# (S330) FISRT-IN-HUMAN OF ALPACA-DERIVED NANOBODY-BASED BISPECIFIC EPITOPE CD5 CAR-T CELLS FOR RELAPSED OR REFRACTORY T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

**Topic:** 01. Acute lymphoblastic leukemia - Biology & translational research

Jing Pan[1, 2], Yuxuan Song*[1, 3], Ang Zhang[4], Su Li[5], Xiaoxia Hu[6]

*[1]State Key Laboratory of Experimental Hematology, Department of Hemato-Oncology and Immunotherapy, Beijing Gobroad Hospital, Beijing, China; [2]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China; [3]School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, China; [4]Department of Comprehensive Basic Experiment, The Ninth Medical Center of Chinese PLA General Hospital, Beijing, China; [5]Department of Hematology, Shanghai Liquan Hospital, Shanghai, China; [6]State Key Laboratory of Medical Genomics, Shanghai Institute of Hematology, National Research Center for Translational Medicine, Shanghai Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China;*

## Background

CD5 is expressed on the tumor cells of several hematological malignancies. We have designed humanized CD5 CAR-T (hCD5 CAR-T) cell for patients with relapsed or refractory T-cell acute lymphoblastic leukemia (r/r T-ALL), showing high remission efficacy (*Pan et al. Nat Med 2025*). However, its delayed peak expansion relative to other CAR-T therapies contributed to infections and tumor progression. Therefore, we developed a novel alpaca-derived nanobody-based bispecific epitope CD5 CAR (NbCD5 CAR) vector to accelerat expansion and improve safety and efficacy. Here we present its safety and efficacy of preclinical trial and a phase 1/2 trial of NbCD5 CAR-T in r/r T-ALL.

## Aims

We aim to accelerate CAR-T cells expansion, improving efficacy and safety in r/r T-ALL by screening novel NbCD5 CAR vector.

## Methods

We constructed an alpaca-derived nanobody library and screened it for CD5-specific nanobodies to engineer NbCD5 CAR-T cells. In contrast to scFv-based hCD5 CAR-T(*Pan et al. Nat Med 2025*), NbCD5 CAR-T uses a 15 kDa alpaca VHH domai for simpler structure and superior penetration. We assessed antigen-specific activation and in vitro cytotoxicity of CAR-T cells. In vivo efficacy was evaluated in Jurkat-engrafted NSG mice by flow cytometry and bioluminescent imaging. Ultimately, the efficacy and safety of NbCD5 CAR-T were evaluated in a multicenter phase 1/2 trial of r/r T-ALL (NCT07070323). The trial used BOIN1/2 design with initial dose $1\times10^6$ (±20%) CAR-T cells/kg and low-dose $5\times10^5$ (±20%) cells/kg. The primary endpoints was safety and efficacy secondary.

## Results

Flow cytometric CD107a degranulation assay confirmed that all CD5-targeting CAR-T cells (excluding controls) were specifically activated by $CD5^+$ target cells, with NbCD5 CAR-T demonstrating superior antigen-dependent activation compared to hCD5 CAR-T.

In vitro cytotoxicity assays, NbCD5 CAR-T cells showed potent, dose-dependent cytotoxicity specifically against $CD5^+$ target cells, significantly outperforming controls; minimal killing activity against $CD5^-$ K562 cells confirmed their antigen specificity.

In Jurkat-engrafted NSG mice, NbCD5 CAR-T on day 7 eradicated tumors and ensured long-term survival, outperforming Mock-T and hCD5 CAR-T.

We then evaluated NbCD5 CAR-T in a clinical trial for r/r T-ALL. The trial enrolled 6 patients (4 were refractory to prior CD7 CAR-T therapy). Three patients who were relapsed post-transplant received CAR-T cells derived from preivous transplant donors (Cohort B) and 3 patients were relapsed after chemotherpies and received CAR-T cells derived from new HLA-matched donors (Cohort C). All patients received $1.0\times10^6$/kg NbCD5 CAR-T cells. Adverse events within 30 days included: Grade 1 CRS (6/6), Grade 2 ICANS (1/6), and Grade 1~2 GVHD (2/6). All patients achieved complete remission by day 30. In a follow-up time of 90 days, 5 patients bridged to allo SCT, 4 patients remained $MRD^-$; 2 patients relapsed: 1 patient CD5dim relapsed at day 88, 1 patient $CD5^-$ relapsed at day 45 and died 6 days later. The peak expansion of CAR-T cells at day 10 (range: 7~21).

## Summary/Conclusion

The nanobody-based CD5 CAR we modified demonstrated excellent antigen specificity, cytotoxicity and efficacy in preliminaryin vitro and in vivo studies. Furthermore, we got the safety and efficacy profiles of NbCD5 CAR-T in r/r T-ALL. Our trial is still ongoing, it requires further investigation of long-term efficacy, safety and immune reconstitution.