

SVILUPPO DI STRATEGIE CAR T DIRETTE CONTRO GLI ANTIGENI GD2 E FAP PER SUPERARE LE BARRIERE TUMORALI E STROMALI NELL'OSTEOSARCOMA

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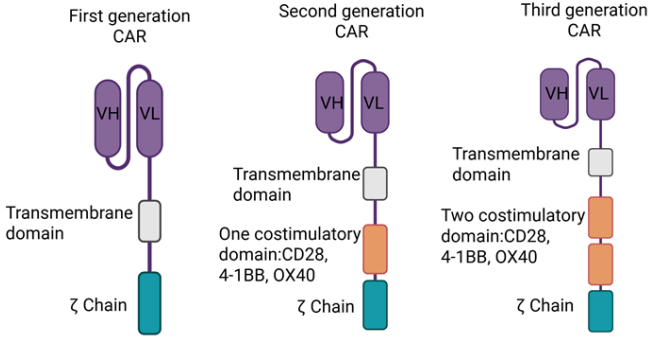
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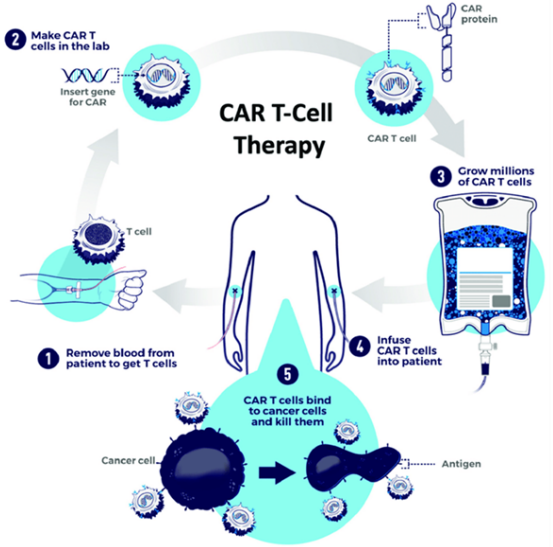
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LECCE, 12-14 ottobre 2026
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Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Chimeric Antigen Receptor (CAR) T-cell therapy





Zachary D. Crees et al. 2021. Cancers, 13,5181

	Kymriah	Yescarta	Tecartus	Breyanzi	Abecma	Carvykti
	01	02	03	04	05	06
	Follicular lymphoma, Diffuse Large B-cell lymphoma, Lymphoblastic Leukemia	Follicular lymphoma or diffuse large B-cell lymphoma	Mantle cell lymphoma or acute lymphoblastic leukemia	Large B-cell lymphoma	Relapsed or refractory multiple myeloma	Relapsed or refractory multiple myeloma

Barriers to CAR T-cell therapy in solid tumors

01 Lack of tumor
antigen heter

02 Inability of
infiltrate with
persist and r
immunosupp

The NEW ENGLAND JOURNAL of MEDICINE

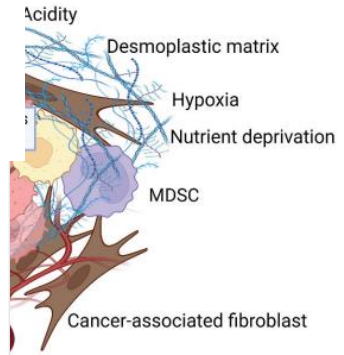
ORIGINAL ARTICLE

GD2-CART01 for Relapsed or Refractory High-Risk Neuroblastoma

Authors: Francesca Del Bufalo, M.D., Biagio De Angelis, Ph.D., Ignazio Caruana, Ph.D., Giada Del Baldo, M.D., Maria A. De Ioris, M.D., Ph.D., Annalisa Serra, M.D., Angela Mastronuzzi, M.D., Ph.D., +28, for the Precision Medicine Team–IRCCS Ospedale Pediatrico Bambino Gesù* Author Info & Affiliations

