BIO_01 - Development and optimization of a protocol for 19BBZ CAR-T cells generation

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Introduction: CAR-T-cell immunotherapy has achieved high response rates in treatment-refractory patients with B-cell malignancies. In this therapy, T cells are isolated from the patient and genetically engineered to express an anti-CD19 CAR (Chimeric Antigen Receptor), expanded *in vitro* and then re-infused into the patient. CAR-T-cell therapy has shown considerable advance in recent years, being approved by regulatory agencies in US, Europe, Japan and more recently in Brazil. Most current methods for CAR-T-cell generation use high cost viral vectors for T-cell genetic modification. To adapt this protocol to our local reality, we are developing simple and less costly manufacturing protocols.

Objective: This work sought to generate a 19BBz CAR-T cells with a short *in vitro* expansion protocol based on the non- viral Sleeping Beauty (SB) transposon-based vector system.

Methodology: The 19BBz CAR sequence was provided by Dr Dario Campana (Memphis, TN) and was cloned in the transposon vector pT4/HB. PBMCs were collected from healthy donors after signed board–approved informed consent. Mononuclear cells were isolated by density gradient centrifugation with Ficoll-Hypaque-1077 and electroporated with plasmids enconding 19BBz CAR and the SB100x transposase. The expansion of CAR-T cells was performed using G-REX culture wells for 8 days. CAR-T cells effector capacity was evaluated *in vitro* by cytotoxicity assay with Calcein-AM-loaded target cells incubated with different ratios of effector cells. For the xenograft mouse model, NOD-SCID IL2R gamma null (NSG) mice were injected on the tail vein with 1x 105 Nalm-6 Luc-GFP cells and treated 2 days later with CAR-T cells or control cells. For *in vivo* imaging, mice were injected i.p. with 75 mg/kg d-luciferin and tumor burden was verified by bioluminescence. For cell analysis, organs were processed and analyzed by flow cytometry.

Results: Using the protocol described herein we generate, starting from 3 $x10^7$ total PBMCs, a mean of 3.7 $x10^6$ CAR-T cells after 8 days of expansion. CAR-T cells generated showed cytotoxic effect against CD19+ leukemia cells *in vitro*. Furthermore, CART-T cells treatment improved overall survival rates of leukemia-engrafted NSG mice by 40% at 2,5 $x10^5$ dose after 37 days and 77% at 5 $x10^5$ CAR-T dose after 86 days of tumor inoculation, leading to a significative reduction in the tumor burden. Finally, infused CAR-T cells persisted for up to 28 days, showing capable of long-term persistence and antitumor response.

Conclusion: The current protocol can generate a cellular product compatible with regulatory requirements and performance to be tested in a phase I clinical assay.

Keywords: CAR-T; Immunotherapy; Transposon