TNFR2 as a Target to Improve CD19-Directed CART Cell Fitness and Antitumor Activity in Large B Cell Lymphoma

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703. Cellular Immunotherapies: Basic and Translational IV

Disclosure

- **Cox:** *Humanigen:* Patents & Royalties.
- **Sakemura:** *Humanigen:* Patents & Royalties.
- **Ding:** *Merck:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *DTRM:* Research Funding; *Octapharma:* Membership on an entity's Board of Directors or advisory committees.
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- **Scholler:** *Gilead Sciences :* Current Employment.
- **Bot:** *Kite, a Gilead Company:* Current Employment; *Gilead Sciences:* Consultancy, Current equity holder in publicly-traded company.
- **Kay:** Pharmacyclics: Membership on an entity's Board of Directors or advisory committees, Research Funding; MEI Pharma: Research Funding; Abbvie: Research Funding; Tolero Pharmaceuticals: Membership on an entity's Board of Directors or advisory committees, Research Funding; Bristol Meyer Squib: Membership on an entity's Board of Directors or advisory committees, Research Funding; Acerta Pharma: Research Funding; Sunesis: Research Funding; Astra Zeneca: Membership on an entity's Board of Directors or advisory committees; Agios Pharma: Membership on an entity's Board of Directors or advisory committees; Cytomx: Membership on an entity's Board of Directors or advisory committees; Morpho-sys: Membership on an entity's Board of Directors or advisory committees; Rigel: Membership on an entity's Board of Directors or advisory committees; Oncotracker: Membership on an entity's Board of Directors or advisory committees; Dava Oncology: Membership on an entity's Board of Directors or advisory committees; Juno Theraputics: Membership on an entity's Board of Directors or advisory committees.
- **Mattie:** *Kite:* Current Employment.
- **Kim:** *Gilead Sciences:* Current equity holder in publicly-traded company; *Kite, a Gilead Company:* Current Employment.
- **Filosto:** *Kite, a Gilead Company:* Current Employment; *Tusk Therapeutics:* Patents & Royalties: or other intellecular property; *Gilead Sciences:* Other: stock or other ownership
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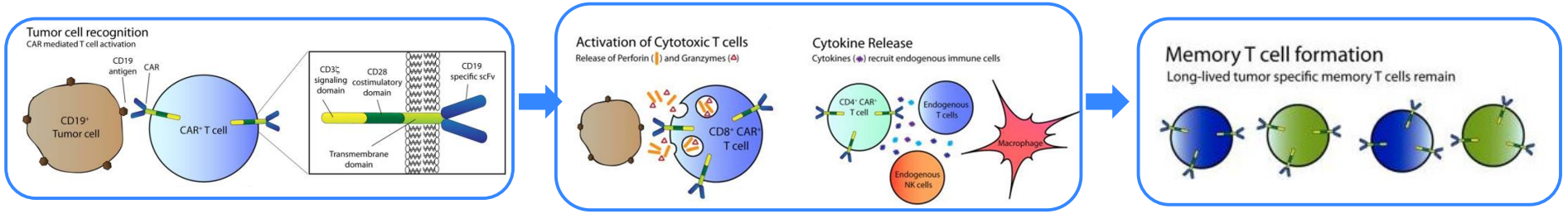
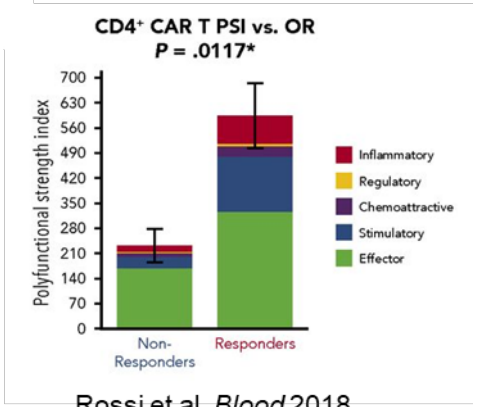
CART cell Fates: activation, expansion and persistence

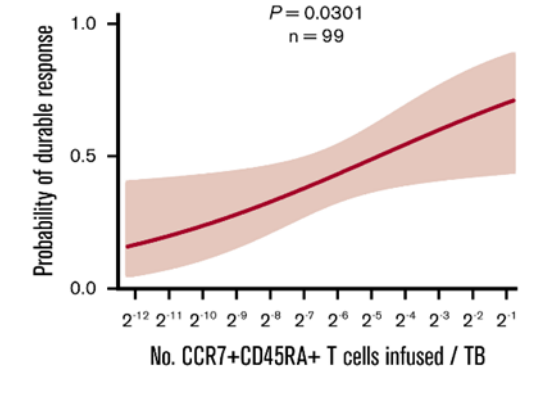


Activation **Expansion** **Persistence**

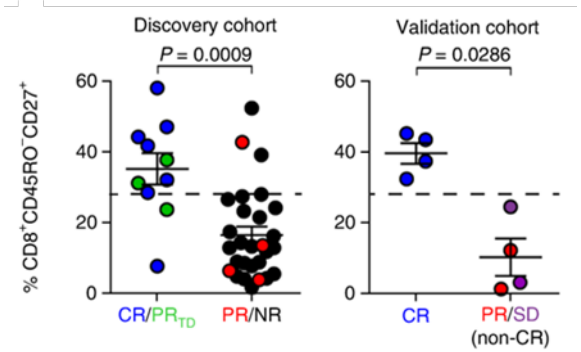
Baseline CART cell fitness is associated with response



Rossi et al, *Blood* 2018



Locke et al, *Blood Advance* 2020



Fraietta et al, *Nature Medicine* 2018

MAYO CLINIC

Figure adapted from Davila ML et al, *Int J Hematol*. 2014

AIMS

1) Study baseline activation of CART cells in healthy donors and patients with lymphoma

