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

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**Introdução**

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

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**Casuística e Métodos**

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 Nluc carrying plasmid. After electroporation, the cells were cultured up to 9 days and the receptor expression analyzed at different times by flow cytometry.

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**Resultados**

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 Nluc CARs respectively (figure 1). 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 10ug of plasmid, obtaining 35.6% and 38.1% positivity for 4D5 (figure 2) and FRP5 Nluc (figure 3) receptors respectively. In the case of the primary T cells, we 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 Nluc 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 Nluc CARs respectively (figure 4).

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**Conclusões**

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 antigen being clinically. The use of piggyBac has potential to facilitate CAR-T cell production alone or in combination with other therapies.

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**Contato**

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