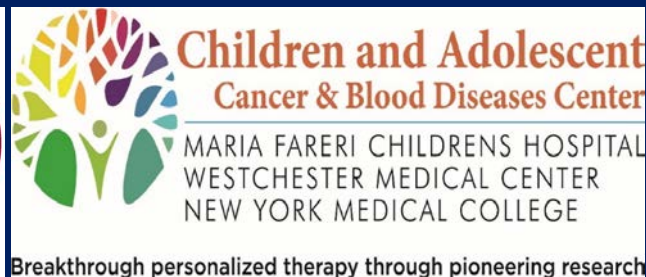
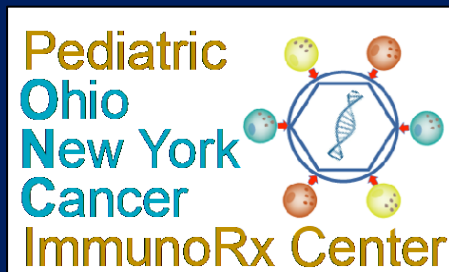


Targeting pediatric solid tumors with anti-MCAM chimeric antigen receptor modified natural killer cells

Ninth Annual PTCTC Educational Meeting
May 3, 2022

Wen Luo, Aliza Gardenswartz, Yaya Chu, Jeremy M. Rosenblum, Janet Ayello, Mario Marcondes, Willem W. Overwijk, Timothy P. Cripe, Kevin A. Cassady, Dean A. Lee, Mitchell S. Cairo

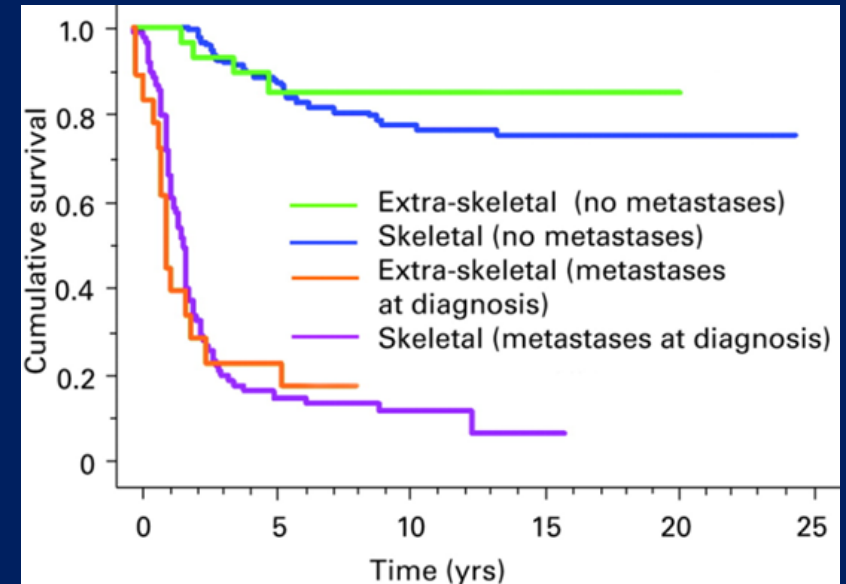


Disclosure

- Funded by NCI Cancer Moonshot
Pediatric Cancer Research Foundation
Alex's Lemonade Stand Foundation
- NKTR-255 provided by Nektar Therapeutics

Ewing sarcoma, osteosarcoma, neuroblastoma

- Malignant solid tumors in CAYA
- Average 5-year OS <25%
- Surgery, radiation, chemotherapy



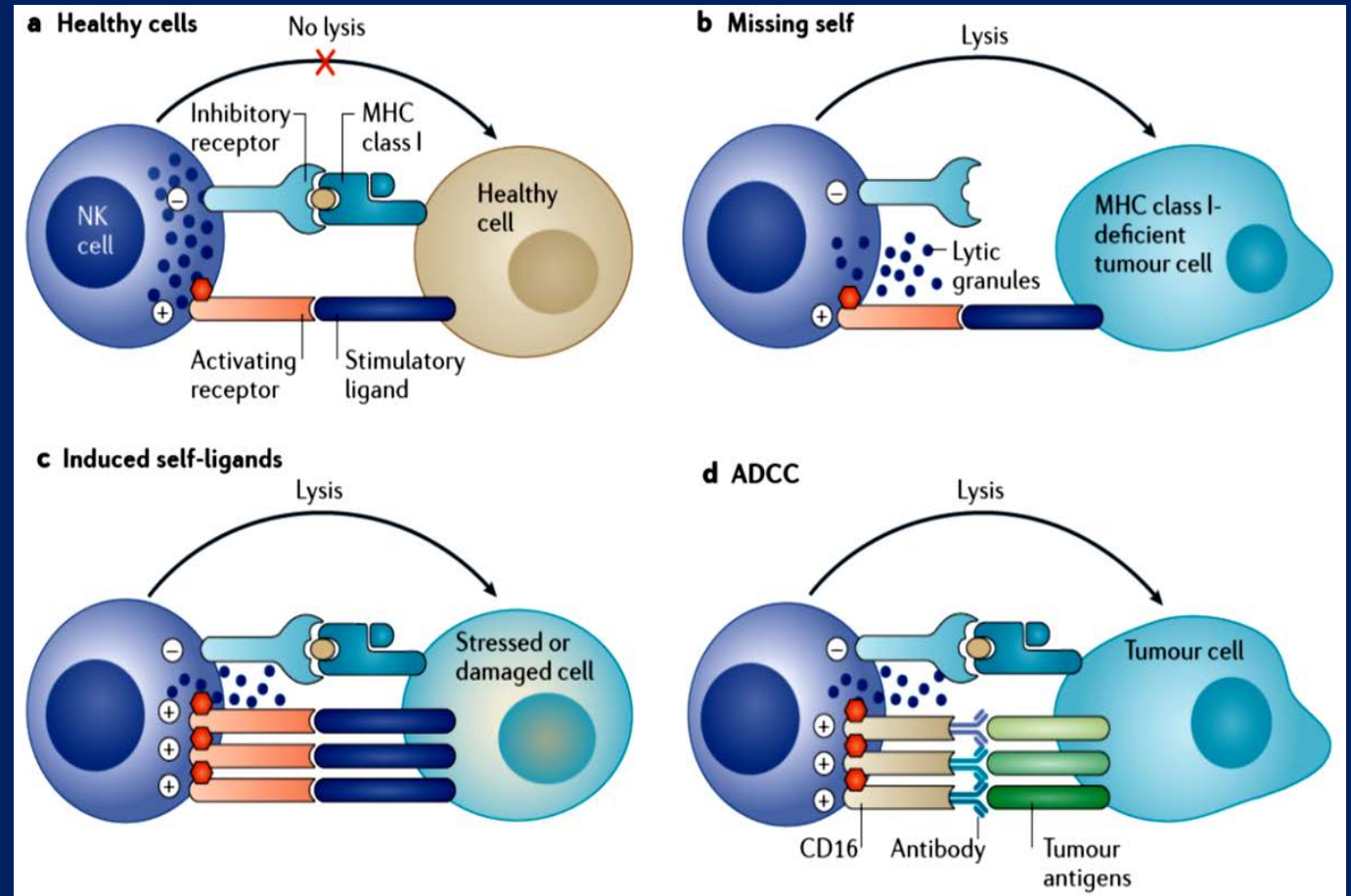
Grunewald TGP et al, 2018
Whittle SB et al, 2017
Isakoff MS et al, 2015
Pradan A et al, 2011
Shankar et al, 2003

Natural Killer (NK) cell

❁ NK cells do not require prior sensitization to target

❁ NK cell functions are balanced by the activating and inhibitory receptors.

