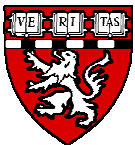
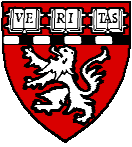


# Engineered T cells: Next-generation cancer immunotherapy

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# Outline



## ■ Introduction

- Adoptive T cell therapy: Engineered CAR-T cells
- Review of recent clinical trials
- Challenges of CAR T cell therapy

## ■ Results

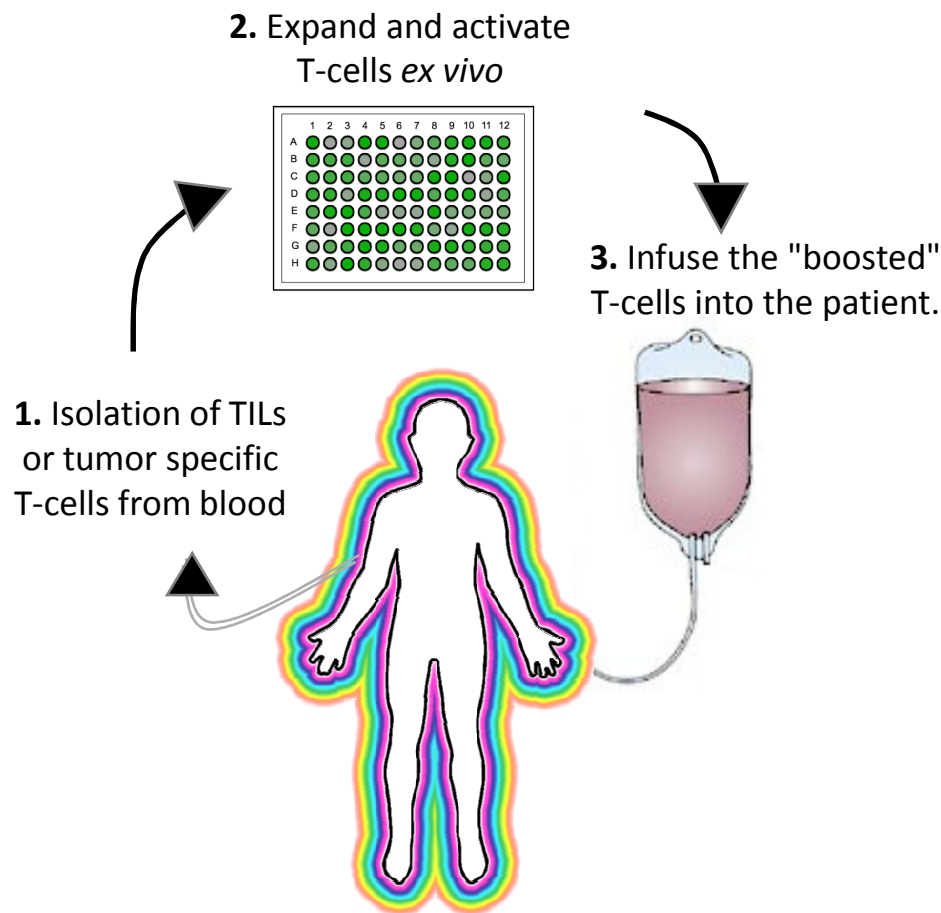
- T cell subsets: Th17 and Th9 cells in cancer immunotherapy

## ■ Summary

- Adoptive T cell therapy: what we learnt and what we should do next



# Overview: Adoptive T cell therapy



## ■ Target therapy with Tumor specific T cells

- Cancer: Melanoma
- Autologous tumor infiltrating lymphocytes (TILs); "Live drug"

## ■ Advantages

- High response rate (>50%),
- Long-term remission,
- Less toxic & gentler to the patient

## ■ Limitation:

- Extraction of TILs,
- Cell manufacturing

## ■ Possible alternate

- T cell Engineering (CAR-T cells)

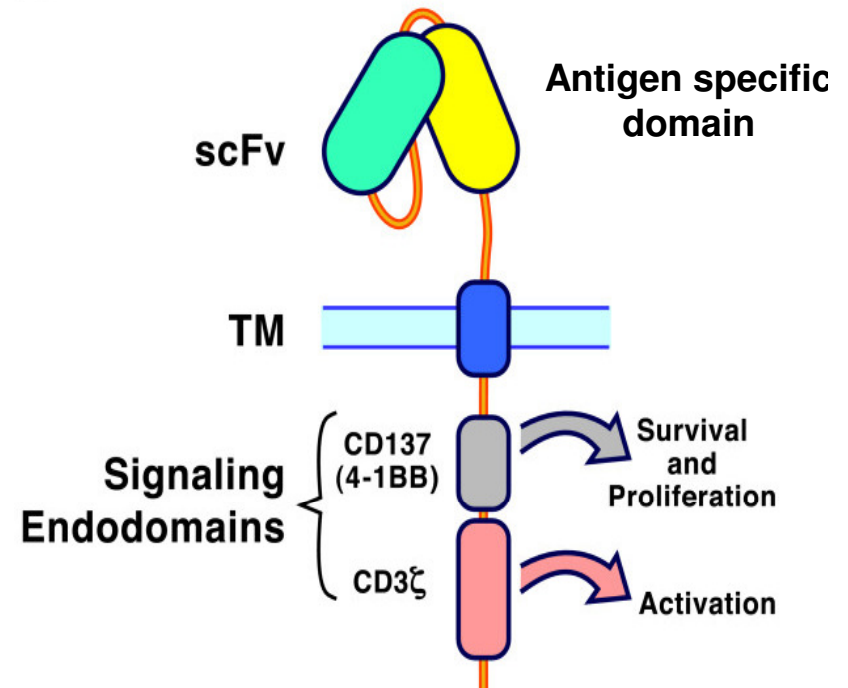


# Adoptive T cell therapy: CAR-T cells



## ■ CAR-T cells (Chimeric antigen receptor-T cells)

- T cells transduced with tumor-specific CAR
- CAR: Single fusion molecule with antigen specificity plus signaling domain
- Three types of CAR: First/second/generations
  - Based on co-stimulatory receptors
- Cancer: Solid tumor & hematological malignancies



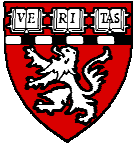
### Advantages of CAR T cells

“Live drug”

Tumor recognition independent of HLA (no HLA typing needed)

Multiple anti-tumor immunomodulators can be engineered

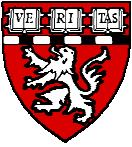
Target variety of antigens (protein, carbohydrate, glycolipid)



