BIO_12 - Generation and characterization of anti-CD19 CAR-T cells overexpressing the protein PHF19

Karina Lôbo Hajdu[1]; Leonardo Ribeiro Batista Silva[1]; Martín Hernán Bonamino[1].
[1]Instituto Nacional do Câncer (INCA).

Introduction: Chimeric antigen receptor (CAR) T cells are genetically modified T lymphocytes that express a synthetic receptor which can recognize surface antigens. This form of immunotherapy gained attention in the last decade due to the success of anti-CD19 CAR-T cells for refractory B-cell malignancies. Two main challenges of CAR-T cell technology are the high cost of production, that limits the amount of patients who can benefit from the treatment, and persistence of functional memory T-cells *in vivo*, which is often restricted by the acquisition of a terminally exhausted phenotype. Non-viral gene delivery systems such as *Sleeping Beauty* (SB) and *Piggybac* (PB) transposon systems have fewer biosafety and infrastructure requirements than viral vectors, representing a good strategy to reduce cost of production. Recent work showed that Phf19, an accessory protein of the Polycomb Repressor Complex 2 (PRC2) can modulate T cell phenotype by downregulating exhaustion-associated transcription factors.

Objective: This work aims to assess the benefit of overexpressing PHF19 on the exhaustion and memory phenotype of 19BBz CAR-T cells generated with SB and PB systems.

Methodology: PHF19 sequence followed by a membrane reporter was cloned in both PT3-19BBZ and PBCAG-19BBZ CARs, transposons for the SB and PB systems, respectively. Jurkat and 293T cell lines were electroporated and western blotting was performed to confirm PHF19 overexpression. Lymphocytes from healthy donors were electroporated with SB100X (SB transposase) or PBCAG-PBase (PB transposase) along with the respective transposon. T cell phenotype was assessed by flow cytometry.

Results: Plasmids encoding 19BBz CAR and Phf19 were validated In Jurkat and 293T cells. Our results in primary lymphocytes show that the SB system cannot successfully integrate the construction, although transient expression occurs. Partial results suggest that overexpressing Phf19 may lead to lower expression of the exhaustion marker TIM-3, while maintaining PD-1 levels.

Conclusion: We hope this work will help develop new strategies to generate cost-effective CAR-T cells while avoiding terminal exhaustion, enhancing long-term efficacy.

Keywords: CAR-T cells; Cancer immunotherapy; Cell therapy