NUOVO METODO DI RANDOM-LIGATION PER LA GENERAZIONE DI COSTRUTTI CAR OTTIMIZZATI PER LE CELLULE NK

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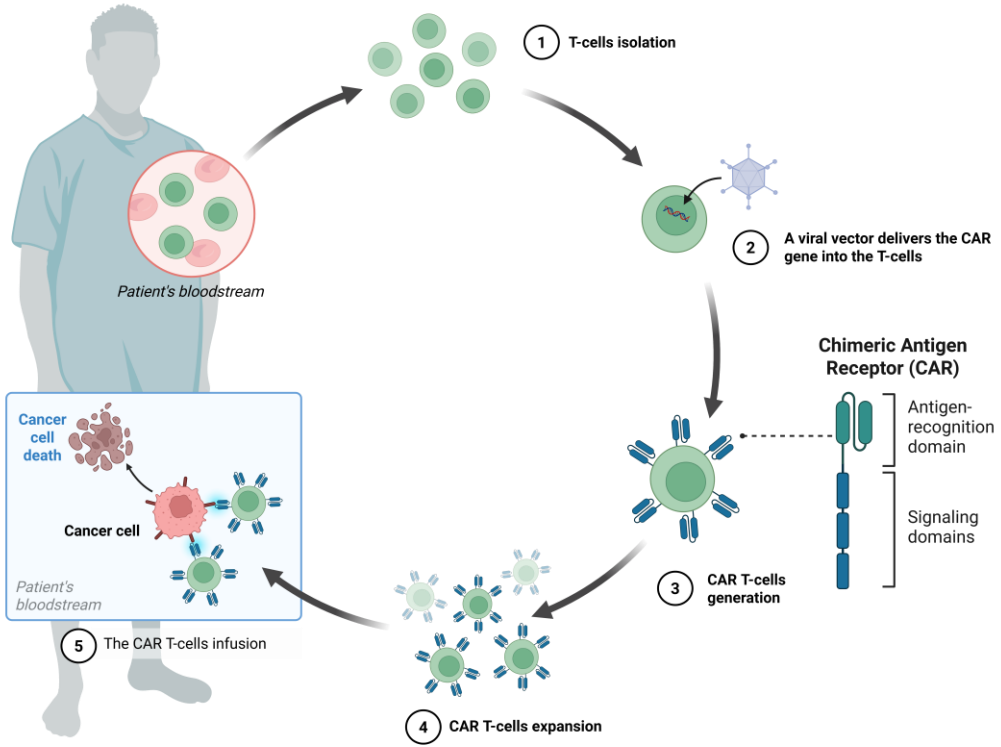
Disclosures of Andrea Sarcinelli

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
NONE	NONE	NONE	NONE	NONE	NONE	NONE	NONE

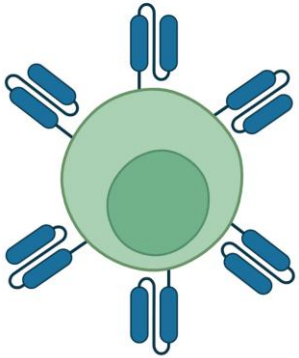
CAR-based immunotherapies



- Ex-vivo gene engineering of patient's cells
- Different generations: 1st, 2nd, 3rd
- Expensive Manufacturing
- Toxicities: CRS, ICANS

CAR-based immunotherapies

CAR T-cells

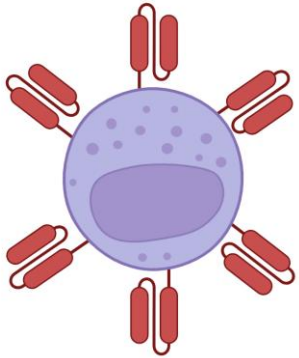


- Higher toxicities
- Autologous or *Allogenic*
- Slow manifacture
- High costs

FDA-approved

CAR T-cell constructs based on T-cells costimulatory domains: CD3z, CD28, CD8, 41BB

CAR NK-cells

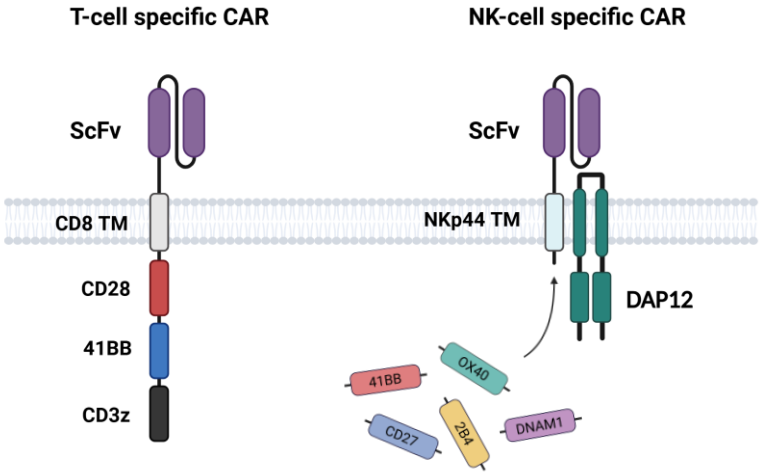


- Less toxicities: NO GvHD
- Allogenic: healthy donors
- Product “Off-the-Shelf”
- CAR-mediated + Innate NK
- Cheaper and Faster

