Targeting immune modulatory pathways in cancer

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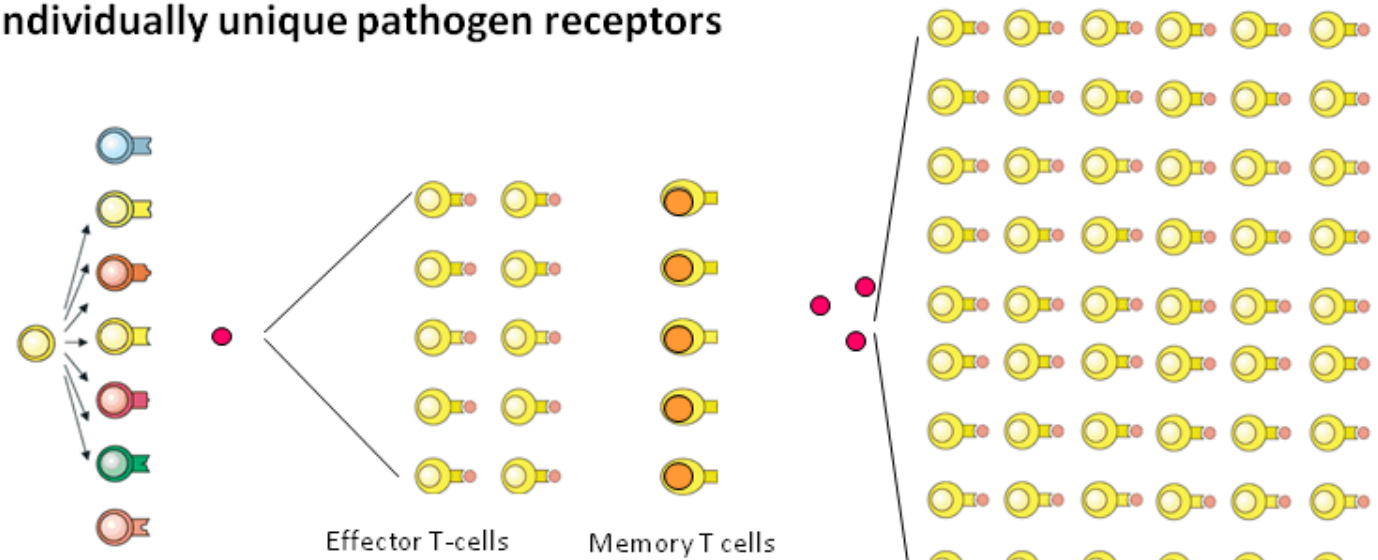
German Cancer Research Center

How is an immune response generated?

T-cells with individually unique pathogen receptors

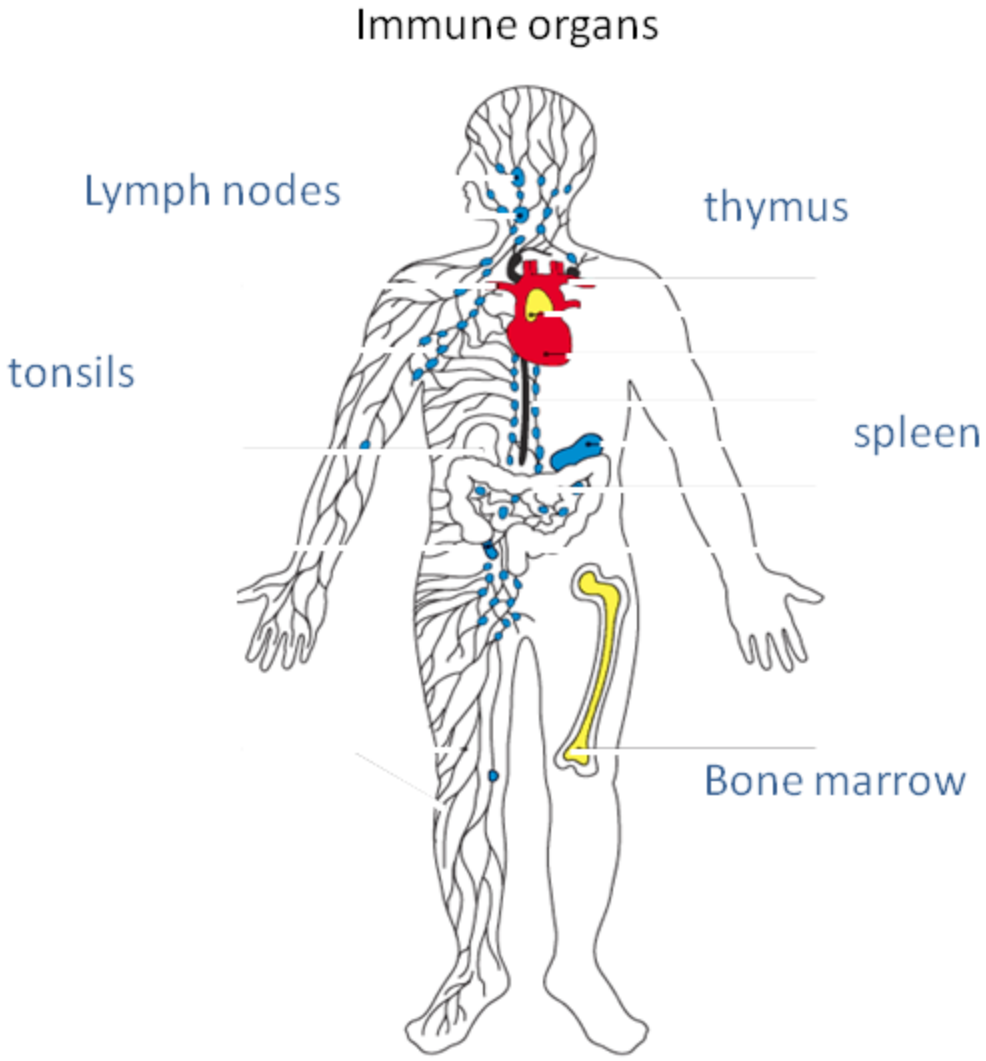


Features:

Effector T-cells	Memory T cells
short lived	long lived
low proliferation	Strong proliferation

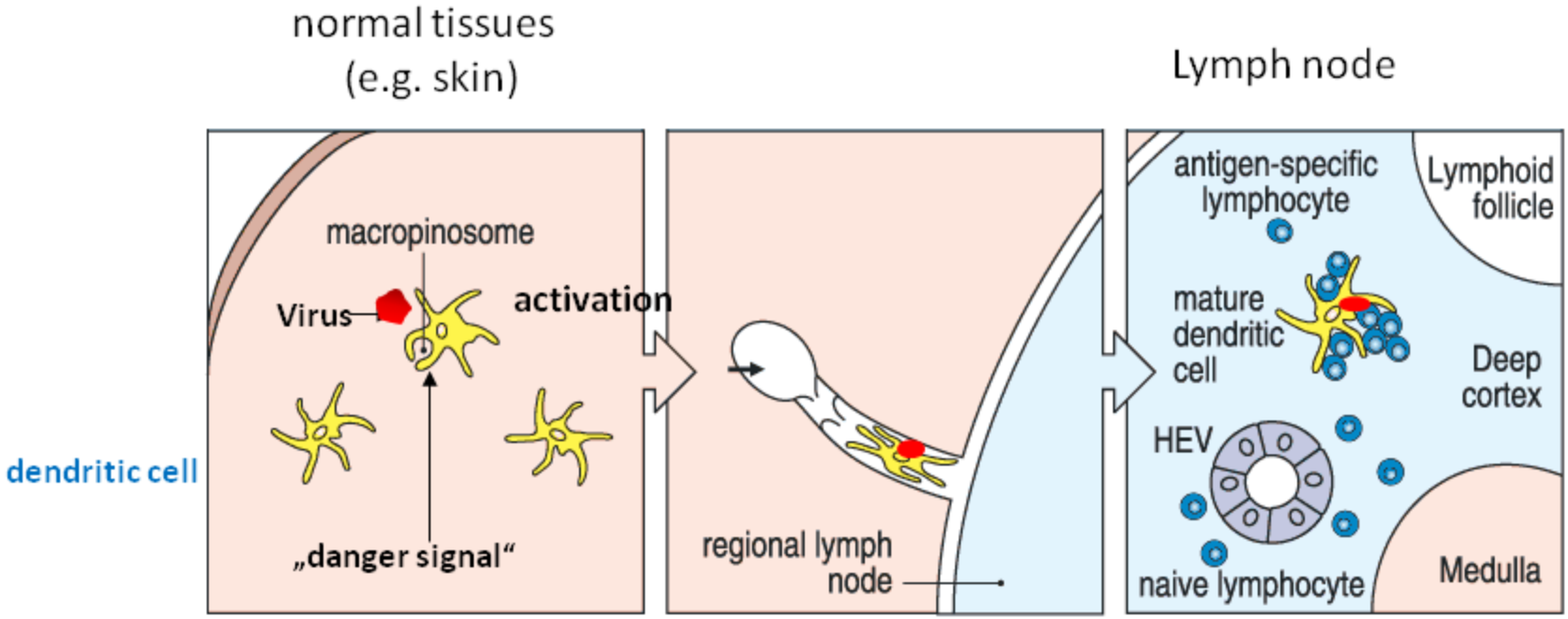
adapted from Janeway, Garlandscience pblsh.

Where is an immune response initiated?



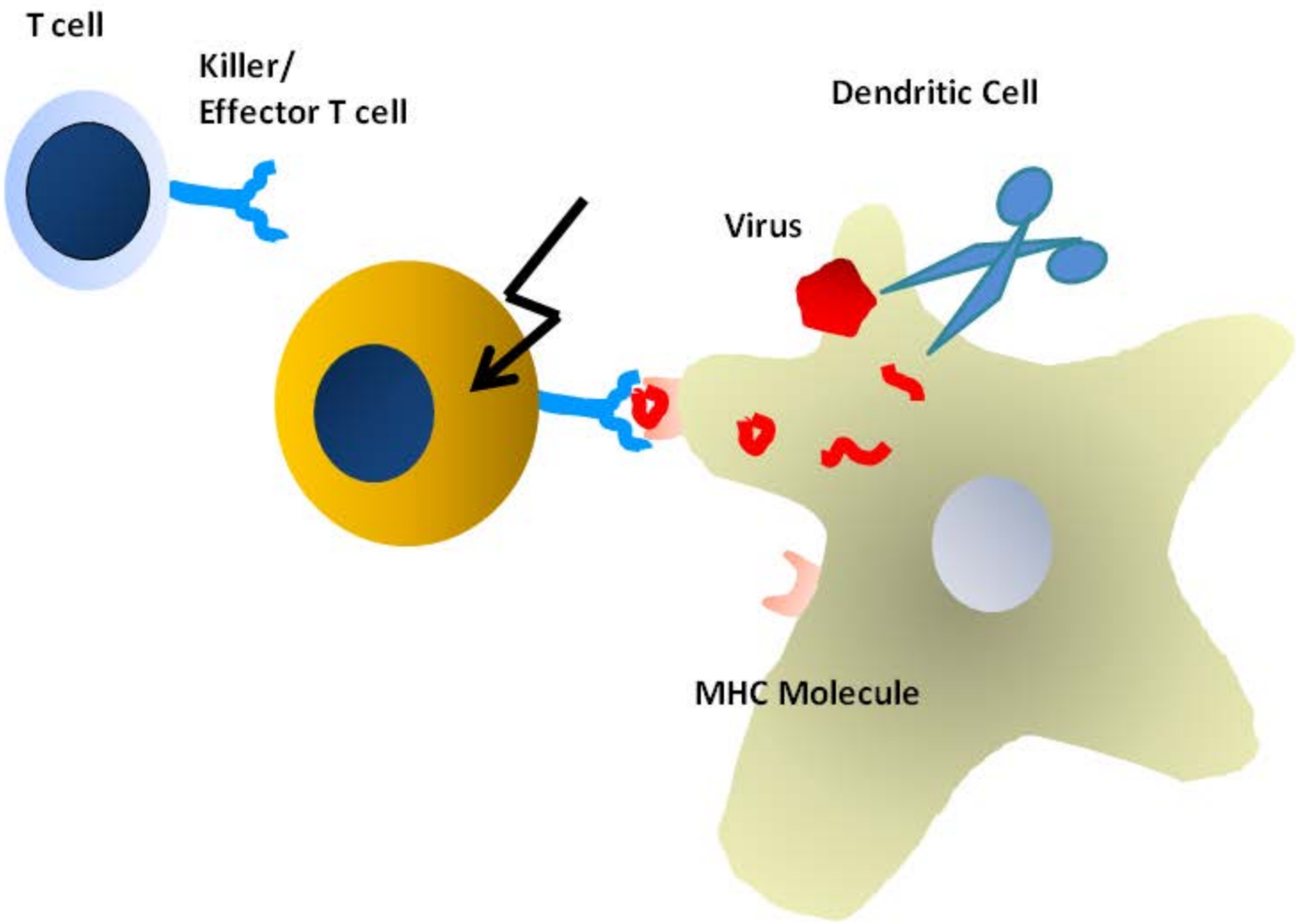
adapted from Janeway, Garlandscience pblsh.