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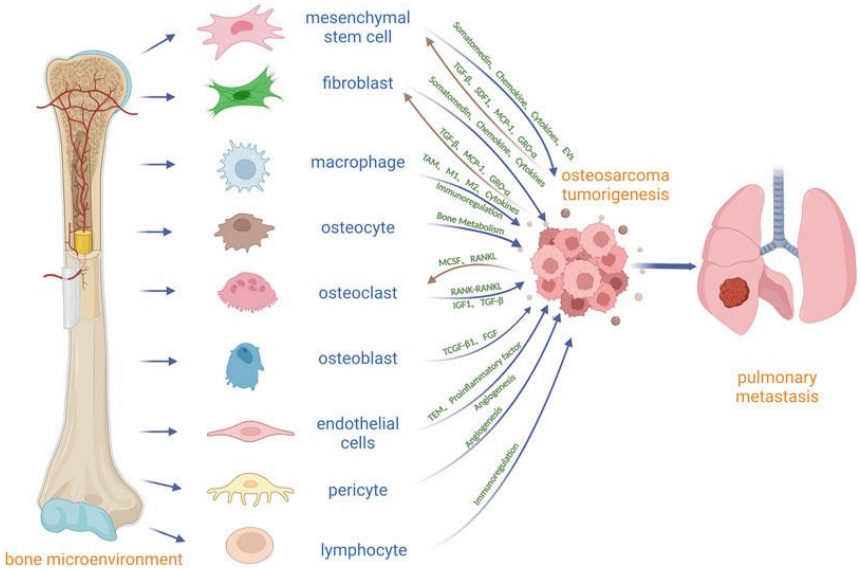
Expert Rev Anticancer Ther. 2025 Jan;25(1):9-2



The cross-talk between mutated tumor cells and the dynamic tumor microenvironment (TME) provides a mechanism and signal network for tumors to survive and progress

Aim of the project

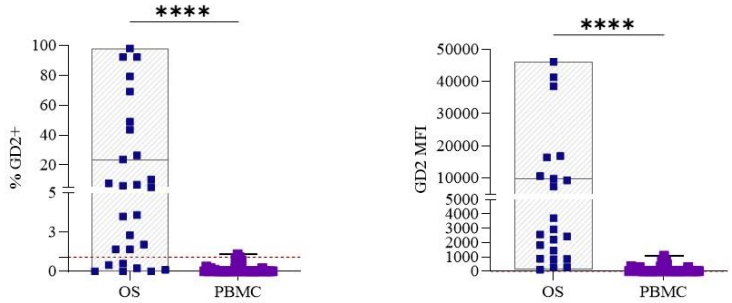
This project aims to evaluate CAR T-cell–based therapeutic strategies for osteosarcoma (OS) by targeting both tumor cells and the TME, specifically with GD2.CAR T-cells and FAP.CAR T-cells.



Front Cell Dev Biol. 2023 May 3;11:1123065.

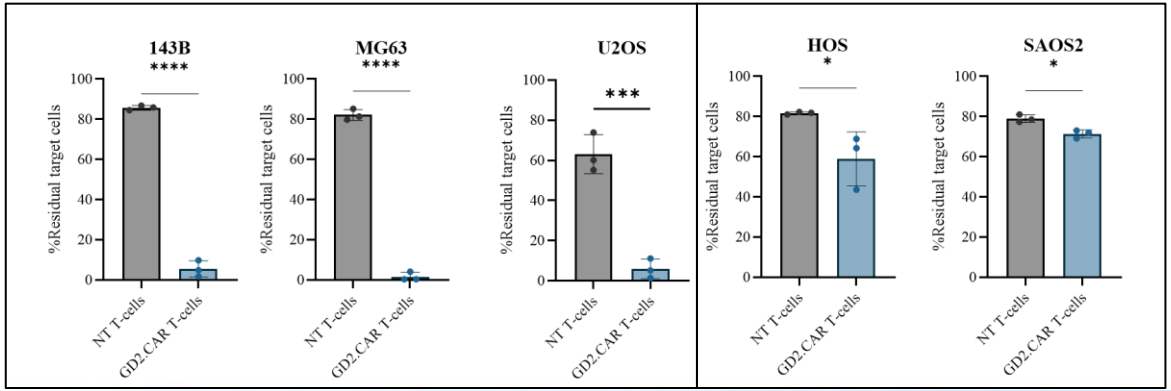
Is GD2 a valid therapeutic target in Osteosarcoma?

Percentage and MFI of GD2 expression levels on tumor biopsies and PBMC



GD2 positive OS cell lines

GD2 negative OS cell lines



Pezzella et al.
Journal of Hematology & Oncology (2024) 17:127
https://doi.org/10.1186/s13045-024-01641-7

