9° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Bologna, Aula "G. Prodi", 19-20 maggio 2025

Harnessing the tumor microenvironment by Genome Editing of T cells

Chiara Bonini

IRCCS San Raffaele Scientific Institute, Milan, Italy

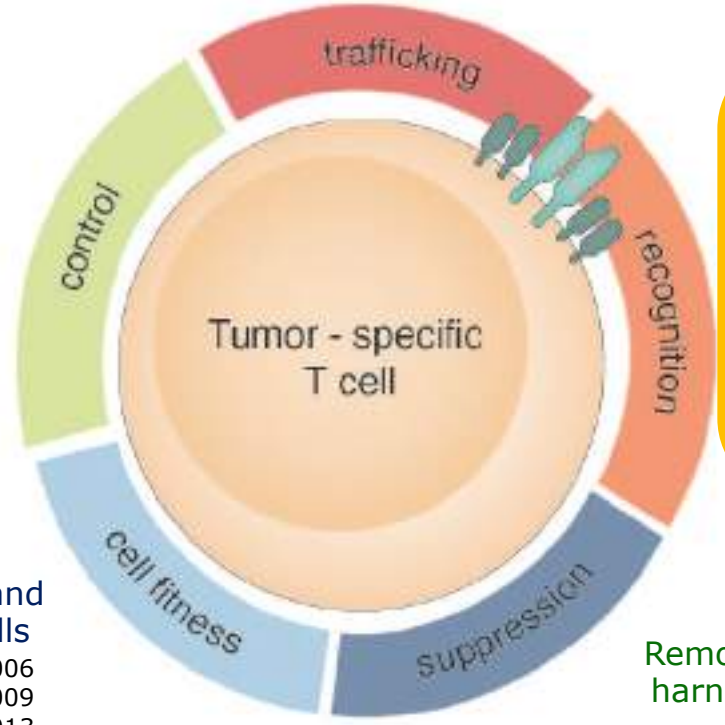
Conflicts of interest

- Research Contract with Intellia Therapeutics
- Member of Advisory Boards/Consultant/Speaker: Intellia, Kite/Gilead, Miltenyi, QuellTx, Janssen, Chroma, Genyo, Smart-immune, GentiBio, Pancancer-T, Alia
- Patents (Adoptive T cell therapy field)

ACT with genetically engineered lymphocytes: challenges

Increasing the safety profile (suicide genes)

Bonini et al, Science 1997
Bonini et al, Nat. Med. 2003
Recchia et al, PNAS 2006
Ciceri et al, Blood 2007
Ciceri, Bonini et al, Lancet Oncol. 2009
Zalmoxis EMA conditional approval 2016
Oliveira et al, STM 2015



Redirecting T cell specificity (CAR & TCR)

Casucci et al, Blood 2013
Norelli et al, Nat Med 2018
Provasi, Genovese et al Nat Med 2012
Mastaglio et al, Blood 2017
Greco et al Sci Transl Med 2022
Ruggiero et al, Sci Transl Med 2022
Potenza, Balestrieri et al. Gut 2023
Manfredi et al., Science Advances 2023
US P.A.N. 12/927,292
Patent N. 9937207

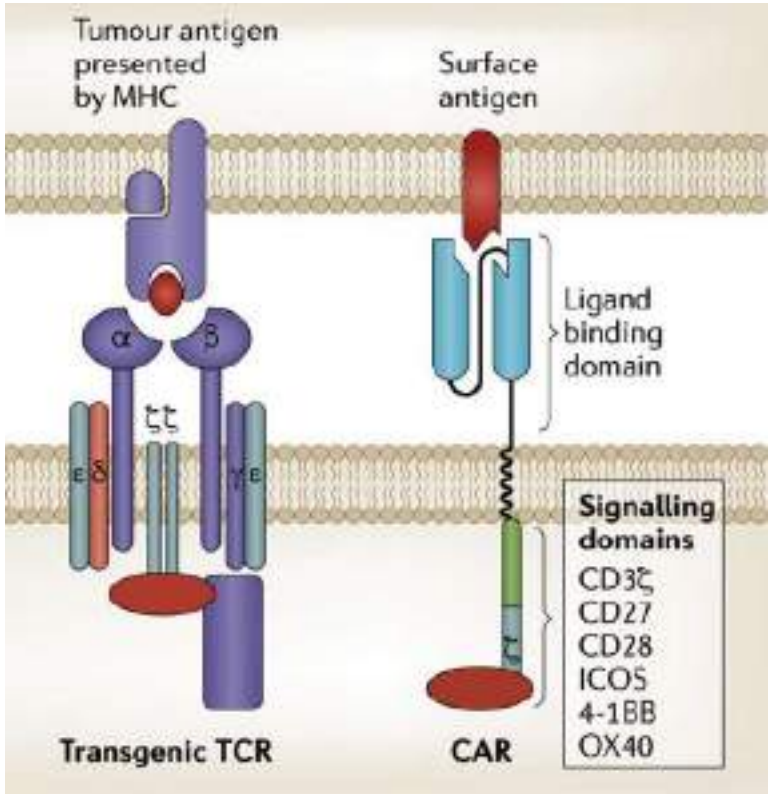
Improving function and persistence of T cells

Bondanza et al, Blood 2006
Kaneko et al, Blood, 2009
Cieri et al, Blood, 2013
Cieri et al, Blood 2015
Oliveira et al, Sci Transl Med 2015
Arcangeli et al., JCI 2022
Bove et al., JITC 2023
WO 2007017915
WO 2021219758

Remodeling of microenvironment harnessing immune suppression

Noviello, Manfredi et al Nat Comm 2019
Toffalori et al, Nat Med 2019
Greco et al, Sci Transl Med 2022
Potenza, Balestrieri et al., Gut 2023
Tassi, Bergamini et al., Front in Imm 2023
Cianciotti et al., Front Imm. 2024
EP21155865.5

Adoptive T cell therapy for cancer: why TCRs?



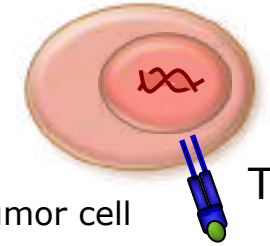
Garber K Nat Biotec 2018

- TCRs can virtually **target every antigen**: tumor associated antigens and neoantigens with relevant roles in oncogenicity
- TCRs have a **higher sensitivity** compared to CARs linked to the proper assembly of the immunological synapse and intracellular signaling machinery
- TCR physiological downstream signaling **prevents** premature **exhaustion** and **drives survival** messages
- Reduced risk of CRS and ICANS

