Recent developments in CAR-T cell therapy

Technical journal club 18.12.2018

Anna Henzi

Recent case report...

nature medicine

Brief Communication | Published: 01 October 2018

Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell

Marco Ruella, Jun Xu, […] J. Joseph Melenhorst

Nature Medicine **24**, 1499–1503 (2018) | Download Citation

Recent case report...

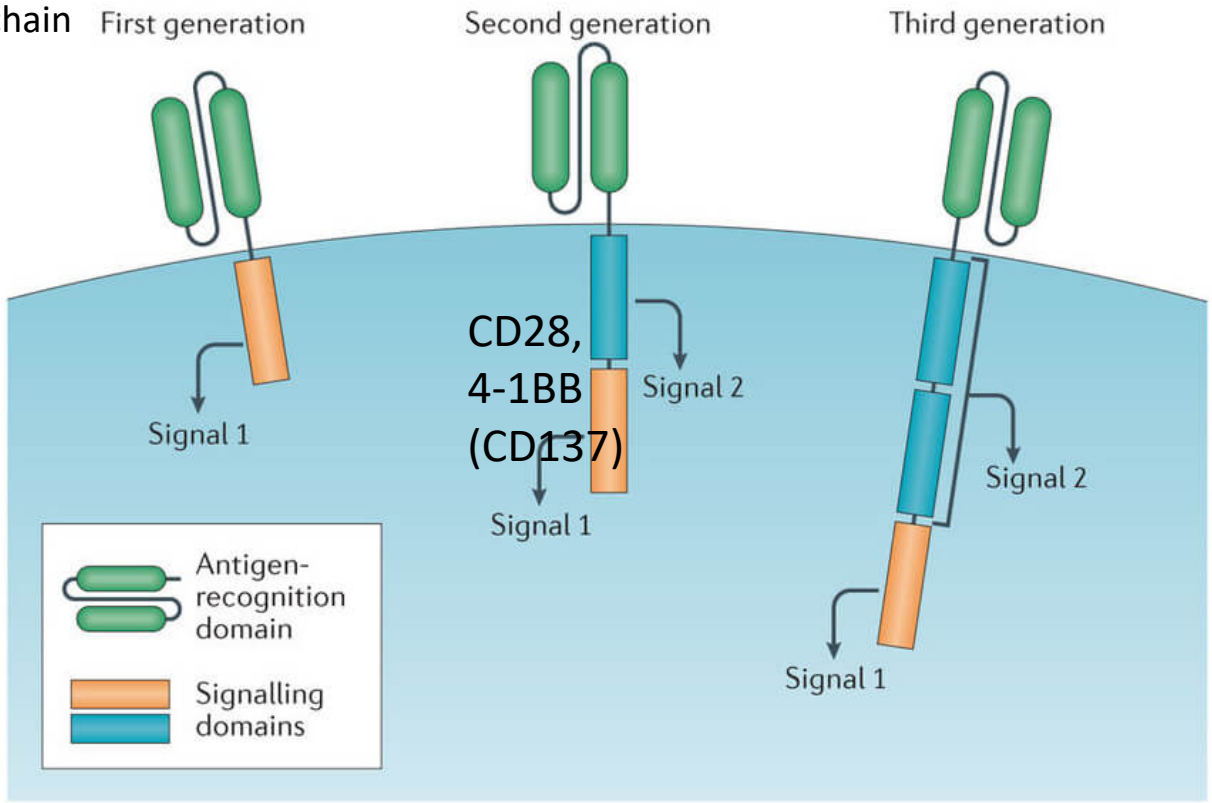
- 20y old patient with B-ALL
- Relapse 9 months after CD19 targeted CAR T cell infusion
- CD19- leukemia with aberrant expression of anti-CD19 CAR

CAR-T cell therapy

- CAR = chimeric antigen receptor
  - Antigen-binding region (scFv)
  - T-cell receptor transmembrane domain
  - Intracellular signaling: CD3ζ chain

Tisangenlecleucel (Kymriah)

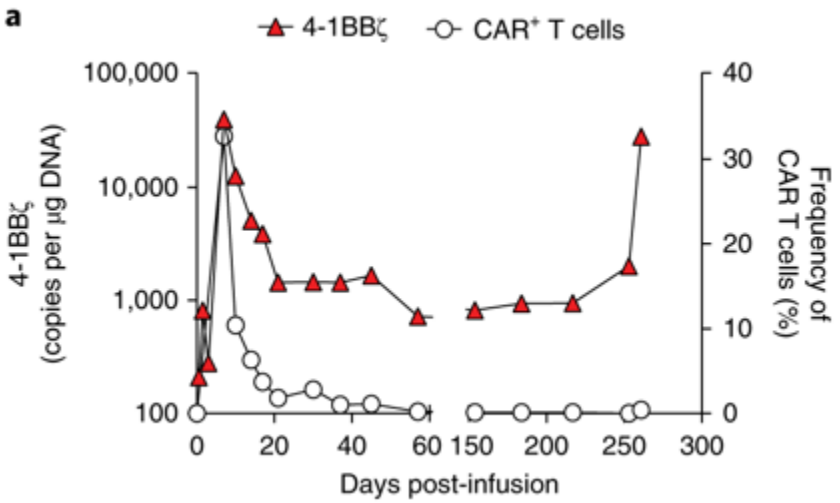
CD19 CAR T cell products for treatment of B cell malignancies
- Autologous lymphocytes

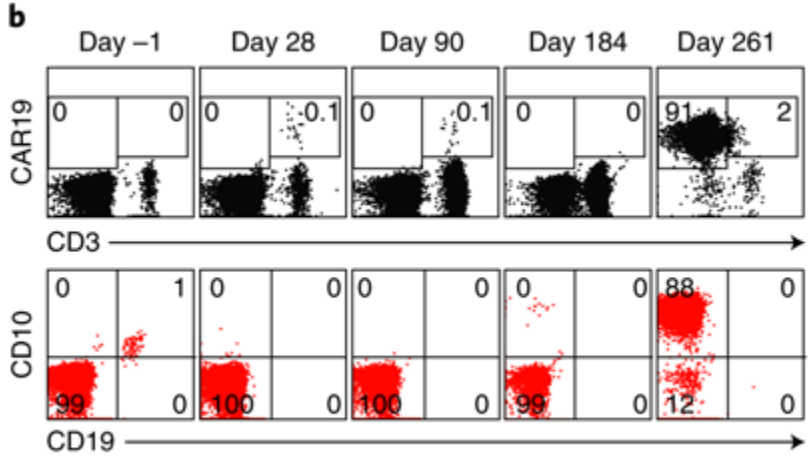


Nature Reviews | Clinical Oncology

Back to the case report...

- Introduction of CAR gene into a single leukemic B cell during T cell manufacturing
- CAR binds to CD19 epitope > masking > resistance





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Nat Nanotechnol. 2017 August ; 12(8): 813–820. doi:10.1038/nnano.2017.57.

In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers

Tyrel T. Smith[1,†], **Sirkka B. Stephan**[1,†], **Howell F. Moffett**[1,†], **Laura E. McKnight**[1], **Weihang Ji**[1], **Diana Reiman**[2], **Emmy Bonagofski**[2], **Martin E. Wohlfahrt**[1], **Smitha P. S. Pillai**[3], and **Matthias T. Stephan**[1,2,4,5,*]

In situ programming of leukaemia-specific T cells using synthethic DNA nanocarriers

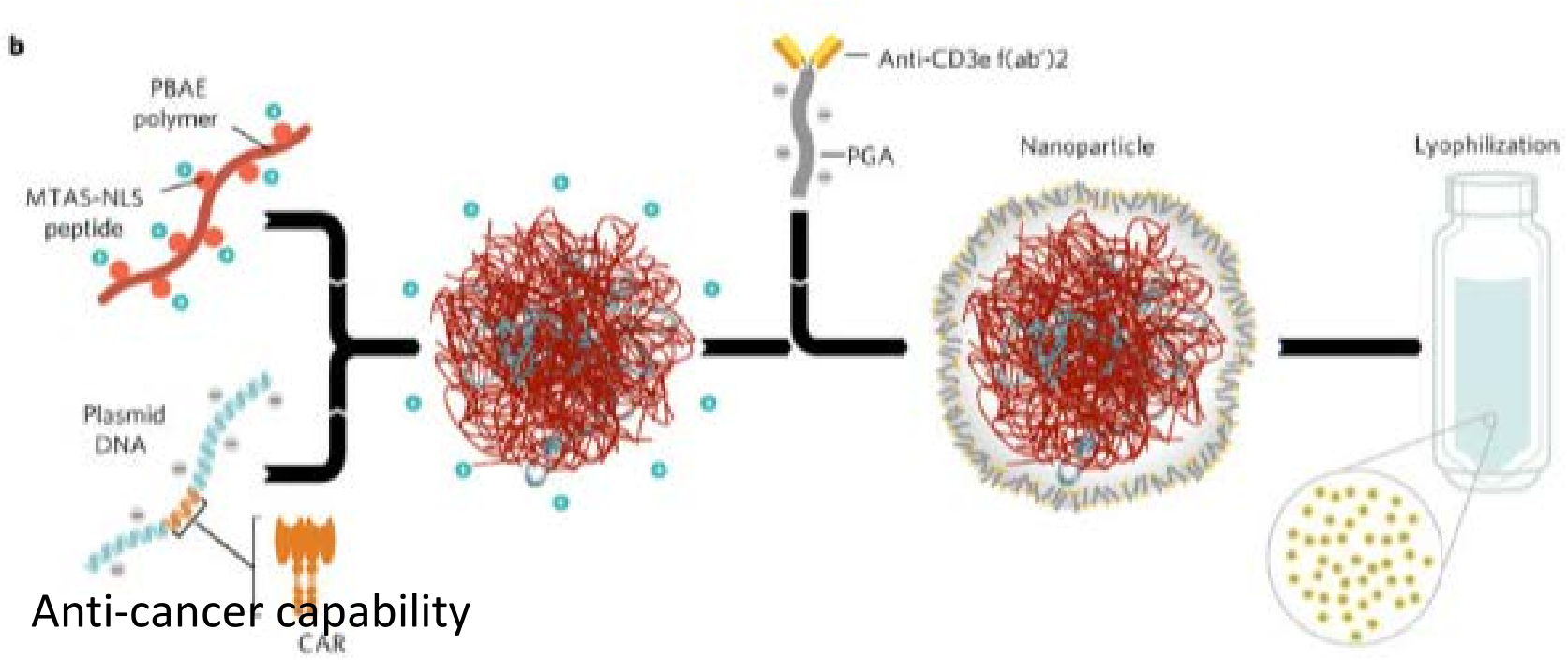
Problem: complex procedures to produce genetically modified lymphocytes

Solution: nanotechnology to make inexpensive DNA carriers > program T cells *in* patient

- inexpensive, quick, specific
- Sufficient quantities for anti-tumor activity

2 Delivery into nucleus

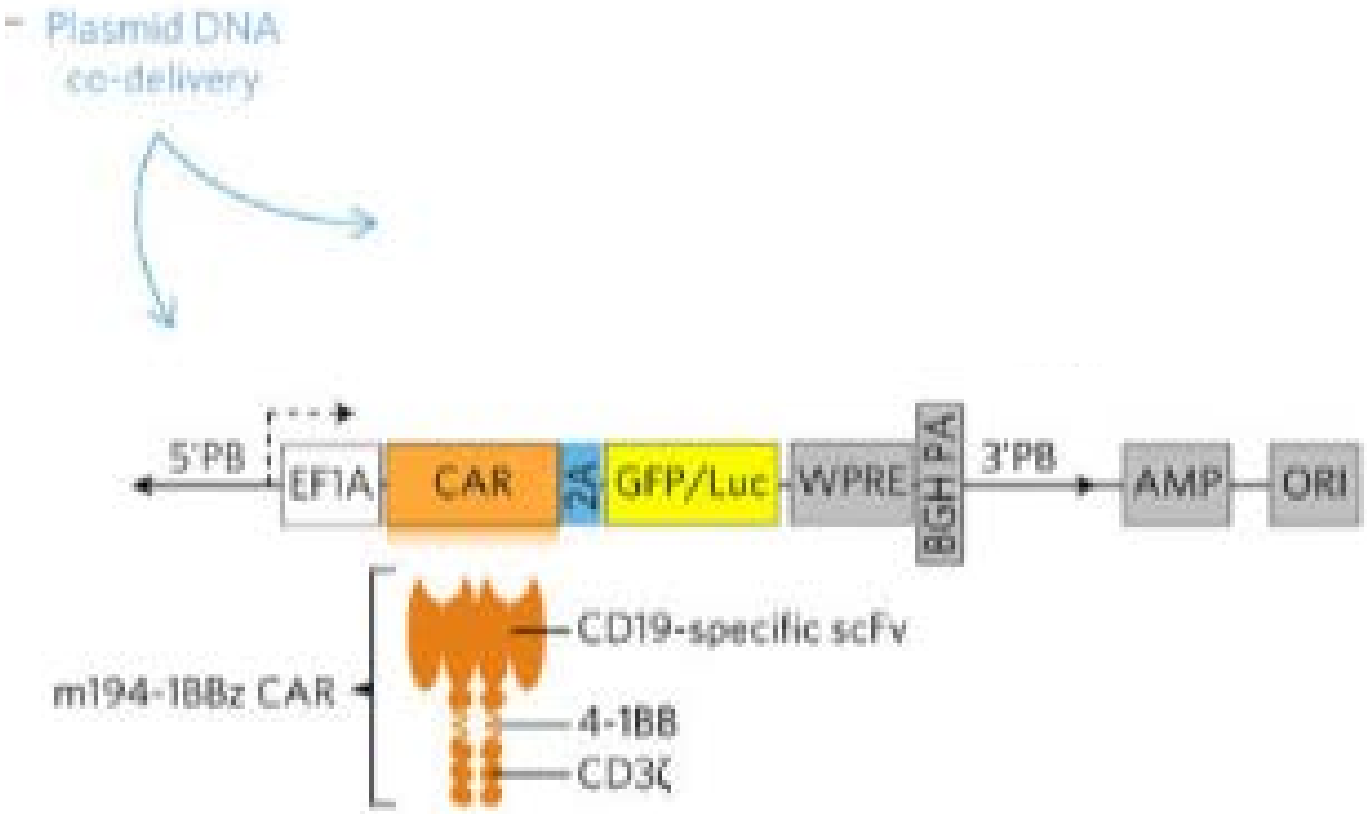
1 Uptake by T cells



Anti-cancer capability

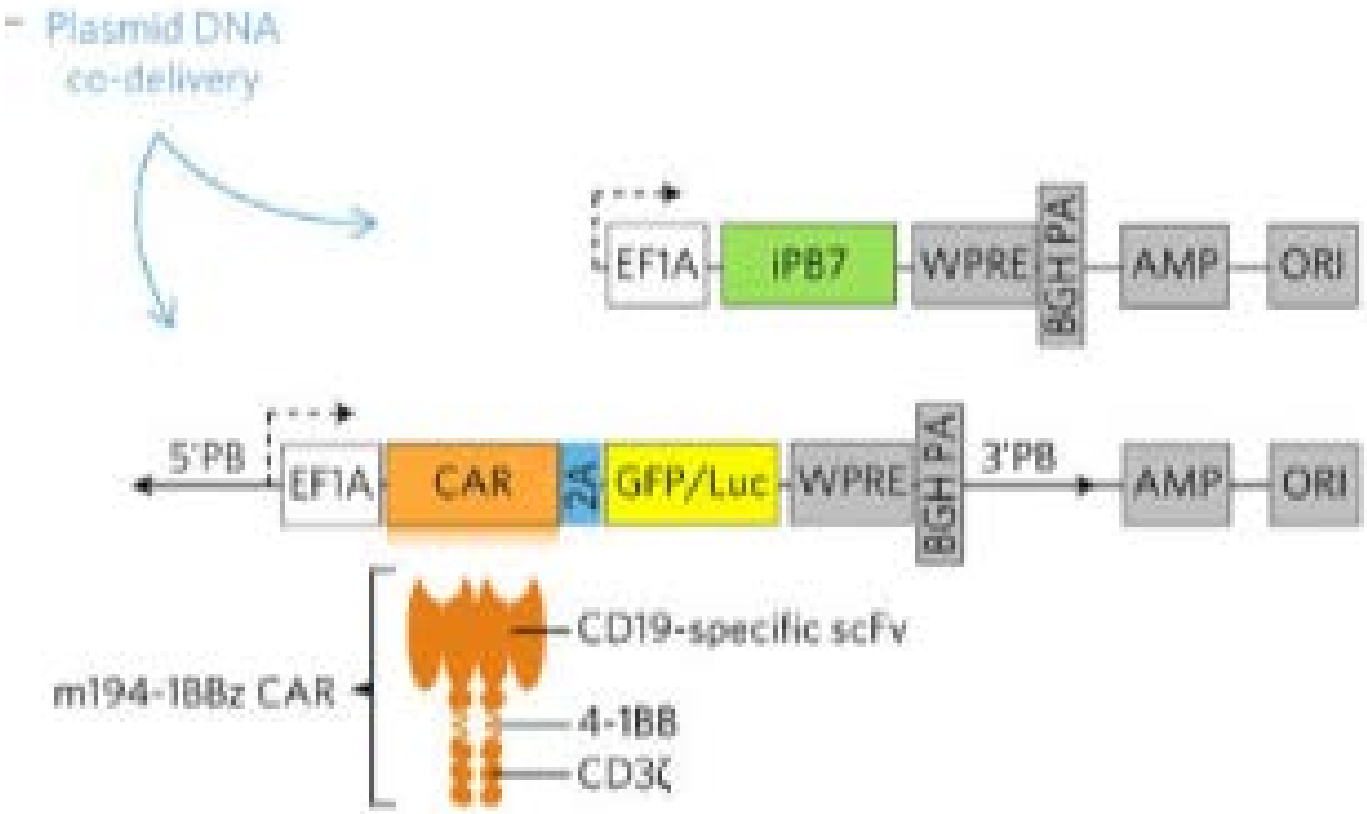
(leukaemia specific CAR, murine)