# Clinical significance of CAR-T cells



Target	CAR	Cancer	Objective response
CD19	CAR:CD28-CD3 $\zeta$	Lymphoma and CLL	N=7: 1CR, 5 PR & 1SD
	CAR:CD137-CD3 $\zeta$	ALL	2CR
	CAR:CD28-CD3 $\zeta$	ALL	5CR
CD20	CAR:CD137-CD28-CD3 $\zeta$	NHL	N=3: 1PR, 2NED
CEA	CAR-CD3 $\zeta$ (1 <sup>st</sup> gen)	Colorectal & breast	N=7: minor responses in two patients
GD2	CAR-CD3 $\zeta$ (1 <sup>st</sup> gen)	Neuroblastoma	N=19: 3CR
ERBB2	CAR:CD28-CD137-CD3 $\zeta$	Colorectal cancer	N=1, patient died



# Challenges of CAR-T cells



## ■ Toxicities

### — On target/off tumor toxicities

- Metastatic colon cancer patient died after 5 days of infusion of ERBB2+CAR-T cells
  - Low levels of ERBB2 express on lung epithelium (lung tox)
- Renal cell carcinoma: 5/11 patients developed liver toxicity

### — Cytokine syndrome

- Elevated levels of pro-inflammatory cytokines
  - Treatable by anti-IL-6mAb and steroids



# Determinants of successful ACT: CAR-T cells



## ■ Tumor target

- Target antigen is critical determinant for efficacy & safety
- Ideal target uniquely express on tumor cells or on cells which are not essential for survival

## ■ Trafficking of CAR T cells to tumor

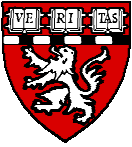
- Expression of addressins
- Route of CAR-T cell infusion
  - Intra-tumoral/intravenous
- Optimal co-stimulation of T cells

## ■ Efficacy & Long-term persistence

- Subtypes of CD4+T cells (Th1, Th2, Th17, Th9 cells),
- CD8+T cells
  - naïve, central memory; long-term
  - effector; active but short lived

## ■ Patient conditioning before ACT

- Reduced-intensity or non-myeloablative
- Increased intensity myelo ablative



# Outline



## ■ Introduction

- Adoptive T cell therapy: focus on engineered CAR-T cells
- Overview of current & investigational CAR therapies
- Challenges of CAR T cell therapy