Clinical trials ongoing

Clinical trials use T-cell–optimized CARs rather than NK-specific constructs.

CAR-design



CAR-NK clinical trials use T-cell–optimized CARs rather than NK-specific constructs

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Integration of T-specific co-stimulatory domains into NK-specific CAR constructs

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Integration using a *Random-Ligation* approach

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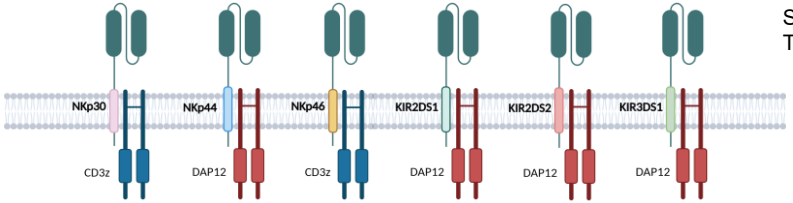
CAR-constructs suitable for T and NK cells

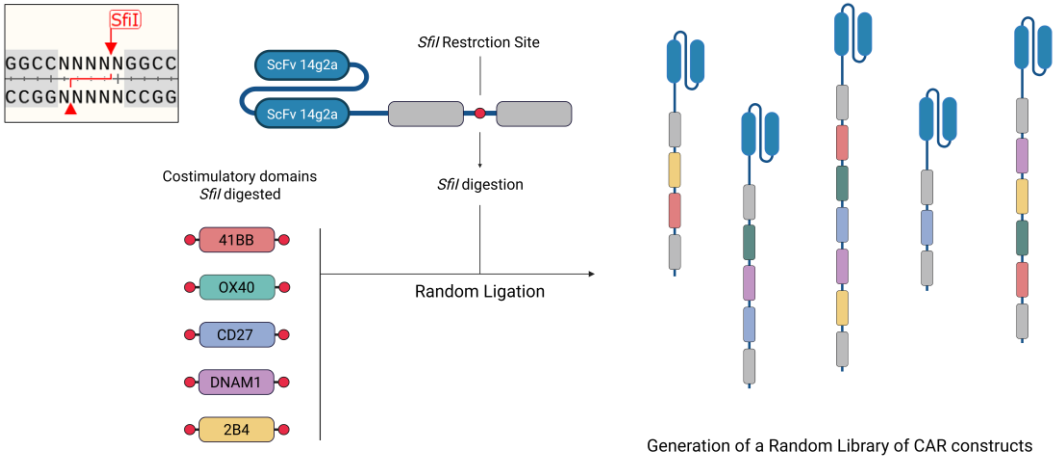
Random Ligation

6 different CAR backbones

Transmembrane	Co-receptors
NKp46	CD3z
NKp30	CD3z
NKp44	DAP12
KIR2DS1	DAP12
KIR2DS2	DAP12
KIR3DS1	DAP12