Nanocarrier



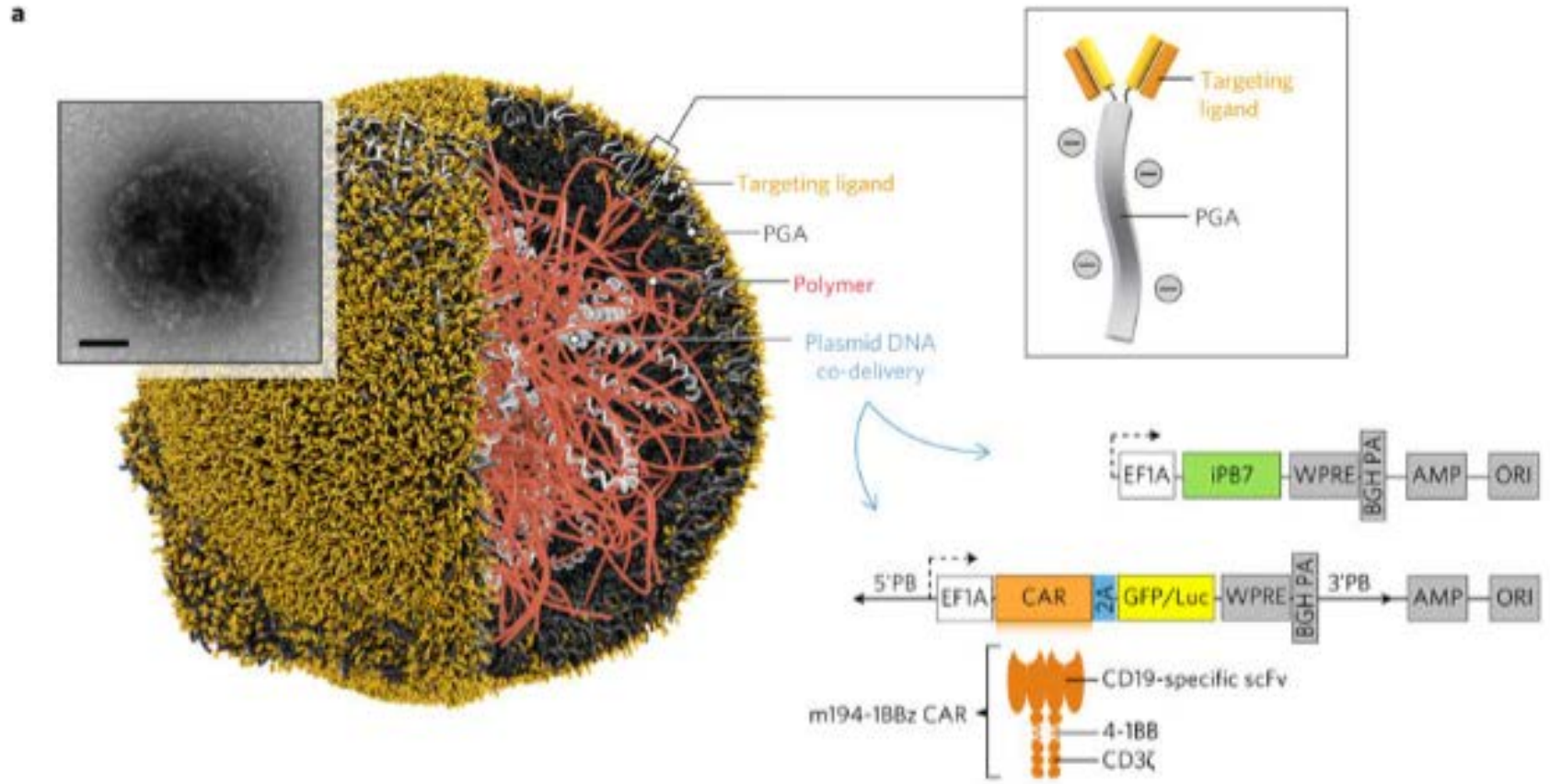
Nanocarrier

Anti-cancer capability



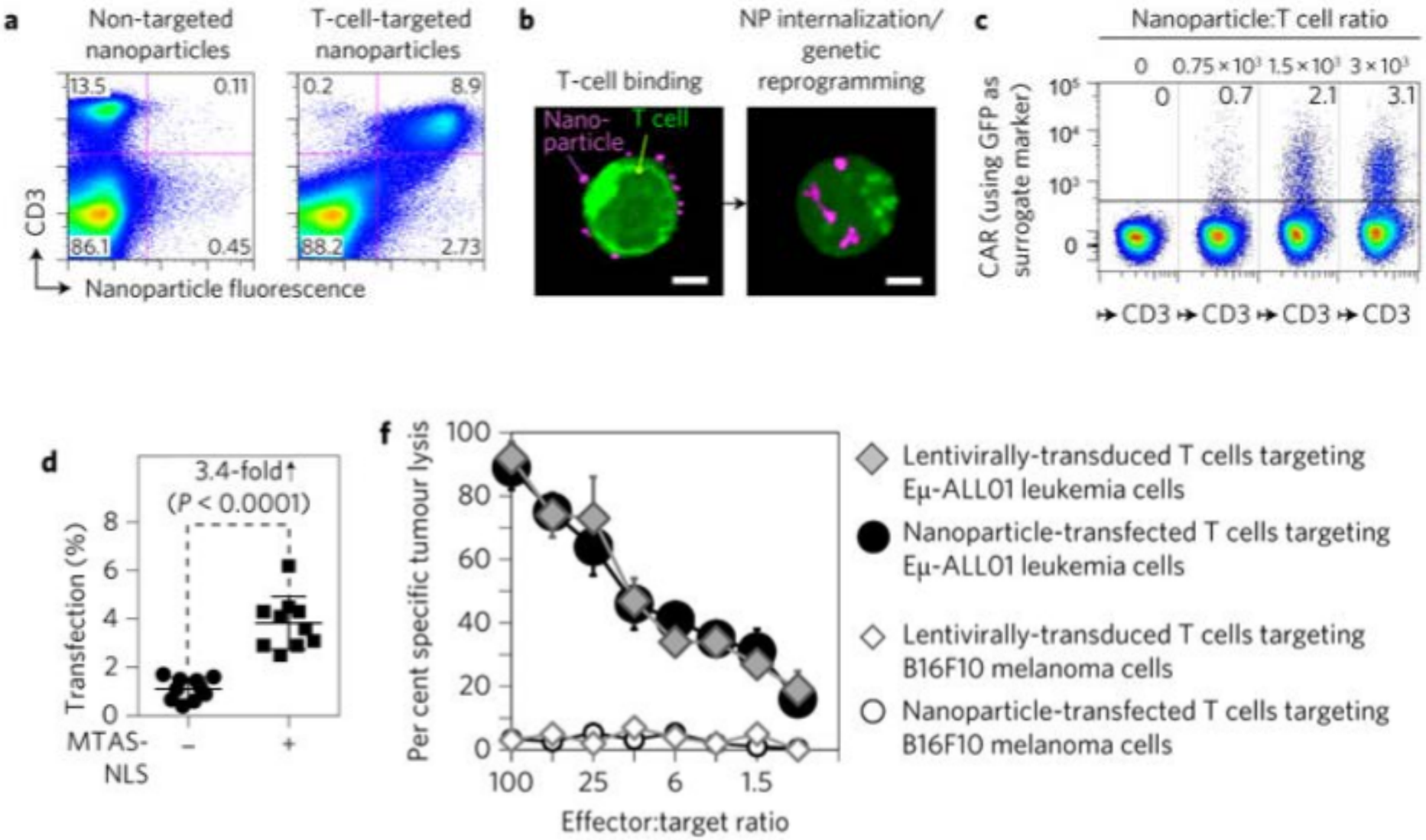
Nanocarrier

Anti cancer capability

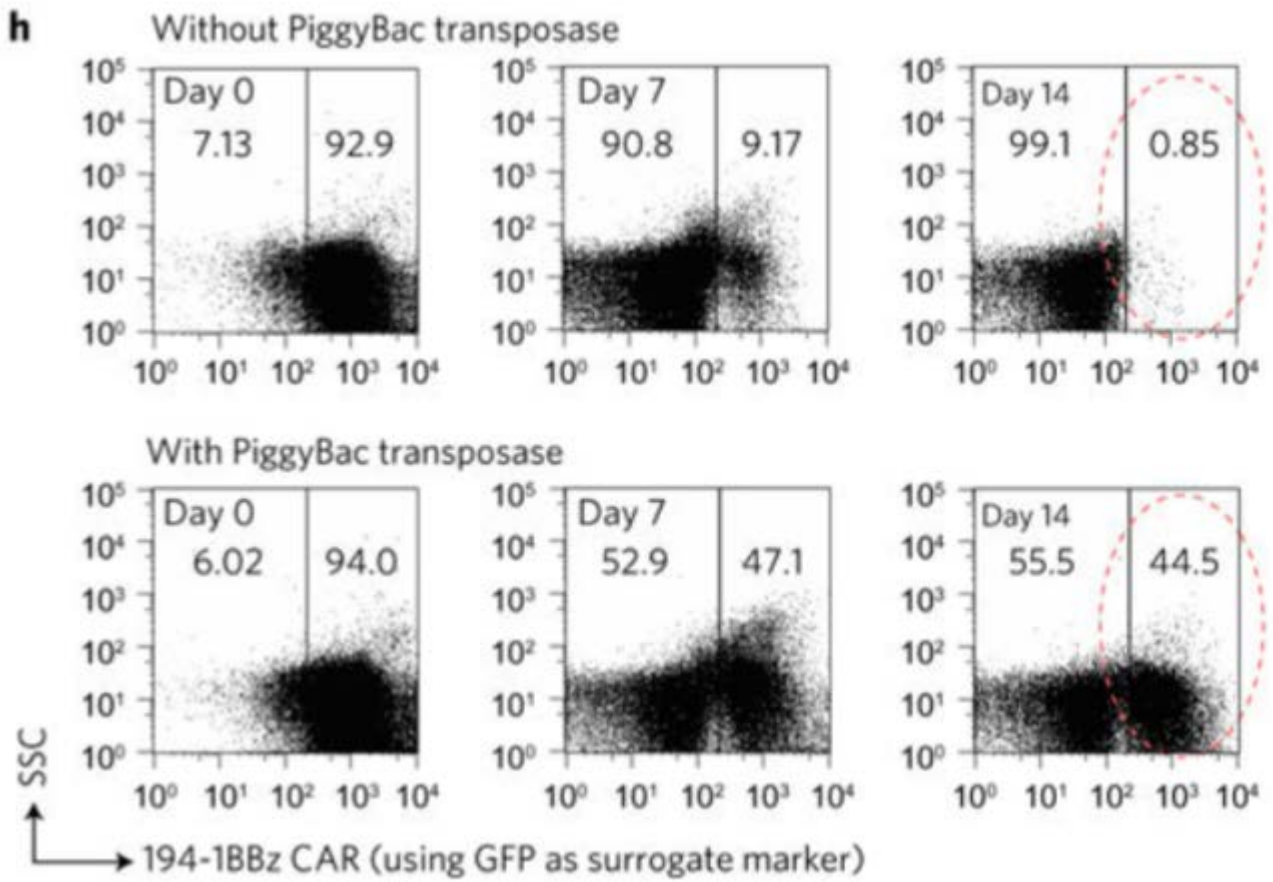


Nanocarrier

Targeting T cells *in vitro*

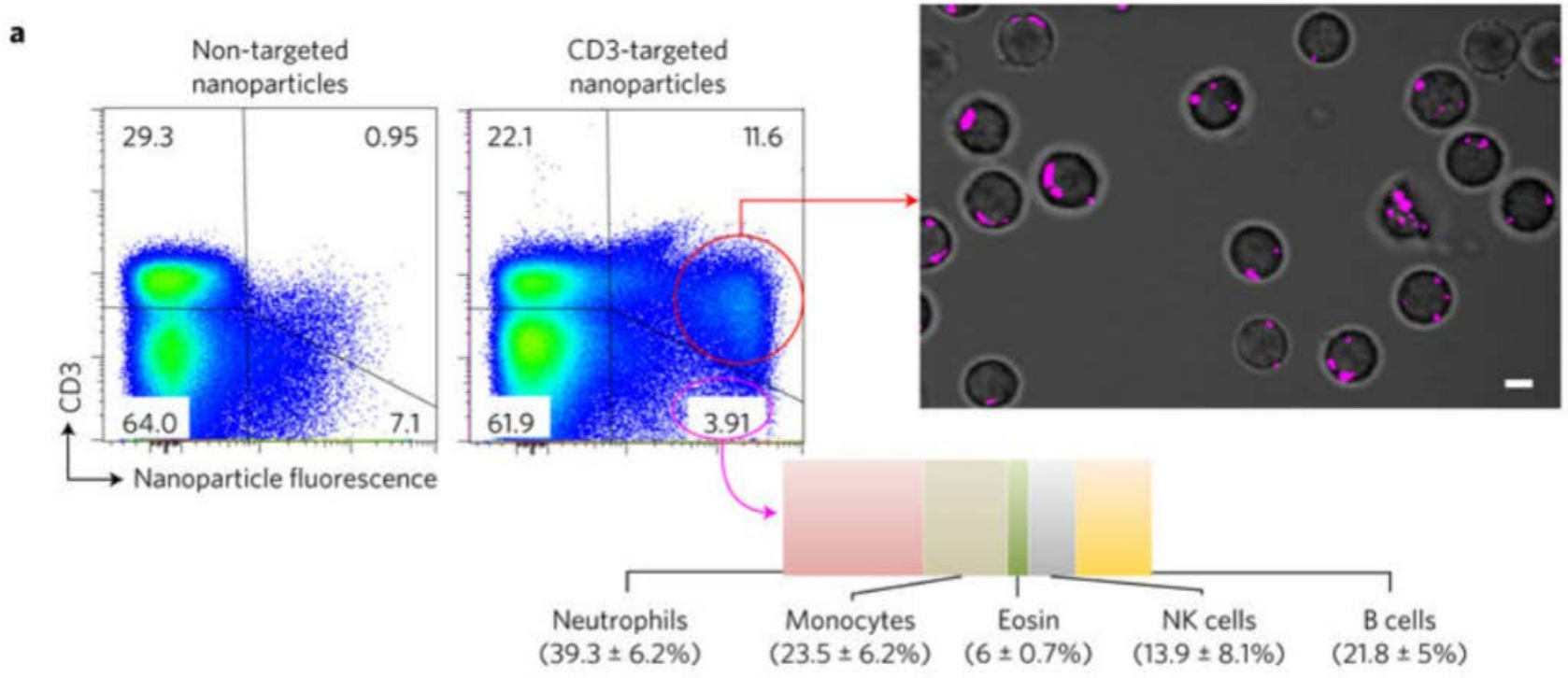


Targeting T cells *in vitro*



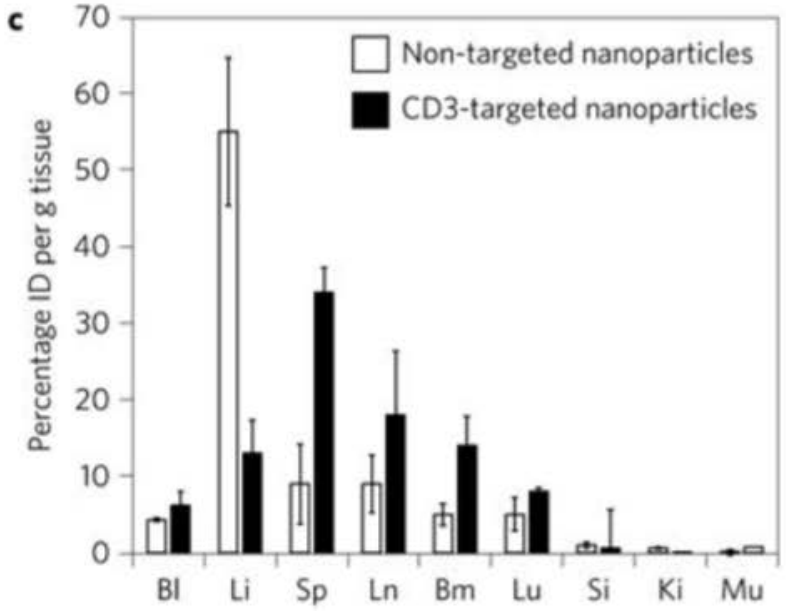
Targeting T cells *in vivo*

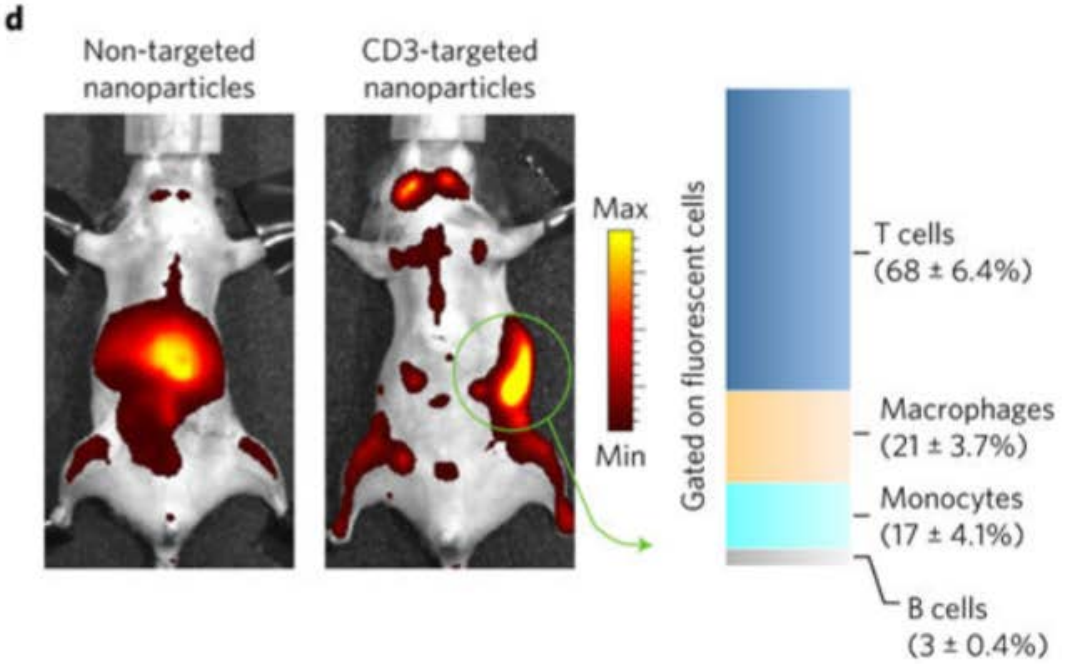
- How exclusively is the targeting of T cells?



