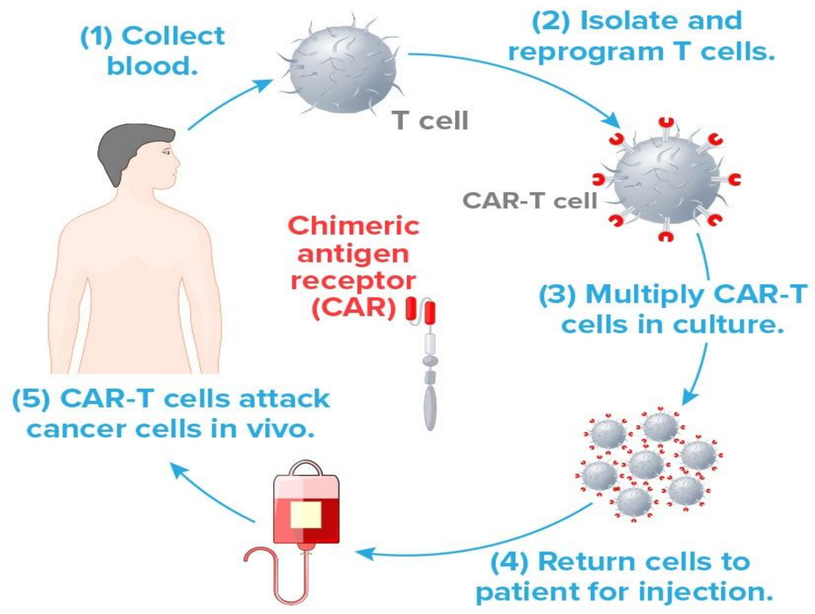
Developing a Novel CD371 Targeting CAR-T Cell Therapy for Acute Myeloid Leukemia

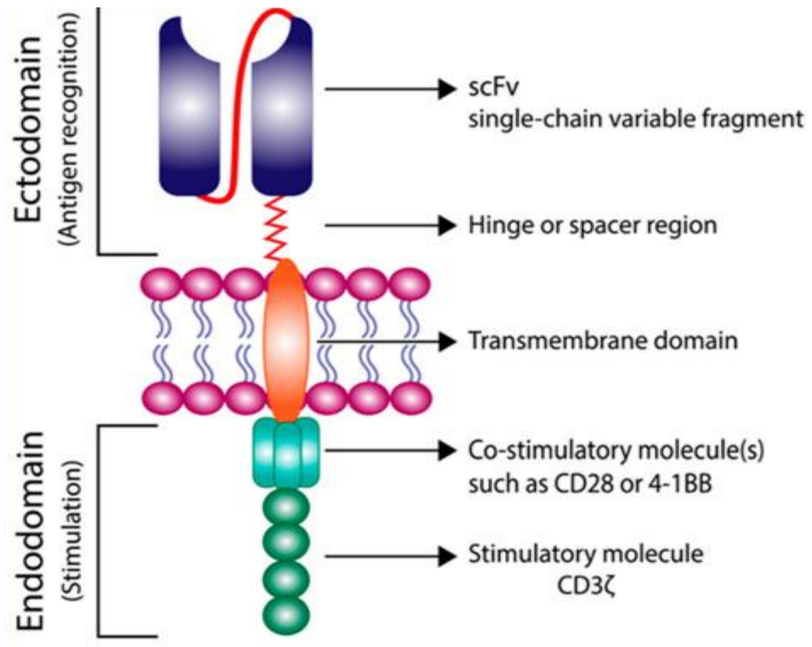
Presenter: Serena Mathew, MD1
serena.mathew@uqconnect.edu.au

INTRODUCTION



(1) **CAR T Cell Therapy**: Patient T cells are genetically engineered in the laboratory to recognize and bind specific antigens on cancer cells. T cells are isolated from patient blood and CARs are inserted on their cell surface. A "living drug", these CAR T cells can now bind and kill cancer cells. After growth and expansion in the lab, the CAR T cells are re-administered to the patient intravenously.

- While novel immunotherapies hold great promise for cancer treatment, chimeric antigen receptor (CAR) T cell therapy poses a challenge for treatment of acute myeloid leukemia (AML).
- Antigens expressed on AML cells are shared with healthy hematopoietic progenitor cells (HSPCs), so targeting them can lead to life threatening toxicity.
- CD371 is a reasonable target due to its expression on leukemic cells and lack of expression on HSPCs.
- In addition to target specificity, orientation between single chain variable fragment (scFV) antibodies may optimize CAR-T cell functioning.



(2) **CAR Design:** A CAR has an ectodomain, transmembrane domain and endodomain. The ectodomain contains a scFV (with heavy and light chain regions connected by a linker) to recognize tumor antigens, and a spacer to provide binding flexibility. The transmembrane domain connects the ecto- and endodomain. The endodomain transduces signal and is composed of one or more co-stimulatory molecules.

METHODS

CAR Design

- We engineered a CD371 targeting CAR in two orientations: B10H4Lmt28z and B10L4Hmt28z, in which the scFVS were synthesized in light-heavy (LH) and heavy-light (HL) orientations.
- These CARs include an anti-human CD371 sequence, human CD28 transmembrane/intracellular domain, and a repeating glycine-serine linker (G4S linker) with 19 amino acids between heavy and light chains.
- Non-functional truncated EGFRt (Et) is also expressed for tracking and selection of engineered T cells.

In Vitro Cytotoxicity Assay

- Cytotoxicity was assessed using a luciferase-based co-culture assay.
- AML tumor cells expressing green fluorescent protein (GFP) were incubated at a 1:20 effector to target ratio over 7 days.
- Bioluminescence of each well was detected using the Incucyte Live-Cell Analysis System.