What happens during an immune response?

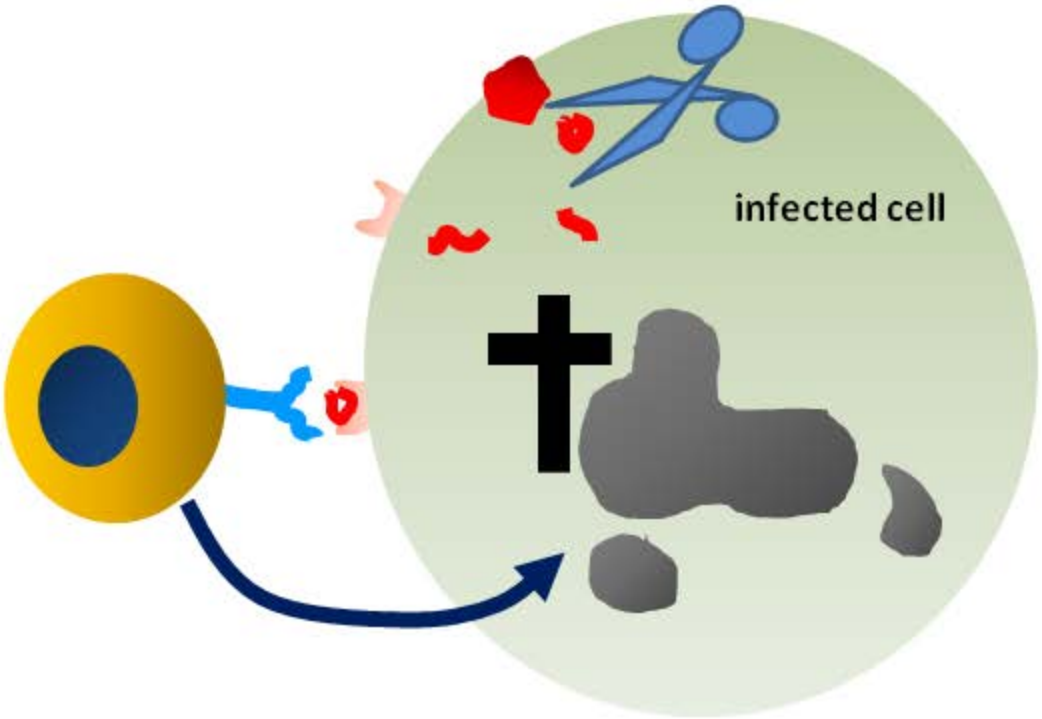


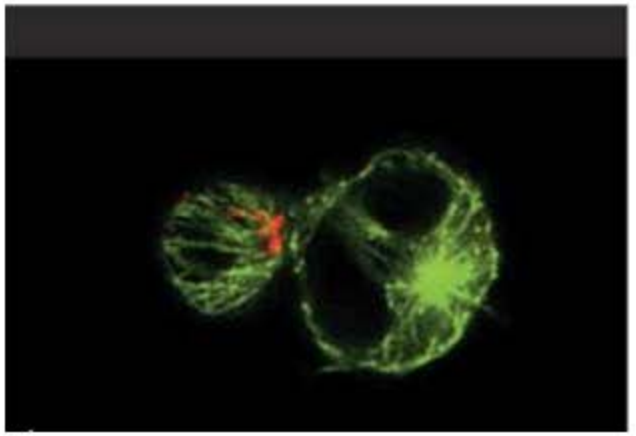
adapted from Janeway, Garlandscience pblsh.

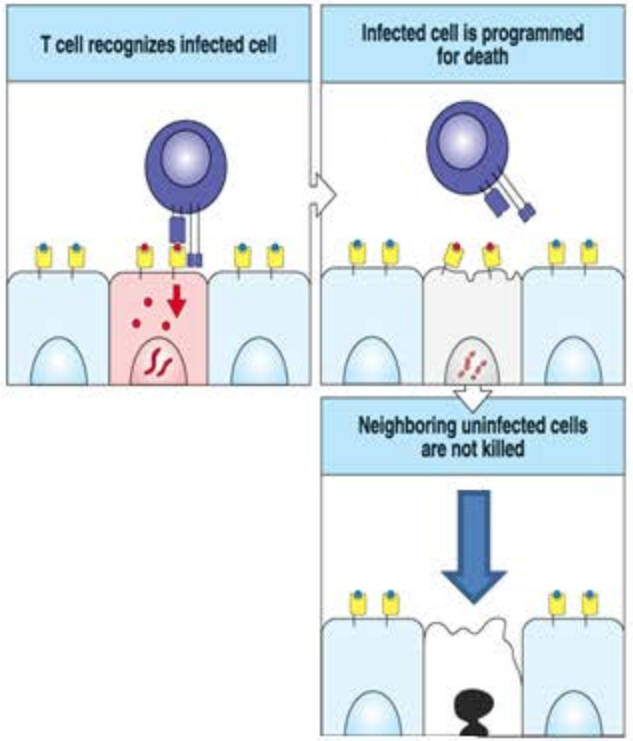
How does a T cell recognize an antigen?



How does a T cell recognize an antigen?



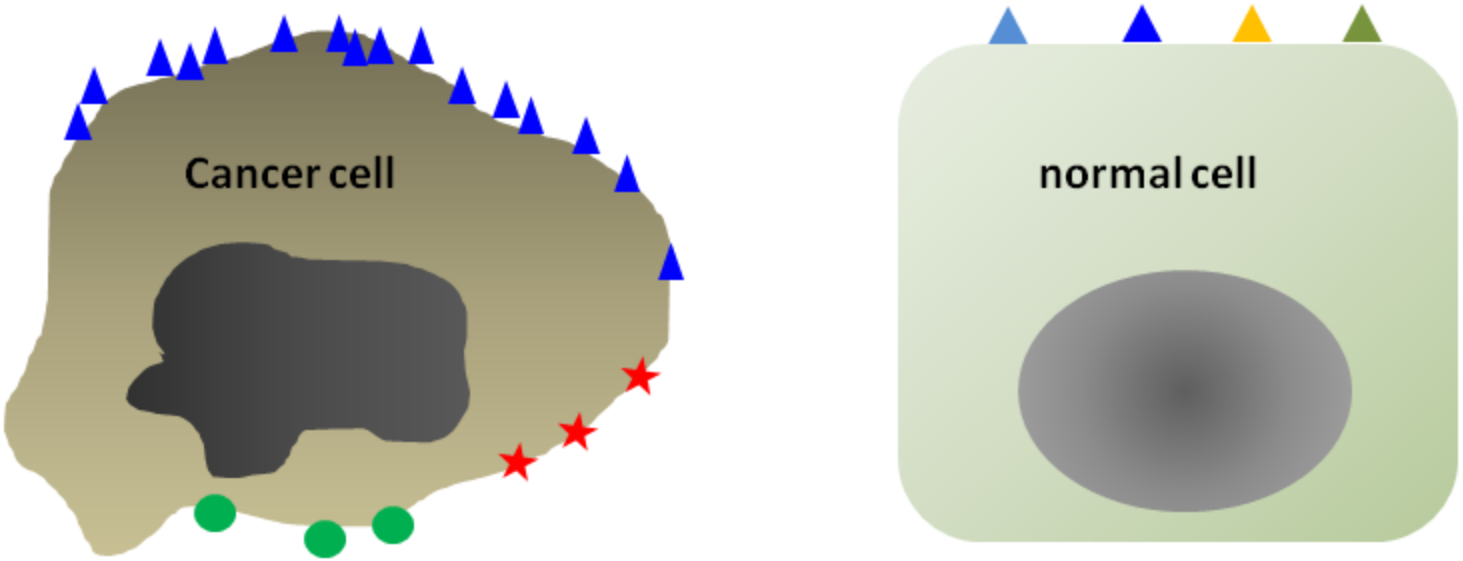




adapted from Janeway, Garlandscience pblsh.

Can the immune system influence tumor disease?

Immunologic target structures (antigens) on tumor cells

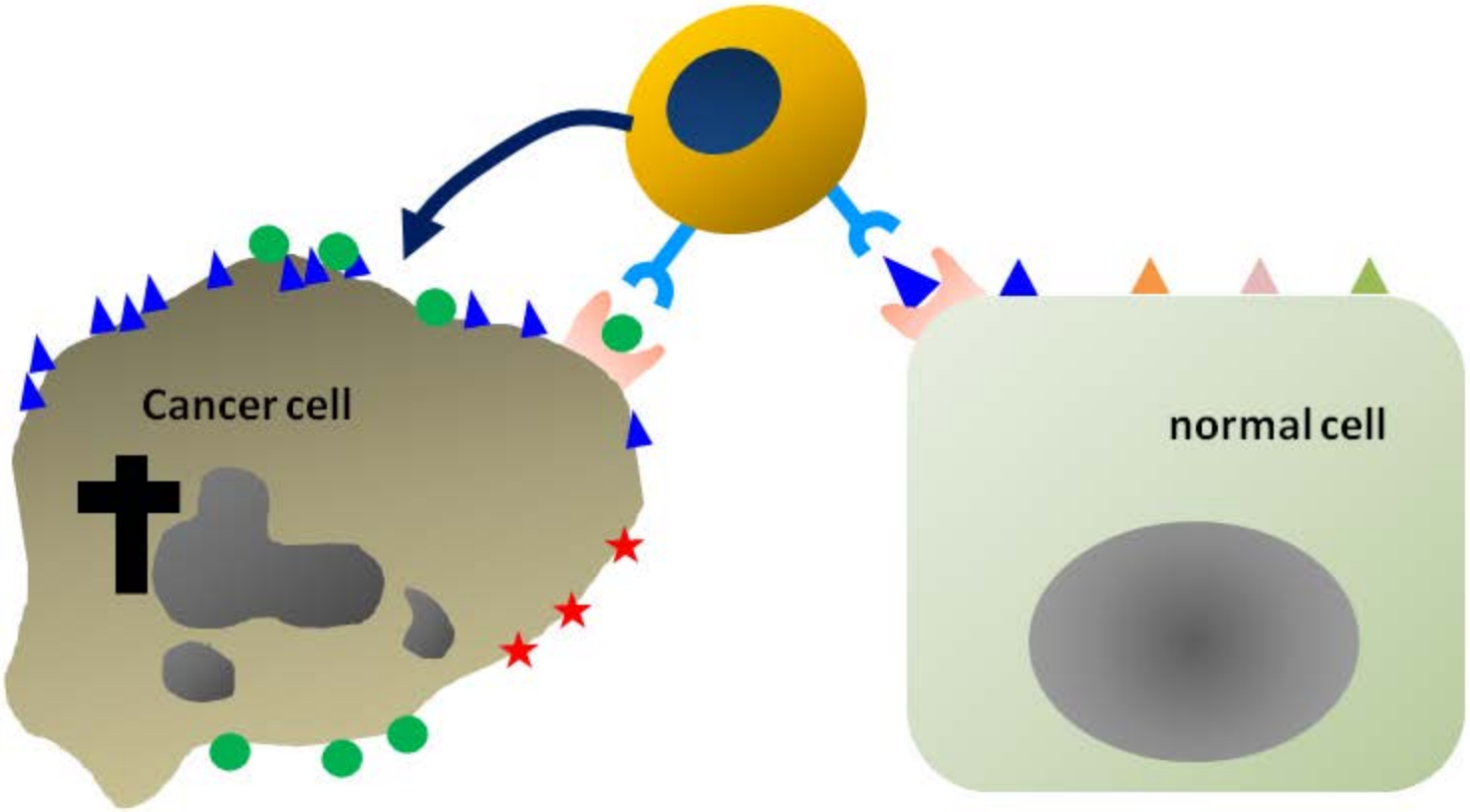


Classes of cancer antigens

- Over expression of „normal“ differentiation antigens („Tumormarker“)
- Mutations
- aberrant expression of embryonic or testicular Genes

