Chimeric Antigen Receptor (CAR) modified T cells using Sleeping Beauty system by electroporation and combination with co-culture expansion has cytotoxic activity *in vitro* and *in vivo*

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INTRODUCTION

In the last year, the first T-cell therapy modified to express the chimeric receptor antigen (CAR) targeting the CD19 molecule, has been approved by the Food and Drug Administration (FDA) to treat refractory or relapsed B-cell lymphoma and acute lymphoblastic leukemia. Currently, these cells are modified by viral vectors, expanded and returned to the patient. However, their use is associated with high production costs and biosafety requirements. Thus, it is important to research alternative methods with low costs and more accessible to transform this immunotherapy into available therapy

OBJECTIVE

We herein describe an alternative protocol involving effectiveness to modify these cells with the Transposon and electroporation mechanism and expansion based on the co-culture model.

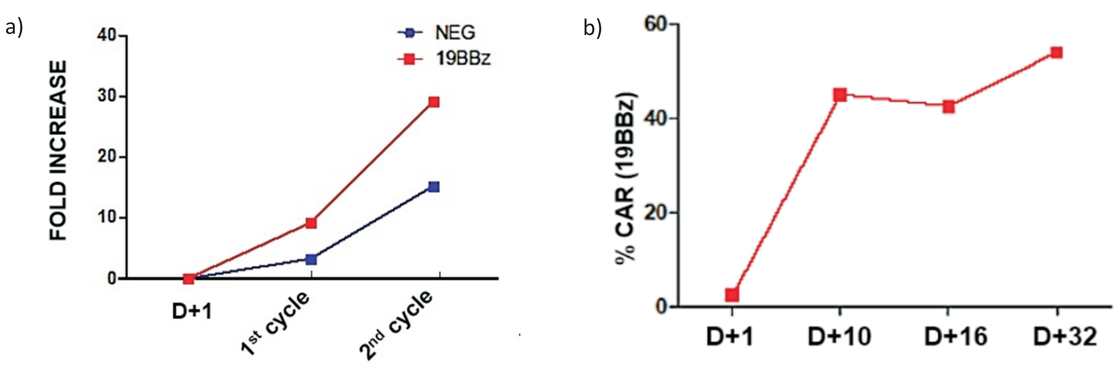
METHODOLOGY

- BCP-ALL RS4;11cells were transduced with a lentiviral vector carrying the GFP gene and GFP positive cells were sorted by FACS.
- Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll and electroporated using the Nucleofector II device combined with plasmids encoding 19BBz CAR in the pT3 SB transposon backbone and SB100x transposase following stimulation up to 3 times with the irradiated EBV transformed LCL cell line L388 cells. The phenotype and cytotoxic activity *in vitro* was evaluated by flow cytometry
- For *in vivo* experiment, tumor cells were inoculated intravenously into NSG mice (8 mice per group) and 19BBz CAR$^+$ T cells were inoculated three days later. Tumor burden was monitored by analyzing GFP$^+$ cells in blood samples every 10 days and in different organs by the end of the experiment by flow cytometry. Weight was monitored twice a week. Animal welfare was monitored daily.
- All human samples were collected after Ethics Committee approval and animal experimentation was performed after approval of the Institutional Research Ethics Committee

RESULTS

19BBz+ and mock T cells showed 28 and 15-fold expansion respectively compared to d+1 values with frequencies of memory cell subpopulations differing between these conditions. NK cell depletion favored the expansion of CD8+CD62L-CCR7+ cells. LAZ388 stimulation favored CAR+ cell expansion, yielding to 60% of 19BBz+ T cells on first cycle (14-20 days). Baseline d+1 CAR expression ranged from 2,5% to 37%. The potential to eliminate B-cell was confirmed in cell lysis assays against Nalm-6, K562, L388 and RS4;11 cells, showing elimination of the CD19+ target cells while sparing K562. L388 cells were also eliminated, indicating anti EBV T cell based responses. In NSG mice engrafted with the B cell leukemia RS4;11, 19BBz+ CAR+ T cells were able to significantly improve survival over non related or Mock treated groups.