Targeting T cells *in vivo*

- Distribution



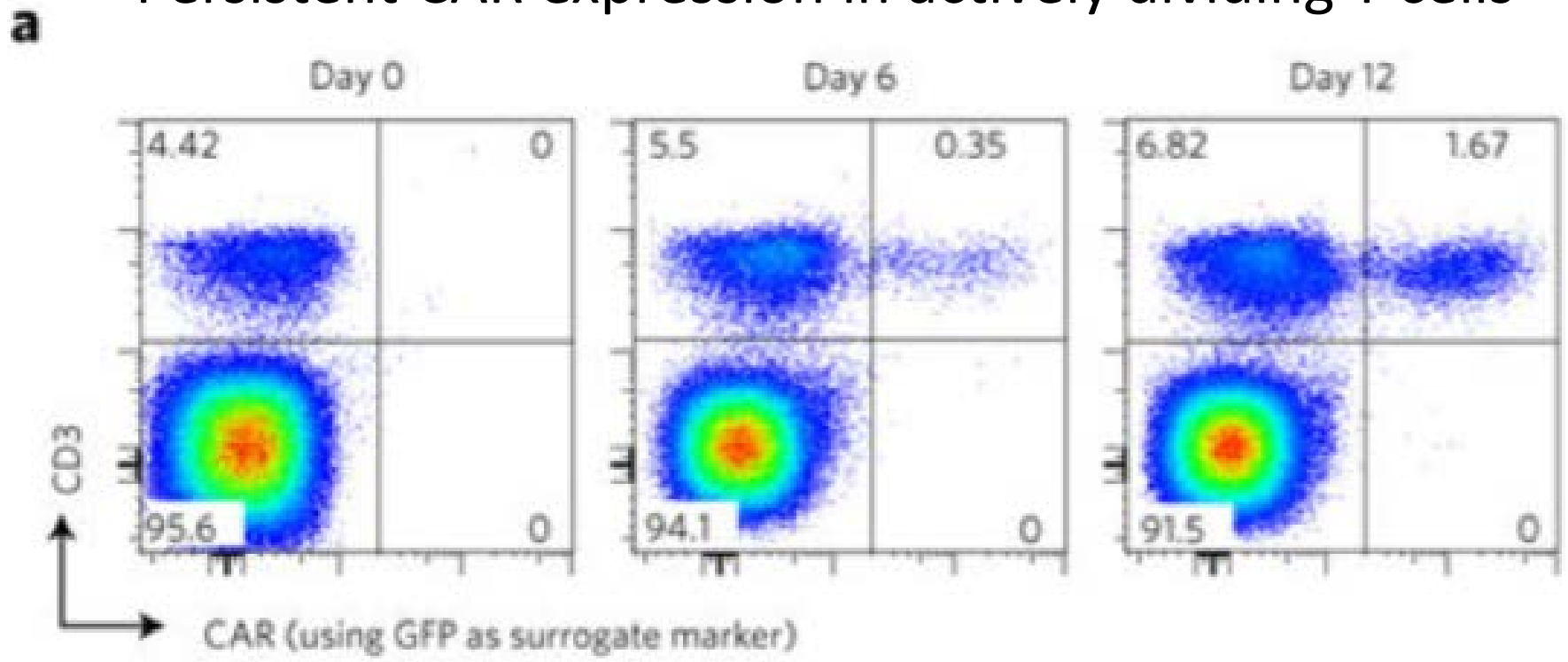


Toxicity of nanocarriers?

- Loaded with anti-P4-1BBz gene > human prostate specific membrane antigen

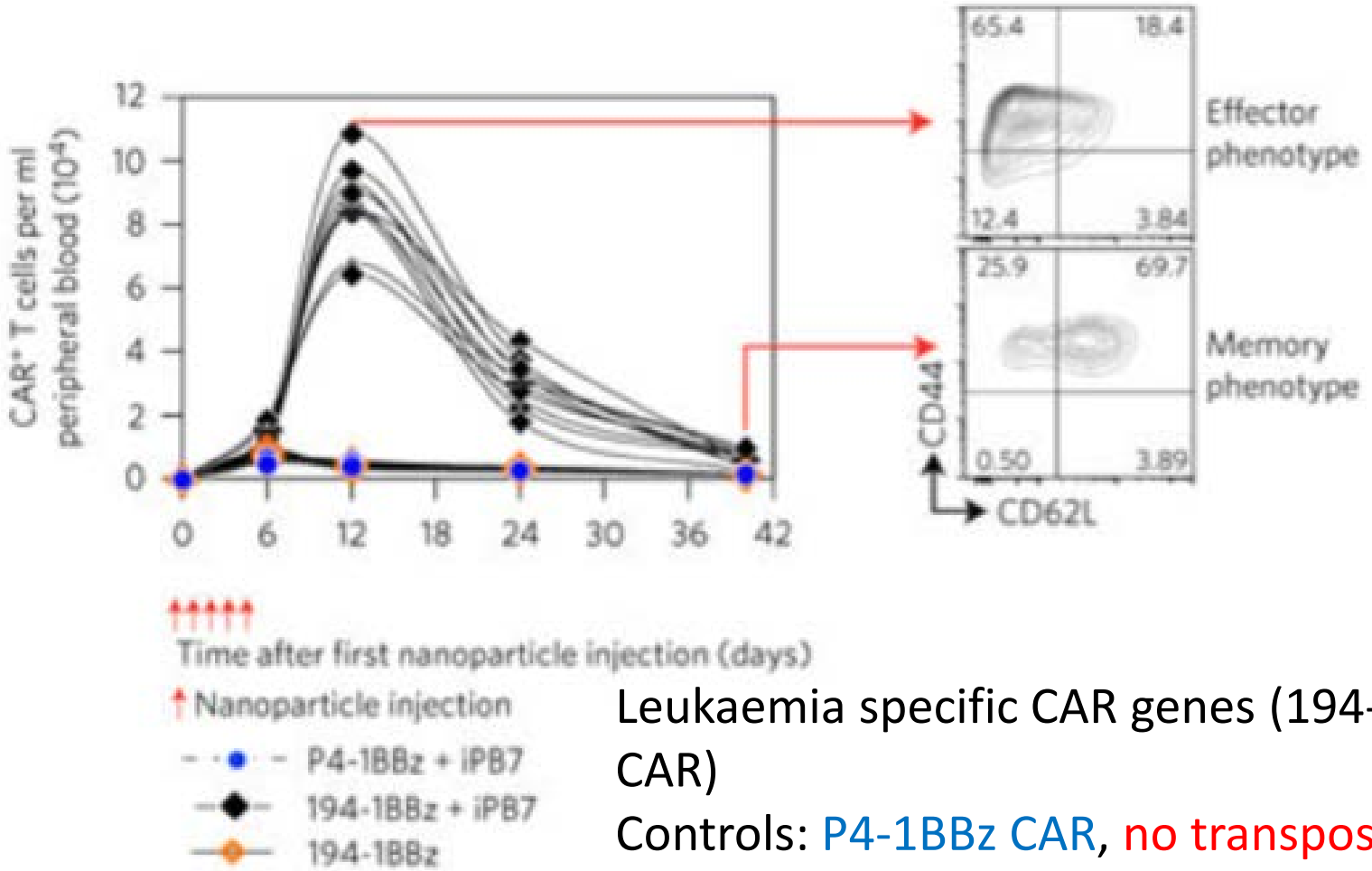
Reprogramming of T cells

- Mouse leukaemia model
- Persistent CAR expression in actively dividing T cells



Reprogramming of T cells

- Antigen required for proliferation
- Memory phenotype

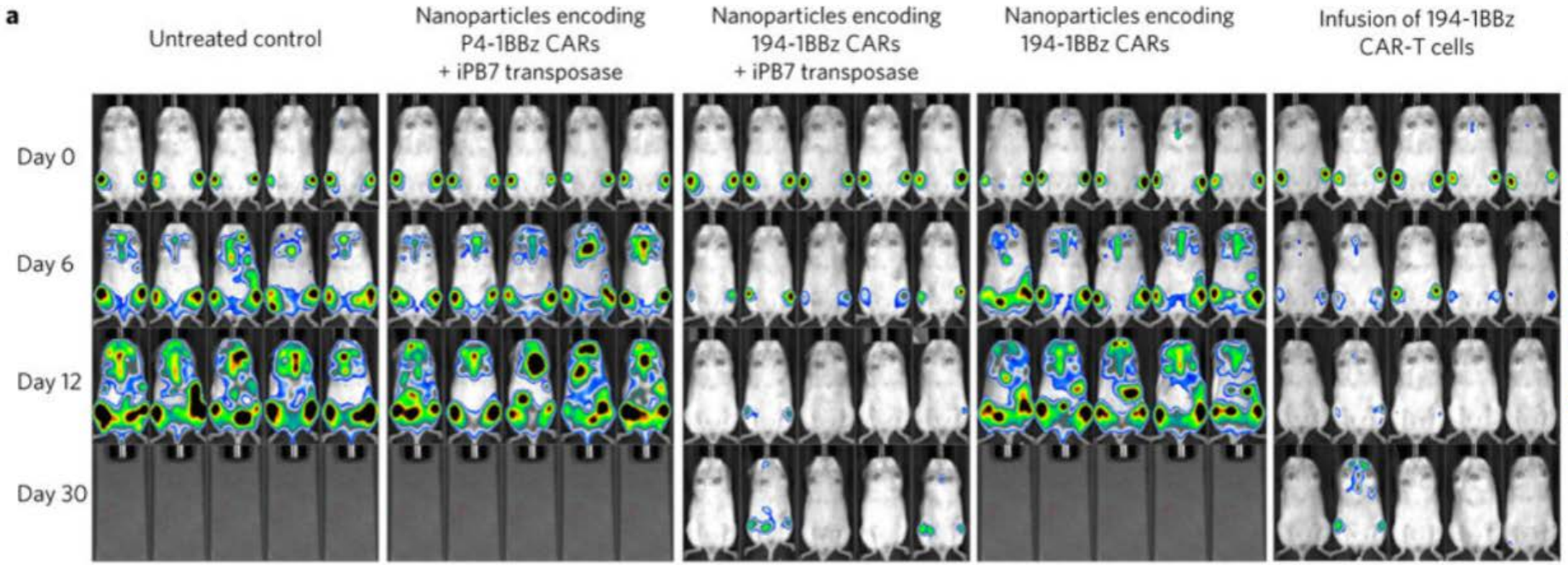


Leukaemia specific CAR genes (194-1BBz CAR)

Controls: P4-1BBz CAR, no transposase

Anti-tumor activity

- Luciferase expressing leukaemia cells (Eμ-ALL01)
- Immunocompetent albino mice



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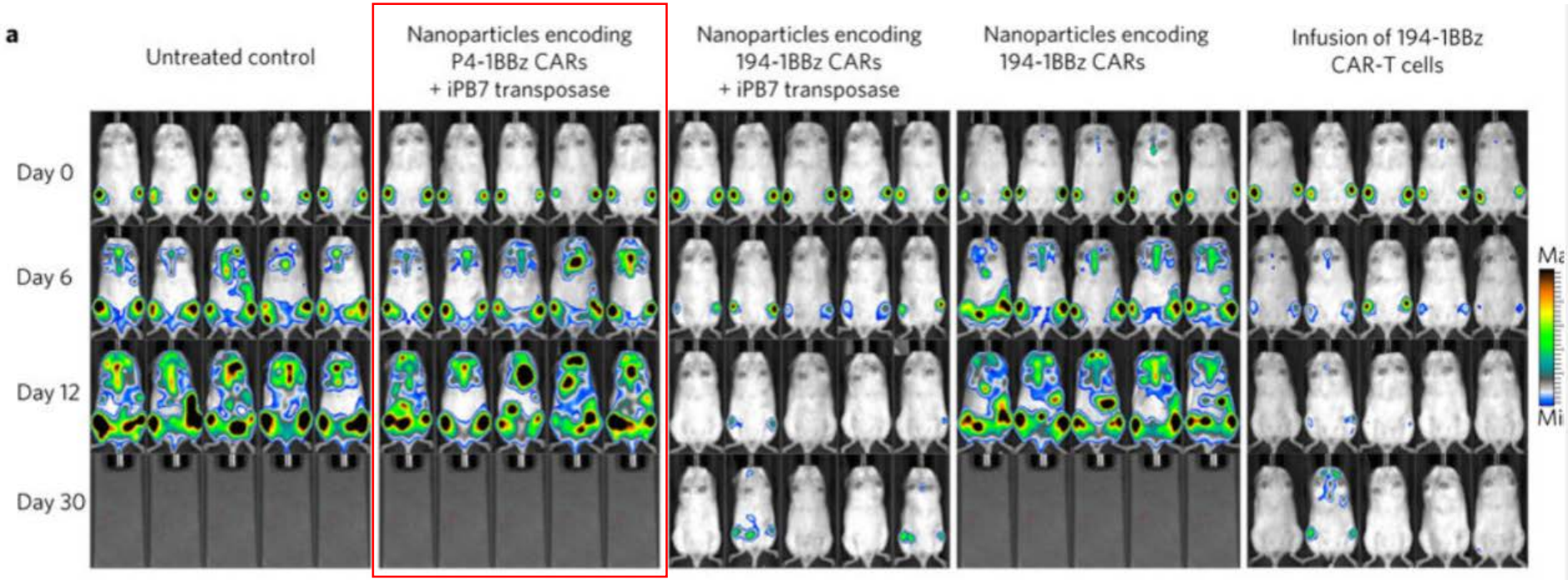

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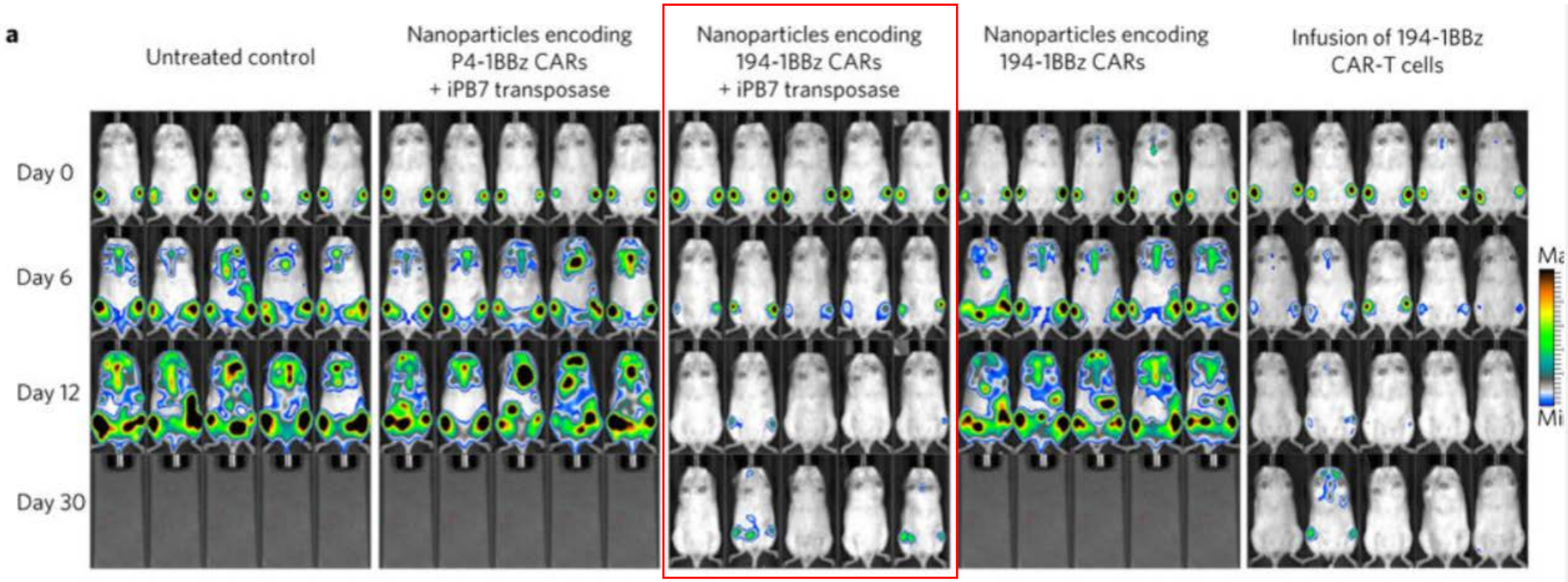

