



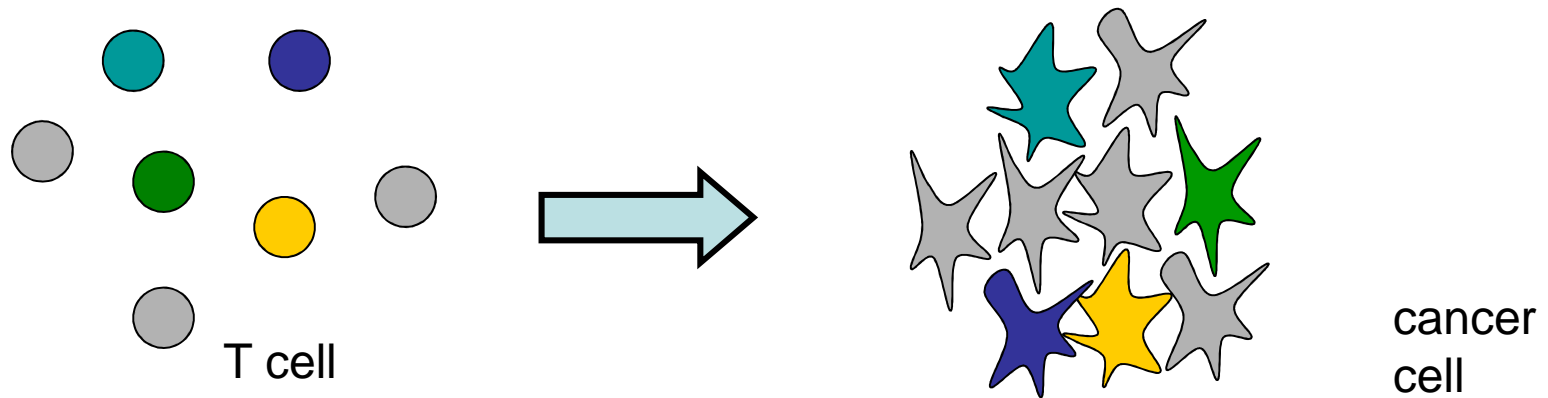
# **CARs and TRUCKs: how engineered T cells become living factories**

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# T cells with engineered pre-defined specificity



## The aim:

To give patient's immune cells specificity  
for targeting autologous cancer cells.



## 1. Targeting leukemia/lymphoma by CAR T cells is clinically successful

## The growing world of CAR T cell trials: a systematic review

Astrid Holzinger<sup>1,2</sup> · Markus Barden<sup>1,2</sup> · Hinrich Abken<sup>1,2</sup>

**Abstract** In recent years, cancer treatment involving adoptive cell therapy with chimeric antigen receptor (CAR)-modified patient's immune cells has attracted growing interest. Using gene transfer techniques, the patient's T cells are modified ex vivo with a CAR which redirects the T cells toward the cancer cells through an antibody-derived binding domain. The T cells are activated by the CAR primary signaling and costimulatory domains. Such "second generation" CAR T cells induced complete remission of B cell malignancies in the long-term. In this fast-moving field with a growing number of engineered T cell products, we list **about 100 currently ongoing trials** here that involve CAR T cells targeting hematopoietic malignancies and solid cancer. Major challenges in the further development of the therapy are briefly discussed.

# 54 CAR T cell trials targeting CD19 (May 2016)

**Table 1** Clinical trials in adoptive cell therapy using second- and third-generation CAR T cells

Target antigen	Disease	CAR	Gene transfer	T cell origin	Infused dose	Preconditioning	Number of patients	Response	PI	Center	Identifier	References
BCMA	Myeloma	4-1BB-CD3 $\xi$	NA	Autologous	NA, split dose				Cohen	Abramson Cancer Center	NCT02546167	
BCMA	Myeloma	CD28-CD3 $\xi$		Autologous	0.3–15 $\times 10^6$ CAR T cells/kg, escalating doses	CTX, FLU			Kochenderfer	NCI	NCT02215967	
CD19	FL	CD28-CD3 $\xi$	RV	Autologous	10 <sup>8</sup> CAR T cells day 1, 3 $\times 10^8$ day 2	CTX/FLU IL-2	1	1 $\times$ PR	Rosenberg	NCI	NCT00924326	[35]
CD19	DLBCL, FL	CD3 $\xi$ , CD28-CD3 $\xi$	RV	Autologous	0.2, 1, 2 $\times 10^8$ CAR T cells/m <sup>2</sup>	None	6	2 $\times$ SD, 4 $\times$ NR	Savoldo	BCM		[7]
CD19	CLL	CD28-CD3 $\xi$	RV	Autologous	1.2–3 $\times 10^7$ CAR T cells/kg, 0.4–1 $\times 10^7$ CAR T cells/kg, split dose over 2 days	3 CLL: none 5 CLL: CTX	8	4 $\times$ NR, 1 $\times$ PR, 2 $\times$ SD	Park	MSKCC	NCT00466531	[14]
CD19	CLL	CD28-CD3 $\xi$	RV	Autologous	0.3–3 $\times 10^7$ CAR T cells/kg	CTX, FLU	4 (8 in total)	1 $\times$ CR, 1 $\times$ SD, 2 $\times$ PR	Rosenberg	NCI	NCT00924326	[15]
CD19	CLL	CD28-CD3 $\xi$	RV	Autologous	1–4 $\times 10^6$ CAR T cells/kg	CTX, FLU	4 (15 in total)	3 $\times$ CR, 1 $\times$ PR	Rosenberg	NCI	NCT00924326	[36]
CD19	CLL	CD28-CD3 $\xi$	RV	Allogeneic, donor derived	1.5, 4.5, 12 $\times 10^7$ T cells/m <sup>2</sup>	None	4	1 $\times$ PR, 1 $\times$ SD	Ramos	BCM	NCT00840853	[8]
CD19	Leukemia	CD28-CD3 $\xi$	RV	Allogeneic, donor derived	0.4–7.8 $\times 10^6$ CAR T cells/kg	None	10	1 $\times$ SD, 2 $\times$ NR, 1 $\times$ CR	Kochenderfer	NCI	NCT01087294	[37]
CD19	CLL			Autologous	NA, split dose	CTX, FLU			Hosing	MDACC	NCT01653717	
CD19	CLL, SLL	4-1BB-CD3 $\xi$	NA	Autologous	1–5 $\times 10^7$ CAR T cells, 1–5 $\times 10^8$ CAR T cells				Frey	Abramson Cancer Center	NCT01747486	
CD19	ALL	4-1BB-CD3 $\xi$	LV	Autologous	0.14–1.2 $\times 10^7$ CAR T cells/kg	1: None, 1: ETO-CTX	2	2 $\times$ CR	Grupp	UPenn	NCT01626495	[25]

**CD19 CAR T cell therapy of B cell leukemia is successful,**

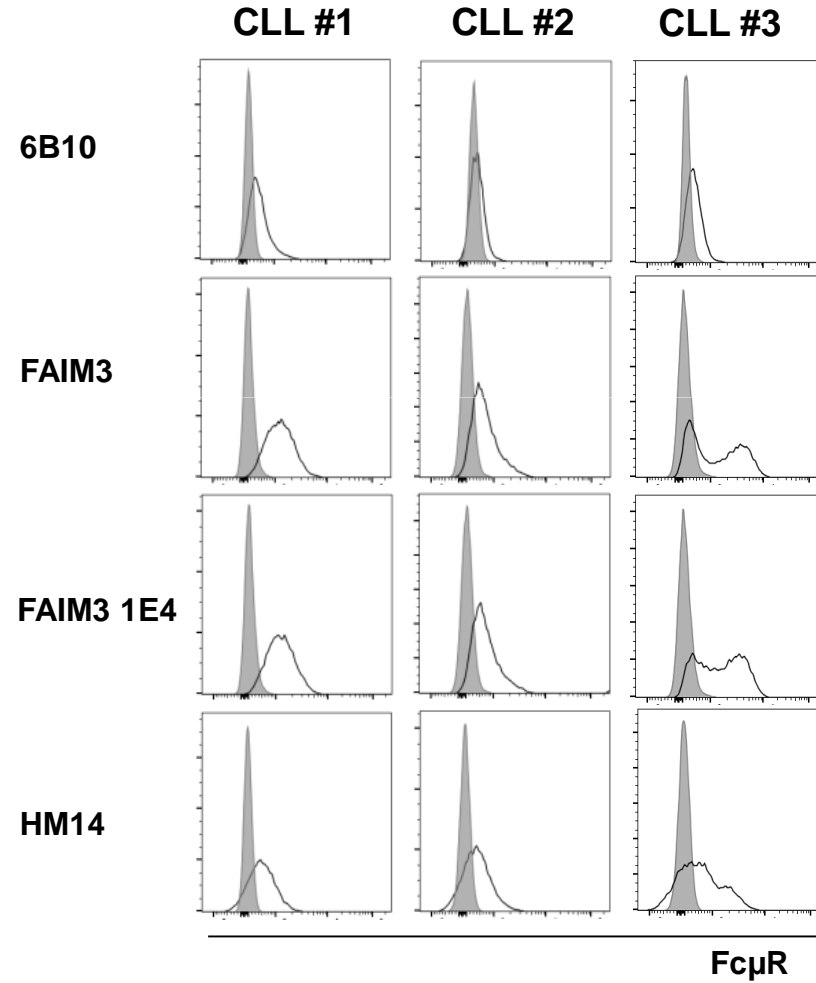
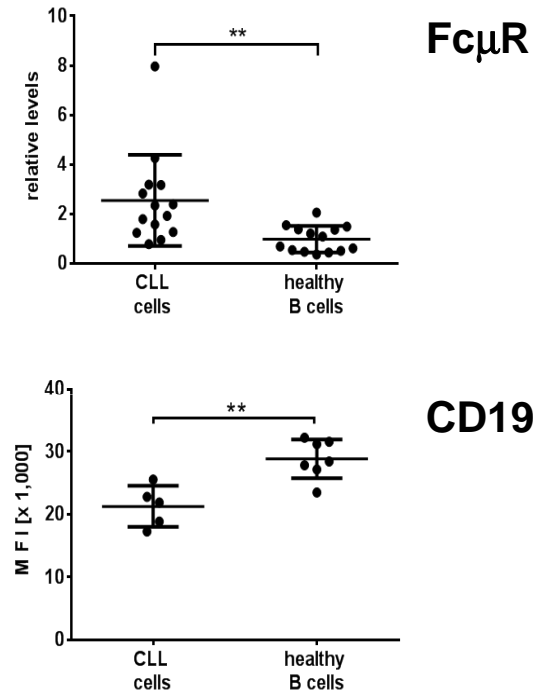
**however,**