- organspecific repertoire of differentiation antigens

Selective immune recognition of a tumor cell

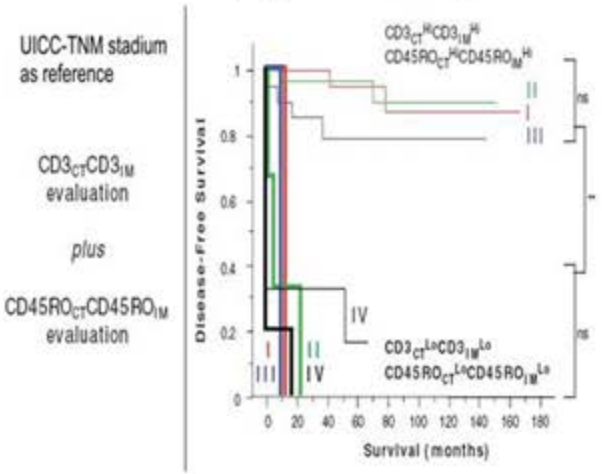


+ tissue damage associated „danger“ signals

Critical hurdles on the way to succesful cancer immunotherapy

Succesful Cancer Immunotherapy

Spontaneous T cell responses determine prognosis of CRC patients



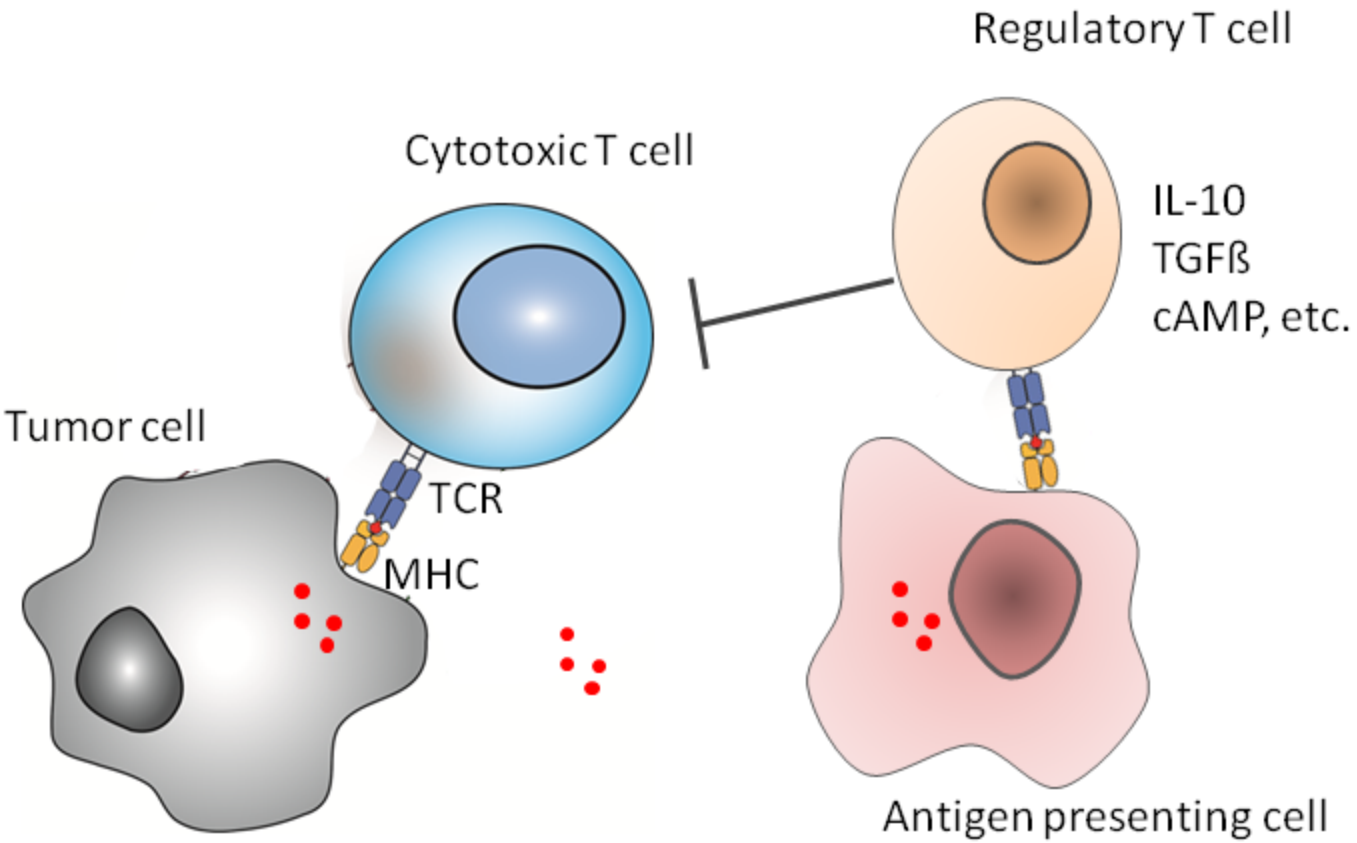
Galon et al., Science 2006, 313:1960-62

Hallmarks of cancer

Regulatorische T Zellen immunmodulatorische Liganden

„Innate" Inflammation TAM, myeloide Zellen

Hanahan et al. Cell. 2011 Mar 4;144(5):646-74



overexpressed antigens

-differentiation related

-Cancer testis

Neoantigens

-individual mutations

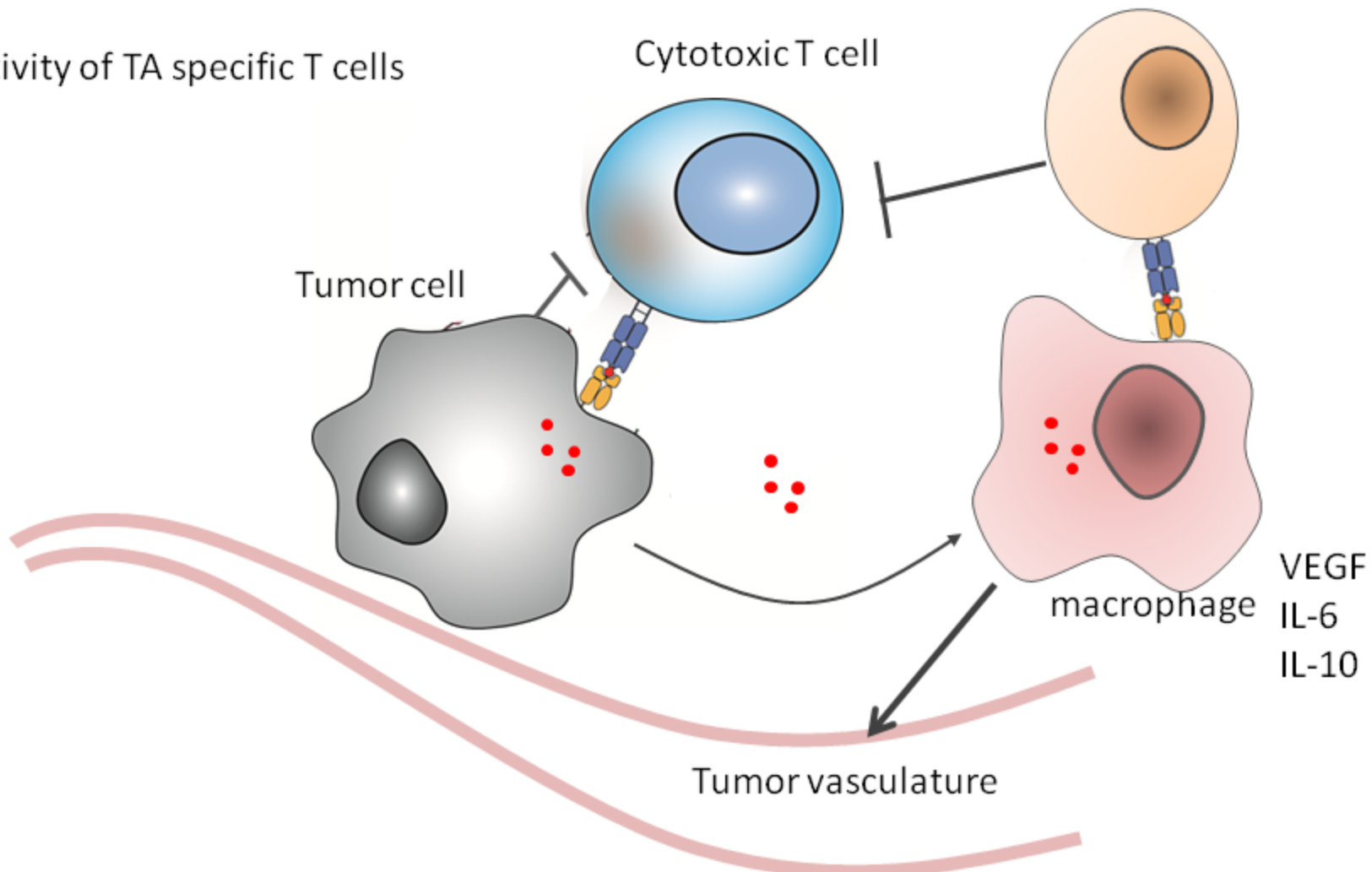
Prerequirements of succesful tumor immune surveillance

- Systemic tumor –specific Teff response

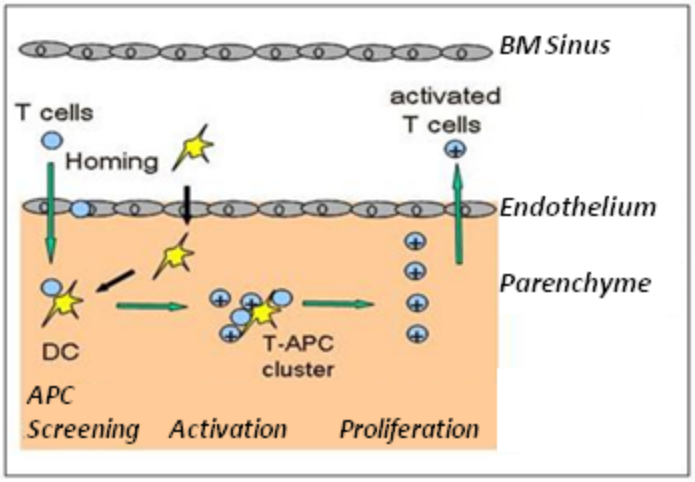
-Immigration of TA specific Teff into tumor tissue

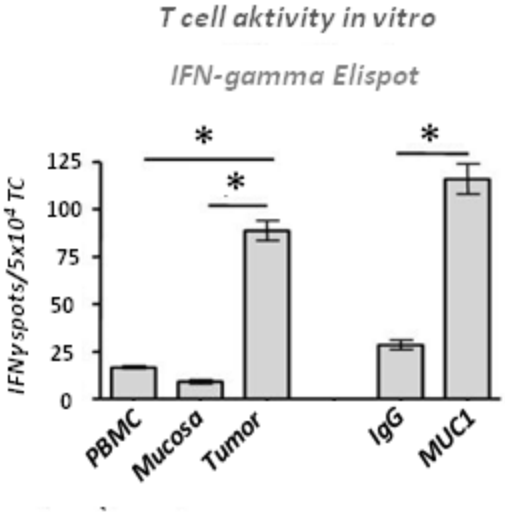
-In situ activity of TA specific T cells

