DEVELOPING "OFF-THE-SHELF" CLL1 CAR-DNT THERAPEUTICS FOR THE R/R ACUTE MYELOID LEUKEMIA

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INTRODUCTION

Patients with relapsed/refractory (R/R) Acute Myeloid Leukemia (AML) have a poor prognosis, with overall survival (OS) estimated at no more than 10% at 3 years(1, 2). Double negative T(DNT) cells are mature CD3 T cells without CD4 and CD8. It could be an ideal cell therapy candidate for AML as they have natural killing toward AML cell lines, and they have been successfully administered to relapsed AML patients with therapeutic benefit without causing GvHD(3,4). In our study, we are developing an allogeneic product of CAR-DNT cells targeting C-type lectin-like molecule-1 (CLL1), a promising target due to its over-expression in primary AML cells and leukemia stem cells, while absence on hematopoietic stem/progenitor cells(5).

AIM

- Develop a novel DNT cell therapy platform for allogeneic use.
- Develop CLL1 CAR-DNT for R/R AML treatment.
- Explore several enhancement strategies to improve the proliferation and persistence of CAR-DNT cells.

METHOD

Screening anti-CLL1 scFvs: Hybridoma clones were identified by FCM, ELISA and Beacon® screening. The selected scFvs were humanized and fused to the CAR construct containing 4-1BB and CD3zeta chain. Specificity toward human CLL1 antigen was verified by the MPA (Membrane Proteome Array) assay.
Testing GvH & HvG of DNT: Mixed lymphocyte reaction was conduced in vitro. Several controls were compared with DNT cells.
Manufacturing CLL1 CAR-DNT cells: Using Juventas's protocol, CAR-DNT cells were made from healthy donors.
Detecting the tumoricidal function in vitro: Effector cells were cocultured with AML cell lines (THP1 and HL60) by different E:T ratio. Repeated killing assay were conducted every other day.

RESULTS

Figure 1: The CLL1 CAR-T by lead humanized CLL1 scFv candidate demonstrated in vivo efficacy. (A) Violin plots illustrate CLL1 expression in AML and normal hematopoietic cells (5). (B) The specificity of lead candidate h1-3Q2 scFv binding to CLL1(CLEC12A) was confirmed by MPA. (C) The schematic diagram of HL60-luc cells transplantation and treatment. (D) Representative bioluminescence images of each group at indicated days.

Fig.1

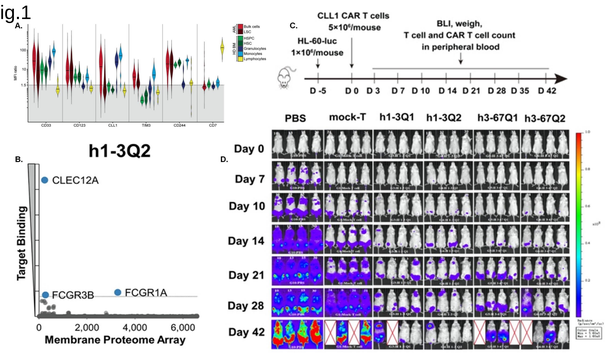


Figure 2: DNT cells show potential for use in allogenic cell therapy. (A) and (C) Schematic diagram of in vitro GvH and HvG assay setup. allo PBMC, allogenic peripheral blood mononuclear cell; allo UCBMC, allogenic umbilical cord blood mononuclear cell; DKO-T, TCR and B2M double knock-out T cell; TKO, TCR knock-out T cell (B) DNT cells do not kill allogenic PBMCs or UCBMCs in co-culturing experiment up to 72 hours, indicating a low risk of GvHD. (D) The killing effect of allo PBMC on control cells gradually increased over time, while no effect on DNT cells even after 192 hours, suggesting that DNT cells may not be easily cleared by host immune cells.