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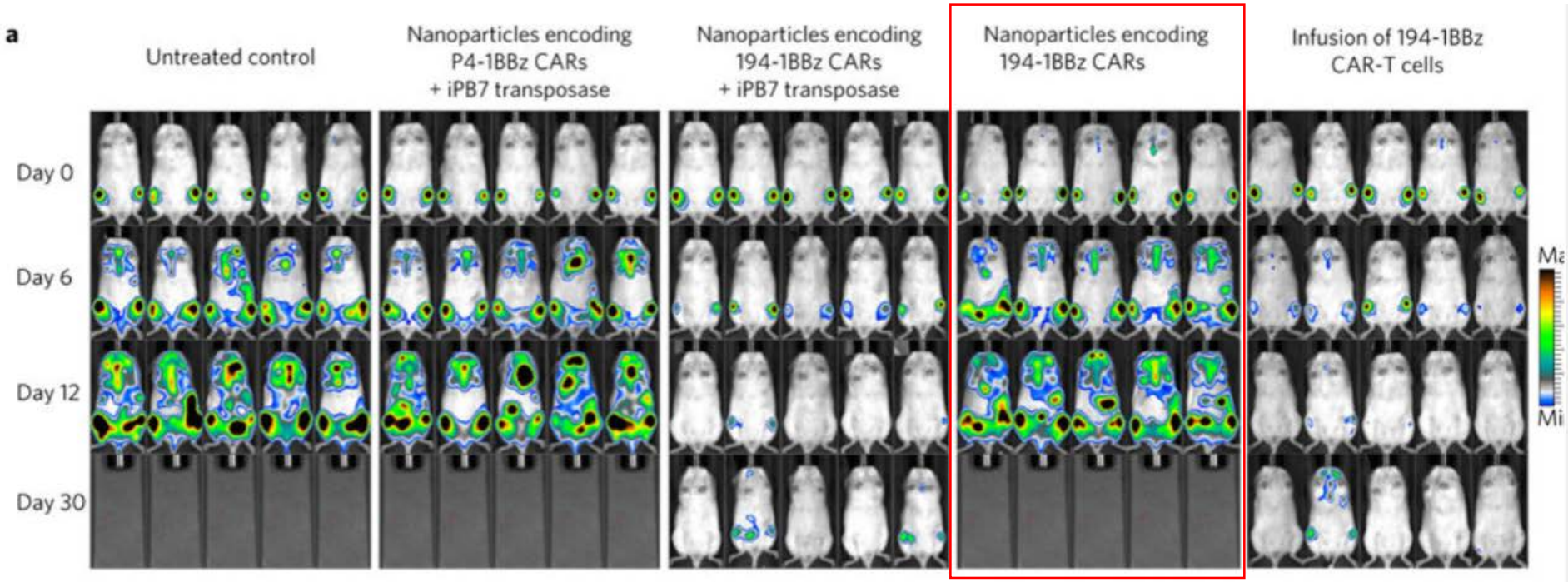

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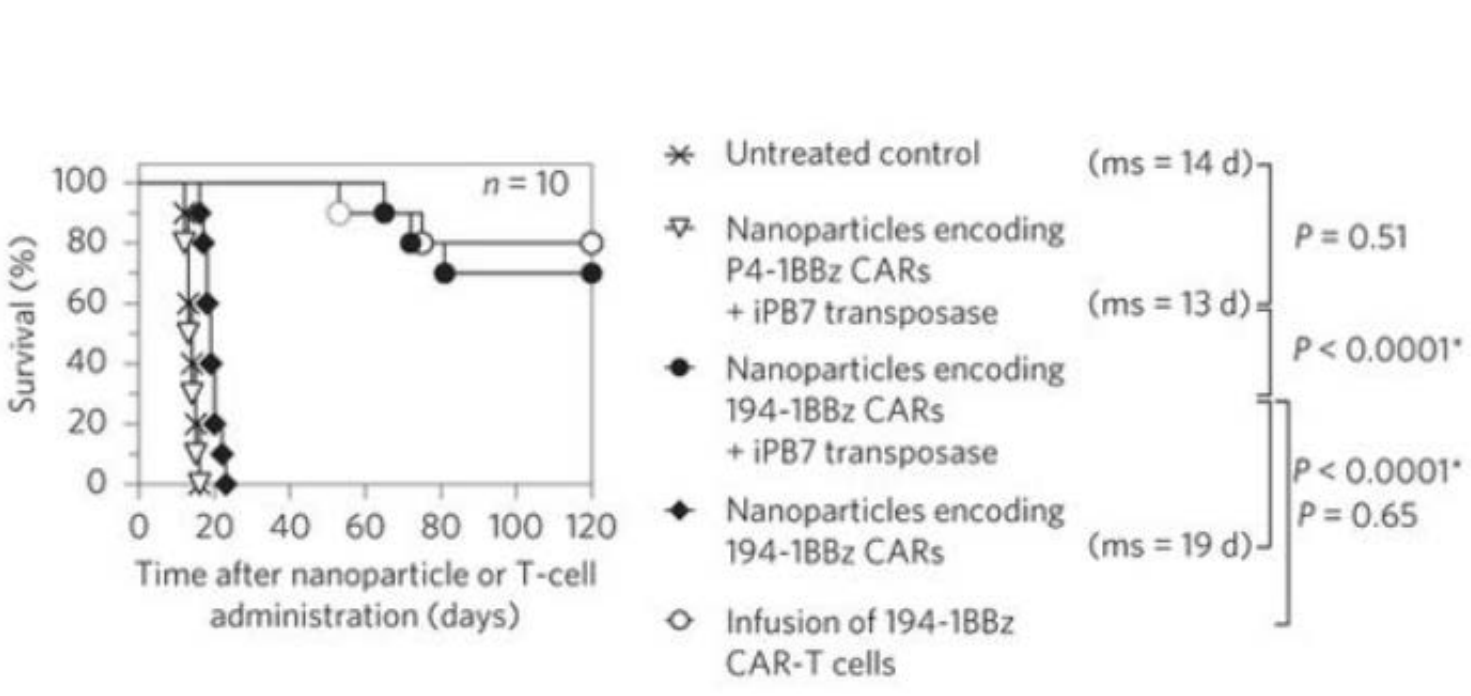


Conclusions

- Nanoparticles carrying genes of CD19 specific CARs can selectively and quickly edit T-cell specificity *in vivo*
- Comparable efficacy to conventional adoptive transfer
- Safety: off-target gene transfer!
- Nanoparticles: easy to manufacture, stable, good long term storage, cheaper

Cell

Volume 173, Issue 6, 31 May 2018, Pages 1426-1438.e11

Article

Universal Chimeric Antigen Receptors for Multiplexed and Logical Control of T Cell Responses

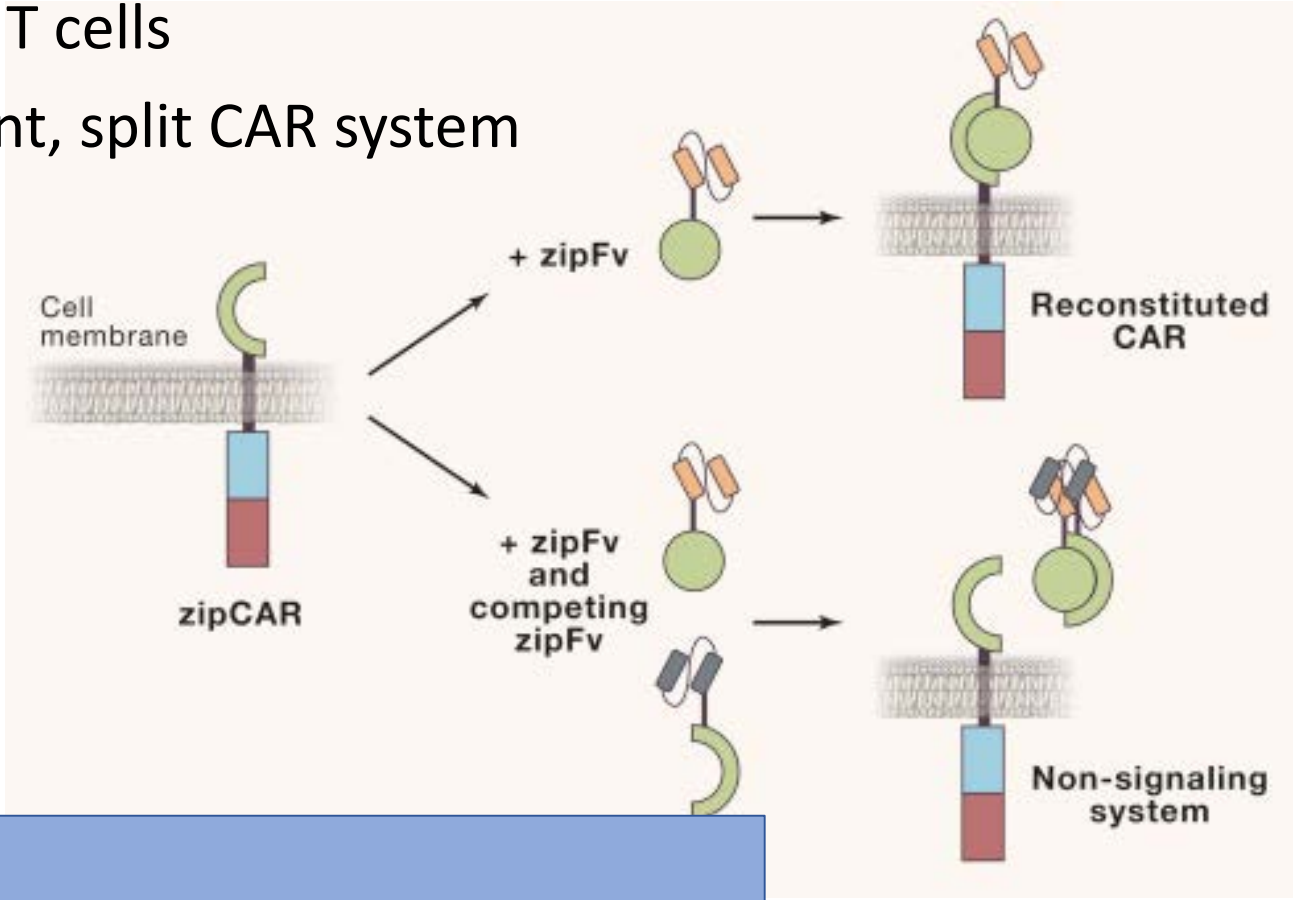
Jang Hwan Cho [1, 2], James J. Collins [3, 4, 5, 6, 7, 8], Wilson W. Wong [1, 2, 9]

Show more

https://doi.org/10.1016/j.cell.2018.03.038

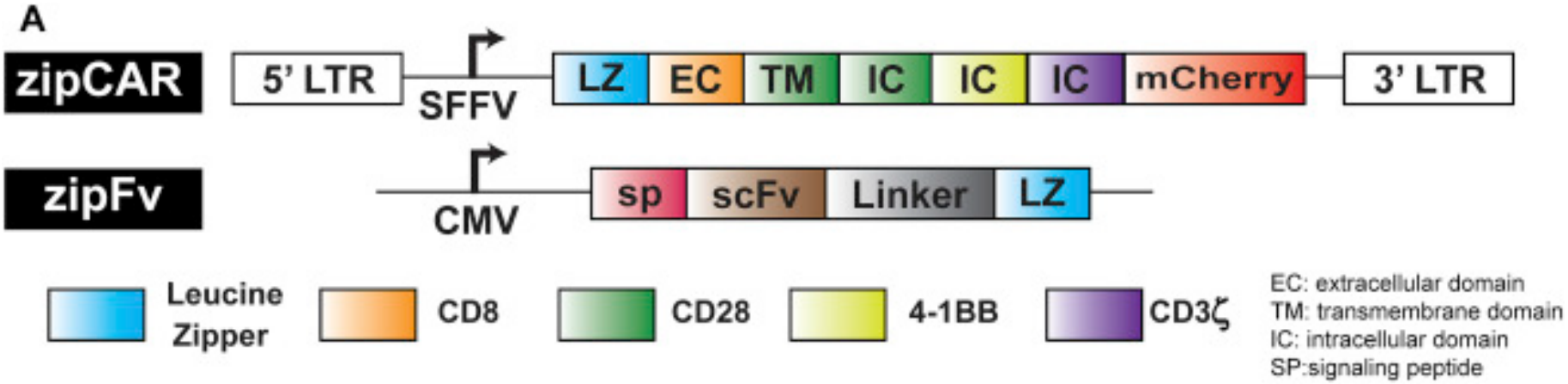
Increasing T cell versatility

- «feature-rich» T cells
- Two-component, split CAR system
  - zipCAR
  - zipFv

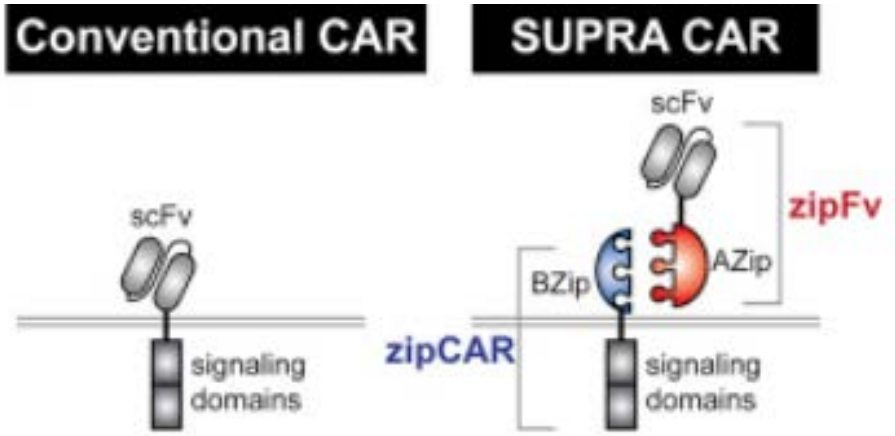


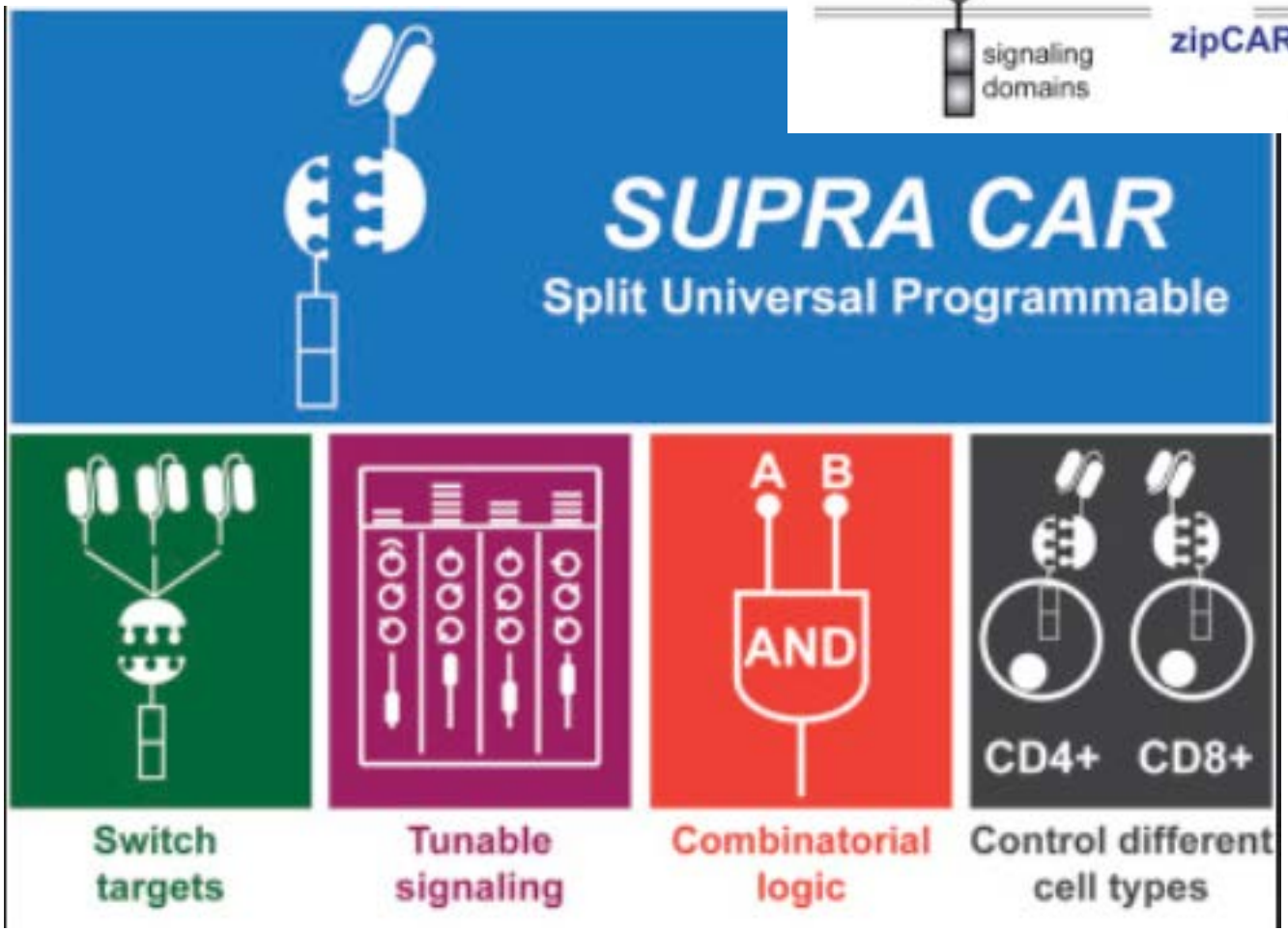
SUPRA CAR
(split, universal, programmable)