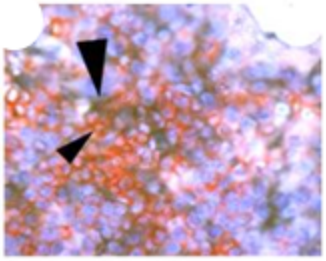
Tumor micromillieu

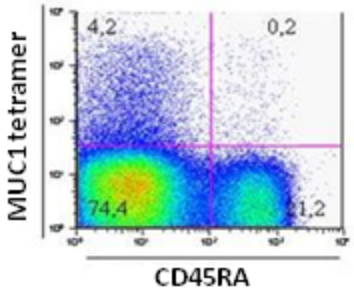


The bone marrow: a site for induction of tumor specific T cells









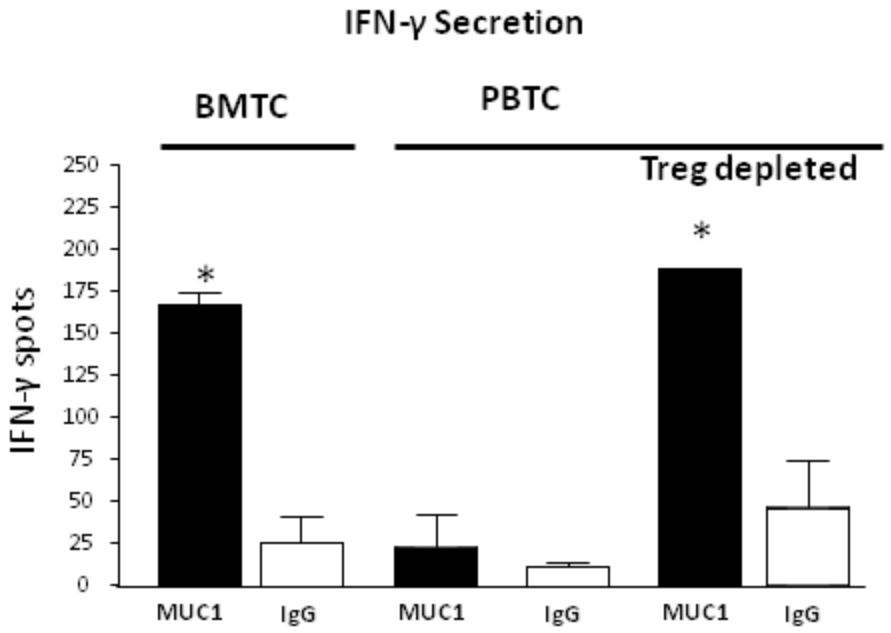
Feuerer, Beckhove et al., Nat. Med. 2001, 2003

Entity	*n*	*TA reactive (%)*
Breast Ca.	*450*	*60%*
Pancreatic Ca.	*180*	*80%*
HNSCC	*150*	*40%*
Colorektal Ca.	*250*	*40%*
Malignant Melanoma	*80*	*50%*
Multiple myeloma	*140*	*42%*
Glioblastoma	*250*	*38%*
	1500	*50%*

Beckhove et al. J.Clin. Invest., 2004, 114:67-76
Steiner et al., J.Clin. Oncol., 2004, 27:1141-9
Feuerer & Beckhove et al., Nat. Med. 2003:1151-7
Feuerer & Beckhove et al., Nat. Med. 2001:7:452-8

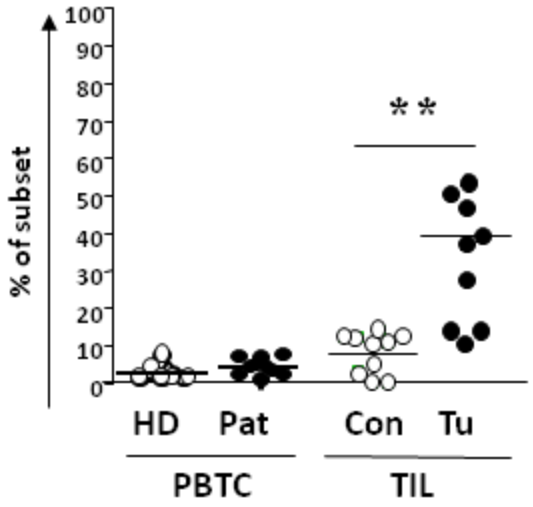
Schmitz-Winnenthal et al., Gastroenterology, 2010:138:1178-88
Domschke et al. Cancer Res., 2009, 69:8420-8
Schmitz-Winnenthal et al., Cancer Res., 2006:65:10079-87
Choi et al., Blood, 2005, 105:2132-4

The Bone Marrow: a Site for Induction of Tumor Specific T-Cell Responses



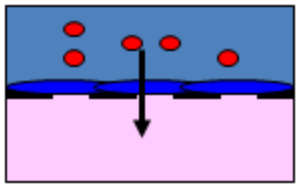
Feuerer, Beckhove et al., Nat. Med. 2001, 2003
Beckhove et al. JCI 2004

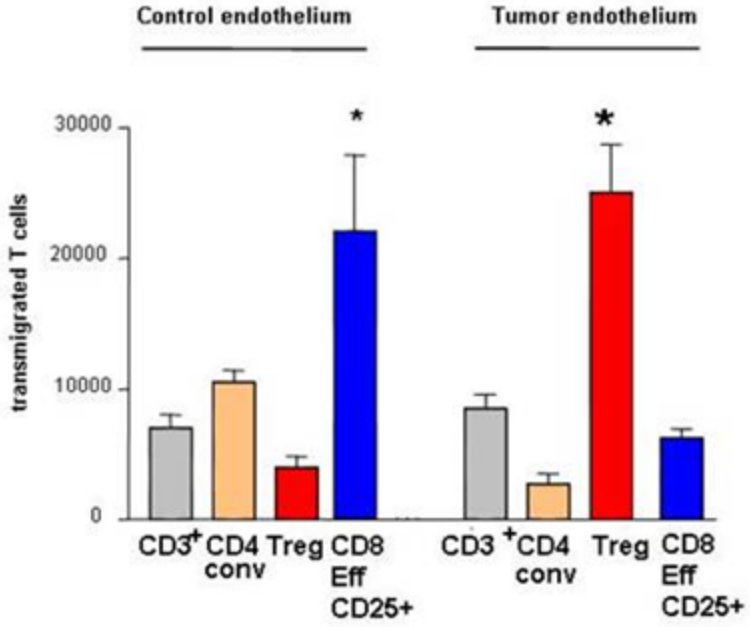
Enrichment of Treg in Pancreatic cancer



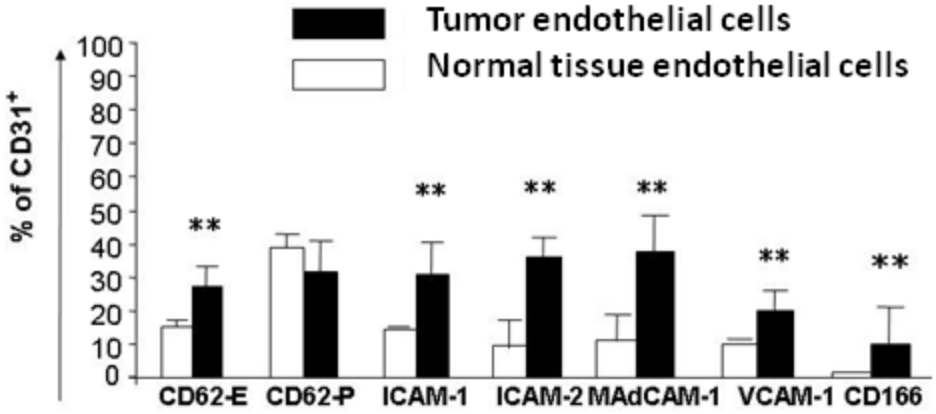
Selective Treg transmigration through tumour endothelium

T-cell transmigration assay





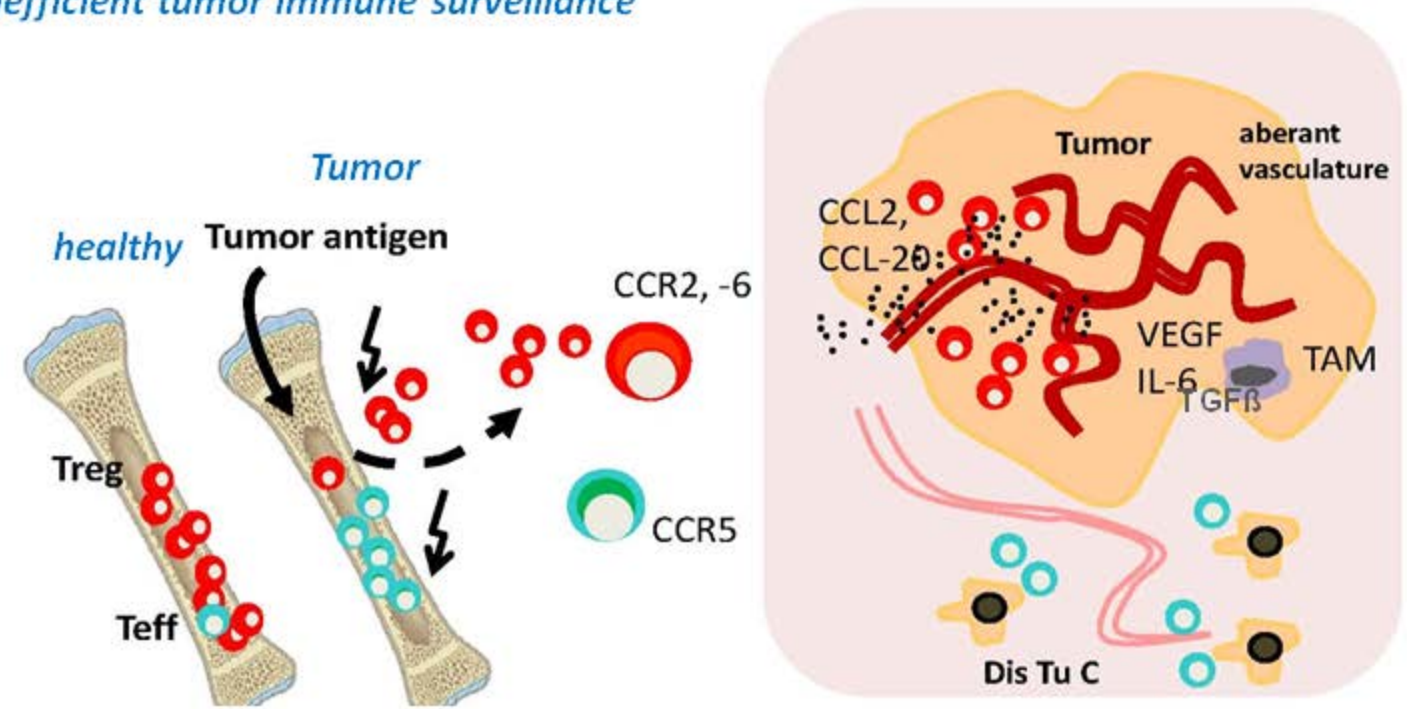
Increased adressin expression in tumour endothelial cells



Nummer et al., JNCI 2007

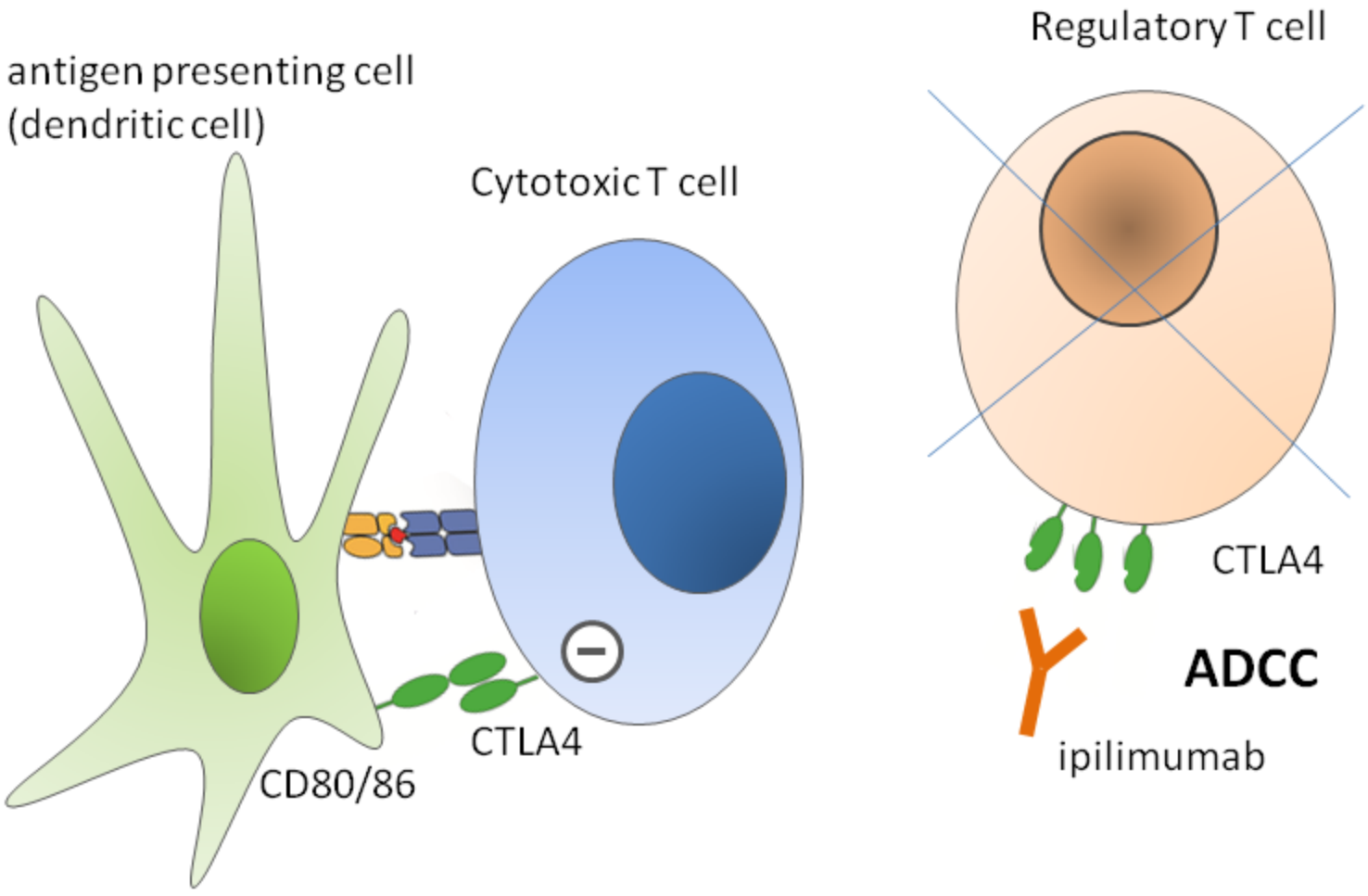
Spontanous T cell response at the expense of Treg enrichment in tumors

