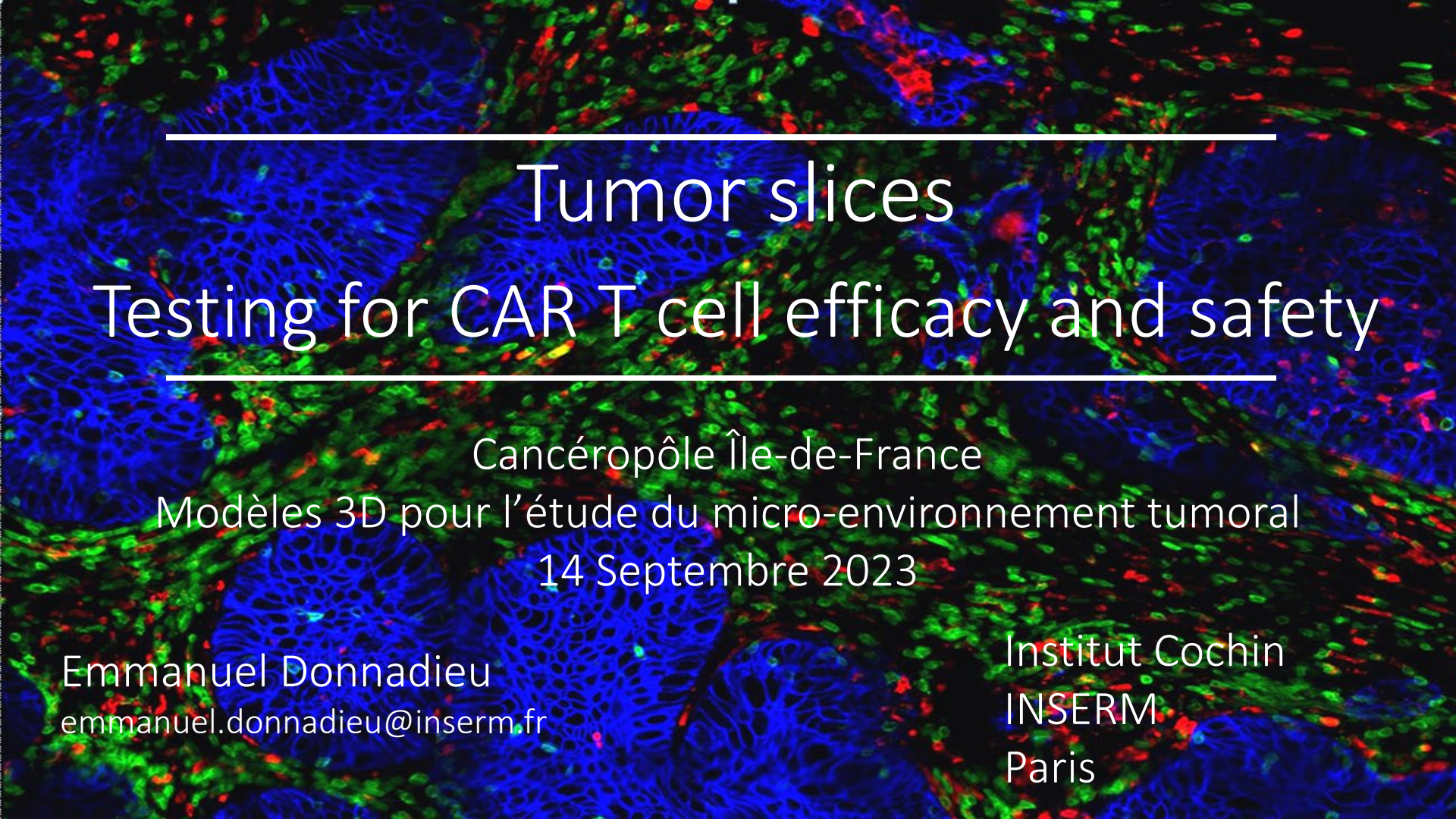


Tumor slices

Testing for CAR T cell efficacy and safety

Cancéropôle Île-de-France

Modèles 3D pour l'étude du micro-environnement tumoral

14 Septembre 2023

Emmanuel Donnadieu
emmanuel.donnadieu@inserm.fr

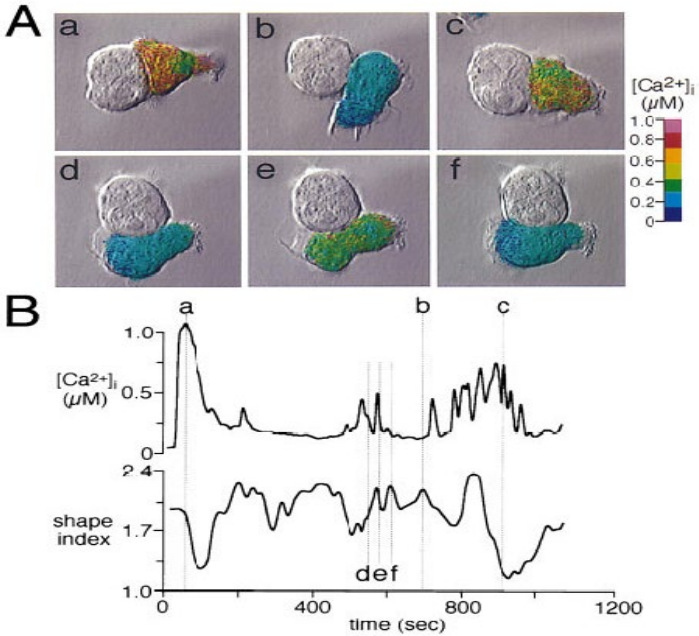
Institut Cochin
INSERM
Paris

Overview of the presentation

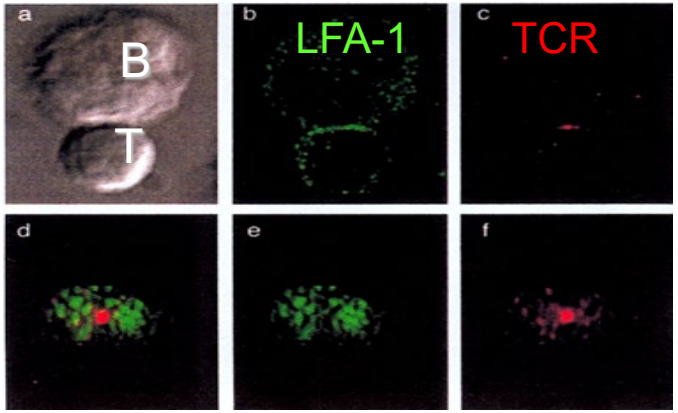
- ✓Tumor slices: History
- ✓Tumor slices: Study of the interactions between T cells and the TME
- ✓Tumor slices: Testing for CAR T cell efficacy and safety

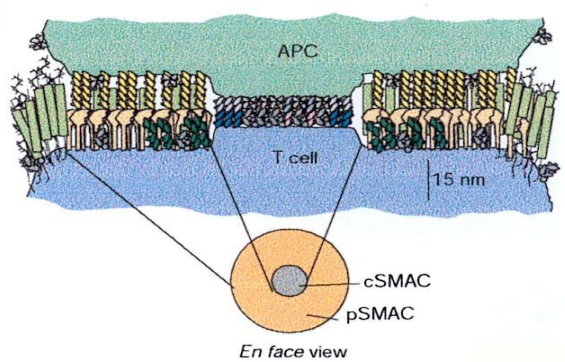
2D in vitro models to image T cells

1990-2000



Negulescu et al. Immunity 1996





Grakoui et al. Science 1999

Imaging immune cells in intact organs

2002

SCIENCE VOL 296 7 JUNE 2002

Two-Photon Imaging of Lymphocyte Motility and Antigen Response in Intact Lymph Node

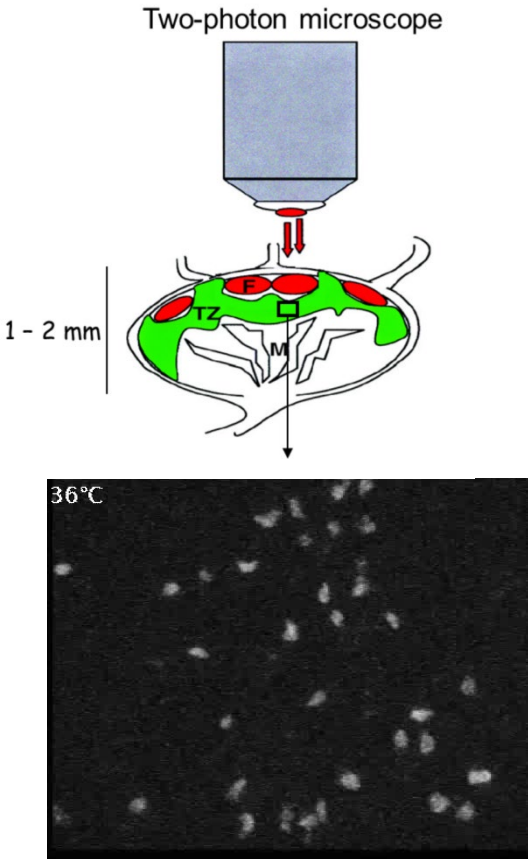
Mark J. Miller,[1] Sindy H. Wei,[1] Ian Parker,[2*] Michael D. Cahalan[1*†]

Dynamic Imaging of T Cell–Dendritic Cell Interactions in Lymph Nodes

Sabine Stoll,[1] Jérôme Delon,[1] Tilmann M. Brotz,[2*] Ronald N. Germain[1†]