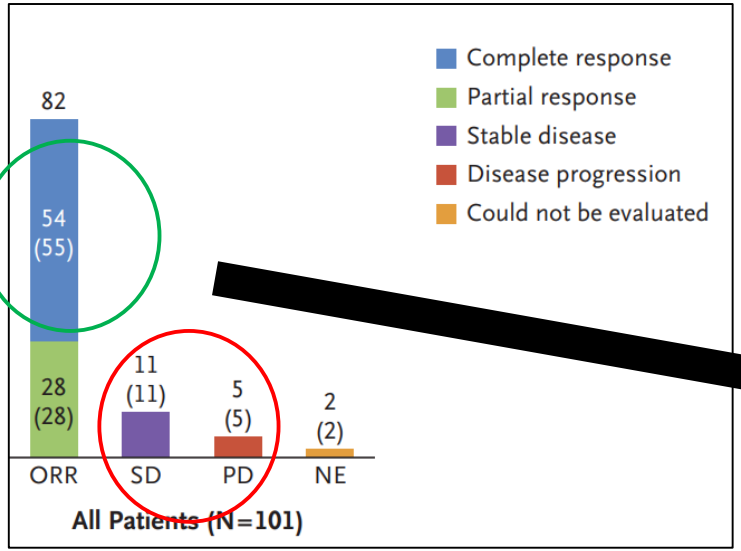
2) Investigate how CART cell activation could impact their fitness and clinical responses

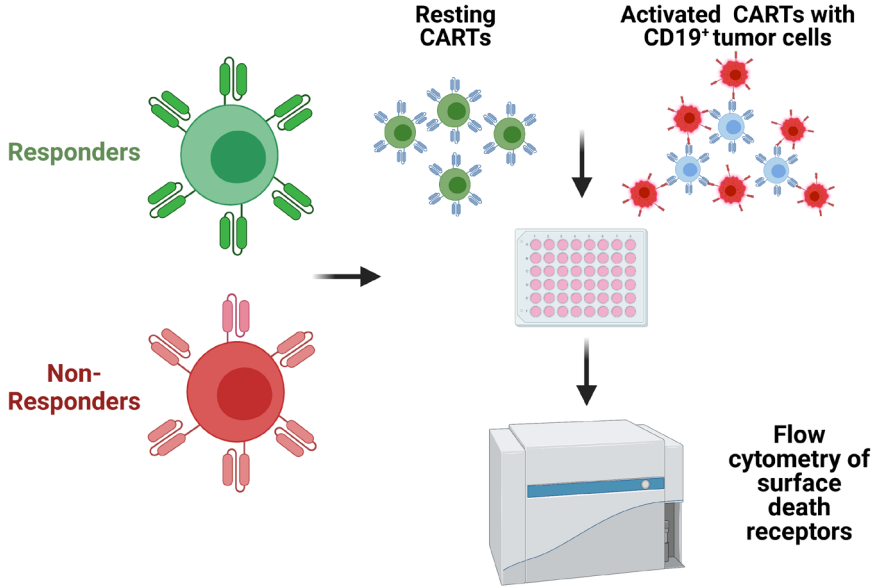
3) Identify targets to modulate CART cell activation, apoptosis, and cytotoxicity to improve anti-tumor activity

METHODS

Zuma-1 clinical trial: led to FDA approval of Axi-Cel (CART19) in lymphoma (101 patients):

- ✓ 17% No Response (SD+PD) → Non-Responders
- ✓ 39% Durable Response (CR) → Responders





Neelapu, S. S., et al., (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*, 377(26), 2531–2544. https://doi.org/10.1056/NEJMoa1707447

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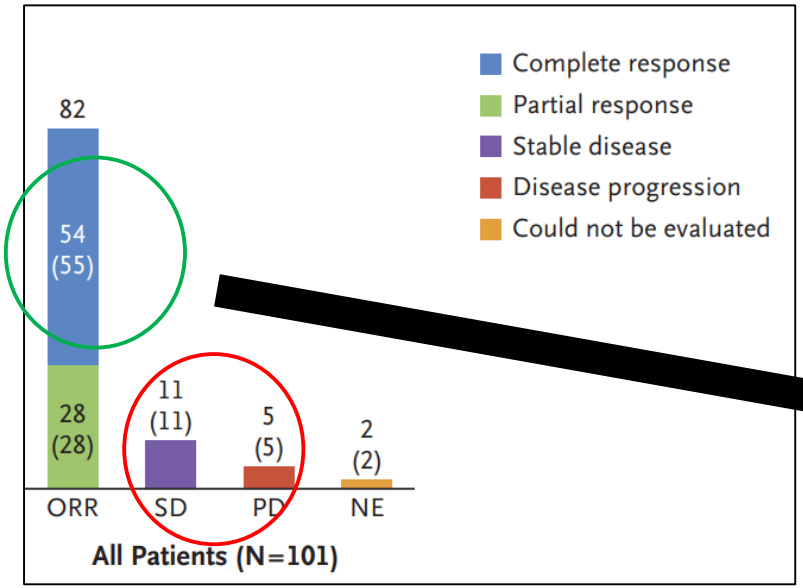
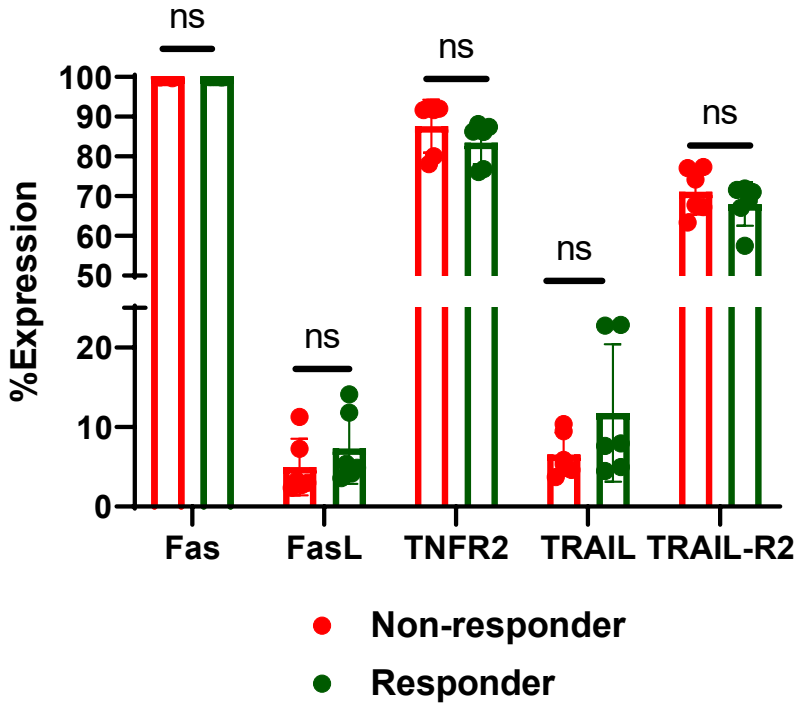
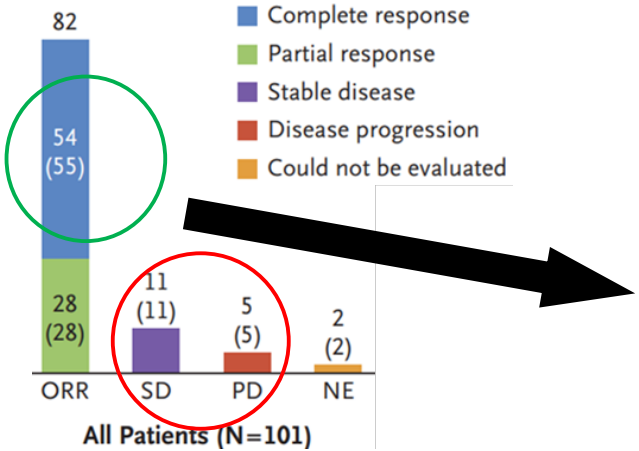
No significant differences in TRAIL, TRAIL-R, FasL, FasL and TNFR2 expression on resting CART cells between responders and non-responders from the Zuma-1 clinical trial



