Abstract: P438

Title: A CLL1 AND CD38 DUAL TARGETING FASTCAR T THERAPY FOR ACUTE MYELOID LEUKEMIA

Abstract Type: Poster Presentation

Topic: Acute myeloid leukemia - Biology & translational research

Background:

AML is a group of heterogeneous hematological malignances with a high incidence and few effective and durable therapeutic options. CAR T-cell therapies targeting either CD33 or CD123 have been under clinical studies but with severe toxicity and limited efficacy. Targeting AML through multiple antigens may overcome antigen escape and improve clinical benefits.

Aims:

To study the preclinical efficacy and safety of CLL1 and CD38 dual targeting FasTCAR T therapy in AML.

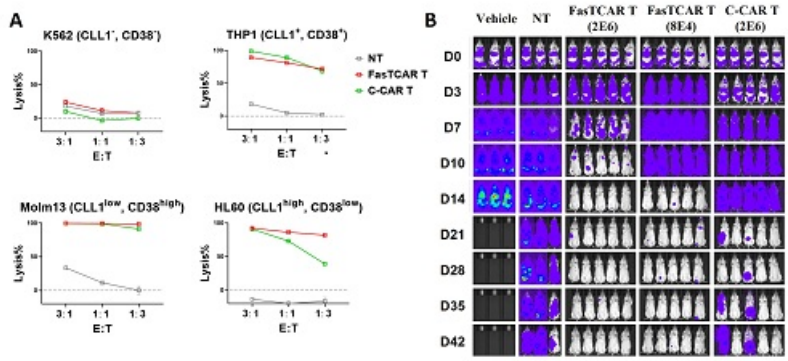
Methods:

CLL1- and CD38-specific antigen binders derived from camelid nanobodies (VHH) were developed in-house. A VHH with a moderate affinity for CD38 was selected to minimize fratricide. The dual targeting CAR T was generated with the FasTCAR platform. Phenotypic and functional characteristics of the FasTCAR T and its conventional counterpart (C-CAR T) were evaluated *in vitro* by co-culturing CAR T cells with AML cell lines and *in vivo* with xenograft mouse models. Off-target binding of VHH was investigated by protein arrays or by co-culturing CAR T cells with human primary cell lines. On-target off-tumor toxicity was investigated by colony forming assay using hematopoietic stem cells (HSCs).

Results:

Compared with C-CAR T, the dual targeting FasTCAR T expressed a younger phenotype by having more stem cell-like memory T cells (TSCM) and showed similar viability and expansion capability as non-transduced T cell (NT) post thaw *in vitro*. The FasTCAR T was able to efficiently and specifically lyse AML cell lines with differing target expression profiles (Figure 1A) and secrete more pro-inflammatory cytokines. The FasTCAR T also elicited more durable and potent responses when repeatedly challenged with target cells. Whereas limited on-target off-tumor toxicity toward HSCs was observed in colony forming assays, no off-target killing of several human primary cell lines was detected. *In vivo* studies revealed that the dual specific FasTCAR T eradicated tumor cells in a disseminated dual antigen-positive AML mouse model with only a fraction (4%) of the dosage of C-CAR T and maintained extremely low tumor burden without relapse even 7 weeks post dosing (Figure 1B). CAR T cells were detectable by flow cytometry in the peripheral blood by day 7 and peaked between days 14 and 21. Animals dosed with dual FasTCAR T did not manifest observable acute toxicity.

Summary/Conclusion: The CLL1 and CD38 dual targeting FasTCAR T cells exhibited specific and effective cytotoxicity in a dose-dependent manner both *in vitro* and *in vivo* with minimal toxicity. The preclinical efficacy and safety data support the clinical development of the dual FasTCAR T in patients with AML.



Keywords: Acute myeloid leukemia, Cellular immunity, CAR-T