In Vivo Survival

- Immunocompromised mice were inoculated with luciferase-expressing tumor cells (U937) on Day 0.
- CAR T cells (donor A1, A2) were injected on day 3, and cell survival was measured via bioluminescence imaging.

RESULTS

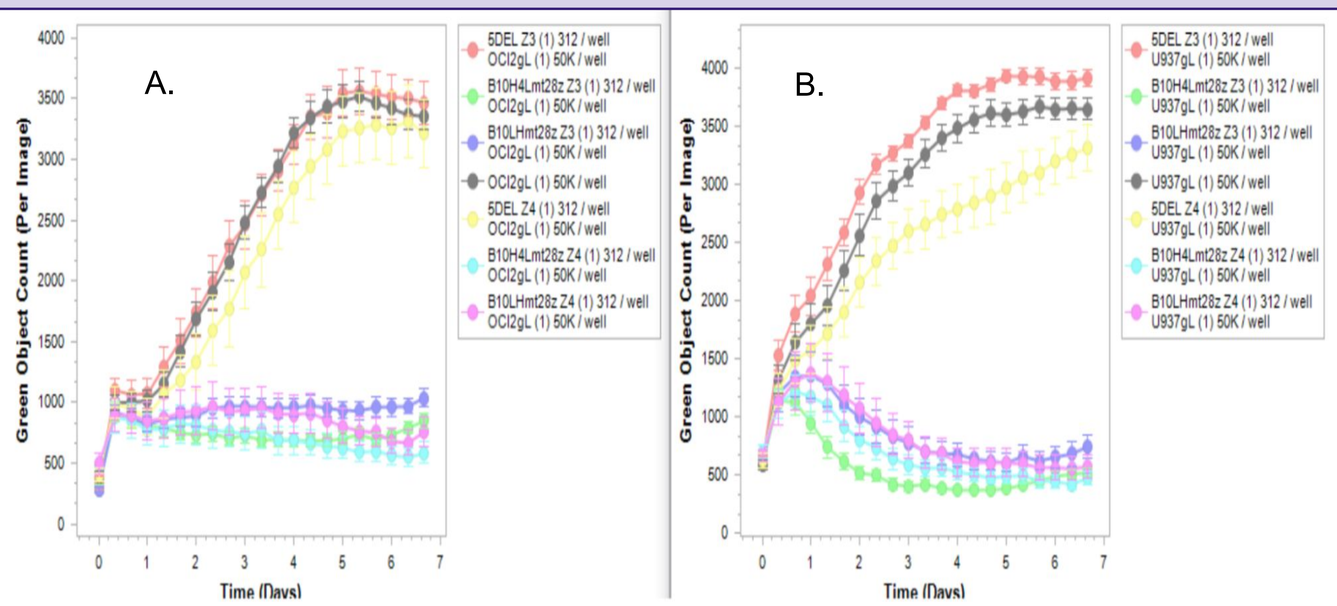


Figure 1. In vitro luciferase based cytotoxicity assay shows enhanced cytotoxicity of LH and HL constructs against OCI2 (A) and U937 (B) AML tumor lines as compared to deleted CAR construct in two donors (Z3, Z4).

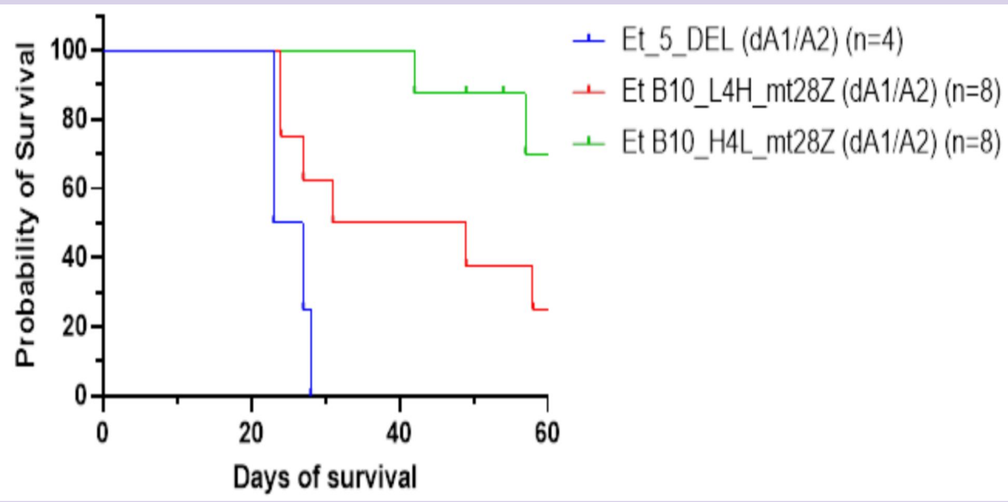


Figure 2. In vivo survival indicates enhanced HL potence as compared to LH and deleted CAR.

DISCUSSION

- We observed that both LH and HL constructs display enhanced killing capacity upon exposure to AML cell lines (**Figure 1**).
- In vivo, both constructs show longer percent survival post T-cell injection.
- Survival was further enhanced in the EtB10H4Lmt28z subset, suggesting HL CAR T cells are more potent than LH (**Figure 2**).
- **Our results suggest that in addition to target specificity, the orientation of V_L/V_H also affects CAR-T efficacy.**
- **We propose that this optimized CAR design could contribute to a novel CD371 targeting CAR-T therapy for the treatment of AML.**

Affiliations and Acknowledgements

This research is currently in progress at Memorial Sloan Kettering Cancer Center in New York.

Anthony F. Daniyan, MD
Memorial Sloan Kettering Cancer Center, New York, NY, USA 10065
Reiner J. Brentjens, MD, PhD
Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA 142

References

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