

A detailed illustration on a dark blue background. In the center, a large, translucent purple and blue shield-like structure represents a CAR-T cell. To its left are several blue, spherical cells with dark blue nuclei, representing healthy cells. To its right, two red, spiky spherical cells (target cells) are shown. One target cell is being destroyed by the CAR-T cell, with a bright blue and white energy burst emanating from the point of contact. The other target cell is further away, with blue energy lines trailing behind it. The overall scene depicts the cytotoxic activity of CAR-T cells against leukemia cells.

## **Novel CD33/CLL-1-directed dual CAR-T cells mediate potent antigen-specific cytolytic activity in mouse models of Acute Myeloid Leukemia (AML)**

M Silva, B Gjerci, H Hoyt, N Patel, A Kharbanda, N Kleinberg, A Thomas, A Halfond, R Williams, J Lydeard, J Scherer, T Chakraborty



## Disclaimer

Mariana Silva is an employee and stockholder of Vor Bio.



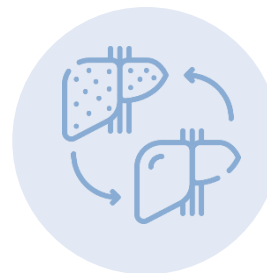
# AML is the Most Common Adult Leukemia



~ 20,000 (US)

~ 26,000 (EU)

People diagnosed with AML annually<sup>1,2,3</sup>



~ 4,000 (US)

~ 7,000 (EU)

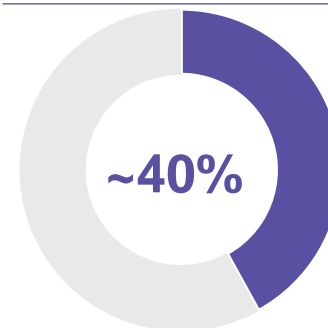
AML Transplants per year <sup>4,5</sup>



50% (US)

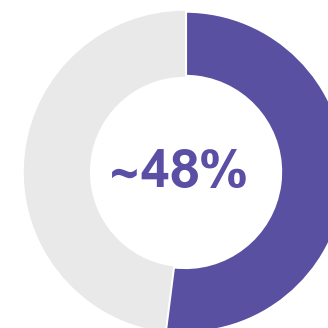
70% (EU)

Increase in # of AML transplants over the last 10 years<sup>5,6</sup>



~ 40% (US)

Post-transplant relapse, with <20% two-year survival<sup>7,8</sup>



~ 48% (EU)

Post-transplant relapse<sup>9</sup>, with <50% two-year survival<sup>10</sup>

1 American Cancer Society 2023

2 Orphanet

3 WordMeter World Population Statistics

4 Passweg et al, Bone Marrow Transplant, 2023

5 Current use and outcome of hematopoietic

stem cell transplantation: CIBMTR summary slides 2022

6. Lee et al, Haematologica 2017

7 Araki et al, JCO 2016

8 Schmid et al, Blood 2012

9 Heinicke et al, Annals of Hematology, 2021

10 Khalil et al, European Journal of Haematology 2017



# Rationale For Multi-Specific CAR-T Cells in AML

## AML is a Heterogenous Disease

- AML across the Patient Population
- Blasts Within Each Patient



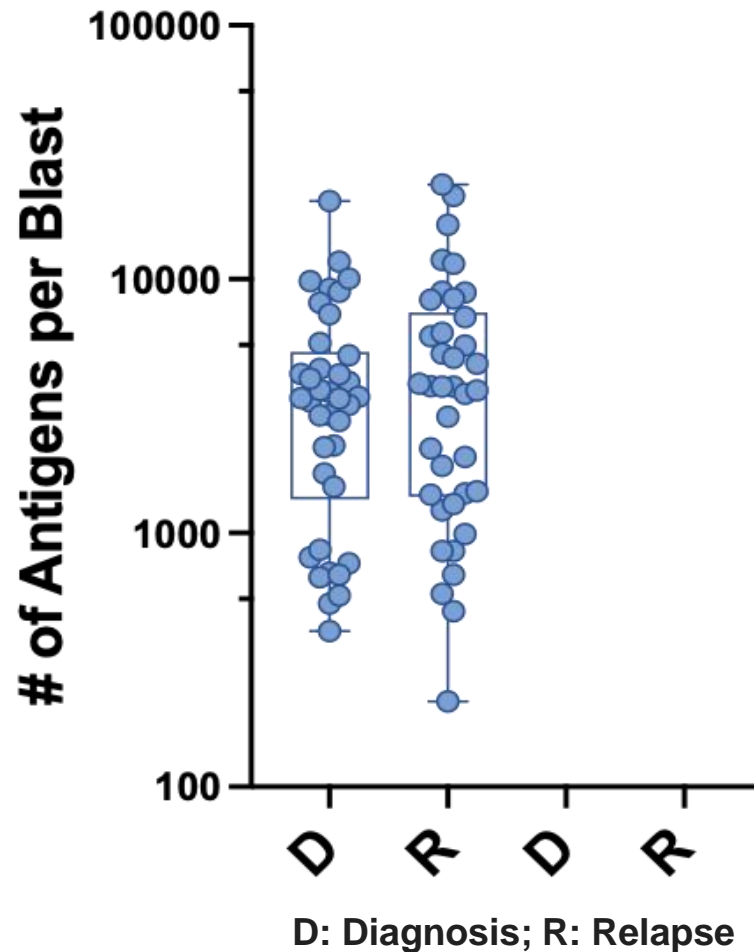
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## AML is a Dynamic Disease

- Antigen Numbers on Blast Surface
- Potential Antigen Escape



(Ung *et al.*, ASH, 2023)



# Rationale For Multi-Specific CAR-T Cells in AML

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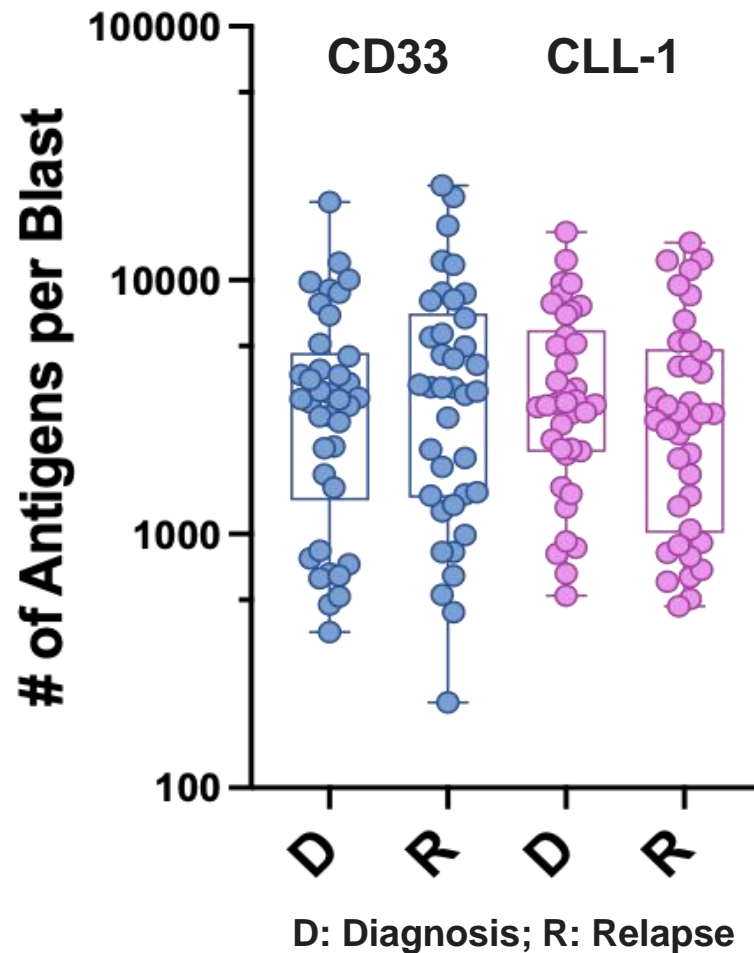
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- CD33 and CLL-1 as Promising Target Pair



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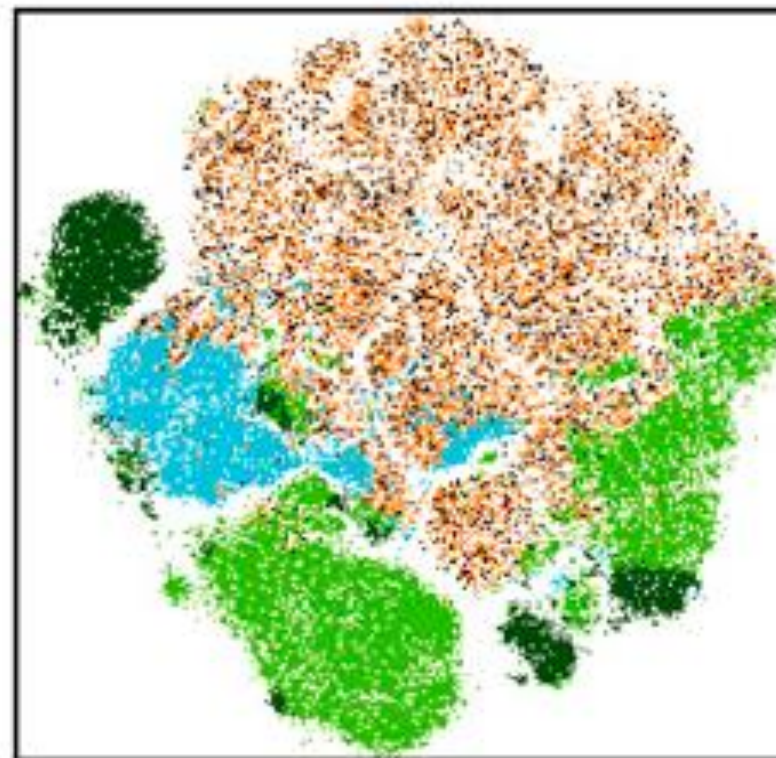
## AML is a Dynamic Disease

