



# Developing Off-The-Shelf CAR NK Cells Targeting CD70: Preclinical and Early Phase Clinical Updates



**Sunil Acharya, PhD**

Principal Research Scientist

Rezvani lab

Stem Cell Transplantation and Cellular Therapy

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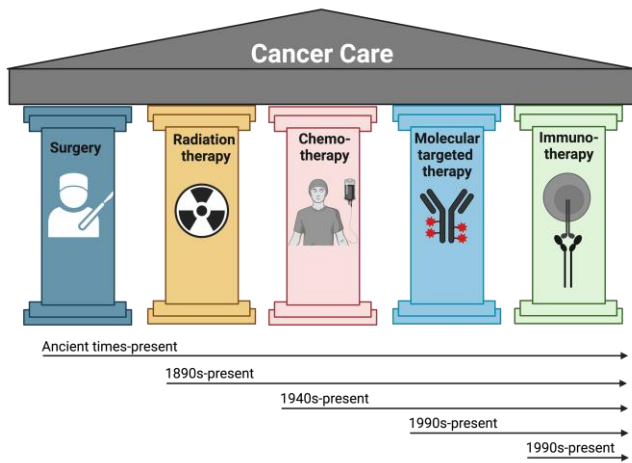
THE UNIVERSITY OF TEXAS  
**MDAnderson**  
**Cancer Center**  
Making Cancer History®

## Disclosures

### **License agreement and research agreement**

Takeda to develop CB-CAR NK cells for the treatment of B-cell malignancies and other cancers, which creates an institutional conflict of interest under MD Anderson policy

# Pillars of Cancer Care

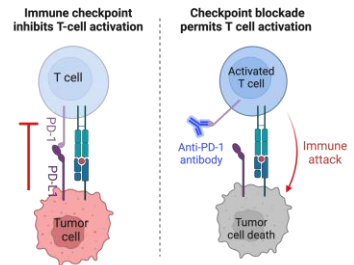


James Allison, PhD

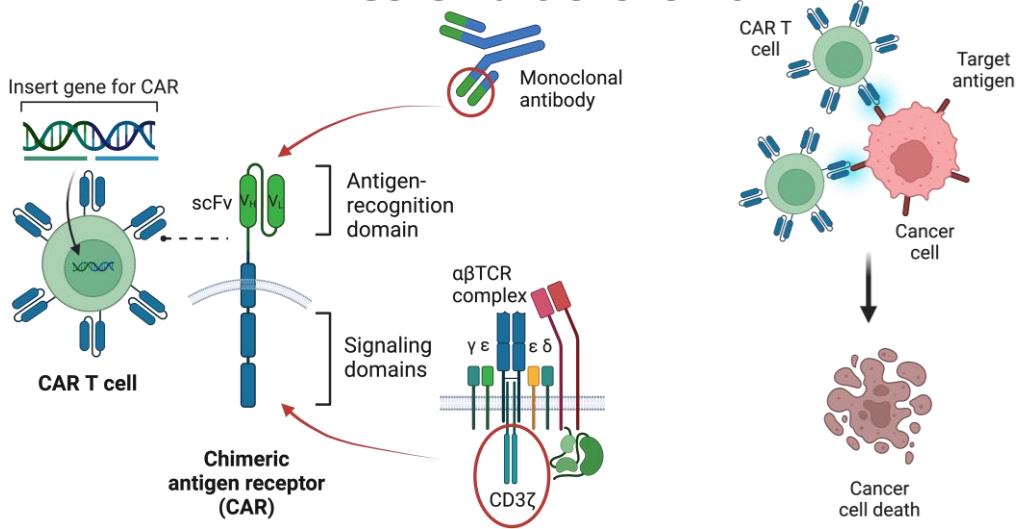


Tasuku Honjo, MD, PhD

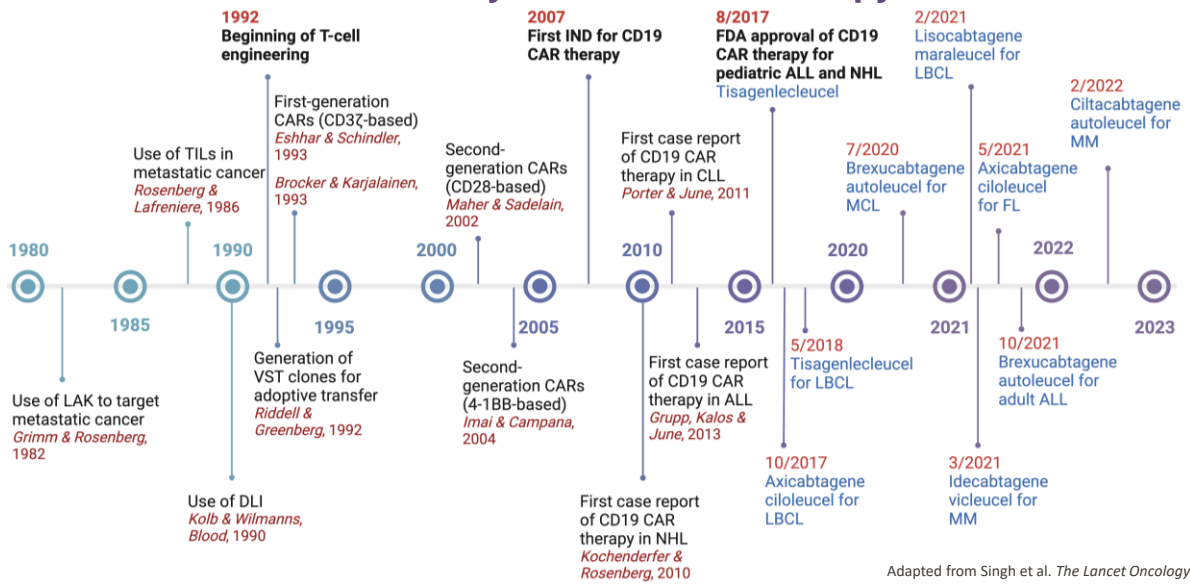
Nobel Prize Medicine & Physiology  
2018



