BIO_01 - Efficient large-scale point-of-care production of affordable anti-CD19 CART-T Cells for leukemia immunotherapy

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Introduction: The manufacturing process of T lymphocytes expressing Chimeric Antigen Receptors (CARs) involves genetic modification, activation, and *in vitro* expansion of patient-derived lymphocytes to produce billions of CAR-T cells for reinfusion. CAR-T cell therapy targeting CD19 has demonstrated high response rates in patients with B-cell malignancies. However, current approved CAR-T therapies entail complex procedures and high costs, potentially hindering their widespread adoption.

Objectives: To develop a non-viral gene delivery system protocol based on the Sleeping Beauty (SB) transposon, enabling the generation of anti-CD19 CAR-T cells with a shorter ex vivo *expansion period*.

Methodology: Peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were isolated via density gradient centrifugation and then electroporated using the 4D-Nucleofector LV System (Lonza) with the PT419BBz CAR vector and SB100x transposase. We utilized Wilson Wolf's gas-permeable membrane G-Rex M1000 bottles to support high-density production of CAR-T cells over a brief culture period of 8 days.

Results: Scaling up the expansion protocol using G-REX bottle culture, starting from about $1.5x10^8$ total PBMCs, yielded a total of $1x10^8$ CAR-T cells, demonstrating our capability for Large-scale production of anti- CD19 CAR-T cells. To assess the efficacy of these CAR-T cells, we established a patient-derived xenograft NSG mouse model (PDX) utilizing primary tumor cells from an acute lymphoblastic leukemia patient. After 47 days, tumor burden in PDX mice (tumor dose 10^6 cells) was confirmed by detecting human CD19+ and CD45+ positive cells in mouse blood by flow cytometry. Next, the PDX mice were treated with $7x10^5$ of anti-CD19 CAR-T cells product. Preliminary results revealed that after 17 days of CAR-T cell treatment, the tumor burden in PDX animals decreased to 0.5% compared to 16.3% in the control group. Additionally, CAR-T cell-treated mice showed increased survival compared to the control group.

Conclusion: This approach not only yields a sufficient number of potent anti-tumor CAR-T cells but also paves the way for large-scale and point-of-care low cost manufacturing, laying the foundation for future clinical trials in patients from INCA.

Keywords: CAR-T cells; Immunotherapy; Point-of-care