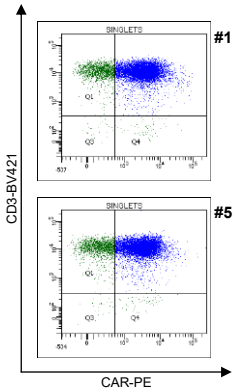
ScFv: 14G2a
Targeting GD2-antigen

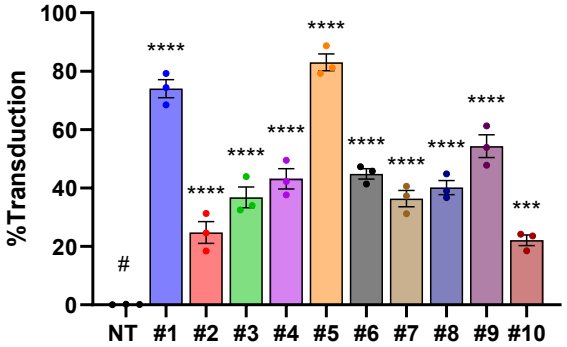


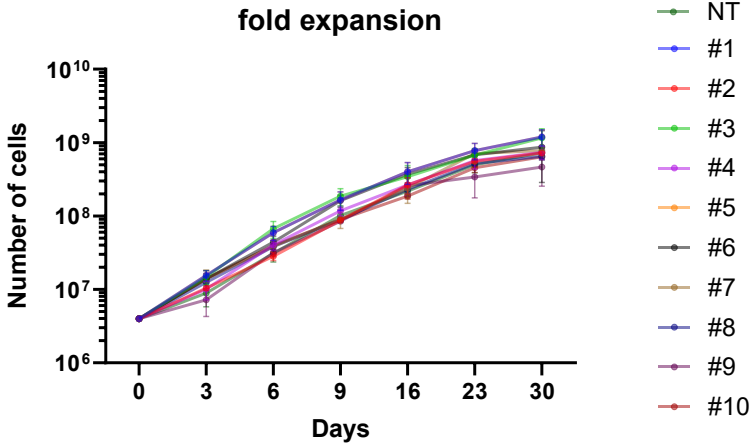
- Directional insertion 5'→ 3'
- Reduced self-ligation

29 CAR constructs were succefully generated

In-vitro characterization on T-cells







In-vitro Antitumor Activity was assessed using IncuCyte S3 live-cell imaging system (Sartorius) on 4 different cell lines: SHSY-5Y, IMR-32, D283, 143b.

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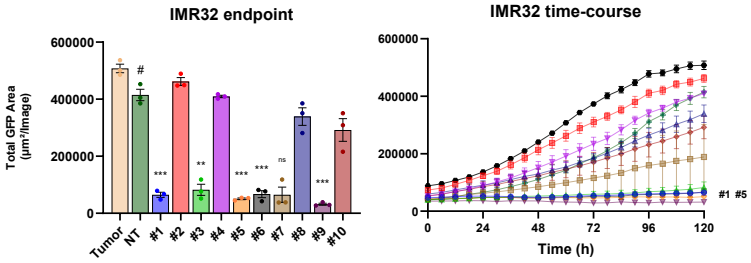
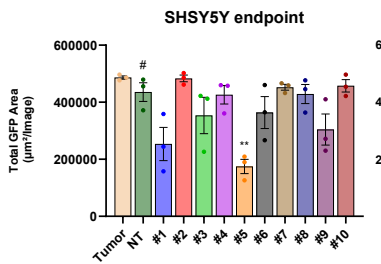
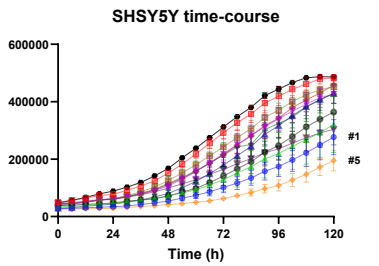
In-vitro characterization on T-cells







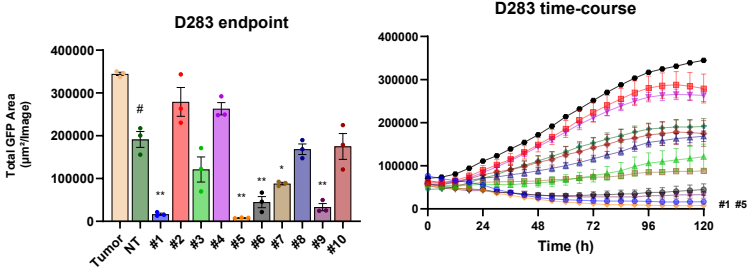
Neuroblastoma

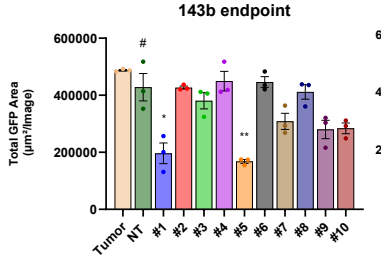
Medulloblastoma

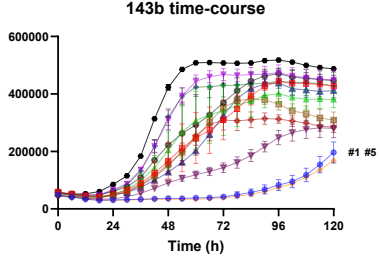
#1 and #5 best CAR constructs on T-cells selected for NK testing

