Engineering Function and Safety into CAR T cells for Cancer Immunotherapy

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20 ans CANCÉROPÔLE Est

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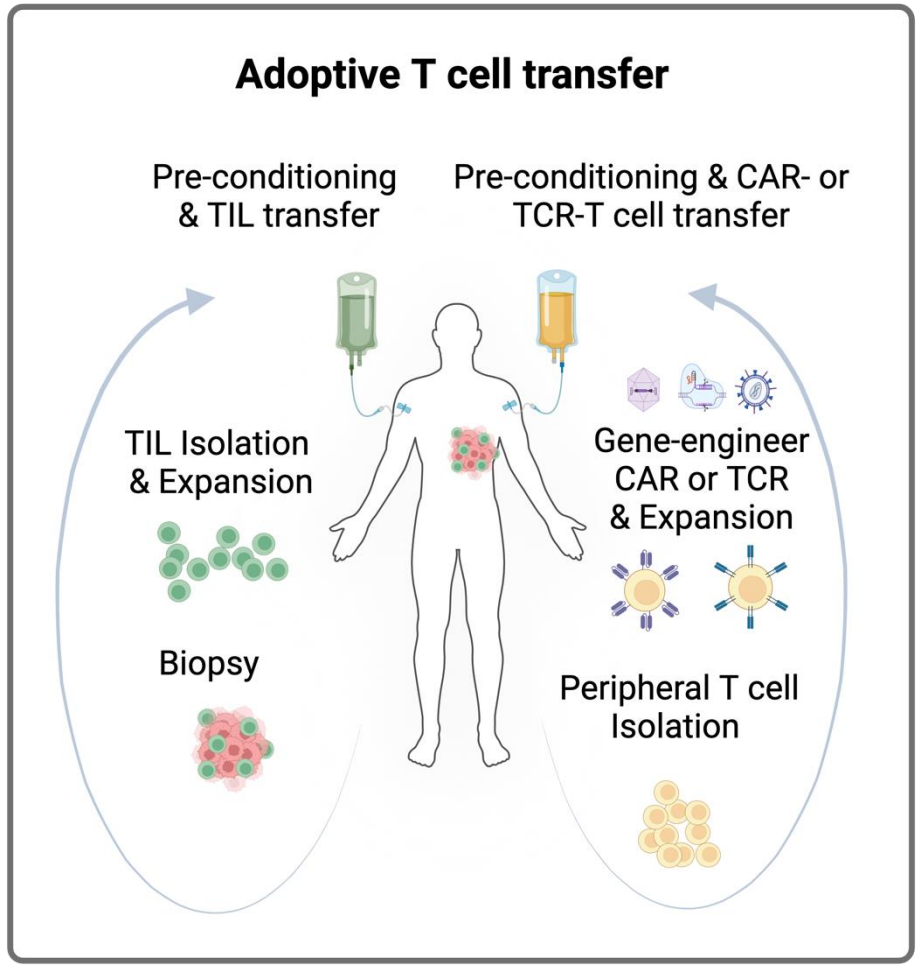
LUDWIG CANCER RESEARCH

Adoptive T cell therapy (ACT) of cancer

7 FDA approved CAR T cell products since 2017

CR of up to 86% for CD19 CAR therapy of B-ALL

TIL Therapy FDA approval against melanoma in Feb 2024 (first developed in the 1980s!)



Emily Whitehead and Dr. Stephan Grupp celebrate Emily's 10-year anniversary of being cancer-free after CAR T-cell therapy. Credit: Children's Hospital of Philadelphia.

Emily Whitehead: first pediatric patient treated with CAR T-cell therapy in 2012

FDA approval of TCR-T for treating sarcoma in June 2024

August 1, 2024

Adaptimmune

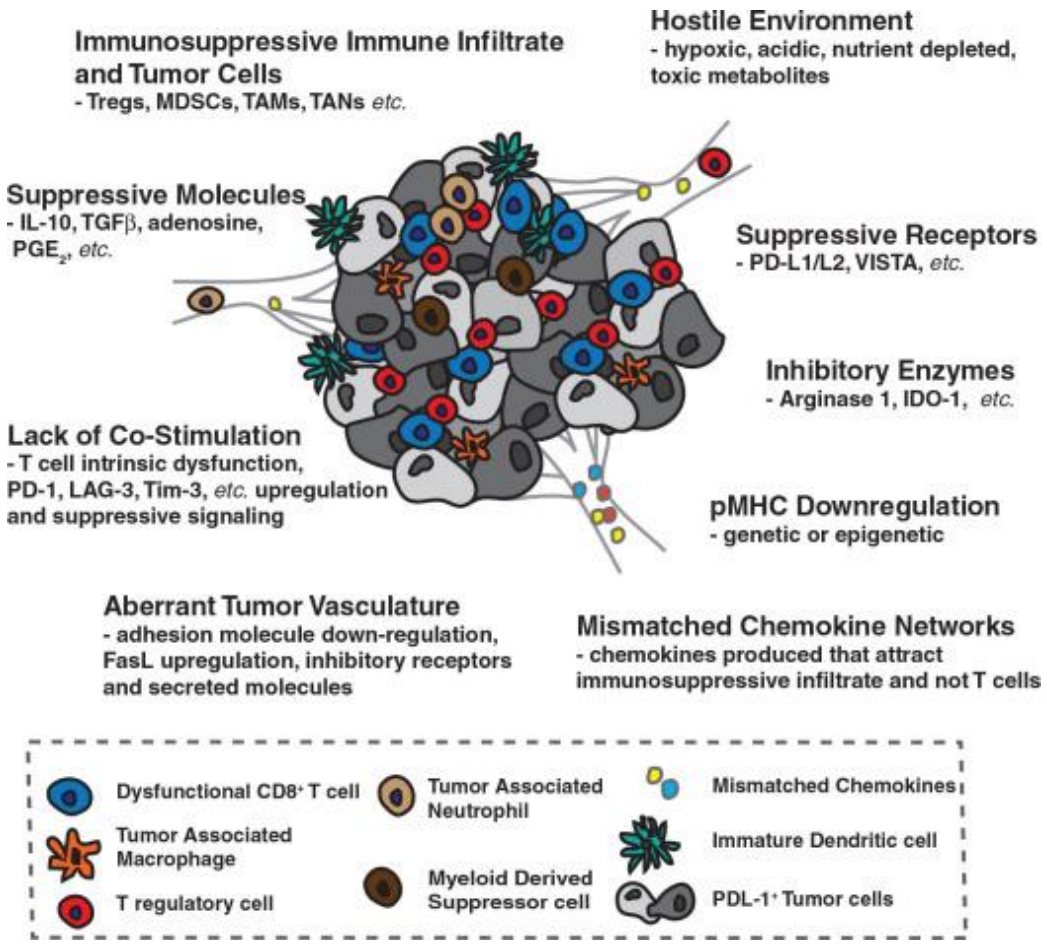
Adaptimmune Receives U.S. FDA Accelerated Approval of TECELRA® (afamitresgene autoleucel), the First Approved Engineered Cell Therapy for a Solid Tumor

Approved for advanced MAGE-A4+synovial sarcoma in adults with certain HLA types who have received prior chemotherapy

TECELRA is the first new treatment option for people with synovial sarcoma in more than a decade

Adaptimmune to hold webcast at https://www.gowebcasting.com/13428 on August 2, at 8:00 a.m. EDT

Challenges to adoptive T cell therapy of solid tumors



- Homing & infiltration/penetration
- Antigen escape
- Chronic antigen exposure & insufficient costimulation (exhaustion)
- Inhibitory receptors & molecules (suppressive infiltrate & tumor cells)
- Limited nutrients & oxygen, low pH, toxic metabolites
- Toxicity (CRS, on-site/off-tumor reactivity etc.)

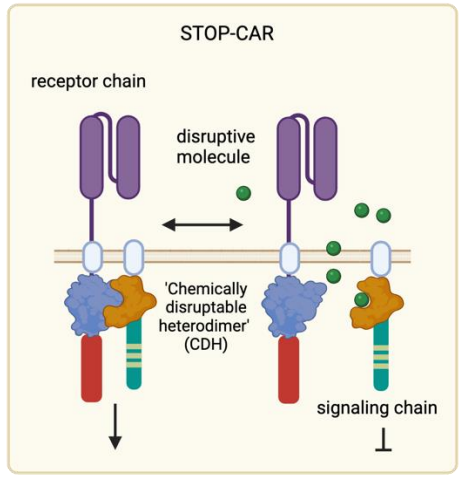
From Lanitis, Dangaj, Irving & Coukos. Mechanisms regulating T-cell infiltration and activity in solid tumors. Annals of Oncology 2017

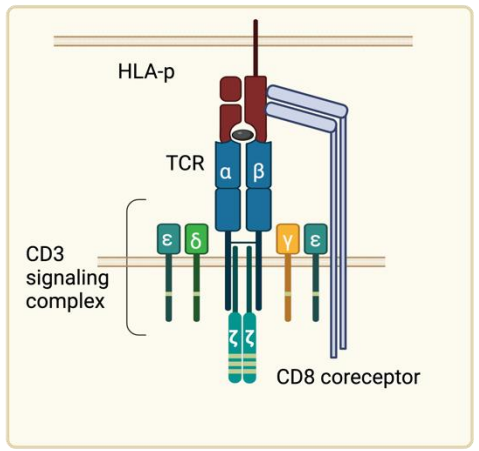
Advantages of engineering T cells for cancer immunotherapy

- T cells can directly kill tumor cells and harness endogenous immune infiltrate (e.g., via IFNγ secretion)
- can be efficiently engineered by both viral and non-viral means to overexpress or knockout a gene(s)
- can be constitutively or inducibly enforced to express multiple genes
- migrate along the vasculature to circulate throughout the body and penetrate deep into tissues
- expand in the patient (best if transferred in a less differentiated state like T_{CM})
- are a ‘living drug’ that can generate memory and thus foster long-term immunity in patients

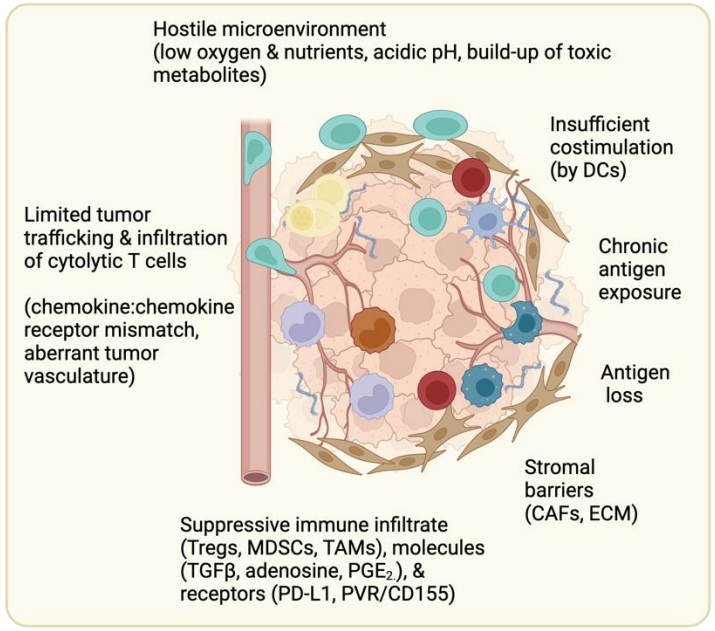
Strategies we are taking for improving T-cell therapies