- Antigen Numbers on Blast Surface
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## AML is Well Characterized

- CD33 and CLL-1 as Promising Target Pair
- > 92% of Blasts Express CD33 and/or CLL-1

### single-cell analysis of AML Blasts



+ / +	61%
+ / -	23%
- / +	9%
- / -	8%

	CD33+ CLL-1+
	CD33+ CLL-1neg
	CD33neg CLL-1+
	CD33neg CLL-1neg

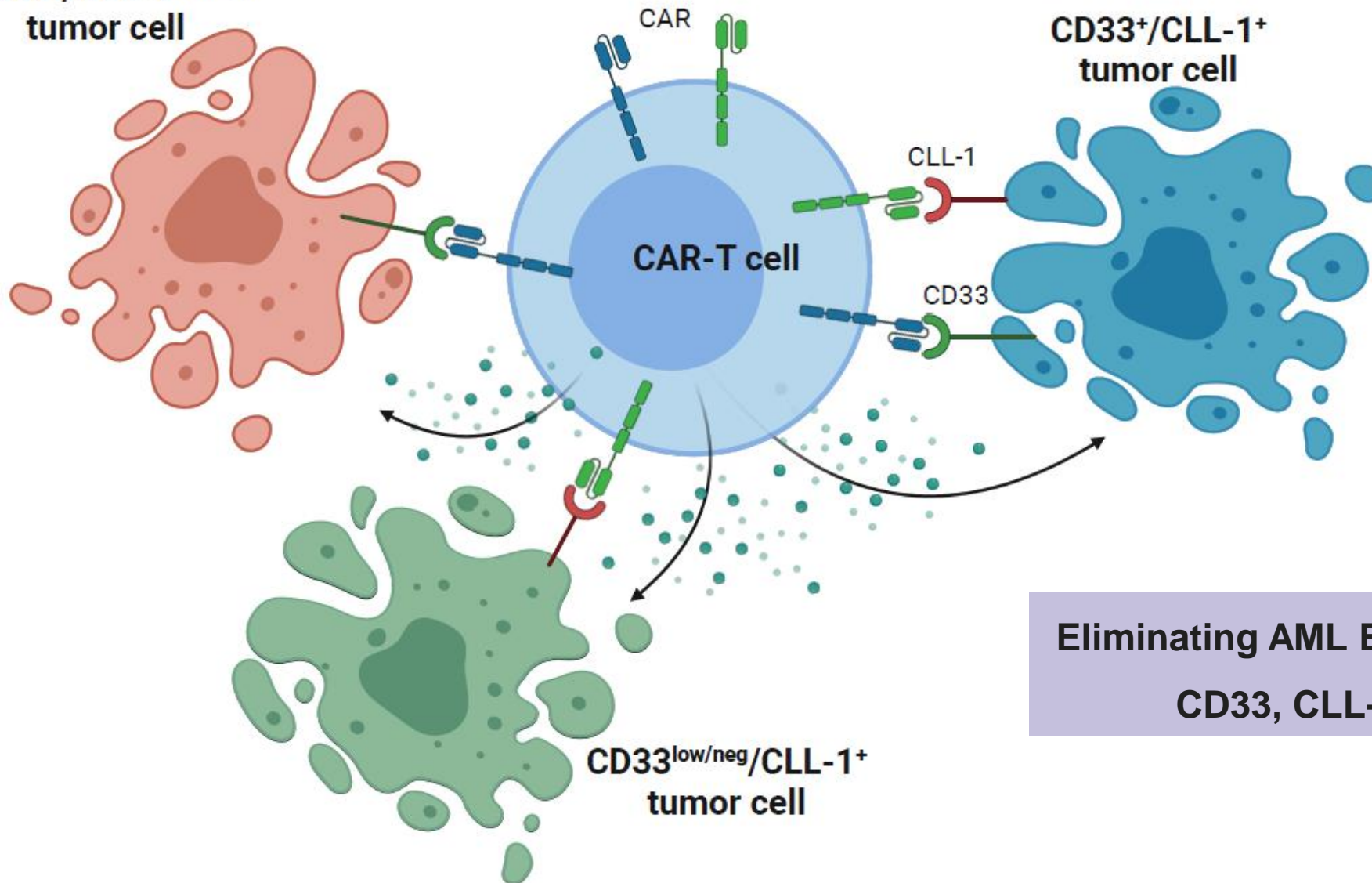
(Willier, *et al.*, 2021)





# Multi-Specific CAR-T cells: “OR” Gate Strategy

CD33<sup>+</sup>/CLL-1<sup>low/neg</sup>  
tumor cell

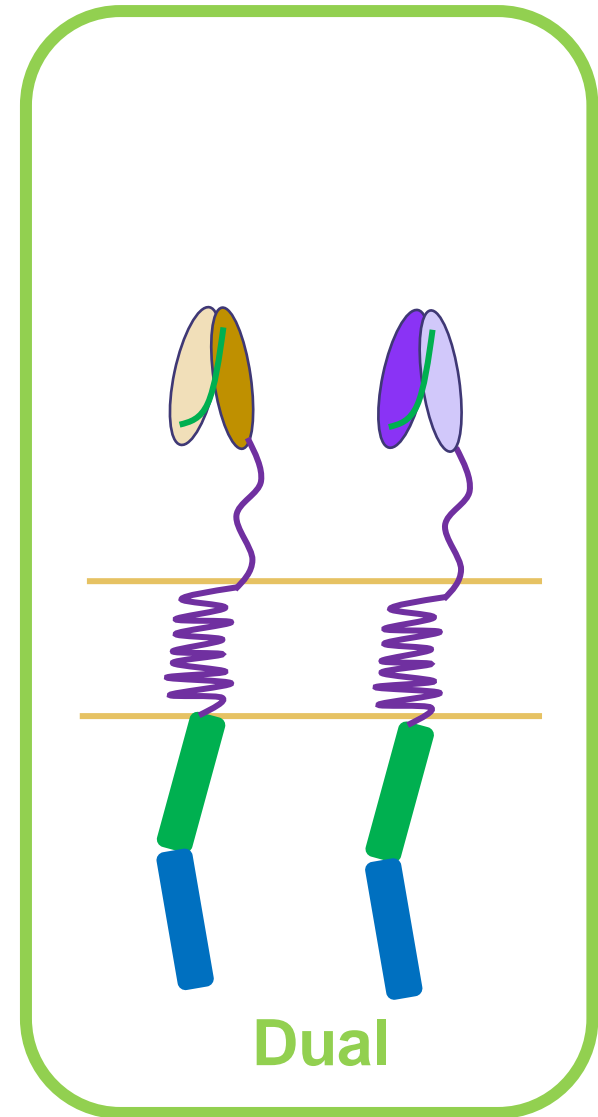
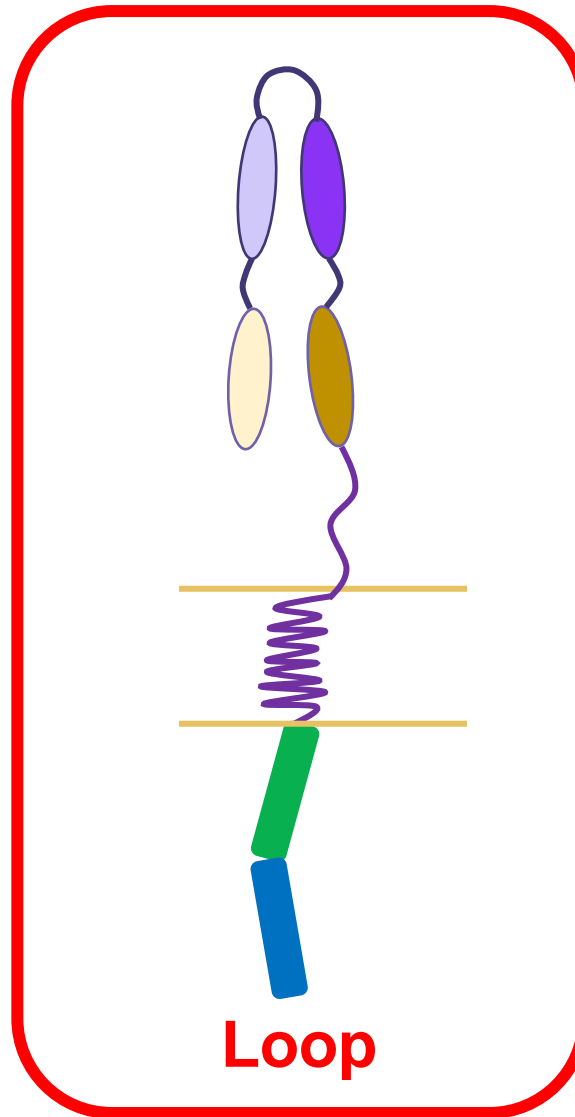
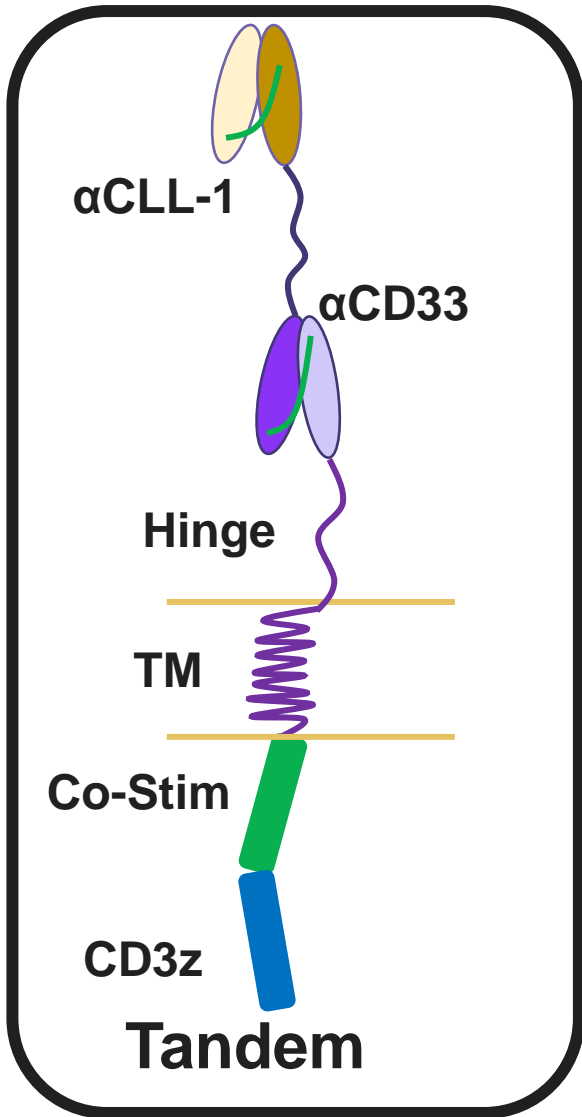


Eliminating AML Blasts Expressing  
CD33, CLL-1, or Both.



