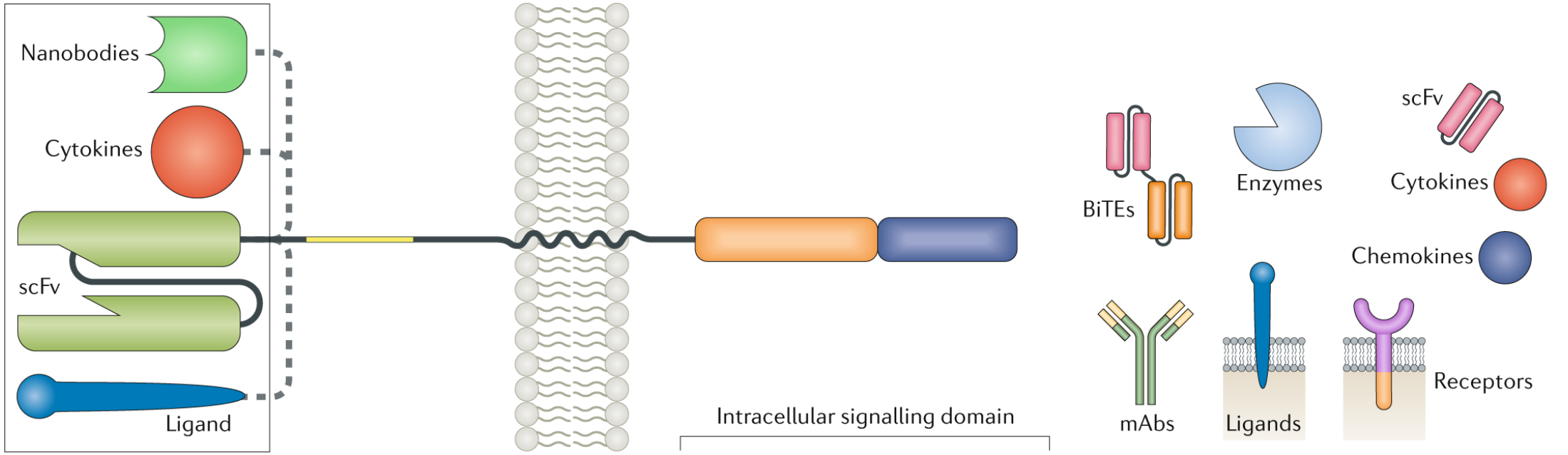
Armoring CAR T cells for solid tumor malignancies

ROSWELL PARK
COMPREHENSIVE CANCER CENTER

Amoring Strategies



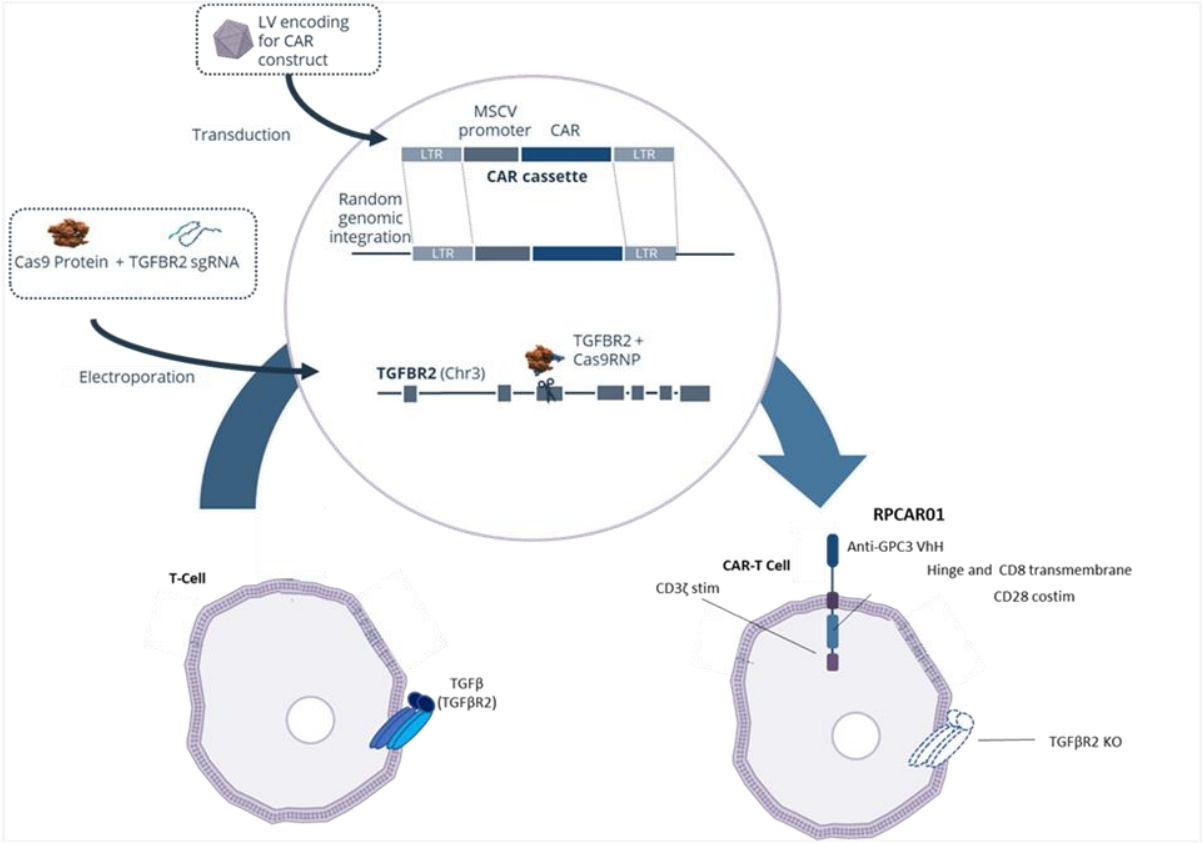
Binding domain	Hinge	Transmembrane domain	Co-stimulation	Activation	Armour
• scFv • Nanobodies (camelid V_HH domains) • Cytokines • Ligands • Peptides (adnectins and DARPins)	• CD8 • CD28 • IgG1 • IgG4	• CD3ζ • CD8α • CD4 • CD28 • ICOS	• CD28 • 4-1BB • OX40 • ICOS • CD27 • MYD88–CD40 • KIR2DS2	• CD3ζ	• Cytokines or chemokines • Cytokine or chemokine receptors • Ligands or receptors • mAbs • BiTEs • scFv • Peptides (IL-1Ra, off-switch receptors or inducible suicide constructs) • Enzymes