Design


A
zipCAR
5' LTR
SFFV
LZ
EC
TM
IC
IC
IC
mCherry
3' LTR
zipFv
CMV
sp
scFv
Linker
LZ
Leucine Zipper
CD8
CD28
4-1BB
CD3ζ
EC: extracellular domain
TM: transmembrane domain
IC: intracellular domain
SP:signaling peptide

Goal

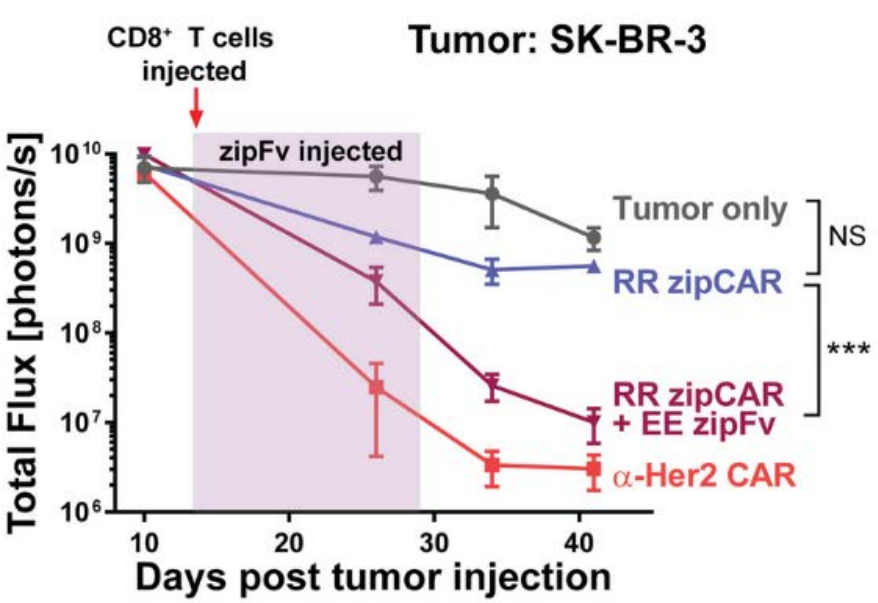

Conventional CAR
SUPRA CAR
scFv
signaling domains
scFv
zipFv
BZip
AZip
zipCAR
signaling domains

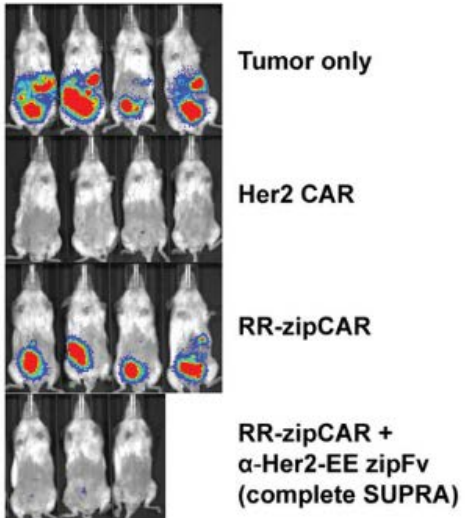

SUPRA CAR
Split Universal Programmable
A B
AND
CD4+ CD8+
Switch targets
Tunable signaling
Combinatorial logic
Control different cell types

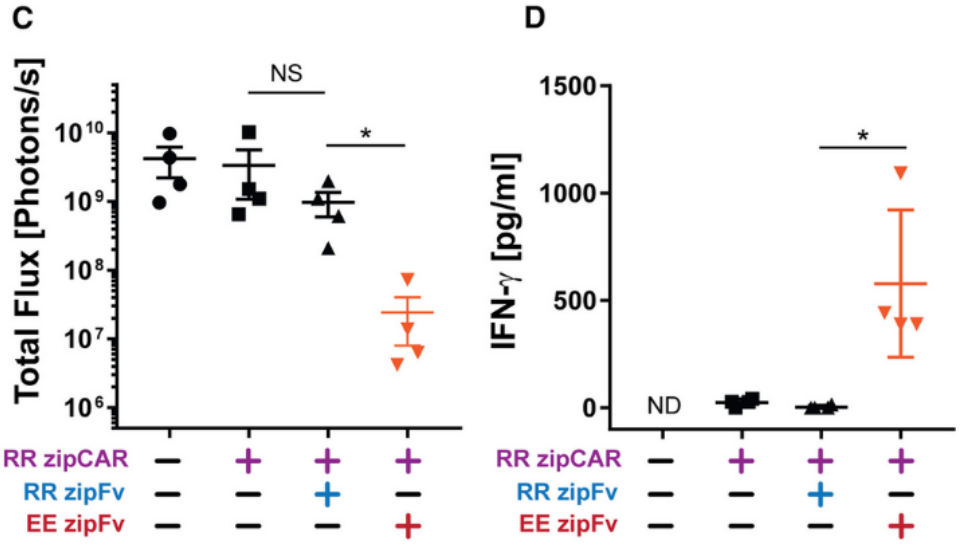
In vivo experiment

- Control tumor growth in mouse xenograft tumor models:
  - Nod/scid/$y^{-/-}$ (NSG) mice
  - Breast cancer cells (i.p.) (SK-BR-3)
- 2 weeks of tumor establishment
- Conventional Her2 CAR vs. RR zipCAR
- Anti-Her2-EE zipFv injected every 2 days for 2 weeks
- *In vivo* imaging of luciferase signal from breast cancer cells

In vivo experiments



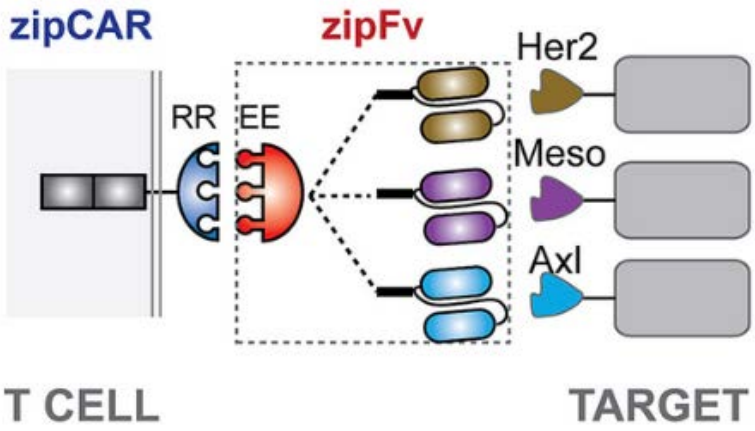




Multiple targets for one CAR

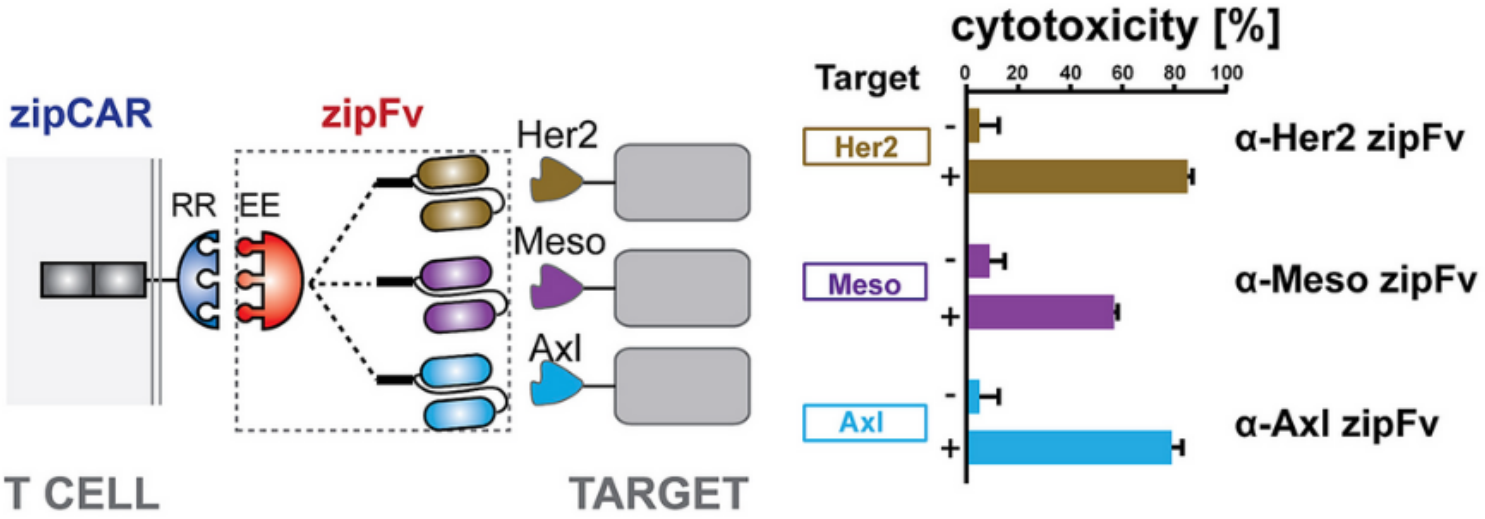
- Problem: antigen specificity not flexible
- Solutions: split CAR

Multiple targets for one CAR



CD8+

Multiple targets for one CAR



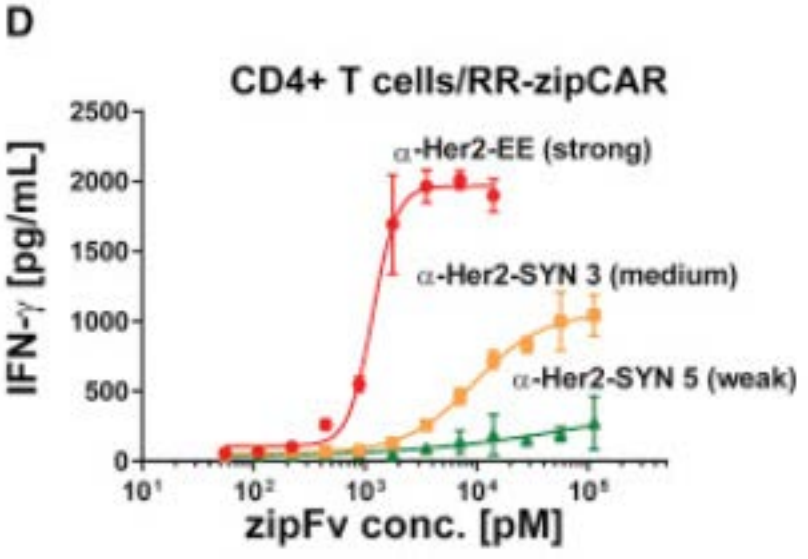
CD8+

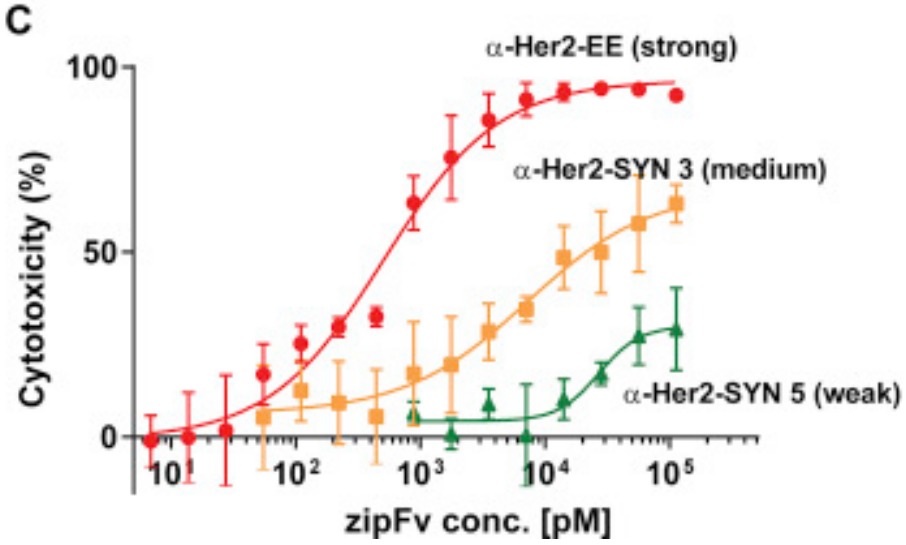
Controlling SUPRA CAR activity

- Adverse event: cytokine release syndrome
- CAR T cell activity cannot be prevented, cytokine release cannot be controlled

- Solutions:
  - **Amount**
  - **affinity**
  - **competition**

Fine-tuning of SUPRA-CARs





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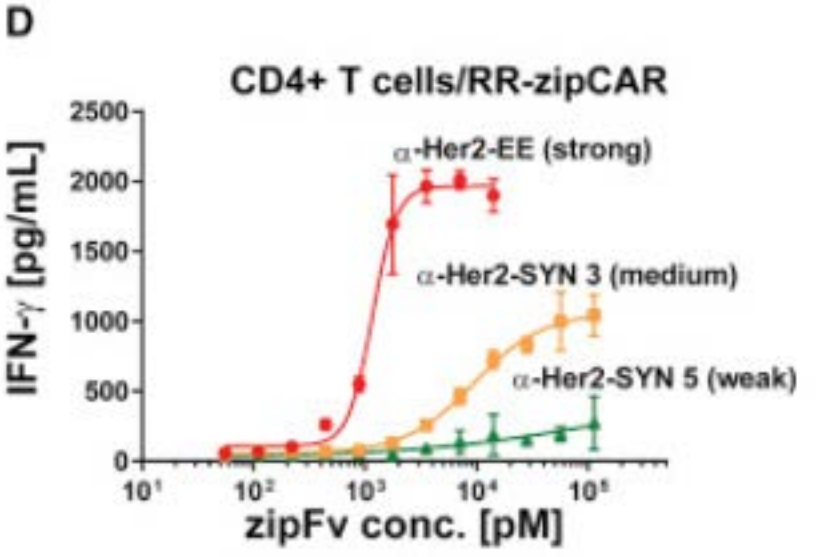