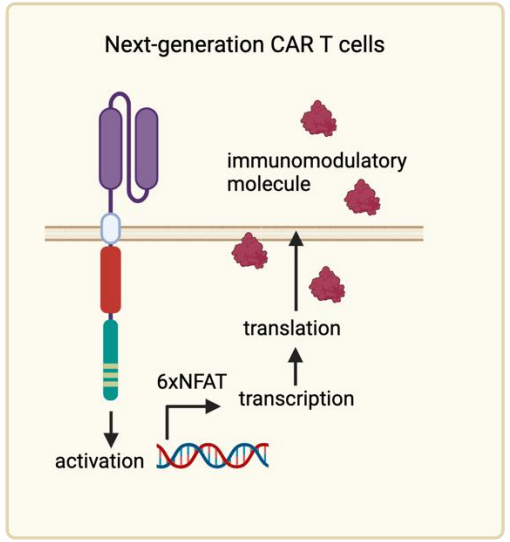
Receptor design



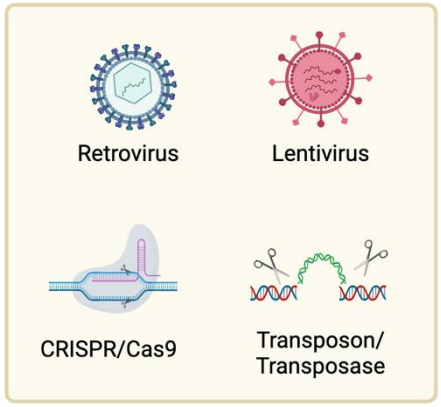


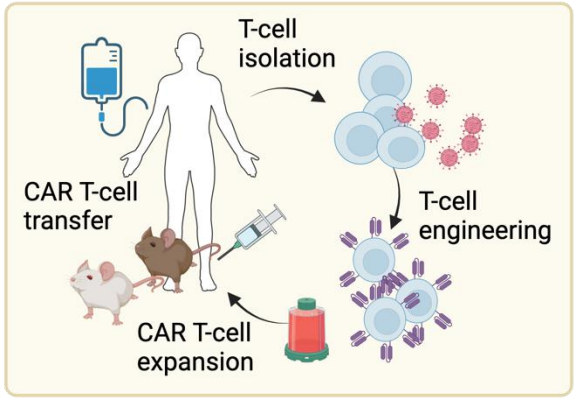
T cell coengineering and combinatorial treatments



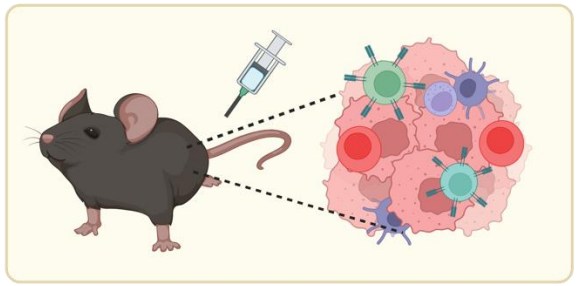


Gene-engineering tools



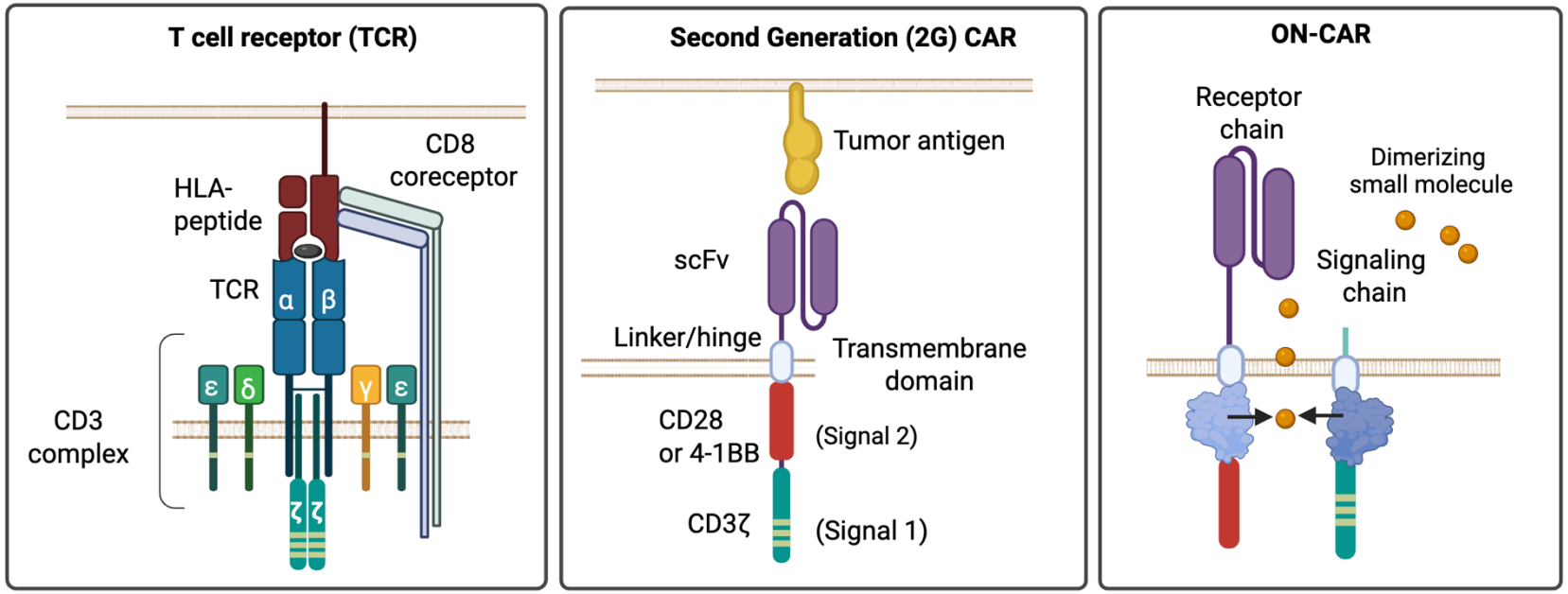


Pre-clinical testing in syngeneic tumor models



IMPROVING CHIMERIC ANTIGEN RECEPTOR (CAR) DESIGN

2G versus remote-control CAR designs



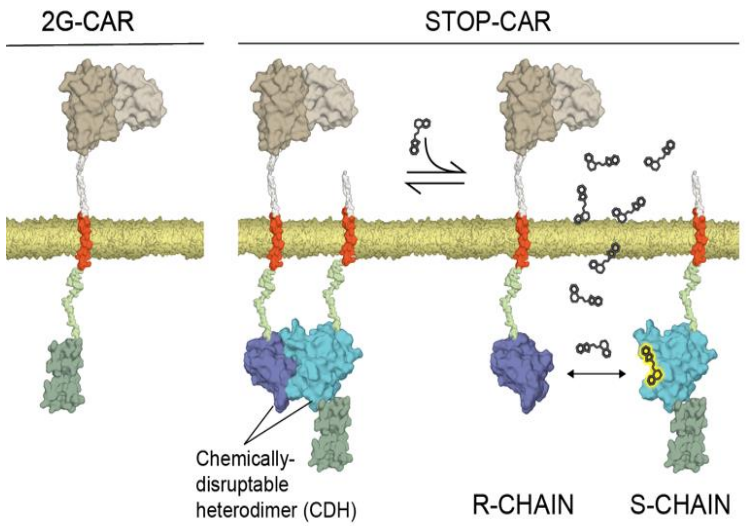
- classic 2G CARs link tumor antigen binding to T-cell activation in a single receptor
- ‘remote control’ CAR designs dissociate tumor antigen binding (scFv) & T-cell activation on 2 separate chains and a small molecule is required to switch on or off remote-control CARs
- remote control CAR designs can help mitigate toxicity and T-cell exhaustion by transient resting

STOP-CAR Design

LETTERS
https://doi.org/10.1038/s41587-019-0403-9 2020
nature biotechnology
There are amendments to this paper

A computationally designed chimeric antigen receptor provides a small-molecule safety switch for T-cell therapy

Greta Giordano-Attianese[1,2,6], Pablo Gainza[3,4,6], Elise Gray-Gaillard[1,2,6], Elisabetta Cribioli[1,2], Sailan Shui[3,4], Seonghoon Kim[5], Mi-Jeong Kwak[5], Sabrina Vollers[3,4], Angel De Jesus Corria Osorio[1,2], Patrick Reichenbach[1,2], Jaume Bonet[3,4], Byung-Ha Oh[5], Melita Irving[1,2,7]*, George Coukos[1,2,7]* and Bruno E. Correia[3,4,7]*

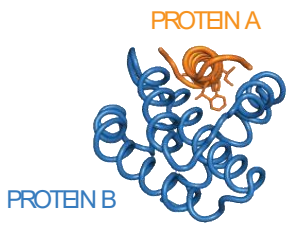


Prof. Bruno Correia

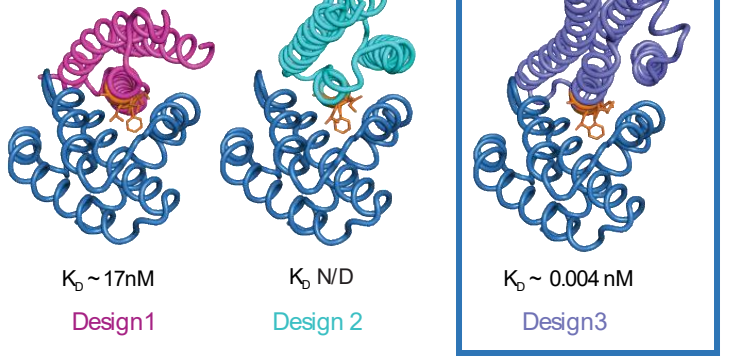
Dr. Pablo Gainza

Chemically disruptable heterodimer (CDH)

- proteins of human origin with minimal deviation from wild-type sequence
- proteins that are well-packed
- proteins that will not interfere with synapse proximal T-cell signaling
- commercial availability of drugs that have a long half-life and are well-tolerated
- design: Bcl-xL and human scaffold protein engrafted with critical binding residues of the BH3 domain of BIM

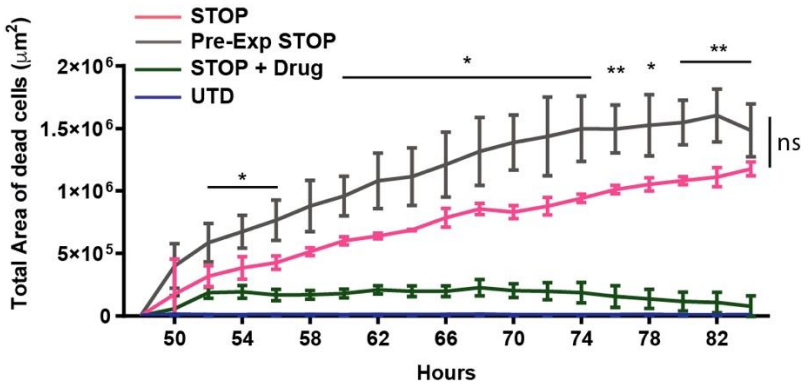


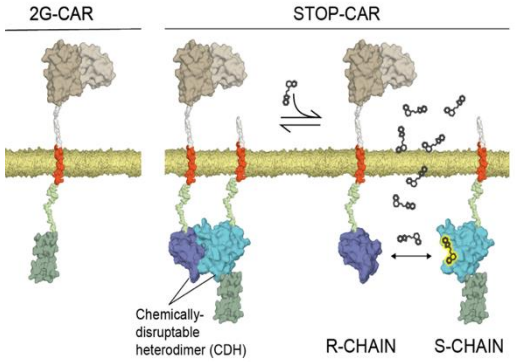
Apolipoprotein E4 scaffold identifed by Motifgraft



STOP-CAR T cells

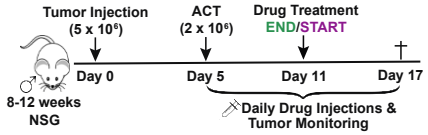
STOP-CAR T cells regain function upon small molecule withdrawal

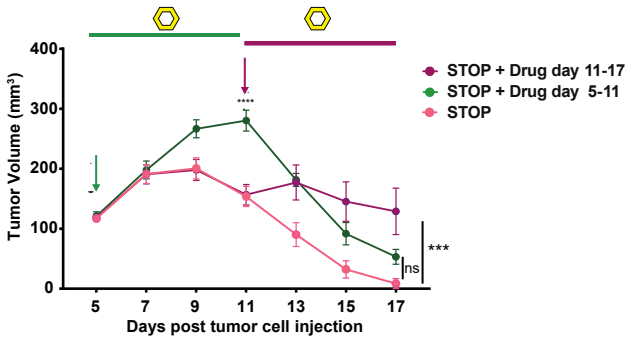




Greta Giordano Attianese

Small molecule co-administration reversibly disrupts tumor control by STOP-CAR but not 2G CAR T cells





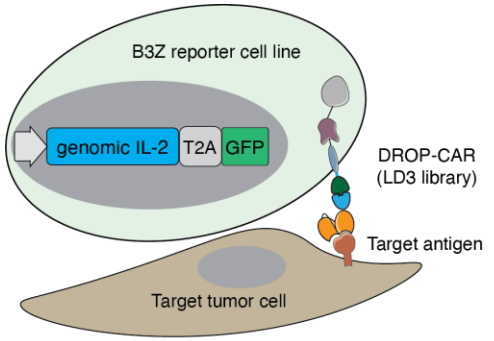
- Disruptive small molecule is not clinically approved (A1155463)
- CDH is of very high affinity – can this interface be optimized for stable CAR assembly & efficient disruption?
- Can the CDH be moved to the extracellular region for disruption with lower concentrations of drug?
- CAR T cell and tumor target cell contact is not broken – would it be better to disrupt it?