**associated with relapse of leukemic cells**

**which lost the targeted antigen**

**CD19 CAR T cell therapy is specific  
but not selective for B leukemic cells**

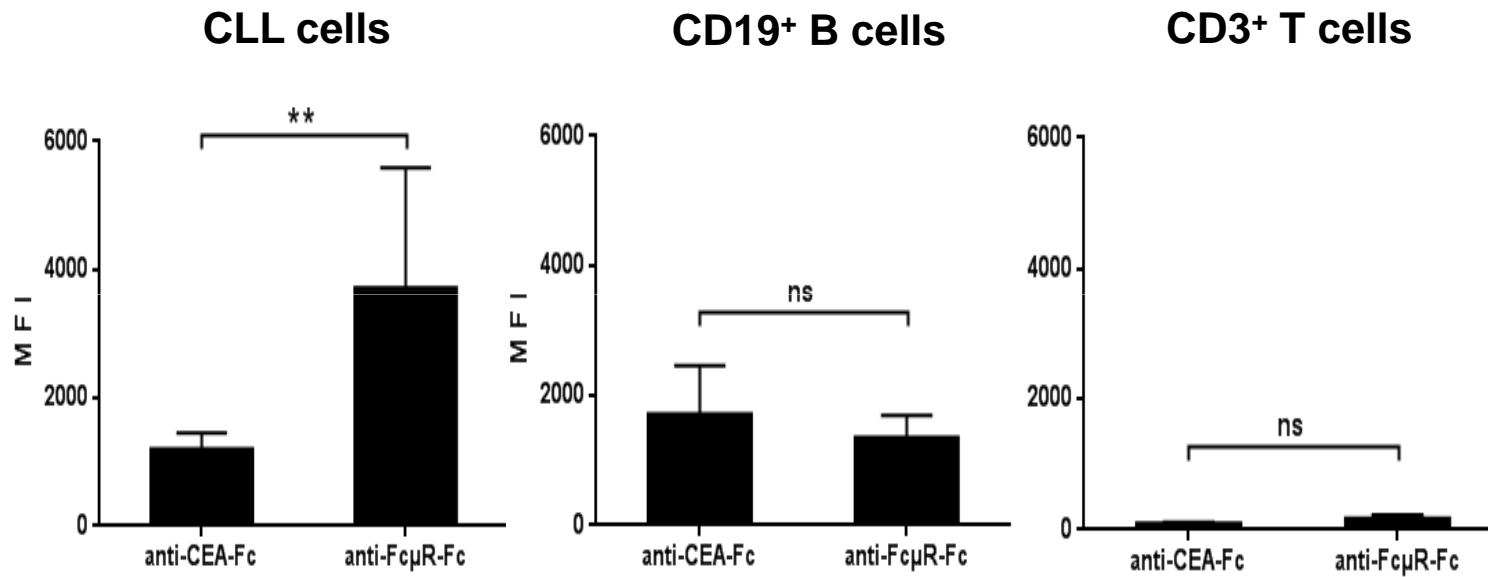
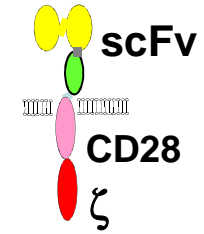
# Fc $\mu$ R may be a good candidate for CAR T cell targeting CLL



Faitschuk et al., Blood (2016)

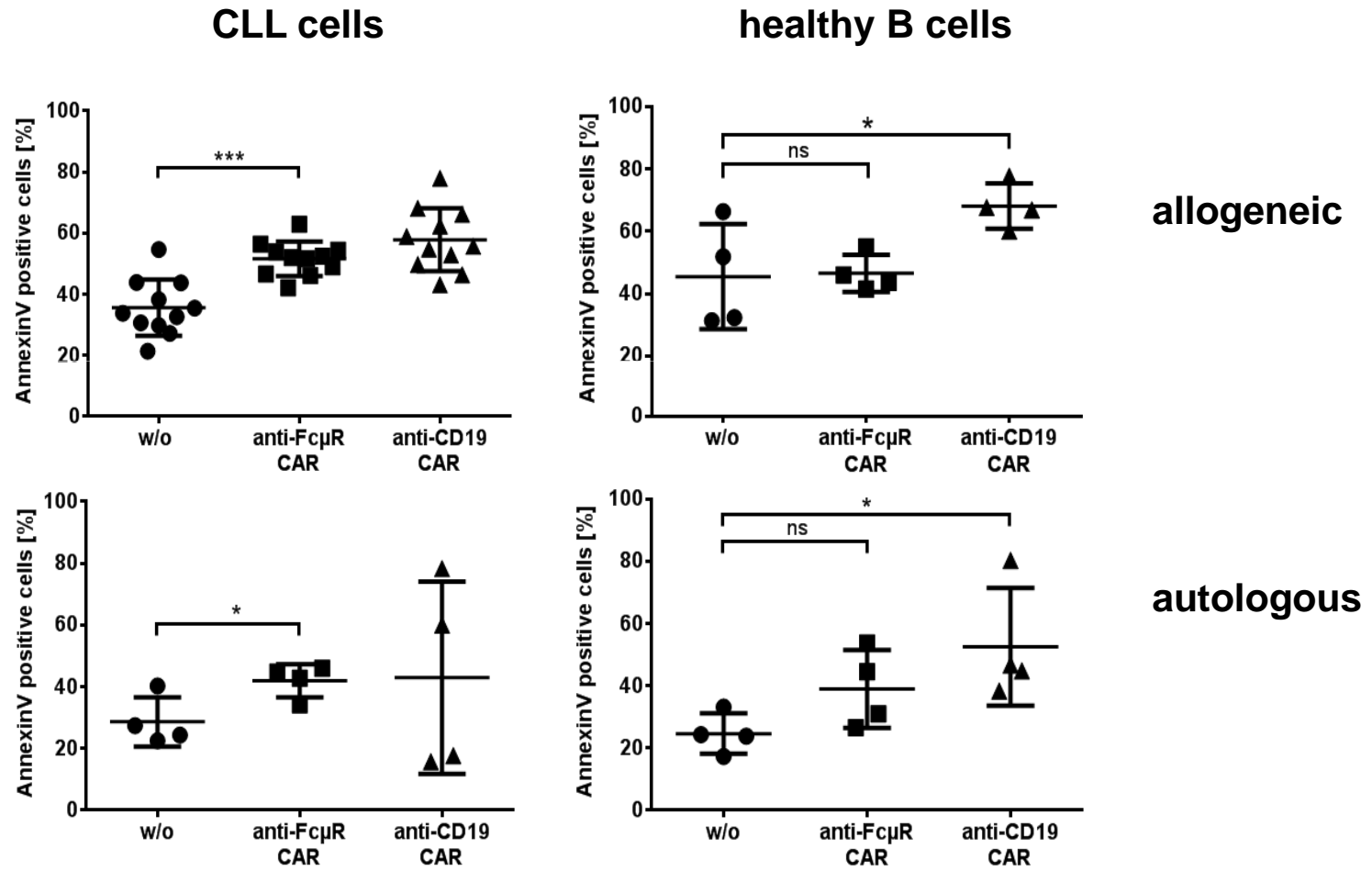


# anti-Fc $\mu$ R CAR

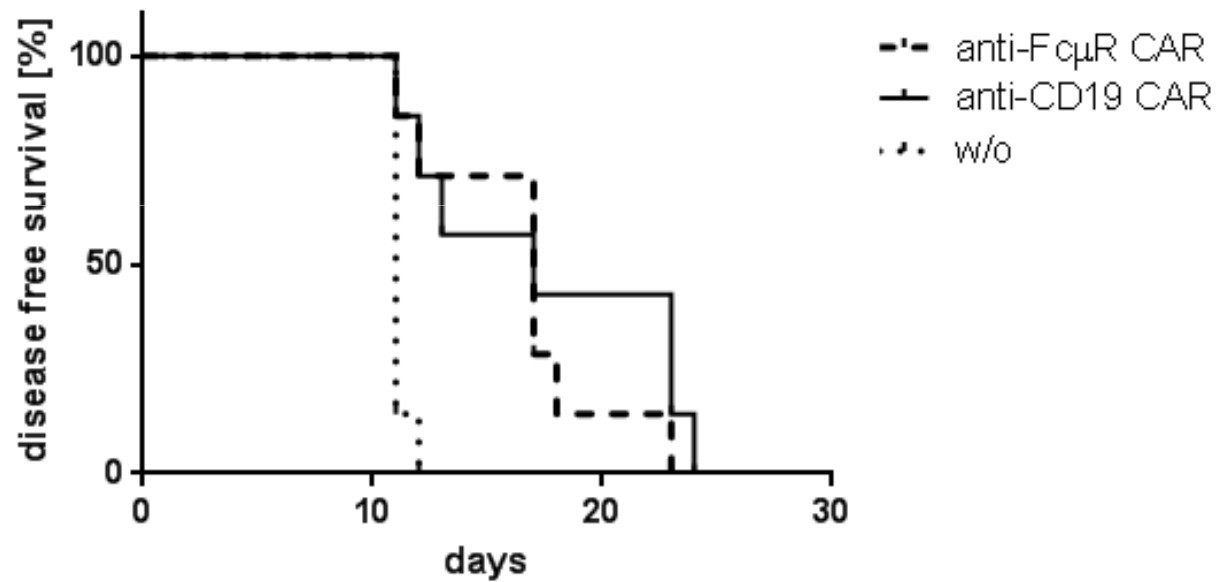


Faitschuk et al., Blood (2016)

# Anti-Fc $\mu$ R CAR T cells eliminate CD19+ CLL cells but not CD19+ B cells



# Anti-Fc $\mu$ R CAR T cells prolong disease free survival in a mouse model as do anti-CD19 CAR T cells



Faitschuk et al., Blood (2016)

