## OTR7. ECTOPIC EXPRESSION OF miR-182 IN CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS.

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**INTRODUCTION** Immunotherapy based strategies use components of the immune system, such as monoclonal antibodies, adoptive cell transfer and genetically modified T cells to respond specifically against cancer cells. B cell malignances have been clinically targeted by Chimeric Antigen Receptors (CARs) modified T lymphocytes. These receptors are derived from Fab portion from antibody, fused to transmembrane and T-cell cytoplasmic signaling domains of the CD3 zeta chain and 4-1BB molecule, called 19BBζ, because they recognize the CD19 expressed in B cell lineage. Good therapeutic responses in hematological tumors have been described with this approach; although there is still field to optimize this response. To increase the antitumor response we explored the association of anti-CD19-41BBzeta CAR (α19BBζ) and microRNAs, described recently as modulators of T cell, such the miR-182.

**OBJECTIVE** We aim co-express CAR and miR-182 in primary T lymphocytes in order to evaluate potential functional improvements in antitumor responses *in vitro*.

**METHODOLOGY** Peripheral blood mononuclear cells from healthy donors were electroporated with bidirectional *Sleeping Beauty* transposon for simultaneous expression of the CAR and miRNA. Gene-modified T cells were expanded and activated *in vitro* by co-culture with irradiated L388 cell line. The expression of 19BBζ miRNAs and targets were evaluated by FACS and RQ-PCR, respectively. *In vitro* expanded T lymphocytes were phenotyped for memory population, CD8 + and CD4 +, and evaluated for *in vitro* and *in vivo* activity of lysis of target pre-B ALL cell line NALM-6.

**RESULTS** Manufacture of T cell using the biderecitonal Sleeping Beauty system has efficiently promoted CAR 19BBζ and miR-182 expression. No difference was observed in lymphocyte subpopulations when miR-182 was overexpressed, however

*in vitro* analysis showed more expansion of this group for the majority of the donors tested. The cytotoxic assay indicated that CAR and miR-182 coexpression was not able to increase the antitumor activity of CAR-modified T lymphocytes.

**CONCLUSION** The co-expression of miR-182 and the CAR 19BBζ seems to impact the rate of T lymphocyte expansion *in vitro* for cells expressing CARs.

**KEYWORDS** immunotherapy, CAR-T cell, miRNAs.