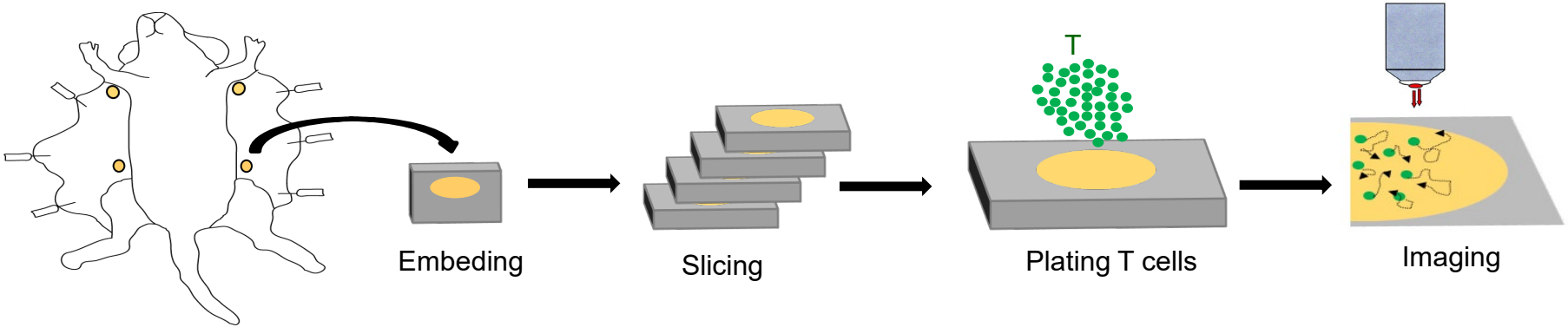
Dynamics of Thymocyte–Stromal Cell Interactions Visualized by Two-Photon Microscopy

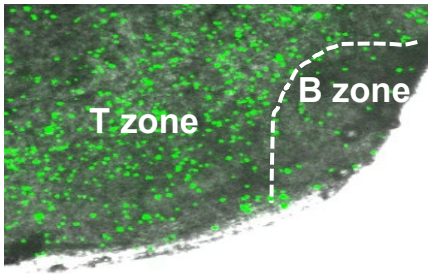
Philippe Bousso,[1] Nirav R. Bhakta,[2*] Richard S. Lewis,[2] Ellen Robey[1†]



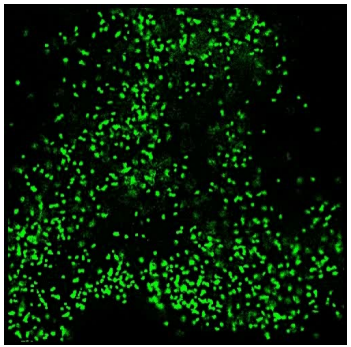
Miller et al. Science 2022

imaging of T cells in murine lymph node slices

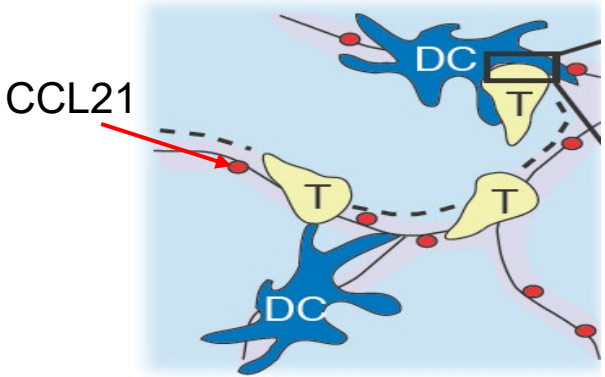




- Localization

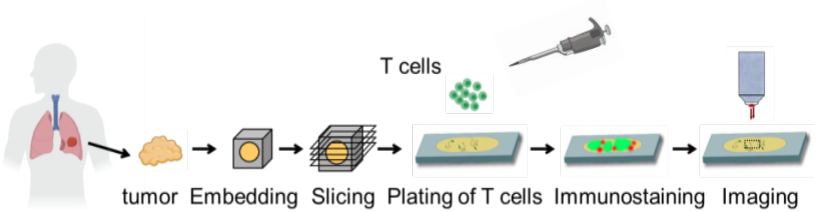


- Motility



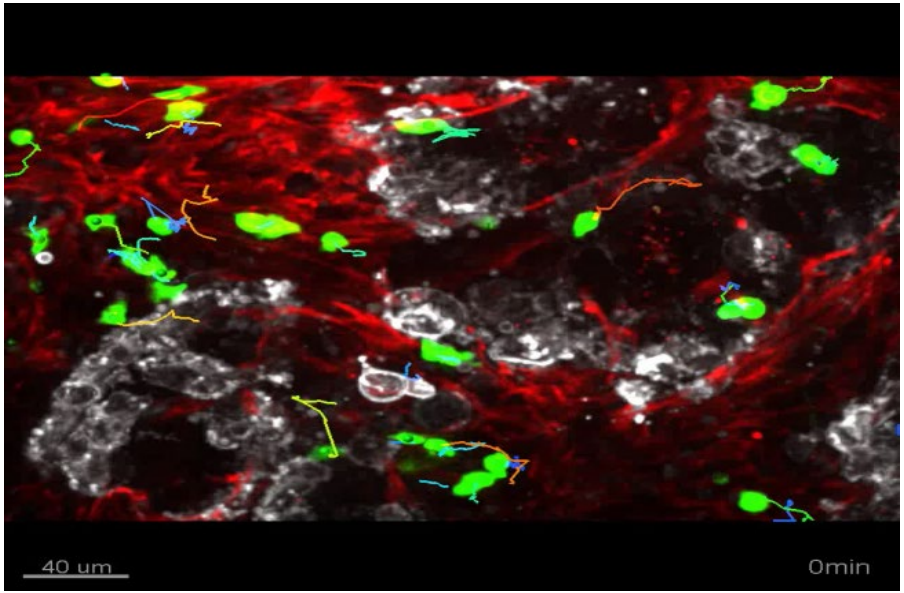
Asperti-Boursin et al. J Exp Med 2007

Monitoring T cell activity in human tumor slices



Human lung tumor slice

T cells Stroma Tumor

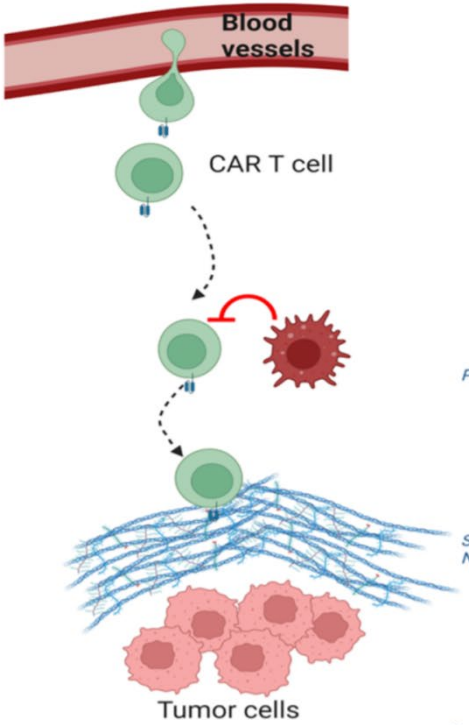


1 image / 20 sec. 20 min

Salmon et al, J clin Invest, 2012

✓ T cell migration is constrained to the stroma

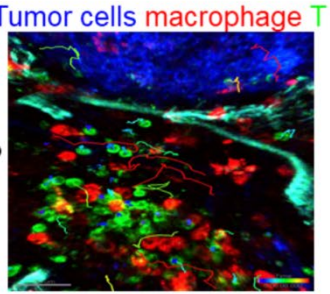
Obstacles to T cell migration and interaction with tumor cells

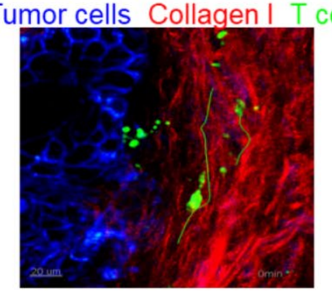


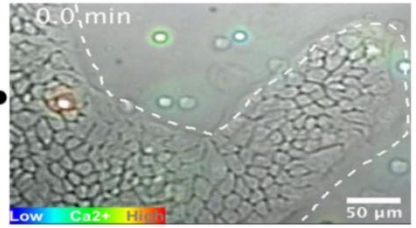
Suppressive macrophages
Peranzoni et al. PNAS 2018

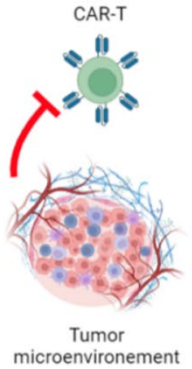
Extracellular matrix
Salmon et al. JCI 2012
Nicolas-Boluda eLife 2021