Fig.2

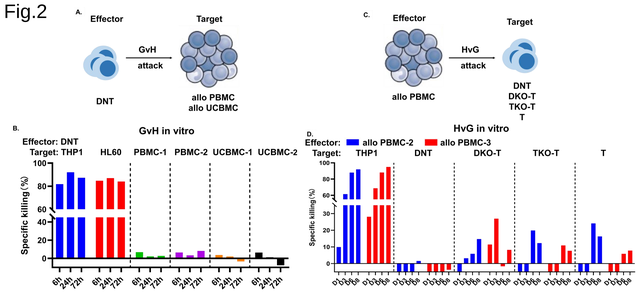


Figure 3: Preparation of CAR-DNT cells and their anti-tumor characteristics. (A) Proliferation of CAR-DNT cells from different donors. (B) CAR-DNT cells can effectively kill tumors losing antigens. (C) AML cell lines exhibit high sensitivity to DNT cells. (D) CLL1 CAR-DNT cells exhibited stronger and faster AML resistance compared with CAR-T cells. (E) show limited proliferation in the repeated killing assays.

Fig.3

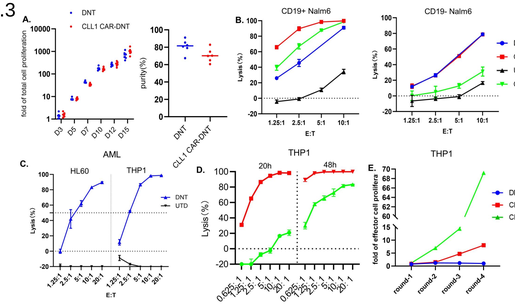


Figure 4: The immune function of CAR-DNT cells is enhanced by Tyrosine kinase inhibitors (TKI). Adding TKI during culture (A) improve the memory phenotype of CAR-DNT and (B) reduce the exhausted marker expression. (C) The schematic diagram of HL60-luc cells transplantation and treatment. (D) Representative bioluminescence images indicate that CAR-T and CAR-DNT with 3 infusions show comparable anti-tumor effects. (E) Pharmacokinetics in blood is tested by Q-PCR and FACS.

Fig.4

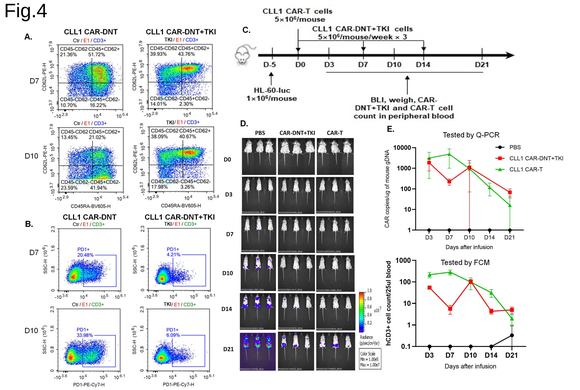
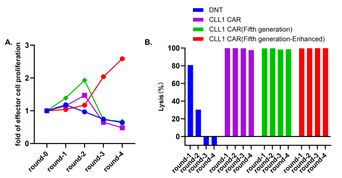


Figure 5: The next-generation CLL1 CAR-DNT cells exhibit improved proliferation (A) and tumor lysis(B) in the repeated killing assays.

Fig.5



CONCLUSIONS

1. We have obtained a humanized scFv specific to human CLL1.
2. DNT cells have the potential to be used as allogenic cell therapy product, they do not show GvHD and HvG in the in-vitro setting.
3. CLL1 CAR-DNT can be extensively proliferated in vitro, with potency killing on AML cell lines and faster tumor clearance. They also kill tumor cell that lack antigen expression. CAR-DNT proliferation would need to be improved.
4. TKI could enhance the immune function of CAR-DNT by increasing the percentage of Tscm and reducing the exhaustion marker PD1 expression.
5. The next-generation CAR-DNT with enhance showed improved proliferation as comparing to second generation CAR-DNT.

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