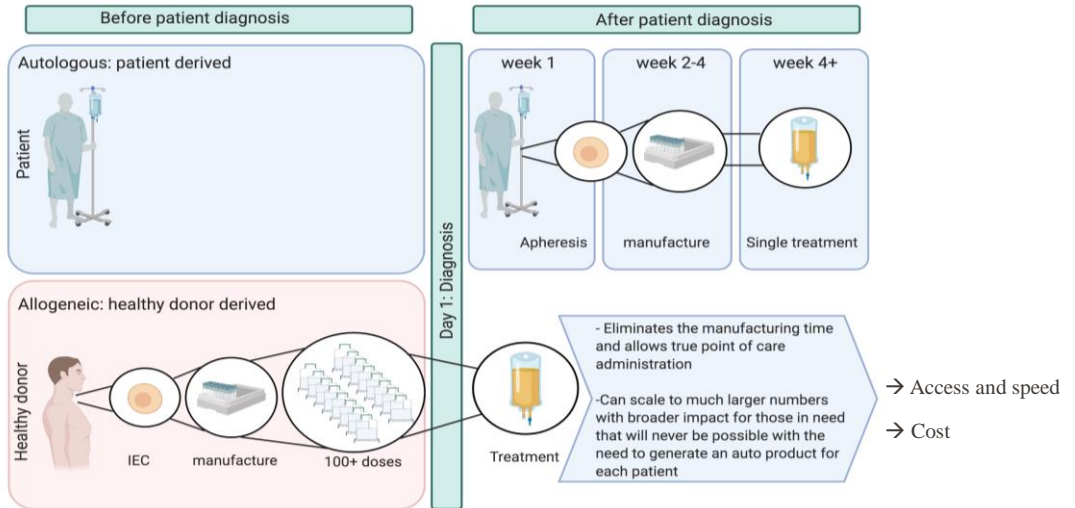
### Gene Transfer of CARs



## The history of CAR T-cell therapy

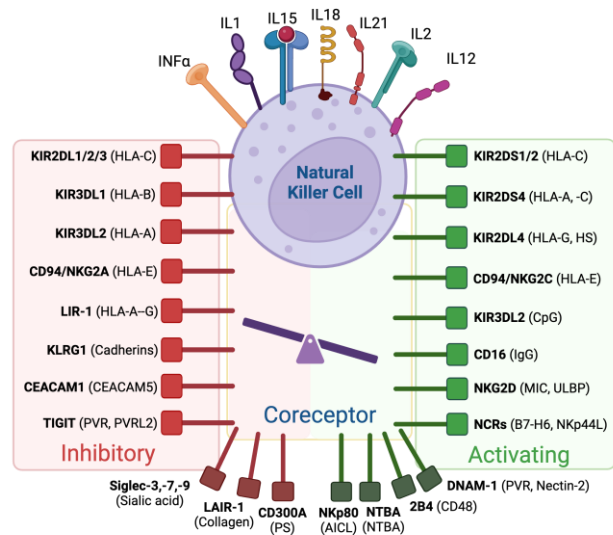


## Reduce the costs and eliminate the logistical hurdles of autologous Immune Effector Cells (IEC)

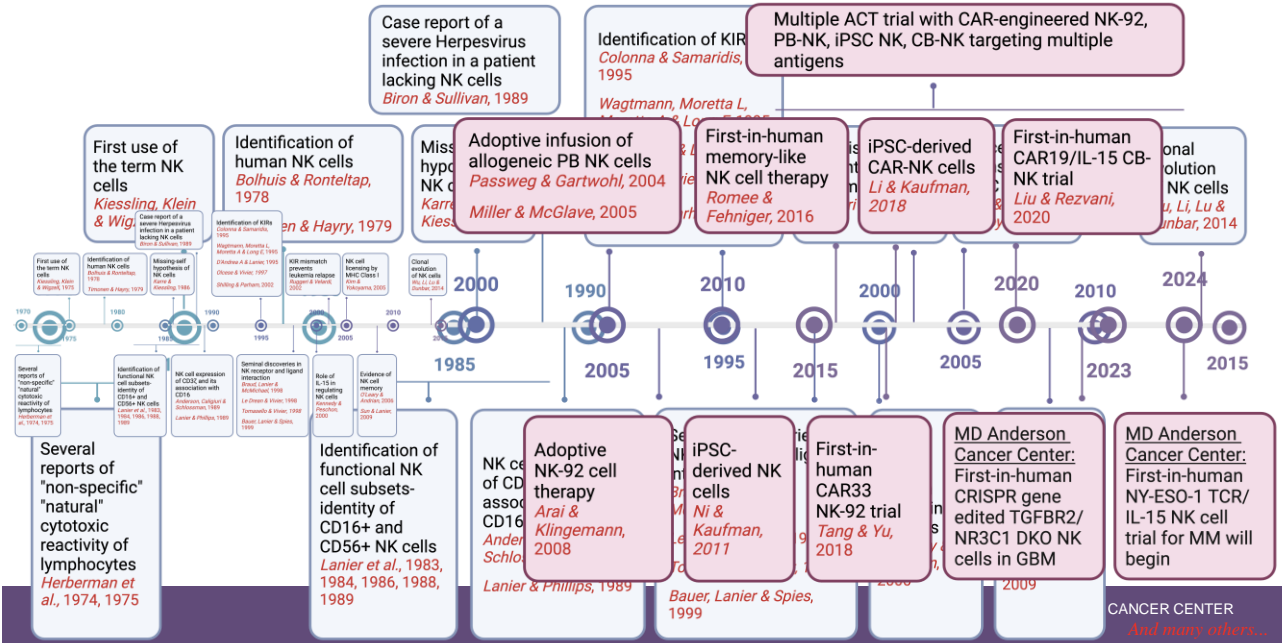


## What are Natural Killer (NK) cells?

- Innate immune system
- CD56+CD3-
- Differentiate in the BM
- No antigen priming
- Primarily in blood
- No/low risk of GVHD
- Recognition takes place through complex array of receptors
- Anti-metastatic activity