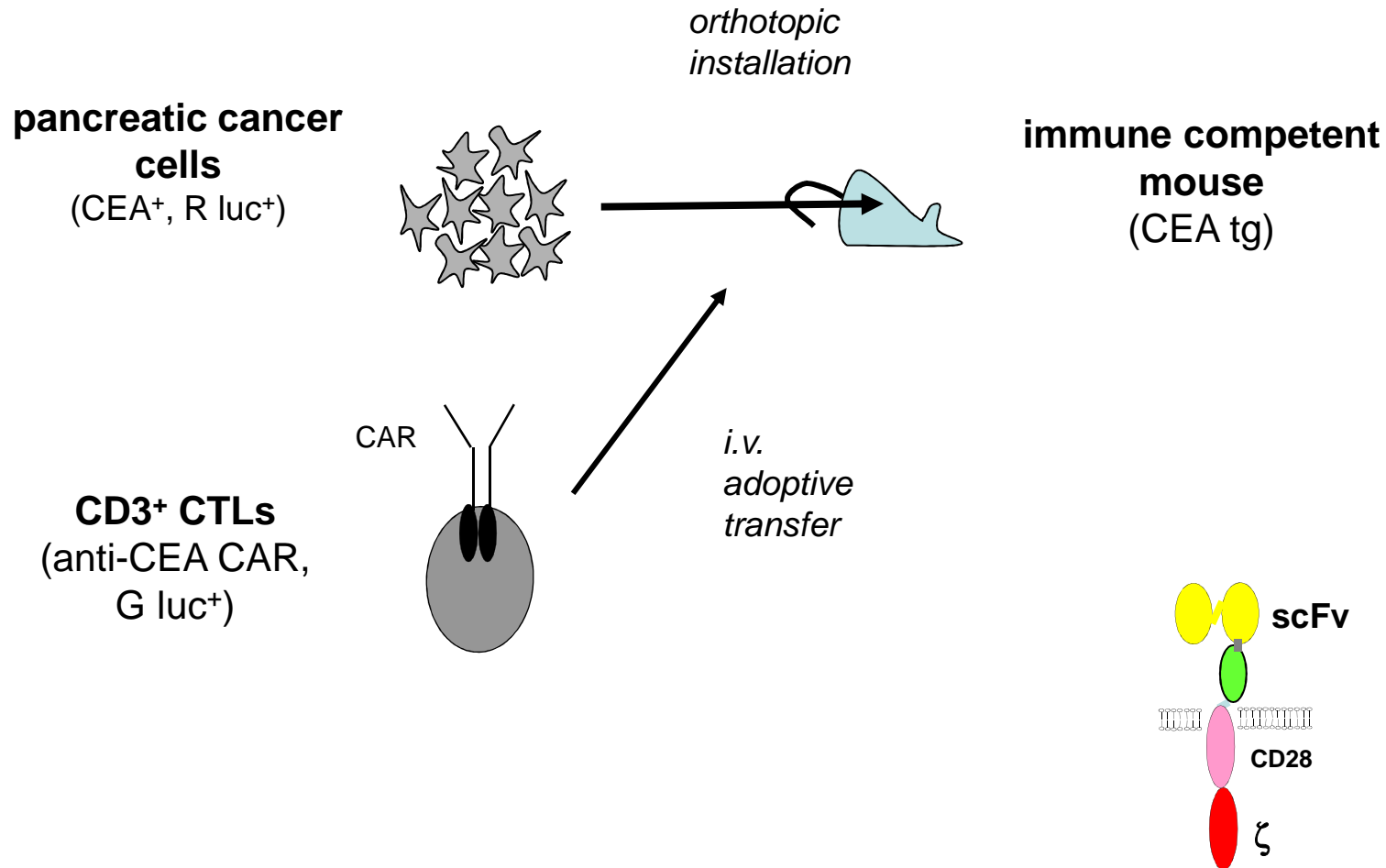


1. „Tumor associated antigens“ are not exclusively expressed by tumor cells.
2. Tumors are extremely heterogenous with respect to targetable surface antigens

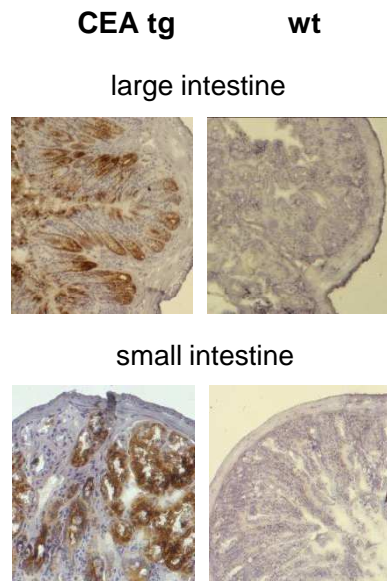
# **CAR T cells for treating adenocarcinoma**

- **CEA is a validated target**
- **T cells engineered with anti-CEA CAR**
- **soluble (serum) CEA does not block anti-CEA CAR mediated T cell activation**
  
- **the same TAA is expressed by healthy tissues**

# CAR T cells to target pancreatic cancer cells in the tolerant, immune competent mouse

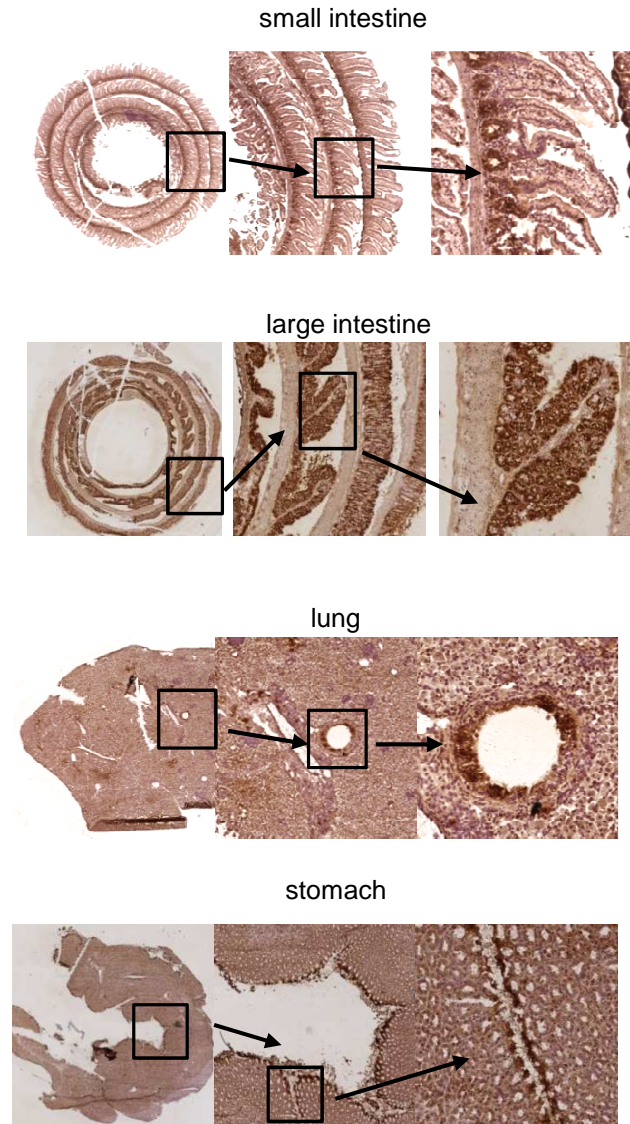
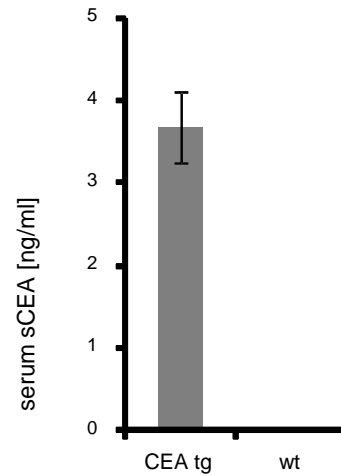


