

SITC 2019

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Society for Immunotherapy of Cancer



Enhancing affinity of CD22-directed CAR T cells increases activation signaling and in vivo response to CD22^{lo} leukemia

Zachary Walsh

Principal Investigator: Terry Fry, MD

11.07.2019



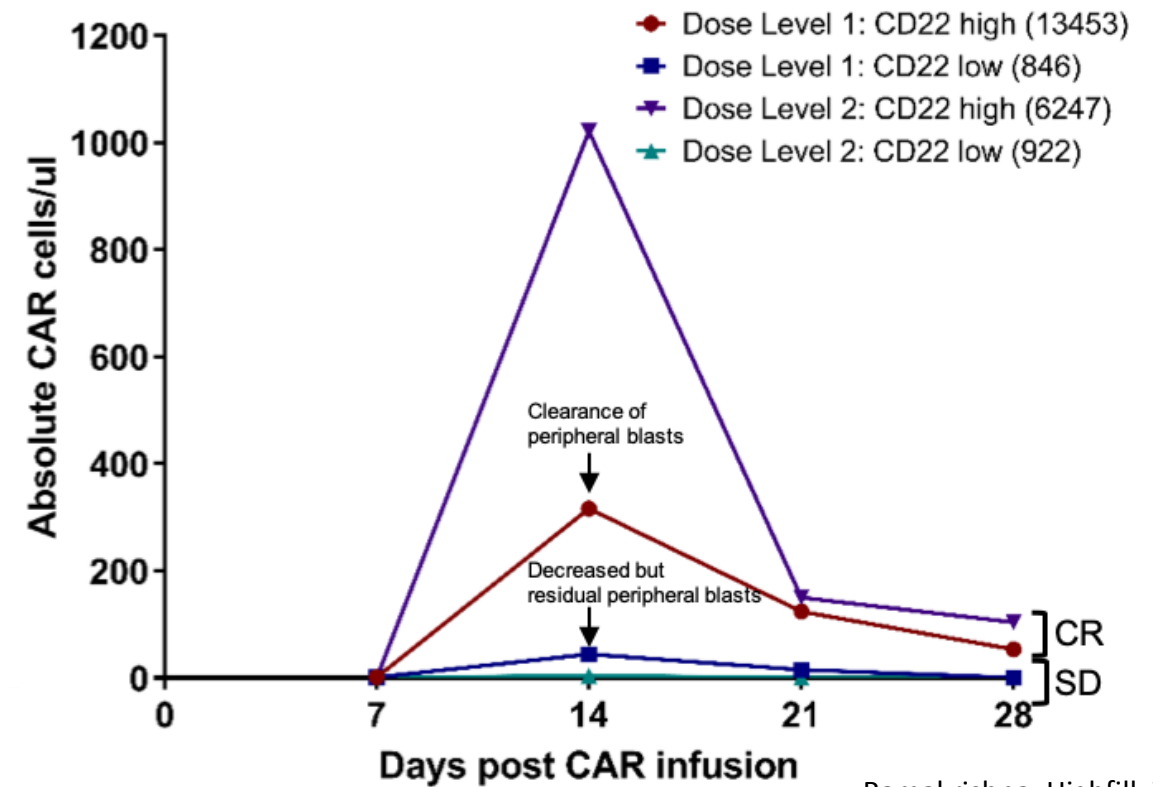
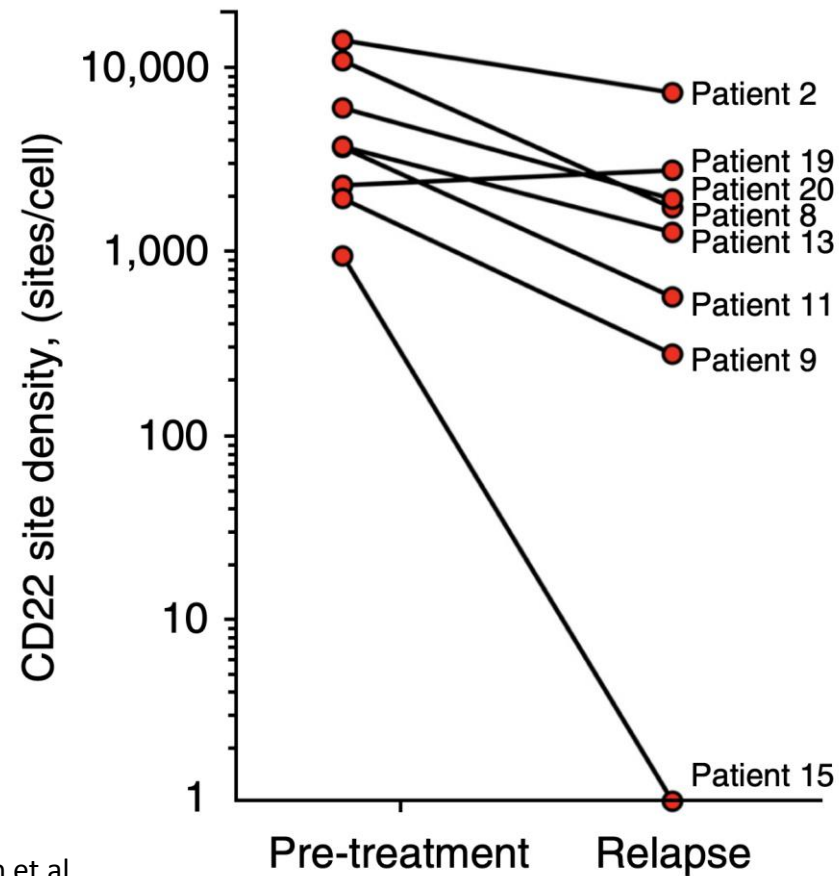
Society for Immunotherapy of Cancer