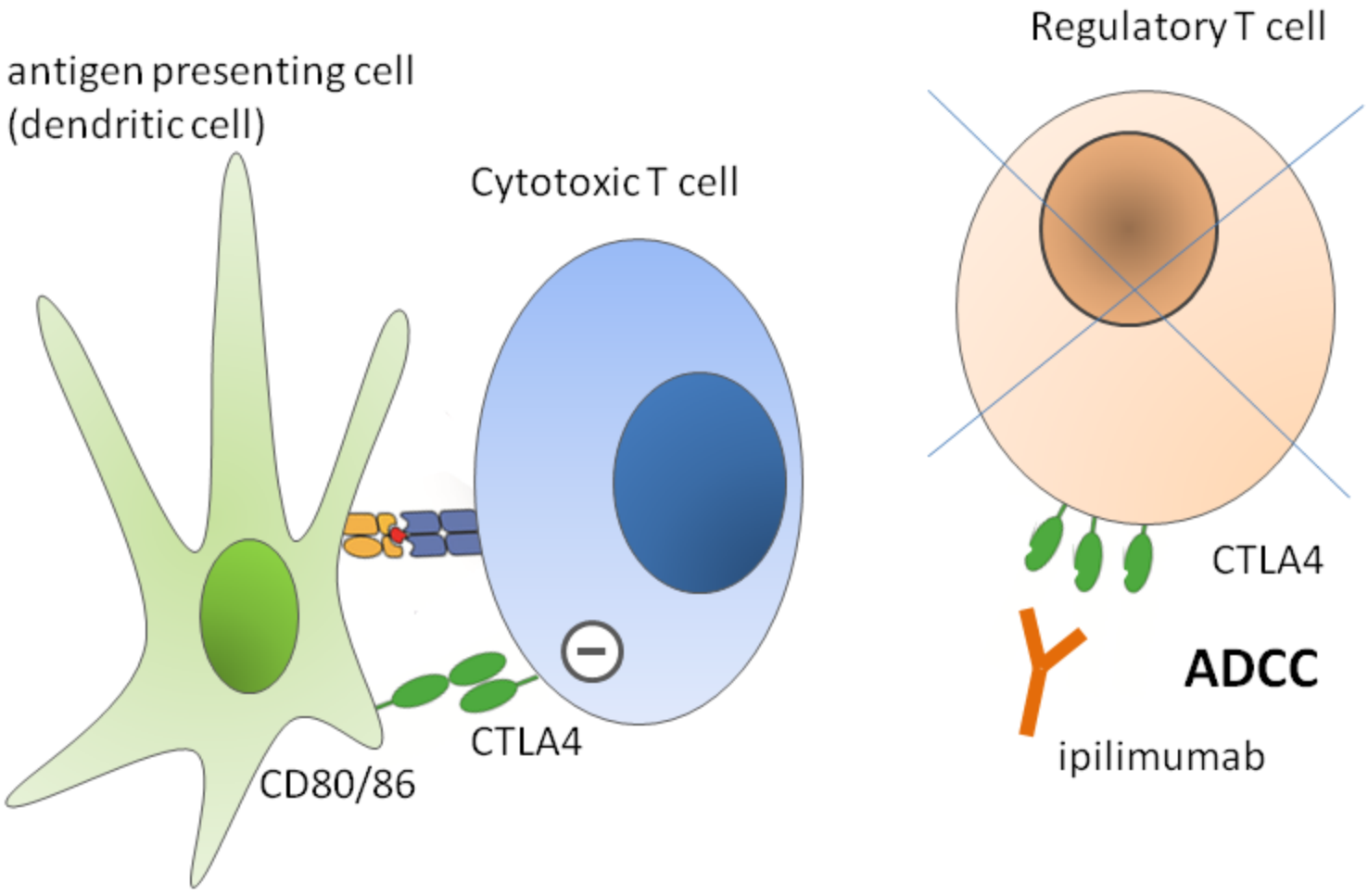
Sequential activation of Treg and Teff in the BM orchestrates inefficient tumor immune surveillance



Bonertz et al. J.Clin.Invest. 2009, 119:3311-21

Klug et al., Cancer Cell, 2013
Schmidt et al., Oncoimmunology, 2013
Bonertz et al. J. Clin. Invest. 2009
Nummer et al., J.N.C.I., 2007


antigen presenting cell
(dendritic cell)
Cytotoxic T cell
CTLA4
CD80/86
Regulatory T cell
CTLA4
ADCC
ipilimumab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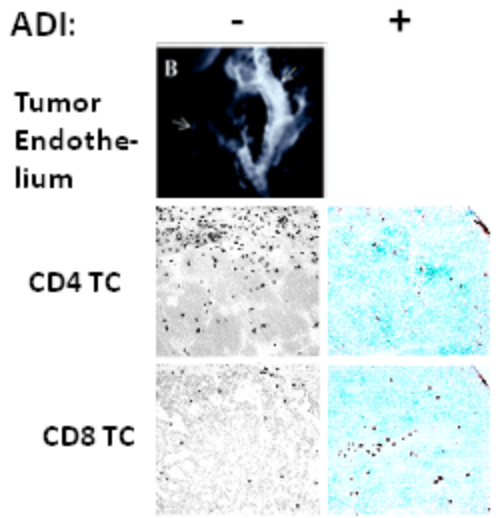
Local Low Dose Irradiation Increases Effector T Cell Infiltration through INOS+ Macrophages

Objective: Modify Tumour Vasculature to Prevent T Cell Recruitment and Improve Teff Immigration

The riptag-5 mouse model of spontaneous insulinoma- a model of vascular anergy

...based on tg rat insulin promotor driven oncogene SV40 large T antigen

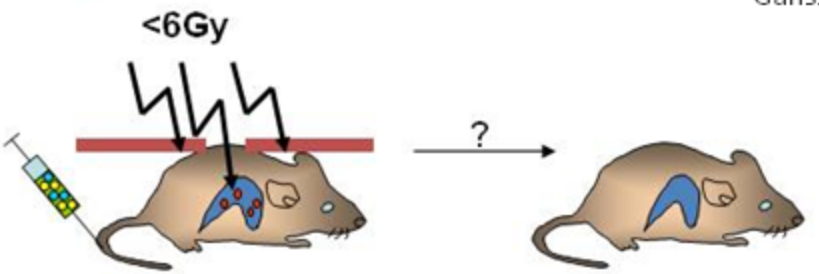


Ganss et al., Cancer Res., 2002

RIP-Tag mouse model of spontaneous insulinoma

Local low dose irradiation

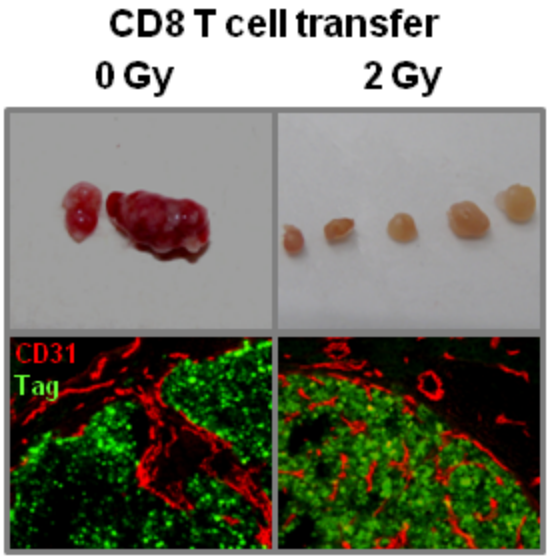
Tumor specific TCRtg donor T cells

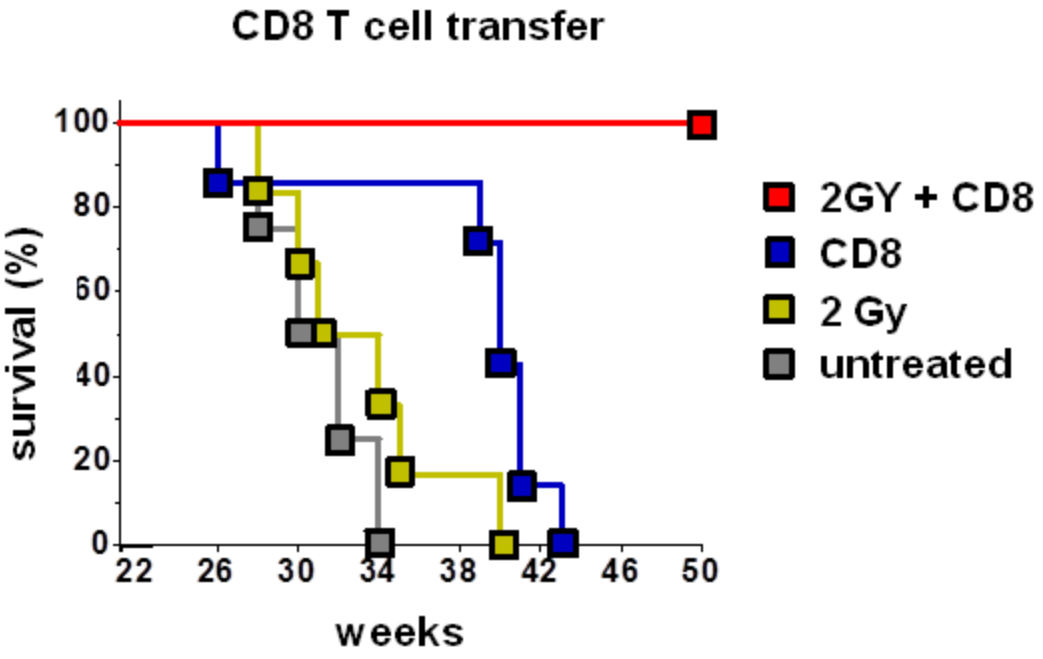


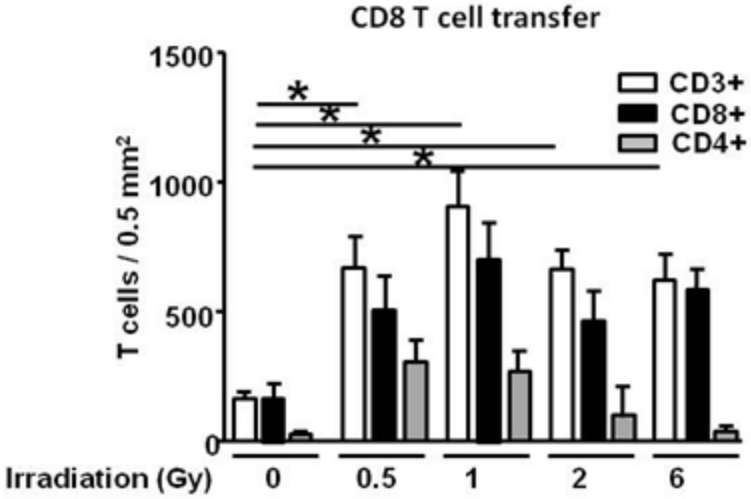
Large T antigen spec. T cells

Local Low Dose Irradiation Increases Effector T Cell Infiltration through INOS+ Macrophages