# The CEA<sub>tg</sub> mouse displays the human pattern in CEA expression

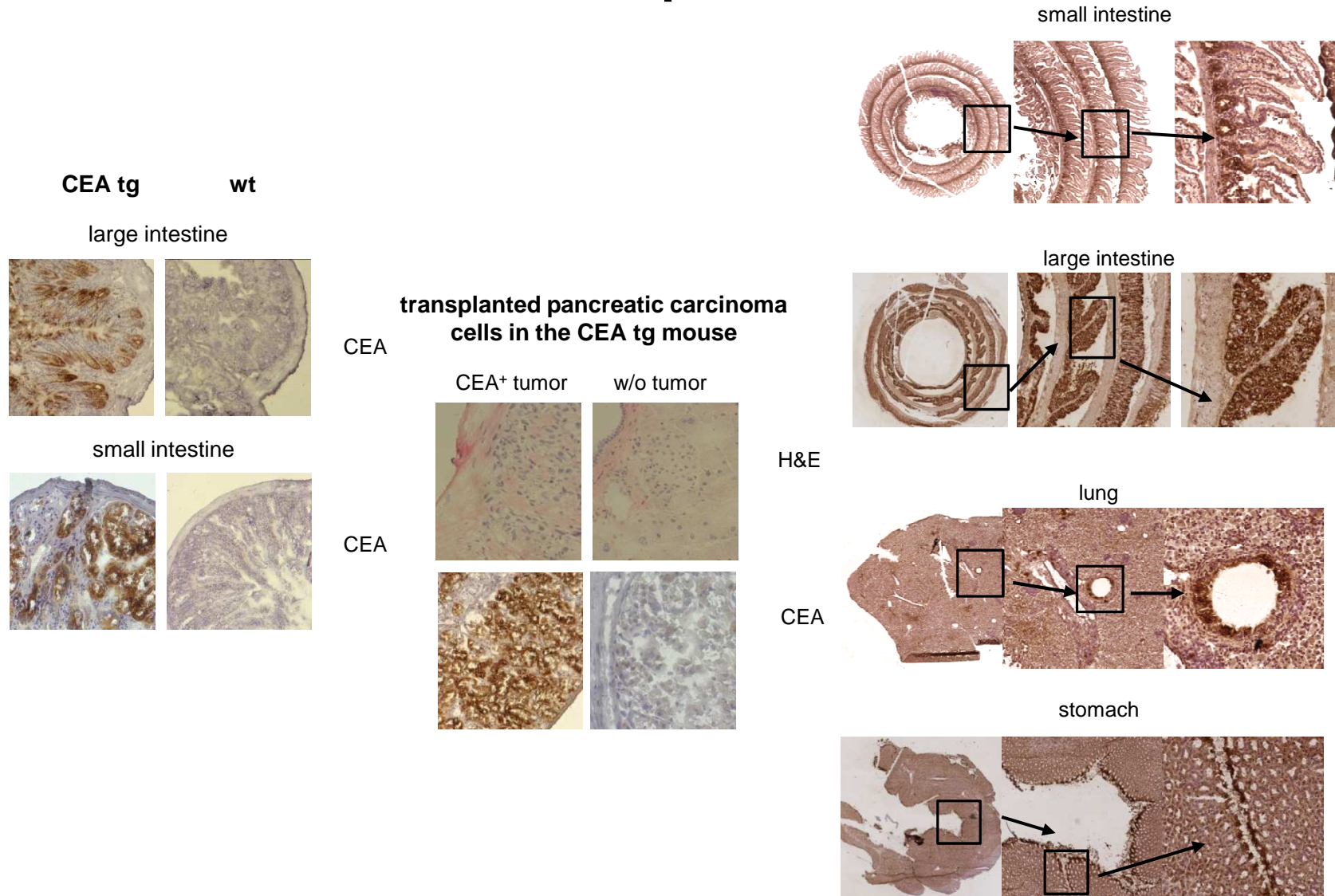


CEA

CEA



# The CEA<sub>tg</sub> mouse displays the human pattern in CEA expression





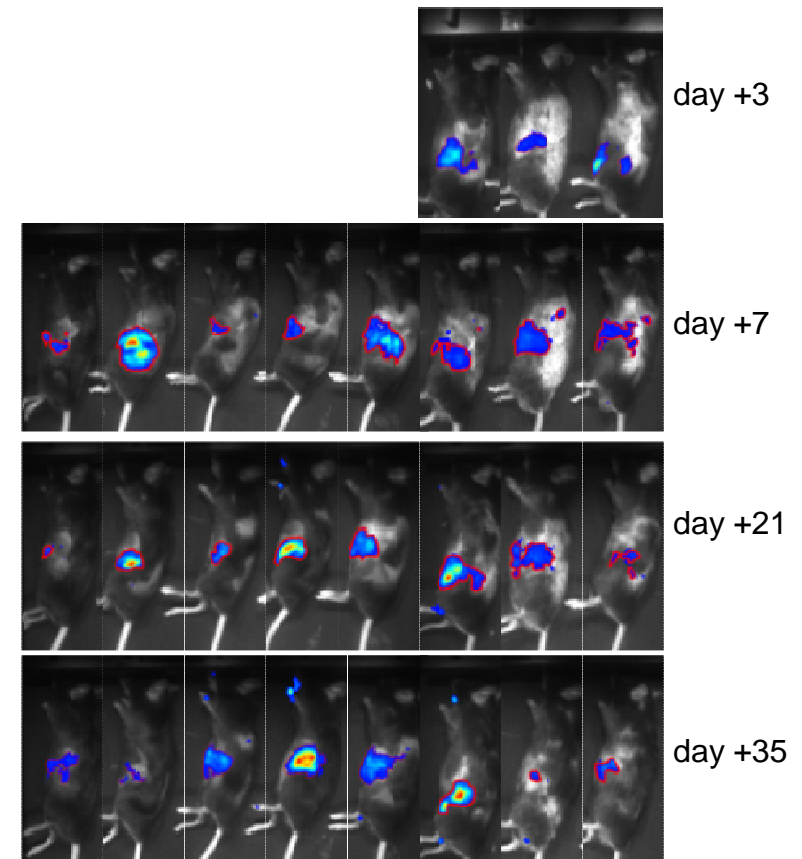
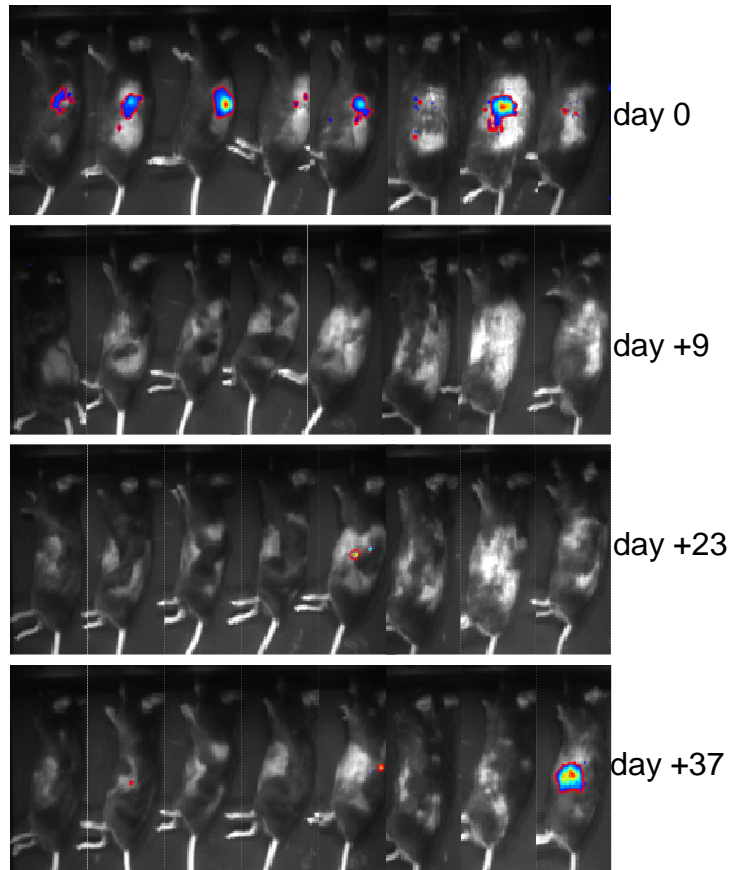
# Imaging tumor and CAR engineered T cells

tumor imaging

T cell imaging

T cells (CAR<sup>+</sup>)  
tumor (CEA<sup>+</sup>)

T cells (CAR<sup>+</sup>)  
tumor (CEA<sup>+</sup>)

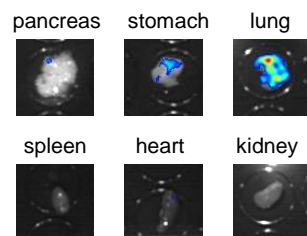
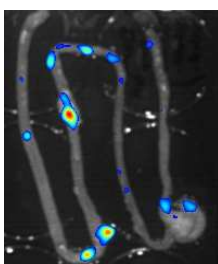


# No severe auto-immunity by anti-CEA CAR T cells

## T cell imaging

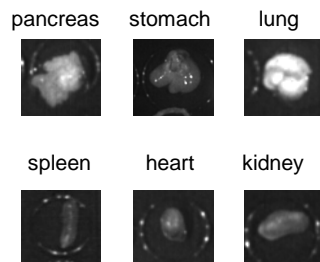
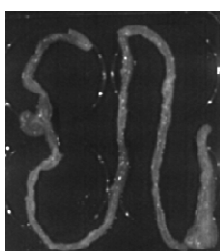
CEAtg mouse treated with anti-CEA CAR T cells

small & large intestine,  
appendix

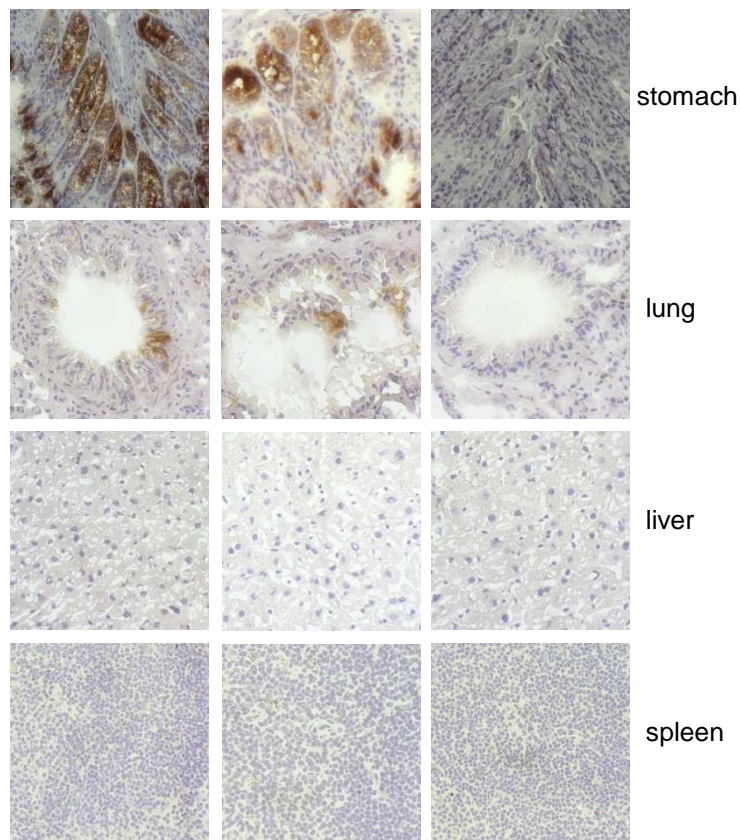


CEAtg mouse treated with T cells w/o CAR

small & large intestine,  
appendix

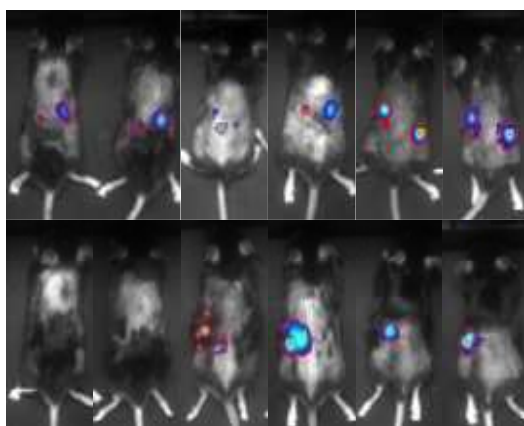


CAR T cells CEA tg mouse	T cells w/o CAR CEA tg mouse	CAR T cells wt mouse
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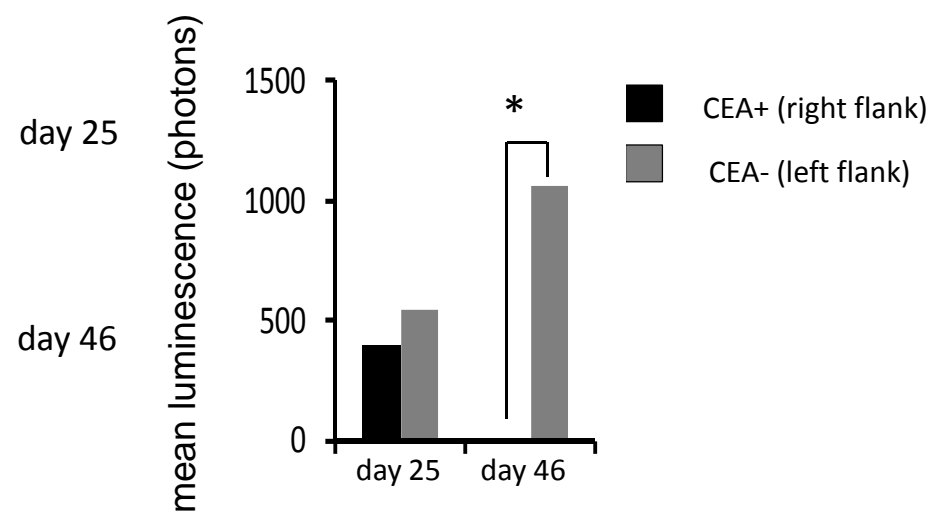