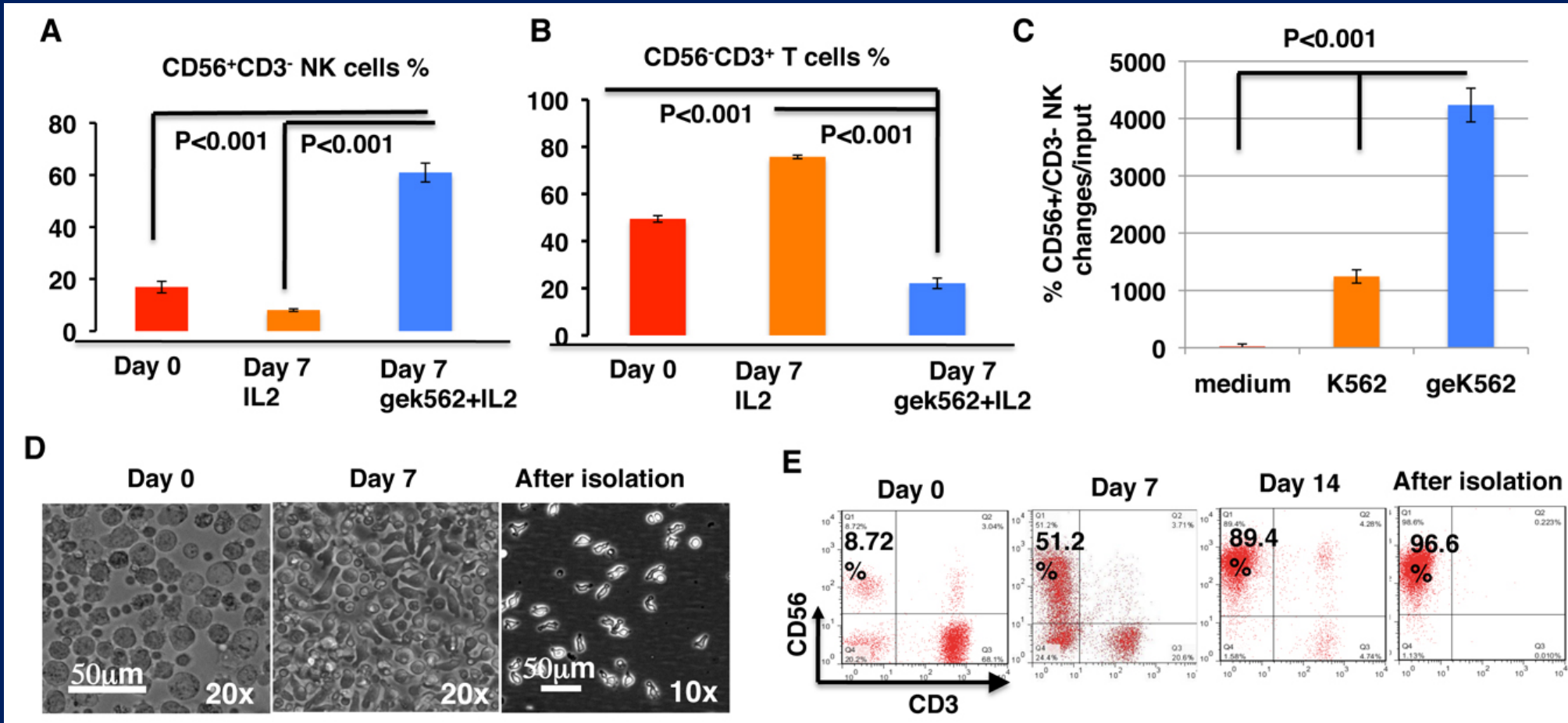
- Farag/Caligiuri, **Bld Rev** 2006
- Raulet *et al. Nature Reviews Immunology* 2006
- Shereck/Cairo *et al. Ped Bld Can* 2007
- Fujisaki *et al Can Res* 2009
- Ruggieri *et al. Science* 2002
- Dunbar *et al. Haematologica* 2008
- Venstrom JM, *et al, N Engl J Med.* 2012
- Murphy/Miller, BBMT, 2012
- Wing Leung *Clin Cancer Res* 2014
- **Morvan/Lanier, Nat Rev Can, 2016**



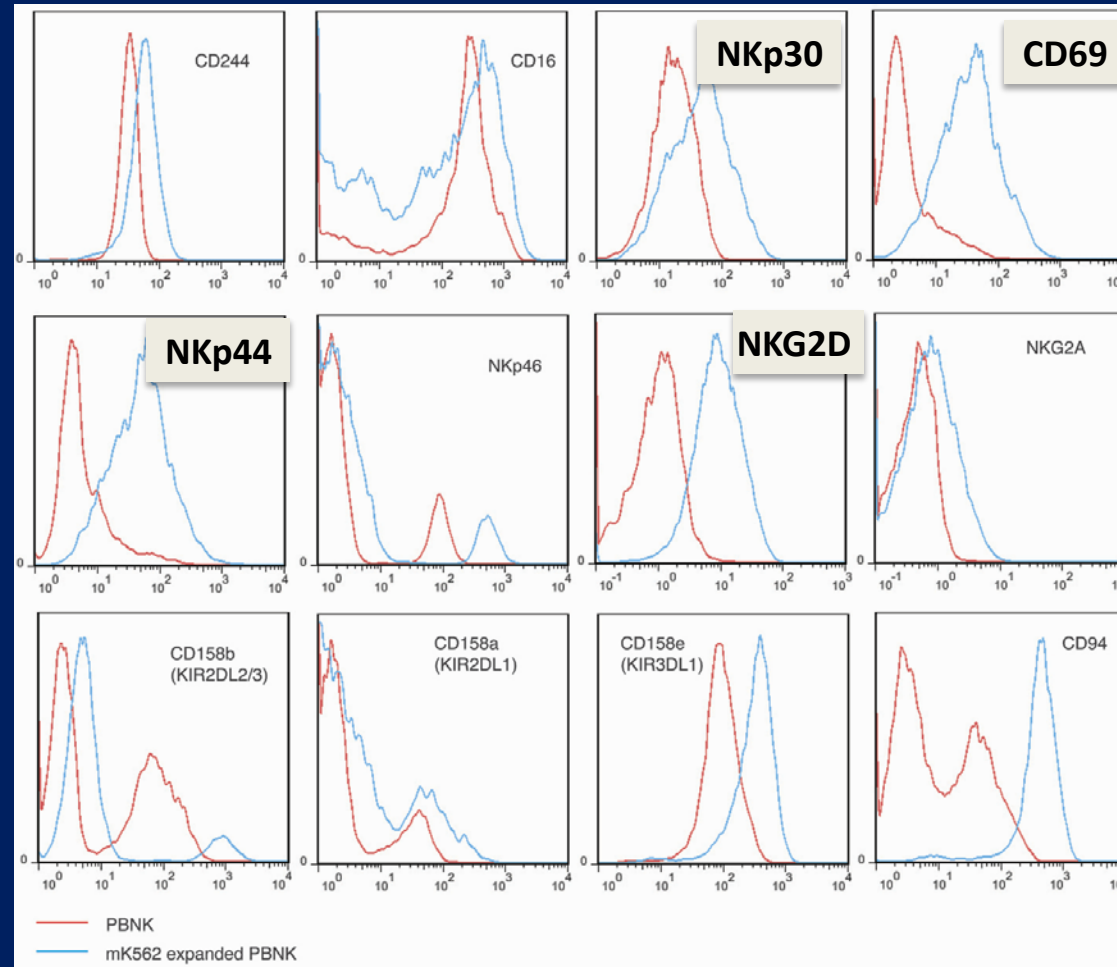
Limitations of NK Cell Tumor Immunity

- Small number of circulating NK Cells
- NK Cell inhibitory receptor induced inhibition
- Poor activation and persistence in-vivo
- Lack of specific tumor antigen targeting

