GPC3 CAR-T cells for patients with solid cancers

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Texas Children's Cancer Center

CAGT CENTER FOR CELL AND GENE THERAPY

Baylor College of Medicine

No Pertinent Disclosures

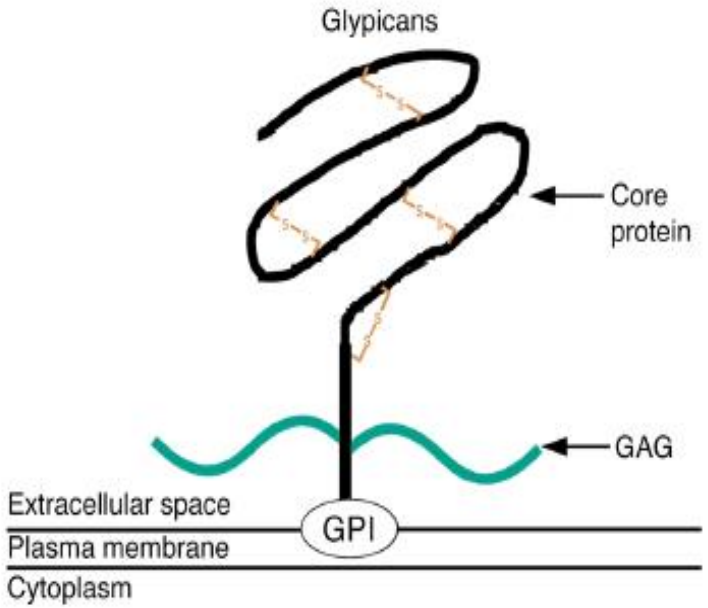
Challenges to treat solid tumors with CAR T cells

- Limited number of promising/safe target antigens **Glypican-3**
- Single chain variable fragments
- Effector cell type
- Limited trafficking
- Limited expansion **IL-15 +/- IL21 co-expression**
- Microenvironment-related factors
  - Presence of inhibitory signals (i.e. metabolites, TGF-β, adenosine, checkpoints…)
  - Lack of supportive signals (IL-7, -15, -21; nutrients, costimulation…)

Examine the evolution of CAR T cells post-infusion in patients

Glypican-3 - Structure and function

- Membrane bound heparan sulfate proteoglycan
- Promotes wnt signaling
- Stimulates cancer cell growth



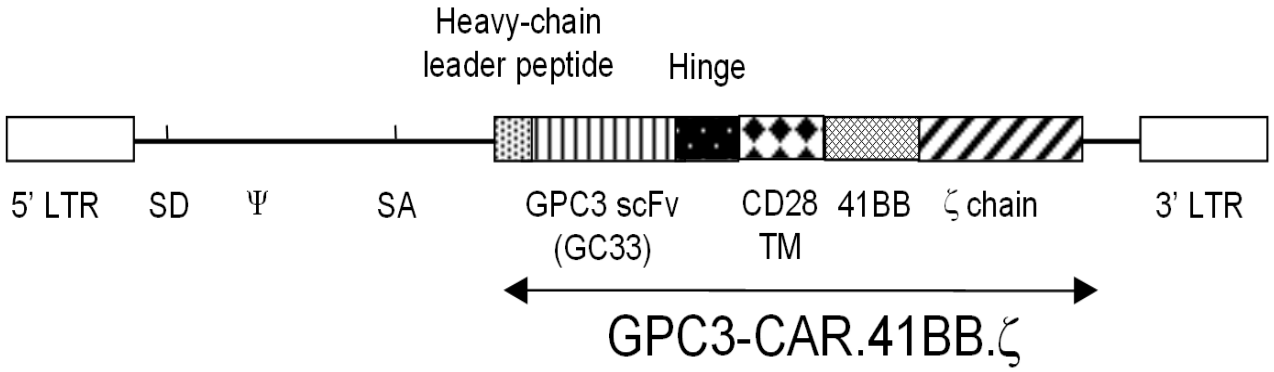
- Hepatoblastoma ~75%
- Hepatocellular carcinoma ~75%
- Yolk sac tumors >90%
- Malignant Rhabdoid tumors >90%
- Wilms tumors ~70%
- Rhabdomyosarcomas ~40%
- Glioblastoma multiforme ?

- Not expressed on healthy, mature tissues

Filmus J Clin Invest 2001, Chan 2013, Tretiakova 2015, Zynger 2010

Clinical program

Retroviral constructs



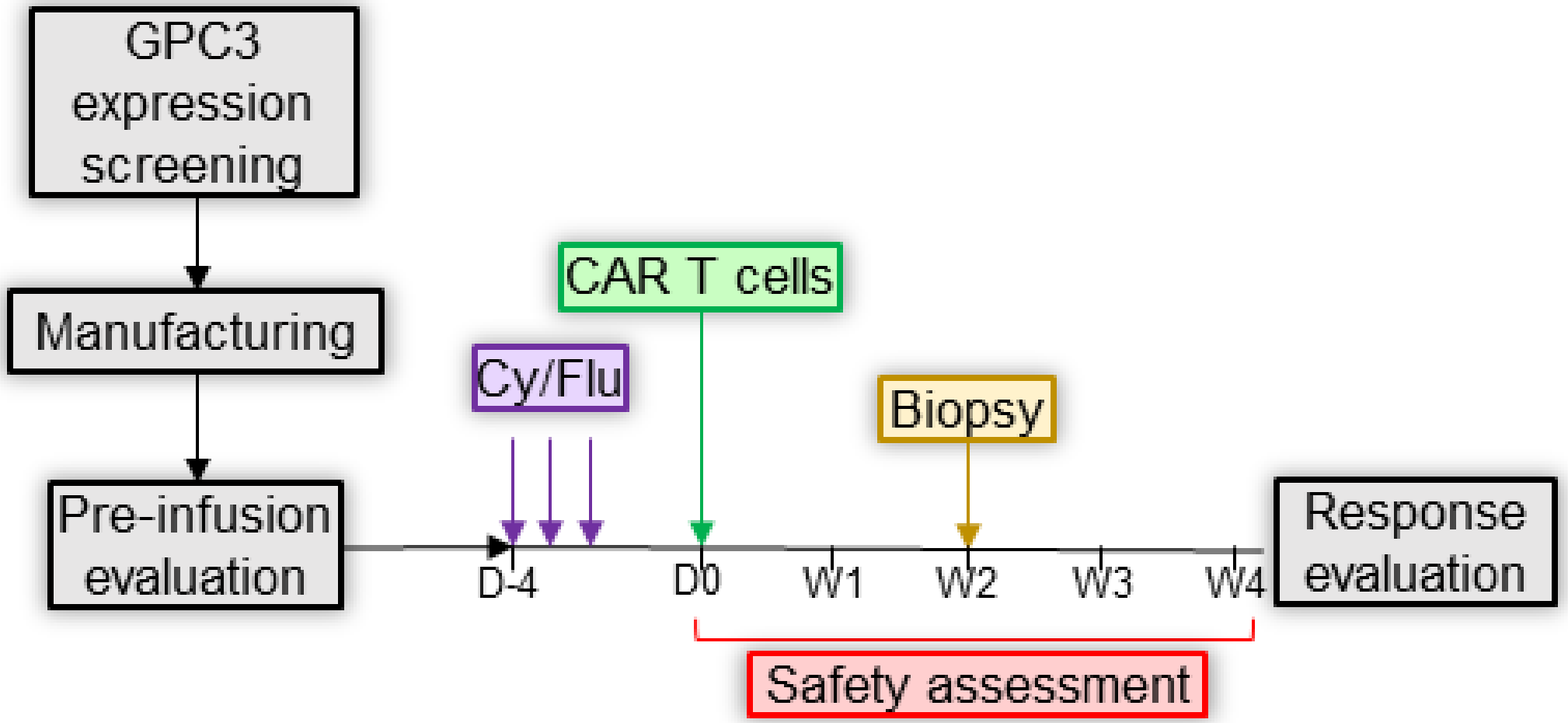
Clinical Trial Schema:

Immunotherapy product	**GPC3-CAR** **Pedi- GAP** **Adult- GLYCAR** **GPC3-CAR T cells**