Lack of adhesion molecules
Kantari-Mimoun et al. CIR 2021



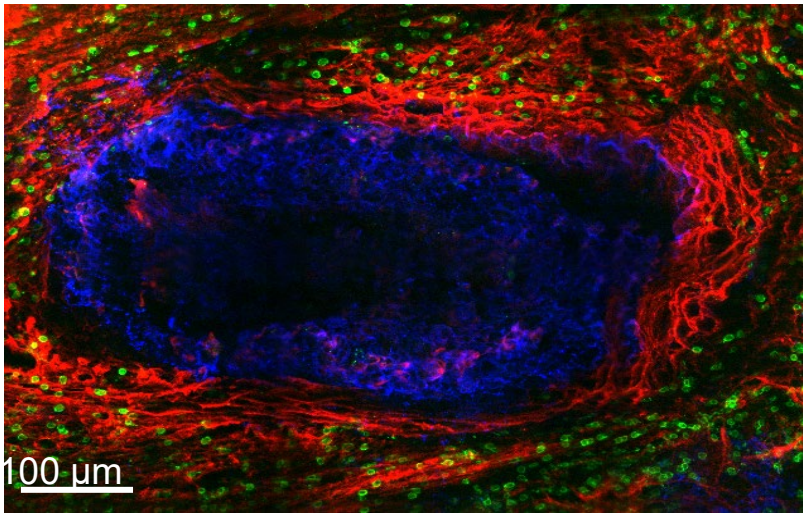


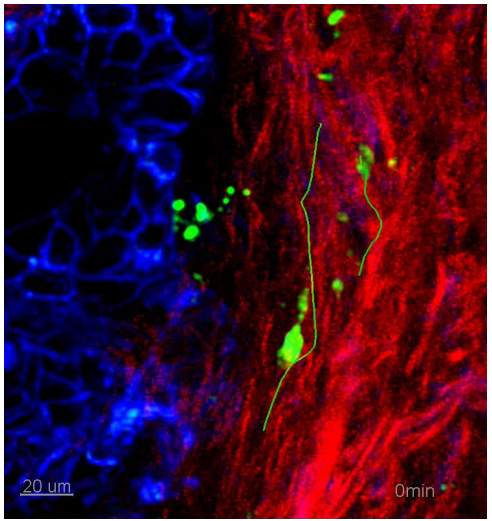


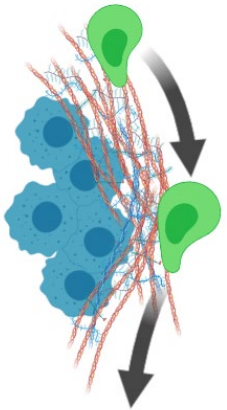


Dense matrix fibers prevent T cells from reaching tumor cells

Collagen I Tumor cells CD3





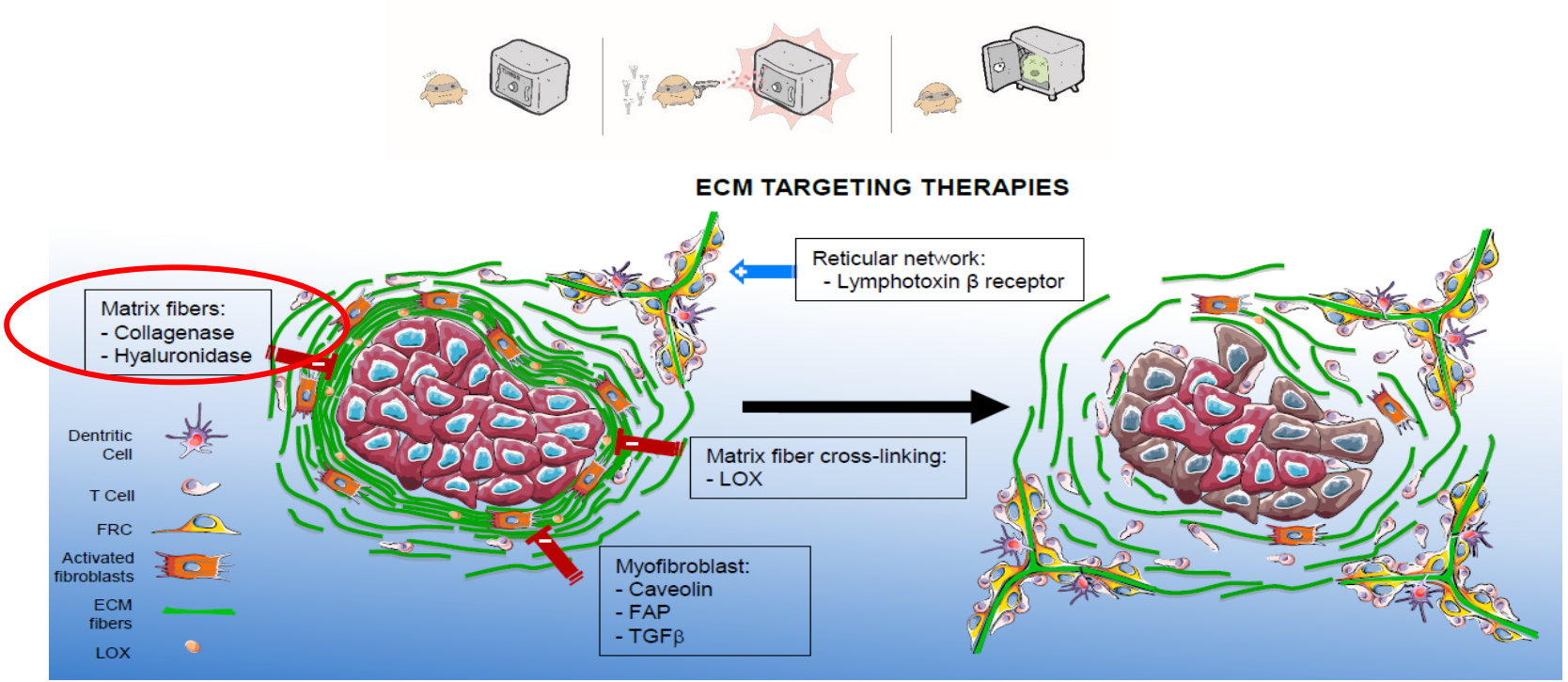


Human pulmonary tumor

Salmon et al, J clin Invest, 2012
Bougherara et al, Front Immunol, 2015

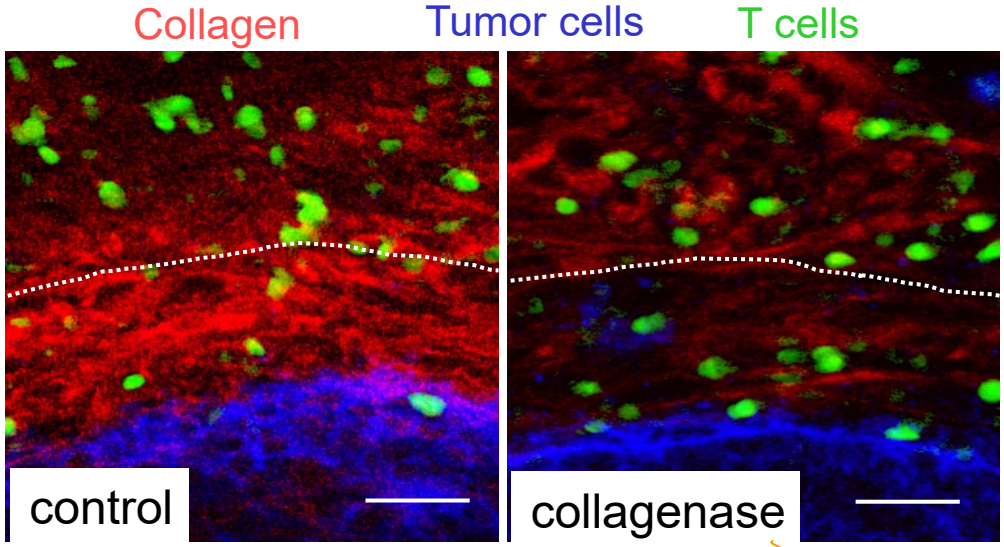
✓ In human lung tumors T cells are sequestred in the stroma

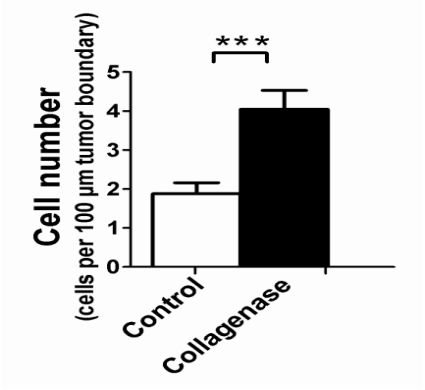
Overcoming a dense stroma: Targeting the tumor ECM