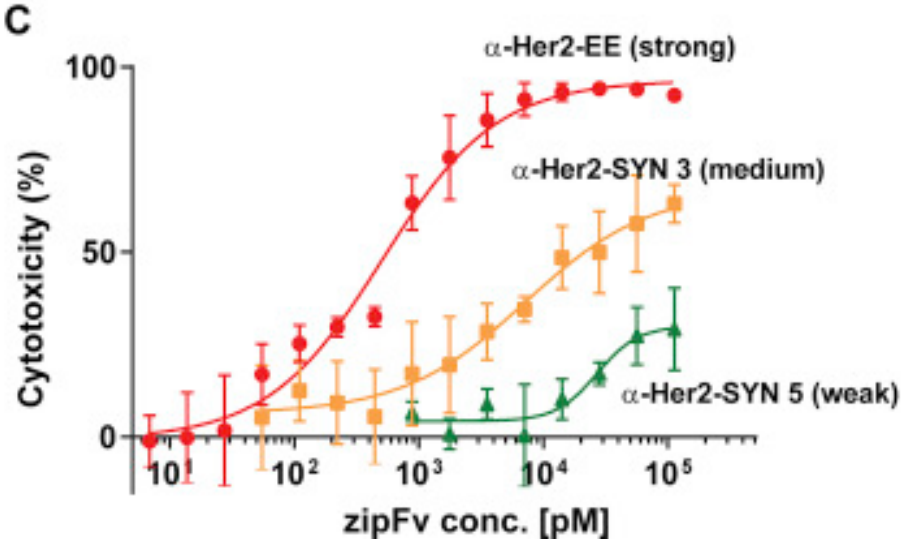

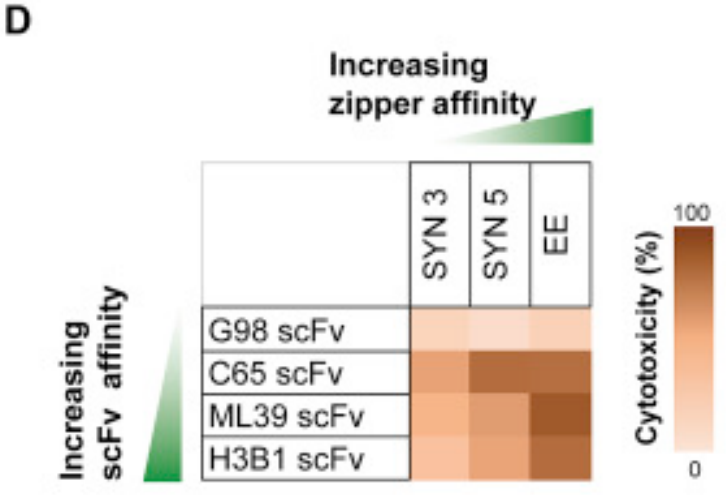

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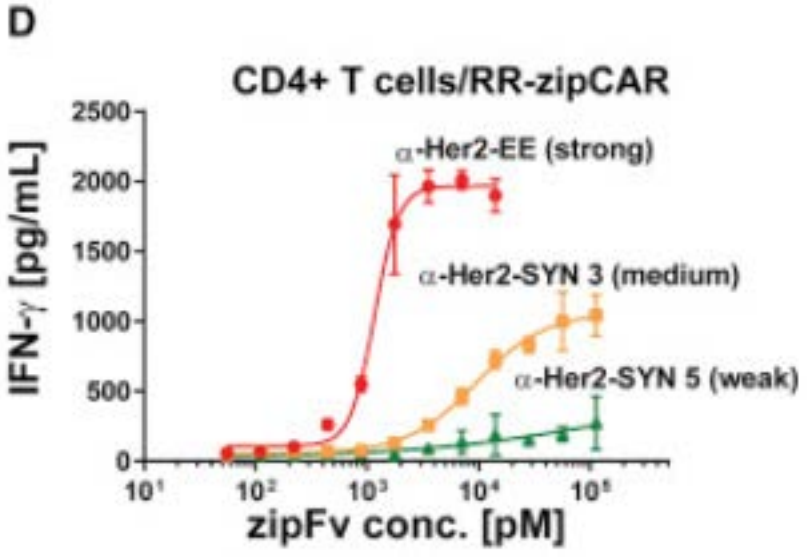
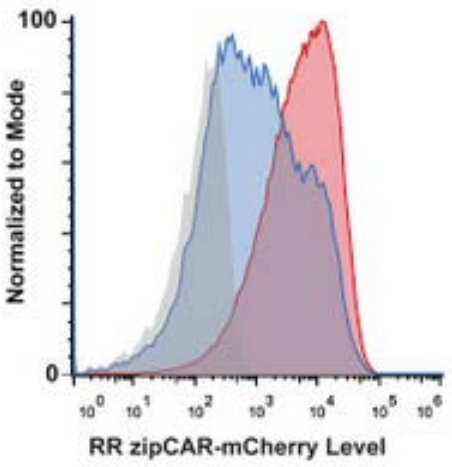
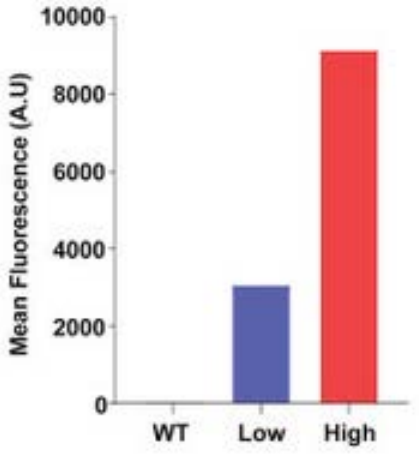
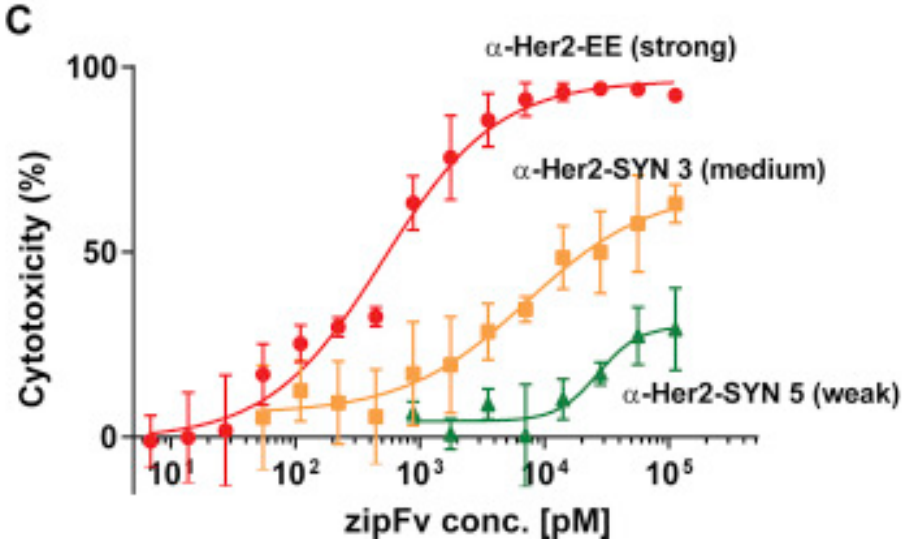
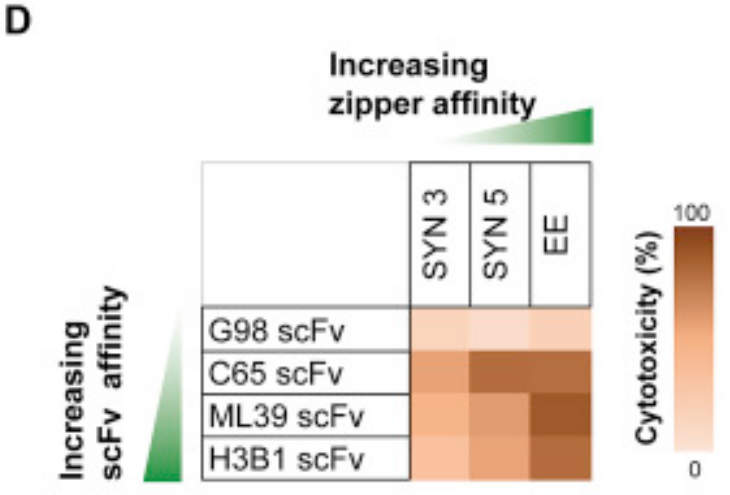


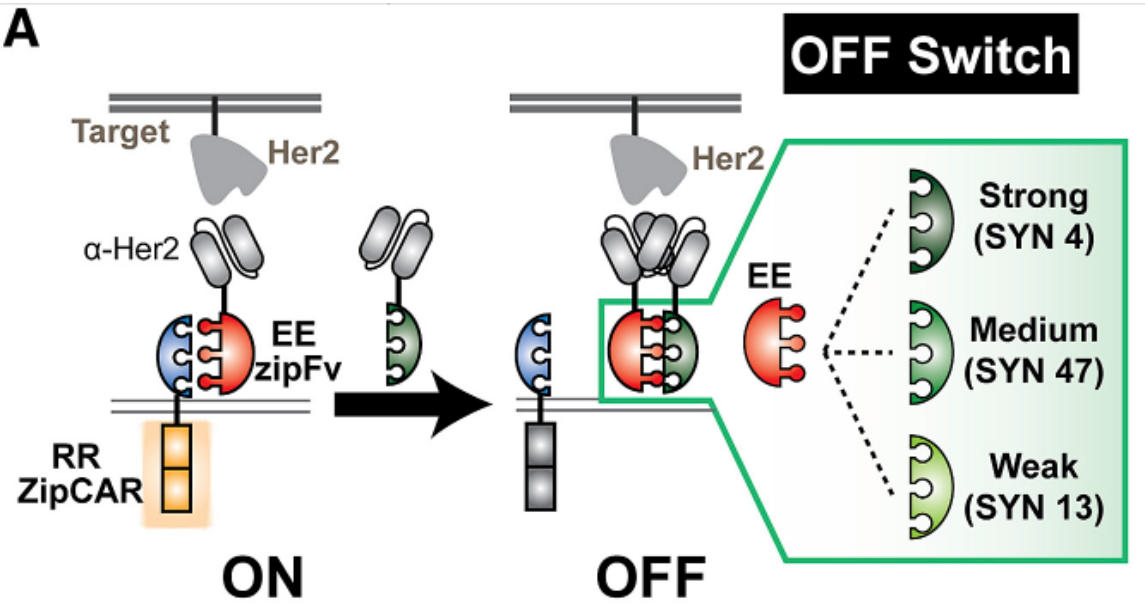








Competitive zipFvs



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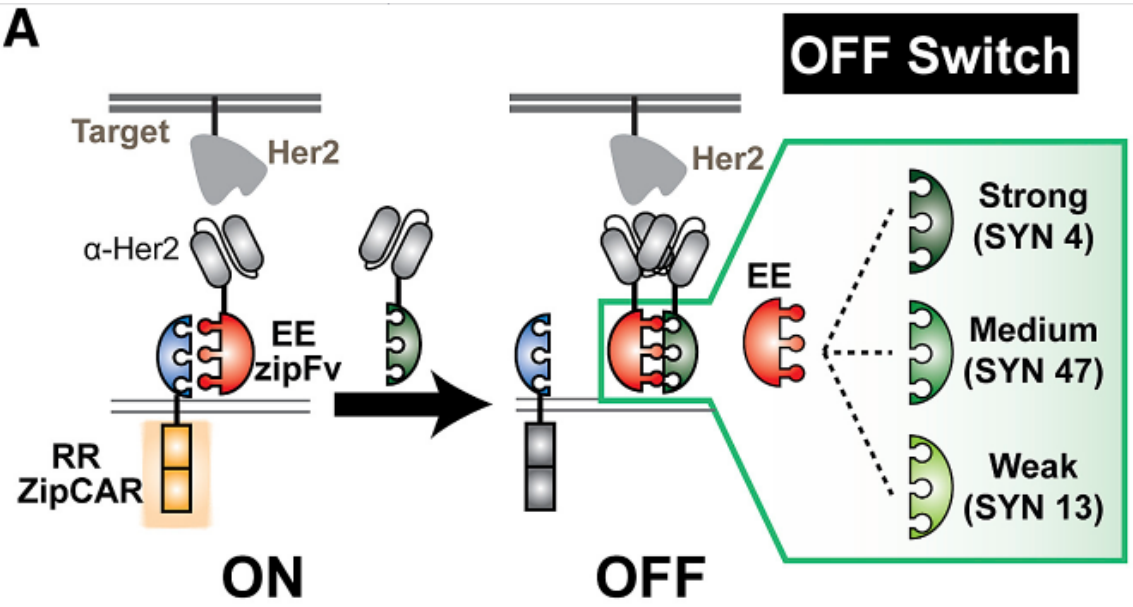

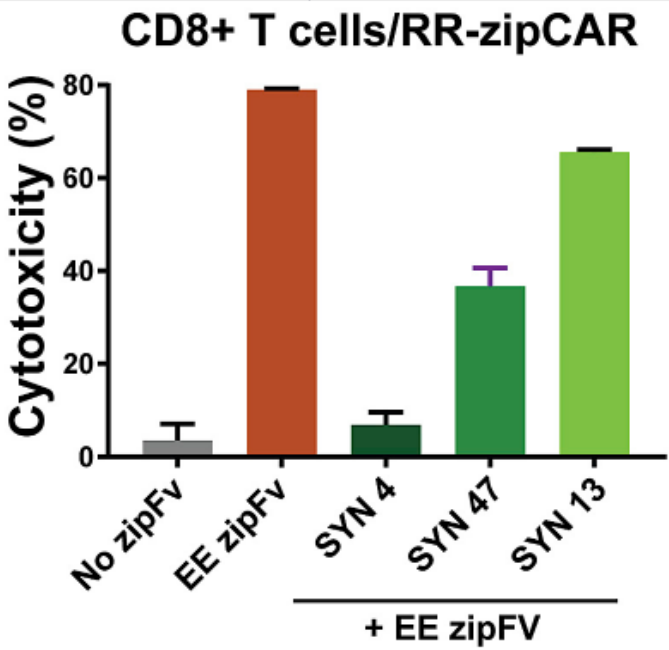

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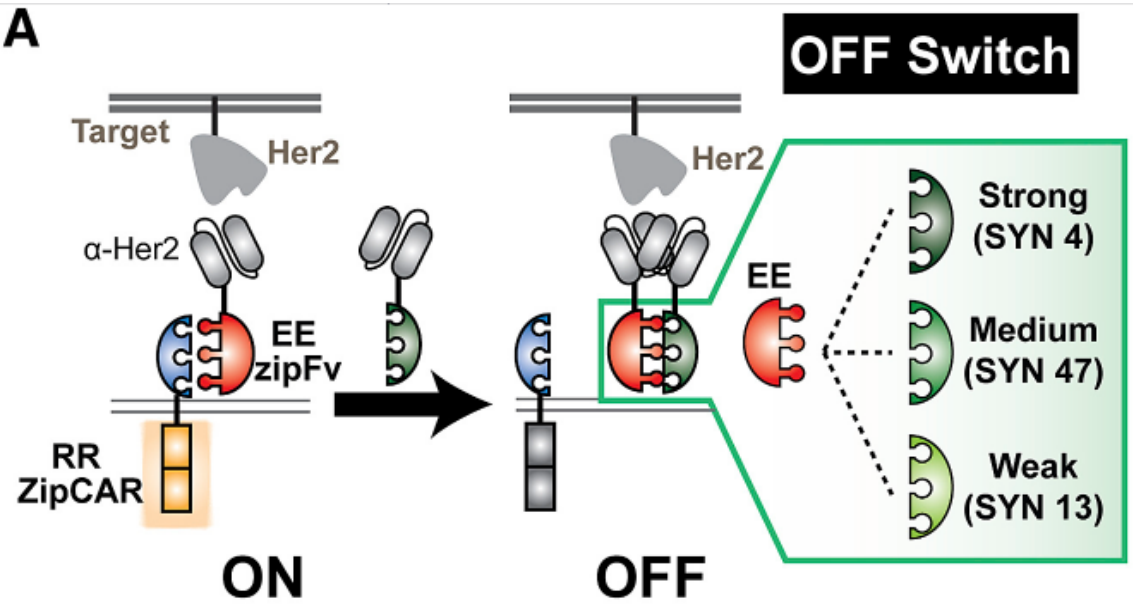
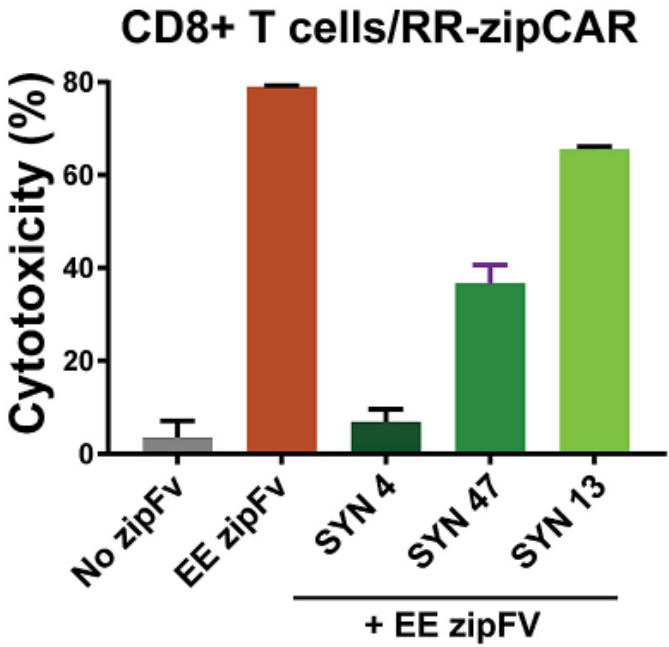
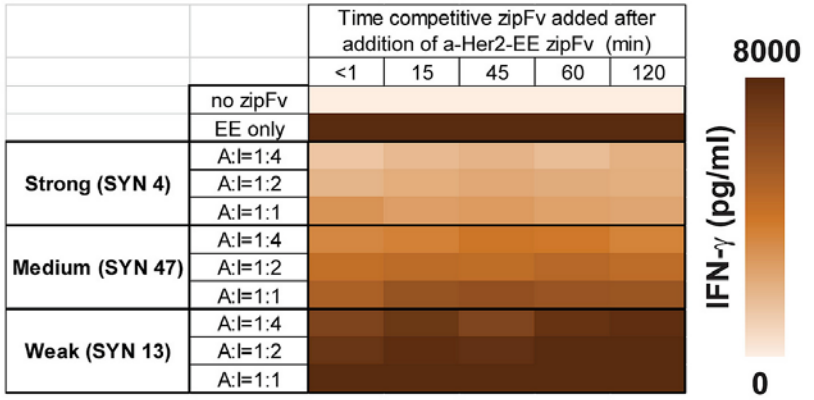


