ORT_15 - Evaluation of different transposon-based genetic modification tools for CAR-T cell therapy

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Introduction: CAR-T cell therapies are now widely spread but pose challenges in terms of costs and access. Non- viral vectors represent an opportunity to generate CAR-T cells with favorable cost-effective profiles. In this work, we compare different systems of non-viral vector-based Transposons aiming to insert the CAR transgene into T cells. Sleeping Beauty (SB) and PiggyBac (PB) transposons represent "cut-and-paste" platforms in which the transposase can "cut" the sequence of interest from the transposon backbone, and "paste" it in host genome, usually and TA (SB) or TTAA (PB) rich sites. Both tools are derived from different organisms and can be assembled using different promoters to drive CAR transcription.

Objectives: To compare different generations of SB transposon systems (PT2, PT3 and PT4) – with a PB-based construct to generate CAR-T cells.

Methodology: Mononuclear cells were isolated and electroporated using the Nucleofector IIb with 20ug SB transposons bearing the MSCV promoter (PT2, PT3 or PT4) which encode 19BBz with 1ug SB100x transposase. For PB, we electroporated 10ug 19BBz PB (CAG promoter) with 20ug PBase transposase. CAR expression and phenotype were measured by cytometry. For the *in vivo* evaluation, NSG mice were inoculated with Nalm-6 tumor and treated with corresponding groups (tumor only, mock, PT2, PT3, PT4 and PB). Tumor burden was measured by bioluminescence.

Results: CAR-T cells produced with all of the SB constructs showed 5-15% of CAR-T cells on day 1 and increase to 25-35% on day 12. However, PB expresses the CAR in approximately 30% of the cells on day 1 and then around 20% on day 12. There was no difference in memory and exhaustion phenotypes. In a *in vivo* model evaluation $3x10^6$ CAR-T cells were inoculated. Tumor burden for PT2, PT3 and PT4 showed to be similar, while the PB group had no tumor, suggesting a complete tumor elimination. However, the survival of all treated groups was similar, despite lack of tumor in the PB group, suggesting that tumor-free mice died due to Graft-versus-host disease as a consequence percentage in this condition. A new *in vivo* experiment normalizing the total number of T cells and the number of CAR-T cells ($2x10^6$), showing that the groups treated with all of the SB versions had similar survival curves while animals in the PB group survived much longer.

Conclusion: We can conclude that the behavior of the CAR expression between SB and PB systems is different, but similar phenotypes. However, although the animals treated with SB showed an improvement in survival compared to the control, the PB group had a greater survival gain. We are further investigating if the different promoters used can play a role in the observed outcomes.

Keywords: CAT-T cells, Non-viral vectores, Immunotherapy