Rationale for deleting TGFβR2 in CAR T cells

TGF-β inhibits many aspects of T cell anti-tumor immunity

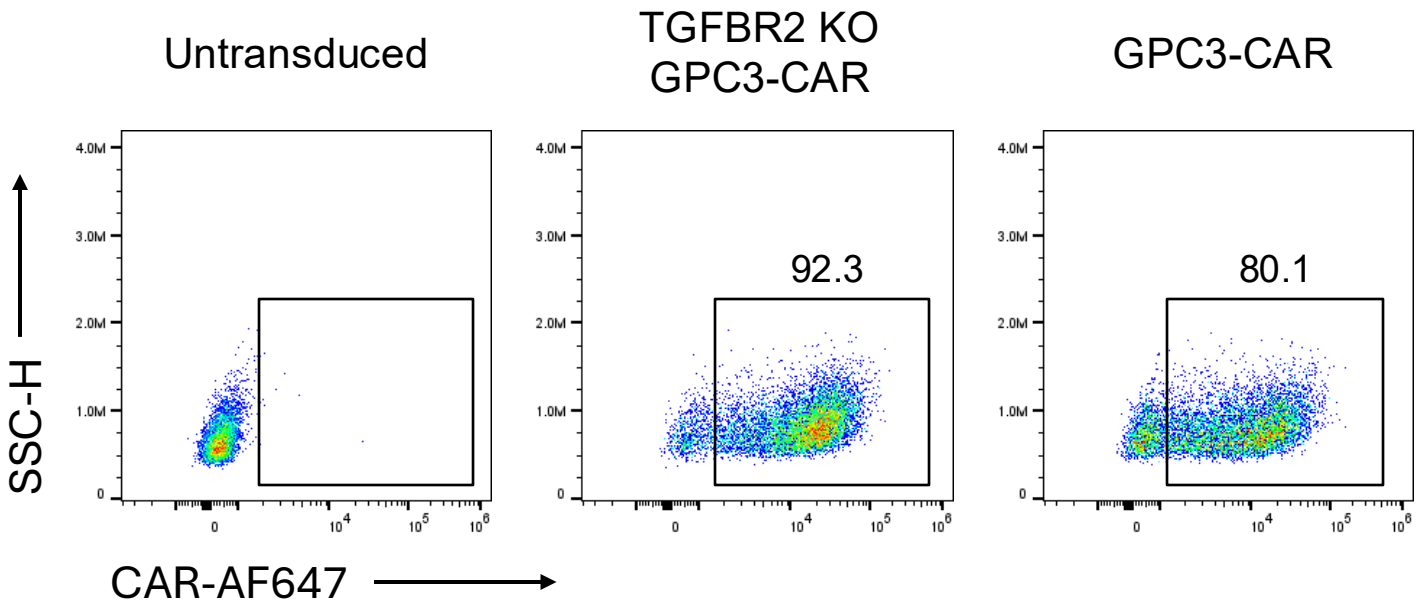
- Transcriptional downregulation of granzymes, perforin, cytotoxins
  Thomas et. al., *Cancer Cell*, 2005
- Inhibition of Thelper cells and promotes Treg differentiation
  Flavell et. al., *Nat Rev Immunol*, 2010
- Direct suppression of T cell activation, production of effector cytokines, cytolysis, proliferation
  de Folmont et. al., *Immuno*, 2021
  Tang et. al., *JCI Insight*, 2020
  Stuber et. al., *JITC*, 2020
  Salmond et. al., *Biology*, 2023
- Promotes inhibitory receptor expression
  Park et. al., *Cancer Discovery*, 2010
- Effect on CAR T cell metabolism is poorly understood

Generation of TGFβR2 KO GPC3-CAR T cells



T cell Isolation	Activation	TransAct removal	Electroporation	Transduction	Transduction duration	Cytokine replenishment	Cryopreservation
Day 0	**D0-D2**	**Day 2-3**	**Day 3**	**Day 3**	**Day 3-5**	**Every 2-3 days**	**Day 9**
T-cells purified through negative selection from PBMCs	TransAct for 48 Hours	Wash T-cells through centrifugation followed by 24 hours rest	RNP targeting TGFβR2 locus	GPC3-CAR LV MOI 3	48 hours	Addition of fresh T-cell media	10% dimethyl sulfoxide (DMSO) cryopreservation buffer

Efficient GPC3-CAR expression and TGFβR2-editing in human T cells



Research Lot Number	By Flow Cytometry CAR+	By AmpSeq TGFβR2 -
RPCAR01-Res-006	60.1	97.4
RPCAR01-Res-007	62.8	92.8
RPCAR01-Res-008	75.5	89
RPCAR01-Res-009	52.2	91.1
RPCAR01-Res-010	61.0	96.7
RPCAR01-Res-011	86.2	96
RPCAR01-Res-012	92.3	97.4
Mean	70.0	94.3
S.D.	13.8	3.13

