BIO_01 - Immune Checkpoint Blockade via PD-L1 Potentiates More CD28-Based than 4-1BB-Based Anti-Carbonic Anhydrase IX Chimeric Antigen Receptor T Cells

Najla Santos Pacheco de Campos[1]; Adriano de Oliveira Beserra[2]; Eloah Rabello Suarez[1].
[1]Universidade Federal do ABC
[2]A.C. Camargo Cancer Center

Introduction: Chimeric antigen receptor (CAR) T cells are capable to be activated directly when in contact with a specific tumor antigen, becoming a new potent strategy against cancer. The complete regression of clear cell renal cell carcinoma (ccRCC) obtained pre-clinically with anti-carbonic anhydrase IX (CAIX) G36 CAR T cells in doses equivalent to ≅10^8 CAR T cells/kg renewed the potential of this target to treat ccRCC and other tumors in hypoxia. The immune checkpoint blockade (ICB) brought durable clinical responses for ccRCC in adjuvant settings and metastatic scenarios, becoming an important pillar treatment.

Objectives: This project tested CD8α/4-1BB compared to CD28-based anti-CAIX CAR T cells releasing anti-programmed cell death ligand-1 (PD-L1) IgG4 for human ccRCC treatment *in vitro* and in an orthotopic NSG mice model *in vivo*.

Methodology: Lentiviruses containing the different CAR constructions to be tested were produced, concentrated, and the transduction efficiency was determined by flow cytometry and IgG secretion. The cytotoxic effects of anti-CAIX CAR T were analyzed by flow cytometry and lactate dehydrogenase activity. The secretion of IL-2 and IFNγ was determined by ELISA. The exhaustion status was determined by flow cytometry. Alanine (ALT) and aspartate (AST) transaminases activity was determined by spectrophotometry.

Results: Anti-CAIX CAR T cells were able to induce around 80% decrease in the viability of ccRCC cells *in vitro*. Using a ≅10^7 CAR PBMCs cells/kg dose in the *in vivo* orthotopic ccRCC model showed that anti-CAIX CAR T cells that release anti-PD-L1 promoted a significant reduction in tumor volume and weight, with the construction with CD28 showing more potent results compared to 4-1BB, preventing the induction of tumor metastases. Considering T cell exhaustion, the constructions with anti-CAIX CD28 CAR and anti-PD-L1 secretion and with 4-1BB CAR with or without anti-PD-L1 secretion showed reduced co-expression of PD-1, TIM-3, CTLA-4, and CD39 in viable tumor-infiltrating T cells. We evaluated the renal and hepatic function of the mice by measuring transaminases and creatinine and did not observe any type of toxicity.

Conclusion: Anti-CAIX CAR T cells secreting anti-PD-L1 can diminish T cell exhaustion and improve CAR T cell treatment of ccRCC *in vivo*, offering exciting new prospects for the treatment of refractory ccRCC and hypoxic tumors.

Keywords: CAR-T cells, Immunotherapy, Point-of-care