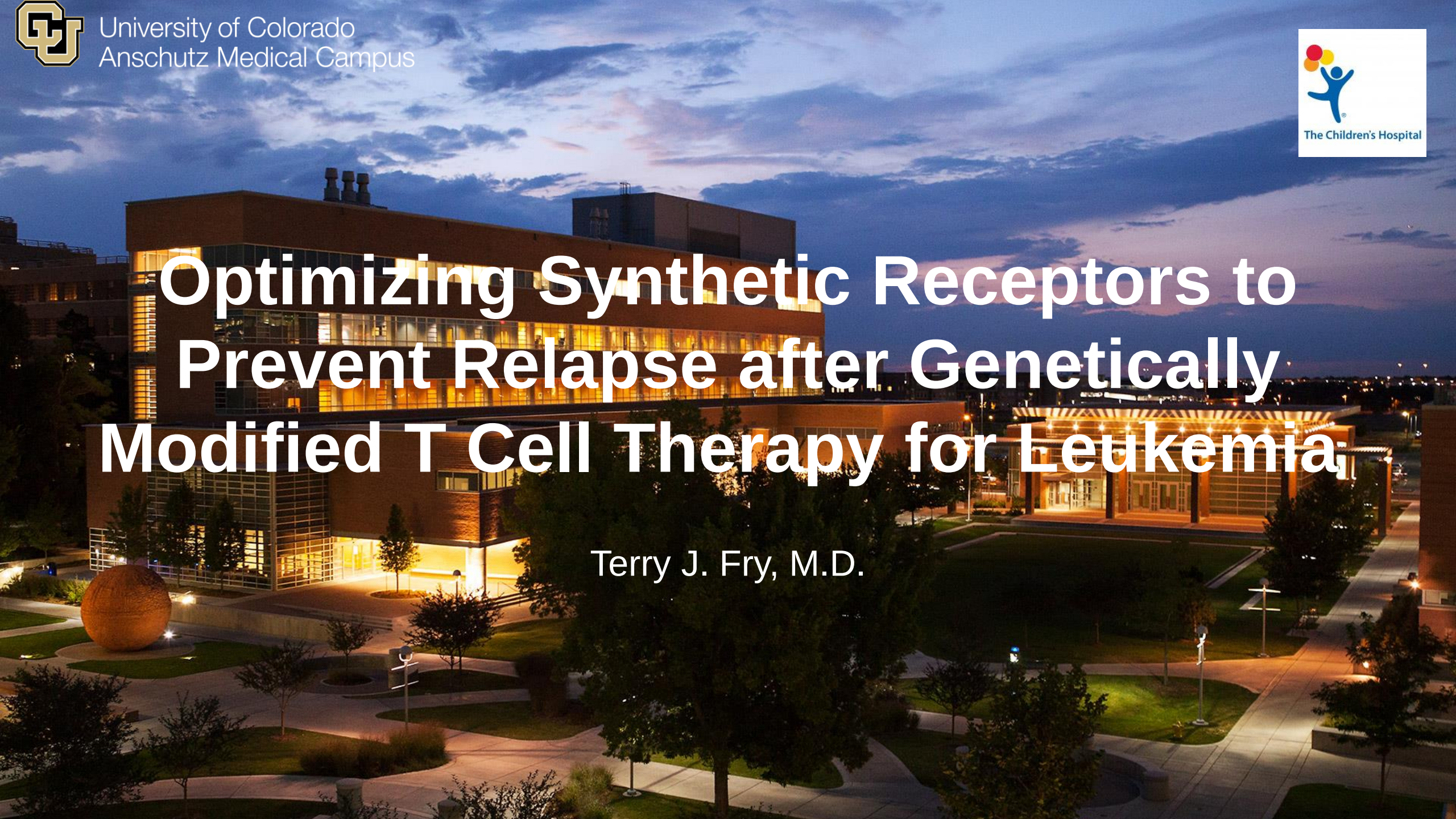



# Optimizing Synthetic Receptors to Prevent Relapse after Genetically Modified T Cell Therapy for Leukemia

Terry J. Fry, M.D.

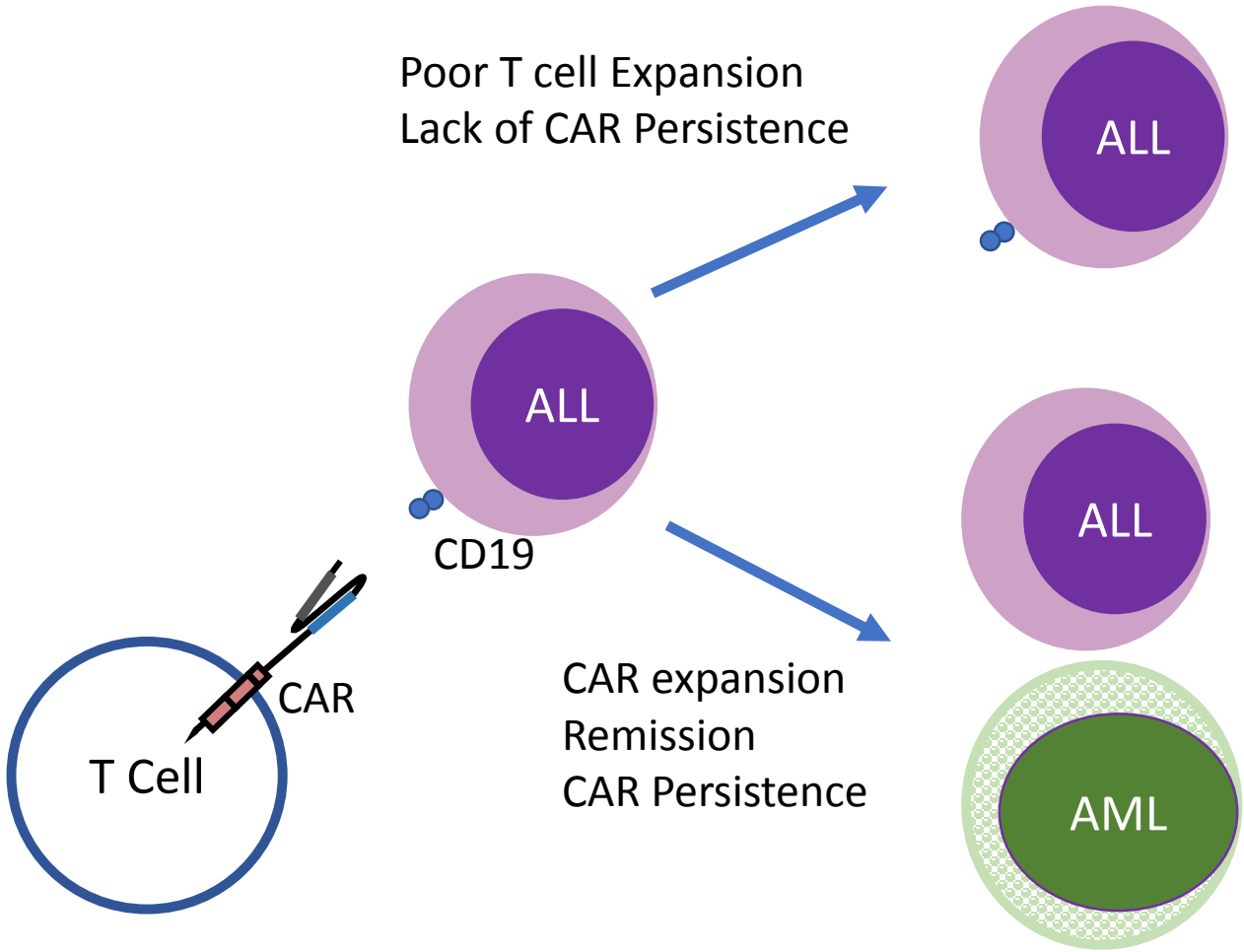
Society for Immunotherapy of Cancer

# Disclosure Information


**Adoptive Cellular Therapies Workshop**
**Terry J. Fry, MD**
**University of Colorado**


**I have no financial interests to disclose.**
**I will not off label use and/or investigational use in my presentation.**

