An *in vivo* model to study Chimeric Antigen Receptor (CAR) and Nivolumab combination therapy in B cell precursor acute lymphoblastic leukemia

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Introduction

In the last decade, there has been an increase in the incidence of cancer with around 14 million new cases in 2012 (WHO). Therefore, the search for new therapies, such as immunotherapy and targeted therapy approaches, has become essential. Furthermore, combinatorial therapeutic strategies may yield even better results and possibly prevent or delay the development of resistant mechanisms fostering tumor cell escape.

Objective

This project aims to establish an in vivo model to study the effect of Chimeric Antigen Receptor (CAR) in combination with PD-1 blocking using Nivolumab in B cell precursor acute lymphoblastic leukemia (BCP-ALL).

Methodology

✓ BCP-ALL RS4;11cells were transduced with a lentiviral vector carrying the GFP gene and positive cells were sorted by FACS.

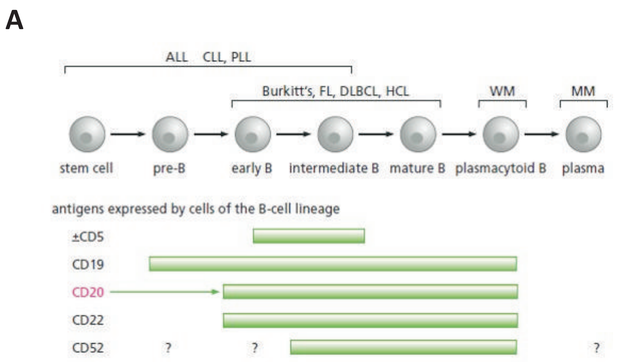
✓ Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque TM centrifugation.

✓ To generate 19BBz CAR+ T cells, PBMCs were electroporated with a Sleeping Beauty transposon-based plasmid carrying the 19BBz CAR construct along with a plasmid carrying the SB100x transposase. Cells were expanded in vitro by stimulation with the EBV+ LAZ388 cell line. An anti-myc monoclonal antibody was used to confirm 19BBz CAR expression by flow cytometry.

✓ For the in vivo experiment, tumor cells were inoculated intravenously into NOD/SCID mice (3-5 mice per group) and 19BBz CAR+ T cells were inoculated two days after tumor cells inoculation. Tumor burden was monitored by analyzing by flow cytometry GFP+ cells in blood samples every 10 days and in different organs by the end of the experiment. Weight was monitored every 7 days. Animal welfare was monitored daily.

✓ All animal experimentation was performed after approval of the Institutional Research Ethics Committee

Figure 1.



(WEINBERG, 2014)

(CHICAYBAM; LAINO SODRÉ; BONAMINO, 2011)

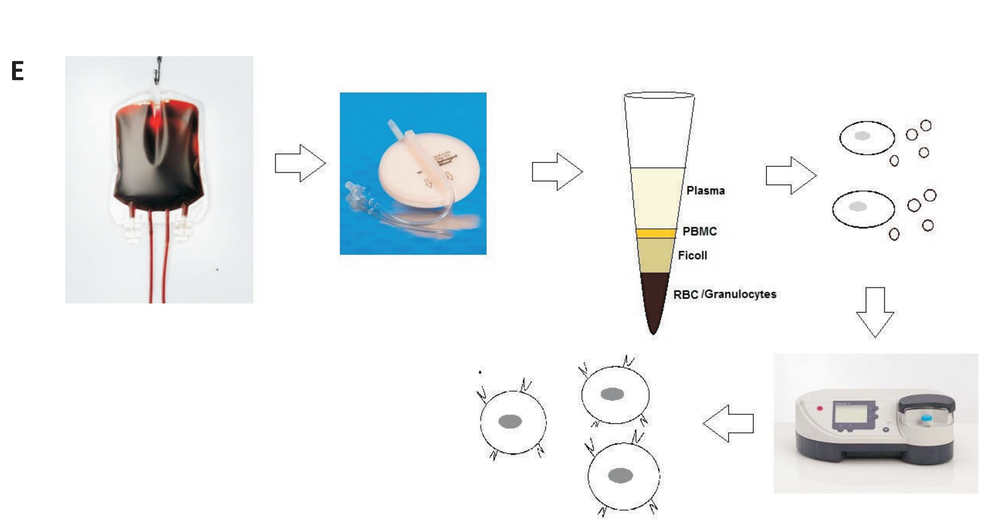


Figure 1. Methodology A) Expression of CD19 antigen by cells of the B-cell lineage. (WEINBERG, 2014). **B)** Schematic representation of a chimeric antigen receptor 19BBz (CHICAYBAM; LAINO SODRÉ; BONAMINO, 2011) **C)** BCP-ALL RS4;11 cells were transduced with a lentiviral expressing GFP . **D)** CAR expression on human T lymphocytes by the time of infusion. **E)** Schematic representation of the ficoll gradient process for the isolation of mononuclear cells and electroporation process for generating lymphocytes carrying 19BBz CAR.