TCR gene EDITING: rational

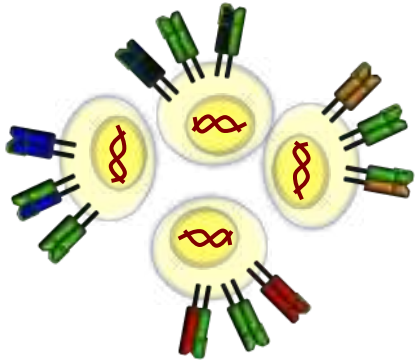
Zinc Finger Nucleases
CRISPR/Cas9

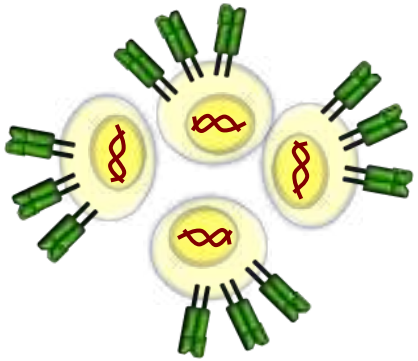
TCR-LV

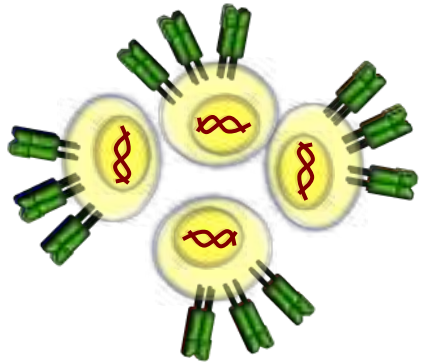


Tumor cell

Tumor antigen



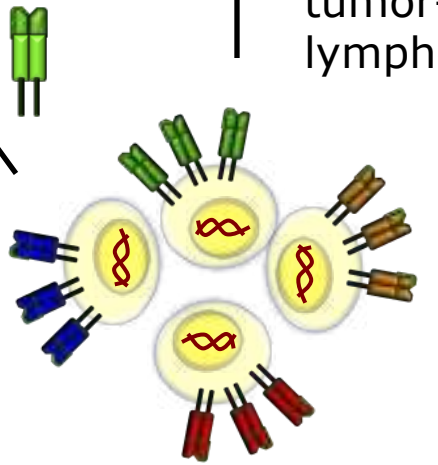




TCR gene transfer

Expansion of tumor-specific lymphocytes

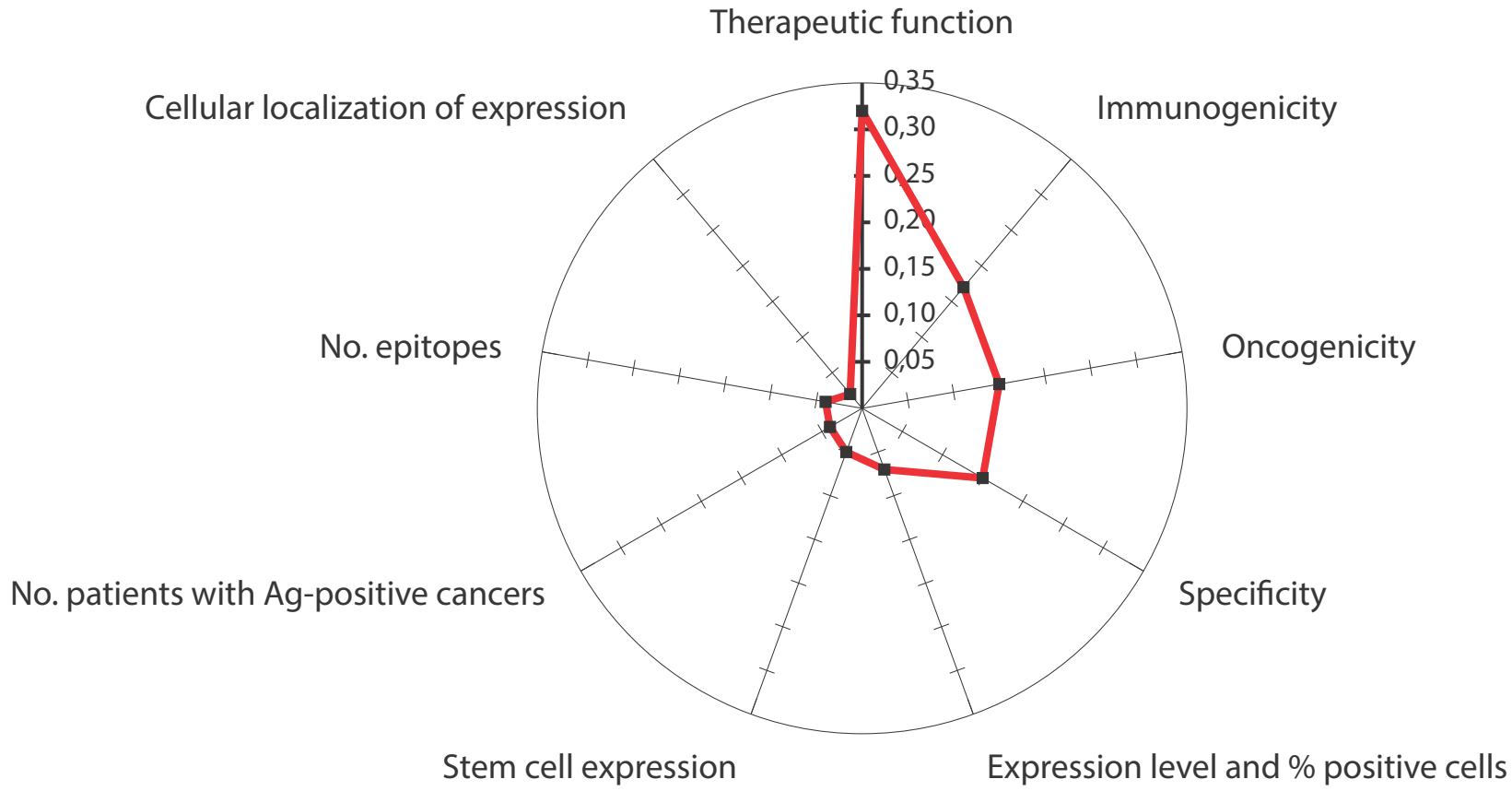
TCR gene EDITING



Provasi, Genovese et al., *Nat Med* 2012
Mastaglio et al., *Blood* 2017
Ruggiero et al., *Sci Transl Med* 2022

Cancer Antigen Pilot Prioritization

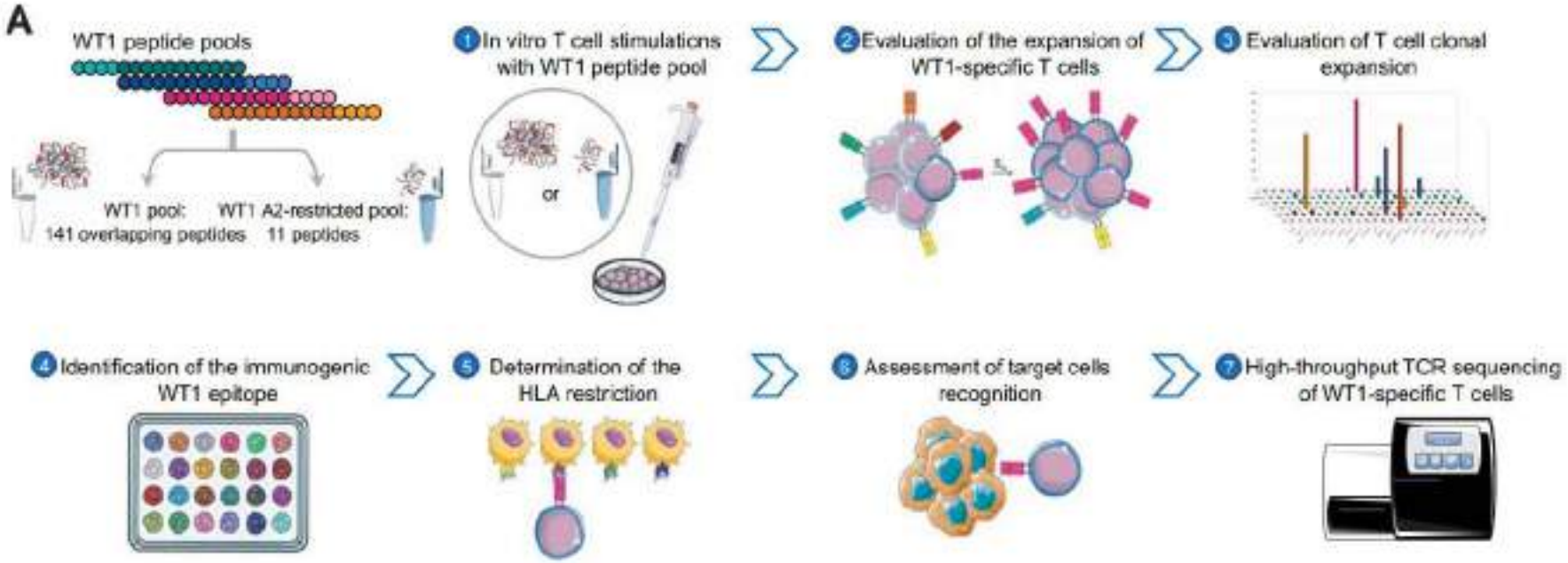
NCI developed a priority-ranked list of cancer antigens based on the likelihood for efficacy in cancer therapy



modified from Cheever M. A., *Hum Canc Bio*, 2009

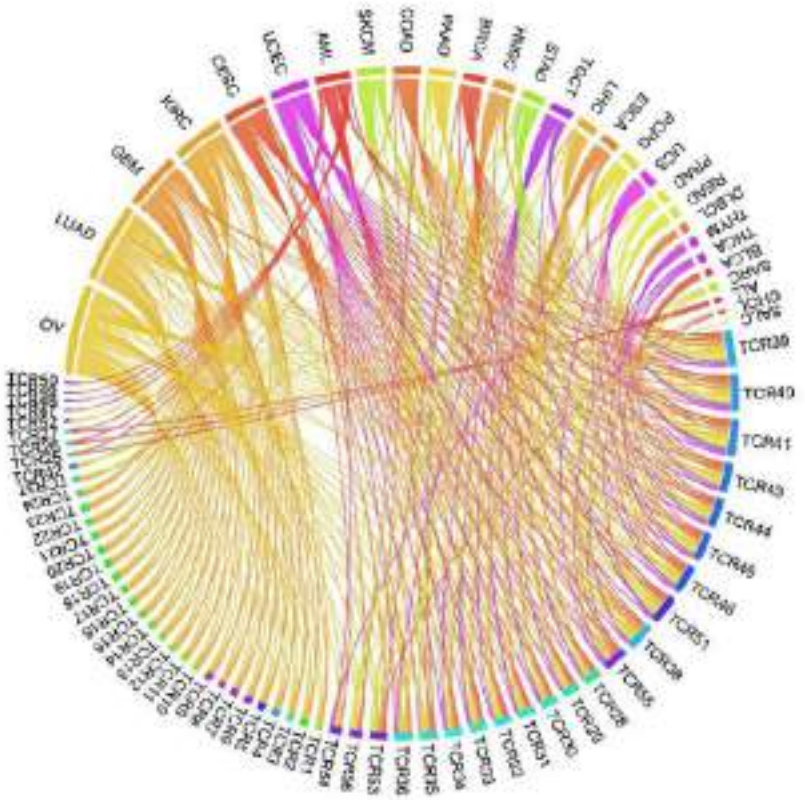
A platform for TCR hunting for adoptive T-cell therapy

Eliana Ruggiero



Ruggiero et al. *Sci Transl Med* 2022

A platform for TCR hunting for adoptive T-cell therapy

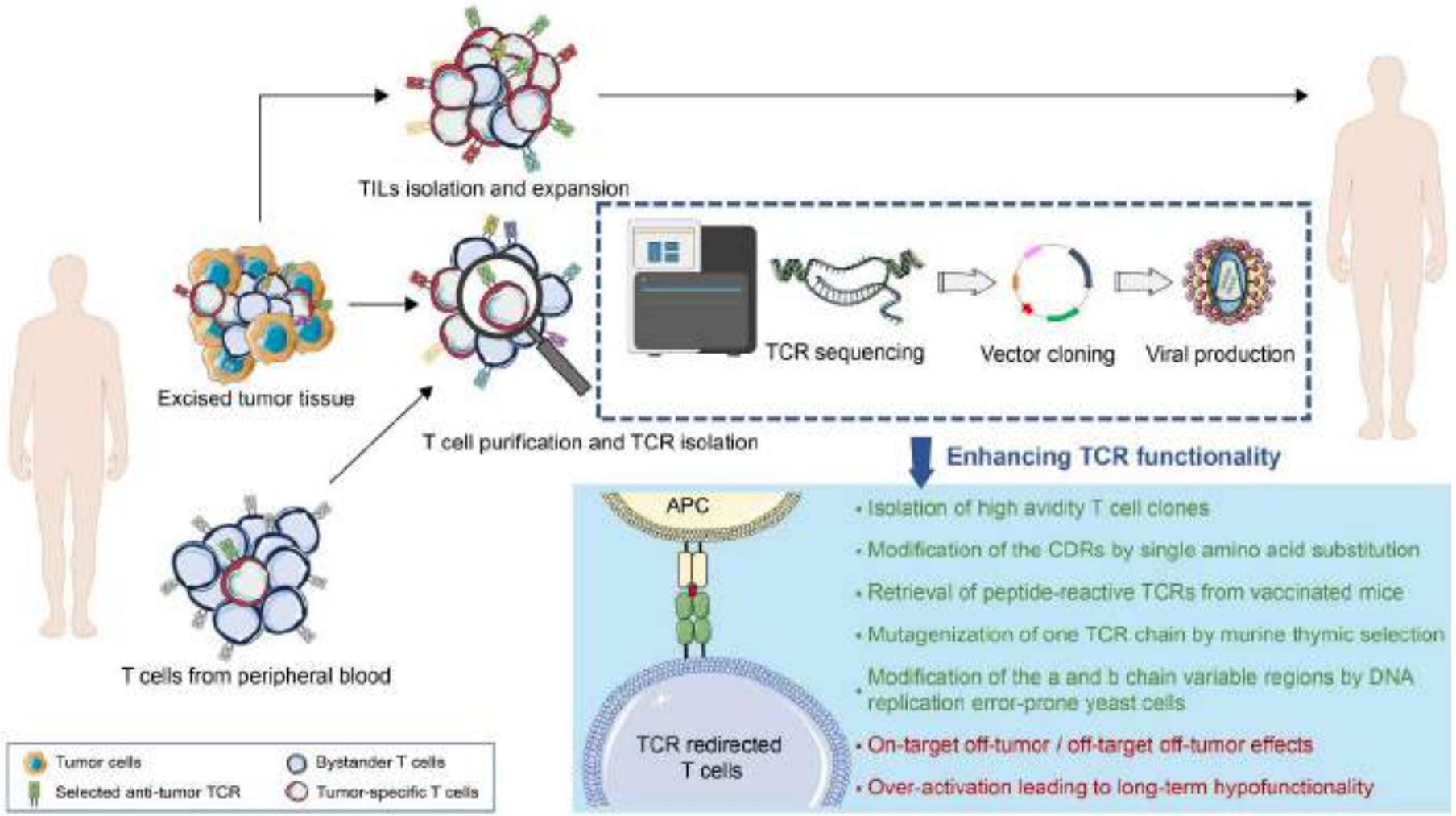


Library of TCRs recognizing peptides restricted to HLA alleles common in the Caucasian population