GPC3-CAR T cells target human hepatoma *in vitro*

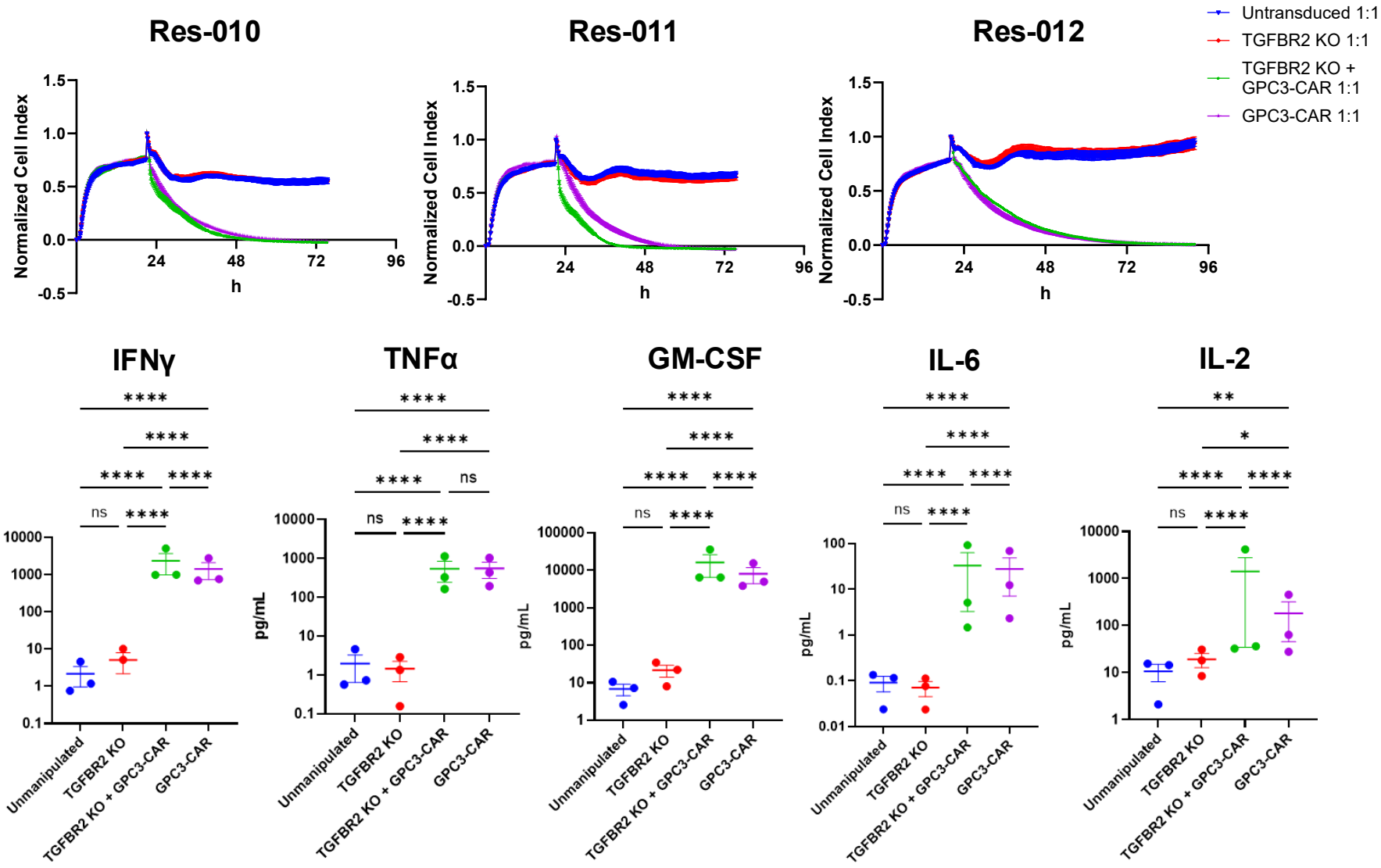
CAR T cell + Hep3B/Huh7 coculture

↓

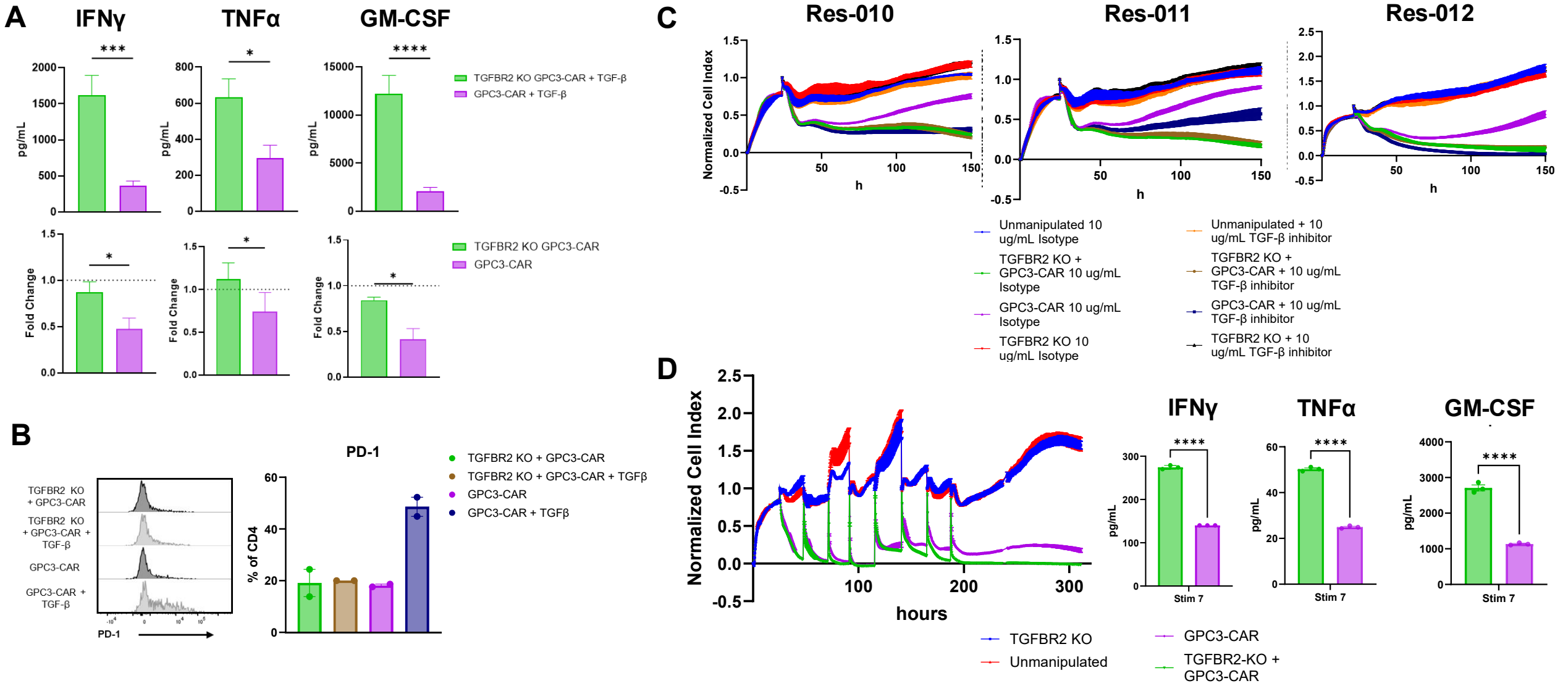
xCELLigence RTCA (cytotoxicity)

↓

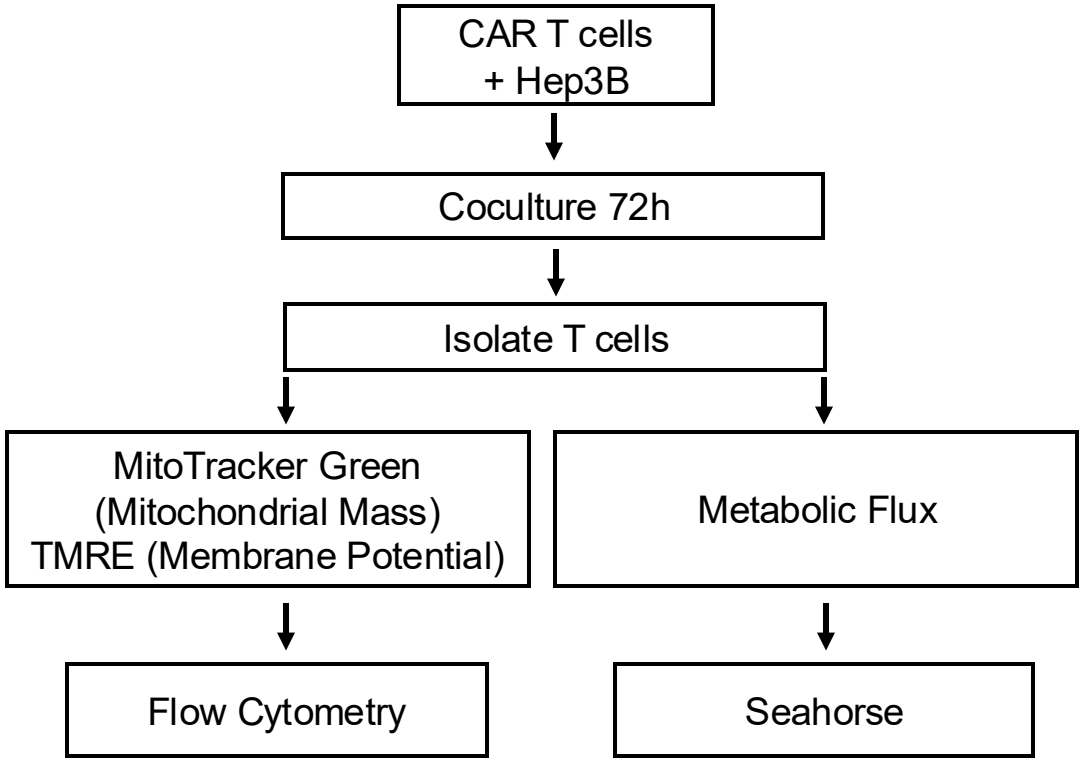
Collect coculture supernatants (cytokines)