Results

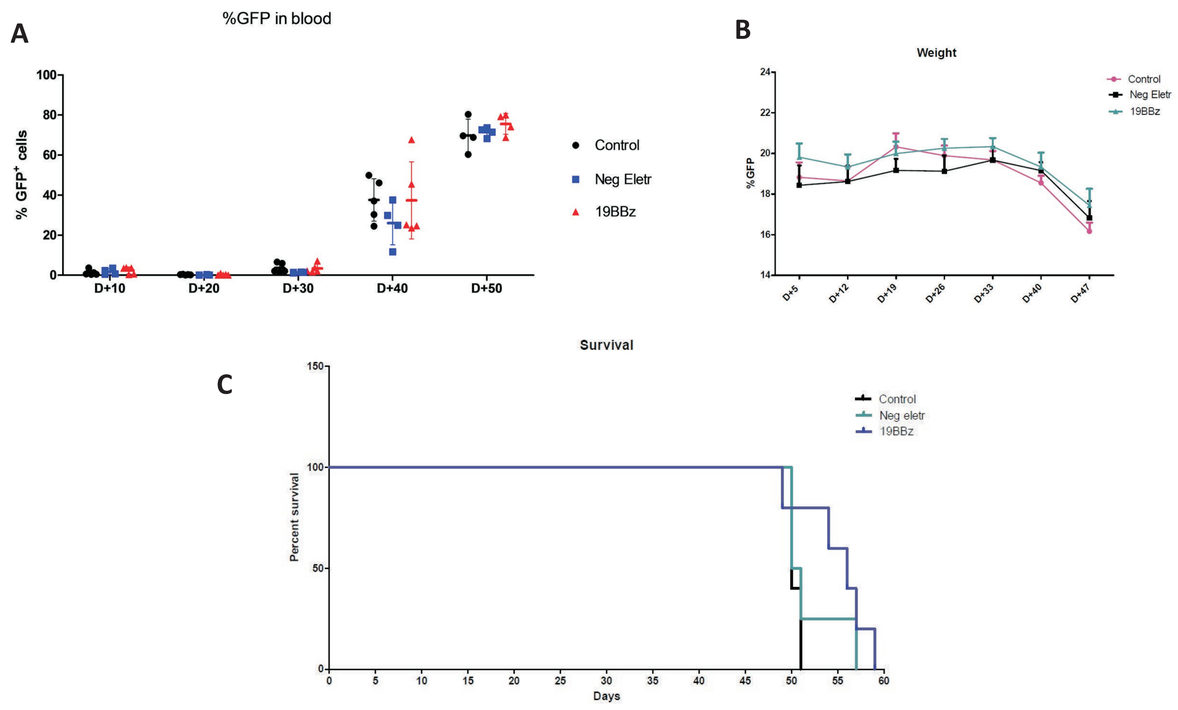


Figure 3. A) Blood samples were collected from mice every 10 days, and GFP expression was checked by flow cytometry. **B)** The weight of each animal was monitored every 7 days. **C)** Kaplan-meier survival curve for mice injected with GFP+ RS4;11 cells. Long-hank test was used to compare groups (p value = 0,2804 between all groups). Control : group inoculated with RS4;11 GFP+ ; Neg Eletr: group inoculated with RS4;11 GFP+ and lymphocytes electroporated without plasmids (mock condition); 19BBz: group inoculated with RS4;11 GFP+ and lymphocytes electroporated with plasmids carrying the 19BBz CAR construct.

Future Perspectives

✓ We will now test if higher numbers of 19BBz CAR+ T lymphocytes can significantly affect tumor burden and improve the survival kinetics of animals. Once the model is established, we will evaluate the anti-tumor effect of Nivolumab in the presence or absence of CAR+ T cells. The complete model will then be used to identify tumor genes that may confer resistance to this combinatorial therapeutic approach using CRISPR/Cas9 genetic screens.

REFERENCES

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WEINBERG, Robert A.. The biology of cancer. 2. ed. New York: Garland Science, 2014. 783 p

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