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Sviluppo preclinico di una terapia CAR-T anti-HER3 per il trattamento dei rabdomiosarcomi pediatrici

Marika Guercio, PhD
Unità di Terapia Genica e Cellulare dei Tumori Pediatrici
Ospedale Pediatrico Bambino Gesù

Milano, 22 e 23 maggio 2026

Rhabdomyosarcoma is a rare pediatric tumor with poor prognosis

- Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, representing 40% of all pediatric soft tissue sarcomas and 3% of all pediatric cancer
- RMS has a mesenchymal origin and occurs in almost every part of the body (soft tissues)
- Morphological (historical) and molecular (more recent) classification, with FP (alveolar) presenting the worst prognosis

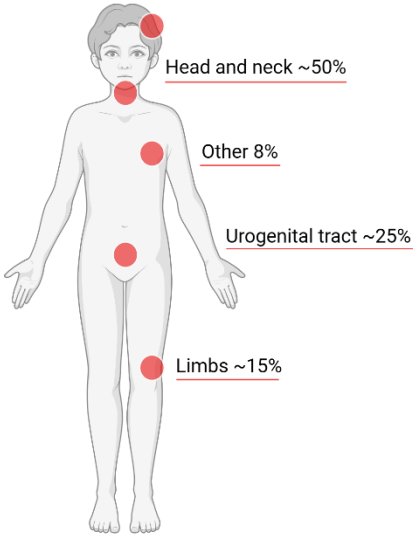
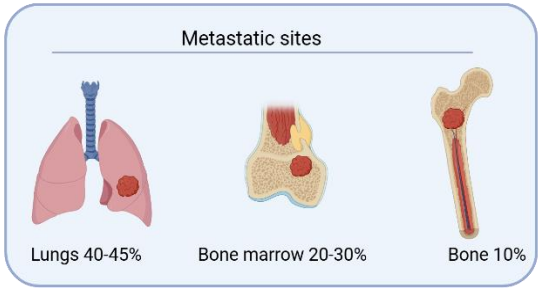


Table 1
Key features

	Age of Presentation	Typical Location	Morphology	Molecular	Methylation Pattern	Prognosis
Embryonal	<10 years	Head and neck & genitourinary tract	Round and spindle cells with variably dense cellularity	Variable	Fusion negative[a]	Variable
Alveolar	10–25 years	Extremities	Round	Majority with *FOXO1* fusion	Fusion positive[a]	Poor
Spindle Cell/ Sclerosing	Adolescent/ young adult (2–94 years)[27]	Head and neck & Paratesticular	Spindle/ sclerosing	*MyoD1* mutation	*MyoD1*	Poor
	<5 years	Chest wall		*VGLL2/NCOA2* fusion	Fusion negative	Favorable
	(11–86 years)[35]	Intraosseous craniofacial skeleton	Spindle and epithelioid	*TFCP2* fusion		Poor
	10–20 years	Not otherwise specified (NOS)	Spindled with embryonal-like areas	Unidentified		
Pleomorphic	50–60 years	Deep soft tissue of extremity	Large, atypical cells	Complex karyotype	Complex sarcomas	Poor

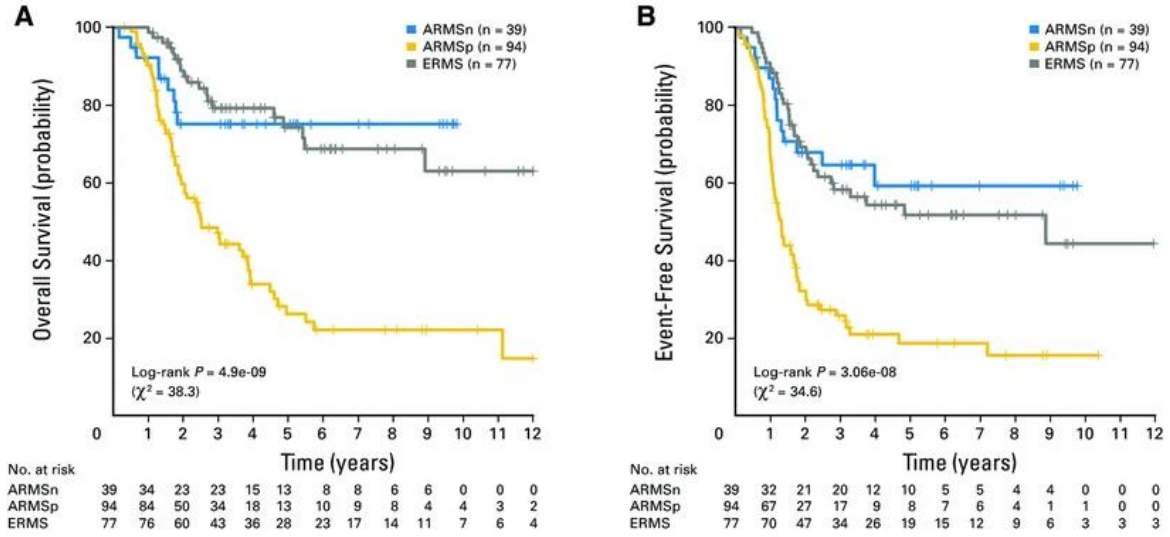
[a] Fusion positive and negative refers to *FOXO1* rearrangement.