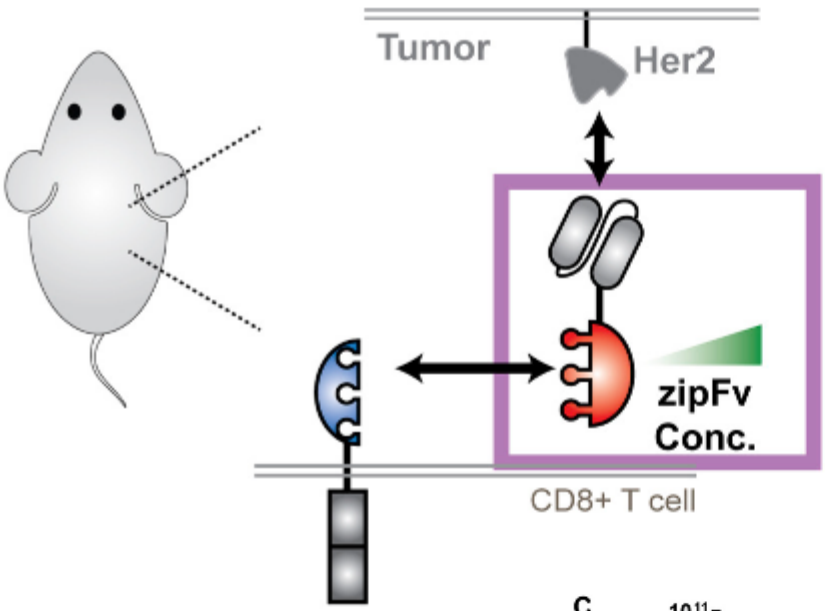


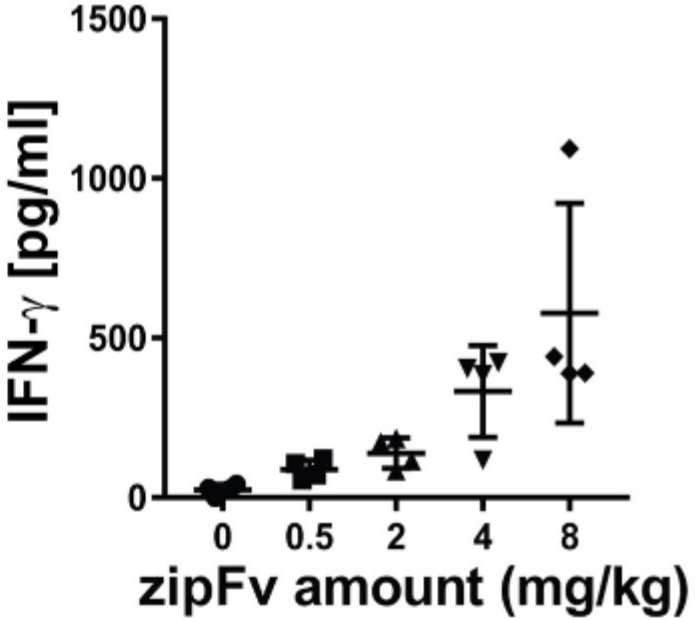


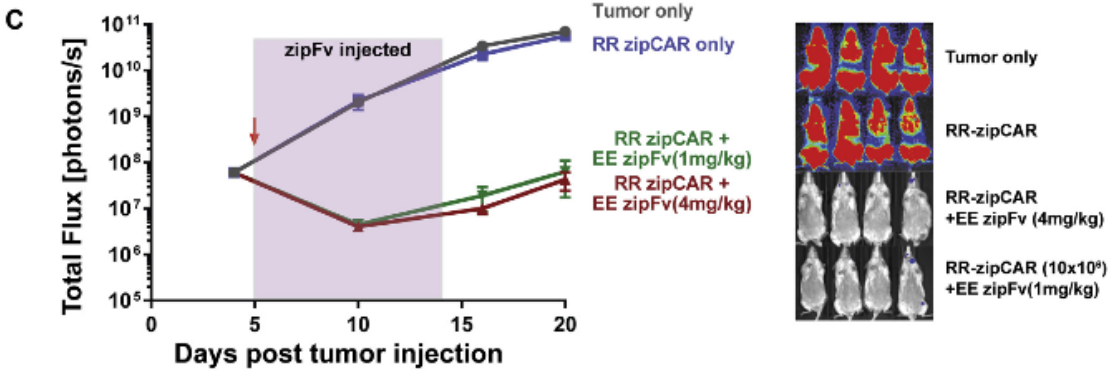
In vivo?

- SK-BR-3 breast cancer model
- Tumor establishment > RR-zipCAR expressing CD8+ T cells + anti-Her2-EE zipFv

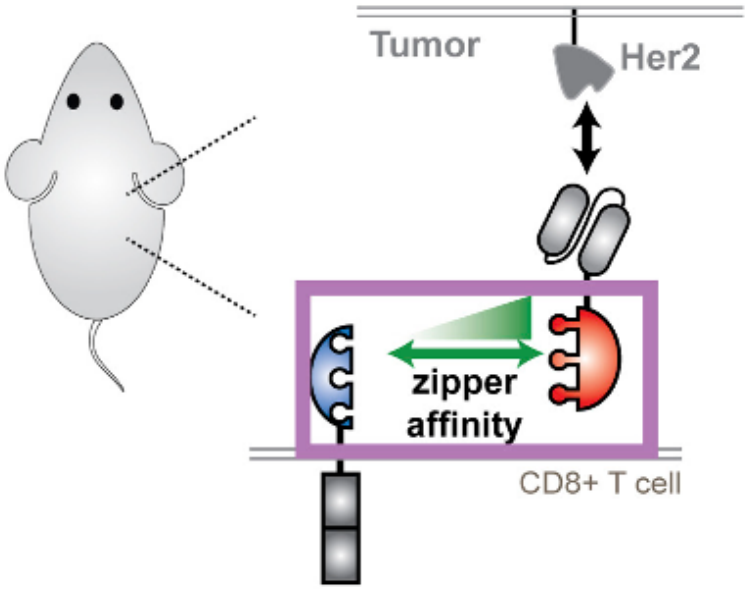
In vivo control of cytokine release

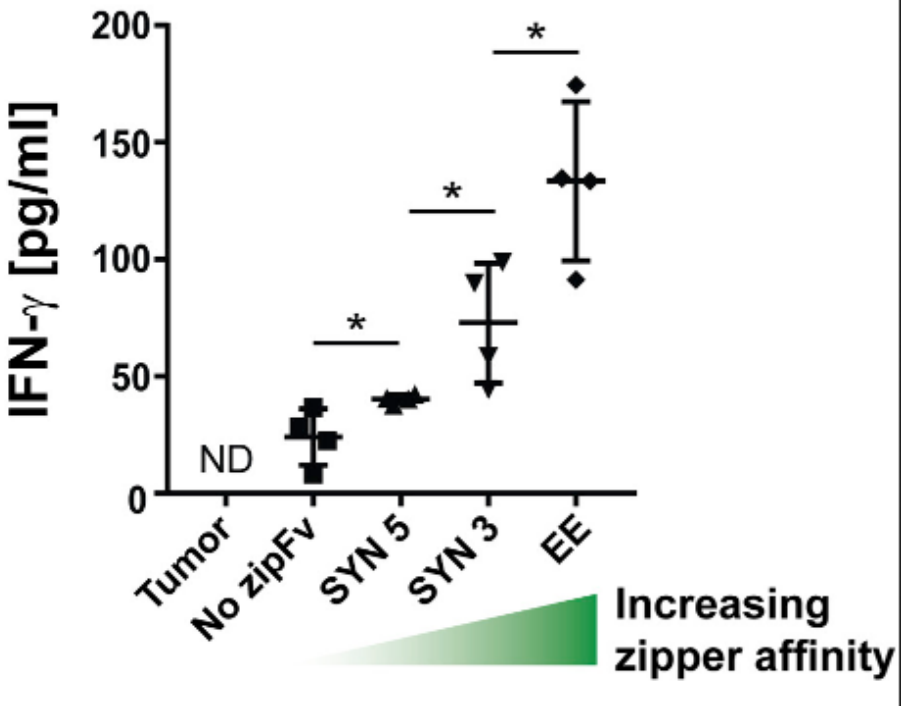






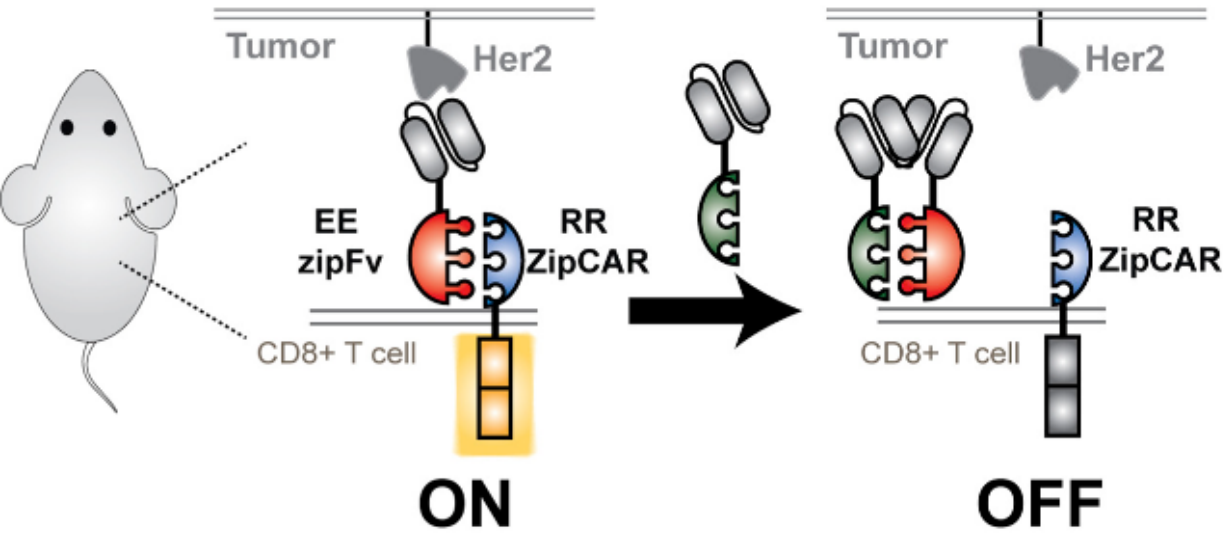
In vivo control of cytokine release



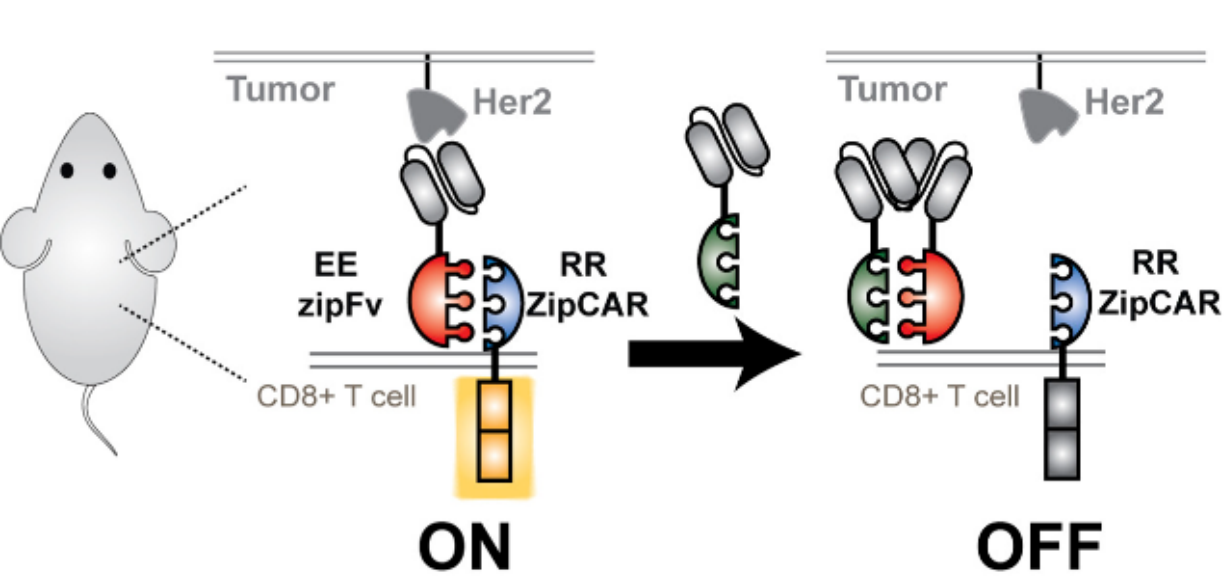


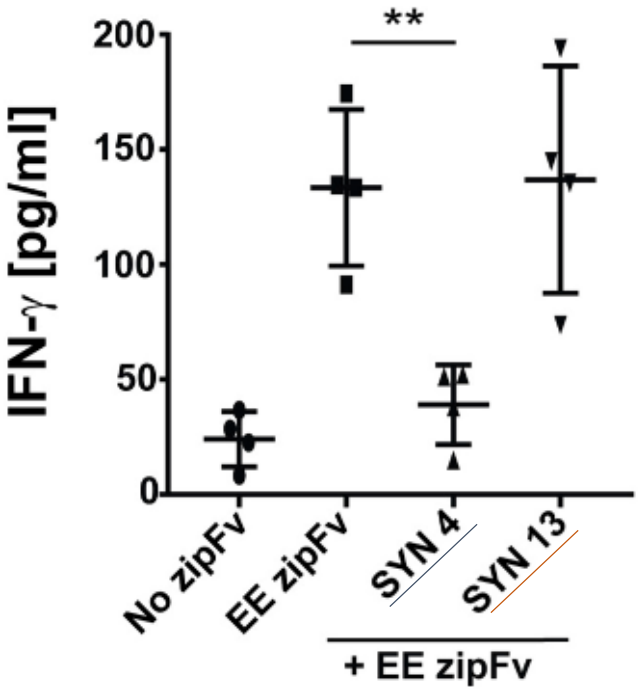
Effect on anti-tumor activity?

In vivo control of cytokine release



In vivo control of cytokine release





SYN4 = competitive
SYN13 = control

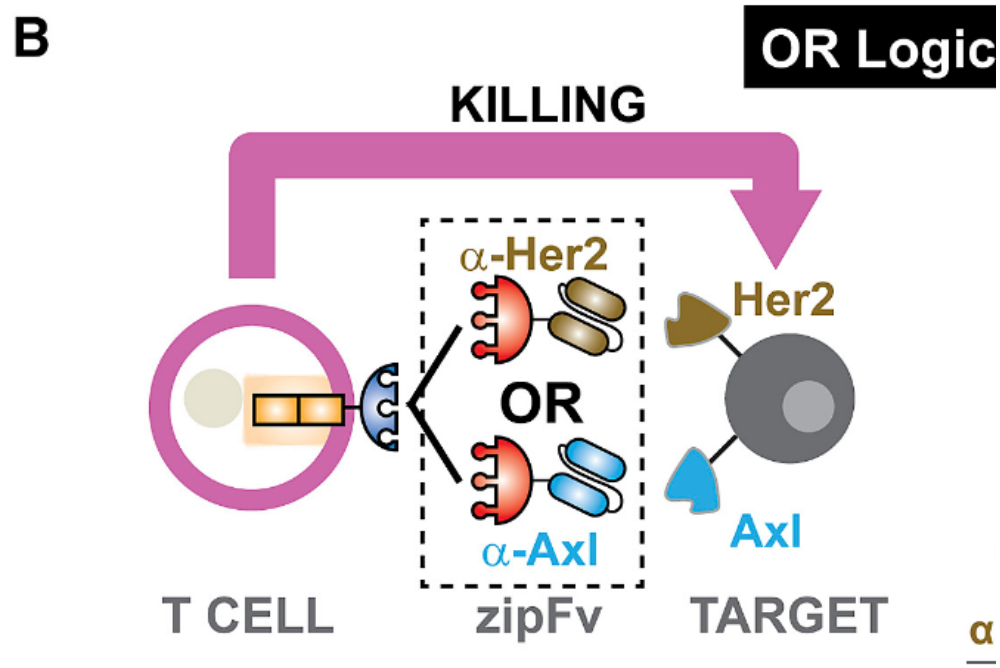
Fast enough in patients?