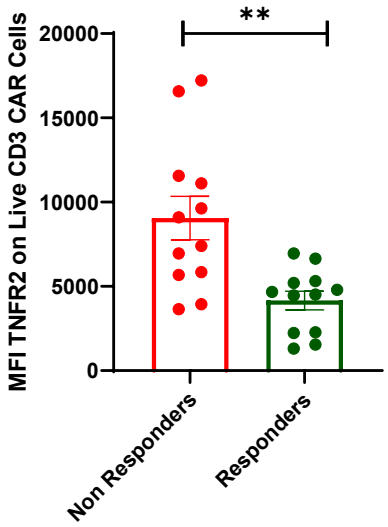


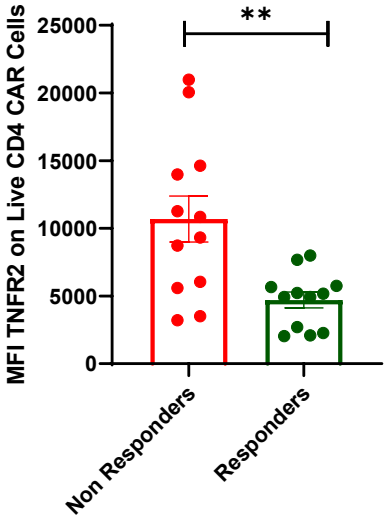
Neelapu, S. S., et al., (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*, 377(26), 2531–2544. https://doi.org/10.1056/NEJMoa1707447

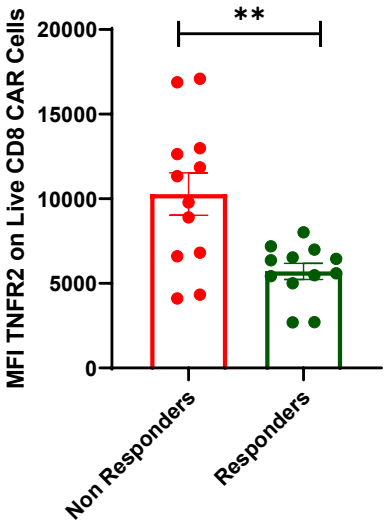
Activated CART cells from CART19 products of non-responders have higher levels of expression of TNFR2 in comparison to responders from the Zuma-1 clinical trial



Neelapu, S. S., et al., 2017*N Engl J Med*

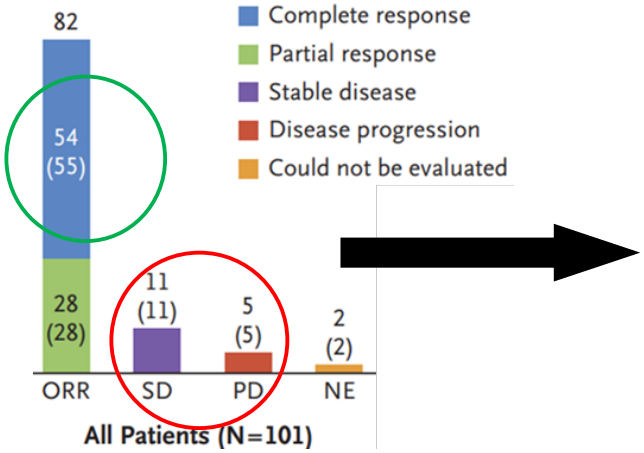




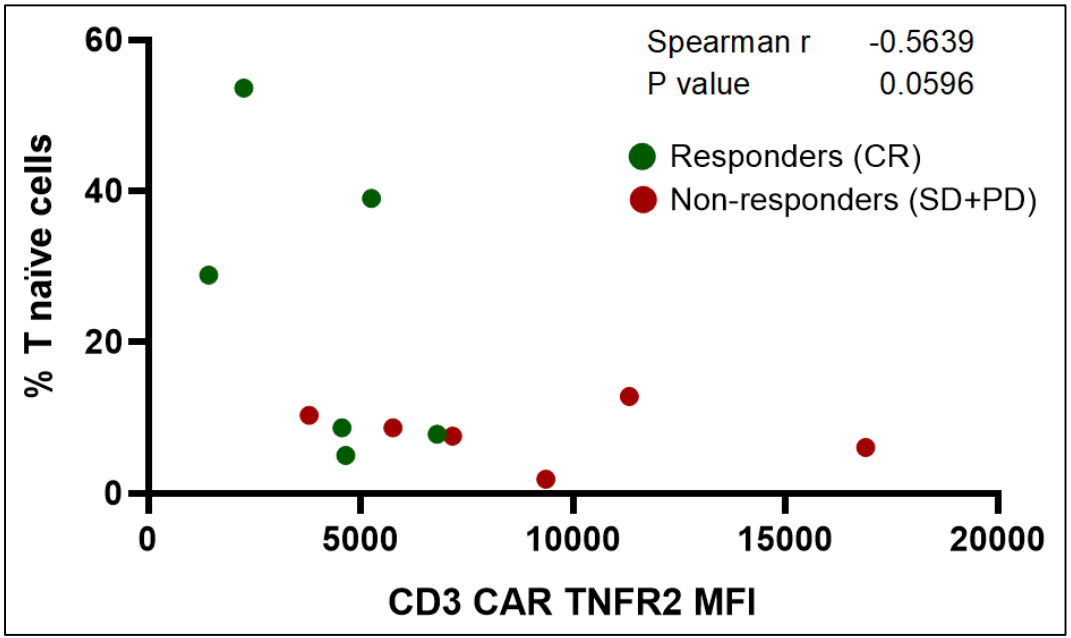


(** p < 0.01; Unpaired T-test)