# NK cells: History

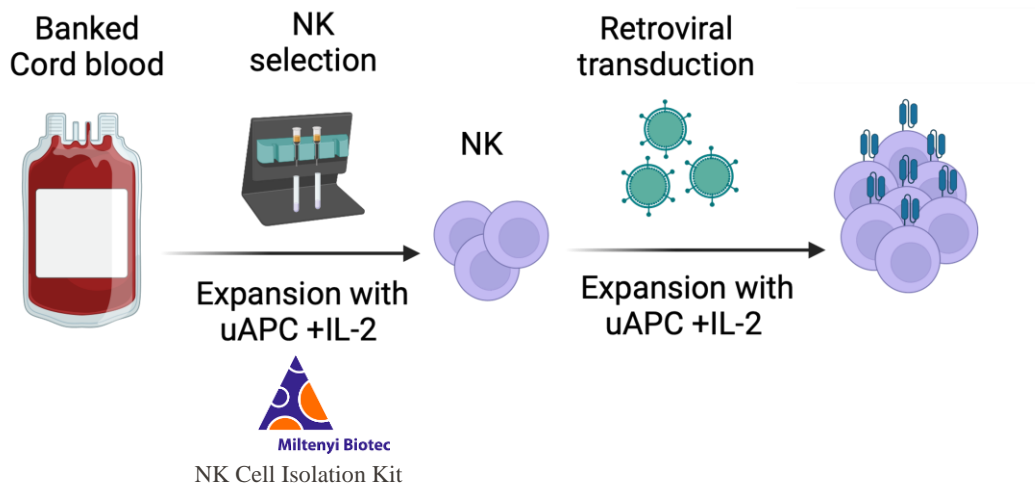




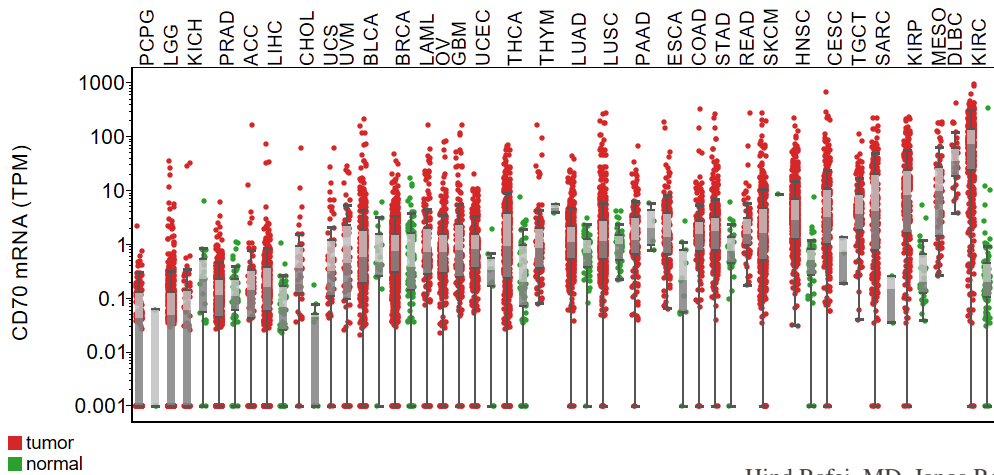
## Introduction

- CAR-NK cells: promising immunotherapy for cancer due to NK cells' **innate ability to kill tumors** and their **safety** in the allogeneic setting
- Build better CAR-NK therapies?
  - Suitable antigen (target)
  - Optimize the CAR construct for better efficacy
- CD70: ligand for CD27 receptor, is an attractive **"pan-cancer antigen"**
- CD70 is only transiently expressed on activated T and B lymphocytes and on DCs.