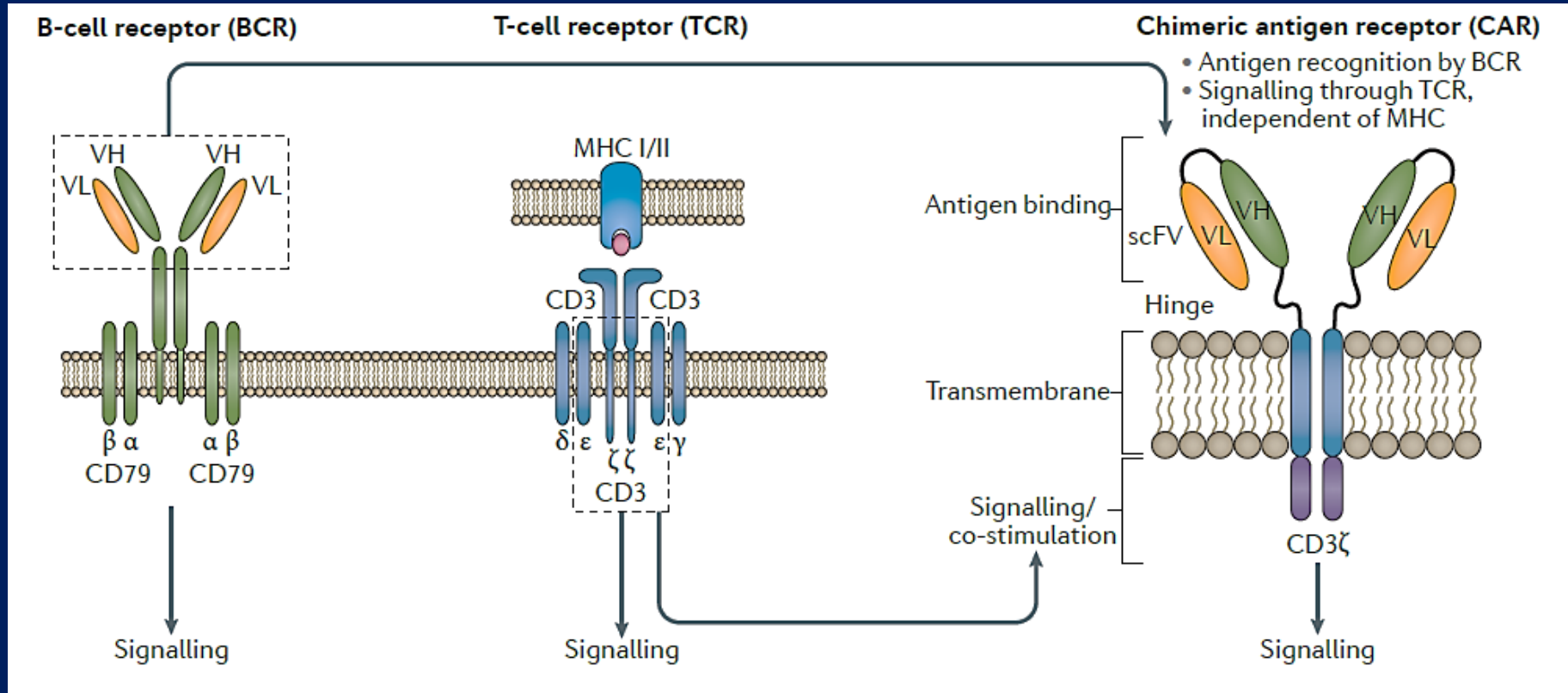
Irradiated genetically modified K562 cells significantly expanded NK cells from whole PBMC cultures



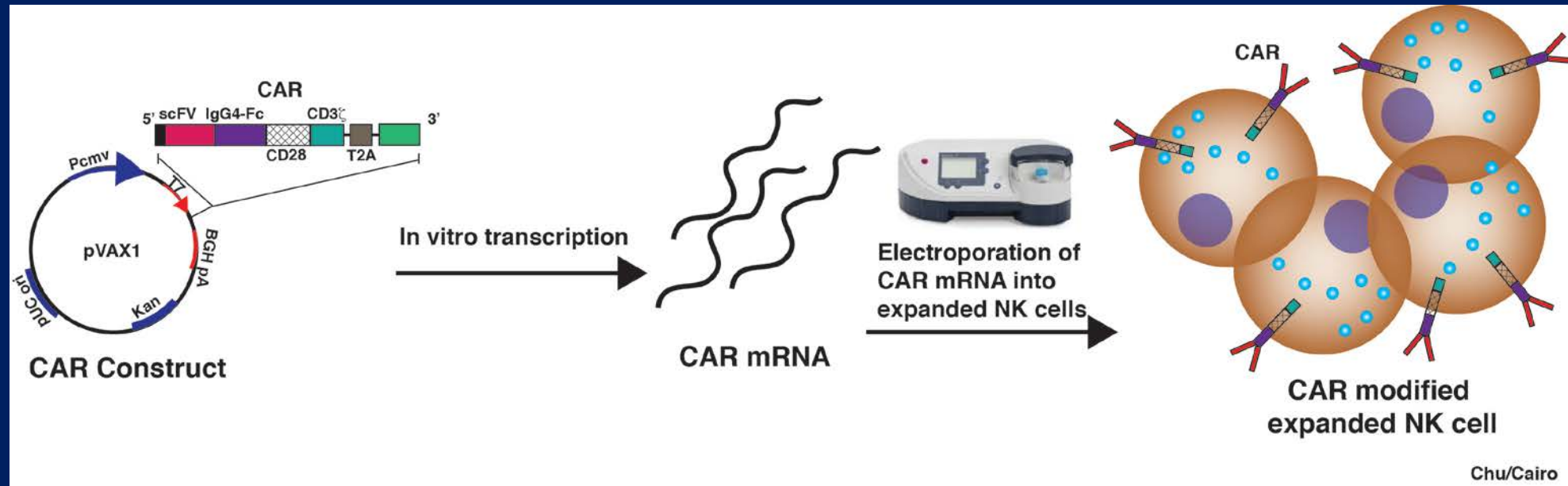
The purified expanded PBNK cells have higher activating receptor expression



Structure of Chimeric Antigen Receptor (CAR)



CAR mRNA Nucleofection (Electroporation)