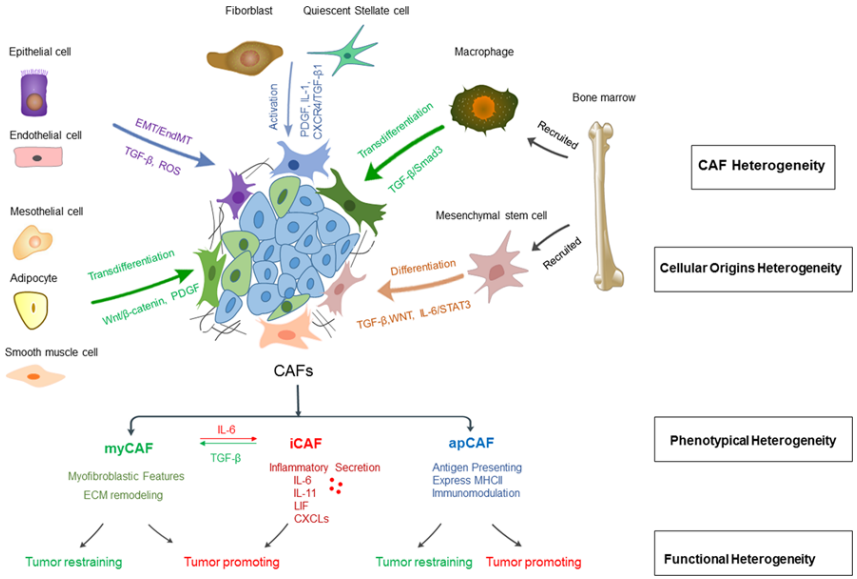
Journal of Hematology & Oncology

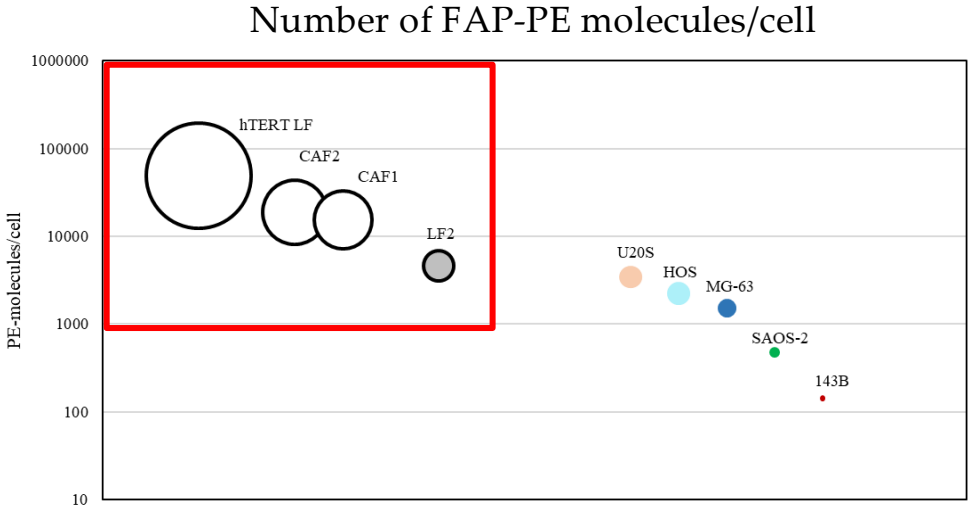
RESEARCH Open Access

Tumor-derived G-CSF induces an immunosuppressive microenvironment in an osteosarcoma model, reducing response to CAR.GD2 T-cells

Michele Pezzella[1†], Concetta Quintarelli[1,2†], Maria C. Quadraccia[1,3], Andrea Sarcinelli[1,4], Simona Manni[1], Laura Iaffaldano[1], Alessio Ottaviani[1], Roselia Ciccone[1], Antonio Camera[1], Maria L. D'Amore[1], Stefano Di Cecca[1], Matilde Sinibaldi[1], Marika Guercio[1], Mariasole Aurigemma[1,3], Pamela De Falco[1], Valentina Fustaino[1], Rossella Rota[1], Silvia Pomella[1], Matteo Cassandri[1], Angela Di Giannatale[1], Chiara Agrati[5], Veronica Bordoni[5], Federica Guarracino[5], Michele Massa[1], Giada Del Baldo[1], Marco Becilli[1], Giuseppe M. Milano[1], Francesca Del Bufalo[1], Franco Locatelli[1,6*†] and Biagio De Angelis[1*†]

Is FAP a valid therapeutic target for the TME?