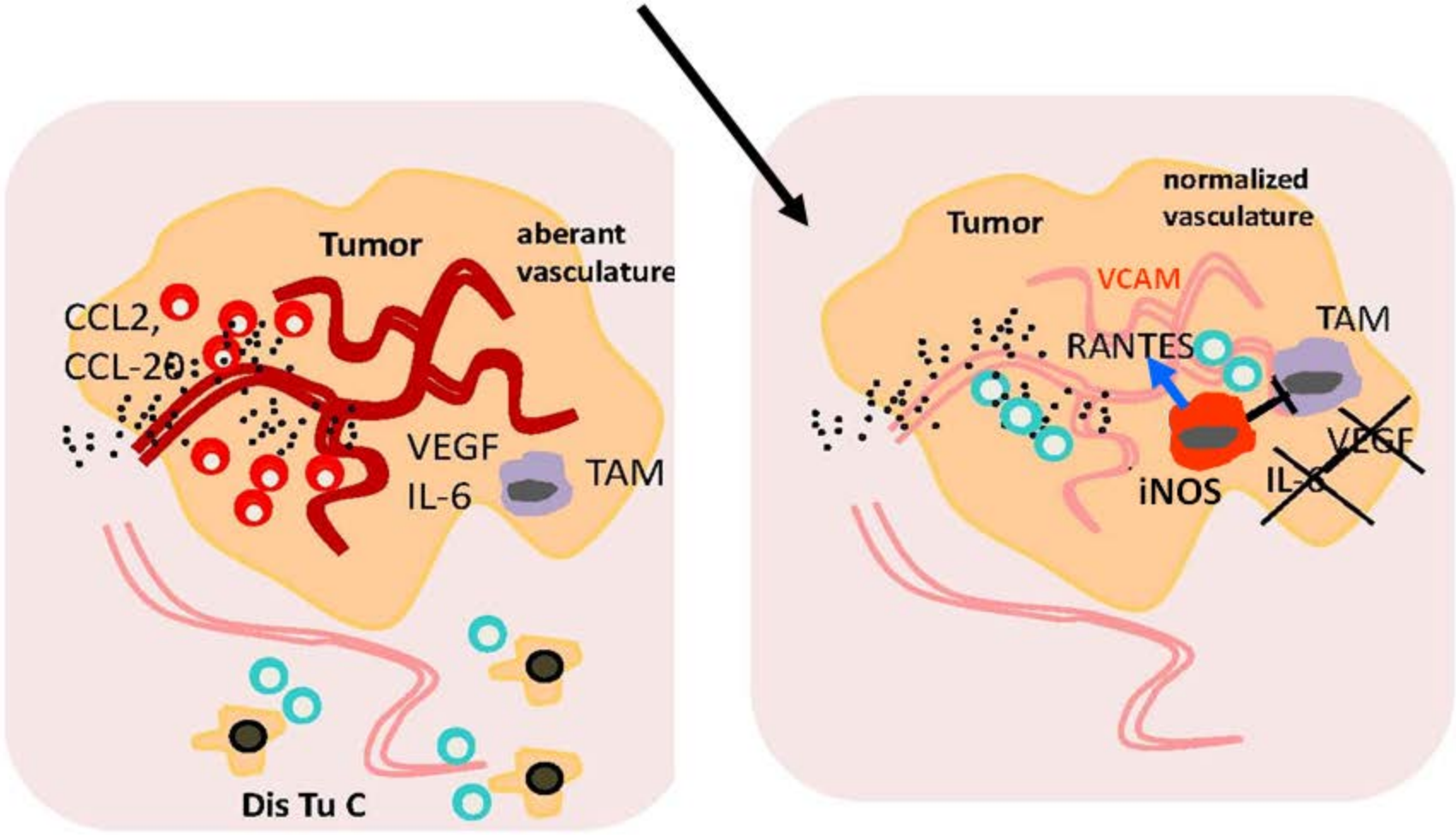
Increased T cell recruitment after local low dose irradiation







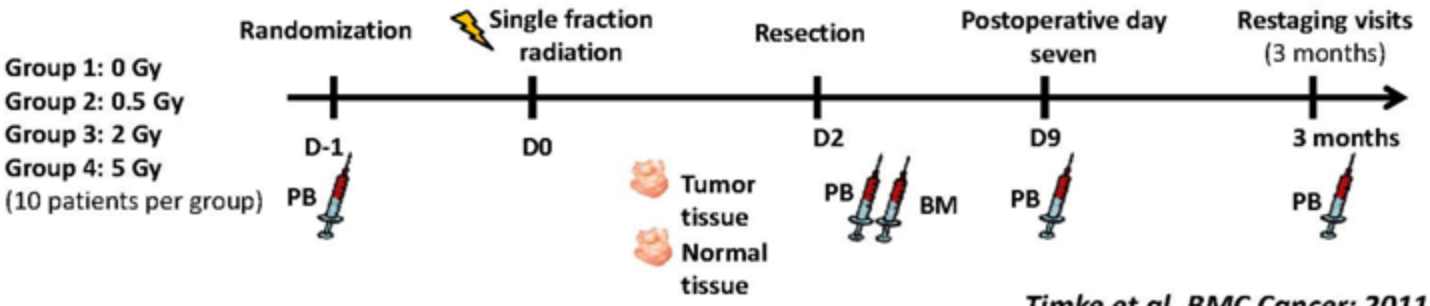
Low dose irradiation



Two clinical trials launched in 2010

2 Randomized controlled phase I/II studies to investigate T cell infiltration after neoadjuvant local low dose radiotherapy in

- **Locally advanced operable pancreatic cancer**
- **Single, operable liver metastases of colorectal cancer**



Timke et al. BMC Cancer; 2011;11:134.

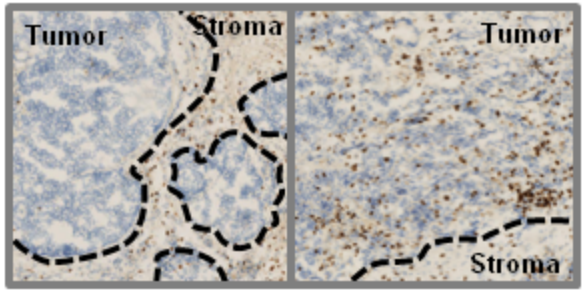
Automated full slide imaging:
Niels Halama & Dirk Jäger,
NCT, Heidelberg

Local Low Dose Irradiation Increases Effector T Cell Infiltration in human tumors

Pancreatic Cancer (PT)

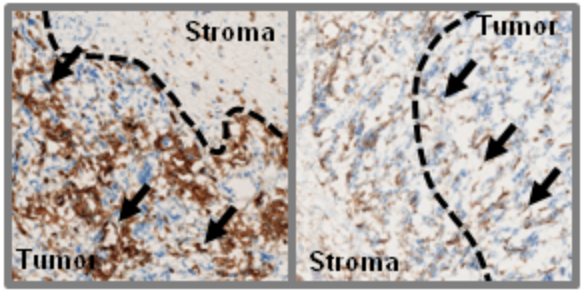
CD8 T cell infiltration

non-irradiated | irradiated

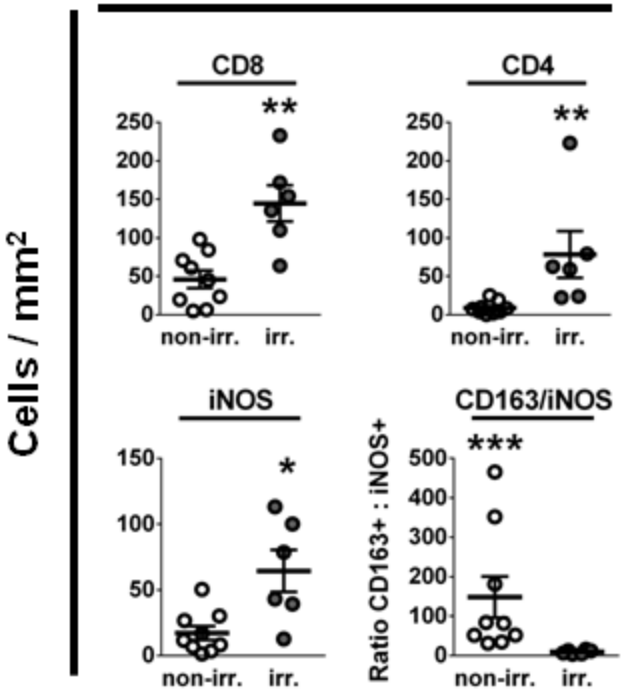


Vessel phenotype (CD31)

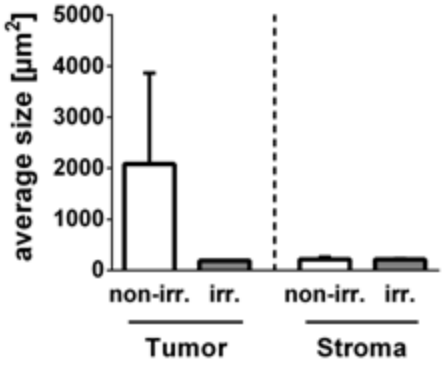
non-irradiated | irradiated



intraepithelial cells

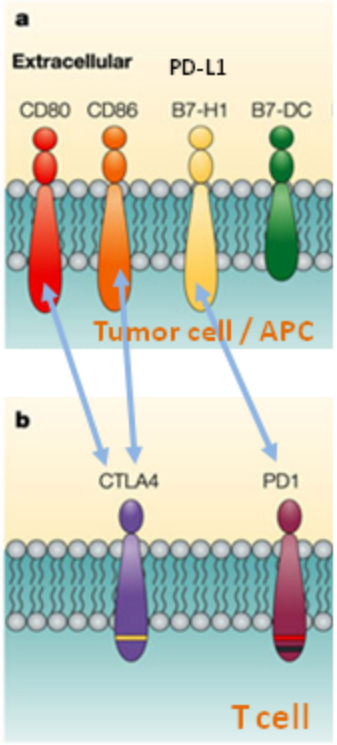


CD31+ vessel phenotype vessel size



CTL-A4 and PD1/PDL-1 are major targets for cancer immunotherapy

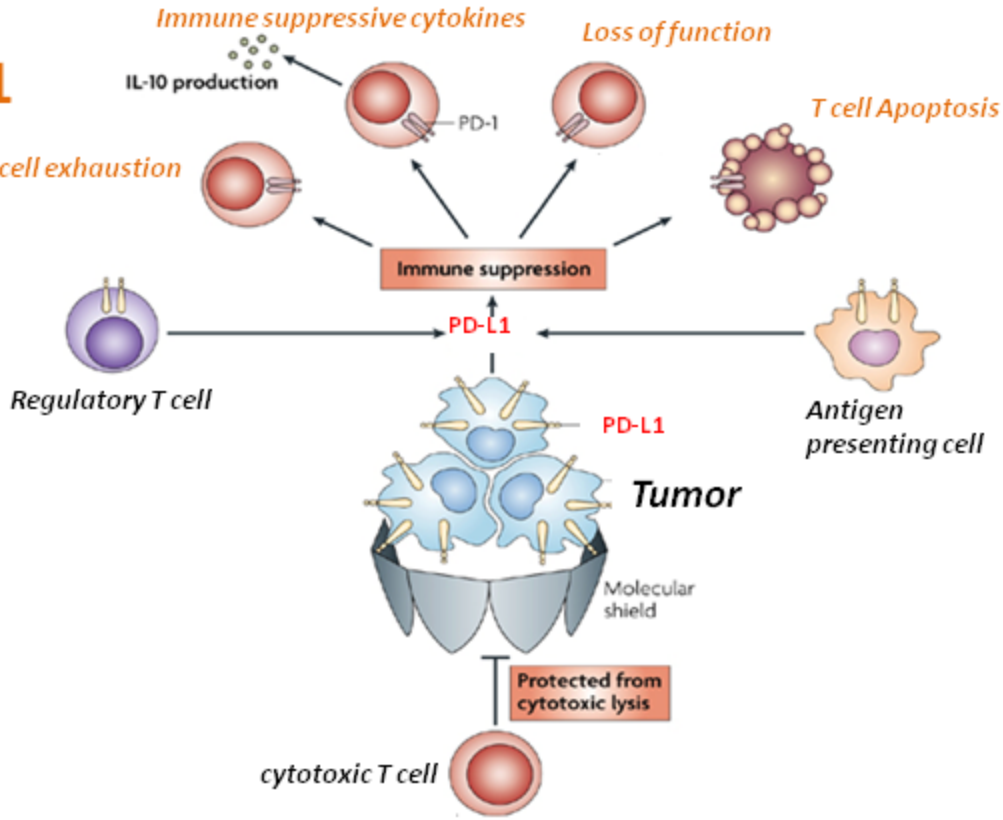
B7 family of immune modulatory molecules and their inhibitory receptors on T cells