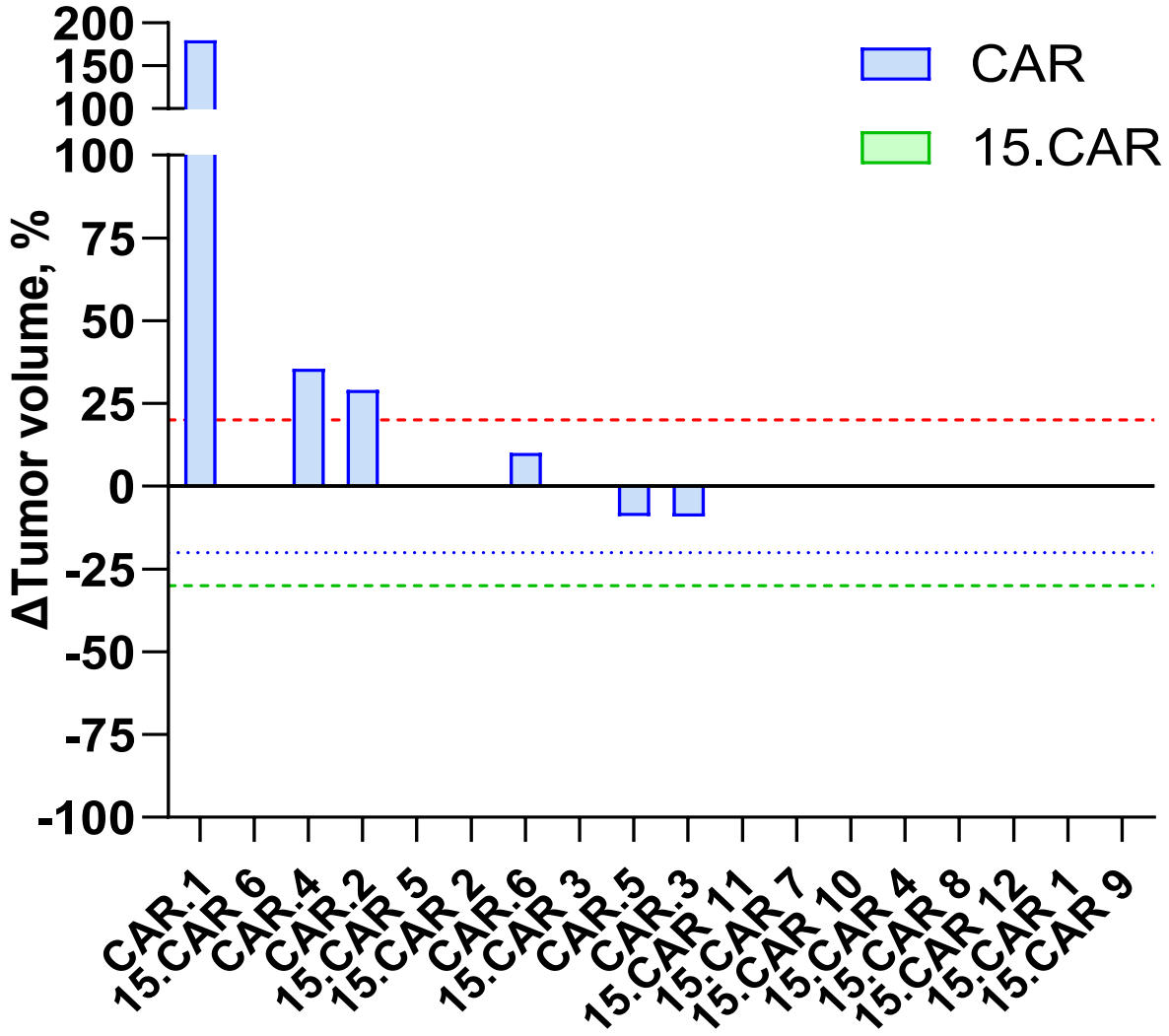
DL1: $1x10^7/m^2$

DL2: $3x10^7/m^2$

Enrollment and treatment of patients



T cells expressing the GPC3-CAR alone have negligible antitumor activity



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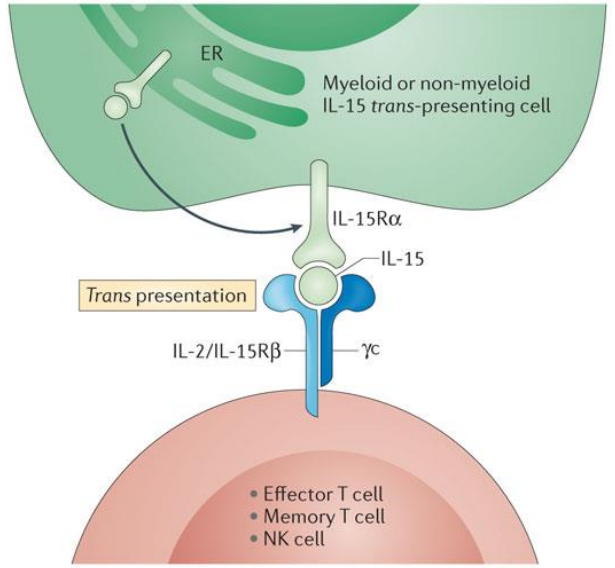
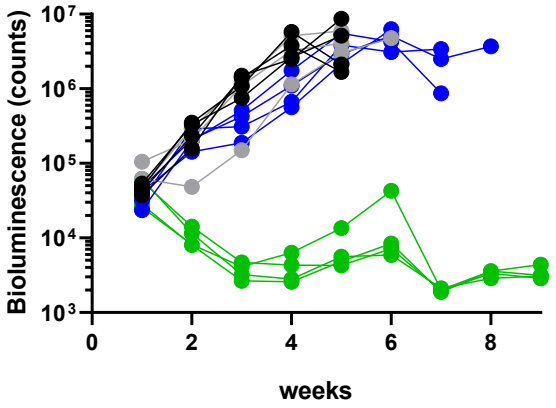
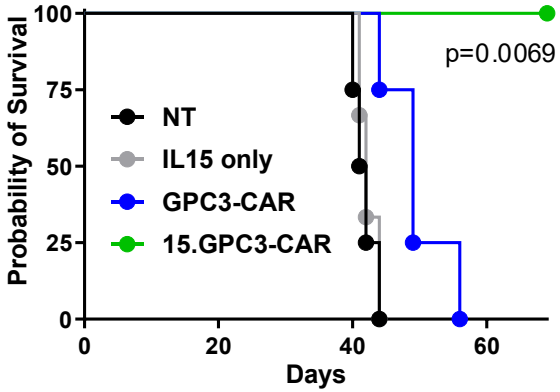
Effects of IL15 on CAR T cells in the preclinical setting

- Important for homeostatic maintenance of CD8+ memory cells
- Enhances *in vivo* antitumor activity of tumor reactive CD8+ cells
- IL-15 expressing CAR T cells - improved persistence and anti-tumor activity in <u>preclinical models</u> of solid and hematologic neoplasms.



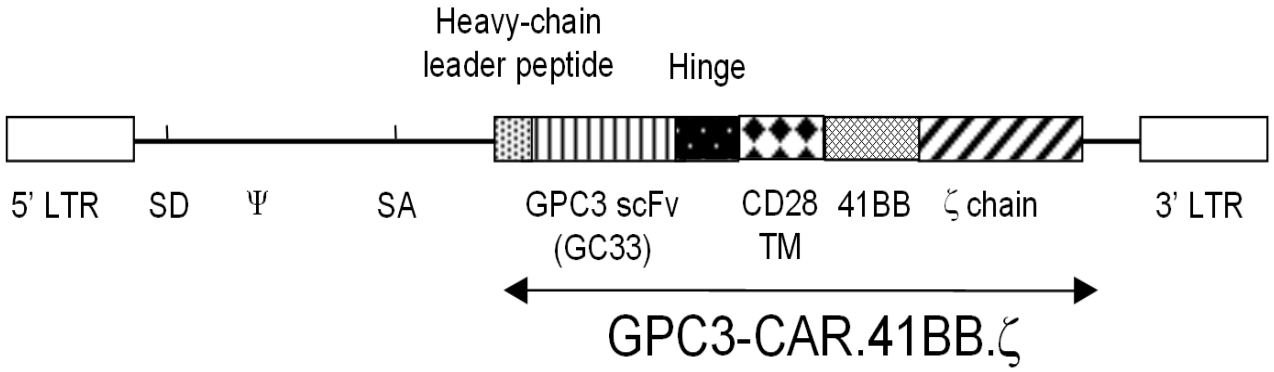


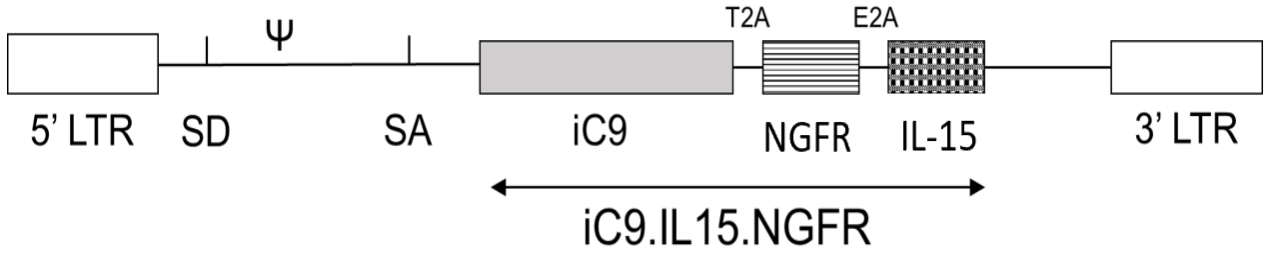


Zhang Immunity 1998; Klebanoff PNAS 2004; Hoyos Leukemia 2010; Hurton PNAS 2016; Batra Canc Imm Res 2020; Jabri Nat Imm 2015

