ORT_29 - Generation of 19bbz CAR-T cells in tcr knockout T-cells

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Introduction: The use of autologous T cells bearing CAR, synthetic constructs that redirect lymphocyte specificity to tumor membrane antigens shows good results, although it presents some limitations. To circumvent these, allogenic CAR-T cells can be made. In order to avoid GVDH, genes encoding the TCR can be knockout (Ko) and the CAR transgene be delivered through a viral approach, transposons, such as Sleeping Beauty (SB), or donor DNA. The use of SB can optimize the production of CAR-T since it is cheaper and less laborious compared to viral assembly and delivery. The use of donor DNA can provide some improvements in the cells generated by knock-in (KI) of the transgene in specific genetic sites.

Objectives: The objective is the generation of allo 19BBz CAR-T cells KO for TCR via CRISPR and CAR+ via SB system or KI.

Methodology: PBMCs were isolated, in some experiments submitted to CD3 purification columns, electroporated in 2B or 4D nucleofector with CRISPR RNPs, SB or donor DNA. The cells KO of the TCR were evaluated by flow cytometry.

Results: The editing system was optimized and tested in Jurkat, which achieved 41 days post-eletroporation 70% of KO, with better results with a reason of 1:3 (Cas9/gRNA). Using PBMC as starting material, the rate of CD3 negative cells was 38%, and after the expansion the rate of KO in T cells was 20%. The SB transposon carrying CAR was co-delivered with the RNP achieving 20% of CD3- cells. The editing system alone shows 9% KO, but when we used it along with SB the KO rate was 21%, with higher CAR expression in the CD3- subpopulation. We used a mock donor DNA sequence, which impaired CAR expression to test the KI to compare it with KO rates. This condition with a donor DNA generated a stable population of 60% of CD3 negative cells throughout the expansion.

Conclusion: We can conclude that the editing system works in the Jurkat, PBMCs, and CD3 purified population. It was possible to generate allo CAR-T cells with the SB system, CD3- population showing advantage in the expression of the CAR molecule. The mock KI promoted higher KO rates, stabling maintaining the CD3- population during the expansion.

Keywords: CRISPR/CAS9, Immunotherapy, CAR-T