BIO_08 - Improvement of CAR-T cell therapy using IL-15 membrane and anti-PD-L1 using different transposon vectors

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Introduction: Inducing the immune system to fight tumors has become one of the alternatives to treat cancer, among them there is the treatment with CAR-T cells, which consists of modifying the patient's T cells to express a chimeric antigen receptor (CAR). CARs will guide T cells to recognize and eliminate the tumor. This therapy has shown promise for some cancers, particularly B-cell-derived leukemia, and lymphoma. Despite its first FDA approval nearly 5 years ago, access to this therapy is still selective as it requires highly specialized and equipped laboratories, and its high cost. One of the main aspects is the use of viral vectors for CAR insertion. An alternative is to use transposon-derived non-viral vectors, such as Sleeping Beauty (SB) or PiggyBac (PB), which have already been shown to be safe and efficient. Furthermore, it is possible to add therapeutic molecules such as cytokines and checkpoint blockers along with the CAR to modulate the immune response and tumor microenvironment.

Objective: This project aims to compare two transposon-based techniques (SB and PB) to promote the expression of the 19BBz CAR transgene in combination with a membrane bound IL-15 and/or an anti-PD-L1 nanobody.

Methodology: Mononuclear cells were isolated using Ficoll and electroporated using Nucleofector IIb with plasmids encoding 19BBz, 19BBzmIL-15, 19BBzPD-L1, 19BBzmIL-15PD-L1 for SB or PB and their corresponding transposases. Cell phenotype was assessed by flow cytometry. The *in vitro* cytotoxicity assay was performed using Calcein-AM dye. For *in vivo*, NSG mice were injected iv. 10^5 Nalm-6 and treated with 0,1-0,5x10^6 CAR-T cells.

Results: The 19BBz has already been validated by our group using the SB approach, however, due to the size of the transgenes combinations, loss of efficacy in gene transfer was observed. We thus decided to use the PB vector to transfer our transgenes cassettes. By doing so, it was possible to detect the expression of mIL-15 and PD-L1 by flow cytometry. Different plasmid DNA rations were tested, and we determined 10ug transposon for 20ug transposase. The expression ranging from 30- 50% on day 1 to 20-30% on day 12 was observed along with expansion of T cells. *In vivo* experiment with NSG mice grafted with Nalm-6 are currently underway with all the conditions. Mice were treated 24h after cell production, without activation and expansion.

Conclusion: The results we obtained so far show that the SB vector does not efficiently insert our larger set of transgenes and that PB is a viable alternative. Using PB, although we can detect the expected transgenes, the expression decays with the passing of the days. Functional *in vitro* and *in vivo* assays are already being carried out.

Keywords: Immunotherapy; CAR-T cell; Transposons