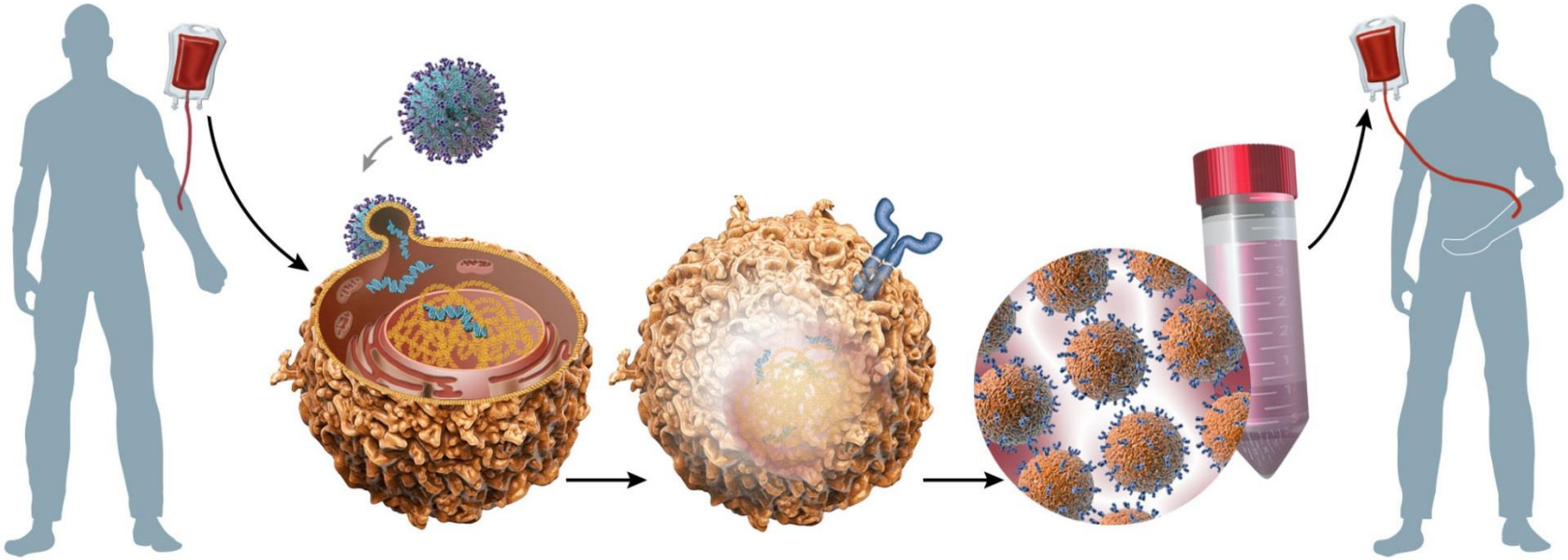
Making the switch to a safer CAR-T cell therapy



HaemaLogiX 2015

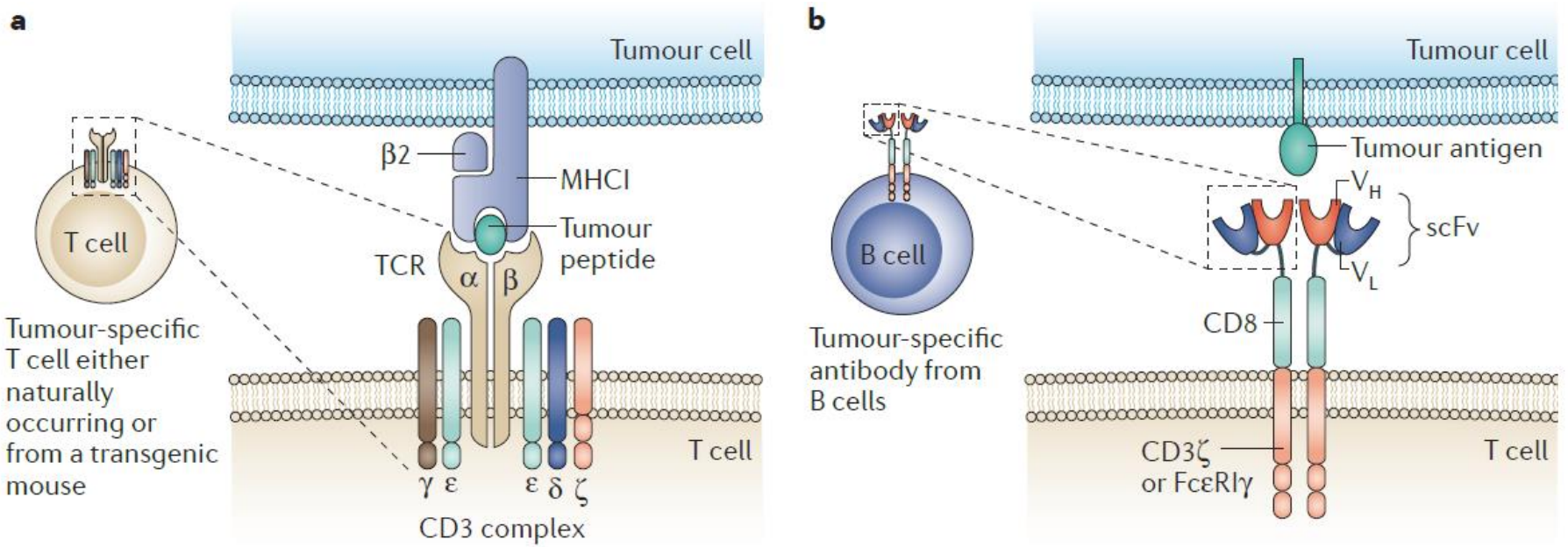
Technical Journal Club May 24th 2016

Christina Müller

Making the switch to a safer CAR-T cell therapy

- **c**himeric **a**ntigen **r**eceptor = CAR

- CAR T cells are generated by lentiviral transduction using a CAR construct
  - extracellular antigen recognition domain; **scFV**
  - hinge and transmembrane domain; **CD8 hinge**
  - intracellular signaling domain; **CD3ζ**



Kershaw et al. 2013, Nature Reviews — Cancer

Making the switch to a safer CAR-T cell therapy

CAR design:

- First generation:

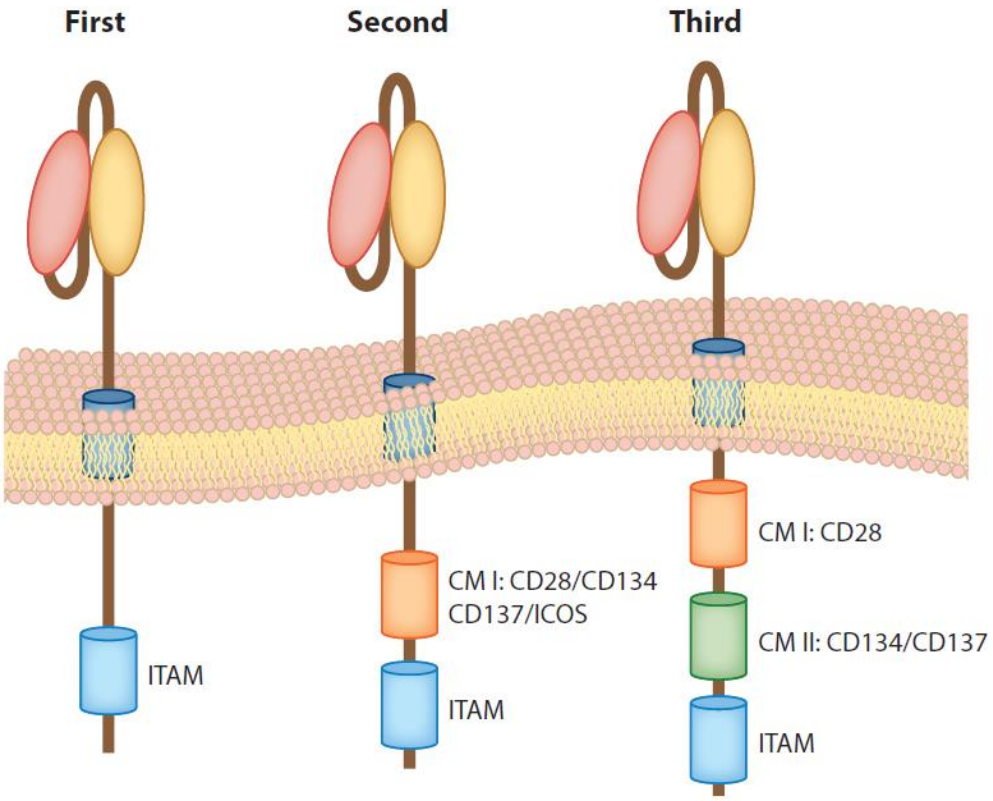
  no co-stimulatory domain → limited efficacy, activation induced cell death, lack of T cell expansion

- Second generation:

  1 co-stimulatory domain → enhanced proliferation and persistence

- Third generation:

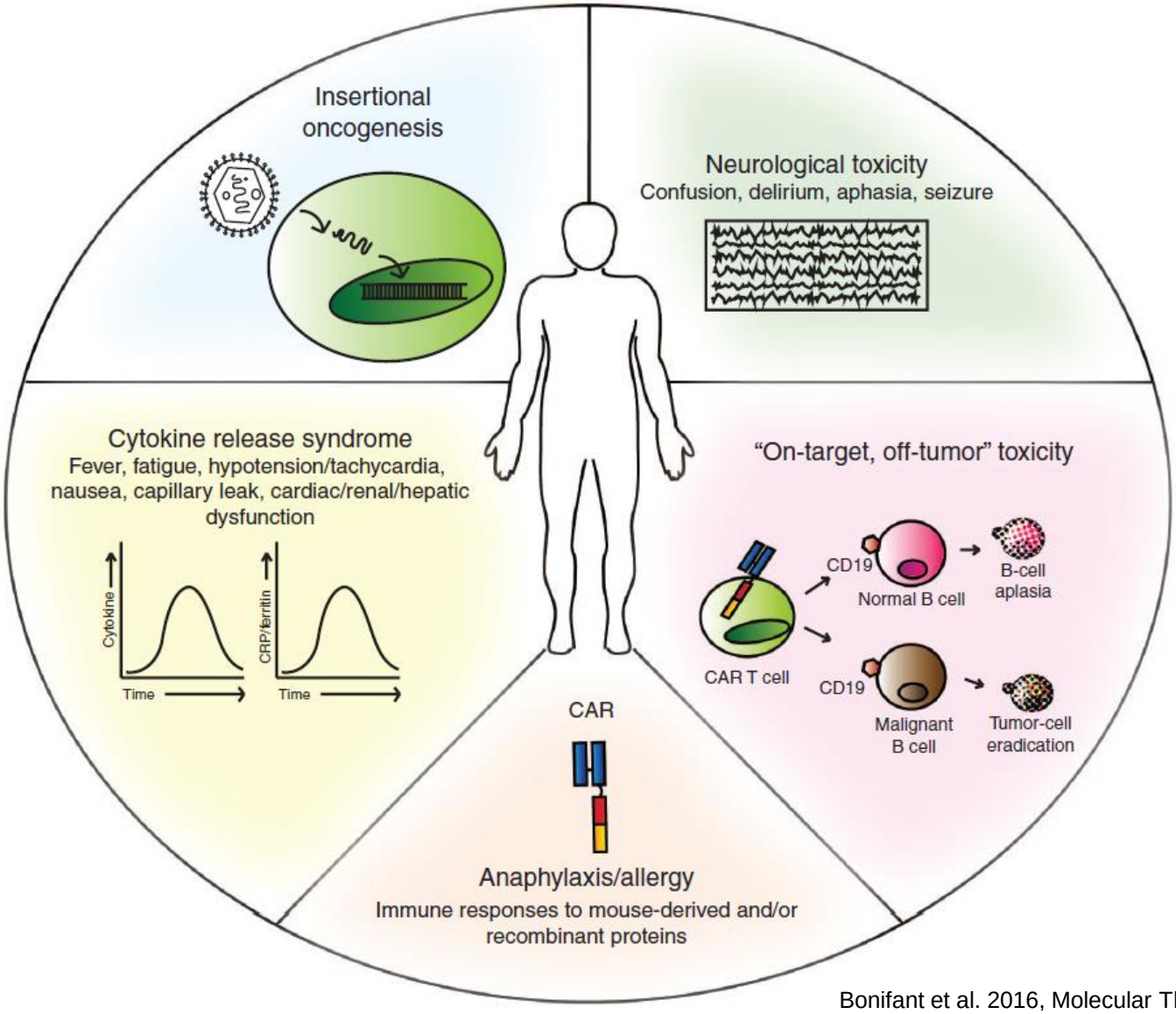
  2 co-stimulatory domains → superior antitumor efficacy



Gross Eshhar et al. 2016, Annu. Rev. Pharmacol. Toxicol.

Making the switch to a safer CAR-T cell therapy

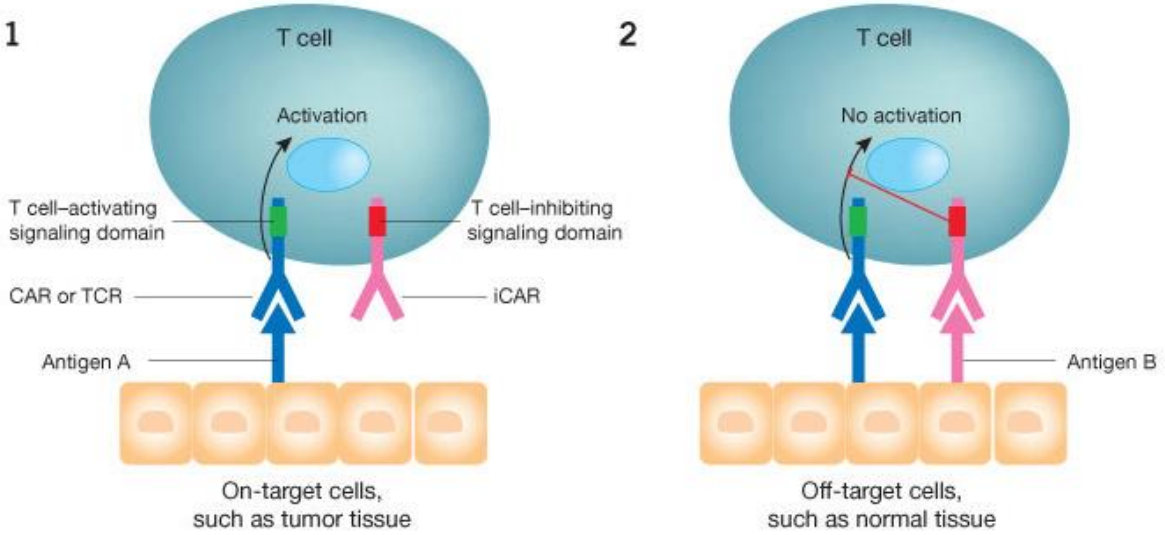
Toxicities of CAR-T cell therapy:



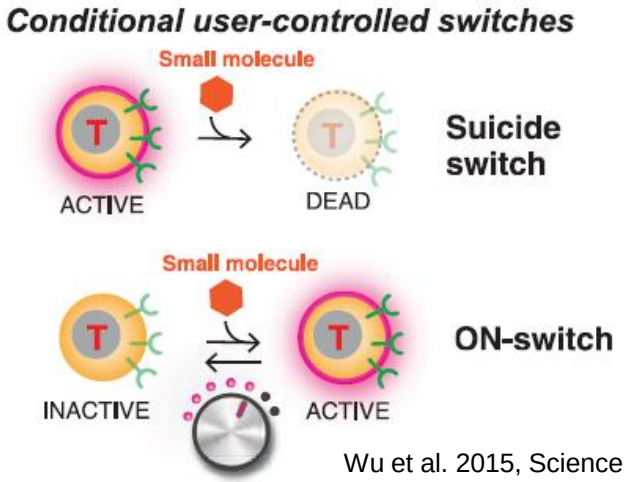
Bonifant et al. 2016, Molecular Therapy — Oncolytics

Making the switch to a safer CAR-T cell therapy

Toxicity management in CAR-T cell therapy:



Baas 2014, SciBX:Science–Business eXchange



Wu et al. 2015, Science

SYNTHETIC BIOLOGY

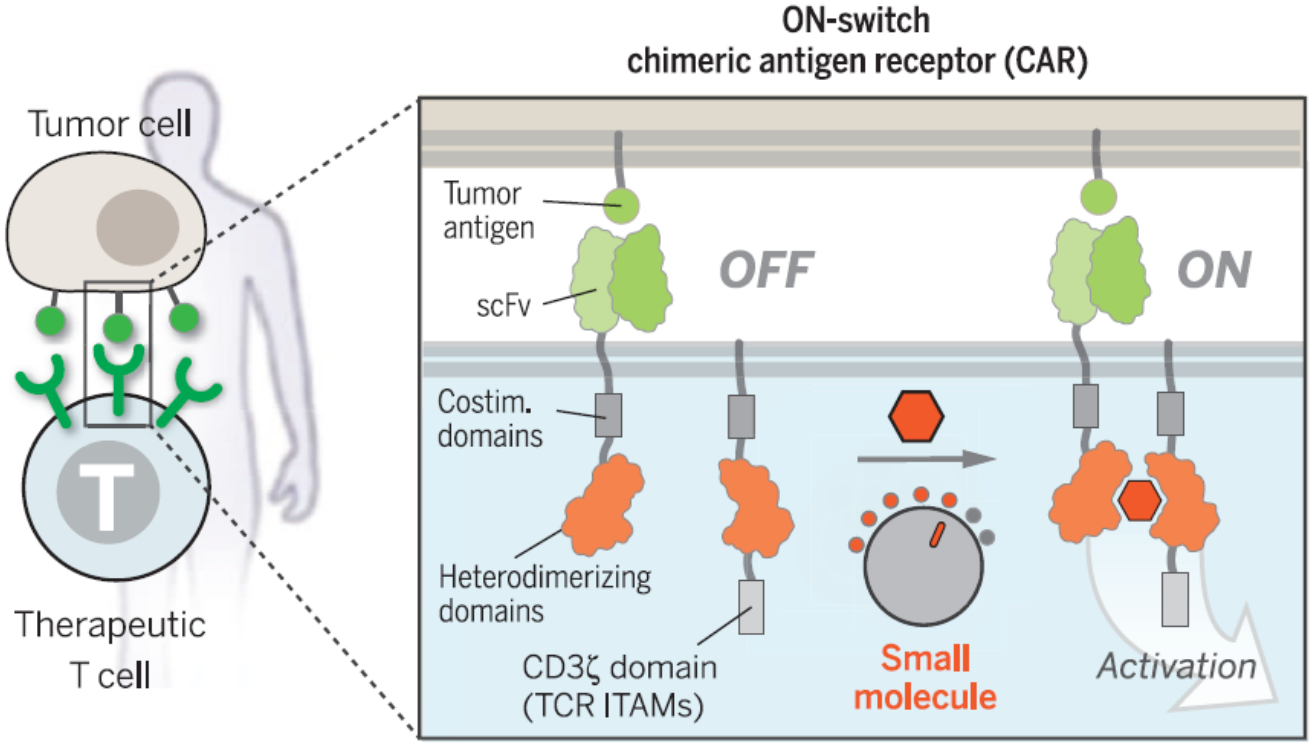
Remote control of therapeutic T cells through a small molecule–gated chimeric receptor