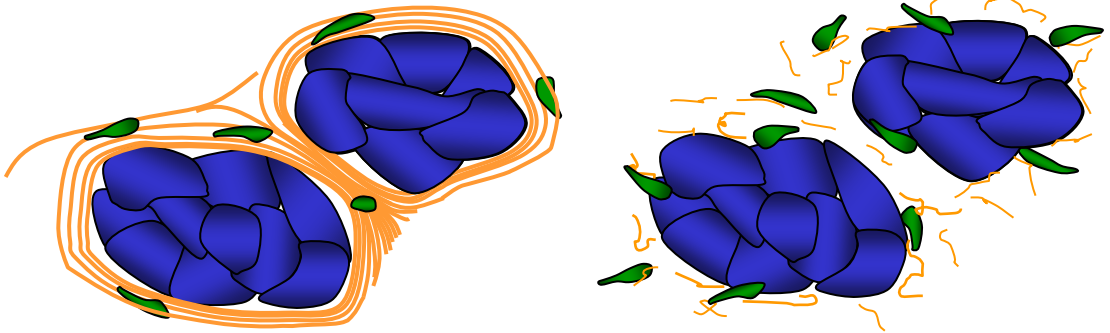


Peranzoni et al, Cell Mol Life Sci, 2013

Collagenase enhances the number of T cells in contact with tumor cells



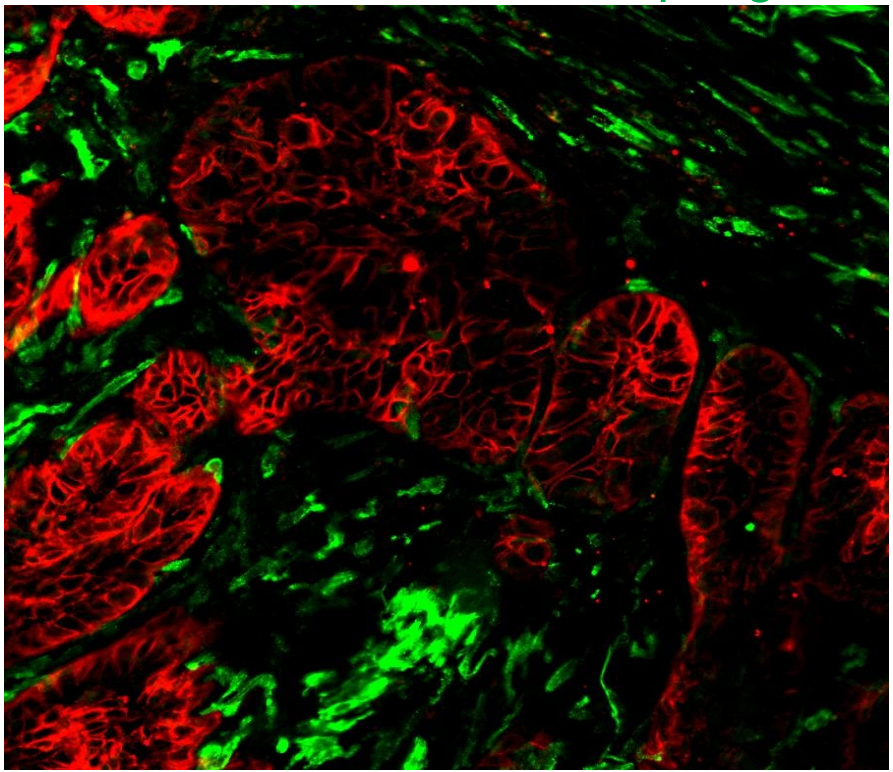




Salmon et al, J clin Invest, 2012

Macrophages are enriched in the tumor stroma of lung tumors

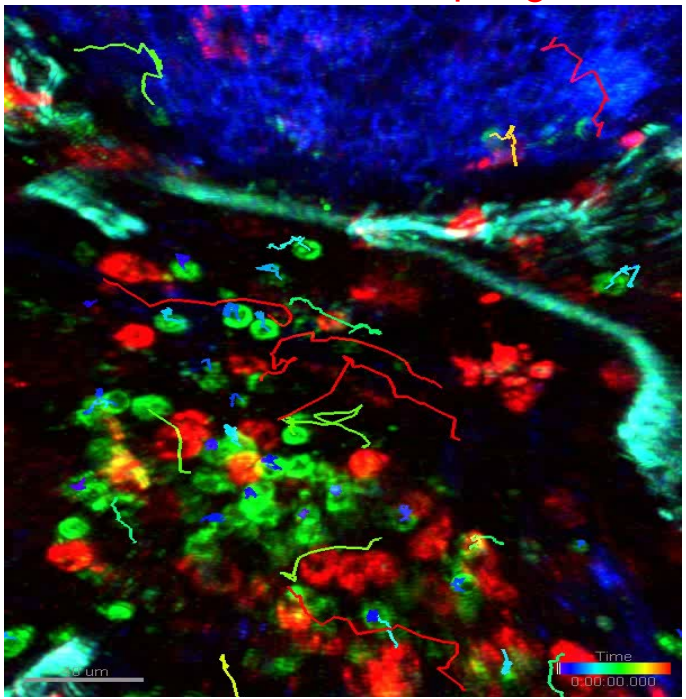
Tumor cells CD206 macrophages



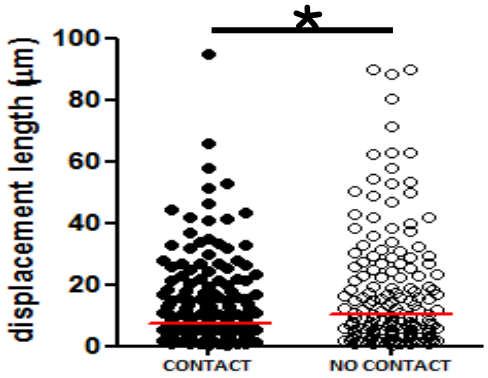
Human pulmonary tumor

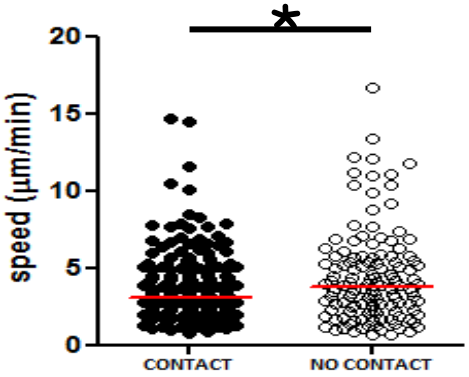
Stable interactions between CD206+ macrophages and CD8+ T cells

Tumor cells CD206 macrophage CD8 T cells



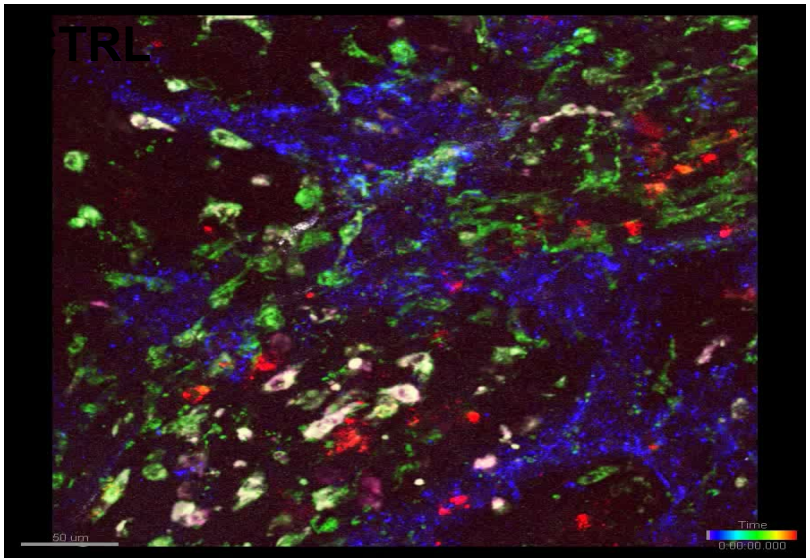
Human pulmonary tumor





Macrophages depletion increases T cell motility

Tumor cells CD8 T cells macrophages



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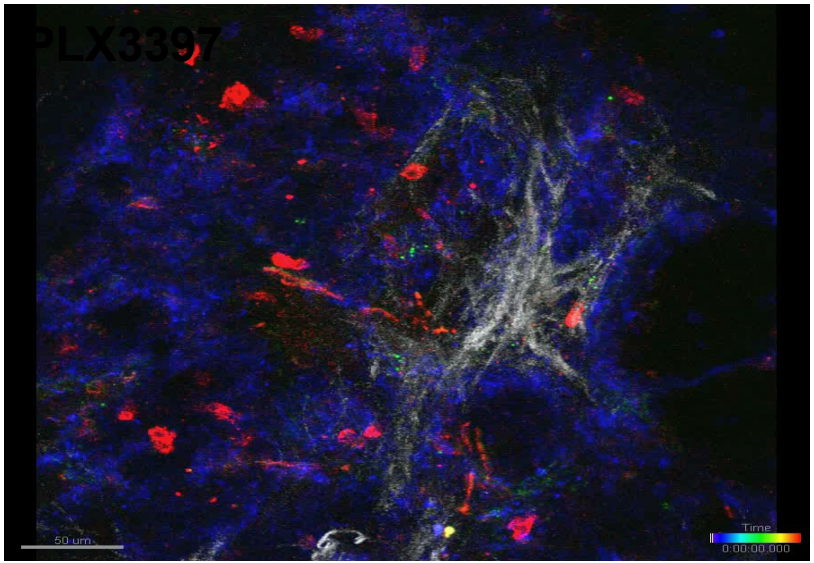
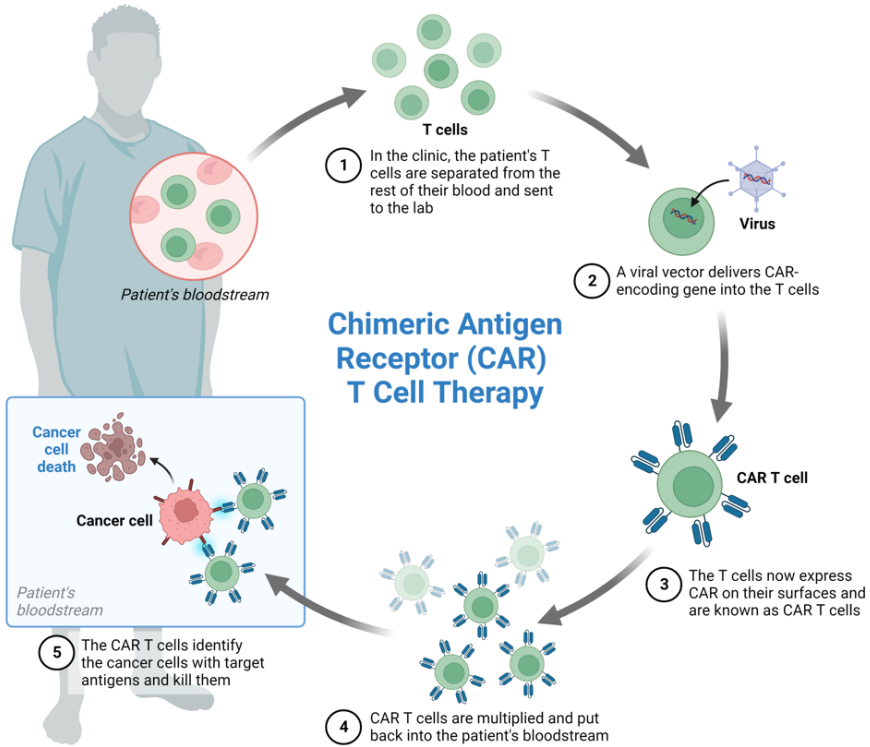


Macrophage-depletion (PLX3397)

PLX3397: *Tyrosine kinase inhibitor of the colony-stimulating factor-1 receptor (CSF1R).*

Peranzoni et al, PNAS, 2018

CAR T cell therapy



Disease	Response Rate
	percent
Leukemia	
B-cell acute lymphoblastic leukemia (in adults)	83–93
B-cell acute lymphoblastic leukemia (in children)	68–90
Chronic lymphocytic leukemia	57–71
Lymphoma	
Diffuse large B-cell lymphoma	64–86
Follicular lymphoma	71
Transformed follicular lymphoma	70–83
Refractory multiple myeloma	25–100