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Disclosures

- I have no actual or potential conflict of interest in relation to this presentation.

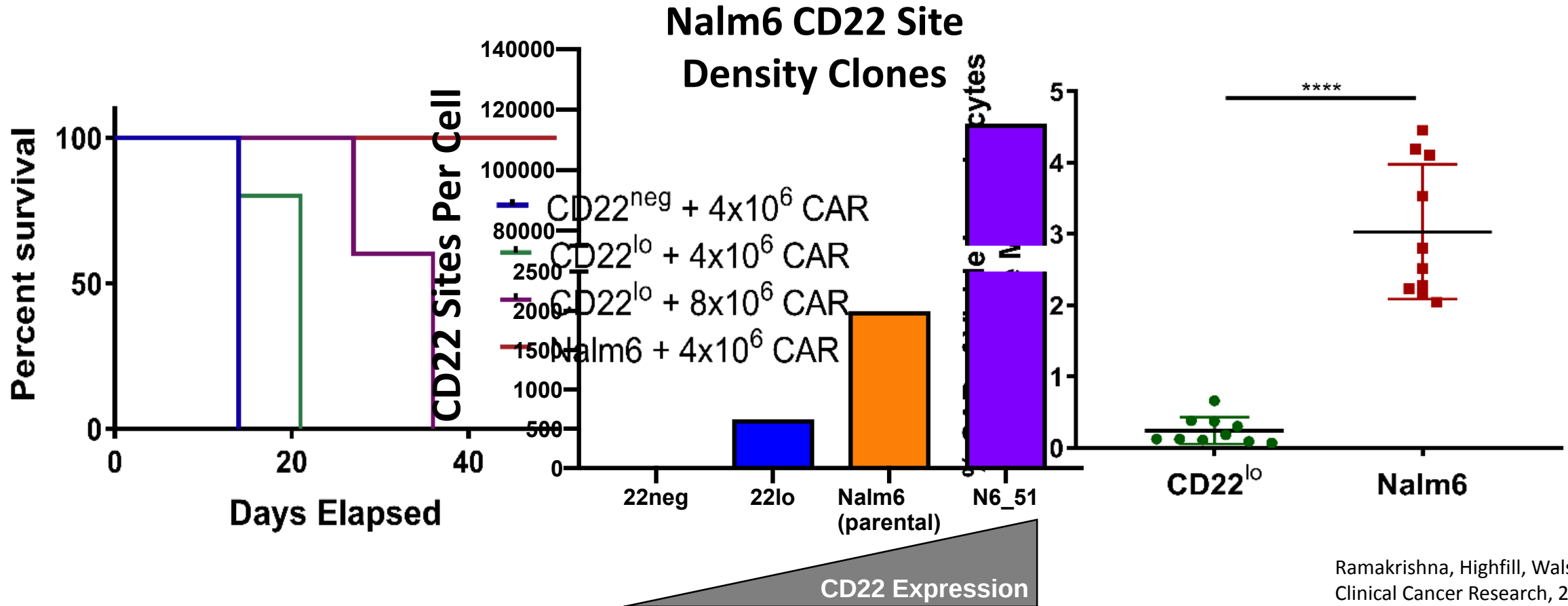
Clinical Findings: Relapse following CD22-CAR therapy is associated with diminished leukemic CD22 expression



Fry and Shah et al.,
Nature Medicine, 2017.