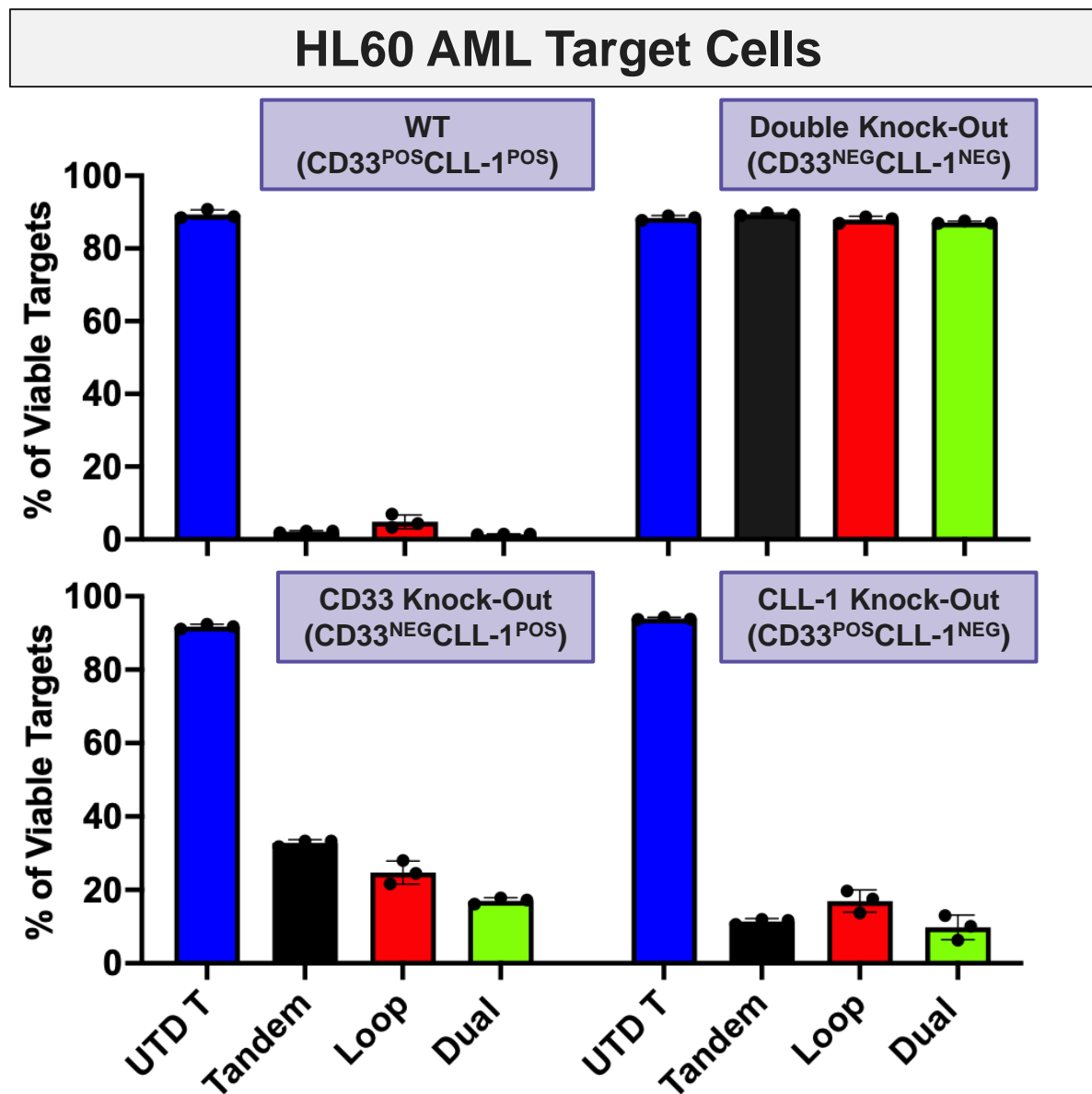
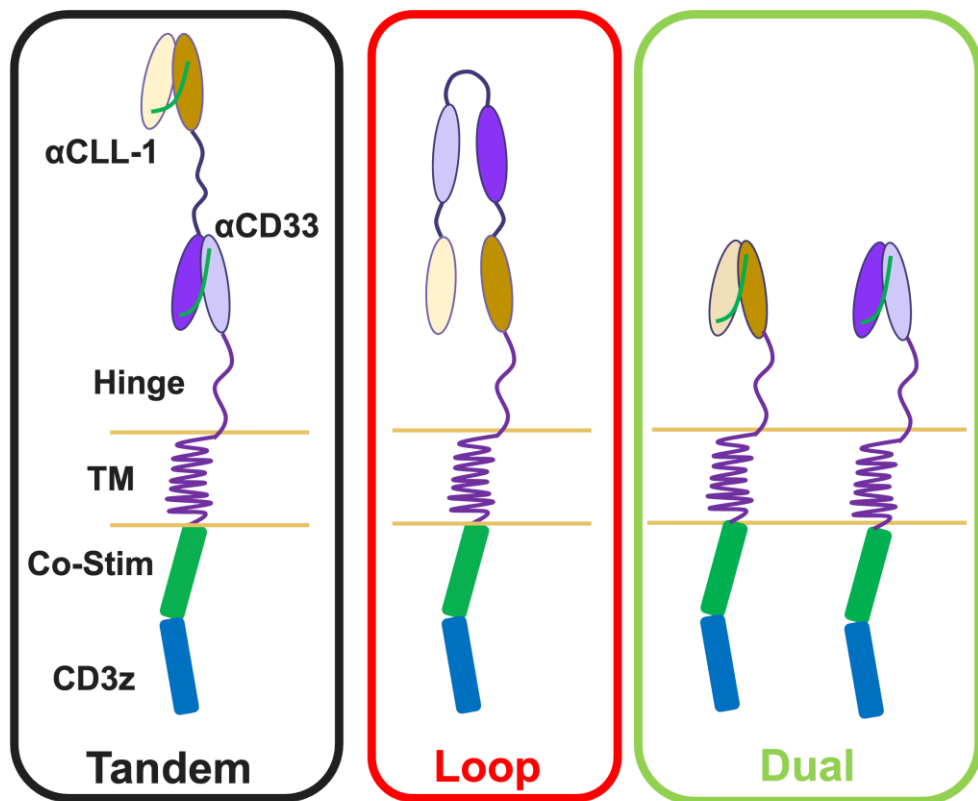