## ■ Results

- Roles of T-helper cell subsets in cancer immunotherapy

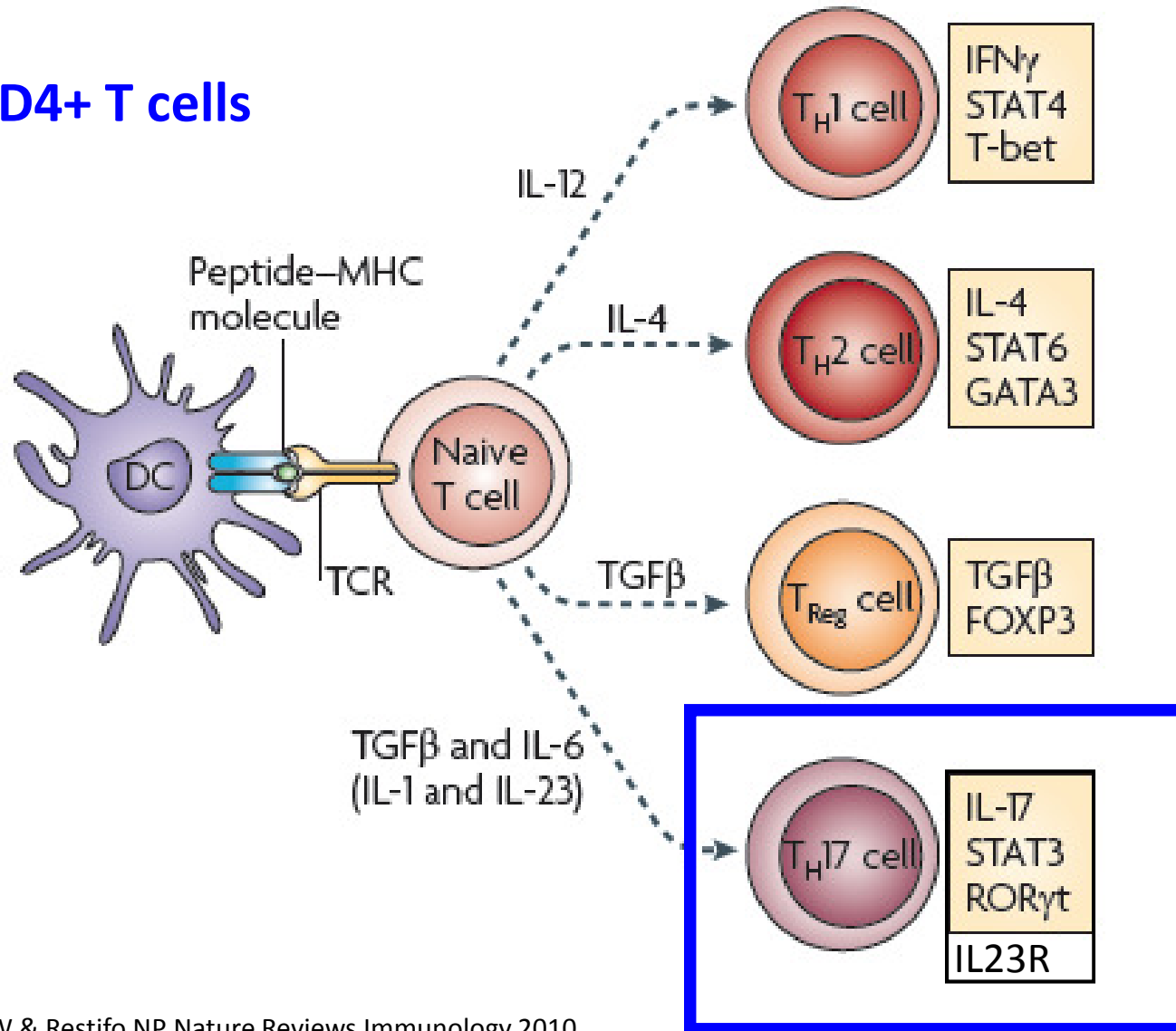
## ■ Summary

- Adoptive T cell therapy: what we learnt and what we should do next



# Adoptive T cell therapy: Right T cell population?

## CD4+ T cells





# Role of Th17 cells in tumor immunity is controversial



## Pro-tumor:

- enhances vascularization/angiogenesis
- promotes metastasis
- promotes growth

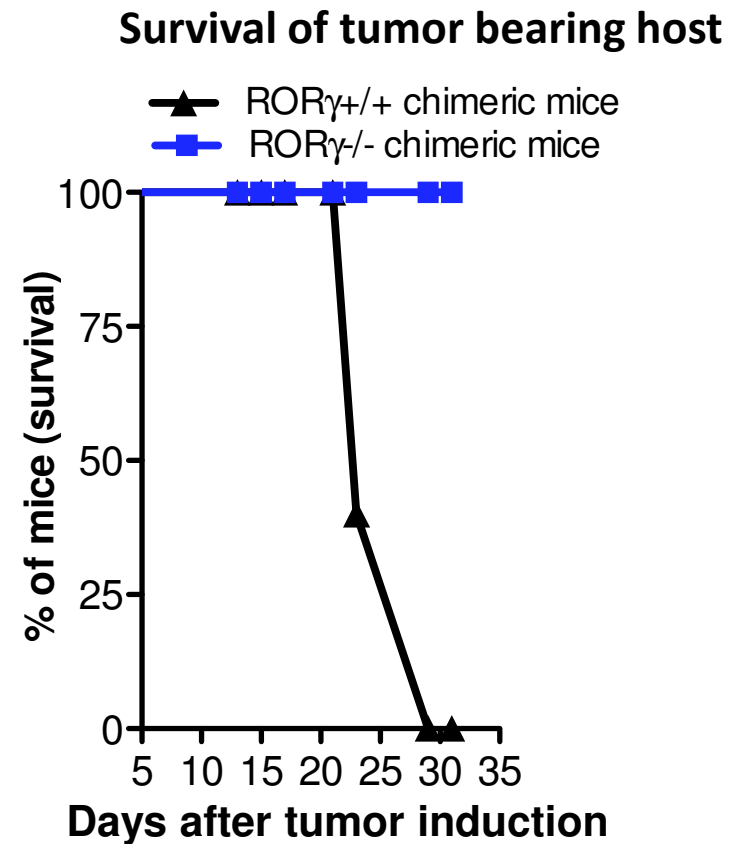
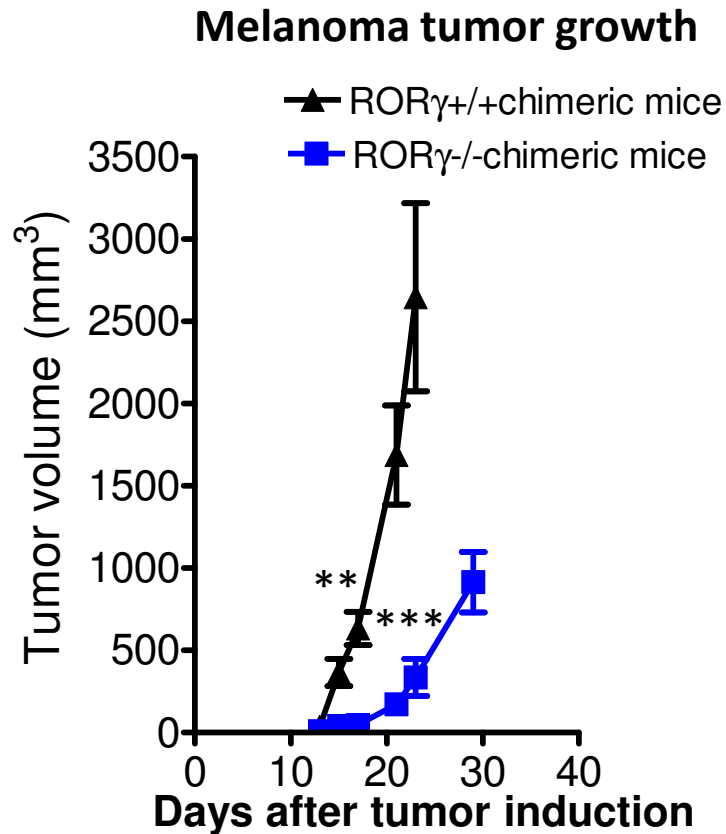
Langowski et al, Nature 2006; Murugayen 2011 J.Immunol; Wang et al. J.Exp.Med 2009

