Efficient LNP delivery of mRNA *in vivo* and *ex vivo* to T and NK cells

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Introduction

Autologous CAR T cell therapy requires the manufacture of bespoke products on an individual patient basis, and current-generation allogeneic CAR T cells require stringent patient lymphodepletion to achieve clinical responses comparable to autologous products. *In vivo* delivery of mRNA encoding therapeutically relevant transgenes such as CARs has great potential for transiently reprogramming patient immune cells in an autologous manner without the need to manufacture the cells *ex vivo,* and without the undesirable effects of stringent lymphodepletion or the added risk of allogeneic product rejection. Lipid nanoparticles (LNPs) are an efficient platform for *in vivo* delivery of RNA. Moreover, *ex vivo* RNA delivery via LNP could provide a platform for base editing CAR-T cells to improve upon current CAR-T manufacturing processes.

Here we describe *ex vivo* and *in vivo* LNP-mediated delivery of mRNA encoding reporter proteins to T and NK cells. Taken together, these data provide a promising approach for LNP-based delivery for *in vivo* CAR-T and -NK cell production as well as an LNP-based strategy for *ex vivo* base editing in CAR manufacturing.

Applications for LNP delivery to T and NK cells

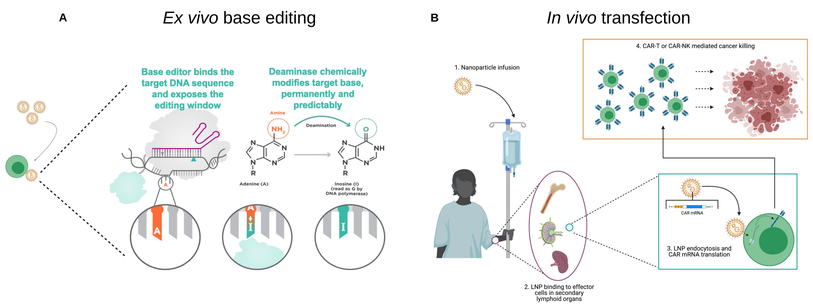

Figure 1. (A) *Ex vivo* delivery of LNP containing guide-RNA and editor mRNA for base editing T cells provides an alternative to current CAR-T manufacturing processes. ABE is a fusion protein comprising an evolved TadA* deaminase (teal) connected to a CRISPR-Cas enzyme (grey). The base editor binds to a target sequence that is complementary to the gRNA (magenta) and exposes a stretch of ssDNA. The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks the opposite strand. The nicked strand is repaired completing the conversion of an A:T to G:C base pair (1-2). **(B)** *In vivo* LNP-mediated delivery of mRNA allows for transient transfection of immune cells with mRNA CAR molecules.

High-throughput barcoding of LNPs enables multiplexed screening of hundreds of LNPs *in vivo* in the same experiment

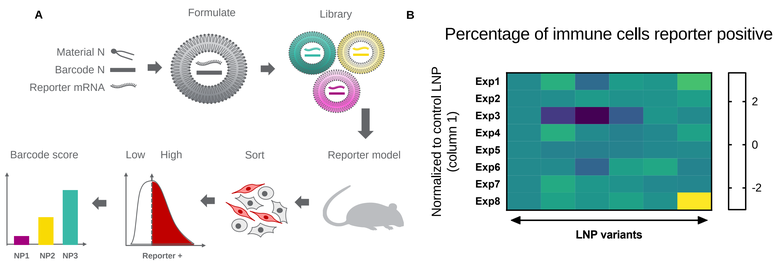

Figure 2. *In vivo* screening of thousands of chemically-distinct LNPs was conducted in a manner similar to previously described (3). In a single experiment, hundreds of LNPs were formulated with various novel ionizable lipid structures to co-encapsulate an mRNA encoding for Cre recombinase and a DNA barcode. LNPs were pooled together and administered into a single cohort of Ai14 Cre-reporter mice. After 72 hours, tissues were isolated and tdTomato-positive cells were FACS isolated. DNA barcodes were recovered from isolated cell populations and sequenced using NGS; after normalization to LNP library administered, LNPs are scored for biodistribution tropism to bone marrow and other tissues **(A)**. LNPs with favorable barcode scores were analyzed across additional *in vivo* experiments. Lead LNPs were selected by percentage of T or NK cells reporter positive normalized to an LNP control **(B)**.

Robust Reporter Expression in T and NK cells *ex vivo*

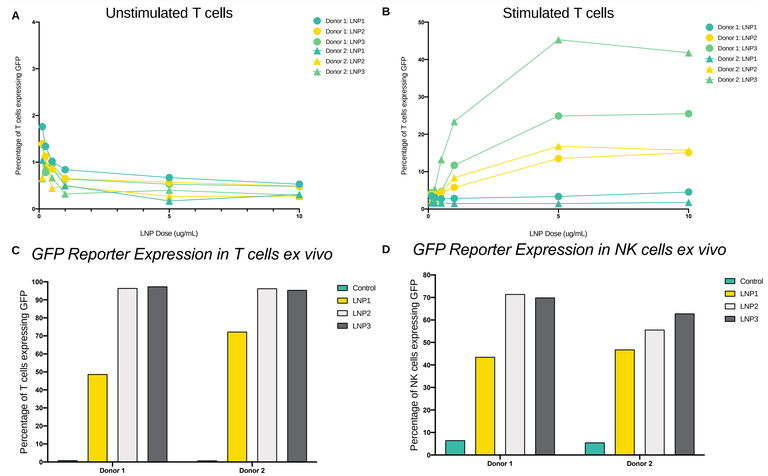

Figure 3. After identification via barcoded screening, LNPs 1-3 were formulated with GFP mRNA. LNPs transfect stimulated human primary T cells *ex vivo* in a dose-dependent manner plateauing at 5ug/mL (**B**). Unstimulated T cells (**A**) demonstrated no increase in GFP expression following LNP administration. Stimulated human T (**C**) and NK (**D**) cells were next transfected under optimized culture conditions and in the presence of serum, yielding over 90% and ~70% GFP expression, respectively.