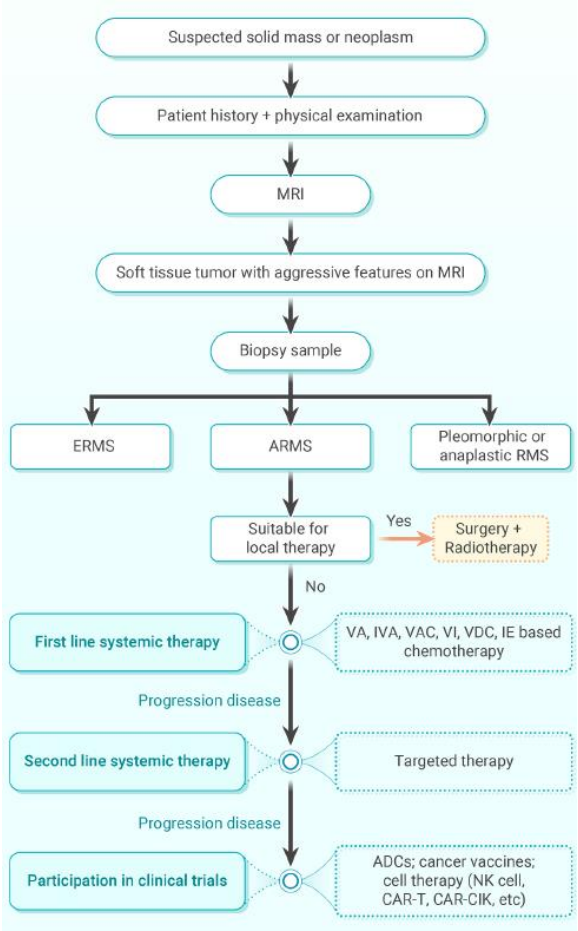


https://doi.org/10.1016/j.xinn.2025.101187

Clinical guidelines for RMS treatment and Targeted Therapies

- Advanced stage and HR refractory disease has poor prognosis with an overall survival of about only 20% despite intensified multimodal treatment
- First-line standard of care remains multidisciplinary (surgery, radiation, and multi-agent chemotherapy like VAC/IVA).
- However, in relapsed or refractory cases, targeted therapies and immunotherapies are actively utilized, either through off-label prescribing or clinical trials.





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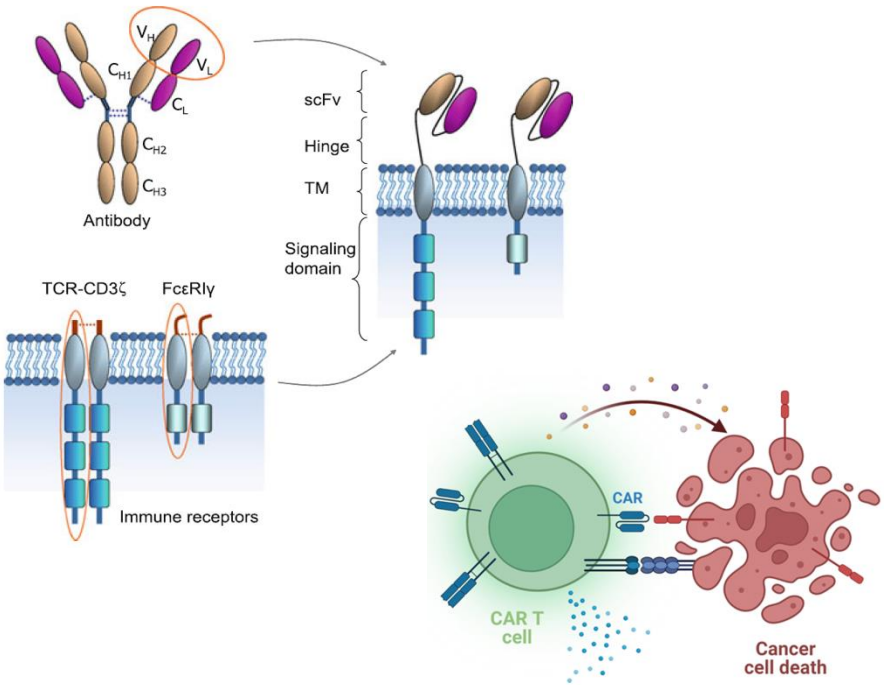
CAR-T cell therapy in rhabdomyosarcoma

Specific antigens of RMS were identified as immune markers for CAR T cell therapy



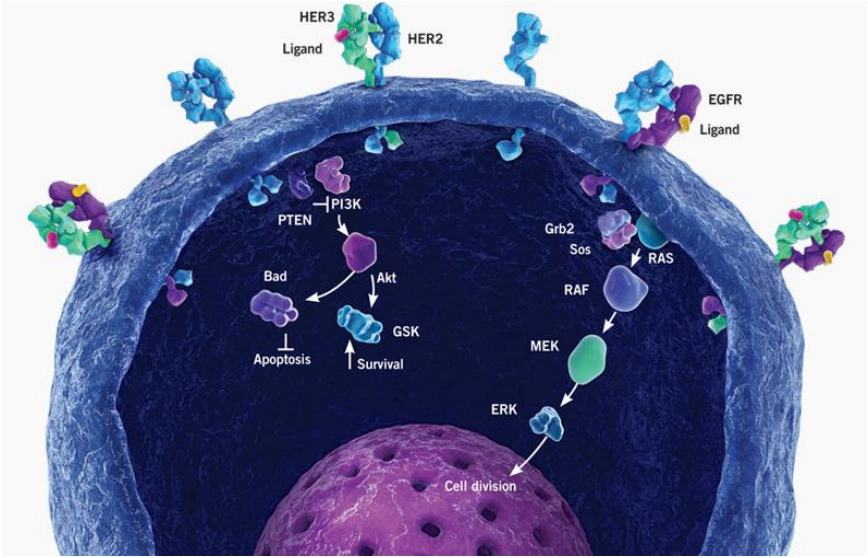
Target Antigen	Developmental Stage
HER2	Clinical evidence (case report)
CD276 (B7-H3)	Clinical trial (Phase I: NCT04483778)
CD56	Clinical evidence (case report)
FGFR4	Preclinical in vivo / Clinical trial initiated (NCT06865664)
PDGFR-α	Preclinical in vivo
EPHB4	Preclinical in vivo
fAChR	Preclinical in vivo
GD2	Preclinical in vivo
αvβ3 Integrin	Preclinical in vitro
IGF1R	Preclinical in vitro
ROR1	Preclinical in vitro