## Anti-tumor:

- enhances tumor immunity by promoting CD8+T cell and DC function

Martin-Orcazzo et al Immunity 2010

# Tumor growth suppression in ROR $\gamma$ <sup>-/-</sup> mice (Th17 cell deficient)

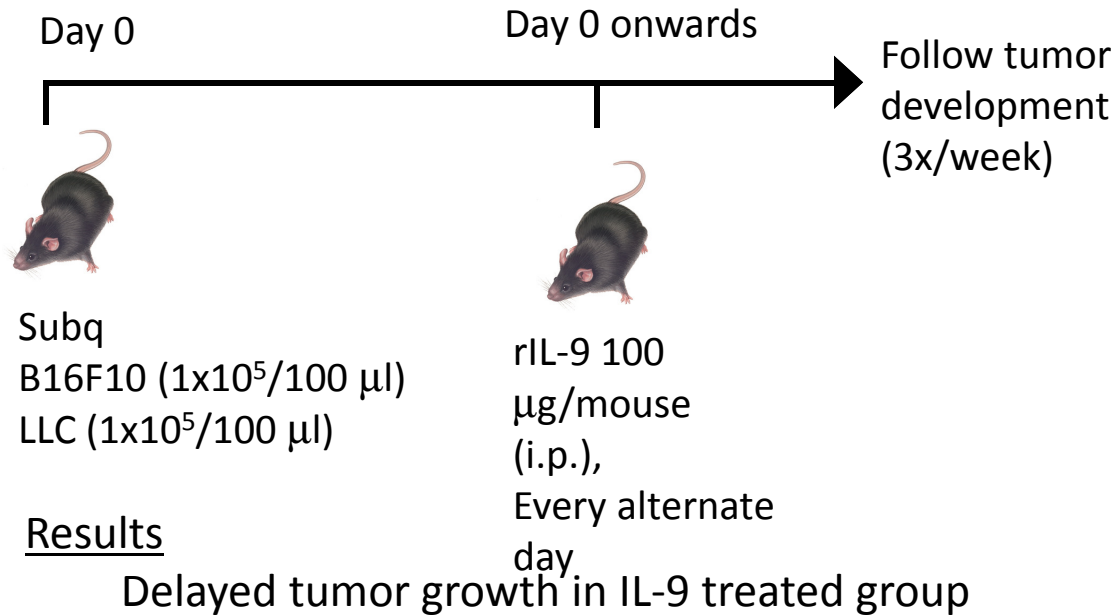


Abrogation of Th17 pathways promotes anti-tumor immune responses



# Treatment with exogenous rIL-9 suppresses tumor growth

## Efficacy study plan

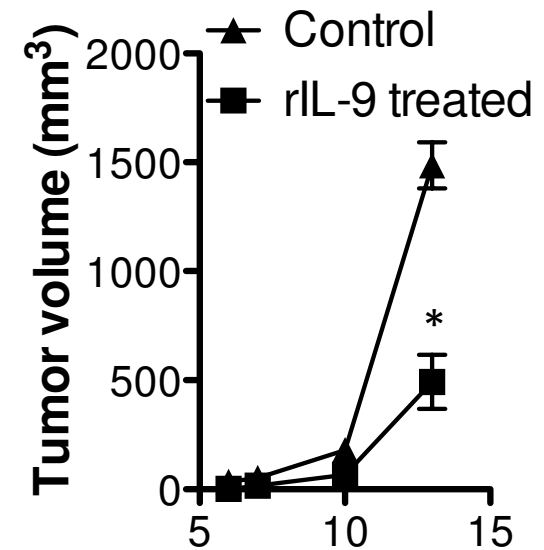


## Results

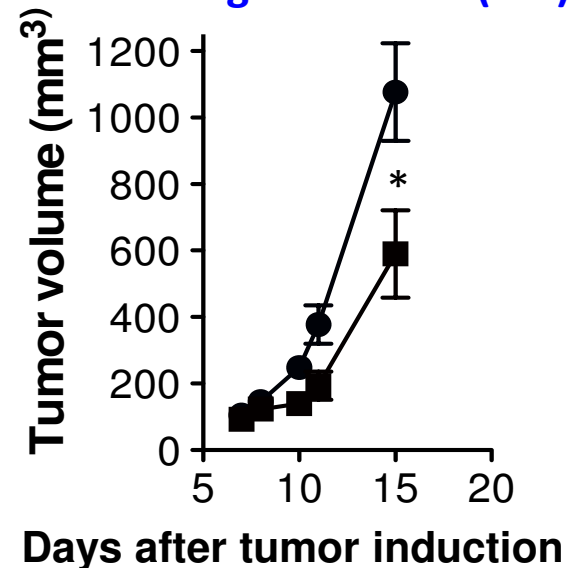
Delayed tumor growth in IL-9 treated group

