# Tuning CAR-T cell fitness through co-stimulatory receptors


Loïc DUPRÉ

Toulouse Institute for Infectious and Inflammatory Diseases (France)

Medical University of Vienna (Austria)




# Scientific and educational cooperation with Brazilian partners

FAPESP France Week 2025

**Co-organization of 5 workshops in Brazil**

- *LymphoRioMove (Fiocruz)*
- *BSI pre-congress courses*

**Training Brazilian fellows in Toulouse**

- *Zilton Vasconcelos*
- *Beatriz Chaves*

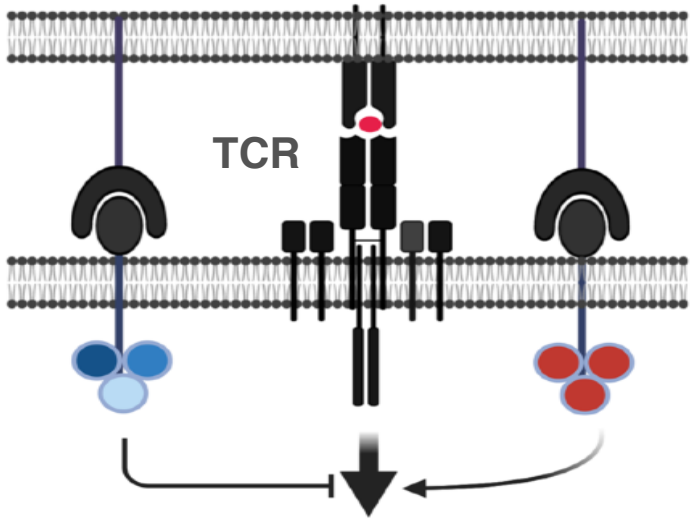
**Currently funded scientific projects**

- *Jose Carlos Alves-Filho*
  *CNRS-USP PhD Joint Program*
- *Helder Nakaya*
  *CAR-T-Fit project (Fondation ARC)*



# Lesourne / Dupré Lab : integrative mapping of lymphocyte signaling and function

Inhibitory receptors = Immune checkpoints

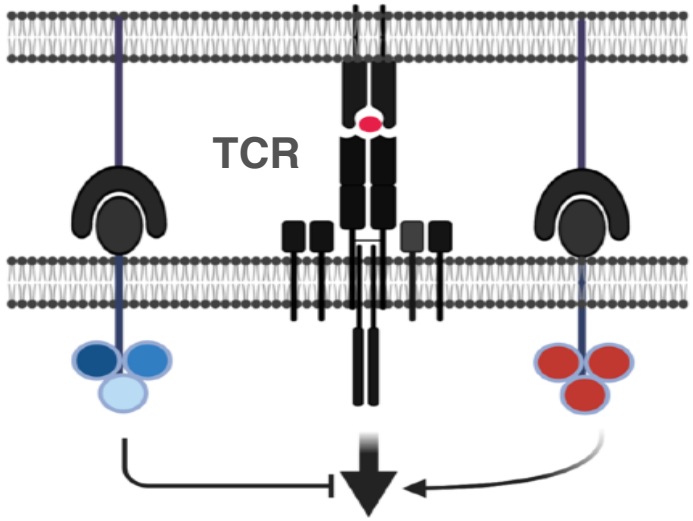



Activatory receptors = Costimulatory Receptors

**Modulation of T cell responses**

# Lesourne / Dupré Lab : integrative mapping of lymphocyte signaling and function

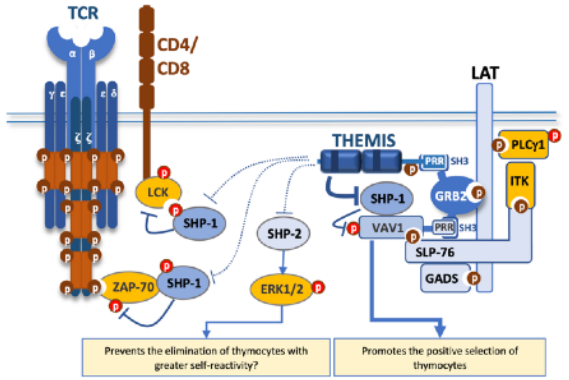
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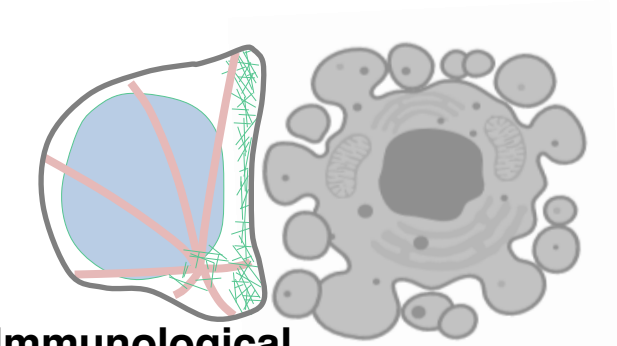

Activatory receptors = Costimulatory Receptors

**Modulation of T cell responses**

## Interactomes and pathways




## T cell activation and function



Immunological synapse

Cytotoxicity

## Pathological models



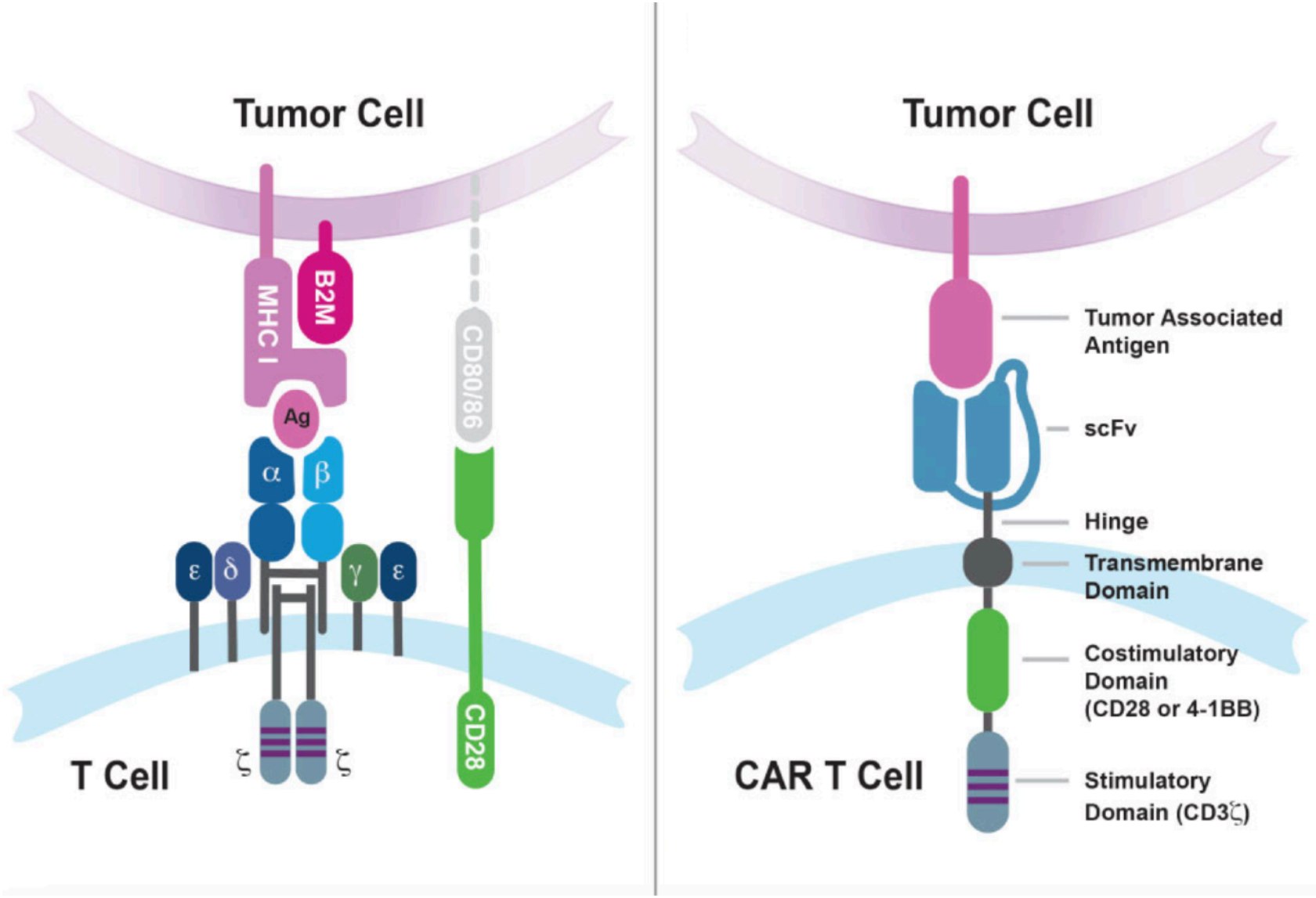
Immunodeficiencies
Multiple myeloma
Multiple sclerosis
Skin pathologies