# Patterns of Relapse Following CAR T cells

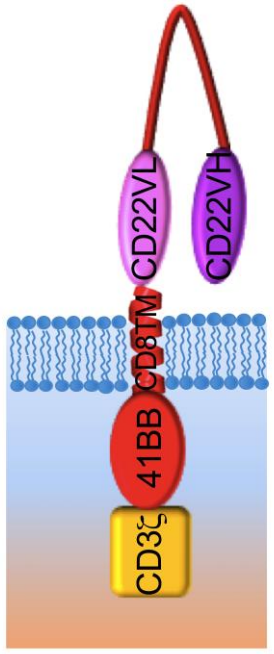


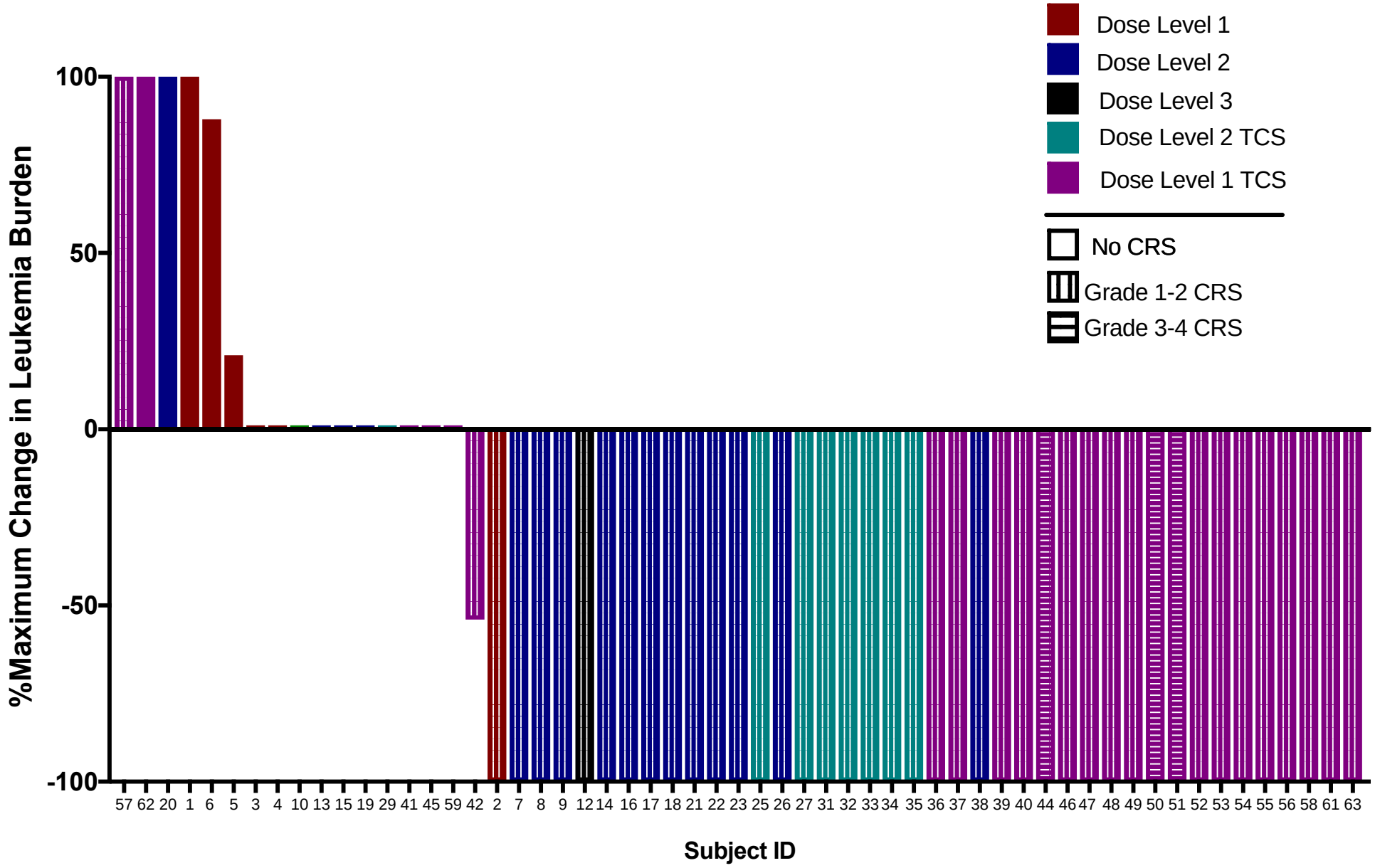

Poor T cell Quality
Suboptimal CAR T cell Biology

Leukemic Resistance

Fry and Shah,
Nature Reviews Clinical Oncology, 2019

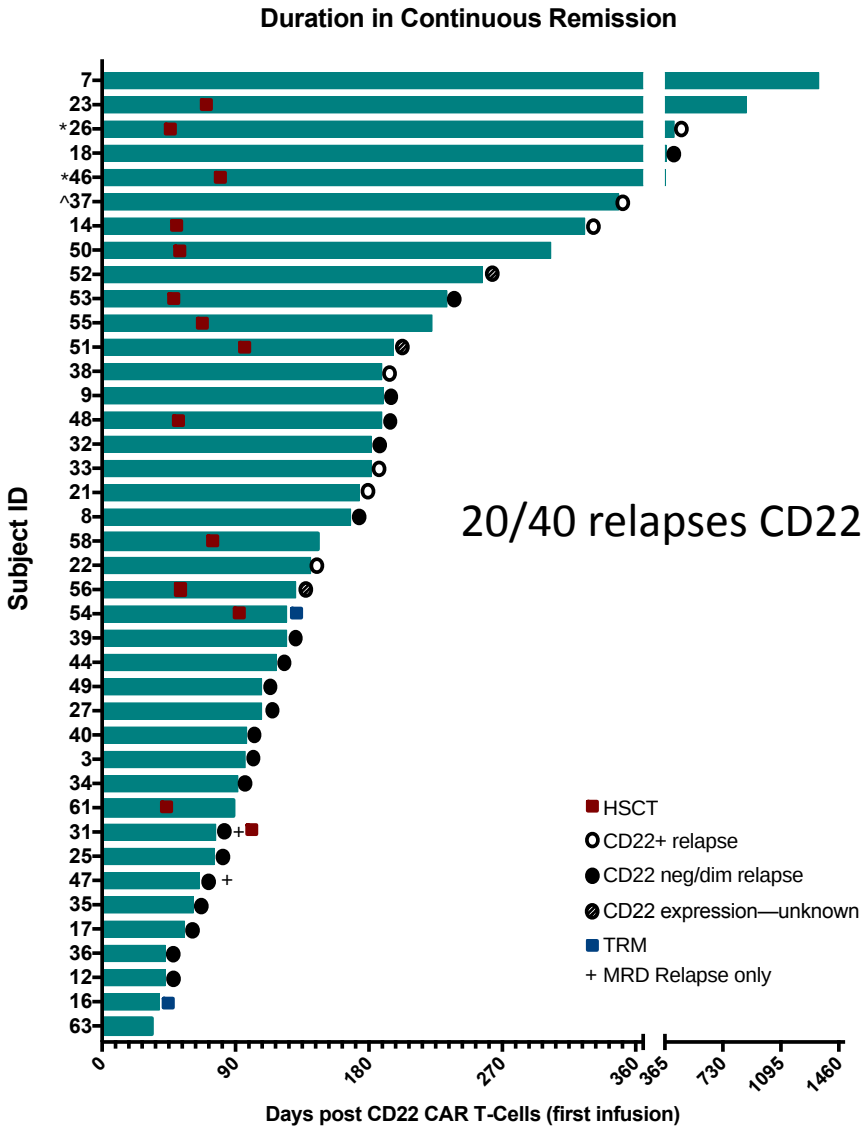
# CD22 targeted CART achieves MRD Negative Remission in Relapsed/Refractory ALL



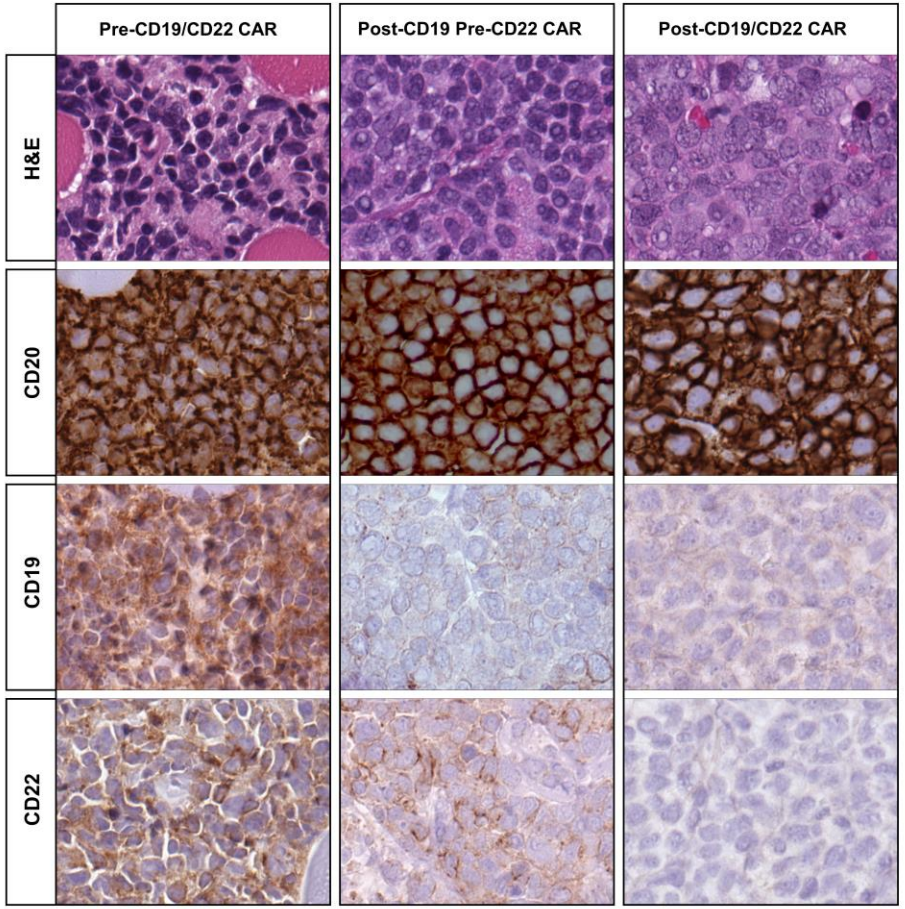




Haso…Orentas, *Blood* 2013
Haso….Fry, *ASH* 2013
Fry/Shah et al., *Nature Medicine 2017*