**IL-9 mediated anti-tumor effects are not limited to melanoma tumor model**

## Melanoma (B16F10)

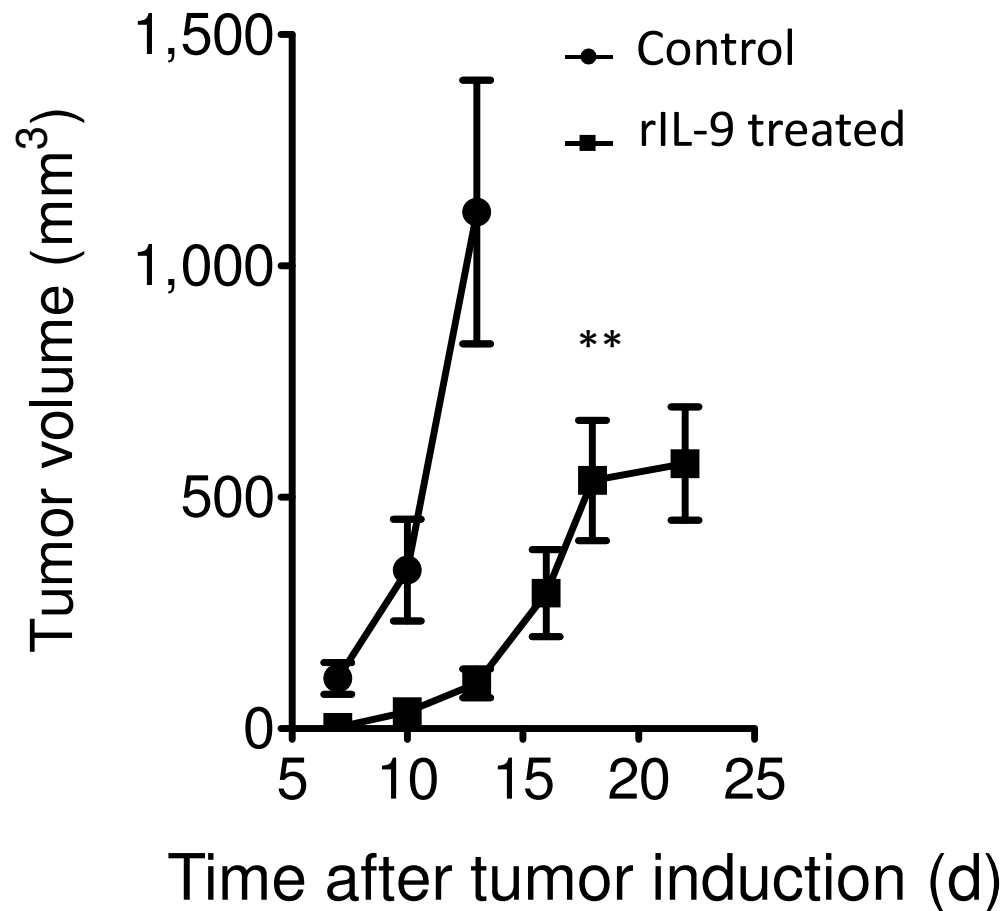


## Lung carcinoma (LLC)



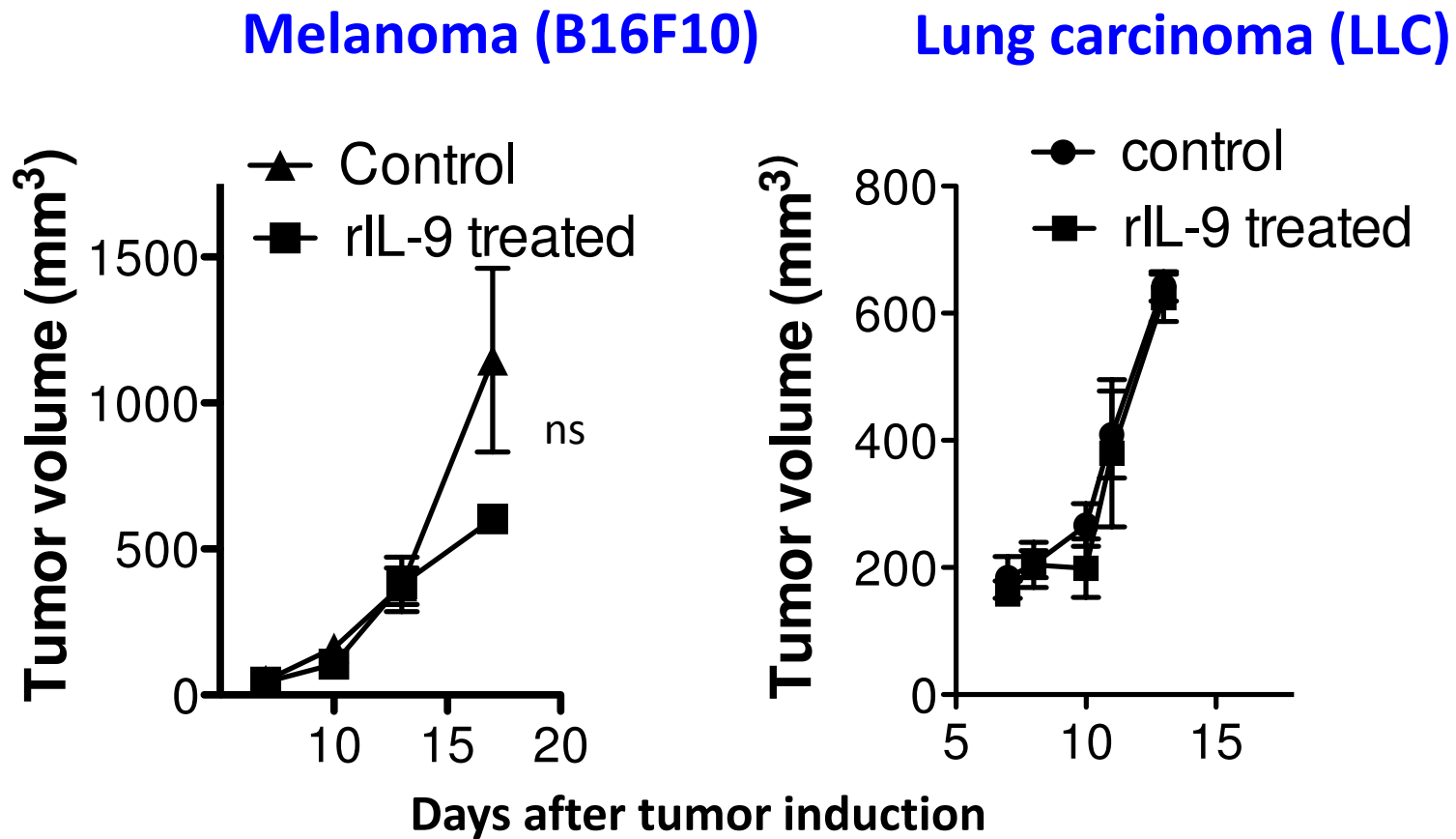
## Effects of rIL-9 on melanoma tumor growth in Rag1<sup>-/-</sup> mice (T cell and B cell deficient host)

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**IL-9 mediated tumor growth suppression is independent of T cells and B cells**

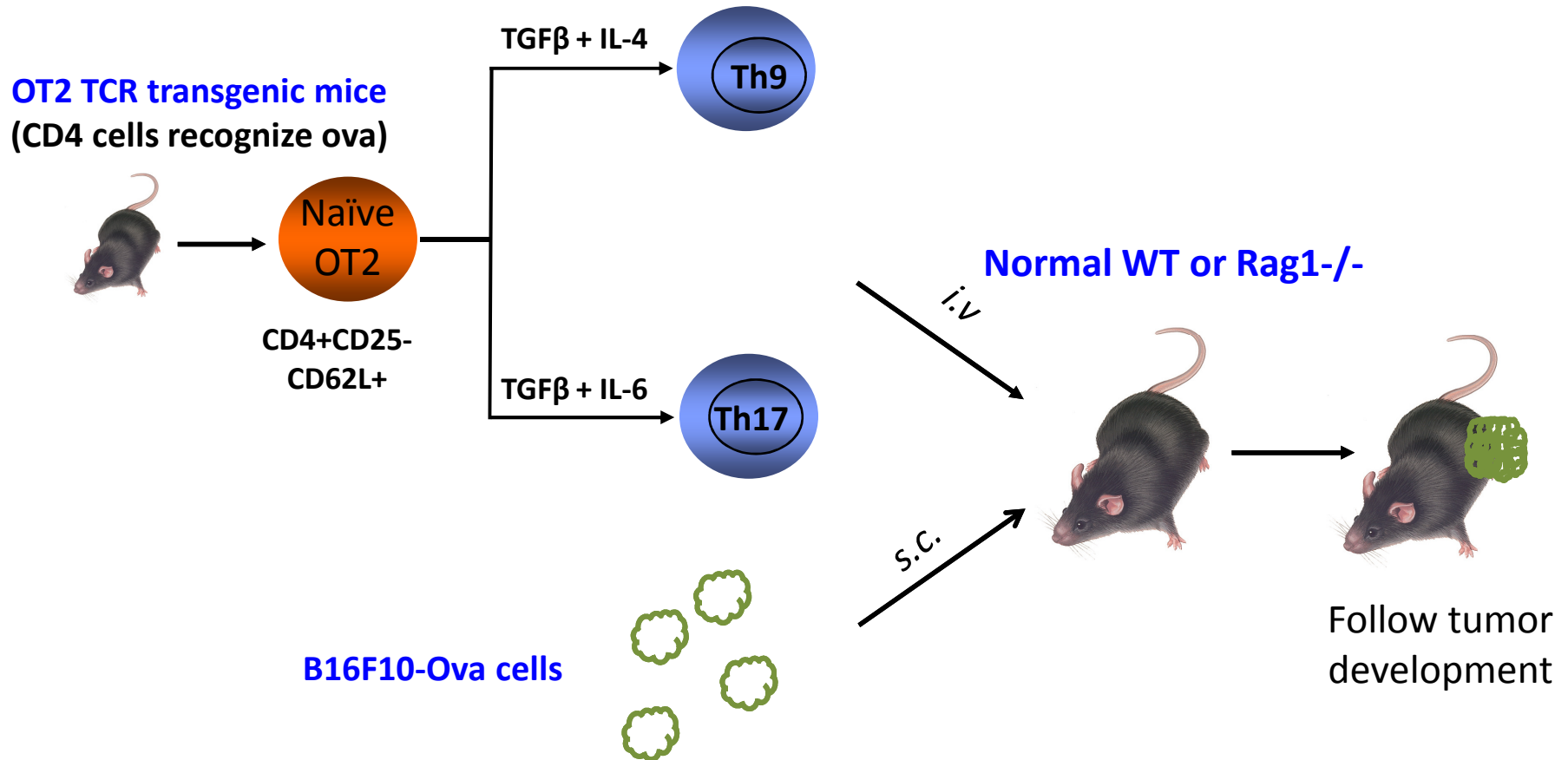
# Effects of rIL-9 on tumor growth in mast cell<sup>-/-</sup> mice



