ORT.22 - Development of CAR T therapy for ALL-B using the point of care approach

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Introduction: The global cancer data released by the GLOBOCAN database showed 18.3 million new cases in 2018. About 440,000 of these cases correspond to leukemia, a cancer that urges for treatment modalities. Recently, CAR T-cell immunotherapy was approved for the treatment of acute B cell leukemias (B-ALL) and some lymphomas with promising results. However, the major drawbacks of CAR-T treatments are the high costs, being prohibitive for many of the patients. We developed an alternative low-cost approach to gene modify T cells to express CAR using the Sleeping Beauty (SB) system and electroporation. In addition, we show that it is not necessary to activate or expand T cells *ex vivo* when using this system, an aspect that renders this approach to a point-of-care (POC) strategy.

Objective: The objective of this present study is to demonstrate that the POC strategy is efficient in NSG animals xenografted with human leukemia when treated with the POC CAR-T cells.

Methodology: Peripheral blood mononuclear cells were isolated using Ficoll and electroporated using the Nucleofector II combined with plasmids enconding 19BBz CAR (in the pT3 SB transposon backbone) and the SB100x transposase. The phenotype was assessed by flow cytometry. The *in vitro* cytotoxicity assay was performed using Calcein-AM dye on target cells incubated with different ratios of effector cells. 8-12-week-old-female-NSG were injected iv. $5x10^6$ RS4;11 GFP and after 3 days were treated with different doses of recently electroporated CAR-T cells. All animal procedures were approved by the Animal Ethics Committee.

Results: The expression of CAR on the first day (d+1) was about 5% -15%. In addition, cell lysis assays against RS4;11 and Nalm-6 on d+1 showed no potential to eliminate the target cells *in vitro*, as expected. The *in vivo* experiments were performed with NSG mice engrafted with RS4;11 B-ALL cells followed by T cell injection 3 days later. Animals treated with 10^7 CAR-T cells (expression of approximately 10% of 19BBz) provided 100% survival when comparing the control and mock (electroporated without plasmids) group. The mock treated group has the ability to eliminate leukemia cells from the peripheral blood and showed better survival as compared to untreated control group. We were able to show a survival improvement even using reduced doses of lymphocytes, such as 10^6 or 10^5. Furthermore, we compared the same donor using the POC approach and expansion of CAR-T cells with anti-CD3/28 for 12 days (clinical methodology nowadays), and the results showed similar, indicating that the POC approach is interchangeable with the labor and cost-intensive expansion protocol.

Conclusion: This methodology proved effective in the animal model and is much cheaper when compared to the approved clinical approach for the use of CAR T cells. This aspect could grant greater access of patients to this very promising treatment strategy.

Keywords: CAR-T cell; Immunotherapy; Leukemia