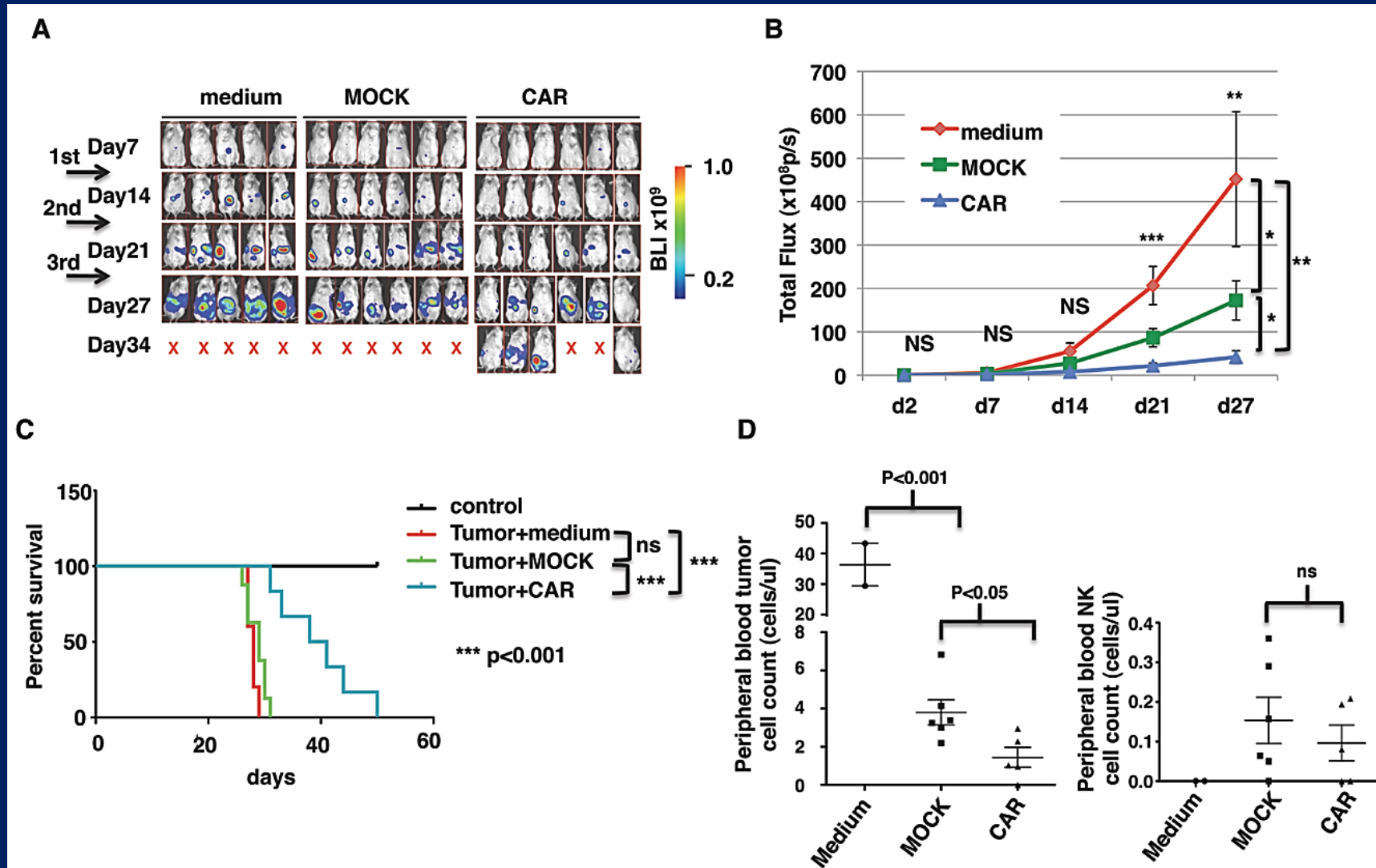


Advantages

- No long-term on-target off-tissue effect
- Not associated with genotoxicity
- Lack of CRS, ICANS, and HLH/MAS toxicities

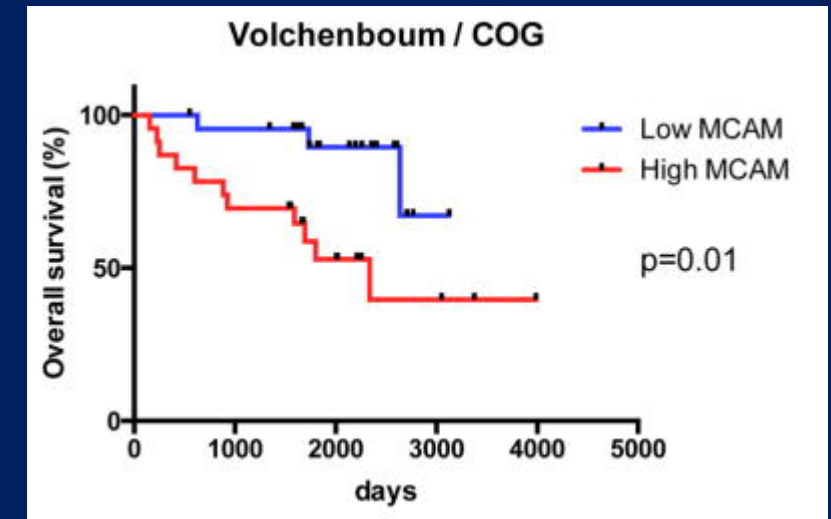
Flower/Chu/Cairo et al, *Methods Mol Bio*, 2016
Chu/Cairo, *Can Immun Res*, 2015

CD20 CAR exPBNK significantly reduced BLI signals in Raji-Luc xenografts and significantly extended survival time in a semi-disseminated model

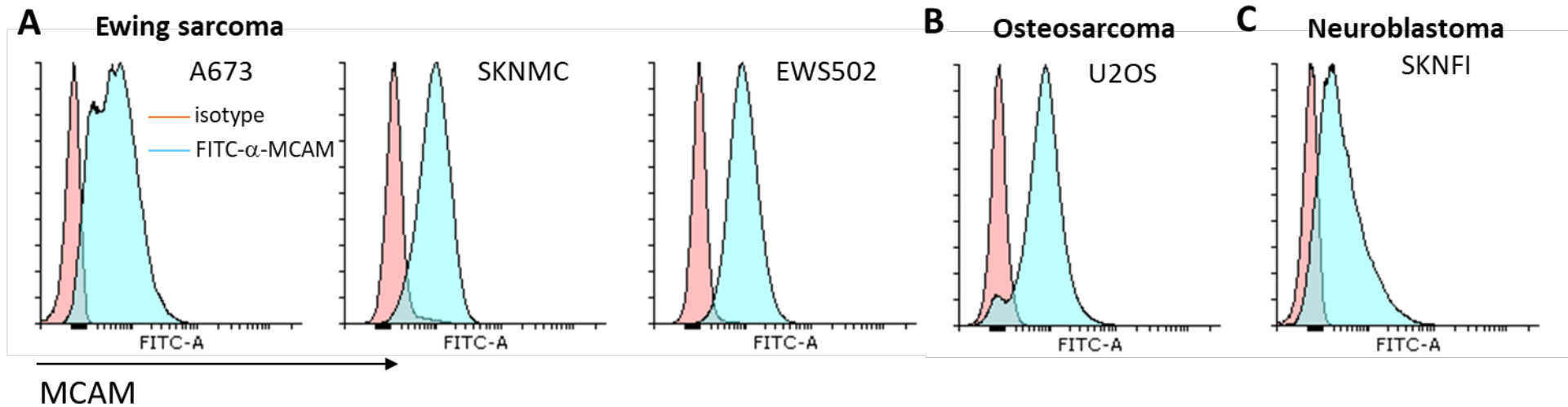


Melanoma Cell Adhesion Molecule (MCAM)

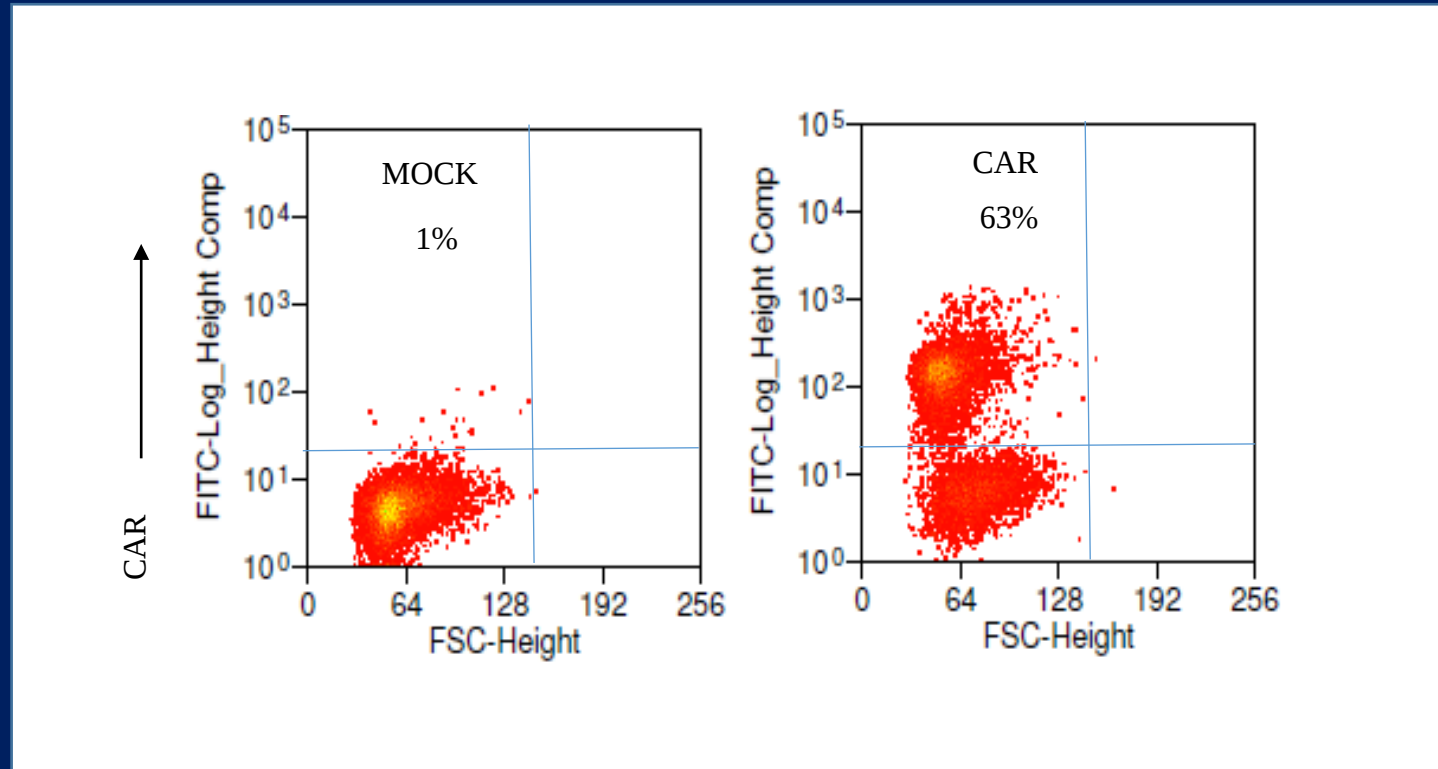
- MCAM/CD146/MUC18, a transmembrane glycoprotein, immuno-globulin superfamily
- Overexpressed on the cell surface in common pediatric cancers
- Promotes Ewing sarcoma cell migration and metastasis
- High level is associated with poor patient survival in Ewing sarcoma
- Highly expressed in the embryo but drastically decreases in mature tissue



MCAM expression in ES, OS and NB cells

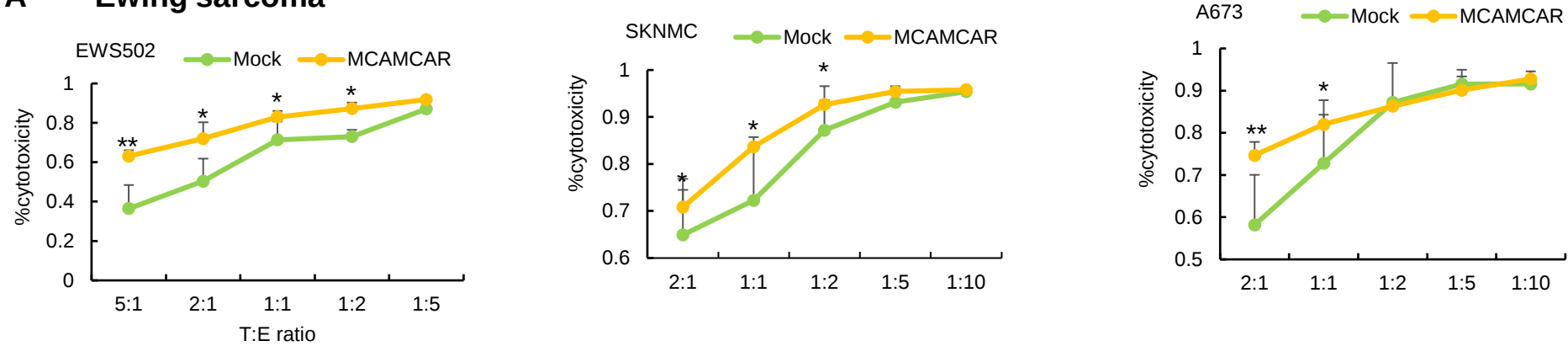


MCAM CAR expression in ex vivo expanded NK

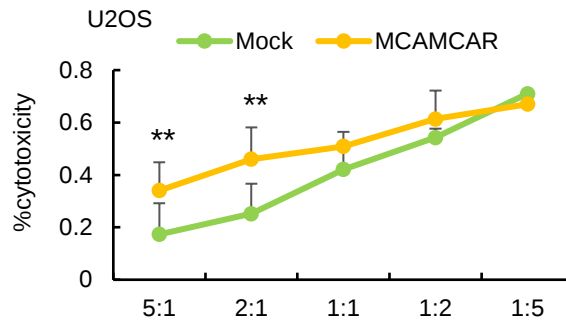


Expression of anti-MCAM CAR significantly enhances NK cytotoxicity against ES, OS and NB

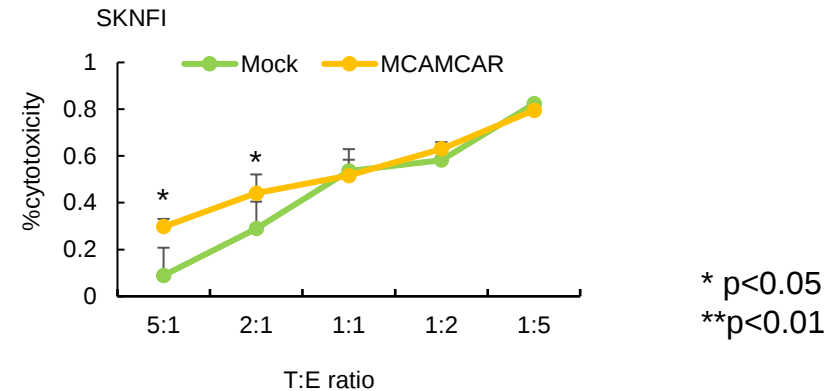
A Ewing sarcoma



B Osteosarcoma

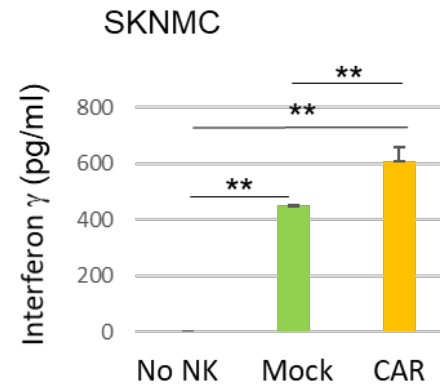
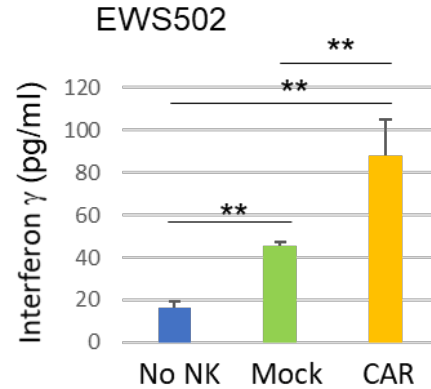
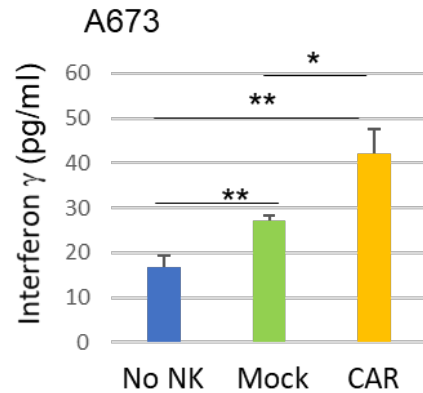


C Neuroblastoma

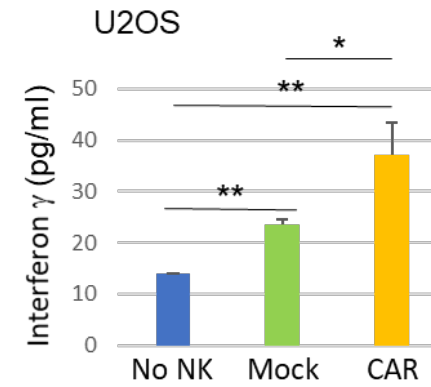


Expression of anti-MCAM CAR significantly enhances NK cytokine secretion

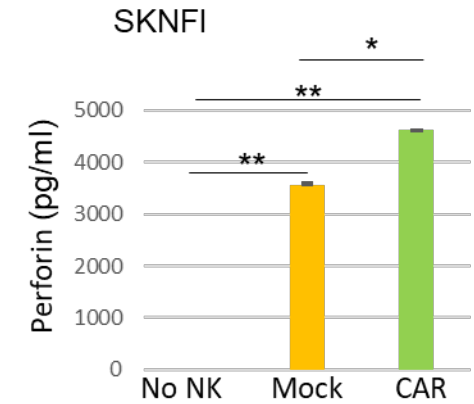
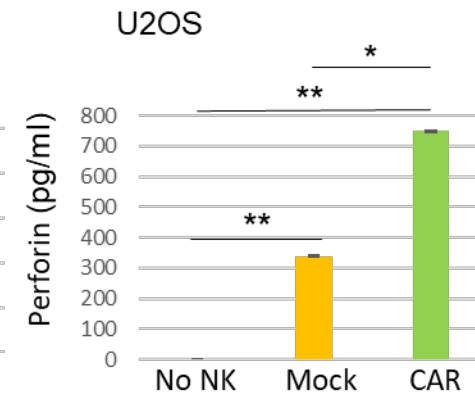
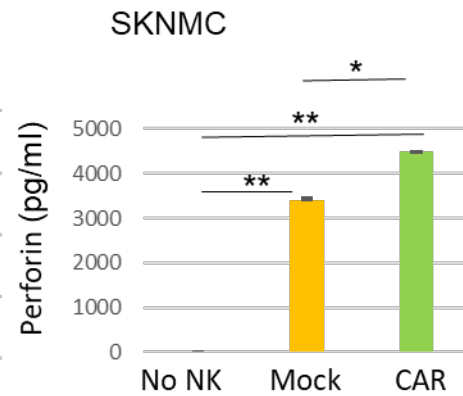
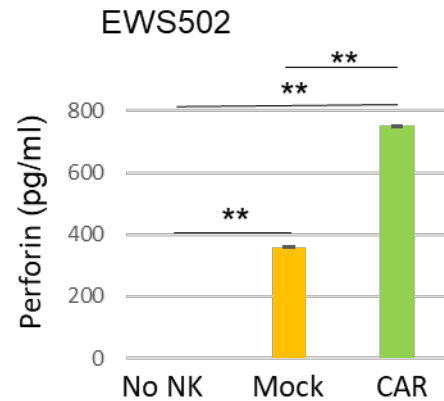
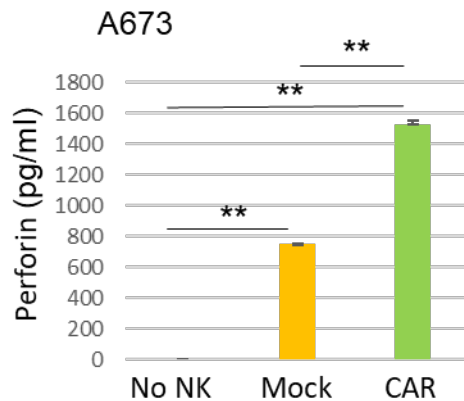
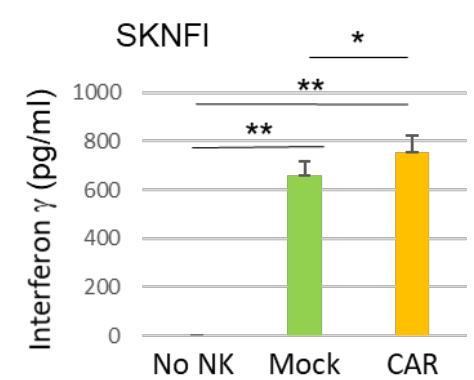
Ewing sarcoma