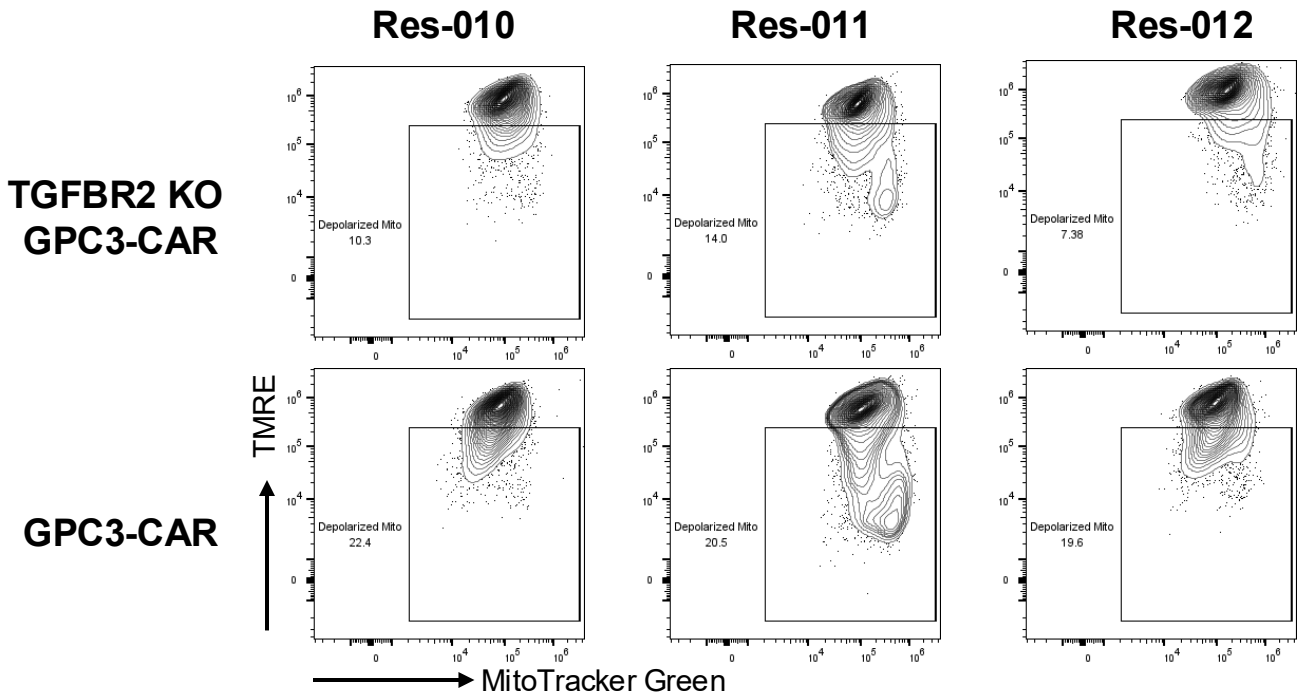


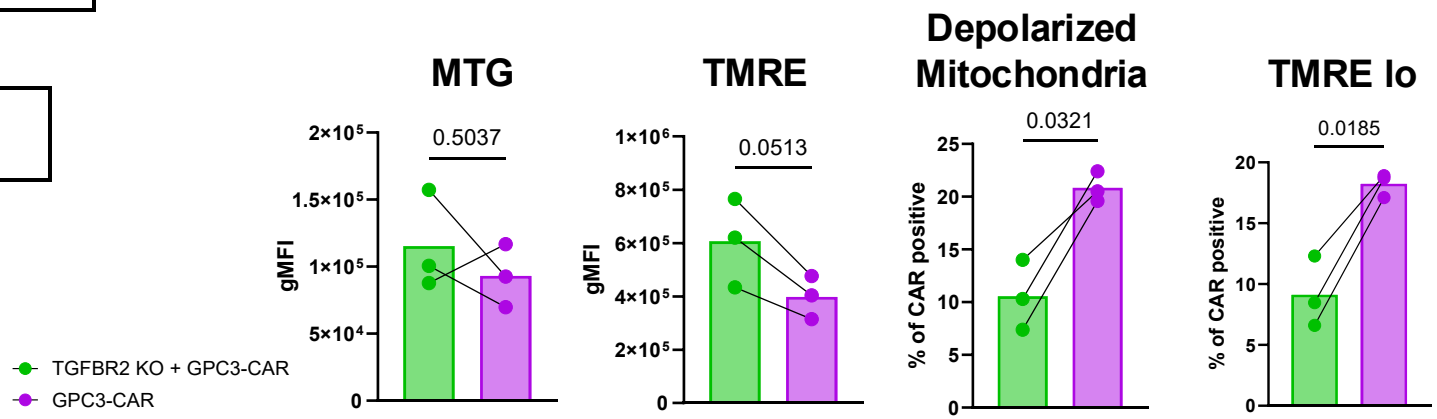
TGFβR2 KO GPC3-CAR T cells resist TGF-β-mediated immunosuppression *in vitro*



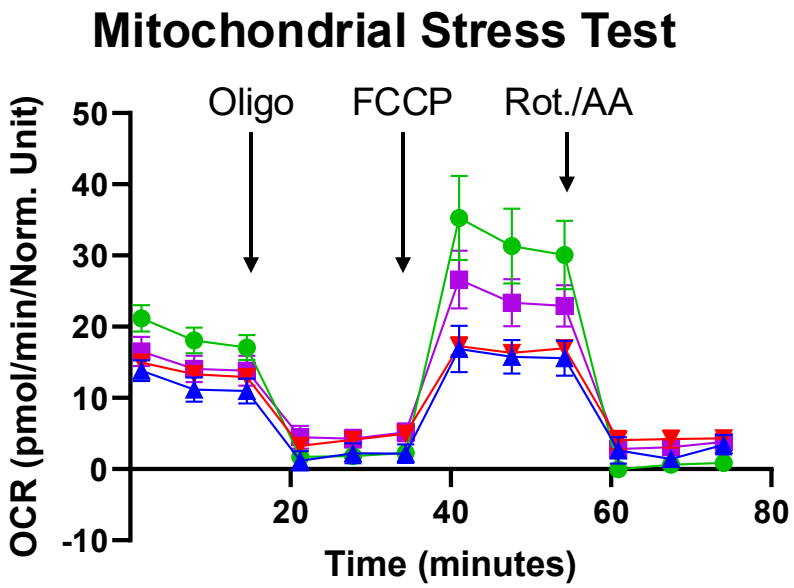
TGFβR2 KO GPC3-CAR T cells accumulate less dysfunctional mitochondria in cocultures with Hep3B