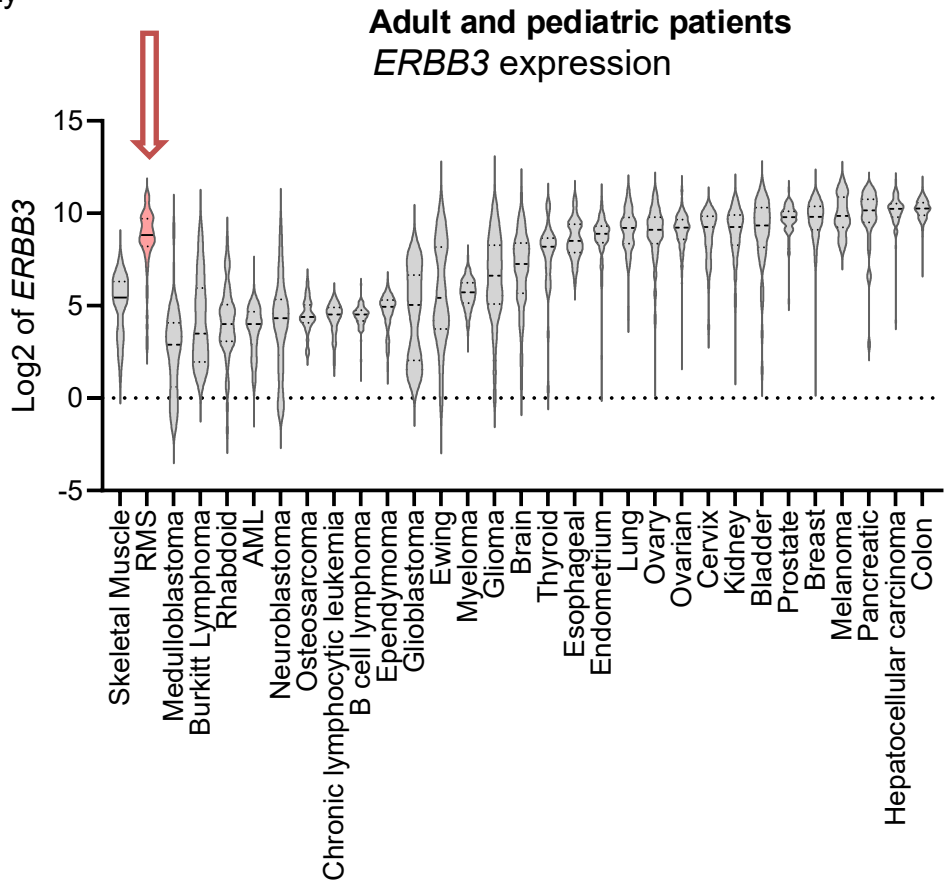
Safarzadeh Kozani P. *et al.*. J Transl Med. 2026 Feb 18;24(1):316. doi: 10.1186/s12967-026-07773-3.

Lipowska-Bhalla et al., 2012 DOI 10.1007/s00262-012-1254-0

HER3 antigen as a novel target for CAR T-cell therapy

- HER3 (or ErbB3) is a receptor tyrosine kinase ubiquitously expressed across various solid tumors;
- It is known driver of resistance to prior targeted therapies;
- Key node in cancer survival and metastasis