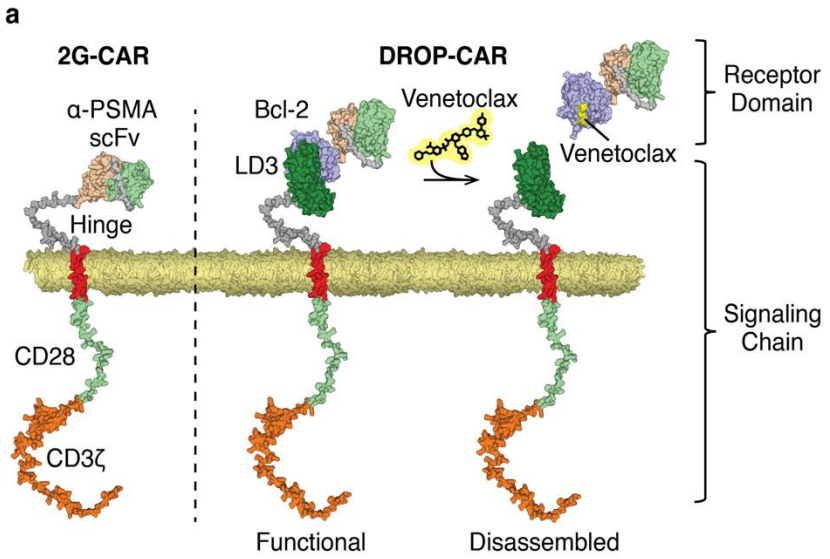
Drug-Regulated Off-switch PPI (DROP)-CARs

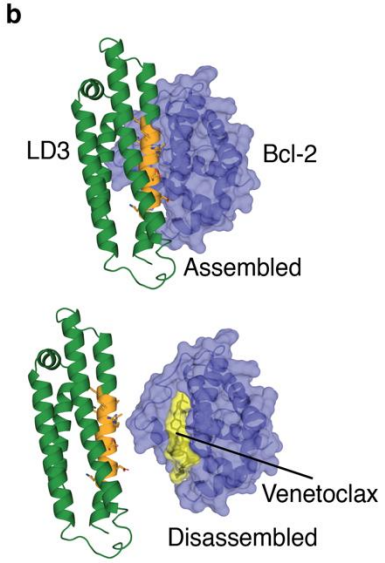
Leo Scheller
(Correia lab, EPFL)

Greta Giordano Attianese

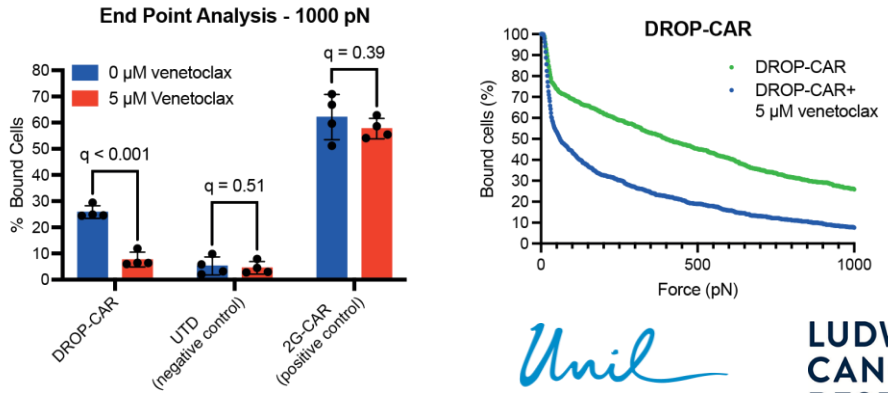
Library screening to develop a stable CDH interface readily disrupted by venetoclax (in collaboration with Sai Reddy lab, ETH)







Avidity measurements (LUMICKS) demonstrate that venetoclax breaks cell-cell contacts (in collaboration with Markus Barden & Hinrich Abken)



Scheller, Giordano Attianese….Correia & Irving (accepted, Nat Chem Biol) doi.org/10.1101/2024.08.06.606454

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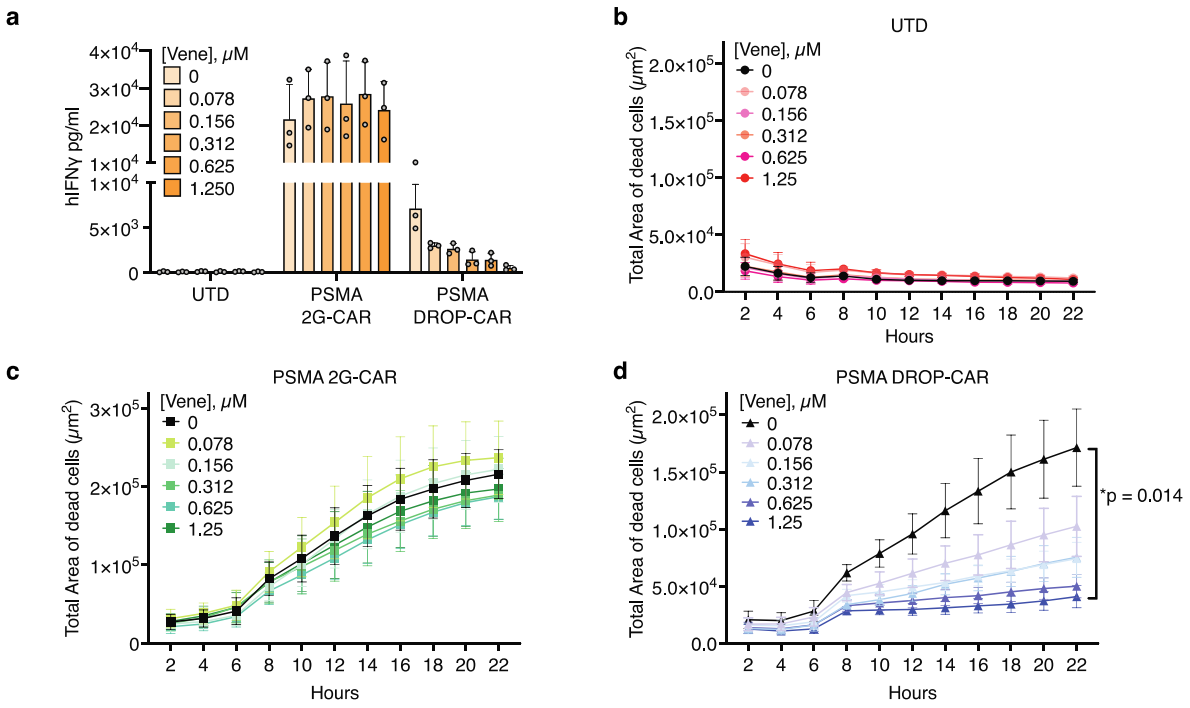
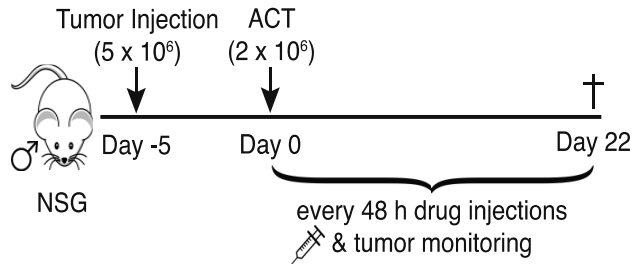
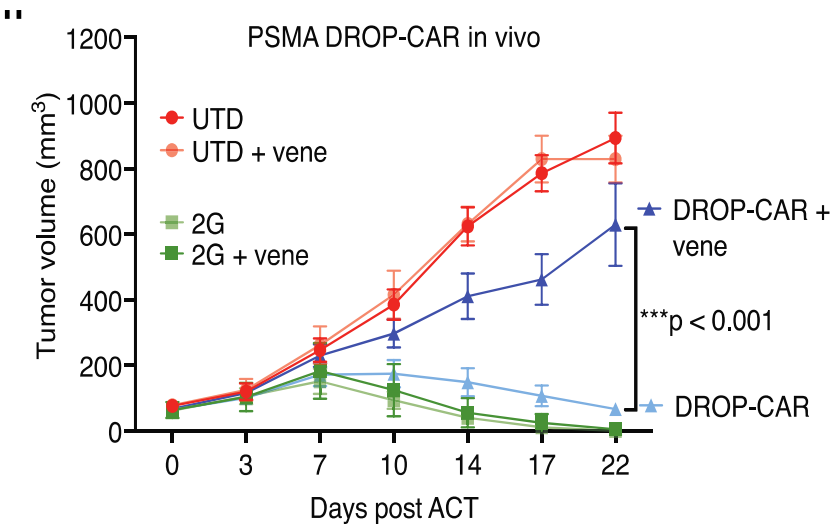
DROP-CAR T cells

Drop-CAR T cells can be efficiently shut-off with venetoclax both in vitro and in vivo

Greta Giordano Attianese







Inducible ON-CAR development: iON-CAR

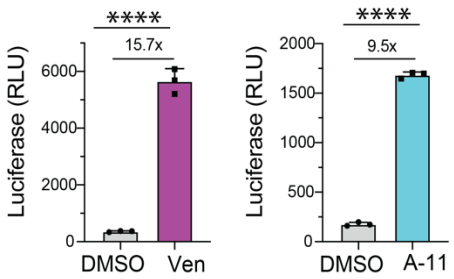
Swiss National Science Foundation

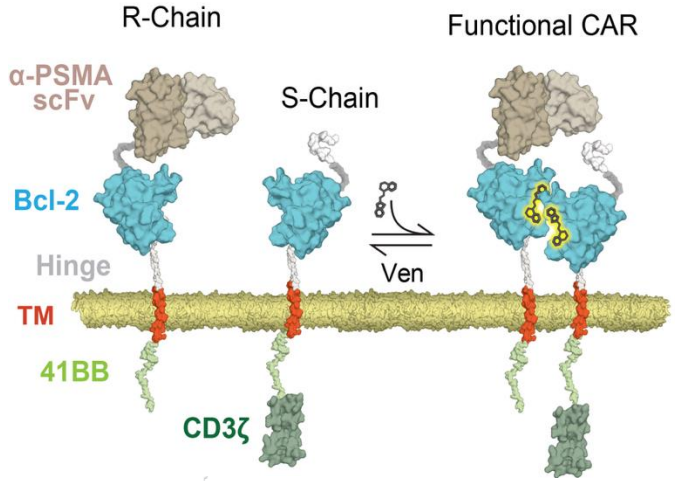
PNAS RESEARCH ARTICLE | APPLIED BIOLOGICAL SCIENCES 2024 OPEN ACCESS

Dual ON/OFF-switch chimeric antigen receptor controlled by two clinically approved drugs