Autoimmunity

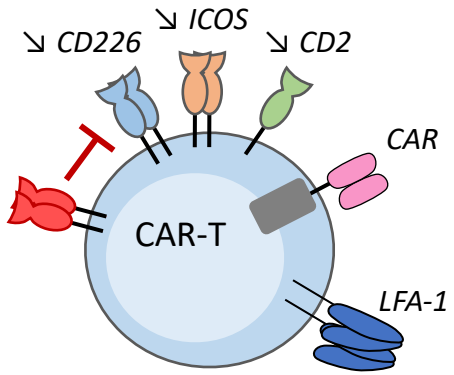
Cancer

# Components of the chimeric antigen receptor of clinically-approved CAR-T cells

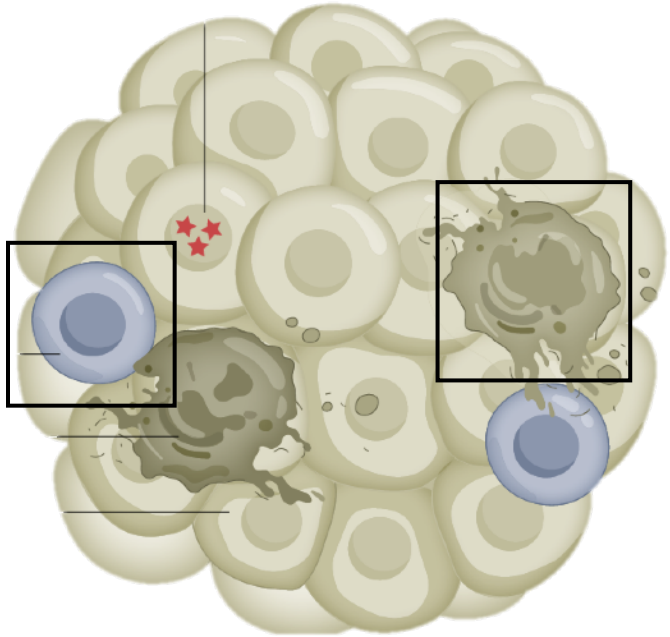


# Challenges currently limiting successful application of CAR-T therapy to solid tumors

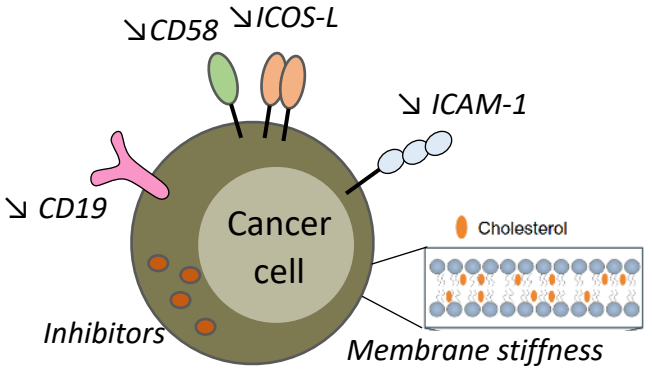
## CAR-T




- Low sensitivity (strong affinity but weak signaling)
- Up-regulation of immune checkpoints and loss of co-stimulatory receptors
- Reduced infiltration of solid tumors
- Reduced survival



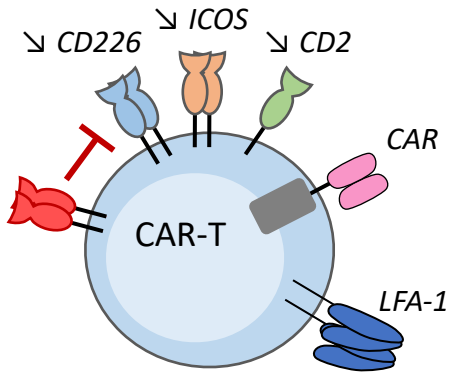
## Cancer cell

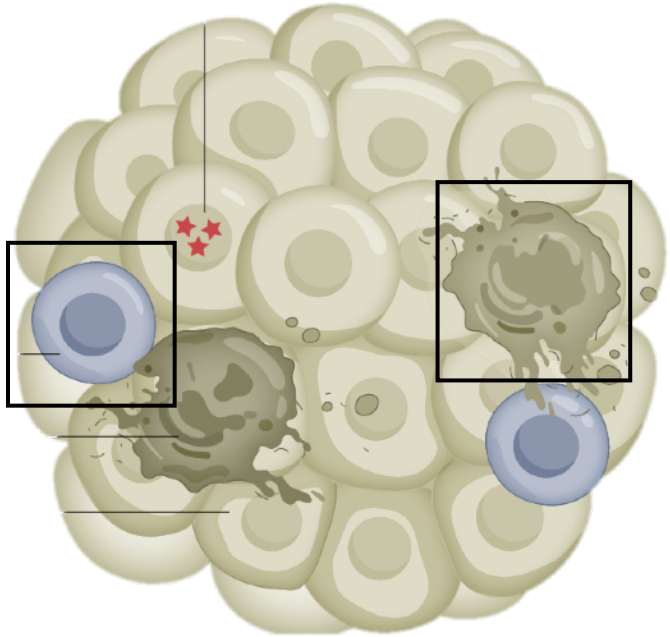



- Down-regulation of targeted antigens
- Down-regulation of co-stimulation ligands (ICAM1, ICOSL…)
- Up-regulation of immune checkpoint ligands (PD-L1)
- Resistance to cytotoxicity (membrane stiffness, lytic molecule inhibitors)

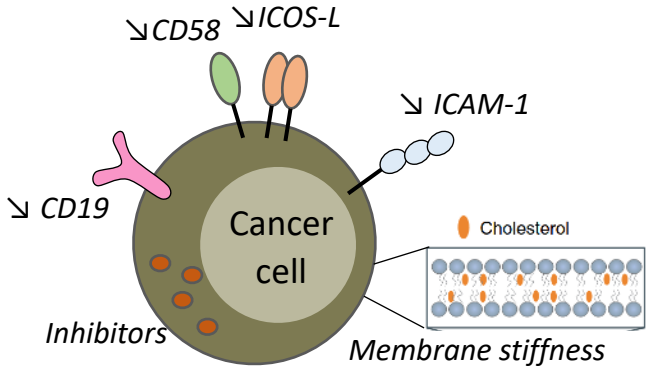
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## CAR-T



## Cancer cell

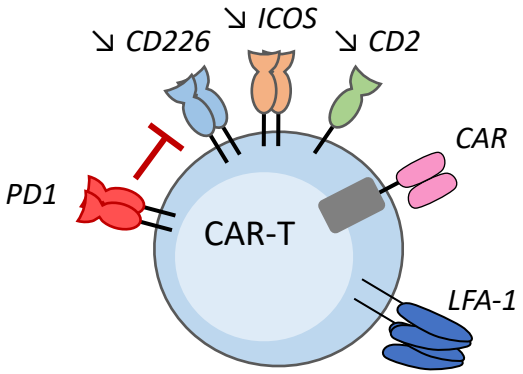



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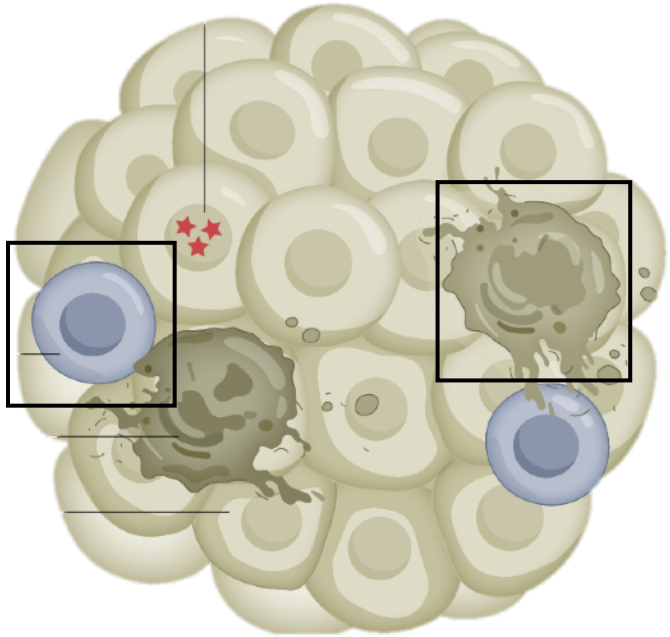
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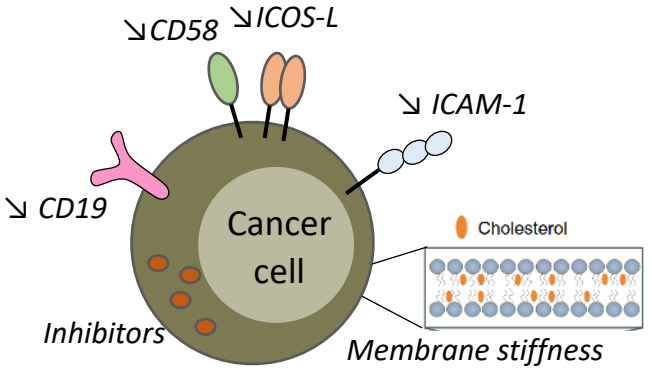
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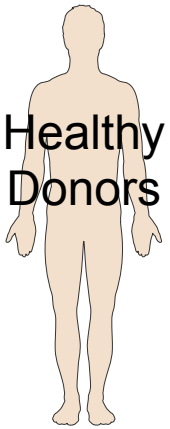