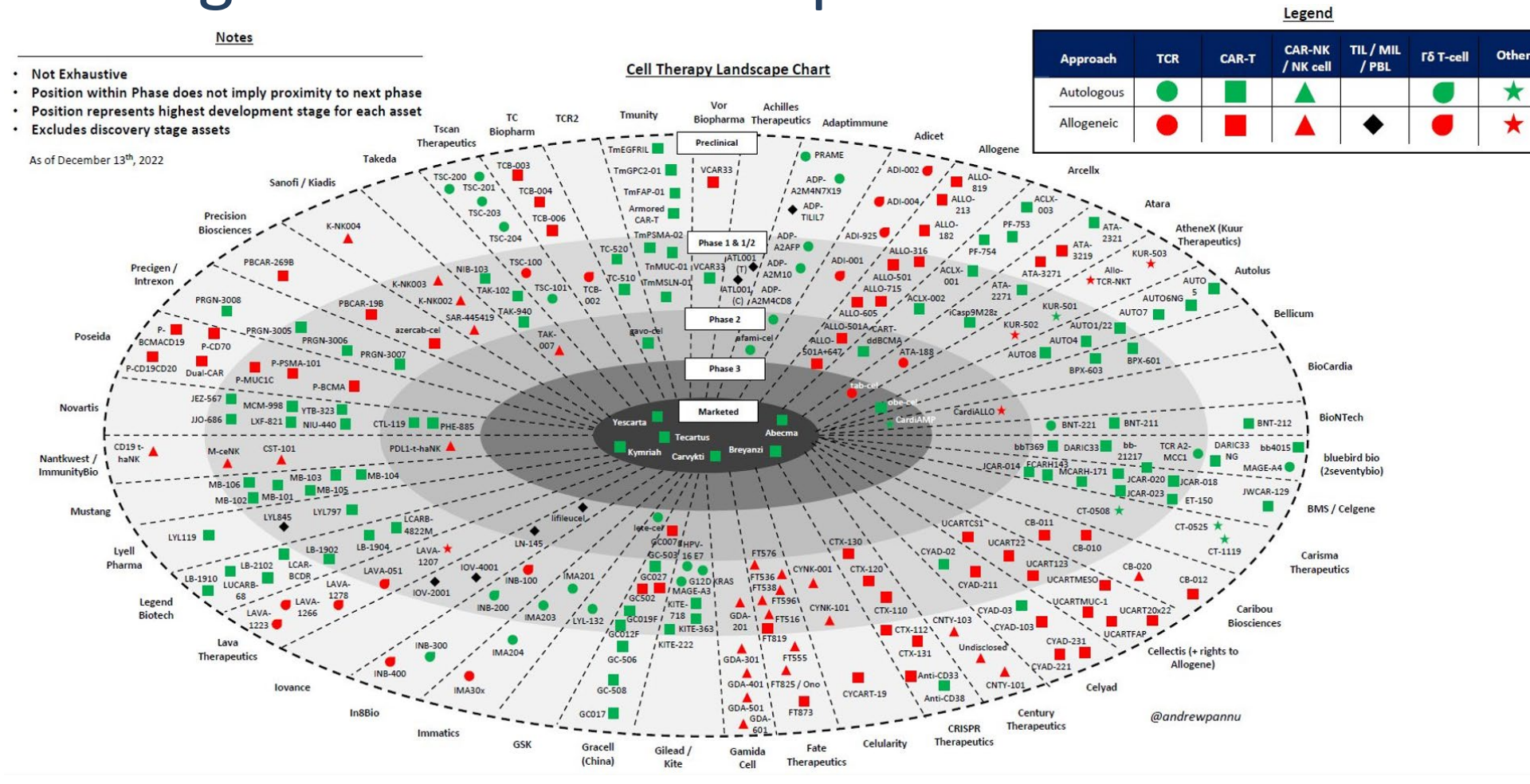
Disease	Response Rate
	percent
Solid tumors	
Glioblastoma	ND
Pancreatic ductal adenocarcinoma	17

*June & Sadelain, **NEJM**, 2017*

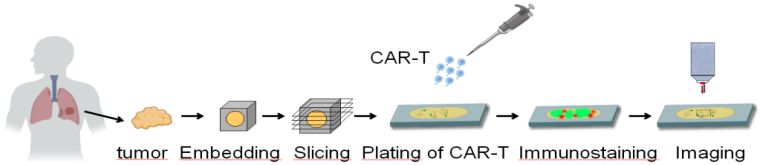
Biorender

- CAR T cells are effective in certain types of B cell malignancies but not yet in solid tumors

A huge number of T cell products to be tested



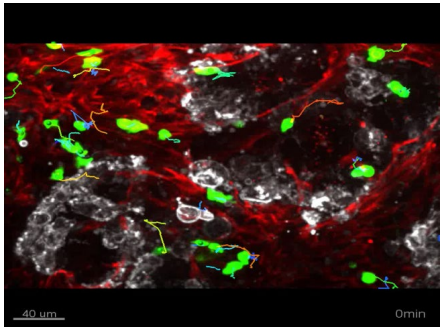
Monitoring CAR T cell activity in human tumor slices