## Isolation of NK cells and generation of CAR NK cells

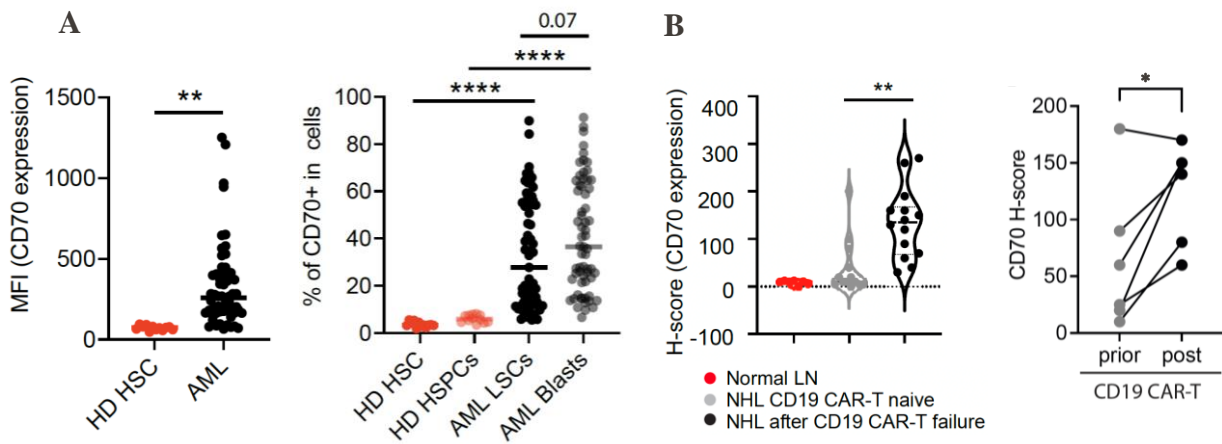


## CD70 expression at mRNA level is higher in various cancers compared to normal tissues

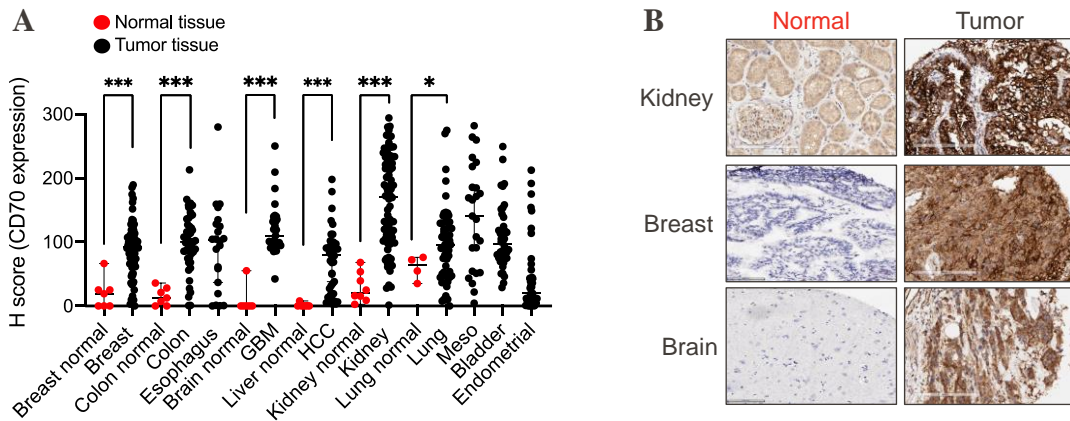


Hind Rafei, MD, Janos Roszik, PhD

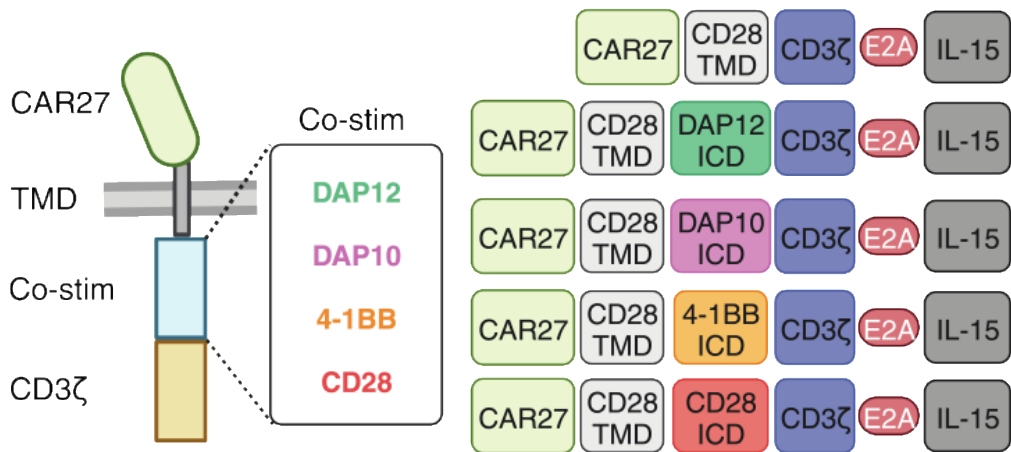
## CD70 expression is higher in AML leukemia stem cells and mature blast cells, as well as B-cell lymphoma following CD19 CAR T failure



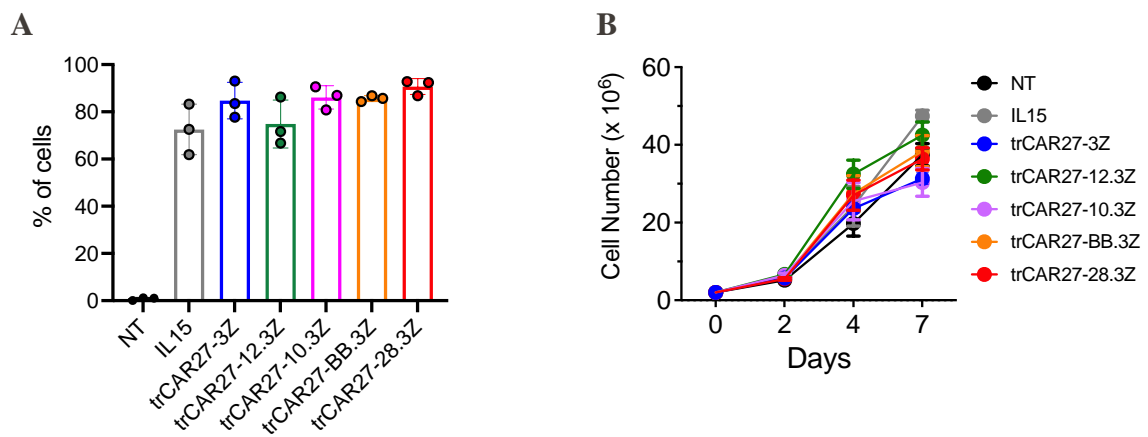
## CD70 expression is higher in various solid cancers compared to normal tissues



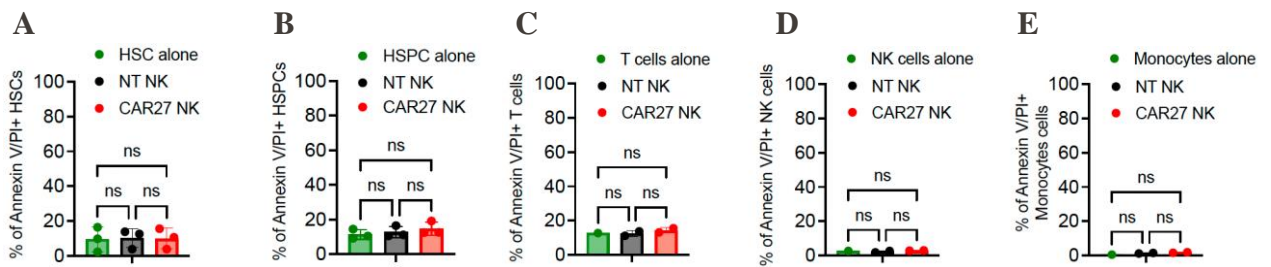
## Designed and tested multiple CD70-targeting CAR constructs based on human CD27 receptor sequence (CAR27 NK cells)