TCRs recognizing antigens expressed by hematological and solid tumors

Ruggiero et al. *Sci Transl Med* 2022
Manfredi et al., *Sci Adv* 2023

TCR hunting for adoptive T-cell therapy



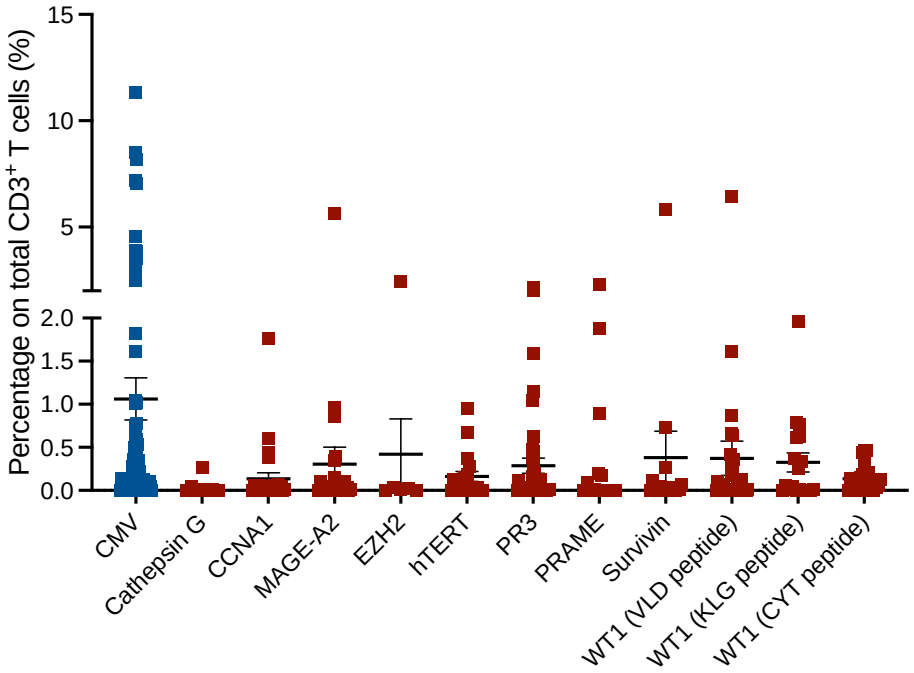
F Manfredi, et al., **Frontiers in Immunol**. *2021*

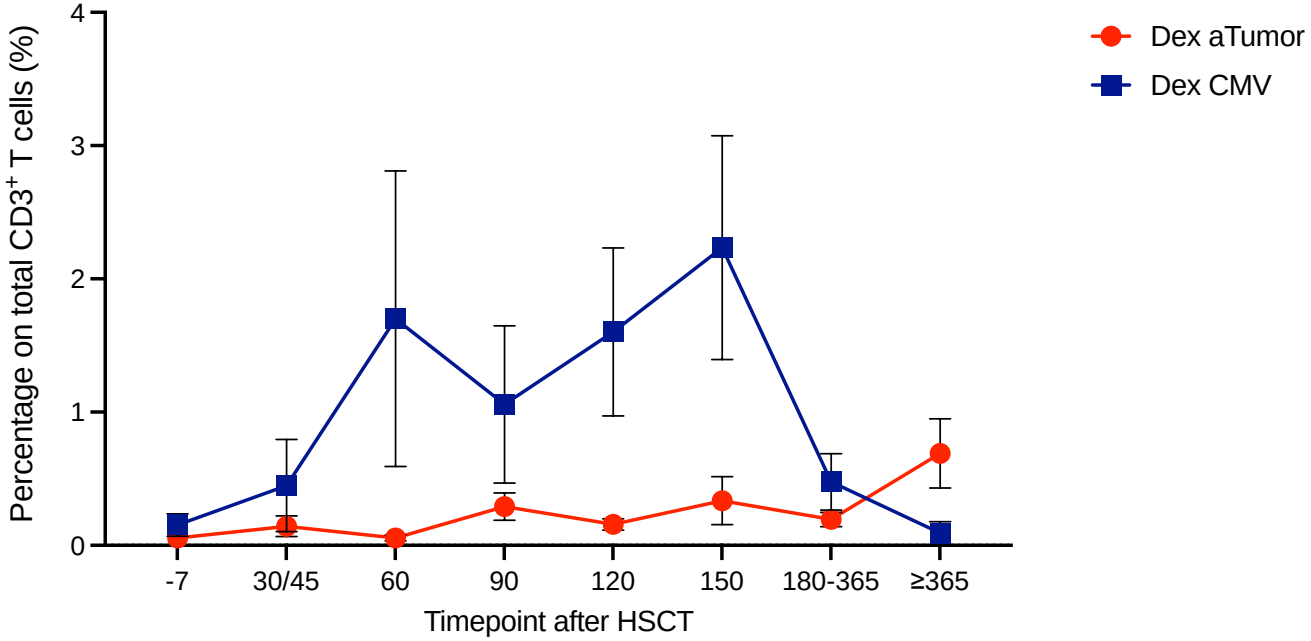
Hunting TAA-specific TCR from cancer patients

Francesco Manfredi

TAA-specific T cells circulate after allo-HSCT and expand in time

Sampling population: N=39 patients affected by blood malignancies who underwent Allo-HSCT

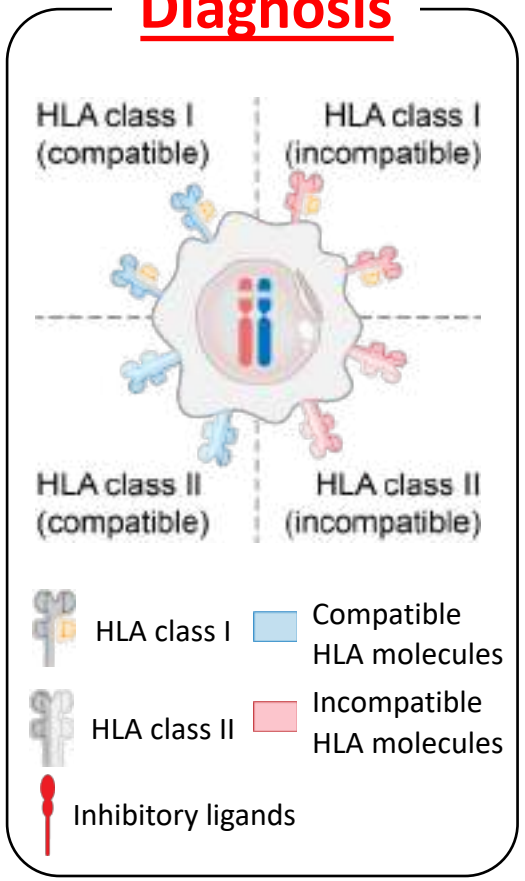




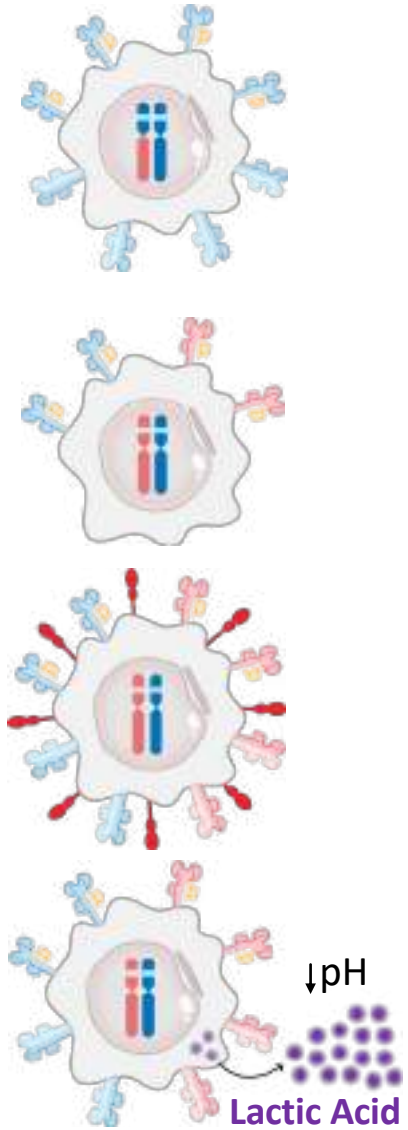
F. Manfredi et al., *Science Advances 2023*

Mechanisms of Post-Transplantation Immune Escape

Diagnosis



Relapse



Genomic

HLA haplotype loss

Vago, NEJM, 2009; Crucitti, Leukemia, 2015; Ahci and Toffalori, Blood, 2017

Non-Genomic

Downregulation of HLA Class II molecules

Christopher, NEJM, 2018; Toffalori, Nat Med, 2019; Gambacorta, Canc Disc, 2022

Upregulation of T cell inhibitory ligands

Toffalori, Nat Med, 2019; Noviello and Manfredi, Nat Comm, 2019

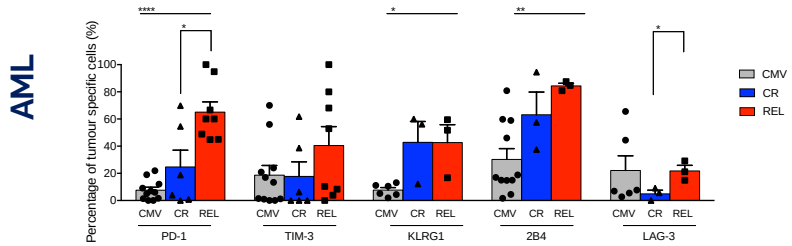
Impairment of T cell metabolic fitness

Uhl, Sci Transl Med, 2020

Exploiting T cell exhaustion for TCR hunting

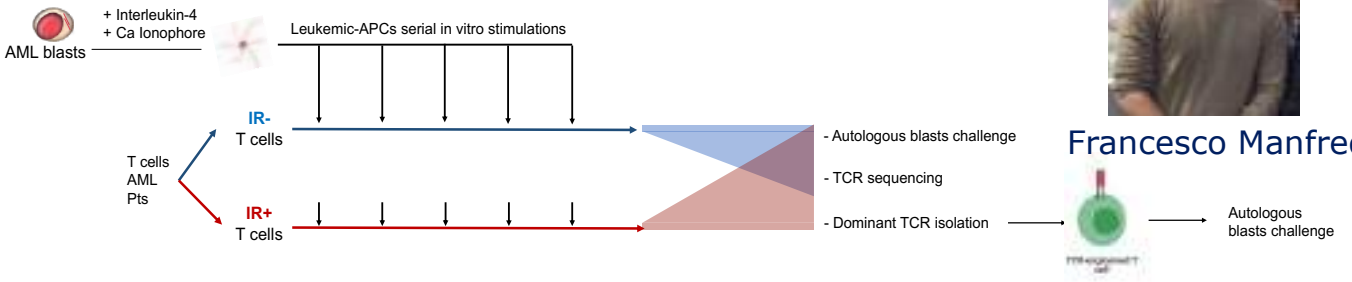
TAA-specific T cells display an exhaustion phentype in hematological malignancies