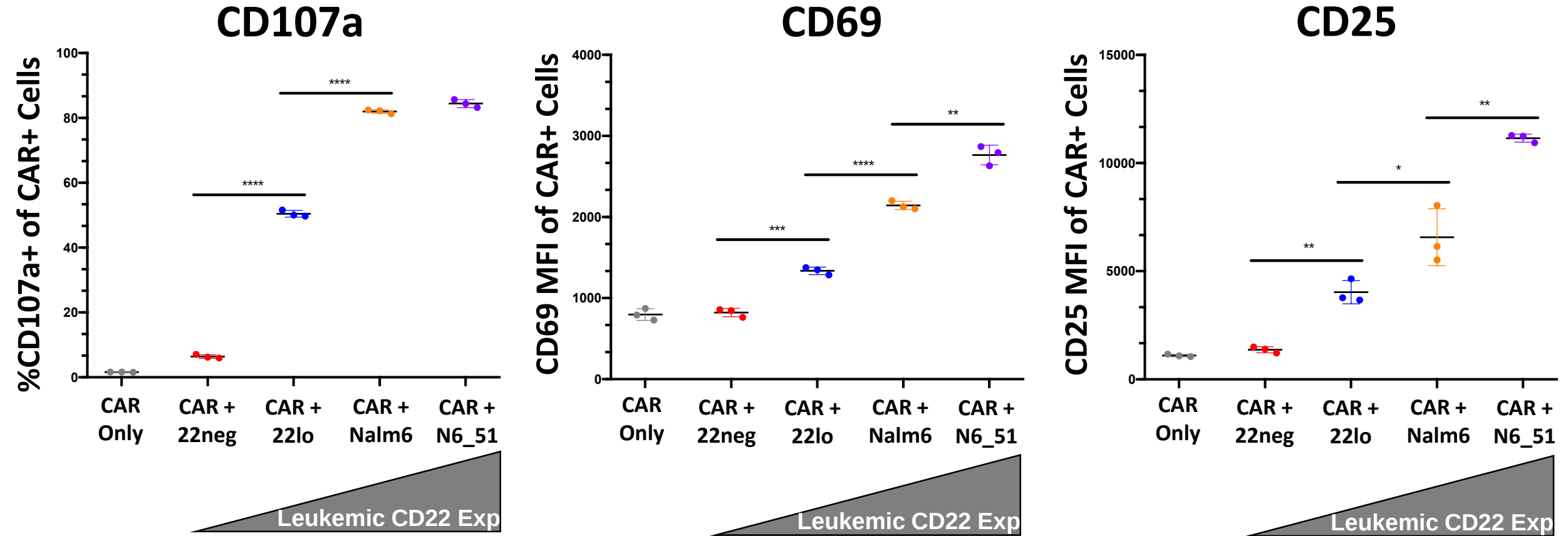
Ramakrishna, Highfill, Walsh et al.,
Clinical Cancer Research, 2019.

Preclinical Correlate: CD22 Antigen Density Impacts Function and Persistence of CD22-CAR T Cells