## CAR27 NK cells showed similar transduction efficiency and no significant difference in cell proliferation



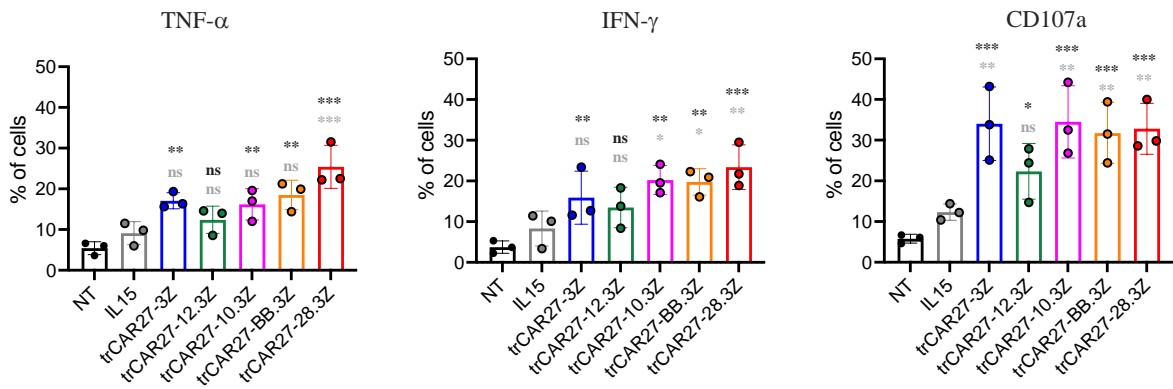
## CAR27 NK cells did not demonstrate toxicity against normal hematopoietic cells





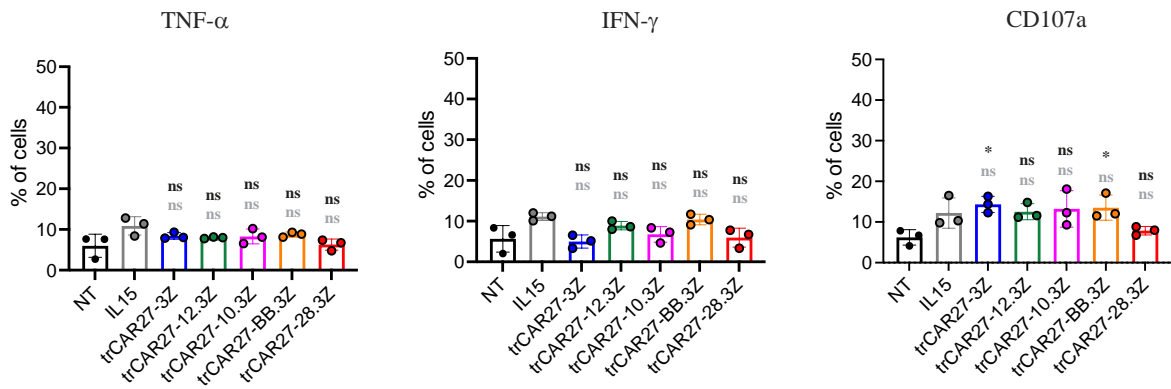
## CAR27 NK cells secreted higher levels of effector cytokines and degranulation marker compared to NT and IL-15 NK cells

### Raji Cells



## Specific interaction between the CD27 receptor and the CD70 antigen on target cells was required for CAR activation

Raji *CD70* KO Cells

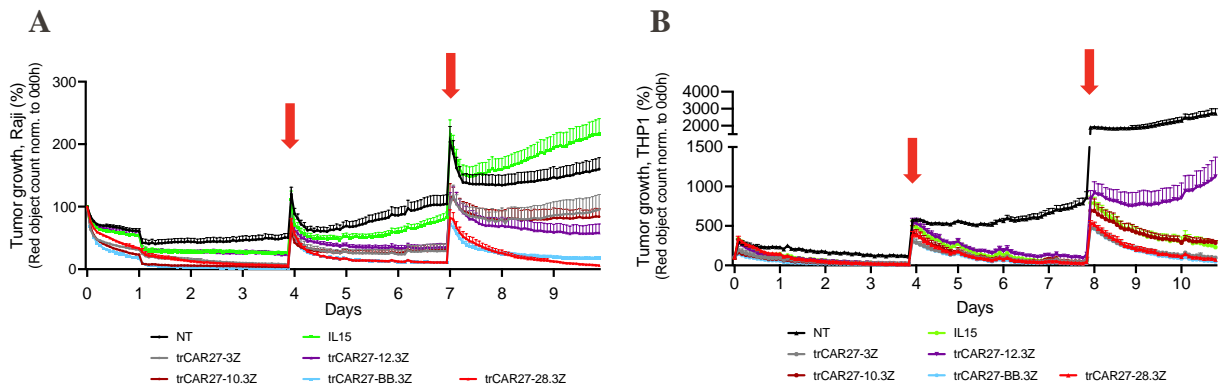


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Acharya et al., *Cancer Discov* (2024)

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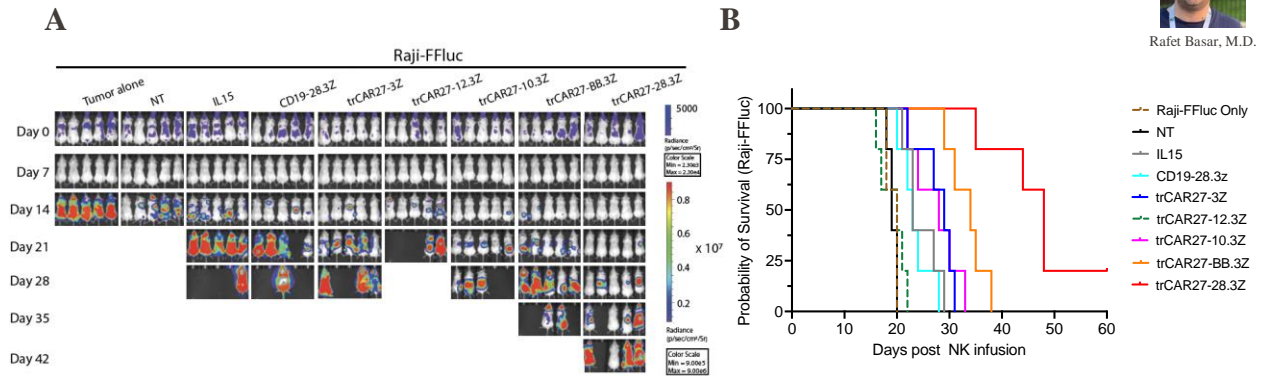
## CAR27 NK cells exerted long term cytotoxicity against Raji and THP-1 cells *in vitro*