Figure 1



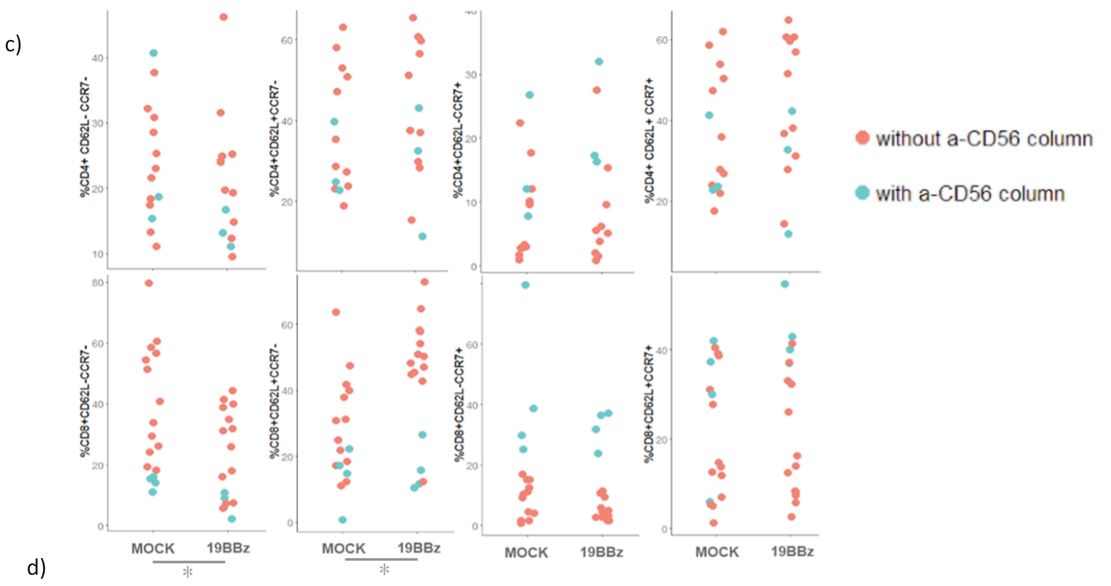


Figure 1: a) Fold increase compared to d+1 of CAR+ T cells from a representative donor during cell expansion after stimulation with L388. **b)** Percentage of CAR+ T cells. **c)** The impact of different CARs and NK cell depletion on lymphocyte populations after the first cycle of expansion. **d)** Target cell lysis measured by chrome release assay. One-way ANOVA statistical test with Tukey post-test was applied. * p<0.05,** p<0.01, *** p<0.001.

Figure 2

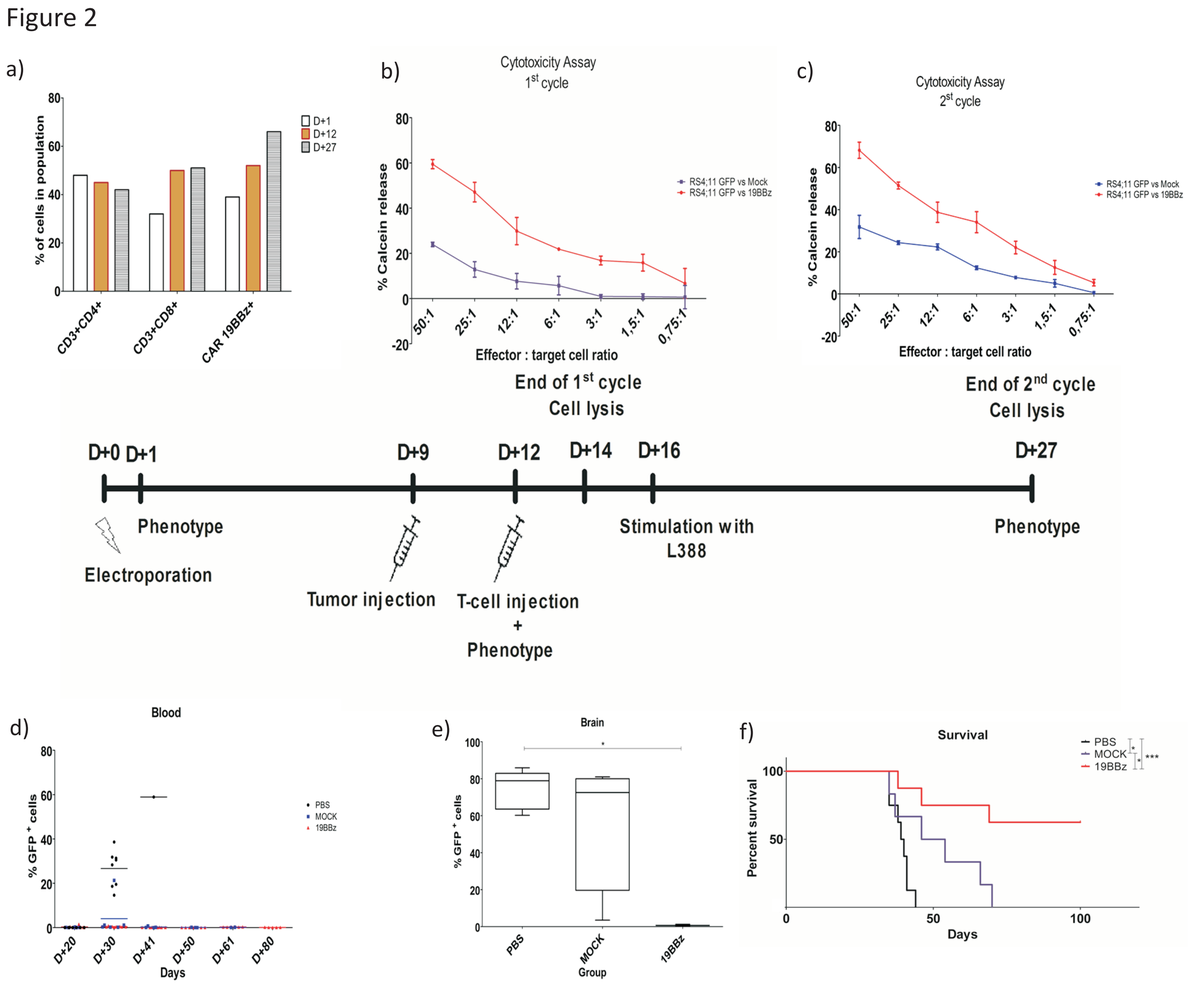


Figure 2 : *In vivo* experiment timeline. a) Phenotype of the T cells throughout the cycles. **b)** Target cell lysis measured by calcein release assay in the first cycle. **c)** Target cell lysis measured by calcein release assay in the second cycle. **d-e)** tumor burden in the blood and brain. **f)** Survival. one-way ANOVA statistical test with Tukey post-test was applied. Logrank test for survival * p<0.05, ** p<0.01,*** p<0.001.

CONCLUSION

The results indicate that 19BBz CAR gene transfer by combining the SB system, electroporation and co-culture with L388 is a simple and straightforward method for inducing long term CAR expression and target specific cytotoxicity both *in vitro* and *in vivo*.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA