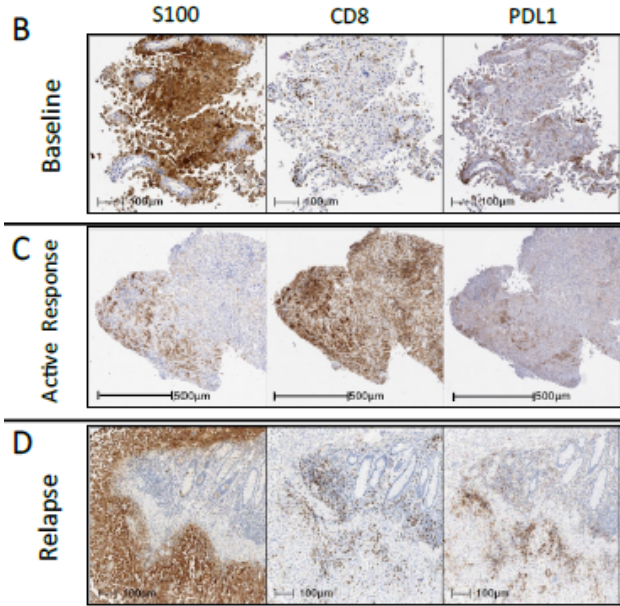
# .....Chemotherapy does induce an immune-rejection response in C3 model.....



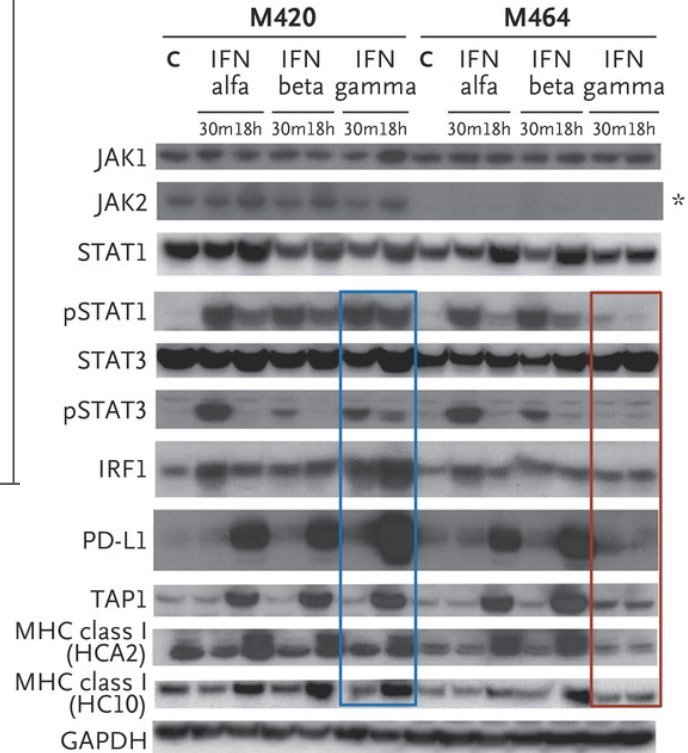
# Immune evasion: the three phases of immuno-editing



# Therapy-induced immune escape



## Western Blot Analysis

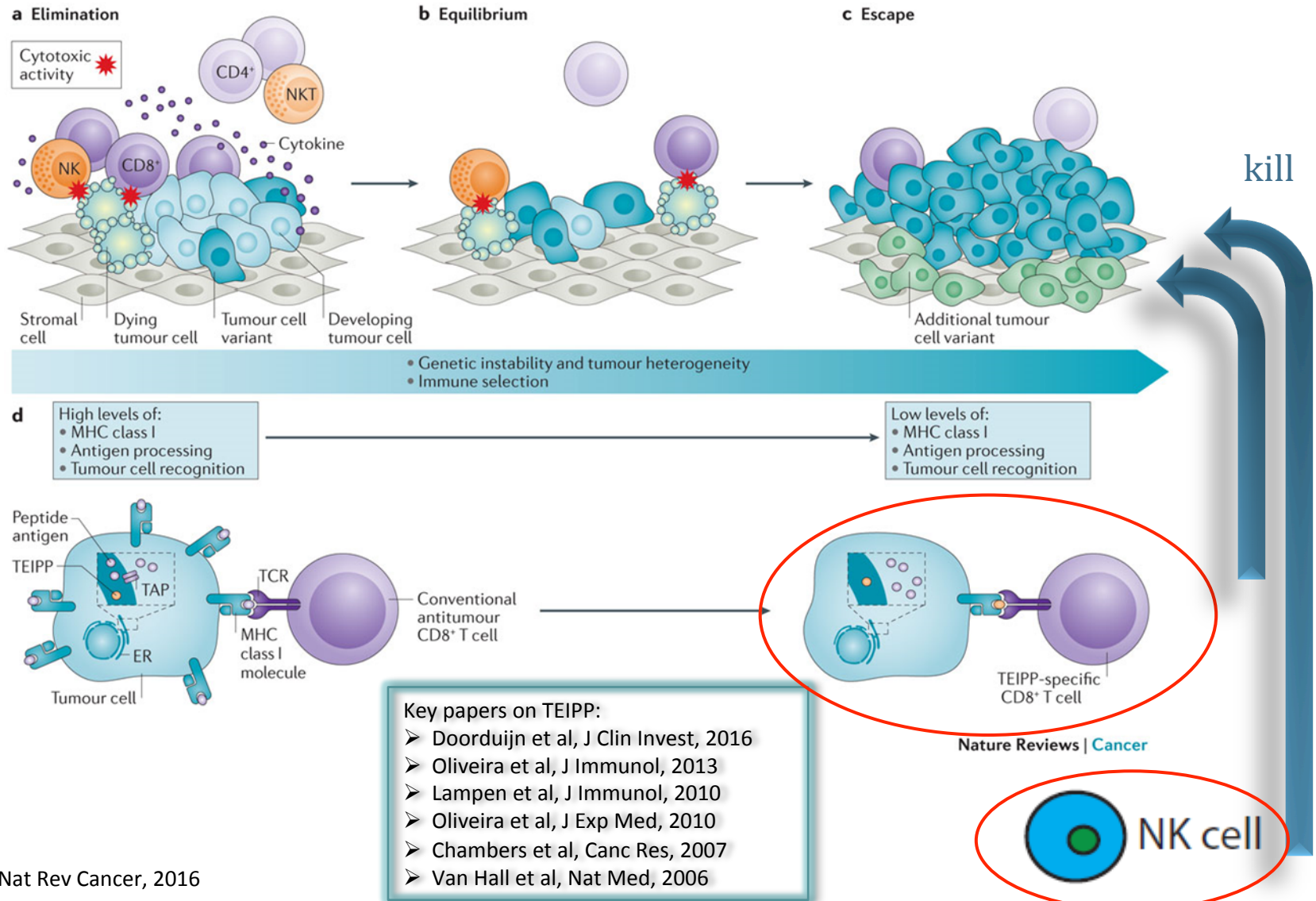


### Relapsing melanoma patients after PD-1 blockade therapy:

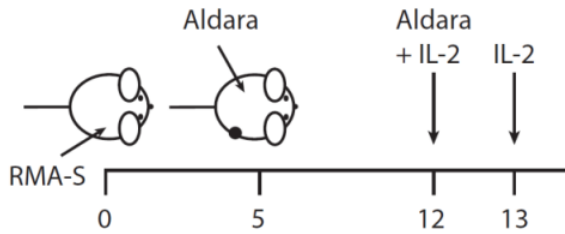
- JAK1 mutation (patient #1)
- JAK2 mutation (patient #2)
- β2M mutation (patient #3)



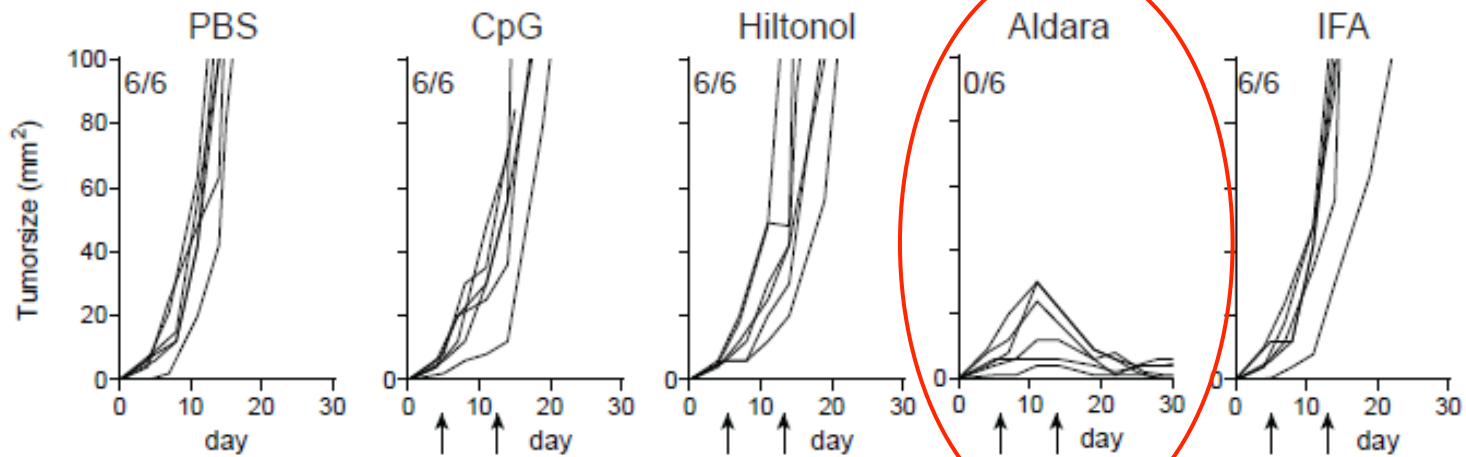
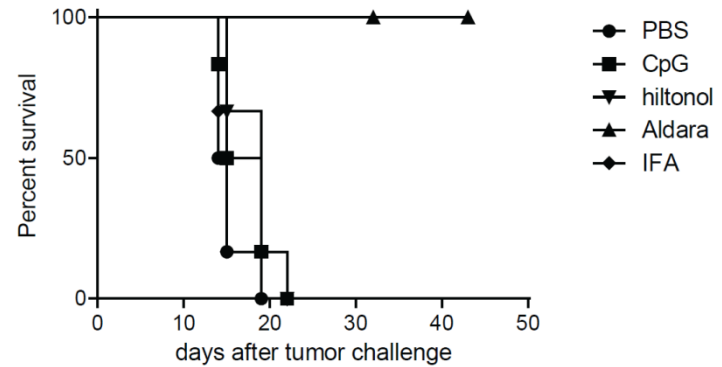
# How to target strongly immuno-edited tumors?



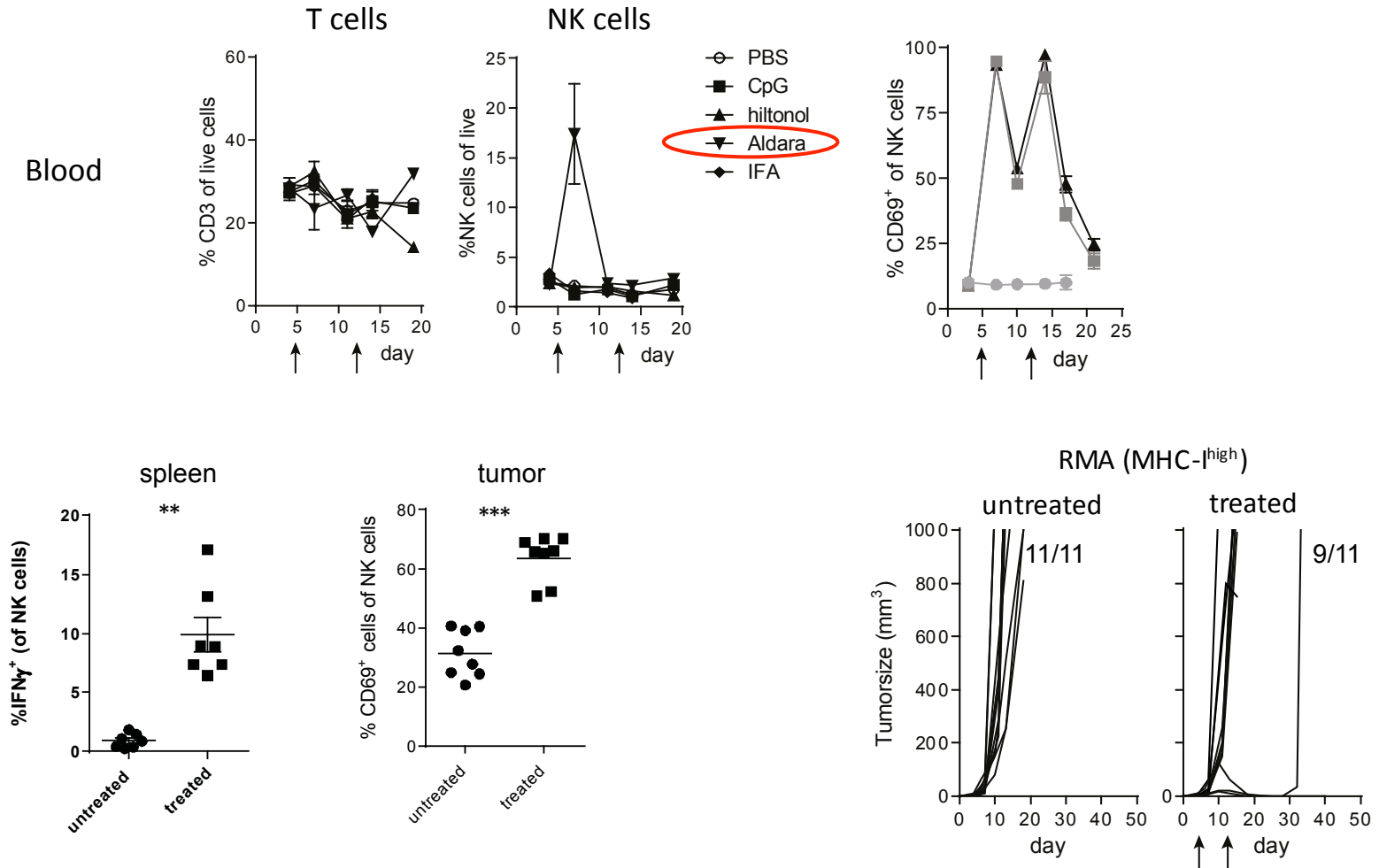
# Treatment of MHC-I<sup>low</sup> tumors by a TLR-7 agonist



Established TAP-negative RMA-S tumors

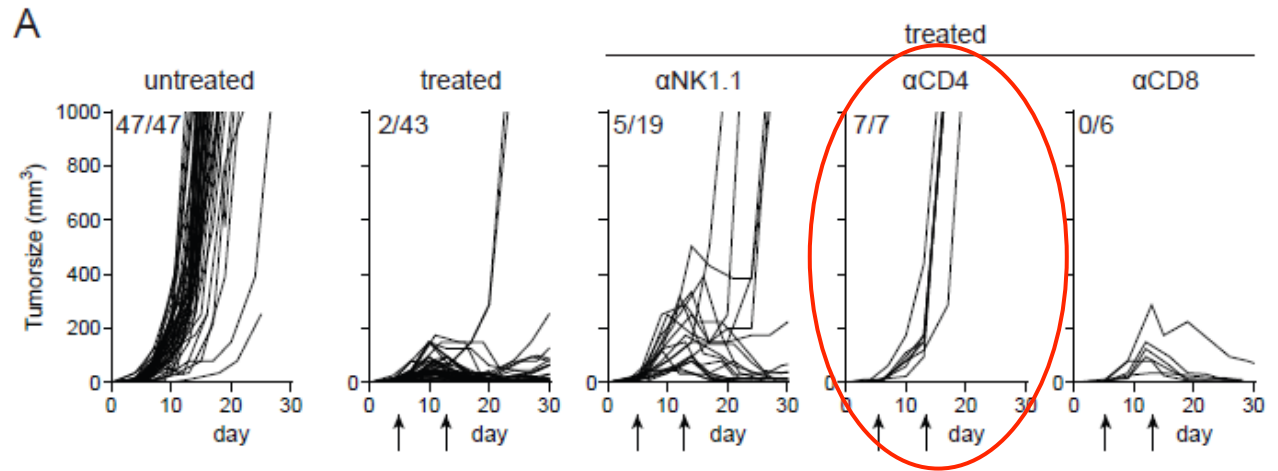


# Importance of NK cells in the TLR-7 agonist-induced response

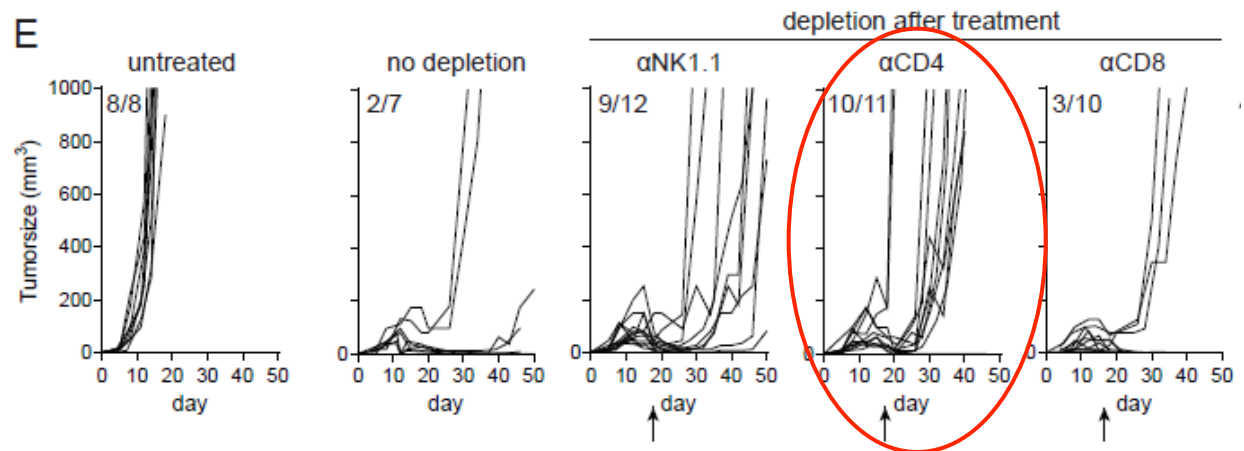


# NK cells and CD4+ T cells are indispensable for regression

Continuous depletion

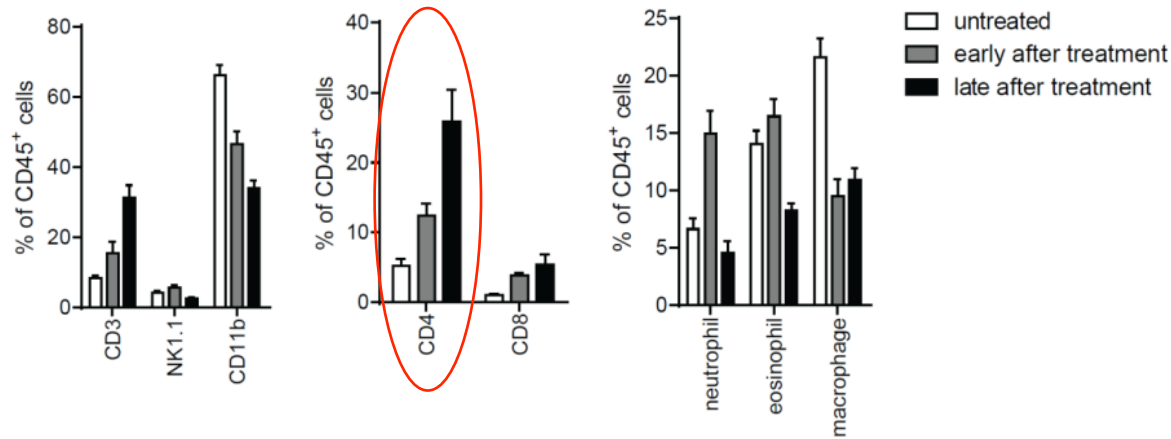


Depletion after regression response

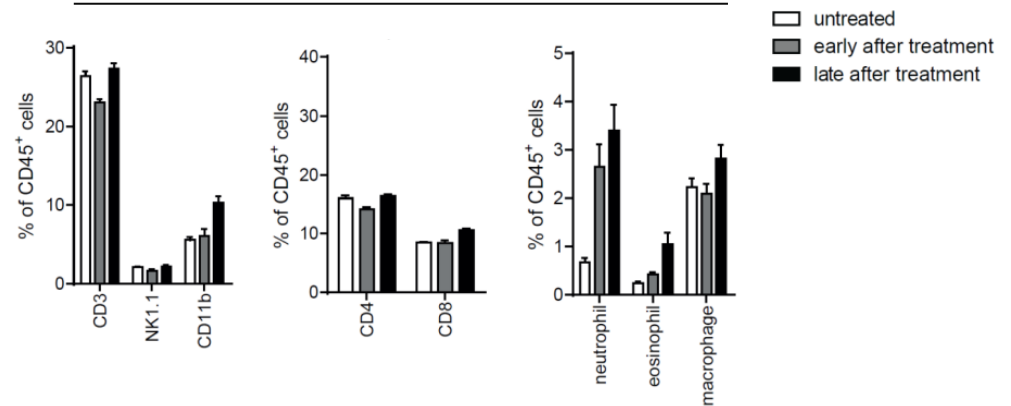


# CD4+ T cells accumulate in MHC-I<sup>low</sup> tumors

## tumor



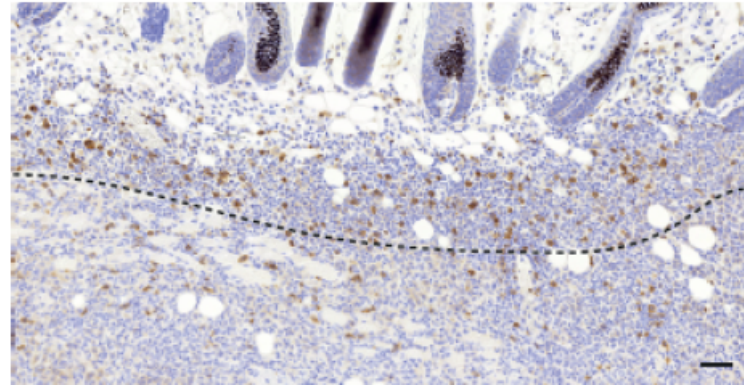
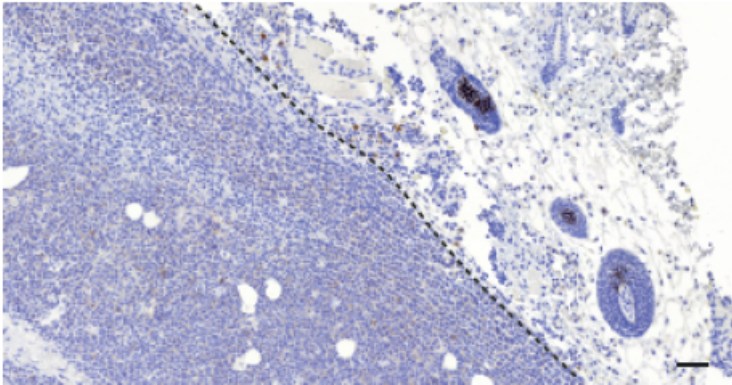
## spleen



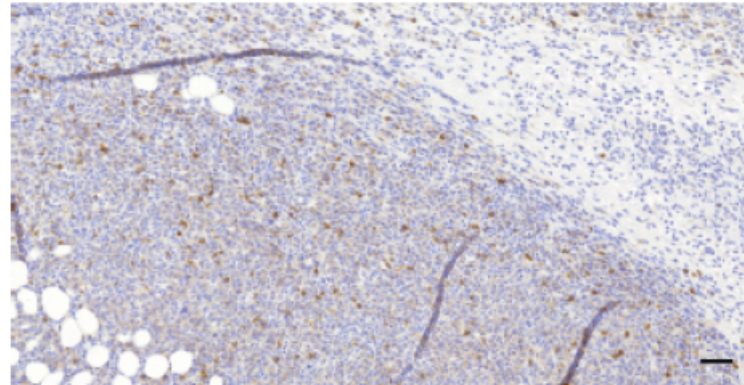
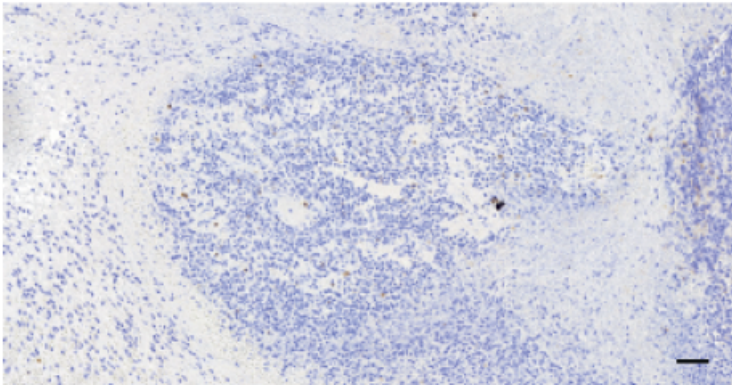
# Intratumoral detection of CD4+ T cells

untreated

treated



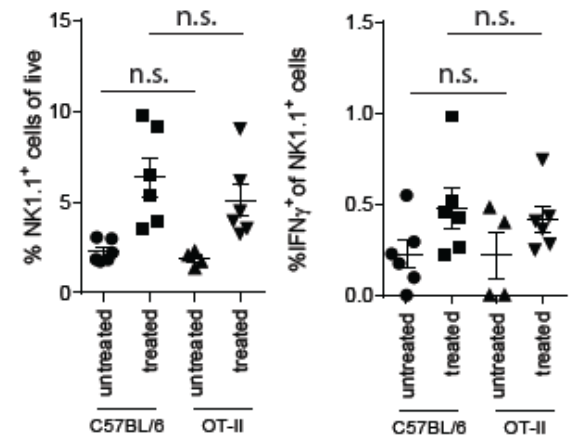
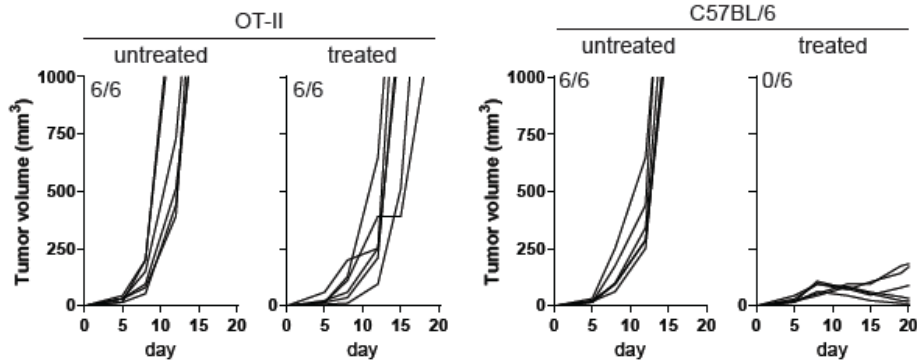
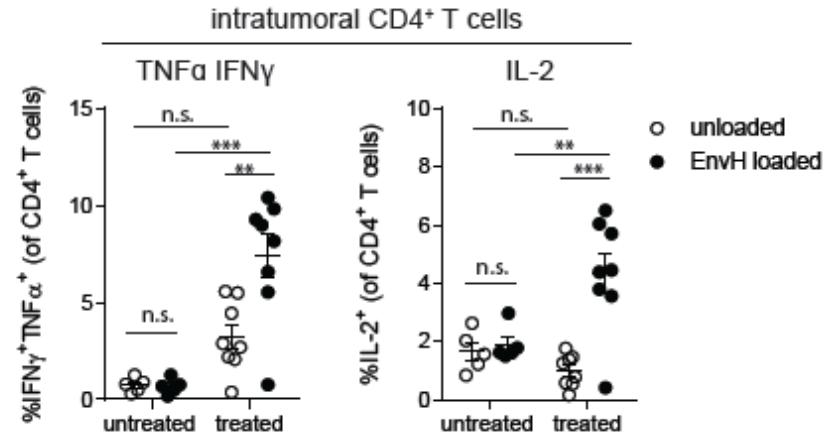
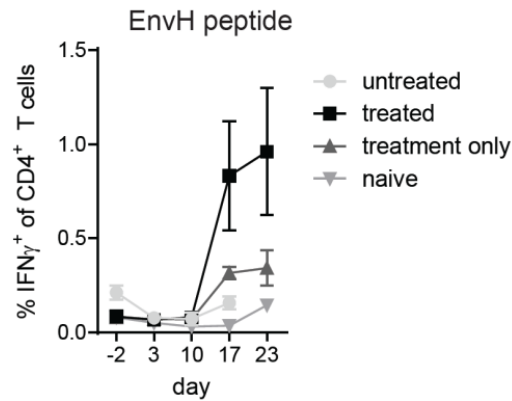
border