Time *Min* *Hours* *Days*

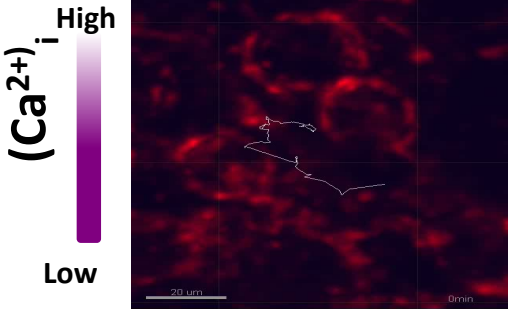
Distribution / Migration

T cells Stroma Tumor



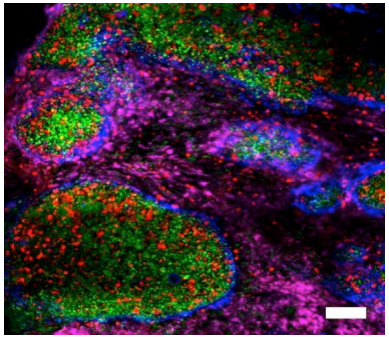
Activation

CAR T Tumor

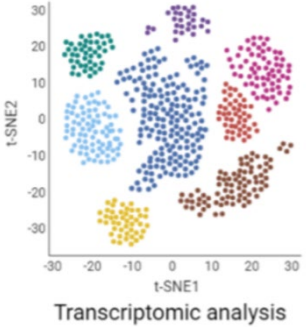


Cytotoxicity

Apoptotic cells CAR T Tumor



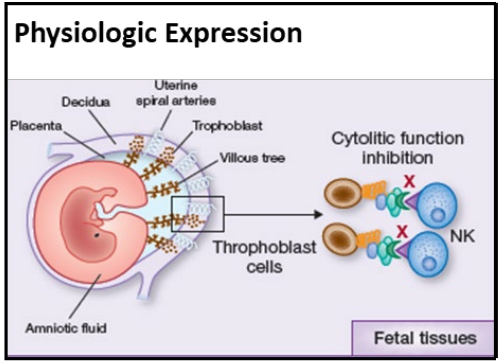
TME remodeling

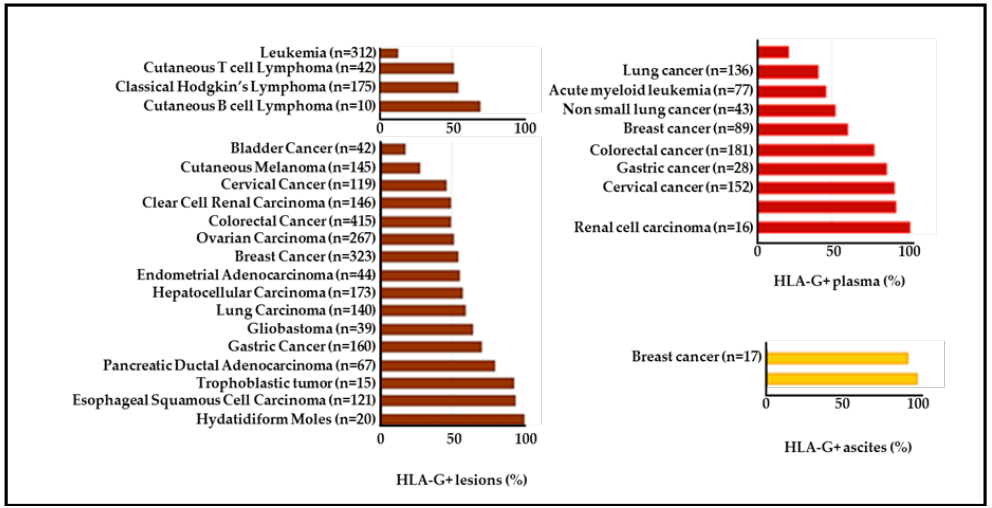


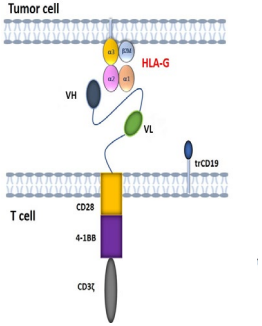
Human lung tumor slice

Irena Rajnpreht's poster

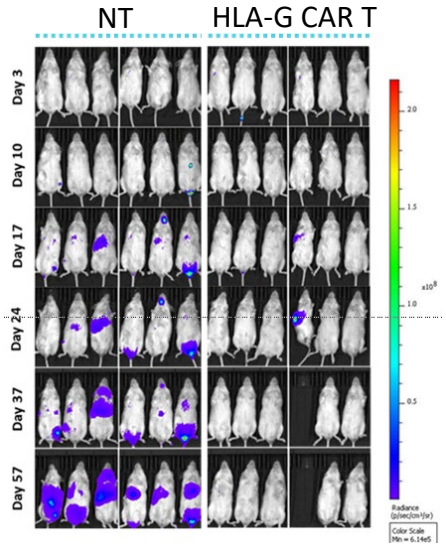
HLA-G: A tumor associated antigen





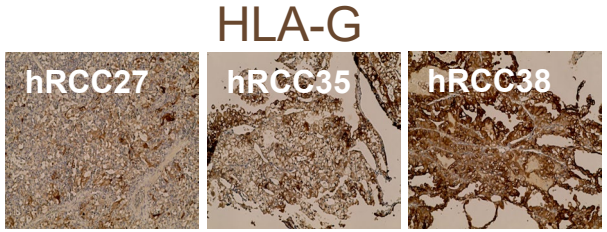


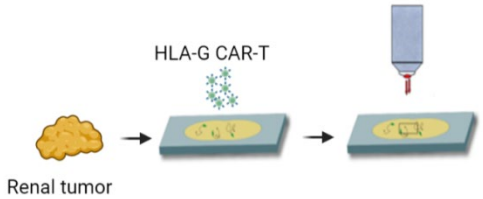
invectys
CANCER IMMUNOTHERAPEUTICS

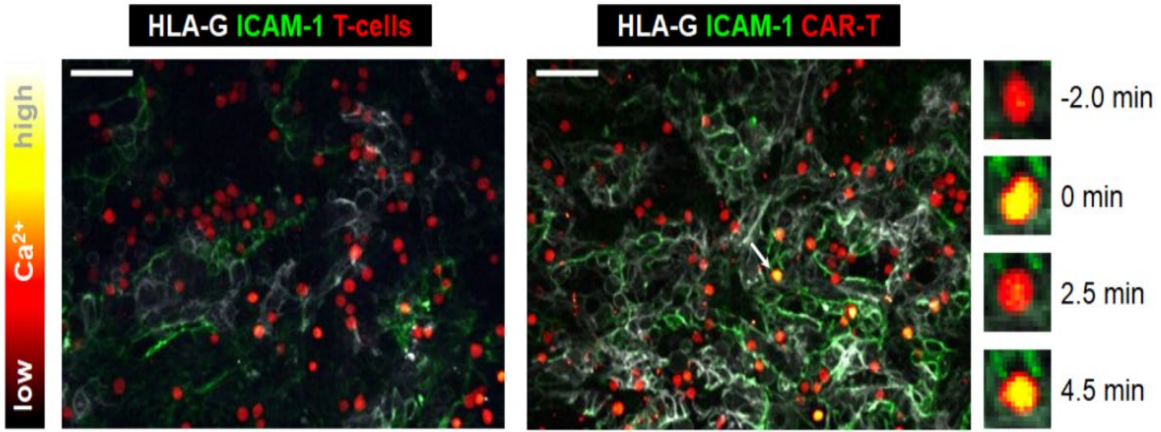


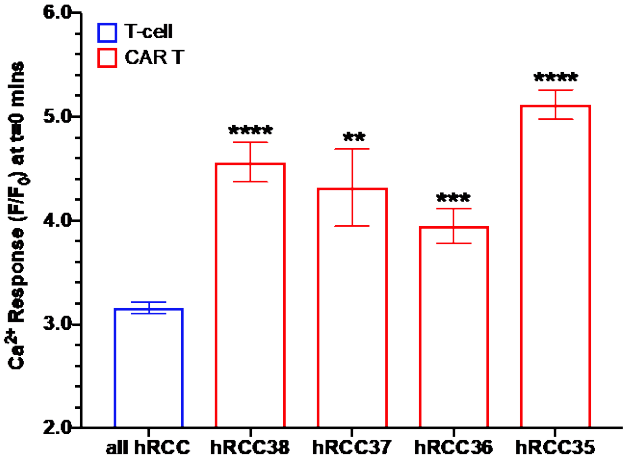
*Anna et al. **JITC**. 2021*

Testing the efficacy of HLA-G CAR T in renal cell carcinomas



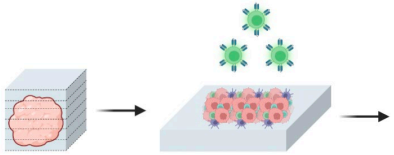






Espie et al. ***Unpublished***

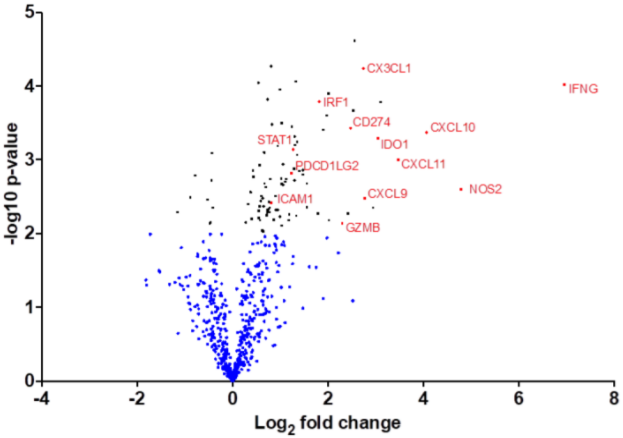
Impact of CAR T cells on the tumor microenvironment

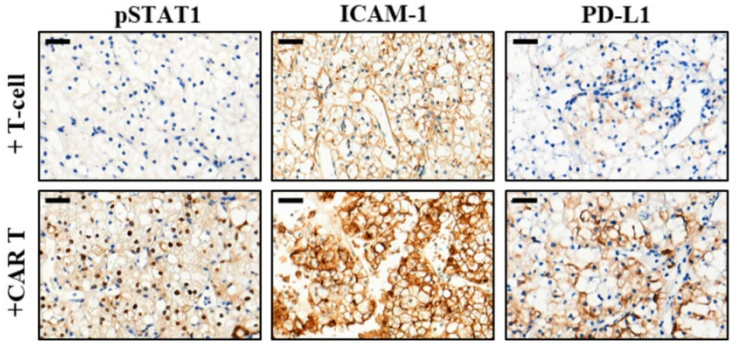


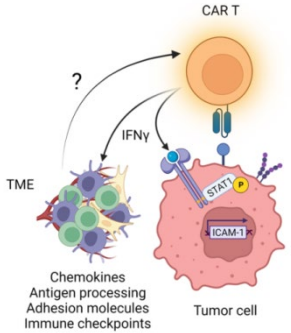
Renal tumor

Co-culture 24hrs
Bulk RNA analysis (nanostring)

CAR T cells vs non-transduced T cells



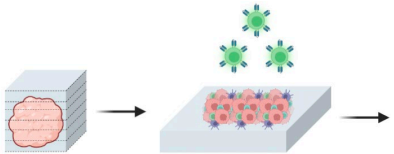




➢ HLA-G CAR T cells reprogram the TME through IFNγ secretion,

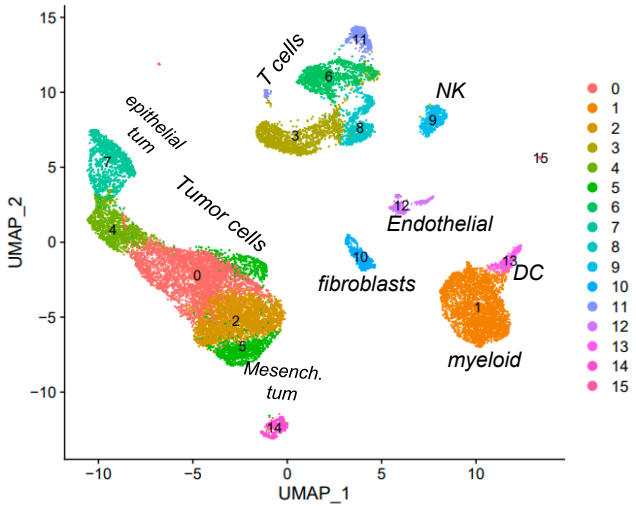
Espie et al. ***Unpublished***

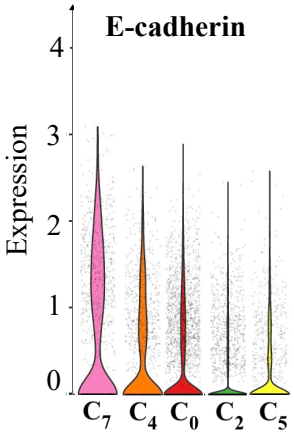
Impact of CAR T cells on tumor cells

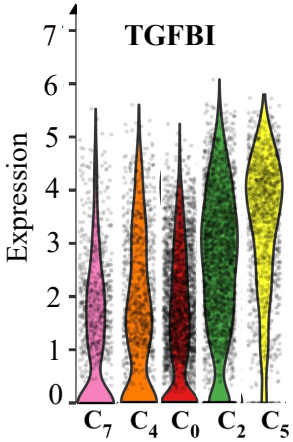


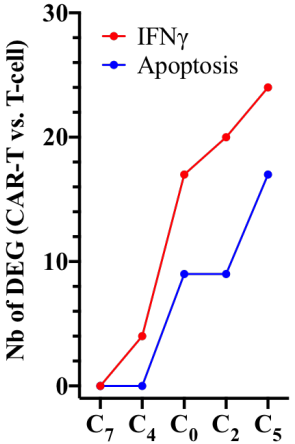
Renal tumor

Co-culture 24hrs
scRNAseq (10x genomics)





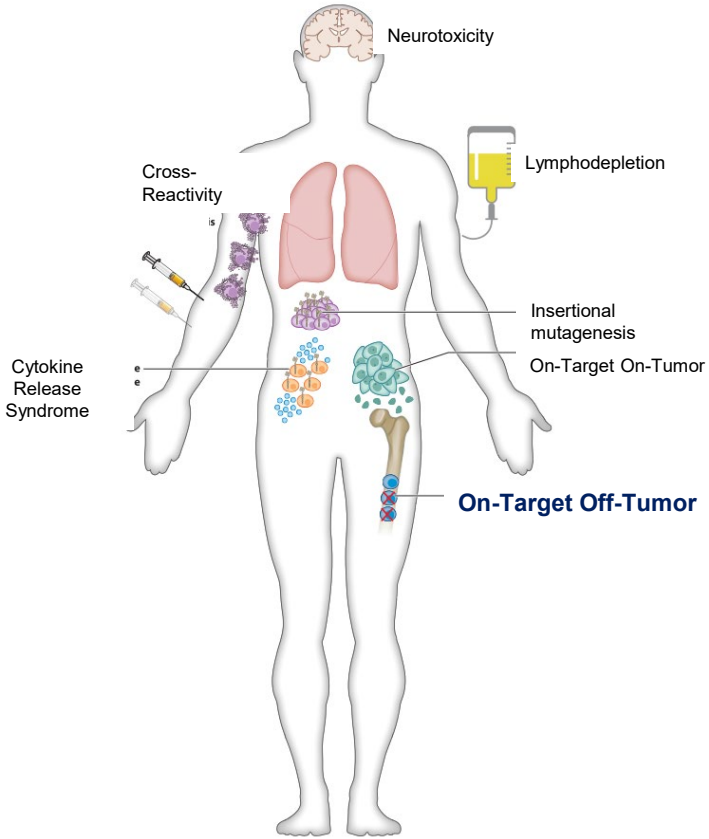




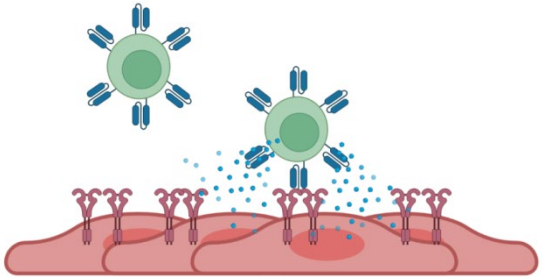
➢ HLA-G CAR T cells reprogram certain types of renal tumor cells