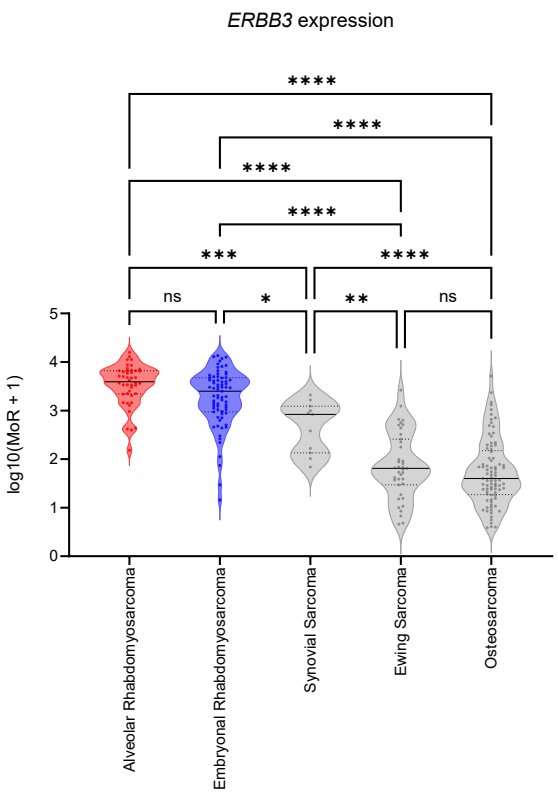


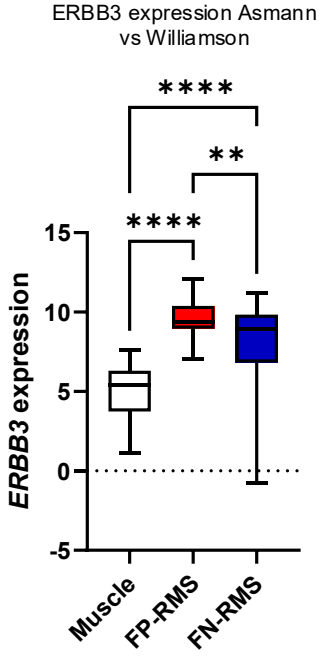


https://doi.org/10.1016/j.esmoop.2023.100790

HER3 antigen is overexpressed in pediatric rhabdomyosarcoma

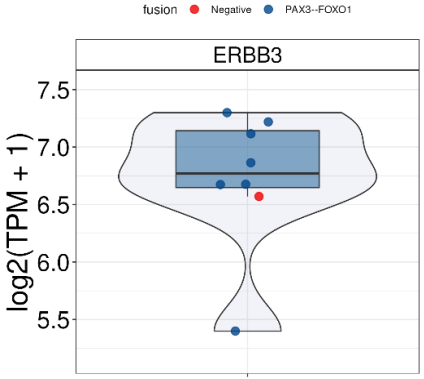
Pediatric patients with sarcomas



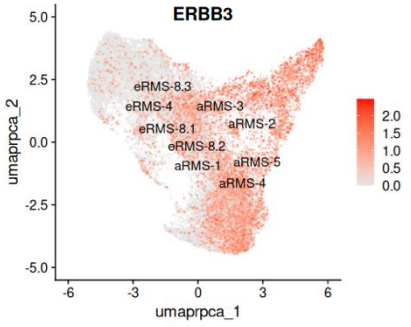


- HER3 is highly expressed in pediatric RMS
- ERBB3 enrichment in FP-RMS
- Validated across bulk and single-cell datasets
- HER3 is a candidate immunotherapeutic target for pediatric RMS

