ORT_07 - Anti-HER2 CAR-T cells evaluation in a ovarian tumor preclinical model

Emmanuel Arthur Albuquerque Aragão; Luiza de Macedo Abdo; Eduardo Mannarino Correia; Layla Vieira; Sabrina Reis; Karina Hajdu; Leonardo Ribeiro; Martín Hernán Bonamino.

1Instituto Nacional de Câncer (INCA)
2Fundação Oswaldo Cruz (FIOCRUZ)

Introduction: Several studies select the HER2 receptor as a good target due to its specific overexpression in solid tumors.

Objectives: The objective of this work was to evaluate and compare the effectiveness in the production of two anti-HER2 CAR (4D5 and FRP5) in peripheral blood mononuclear cells (PBMCs), as well as their in vivo antitumor capacity.

Methodology: For this, PMBCs were isolated and electroporated with 2:1 transposase:transposon to CAR 4D5 or FRP5. Then, cells were cultured for up to 12 days, and receptor expression, memory, and exhaustion phenotype were analyzed at different times by flow cytometry. For in vivo antitumor evaluation, 3x10^6 SK-OV-3 cells (ovarian adenocarcinoma) were injected into the right flank of NSG mice; the animals were treated in different routes with CAR-T cells, and tumor volume and bioluminescence were monitored.

Results: A constant expression of both receptors was observed, reaching an average of 25% of CAR+ cells 12 days after transduction. On the eighth day of expansion, lymphocytes with different receptors exhibited a predominant phenotype of central memory (CD45RO+ CCR7+), followed by effector (CD45RO+ CCR7-) in the CD4+ and CD8+ subpopulations, with low levels of exhaustion receptors, especially in the CD8+ population expressing the FRP5 clone. In the in vivo assay, 8.9x10^6 total cells (1.3x10^6 CAR+) injected peritumorally into medium-sized tumors led to a regression in the CAR-4D5 group, albeit with deaths due to necrosis and adverse symptoms; in contrast, the CAR-FRP5 group was not responsive. A new experiment with 5x10^6 total cells (0.65x10^6 4D5 CAR+) in mice inoculated with tumors in earlier stages, led to complete remission in peritumoral treatment, and partial in intraperitoneal and intravenous treatment.

Conclusion: In summary, the two anti-HER2 CARs evaluated showed consistent expression in PBMCs from different donors, with a predominant central memory phenotype and low frequency of expression of inhibitory receptors. Functionally, the anti-HER2 4D5 CAR proved to be effective in the treatment of a solid tumor model of ovarian cancer, especially via the peritumoral route. In the future, the immunosuppressive context in the effectiveness of the treatment and possible adjuvant therapies to CAR will be evaluated.

Keywords: CAR-T cells, Immunotherapy, HER-2, Ovarian Cancer