312 IL7 INCREASES TARGETED LIPID NANOPARTICLE-MEDIATED MRNA PROTEIN EXPRESSION IN T CELLS *IN VITRO* AND *IN SITU* BY ENHANCING T CELL TRANSLATION

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Background Chimeric Antigen Receptor T cells (CARTs) are a powerful anti-cancer therapy, demonstrating success in hematologic malignancies. The development of targeted lipid nanoparticle-mRNA (tLNP-mRNA) therapeutics has allowed for the generation of CARTs *in situ* and may resolve several challenges of conventional *ex vivo* viral-engineered CAR T cell products including scalability, access and mulitplexing. The *in situ* T cell transfection rate achieved by tLNP-mRNA varies from 4–20% of T cells expressing the protein of interest. As tLNP-mRNA platform efficacy may critically depend on the number, metabolic state, and localization of engineered T cells, we investigated whether cytokines could enhance protein expression.

Methods We used tLNPs that target CD5, a marker expressed highly on mouse and human T cells. These tLNPs carried the mRNA for the reporter protein mCherry or encoded a fibroblast activated protein (FAP) targeted CAR. Mouse and human T cells were cultured with IL2, IL7, IL15 or activated using αCD3/CD28. CD5-mCherry-tLNPs or CD5-FAPCAR-tLNPs were added and protein expression was detected using flow cytometry. For *in vivo* studies, C57BL/6 mice were pretreated with IL7, injected with tLNPs and sacrificed 24 hours later. To analyze the T cell transcriptome, $CD8^+$ T cells were isolated from mouse spleens and cultured with IL2, IL7 or IL15 for 48 hours before being sequenced.

Results We found that CD5-mCherry-tLNPs induced protein expression on 10% of resting T cells *in vivo* and ~15% of T cells *in vivo*. Culturing mouse and human T cells with IL7 significantly improved CD5-mCherry-tLNPs protein expression *in vitro*. This also occurred in the *in vivo* setting as pre-treating mice with IL7 elevated both the proportion and total number of mCherry expressing T cells. FAPCAR expression was also increased by combining CD5-FAPCAR-tLNPs with recombinant IL7. Transcriptomic analysis showed IL7 selectively increased pathways associated with protein translation. The significance of these transcriptomic changes was demonstrated by showing that after electroporation with mRNA, T cells cultured in IL7 produced more protein compared to IL2 or IL15.

Conclusions T cells can be engineered *in situ* using CD5-targeted tLNPs and IL7 increases the protein expression induced by tLNPs. Our data suggests that the upregulation of translation-associated pathways in T cells by IL7 could be exploited to improve the expression of proteins *in situ* after tLNP administration. This provides a novel paradigm through which a T cell activating cytokine, instead of lymphodepletion, can potentiate in situ CAR T cell therapy.

Ethics Approval This study was approved by The University of Pennsylvania's Ethics Board; approval number 806099.

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