# Optimal Multi-Specific CAR Format





# Dual CAR-T Cells Displayed The Highest *in vitro* Cytotoxicity



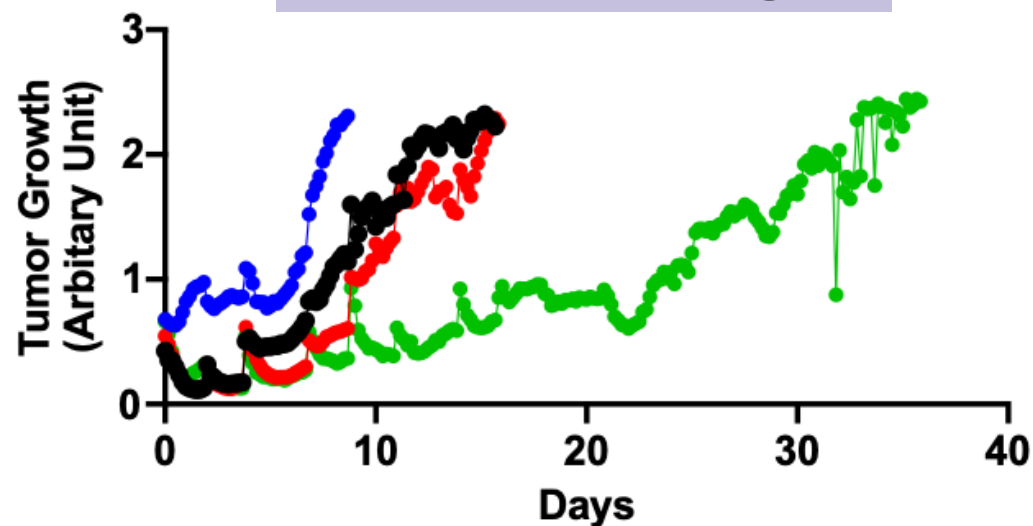


# Dual CAR-T Cells Displayed The Greatest Long-term Persistence

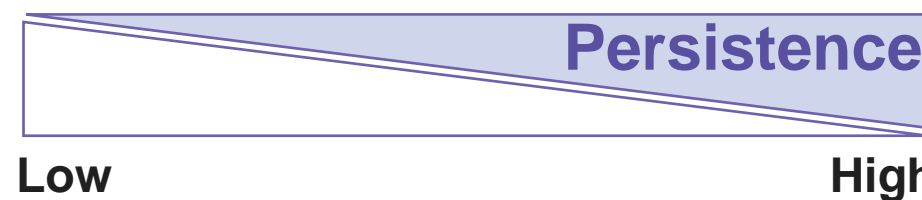
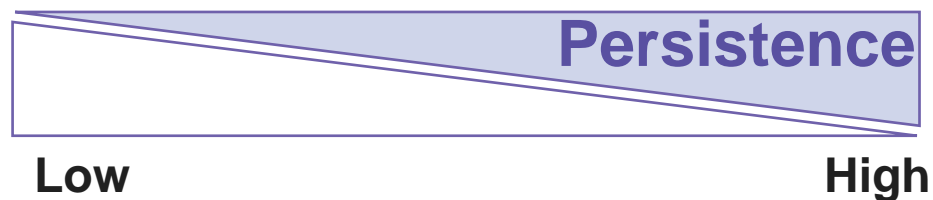
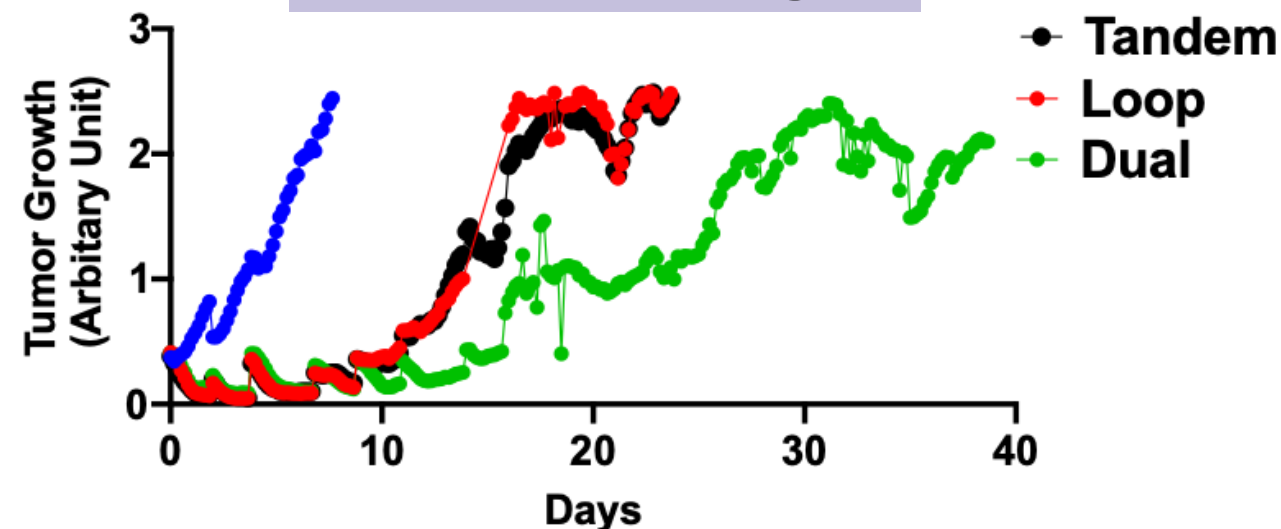
Multi-Specific Dual CAR-T cells Exhibited Improved Antigen-dependent Cytolytic Activity With Increased Persistence *in vitro*.



HL60-CD33KO Targets



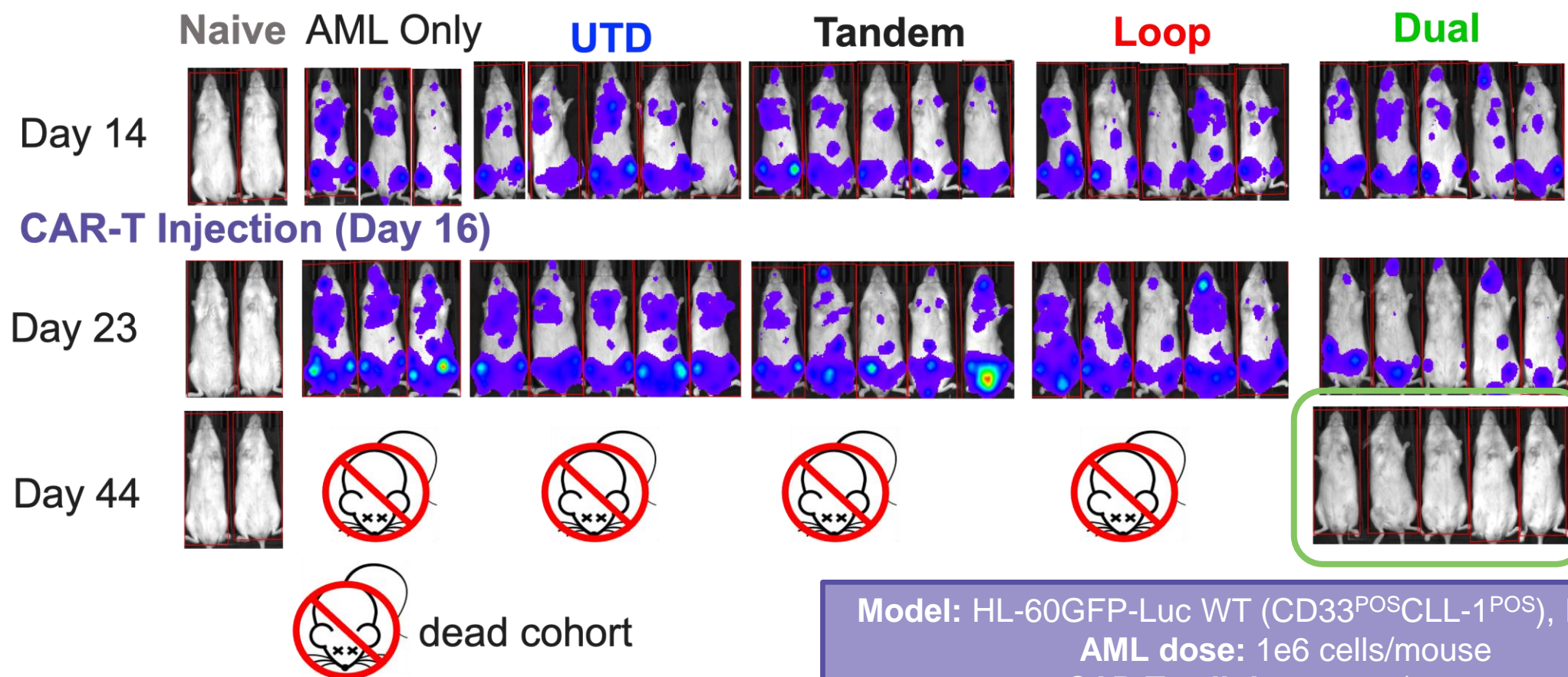
HL60 CLL-1KO Targets





# Dual CAR-T Cells Display Robust and Rapid *in vivo* Activity

Multi-Specific Dual CAR-T cells Showed Potent and Sustained Activity *in vivo*.

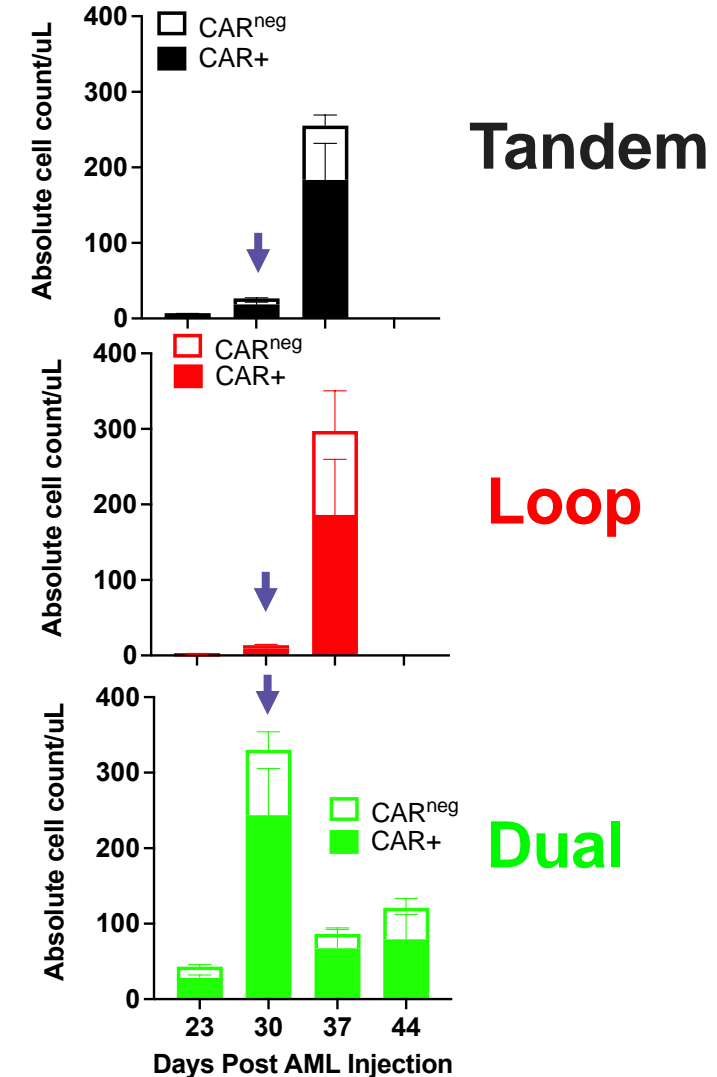
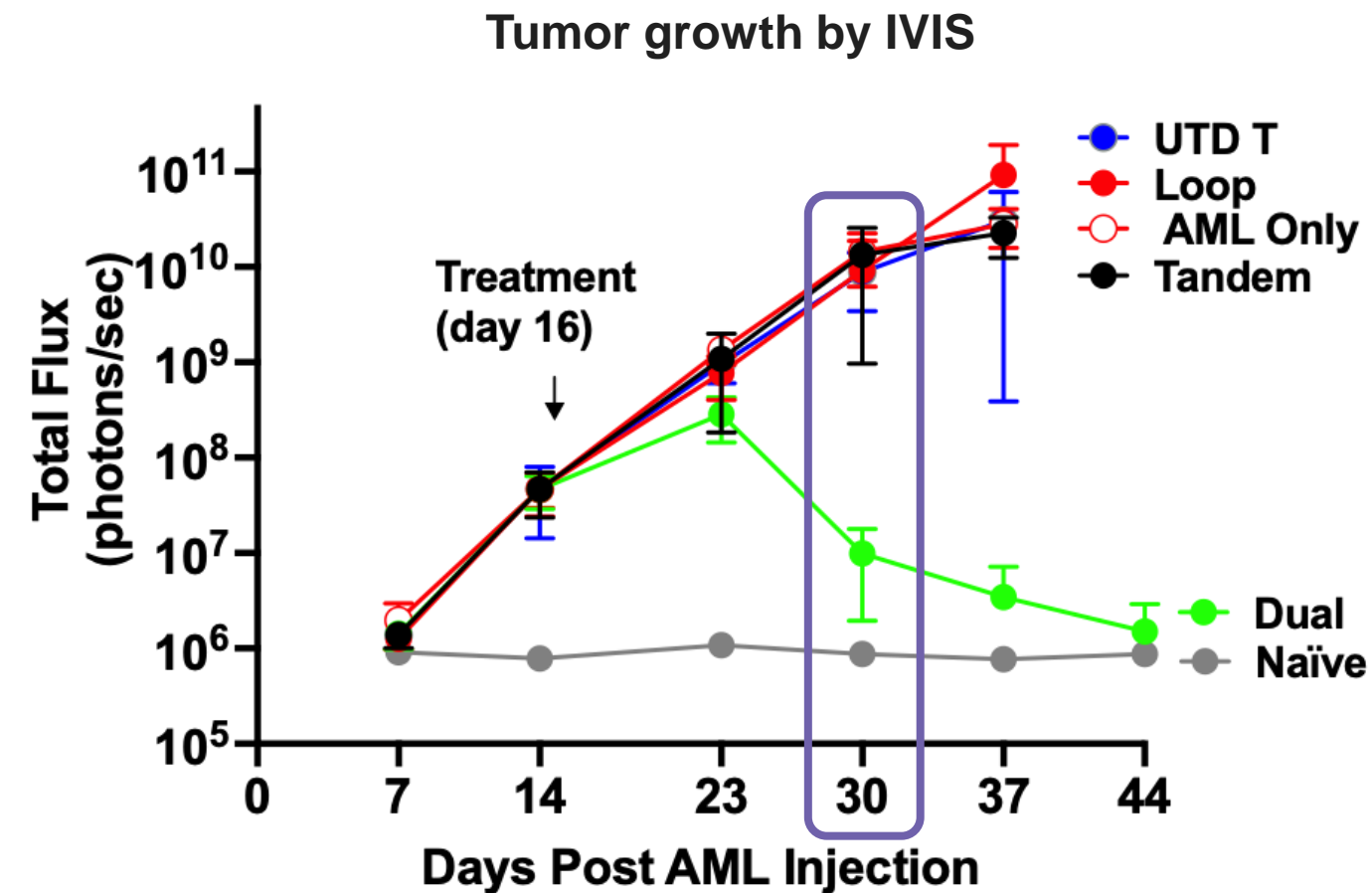