Osteosarcoma

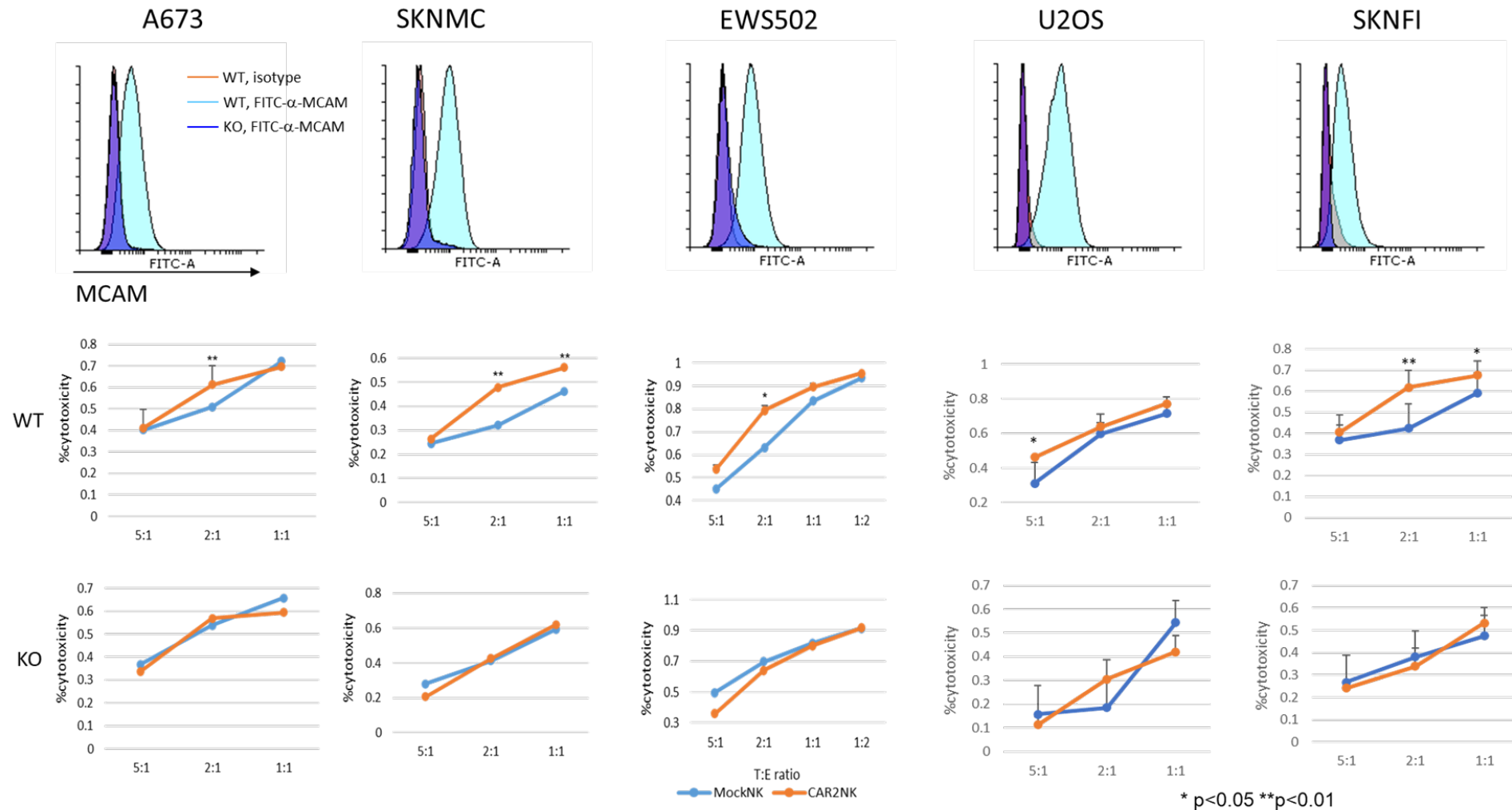


Neuroblastoma

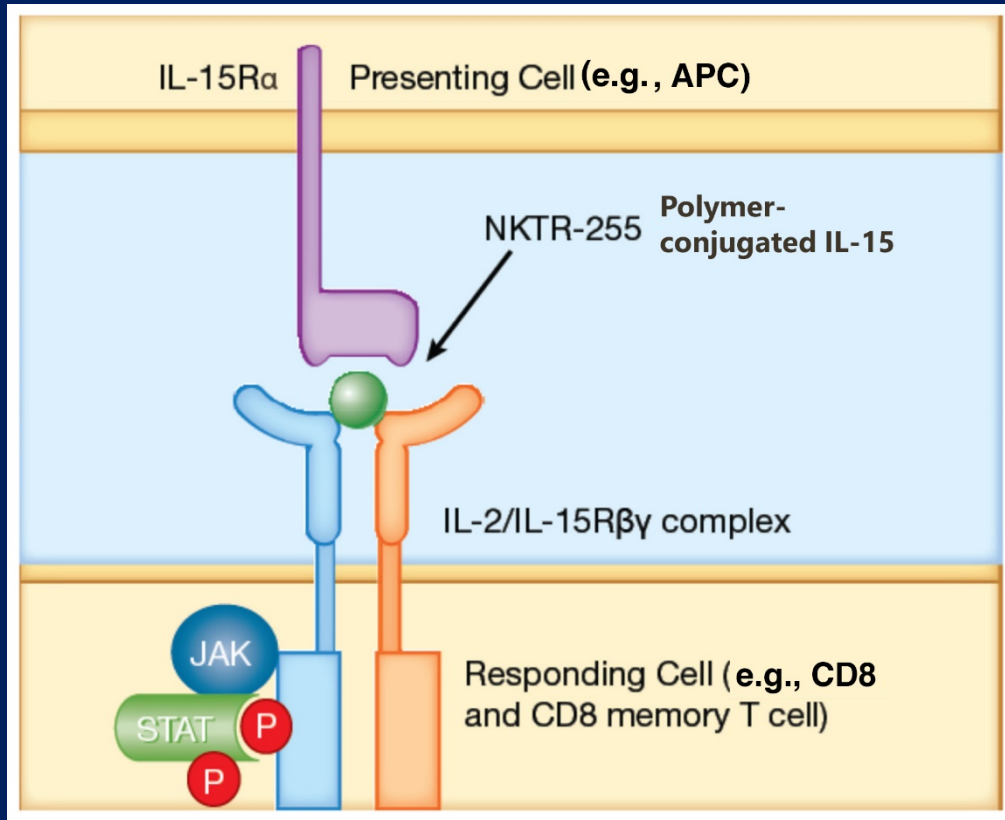


* p < 0.05 ** p < 0.01

Specific targeting of MCAM+ tumor cells by anti-MCAM CAR NK cells

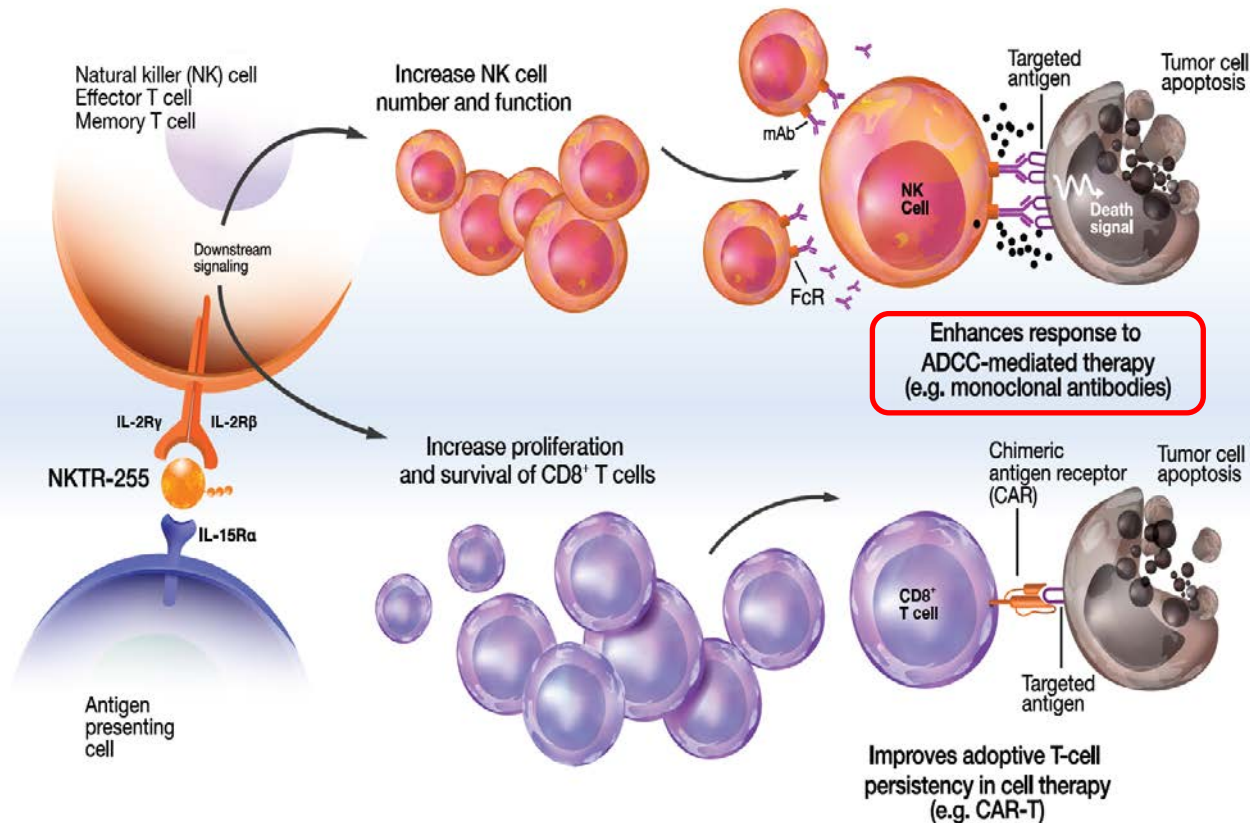


Interleukin-15 (IL-15)