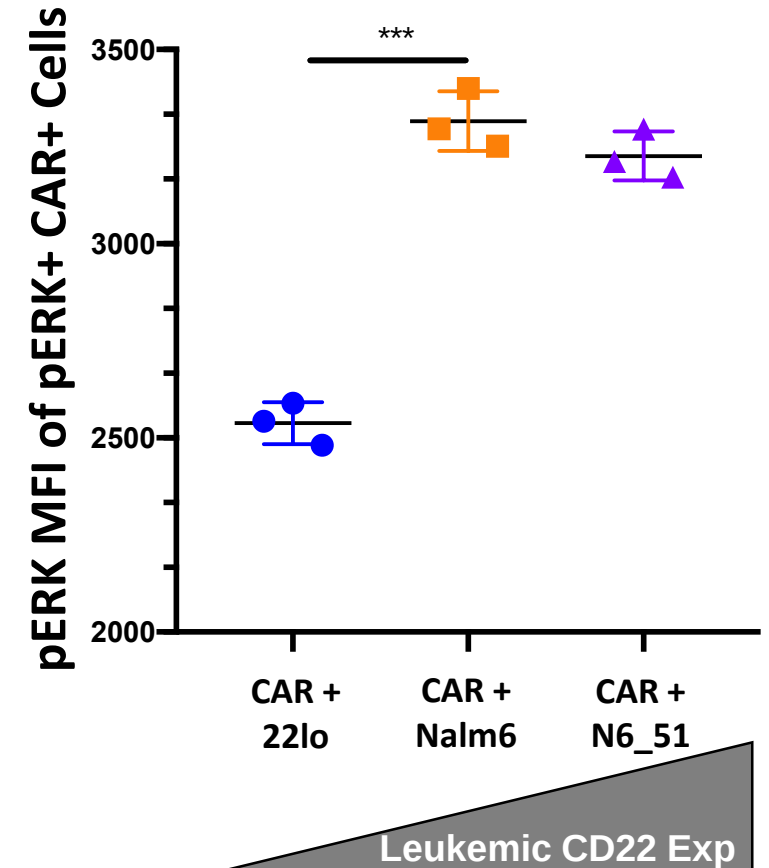
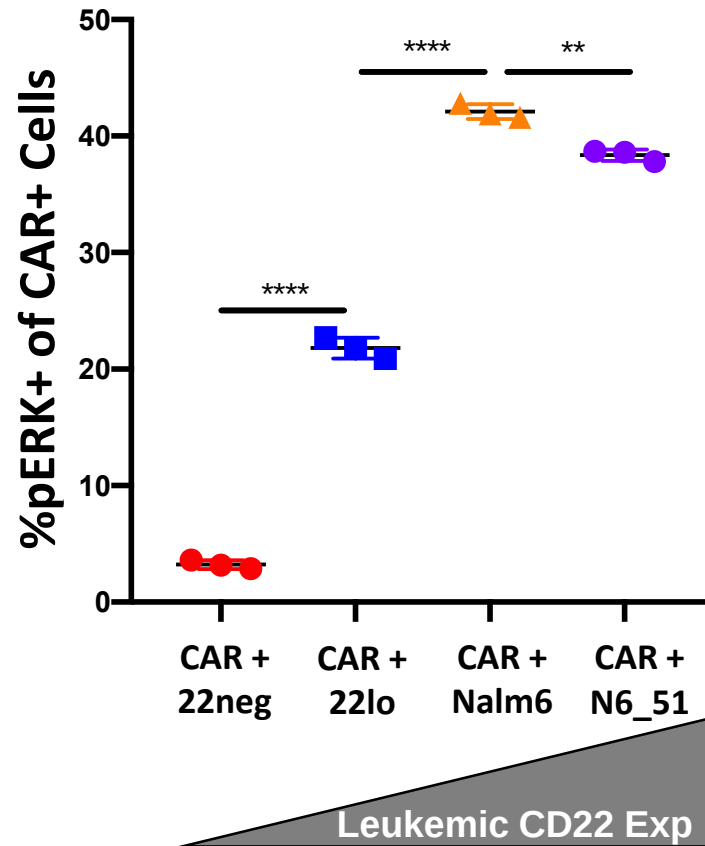
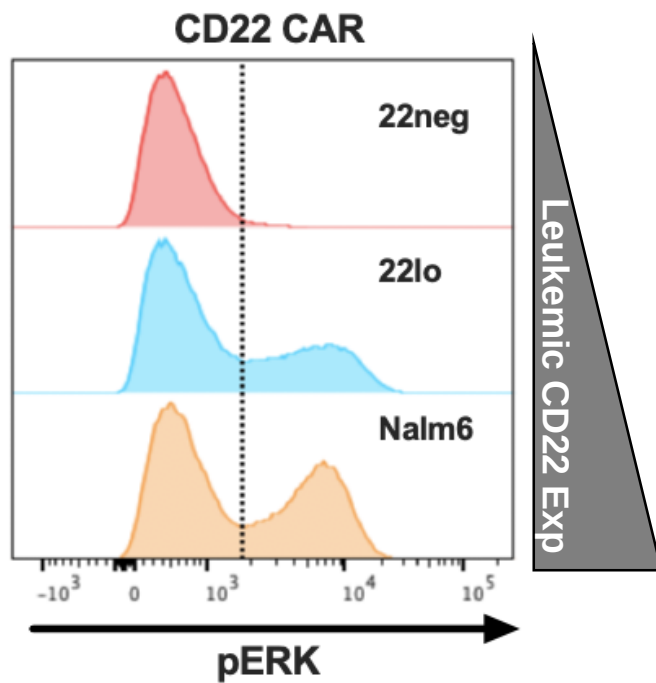


Ramakrishna, Highfill, Walsh et al.,
Clinical Cancer Research, 2019.

Mechanistic Basis: Early CD22-CAR Activation is Impacted by CD22 Antigen Density