Model: HL-60GFP-Luc WT (CD33<sup>POS</sup>CLL-1<sup>POS</sup>), NSG mice  
AML dose: 1e6 cells/mouse  
CAR T cell dose: 5e6 /mouse  
Time of CAR T infusion: day 16



# Dual Format CAR-T Cells Display Rapid *in vivo* Activity

Multi-Specific Dual CAR-T cells Displayed the Earliest Peak of Expansion and Long-Term Persistence *in vivo*.



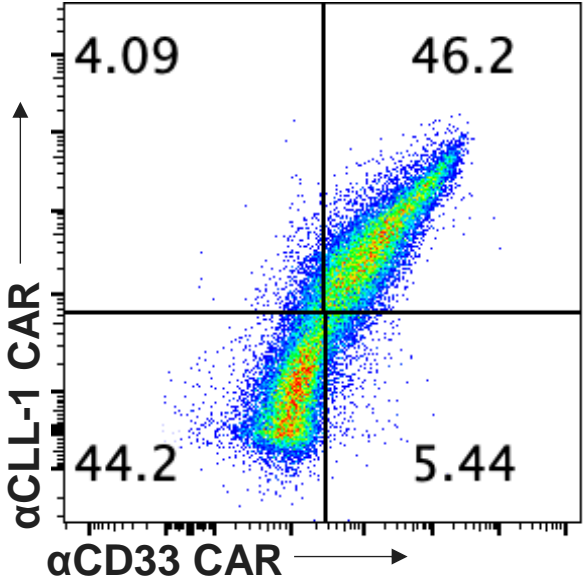
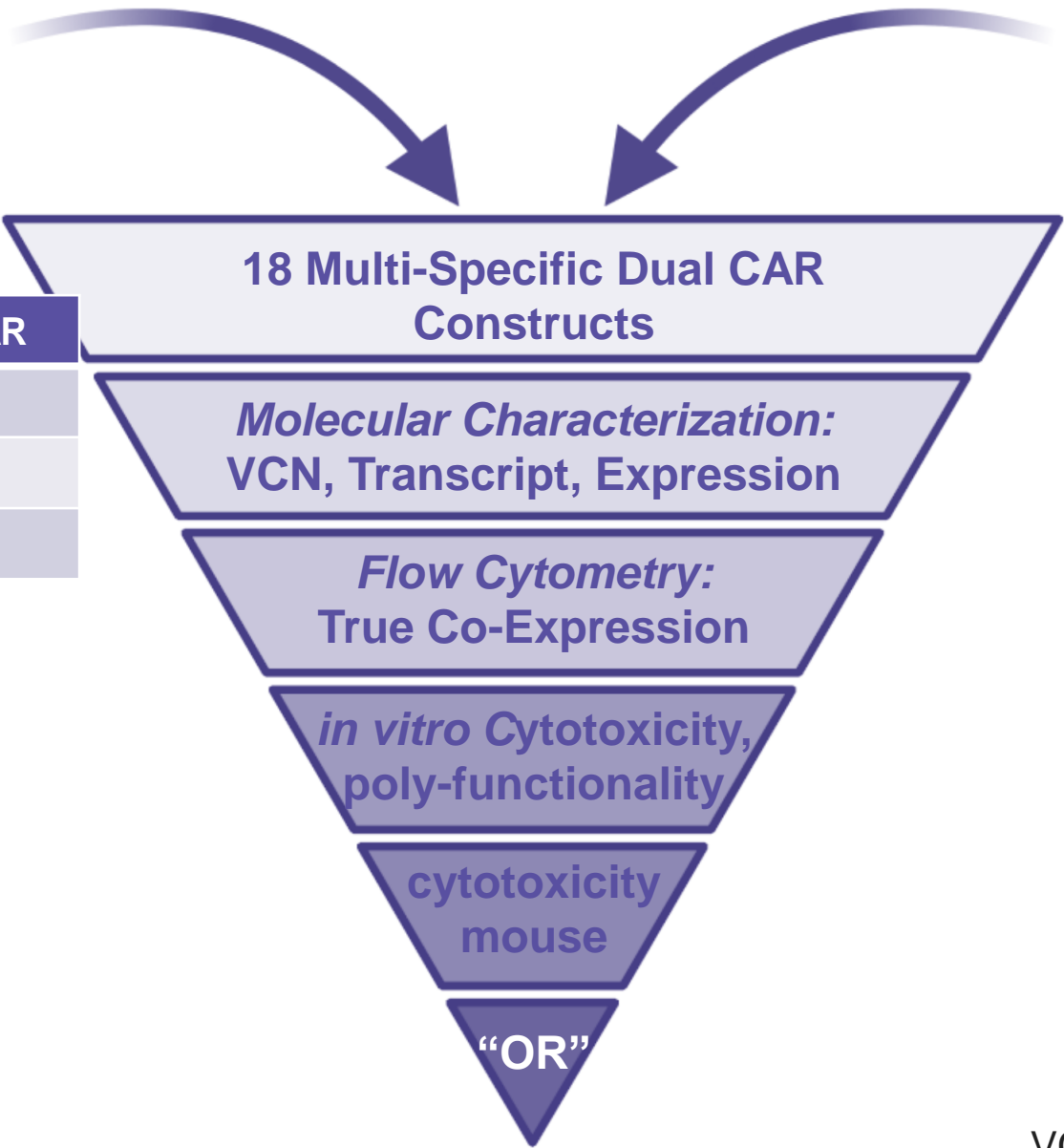