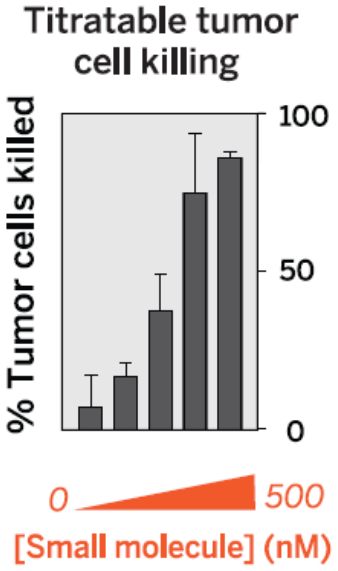
Chia-Yung Wu, Kole T. Roybal, Elias M. Puchner, James Onuffer,*
Wendell A. Lim*

Making the switch to a safer CAR-T cell therapy

ON-switch split CAR design:

- antigen binding domain and intracellular signaling domain are separately expressed polypeptides
- formation of a functional receptor complex requires heterodimerization of these polypeptides





Making the switch to a safer CAR-T cell therapy

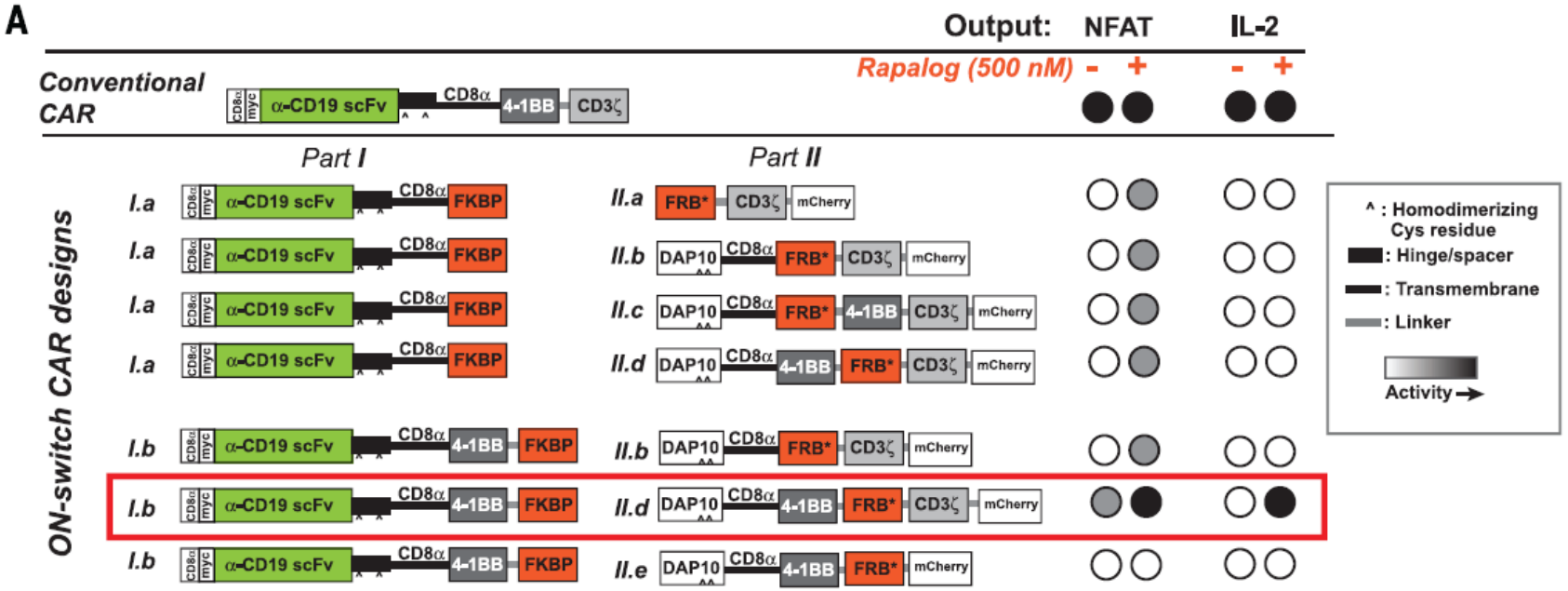
Important features for the design of an ON-switch CAR:

- Receptor still needs to be dependent on specific tumor antigen recognition, while small molecule or antigen alone should not activate
- Therapeutic activity should be titratable
- Timing of CAR T cell response should be reversibly controllable

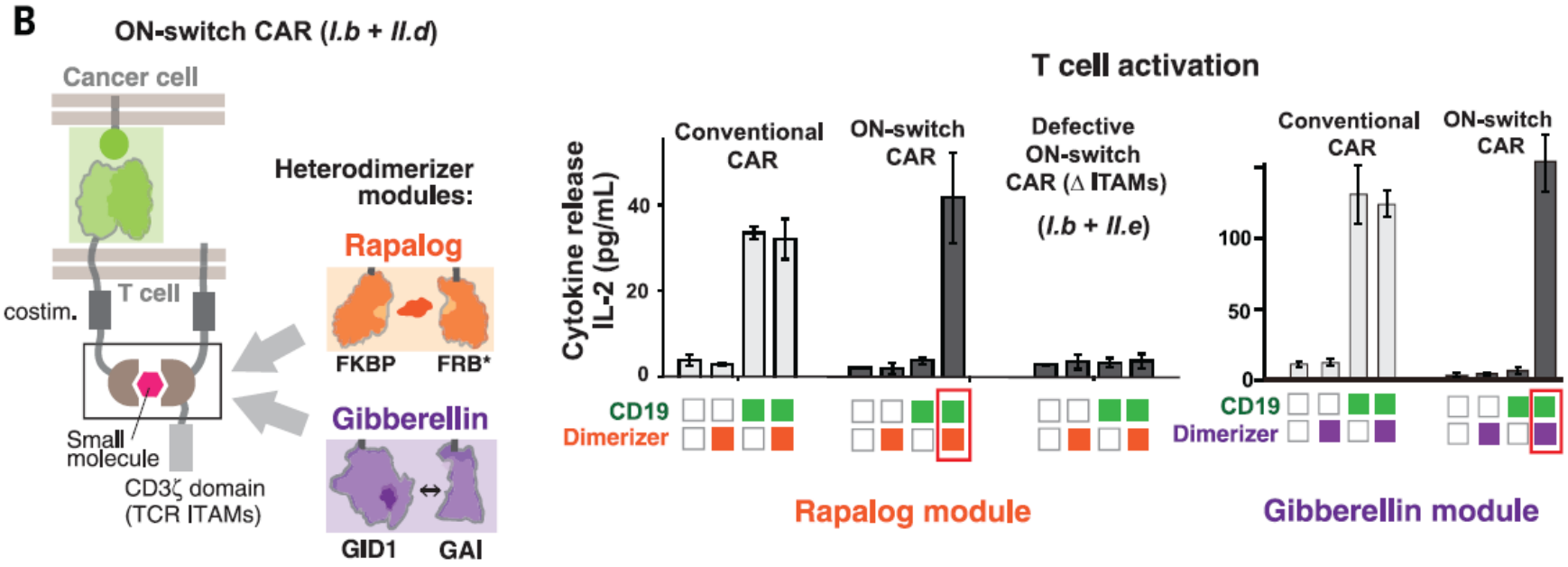
Making the switch to a safer CAR-T cell therapy

- Heterodimerizing components:
  - FK506 binding protein (**FKBP**) domain
  - **FRB*** = mutant of FKBP-rapamycin binding domain

    → heterodimerization in the presence of the small molecule **"rapalog"**
- Screening of candidates in transduced Jurkat cells by looking at
  - activity of a synthetic promoter composed of multiple NFAT response elements
  - IL-2 secretion

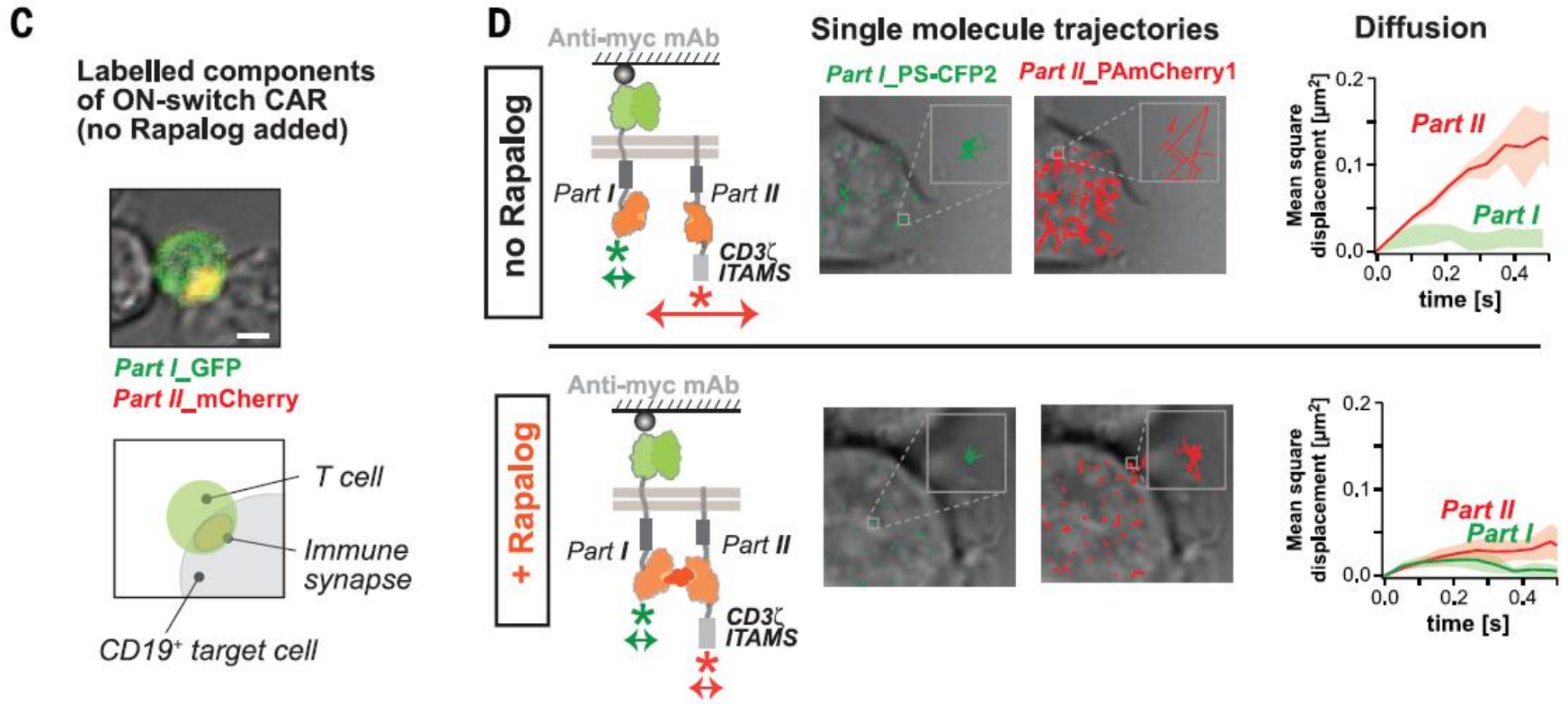


ON-switch CAR design with different heterodimerization systems:



- CAR T cells with rapalog and gibberelic acid based dimerization systems are only activated in the presence of the targeted antigen **and** ON-switch molecule

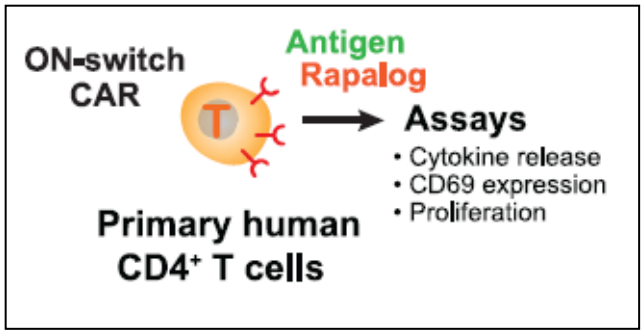
Localization of ON-switch CAR components with and without rapalog:

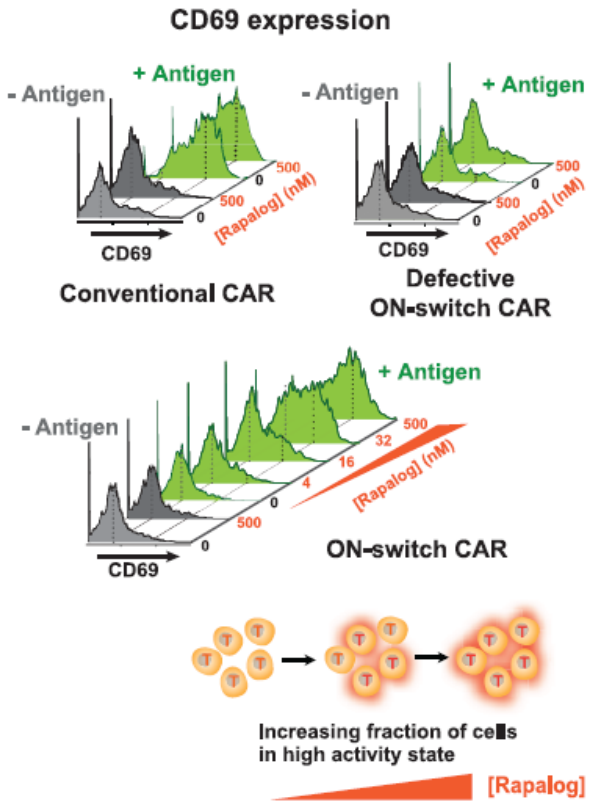


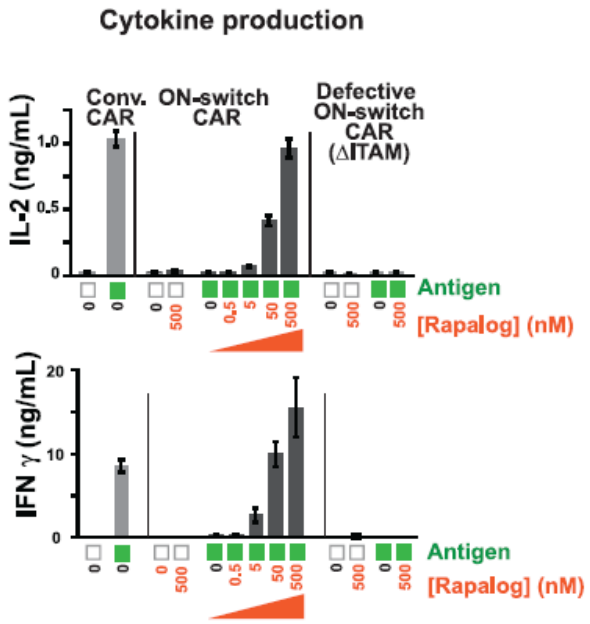
- part I and part II co-localize at macroscopic level
- PALM (**p**hoto**a**ctivated **l**ocalization **m**icroscopy) for analysis on single molecule level → both components are not physically associated in the absence of rapalog

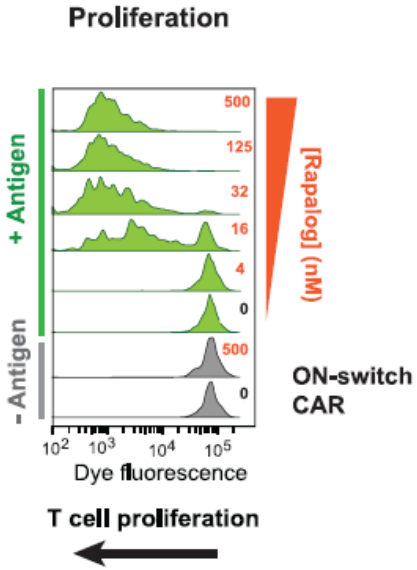
Making the switch to a safer CAR-T cell therapy

Effectiveness of ON-switch CAR cells:



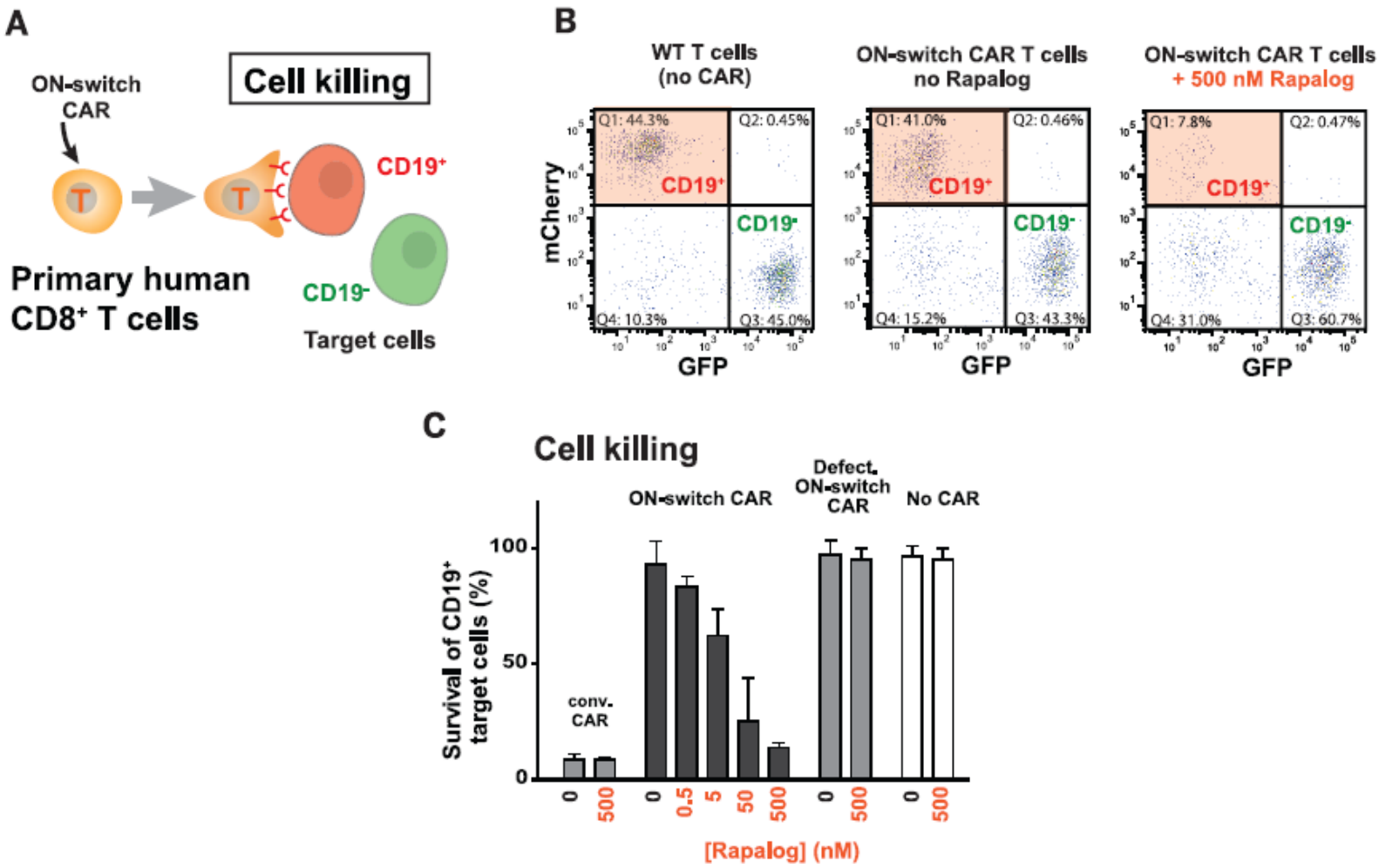






- small molecule activation of CAR transduced primary human CD4+ T cells is titratable