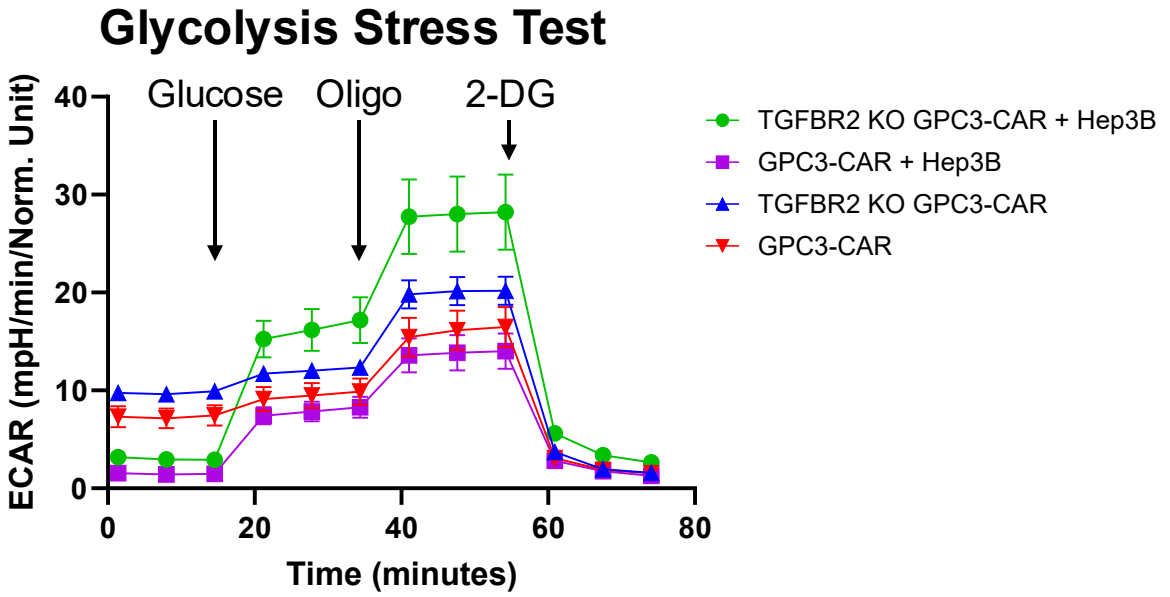


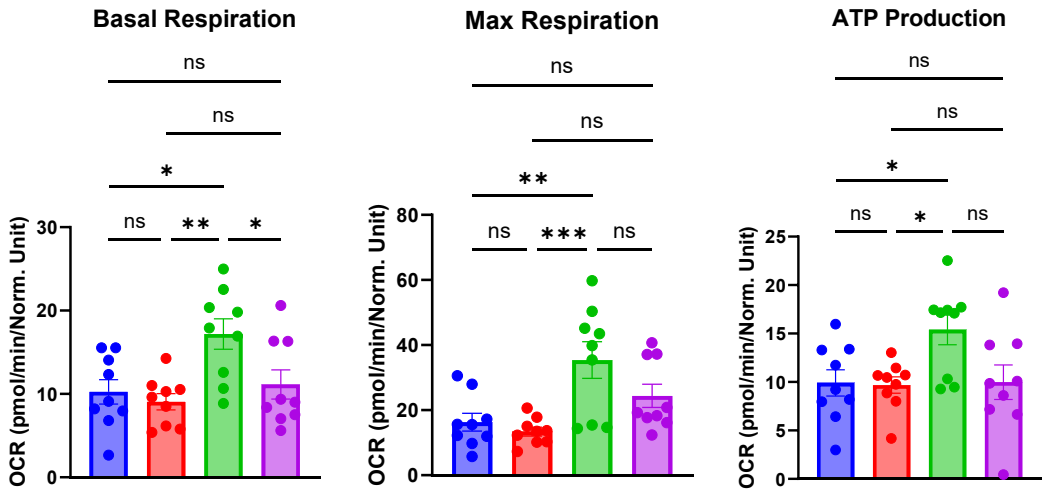


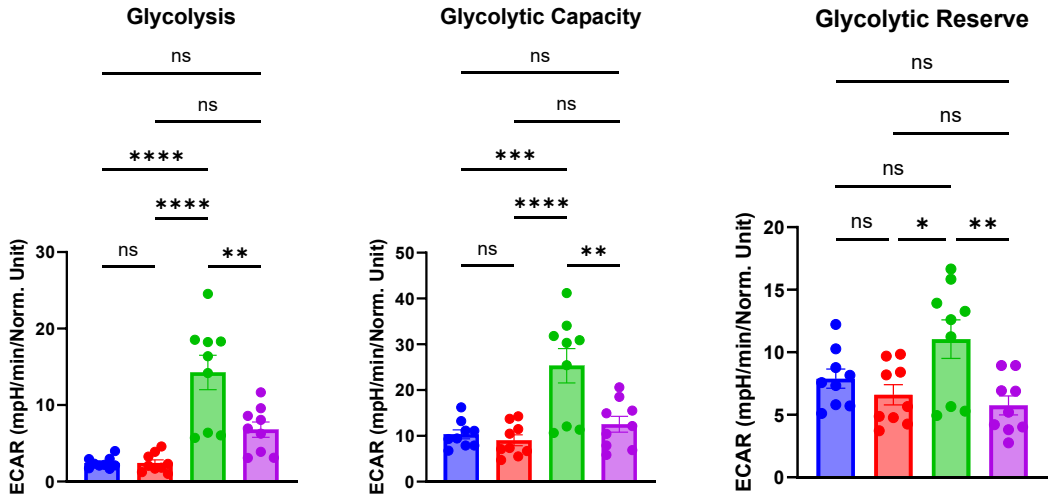


TGFβR2 KO GPC3-CAR T cells resist metabolic suppression after coculture with Hep3B cells









Trial Schema

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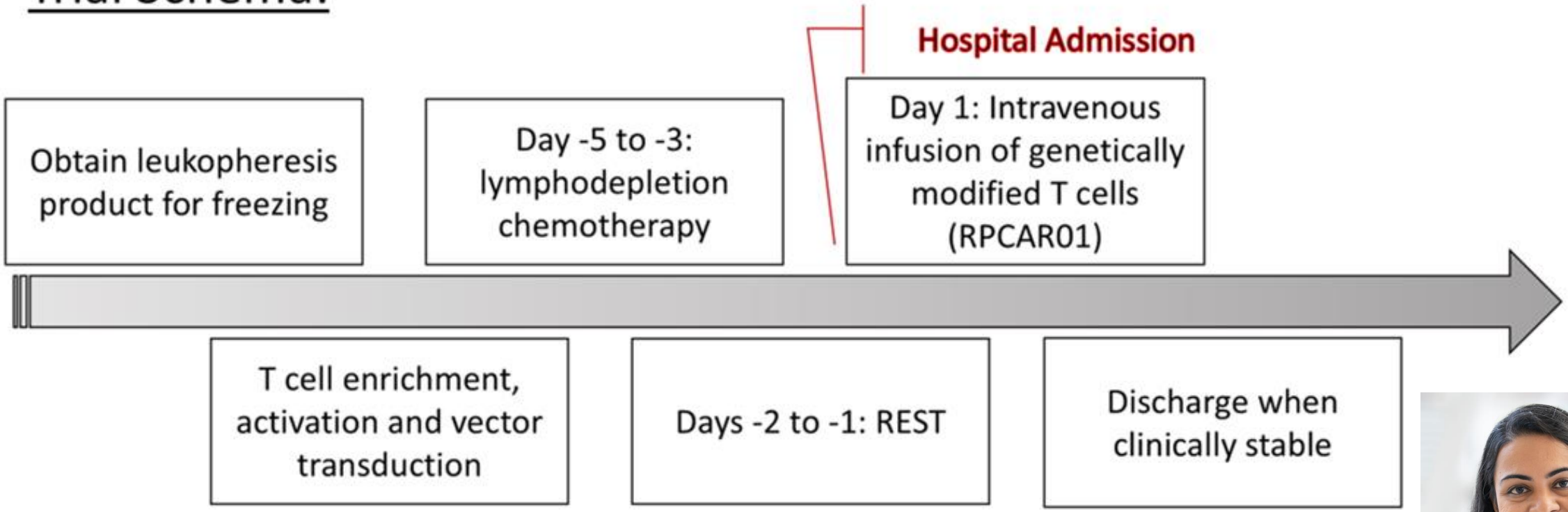
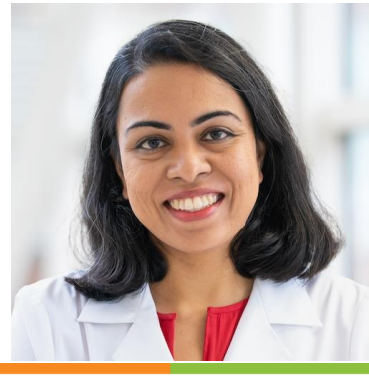
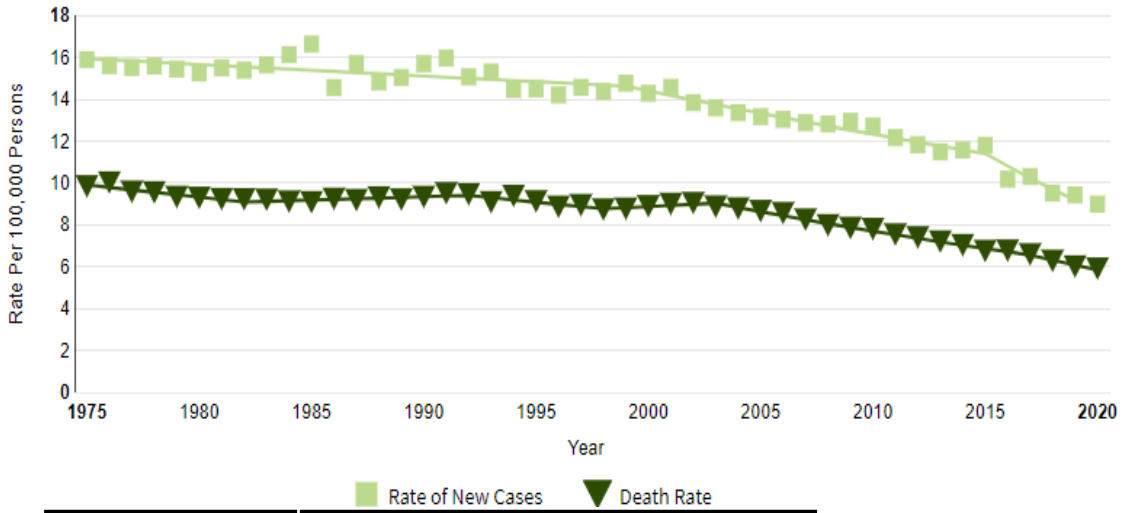