# Generation and Screening of 18 Multi-Specific Dual CAR Constructs

3 combinations  
of co-stimulatory  
domains

3 αCLL-1 CARs  
2 αCD33 CARs

	CLL-1 CAR	CD33 CAR
Dual 1	CD28	4-1BB
Dual 2	4-1BB	CD28
Dual 3	4-1BB	4-1BB

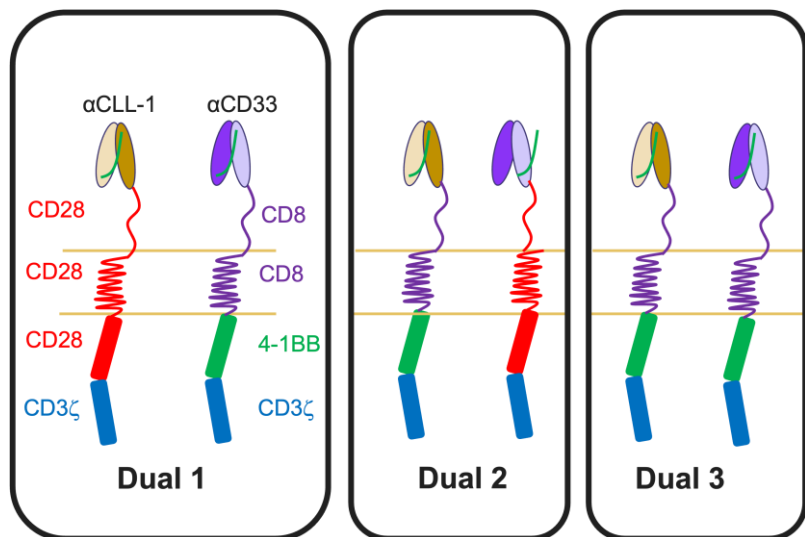






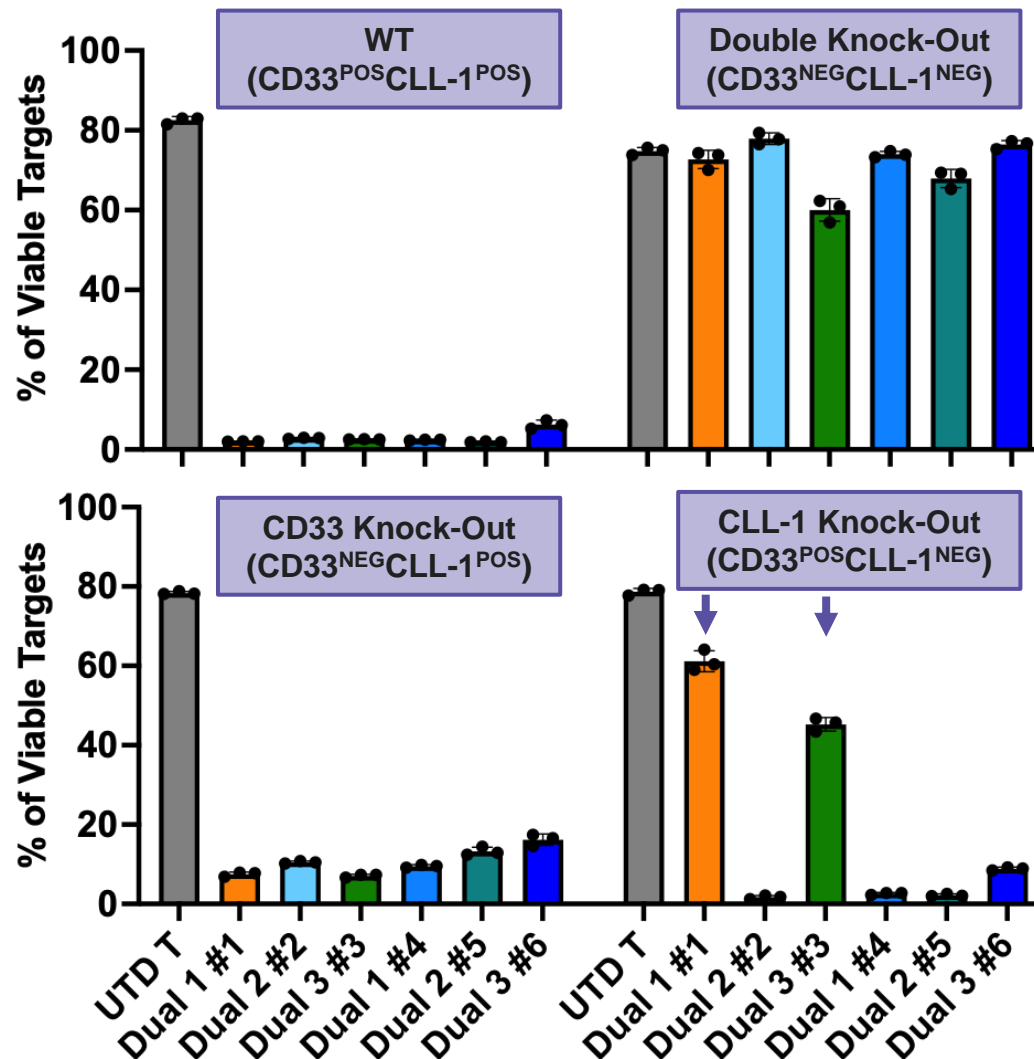
# Selection of 12 Dual Candidates Based on *in vitro* Cytolytic Activity

CD33-specific *in vitro* Activity Distinguished Successful Candidates.



	CLL-1 CAR	CD33 CAR
Dual 1	CD28	4-1BB
Dual 2	4-1BB	CD28
Dual 3	4-1BB	4-1BB

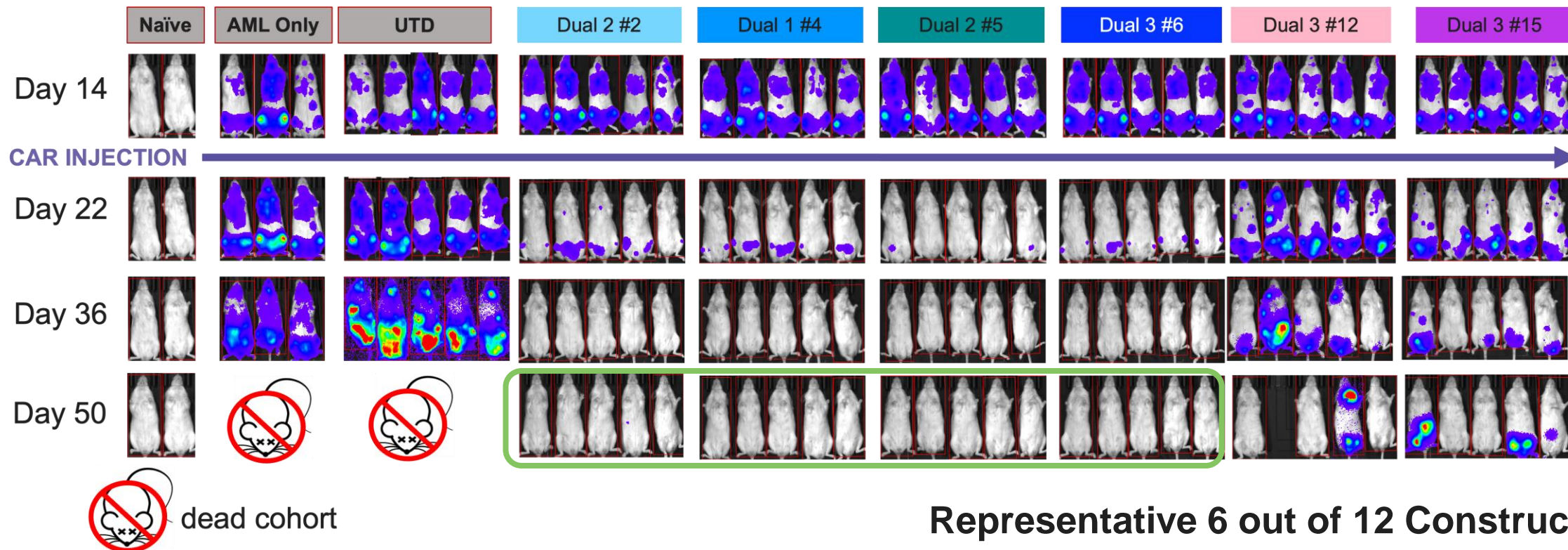
Representative 6 out of 18  
Constructs





# Lead Constructs Showed Potent and Persistent Activity *in vivo*

Immediate and Complete Killing of CD33/CLL-1 double-positive AML cells.



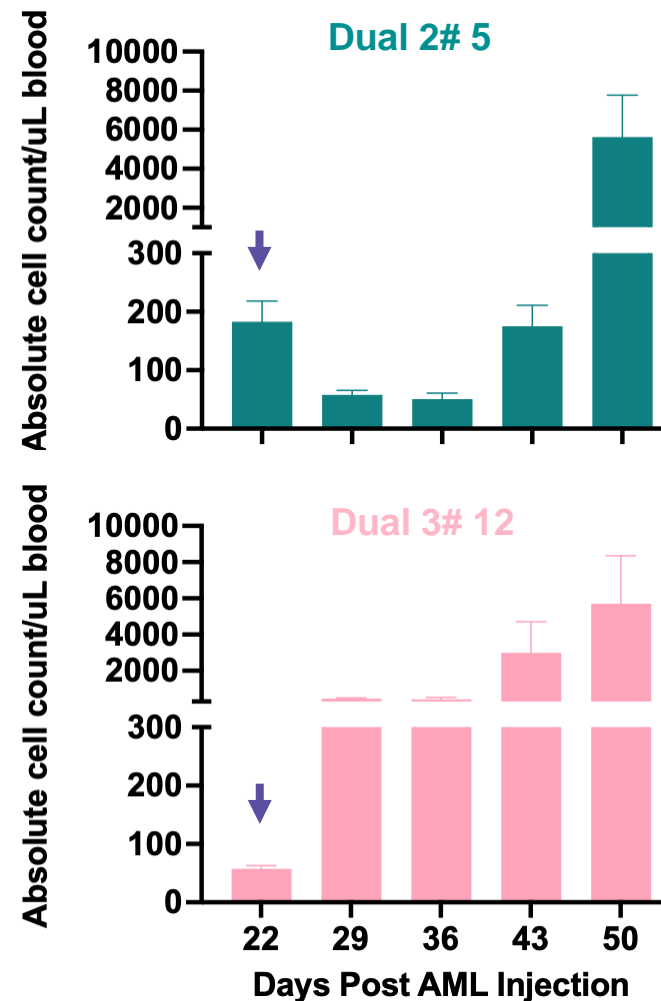
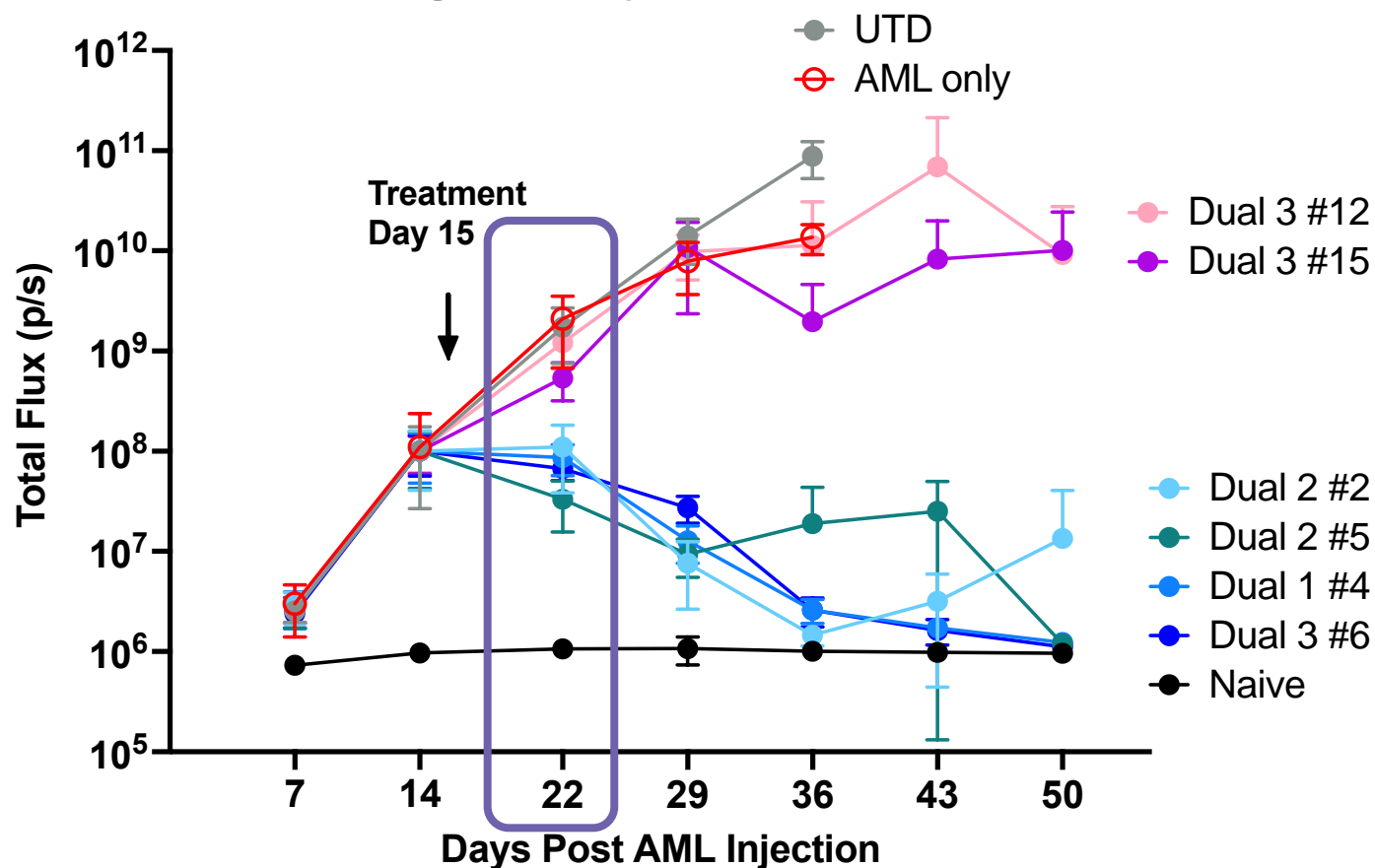
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Time of CAR T infusion: day 15