# CD22 BBz CAR:
# Relapse associated with CD22 modulation



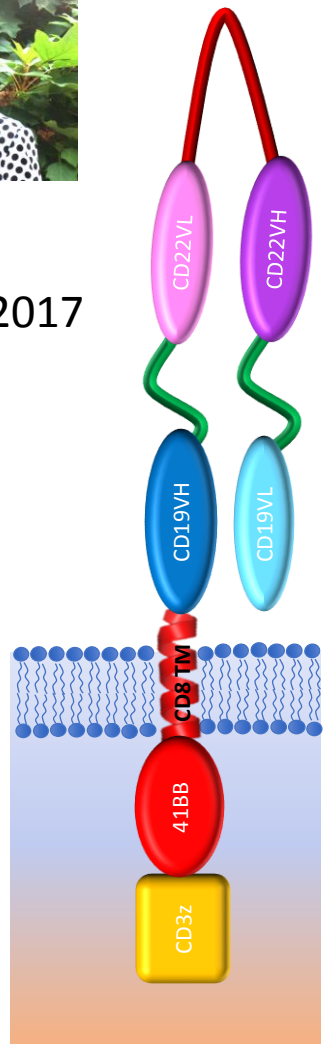

20/40 relapses CD22 dim/neg

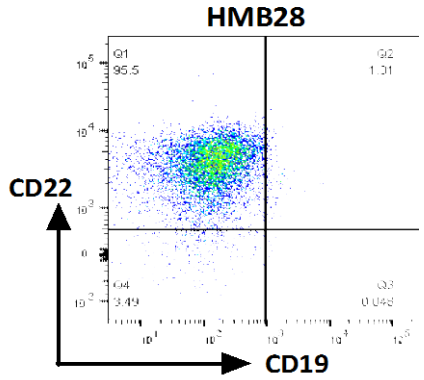


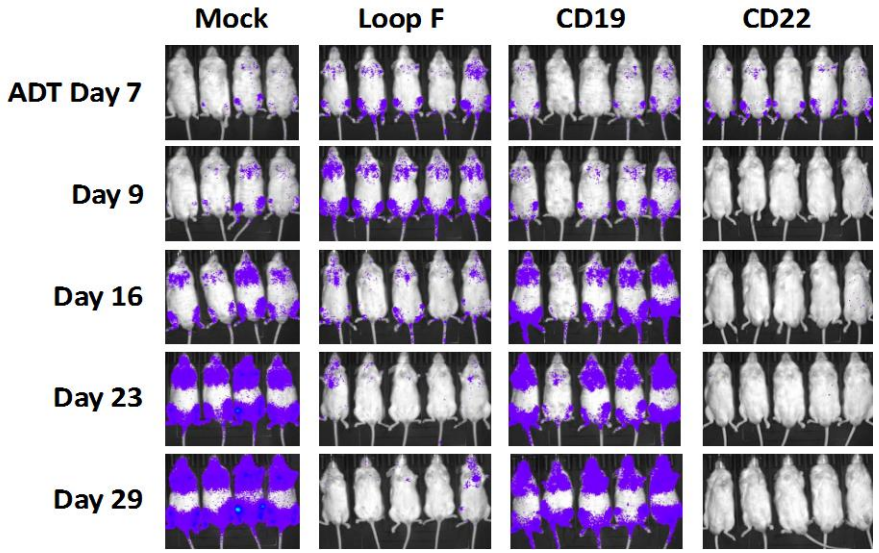

Shalabi…..Fry, Shah, Hematologica, 2017

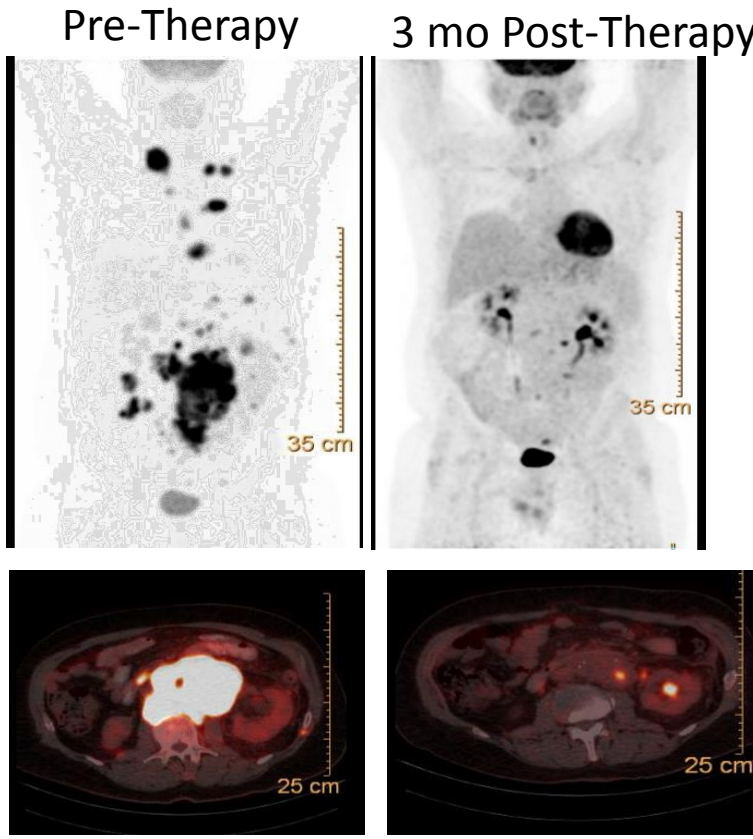
# Development of an Active CD19/CD22 Bivalent CAR

Nature Medicine, 2017
Molecular Therapy Oncolytics, 2018



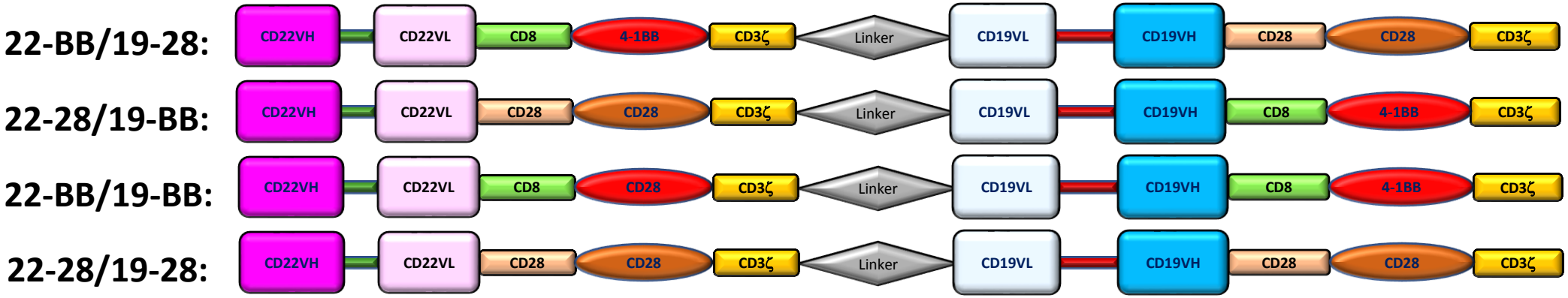



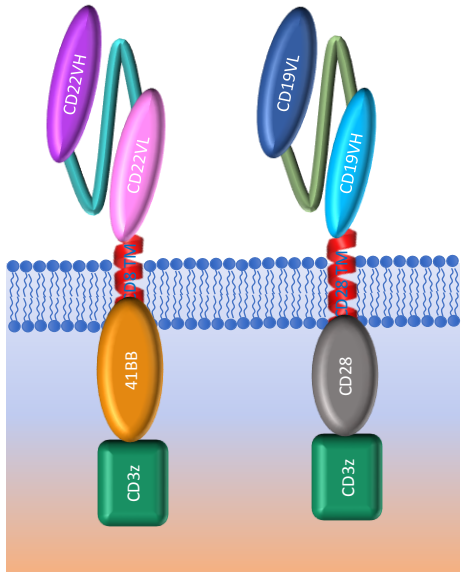



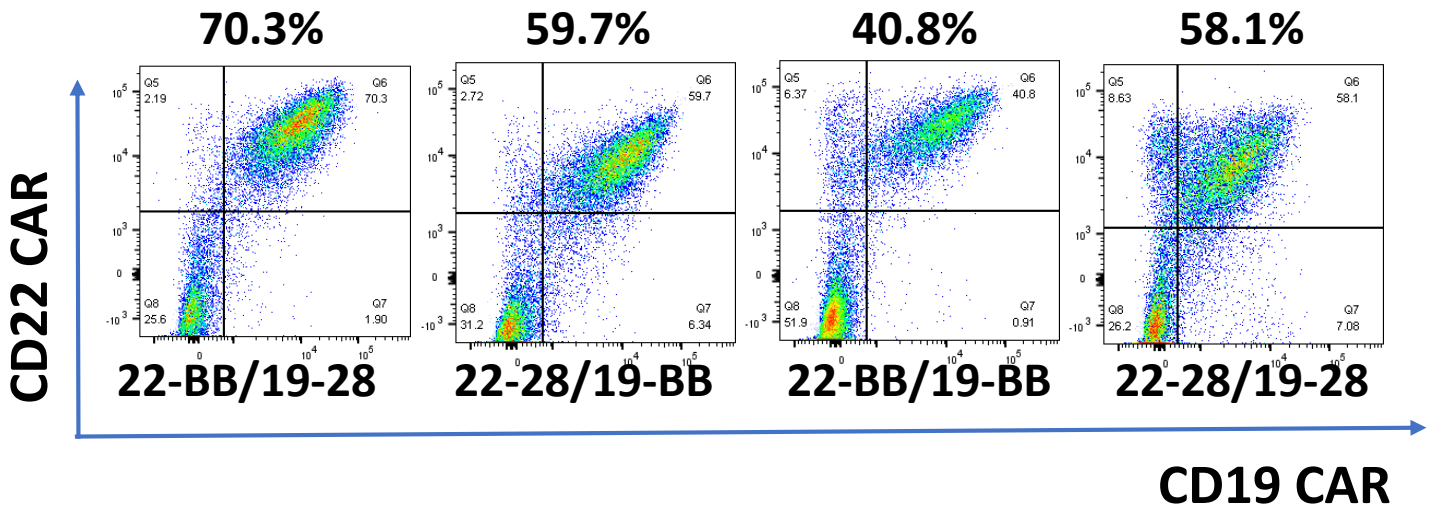

Hossain et al, ASH 2018, Abstract 490.
Schultz et al, ASH 2018, Abstract 898.