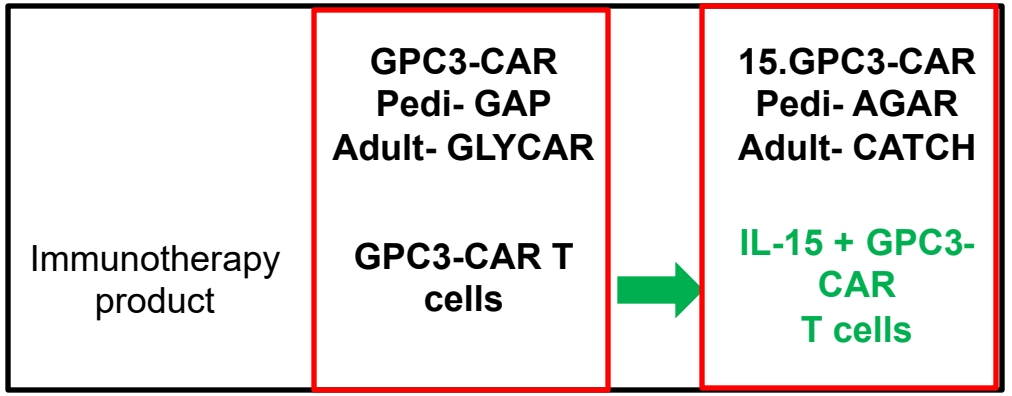
Clinical program

Retroviral constructs





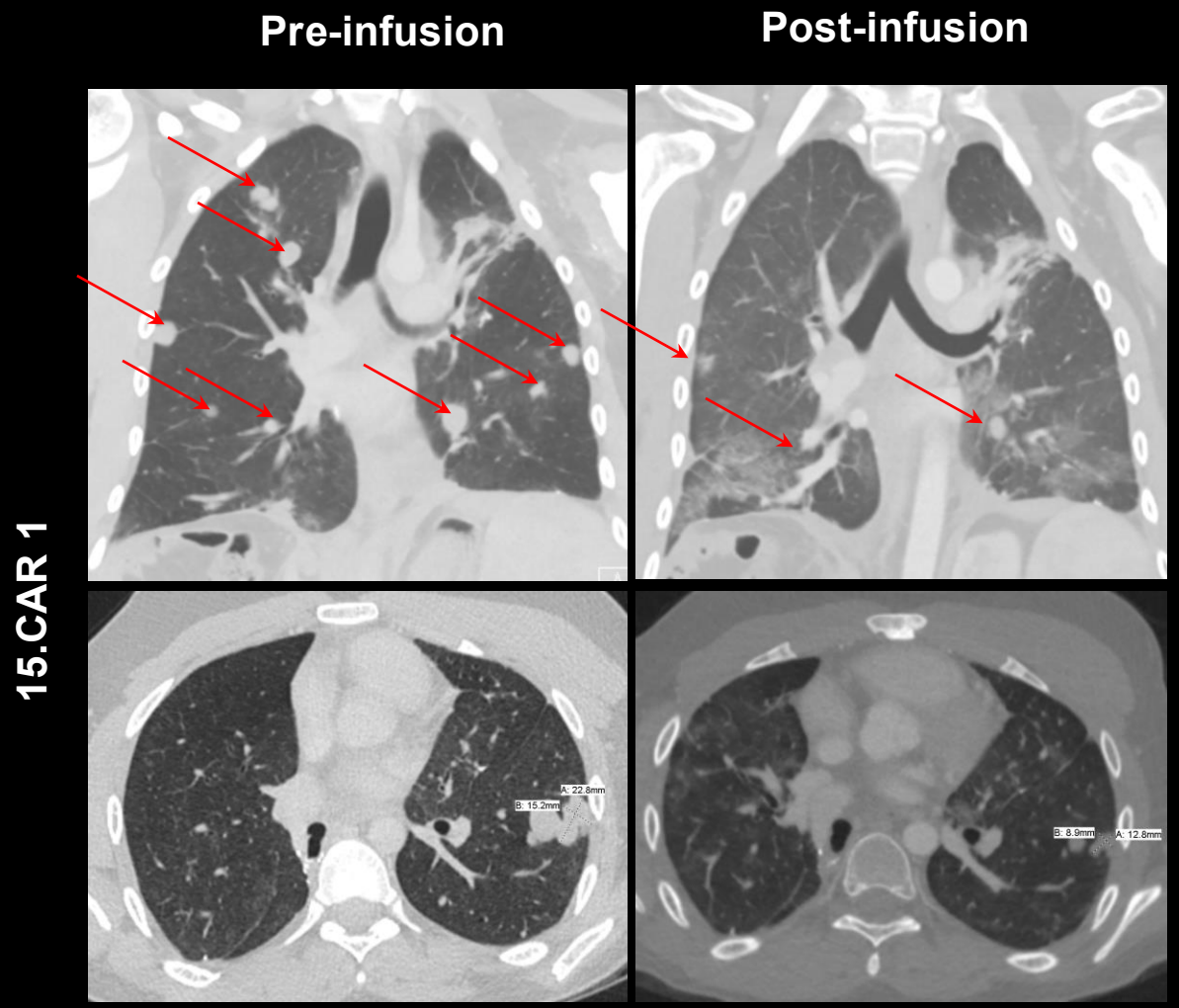
Clinical Trial Schema:

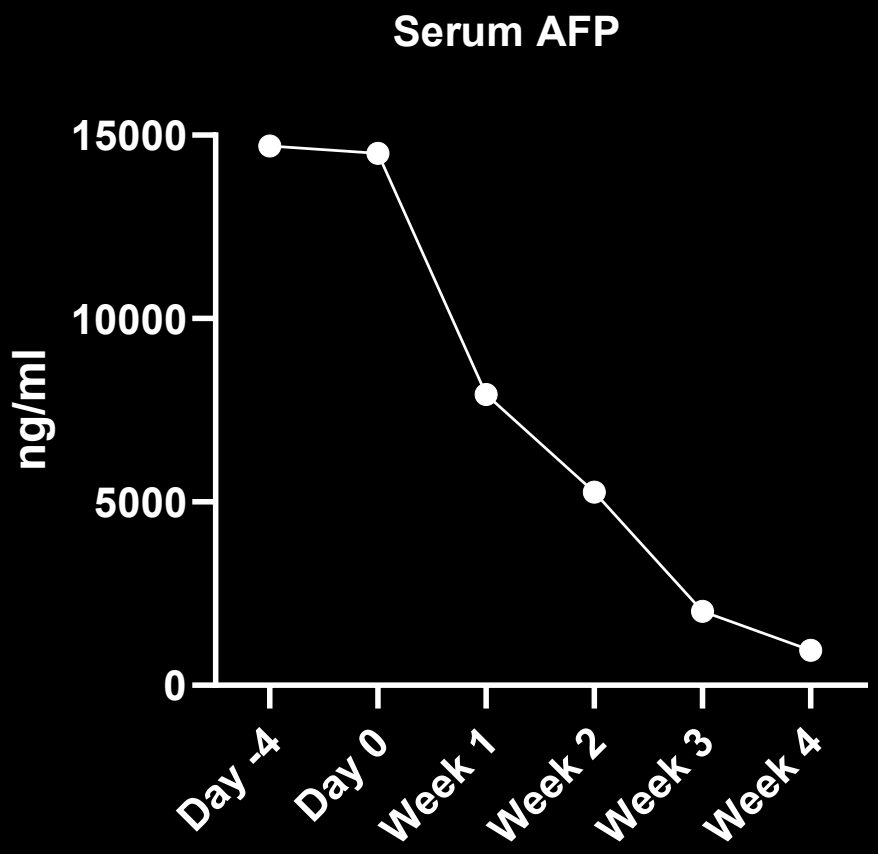


DL1: $1x10^7/m^2$

DL2: $3x10^7/m^2$ DL1: $3x10^7/m^2$

IL15 co-expressing GPC3-CAR T cells induce antitumor responses in the lungs





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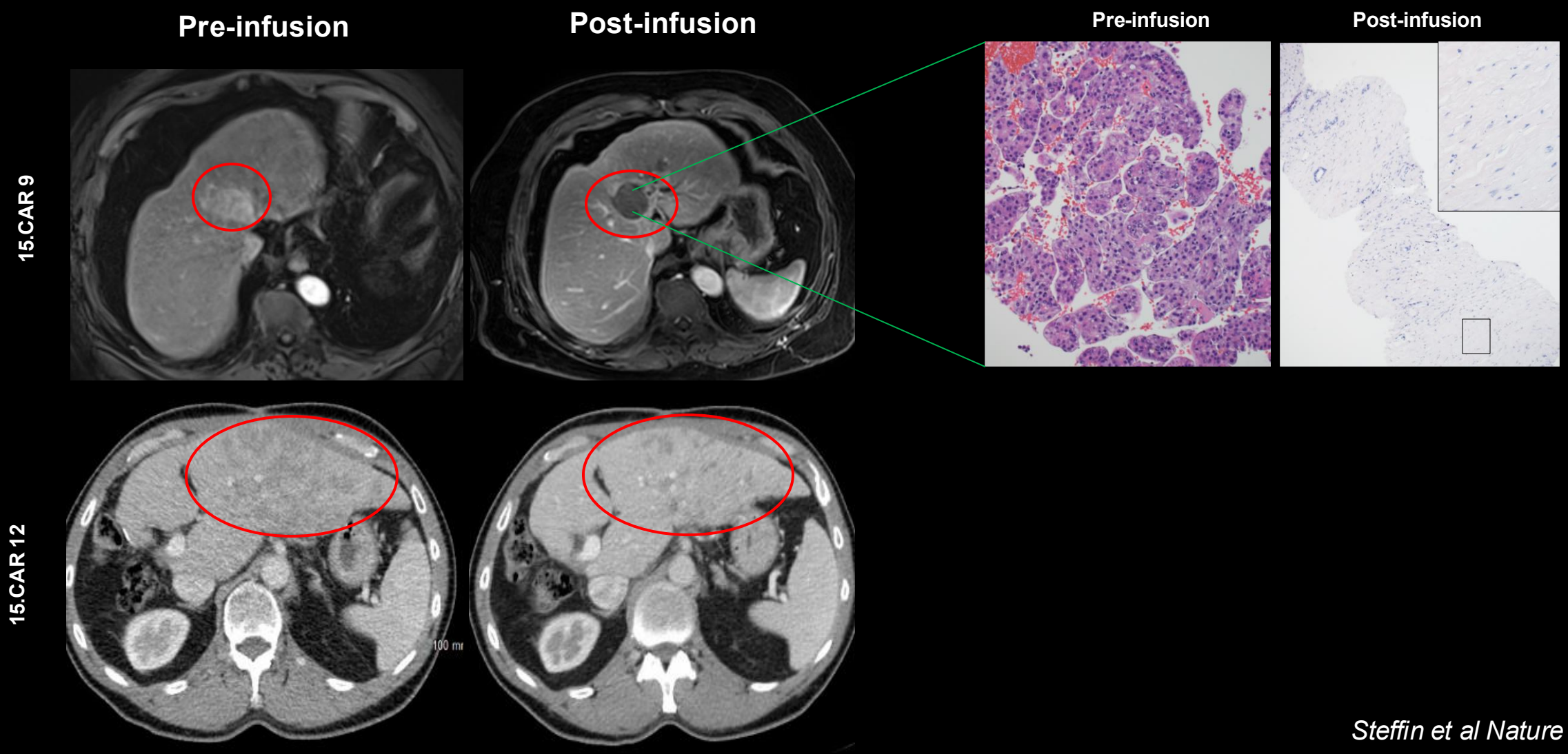
IL15 co-expressing GPC3-CAR T cells induce antitumor responses in the Liver



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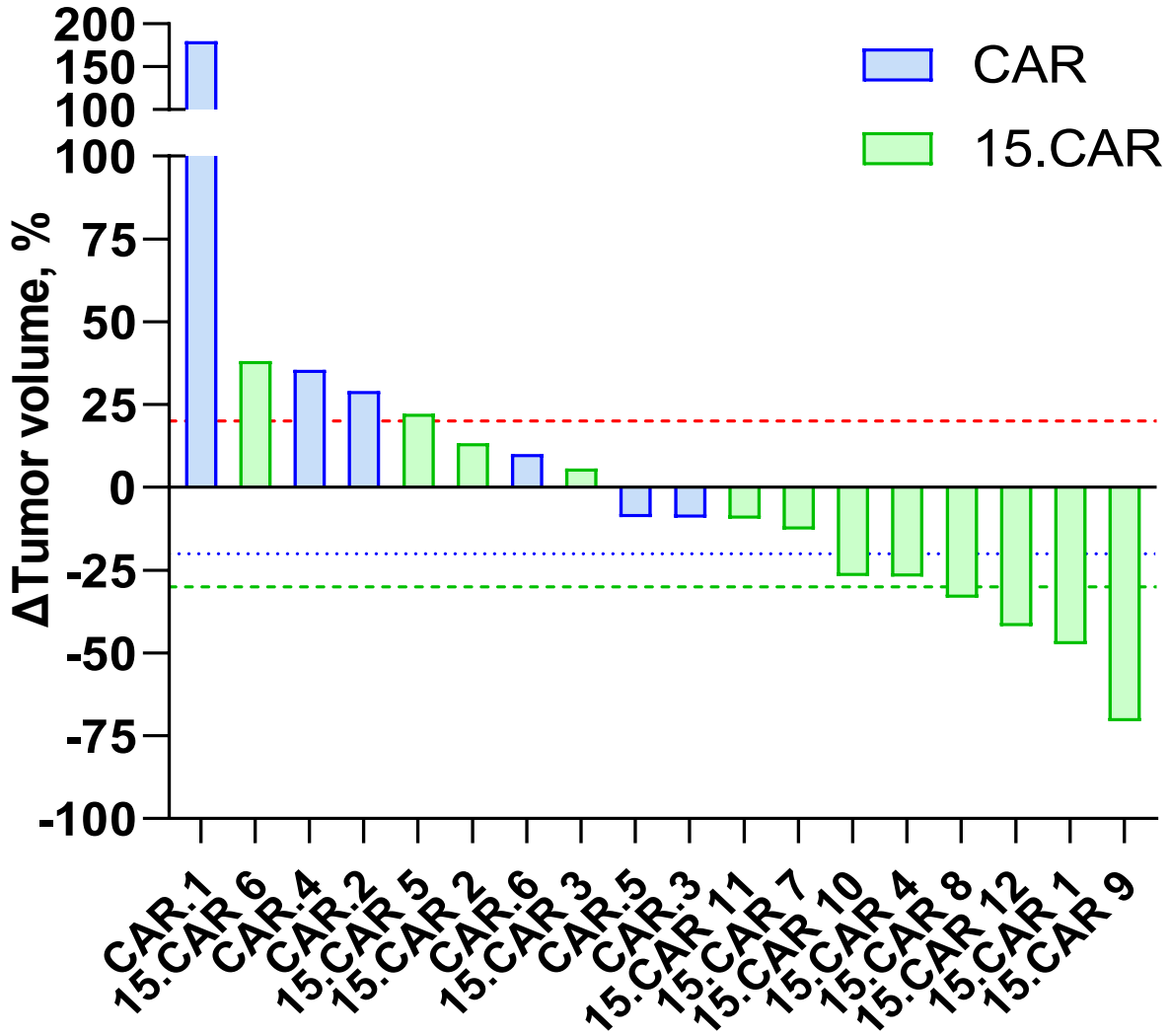
IL15 enhances the antitumor activity of GPC3-CAR T cells