Bulk RNA-seq
OPBG RMS cohort

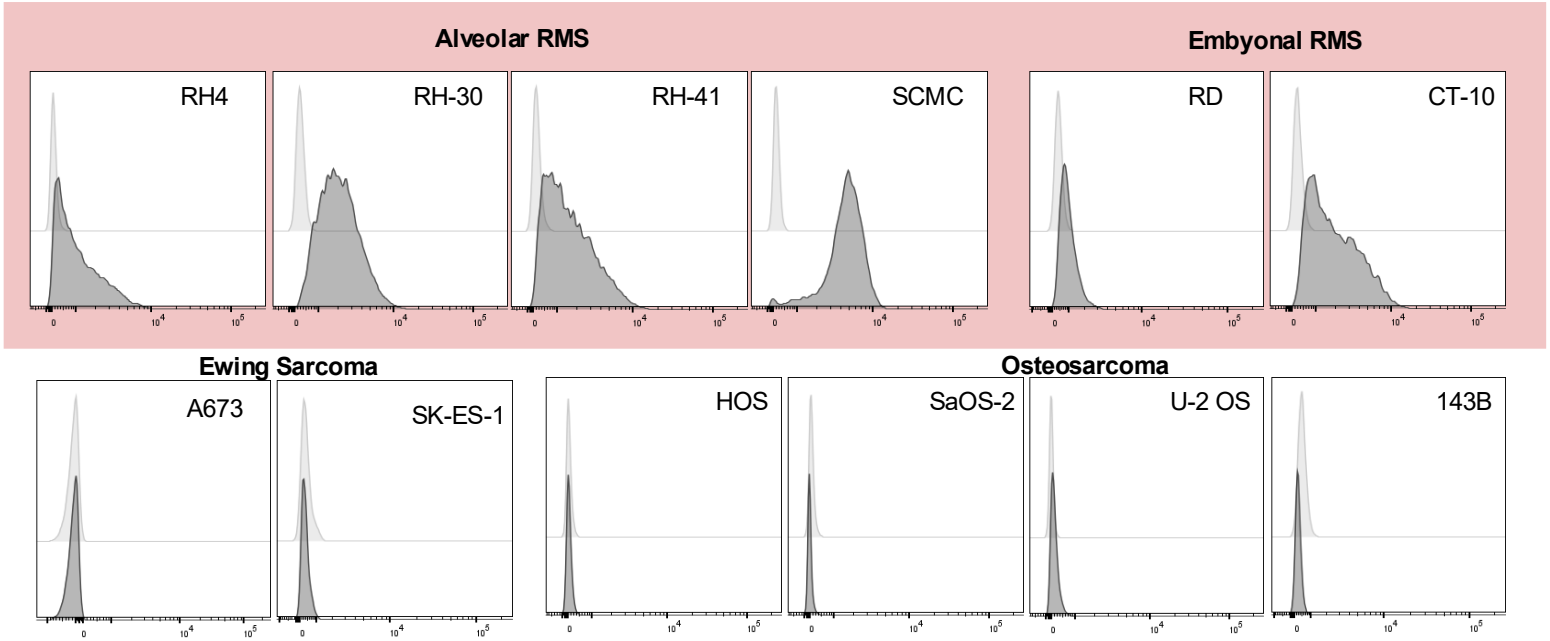


scRNA-seq
PDX model

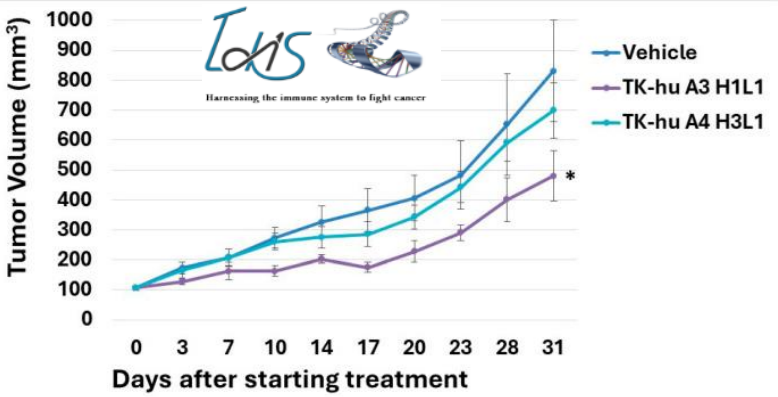


HER3 antigen is overexpressed in rhabdomyosarcoma cell lines

- HER3 is selectively overexpressed in rhabdomyosarcoma cell lines compared with other pediatric sarcoma models.
- Flow cytometry analysis demonstrates strong surface HER3 expression, particularly in alveolar RMS cell lines.
- These data support HER3 as a potential therapeutic target in RMS.

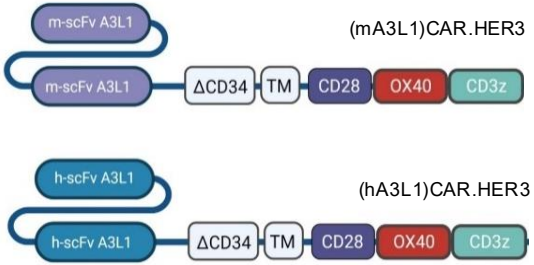


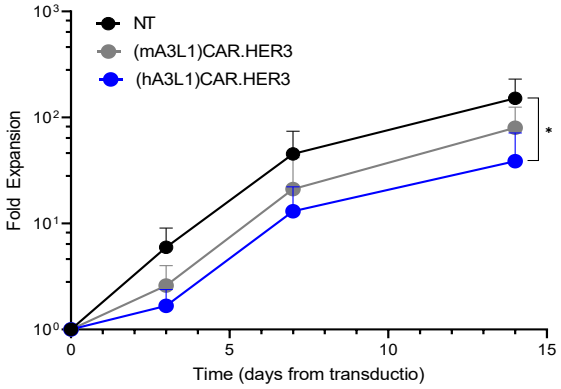
Development of novel αHER3 mAbs and CAR-T constructs

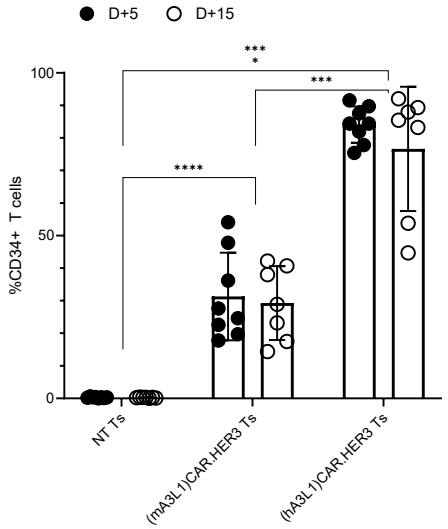


- TK-hu A3-H1L1 showed a clear anti-tumor effect *in vivo*, slowing the growth of BxPC-3 pancreatic cancer xenografts NSG mice
- Both murine and human A3L1 CAR.HER3 T-cells expanded similarly *in vitro* upon cytokine stimulation, indicating preserved proliferative capacity after engineering.
- However, the murine mA3L1 construct yielded lower transduction efficiency than the human hA3L1 version.

