Ionizable Lipid Nanoparticles with Integrated Immune Checkpoint Inhibition for mRNA CAR T Cell Engineering
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Statement of Purpose: Chimeric antigen receptor (CAR) T cell therapy has demonstrated clinical success in recent years, attaining FDA approval for the treatment of B cell lymphoma, acute lymphoblastic leukemia (ALL), and mantle cell lymphoma. Our group has previously reported the design of ionizable lipid nanoparticles (LNPs) encapsulating mRNA for ex vivo engineering of T cells with transient CAR expression. Programmed cell death protein 1 (PD-1) is an immune checkpoint receptor expressed on T cells, with a well-established role in cancer immune evasion and reduced CAR T therapeutic efficacy. Herein, LNPs co-encapsulating mRNA and siRNA are developed for simultaneous potent gene expression and knockdown in T cells and used to deliver CAR mRNA and PD-1 siRNA to primary human T cells ex vivo for applications in immunotherapy (Figure 1a).

Methods: A library of LNPs was designed with formulations varying along a continuum from “siRNA-like” to “mRNA-like” to identify excipient compositions for enhanced mRNA and siRNA delivery in Jurkat (immortalized human T) cells. Following optimization of nucleic acid cargo composition, transfection kinetics of a top-performing LNP formulation were investigated in a time-course study. This lead formulation was then adapted for the delivery of CAR mRNA and PD-1 siRNA to primary human T cells, with CAR expression and PD-1 knockdown quantified via flow cytometry and CAR-T functionality assessed via a coculture killing assay.

Results: In the initial screen of excipients in Jurkats, the LNP most closely resembling typical mRNA formulations demonstrated the greatest delivery of both mRNA and siRNA (Figure 1b-c), with subsequent adjustments to nucleic acid ratios resulting in further improvement of both mRNA and siRNA delivery. A time-course study revealed durable mRNA expression (at least 8 d) and high transfection efficiency (up to 98%) in primary human T cells (Figure 1d-e). Subsequent transfection of primary human T cells with LNPs encapsulating CAR mRNA and PD-1 siRNA resulted in roughly 85% CAR positivity (Figure 1f) and a nearly 70% reduction in PD-1 expression (Figure 1g).

Conclusions: This work reports the development of an LNP platform for the potent simultaneous delivery of mRNA and siRNA to primary human T cells ex vivo. These LNPs show promise to simultaneously transiently express and inhibit proteins and factors in T cells for a number of immunoengineering applications, including in the development of improved cancer immunotherapies.

Figure 1: (a) Schematic overview of LNP-mediated dual RNA delivery for CAR T cell cancer immunotherapy. (b) mCherry expression and (e) EGFP knockdown in EGFP-expressing Jurkats identifies excipients for codelivery of mRNA and siRNA. (d) EGFP and (e) PD-1 expression over time in primary human T cells treated with LNPs co-encapsulating EGFP mRNA and PD-1 siRNA. (f) CAR and (g) PD-1 expression in primary human T cells treated with LNPs co-encapsulating CAR mRNA and PD-1 siRNA.

References