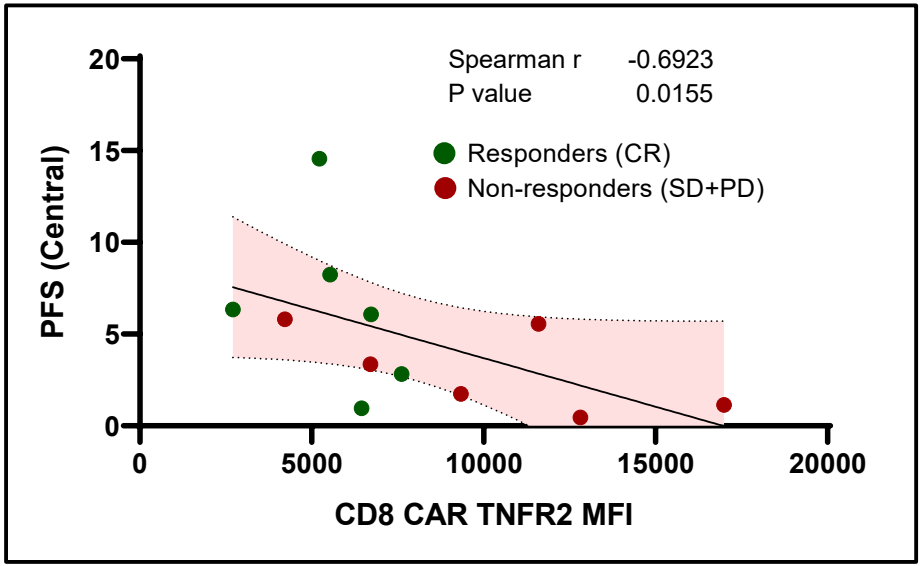
Correlation between TNFR2 expression and CART cell phenotype in responders vs non-responders

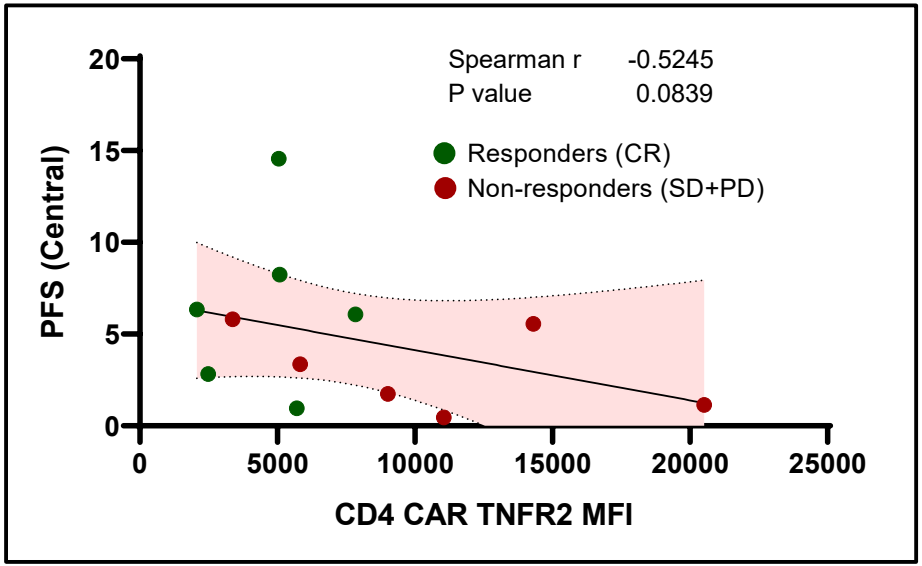


Neelapu, S. S., et al., 2017*N Engl J Med*



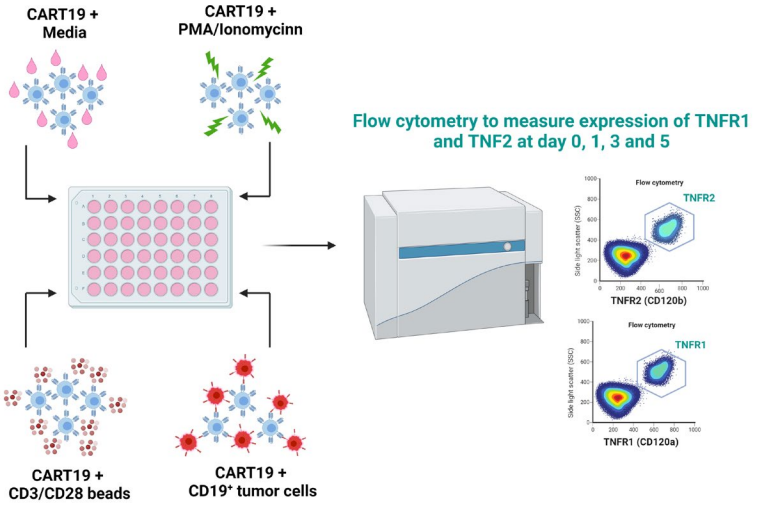
Association of TNFR2 expression on CART19 cells with progression free survival (PFS) in the clinic





What is the role that TNFR2 plays on CART cell activation and dysfunction?

