



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

**Mitochondrial Isocitrate Dehydrogenase Inhibition Enhances CAR T-Cell Function By Restraining Antioxidant Metabolism and Histone Acetylation**Xiaohui Si, PhD<sup>1</sup>, Mi Shao<sup>2</sup>, Xinyi Teng<sup>2</sup>, Yue Huang<sup>3</sup>, Tianning Gu<sup>4</sup>, Gang Xiao, PhD<sup>5</sup>, He Huang<sup>6</sup><sup>1</sup> The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China<sup>2</sup> Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Med, Hangzhou, CHN<sup>3</sup> The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China<sup>4</sup> Bone Marrow Transplantation Center, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China<sup>5</sup> School of Medicine, Zhejiang University, Hangzhou, China<sup>6</sup> The First Affiliated Hospital, College of Medicine, Zhejiang University, Hematology, Hangzhou, China

**Introduction:** The efficacy of chimeric antigen receptor (CAR) T-cell therapy is hampered by relapse in hematologic malignancies and by hyporesponsiveness in solid tumors. Mitochondria are vital for the regulation of memory T-cell formation or exhaustion. A mitochondria-related compound screening was performed and we found that the FDA-approved isocitrate dehydrogenase 2 (IDH2) inhibitor enasidenib enhances long-lived memory CAR T-cell formation, and improves tumor clearance *in vivo*. IDH2 as the key component of the tricarboxylic acid (TCA) cycle, we hypothesized that IDH2 inhibition might promote CAR T-cell persistence by reprogramming the metabolism. The study aims to systematically evaluate the role and the mechanism of IDH2 inhibition on CAR T cells, and explore the application of enasidenib in CAR T-cell therapy.

**Methods and Results:** To identify mitochondrial components that affect the long-term efficacy of CAR T-cells, we performed an *in vitro* screening using a mitochondria-related compound library based on the enrichment of the CD62L<sup>+</sup> CAR T-cell subset, which mainly contains T memory stem cells (T<sub>SCM</sub>) and central memory T cells (T<sub>CM</sub>). Among several candidate compounds that promoted memory CAR T-cell formation, enasidenib (ENA), an inhibitor of both wild-type and mutant IDH2 enzymes, was the most effective one. The proportion of CD62L has increased by over 20%. To determine the effect of ENA on exhaustion induced by tonic CAR signaling and tumor antigen stimulation, we measured the expression of inhibitory receptors PD-1, TIM-3, and LAG-3 and the level of apoptosis in freshly expanded CAR T cells and B-ALL encountered CAR T cells. CAR T cells treated with ENA exhibited reduced surface levels of those inhibitory receptors. We measured the production of granzyme B and IFN $\gamma$ , and tumor rechallenge assay in CAR T cells after ENA treatment to assess the effector function, ENA treated CAR T cells exerted enhanced and sustained cytotoxicity in a tumor rechallenge assay at an extreme E:T ratio of 1:10. These results were observed not only in CD19-41BB $\zeta$  CAR T cells, but also in CD19-28 $\zeta$  and GD2-28 $\zeta$  CAR T cells. CAR T cells expanded in the presence of ENA significantly prolonged survival of recipients after Nalm-6 infusion ( $p < 0.0001$ ), extended *in vivo* ENA treatment further enhanced CAR T-cell expansion and tumor suppression. The median survival was extended from 45 to 67.5 days. Consistent with the effect of ENA treatment, IDH2 knockdown increased the proportions of the T<sub>SCM</sub>/T<sub>n</sub> and T<sub>CM</sub> subsets, and alleviated CAR T-cell exhaustion. IDH2-knockdown CAR T cells had sustained cytotoxicity and higher CD62L expression after rounds of killing B-ALL cells *in vitro*.

To systematically explore the effects of IDH2 inhibition on the metabolism of CAR T cells, relative metabolite amounts was measured using mass spectrometry (MS). IDH2-inhibited CAR T cells exhibited a substantial reduction of metabolites in the TCA cycle, including succinate, fumarate, and malate, and in the glycolysis pathway, including lactate, phosphoenolpyruvate. Mechanistically, IDH2 inhibition reprogram central carbon metabolism of CAR T cells by redirects glucose carbon utilization from glycolysis to the pentose phosphate pathway. In addition, IDH2 limits cytosolic acetyl-CoA level to prevent histone acetylation that promotes memory cell formation.

**Significance:** Our study indicates that metabolic intervention in CAR T cells with the FDA-approved IDH2 inhibitor enasidenib can advance current treatment, with better tumor eradication and CAR T-cell persistence.

**Disclosures** No relevant conflicts of interest to declare.

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