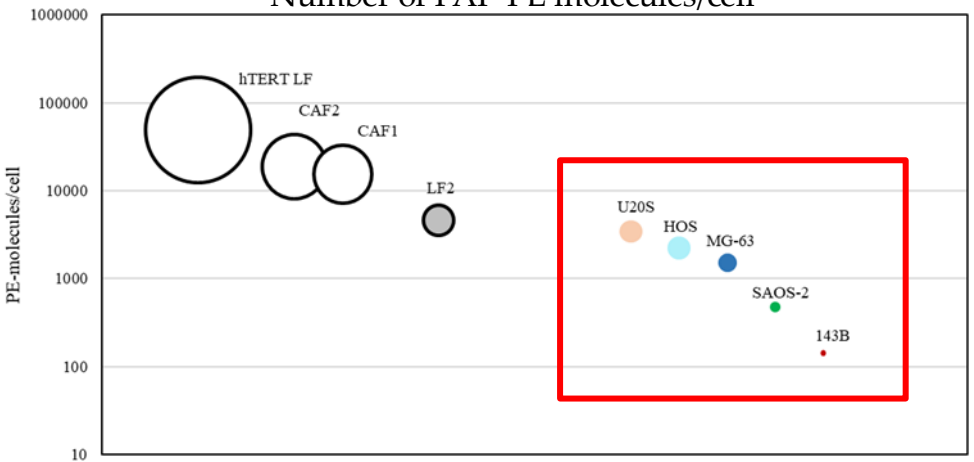




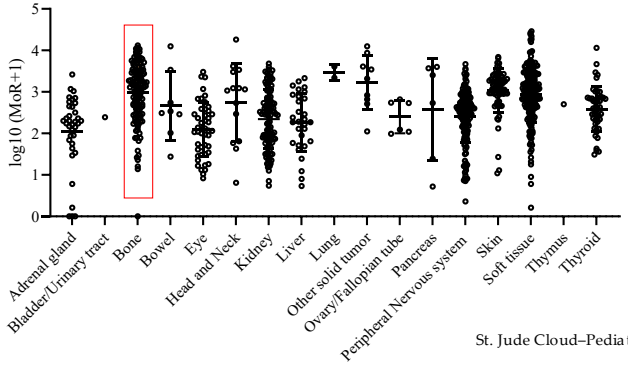
Exp Mol Med. 2023 Jul;55(7):1322-1322. 10.1038/s12276-023-01013-0

Could FAP be a therapeutic target for the tumor?

Number of FAP-PE molecules/cell



Scatter plot of RNA-seq data of
FAP expression across pediatric solid tumors



St. Jude Cloud–Pediatric Cancer Data Portal

FAP has been identified as a promising therapeutic target in multiple solid tumors

• Several Phase I and Phase II clinical trials are currently ongoing to evaluate the safety and feasibility of FAP-targeted therapies in solid tumors, including:

- Eli Lilly and Company – Phase I study (**NCT07213791**)
- Novartis Pharmaceuticals – Phase I/II study (**NCT04939610**)

•An early Phase I clinical trial conducted by the University of Zurich (**NCT01722149**) demonstrated a favorable safety profile of FAP-directed CAR T-cell therapy in patients with Malignant Pleural Mesothelioma.

Core elements of the project

Investigating the role of CAFs in OS progression

Investigating the role of CAFs in OS resistance to CAR T-cell therapy

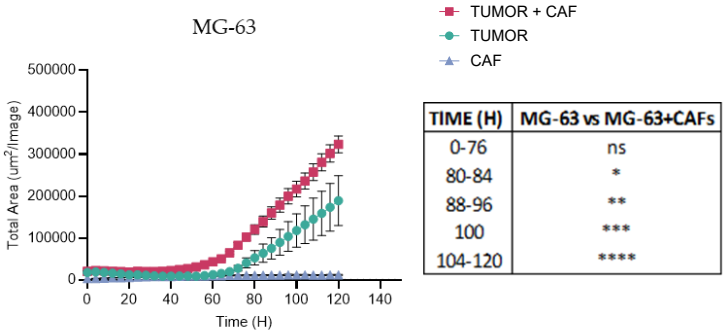


Evaluating the antitumor activity of FAP.CAR T-cells against CAFs and tumor cells

Evaluating the synergistic potential of combined GD2.CAR and FAP.CAR T-cell therapies in preclinical models

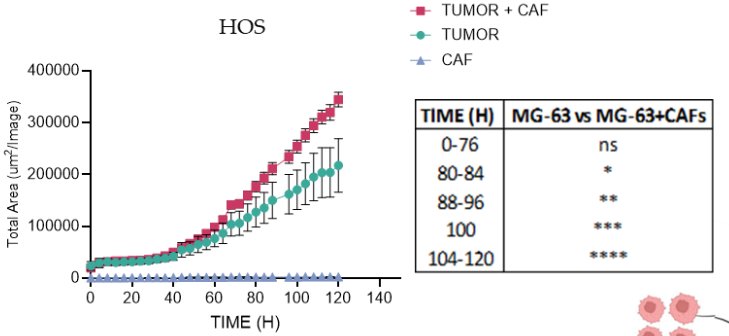
Investigating the role of CAFs in OS

MG-63



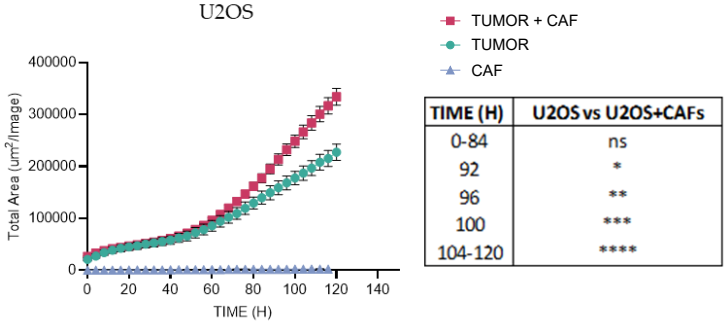
TIME (H)	MG-63 vs MG-63+CAFs
0-76	ns
80-84	*
88-96	**
100	***
104-120	****