## Cancer cell



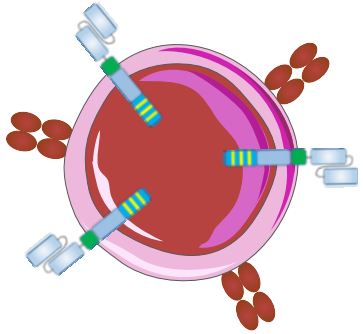

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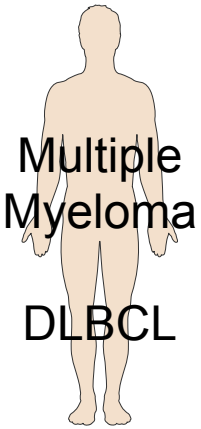
# Do CAR receptors mediate optimal cytotoxic activity ?



Healthy Donors

**Experimental**
EGFR CAR-T





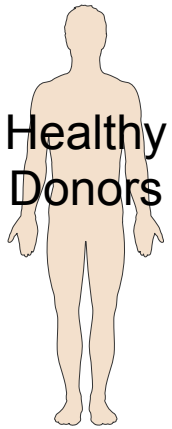
Multiple Myeloma

DLBCL

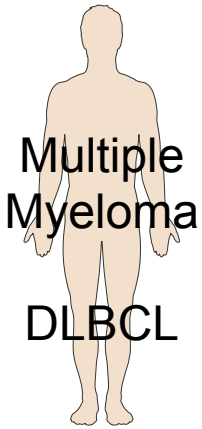
**Clinical products**
BCMA CAR-T
CD19 CAR-T

Emmanuel Donnadieu, Gustave Roussy, Paris
Loïc Ysebaert, IUCT-Oncopole, Toulouse
Ludovic Martinet, CRCT, Toulouse

# Do CAR receptors mediate optimal cytotoxic activity ?



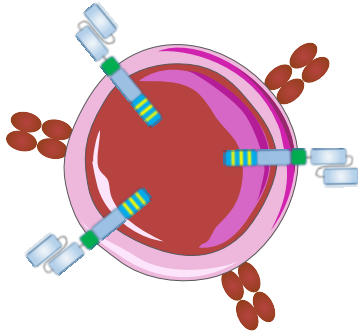
Healthy Donors



Multiple Myeloma

DLBCL

**Experimental**
EGFR CAR-T



**Clinical products**
BCMA CAR-T
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**TCR *vs* CAR** engagement



**OKT3**

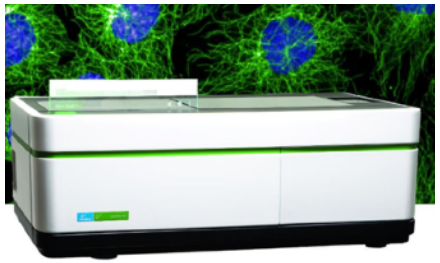


**rhEGFR-Fc**

**Analysis of cytotoxic activity**



Flow cytometry

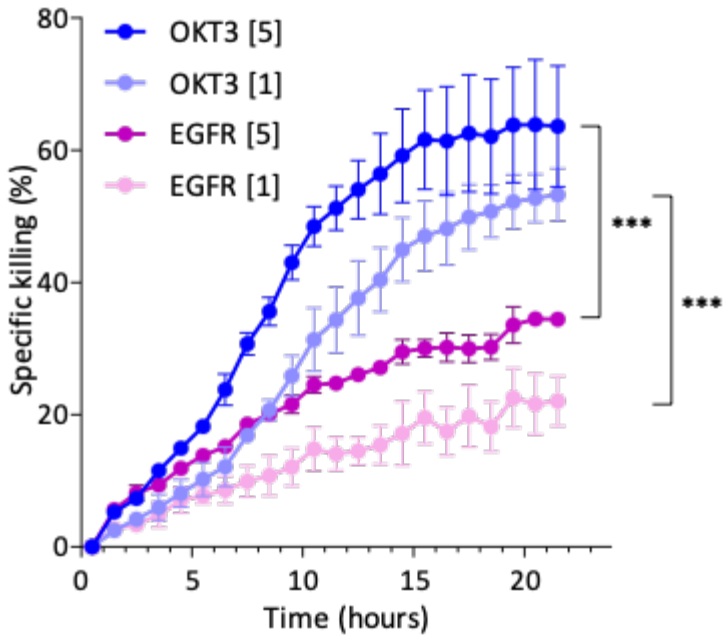


High throughput confocal microscopy

Emmanuel Donnadieu, Gustave Roussy, Paris
Loïc Ysebaert, IUCT-Oncopole, Toulouse
Ludovic Martinet, CRCT, Toulouse