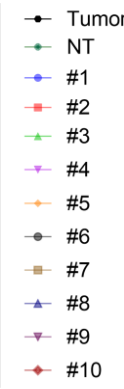
Neuroblastoma

Osteosarcoma









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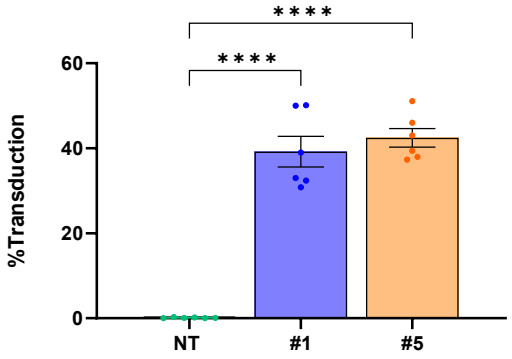
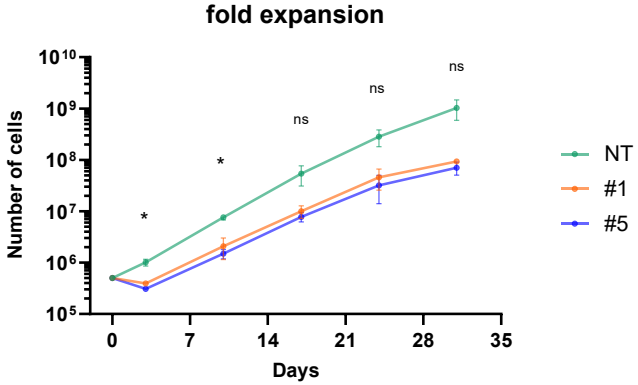
In-vitro characterization on NK-cells





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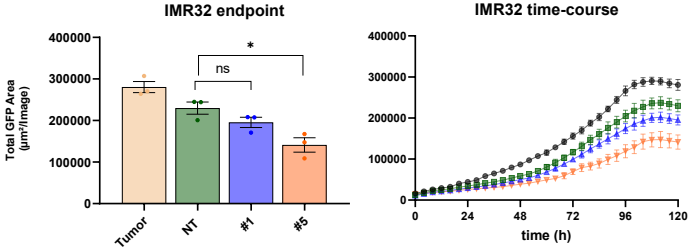
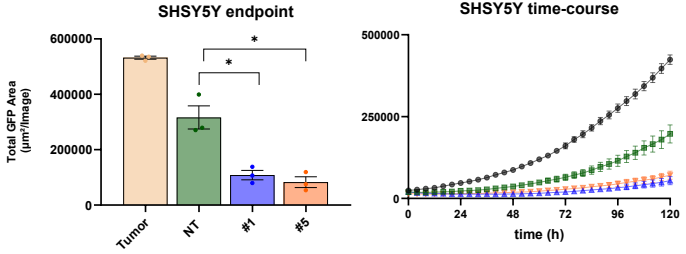
In-vitro characterization on NK-cells

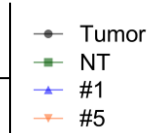
STEP 2: Antitumor assays CAR NK-cells (E:T → 1:2)

Neuroblastoma

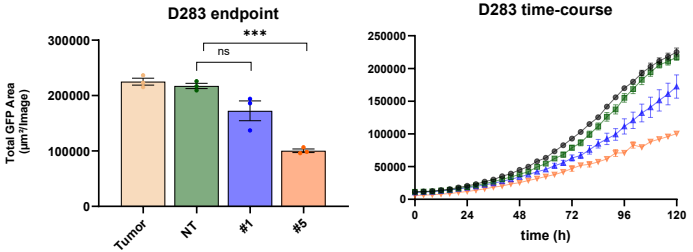


Neuroblastoma

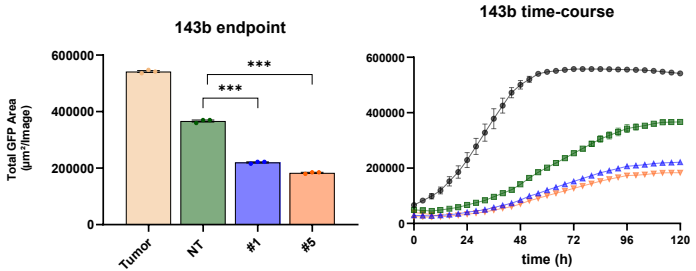




Medulloblastoma



Osteosarcoma



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Conclusions

- Efficient method based on *SfiI* RE able to generate a random library of CAR constructs with a high variability (N=29)
- CAR constructs are functionally active in both T and NK cells (#1 and #5 best candidates)
- Co-stimulatory domains efficiently worked *in-vitro* and they can be exploited for CAR design

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FUTURE PROPECTIVES

- To assess CAR activity using *in-vivo* experiments with T and NK cells
- Expand the library of CAR constructs
- Validate more co-stimulatory domains to integrate into the CAR design
- Optimization of *SfiI* cloning method

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In-vitro characterization on NK-cells

STEP 1: Potency assays NT NK-cells