Mechanistic Basis: ERK Phosphorylation Correlates with CD22-CAR Efficacy and is Dependent on CD22 Antigen Density



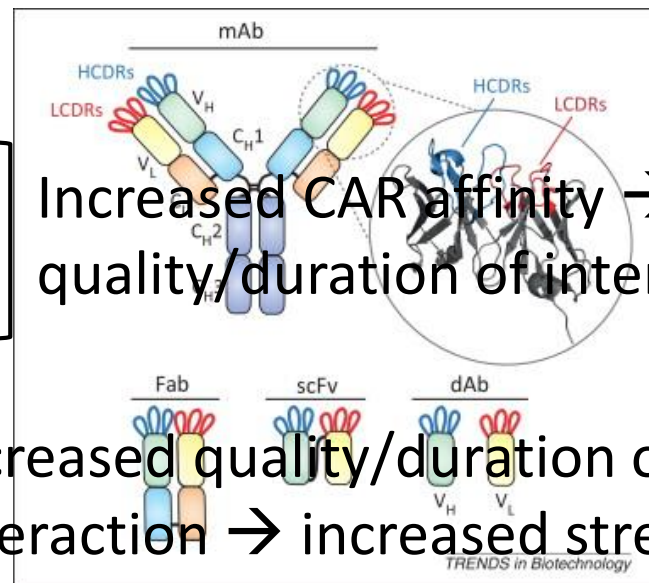
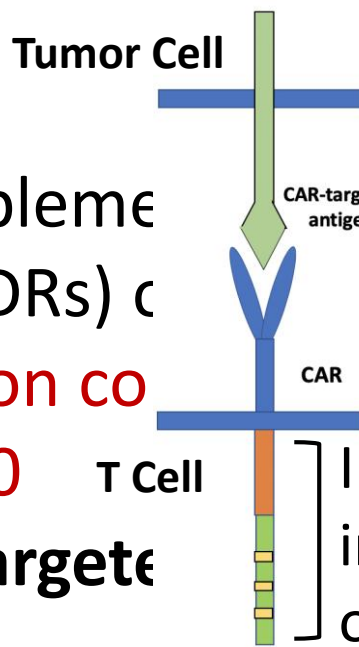
Increasing CAR scFv Affinity to Address Low Antigen Density

Why?