# CAR receptors are suboptimal activators of cytotoxic activity

## Cytotoxicity

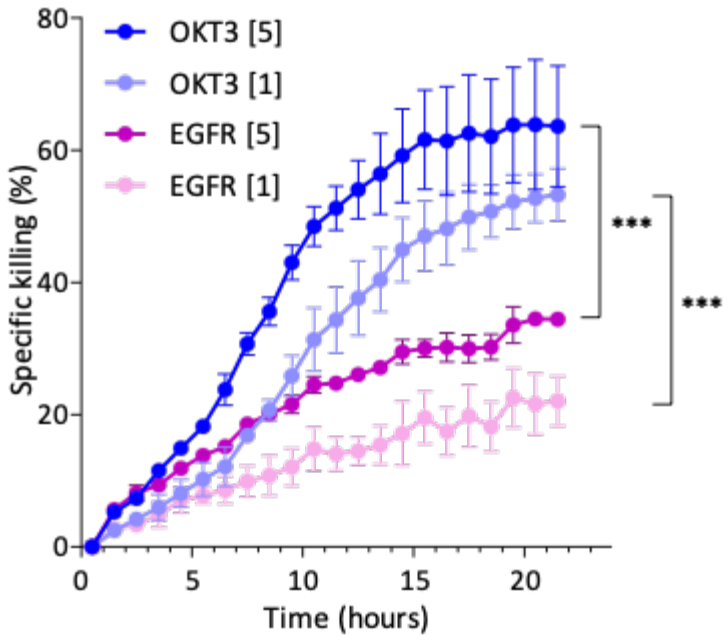




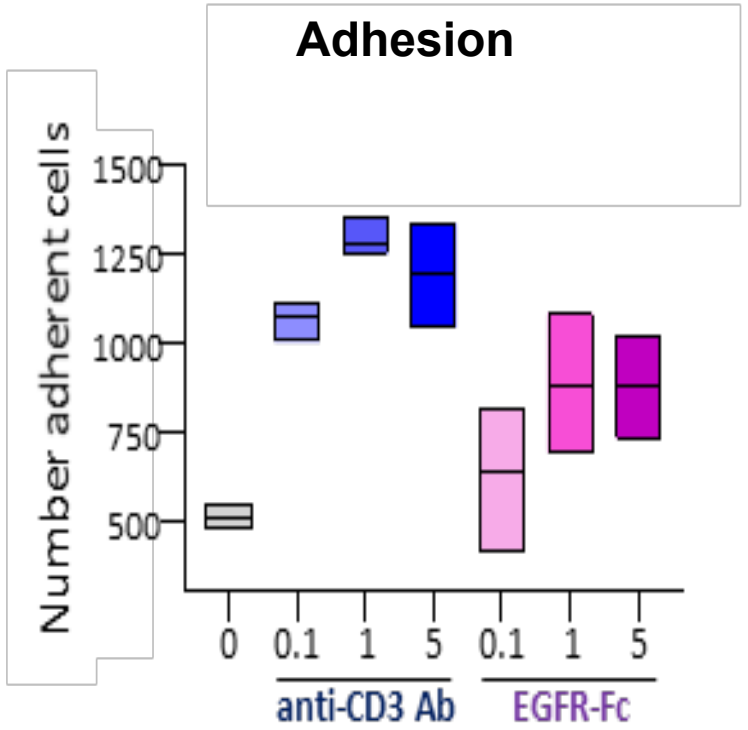

Clément
BOUISSET

# CAR receptors are suboptimal activators of cytotoxic activity

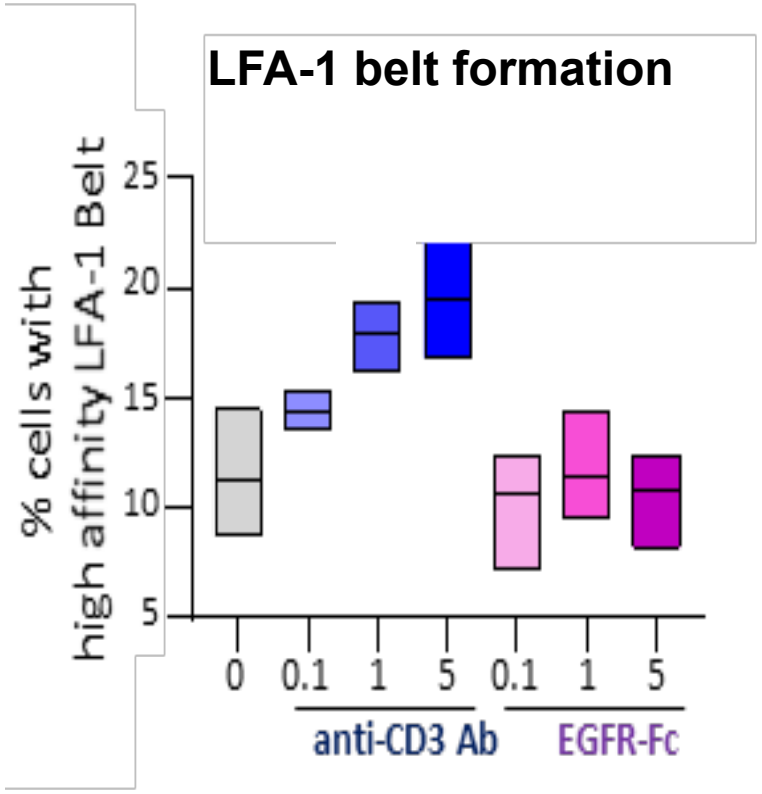
## Cytotoxicity




## Adhesion



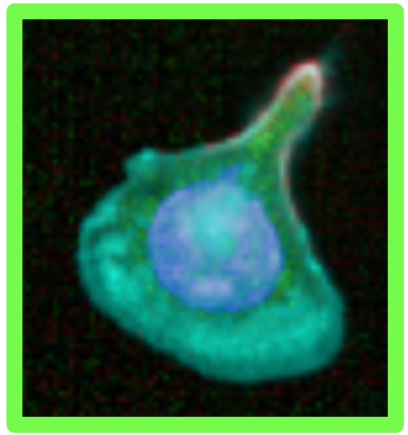

## LFA-1 belt formation

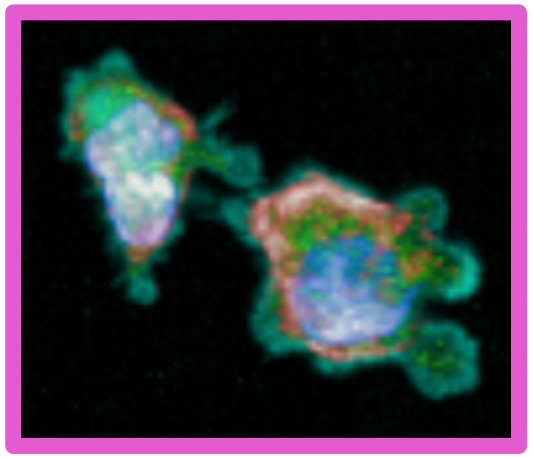


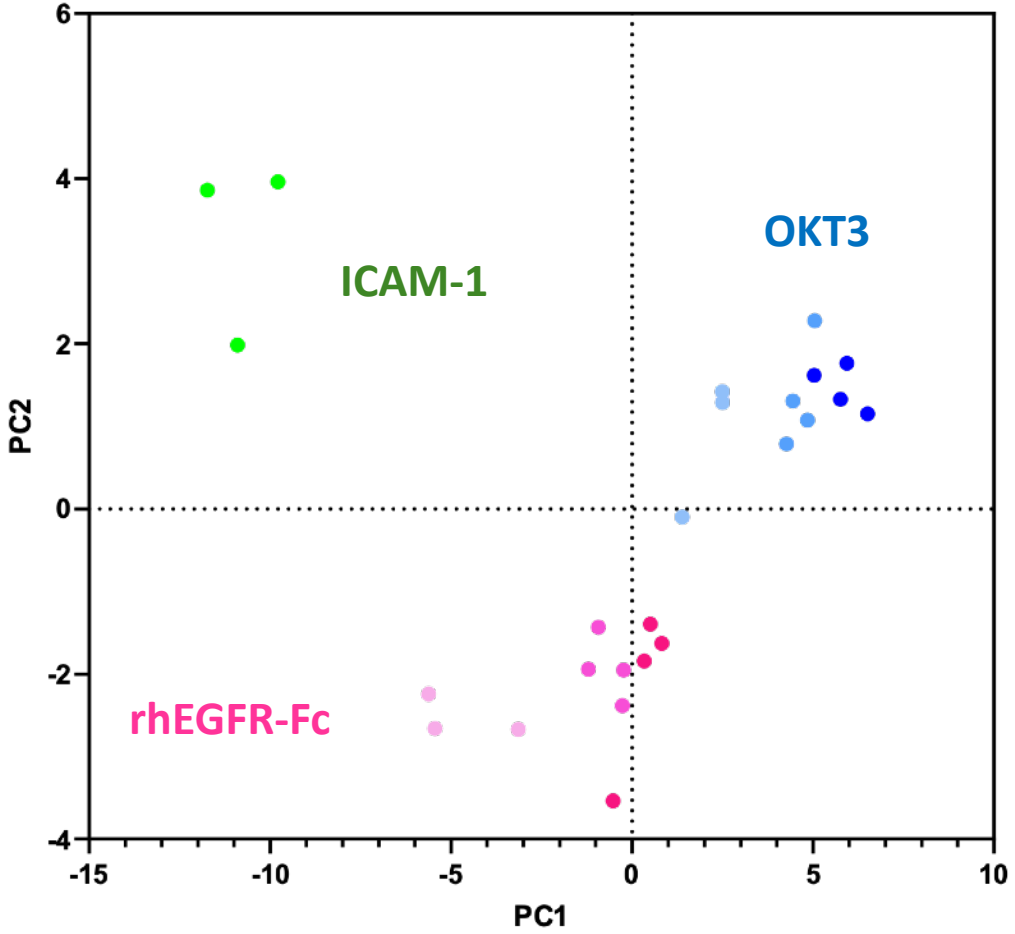


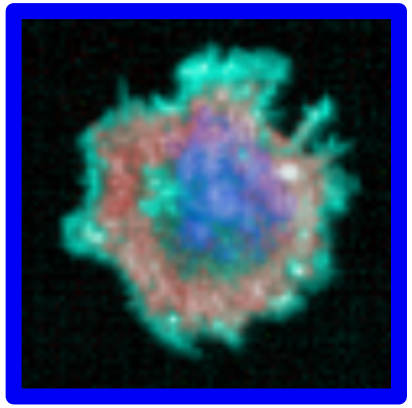