# CAR T cells establish secondary tumor rejection

## secondary tumor challenge



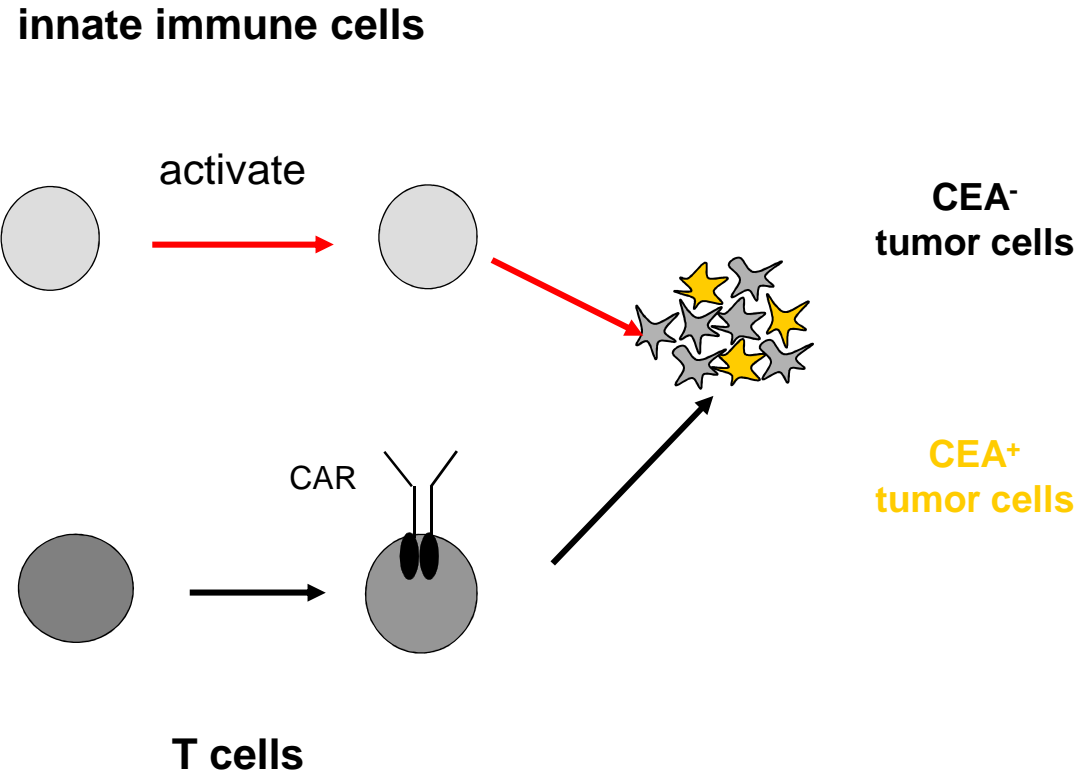
left flank: CEA<sup>-</sup> tumour  
right flank: CEA<sup>+</sup> tumour



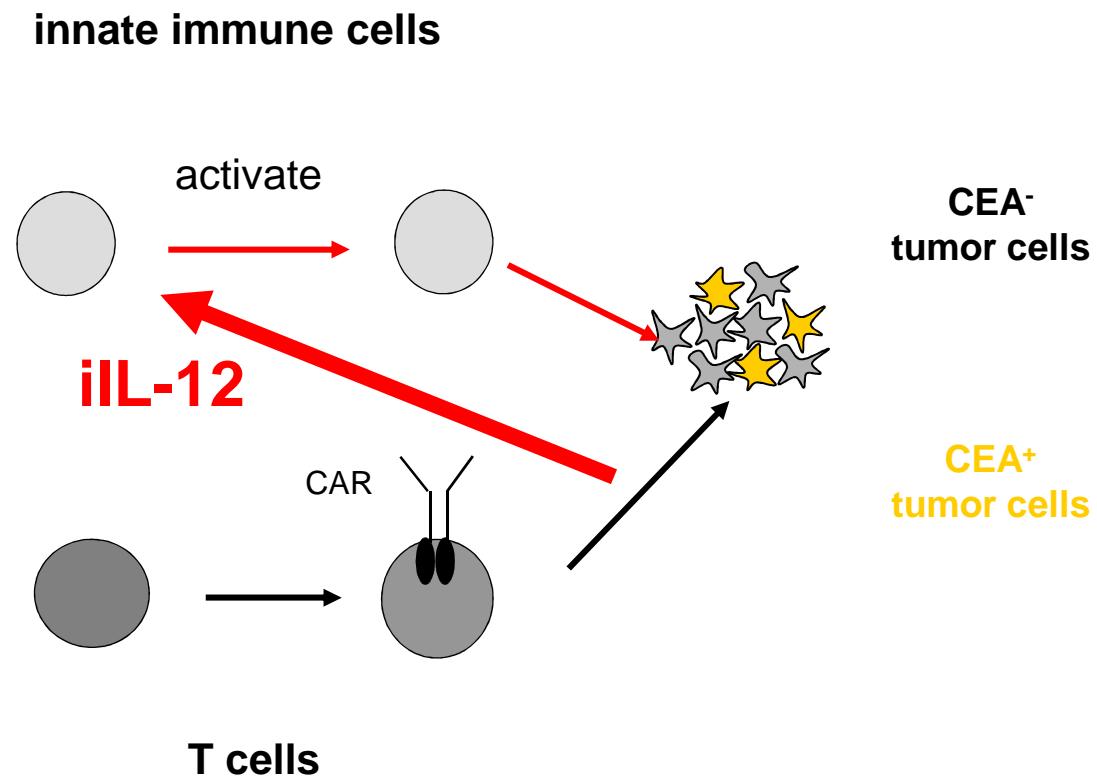


1. „Tumor associated antigens“ are not exclusively expressed by tumor cells.
2. Tumors are extremely heterogenous with respect to targetable surface antigens

# How to activate innate immune cells in the targeted tumor lesion for an anti-tumor attack?



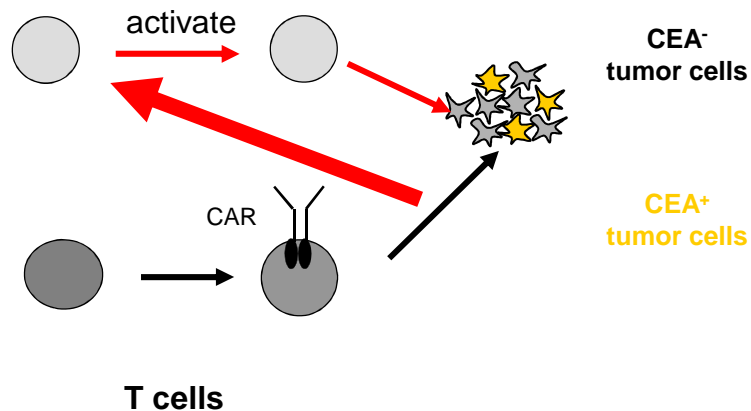
# How to activate innate immune cells in the targeted tumor lesion for an anti-tumor attack?



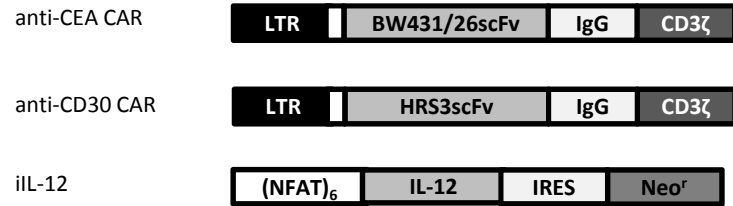
# Why IL12?

- recruits innate and adaptive effector cells
- activates T cells, NK cells, CD11b<sup>+</sup> myeloid derived cells
- promotes T<sub>H</sub>1 cell polarization and reverses T<sub>H</sub>2 polarization
- improves MHC class I presentation
- increases IP-10, MIG chemokine secretion
- alters extracellular matrix (MMPs ↓, VEGF ↓, endothelial cell adhesion molecules ↓)
- decreases angiogenesis

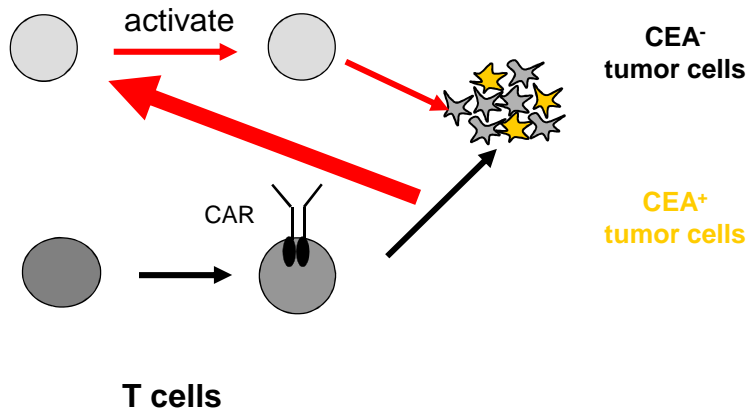
## innate immune cells



# T cells engineered with CAR inducible IL-12

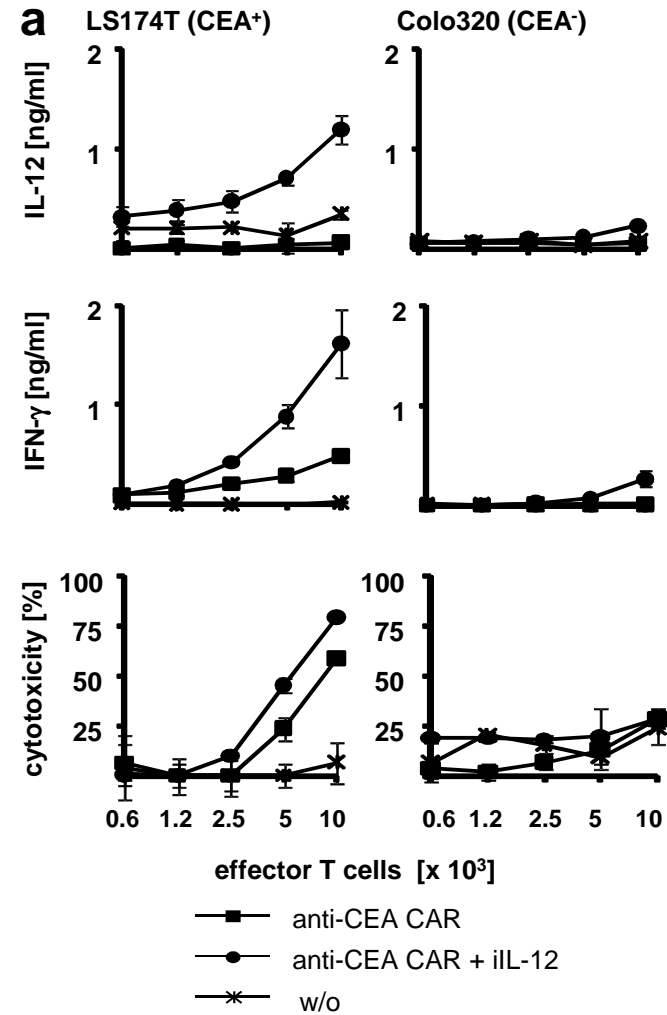
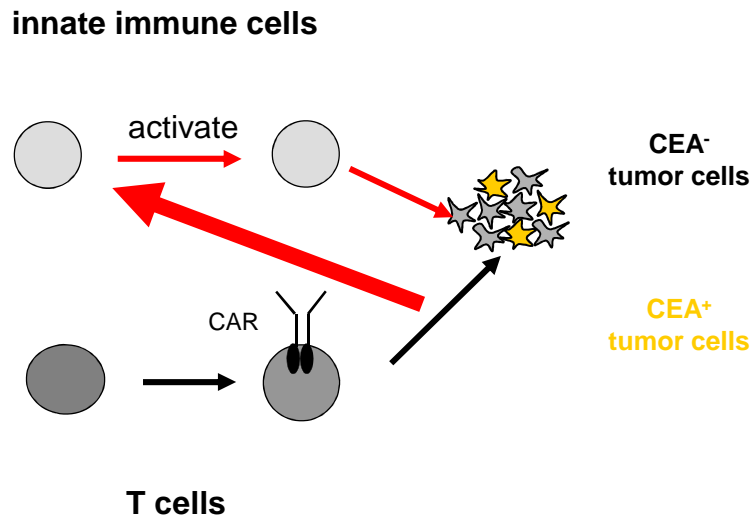
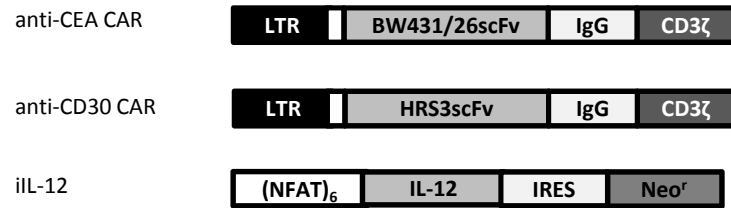