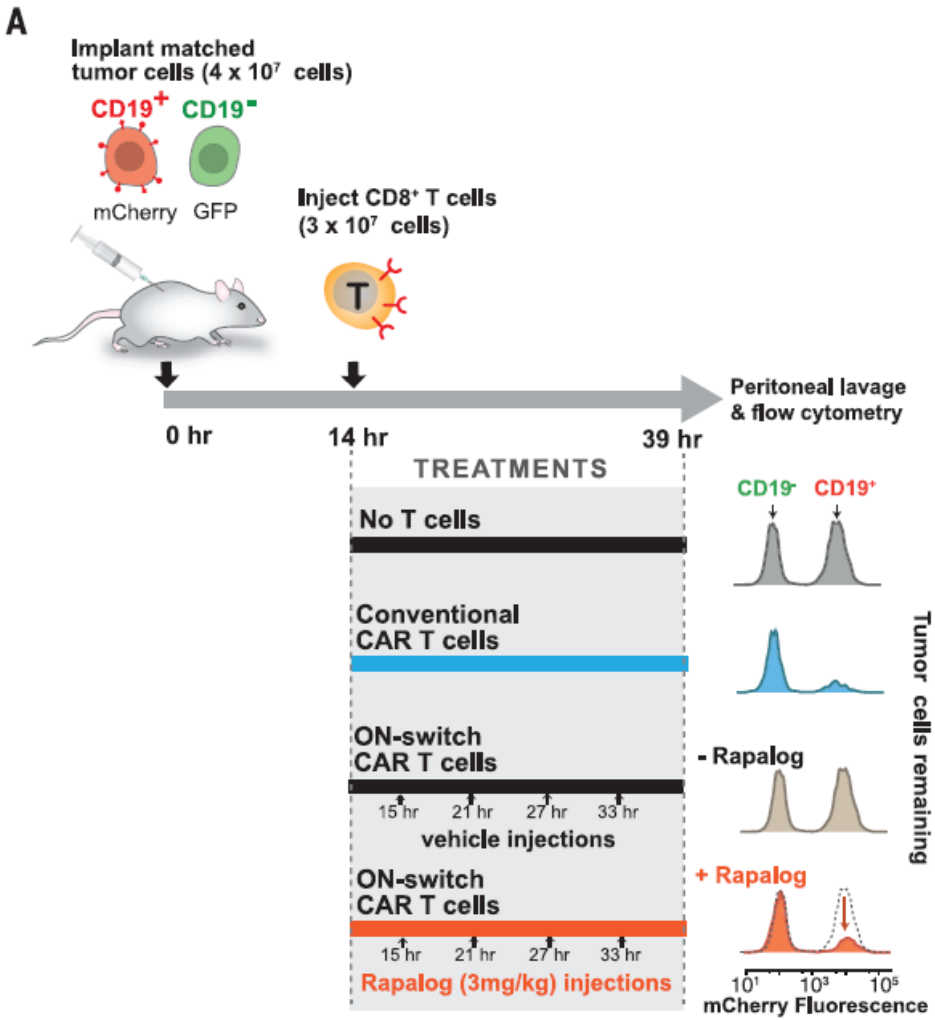
Effectiveness of ON-switch CAR cells:

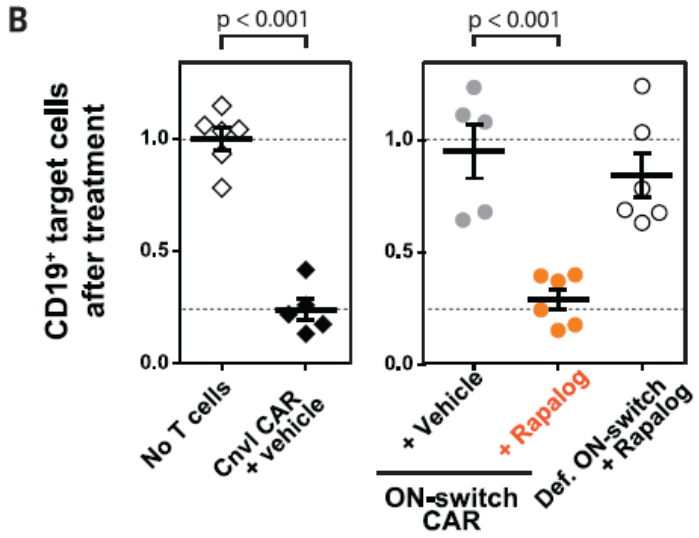


- Antigen-specific and titratable killing of target cell population

Making the switch to a safer CAR-T cell therapy

Effectiveness of ON-switch CAR cells:





- Rapalog dependent killing of target cells by ON-switch CAR T cells *in vivo*

Summary ON-switch CAR cells:

- strategy to combine autonomous control with user control for engineering safer therapeutic immune cells

Pros:

- selective regulation in temporal and titratable manner
- this strategy requires no elimination of the therapeutic immune cells after treatment

  → persistence and re-activation in case of relapse

Cons:

- small-molecule chosen (rapalog) showed suboptimal pharmacokinetic properties
- Single target based therapy → risk of potential escape
- Distribution and localization of CAR-T cells and small-molecule switch?

PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Versatile strategy for controlling the specificity and activity of engineered T cells

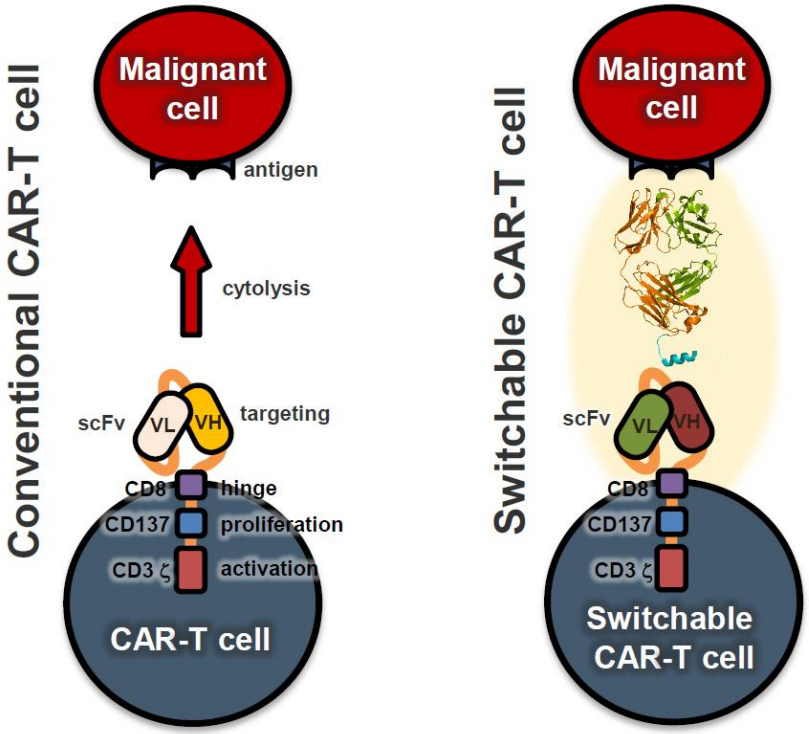
Jennifer S. Y. Ma[a,1], Ji Young Kim[a], Stephanie A. Kazane[a,2], Sei-hyun Choi[b], Hwa Young Yun[b,3], Min Soo Kim[a,4], David T. Rodgers[a], Holly M. Pugh[a], Oded Singer[a], Sophie B. Sun[a], Bryan R. Fonslow[c,d], James N. Kochenderfer[e], Timothy M. Wright[a], Peter G. Schultz[a,b,5], Travis S. Young[a,5], Chan Hyuk Kim[a,5], and Yu Cao[b,1]

[a]Department of Biology, California Institute for Biomedical Research, La Jolla, CA 92037; [b]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037; [c]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037; [d]SCIEX Separations, Brea, CA 92821; and [e]Experimental Transplantation and Immunology Branch, National Institutes of Health, National Cancer Institute, Bethesda, MD 20892

Making the switch to a safer CAR-T cell therapy

Switchable CAR-T cells:

- uncoupling of **antigen recognition domain** (scFv) and the **CD8 hinge, CD3ζ** part by the use of soluble intermediary switch molecules comprised of
  - tumor targeting antibody or small molecule ligand
  - second part providing specific binding only to CAR

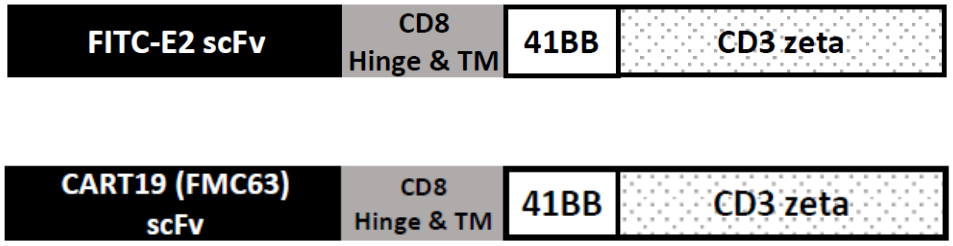


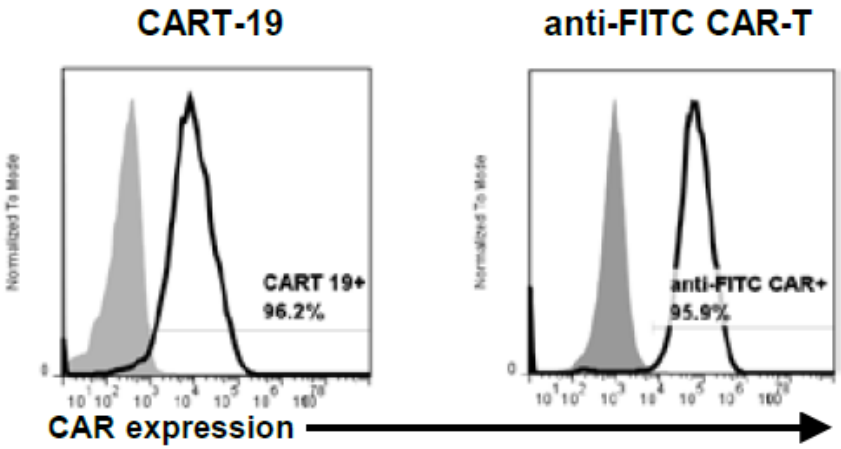
Rodgers et al. 2016, PNAS

Making the switch to a safer CAR-T cell therapy

Design and Synthesis of sCAR-T cell:

- Specificity redirection of CAR T cell by generating CAR T cells recognizing the synthetic dye FITC
  - full sequence of anti FITC-E2 scFv was inserted into a second generation CAR expression cassette



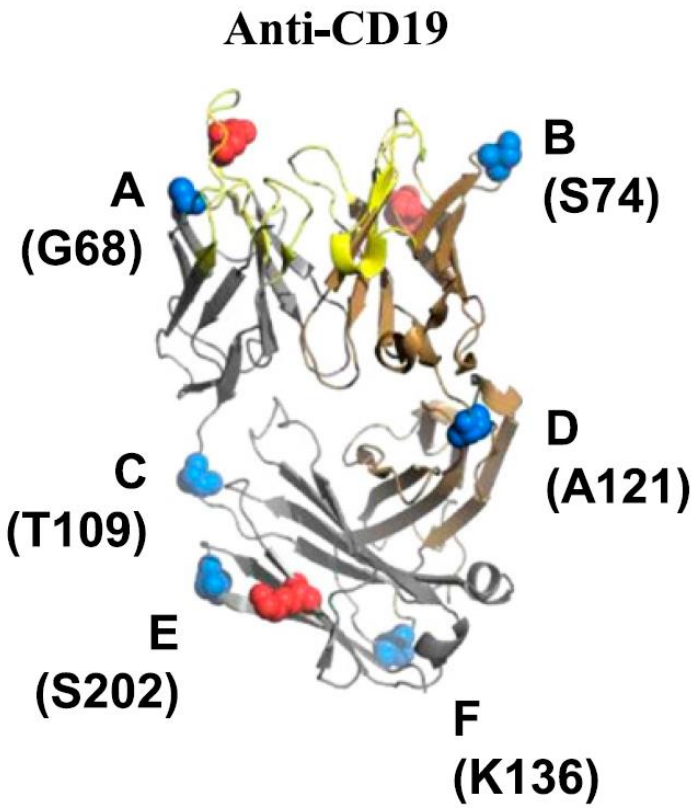


Making the switch to a safer CAR-T cell therapy

Design and Synthesis of switch molecule:

- Switch based on Fab format using a anti-CD19-specific monoclonal Ab
- FITC conjugation of anti-CD19 Fab
  - site-specific
  - random

➢ To study effect of FITC conjugation site on distance and geometry of the pseudoimmunological synapse



Making the switch to a safer CAR-T cell therapy

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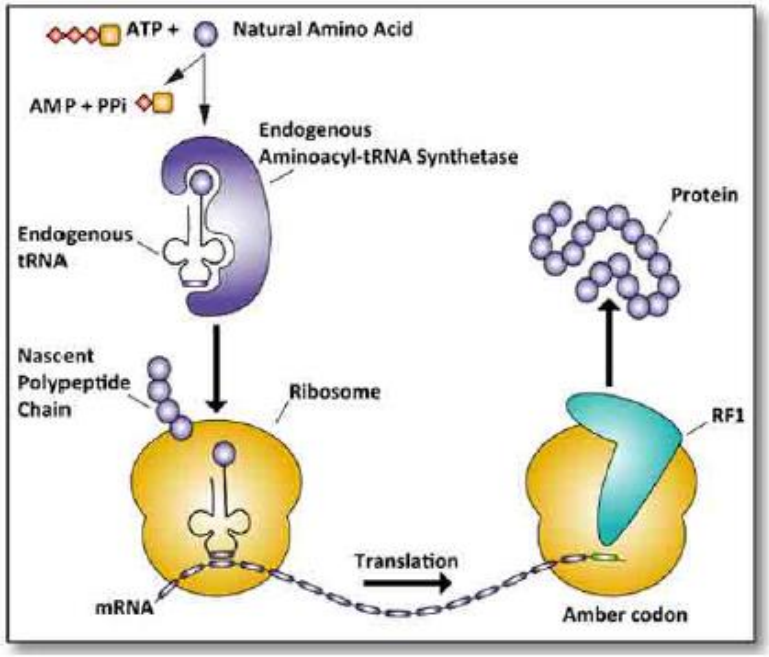
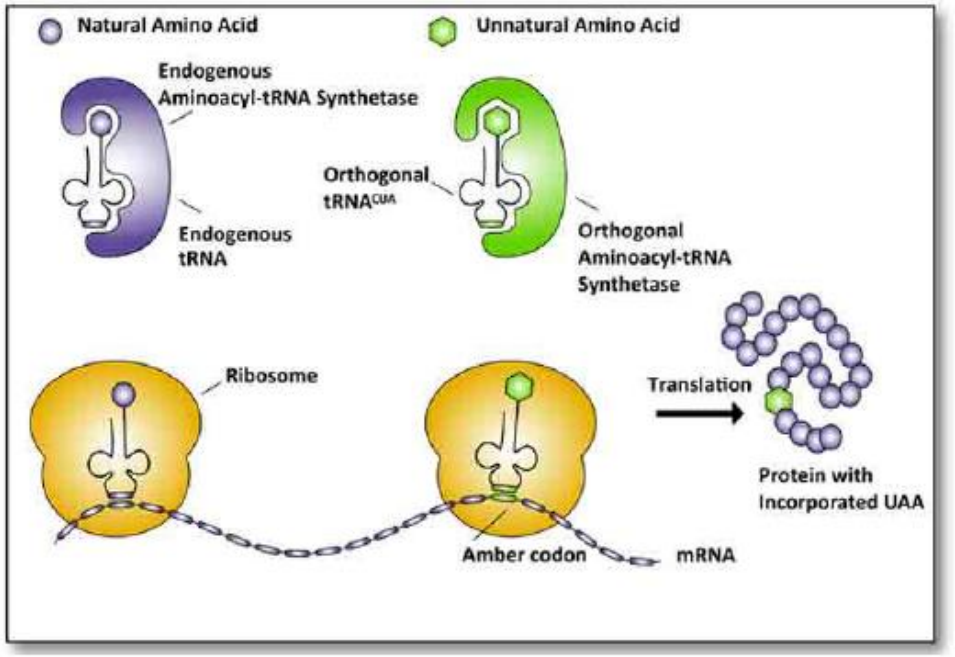
Incorporation of noncanonical amino acids with bio-orthogonal chemical reactivity





Wals and Ovaa 2014, Frontiers in Chemistry

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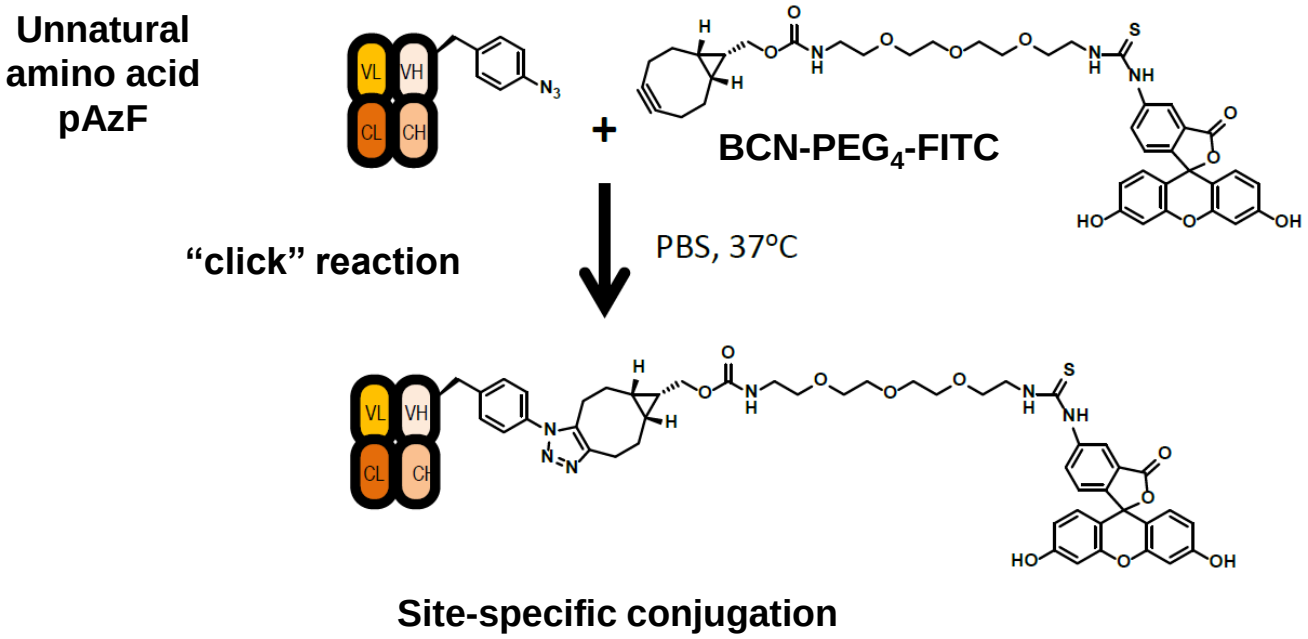

Making the switch to a safer CAR-T cell therapy

Design and Synthesis of switch molecule:

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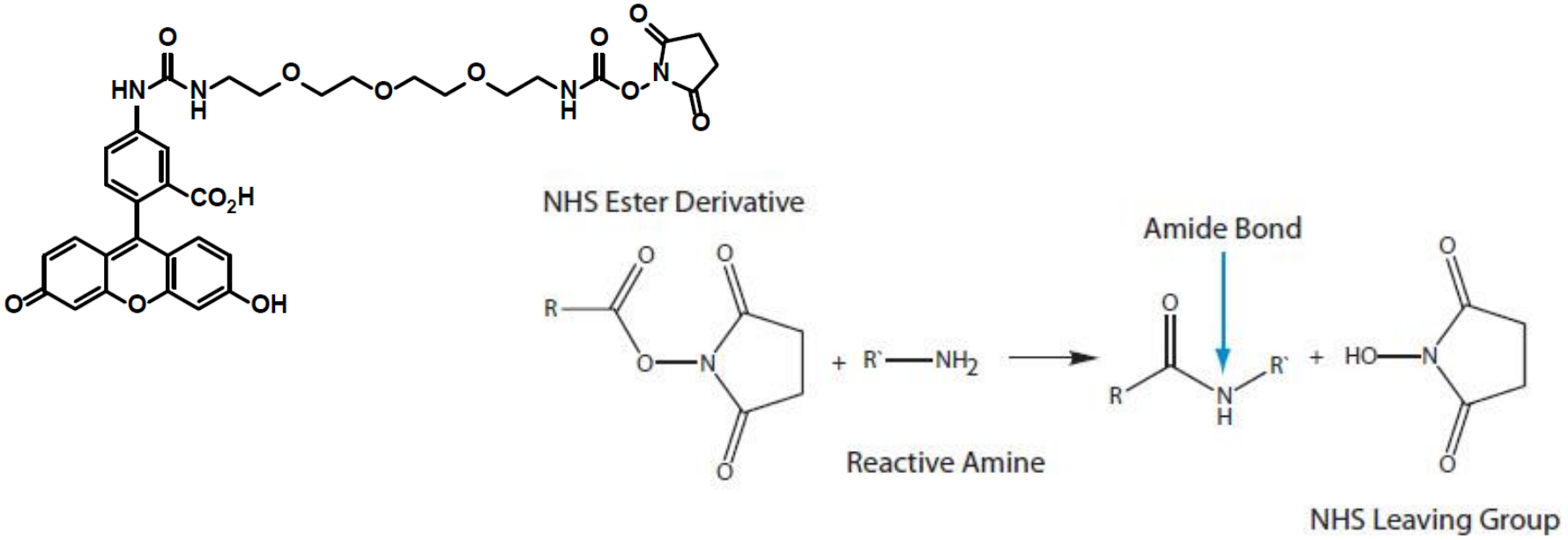
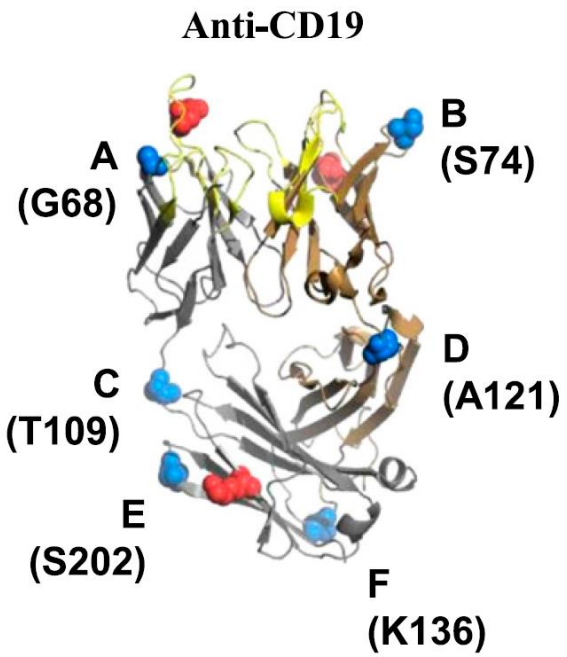
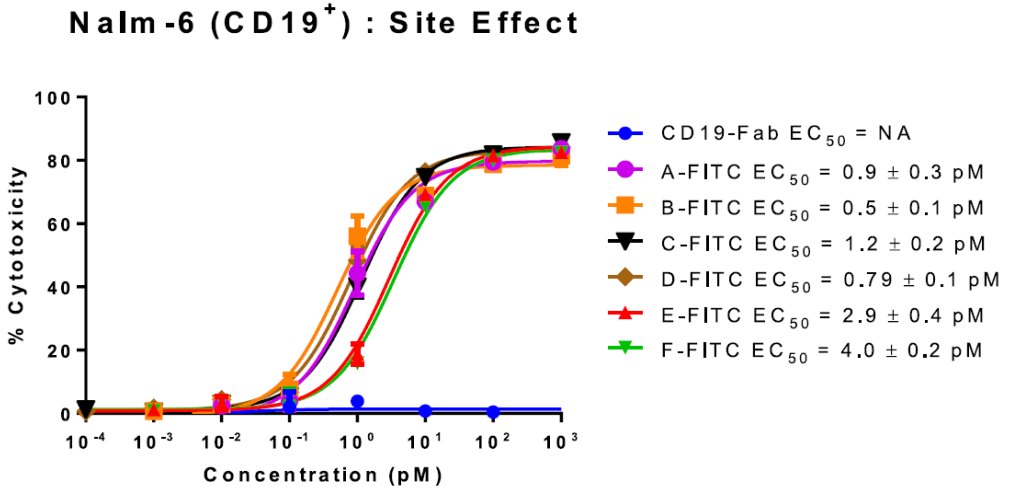
Amine-reactive Crosslinker Chemistry



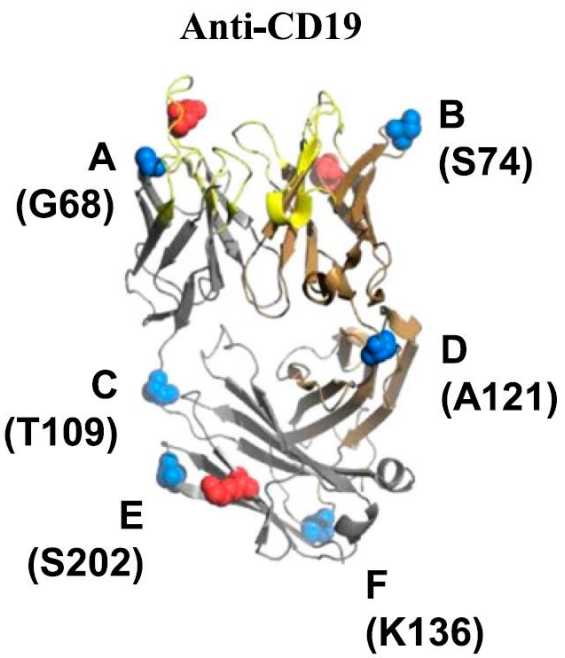
Effect of FITC conjugation on sCAR-T cells:

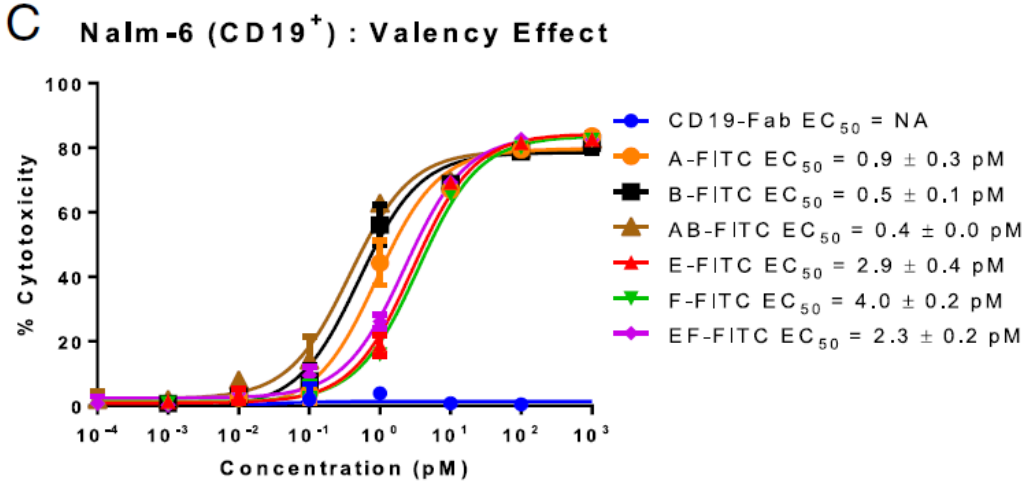


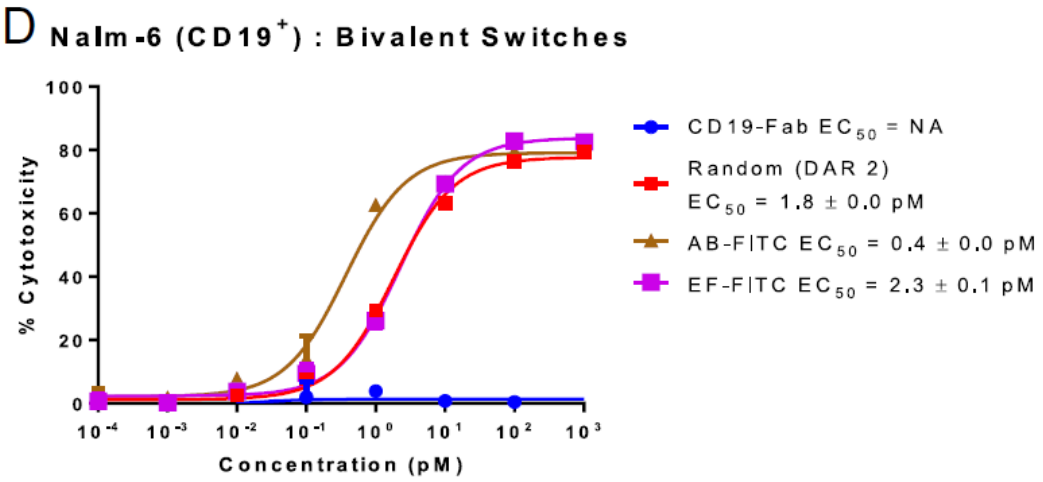


- Highly potent lytic activity for all constructs
- Anti-CD19-switch with FITC conjugations proximal to antigen binding region are more potent

Effect of FITC conjugation on sCAR-T cells:



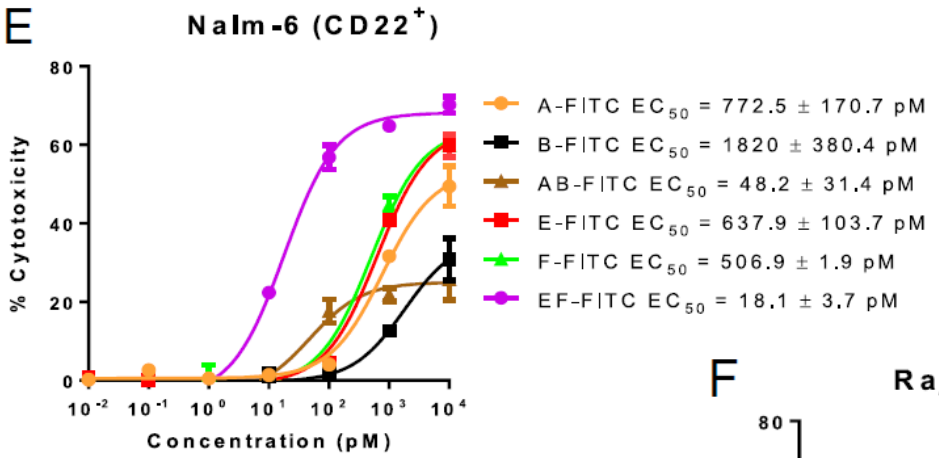


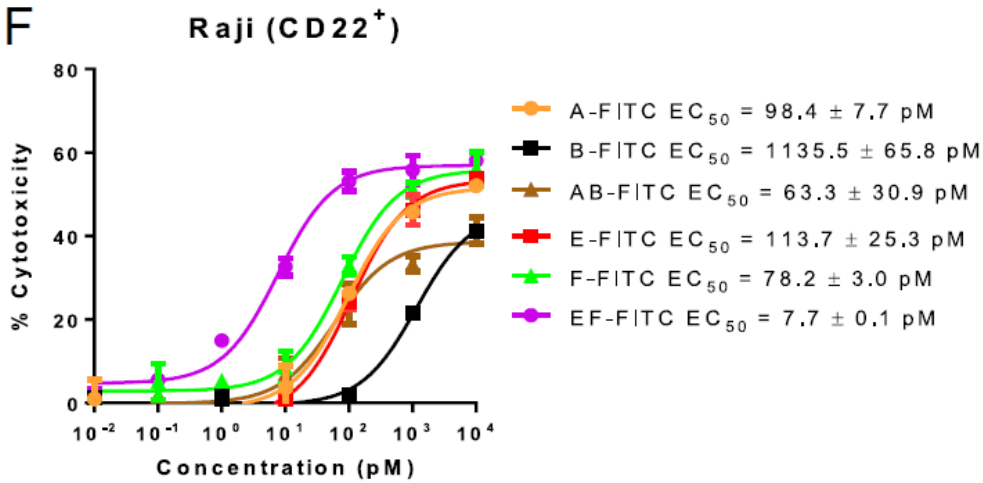


- Bivalent switches more potent than monovalent switches

  → site and number of conjugations affect CAR-T activity

Targeting of other tumor antigens (CD22) using the same sCAR-T cell:



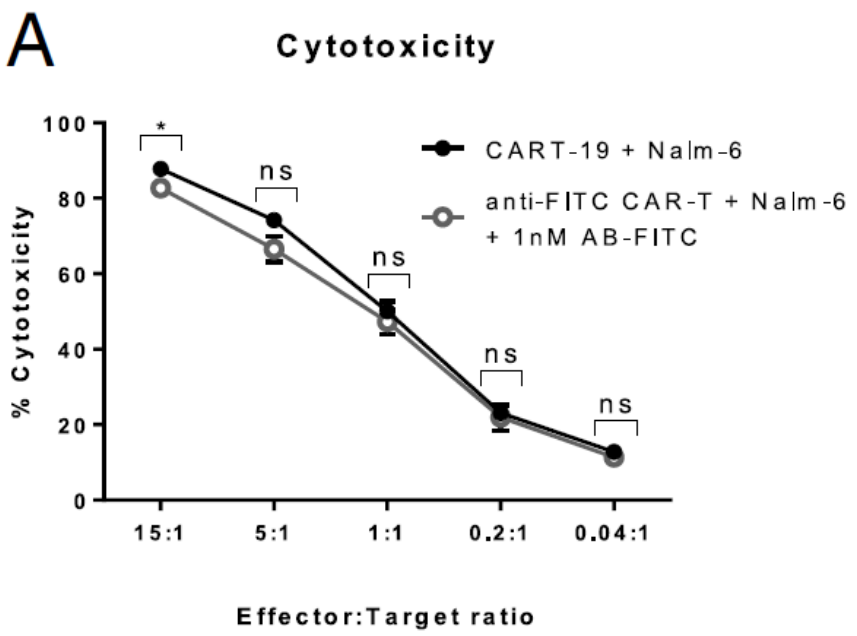


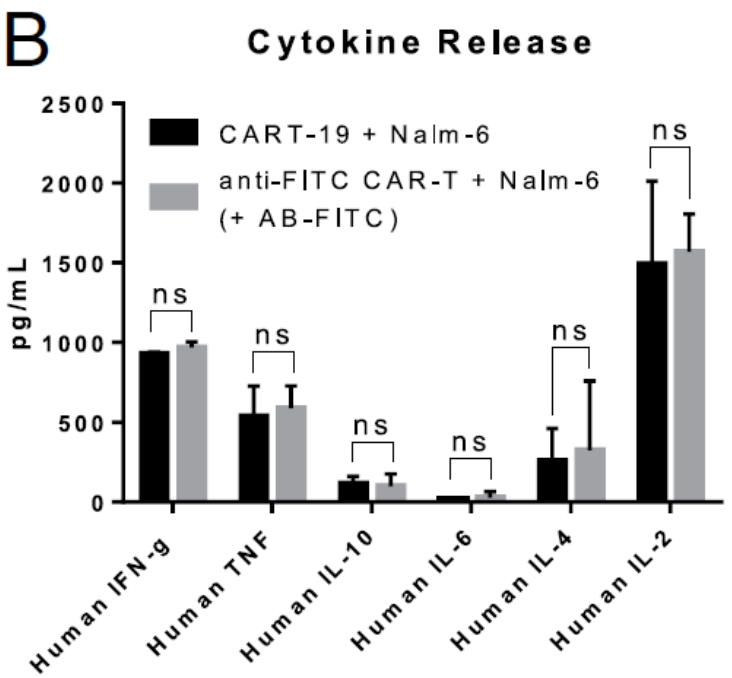
- Anti-CD22-switch with FITC conjugations distal to antigen binding region are more potent

  → distinct geometrics are required for each individual antigen-antibody interaction for optimal effector functions

Effectiveness of sCAR-T cells with optimized anti-CD19 AB-FITC switch

in vitro:

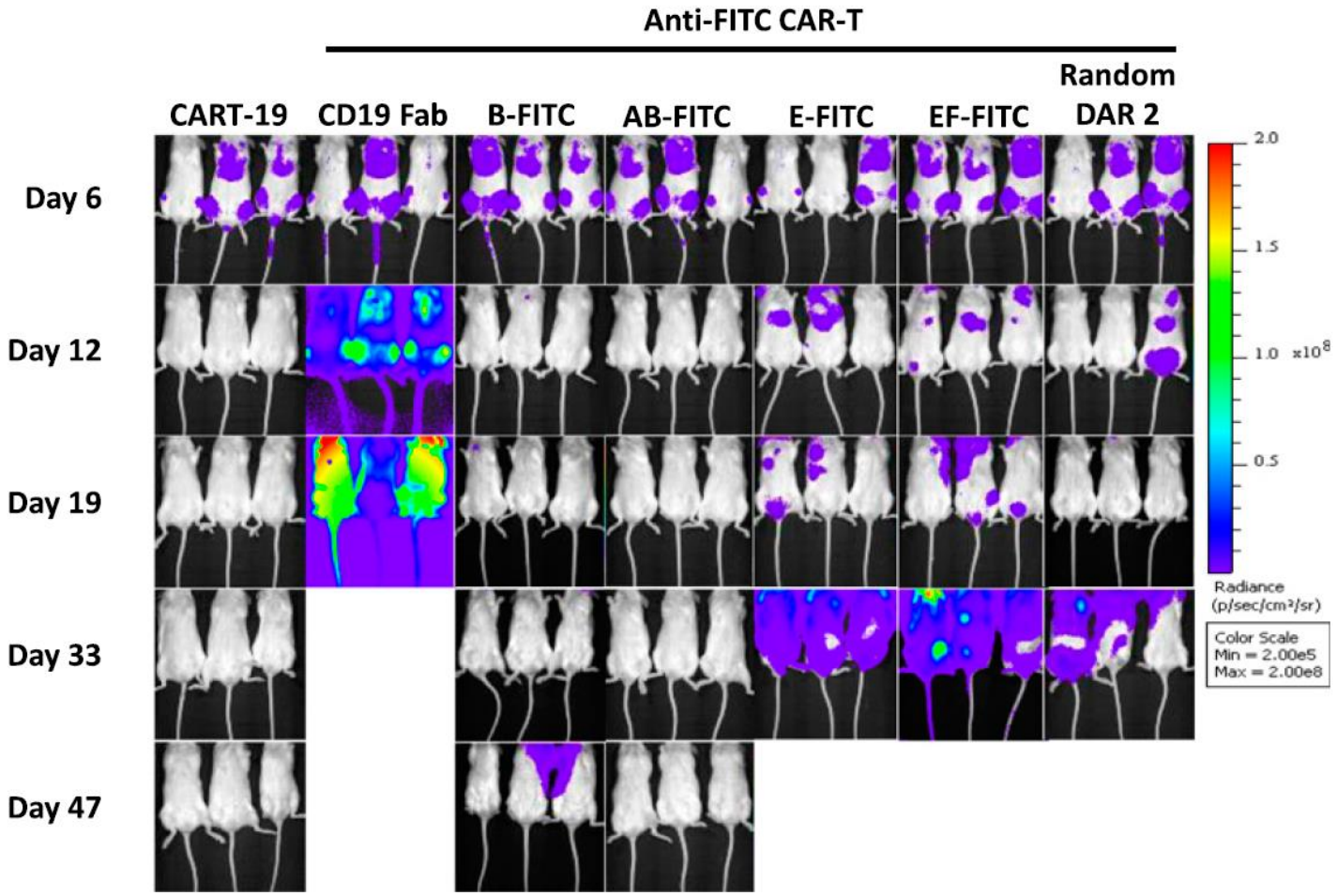




- Anti-FITC CAR-T cells in conjugation with optimized anti-CD19 AB switch are comparable to conventional anti-CD19 CAR-T cells
  - in electing tumor-specific effector functions
  - inducing costimulatory signaling

