Teaching Old Lipids New Tricks: Engineering LNP Composition for Extrahepatic Delivery of DNA for Lung Cancer Imaging and Treatment

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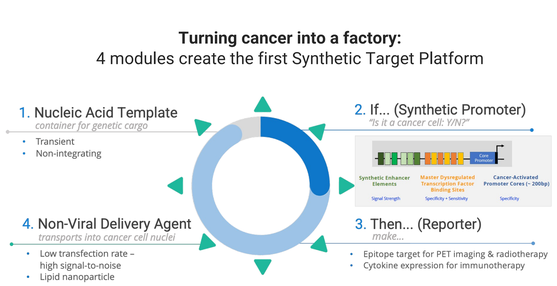
Background

Early detection of lung cancer significantly improves survival rates. Yet only 27% of all lung cancers are diagnosed at Stages 1-2. This is partly due to the lack of highly accurate, non-invasive diagnostic imaging methods that can differentiate malignant cancers from benign lesions. To overcome this limitation, Earli's platform uses cancer-activated DNA vectors to force tumors to produce synthetic biomarkers. Unlike most gene therapy applications, transfection of a tiny fraction of tumor cells often can produce sufficient biomarker expression for diagnostic imaging, provided there is minimal expression in normal cells.

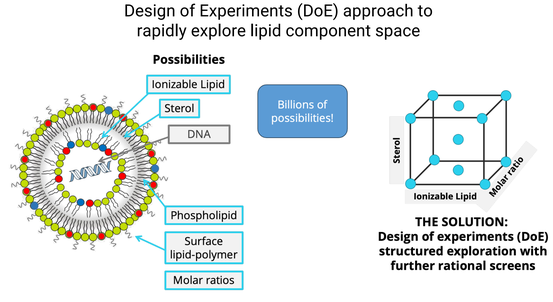
This necessitates the development of nanoparticles that can deliver DNA to lung tumors. While lipid nanoparticles (LNPs) have achieved clinical success for the delivery of RNA to hepatocytes, delivering DNA to lung tumors entails solving two fundamental challenges: preventing rapid clearance of intravenously injected LNPs by cells in the liver and spleen, and delivering DNA into the nucleus of tumor cells for gene expression.

Earli Delivery Platform

Earli has built a Synthetic Target expression platform using backbone-modified plasmid DNA vectors containing synthetic promoter elements that activate *only* in truly malignant cancer cells, rather than normal cells or benign lesions. This forces cancer cells to express reporter proteins, enabling early detection and treatment. These DNA vectors are delivered using non-viral Lipid Nanoparticles (LNPs).

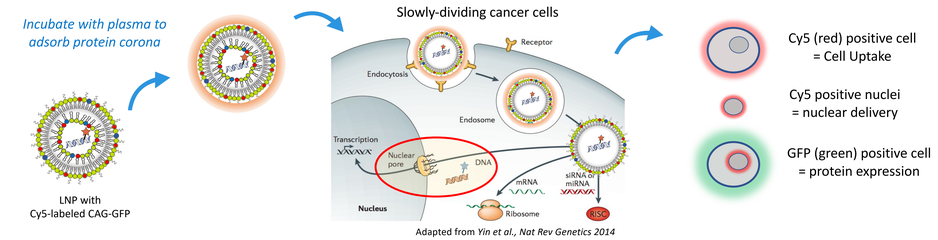


Earli's delivery platform is built to *engineer* LNPs for extrahepatic delivery of DNA. To achieve this, we undertook extensive optimization of LNP compositions using a structured, DoE-driven approach. As a subset of this larger effort, in this work we report on formulations of the ionizable lipid DLin-MC3-DMA (MC3 – vide *Jayaraman et al. Angew. Chem 2012*), which is a component of the FDA-approved siRNA drug patisiran. Whereas conventional patisiran-like MC3 LNPs are strongly liver-tropic, here we describe the development of engineered MC3 LNPs that produce strong DNA expression in tumors rather than the liver.



A high-throughput *in vitro* screening assay for cell uptake and nuclear delivery

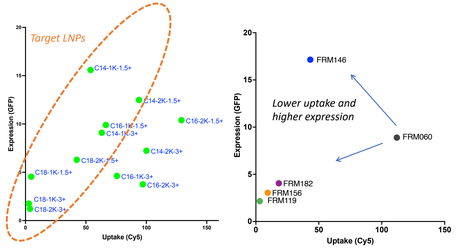
To model DNA delivery to the nucleus of cells, we developed an *in vitro* assay based on serum-starved, slowly dividing cell lines, including H1299 cells derived from lung tumors, and screened LNP formulations encapsulating dye-labeled DNA expressing GFP. LNPs were incubated with plasma to facilitate adsorption of a protein corona, which more closely mimics *in vivo* LNP uptake and trafficking mechanisms.