HOS



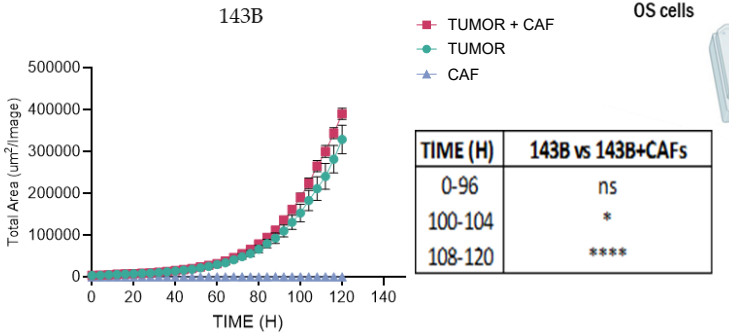
TIME (H)	MG-63 vs MG-63+CAFs
0-76	ns
80-84	*
88-96	**
100	***
104-120	****

U2OS

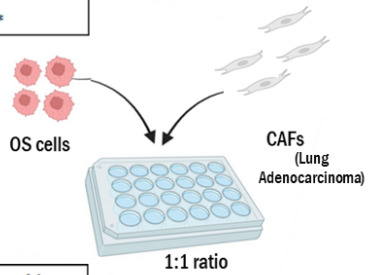


TIME (H)	U2OS vs U2OS+CAFs
0-84	ns
92	*
96	**
100	***
104-120	****

143B



TIME (H)	143B vs 143B+CAFs
0-96	ns
100-104	*
108-120	****

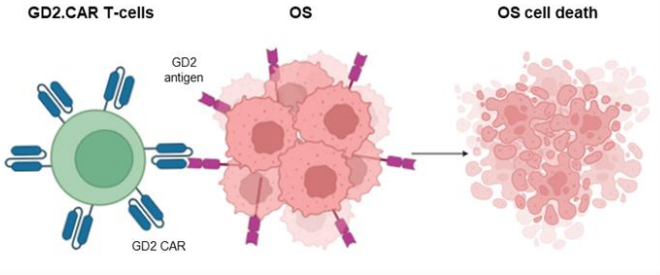


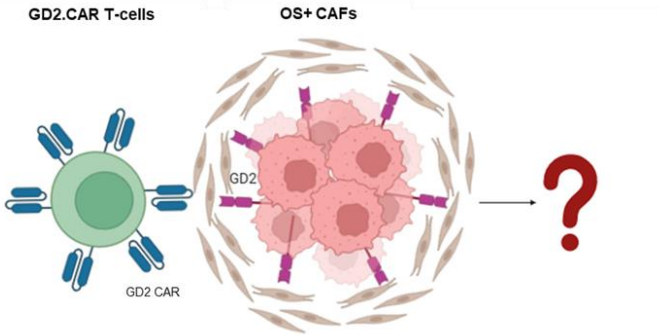


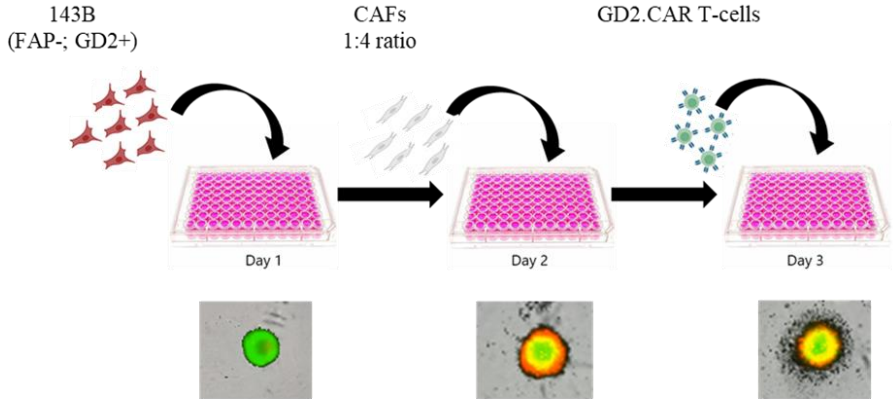
Incucyte Live-Cell Analysis System

2D live imaging analysis of co-cultures reveal that CAFs significantly enhance OS cell proliferation.

