ORT_16 - Piggybac transposon-based production of anti-HER2 CAR T cell

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Introduction: The PiggyBac (PB) system consists in a non-virus transposon/transposase gene delivering tool. Chimeric antigen receptors (CARs) are molecules capable of redirecting immune cells against a specific tumor antigen (Chicaybam et al, 2020). On the current study, the system was used to induce the expression of anti-HER2 CARs based on two different scFvs: 4D5 and FRP5.

Objective: The aim of this work is to evaluate the transposition efficacy of the CARs on different cell lines using the PB system.

Methodology: The two clones of anti-HER2 CARs were synthetized and cloned into the PBCAG plasmid vector. For transposition, HEK 293FT, JUKART cell lines and primary T cell were electroporated with the transposase and the 4D5 or FRP5 carrying plasmid. After electroporation, the cells were cultured up to 9 days and the receptor expression analyzed at different times by flow cytometry.

Results: The HEK 293 FT cell line were analyzed 24 hours after the transfection with 2 ug of transposon, exhibiting an expression of 31,3% and 14,9% for 4D5 and FRP5 CARs respectively. For the JUKART cell line, we used 5ug, 10ug or 20ug PB plasmid concentrations and evaluated CAR expression 24 hours after transduction. We noted a higher expression of the receptors when using 10 ug of plasmid, obtaining 35,6% and 38,1% positivity for 4D5 and FRP5 receptors respectively. In the case of the primary T cells, were used an 10ug:5ug transposon:transposase concentration ratio gene-modifying cells from 3 different healthy donors, and the CAR expression was analyzed 1, 6 and 9 days post electroporation. The cells exhibited a decrease in the 4D5 and FRP5 receptors expression from day 1 to 6 after electroporation, getting more stable by day 9, with 8,94% and 5,06% average expression of 4D5 and FRP5 CARs respectively.

Conclusion: These results indicate the efficacy of the PB system to induce the CAR expression on different cell types. This approach has several advantages, such higher transposition efficiency, long-term expression and cargo capacity. The HER2 antigen is shared among several tumor types with CAR-Ts for this antingen being clinically. The use of piggyBac has potential to facilitate CAR-T cell production alone or in combination with other therapies.

Keywords: CAR-T cells; Piggybac; HER2