n=18

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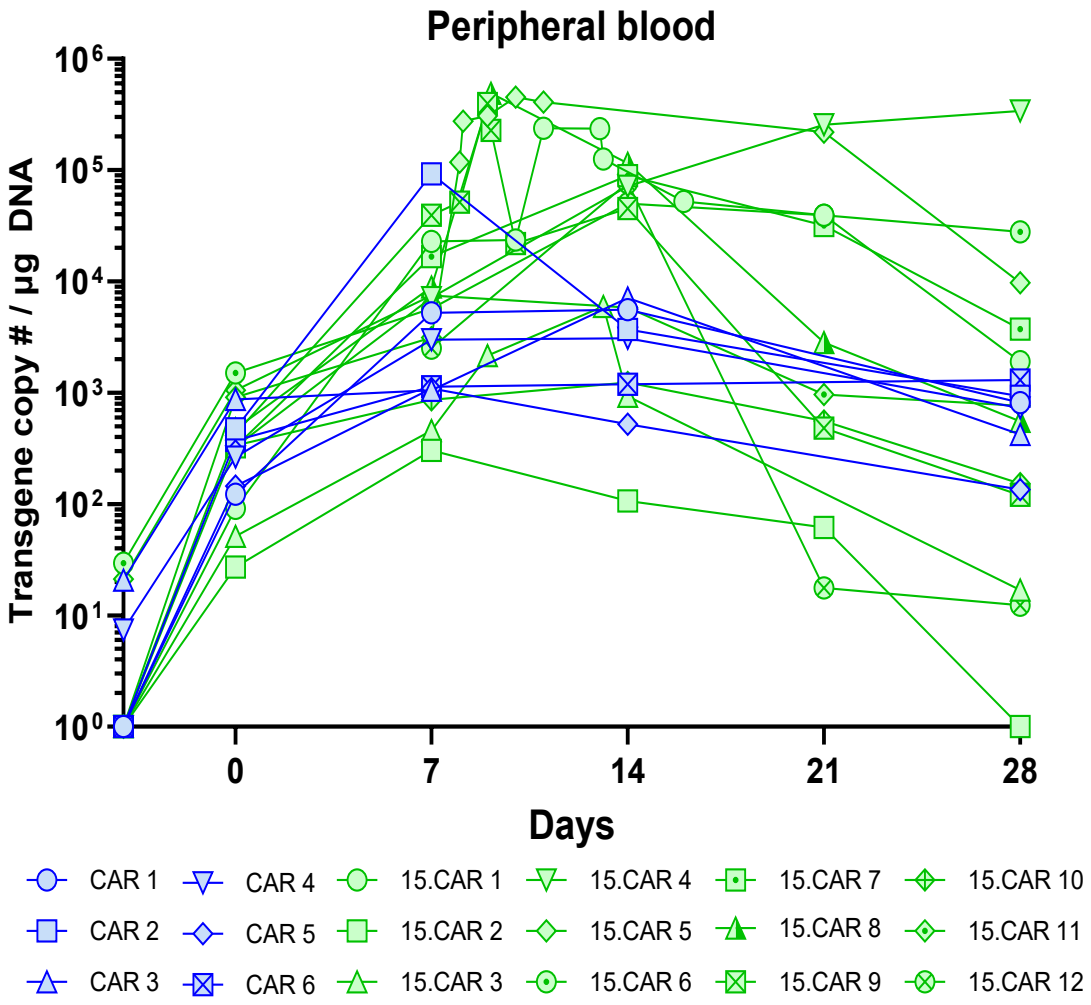
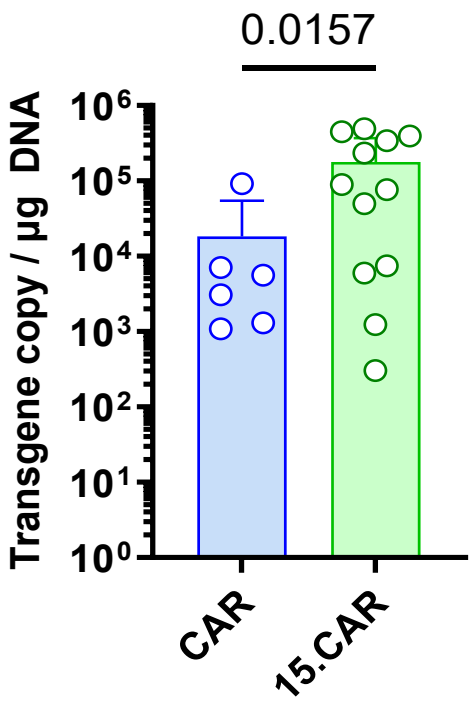
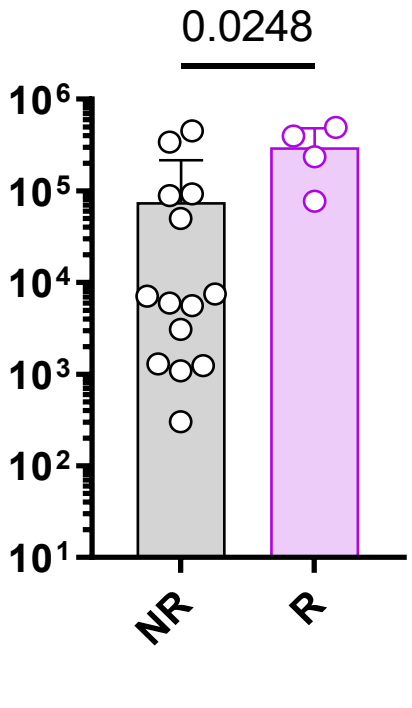
Expansion of CAR T cells is improved with IL15 co-expression







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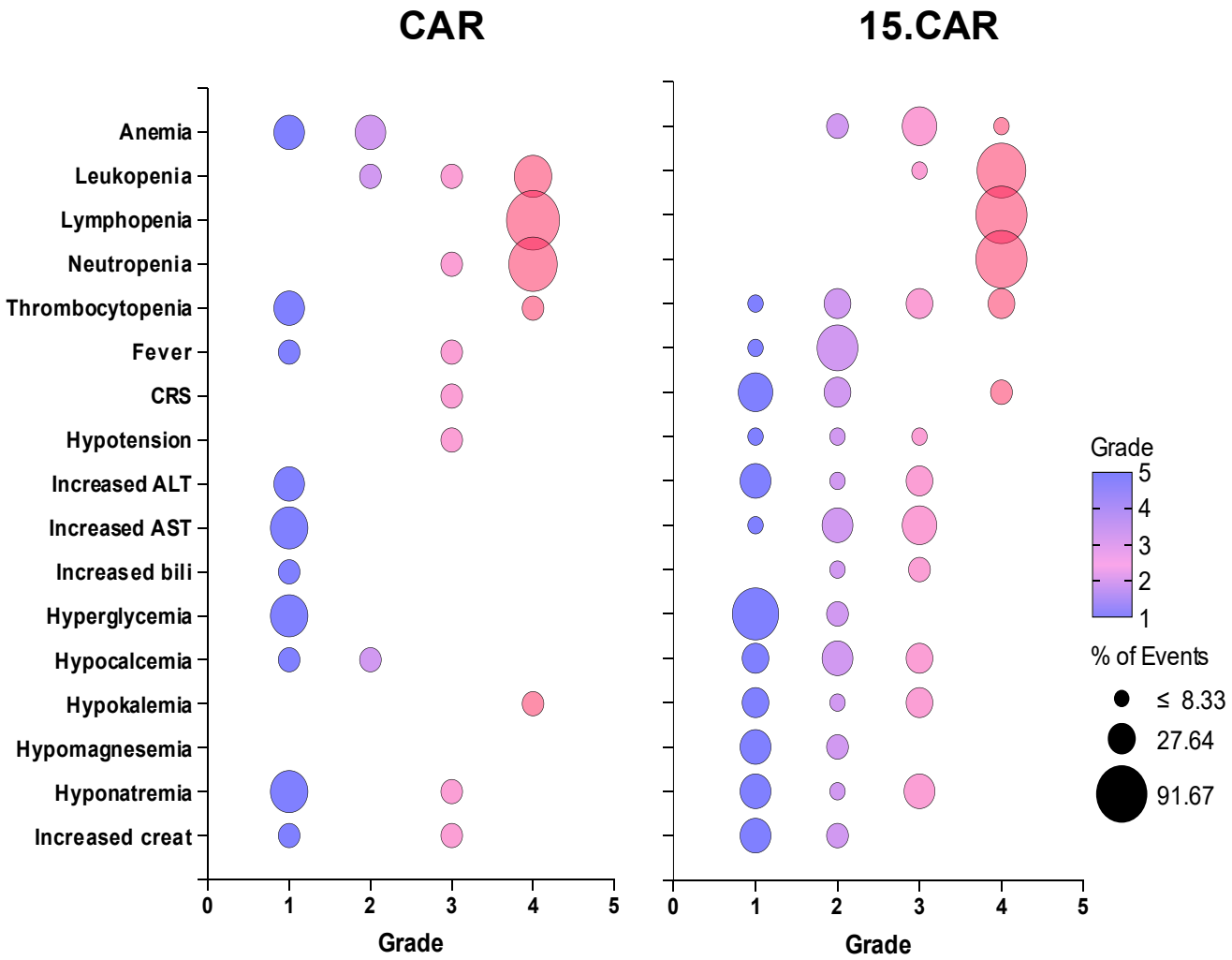
Increased frequency of CRS observed in 15.CAR T group



CRS - CAR vs 15.CAR cohort

RR= 3.3, (95% confidence interval 1.226 to 9.723, p=0.043).