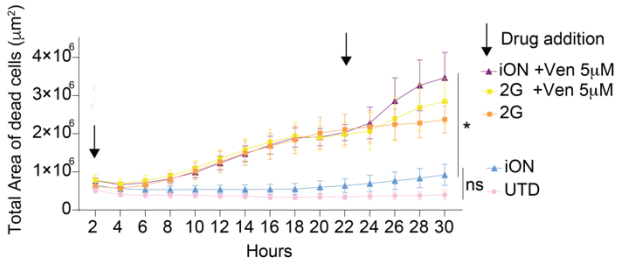
Greta Maria Paola Giordano Attianese[a], Sailan Shui[b,c], Elisabetta Cribioli[a], Melanie Triboulet[a], Leo Scheller[b,c], Morteza Hafezi[a], Patrick Reichenbach[a], Pablo Gainza[a,c], Sandrine Georgeon[b,c], Bruno E. Correia[b,c,1], and Melita Irving[a,1]

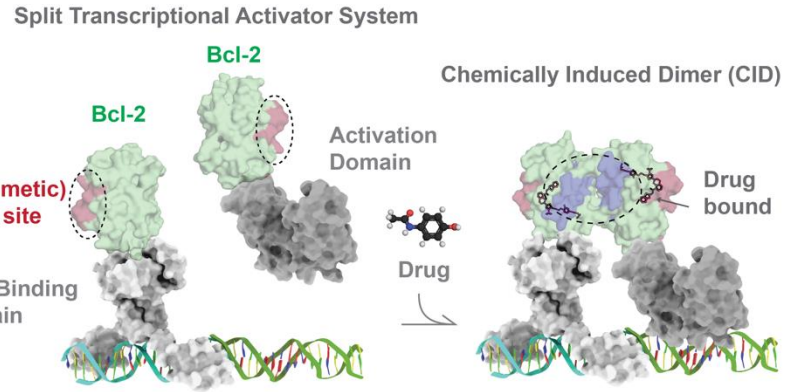
Sailan Shu
(Correia lab, EPFL)

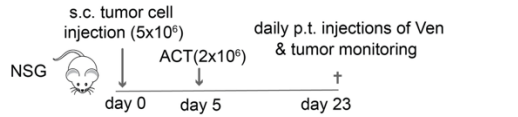


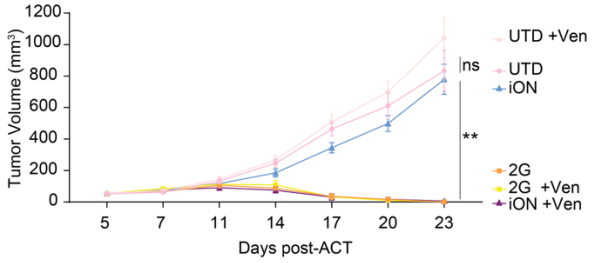


Greta Giordano Attianese



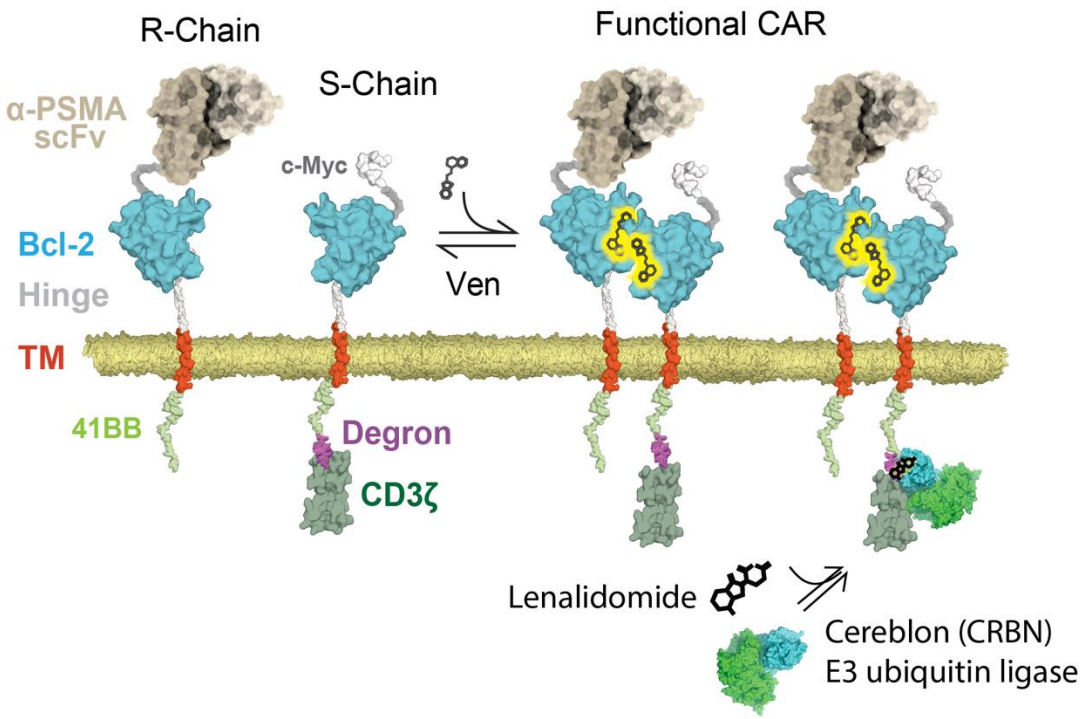


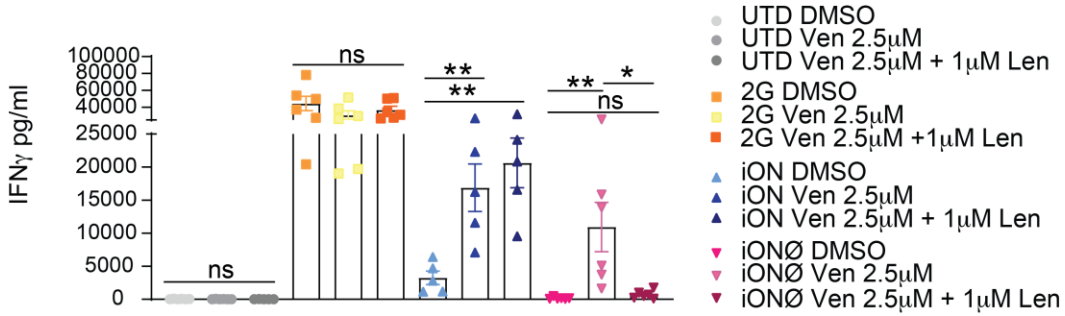




- ON-CAR T cells are only responsive to target tumor cells in the presence of venetoclax
- ON-CAR T cells lose reactivity against target cells 48 h post venetoclax withdrawal

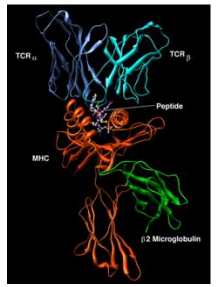
All-in-one inducible ON-OFF CAR design: iONθ-CAR





- A degron was introduced into the S-chain (inspired by by Jan et al 2021, STM & Carbonneau et al 2021, JChemBiol
- iONθ -CAR T cells are only responsive against target tumor cells in the presence of venetoclax
- iONθ -CAR T cells can be shut off within 4-6 hours of lenalidomide administration

TCR affinity optimization and enforced chain pairing for augmetning T-cell function



By computational, structure-based design we developed a panel of increasing affinity TCRs

	CDR2α				CDR2β					CDR3β						
	51	52	53	54	49	50	51	52	53	96	97	98	99	K_D (μM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	$t_{1/2}$
V49I					I									nd	nd	nd	nd
WT	Q	S	S	Q	V	G	A	G	I	G	A	A	G	21.4	1.1×10^4	0.23	3
β-G50A						A								4.62	1.49×10^4	0.069	10
β-A97L											L			2.69	2.26×10^4	0.061	11.4
β-G50A+A51E (DM-β)						A	E							1.91	2.35×10^4	0.045	15.4
β-G50A+A51E+A97L (TM-β)						A	E				L			0.91	1.43×10^4	0.013	53.3
α-S53W + B-G50A+A51E (TM-α)		W				A	E							0.4	12.1×10^4	0.048	14.4
α-S53W + β-G50A+A51E+A97L (QM-α)		W				A	E				L			0.14	10.9×10^4	0.015	46.2
β-G50A+A51I+G52Q+I53T (wtc51m)						A	I	Q	T					0.015	8.5×10^4	0.0013	533.2

Affinity: subphysiologic; natural; upper natural limit; borderline supraphysiologic; low supraphysiologic; extreme supraphysiologic

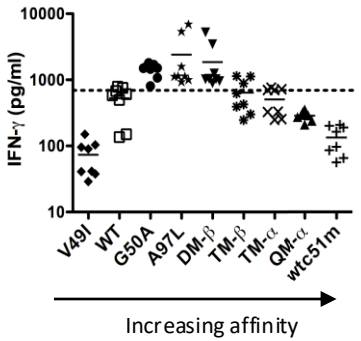
Prof. Olivier Michielin

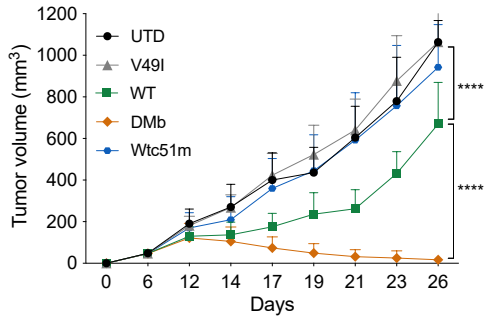
Prof. Vincent Zoete

Adapted from Schmid, Irving et al, JI 2010 and Irving et al, JBC 2012

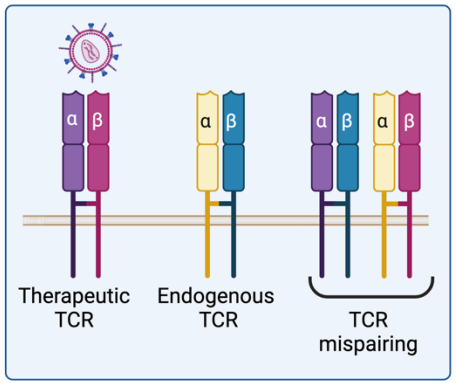
TCRs in the upper range of natural affinity (5-1 μM) augmented the function of T cells, both in vitro and in vivo

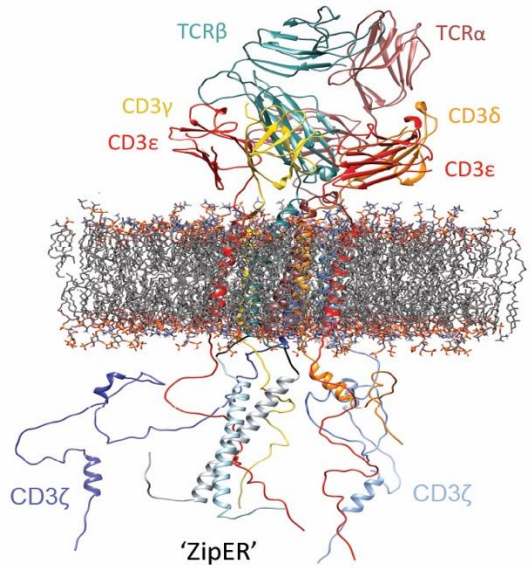
Beyond the 'affinity' threshold T-cell function was abrogated (impaired serially triggering)

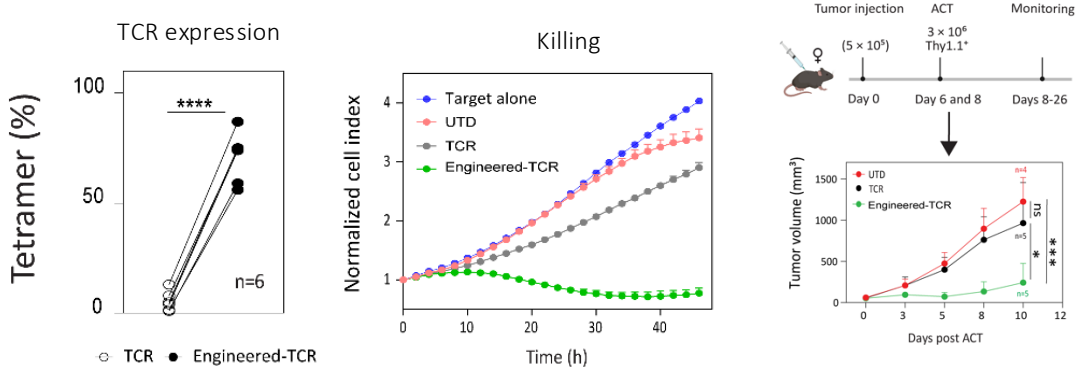




Morteza Hafezi



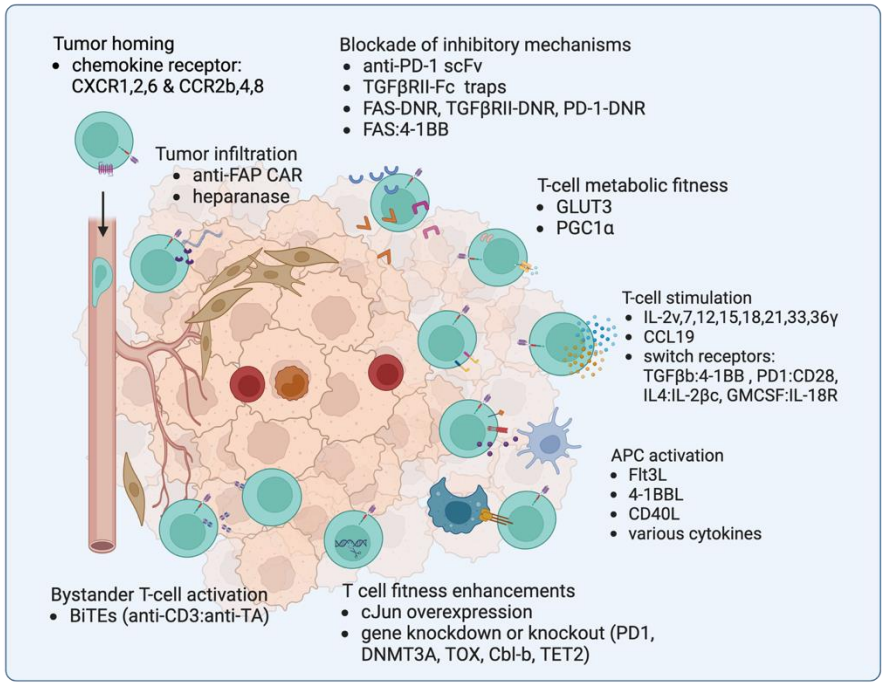




Hafezi et al, manuscript in revisions, Nature Biotechnology

T CELL COENGINEERING STRATEGIES

T-cell coengineering solutions to counteract the suppressive TME



From Giordano Attianese, Ash & Irving. Coengineering specificity, safety and function into T cells for cancer immunotherapy, Immunological Reviews 2023.

- support transferred T cells and/or promote endogenous immunity (*i.e.*, TME re-programming)
- gene-overexpression
- gene-knock-down
- inducible systems for enhanced safety

- **Inhibitory receptors & molecules**: coengineer T cells to secrete decoys or switch receptors, or immunomodulatory molecules to reprogram the TME (e.g. TGFβ decoy, switch receptor, dominant negative receptor or preprogramming M2 to M1 macrophages etc.)
- **Insufficient costimulation**: activate APCs: coengineer T cells to to express costimulatory ligands like CD40L, or to secrete various cytokines
- **T-cell exhaustion:** gene knockouts or transcription factor overexpression, CAR design that can allow transient resting (e.g. remote-control ON- or OFF-switches)
- **Poor metabolic fitness:** overexpression of transporters (i.e. for amino acids or glucose), or of PGC1α to stimulate mitochondrial biogenesis etc.
- **Toxicity:** ON- or OFF-switch CAR designs (transient rest can also abrogate exhaustion) and suicide switches
- **Homing & infiltration:** coengineer T cells with chemokine receptor and target the stroma (e.g. αFAP CAR)
- **Antigen escape:** coengineer T cells to secrete BiTEs to activate bystander T cells

Optimizing tools for pre-clinical testing in syngeneic tumor models

Evros Lanitis

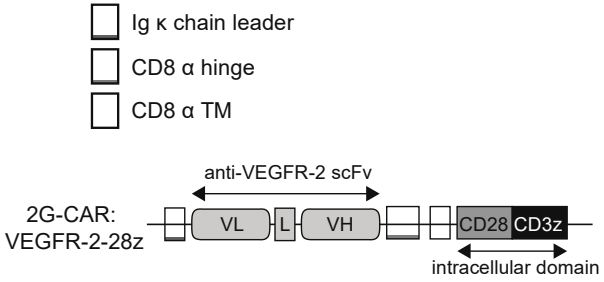
JEM Journal of Experimental Medicine

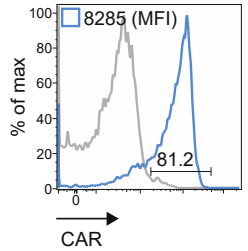
TECHNICAL ADVANCES AND RESOURCES 2021

Optimized gene engineering of murine CAR-T cells reveals the beneficial effects of IL-15 coexpression

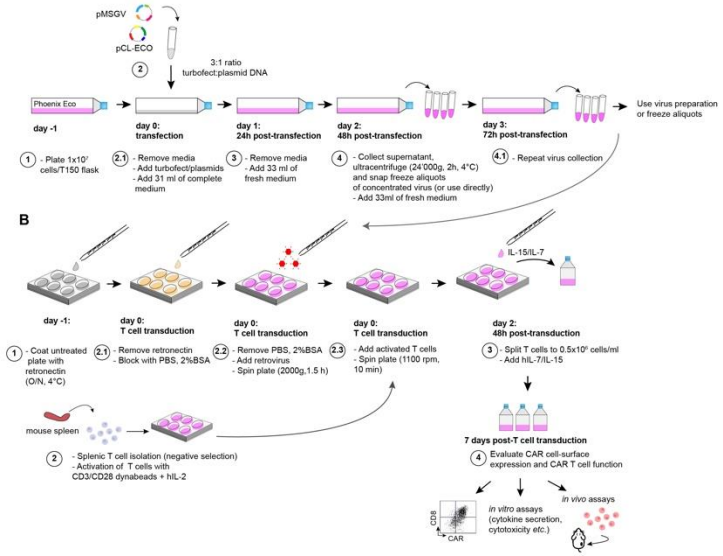
Evripidis Lanitis[1], Giorgia Rota[1], Paris Kosti[1], Catherine Ronet[1], Aodrenn Spill, Bili Seijo[1], Pedro Romero[2], Denarda Dangaj[1], George Coukos[1*], and Melita Irving[1*]

High 2G CAR transduction efficiency

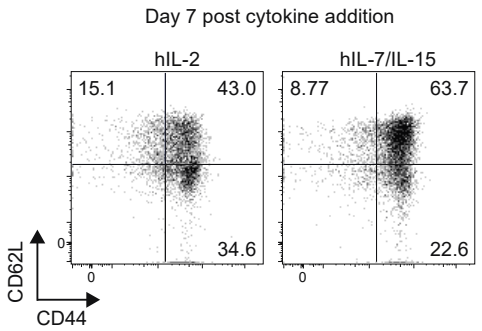




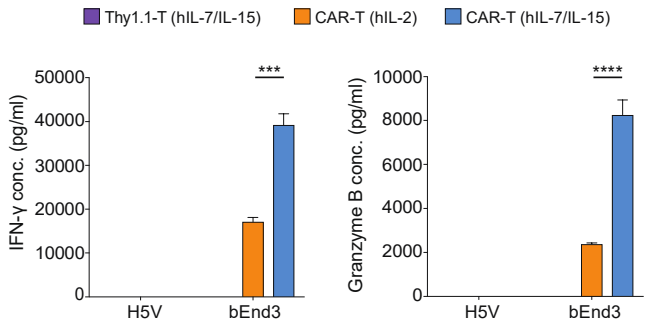
Optimized retrovirus production and transduction protocol



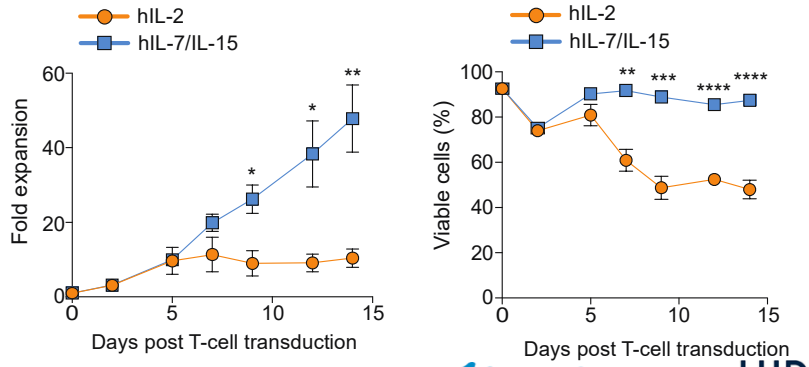
Higher proportion of central memory T cells (IL-7&15@10ng/mL vs IL-2@50IU/mL)



Higher cytokine production



Higher fold-expansion and viability



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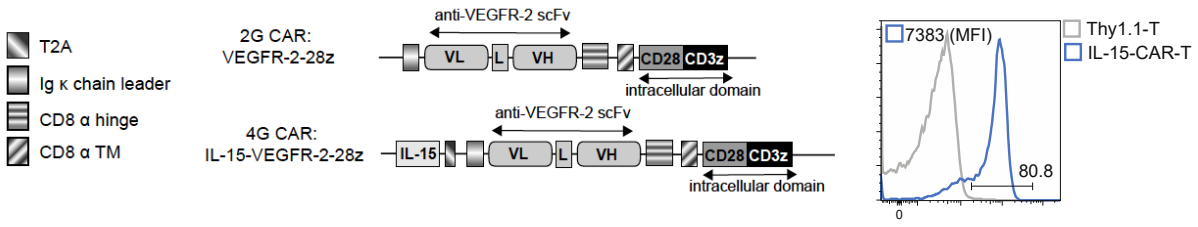
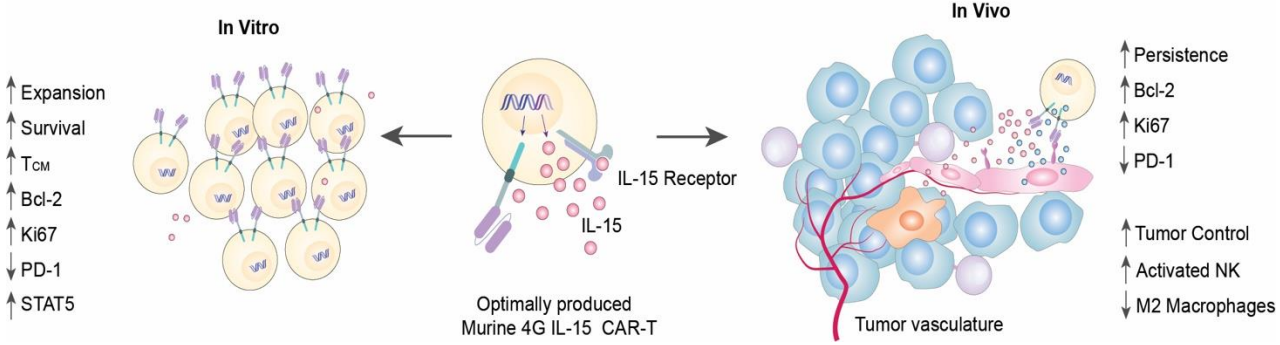
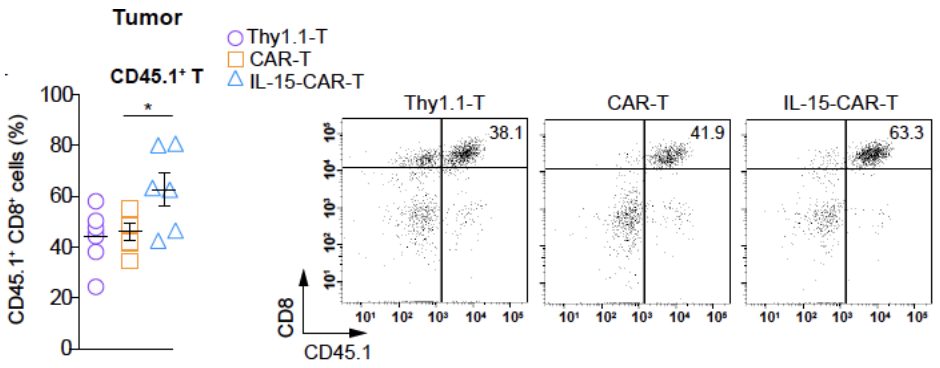
Benefits of IL-15 coengineering

High 4G CAR transduction efficiency

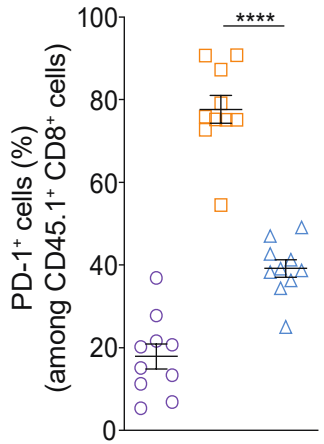




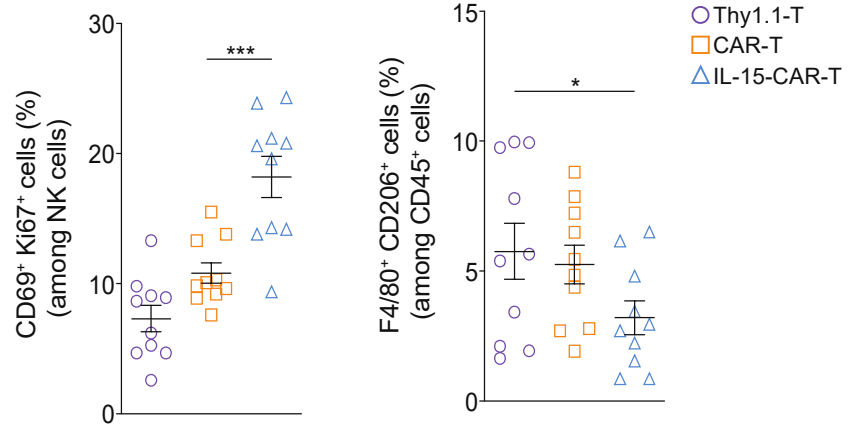
Higher levels of tumor infiltrating 4G CAR T cells



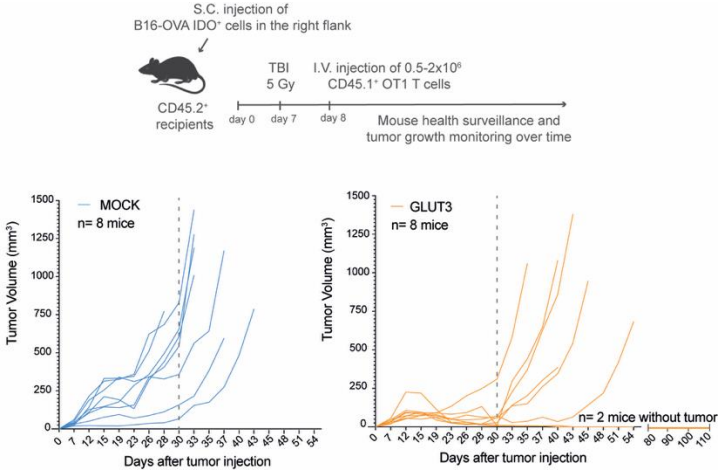
Lower PD1 expression by tumor infiltrating 4G CAR T cells

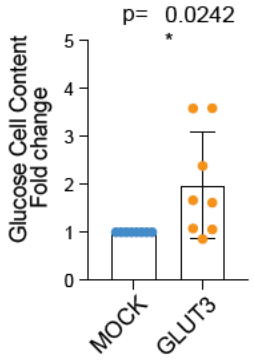


TME re-programming : higher levels of activated NK cells and fewer M2 macrophages



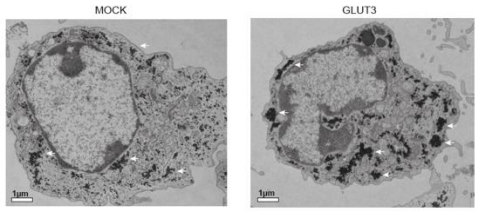
Overcoming glucose competition in the TME: enforced GLUT3 expression

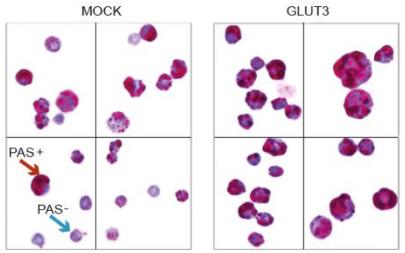


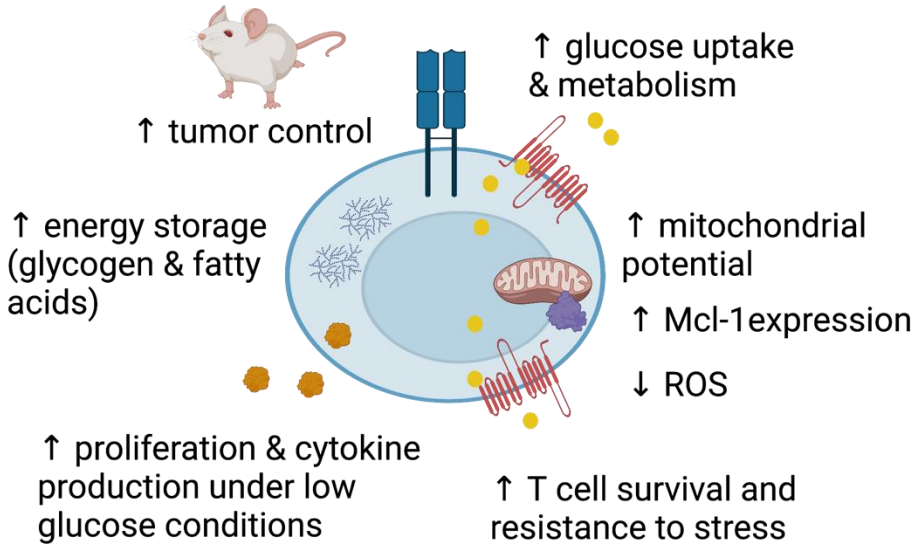


Elisabetta Cribioli

Cribioli,…..Irving* & Coukos*, Enforcing GLUT3 expression in CD8+ T cells improves fitness and tumor control by promoting glucose uptake and energy storage, Frontiers in Immunology 2022

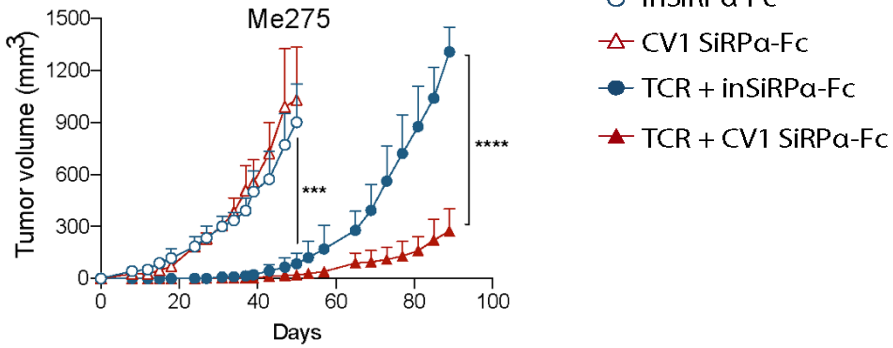






Targeting the CD47/SiRPα ‘don’t eat me’ axis in tumors with CV1 decoy-engineered T cells

- CD47 prevents phagocytosis of healthy cells and has been coopted by my cancers as an innate immune checkpoint
- A2/NY TCR-T cells coengineered to secrete a high affinity variant (CV1) of SiRPα–Fc robustly control tumor outgrowth



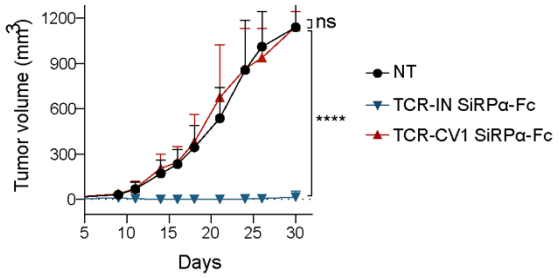
Evangelos Stefanidis

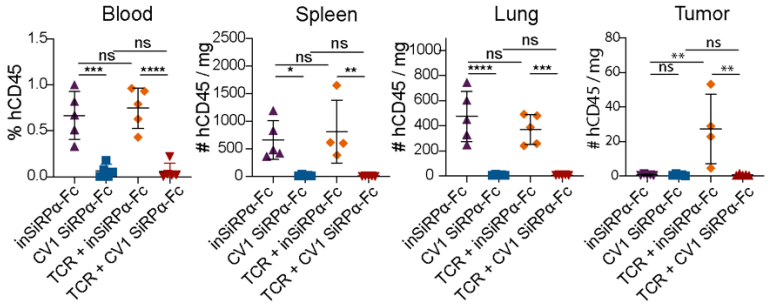
The Journal of Clinical Investigation
RESEARCH ARTICLE
2024

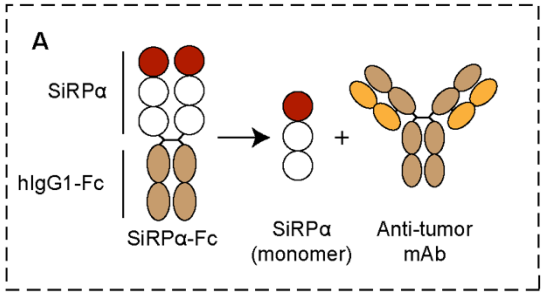
Combining SiRPα decoy-coengineered T cells and antibodies augments macrophage-mediated phagocytosis of tumor cells

Evangelos Stefanidis,[1,2] Aikaterini Semilietof,[1,3] Julien Pujol,[1] Bili Seijo,[1] Kirsten Scholten,[1] Vincent Zoete,[1,3] Olivier Michielin,[1,3,4] Raphael Sandaltzopoulos,[2] George Coukos,[1] and Melita Irving[1]

- The coengineered T cells failed in a subcutaneous tumor model
- The problem: T cells become coated by CV1–Fc and are depleted by macrophages, both in xenograft and syngeneic tumor models

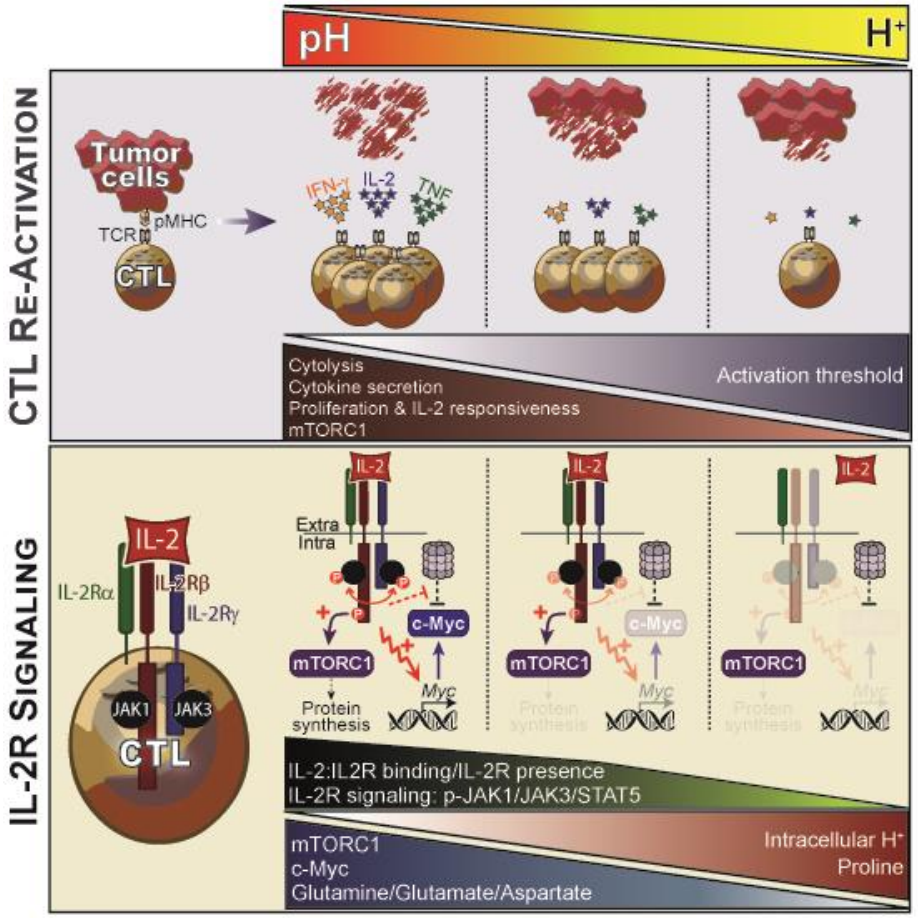






SiRPα decoy = blocks ‘don’t eat me’
Fc = ‘come eat me’

Suppression of T cells under acidic conditions



Romain Vuillefroy de Silly

Article 2024

THE EMBO JOURNAL

Acidity suppresses CD8 + T-cell function by perturbing IL-2, mTORC1, and c-Myc signaling

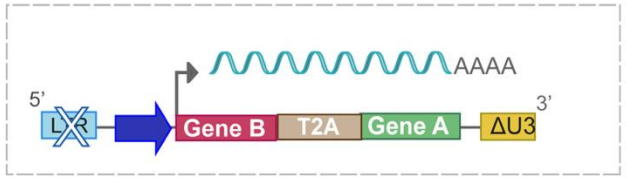
Romain Vuillefroy de Silly, Laetitia Pericou, Bili Seijo, Isaac Crespo & Melita Irving

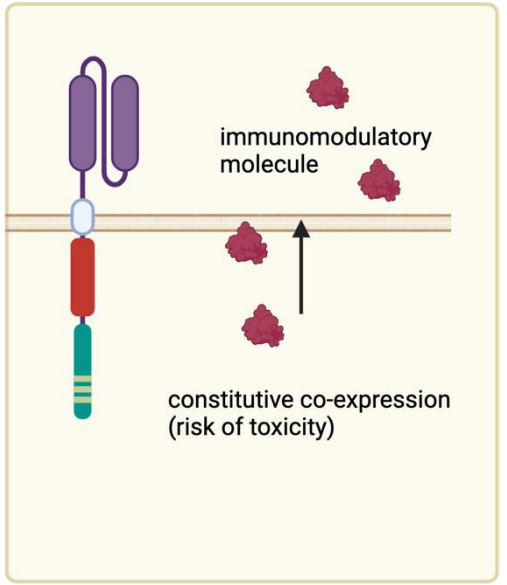
- Extracellular acidity ↓ intracellular pH
- Upon re-activation, acidity significantly ↓ $CD8^+$ T-cell proliferation, cytokine production and cytotoxicity (for low affinity TCR)
- Upon re-activation, acidity ↑ activation threshold
- Lowering pH reduces IL-2/IL-2R binding leading to ↓ global IL-2R signaling
- Proliferation defects are linked to ↓ IL-2 responsiveness
- Acidity ↓ c-Myc accumulation and mTORC1 pathway
- Acidity ↓ glutamine/glutamate/aspartate drop & ↑ proline

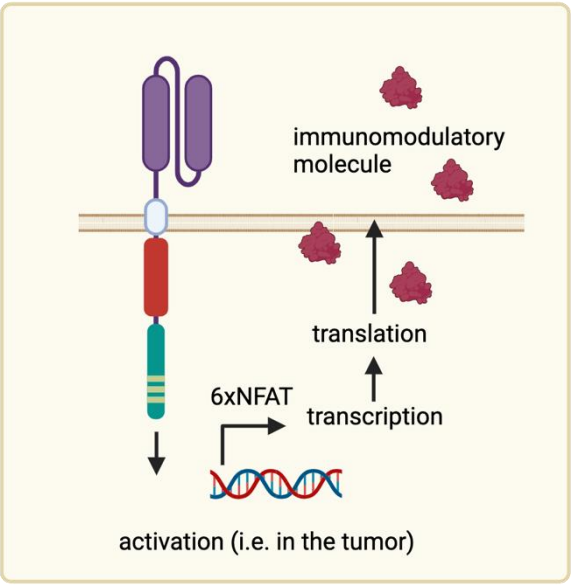
OPTIMIZATION OF ENGINEERING TOOLS

Viral vectors for coengineering T cells

sense configuration; dual constitutive co-expression, post-integration







T2A, P2A…or IRES for constitutive co-expression:

IRES: disadvantage is its large size and lower expression of downstream gene

2A: disadvantage is undesired biological effects of the additional peptide residues that can be left behind either the upstream or the downstream ORF

Limiting transgene expression to the TME

Patrick Reichenbach &
Greta Giordano Attianese

nature biomedical engineering 2023

Article https://doi.org/10.1038/s41551-023-01013-5

A lentiviral vector for the production of T cells with an inducible transgene and a constitutively expressed tumour-targeting receptor

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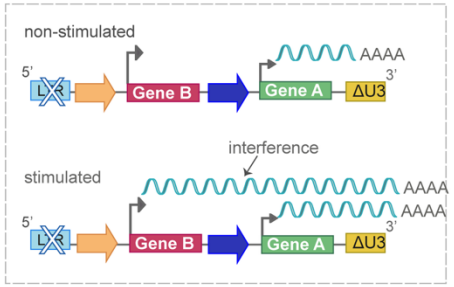
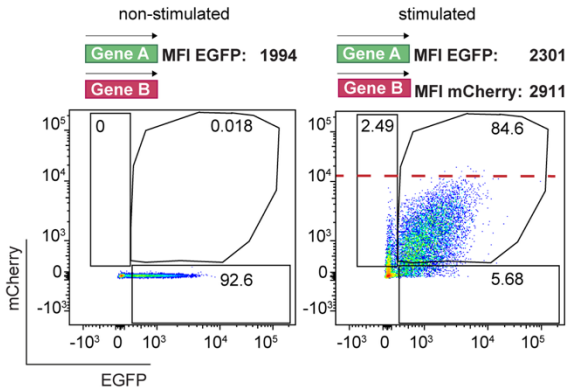
Patrick Reichenbach[1,2], Greta Maria Paola Giordano Attianese[1,2], Khaoula Ouchen[1], Elisabetta Cribioli[1], Melanie Triboulet[1], Sarah Ash[1], Margaux Saillard[1], Romain Vuillefroy de Silly[1], George Coukos[1] & Melita Irving[1]

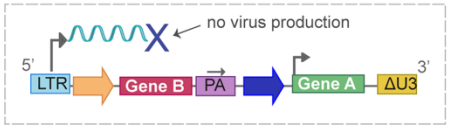
Interference

sense configuration, post-integration



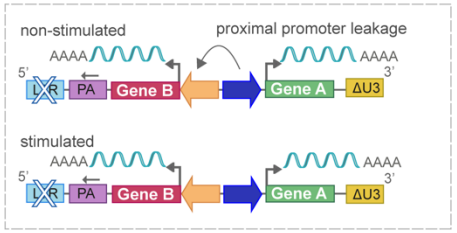


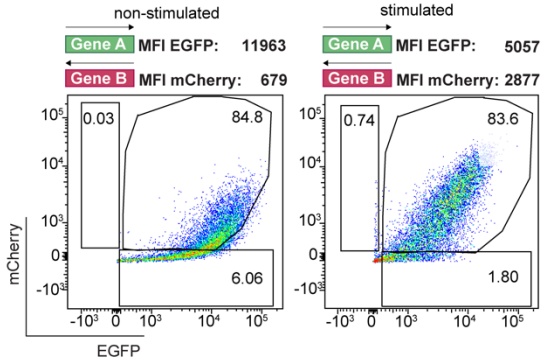
virus production in HEK293T



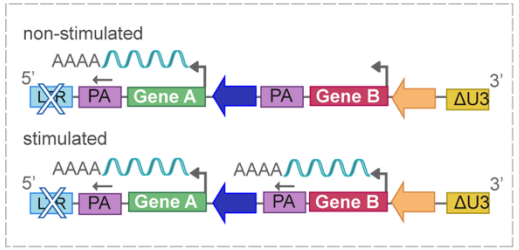
Promoter leakiness

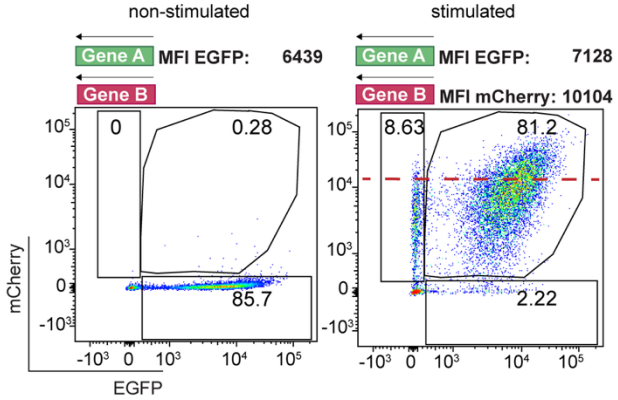
bi-directional configuration, post-integration



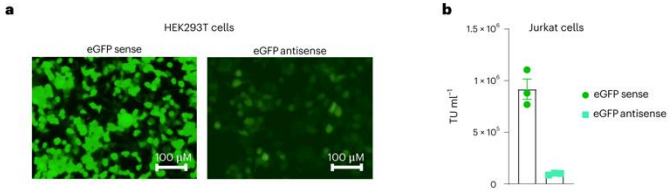


anti-sense configuration, post-integration





Best configuration but very low viral titers!!



Legend

- Consitutive Promoter (PGK)
- Inducible Promoter (6xNFAT)
- PA Polyadenylation signal
- LTR Long Terminal Repeat
- Gene A *EGFP*
- Gene B *mCherry*
- ΔU3 Truncated 3' LTR
- AAAA Poly(A) tail
- LR LTR Post Integration
- RNA

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recherche suisse contre le cancer
ricerca svizzera contro il cancro
swiss cancer research

ISREC
Fondation Recherche Cancer

Fondazione Teofilo Rossi di Montelera e di Premuda

Innosuisse

pactt technology transfer

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Prof. Sai Reddy (ETH Basel)

Flow cytometry, microscopy and in vivo platforms at UNIL

Figures generated with BioRender

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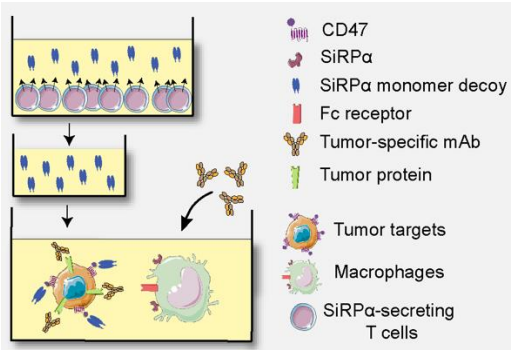
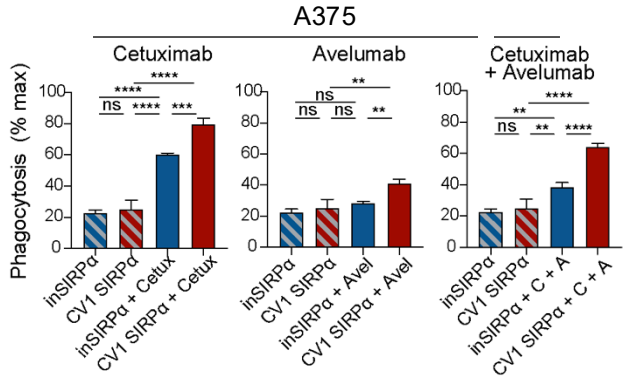
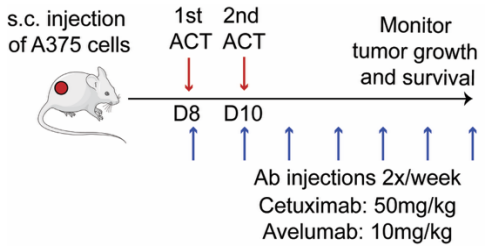
Targeting the CD47/SiRPα 'don't eat me' axis in tumors with decoy-engineered T cells

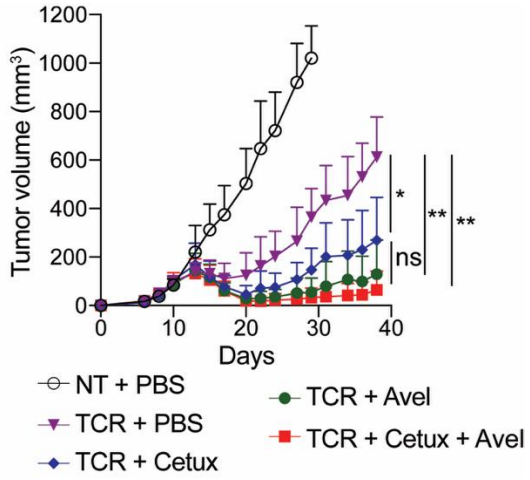
- CV1 (high-affinity SiRPα with no Fc tail) decoys secreted by T cells synergize with tumor-targeted Abs to improve ADCP by macrophages of tumor cells in vitro



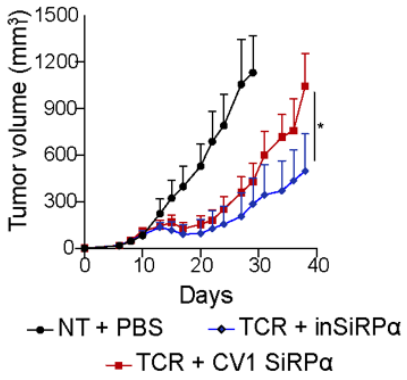


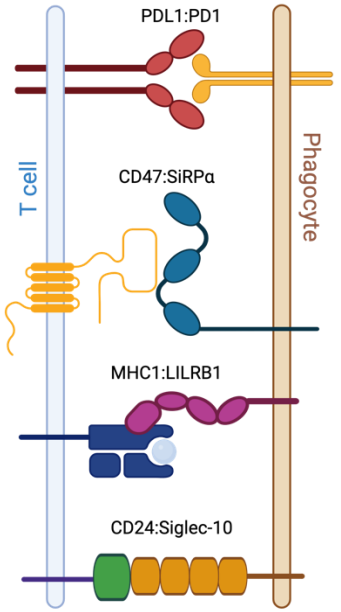
- Cetuximab (αEGFR) and Avelumab (αPDL1) augment tumor control upon co-administration with TCR-T cells

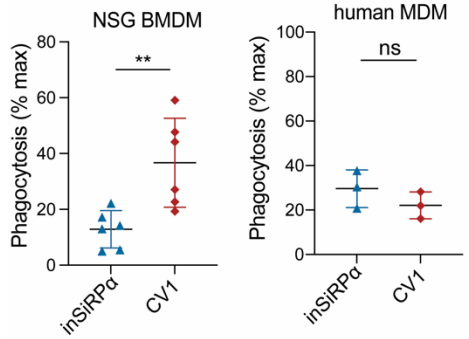


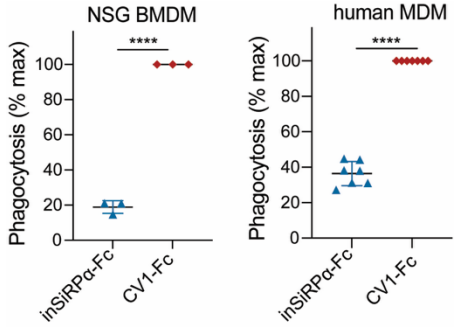


- TCR-T cells + CV1 decoy do worse in vivo than in combination with an inactive decoy
- Murine but not human macrophages phagocytose human T cells coated in CV1
- Both murine and human macrophages phagocytose T cells coated in CV1-Fc





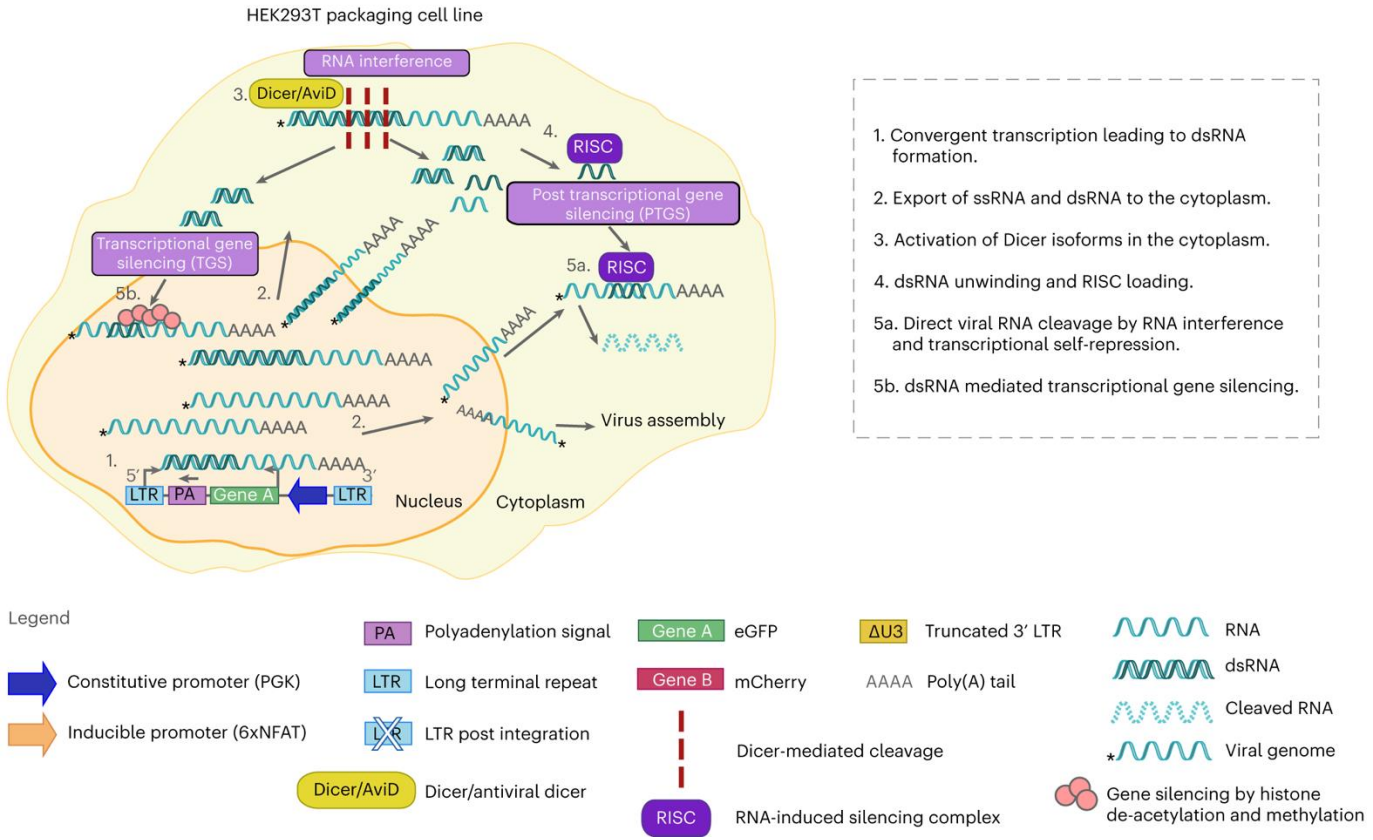




- *Phagocytic threshold* different between mouse and human phagocytes (other active 'don't eat me signals at play for human-human system)
- Potential for translation to humans

Low viral liters for dual inverted lentiviral vector

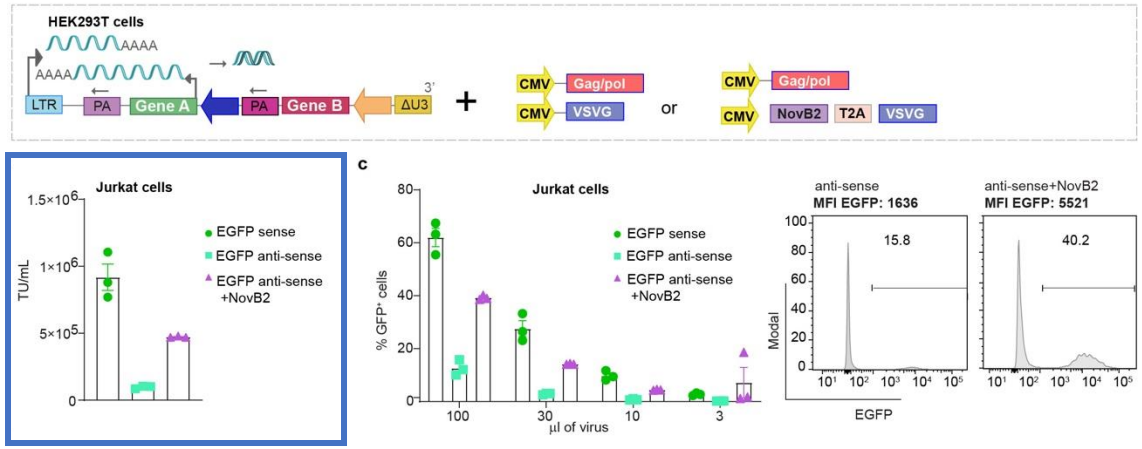
Low viral titers likely caused by convergent transcription resulting in Dicer-mediated cleavage of dsRNA & low levels of viral genome for packaging



The production of second-generation lentivirus relies upon the co-transfection of (i) a transfer, (ii) packaging, and (iii) envelope vector into a producer cell line like HEK293T cells

Solutions for augmenting lentiviral titers

1) Coexpression of NovB2 (a protein from Nodamura virus B2) on the envelope plasmid to block RNA interference in mammalian cells



2) Encode a CMV promoter at the LTR (which comprises 4x NF-kB motifs): TNFα (and IL-1β, camptothecin, and phorbol ester (PMA)) can efficiently activate NF-kB in a dose-dependent manner