Effectiveness of sCAR-T cells with optimized anti-CD19 AB-FITC switch

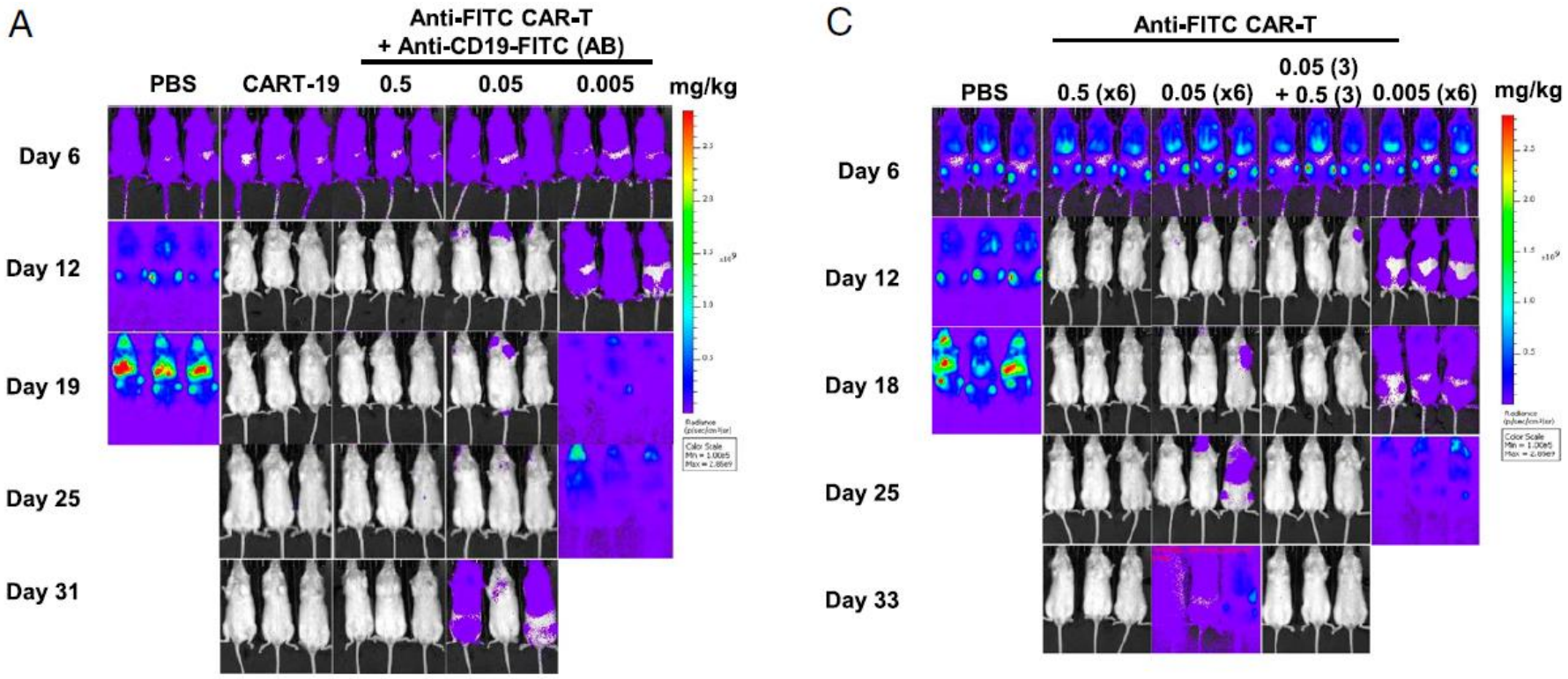
in vivo:



- Anti-FITC CAR-T cells in conjugation with optimized anti-CD19 AB switch are able to clear tumor cells *in vivo*

Making the switch to a safer CAR-T cell therapy

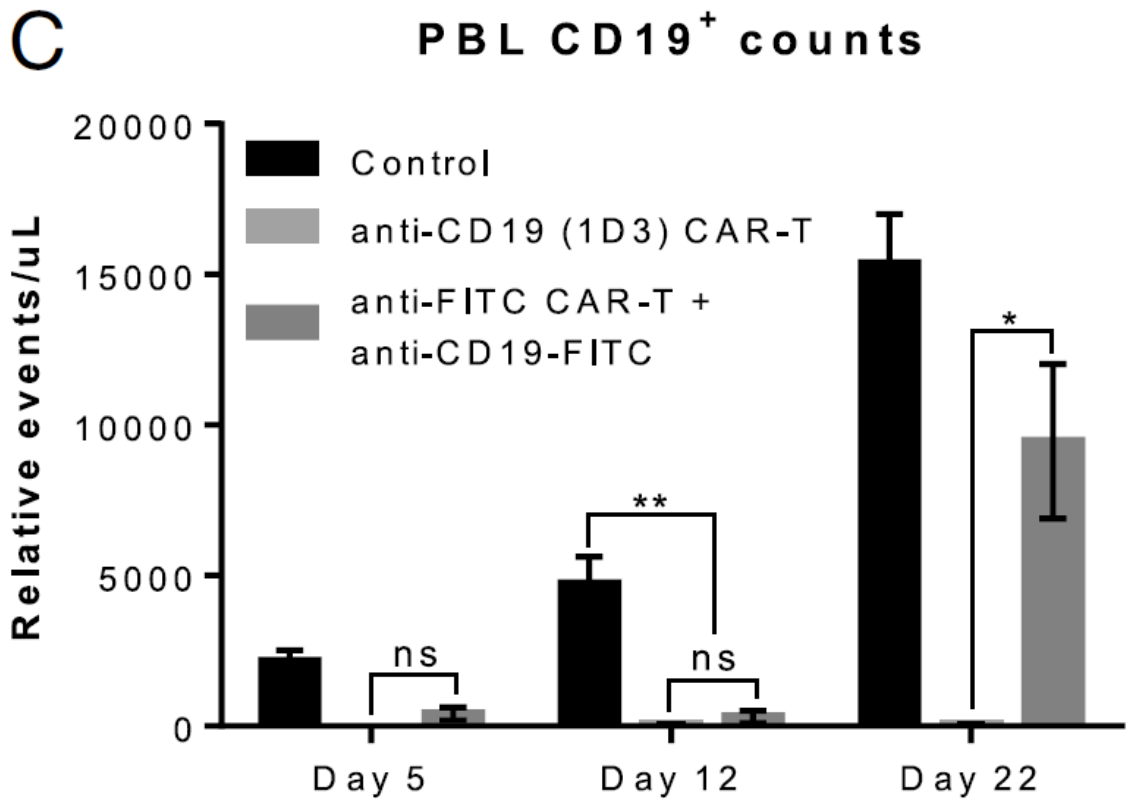
In vivo control of sCAR-T cell activity:



- Anti-FITC CAR-T cells in conjugation with optimized anti-CD19 AB switch can used to decrease the risk of tumor lysis syndrome

***In vivo* termination of persistent B cell ablation by stopping of switch dosing:**

- mouse sCAR-T cell generated using splenocytes from C57BL/6 mice



- Repopulation of $CD19^+$ cells in the peripheral blood upon termination of switch treatment → sCAR-T cell response can be “turned-off”

PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies

David T. Rodgers[a], Magdalena Mazagova[a], Eric N. Hampton[a], Yu Cao[b], Nitya S. Ramadoss[a,1], Ian R. Hardy[a,2], Andrew Schulman[a], Juanjuan Du[a], Feng Wang[a], Oded Singer[a,3], Jennifer Ma[a], Vanessa Nunez[a], Jiayin Shen[a,4], Ashley K. Woods[a], Timothy M. Wright[a], Peter G. Schultz[a,b,5], Chan Hyuk Kim[a,5], and Travis S. Young[a,5]

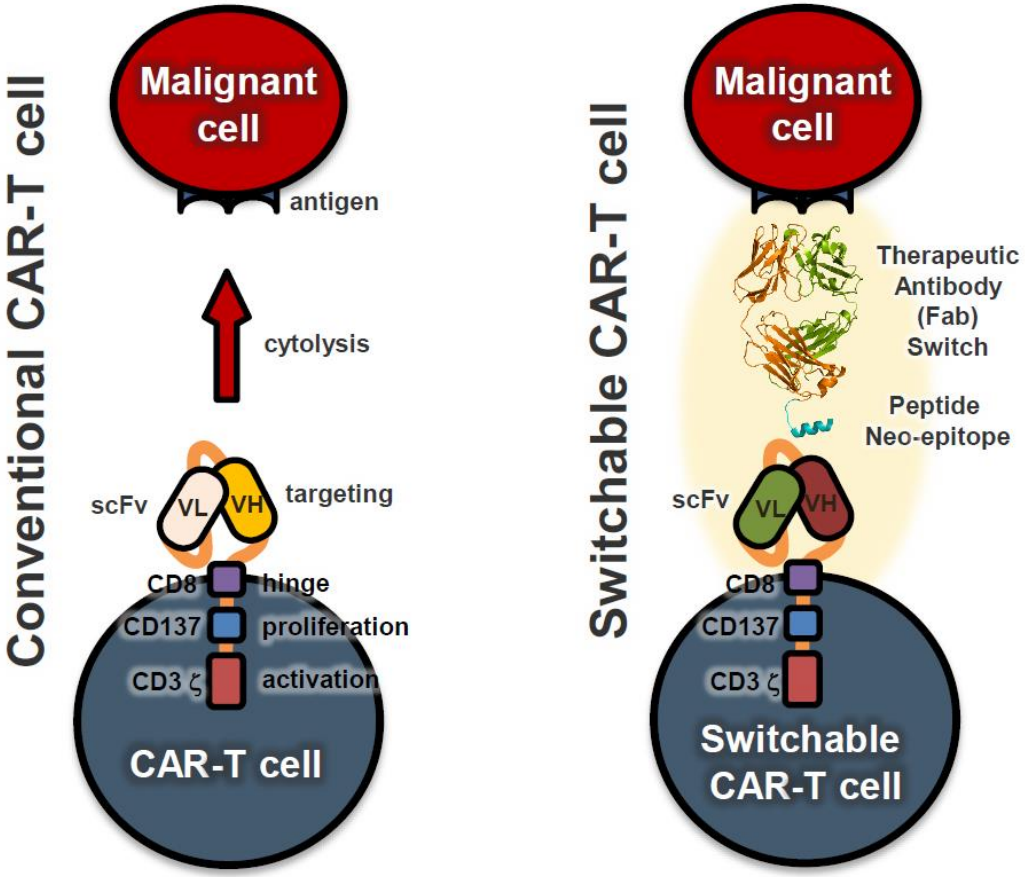
[a]Department of Biology, California Institute for Biomedical Research, La Jolla, CA 92019; and [b]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037

Making the switch to a safer CAR-T cell therapy

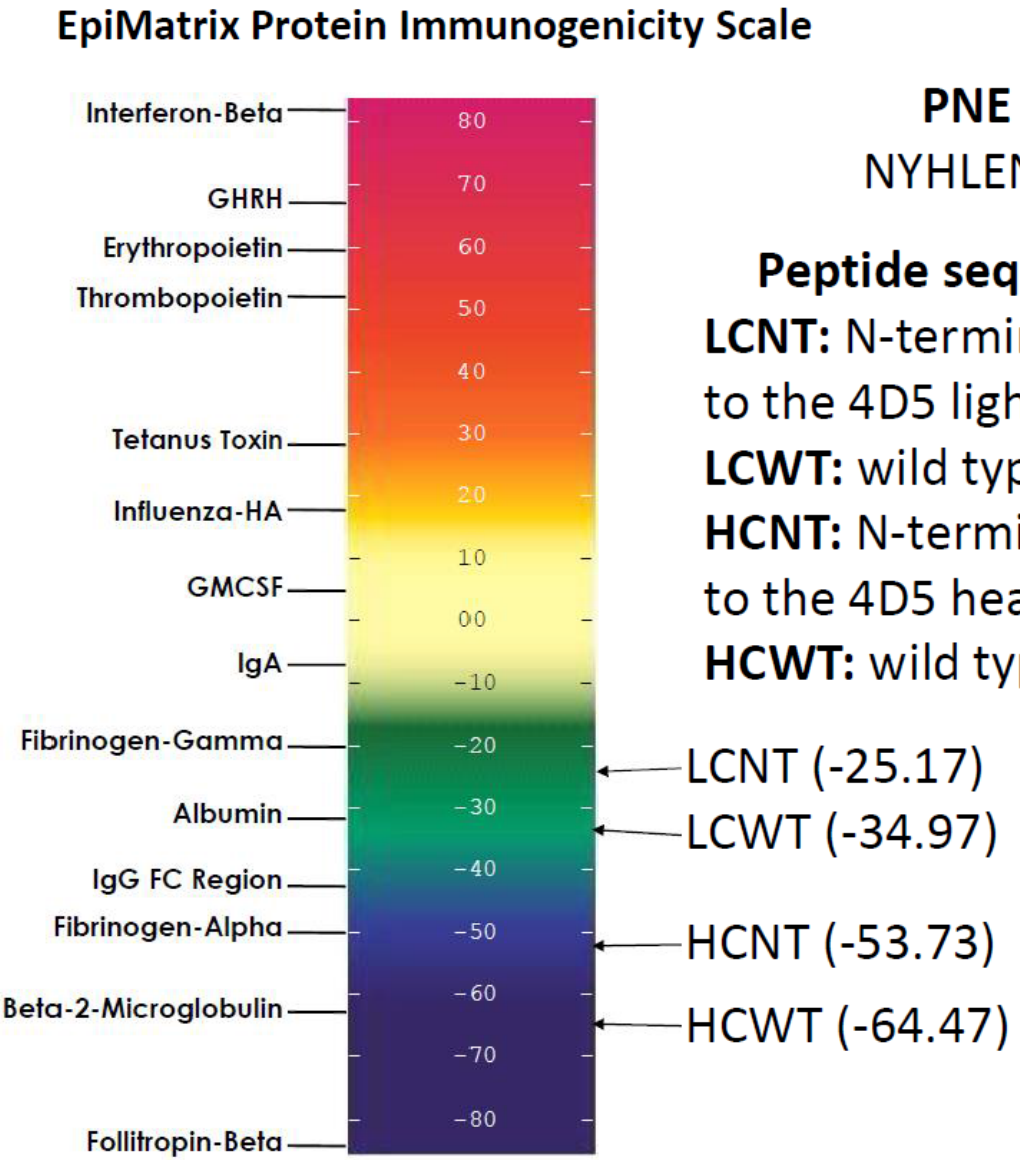
Switchable CAR-T cells:

- Retargeting of CAR-T cells by the introduction of peptide-neo epitopes (PNE) in an antigen-specific antibody (anti-CD19)

  - 14aa PNE sequence from the yeast transcription factor GCN4



In silico immunogenicity assay of PNE:



PNE peptide:
NYHLENEVARLKKL

Peptide sequences analyzed:

LCNT: N-terminal graft of the PNE to the 4D5 light chain

LCWT: wild type 4D5 light chain

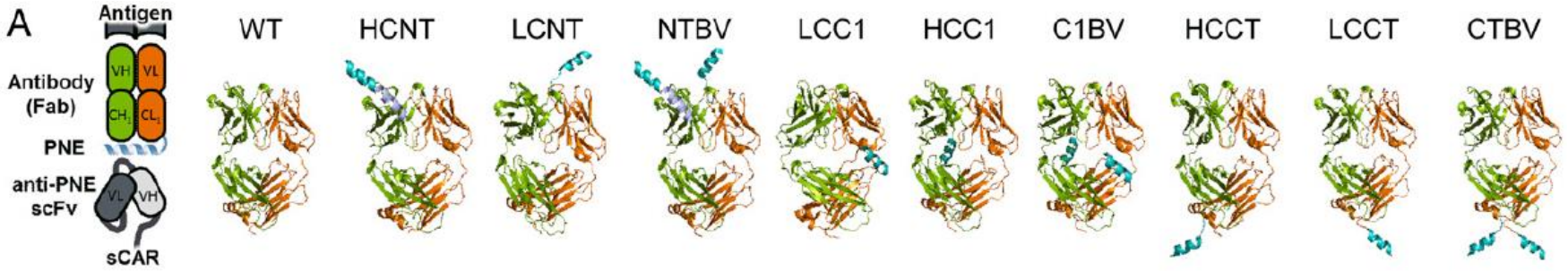
HCNT: N-terminal graft of the PNE to the 4D5 heavy chain

HCWT: wild type 4D5 heavy chain

Making the switch to a safer CAR-T cell therapy

Design and Synthesis of switch molecule:

- Gene fragments encoding anti-CD19 or anti-CD20 heavy and light chains with or without PNE engraftment were synthesized



Anti-CD19 FMC63 Light chain LCC1 (graft and linker underlined):

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

DIATYFCQQGNTLPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ

ESVTEQDSGGGGSNYHLENEVARLKKLGGGGSDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