Clément BOUISSET

# Synapse architecture of CAR-T cells is altered upon CAR engagement



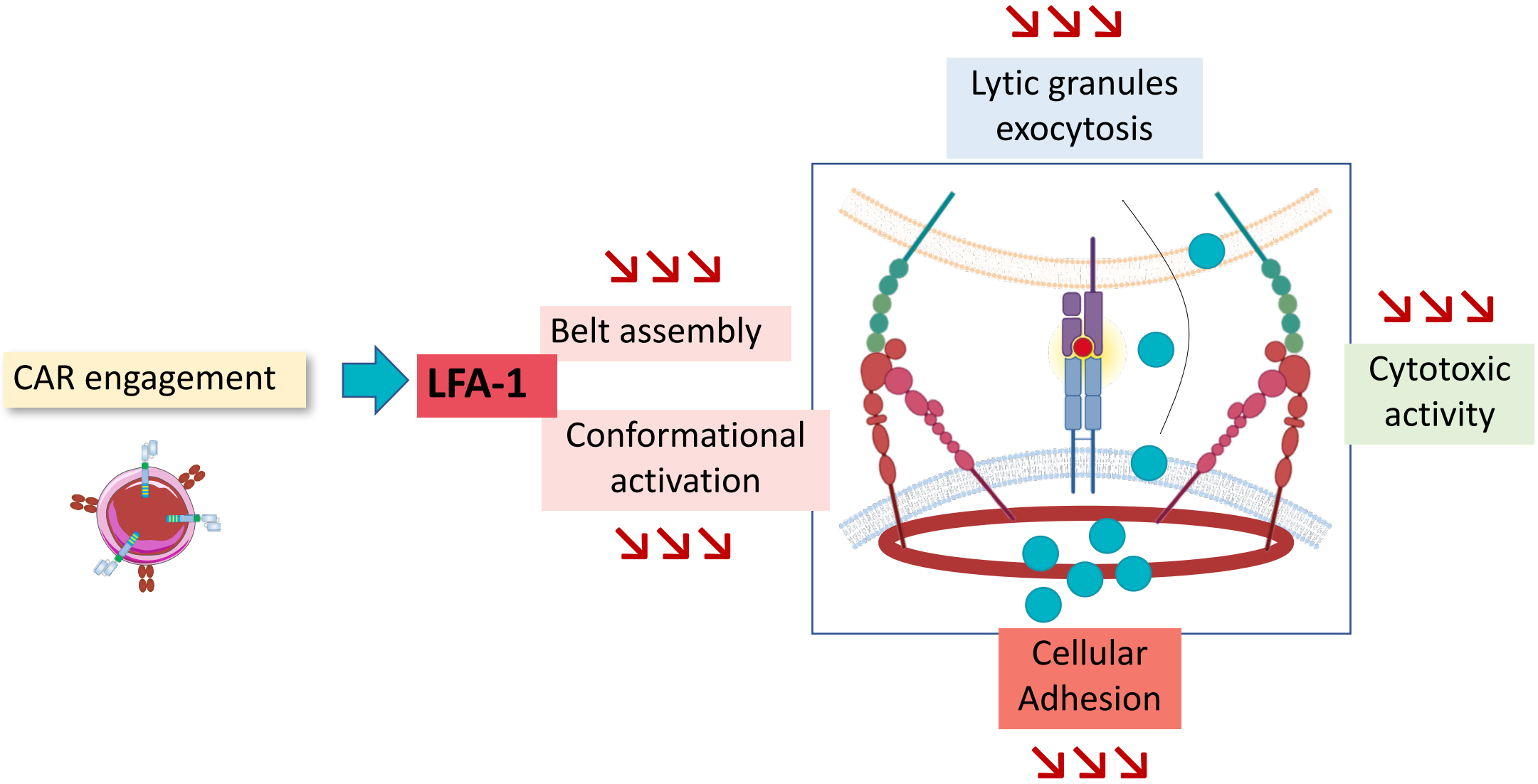




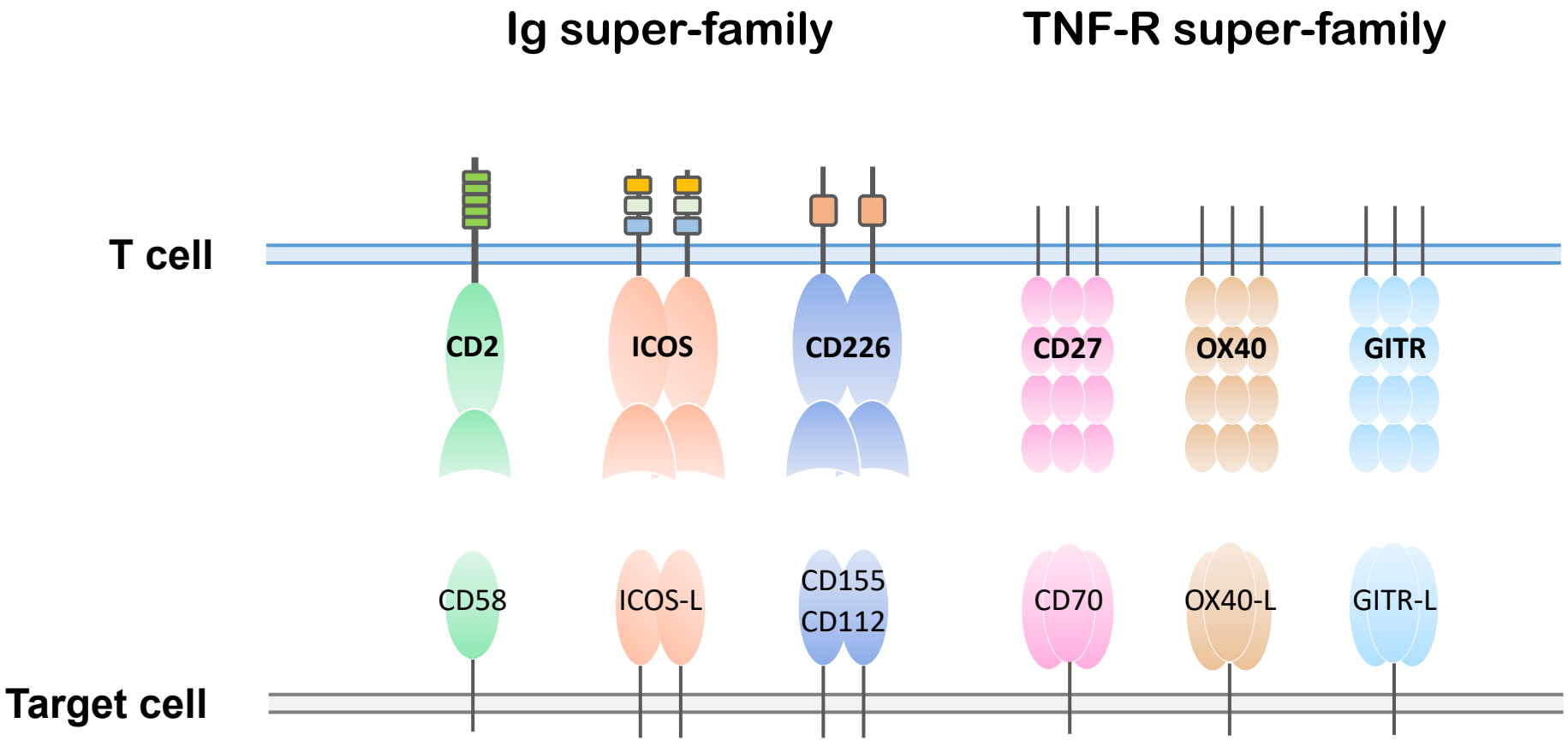



F-Actin
Open LFA-1
GFP
DAPI

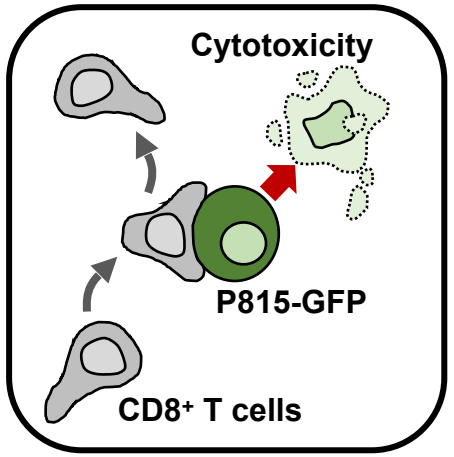
# Suboptimal LFA-1 activation and cytotoxic activity upon CAR stimulation



# Exploiting co-stimulatory receptors to improve CAR-T cell function



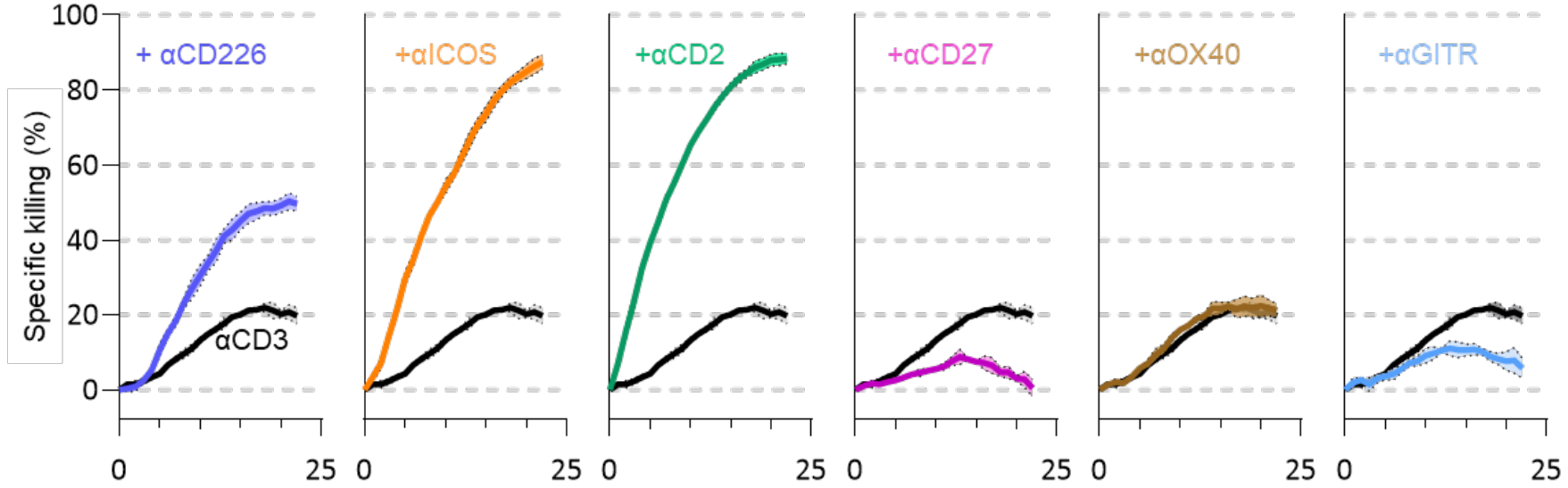
# Co-stimulatory receptors of the Ig super-family enhance cytotoxicity in CD8+ T cells



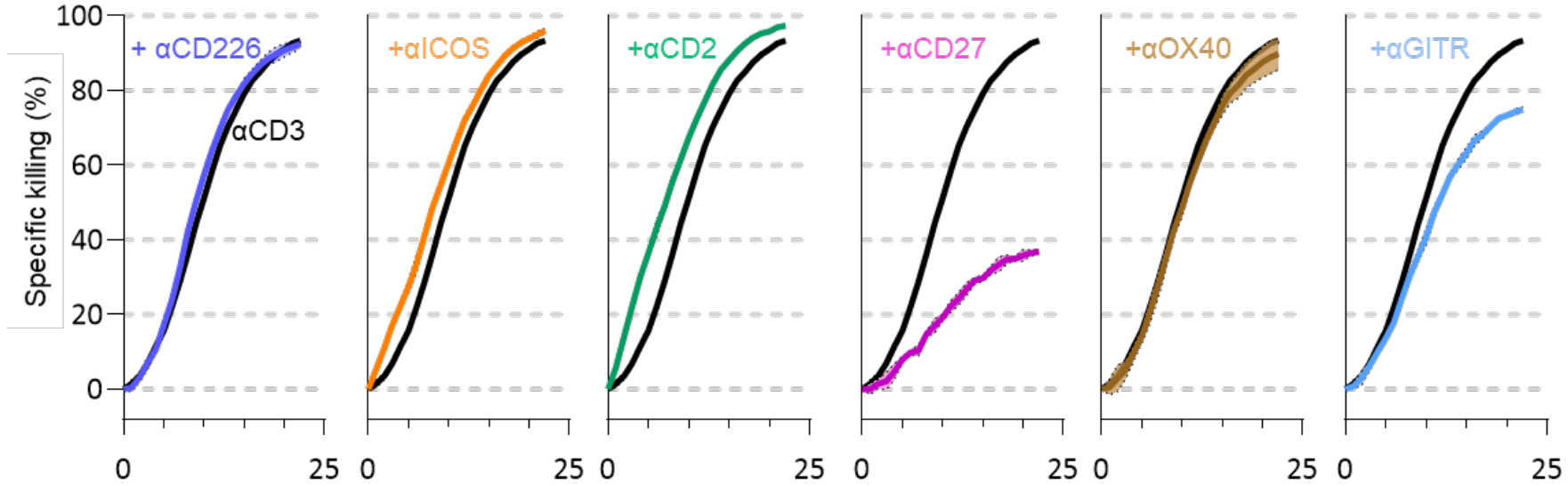



Guilhen PRUNIER

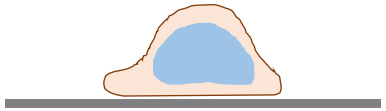
**Suboptimal** TCR engagement



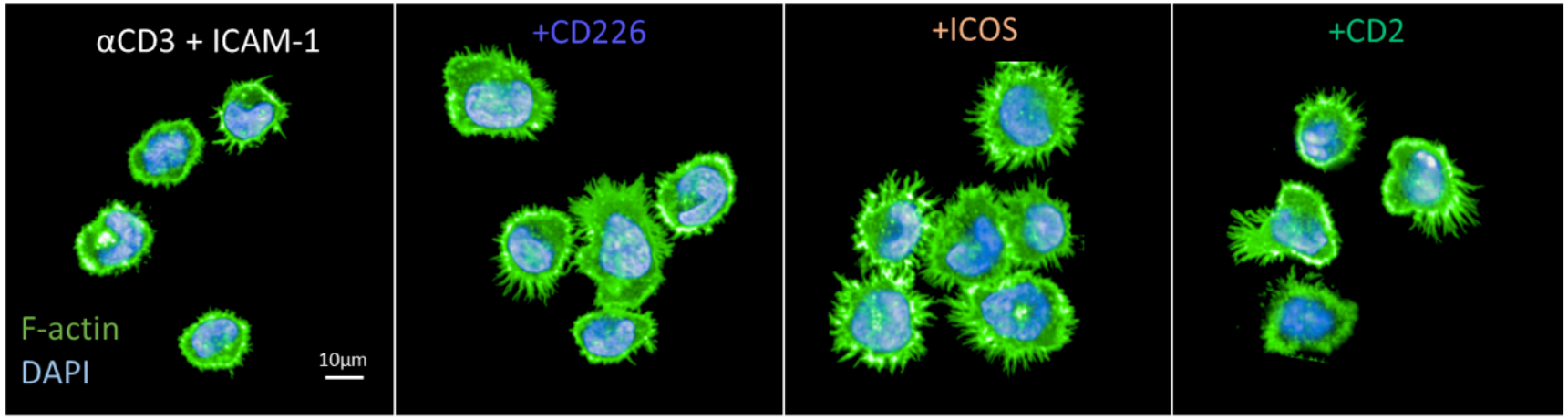

**Saturating** TCR engagement

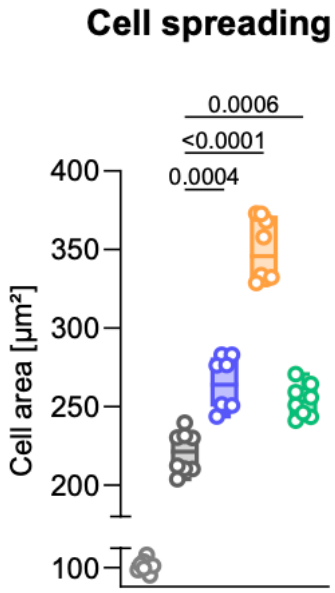


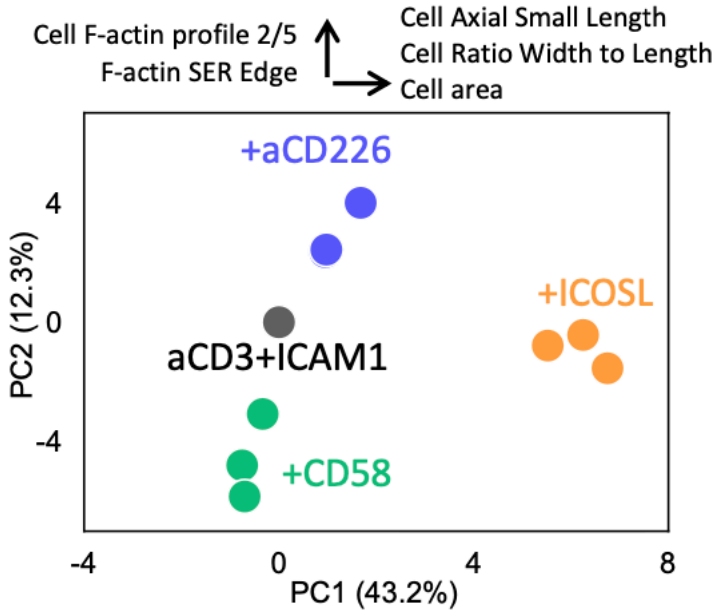
# Unique property of ICOS to promote synaptic spreading



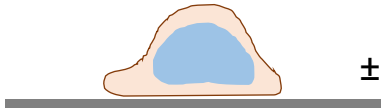
± αCD3 ± ICAM-1 ± Co-stim



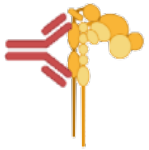



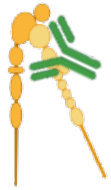
# Unique property of CD226 to promote LFA-1 belt assembly



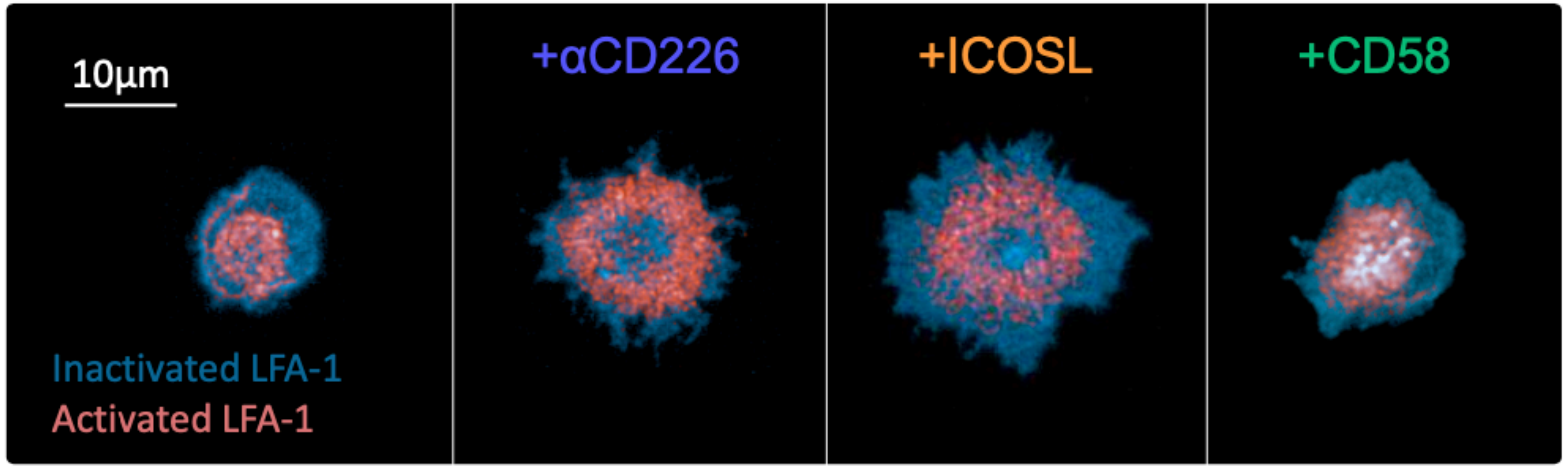
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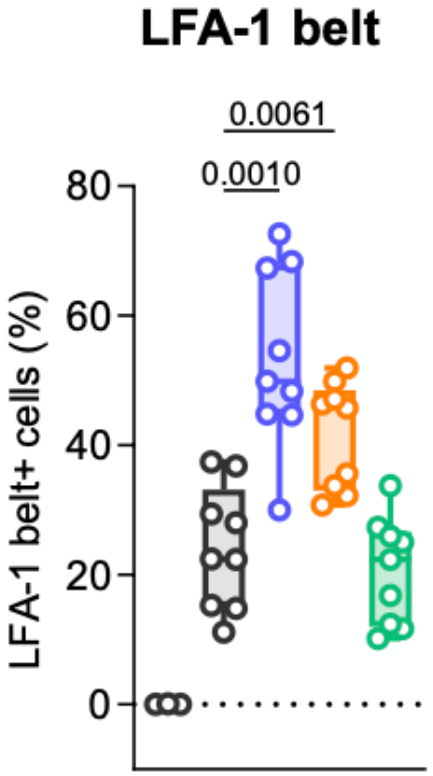


Low-affinity LFA-1
(Hi-111 Ab)

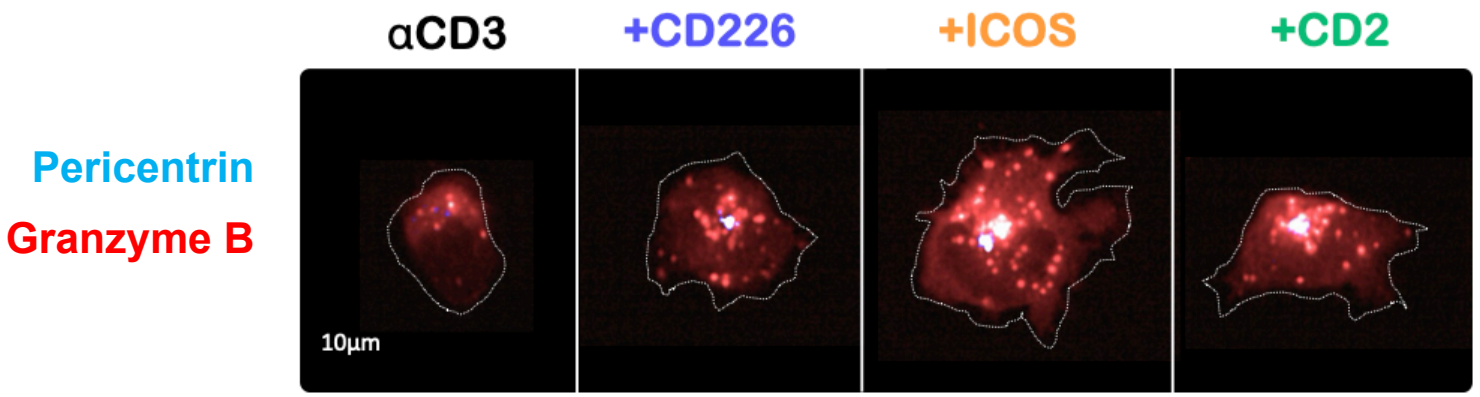


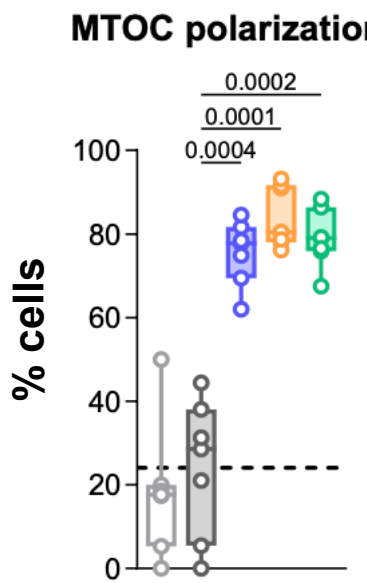
High-affinity LFA-1
(m24 Ab)