# Achieving Uniform Expression of Two CARS using Bicistronic Vectors

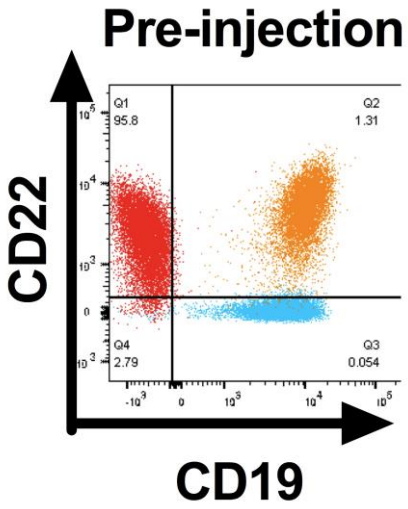



Translation

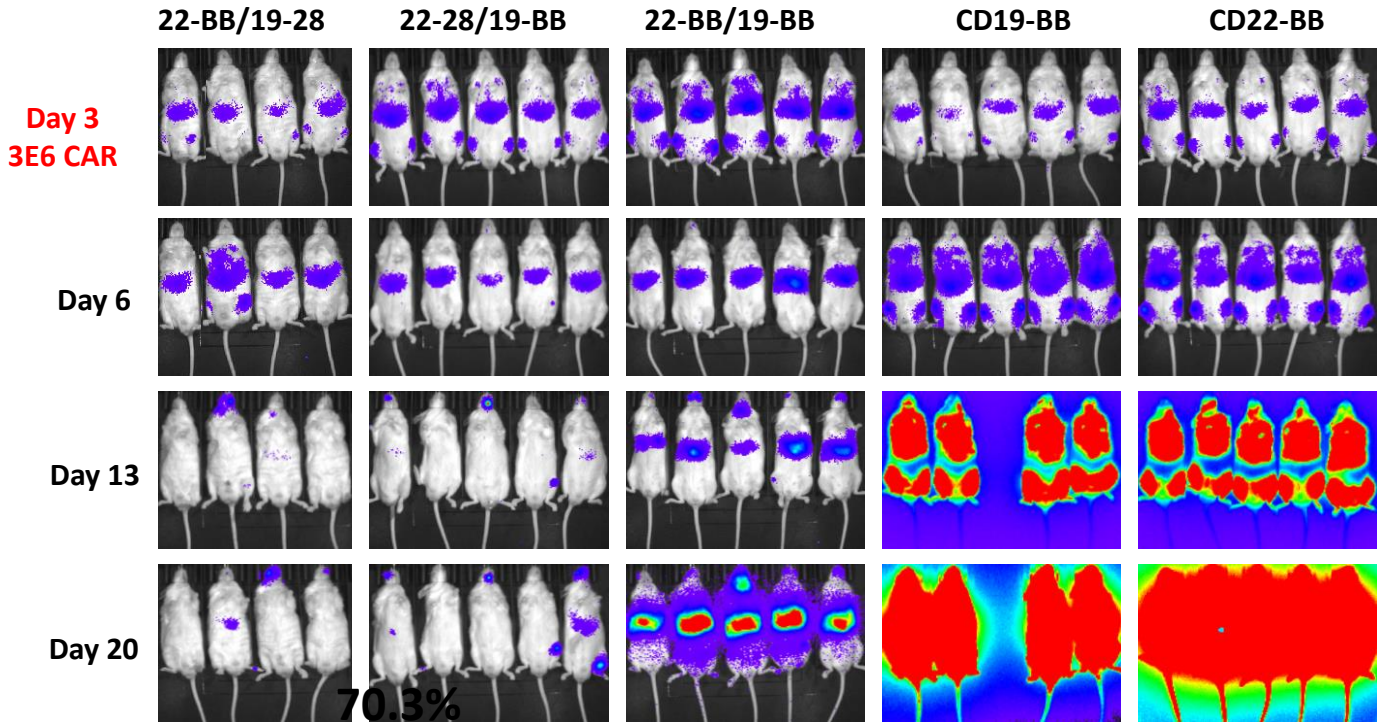


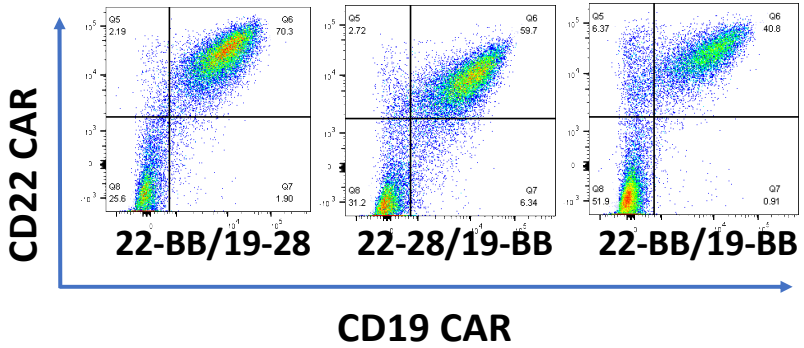



# Dual CAR expressing T cells mediate potent single antigen activity

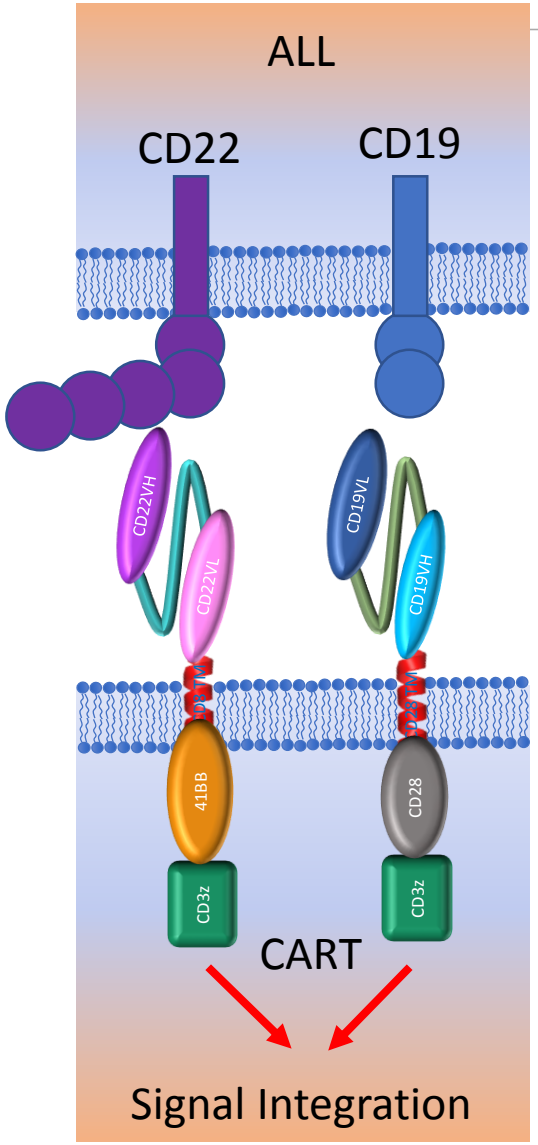


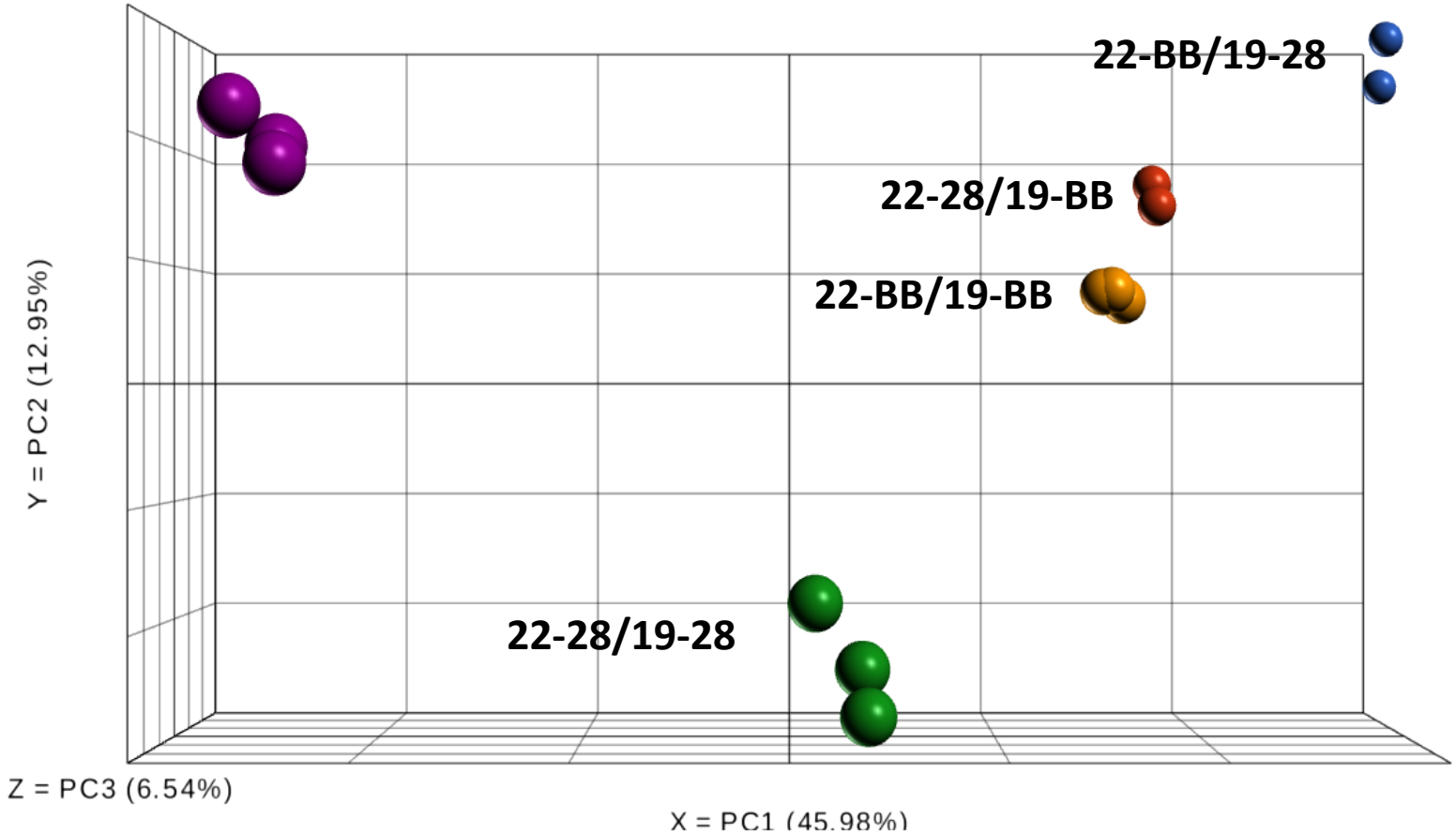

D0: 1E5 NALM6-CD19neg + 1E5 NALM6-CD22neg + 1E5 NALM6



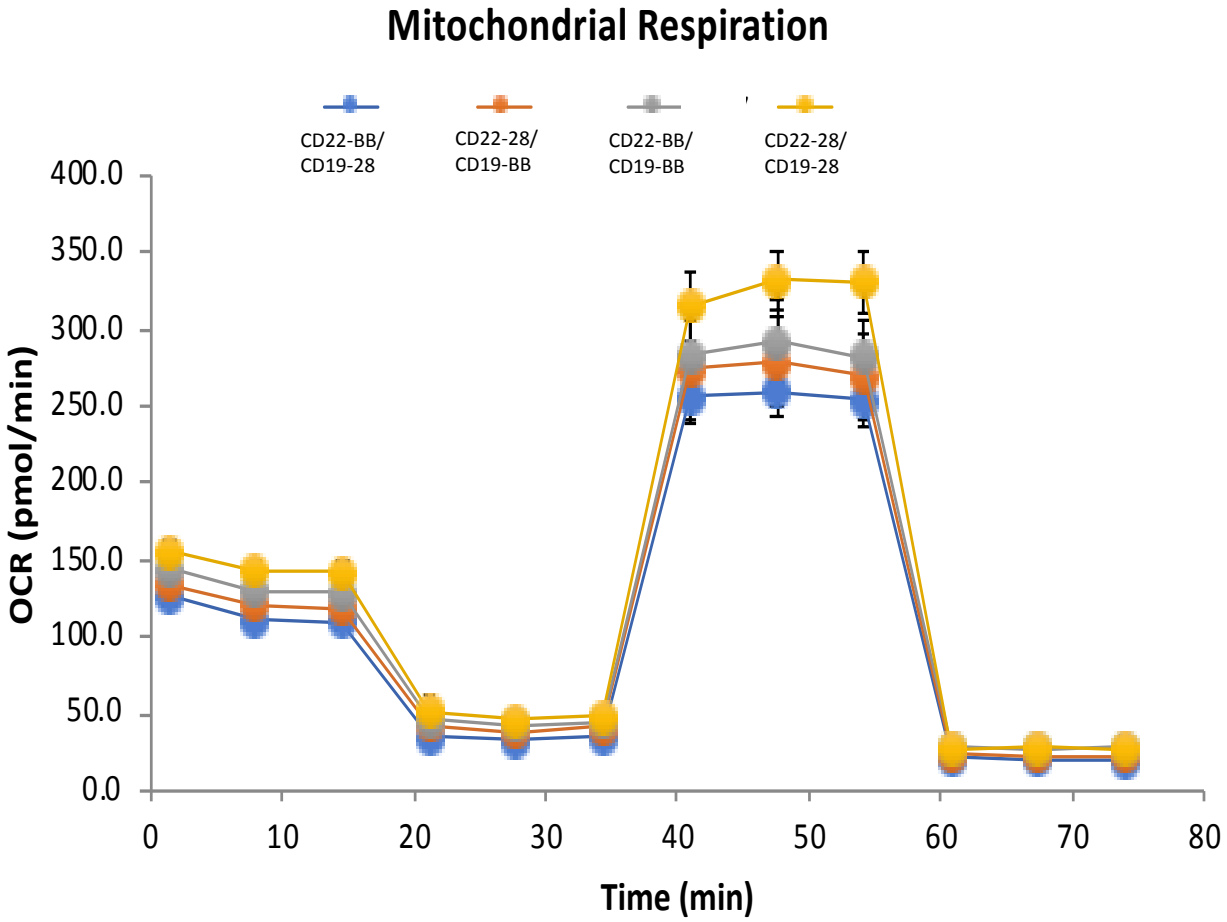