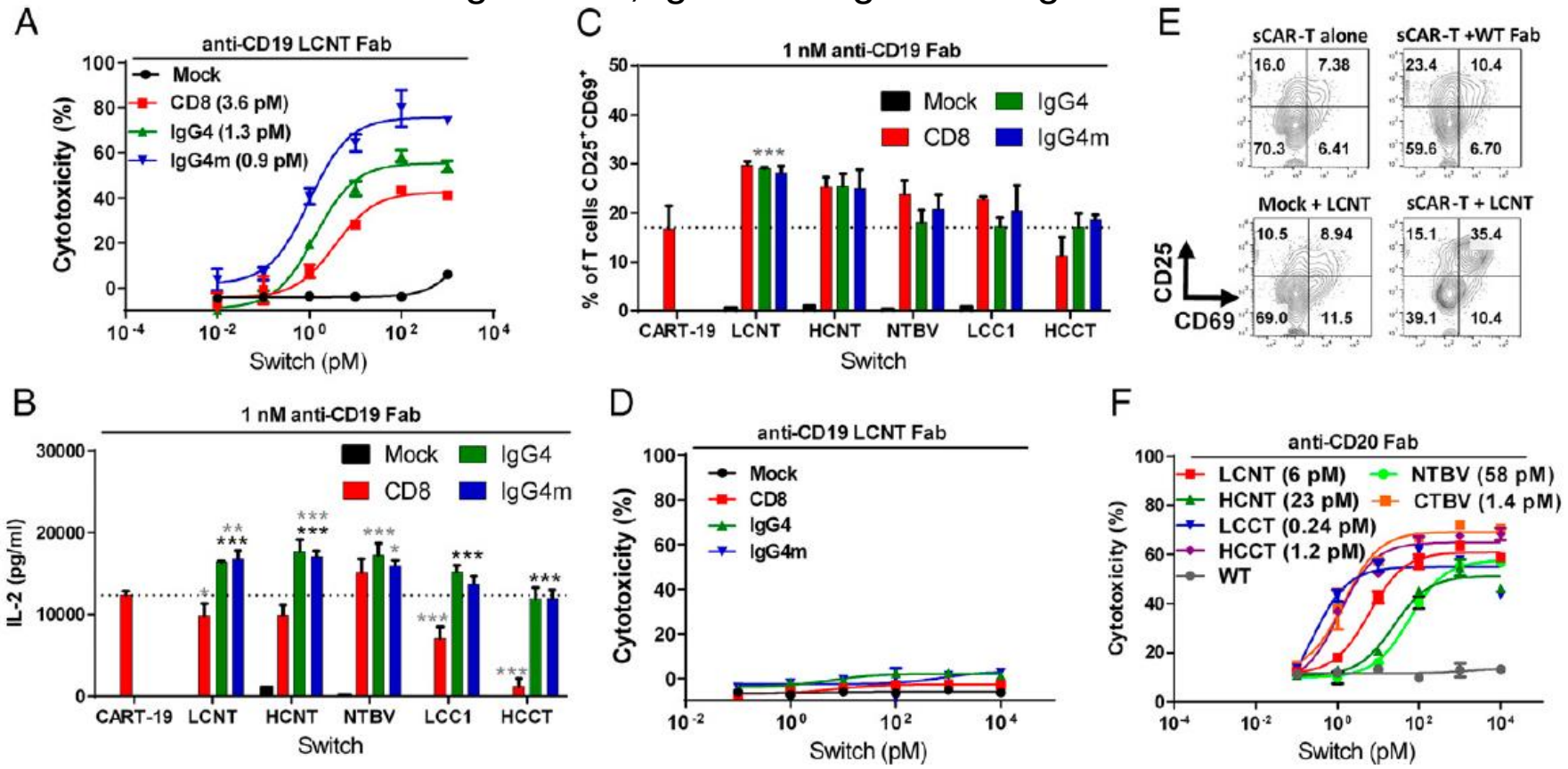
linker sequence

PNE

Effect of hinge region design on sCAR-T cells *in vitro:*

- sCAR-T cells with different hinge length

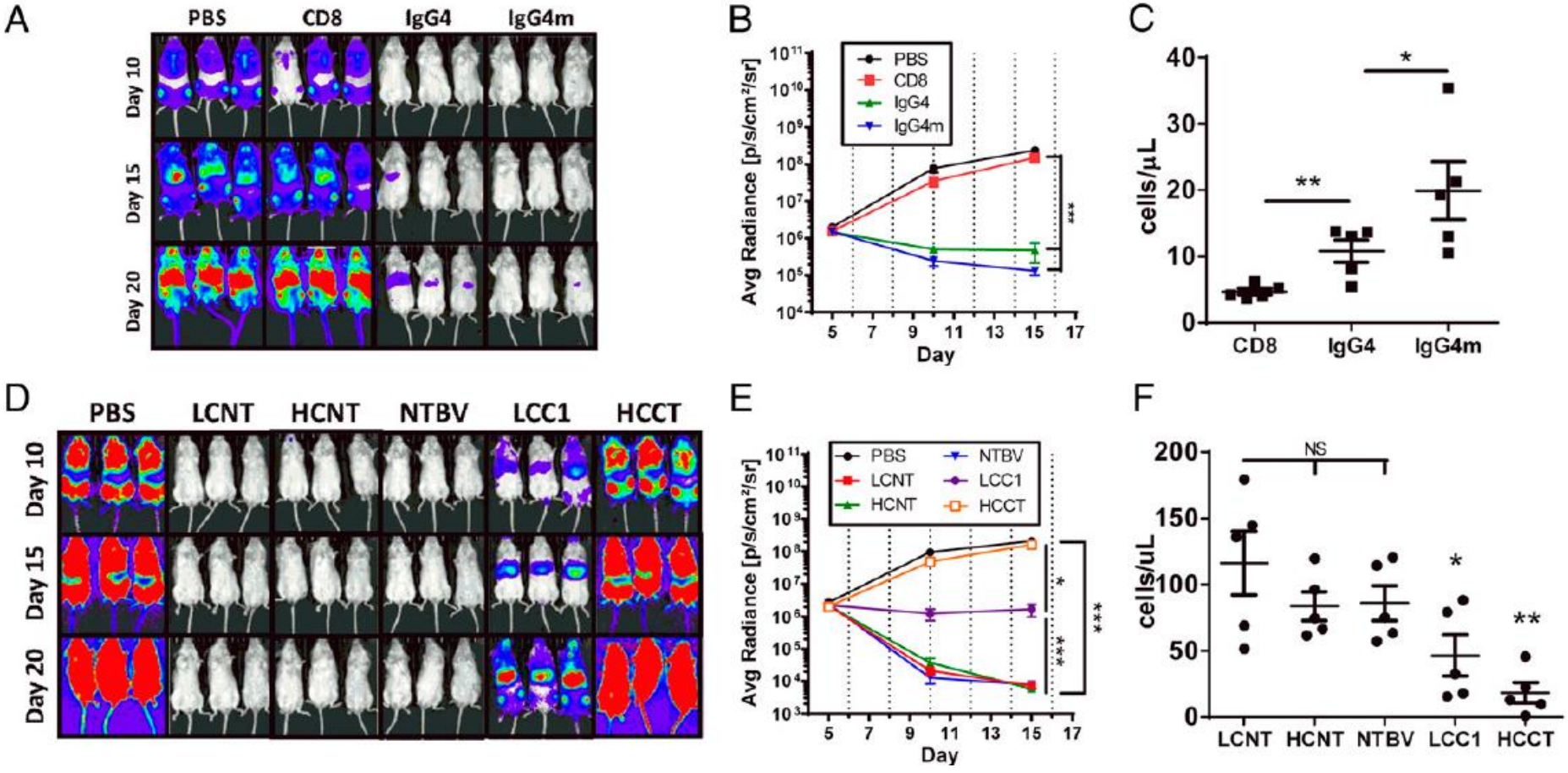
CD8 hinge 45AA; IgG4 and IgG4m hinge 12AA



- Increased sCAR-T cell activation through shorter hinge regions (IgG4) and dimerization of sCAR upon interchain sCAR formation (IgG4m)

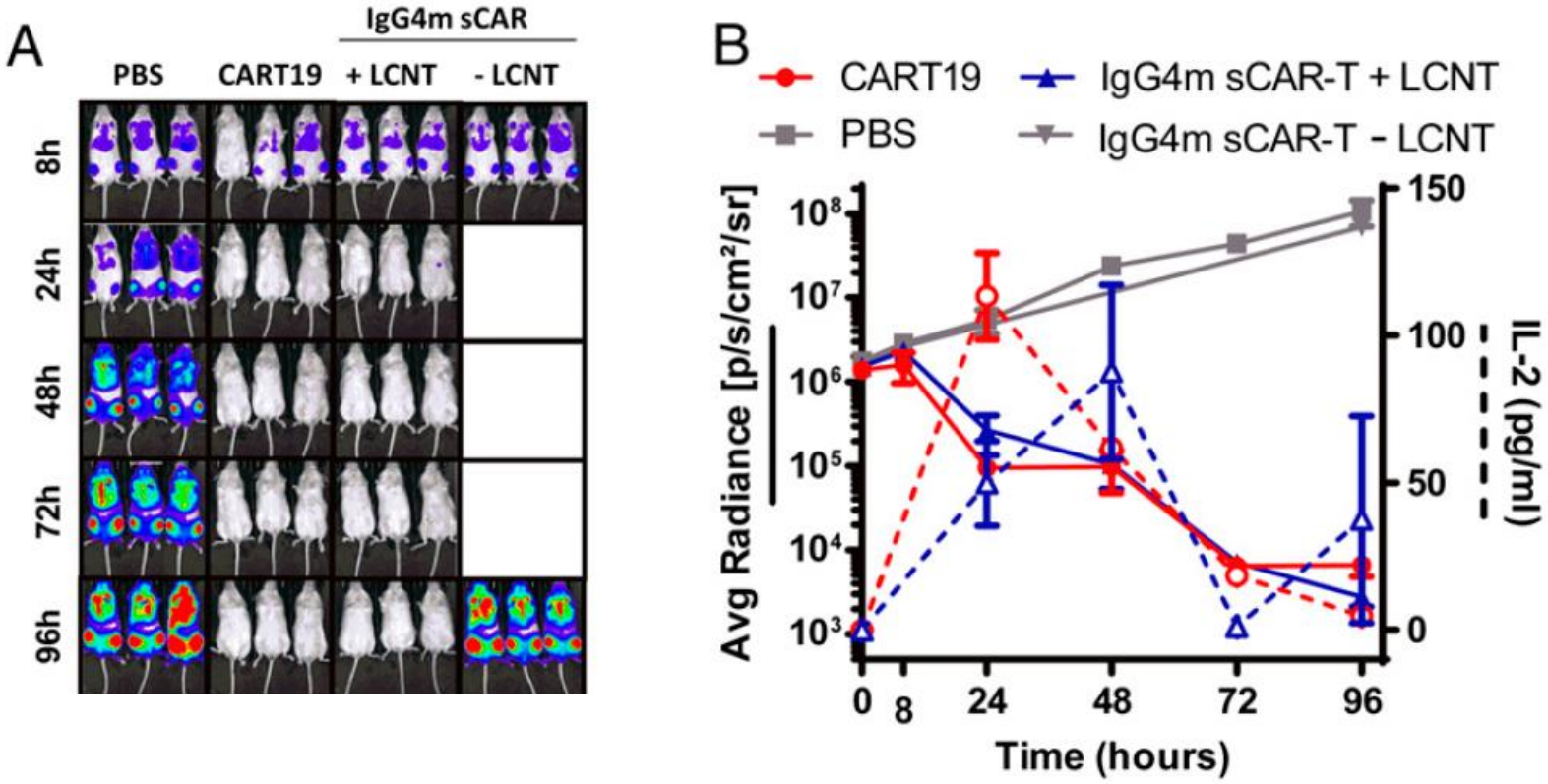
Making the switch to a safer CAR-T cell therapy

Effectiveness of sCAR-T cells with different switch and hinge designs *in vivo:*



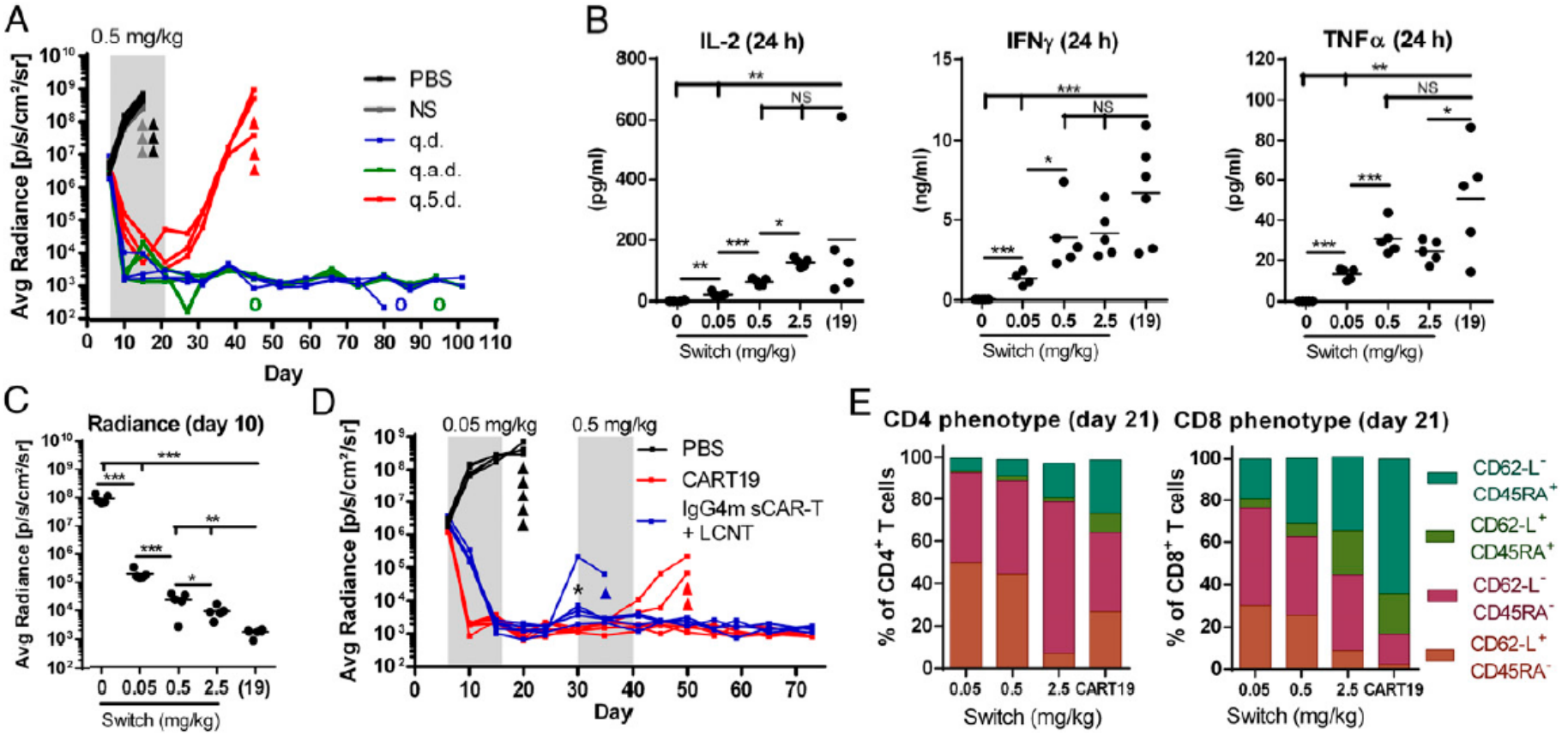
- Most effective *in vivo* tumor clearance by sCAR-T cells with IgG4m hinge region and anti-CD19 switches with NT fused PNE

sCAR-T cell expansion during tumor clearance *in vivo:*



- *in vivo* tumor clearance by sCAR-T cells with IgG4m hinge region and anti-CD19-NT-PNE switch comparable to conventional anti-CD19 CAR-T cells

Dose dependent control of sCAR-T cell activity *in vivo:*



- switch dose is able to control activity, cytokine release and phenotype of CAR-T cells *in vivo*

Summary switchable CAR cells:

- strategy to combine autonomous control with user control for engineering safer therapeutic immune cells

Pros:

- selective regulation in temporal and titratable manner
- this strategy requires no elimination of the therapeutic immune cells after treatment

  → persistence and re-activation in case of relapse
- Modularity allows the use of one sCAR-T cell with different switches targeting various antigens

Cons:

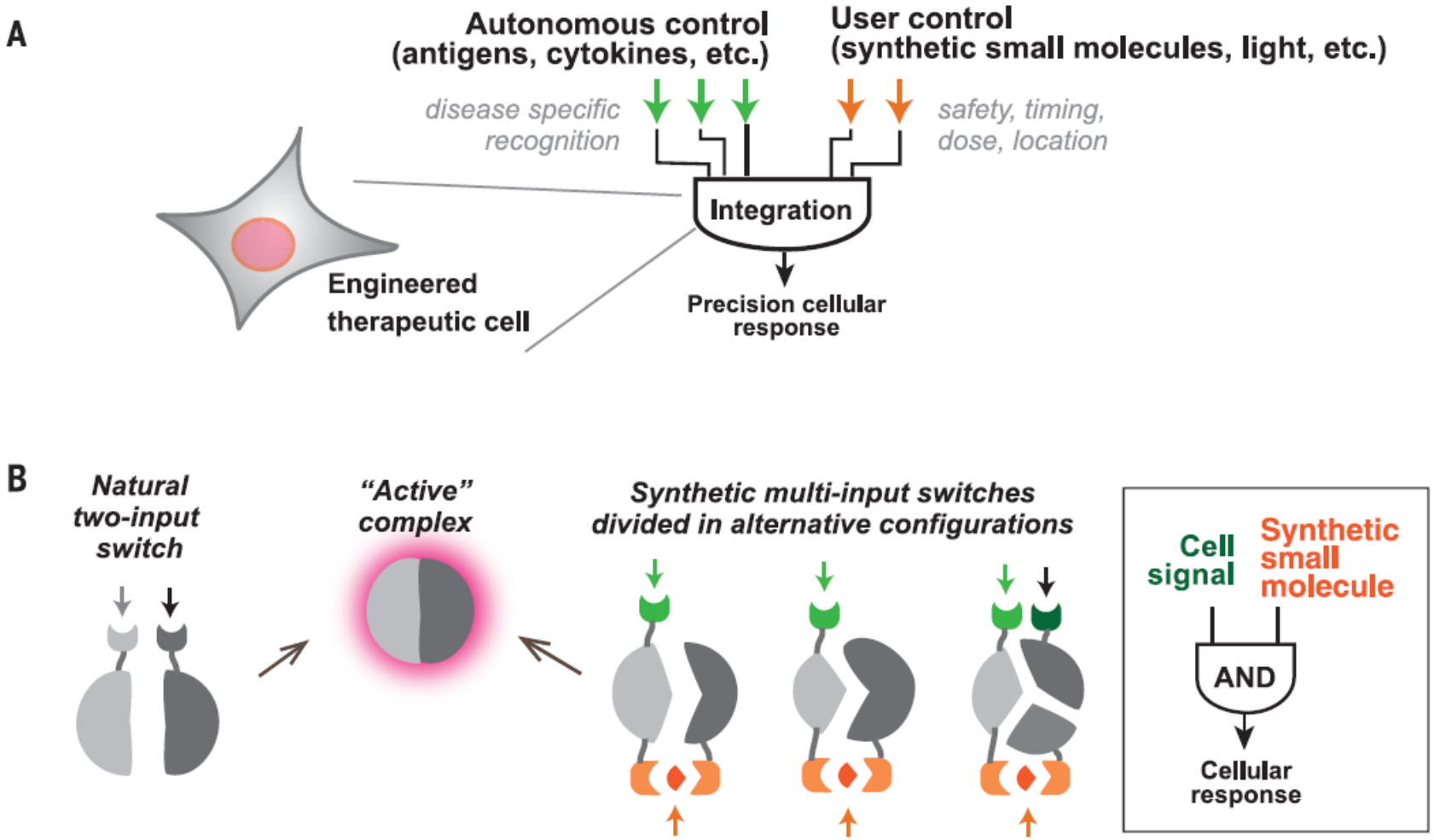
- Distribution and localization of CAR-T cell and switch?

Thank you for your attention!!!

Thank you for your attention!!!