Investigating the role of CAFs in OS resistance to CAR T-cell therapy

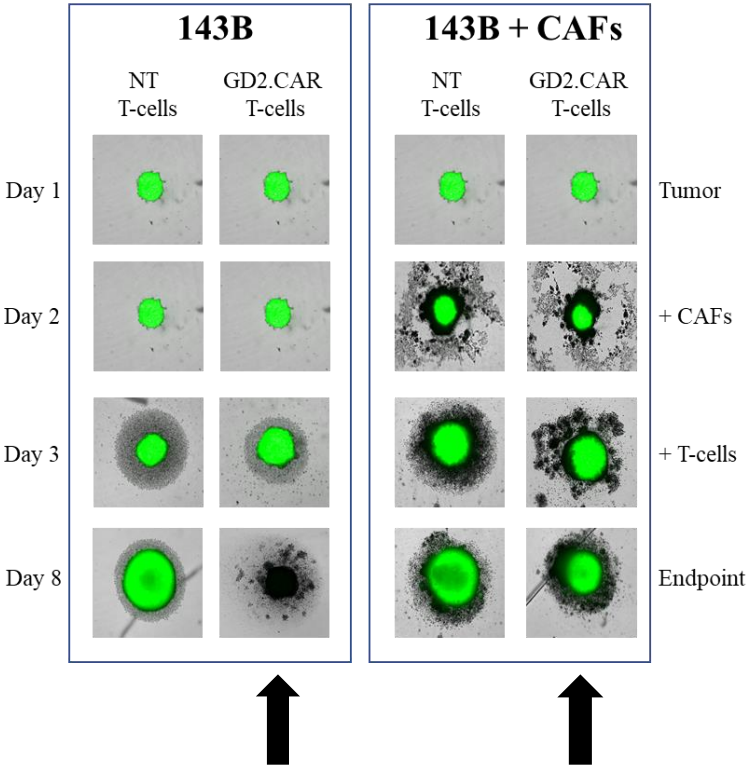


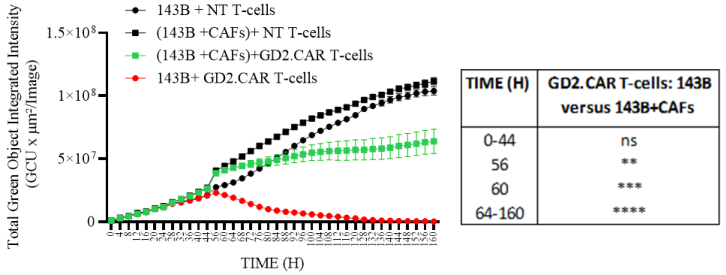




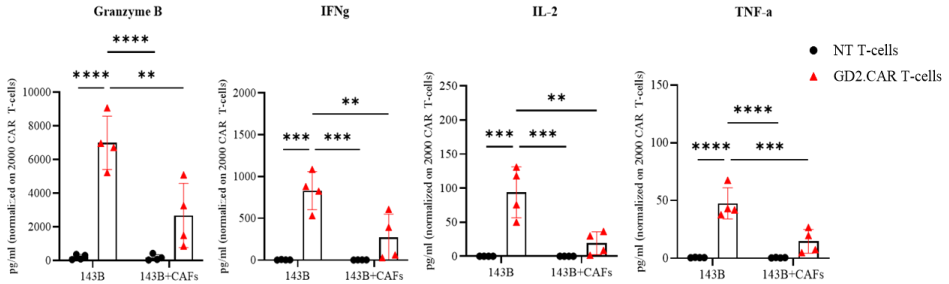
Development of a 3D *in vitro* spheroid model of tumor–CAF interactions to mimic the immunosuppressive TME in OS.

Investigating the role of CAFs in OS resistance to CAR T-cell therapy





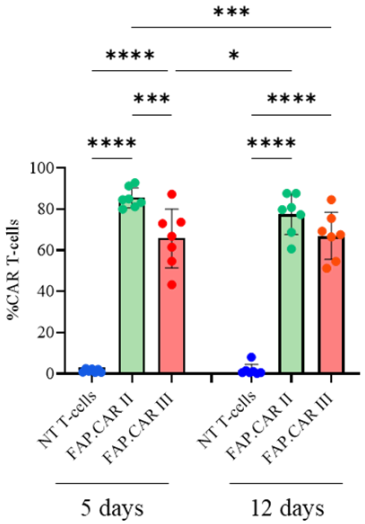
TIME (H)	GD2.CAR T-cells: 143B versus 143B+CAFs
0-44	ns
56	**
60	***
64-160	****

