OTR.20 - Production of chimeric antigen receptor (CAR)-T cells for preclinical testing using non-viral transposon vectors and a lymphoblastoid cell line (LCL)

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Introduction:

CAR T cell immunotherapy for treatment of cancer is showing promising results in clinical trials targeting the B cell restricted CD19 antigen. Patients in clinical trials harboring B cell malignancies experienced overall response rates of 73%, with pediatric B cell acute lymphoblastic leukemia (B-ALL) patients showing the highest response rates (93%). However, there is no standard protocol for the generation of CAR-modified T cells, with different genetic modification vectors and expansion protocols being used. Viral vectors insert the transgene in the genome of the cell, providing long term CAR expression. However, their use is associated with high production costs and cumbersome quality controls, impacting on the final cost of CAR-T cell therapies. Sleeping Beauty (SB) transposon system consist of plasmid-based integrative vectors that, through a cut-and-paste mechanism catalyzed by a transposase, recognize inverted terminal repeats flanking the transgene and insert it in the target cell genome. We show here that this system, combined with LCL-based T cell expansion can be used to efficiently transfect primary T lymphocytes and induce long term CAR expression throughout T cell expansion.

Objective:

To develop a protocol to expand SB-transfected CAR T cells using a LCL.

Methodology:

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll and transfected using Nucleofector II electroporator. The plasmids encoding the anti-CD19 CAR and transposase (SB100X) used in the transfection were mixed based on a pre-defined optimized proportion. After transfection the cells were stimulated with the L388 LCL, a mature B cell line that expresses the target antigen (CD19) and costimulatory receptors. CAR T cells were re-stimulated up to 3 times with L388 and T cell phenotype (CAR expression, memory markers) and effector function were evaluated at the end of each cycle.

Results:

Electroporation of PBMCs with transposon plasmid decreased viability and altered the frequency of memory subpopulations when compared to the mock (electroporated without plasmid) condition. However, CAR expression rescued the electroporated lymphocytes and these cells showed increased proliferation compared to mock control (28-fold vs 15-fold expansion; 19BBz vs mock). Moreover, CAR+ lymphocytes showed an increased frequency by the end of the stimulation cycle compared to d1 post electroporation, suggesting that CAR signaling enhances the activation and proliferation of these cells. NK cell depletion prior to L388 stimulation altered the composition of memory subpopulations, favoring the expansion of CD8+ CD62L- CCR7+ cells.

Conclusion:

The results showed that electroporation using the SB system is a simple and cost-effective method for inducing long-term CAR expression in T lymphocytes. Expansion of gene modified T cells was possible by using the L388, providing up to 3 cycles of stimulations and reaching the required cell number for preclinical testing. Experiments using B cell leukemia models in immunodeficient mice are underway to evaluate CAR T cell function *in vivo*.

Keywords: T lymphocytes; Immunotherapy; Chimeric antigen receptor