# Signal integration in Dual CAR Expressing T cells results in distinct profiles depending on binder/costim combinations

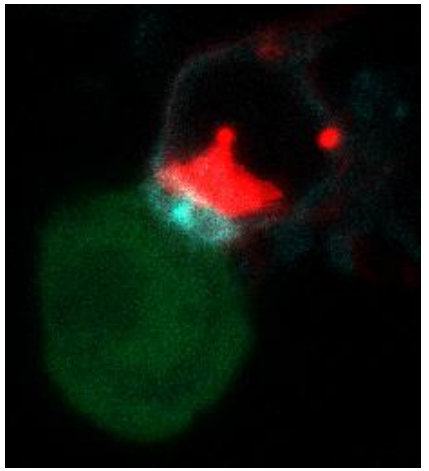


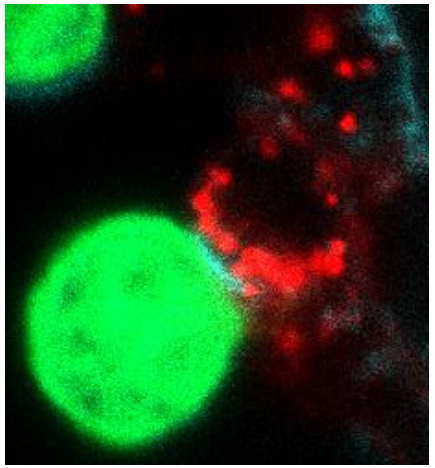
# Signal integration in Dual CAR Expressing T cells results in distinct profiles depending on binder/costim combinations




**22-BB/19-28**



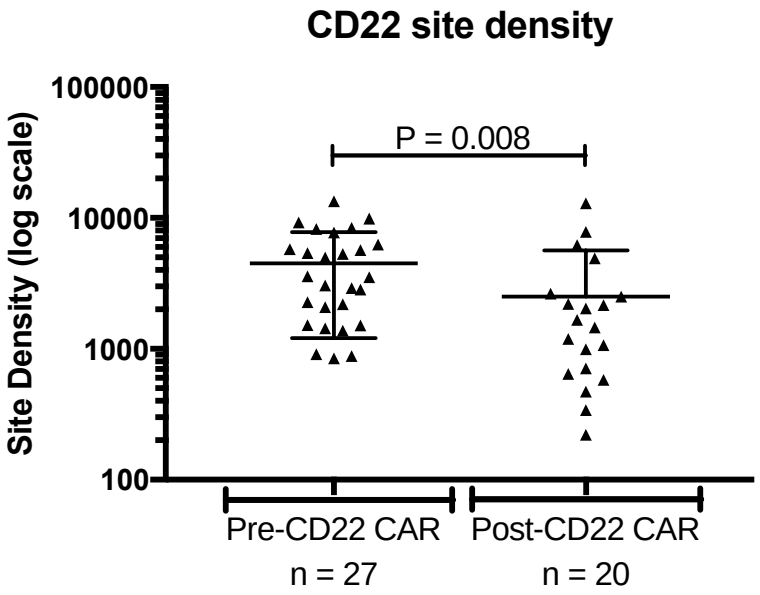
**22-28/19-BB**

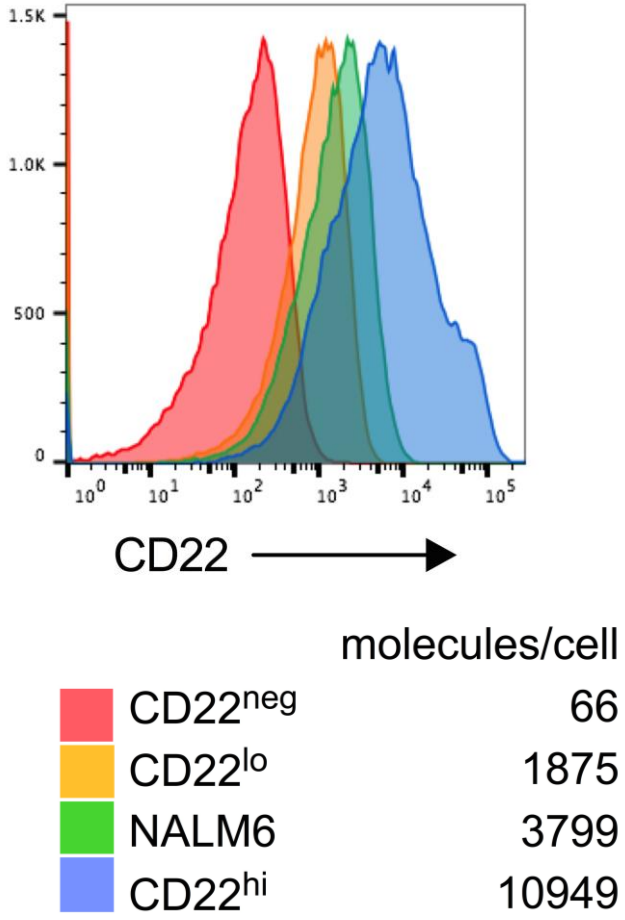


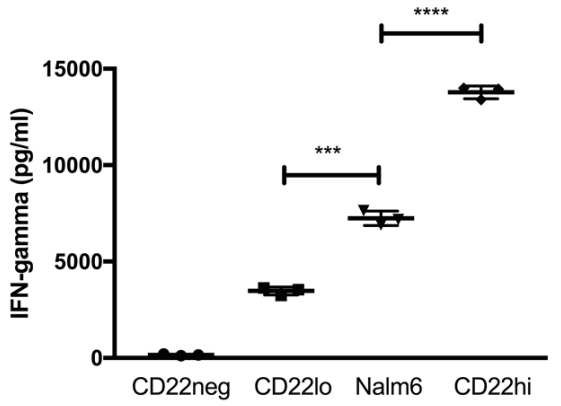
GFP: Nalm6
Red: CD22 CAR
Blue: CD19 CAR

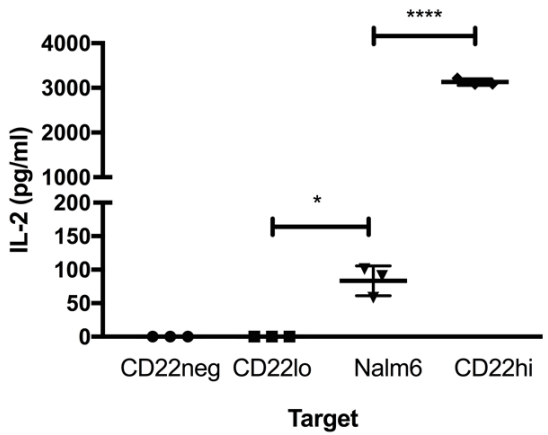
# Target antigen Density Impacts CAR T cell Functionality