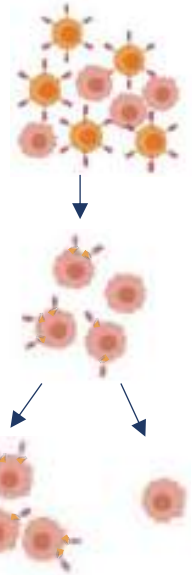
Noviello, Manfredi et al. Nat Comm 2019



Maddalena Noviello

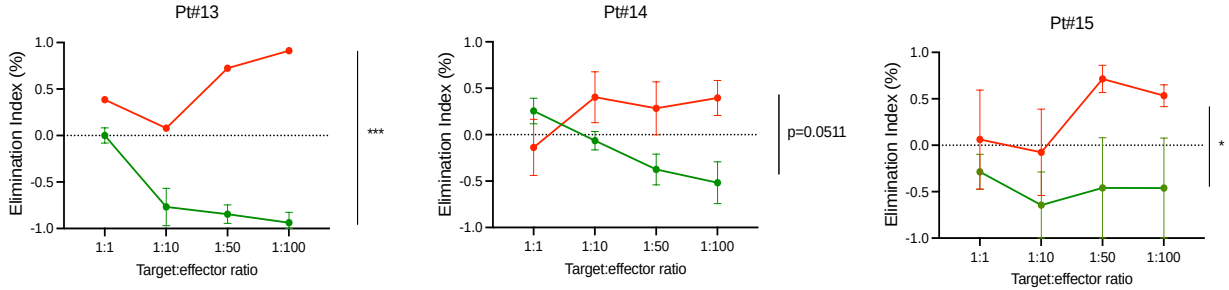
Francesco Manfredi

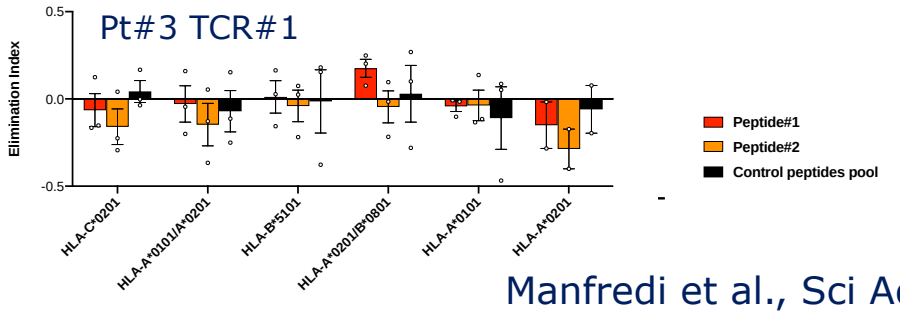




1. **Co-culture** of TCR-edited with orphan TCRs with autologous target blasts
2. **Target blasts** column magnetic **sorting**
3. **CD3⁺ and CD3⁻ target blast fractions sorting** by FACS
4. **Ligandome** analysis

Coll. with V. Cerullo & S. Feola



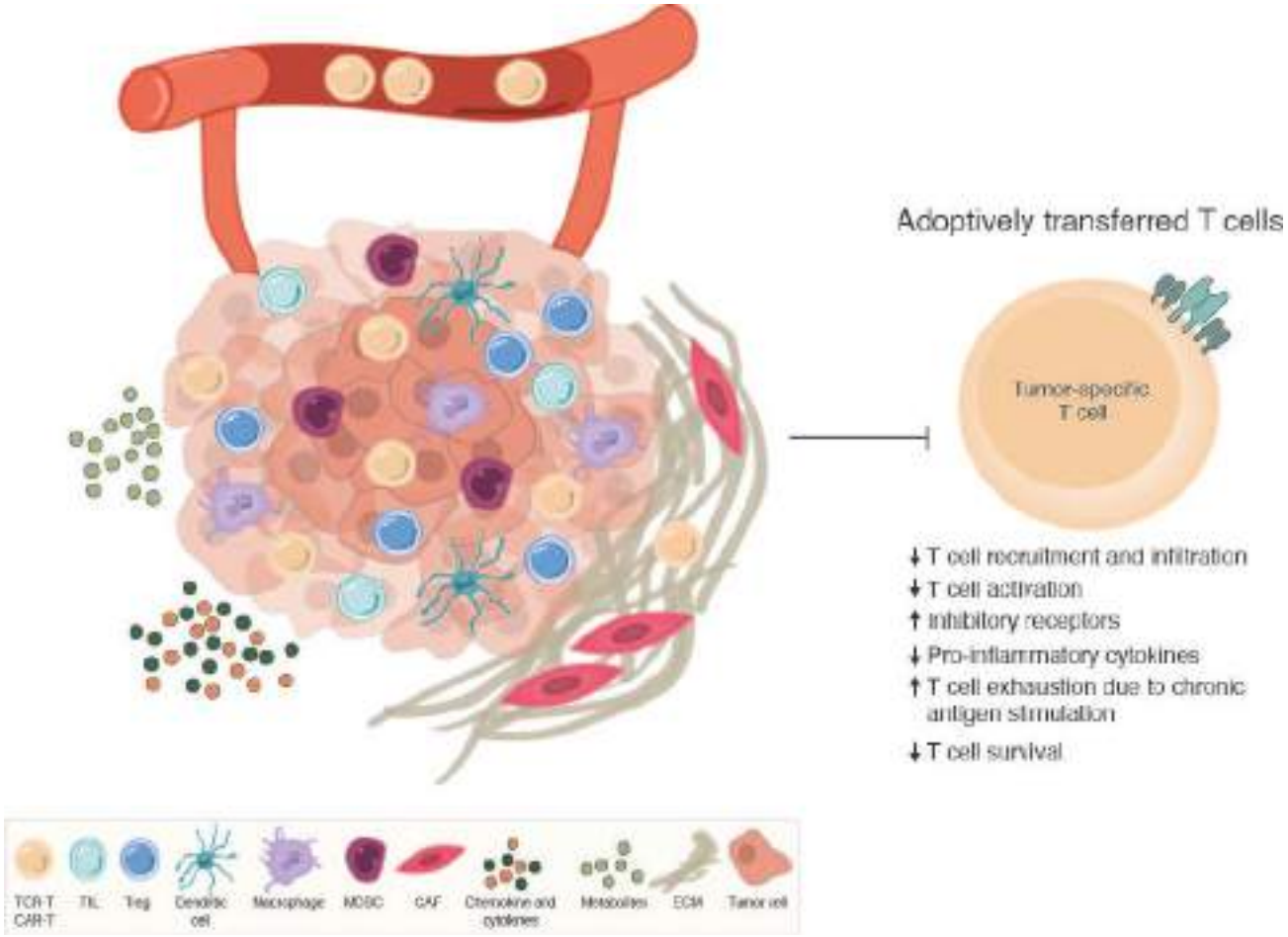


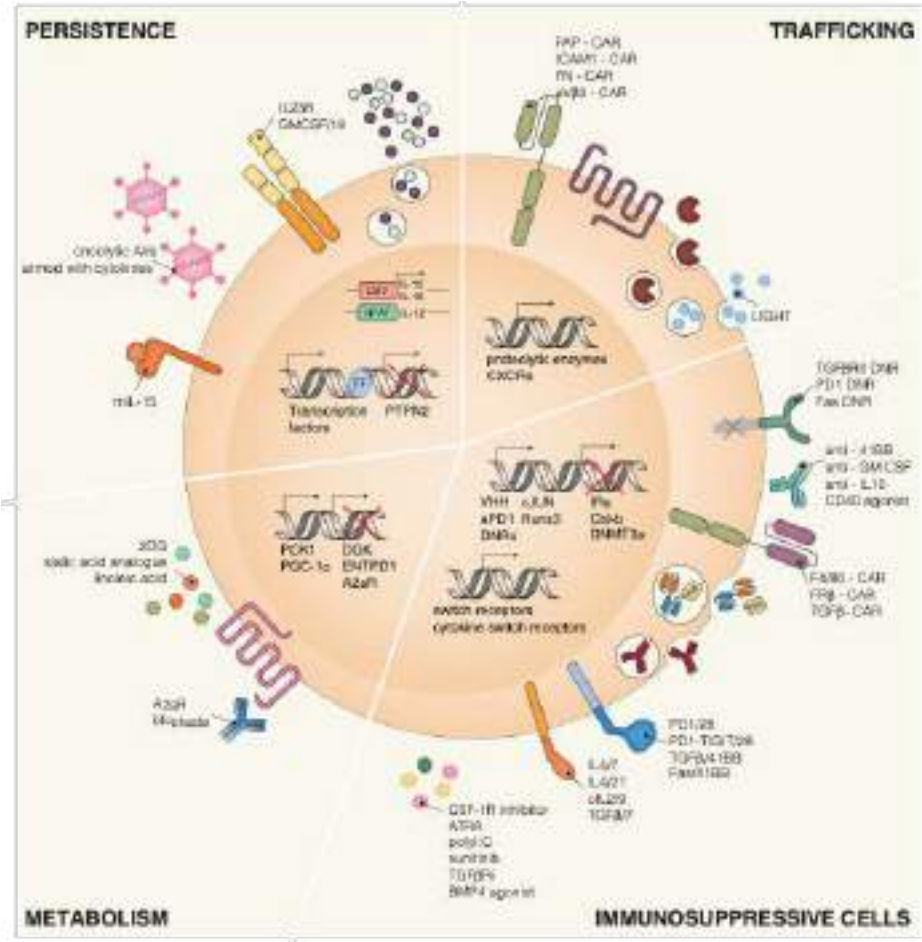
Manfredi et al., Sci Adv 2023

Conclusion 1

- Pipeline for the isolation of tumor-specific TCRs from healthy donors and cancer patients' circulating T cells
- Simultaneous editing of endogenous TCR α and β chain genes using CRISPR/Cas9 technology (efficiency >90%)
- Transduction of T-cells with lentiviral vectors encoding tumor specific-specific TCRs (efficiency >95% of $CD8^+$ T cells)
- Targeted TCR KI in TRAC and simultaneous TRBC KO (efficiency 70-80%)
- TCR edited T-cells specifically and efficiently kill primary $Antigen^+$ leukemic blasts in vitro and in vivo

