Study of Affinity Tuning in AML-Targeted CAR-NK Cells

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Background

- AML accounts for 15-20% of all pediatric leukemias with a dismal prognosis in the relapsed and refractory setting.
- NK cells are lymphocytes of the innate immune system that can be engineered with Chimeric Antigen Receptors (CARs).
- CD123 is a target antigen expressed on AML and healthy myeloid progenitor cells with variable expression density.
- Affinity-modification can be used to optimize CAR-effector activation for more specific leukemia-directed cytotoxicity.

Objective

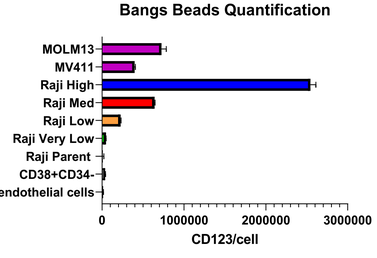
Hypothesis: Lower affinity CAR-NK cells will demonstrate improved discrimination between healthy tissue and AML with enhanced targeted cytotoxicity through serial killing.

Aim 1: To characterize the effect of single chain variable fragment (scFv) affinity modification on CAR-NK cell activation and cytotoxicity.

Aim 2: To relate CAR affinity to CAR-NK cell persistence and anti-tumor activity in vivo.

Target Cell CD123 Expression

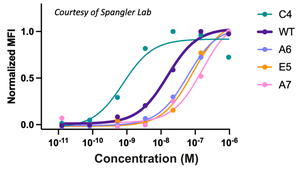
Raji cells can be engineered to express CD123. CD123-negative Raji cells were engineered to express CD123 and single-cell sorted to select for very low, low, medium, and high antigen density clones. CD123 surface expression was quantified using Bangs Beads (Bangs Laboratories, Inc.)



Variable Affinity CAR-NK Cells

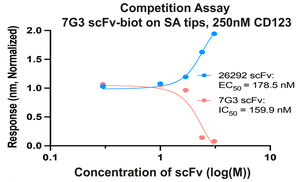
A.

26292 Variants	Kd (nM) Yeast	Fold WT Yeast
C4	0.79	0.05
WT	15	-
A6	59	3.93
E5	82	5.47
A7	160	10.67

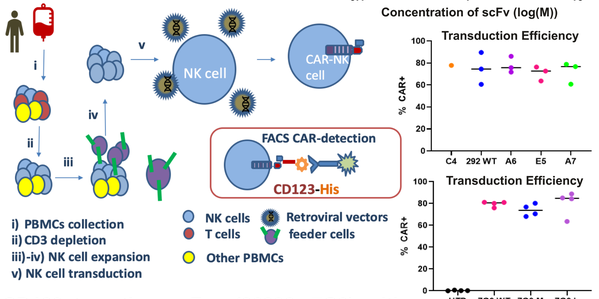


B.

7G3 Variants	Kd (nM) Octet	Fold WT Octet
7G3 WT	8.8	1
7G3 M	24	2.7
7G3 L	65	7.4

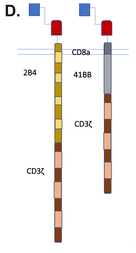


C.



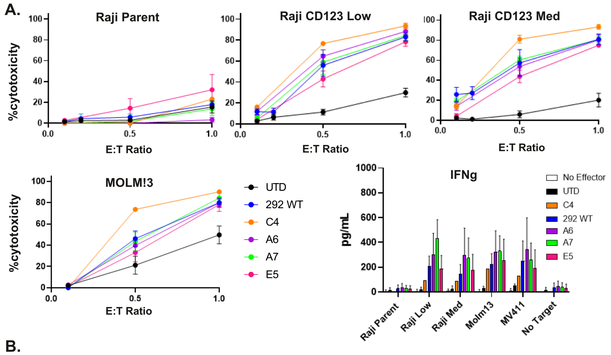
CD123 targeting scFvs (26292, 7G3) with a range of affinity can be produced and cloned into CARs with high expression in primary NK cells derived from different donors. A) Panel of 26292 variant scFvs with relative binding affinities. **B)** Panel of 7G3 variant scFvs with noncompetitive binding of CD123 relative to 26292. **C)** Schematic of NK cell isolation from healthy donor PBMCs and CAR transduction efficiencies. **D)** CAR backbones with intracellular with 41BB.TCRζ and 2B4.TCRζ domains.

D.

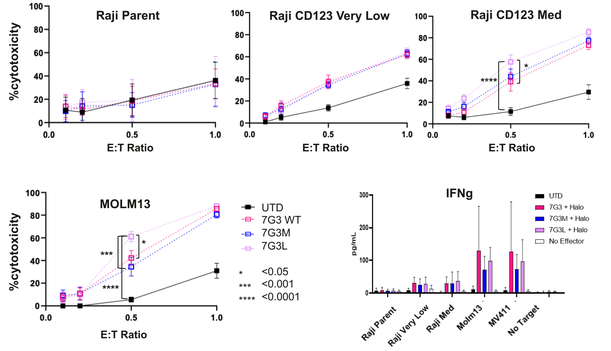


Short-term functional activity of variable affinity CAR NK-Cells

A.

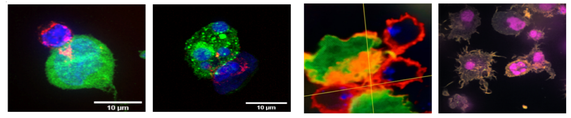


B.



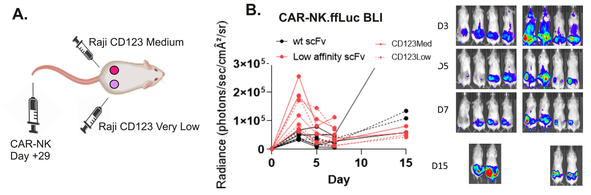
CAR-NK cells with variable affinity 26292 and 7G3 scFvs in 41BB.TCRζ CARs show differences in short-term cytotoxicity. A) 26292 short-term cytotoxicity and IFNg secretion in response to CD123 expressing targets. **B)** 7G3 short-term cytotoxicity and IFNg secretion.

Imaging the Immunological Synapse



Fluorescence based imaging can capture CAR-NK and target cell interactions at high resolution. Representative spinning-disk confocal fluorescent microscope images of CAR-NK and target cell interface.

CAR-NK Cell Trafficking to CD123+ Raji



CAR-NK Cells Traffic to CD123+ Raji tumors. A) Experimental schematic. **B)** Firefly Luciferase tagged CAR-NK cell localization.

Conclusions

scFvs with a defined range of affinity can be expressed on NK cells as CAR extracellular domains. High affinity 26292 may have superior short-term functional activity against CD123+ targets. Low affinity 7G3 has superior short-term functional activity against MOLM13 and Raji.CD123Med. 7G3L CAR-NK cells expand early and may traffic preferentially to Raji.CD123.Med tumors in NSG mice.

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