- Hypothesis: improve strength of CAR activation signaling (measured by pERK) and enhance cytotoxicity

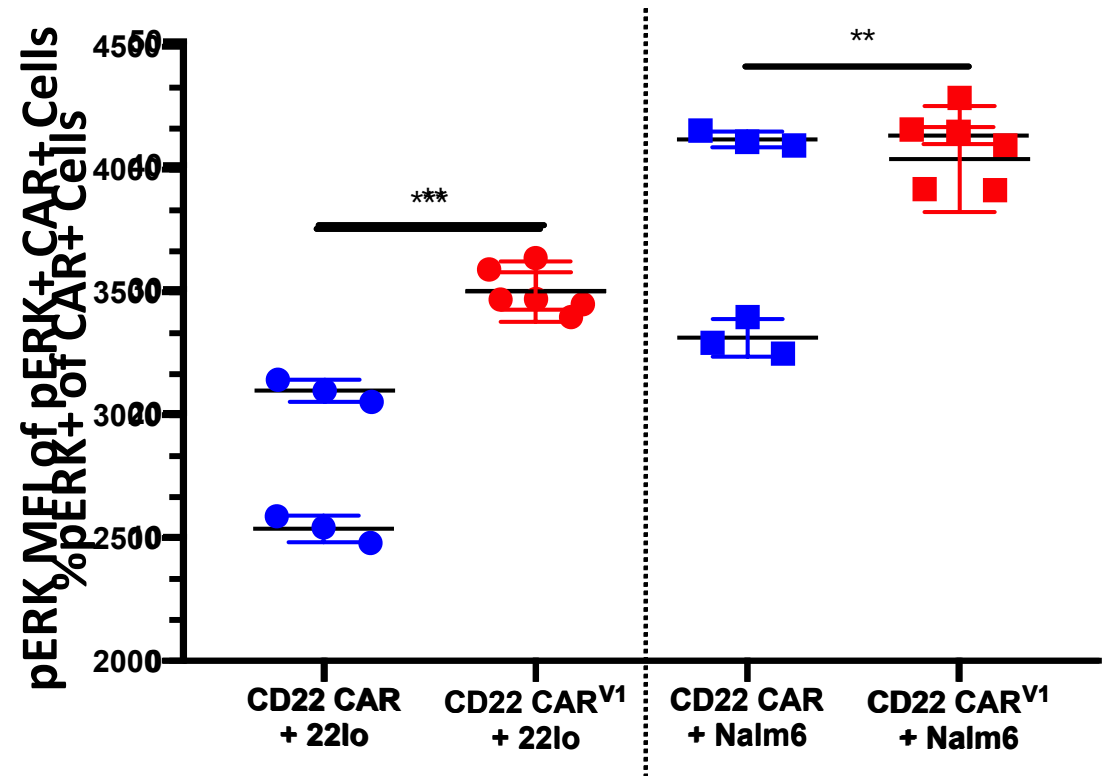
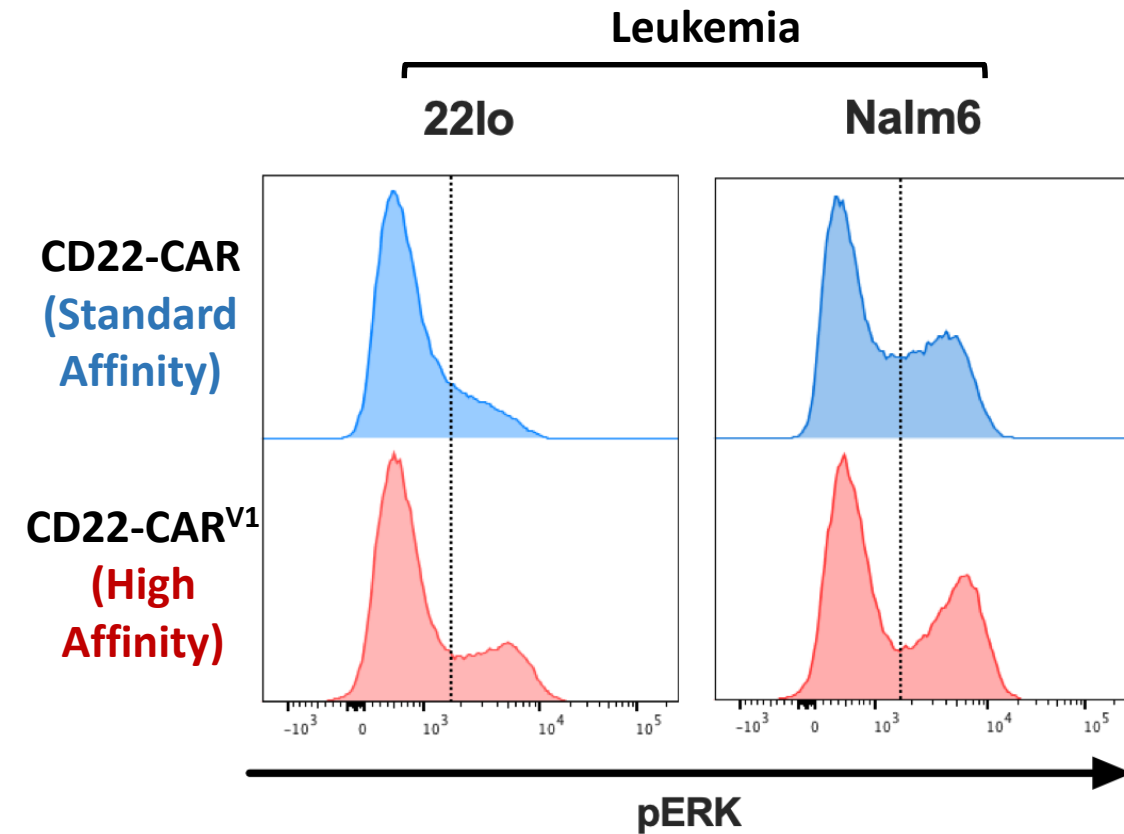
How?