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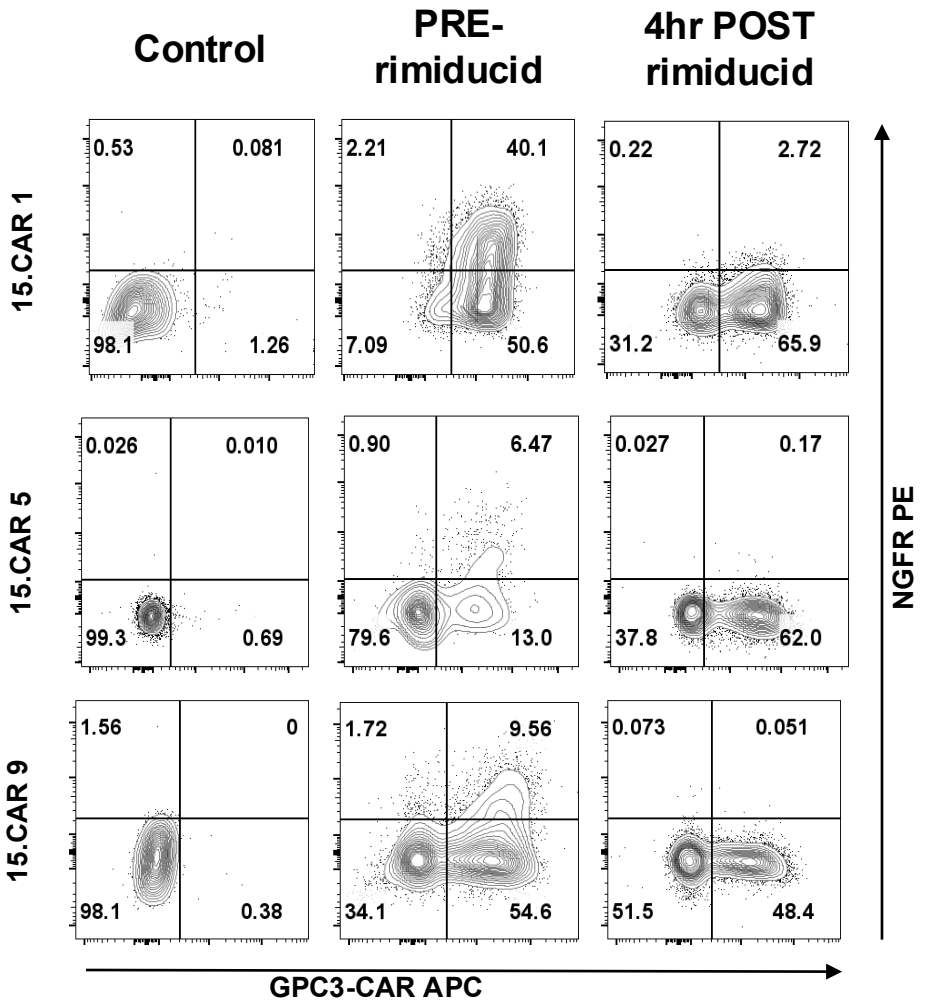
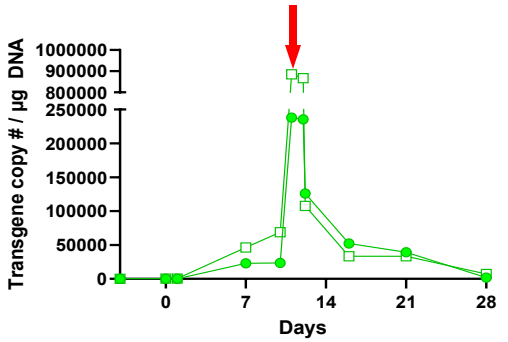
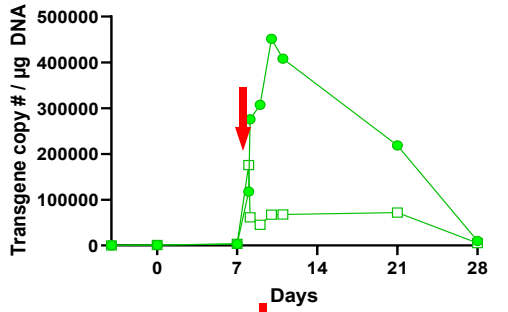
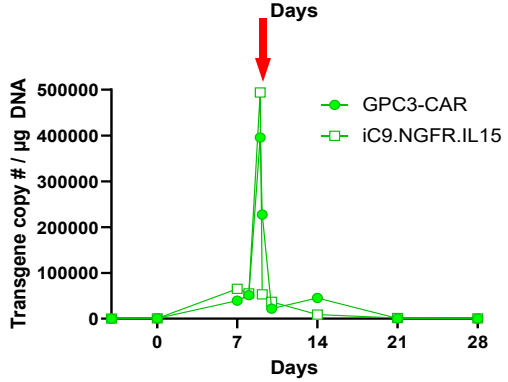
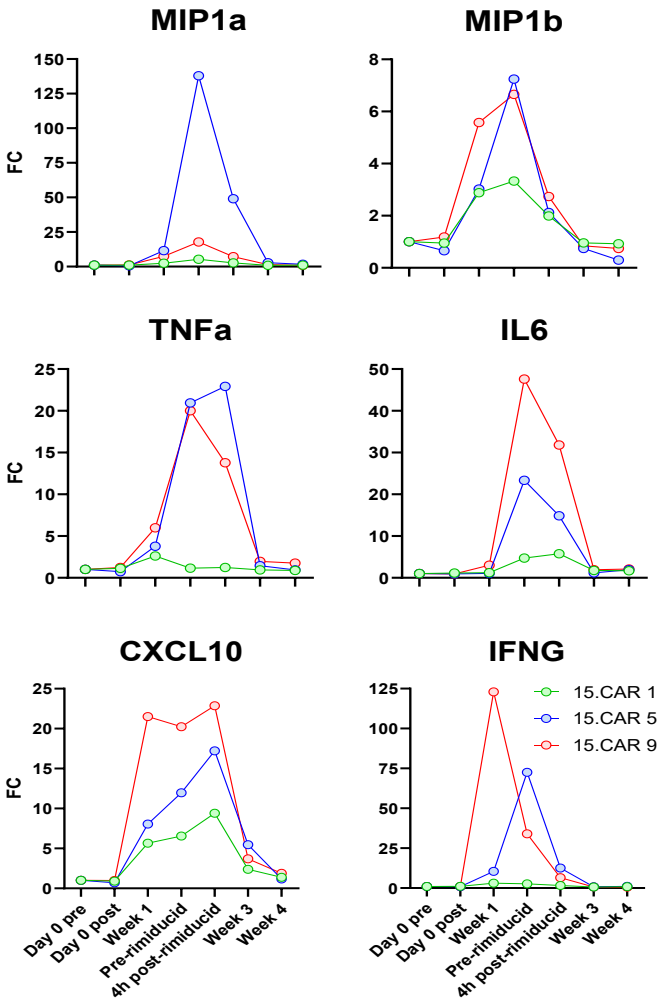
IL1/6 inhibition resistant CRS rapidly stopped by the inducible caspase 9 safety switch











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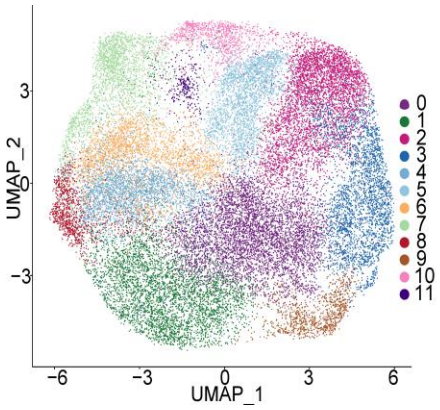
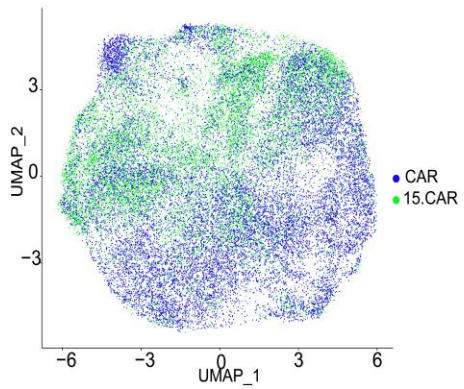
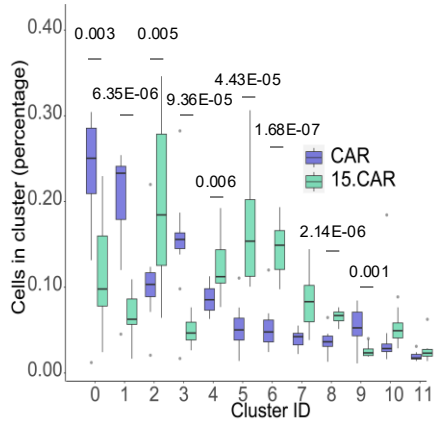
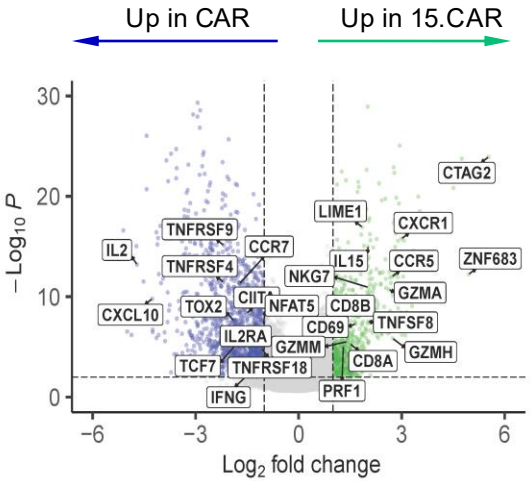
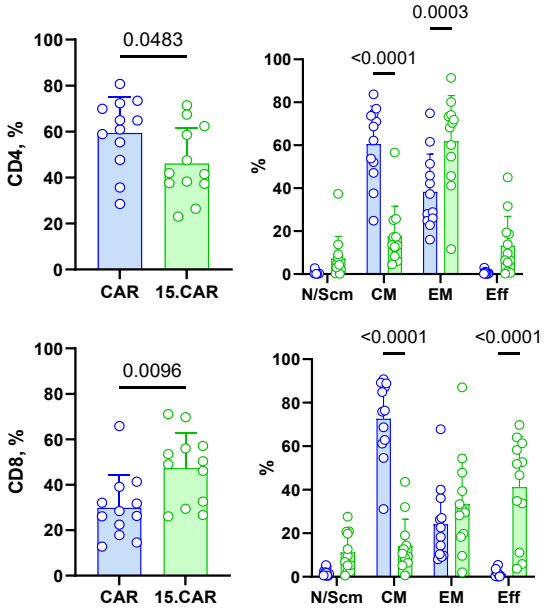
IL15 co-expression changes the gene expression profile of CAR T cell products

