Mobilize LNPs: A novel platform for in vivo targeted delivery of RNA therapeutics to T cells

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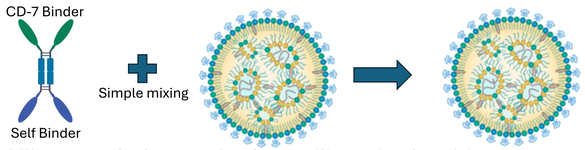
Introduction

Lipid nanoparticles (LNPs) have cemented their utility as efficient and effective delivery systems for RNA-based therapeutics with successful clinical translation as COVID vaccines and hepatic delivery. However, their usefulness in delivering to extra hepatic tissues suffers several technological challenges and warrants further innovation. We have developed *Mobilize* as a versatile and modular extra hepatic targeted delivery platform that is designed to resolve these challenges through a simple but unique approach.

Chimeric Antigen Receptors (CAR) T cell therapy harnesses the power of engineered receptors to redirect lymphocytes of interest (T cells) for the elimination of target cells expressing an antigen of interest. Traditionally, CART therapy involves ex vivo manipulation of T cells, which is complex, expensive, difficult to manufacture, time intensive and involves lymphodepletion. Therefore, there is growing interest in technologies for T cell manipulation *in vivo*. Herein, we demonstrate application of our *Mobilize* platform for targeted *in vivo* CART.

CD-7 Targeted Mobilize LNPs

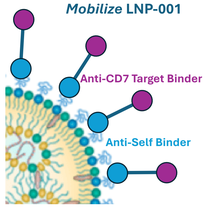


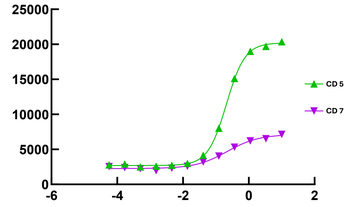
Mobilize as a platform technology utilizes simple mixing to generate targeted delivery system (*Mobilize* LNPs). The simple mixing enables seamless and scalable manufacturing processes including inline mixing, hold incubation and subsequent downstream purification.

The LNP surface coating consists of bifunctional antibody binders capable of binding to T cells, in addition to LNP components. The self & target binding capability of bifunctional binders was evaluated by ELISA.

As proof of concept, we used Green Fluorescent Protein (GFP) encoding circular RNA (circRNA) as model reporter cargo for targeted delivery to T cell. The tLNPs were characterized for their size, polydispersity index (PDI), surface charge, encapsulation efficiency (EE), and stability. We evaluated the delivery efficiency of *Mobilize* LNP in multiple cell-based systems (Jurkat cells, human PBMC and T cells) before confirming in vivo targeted delivery capability in humanized mouse model and non-human primates (NHP).

CD-7 Targeted *Mobilize* LNPs show excellent physicochemical characteristics



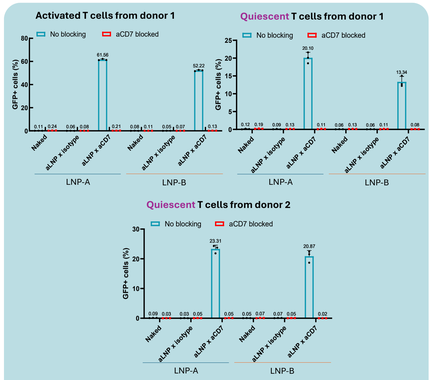


- We have developed 3 unique bifunctional binders to impart T cell specific targeting capability to the LNPs
- Binders retain their self-binding capability in the bifunctional format

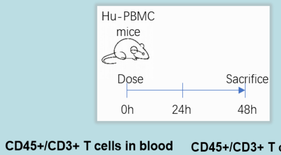
Sample	Z-average (nm)	PDI	EE%
Standard LNP	84.59	0.13	97.3
Mobilize LNP-001	87.61	0.16	97.3
Mobilize LNP-002	92.48	0.10	97.7

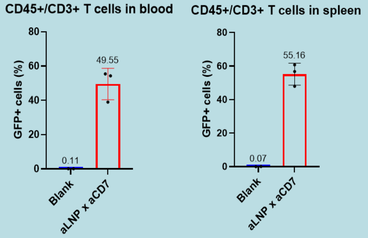
- *Mobilize* LNPs consistently demonstrated excellent physicochemical properties (size <100 nm, PDI <0.1 and EE >95%) and colloidal stability under refrigerated as well as frozen conditions.

CD-7 Targeted LNPs show T cell specific delivery *in vitro* and *in vivo*

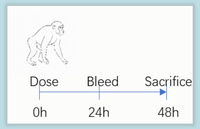


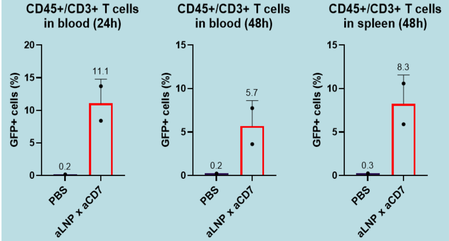
- CD7-targeted *Mobilize* platform showed **~60%** positive expression in T-cells in vitro
- CD7-targeted *Mobilize* platform demonstrated **~20%** positive expression in unstimulated T-cells from two different donors





Mobilize LNPs with human/cynomolgus monkey cross-reactive CD7 binder efficiently deliver to quiescent T-cells in hu-PBMC mice at a dose of ***0.2 mpk***

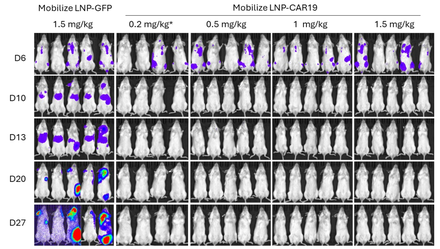


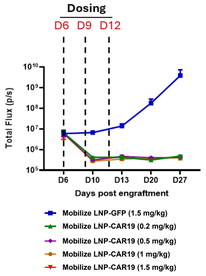


Mobilize LNPs successfully deliver to quiescent non-human primate (NHP) T-cells in vivo at a dose of ***1 mpk.***

No overt toxicity observed in doses up to ***2 mpk***

CD-7 targeted LNPs show Efficacy





We validated activity of these LNPs in Jurkat, human PMBCs, and human T cells achieving ~100%, ~70% ~35% delivery respectively. We then tested in vivo delivery efficiency of the *Mobilize* LNPs in humanized mice and achieved nearly 55% positive quiescent T-cells at a very low dose. We finally dosed the *Mobilize* LNPs to non-human primates (NHPs) to achieve successful delivery in ~10% quiescent T-cells through a single dose with no apparent toxicity at any tested dose.

Conclusions

The *Mobilize* platform consistently demonstrates the ability to both (1) detarget the liver, (thus avoiding passive LNP sinks), and (2) specifically deliver to T-cells in vitro and in vivo in multiple models. The inherent design of the *Mobilize* platform elegantly addresses the challenges in functionalizing LNPs with targeting ligands for extra hepatic delivery.

The process behind *Mobilize* technology maintains platform versality, stability, robustness, and delivery efficiency. Our data indicates that *Mobilize* has strong potential as an in vivo delivery platform of RNA therapeutics to extrahepatic tissues.