Engineering T cells to hinder T cell immunosuppression at the tumor microenvironment

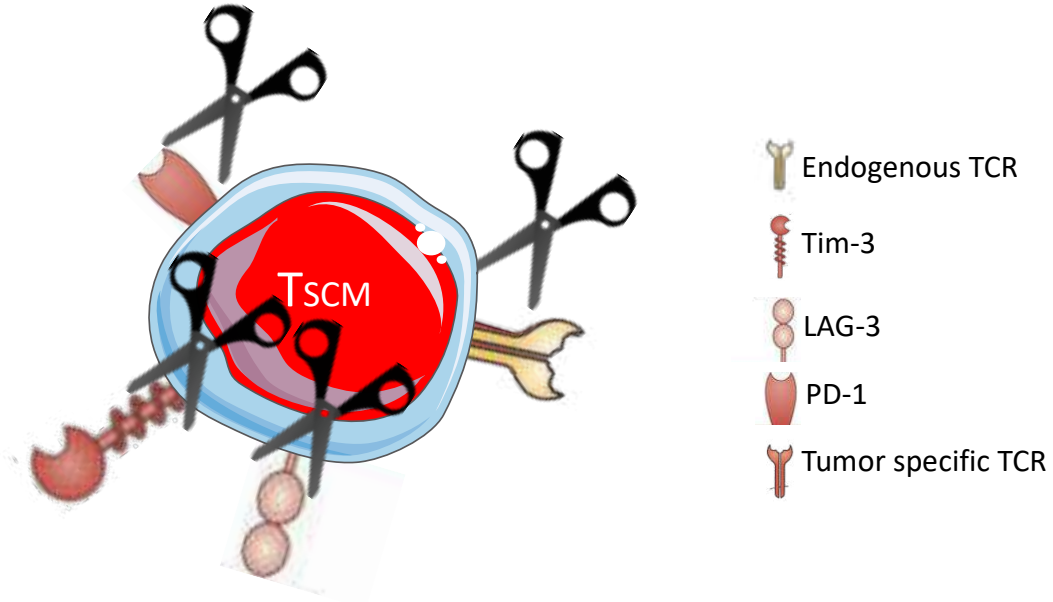




Spiga, et al., *Seminars in Immunopathology*, in press

Adoptive T-cell therapy for cancer:
Overcoming T cell exhaustion with genome editing

To **design** and **develop** a panel of **CRISPR/Cas9** molecules targeting genes encoding **inhibitory receptors** in order to enhance the anti-tumor activity of **TCR-edited memory stem T cells**



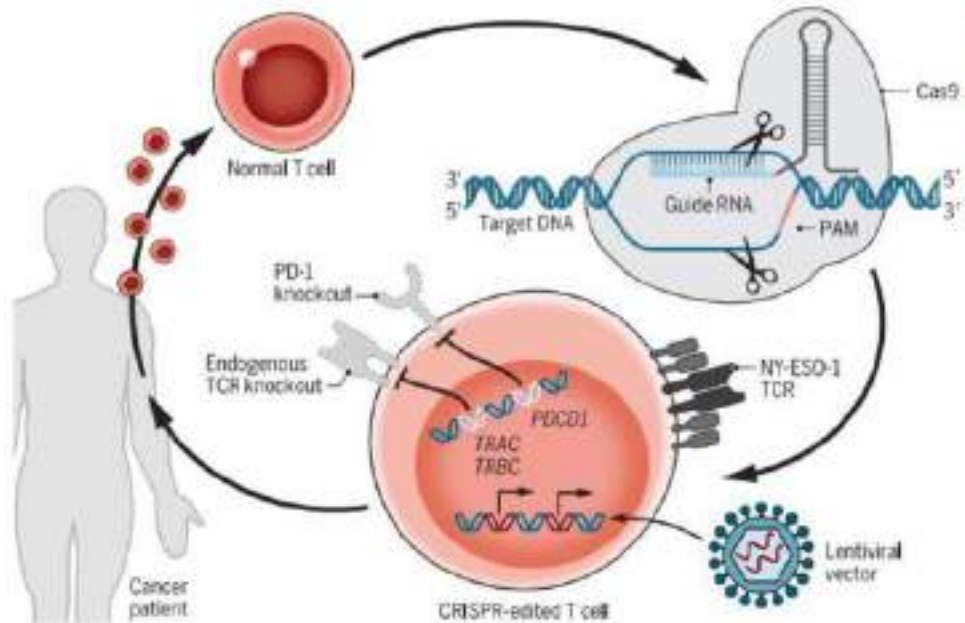
Beatrice Cianciotti,
Zulma Magnani,
Eliana Ruggiero

RESEARCH ARTICLE SUMMARY

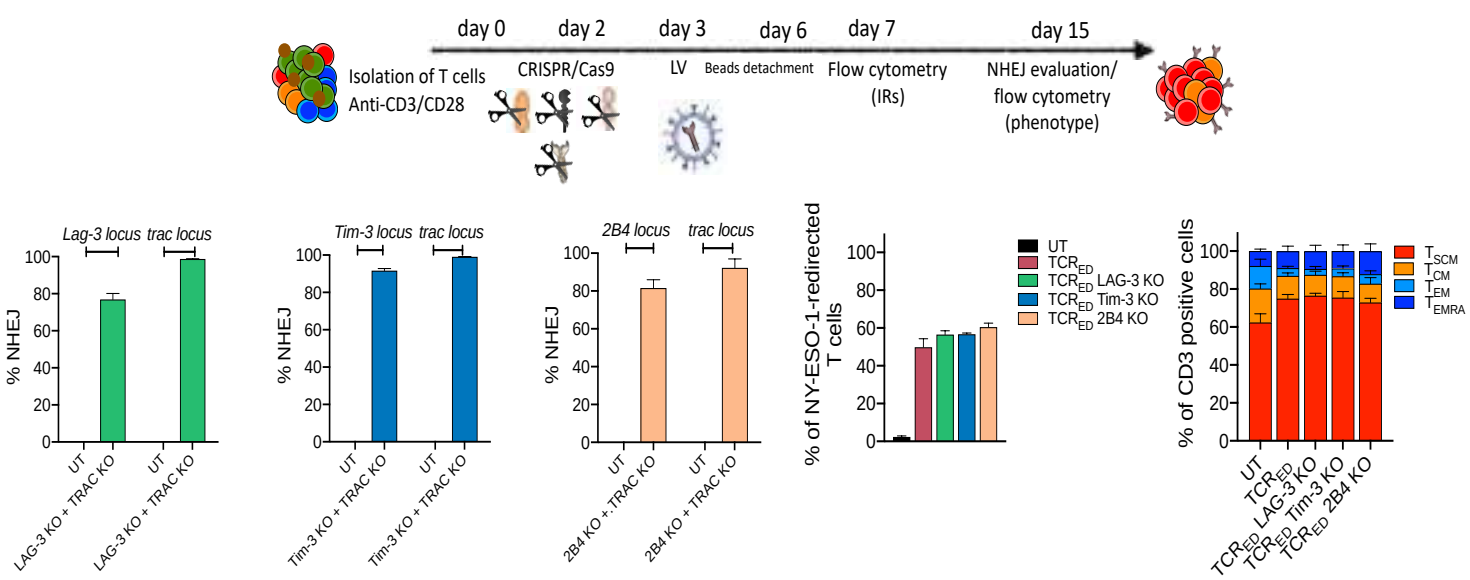
CLINICAL TRIALS

CRISPR-engineered T cells in patients with refractory cancer

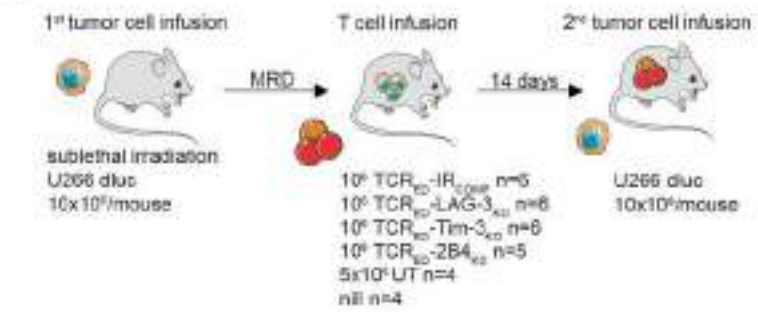
Edward A. Stadtmauer*†, Joseph A. Fraietta*, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tian, Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne Shea, Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagne, January Salas-Mckee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadlowsky, Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno, Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpathy, Howard Y. Chang, Yangbing Zhao, Simon F. Lacey*, Carl H. June*†

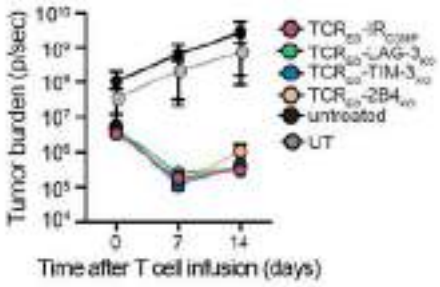


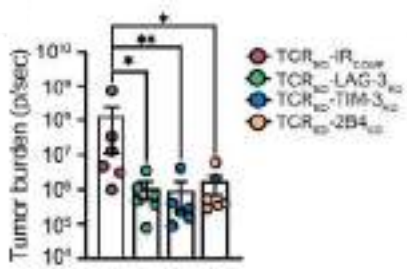
TCR_{ED}-IR_{KO} cells outperform TCR_{ED}-IR_{COMP} T cells *in vivo* upon tumor rechallenge