- Point Mutations in Complement Determining Regions (CDRs) c
- **Decrease scFv dissociation constant (K_D) by a factor of $\sim 1,000$**
- **Same CD22 epitope is targeted**

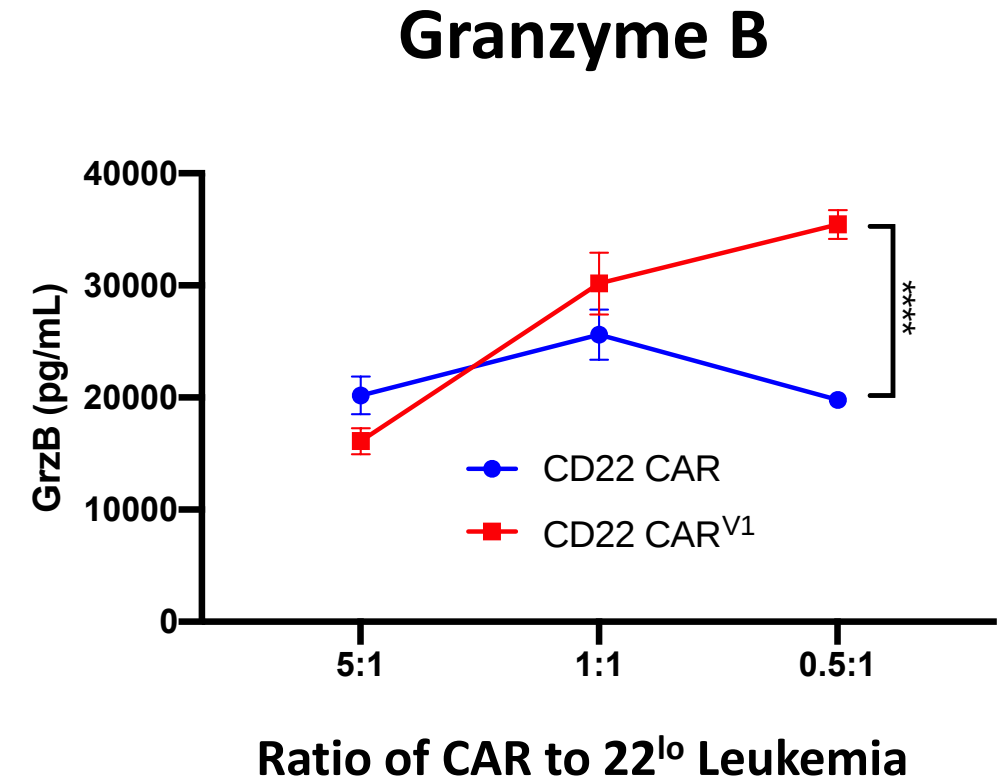
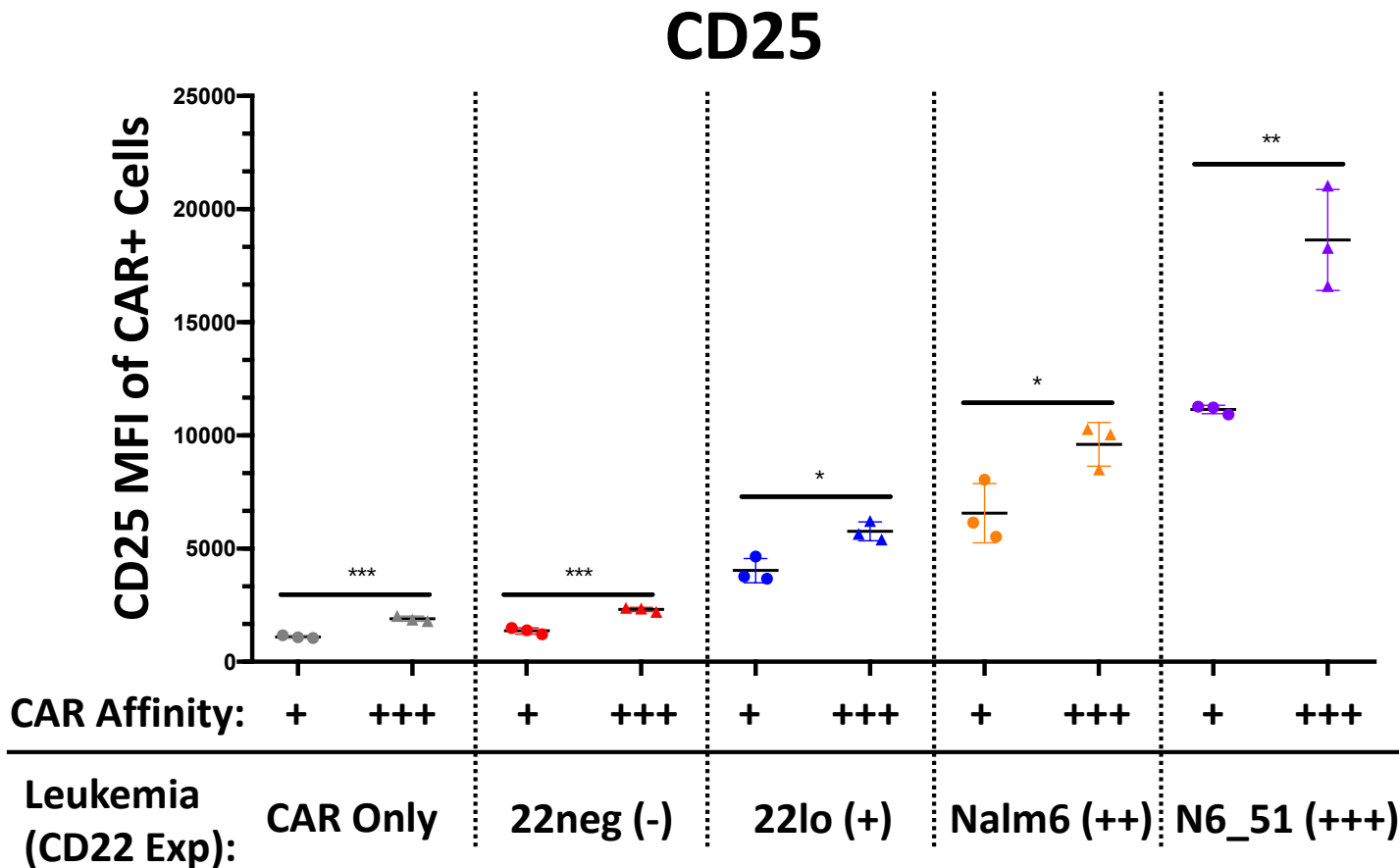