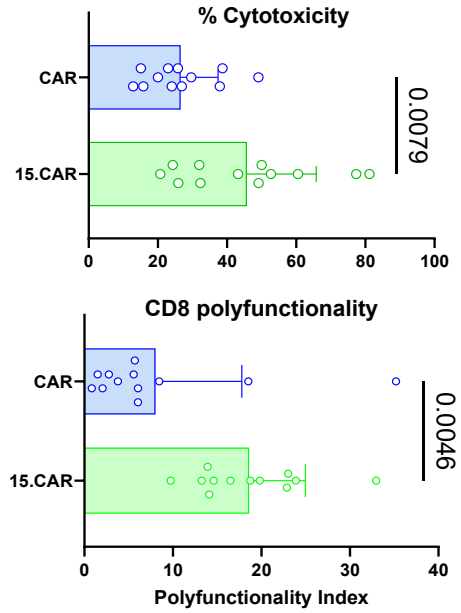


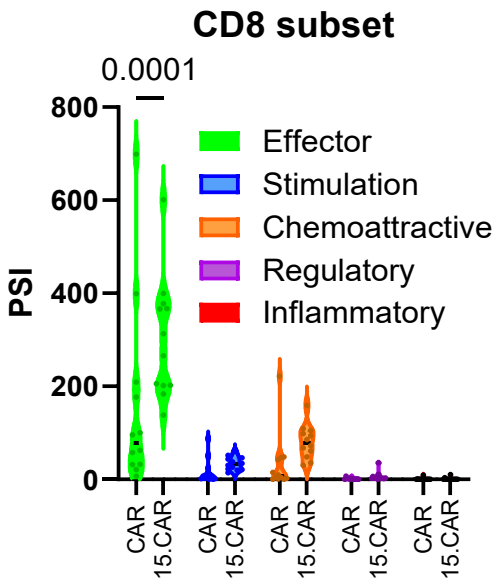












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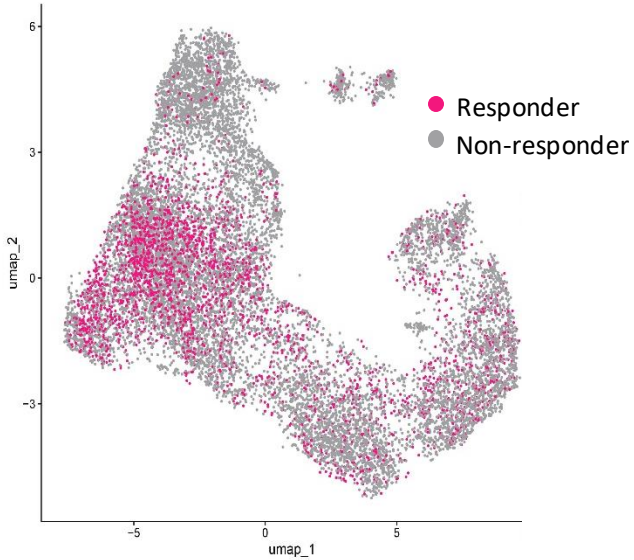
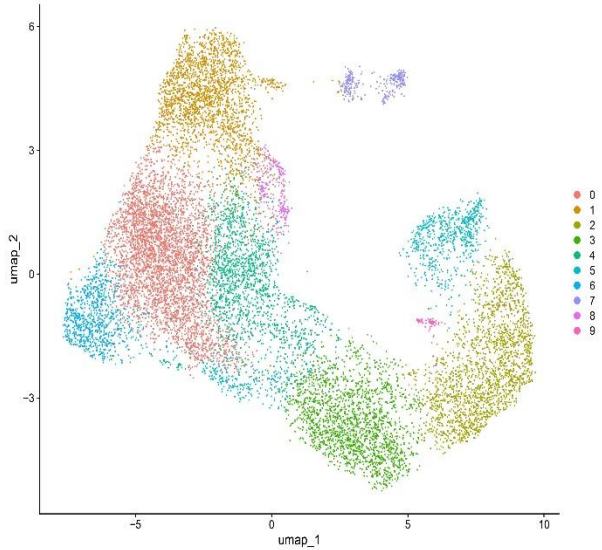
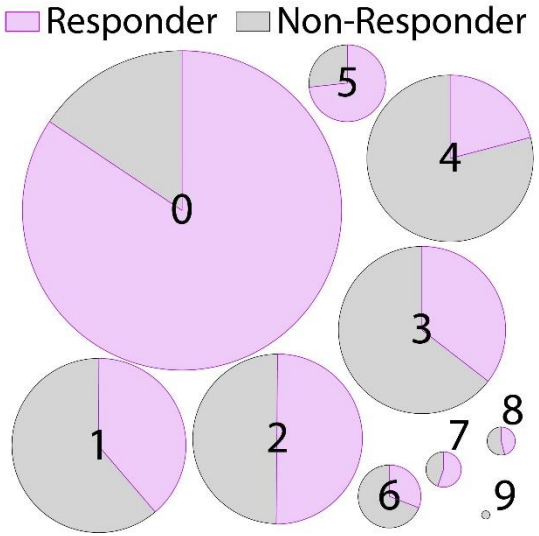
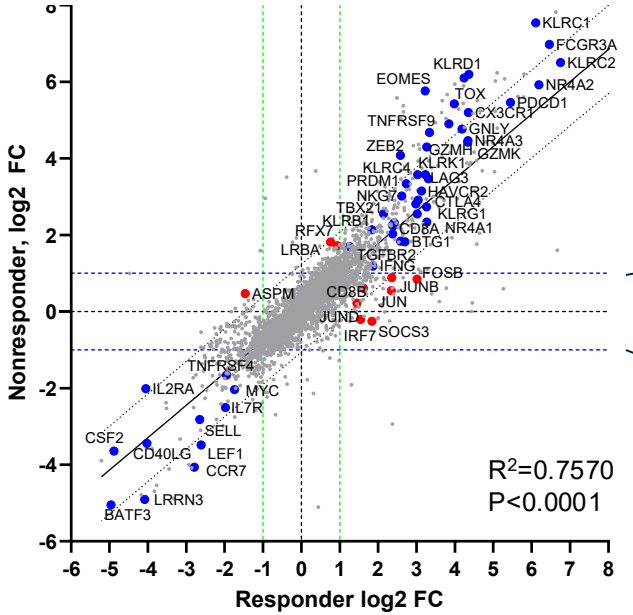
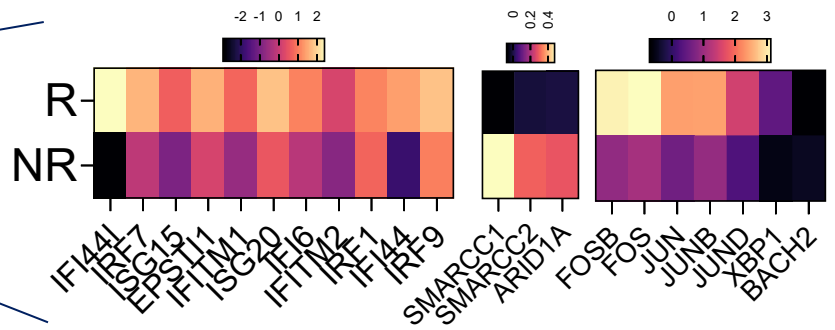
Tumor infiltrating CAR T cells maintain JUN/FOS and upregulate Type I IFN gene-signature











Steffin et al Nature 2025.

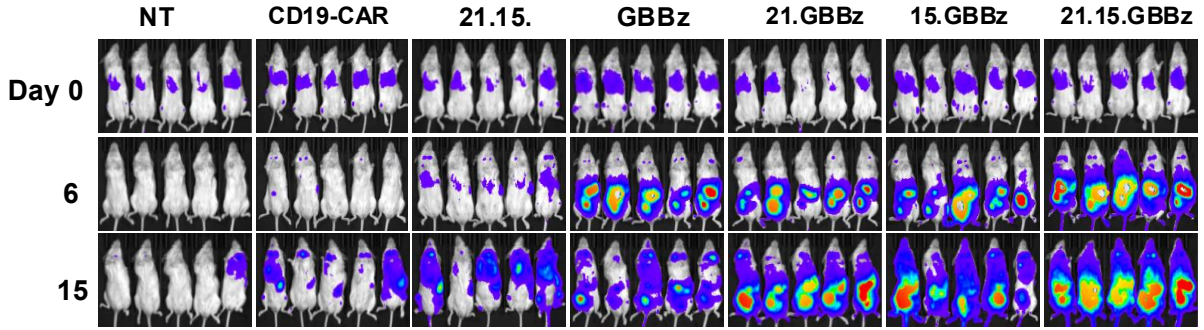
Can we improve IL15.GPC3-CAR T cells?