Making the switch to a safer CAR-T cell therapy

Strategies for engineering therapeutic cells with autonomous and user control:

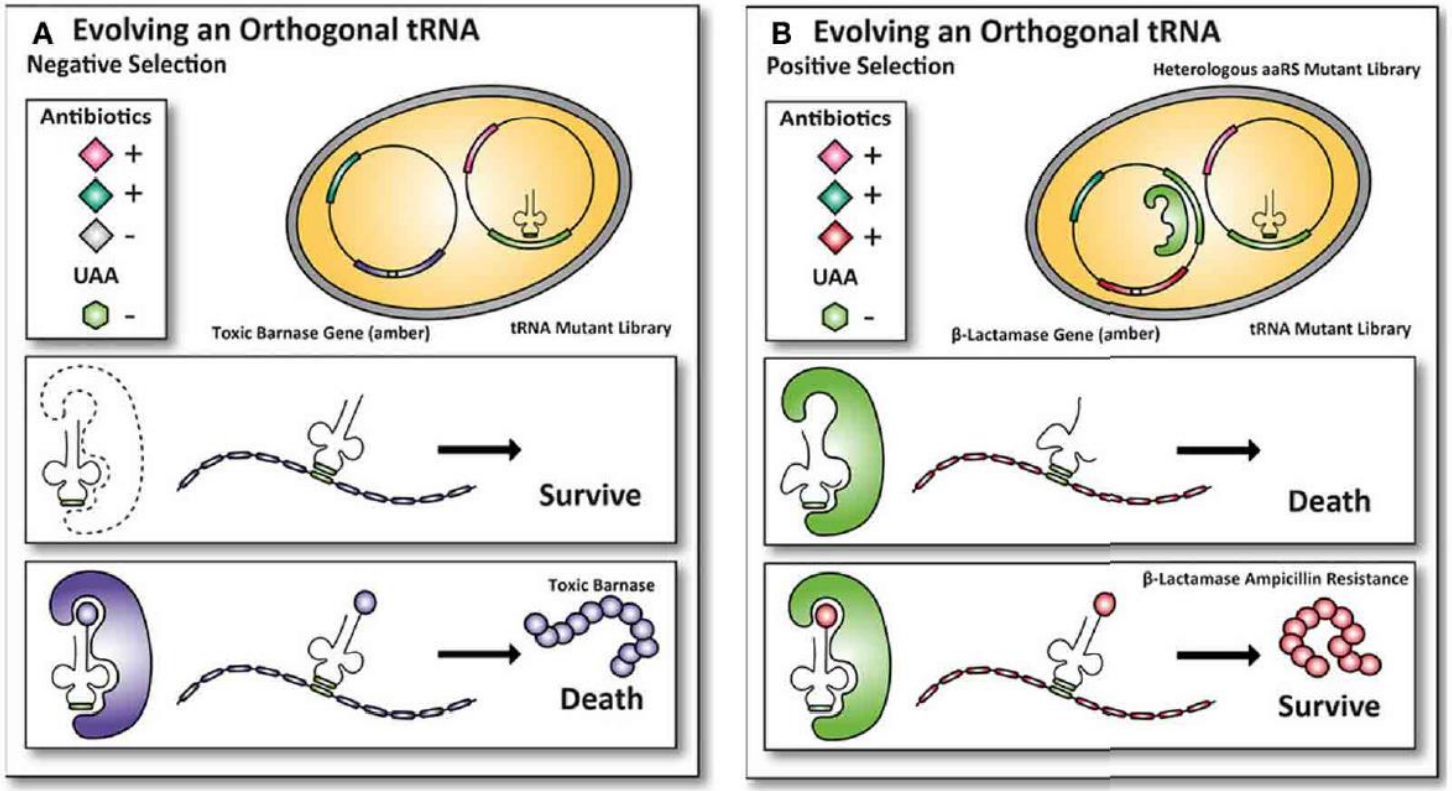


Making the switch to a safer CAR-T cell therapy

Design and Synthesis of switch molecule:

- Switch based on Fab format using a anti-CD19-specific monoclonal Ab
- FITC conjugation of anti-CD-19 Fab
  - site-specific

Incorporation of noncanonical amino acids with bio-orthogonal chemical reactivity



Wals and Ovaa 2014, Frontiers in Chemistry

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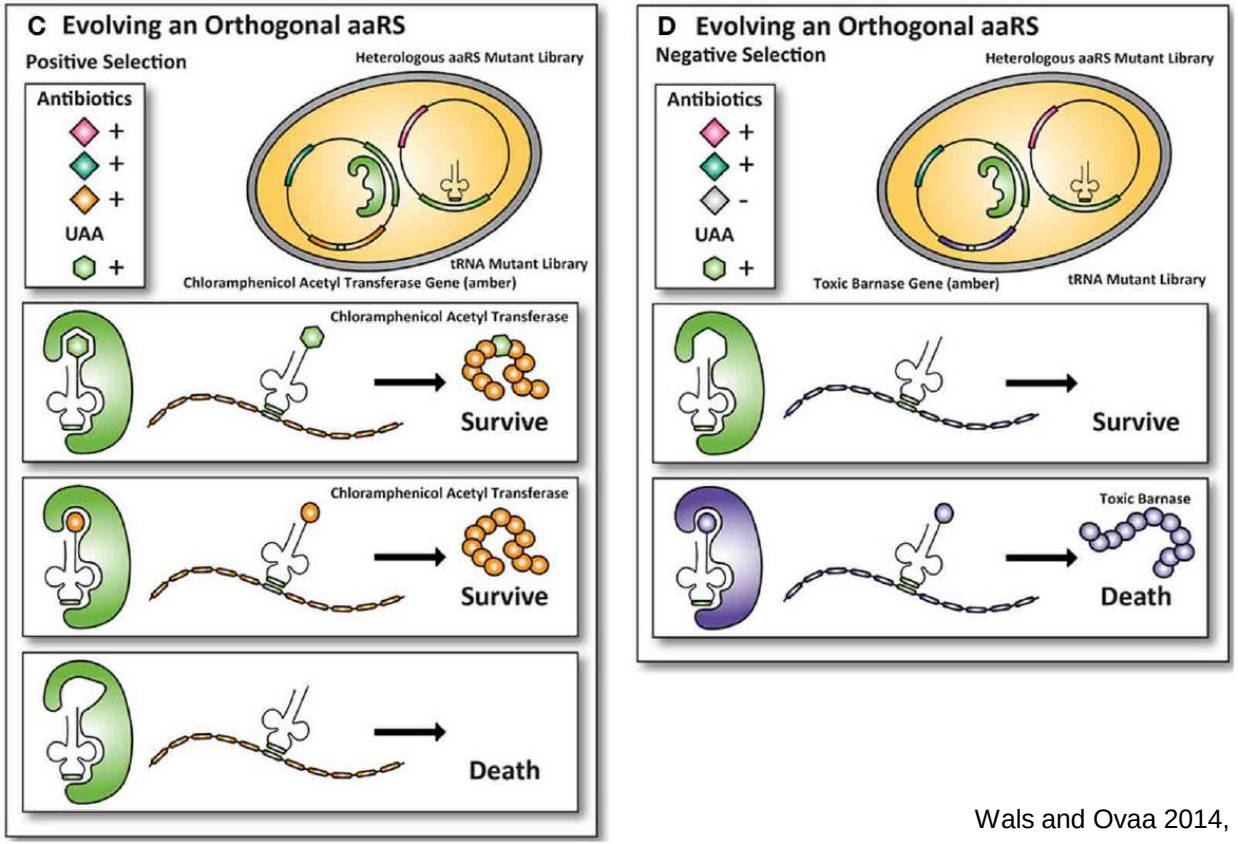
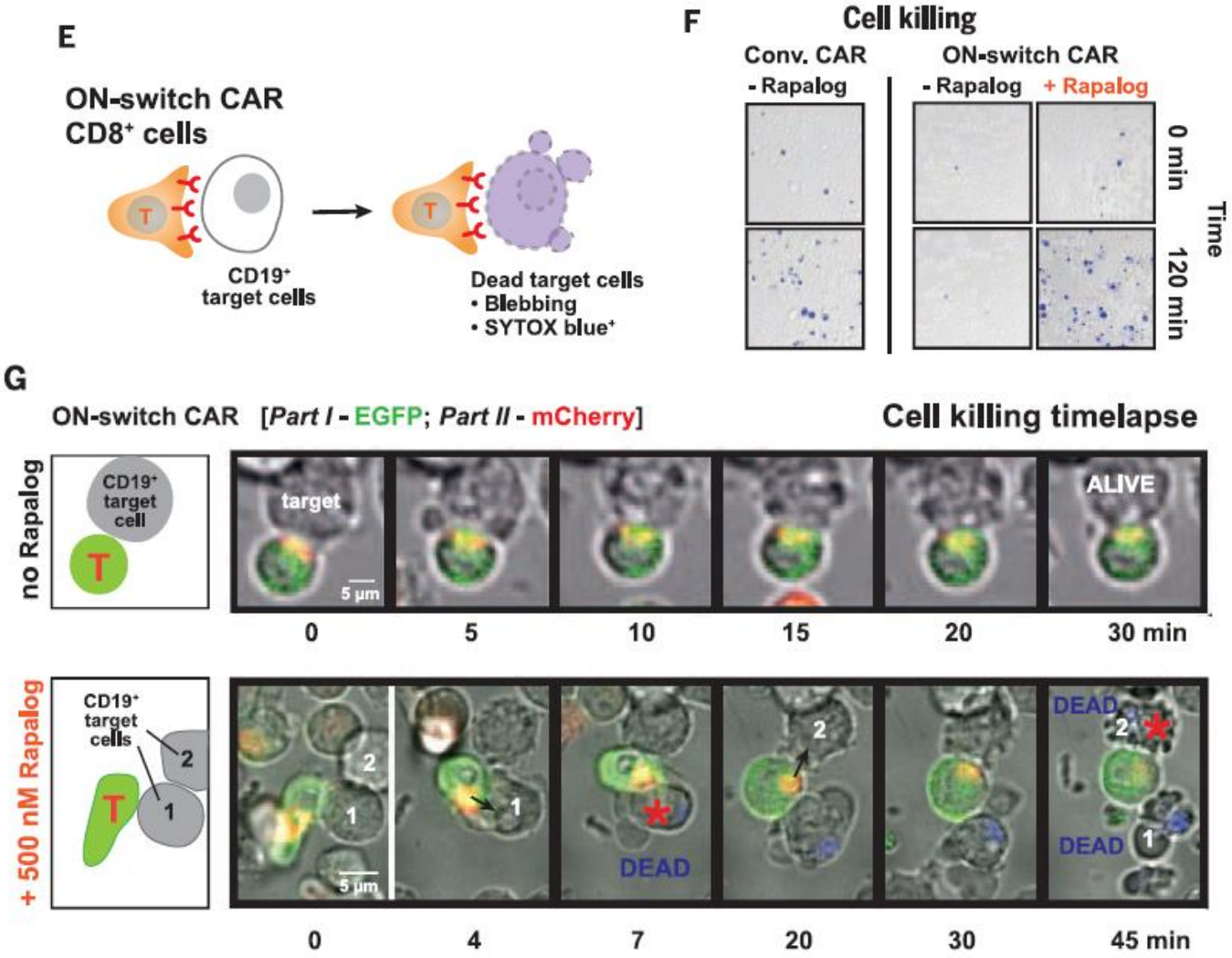


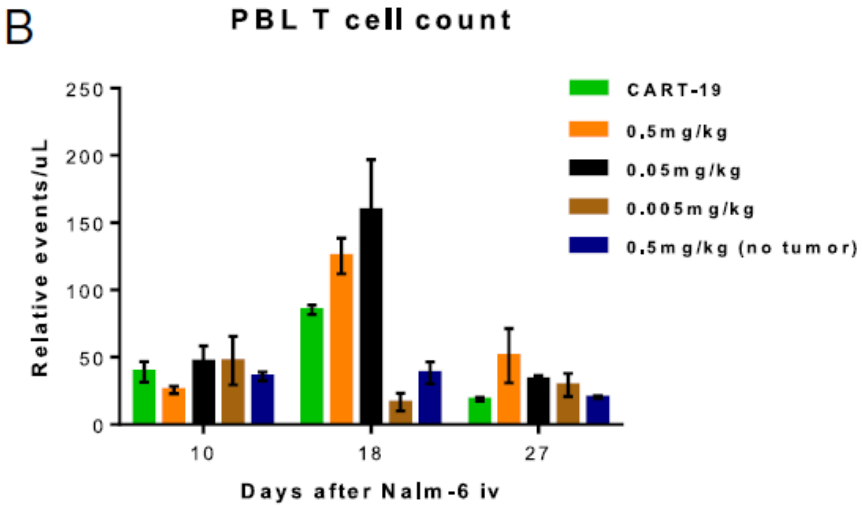
Wals and Ovaa 2014, Frontiers in Chemistry

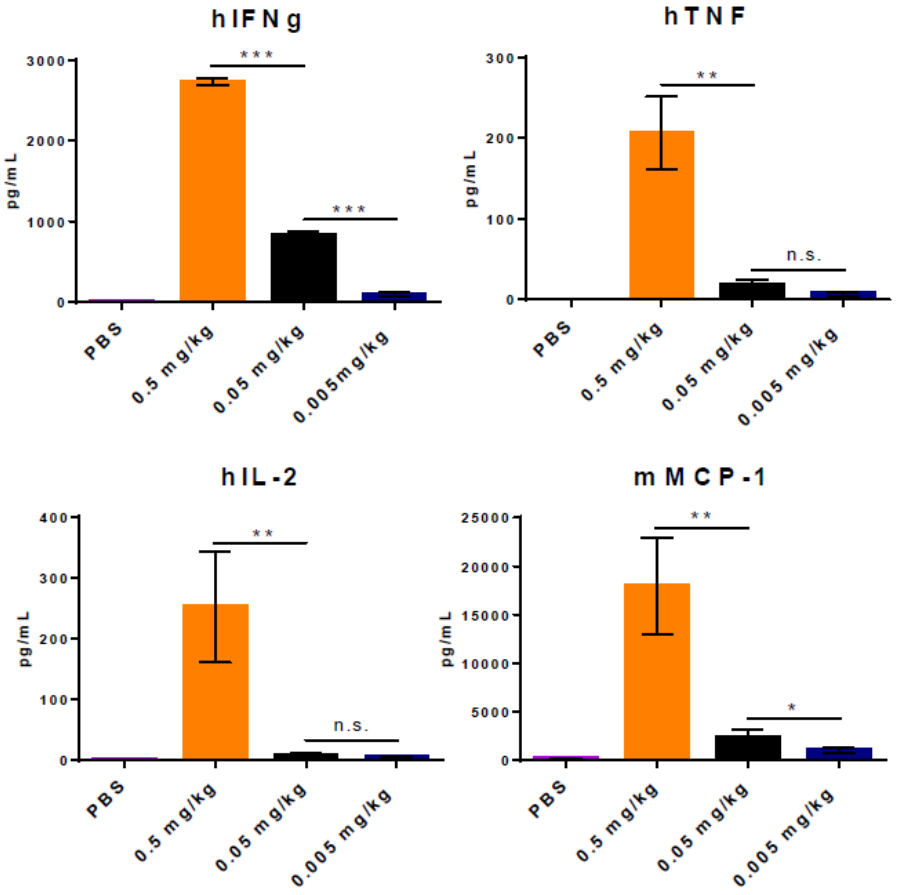
Effectiveness of ON-switch CAR cells:



- Antigen-specific and titratable killing of target cell population

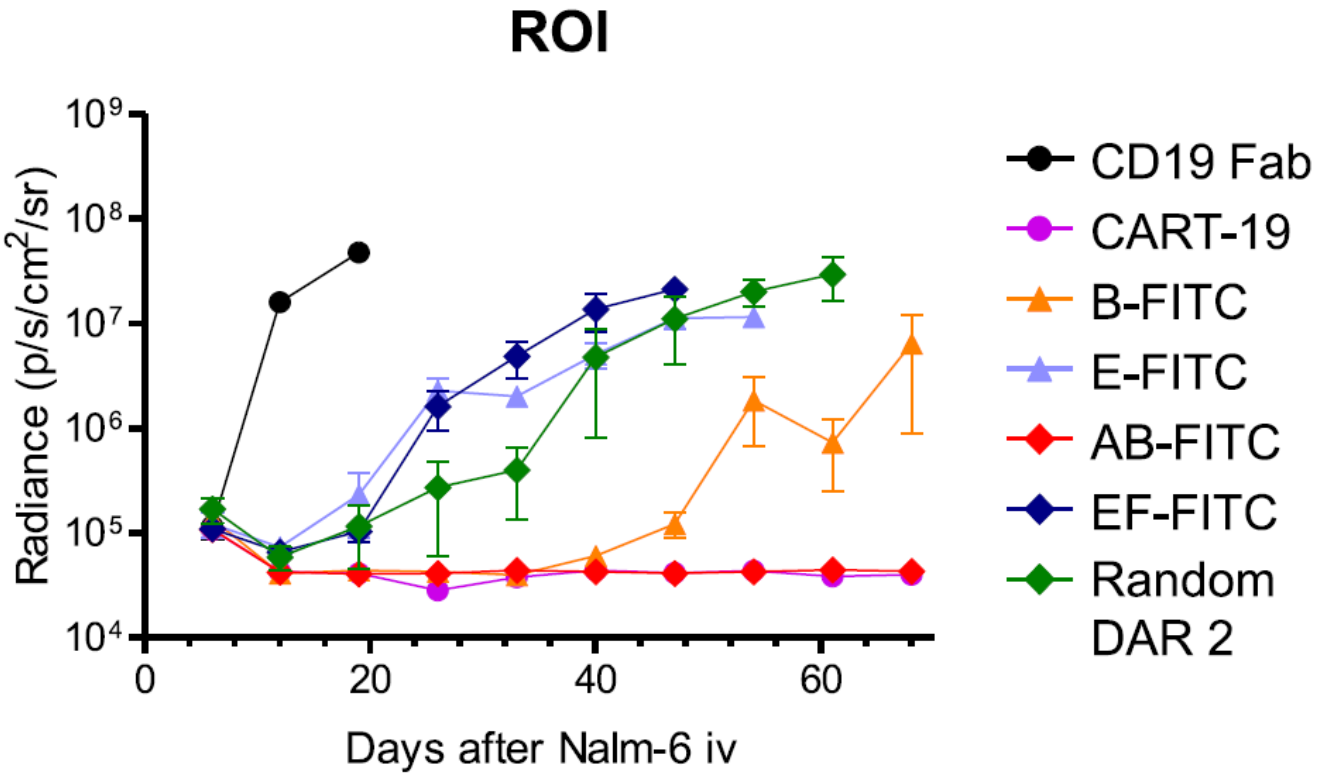
In vivo control of sCAR-T cell activity:





- specific T cell expansion upon treatment with sCAR-T cell and anti-CD19 AB switch
- dose dependent of cytokines

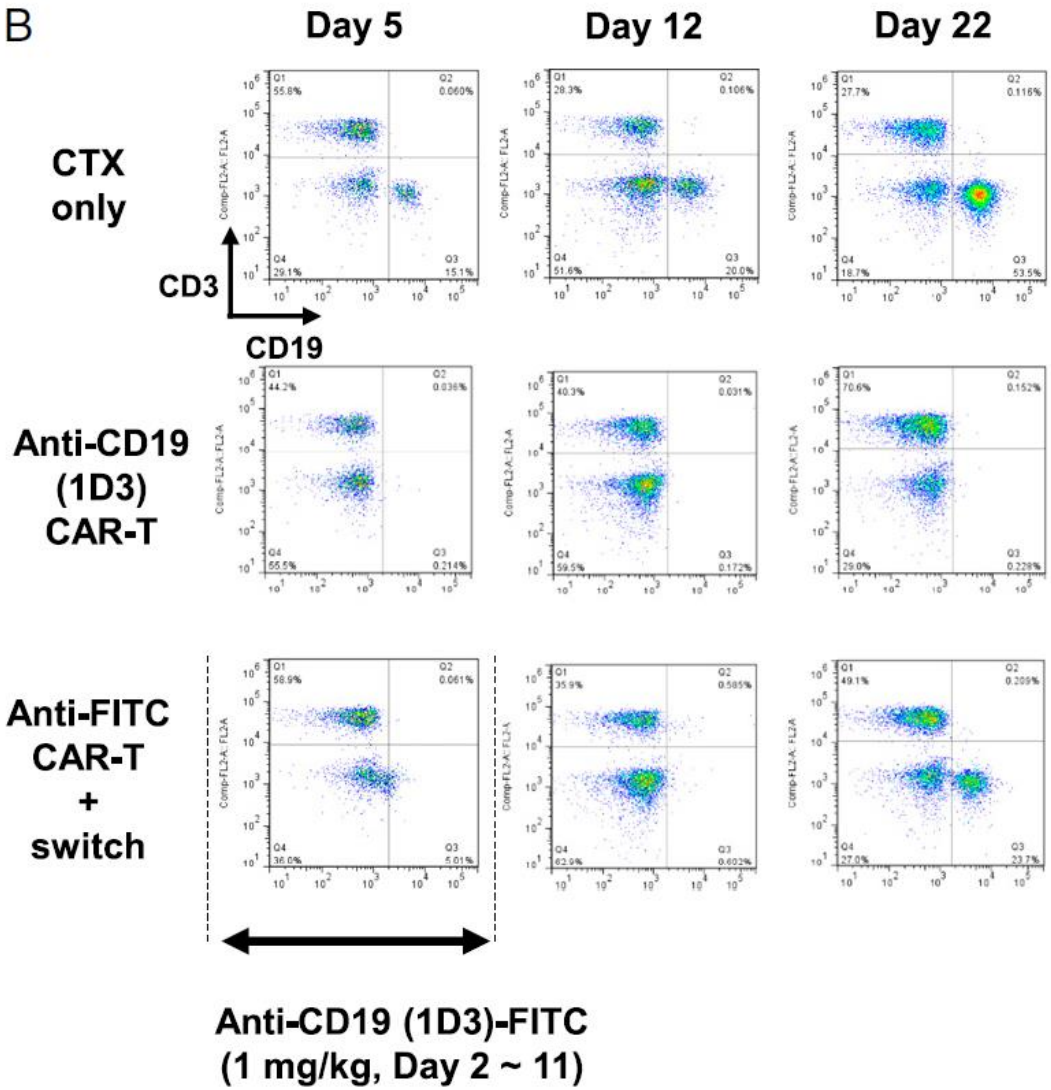
Effectiveness of sCAR-T cells with optimized anti-CD19 AB-FITC switch *in vivo:*