Lee et al., Trends In Biotechnology, 2013.

High-Affinity CD22-CAR^{V1} Demonstrates Improved Distal Activation Signaling



High-Affinity CD22-CAR^{V1} Demonstrates Improved *In Vitro* Activation and Cytotoxicity



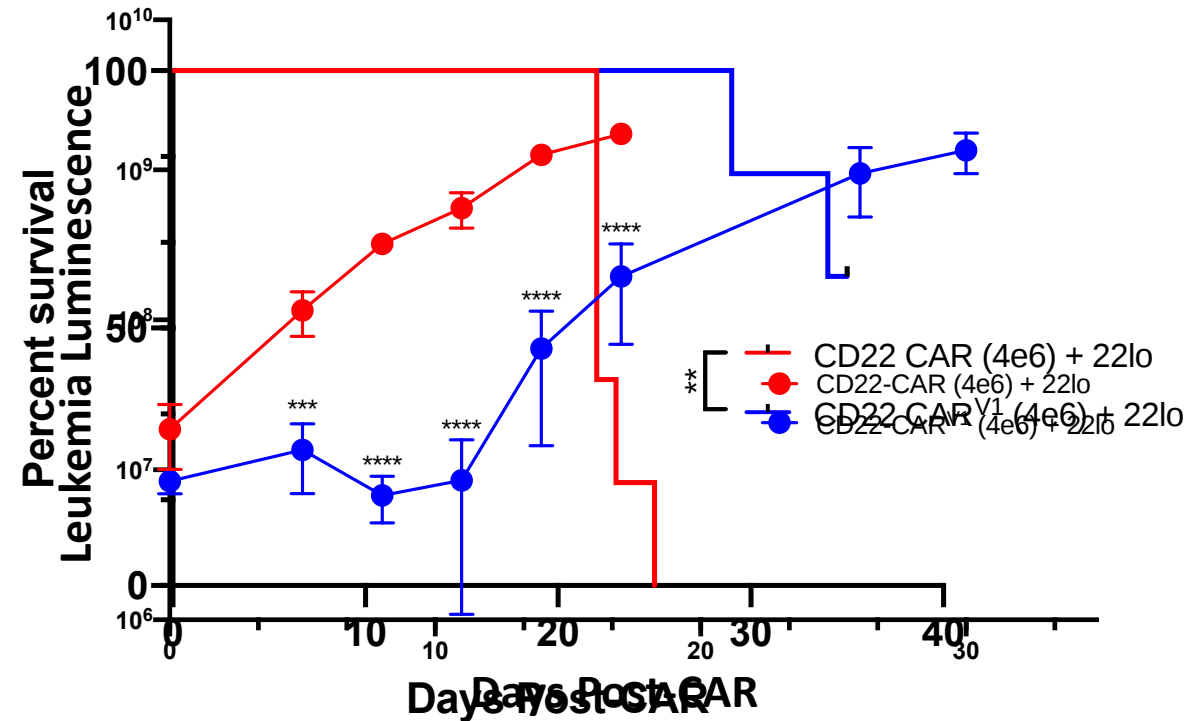
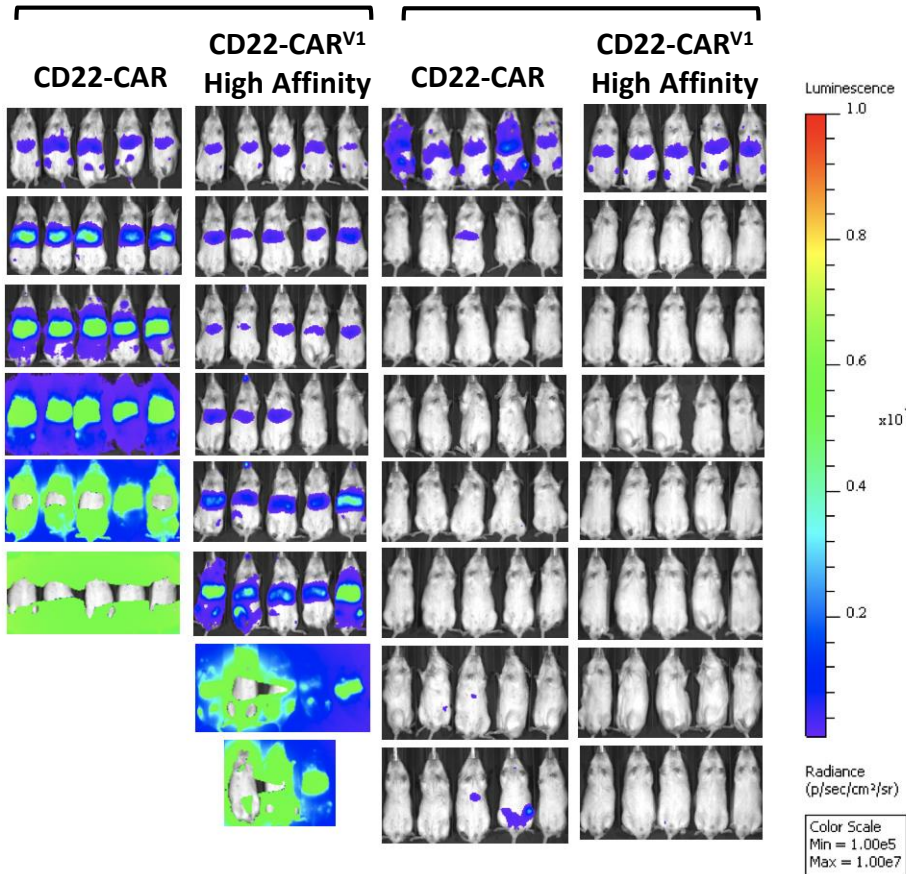
High-Affinity CD22-CAR^{V1} Demonstrates Improved *In Vivo* Efficacy

Leukemia (1e6):

22^{lo}

Nalm6

CAR T (4e6):



Conclusions

- **Enhancing scFv affinity is a rational and potentially effective strategy to improve CAR T efficacy against refractory, antigen-low leukemias**
- ERK phosphorylation is correlated with CD22-CAR efficacy
- Increasing affinity of CD22-CAR scFv (CD22-CAR^{V1}) through CDR point mutations increases ERK phosphorylation in response to antigen
 - CD22-CAR^{V1} demonstrate improved *in vitro* activation and greater *in vivo* efficacy against CD22^{lo} ALL

Future Directions

- **Clinical evaluation of CD22-CAR^{V1}**
- Determine whether inducing increased pERK can improve CD22-CAR efficacy
- Incorporation of CD22-CAR^{V1} scFv into multi-specific CAR platforms
- Examine immune synapse of high-affinity CD22-CAR^{V1} vs. standard-affinity CD22-CAR
 - Improved dwell-time on CD22^{lo} leukemia cells?
 - Improved synapse quality?

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Patients and Families