IL-15 and IL-21 could have synergistic anti-tumor function *in vivo*

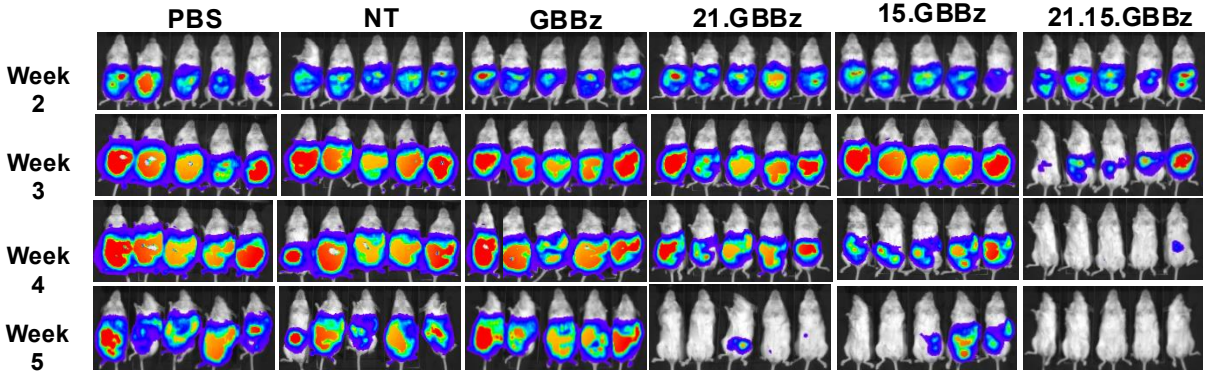
- **IL15:**
  - homeostatic maintenance of **CD8+ memory cells**
  - Enhances *in vivo* **antitumor activity** of **CD8+ cells**
  - Prior studies demonstrate improved persistence and anti-tumor activity
- **IL21:**
  - Inhibitory effects on T cell differentiation
  - IL-21 co-expressing CAR – T cells improve anti-tumor activity *in vivo*
  - IL-21 increases the proportion of stem cell subsets in products
- **IL15/21:**
  - Maintains the expression of **T cell factor -1 (TCF-1),** a transcription factor critical for T cell development and survival

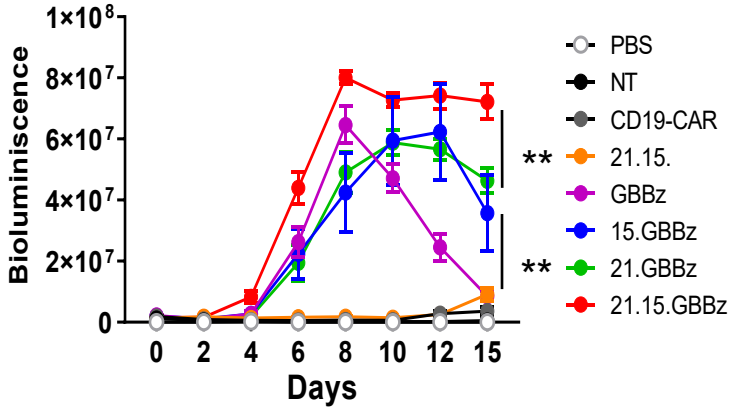
Co-expression of both IL-15 and IL-21 in GPC3-CAR T cells induces superior antitumor activity at low CAR T cell doses

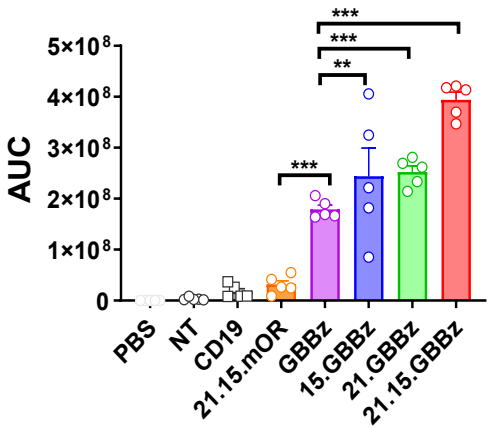
CAR T cell bioluminescence

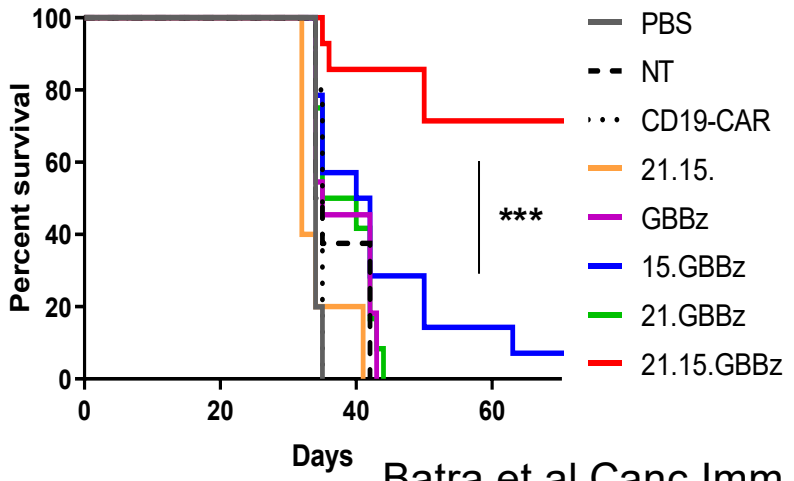


Tumor bioluminescence







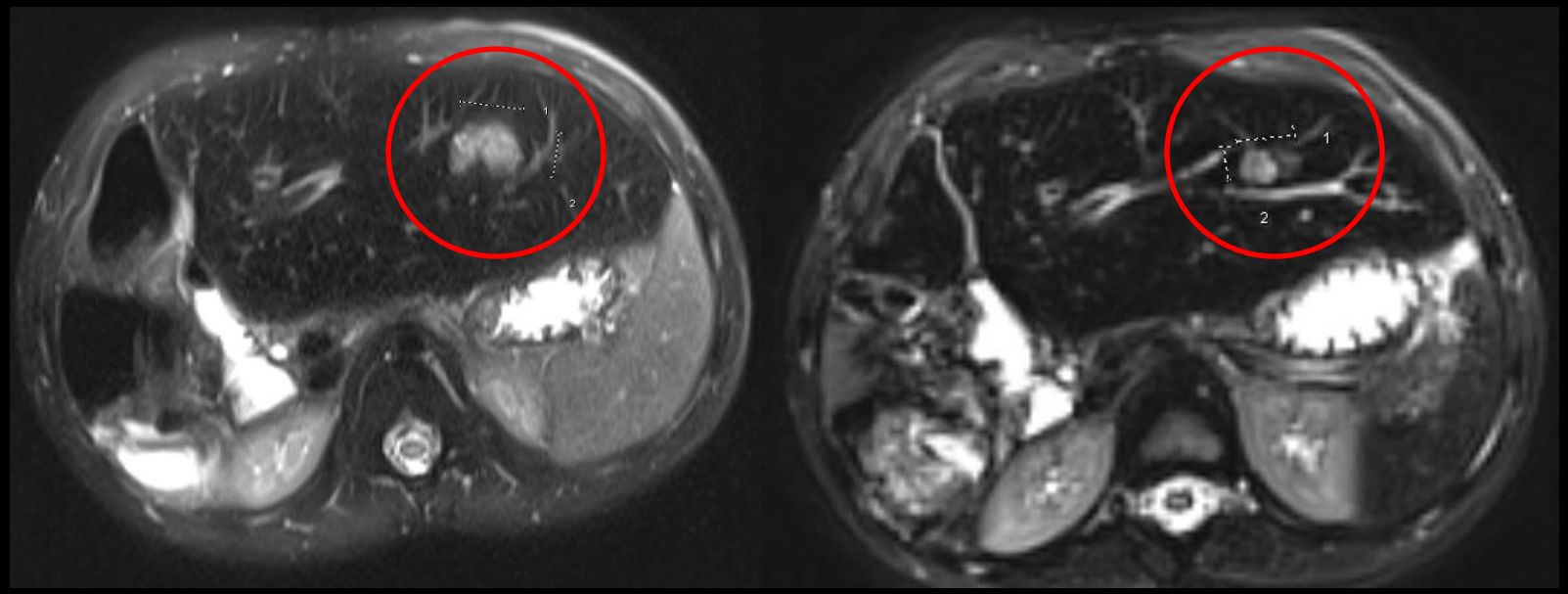


Batra et al Canc Imm Res 2020

Children treated on 21.15.GPC3 CAR exhibit promising responses to therapy

Pre-Infusion

Post-Infusion



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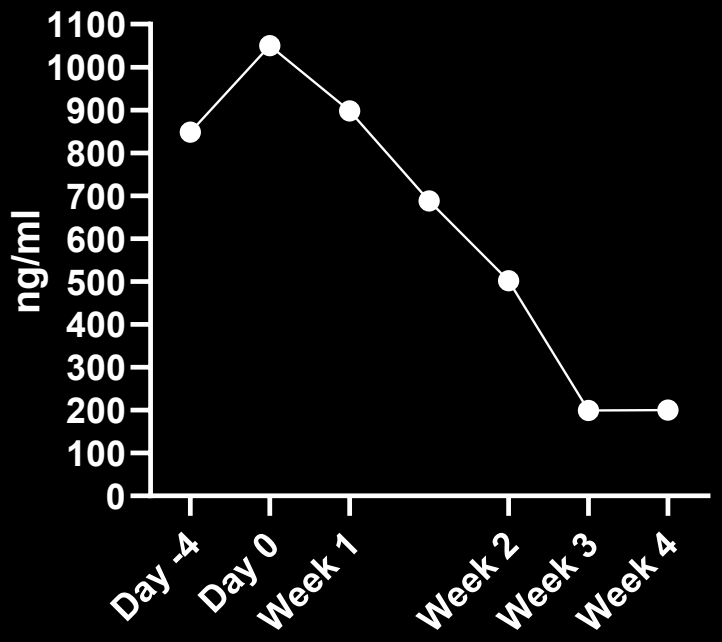