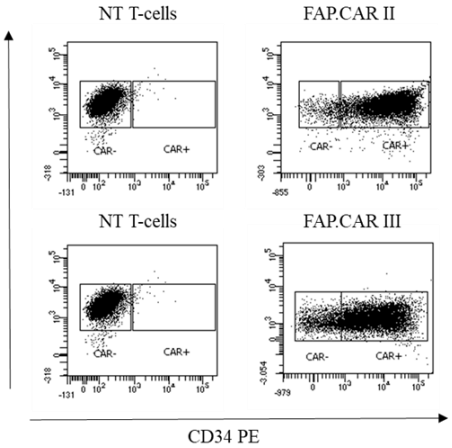


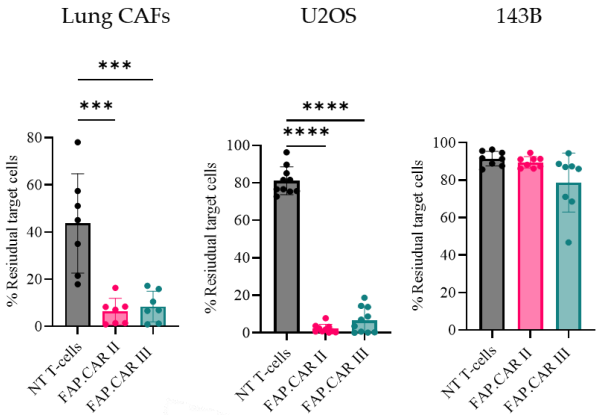
CAFs limit GD2.CAR T-cell efficacy in 3D OS spheroids, reducing tumor killing and effector cytokine release

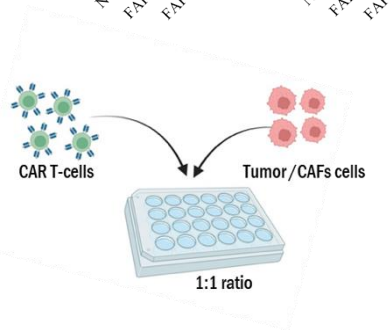
Evaluating the antitumor activity of FAP.CAR T-cells against CAFs and tumor cells



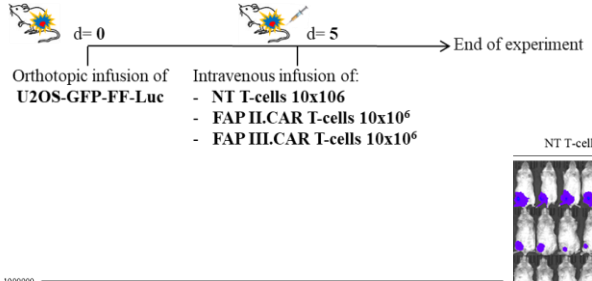




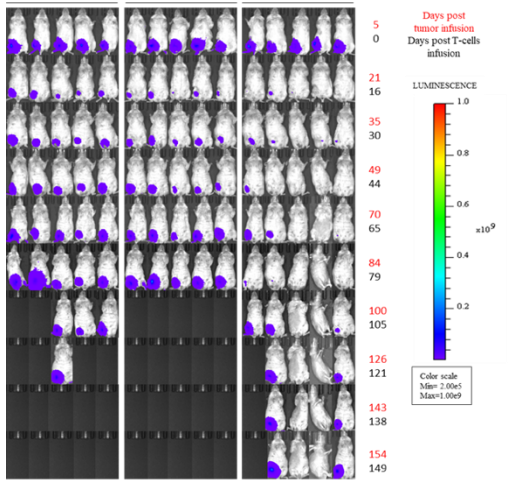


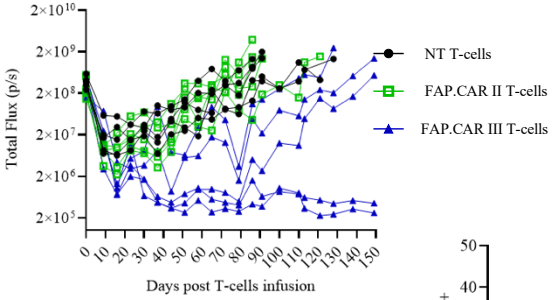


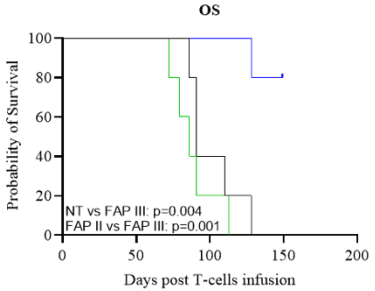
In *vivo* antitumor activity of FAP.CAR T-cells against U2OS OS cells