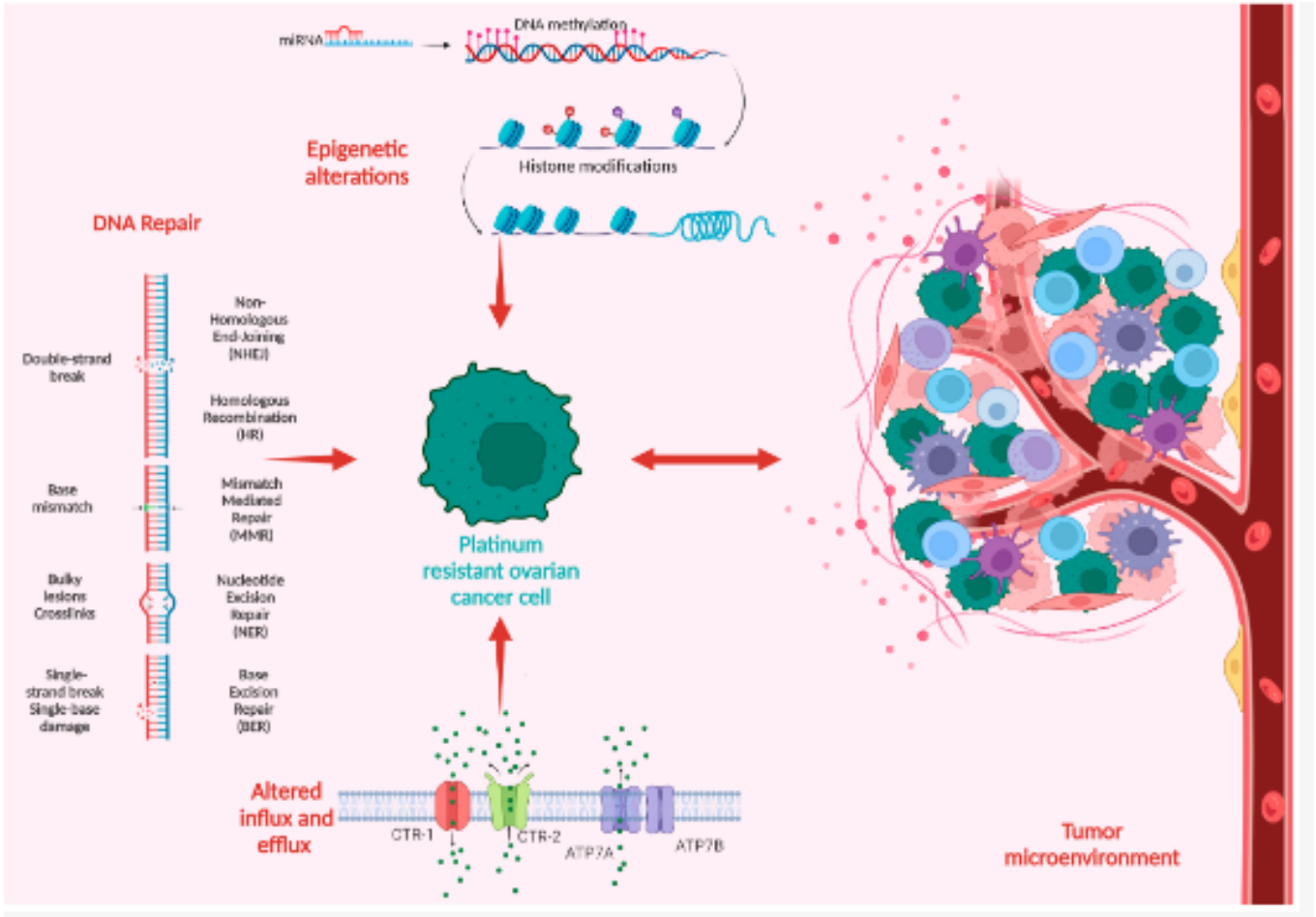
Novel therapies needed for ovarian cancer

- Most lethal gynecologic malignancy
- Deaths predicted to raise by 67% by 2035



Stage at diagnosis	5-year survival
I	89%
II	71%
III	41%
IV	20%

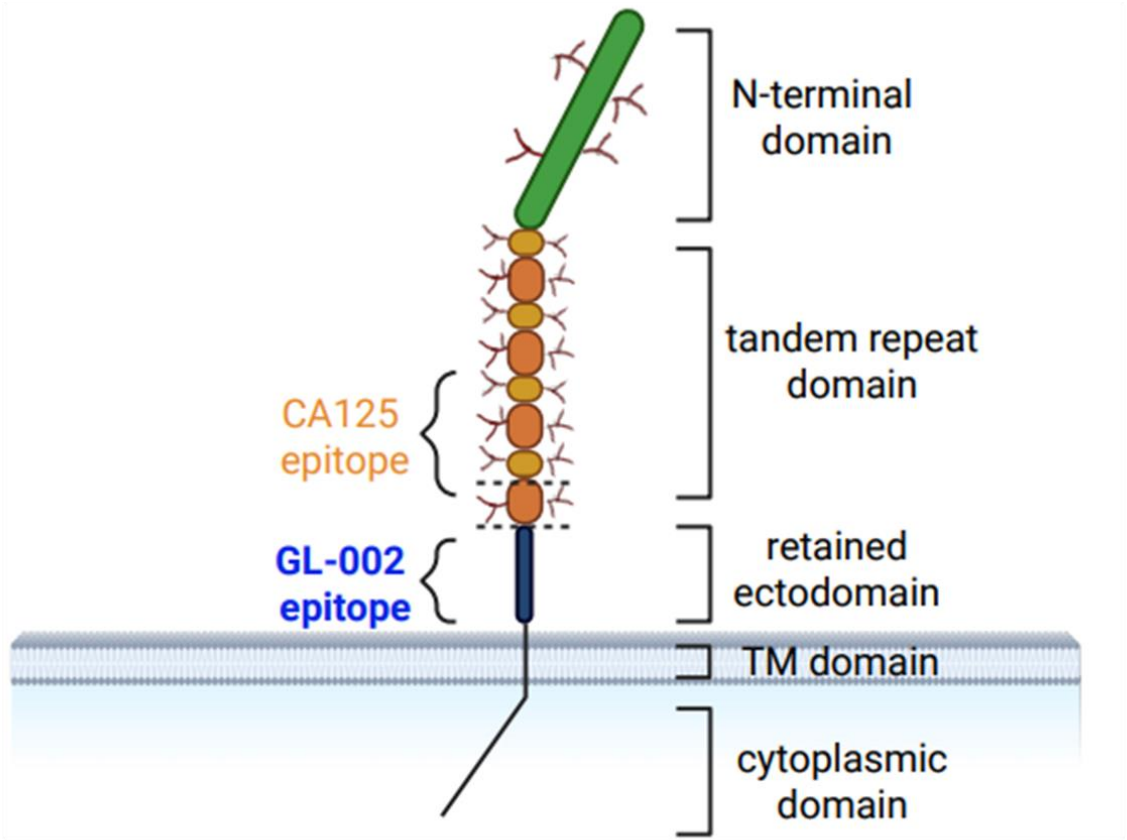
70% of diagnoses



Cancer.gov; Havasi, A., et al. 2023 MDPI

MUC16 is overexpressed in HGSOC

A.



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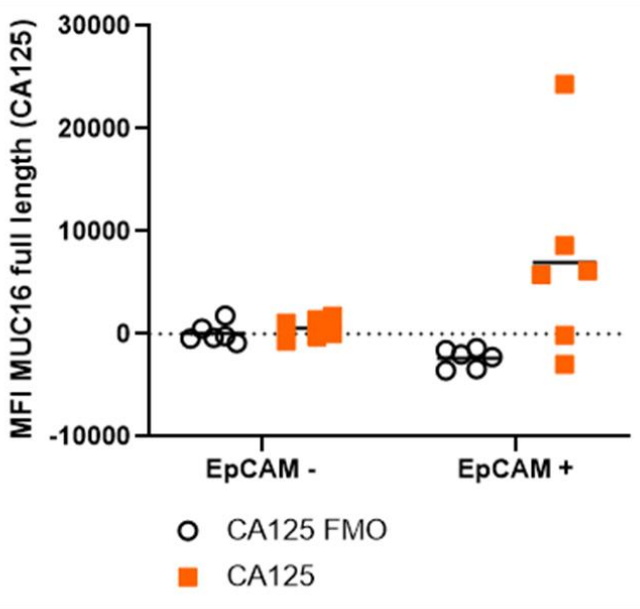