# CAR27 NK cells with CD28 costim showed superior anti-tumor activity and survival in a Raji (Burkitt lymphoma) mouse model



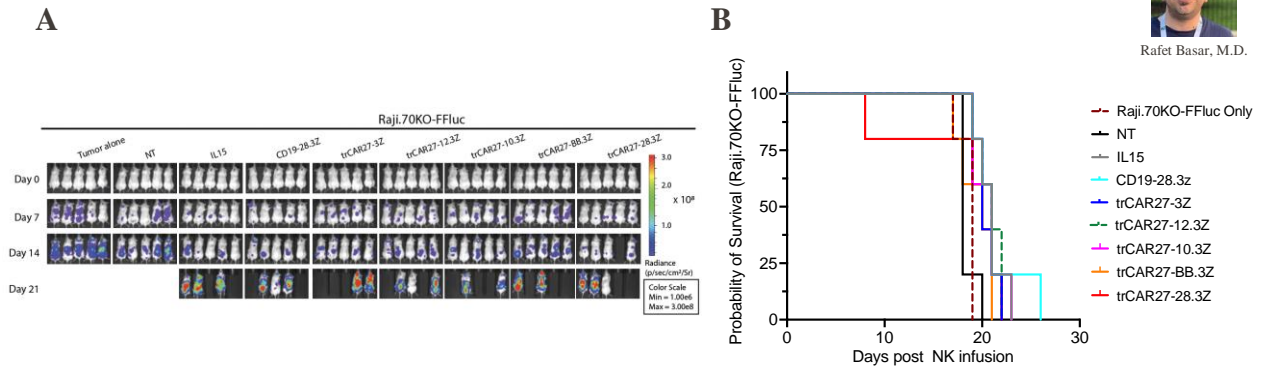
Rafet Basar, M.D.



## Superior anti-tumor activity and survival of CAR27 NK cells with CD28 costim was antigen dependent



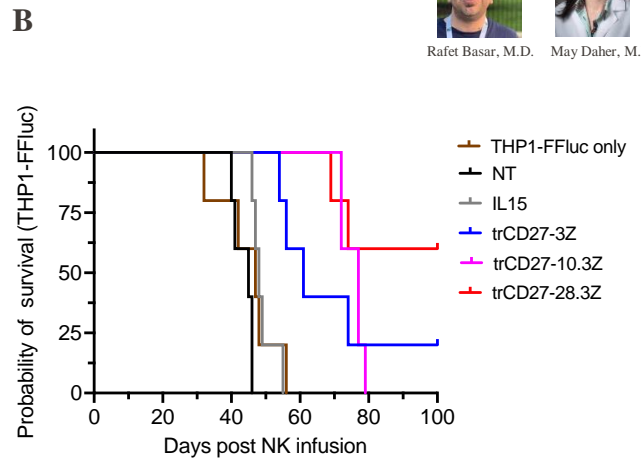
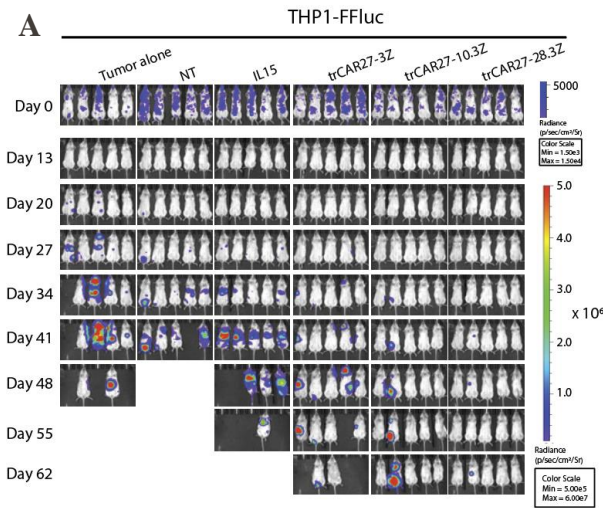
Rafet Basar, M.D.



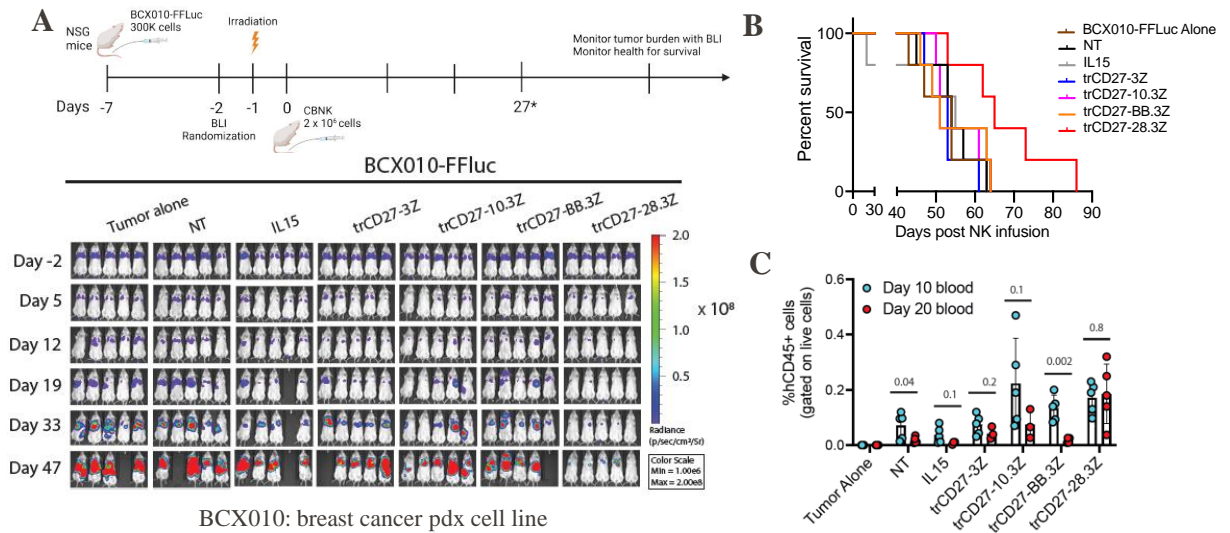
# CAR27 NK cells with CD28 costim showed superior anti-tumor activity and survival in an THP-1 (AML) mouse model



Rafet Basar, M.D. May Daher, M.D.