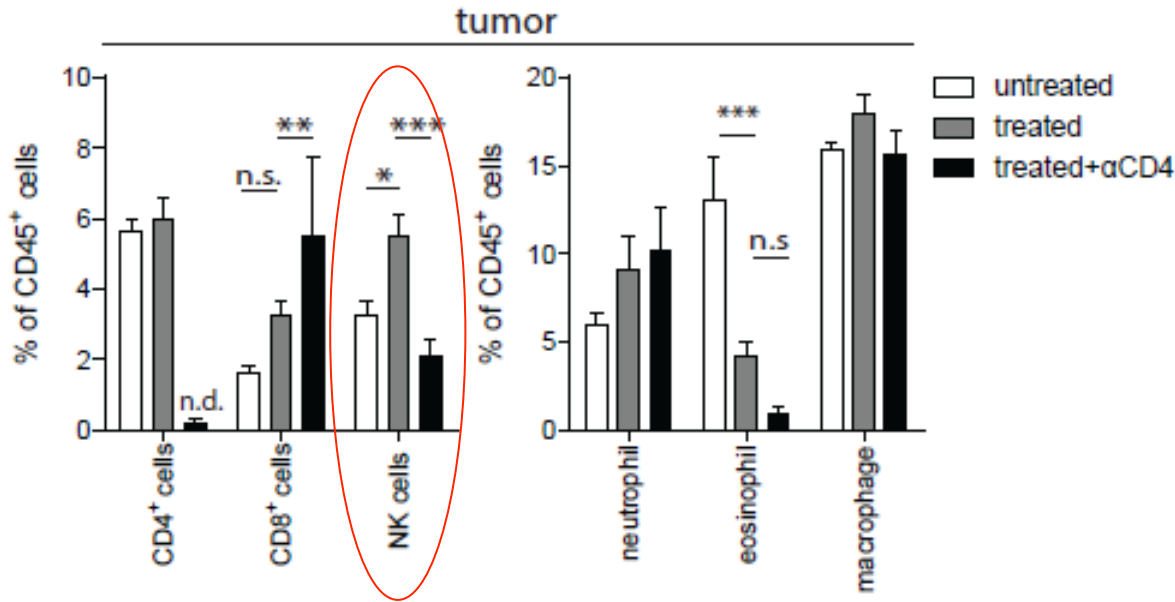
center

# TLR-7 agonist indirectly induces tumor-specific CD4 T cells

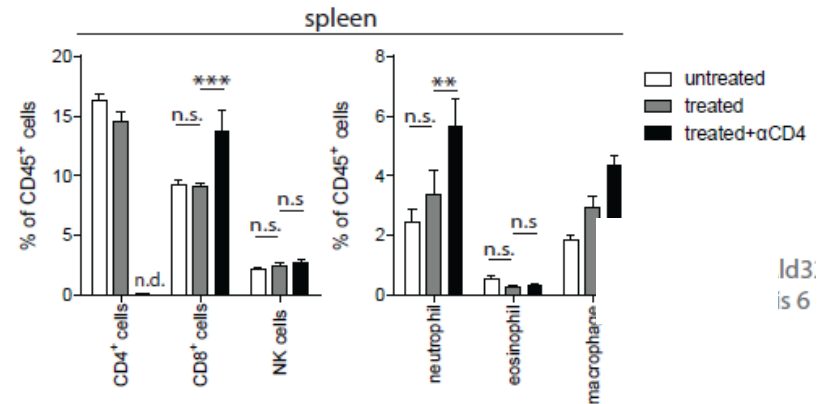
Blood



# How do CD4+ T cells help NK attack?

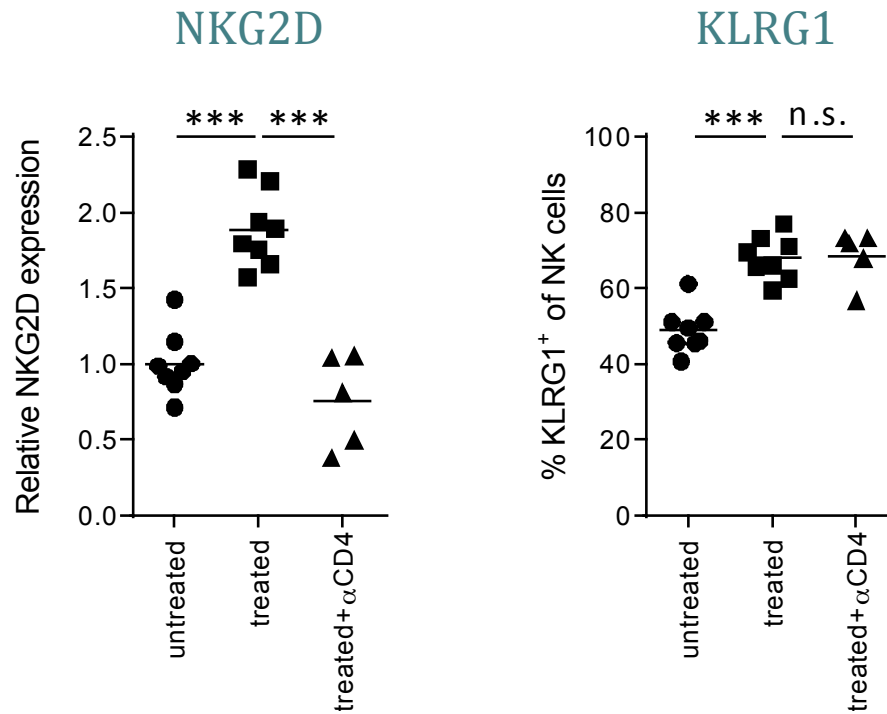


CD4+ T cells **recruit NK cells** to the tumor





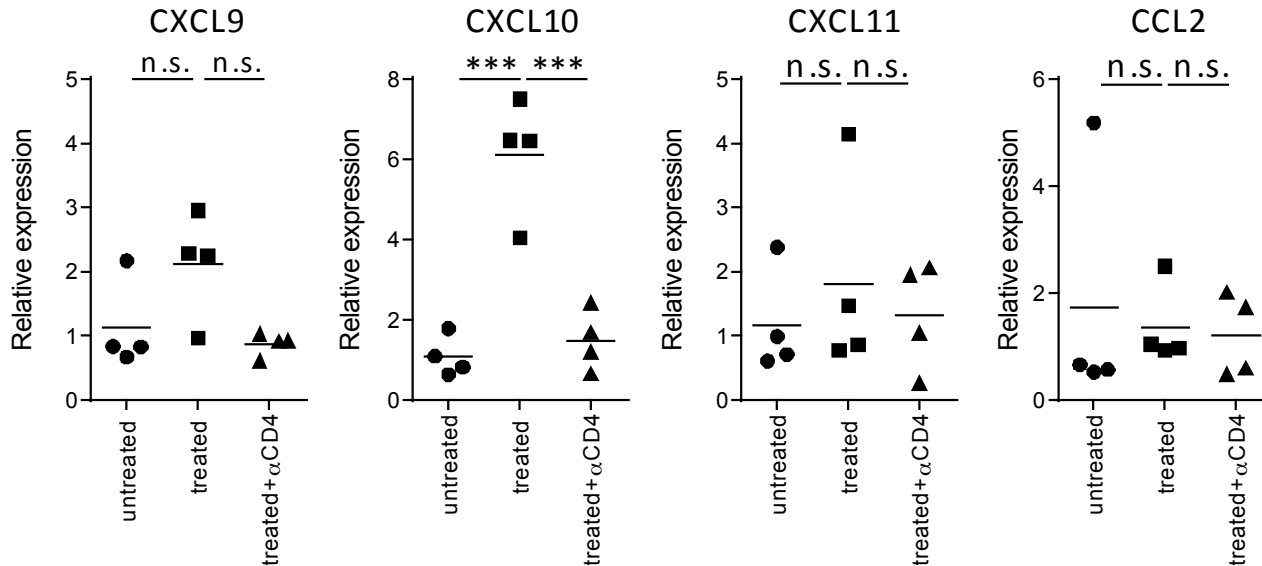
# How do NK cells and CD4+ T cells collaborate?