TNFR2, but not TNFR1 is highly upregulated upon CART19 antigen specific stimulation



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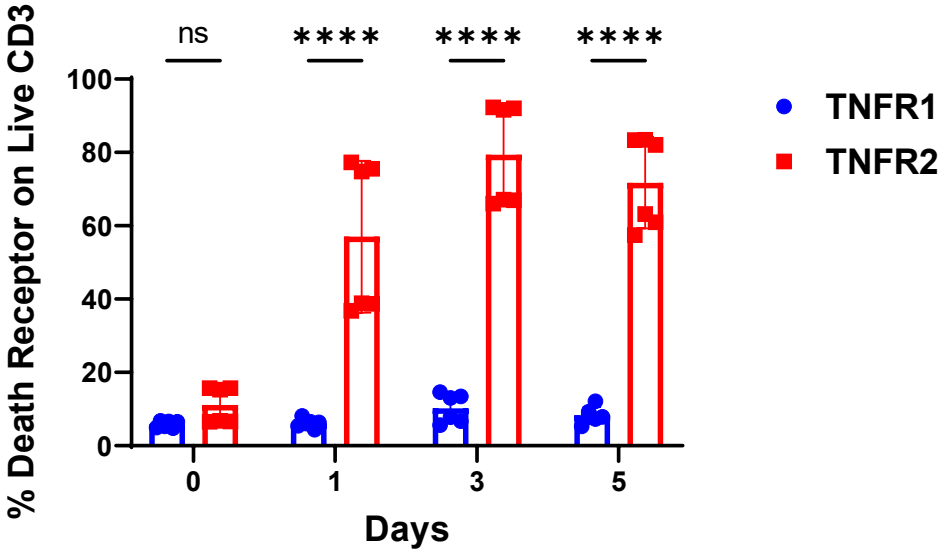
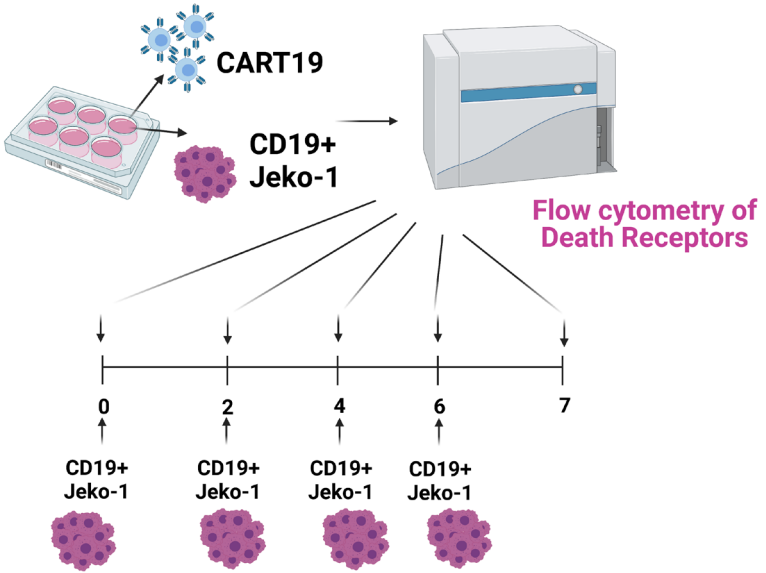


(ns= not significant, **** p < 0.0001; two-way ANOVA)

TNFR2 is persistently elevated on CART19 cells from healthy donors using an extended *in vitro* culture model



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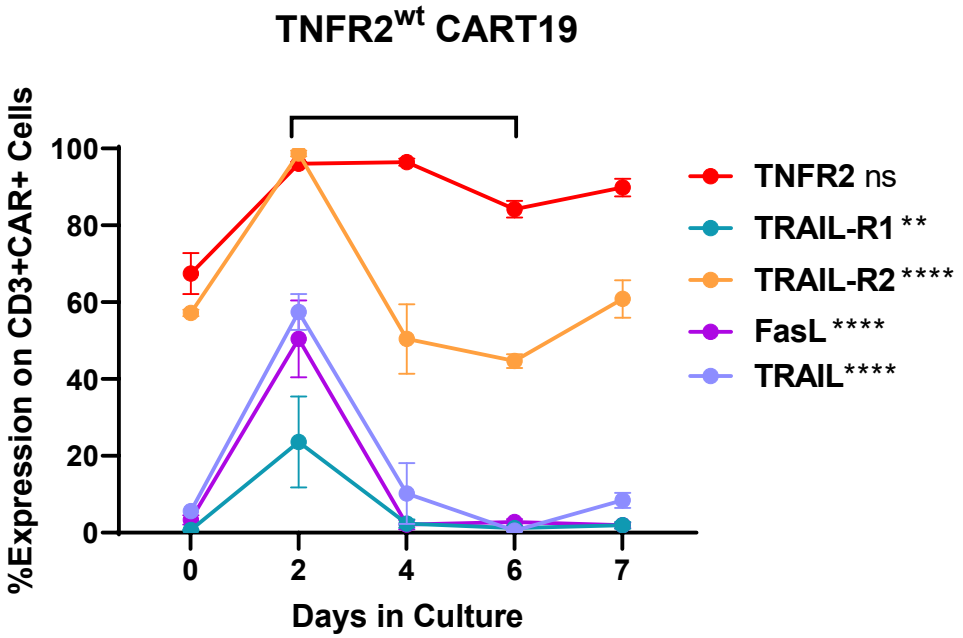
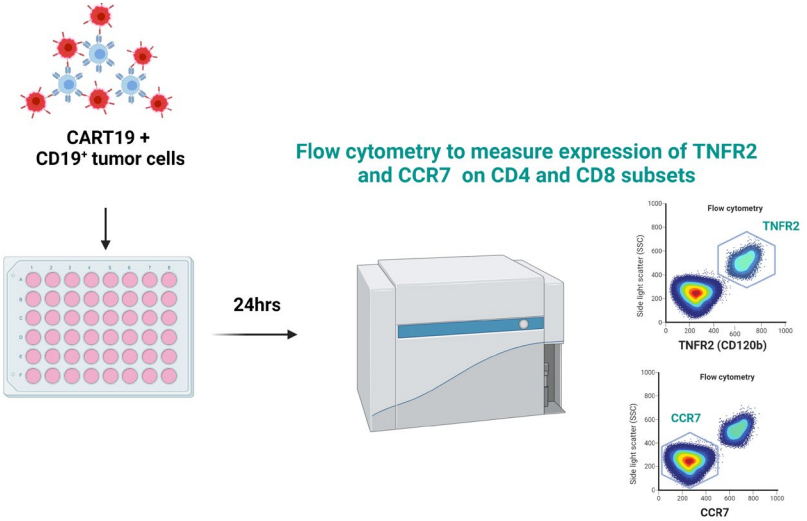