doi: 10.3390/antib14040084

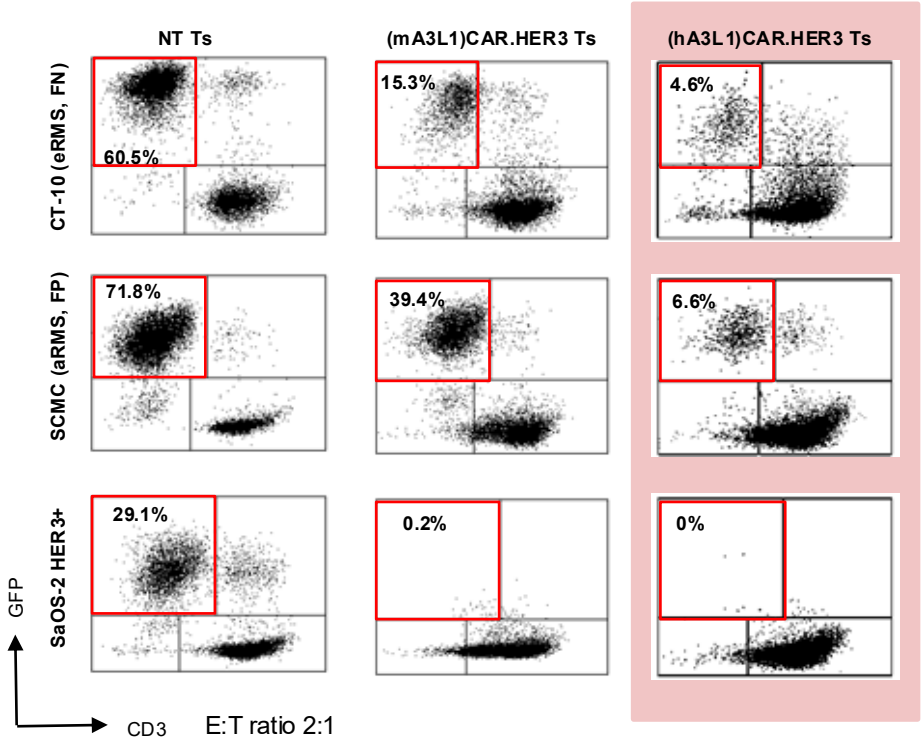


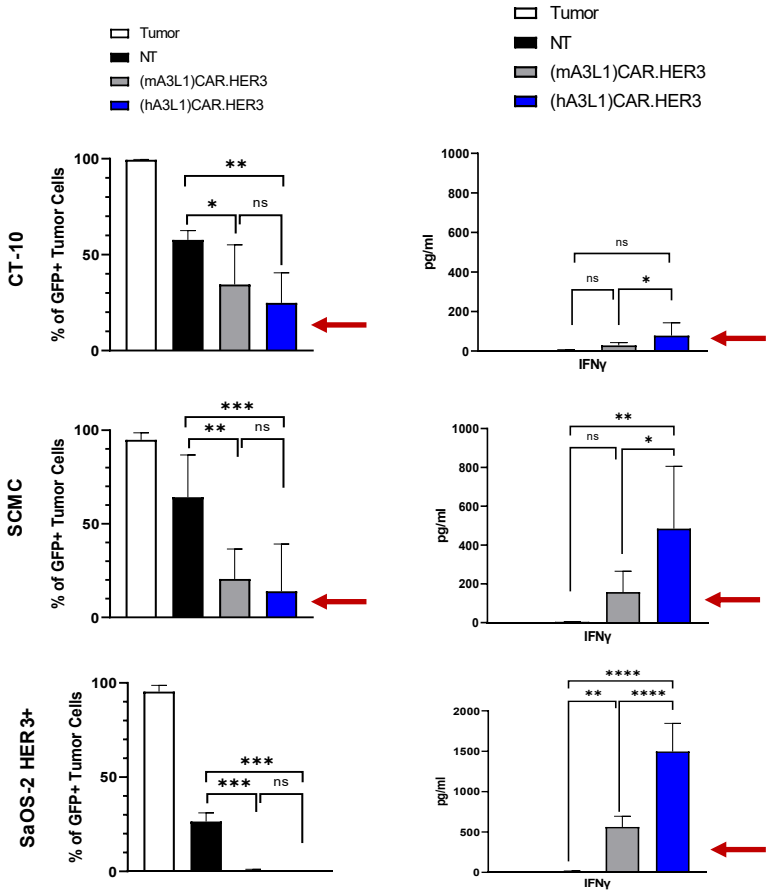




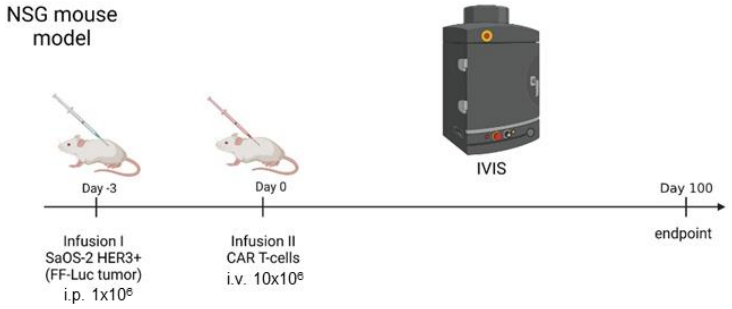
CAR.HER3 T-cells exert a significant antitumor activity against HER3+ tumor cell lines

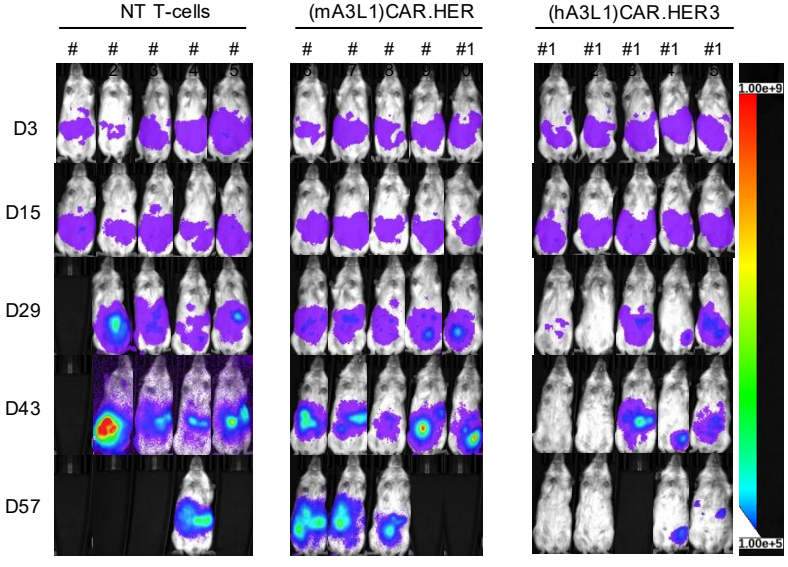
- CAR.HER3 T cells significantly reduced HER3+ tumor cell viability and induced robust cytotoxic and cytokine responses in co-culture assays