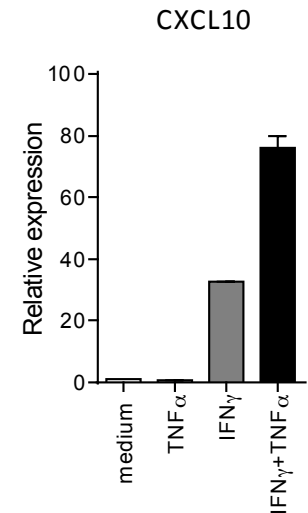
CD4+ T cells **activate** NK cells in the tumor

# What causes the local accumulation of CD4+ T cells?

## In situ



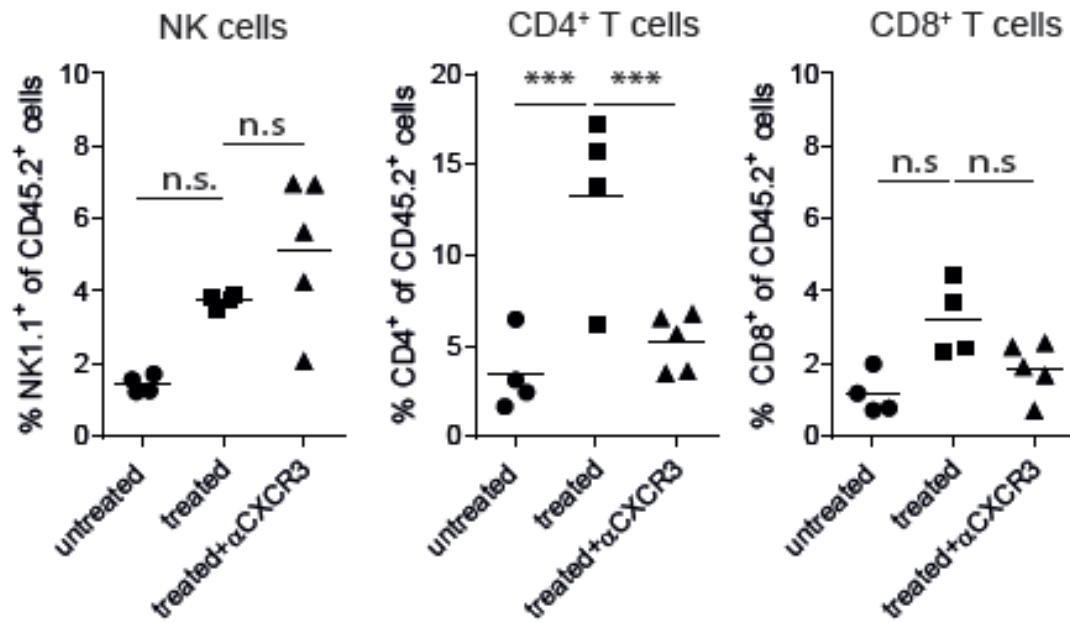
## In vitro



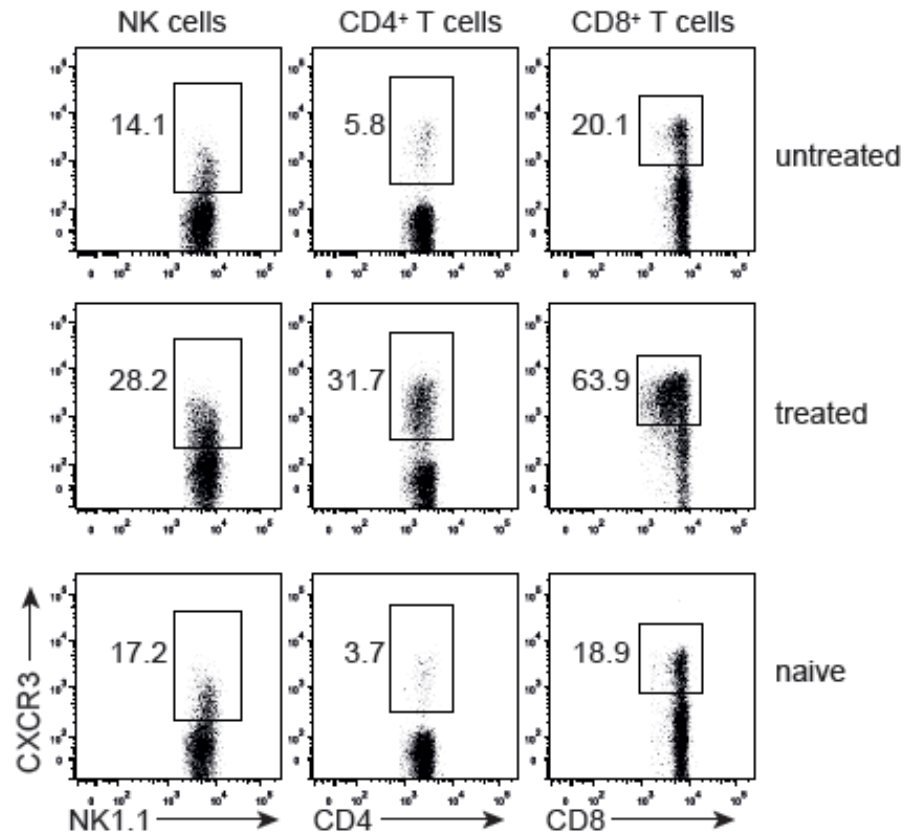
### CXCL9, CXCL10 & CXCL11:

- IFN $\gamma$ -induced chemokines
- Involved in migration of T cells and NK cells to site of infection
- Engage the receptor CXCR3 on blood cells