## innate immune cells



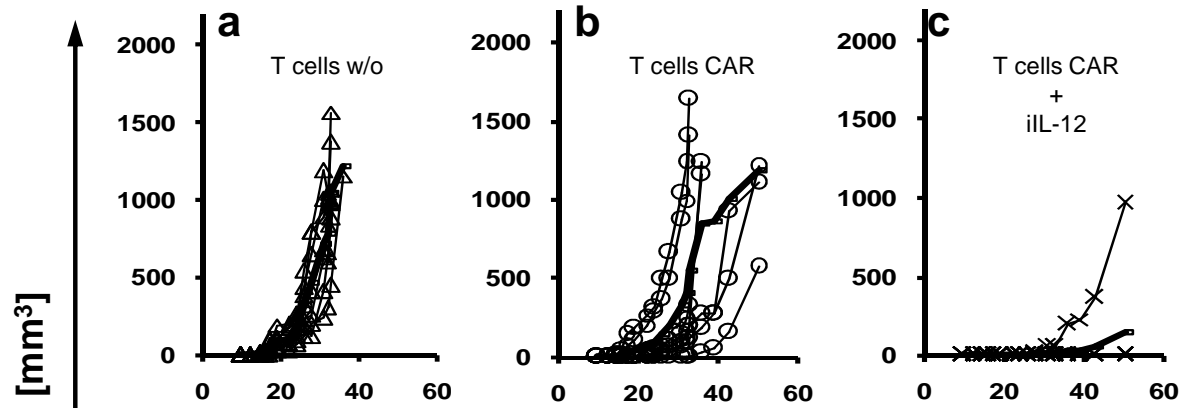


# T cells engineered with CAR inducible IL-12

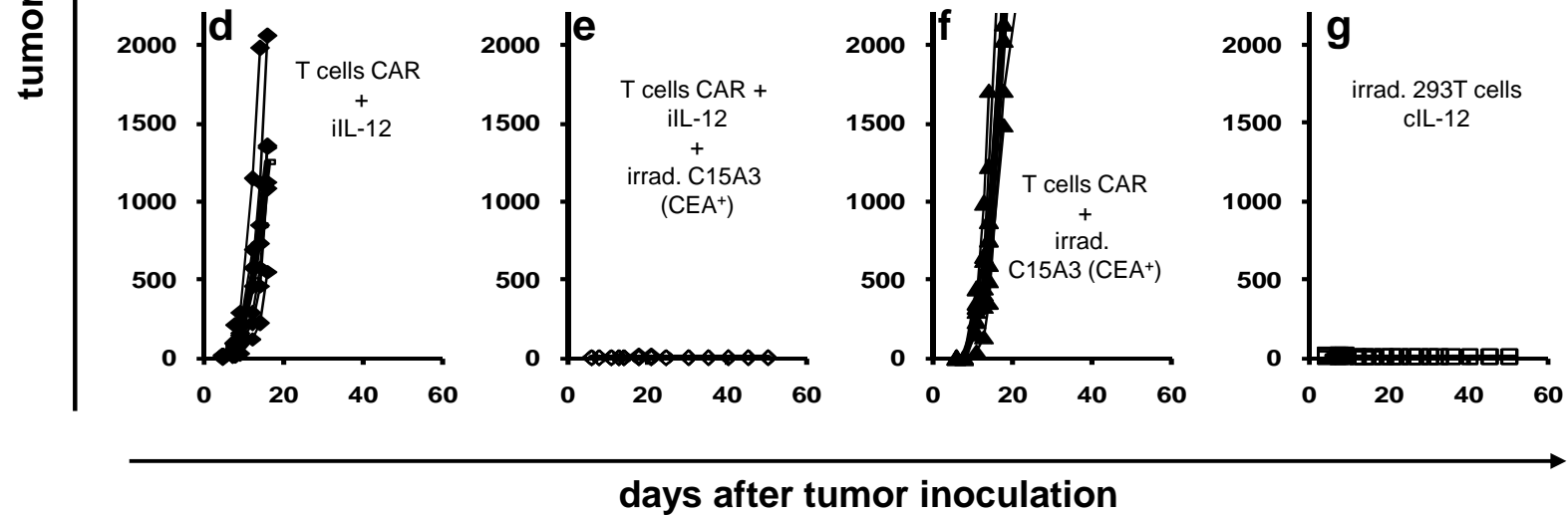


# T cells engineered with CAR inducible IL-12

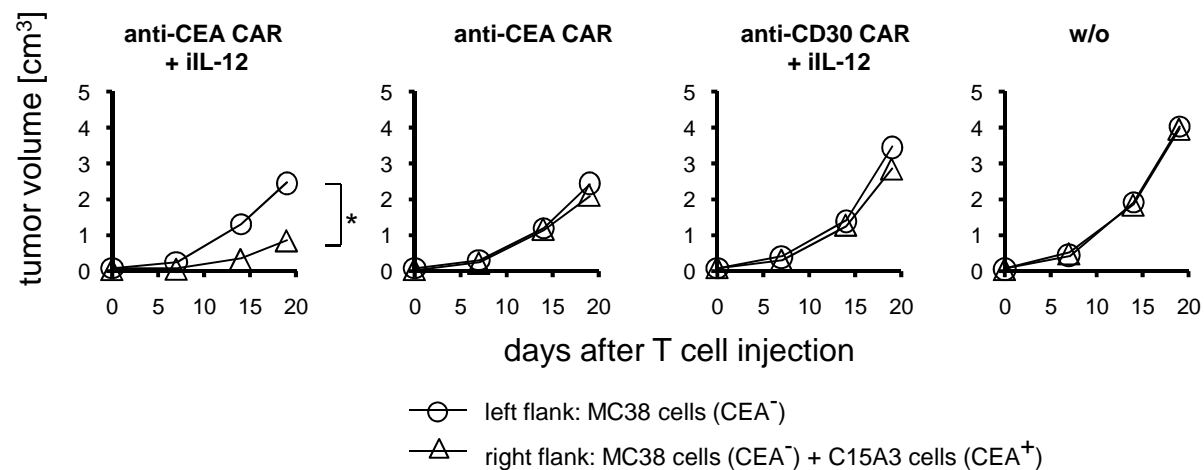
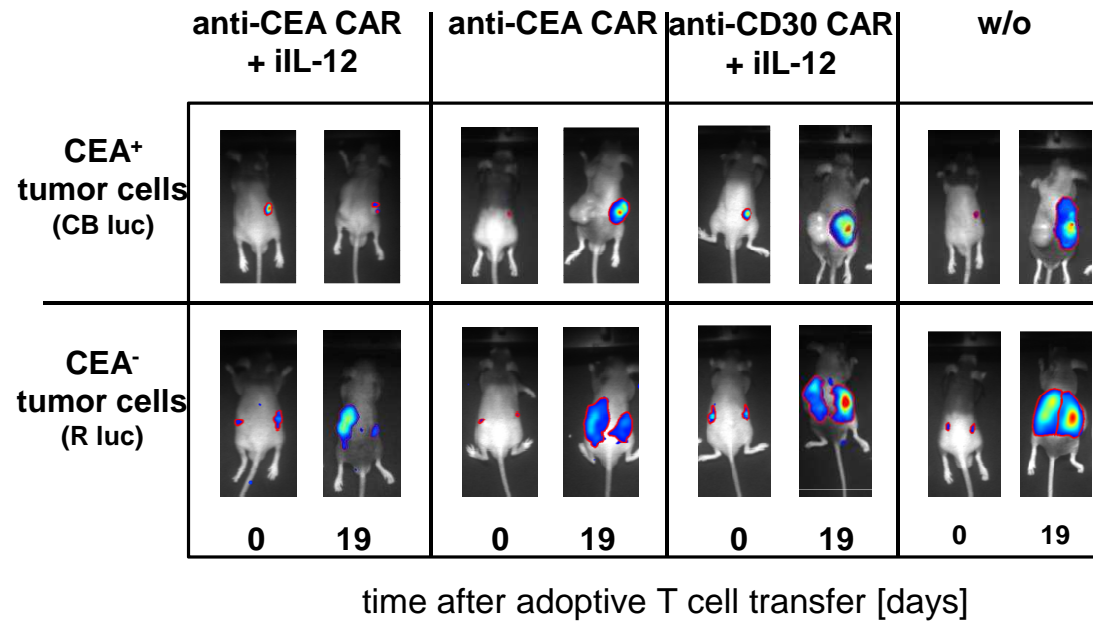
## C15A3 (CEA<sup>+</sup>)