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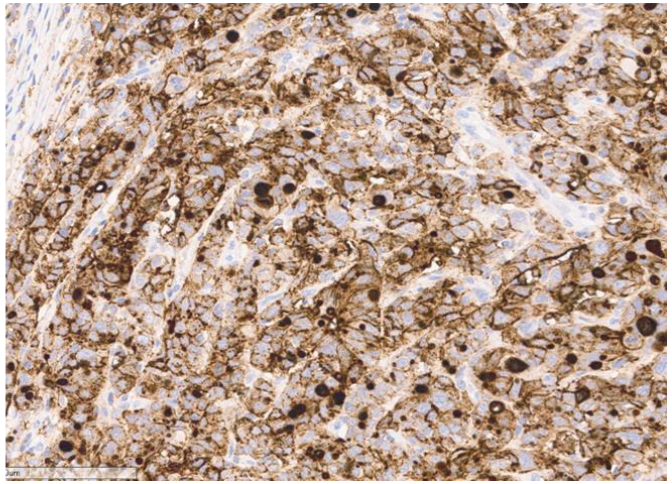
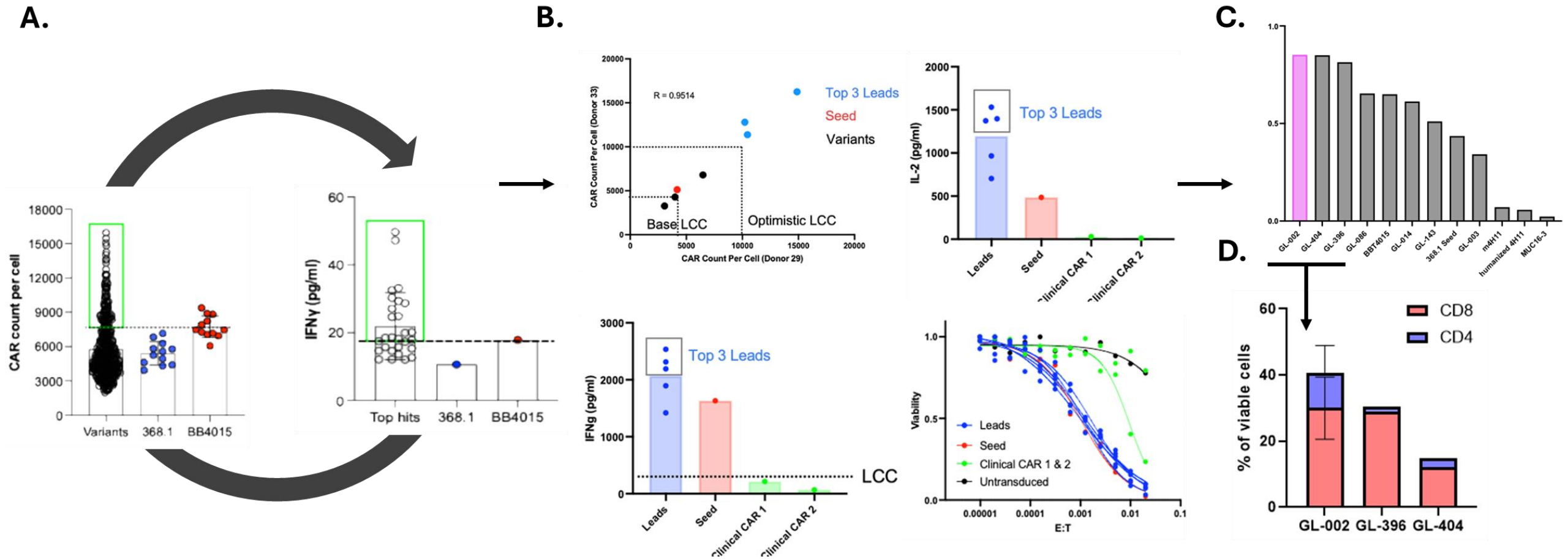
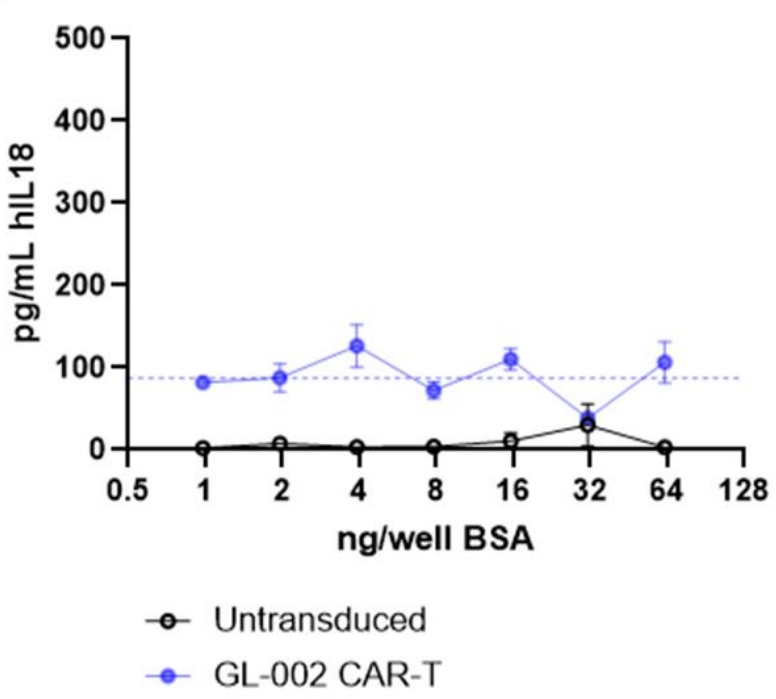
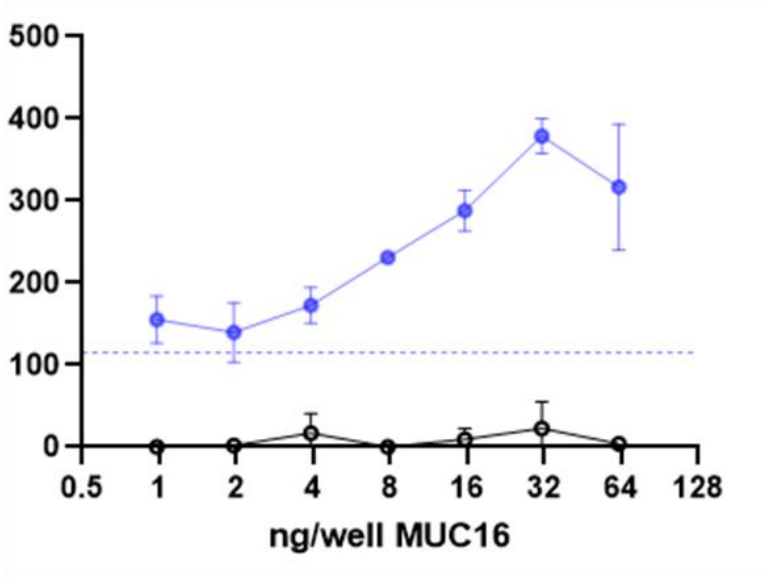


Novel MUC16 binders yielded through AI assisted protein evolution

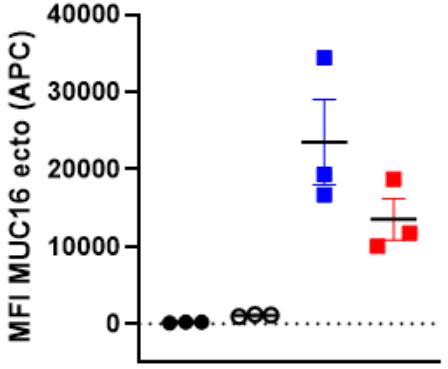


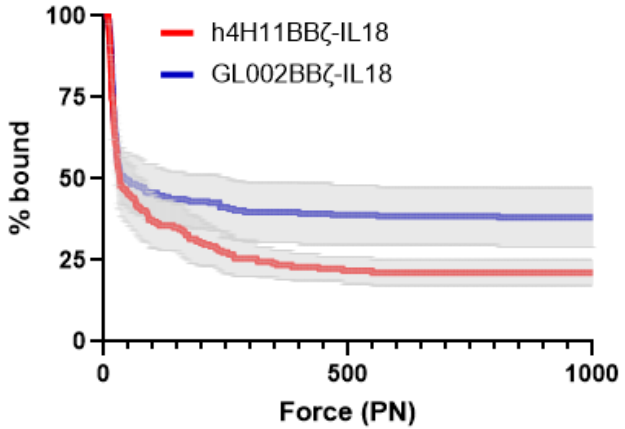
GL-002/IL-18 CAR-T cells secrete IL-18 in an antigen dependent manner