Logical operation

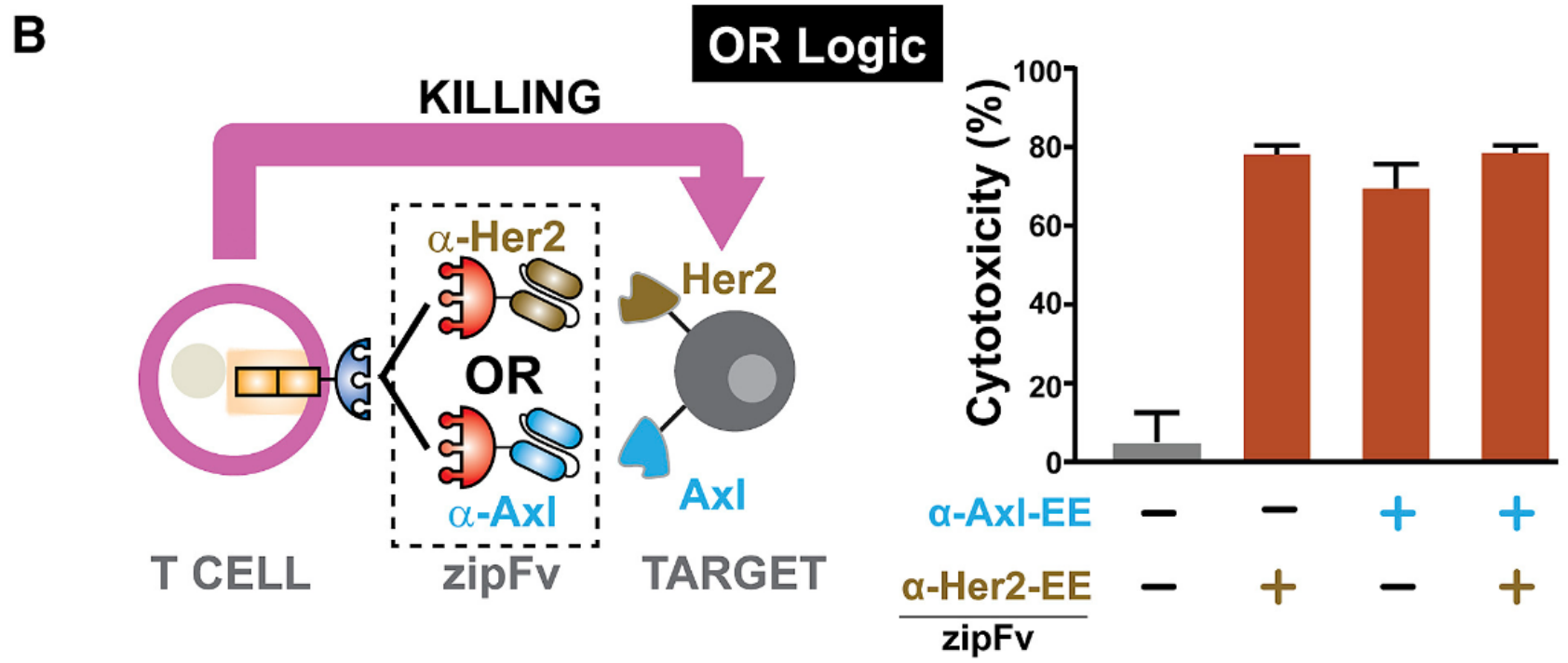
- Problem: antigen escape

- Solutions:
  - New zipFv
  - «OR» operation

Logical operation

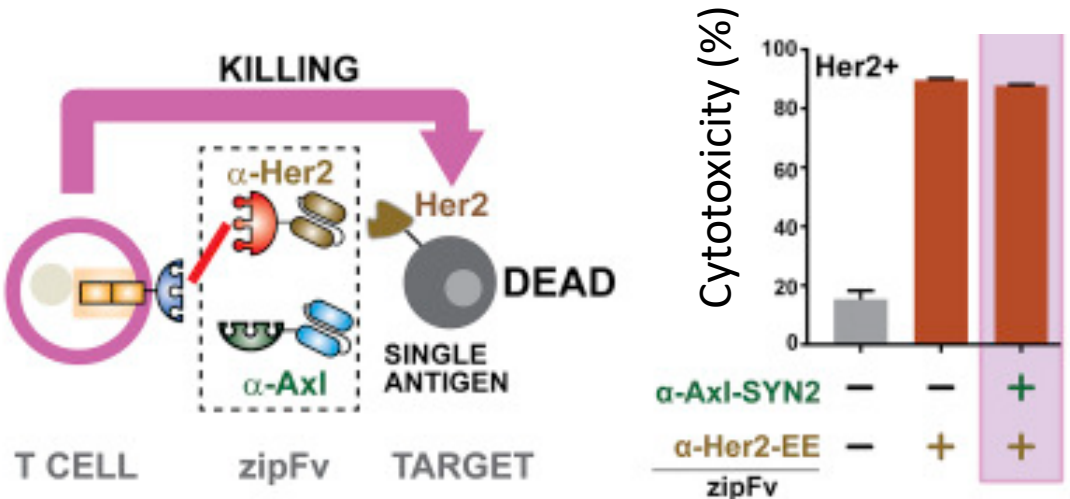


Logical operation



Logical operation

- Problem: Identification of single tumor *specific* antigen
- Example: can we target Her2 cells and spare cells that express both Her2 and Axl?



Logical operation

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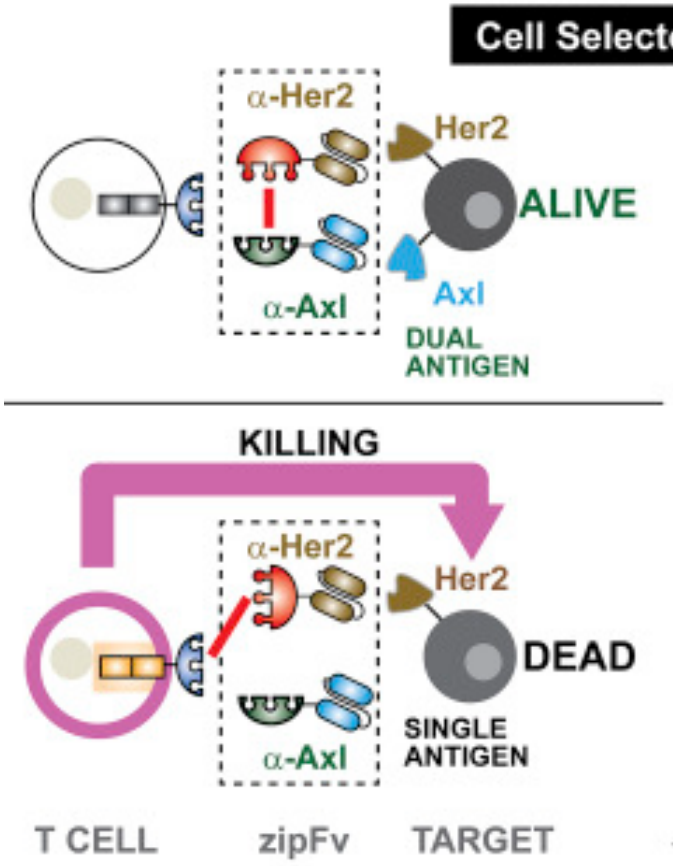
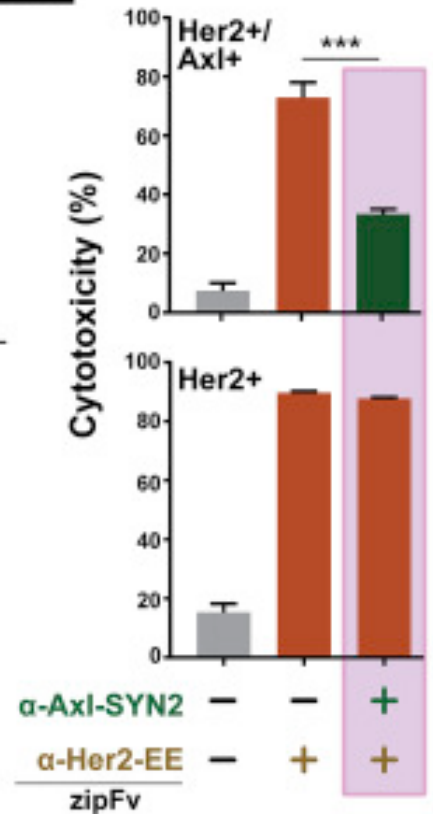




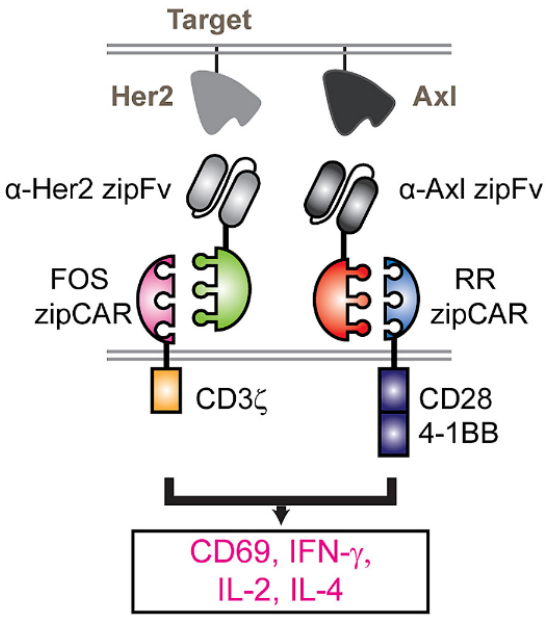
Cells *pretreated* with anti-Axl zipFv

Affinity: prevention of cytotoxicity only with high affinity zipFv

In vivo?

Independent control of signaling domains

- Orthogonal SUPRA CARs can control distinct signaling pathways in same cell



Independent control of signaling domains

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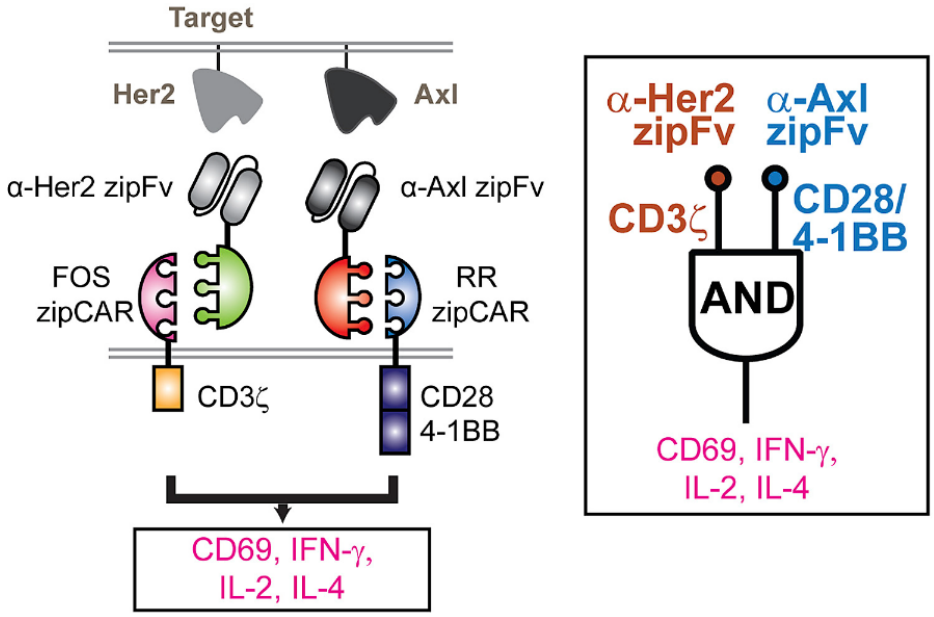

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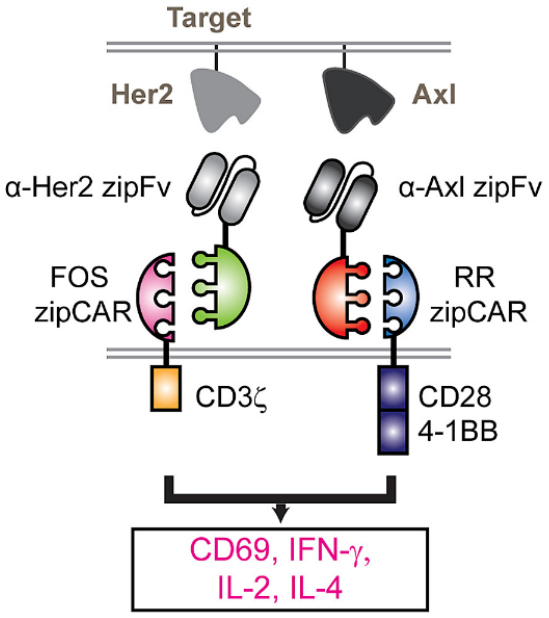
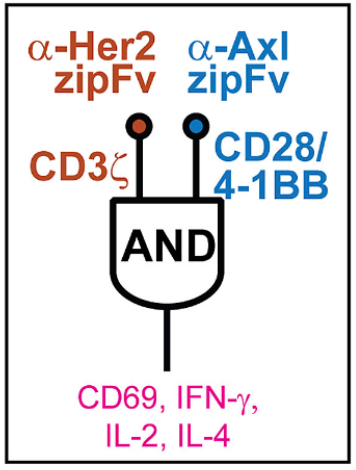
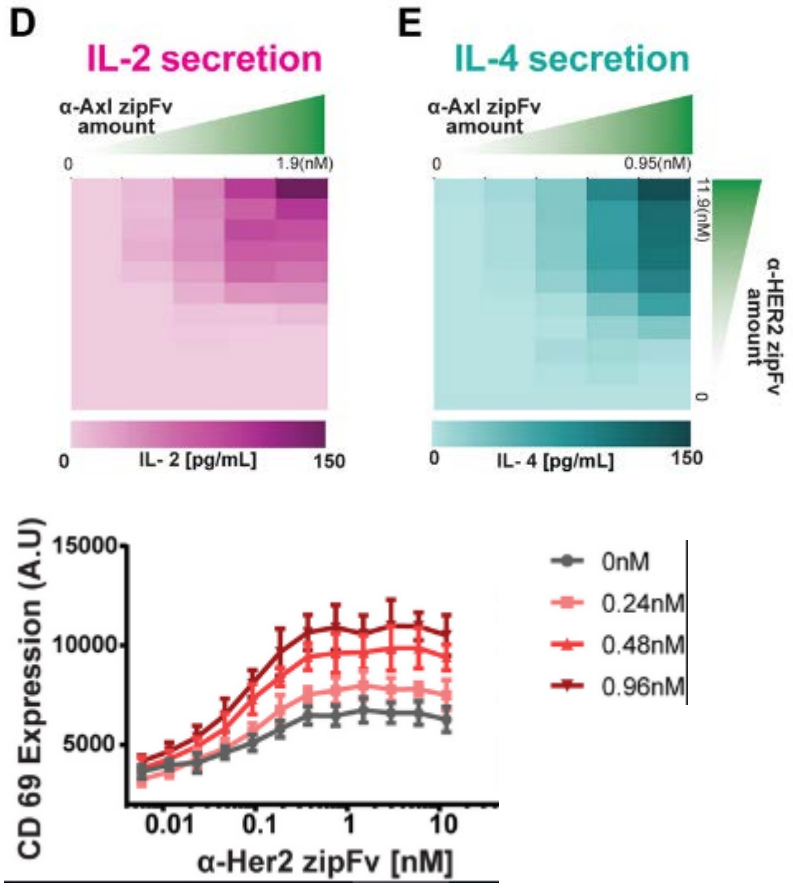






Advantages - Limitations

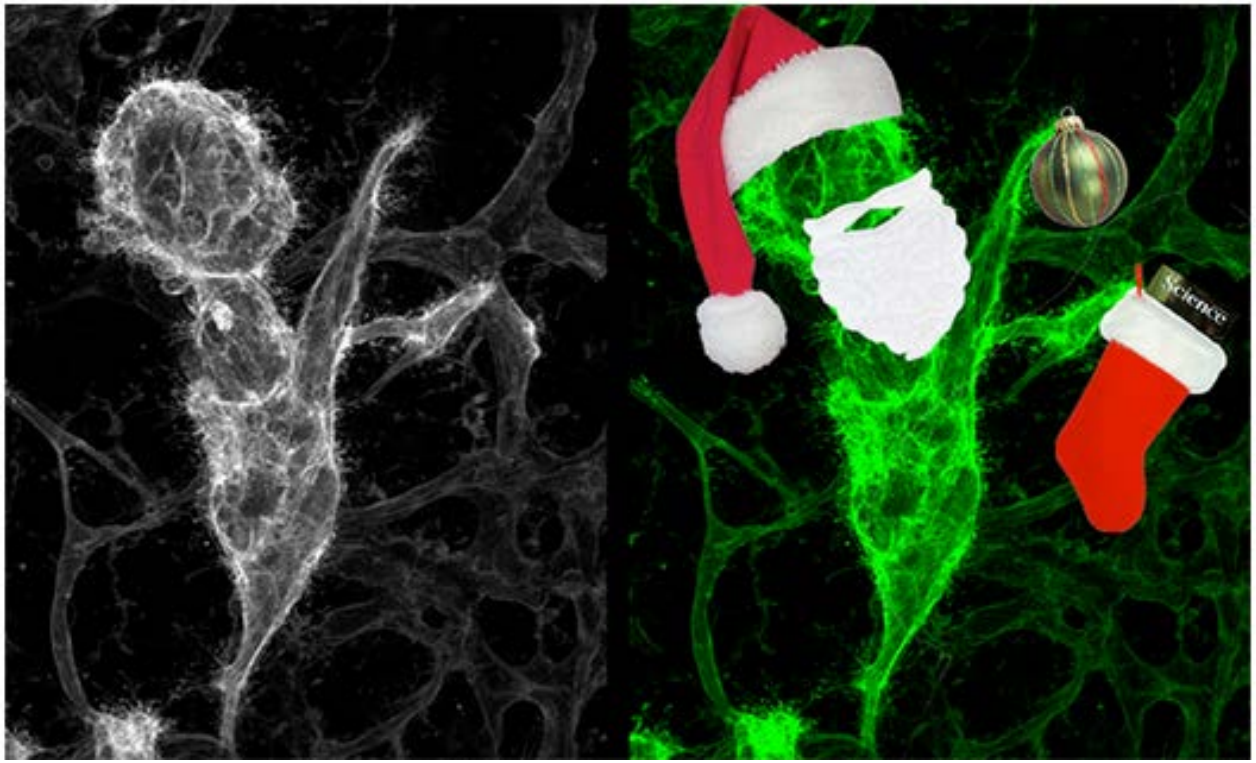
- Platform with improved precision, tunability and controllability
- As good as conventional CAR-T cells – but not better
- Simple change of target
- Combinatorial logic
- Flexible - but additional parts
- Modulation in patient? How precise? How fast?
- Short serum half live of zipFv – temporal control vs. Loss of acvitity

Recent developments in CAR T cell therapy - summary

- More patients, more malignancies
  - Engineering solutions for adjustable and robust control of cellular function
  - Less complex, cheaper solutions

Thank you!

…. and Merry Christmas ☺



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