ELSEVIER

Blood 142 (2023) 2062–2063

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Bio-Orthogonally Redirected Bispecific Lentiviral Vectors for In Vivo CAR-T Cell Generation

Heng Mei[1,2], *Zhaozhao Chen, Doctor*[3,4], *Yu Hu*[5,2,5], *Heng Mei*[6,5]

[1] Department of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[2] Hubei Clinical and Research Centre of Thrombosis and Haemostasis, Wuhan, China
[3] Hubei Clinical Medical Center of Cell Therapy for Neoplastic Disease, Wuhan, China
[4] Institute of Hematology, Union Hospital„ Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[5] Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[6] Union hospital of Huazhong University of Science and Technology, Wuhan, CHN

Background: Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a remarkably efficacious treatment modality in recent years for refractory and relapsed hematopoietic malignancies. However, the exorbitant cost and intricate manufacturing procedure of *ex vivo* CAR-T cell generation have impeded its broader application. In vivo induced CAR-T therapy offers the potential to circumvent cumbersome manufacturing logistics, thereby providing novel insights into the field of CAR-T treatment. In this study, we have developed a bio-orthogonally redirected bispecific lentiviral vector platform for the reprogramming of circulating T lymphocytes into CAR-T cells and subsequent elimination of tumor cells *in vivo*.
Methods: To engineer dual-targeting lentiviral vectors (D-LVs), the packing cell lines 293T was labelled with azide groups by glycometabolic bio-orthogonal chemistry (designated N $_3$-293T) firstly, and a CD3 promoter (CD3p) was fused to CAR transfer plasmid. Secondly, azide groups modified LVs were further surface engineered with anti-CD3 antibody (OKT3) via click chemistry. The conjunction of OKT3 and was analyzed using dot immunobinding assay, confocal microscopy and the *in vitro* transduction efficiency was evaluated using flow cytometry. To demonstrate the ability of targeted transduction and specific cytotoxicity *in vivo*, D-LVs were intravenously infused into humanized NOD-scid-IL2Rγnull (huNSG) mice engrafted with Nalm6-luc cells. Subsequently, the tumor burden was monitored using a noninvasive bioluminescence imaging system and the *in-vivo* CD19 -CAR-T cells existence were detected by flow cytometry.
Results: By displaying OKT3 on the single lentiviral surface and fused CD3p into the key construct, we could achieve targeted delivery of CD19-CAR genes to T cells both *in vitro* and *in vivo*.
Conclusion: These results demonstrate the great potential applications of this engineered lentiviral system as a new strategy for inducing CAR-T immunotherapy *in vivo* and a promising approach for leading personalized treatment.

Disclosures No relevant conflicts of interest to declare.

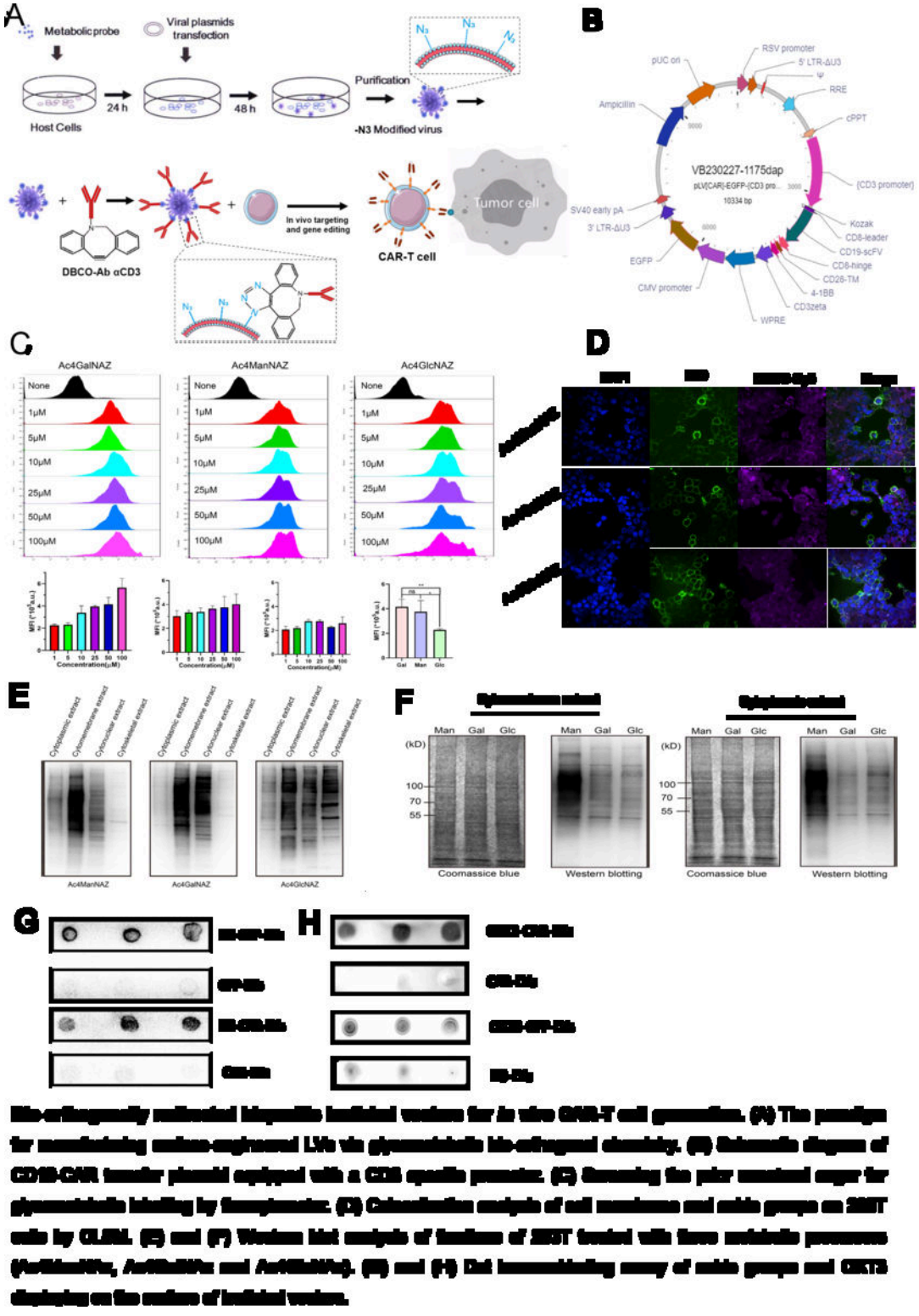

Figure 1

https://doi.org/10.1182/blood-2023-181855