Efficient LNP delivery to mouse T cells *in vivo*

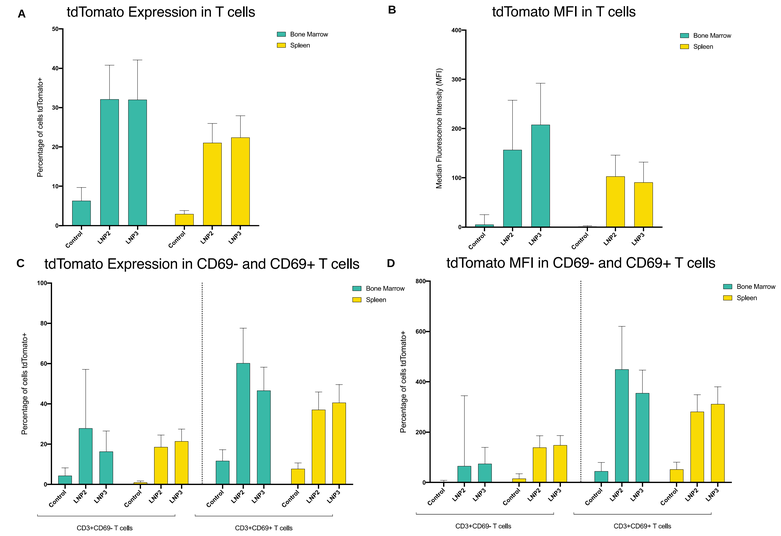

Figure 4. The biodistribution of LNP2 and LNP3 were assessed by delivery of cre recombinase mRNA in a cre-reporter mouse model (Ai14), which expresses the fluorescent protein tdTomato under a constitutive CAG promoter following cre-mediated gene editing. Ai14 mice were dosed with 1.0 mg/kg of LNPs containing cre recombinase mRNA. After 48 hours, the percentage (**A**) and MFI (**B**) of tdTomato expression in T cells in bone marrow and spleen were measured using flow cytometry. T cells expressing CD69, an activation marker, demonstrated increased LNP delivery via percentage (**C**) and MFI (**D**).

LNP transfection of human T and NK cells in humanized NSG-CD34+ mice

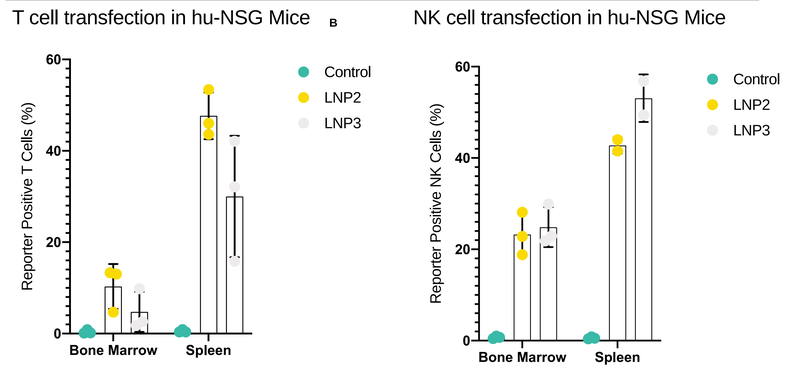

Figure 5. LNPs were administered to humanized NSG mice at a dose of 1.0 mg/kg reporter mRNA. 24 hours after LNP administration, reporter protein expression was detected in T cells (**A**) and NK cells (**B**) in bone marrow and spleen via flow cytometry.

Non-human primate T cells express reporter protein following IV LNP infusion

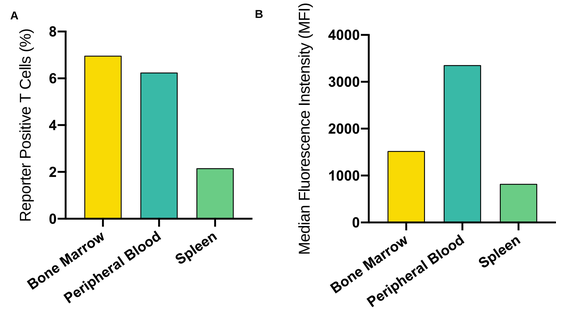

Figure 6. Cynomolgus macaque were dosed via intravenous infusion with LNP. (**A**) Percent reporter-positive CD3+ T cells and (**B**) reporter expression (Mean Fluorescence Intensity; MFI) was measured after 24 hours by flow cytometry.

Conclusions

Multiplexed *in vivo* lipid nanoparticle screening enabled our identification of LNPs with favorable expression profiles in target cells of interest. We validated transfection efficiency of these lead LNPs in immune cells *ex vivo* and *in vivo* in murine, humanized mouse, and NHP models. Efficient *in vivo* delivery of mRNA to T and NK cells could enable transient autologous CAR-T or CAR-NK cell production, negating the requirement for costly *ex vivo* manufacturing. Furthermore, *ex vivo* LNP delivery opens the door for an alternative approach to delivering nucleic acids in CAR-T manufacturing. Work is ongoing for optimizing LNP-mediated base editing and for improving transfection efficiency and specificity of lipid nanoparticles *in vivo* using ligand-targeted LNPs.

References and Disclosures

1. Gaudelli et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 551:464–471 (2017)
2. Gaudelli, N.M., et al. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat Biotechnol (2020).
3. Sago, C., et al. High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. PNAS (2018).

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