Immune modulatory ligands

Inhibitory receptors

PD-L1



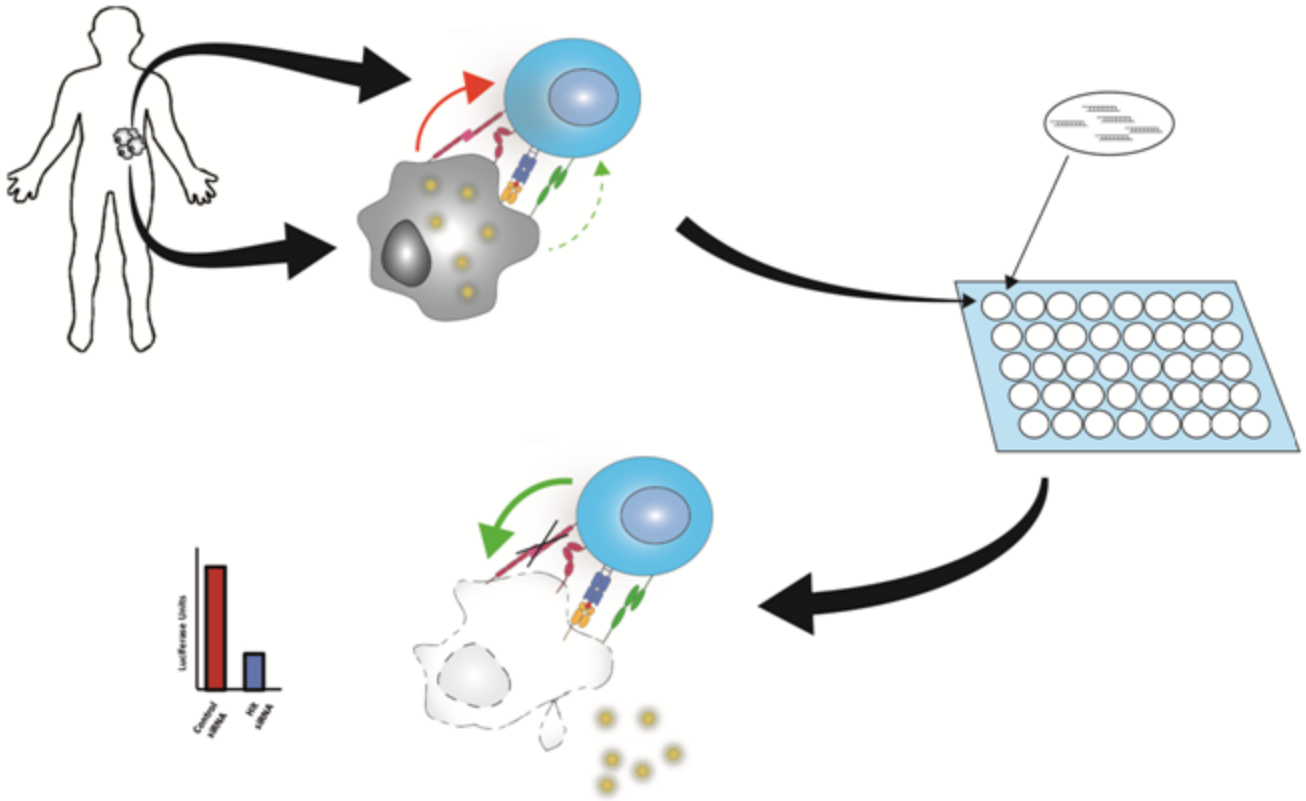
PDL1 not expressed on all tumors
treatment failure despite PDL1 expression

Are there more immune regulatory ligands?

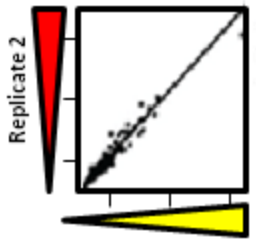
Ligand or receptor inhibition through blocking antibodies restores T cell activity against tumor cells

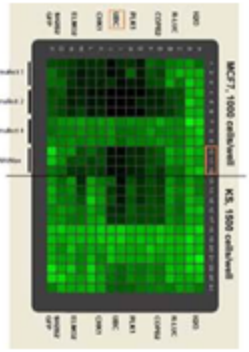
adapted from Nature Reviews Immunology

High-throughput RNAi screen with syngenic TILs and melanoma cells;
1.500 genes



Data Analysis





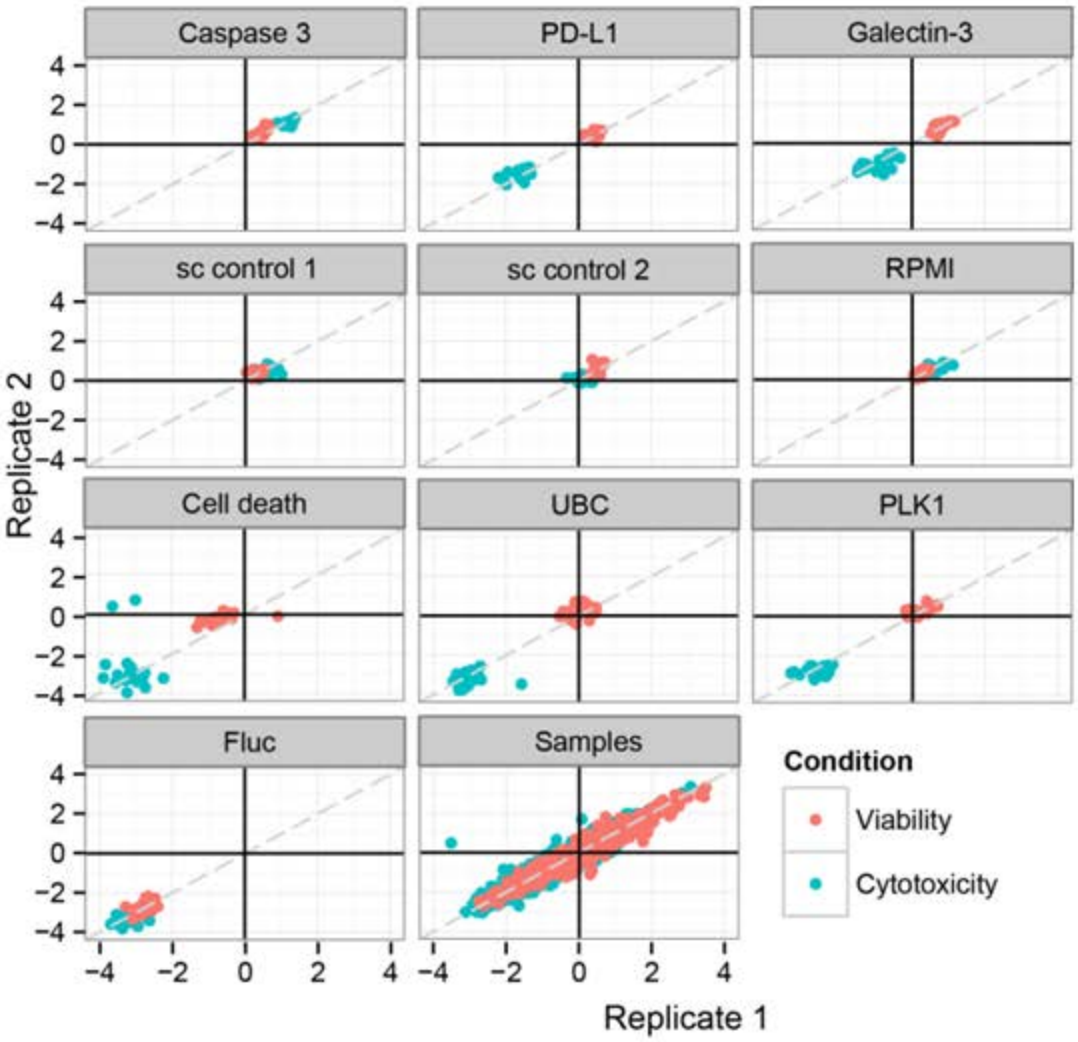
Hit Selection

Candidate Validation:

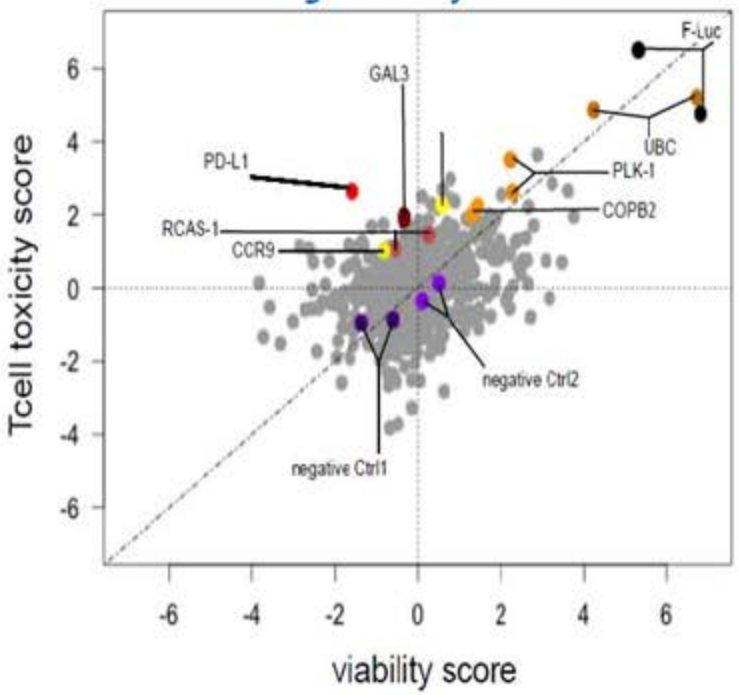
Re-tests

Secondary Assays

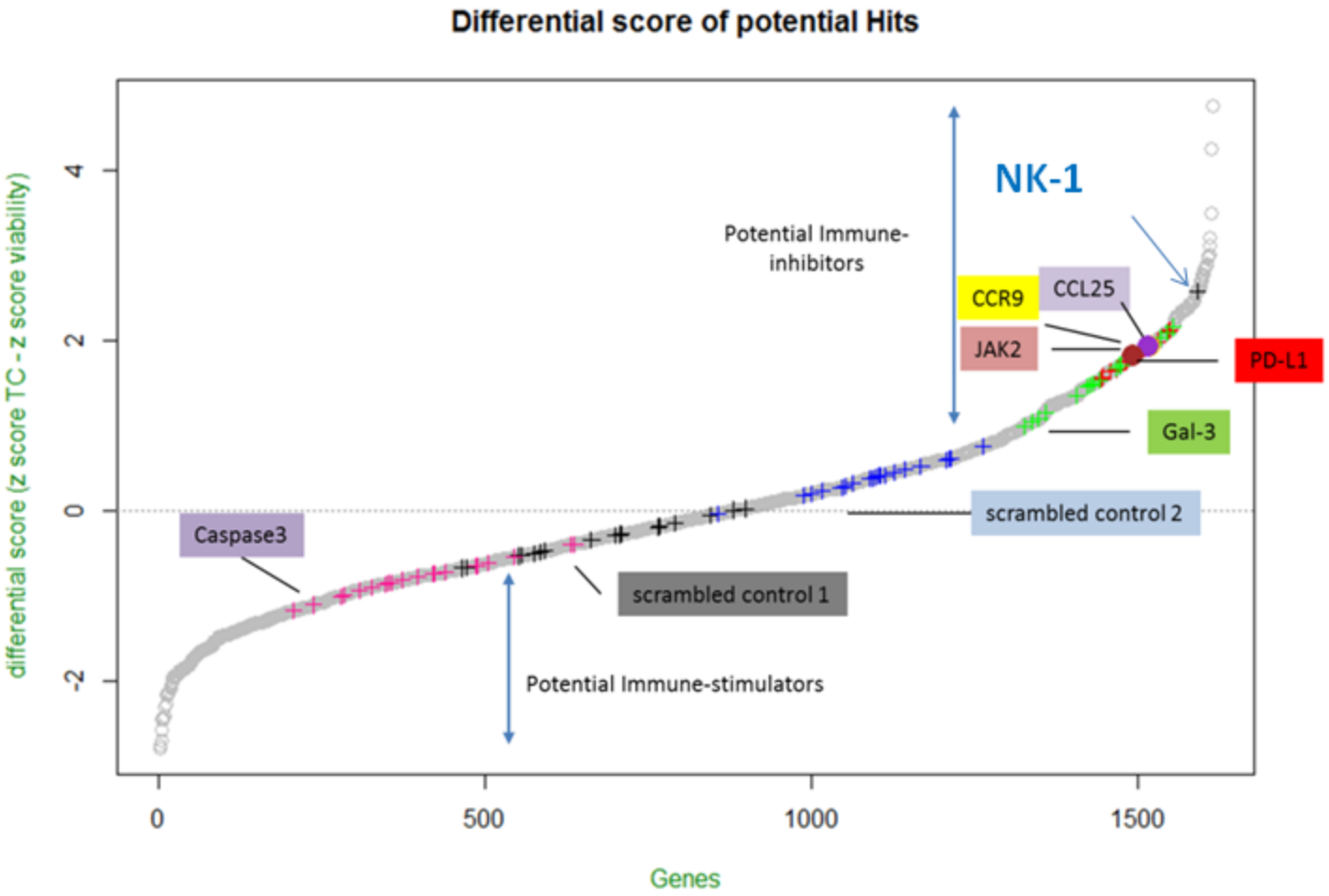
Screen performance



Target Identifikation

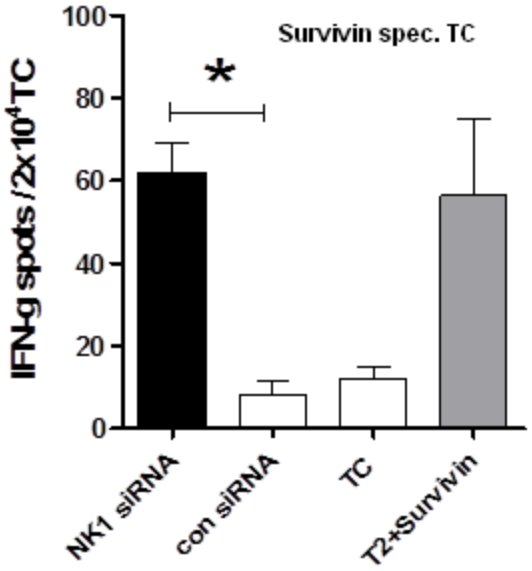


- Spearman correlation for cytotoxicity = 0.92, for viability = 0.96

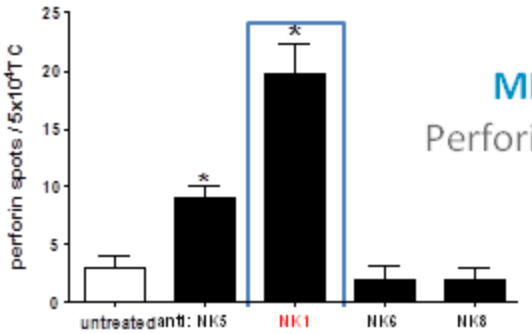


- Low z-score: Potential immune-stimulatory molecule
- High z-score: Potential immune-inhibitory molecule

NK1 Blockade Leads to a Reactivation of T-Cells *In Vitro*



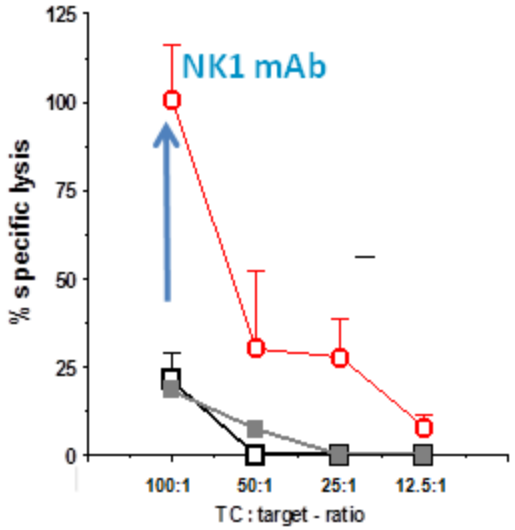
autolog. MM cells + **Pat-derived CD8⁺ T-cells**



MM cells

Perforin (ELISpot)

MCF-7-C6 cells **+ Her2/neu-specific T-cells**



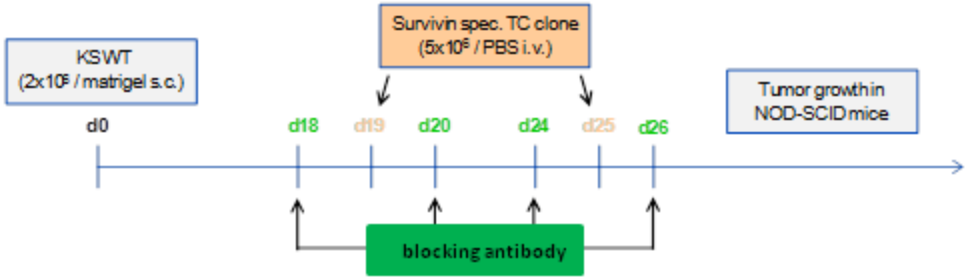
Breast cancer cells

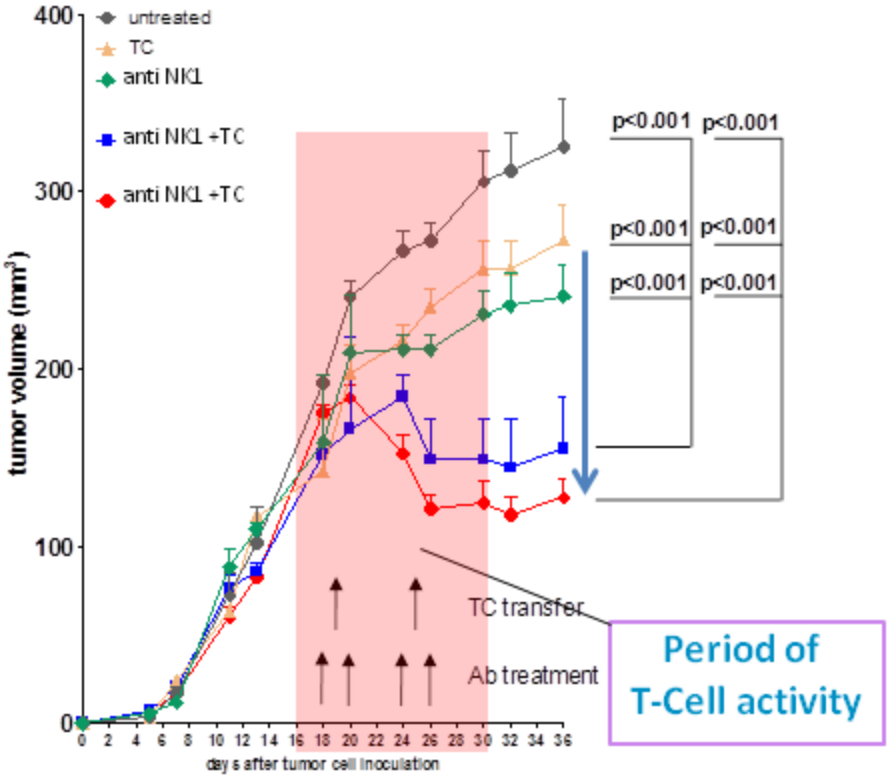
Tumor cell lysis

(^{51}Cr kill assay)

NK1 blockade induces tumor Growth Inhibition *In Vivo*

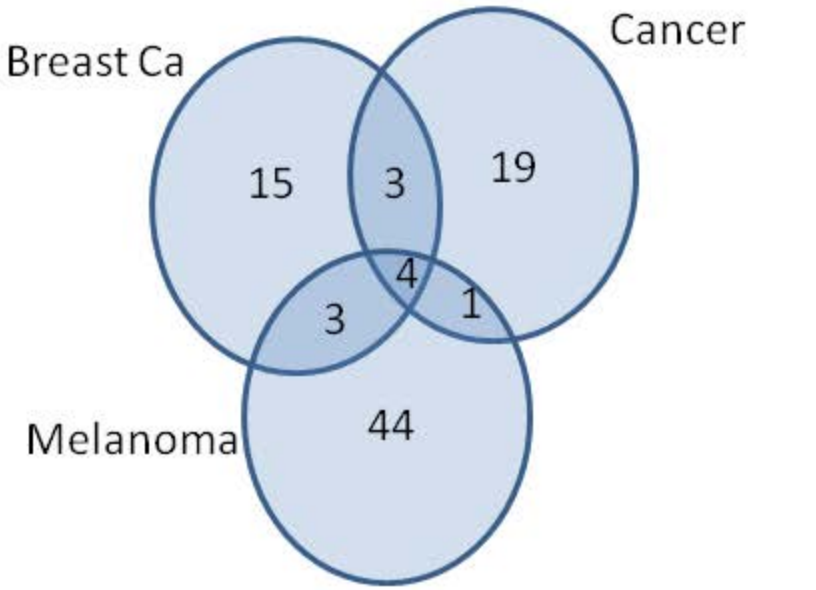
- NOD-SCID mice
- Human KS breast cancer cells
- anti-NK1 mAb
- Adoptive transfer of human survivin-specific T-cells

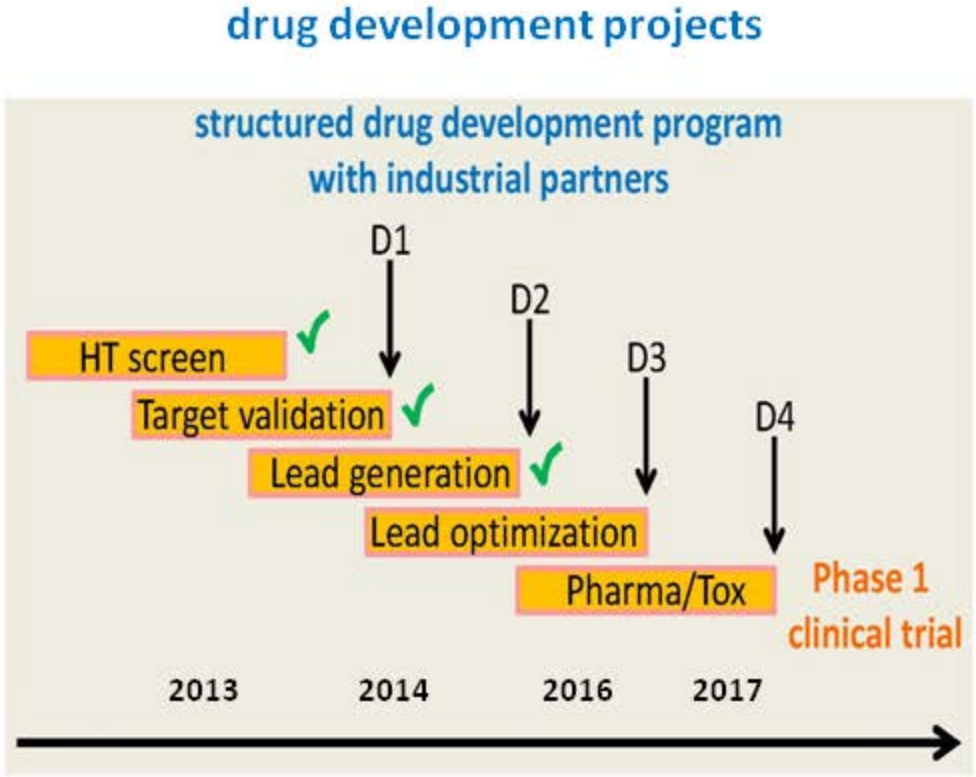




Total hits (of 1.500 gene library)

Breast cancer	melanoma	pancreatic cancer
25 candidates	52 candidates	27 candidates





Conclusions

Succesful cancer immunotherapy requires intervention at several checkpoints that need to be adapted according to an individuum`s immune constitution

- systemic Induction of T cell response (e.g. Treg depletion, vaccine, T cell transfer)

- succesful targeting of T cells into tumor tissue (conversion of anergic tumor vasculature)

- increase of T cell activity in situ (immune check point blockade, Treg depletion)

The immune system exploits multiple immune regulatory ligands on organ cells in order to evade autoimmune disorders

-In the context of cancer therapy these impose considerable complexity
and need for personalized combinations according to individual expression patterns