# Lead Constructs Showed Potent and Persistent Activity *in vivo*

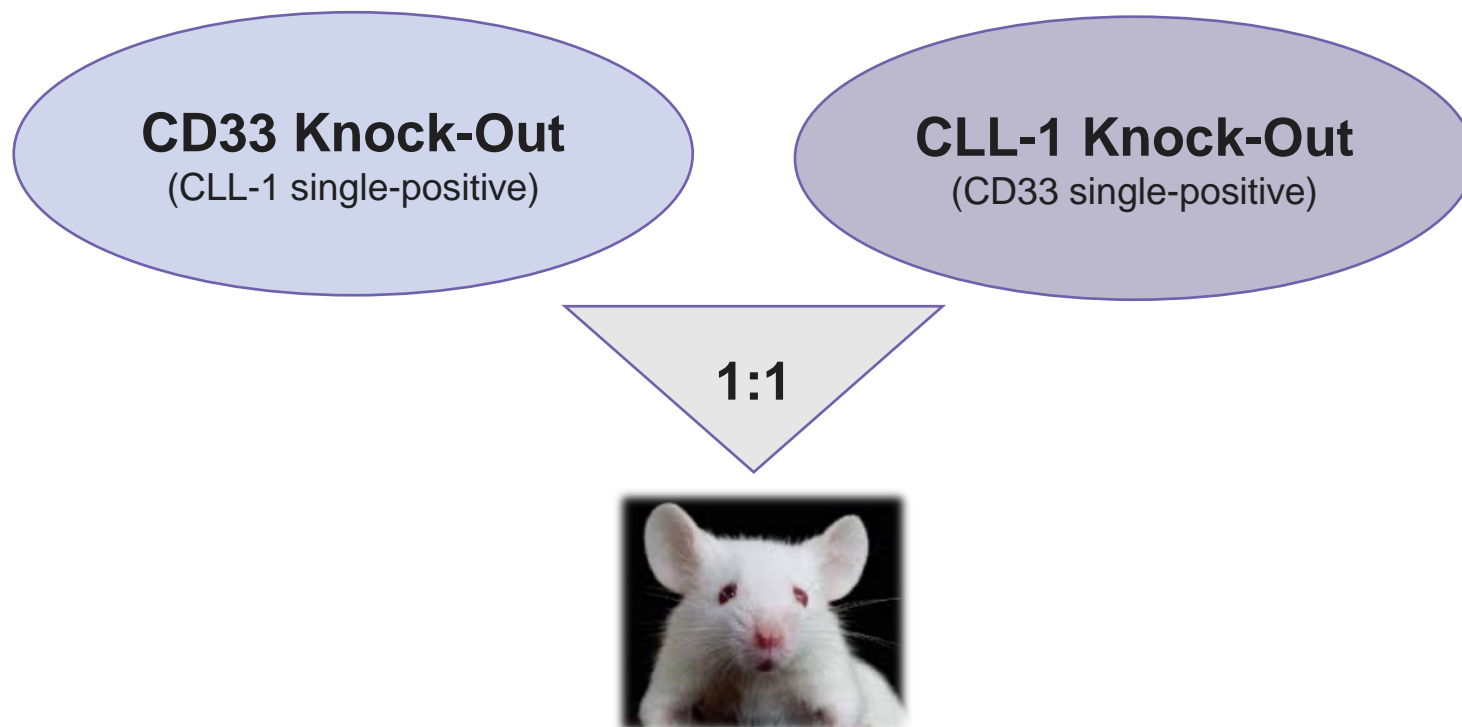
Lead Construct Shows the Fastest *in Vivo* Anti-Leukemic Activity, With Early and Reduced T cell Expansion.

Tumor growth by IVIS





# OR-Gate Validation in Model with Mixed Single-Positive AML



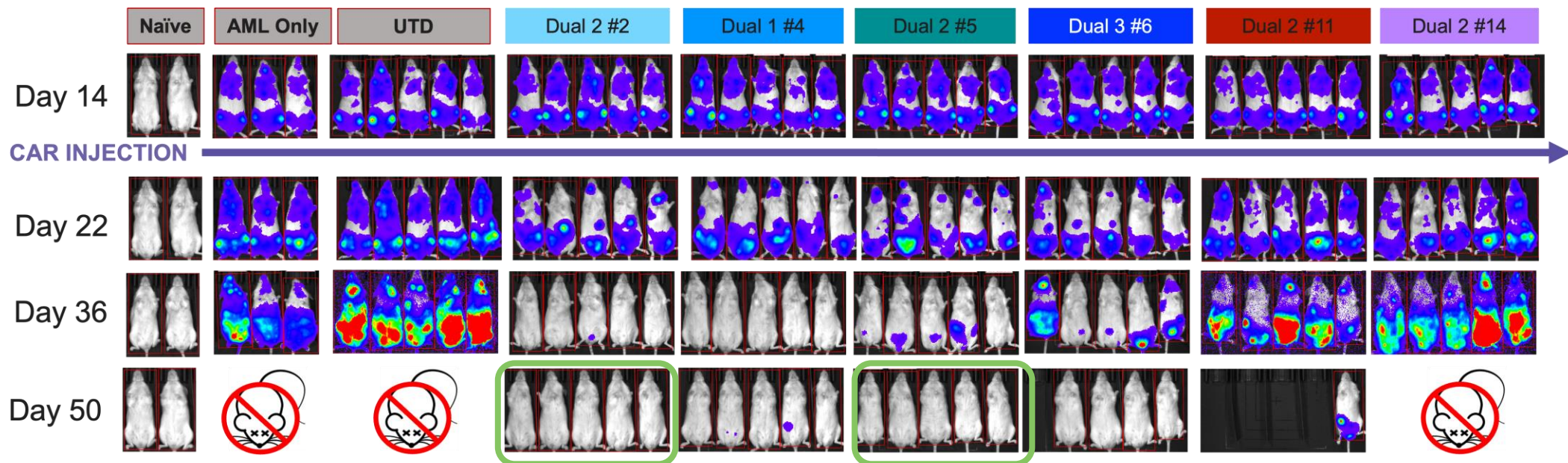
**Model:** CLL-1<sup>KO</sup>HL60-GFP-Luc + CD33<sup>KO</sup>HL60-GFP-Luc, NSG mice  
**AML dose:** 1e6 cells/mouse  
**CAR T cell dose:** 5e6 /mouse  
**Time of CAR T infusion:** day 15





# OR-Gate Validation in Model with Mixed Single-Positive AML

Immediate and Complete Killing of Mixed CD33 + CLL-1 **single-positive** AML cells.



dead cohort

CD33 Knock-Out  
(CLL-1 single-positive)

CLL-1 Knock-Out  
(CD33 single-positive)