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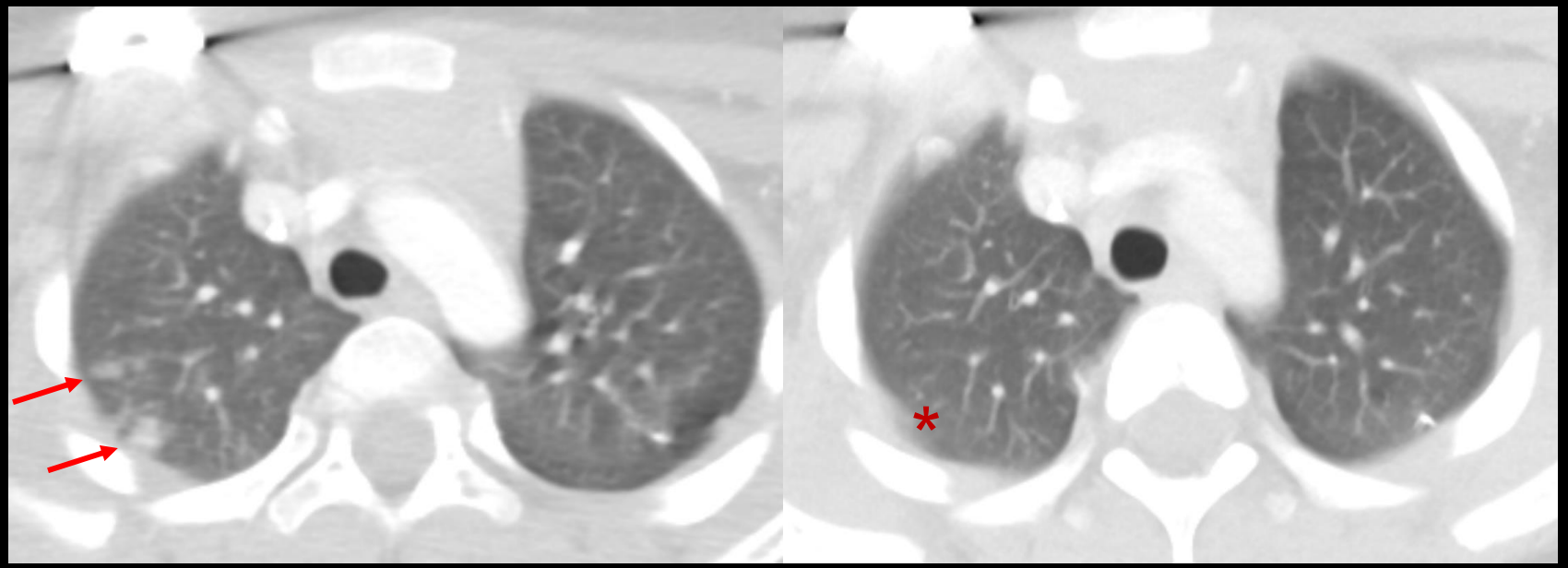
Children treated on 21.15.GPC3 CAR exhibit promising responses to therapy

Pre-Infusion

Post-Infusion



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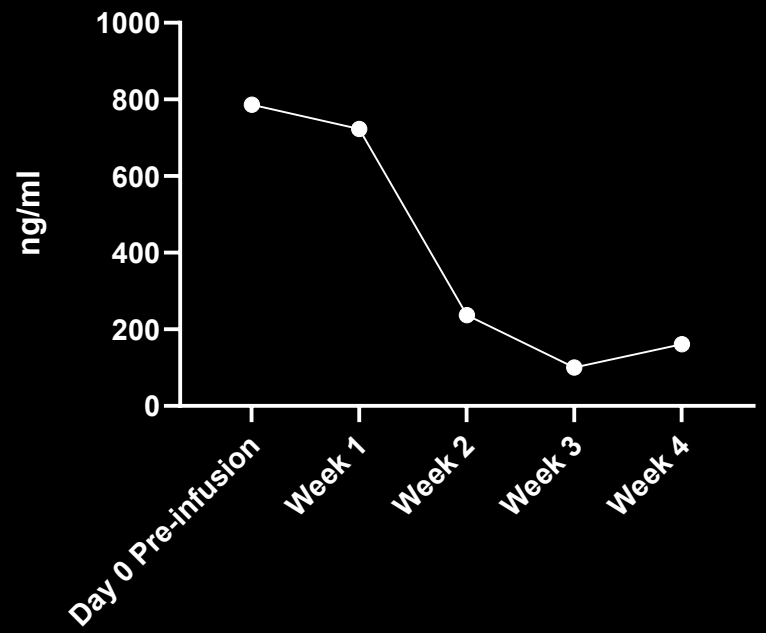


* Complete Resolution

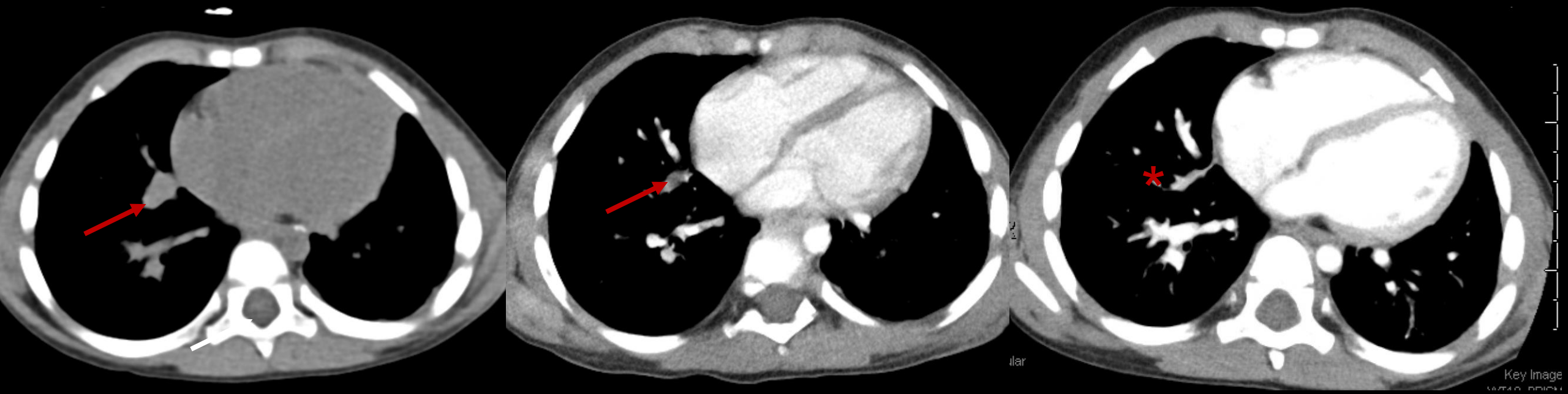
Unpublished, do not post.

Children treated on 21.15.GPC3 CAR exhibit promising responses to therapy

Pre-Infusion

4 weeks post Infusion

4 weeks post Infusion #2



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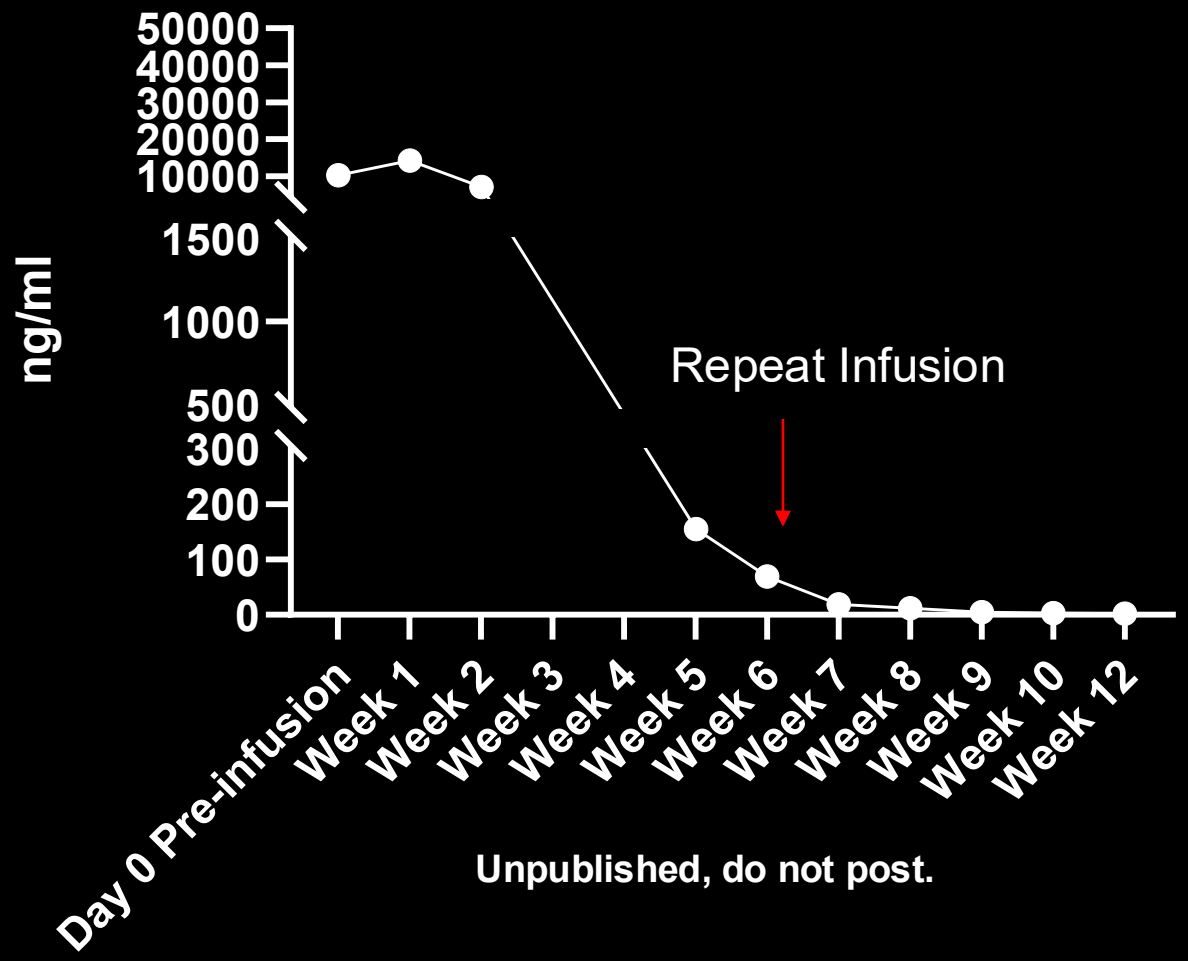
*Fibrotic components
(Non-viable tumor)

Serum AFP normalization in CR Patient



Unpublished, do not post.

Final conclusions and future directions

- GPC3 is a safe target for mpx solid tumors
- Increase in CRS can be modulated with IL1/6 inhibition or with iC9
- IL15 enhances the expansion and antitumor activity of GPC3-CAR T cells in patients.
- Fundamental gene expression evolution of tumor infiltrating CAR T cells
  - NK-like effector differentiation with improved proliferative capacity.
  - Expression of Type I IFN and FOS/JUN family members associated with antitumor responses.
- While early, can IL15 and -21 dual-expression safely enhance the antitumor activity of GPC3-CAR T cells in patients (enrolling patients)

Thank you

PATIENTS, FAMILIES and REFERRING PHYSICIANS

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NCI / NIH, American Cancer Society, Cancer Prevention and Research Institute of Texas, The V Foundation, Alex's Lemonade Stand Foundation, Cookies for Kids Cancer

CAGT CENTER FOR CELL AND GENE THERAPY

Texas Children's Cancer Center

Baylor College of Medicine