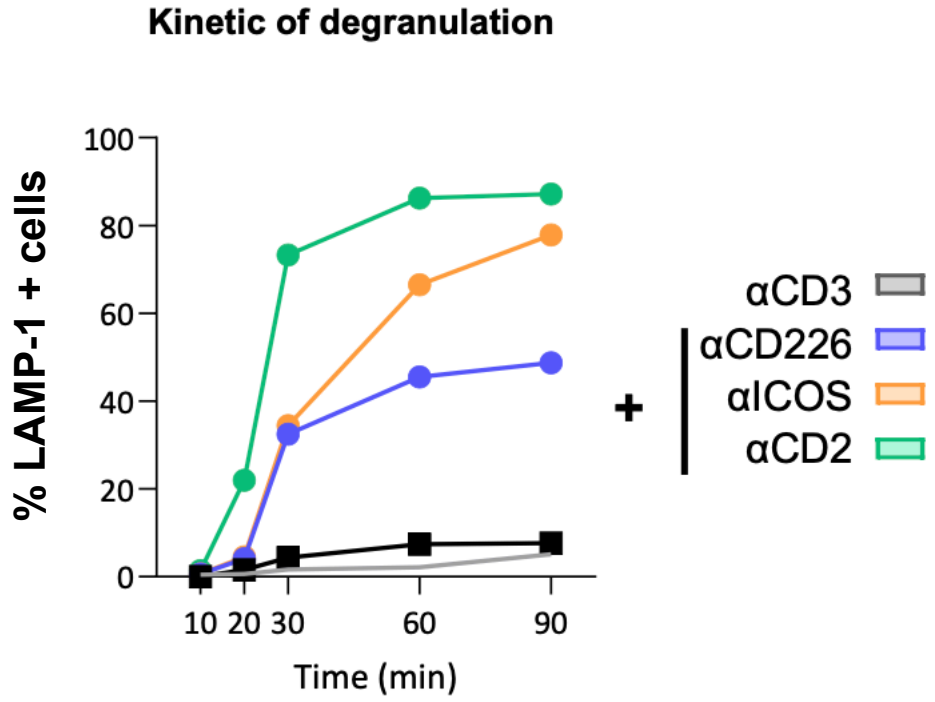


# Unique property of CD2 to promote rapid degranulation

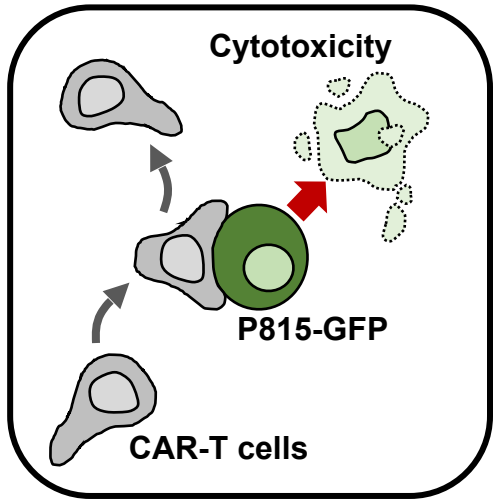


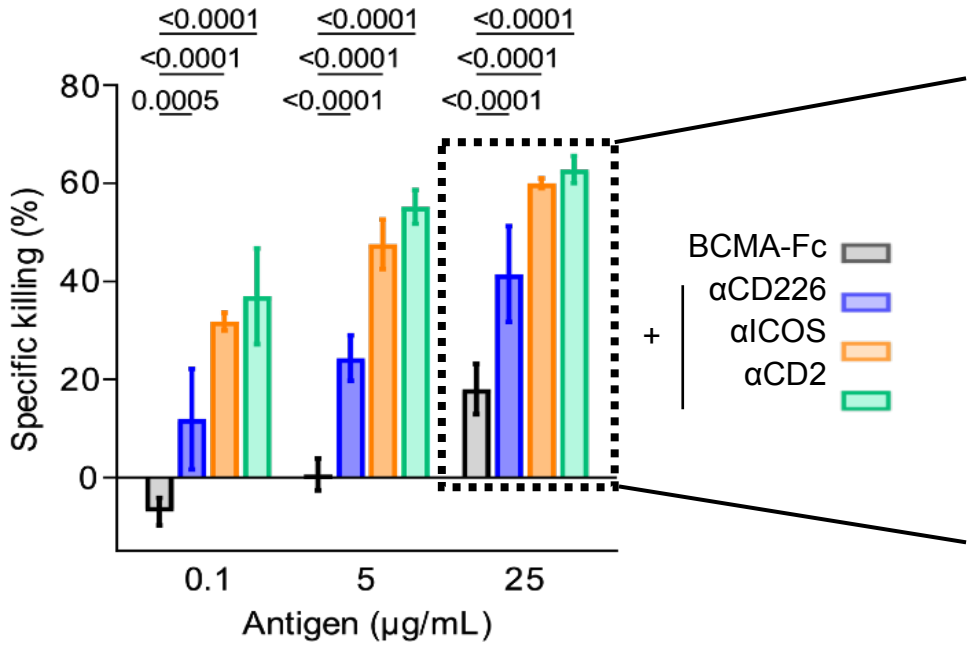



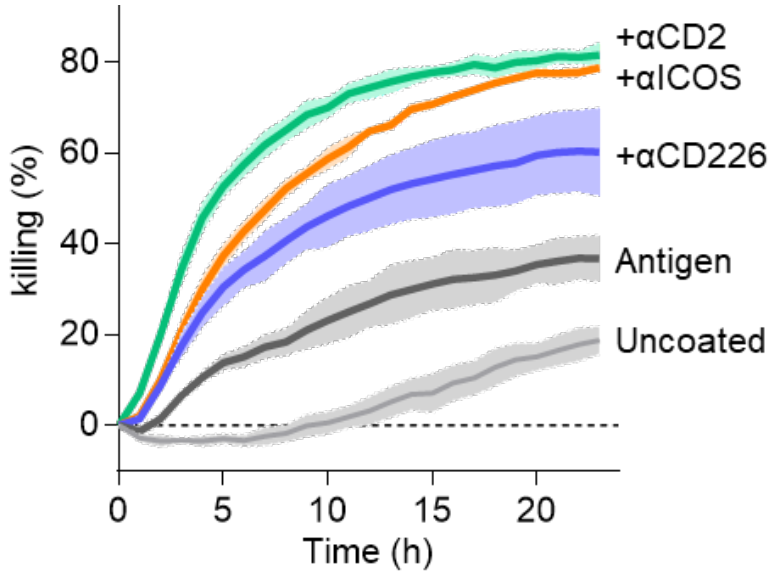
# The CAR can « exploit» co-stimulatory receptors to stimulate cytotoxic function

## Cytotoxicity

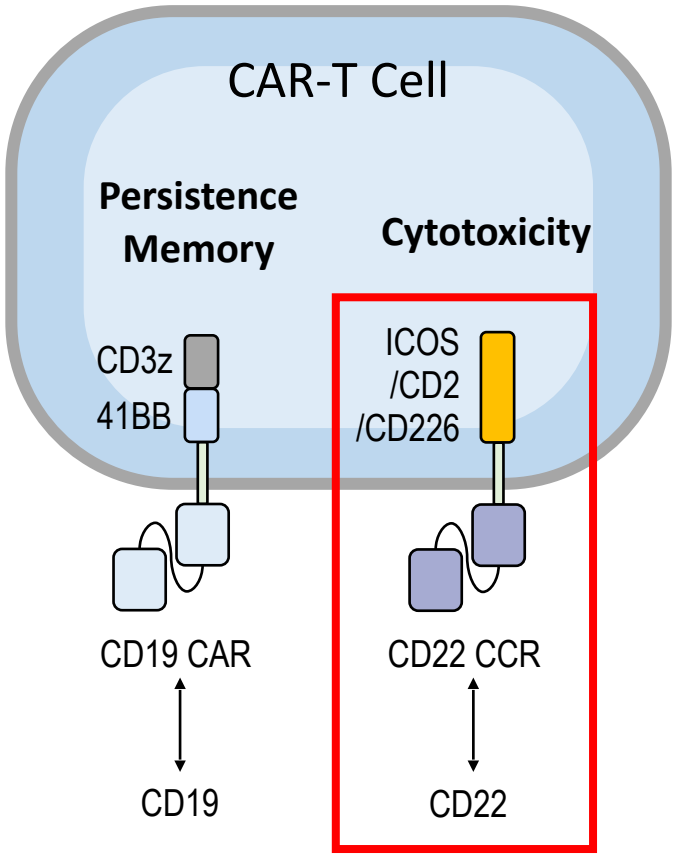


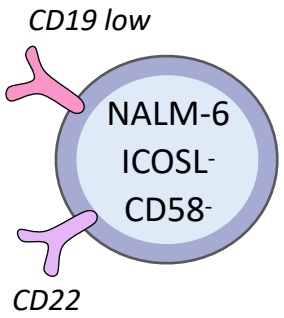

## Specific killing (optimal CAR engagement)

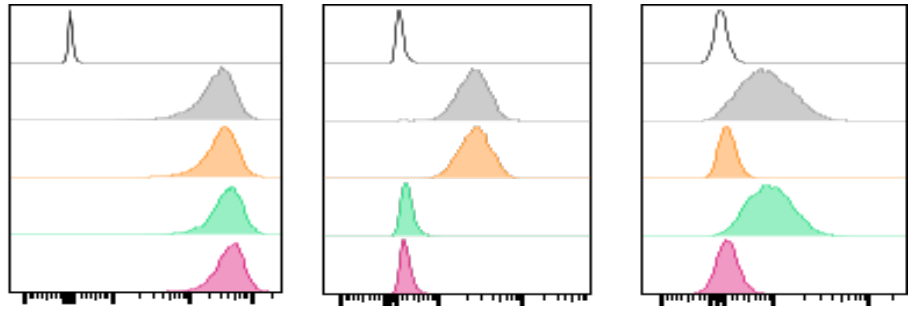


# Ongoing: assessing CAR-T cells equipped with chimeric co-stimulatory receptors

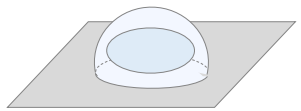


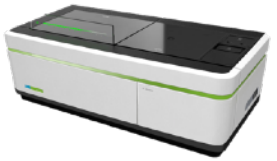

Cytotoxic activity against multi-resistant cancer cells




High content cell imaging pipeline





Immune synapse assembly

**Sylvain Simon**

Fred Hutch Cancer Center

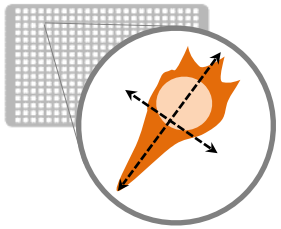
# Ongoing: monitoring pre-treatment T cell fitness to predict CAR-T therapy efficacy

Working hypothesis:

**The functional properties of the T cells used to generate CAR-T cell products affect treatment efficacy**



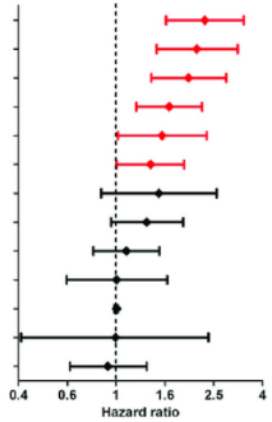
PBMC – LBCL patients
*at apheresis*



High-content
cell imaging



Functional signature




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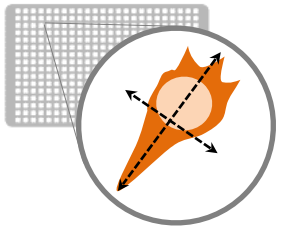
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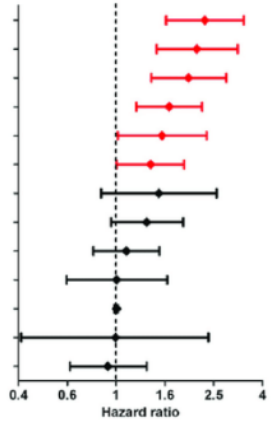
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***Predict which patients might not respond to CAR-T cell therapy***

***Identify functional parameters associated with treatment failure***

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