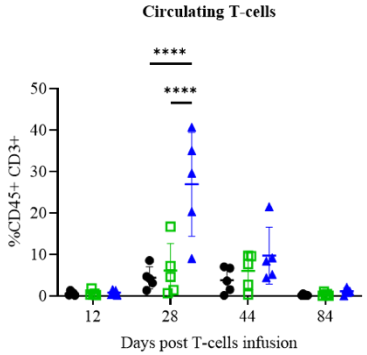






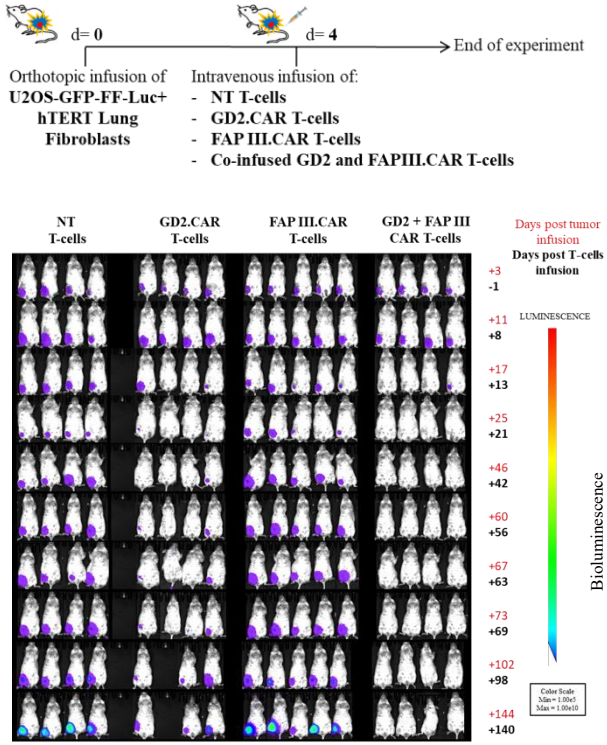


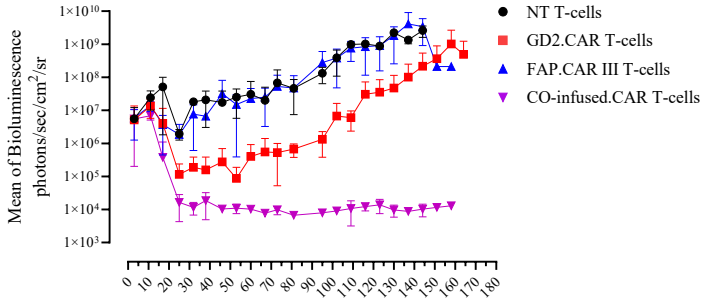


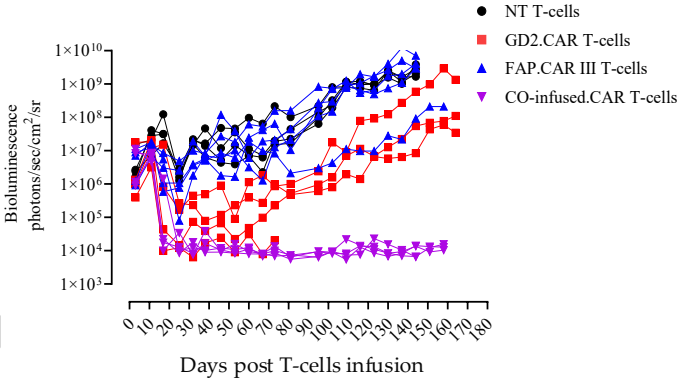


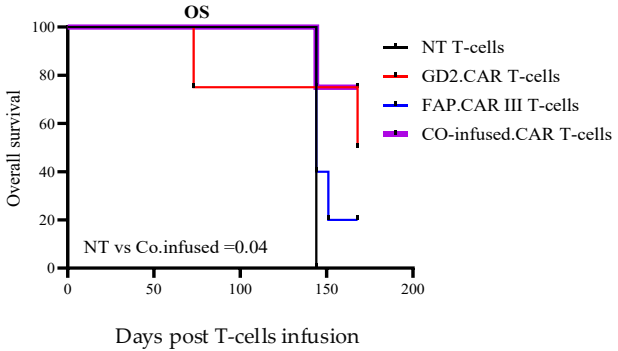
Only FAP.CAR III T-cells expand in U2OS (FAP-low) tumors, prolonging animal survival despite tumor recurrence

In vivo synergistic antitumor activity of GD2- and FAP-CAR T-cells in a U2OS/hTERT Lung Fibroblast co-injection model









Conclusions

- **OS cells show increased growth in co-culture with CAFs.**

- **The TME limits CAR T-cell efficacy in OS**: CAFs create a stromal barrier that impairs GD2.CAR T-cell infiltration and function in 3D spheroid models.

- **FAP.CAR T-cells efficiently eradicate FAP$^+$ target cells**, while only third-generation FAP.CAR T-cells show enhanced *in vivo* persistence.

- **Dual targeting of tumor and stroma enhances antitumor activity**: combined GD2. and FAP.CAR T-cell therapy overcomes stromal resistance and promotes complete tumor eradication in *in vivo* models.

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Prof. Soheil Meshinchi

Thank you for your attention!