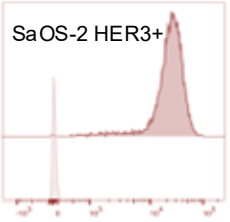


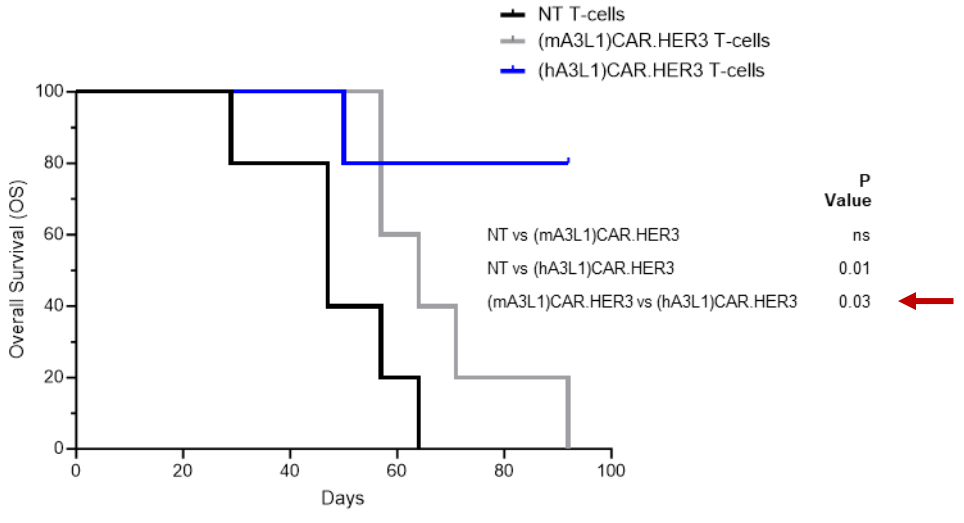
CAR.HER3 T-cells exert a significant antitumor in an *in vivo* model expressing exogenous levels of the antigen





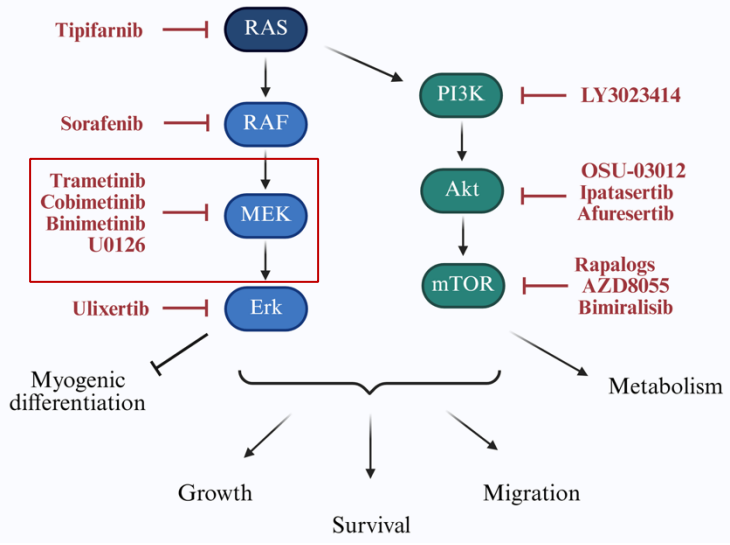
In HER3-high transgenic SaOS-2 model, the humanized scFv CAR-HER3 showed stronger *in vivo* tumor control and survival benefit than the murine scFv CAR-HER3



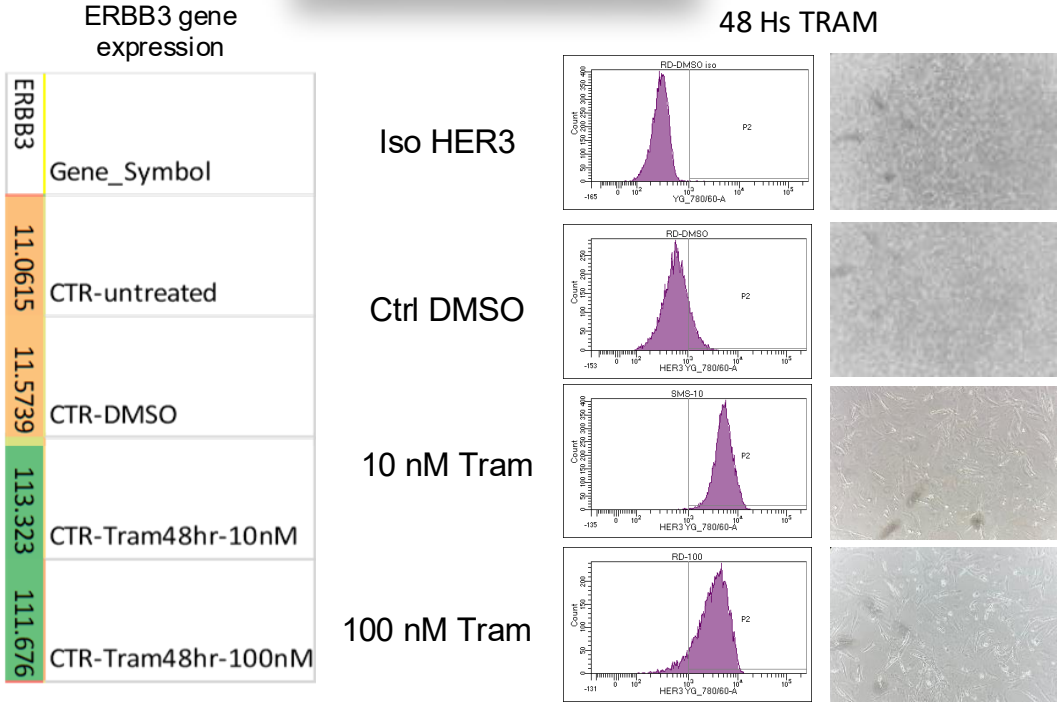


ERBB3 expression is induced upon Trametinib treatment in FN-RMS

- Trametinib is a MEK inhibitor and pro-differentiation drug (reduced RMS proliferation)
- ERBB3 is upregulated after MEK inhibition both is FN-RMS and FP RMS cell limes
- No ERBB upregulation on T-cells upon Trametinib exposure