IL-9 mediated tumor growth suppression is dependent of mast cells

# Engineering Th9 cells: TAA specific Tumor model

## Engineer tumor specific Th9 cells

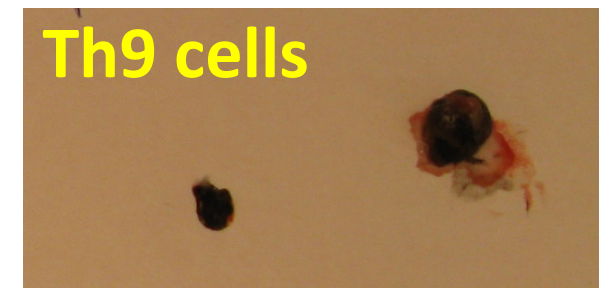
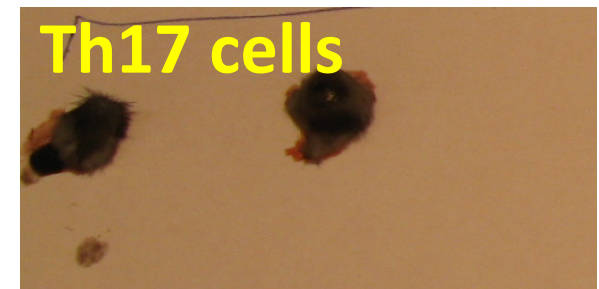
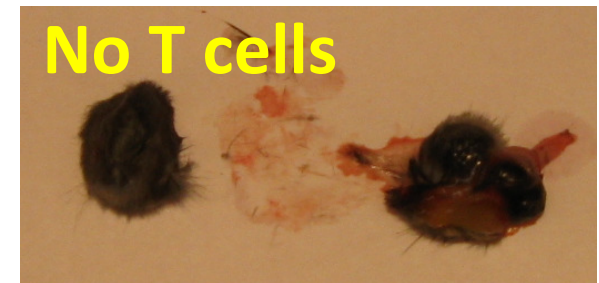
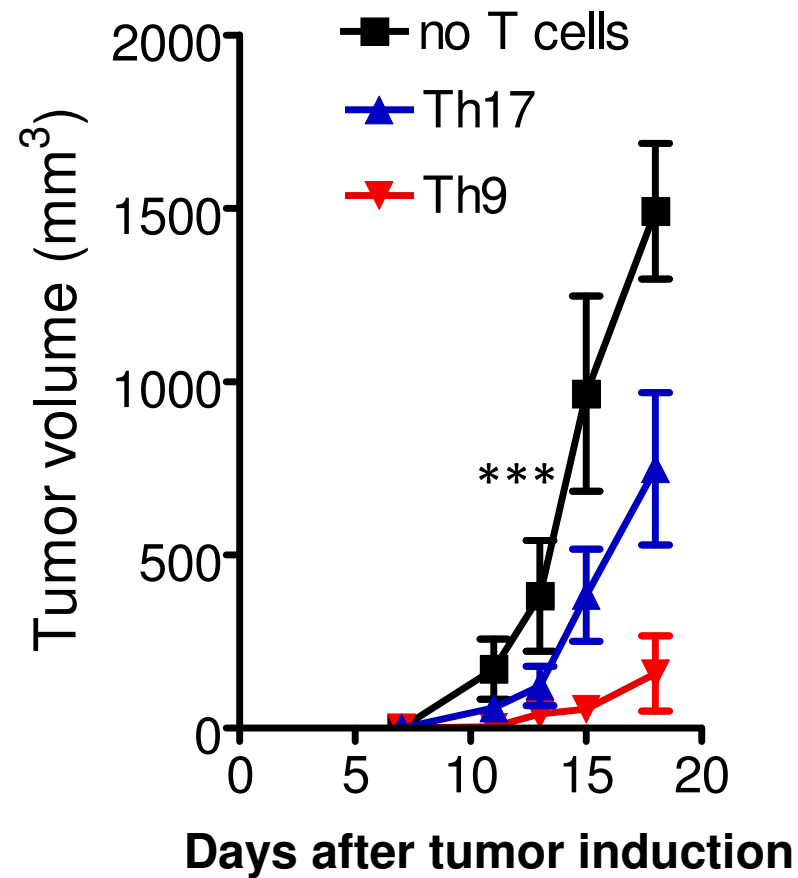


Generation of Ovalbumin expressing B16 tumor cells  
(Lentiviral method)



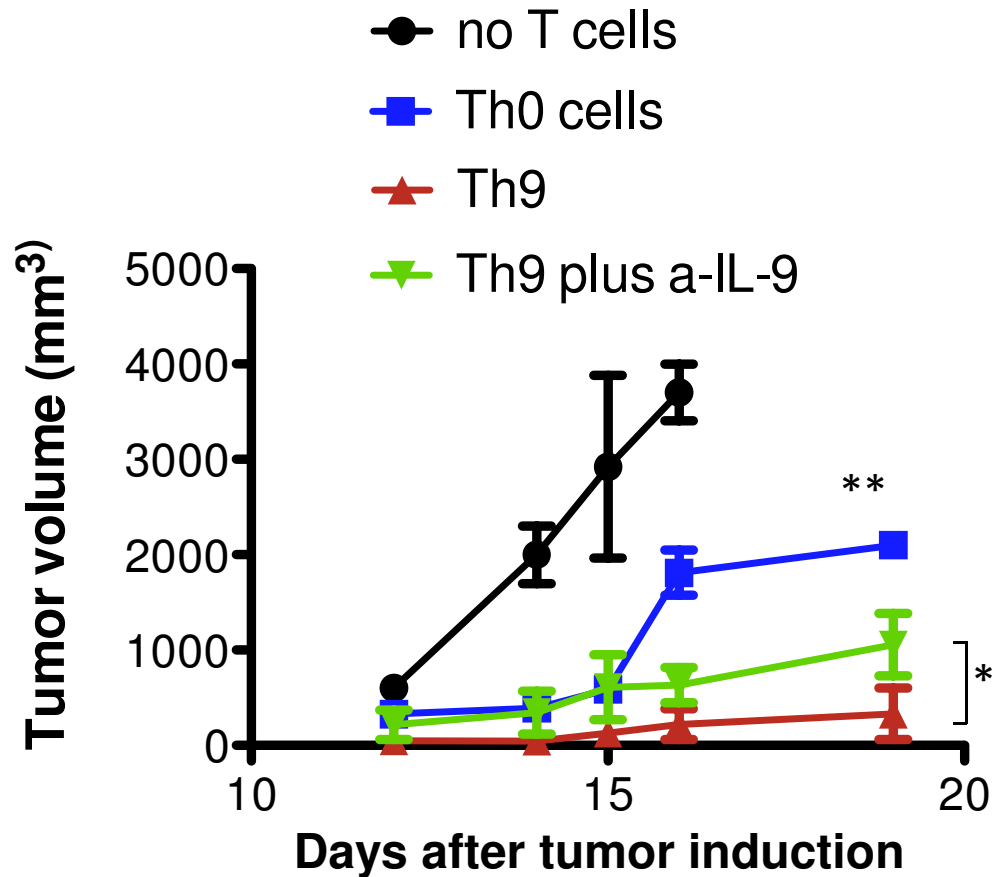
# Treatment with Th9 cells suppresses tumor growth