Sneha Ramakrishna



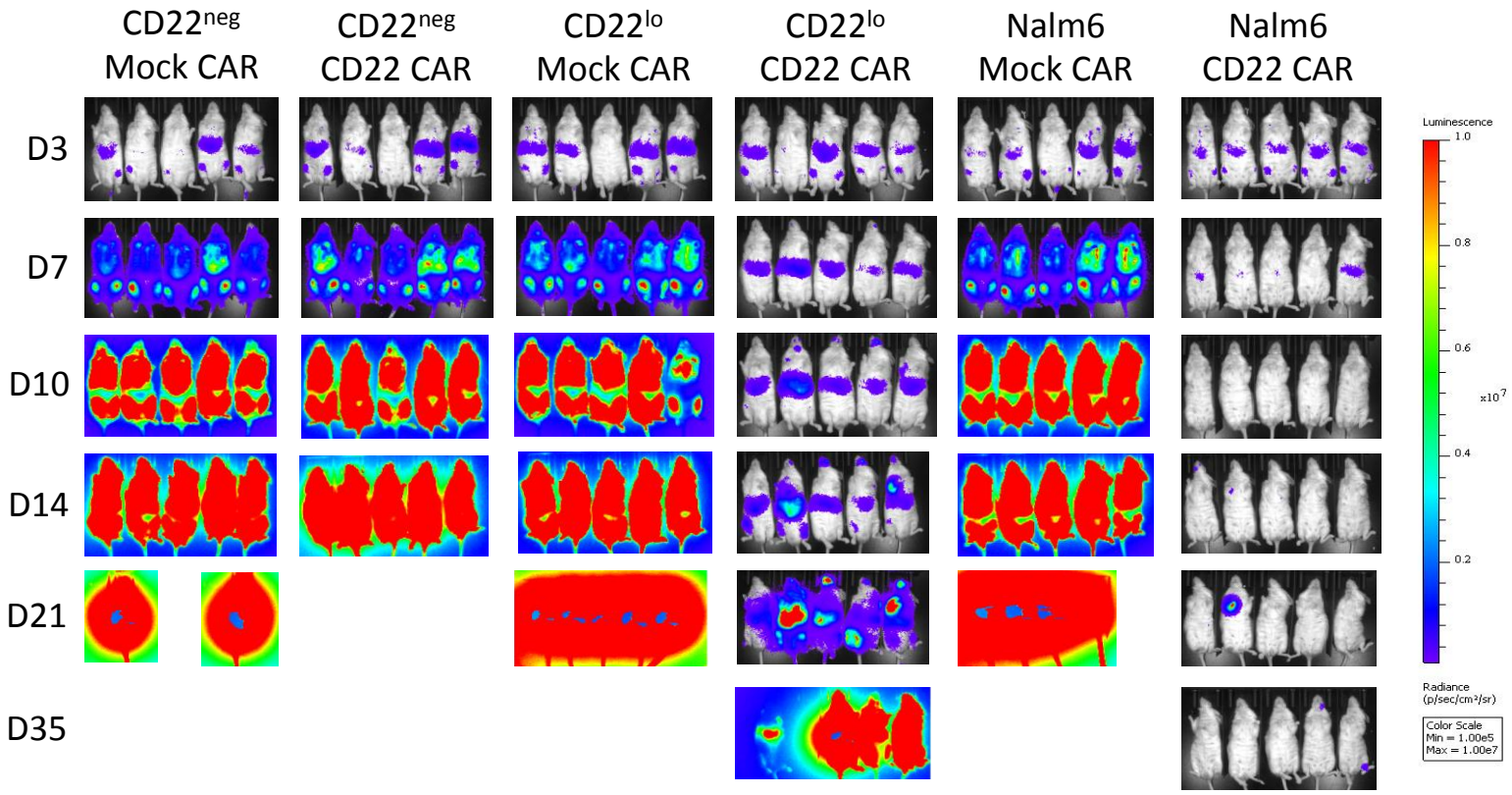



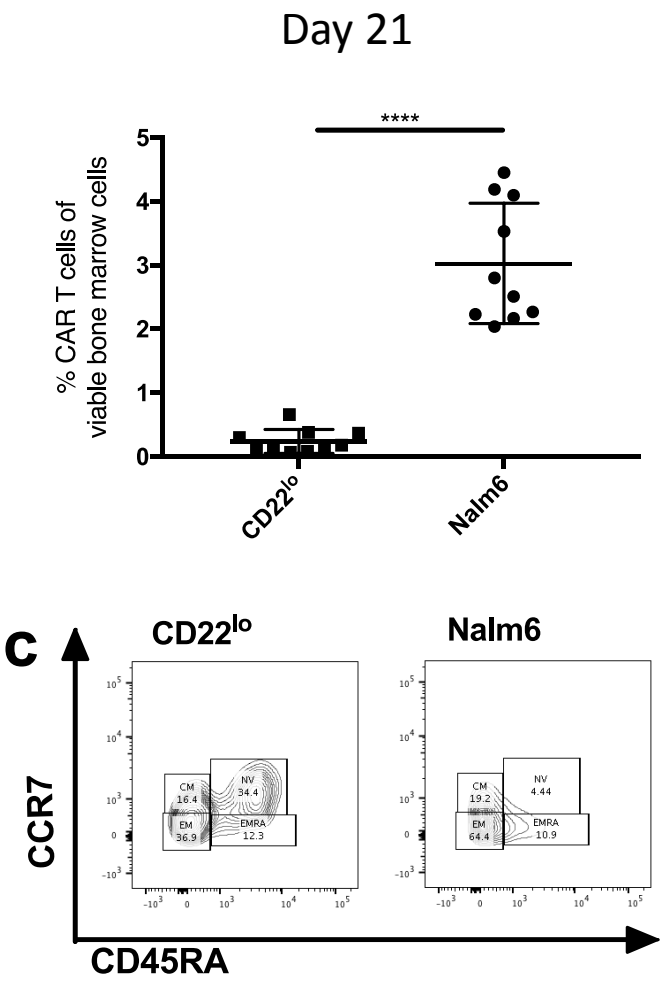




Ramakrishna et al, Clinical Cancer Research, 2019

# Target antigen Density Negatively Impacts *in vivo* CAR Potency and Persistence




Ramakrishna et al, Clinical Cancer Research, 2019

# Antigen density impacts signaling

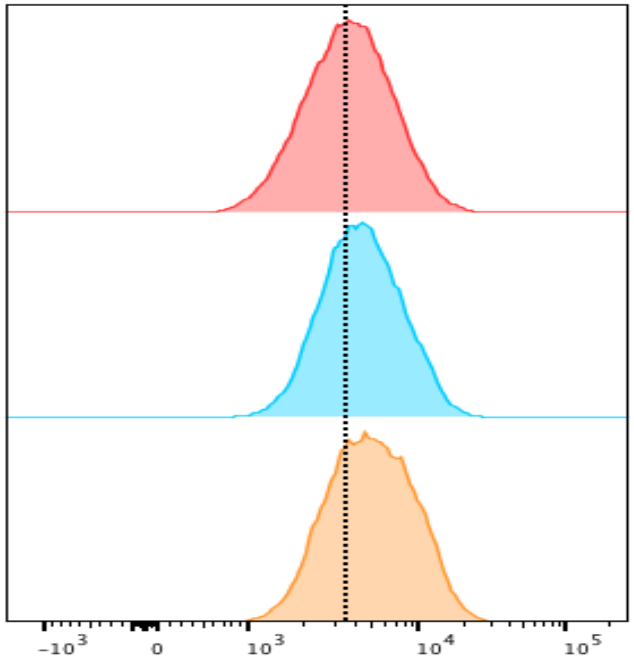
Eric Kohler and Zach Walsh

Stimulation

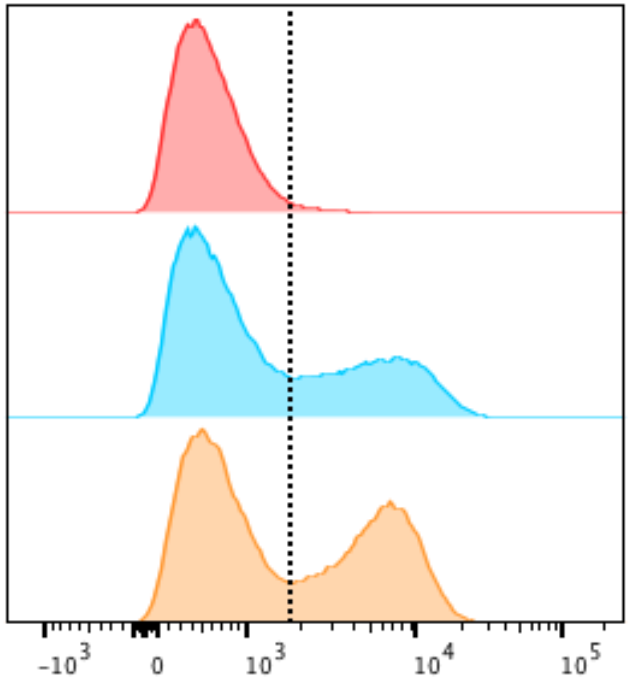
22neg

22lo

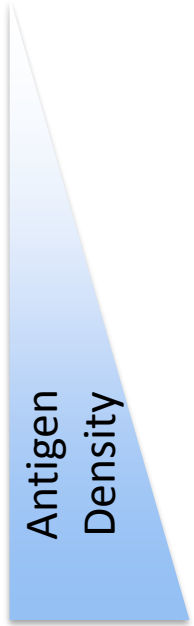
Nalm6




p-P38

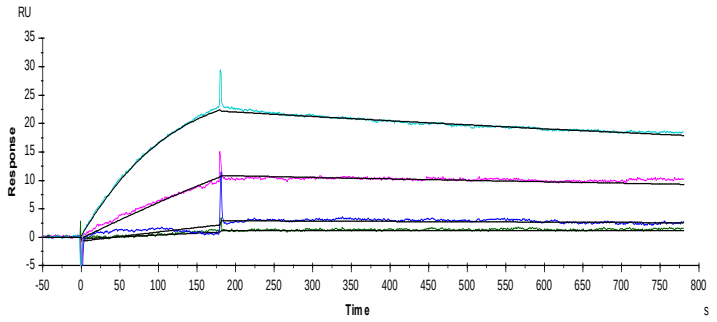



p-ERK



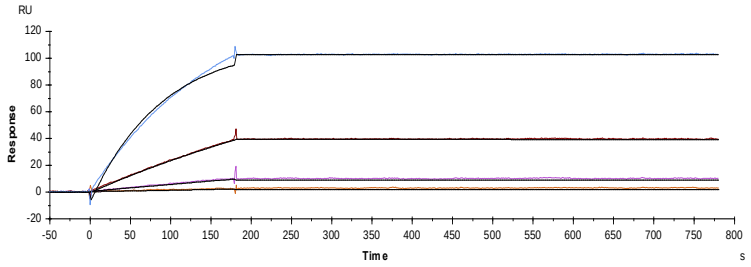
# Increased binder affinity impacts *in vitro* cytokine production