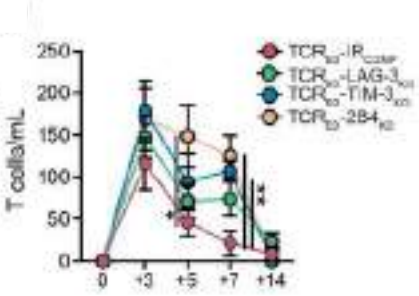


Beatrice Cianciotti



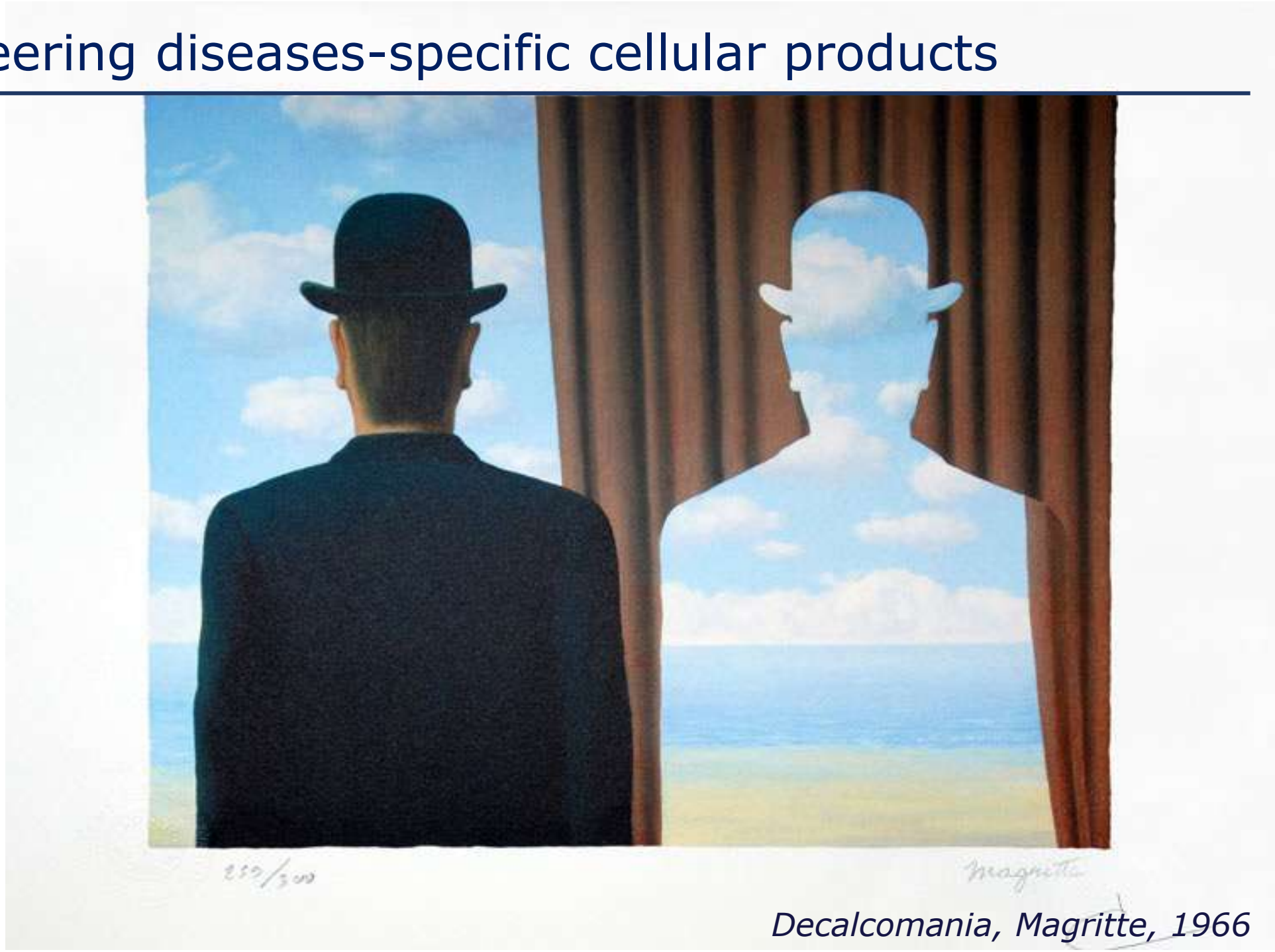






Cianciotti et al. *Front Immunol* 2024

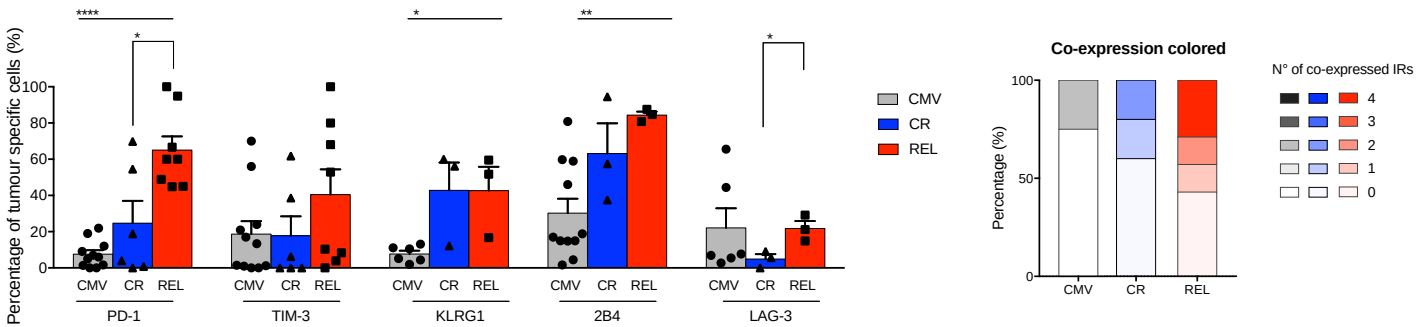
Engineering diseases-specific cellular products



Decalcomania, Magritte, 1966

Tumor-specific T cells are profoundly exhausted in tumor patients

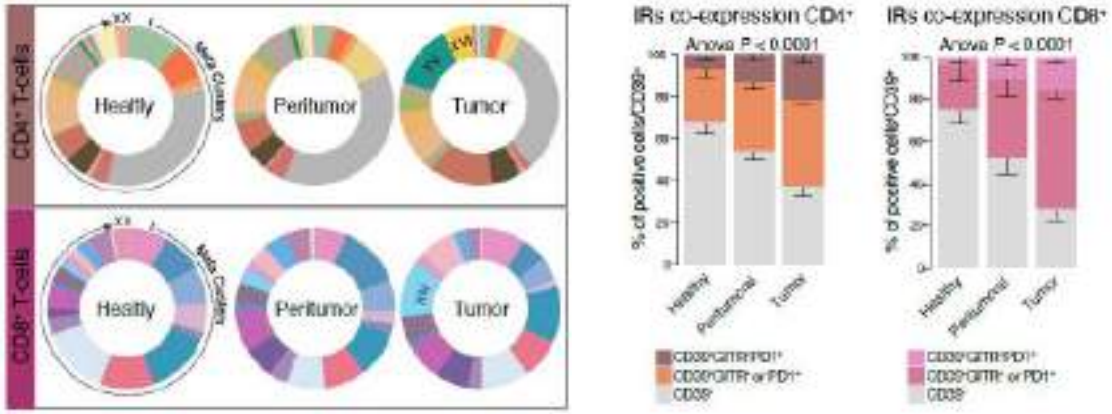
AML



Maddalena Noviello
Francesco Manfredi

Noviello, Manfredi et al., *Nat. Comm.* 2019

CRC



Alessia Potenza

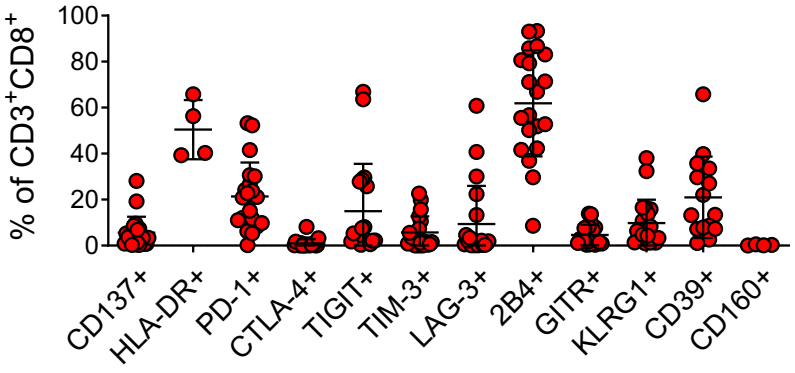
Chiara Balestrieri

Potenza, Balestrieri et al. *GUT 2023*

EOC

PD-1

TIM-3



Alice Bergamini.

Elena Tassi

Chiara Maffia

Tassi, Bergamini et al., *Front Imm 2024*

UniSR
Università Vita-Salute San Raffaele

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

Advanced immune gene and cell therapies for CRC and PDAC liver metastases

Chiara Bonini

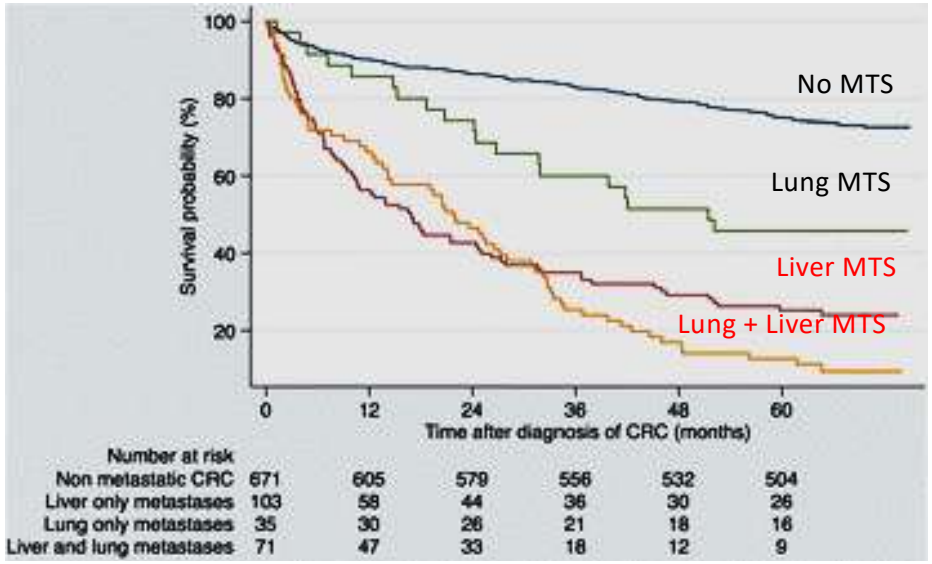
AIRC5x1000 Cross-Fertilization Meeting
January 20th, 2023