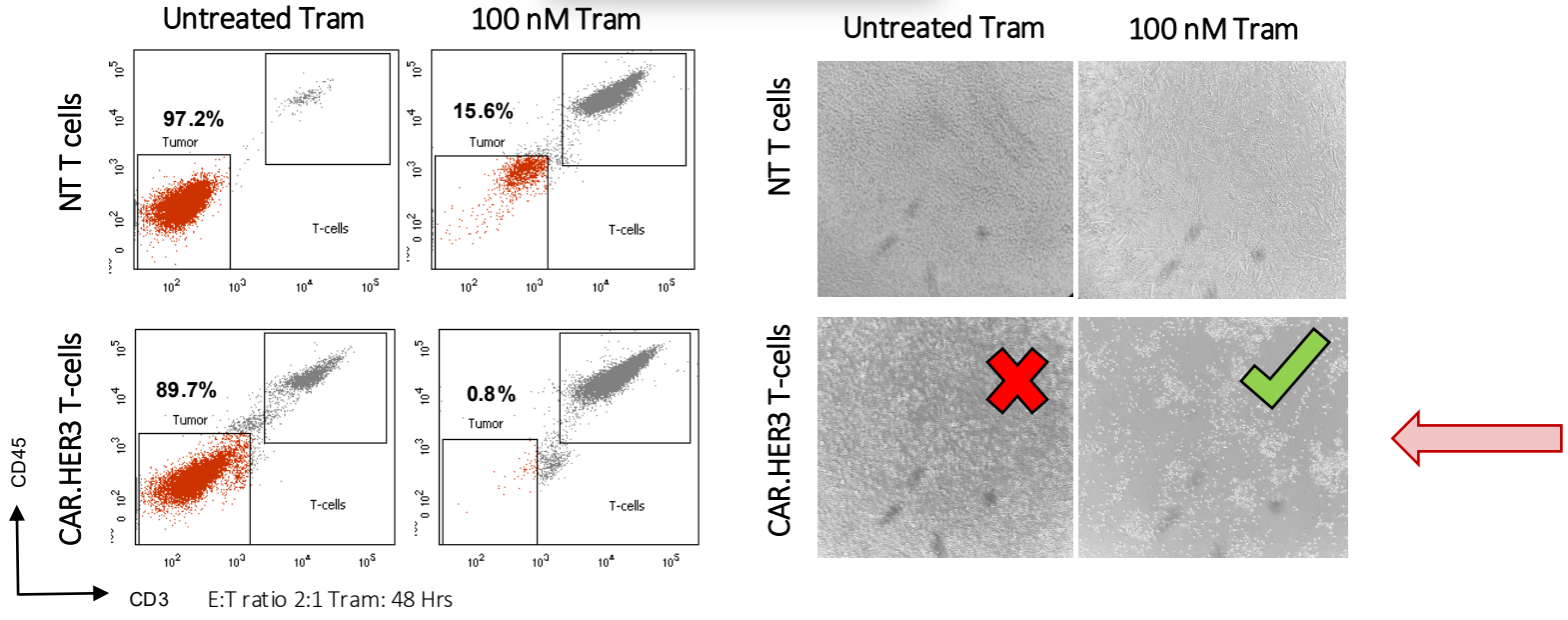
SMS-CTR, eRMS (FN)



Trametinib Potentiates the Antitumor Activity of CAR.HER3 T-cells *in vivo*

- CAR.HER3 plus Trametinib provides superior antitumor activity compared to CAR-T cells alone
- Dual targeting with CAR.HER3 and Trametinib improves tumor control and may limit resistance in RMS

SMS-CTR, eRMS (FN)



Conclusions and future perspectives

- **HER3 is a promising target in pediatric RMS**, with consistent expression across patient-derived datasets and tumor cell lines, supporting its exploitation for CAR-T cell therapy.

- **CAR.HER3 T-cells showed potent *in vitro* antitumor activity**, while *in vivo* efficacy was more evident in HER3-induced models, with the humanized scFv construct outperforming the murine version in tumor control and survival.

- **MEK inhibition may further enhance targetability**, as Trametinib increased ERBB3 expression in RMS cells but not in human T cells, providing a rationale for combination treatment.

Next Steps

- Optimizing CAR affinity for HER3-low tumors
- Validating efficacy in orthotopic RMS *in vivo* models with endogenous expression of HER3 + Trametinib
- Defining persistence, exhaustion, and safety profiles before clinical translation
- Multitarget strategy to avoid tumor escape and resistance

Acknowledgments

Ospedale Pediatrico Bambino Gesù, IRCCS
Department of Pediatric Hematology and Oncology
Prof. Franco Locatelli

Cell and Gene Therapy Unit
Prof. Concetta Quintarelli
Biagio De Angelis
Michele Pezzella
Biancamaria Cembrola
Roselia Ciccone
Simona Caruso
Manuela Ceccarelli
Jenny Leopoldina Smith
Simona Manni
Mariasole Aurigemma

Onco-Haematology Clinical & Research Unit
Giuseppe Maria Milano
Francesca Del Bufalo
Francesca Benini
Sofia Rahman

Epigenetics of Pediatric Sarcoma's Lab
Rossella Rota
Silvia Pomella
Marika Attili

Pathology Unit
Prof. Rita Alaggio
Sabina Barresi
Stracuzzi Alessandra

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