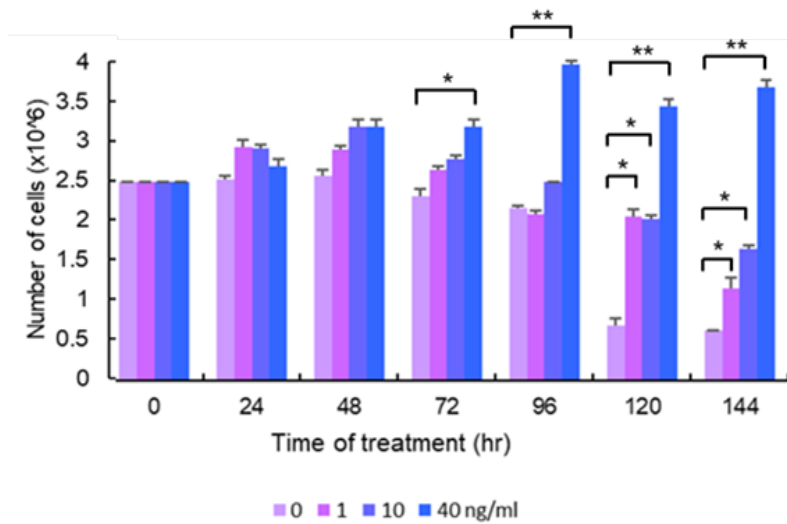
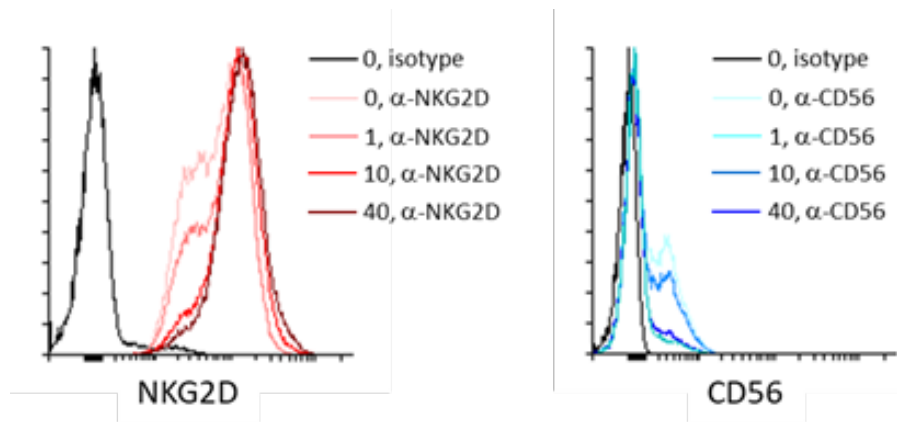
- IL-15 is a pleiotropic cytokine with roles in innate and adaptive immunity.
- Identified by NCI as one of the most promising immuno-oncology agents.
- Key role in formation and maintenance of immunological memory.
- Essential factor for NK (Natural Killer) cell development and homeostasis.
- In vitro, IL-15 can reverse tumor-induced NK cell dysfunction.

NKTR-255

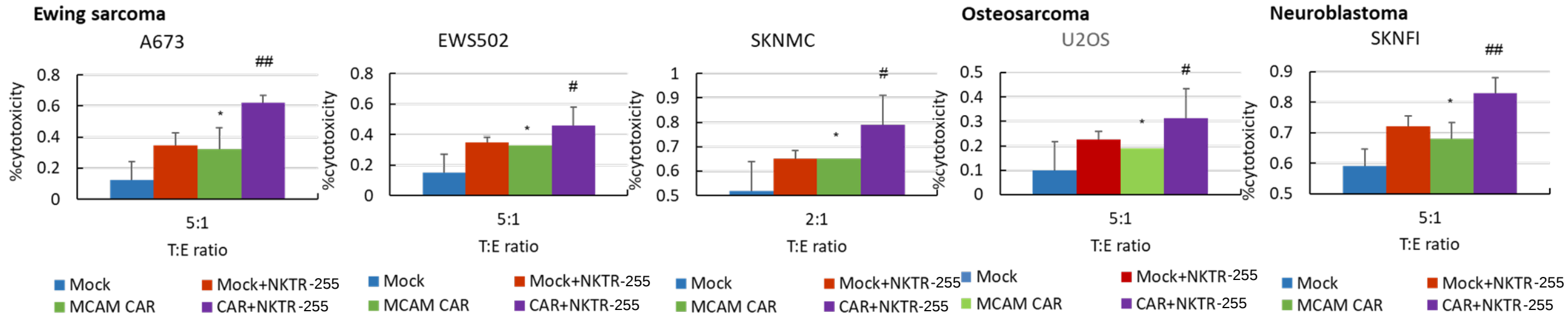


- Polymer-conjugated recombinant human IL-15 agonist
- Retains the full spectrum of IL-15 biology but with a significantly longer half-life compared to natural IL-15
- Activates the IL-15 pathway and stimulates proliferation and survival of NK cells
- Currently in early phase clinical trials in combination with various monoclonal antibodies in patients with hematological malignancies and solid tumors

Effect of NKTR-255 on NK activation and expansion/maintenance



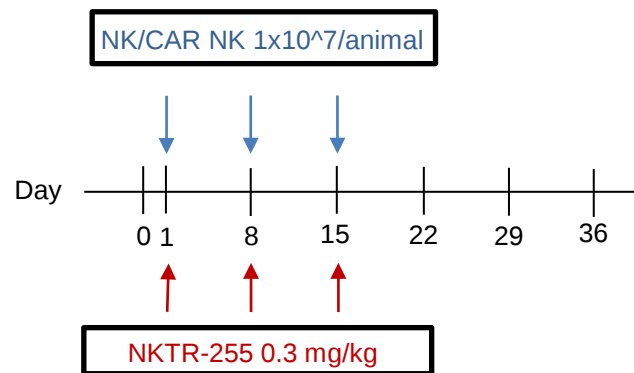
NKTR-255 further enhances anti-MCAM CAR NK cytotoxicity against ES, OS and NB



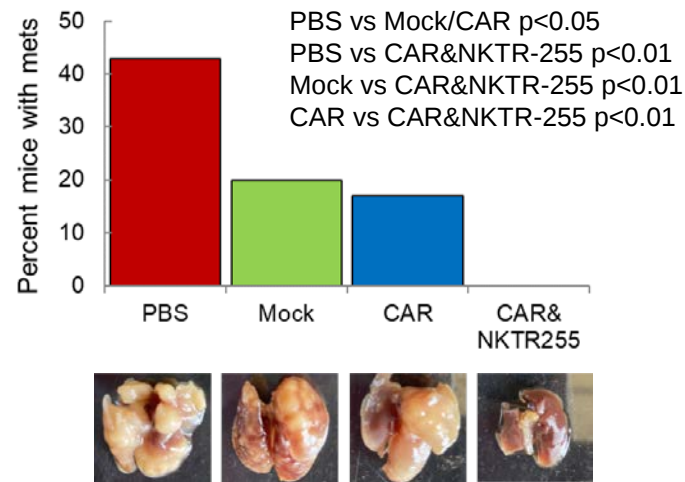
* p<0.05 Mock vs CAR # p<0.05 CAR vs CAR+NKTR-255 ## p<0.01 CAR vs CAR+NKTR-255

Anti-MCAM CAR NK alone or in combination with NKTR-255 significantly decreases lung metastasis and prolongs animal survival in an ES orthotopic mouse model

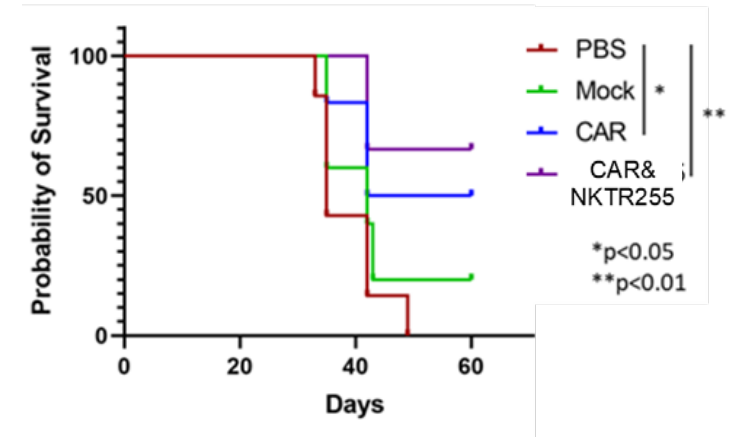
A. Administration schedule



B. Lung Metastasis



C. Animal Survival



Summary

- Expression of anti-MCAM CAR significantly enhances NK cytotoxicity against ES, OS and NB
- Expression of anti-MCAM CAR significantly enhances NK cytokine secretion
- Enhanced cytotoxicity of the anti-MCAM CAR NK cell is due to specific targeting of MCAM
- NKTR-255 enhances expression of NK activating receptors, stimulates NK proliferation and sustains NK expansion
- NKTR-255 further enhances anti-MCAM CAR NK cytotoxicity against ES, OS and NB in vitro
- Anti-MCAM CAR NK alone or in combination with NKTR-255 significantly decreases lung metastasis and prolongs animal survival in an ES orthotopic mouse model

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