AIRC

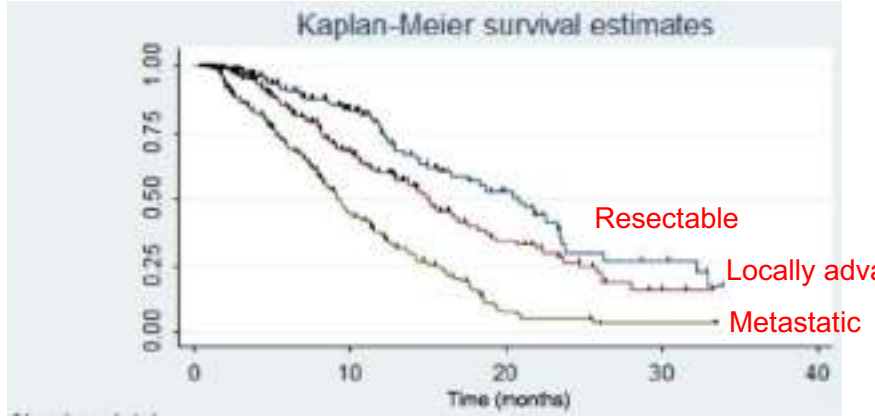
5x1000
x AIRC = RICERCA

The Unmet Clinical Need

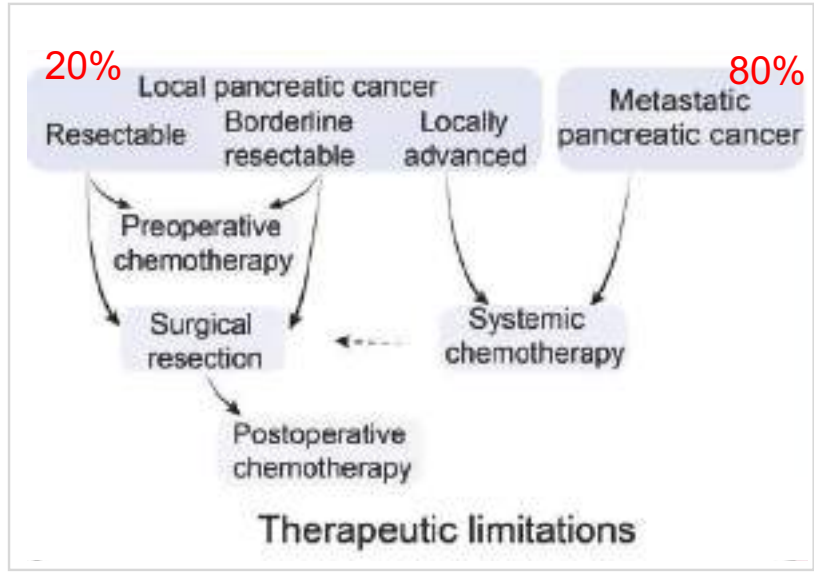
Liver MTS are unfavorable prognostic factors in both CRC and PDAC



CRC Patients	Median OS (months)	OS (5 years)
Liver MTS- resected	65.3	55%
Unresected	26.7	19,5%
HR	0.35	



Maulat et al. 2020



Modified from Wang et al. 2021

A Team with recognized Expertise: USR/OSR Research Units

Clinical facility with extensive experience in clinical translation of cell and gene therapy products

Dedicated Surgery Units & Oncology Units

Institutional Banking
Clinical Trial Office
Clinical facility

CLINICAL UNITS

L. Aldrighetti
F. De Cobelli
C. Doglioni
M. Falconi
M.Reni

EXTERNAL ADVISORY BOARD

Hana Algül, D
Dirk Busch, D
Luca Gianni, I
Nicola Valeri, UK

CELL AND GENE THERAPY UNITS

C. Bonini
M. Casucci
A. Mondino
L. Naldini
R. Ostuni

Frontline gene transfer platforms:
LV, IDLV, RV, miRNA-T ZFN, mRNA Talen, CRISPR

Innovative strategies:
TCR GENE EDITING,
CAR-BASED THERAPY,
HSC-BASED GENE THERAPY)

TUMOR IMMUNOLOGY UNITS

A. Corti
P. Dellabona
M.P. Protti
G. Tonon

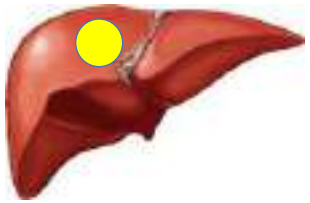
Benchmark studies in:
TUMOR IMMUNOLOGY,
CANCER IMMUNOEDITING,
TME,
TUMOR ANTIGEN DISCOVERY

INNOVATIVE ANIMAL MODEL UNITS

L. Guidotti
M. Iannacone
L. Piemonti
G. Sitia

Innovative models in:
LIVER ONCOIMMUNOLOGY,
PDAC/CRC LIVER METASTASES,
IN VIVO IMAGING

The Research Plan



Program1(LiMeT Protocol)

Multidimensional investigation of metastatic TME by WES, RNA-Seq, scRNA-Seq, HD Flowcytometry, tissue IF and spatial-resolved omics

Identification of new CAR and TCR tumor targets

Identification of druggable immuno-suppressive pathways active in the metastatic TME

Program 2

Adoptive cell therapy with tumor-retargeted T cells (TCRs; CARs)

Combinatorial therapy (ACT + targeted cytokine delivery)

Program 3

Direct cytokine gene therapy (LV injection)

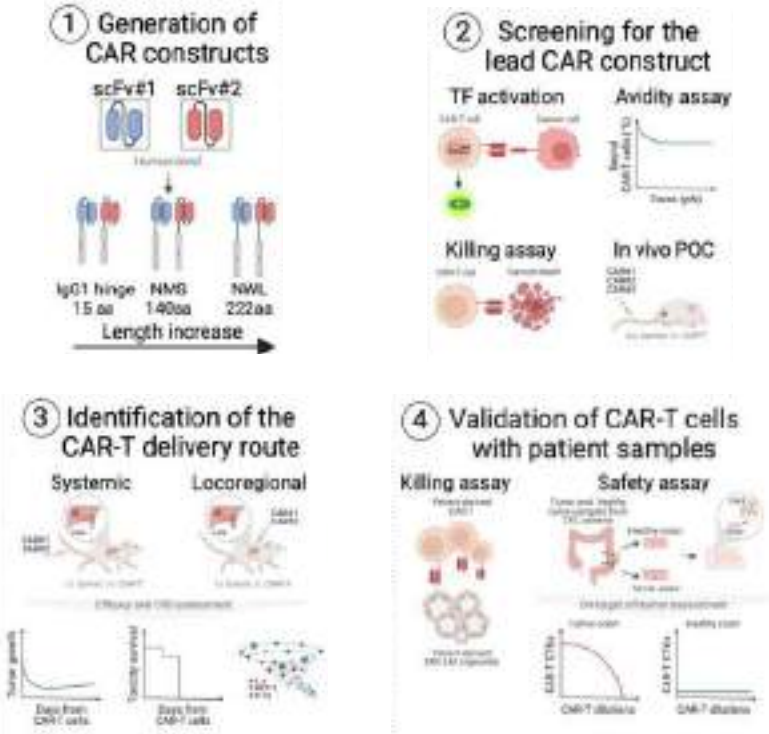
Program 4

Toward clinical testing of innovative ATMPs

CDH17 CAR-T cells are potent and safe

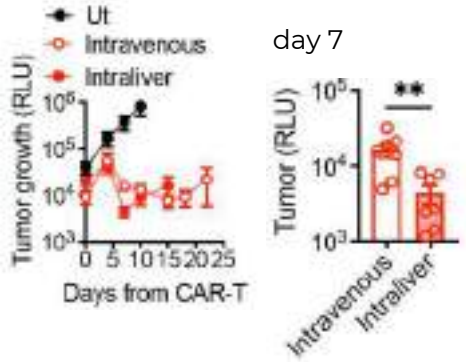
Greco B*, El Khoury R* et al, Science Transl Med, In press; PCT/EP2023/081532