## CAR27 NK cells with CD28 costim showed superior anti-tumor activity and survival in a BCX010 mouse model

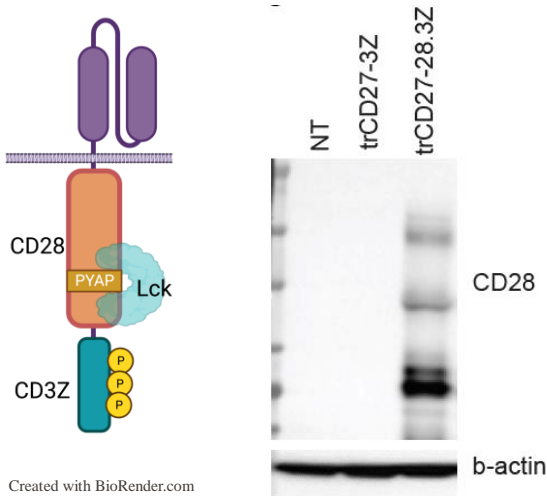


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Acharya et al., *Cancer Discov* (2024)

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## CD28 an important costimulatory molecule in T cells; however, CD28 is mostly absent in mature NK cells including CB NK cells



**Human Natural Killer Cell Committed Thymocytes and Their Relation to the T Cell Lineage**  
 By María José Sánchez, Hergen Spits, Lewis L. Lanier, and Joseph H. Phillips  
 From the DNAX Research Institute for Molecular and Cellular Biology, Department of Immunology, Palo Alto, California 94304

[Proc Natl Acad Sci U S A](#), 2006 Jul 5; 103(27): 10346–10351. PMID: PMC1502460  
 Published online 2006 Jun 26. doi: [10.1073/pnas.0604236103](#) PMID: 16801532

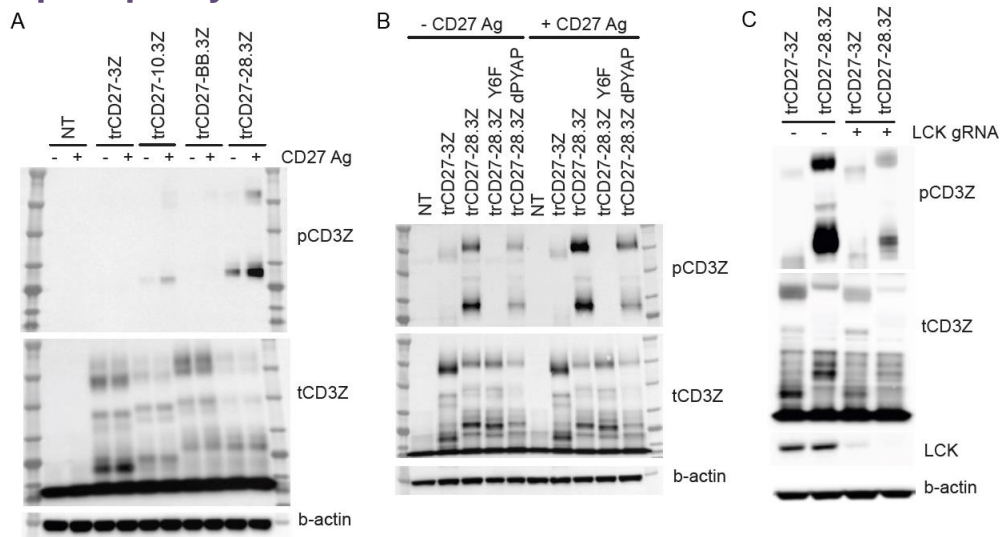
CD28-stimulated ERK2 phosphorylation is required for polarization of the microtubule organizing center and granules in YTS NK cells  
 Xi Chen, David S. J. Allan, Konrad Krzewski, Baoxue Ge, Hernan Kopcow, and Jack L. Strominger

[Cancer Immunol Res](#). Author manuscript; available in PMC 2019 Dec 1. PMID: PMC6548580  
 Published in final edited form as:  
[Cancer Immunol Res](#). 2019 Jun; 7(6): 939–951. NIHMSID: NIHMS1528043  
 Published online 2019 Apr 24. doi: [10.1158/2326-6066.CCR-18-0733](#) PMID: 31018857

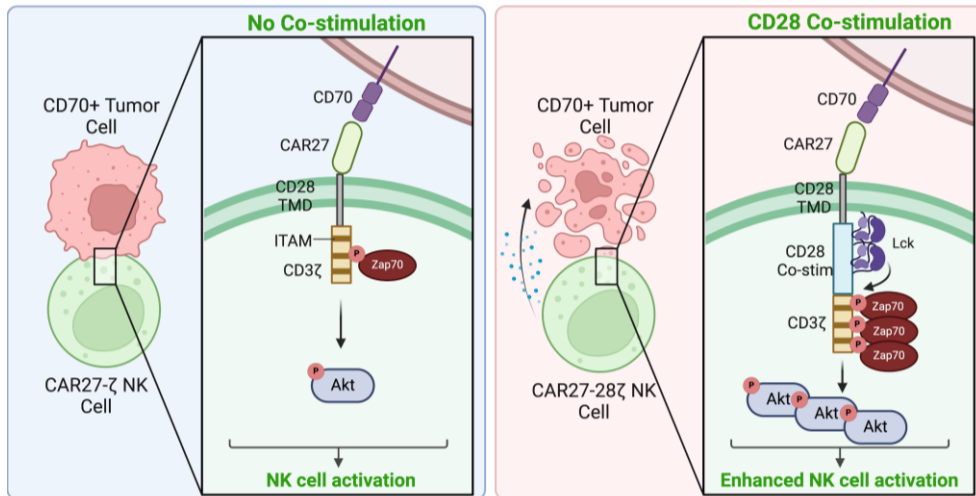
CD28 homolog is a strong activator of natural killer cells for lysis of B7H7<sup>+</sup> tumor cells  
 Xiaoxuan Zhuang<sup>1</sup> and Eric O Long<sup>1,2</sup>



