

442-B

IL-18-ENHANCED NON-ACTIVATED CAR T-CELLS FOR IMPROVED METABOLIC FUNCTION AND THERAPEUTIC EFFICACY

¹Joseph Durgin, ¹Ashwin Sannecy, ¹Nandana Mukherjee, ¹Xiaoling Jin, ¹Andre Kelly, ¹Alexander Shestov, ²Jakub Svoboda, ¹Joseph A Fraietta, ³Carl H June, ¹Roddy O'Connor, ¹Saba Ghassemi*. ¹Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; ²Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA; ³University of Pennsylvania, Philadelphia, PA, USA

Background The success of CAR T-cell immunotherapy depends on the differentiation status and metabolic fitness of the CAR T-cell product. In current CART manufacturing protocols, T-cell activation leads to irreversible differentiation. Our recent work showed that nonactivated CAR T-cells can be generated within 24 hours, eliminating the need for activation or *ex vivo* expansion. We assert this process retains the maximal fitness of the CAR T-cell product.

Interleukin 18 (IL-18) is a proinflammatory cytokine that regulates T-cell activation and expansion. In adoptively transferred CAR T-cells, IL-18 supports the survival and CAR T-cells persistence, an important determinant of efficacy following adoptive transfer.^{1 2} IL-18 enhances the cytotoxic activity of T-cells, as well as their ability to produce IFN γ , which can further amplify the immune response.

Methods In a first-in-human clinical trial, we show that CD19-specific CAR T-cells expanded for 3 days only, co-expressing human IL-18 (CART19-IL18), result in 100% overall response rates in patients that had relapsed after or refractory to prior CAR T-cell therapy.³ Building on these promising results, we examined if IL-18 enhances the potency of nonactivated CAR T-cell in xenograft models of hematologic and solid tumor malignancies.

Results We show that IL-18 enhances anti-tumor function and durability in non-activated CAR T-cells compared with non-activated CAR T-cells expressing a control transgene (CART-GFP). To understand the mechanisms underlying the functional benefit of IL-18, we compared the metabolite content of CART19-GFP to those expressing IL-18 (CART19-IL18). Using an untargeted metabolomic screen, we show that IL18-expressing CAR T-cells produce 2-hydroxyglutarate (2HG) 8-fold higher than control CAR T-cells. Using LC-MS, we also compared the serum metabolome of patients before receiving, and 7 days after CAR T infusion. We found that the glycolytic intermediates glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) were significantly enriched in the serum of patients receiving IL-18-expressing CAR T-cells. Remarkably, the end product of glycolysis-lactic acid, was similar pre and post (day 7) infusion. These findings imply an efficient path of glucose metabolism (potentially mitochondrial) that limits the production and systemic accumulation of lactic acid.

Conclusions Understanding how IL-18 enhances CAR T-cell efficacy will improve the therapeutic potential of CARTs further. Developing approaches to boost nonactivated CAR T-cell function and long-term persistence will raise its therapeutic impact further. Importantly, our studies have the potential to significantly improve the efficacy and accessibility of CAR T-cell therapy for cancer patients.

REFERENCES

1. Chmielewski M, Abken H. CAR T cells releasing IL-18 convert to T-Bet(high) FoxO1(low) effectors that exhibit augmented activity against advanced solid tumors. *Cell Rep*. 2017;**21**:3205–3219.
2. Hu B, *et al*. Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. *Cell Rep*. 2017;**20**:3025–3033.

3. Svoboda J, *et al*. Interleukin-18 secreting autologous anti-CD19 CAR T-cells (huCART19-IL18) in patients with non-hodgkin lymphomas relapsed or refractory to prior CAR T-cell therapy. *Blood*. 2022;**140**:4612–4614.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.0442-B>