## Immunocompetent host (Wild type)



# Th9 cell therapy: efficacy studies in immunodeficient host

Rag1<sup>-/-</sup> (T cells and B cells deficient)



Th9 cells suppresses tumor growth independent of T cell and B cell presence

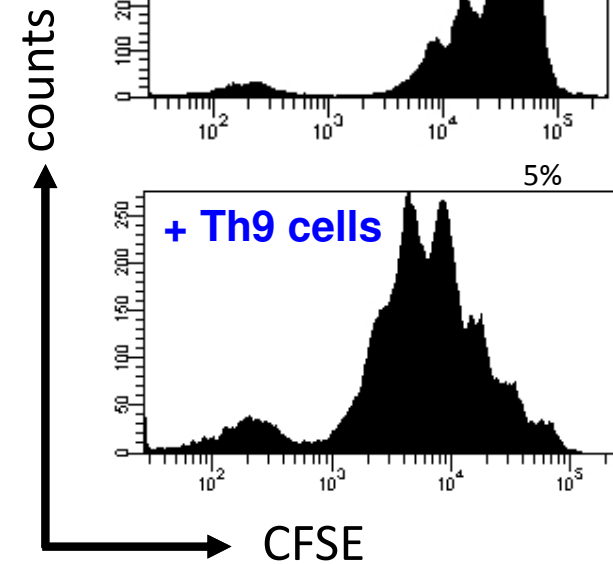
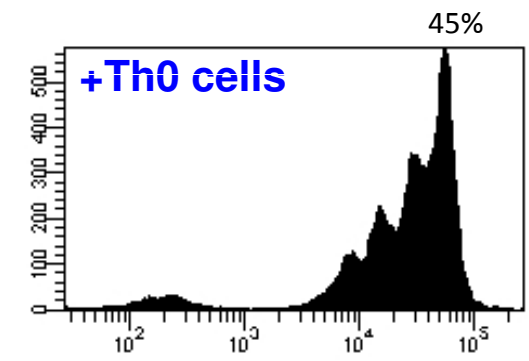
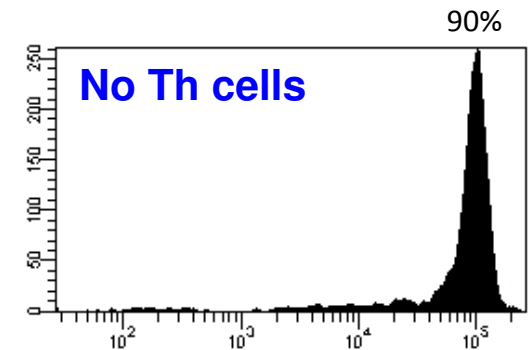
# Mechanism of anti-tumor effects of Th9 cells

## Effects of Th9 cells on CD8+T cells (OT-1 cells) proliferation

CFSE labeled OT1-CD8-T cells + irradiated B16-Ova cells  $\pm$  OT2-Th0 or OT2-Th9 cells

3 days

Evaluation of OT1-CD8-T cells by flow cytometry



**Presence of Th9 cells promotes the OT1-CD8-T cells proliferation.**

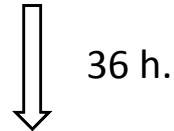
OT1 cells: ovalbumin specific CD8+T cells  
CFSE: Carboxyfluorescein succinimidyl ester

# Mechanism of anti-tumor effects of Th9 cells

## Examining Cytotoxic activity of Th9 cells

5mM CFSE labeled B16F10-ova cells + 0.5 mM CFSE labeled EL-4 cells

± OT2-Th0 or OT2-Th9

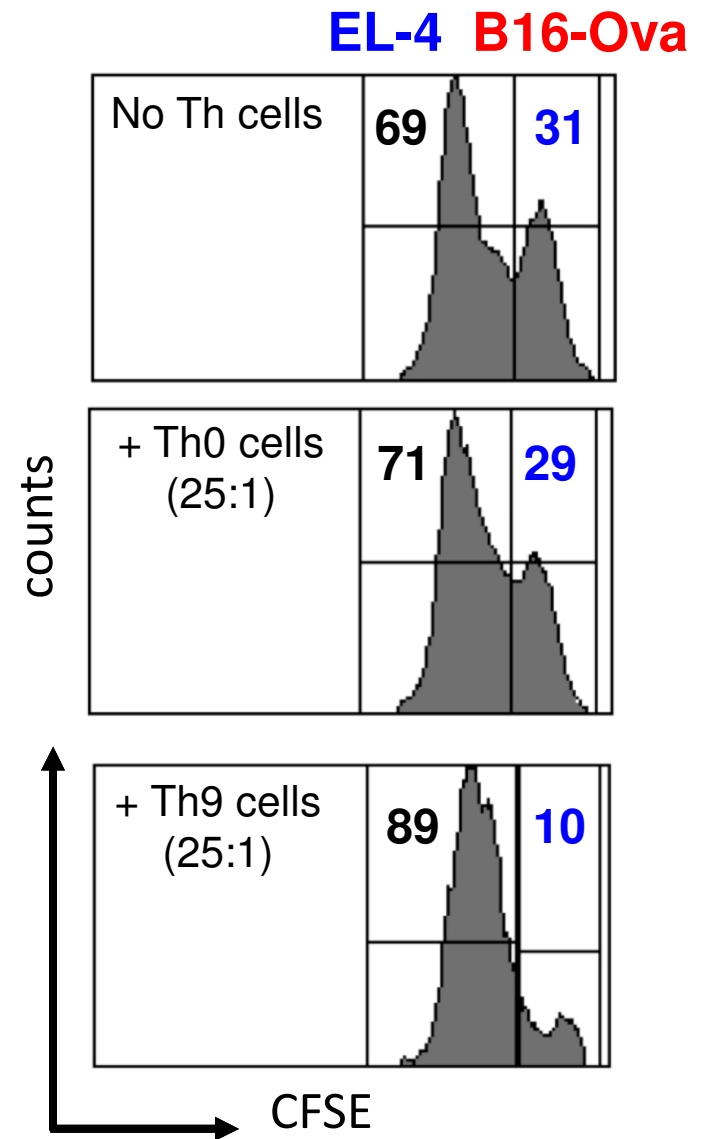


Tumor cell lysis was measured by flow cytometry.