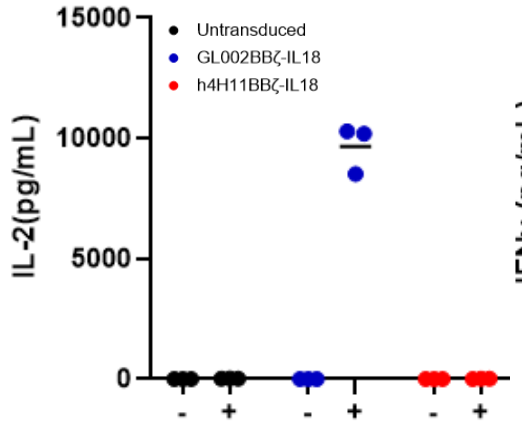


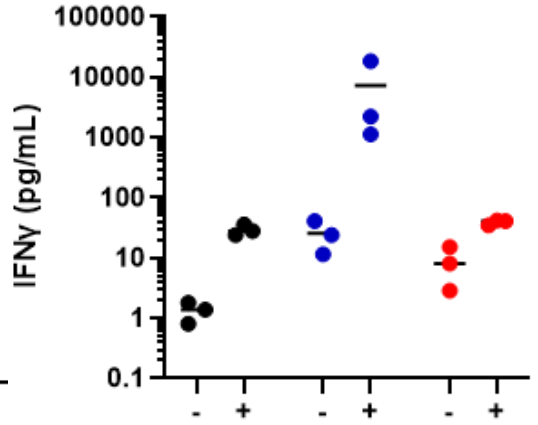


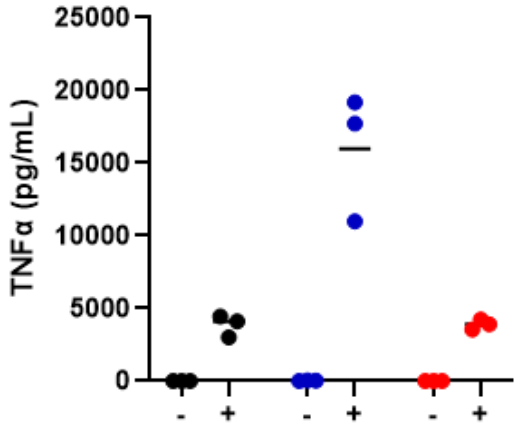
GL-002 CAR-T cells outperform previous MUC16 targeting CAR-T cells *in vitro*

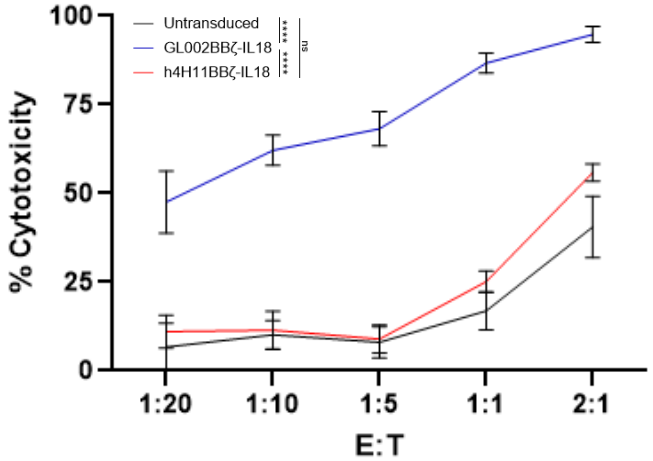






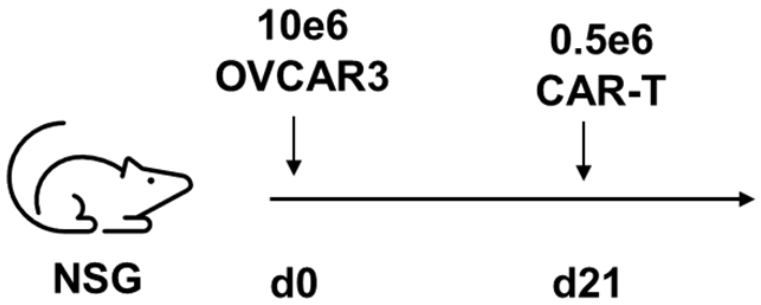






GL-002 CAR-T display anti-tumor efficacy *in vivo* and on additional MUC16+ tumor lines

A.



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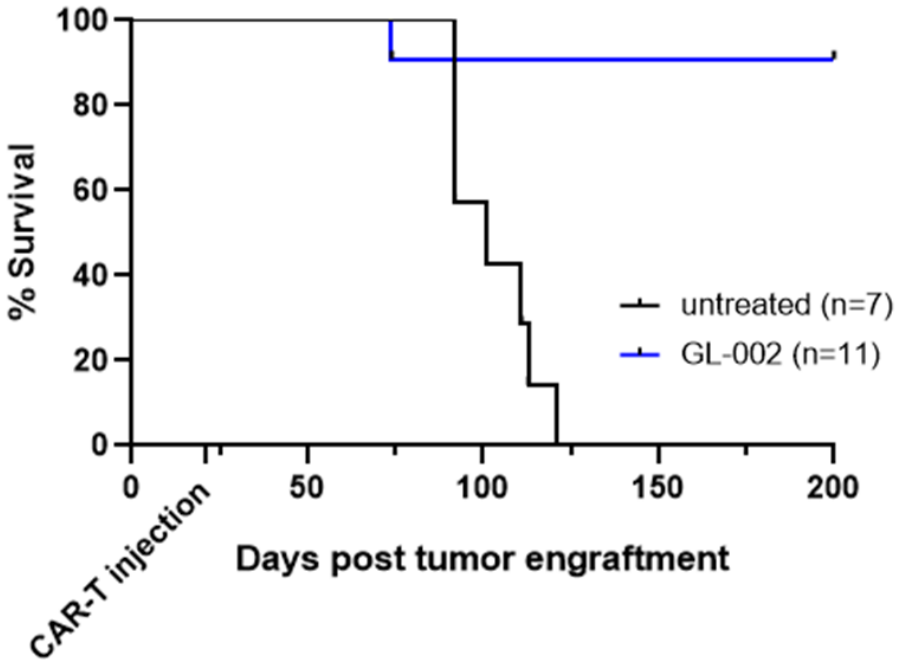

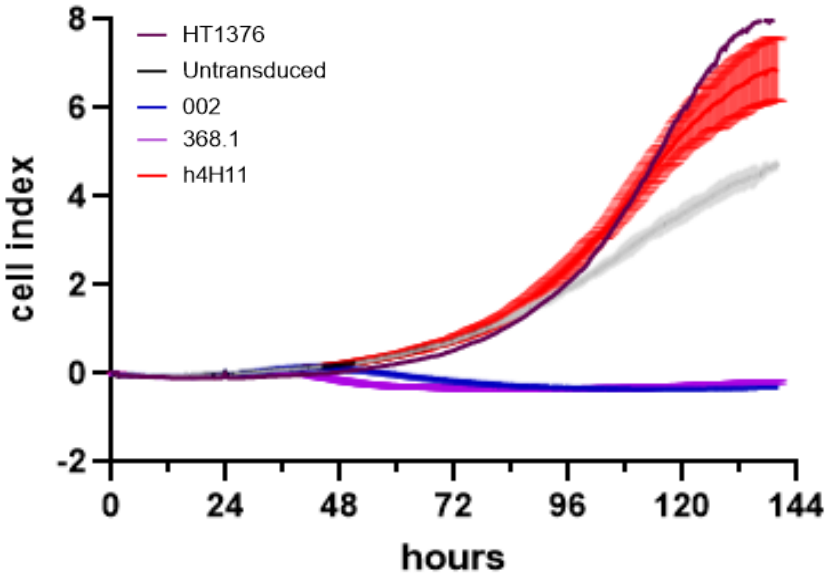

Phase I trial of IV vs IP Armored CAR T cells

IND submission in November 2025

- Hope to initiate enrollment in Q1 2026

Questions