(ns= not significant, ** p < 0.01**** p < 0.0001; two-way ANOVA)

TNFR2 expression is inversely correlated with CCR7 expression in healthy donor CART cells



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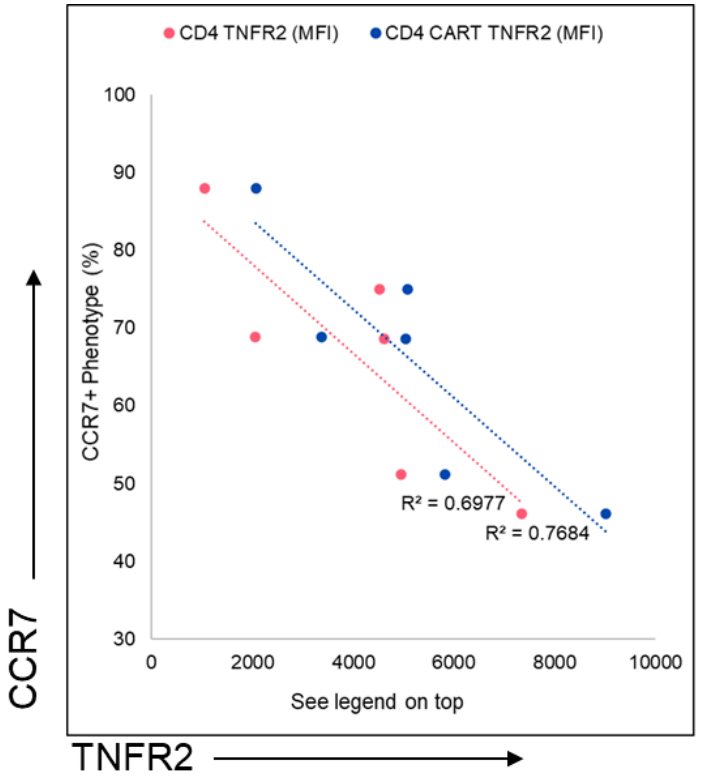
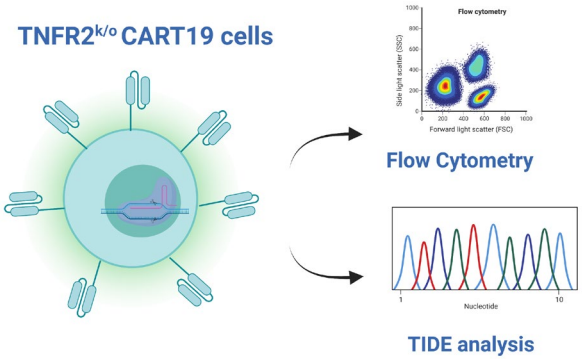
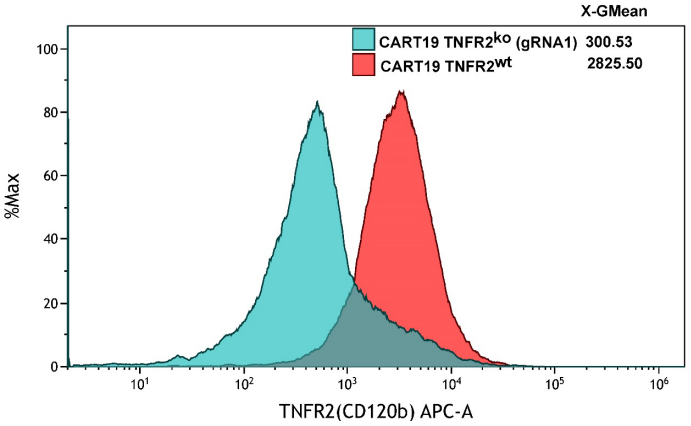


Disruption of TNFR2 in CART19 cells using CRISPR/Cas9





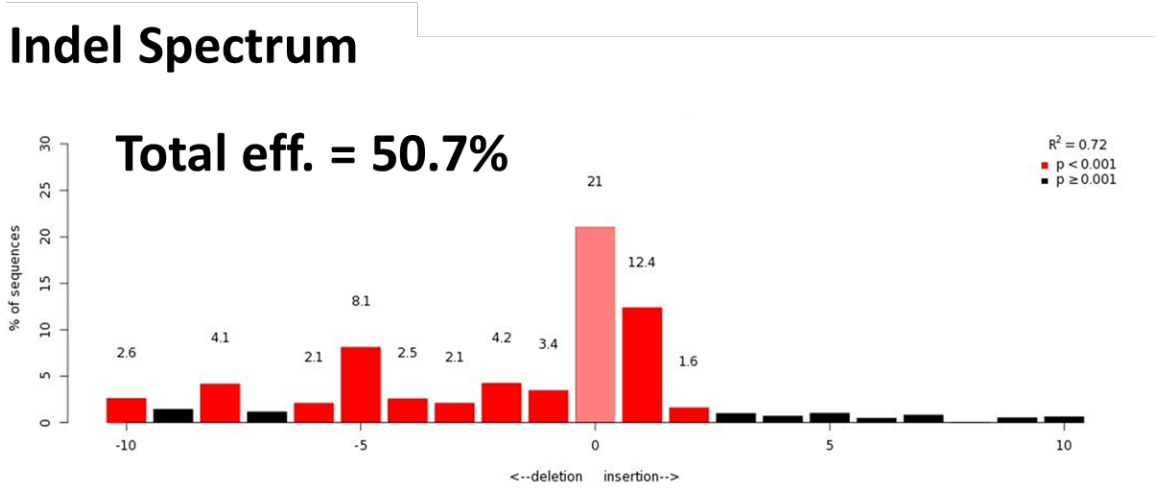
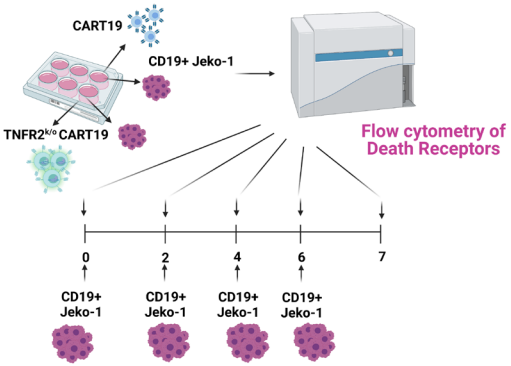