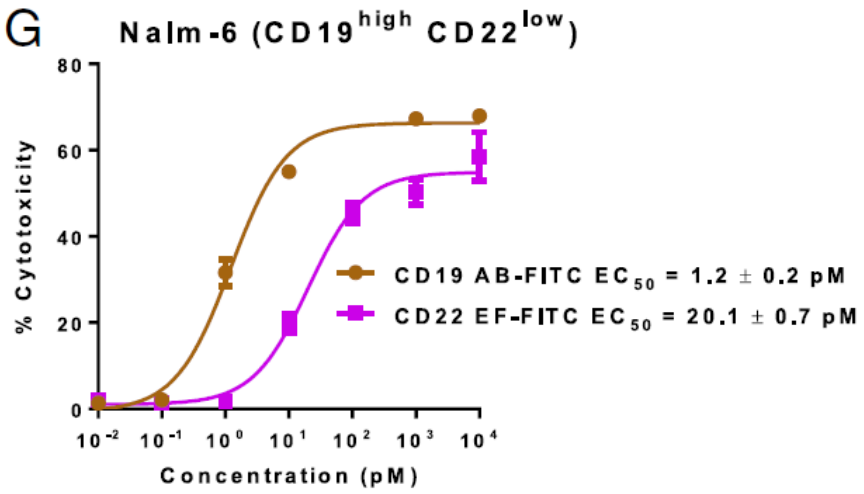
- Anti-FITC CAR-T cells in conjugation with optimized anti-CD19 AB switch are comparable to conventional anti-CD19 CAR-T cells

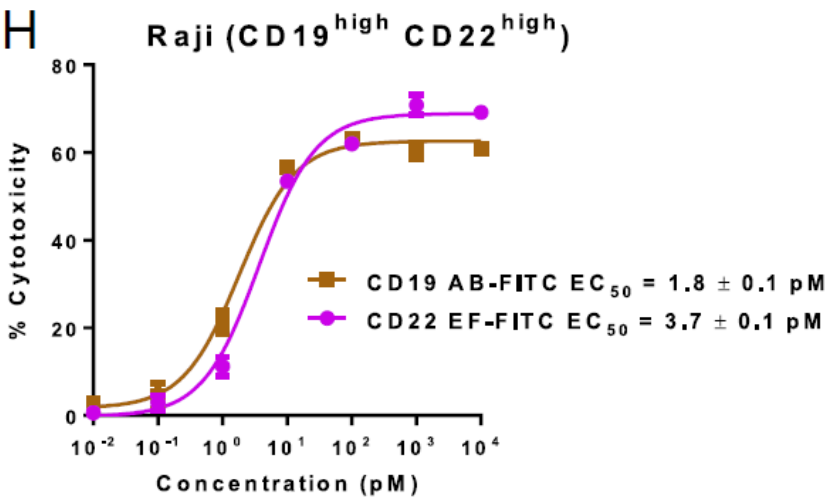
***In vivo* termination of persistent B cell ablation by stopping of switch dosing:**

- mouse sCAR-T cell generated using splenocytes from C57BL/6 mice

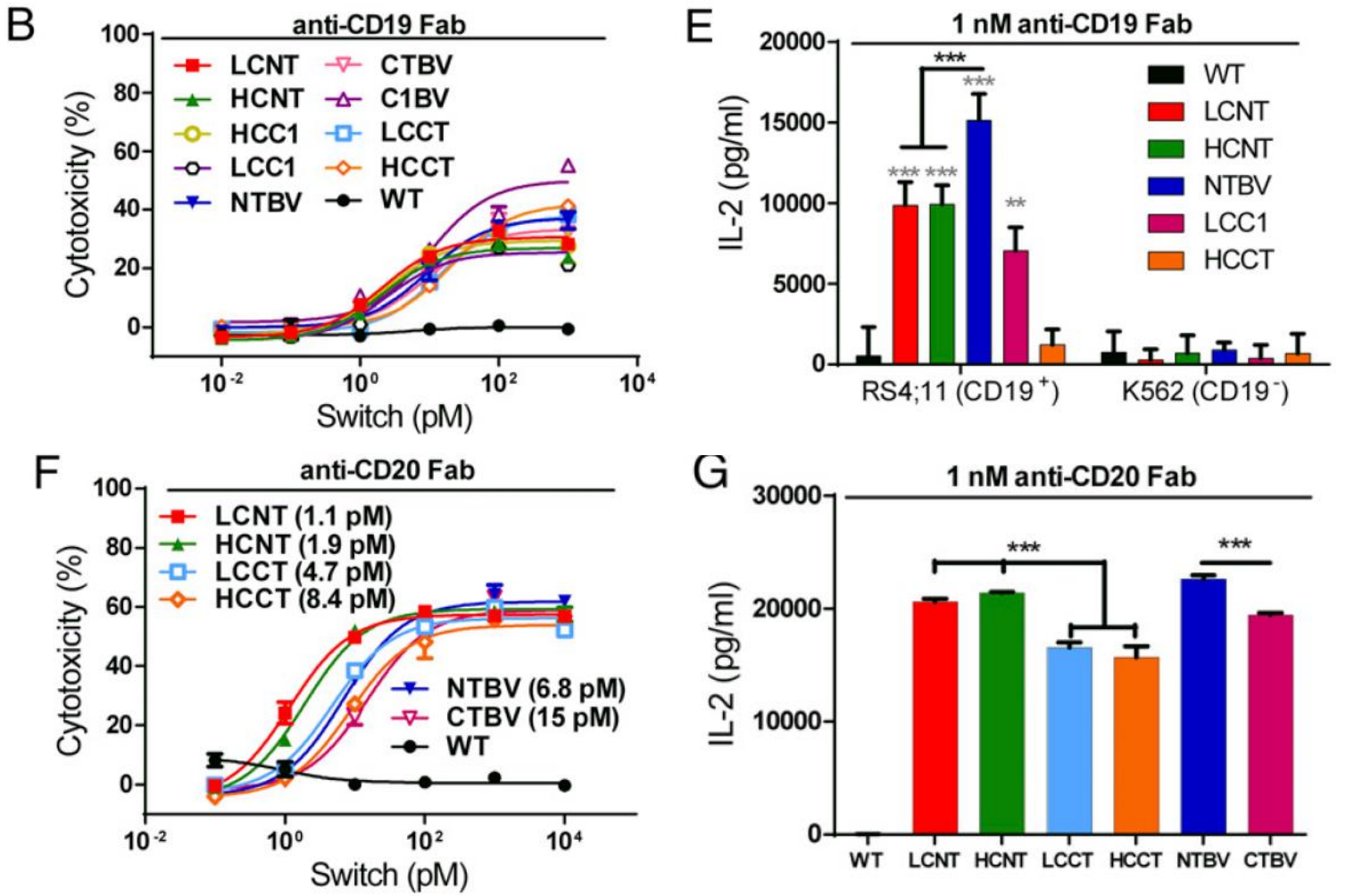


Targeting of other tumor antigens (CD22) using the same anti-FITC CAR T cell:



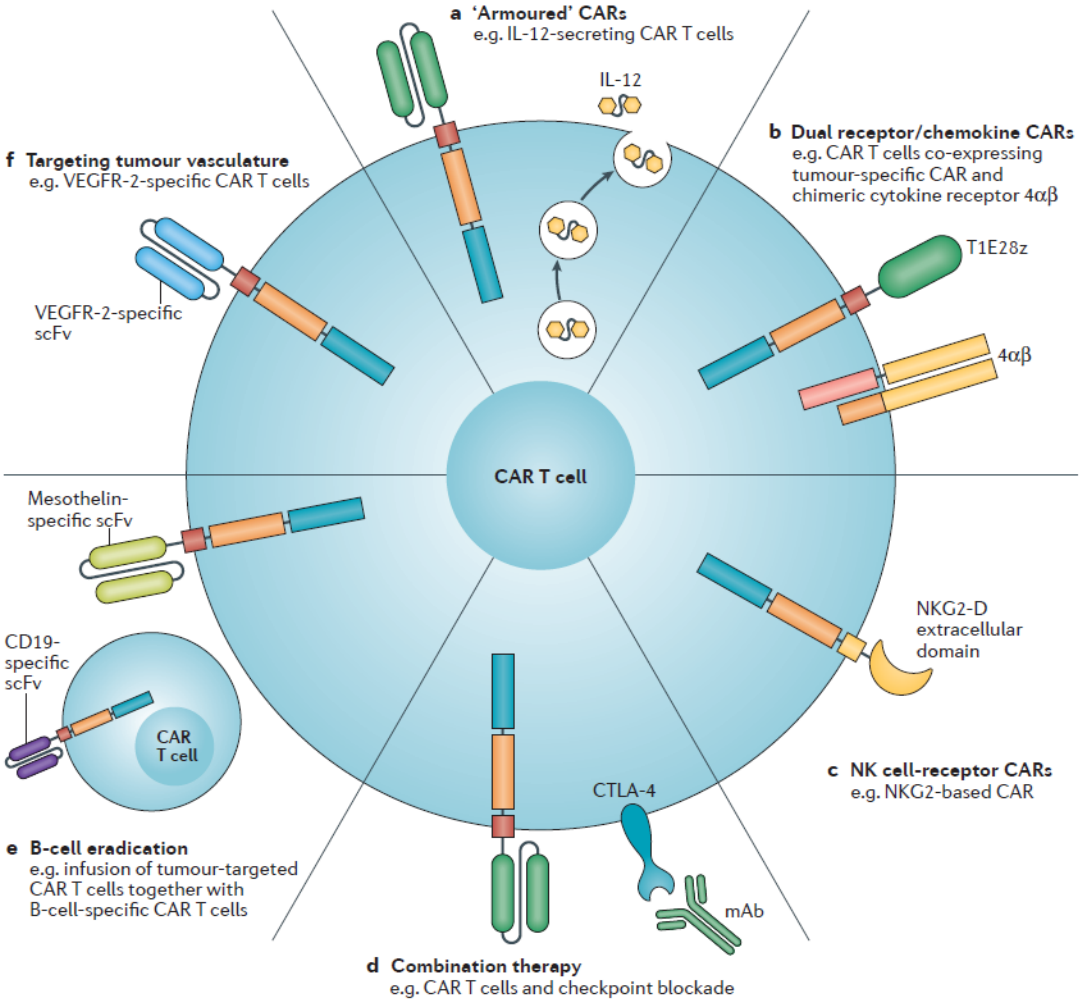


Effect of PNE conjugation on sCAR-T cells:



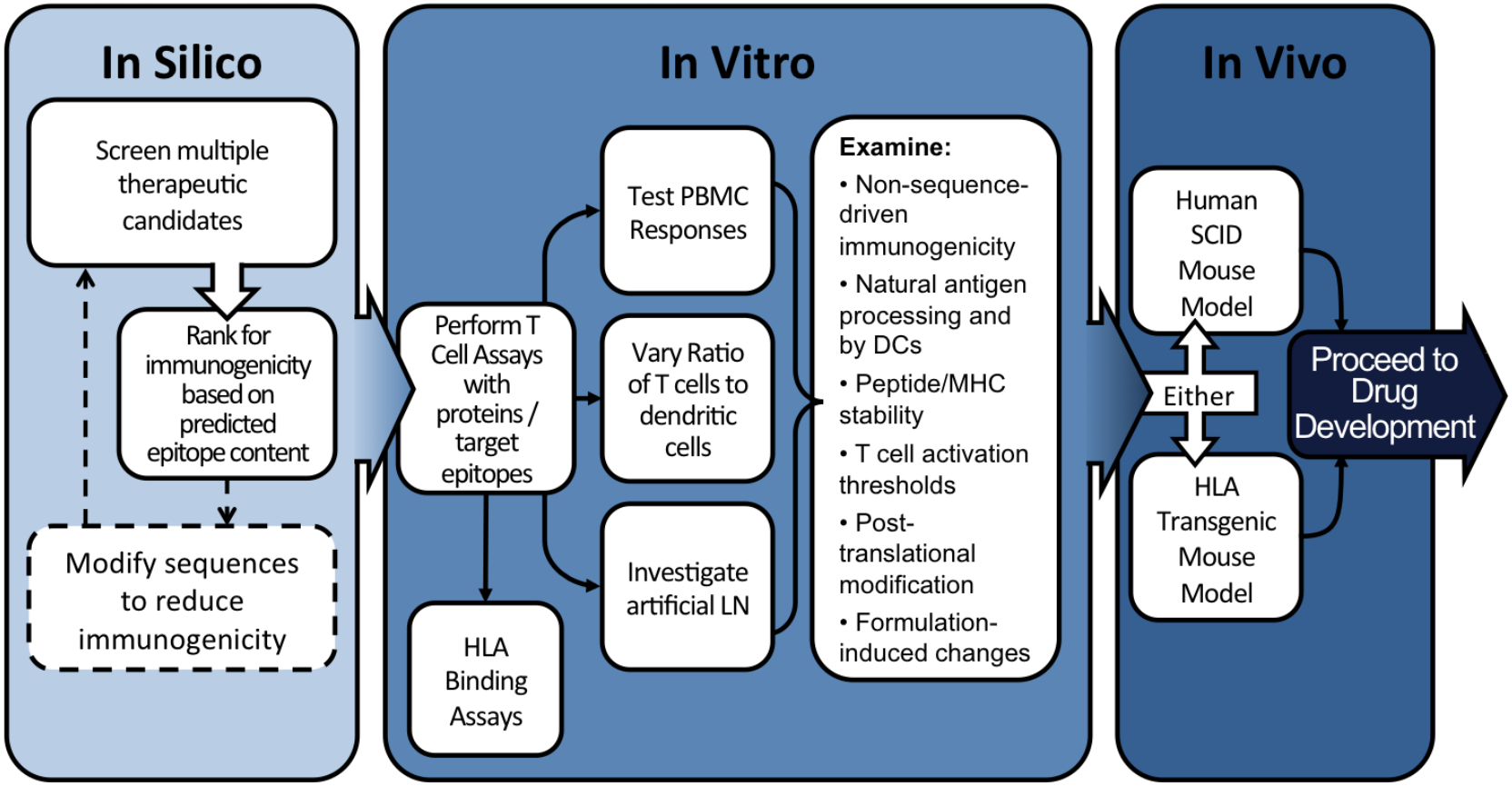
- Anti-CD19 – and anti-CD20 switches with PNE conjugations proximal (NT) to antigen binding region are more potent → decreased distance EC and TC?

Making the switch to a safer CAR-T cell therapy



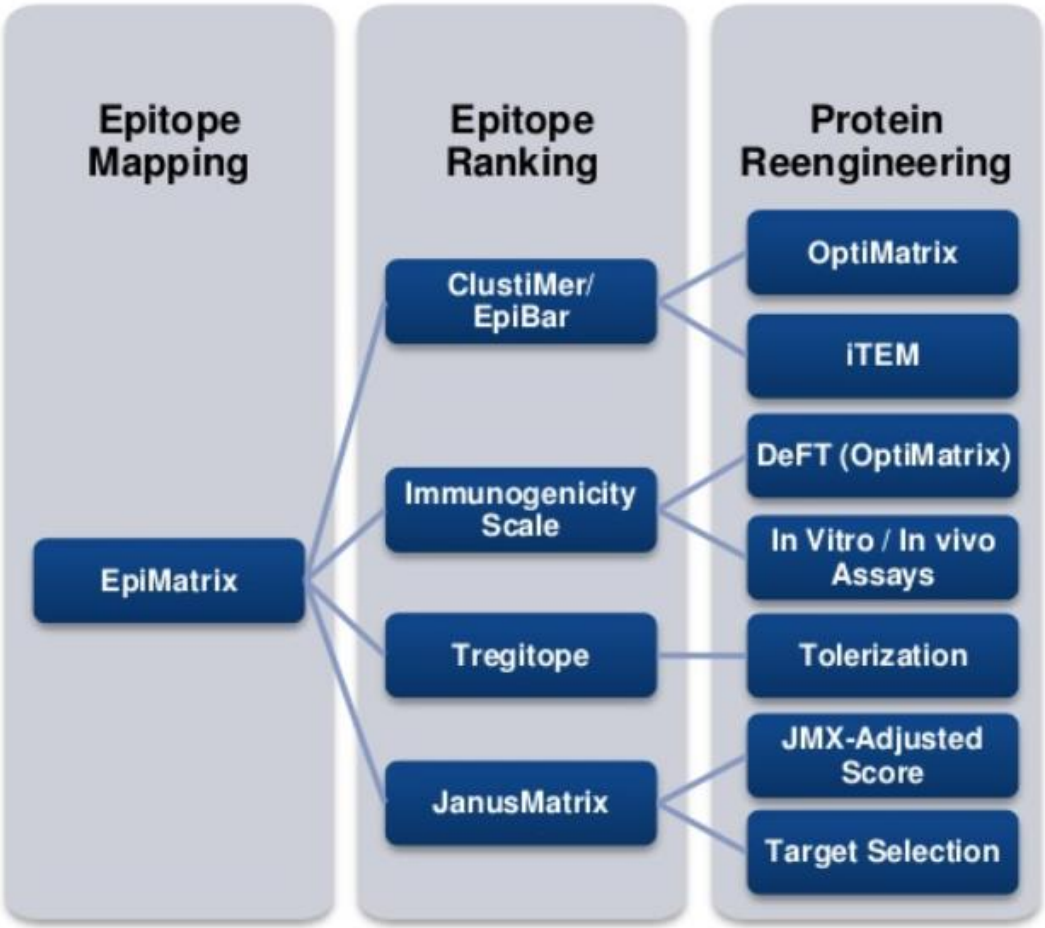
Jackson et al. 2016, Nature Reviews — Clinical Oncology

In silico immunogenicity assay of PNE



In silico immunogenicity assay of PNE

ISPRI is an ***integrated, interactive set of tools*** specifically designed for immunogenicity analysis. ISPRI provides the **depth of analysis** necessary to accurately predict clinical immunogenicity.



Making the switch to a safer CAR-T cell therapy

In silico immunogenicity assay of PNE