Espie et al. ***Unpublished***

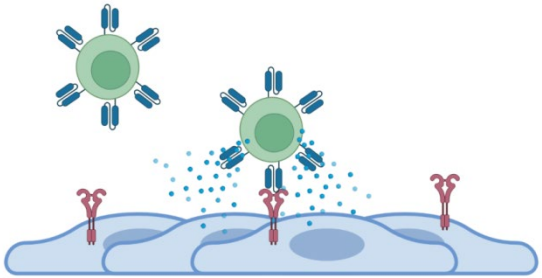
Toxicities induced by CAR T cells



On-target off-tumor



Tumor cells



Healthy cells

Destruction of healthy cells that express the target

Potential risks of on-target off-tumor toxicity

Molecular Therapy 2010

Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing *ERBB2*

Richard A Morgan[1], James C Yang[1], Mio Kitano[1], Mark E Dudley[1], Carolyn M Laurencot[1] and Steven A Rosenberg[1]

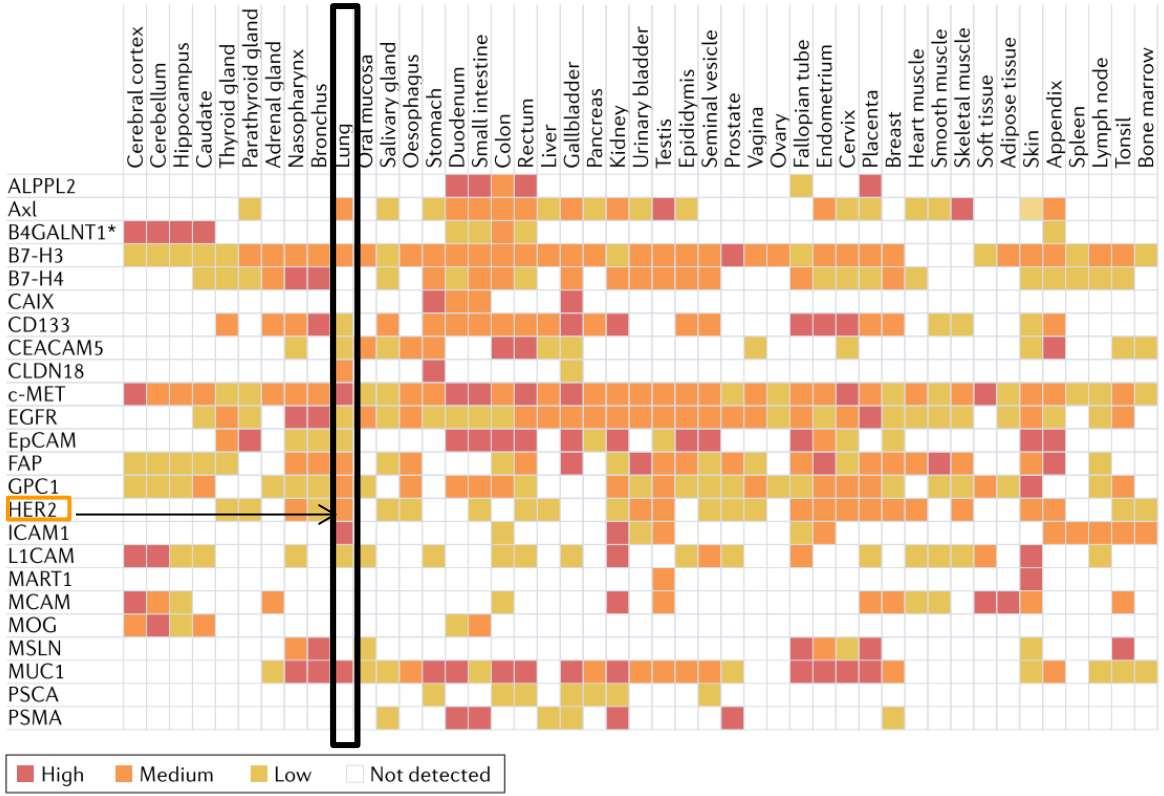
[1]*Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

In an attempt to treat cancer patients with *ERBB2* overexpressing tumors, we developed a chimeric antigen receptor (CAR) based on the widely used humanized monoclonal antibody (mAb) Trastuzumab (Herceptin). An optimized CAR vector containing CD28, 4-1BB, and CD3ζ signaling moieties was assembled in a γ-retroviral vector and used to transduce autologous peripheral blood lymphocytes (PBLs) from a patient with colon cancer metastatic to the lungs and liver, refractory to multiple standard treatments. The gene transfer efficiency into autologous T cells was 79% CAR^+ in $CD3^+$ cells and these cells demonstrated high-specific reactivity in *in vitro* coculture assays. Following completion of nonmyeloablative conditioning, the patient received 10^{10} cells intravenously. Within 15 minutes after cell infusion the patient experienced respiratory distress, and displayed a dramatic pulmonary infiltrate on chest X-ray. She was intubated and despite intensive medical intervention the patient died 5 days after treatment. Serum samples after cell infusion showed marked increases in interferon-γ (IFN-γ), granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-10, consistent with a cytokine storm. We speculate that the large number of administered cells localized to the lung immediately following infusion and were triggered to release cytokine by the recognition of low levels of ERBB2 on lung epithelial cells.

binding. *ERBB2* overexpression/amplification occurs in ~15–25% of human breast cancer patients, and is associated with more aggressive disease.[4] A proportion of other human cancers are also associated with *ERBB2* gene amplification and protein overexpression; including cancers of the colon, ovary, stomach, kidney, melanoma, and others.[5–7] Investigation of agents that target the *ERBB2* protein led to the development of Trastuzumab (Herceptin), a humanized monoclonal antibody (mAb) that binds to the extracellular domain of the receptor.[8] Trastuzumab has been shown to be of clinical benefit for metastatic breast cancer patients with *ERBB2* overexpression/amplification, either alone or in combination with chemotherapy regimens.[9,10] *ERBB2* has also been the target of several cancer vaccine trials,[11–13] as well as, adoptive cell therapy using anti-ERBB2 cytotoxic T lymphocyte lines.[14]

Adoptive cell therapy has emerged as the most effective treatment for patients with metastatic melanoma. Adoptive cell therapy using tumor-reactive autologous tumor infiltrating lymphocytes (TIL) in combination with nonmyeloablative but lymphodepleting conditioning resulted in 50% objective clinical regression in melanoma patients.[15] Intensifying the lymphodepletion by adding total-body irradiation to the chemotherapy conditioning regimen improved the objective response rate to 72%.[16] This potent therapy, however, has been limited by the requisite surgery to procure tumor-reactive TIL, by *ex vivo* identification and expansion of these cells, and by the failure to reproducibly isolate similar cells from common epithelial tumors.

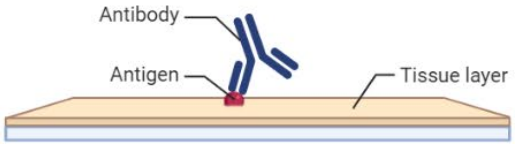
The transfer of genes into primary human lymphocytes permits the introduction of tumor antigen receptor molecules that can endow the engineered cell with antitumor specificity.[17–19] We



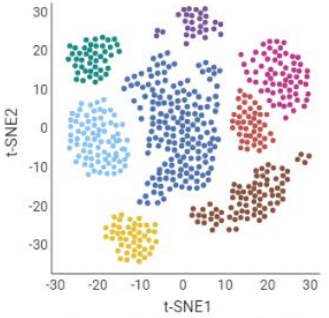
Flugel et al., ***Nat. Rev. Clin. Oncol***. *2023*