1:1



Final 6 CAR Candidates

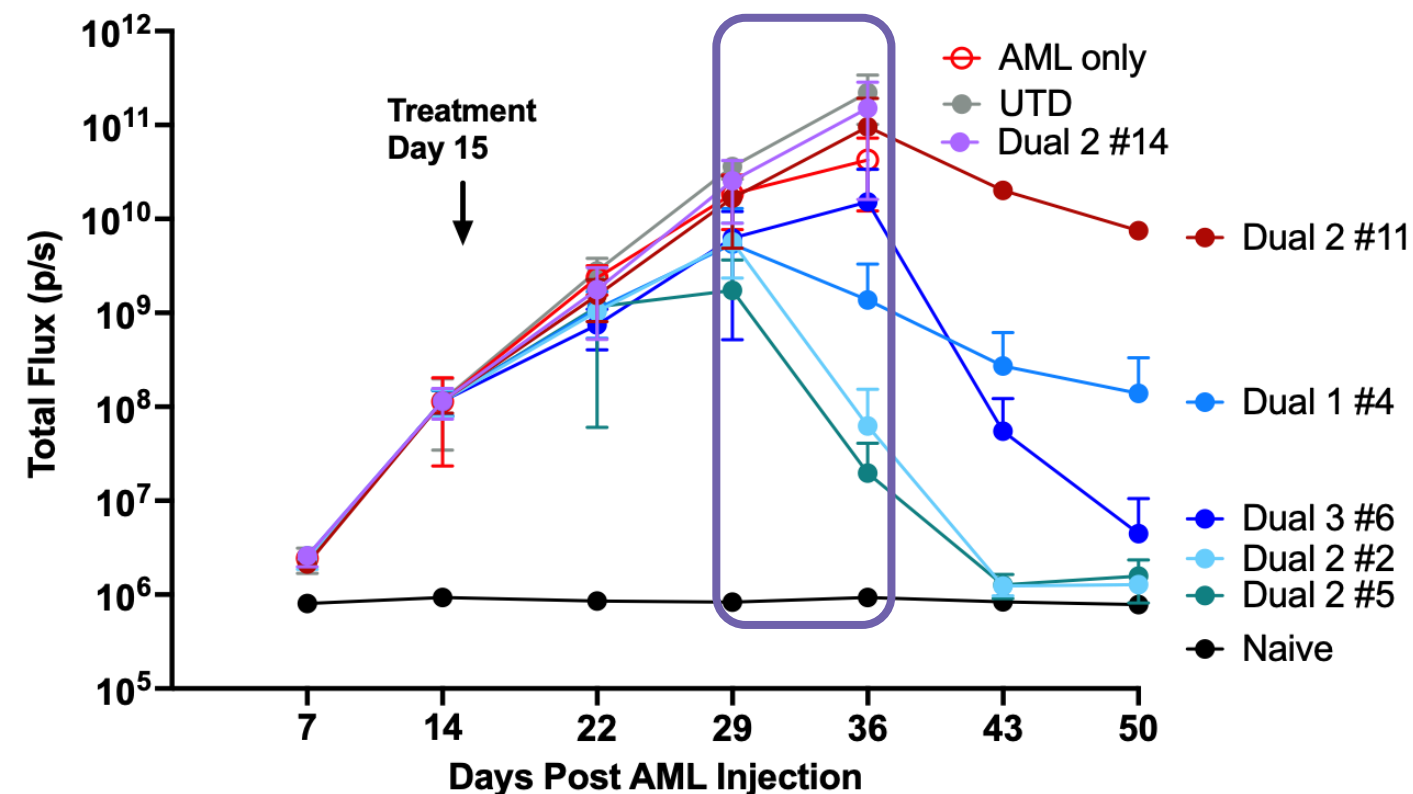
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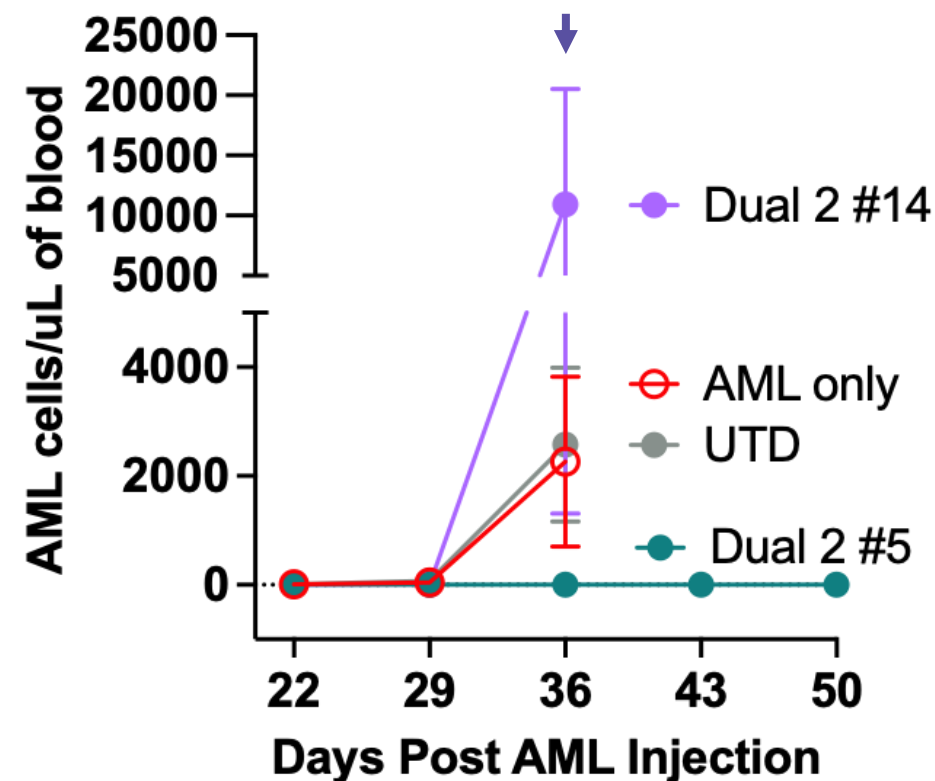
# Fast Activity With Complete AML Clearance by Lead Constructs

Lead Constructs Showed Fast and Sustained Activity With Complete AML Clearance in the Hematopoietic Compartments

Tumor growth by IVIS



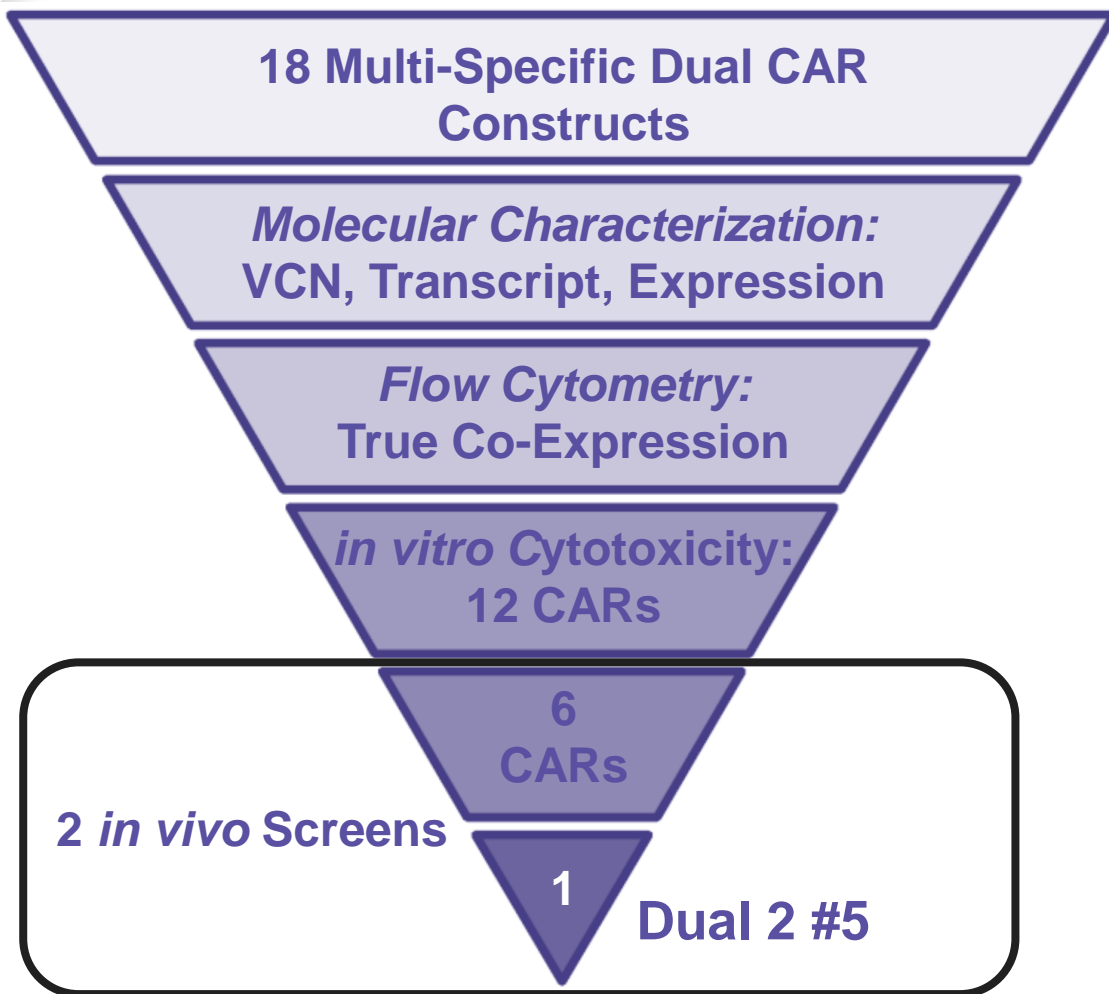
AML cells in Circulation







# Conclusions



- Dual targeting of CD33 and CLL-1 can cover a **larger patient population** and address blast heterogeneity.
- **Dual Format** was identified as optimal for the CD33 and CLL-1 target pair and **18 constructs** have been tested *in vitro* and *in vivo* for potency and specificity:
  - **Lead CAR construct showed potent, fast, and sustained *in vivo* anti-leukemic activity in an OR-gated fashion.**
- Further development of lead construct for a **bridge-to-transplant** clinical setting.



# Acknowledgements



B Gjerci, H Hoyt, N Patel,  
A Kharbanda, N Kleinberg,  
A Thomas, A Halfond,  
R Williams, J Lydeard,  
J Scherer, T Chakraborty

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