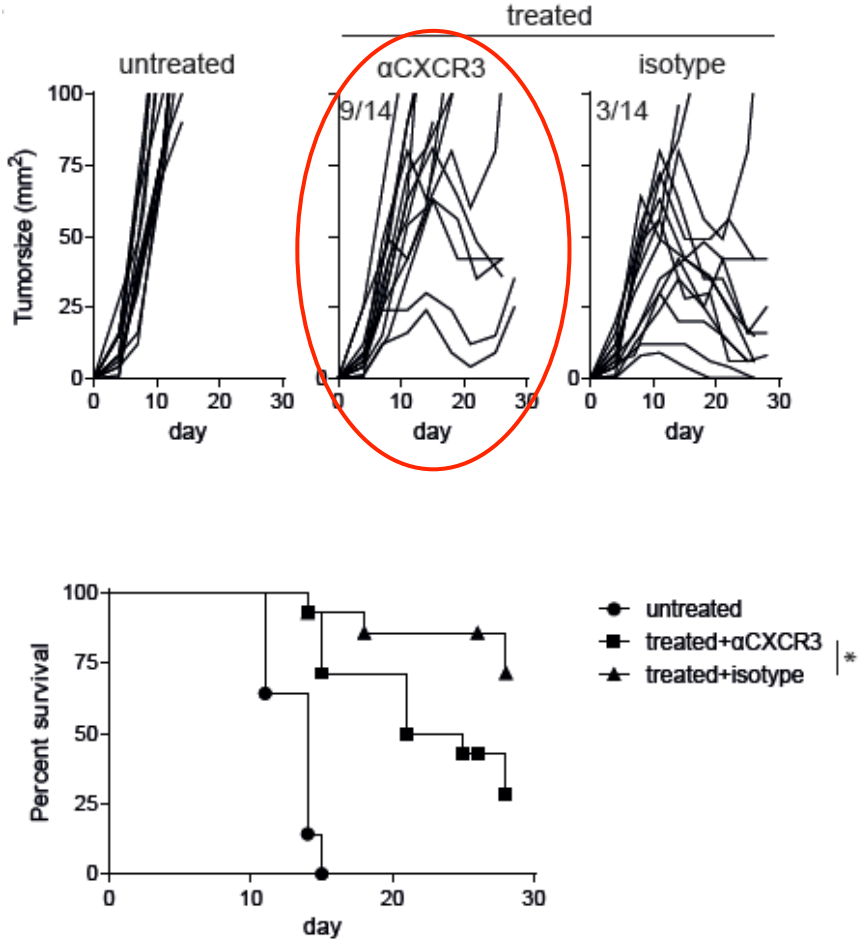
# CXCR3 blockade prevents tumor influx of T cells



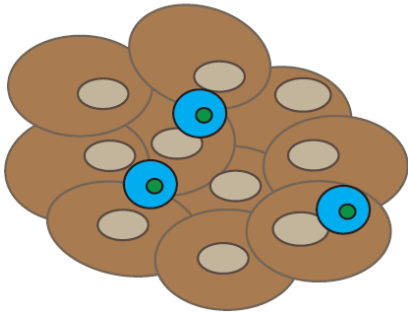
# TLR-7 agonist induces a transient induction of CXCR3 on blood lymphocytes



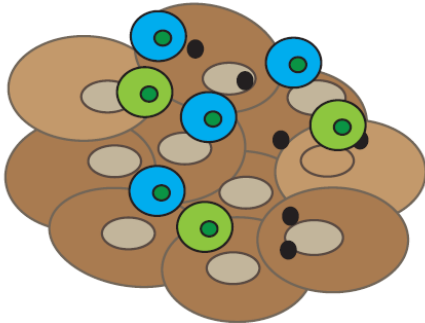
# CXCR3 blocking prevents tumor regressions



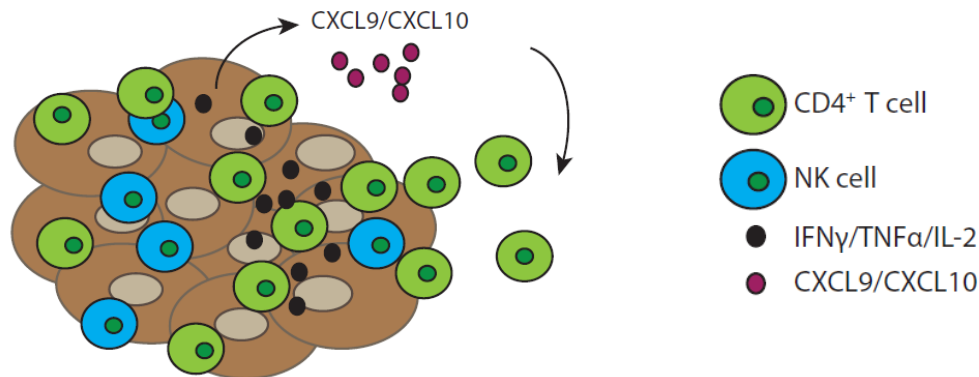
1. NK cells infiltrate RMA-S tumor, release tumor antigens



2. Priming CD4<sup>+</sup>T cells, release cytokines intratumoral



3. Increased infiltration CD4<sup>+</sup>T cells: NK recruitment and activation



## Working model

### Sequence of events:

1. TRL-7 agonist systemically activates NK cells
2. Initial kill of MHC-I<sup>low</sup> tumor cells and IFN $\gamma$  by NK cells
3. Release tumor antigens and priming of CD4<sup>+</sup> T cells
4. Homing of tumor-specific CD4<sup>+</sup> T cells
5. Self-perpetuating accumulation through CXCL10-CXCR3
6. Local support of NK cell recruitment and activation
7. Total rejection of MHC-I<sup>low</sup> tumors

## Conclusions II

- TLR-7 agonist systemically activates NK cells and leads to indirect activation of tumor-specific CD4 and CD8 T cells.
- CD8 T cells are induced, but not important for tumor eradication: MHC-I<sup>low</sup> tumors are resistant.
- Accumulation of intratumoral CD4 T cells is mediated through CXCL10-CXCR3 axis.
- Local support of NK cells by tumor-specific CD4 T cells is crucial for the eradication of MHC-I<sup>low</sup> tumors.

### Lessons:

- Striking low frequencies of NK cells in solid tumors might still be very functional.
- Therapy-induced CD4 T cells are crucial for immuno-edited tumors.

# Acknowledgements

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- Marjolein Sluiter
- Bianca Querido
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- Ferry Ossendorp
- Kees Melief