Figure created with BioRender.com

TNFR2$^{k/o}$ CART19 cells in an extended coculture following repeated antigen specific stimulation



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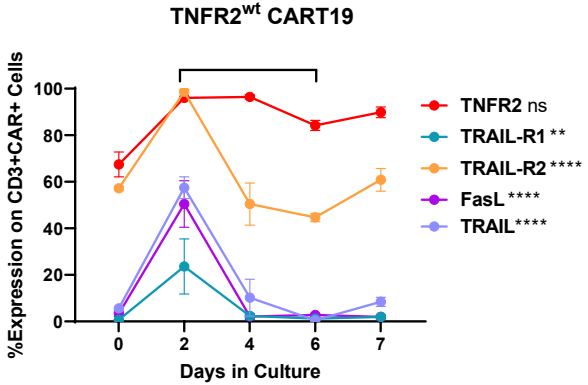
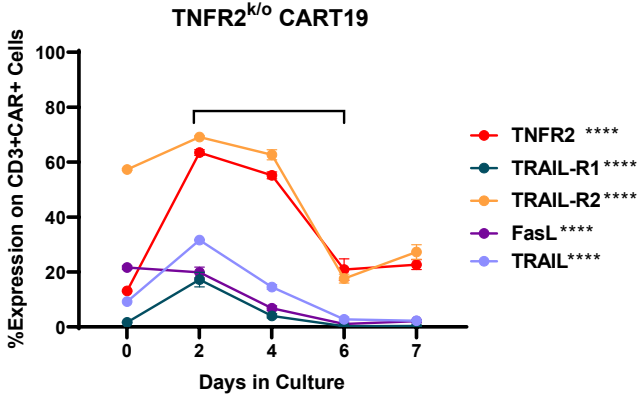
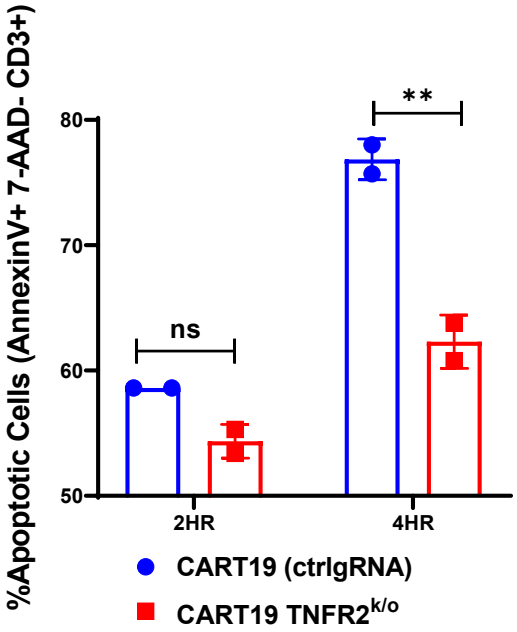
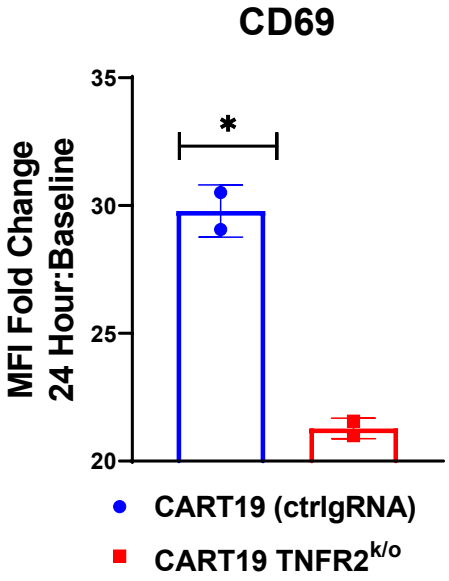


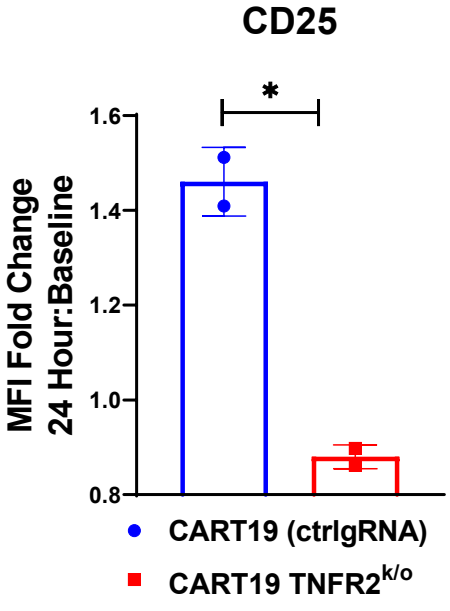


(ns= not significant , ** p < 0.01 , **** p < 0.0001; two-way ANOVA)

CRISPR/Cas9 mediated disruption of TNFR2 in CART19 cells results in increased survival and decreased activation markers