Rational workflow for CAR selection

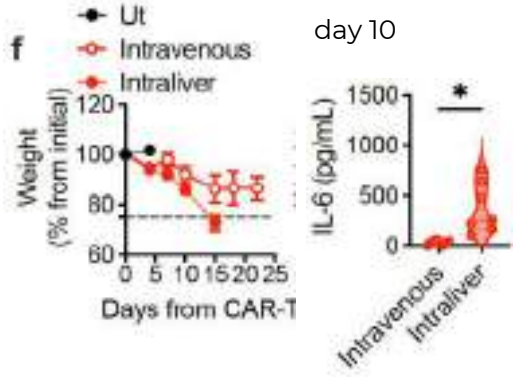


Systemic delivery: potent and safe

Antitumor activity

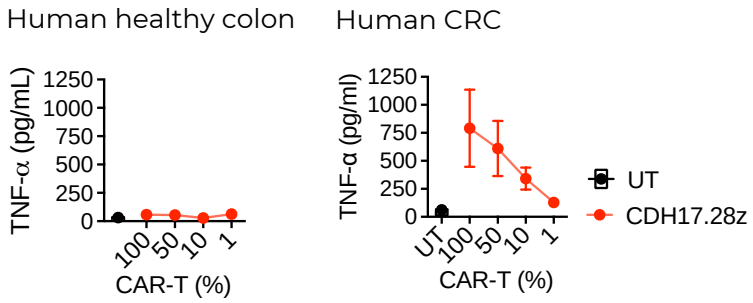


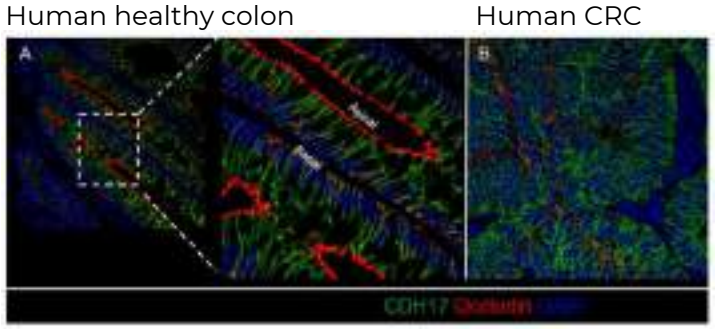
Cytokine release syndrome



Low risk to cause OTOT toxicity

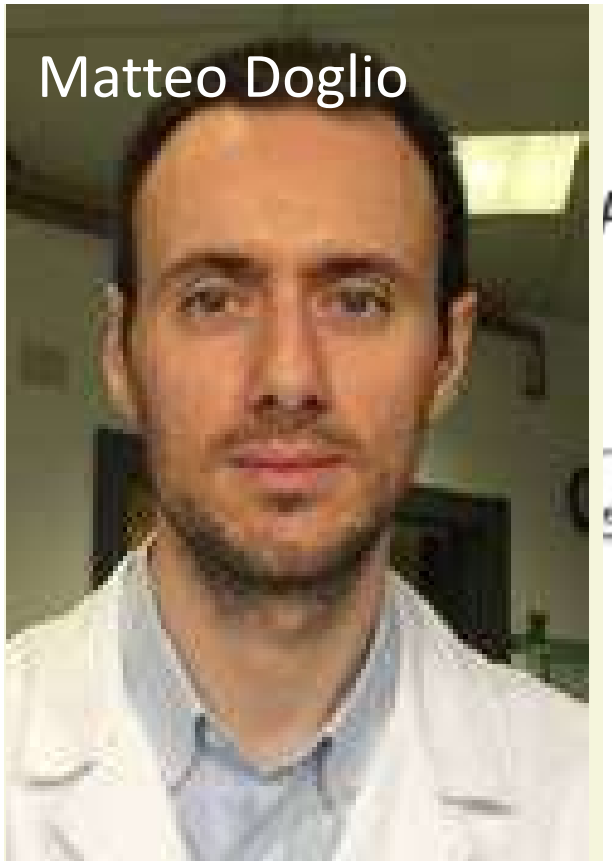
OTOT: On-target Off-tumor

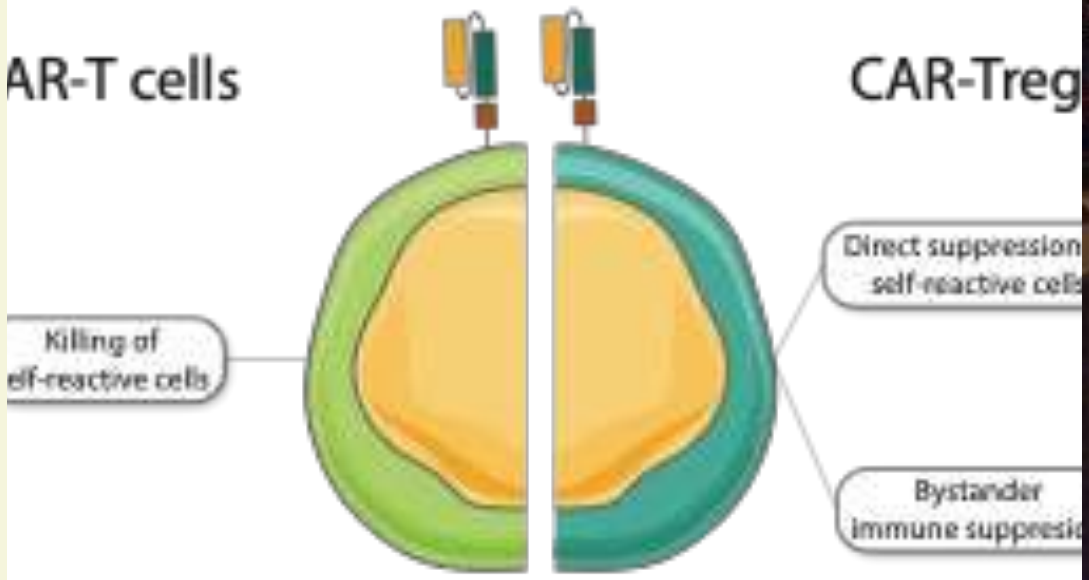




Innovative Immunotherapies Unit, OSR

Gene engineering Tregs to tame autoimmunity





New insights in systemic lupus erythematosus: From regulatory T cells to CAR-T-cell strategies

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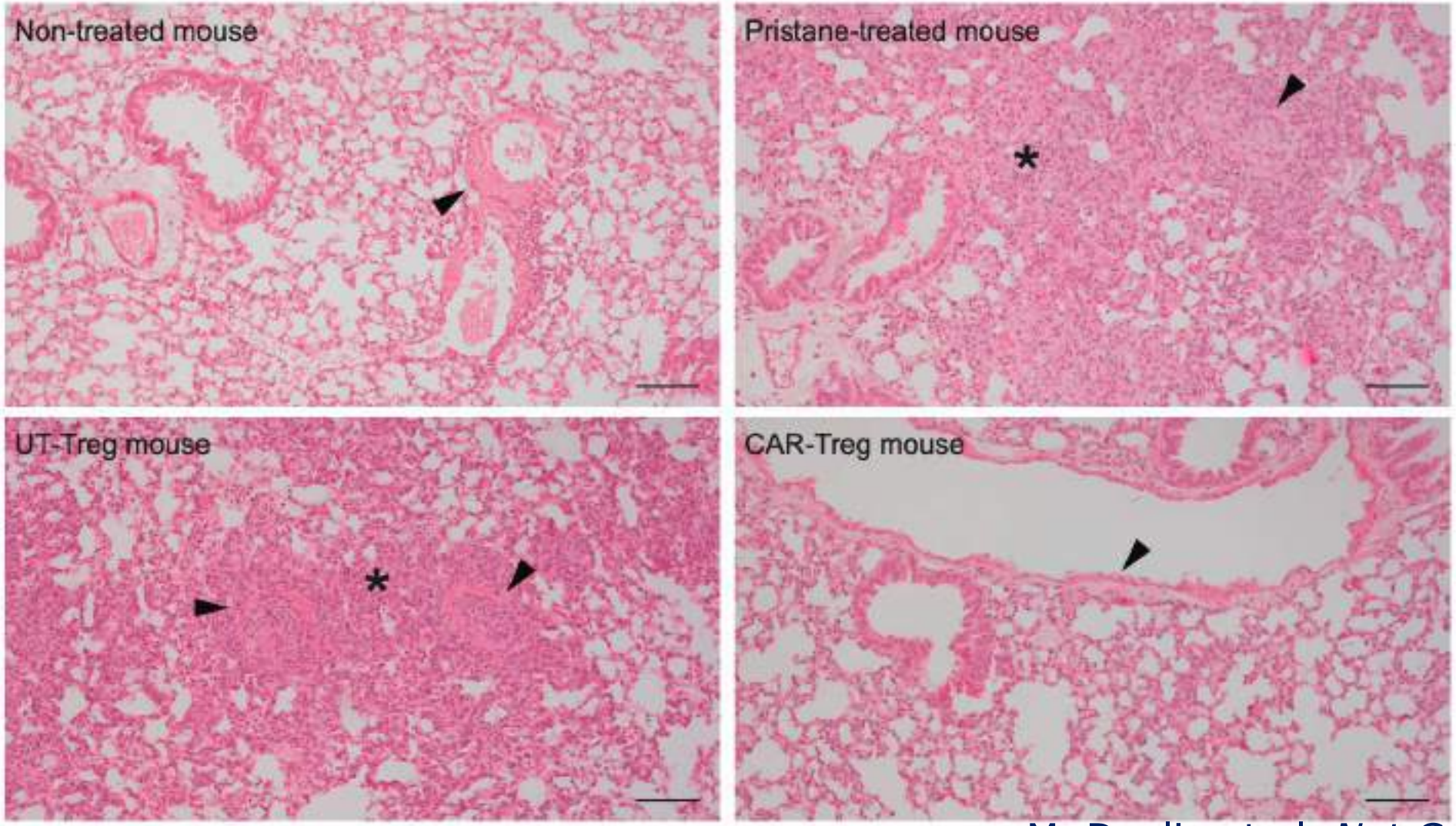
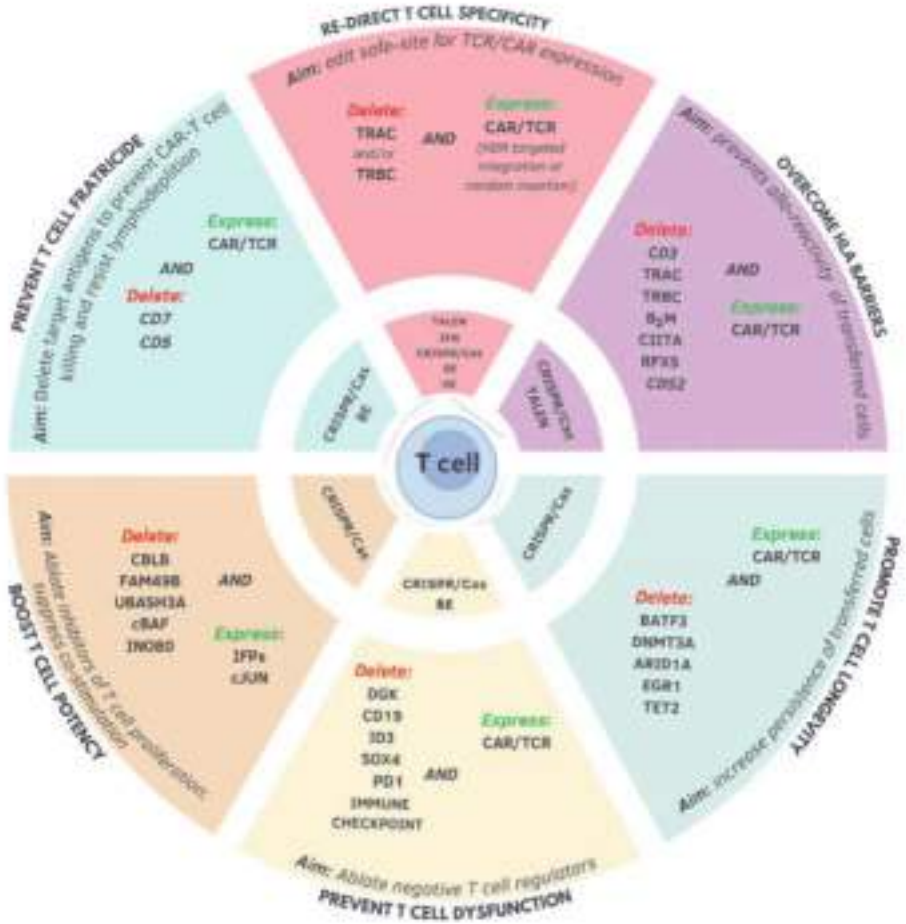
Absence of granulomas in CAR-T regs treated mice



M. Doglio et al, *Nat Comm* 2024

T-cell genome editing for cancer immunotherapy



Bonini, Chapuis, Hudecek, Guedan, Magnani, Qasim. Human Gene Therapy 2023

Acknowledgments

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Massimo Bernardi
Consuelo Corti
and all MDs, Nurses, Data Managers

Unit Immunogenetics, Leukemia Genomics and Immunoiology
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Laura Zito
and all

Innovative Therapy Unit
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