CD22 CAR

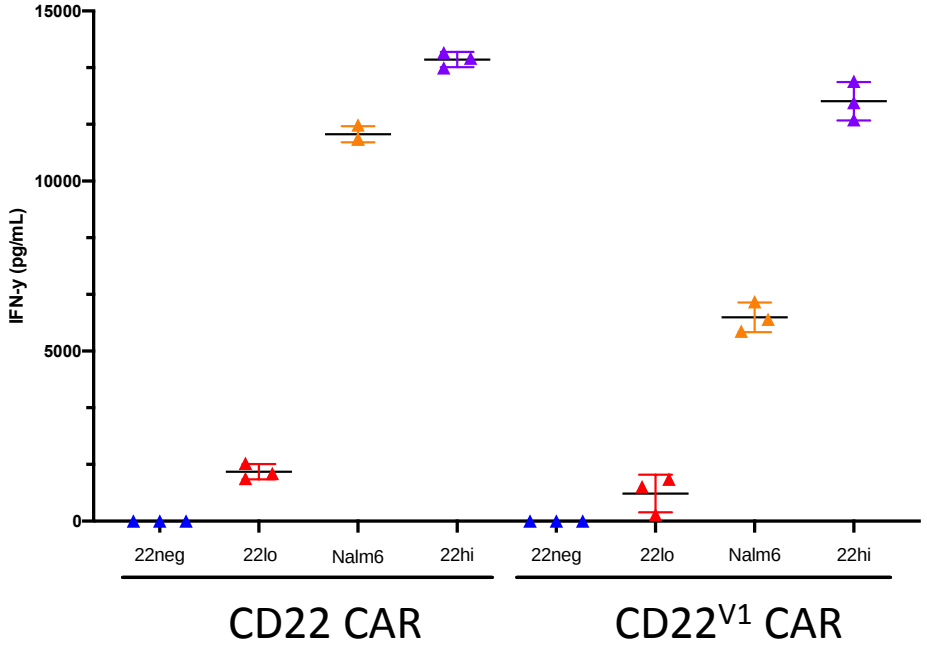


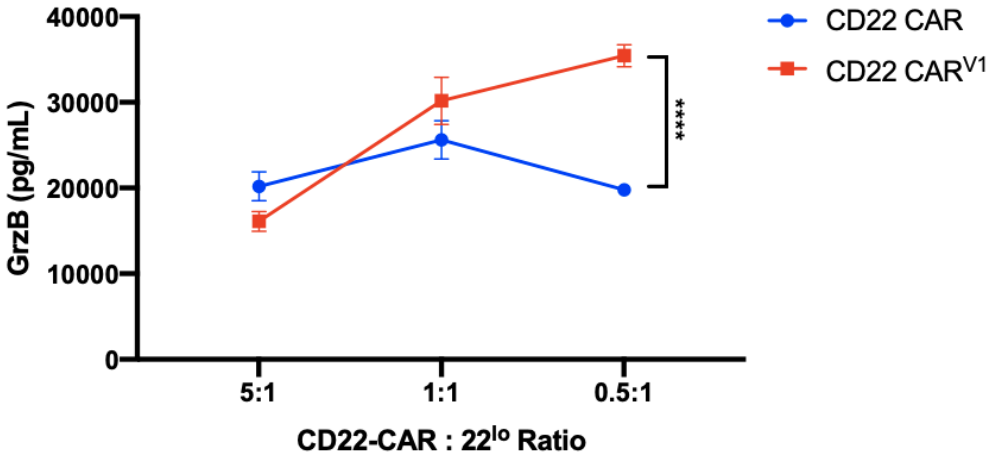

ka1=1.32x10$^{5}$ (1/Ms)
kd1=4.2x10$^{-4}$ (1/s)
KD=3.1x10$^{-9}$ M

CD22$^{V1}$ CAR



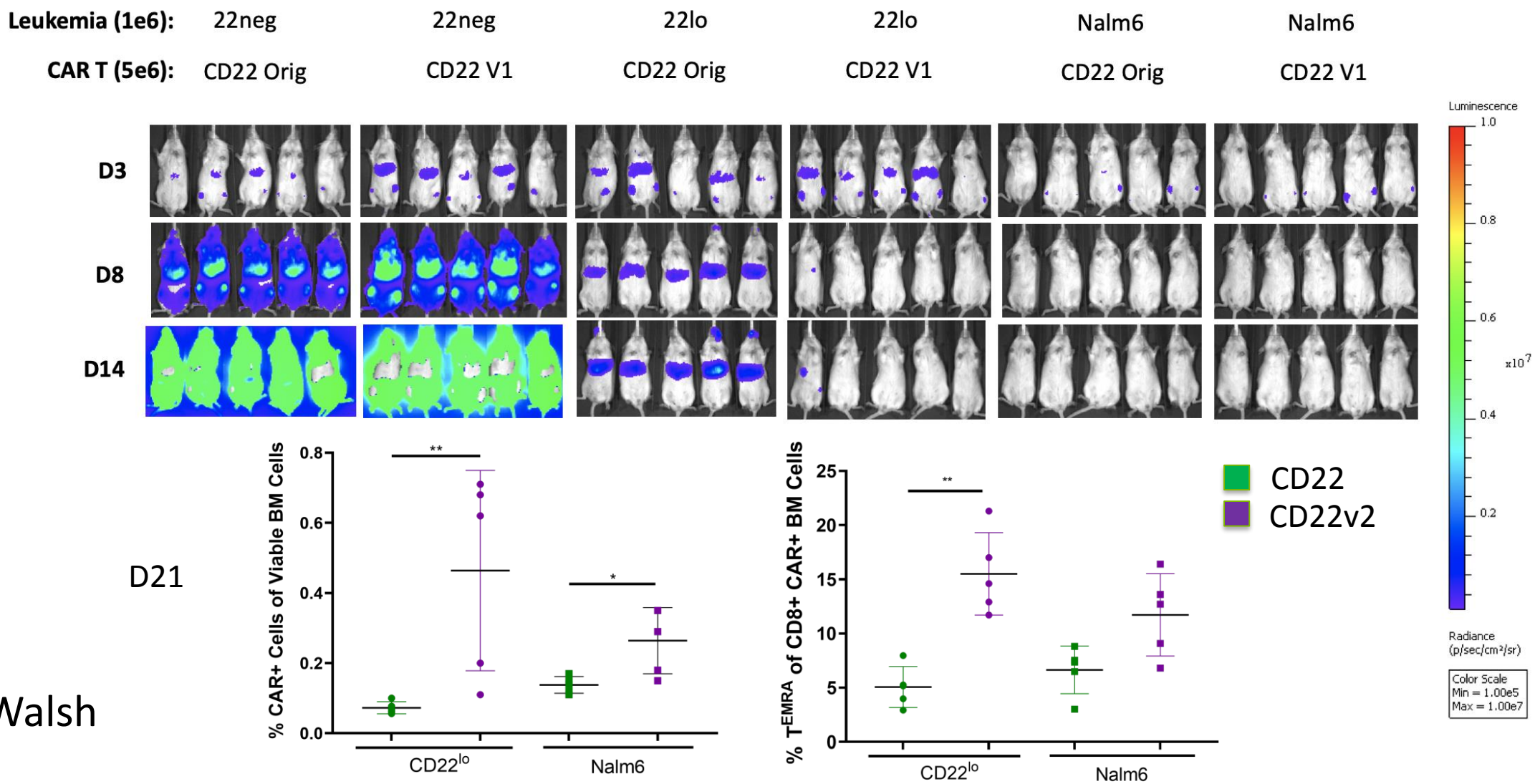

ka1=5.63x10$^{5}$ (1/Ms)
kd1=1.2x10$^{-6}$ (1/s)
KD=3.1x10$^{-9}$ M



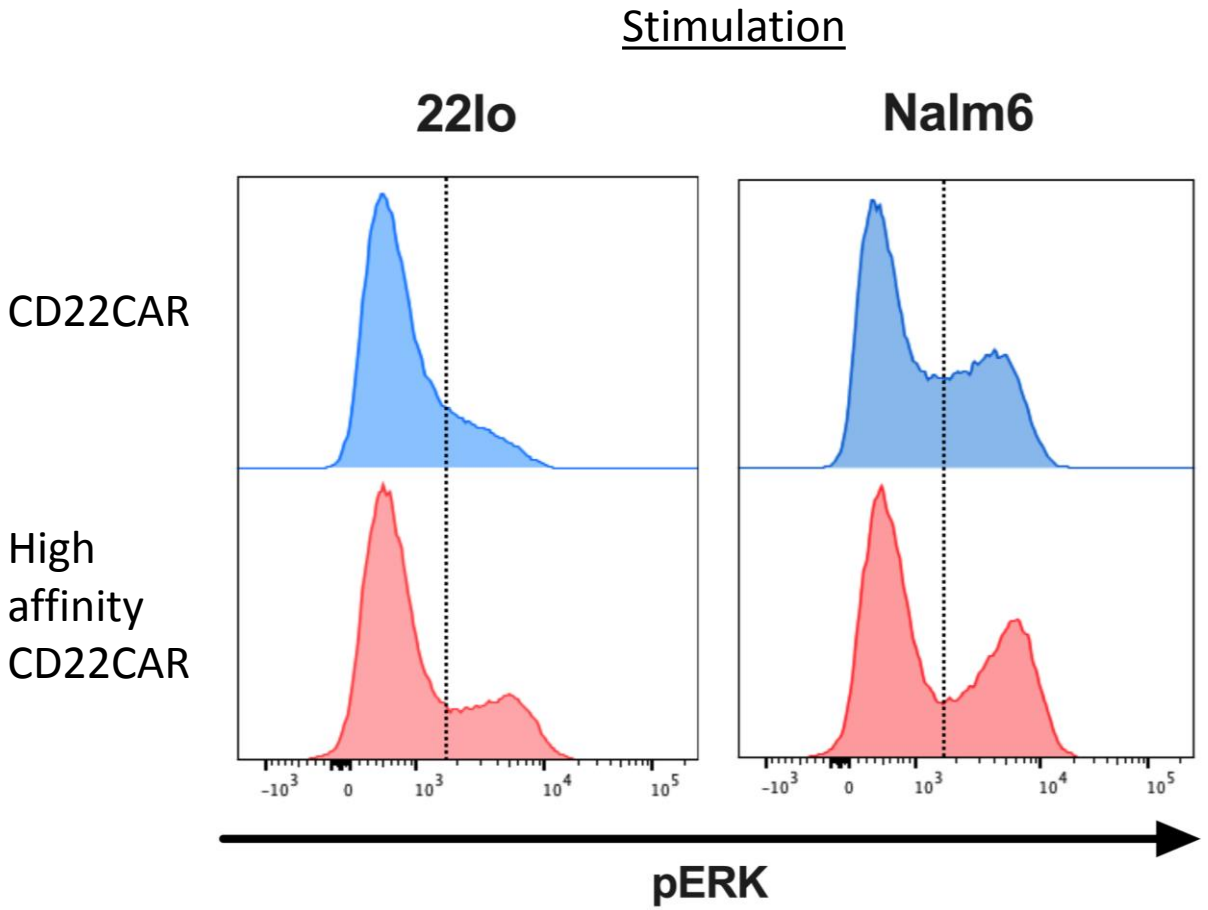

Yang Feng, Mitko Dimitrov

# Enhancing CD22 scFv affinity improves *in vivo* clearance of $CD22^{lo}$ leukemia and CAR persistence

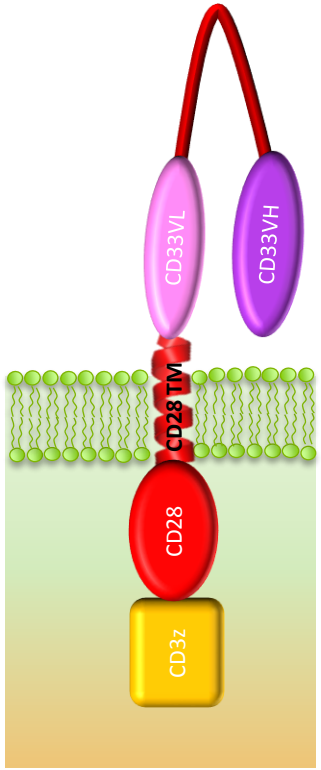



Zach Walsh

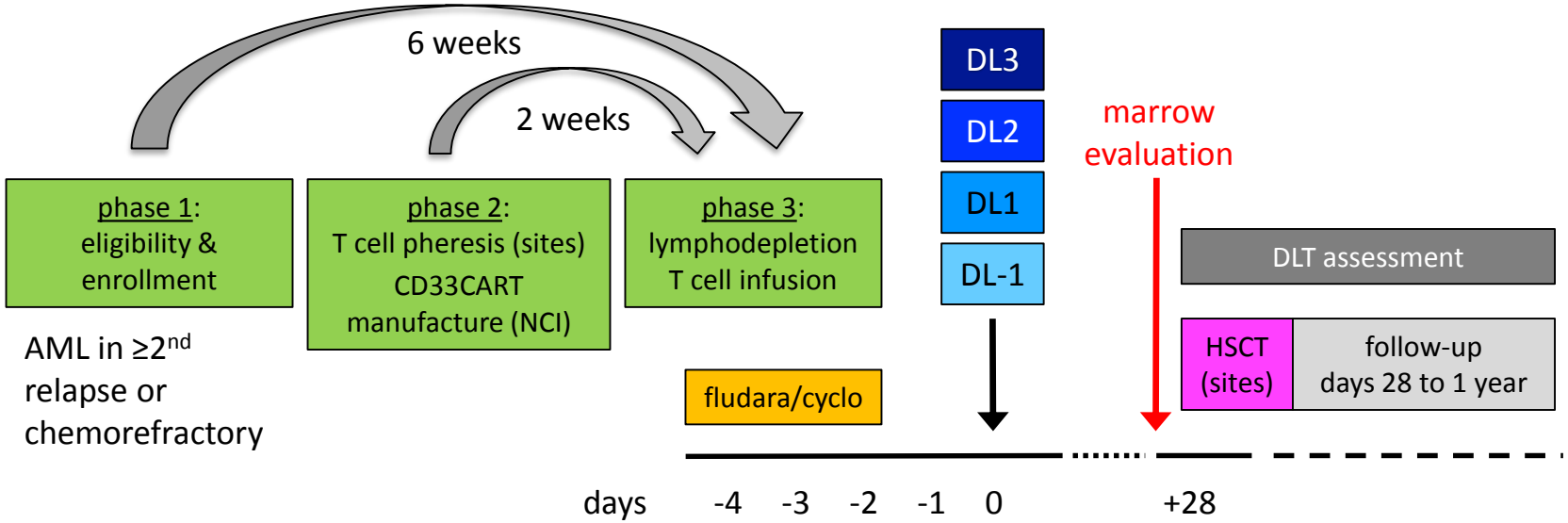
# Enhancing CD22 scFv affinity recruits more T cells with phosphorylated ERK



# First-in-Child CD33CART Phase 1 Trial




Haiying Qin
Lila Yang




AML in ≥2nd relapse or chemorefractory

Primary sites
NCI (Shah)
CHOP (Aplenc, Tasian)

Expansion sites
CHCO (Fry)
CHLA (Pulsipher)
DFCI (Kean)
Seattle (Tarlock, Summers)

fludarabine 25 mg/m$^2$ IV x 3 days -4 to -2
cyclophosphamide 900 mg/m$^2$ IV day -2

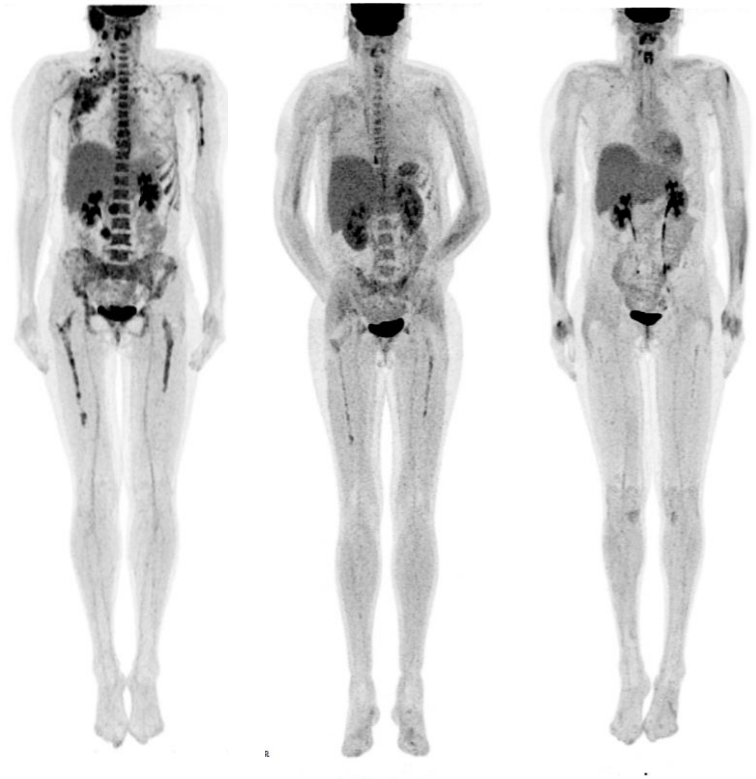
DL-1: 1 x 10$^5$ CD33 CAR+ T cells/kg (±20%)
**DL1: 3 x 10$^5$ CD33 CAR+ T cells/kg** (±20%)
DL2: 1 x 10$^6$ CD33 CAR+ T cells/kg (±20%)
DL3: 3 x 10$^6$ CD33 CAR+ T cells/kg (±20%)

n = ~24 patients over 36 months

Trial open at NCI

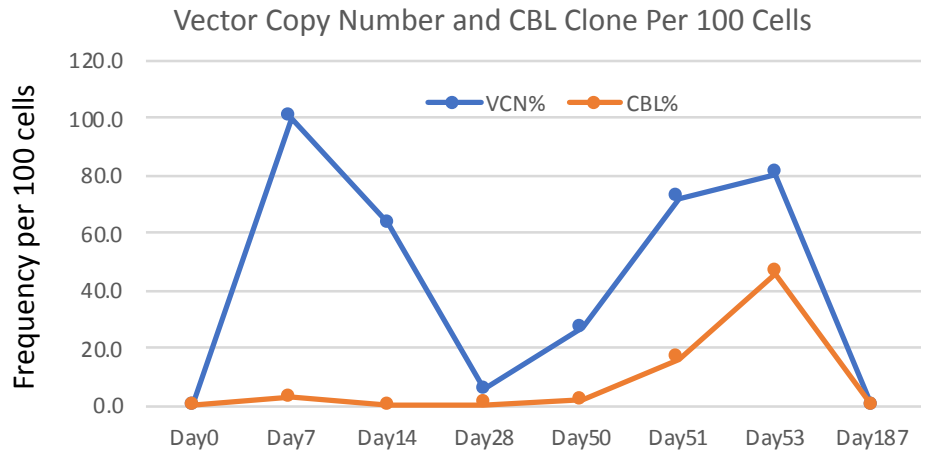
pediatric blood & marrow transplant consortium
NATIONAL MARROW DONOR PROGRAM
BE THE MATCH
CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

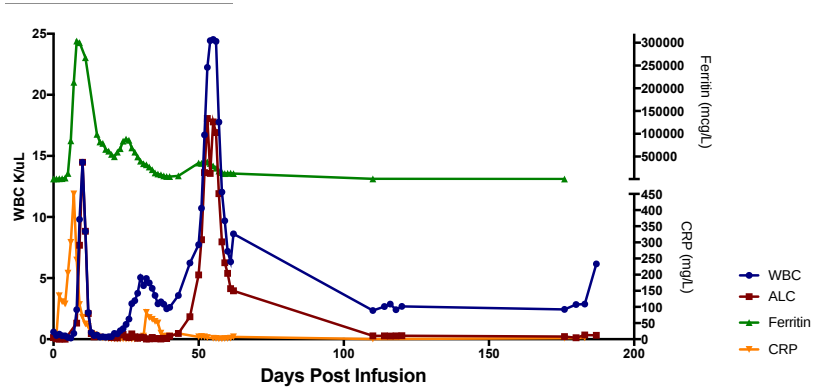
# CD22 CAR Insertion site can drive clonal expansion and clinical activity

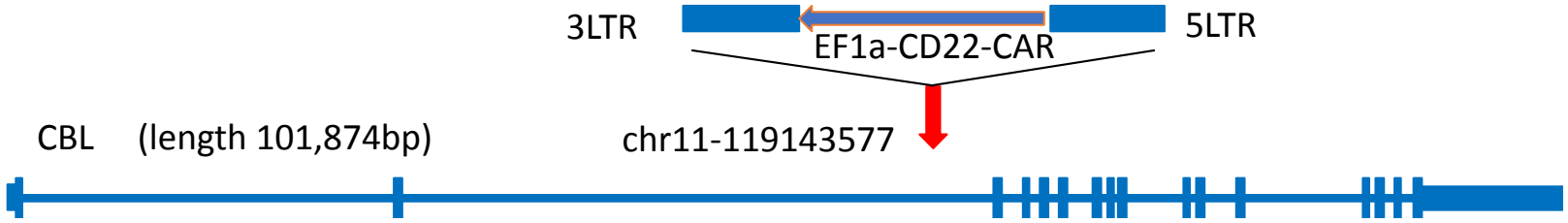


Pre-Treatment  Day + 30  Day +90

| Day Post CAR T-Cell Infusion | % of T-cells in the Peripheral Blood that are CAR+ |
|---|---|
| 8 | 97.8 |
| 13 | 81 |
| 20 | 65 |
| 28 | 80 |
| 50 | 88.4 |
| 110 | 23.8 |




Shah et al, Blood Advances, in press

# Challenges/Opportunities

- Single antigens comparable to CD19 will be difficult to find
- Antigen modulation as well as more complex patterns of cancer resistance will frequently emerge
- This will be more frequent with diseases associated with inherent heterogeneity
- The current CAR formats do not fully recapitulate T cell biology
- Pre-clinical models often poorly predictive of activity in humans
- As synthetic receptors, the ability to modify is almost endless
  - Binding domains, signaling domains, multiplexing

# Acknowledgments


**NCI, Pediatric Oncology Branch and CPS**

- Nirali Shah
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- Jack Shern

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- Savannah Ross
- Jen Cimons
- Zach Walsh
- Lillie Leach
- Christine Meadows
- Michael Yarnell
- Kole DeGolier
- Zach Graff

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- Dave Stroncek

Patients and Families