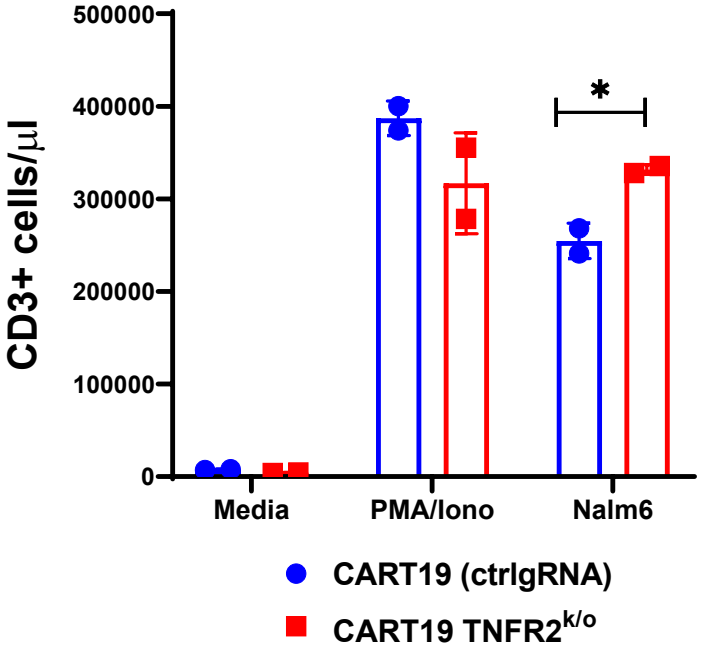


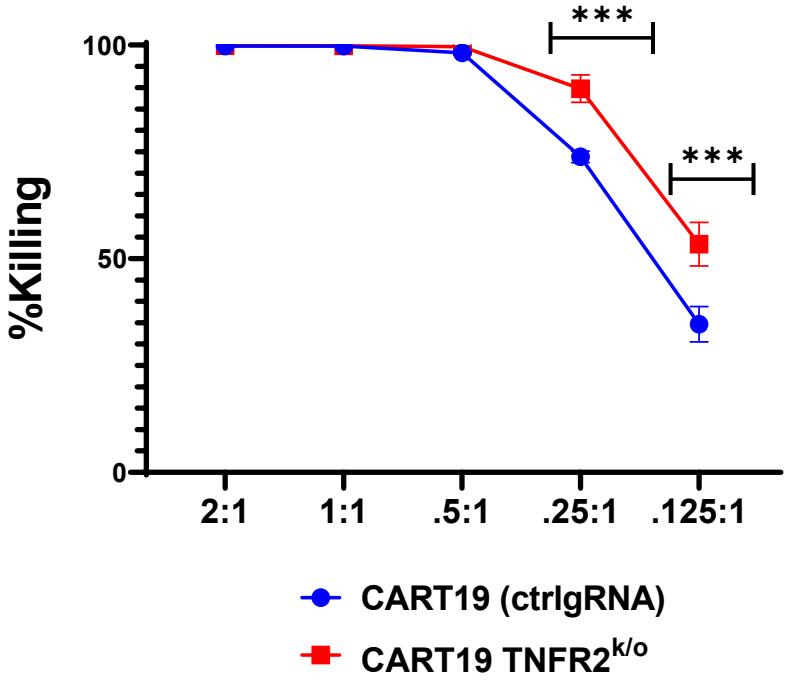




(ns= not significant, * p<0.05 ** p < 0.01; two-way ANOVA)

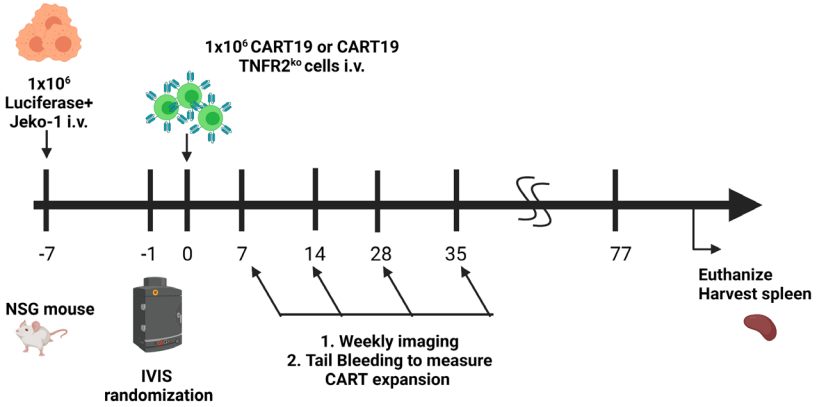
Antigen specific proliferation and cytotoxicity are enhanced in TNFR2$^{k/o}$CART19 in comparison to control CART19 cells





* p-value <0.05, *** p< 0.001, **** p<0.0001. Two-way ANOVA)

TNFR2$^{k/o}$CART19 cells improved CART cell expansion, enhanced anti-tumor activity and proliferation *in vivo*



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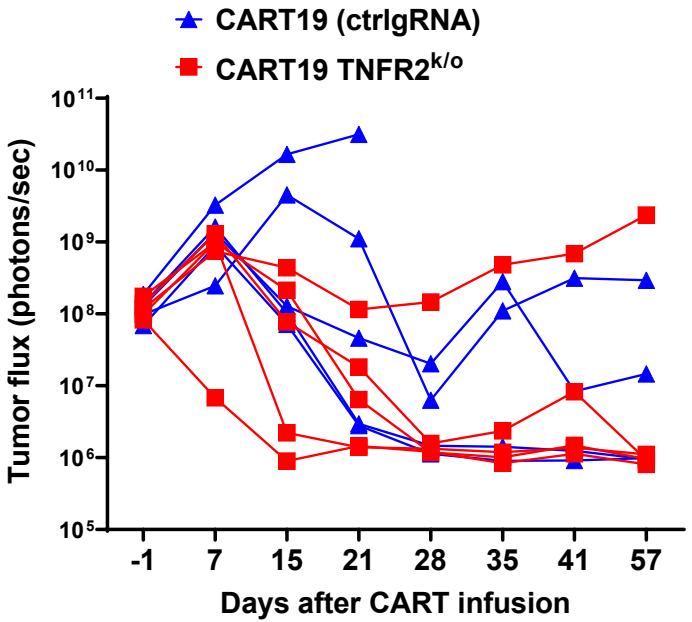

Summary

- ✓ TNFR2 expression on CART19 Axi-Cel products:
  - ✓ Is associated with poor response and reduced PFS
  - ✓ Is associated with differentiation to CART19 effector phenotype
- ✓ TNFR2 expression on healthy donor CART19 cells:
  - ✓ Is stably upregulated following antigen specific stimulation
  - ✓ Remains upregulated following repeated antigen specific stimulation
  - ✓ Is inversely correlated with CCR7 expression
- ✓ Generation of TNFR2$^{k/o}$CART19 via CRISPR/Cas9 results in decreased early activation and enhanced cytotoxicity and tumor control in comparison to control CART19 cells

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Discussion and Questions

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