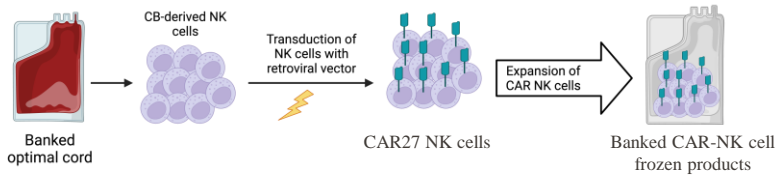
## LCK recruitment to CD28 endo-domain increased CD3 $\zeta$ phosphorylation in CAR27 NK cells with CD28 costim



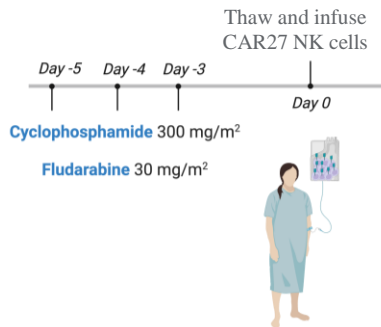
**LCK recruitment to CD28 endo-domain increased CD3 $\zeta$  phosphorylation and facilitates the interaction with ZAP70 and intensify subsequent downstream signaling cascade**



## Clinical translation: Phase I/II clinical trial evaluating the safety and efficacy of CAR27 NK cells in liquid and solid tumors



250 patient doses manufactured and frozen from two cord blood units  
 CAR transduction efficiency 58% and 77%



**Basket trial in hematologic malignancies approved by IRB and FDA (NCT05092451, IND 27757)- dose level 3 complete**

6 dose levels:

- Dose level -1: 4.0 E+6
- Dose level 1: 8.0 E+6
- Dose level 2: 4.0 E+7
- Dose level 3: 8.0 E+7
- Dose level 4: 4.0 E+8
- Dose level 5: 8.0 E+8
- Dose level 6: 4.0 E+9



David Marin, MD  
 Stem Cell Transplant and Cellular Therapy



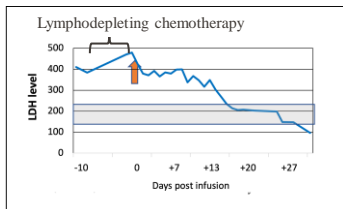
David Hong, MD  
 Department of Investigational Cancer Therapeutics

**Clinical protocol in renal cell carcinoma, mesothelioma, and osteosarcoma approved by IRB and FDA (NCT05703854, IND 29057)**

## Patient response to truly off-the-shelf HLA-mismatched, cryopreserved CAR70/IL-15 NK cells-patient with classical HL

24 yr old male  
 Diagnosed with Stage IV classical HL – widespread LN and bones

- ABVD x 6 → CR
- 3 months later - relapsed disease
- GDP x 2 → CR → ASCT
- 1 month post ASCT- relapsed HL
- Brentuximab + Nivo → CR
- 2 months later- Haplo-SCT (Flu/Cy/TBI)- complicated by cGVHD eyes and mouth
- 10 months later- relapsed
- Camidanlumab (anti-CD25 ADC) – NR
- RT left flank/kidney
- CAR-NK cell infusion (**Flat dose: 8M NK cells**)



### Patient with classical HL

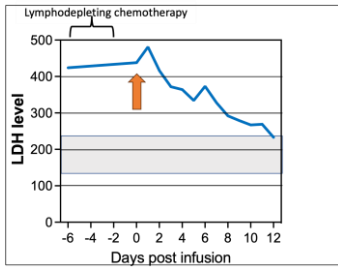


Currently at dose level 4  
 Responses observed in 8/10 patients (7 of 8 with HL)

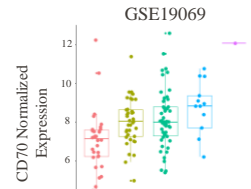
## Patient response to truly off-the-shelf HLA-mismatched, cryopreserved CAR70/IL-15 NK cells-patient with refractory CTCL

Patient with refractory cutaneous T-cell lymphoma (CTCL)

- S/p EPOCH, BV-CHP, Romidepsin, ICE, pralatrexate, Duvelisib, GDP, mogamulizumab
- **CAR-NK cell infusion (First patient treated at dose level 4: 4E8)**
- No toxicity



- Anaplastic Large Cell Lymphoma
- Angioimmunoblastic T-cell lymphoma
- Peripheral T-cell lymphoma, unspecified
- Adult T-cell leukemia/lymphoma
- T-cell line, Sezary Syndrome



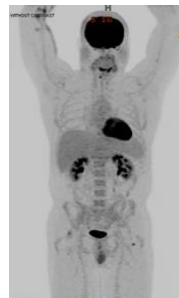
Hind Rafei, M.D., MS

Pre-infusion

D30 post-infusion

Pre-infusion

Post-infusion



Photographs obtained with patient consent

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Confidential, unpublished data

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## Conclusions

- CD70 is an attractive target for the application of CAR NK cellular therapy against hematologic malignancies and solid cancers
- Generated multiple CAR constructs against CD70 and performed various *in vitro* and *in vivo* screening assays
- Compared to NT NK cells, all CD70 CAR NK cells showed enhanced cytotoxicity against CD70 positive cancer cells, both *in vitro* and *in vivo*. However, a trCD27 CAR construct with CD28 co-stimulatory domain showed superior anti-tumor activity and survival in various mouse models
- LCK recruitment to CD28 endo-domain increased CAR specific CD3 $\zeta$  phosphorylation in CAR NK cells
- Initiated a phase I clinical trial evaluating the safety and efficacy of cytokine induced CAR27 NK cells for hematologic malignancies ([NCT05092451](#)) as well as solid cancers ([NCT05703854](#)).

# Team Effort

Research lab team

GMP team



Katy Rezvani, MD, PhD



MD Anderson Moon Shots Program



Patients and their families

**Thank you for your attention!**

**Any Questions?**