Tools to assess target antigen expression in human cells and tissues

Cell microarray assay



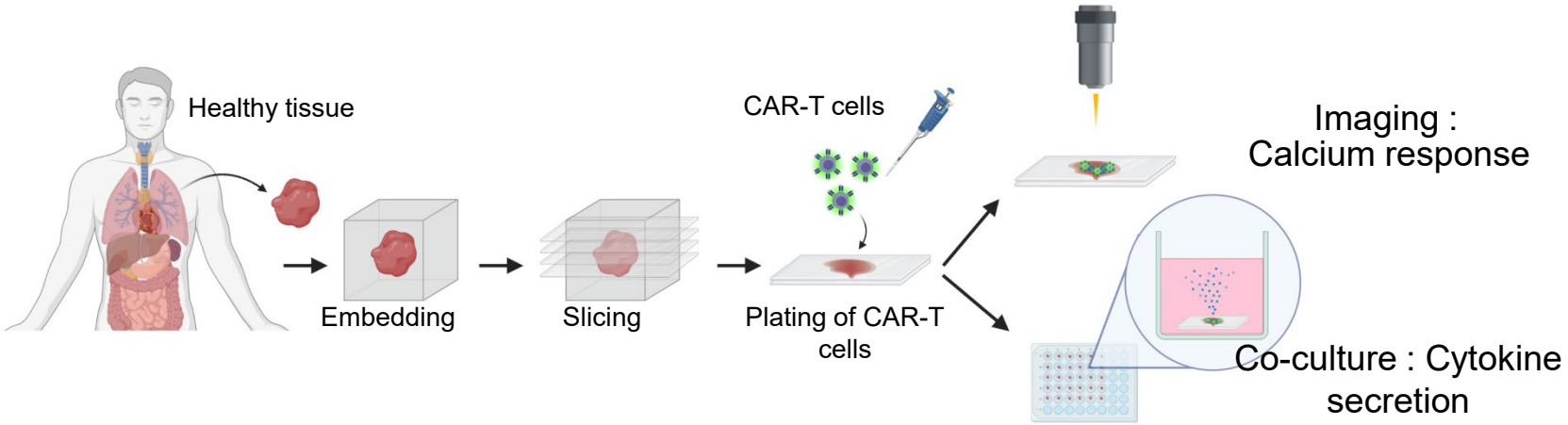
Immunohistochemistry



Transcriptomic analysis

- Not sufficient to prove that CAR recognition will have deleterious effects

Testing CAR T cell reactivity against healthy tissues



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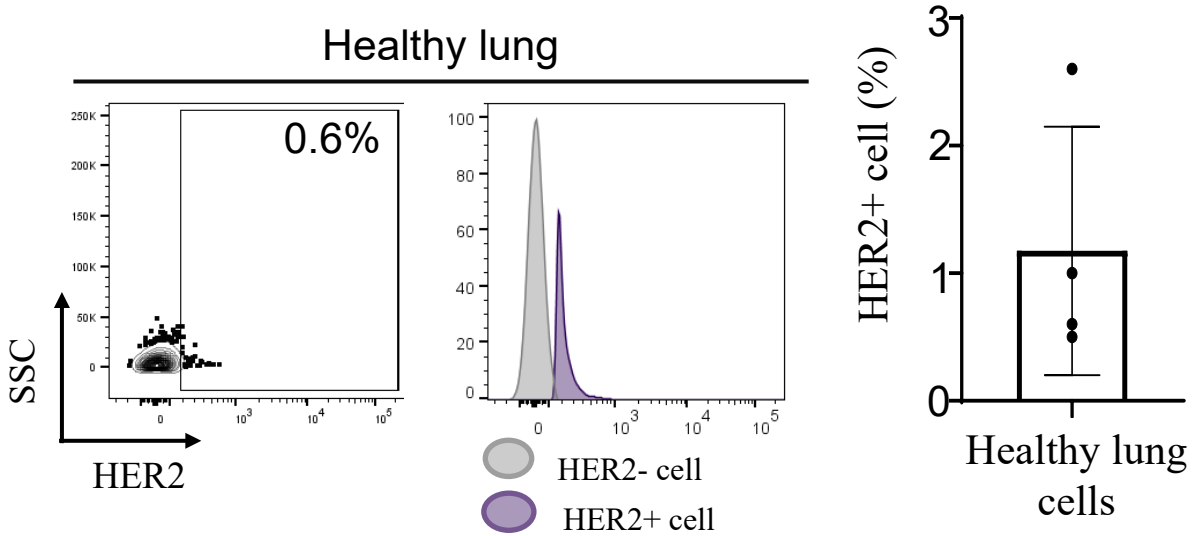
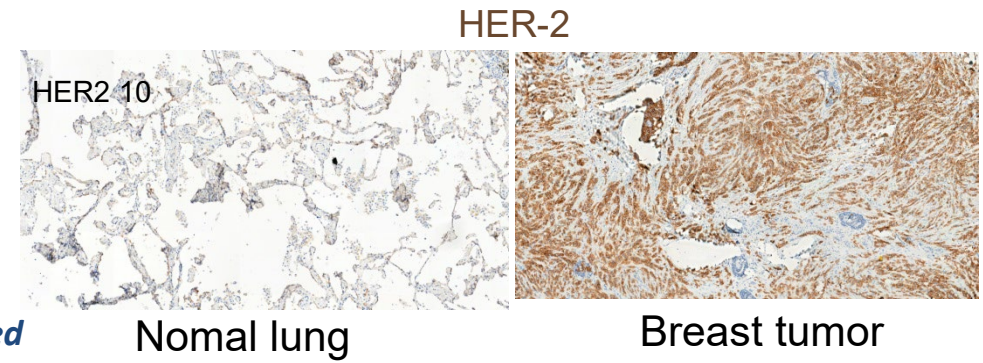
LUPO-MANSUET Audrey

T²EVOLVE

A preclinical platform that allows maintenance of intact fresh human tissues to assess on-target off-tumor toxicity of CAR T cells

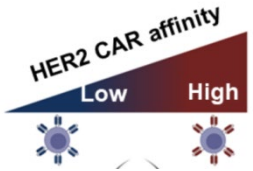
Healthy lungs contains few low-expressing HER2^{+} cells

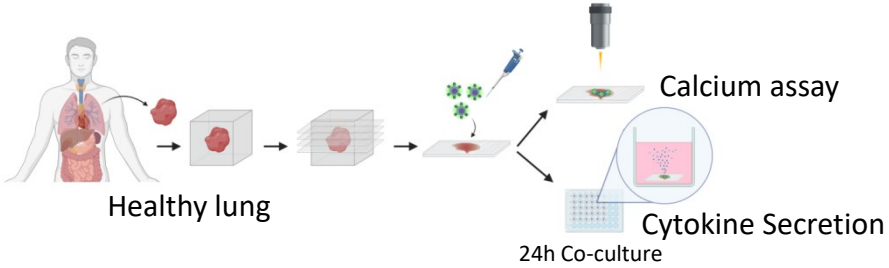


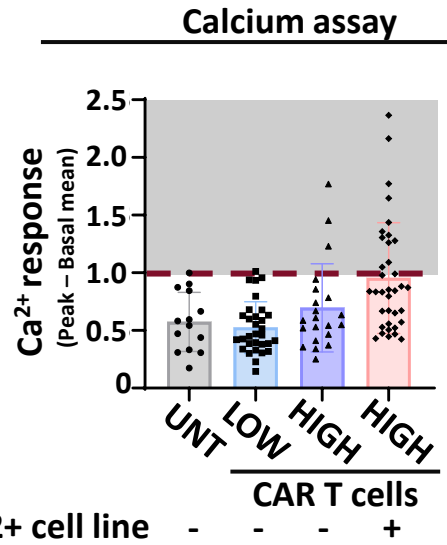


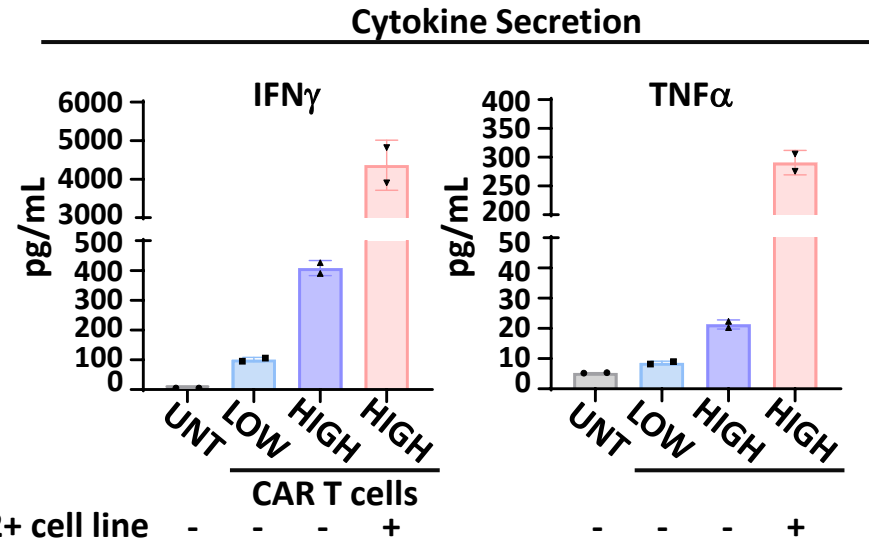
Machado et al. ***Unpublished***

HER-2 CAR-T activity in lung slices







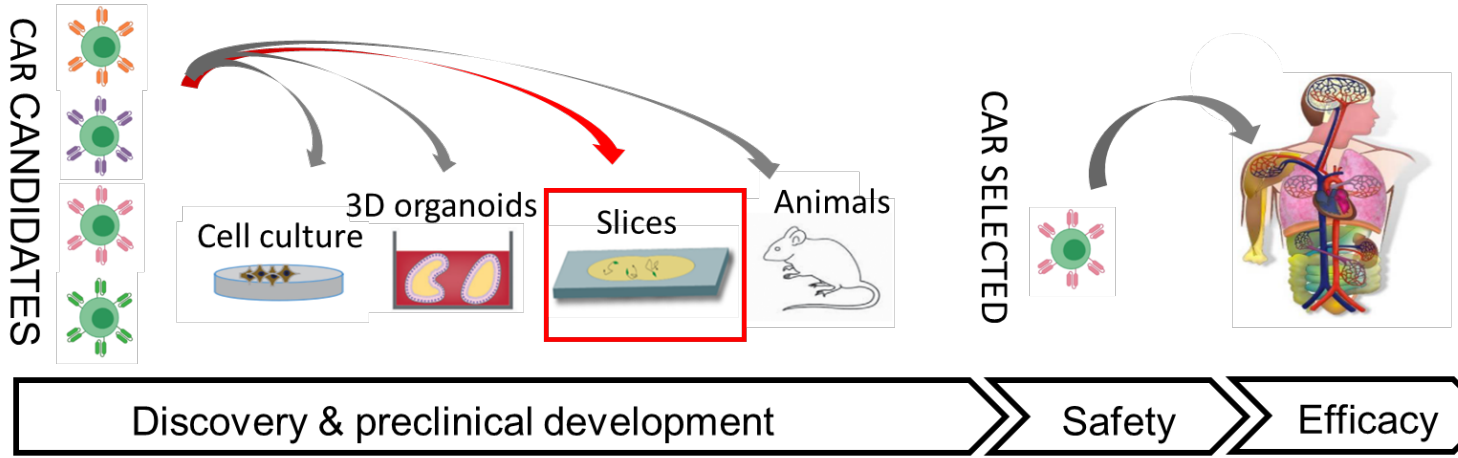


High affinity HER2 CAR-T generate a stronger response than low affinity HER2 CAR-T

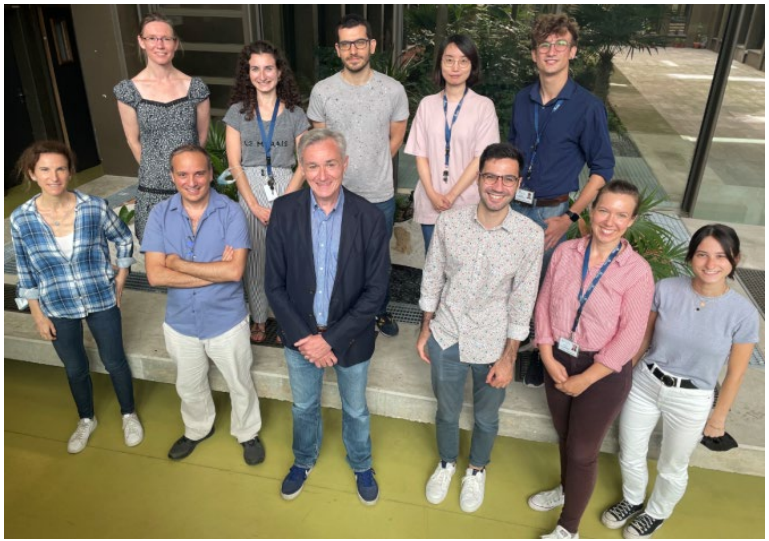
Machado et al. ***Unpublished***

The tissue slice assay: a new model to test/predict the safety/efficacy of engineered T cells

Advantages	Preserved tumor organisation Short generation time Good access to the content of the tissue Amenable to imaging microscopy
Limitations	Possible loss of important components during slicing, O2… Difficult to maintain several days in culture



Cancer and Immune Response Team



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Funding

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