**Th9 cells lyse tumor cells in antigen/  
target dependent manner**

Target cells: B16-Ova

B16 ova specific Th cells: OT2-Th cells

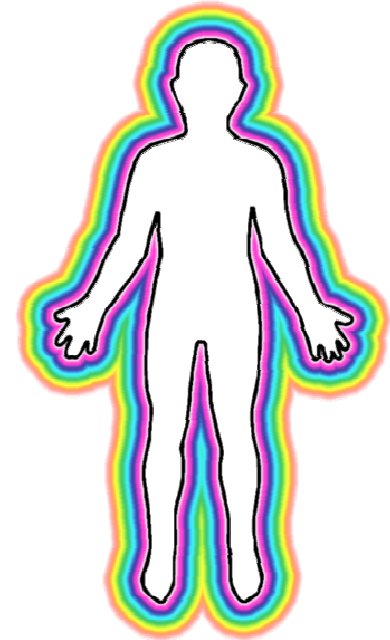


# What is the relevance of these findings in human?

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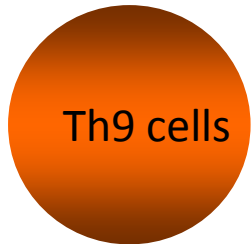


**Translational research**





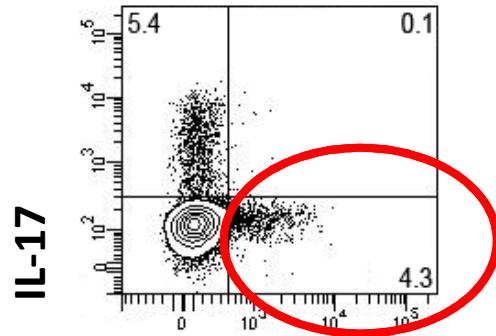
# Th9 cells are present in human “skin” and “blood”



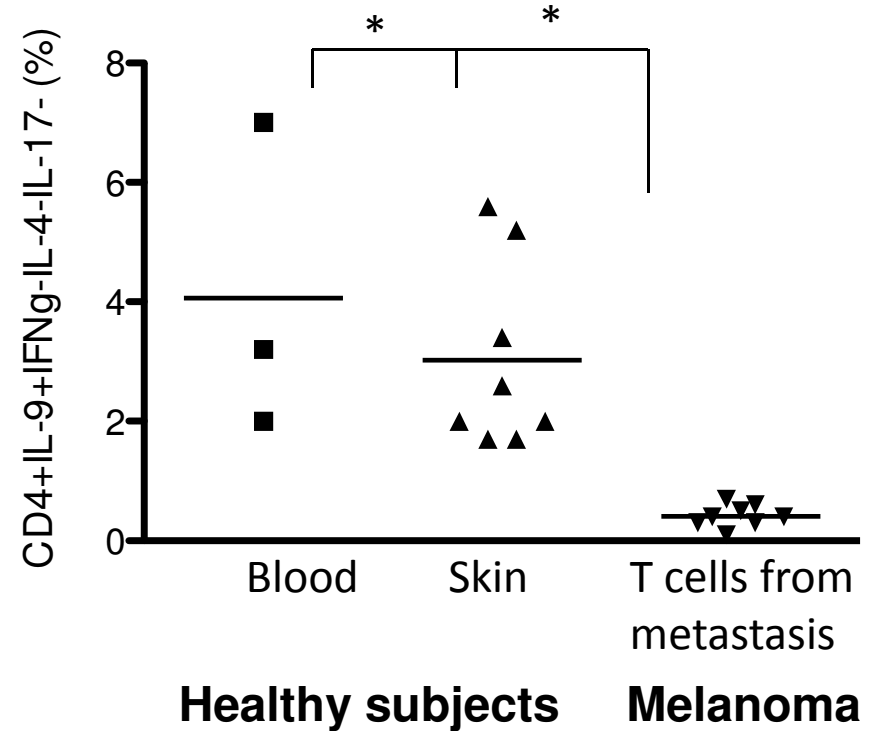
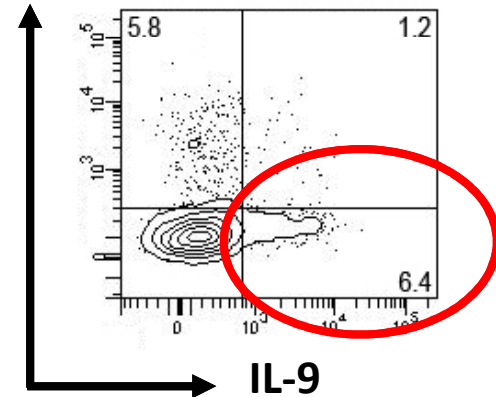
IL-9  
PU.1

Gate on CD4+IFN $\gamma$ -IL-4-

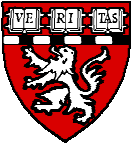
Skin



Blood



Th9 cells are not just a murine phenomenon



# Summary



## ■ CAR-T cells

- T cells transduced with tumor-specific Chimeric Antigen Receptor (CAR)
- Tumor recognition independent of HLA (no HLA typing needed)
- Target: variety of tumor antigens (protein, carbohydrate, glycolipid)
- High response rate (up to 88%): pre-clinical and clinical findings

## ■ Limitation of CAR-T cells

- Toxicities
  - On target/off tumor toxicities
  - Cytokine syndrome
- Tumor microenvironment
  - Presence of MDSCs & Treg in tumor
  - Immunosuppressive agents

## Results & Conclusion

- IL-9 is a novel anti-tumor cytokine and anti-tumor effects are mediated via mast cells
- Th9 cells are the most superior anti-tumor Th cells
- Th9 cells exists in human: not just murine phenomenon
- Strategies that promotes IL-9 production will be a critical for the development of robust treatment for melanoma and lung carcinoma.



# Acknowledgement



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