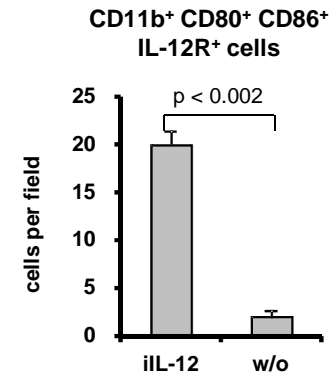
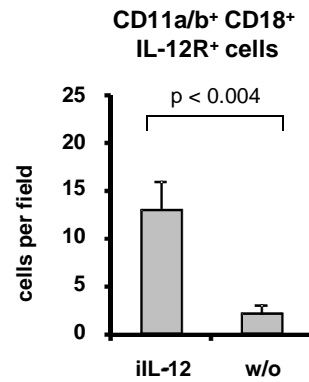
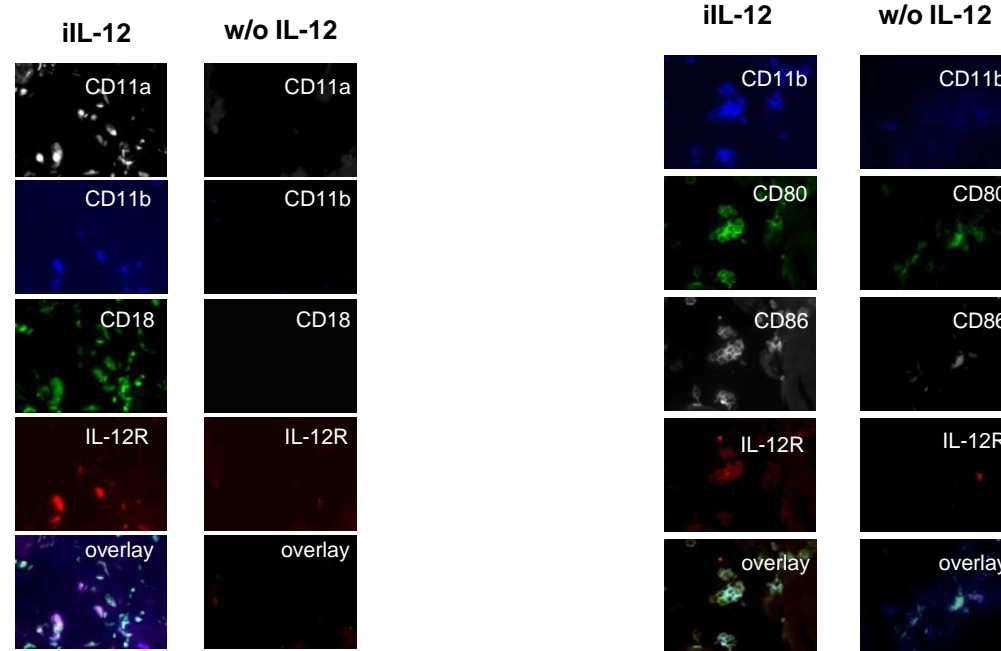
## MC38 (CEA<sup>-</sup>)



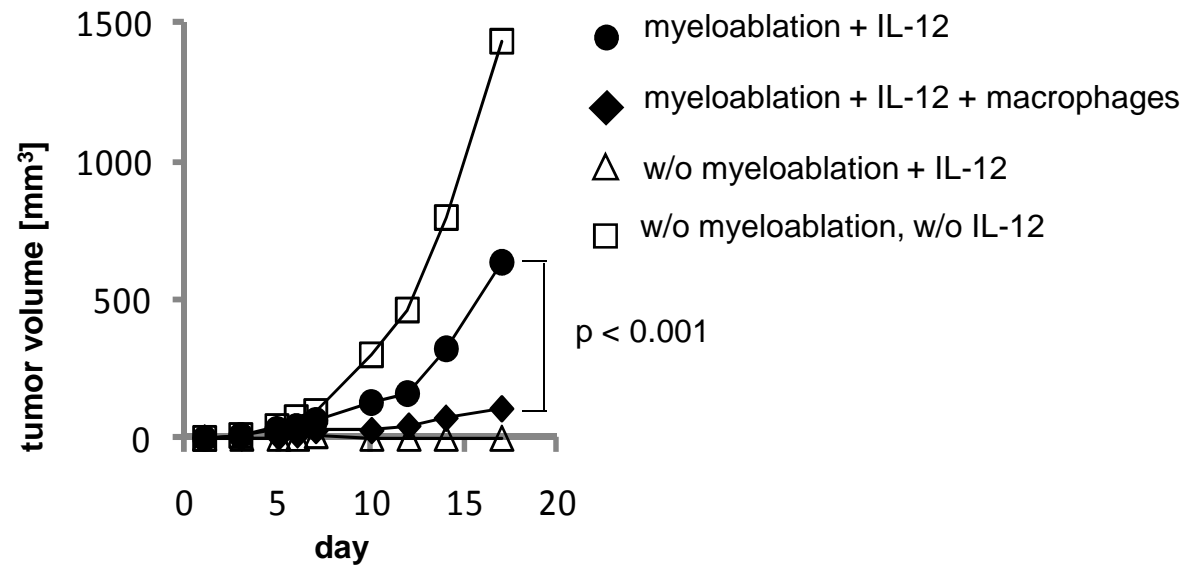
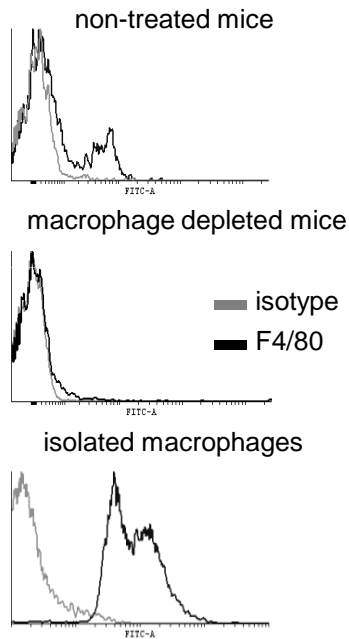
# T cells engineered with CAR inducible IL-12 mediate control of CEA<sup>-</sup> cancer cells in CEA<sup>+</sup> tumors



# Activated macrophages in tumor lesions treated with CAR iL-12 T cells

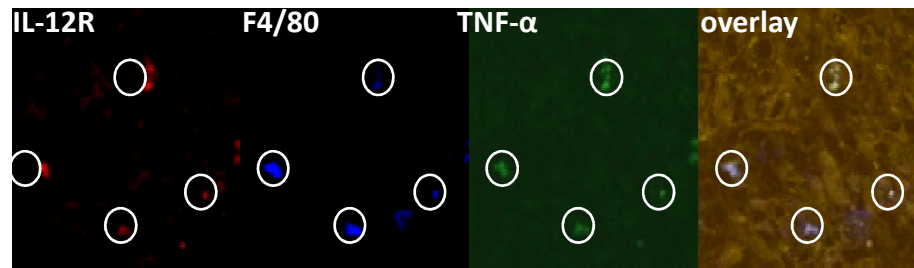


# Activated macrophages are involved in killing CEA<sup>-</sup> tumor cells



# Activated tissue macrophages in tumors produce TNF- $\alpha$

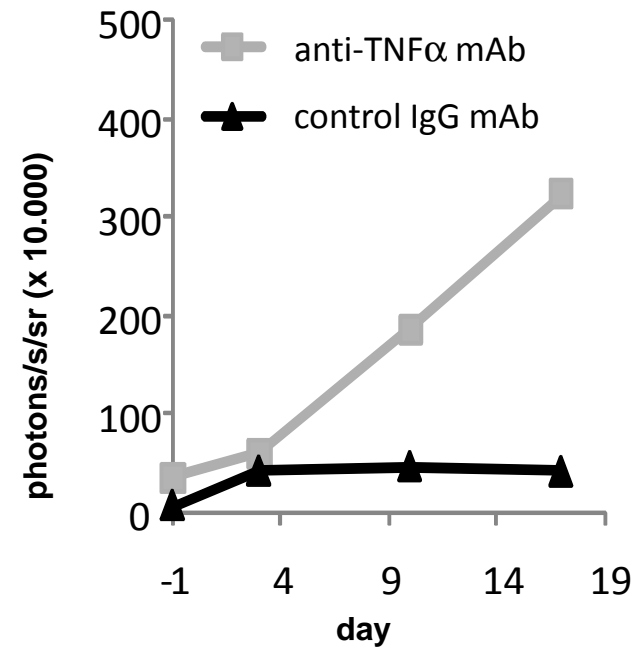
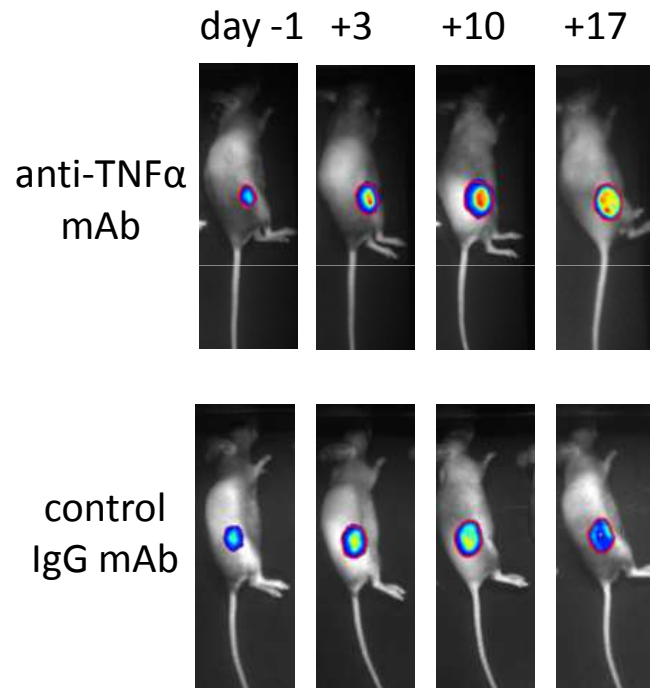
CAR iIL-12 T cell  
treated tumor



