ORT_11 - Generation of allogeneic 19BBz CAR-T using CRISPRGeneration of allogeneic 19BBz CAR-T using CRISPR

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Introduction: CAR-T cell immunotherapy despite being a promising technique, which redirects the T lymphocyte response to a specific tumor target through a chimeric antigen receptor (CAR), this therapy presents some limitations. These include manufacturing and use restricted to a single patient, low quality of starting material, manufacturing delays, and high cost (since most use viral vectors and are autologous). Thus, the approach of generating allogeneic CAR-T cells emerges, using non-viral vectors and CRISPR. In this way, genes involved in the graft-versus-host disease and in immunorejection can be knocked out, and the CAR sequence inserted using transposons, such as Sleeping Beauty (SB), or site-specifically recombined for the TCR alpha chain (KI), producing cells with less potential to cause graft versus host disease and still able to recognize the tumor.

Objectives: Therefore, the proposal of the present study is the generation of "universal" off the shelf CAR-T 19BBz cells using CRISPR and Sleeping Beauty.

Methodology: Cells were separated by density gradient and purified for CD3+ population. They were electroporated in Lonza 4D system with the components to the formation of the groups The modifications were analyzed by flow cytometry. *In vitro* assay was performed with a co-culture of CAR-T cells and Nalm6 tumor modeland the killing was analyzed by flow cytometry. Animals were inoculated with the tumor cells and treated 2 days after with CAR-T.

Results: The KI, SB, and RNP+SB groups exhibited antitumoral activity in vitro, with the lysis capability of RNP+SB being equal to or better than SB. The KI group received half the CAR dosage, so its antitumoral potential was lower compared to SB and RNP+SB. ELISA assays were performed to analyze cytokines that act on the anti-tumor activity by CAR-T cells, such as IFN-gamma and TNF-alpha, and the one that stimulates cell proliferation (IL-2). These were increased in the SB, KI, and RNP+SB groups, compared to the Mock (non-gene- modified) group. Additionally, a low percentage of regulatory T cells (Tregs) was observed. *In vivo*, the KI group stood out, presenting greater anti-tumor activity than the SB group. The RNP+SB group received nearly half the dosage of CAR-T cells per animal compared to the KI and SB groups, exhibiting reduced anti-tumor activity in comparison to both SB and KI.

Conclusion: Therefore, the generation of functional allogeneic CAR-T cells using CRISPR and Sleeping Beauty was achieved, showing potential to circumvent limitations related to autologous CAR-T cells.

Keywords: CAR-T; CRISPR; Sleeping Beauty