CAR T cell  
treated tumor



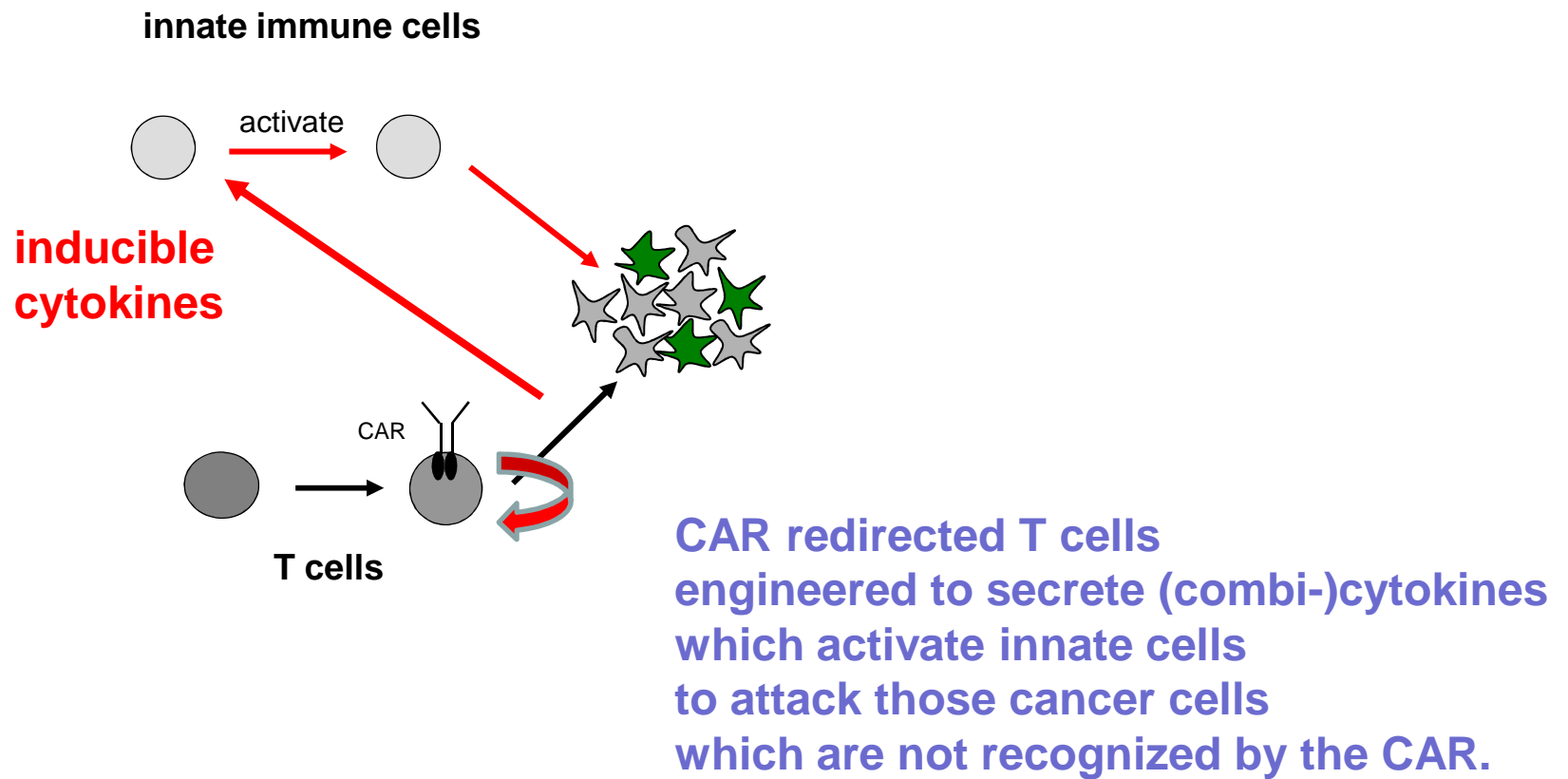
# Activated tissue macrophages kill CEA<sup>-</sup> tumor cells through TNF- $\alpha$



# Other inducible effector molecules?

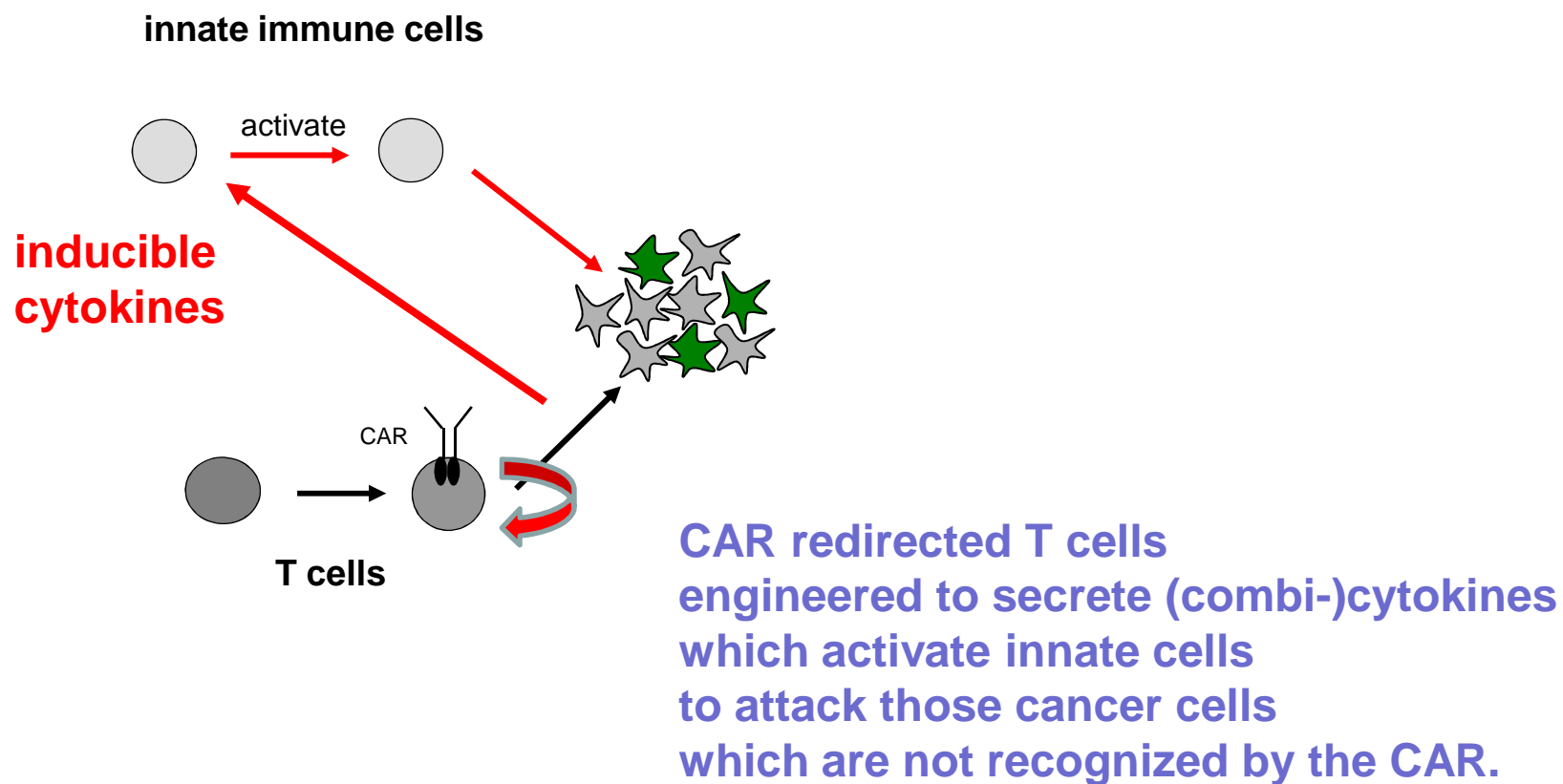


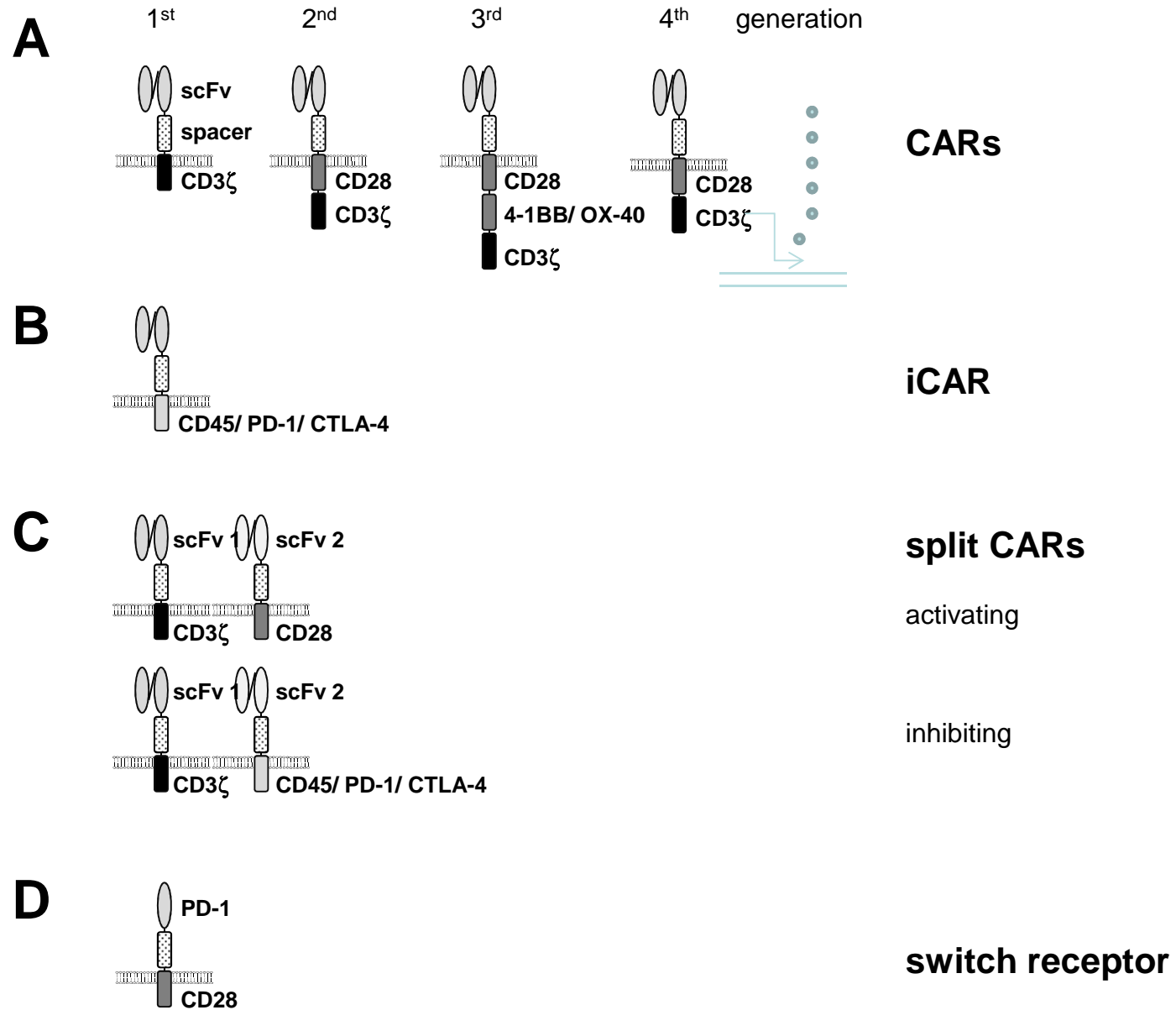




# TRUCKs:

T cells redirected for antigen-unrestricted cytokine-initiated killing





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B. Seliger, Halle  
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BMBF