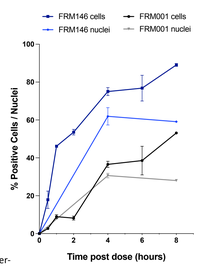
Adapted from *Yin et al., Nat Rev Genetics 2014*

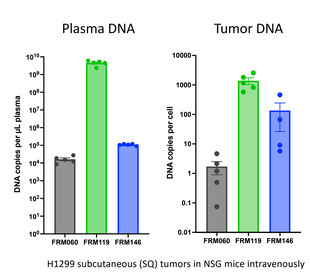
Flexible surfaces achieve a balance of long circulation and high transfection

We hypothesized that reducing LNP clearance by the reticulo-endothelial system would allow for extended blood circulation and increase the likelihood of delivery to tumors in distal organ sites. Therefore, we screened a large library of polymer-lipids to identify LNPs capable of transfecting cells *in vitro*, but with relatively low cell uptake, potentially indicating lower opsonization by plasma proteins. These formulations (e.g. FRM119) achieved long circulation in mice but resulted in relatively poor transfection in H1299 cells *in vitro*. We then engineered LNPs with **flexible surfaces** (e.g. FRM146) that exhibited more rapid cell uptake and nuclear delivery *in vitro*, potentially due to more dynamic rearrangements of the polymer-lipid inside cells. In addition, these formulations exhibited longer plasma pharmacokinetics (PK) than patisiran-like LNP formulations (e.g. FRM060 or FRM001) in mice. Increased plasma PK correlated with increased tumor exposure to DNA, verifying the ability of longer-circulating LNPs to deliver more DNA to tumors.



H1299 cells treated with 1-10 pg/cell CAG-GFP DNA-LNP, analyzed after 24h. A large library of polymer-lipid variants and compositions were screened against FRM001, FRM055, and FRM060, which are patisiran-like MC3 LNP compositions that differ only in helper lipid type and DMG-PEG2000 mol %.

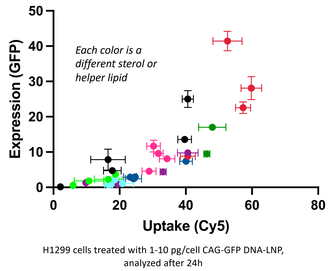




H1299 subcutaneous (SQ) tumors in NSG mice intravenously (i.v.) dosed with DNA-LNP at 1.4 mg/kg, analyzed after 48h

DoE-driven core engineering to further increase transfection of long-circulating LNPs

We identified FRM156 as an LNP with a flexible surface that balanced long circulation with the ability to transfect cells (data not shown). We then sought further improvements in transfection by optimizing core lipid components such as sterols and helper lipids that are known to influence the efficiency of LNP endosomal escape. High-throughput screening identified several compositions that produced significant increases in GFP expression compared to FRM156 *in vitro*. While some of these formulations such as FRM214 did reduce *in vivo* plasma PK, we were able to engineer further improvements such as FRM225 that rescued these effects and resulted in strong DNA expression in multiple lung cancer xenograft tumor models in mice.



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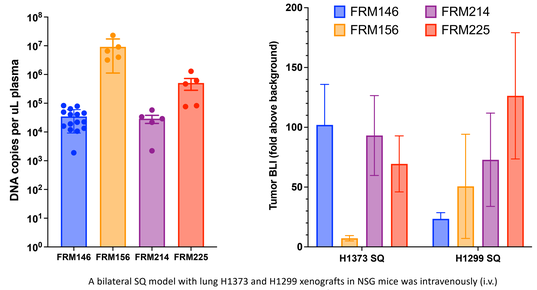
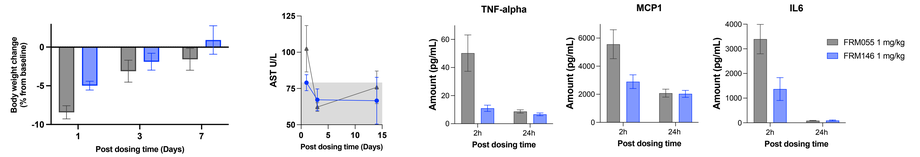


A bilateral SQ model with lung H1373 and H1299 xenografts in NSG mice was intravenously (i.v.) dosed with CAG-FLUC DNA-LNP at 1.4 mg/kg and analyzed after 48h

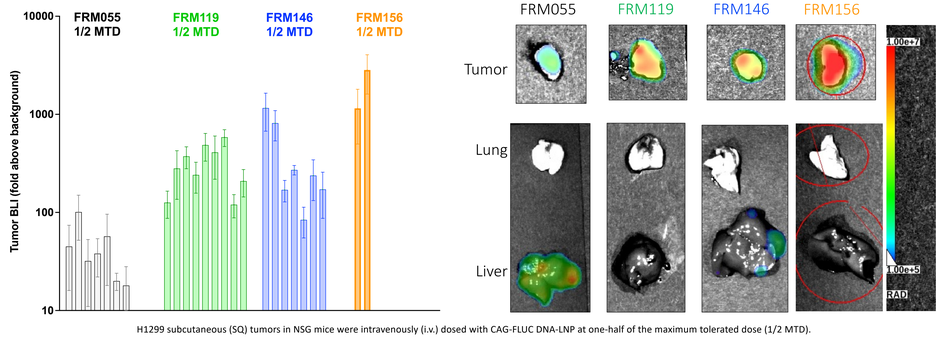
Earli LNPs are better tolerated and have lower immune stimulation than liver-tropic LNPs

We hypothesized that altering LNP biodistribution and clearance mechanisms could alter the tolerability of LNPs *in vivo* due to reduced uptake by liver and immune cells. Indeed, we determined that LNPs with flexible surfaces such as FRM146 trended towards lower body weight loss, lower elevations of liver enzymes such as AST, and lower immunotoxicity as measured by acute cytokine elevations post i.v. DNA-LNP administration.



Earli LNPs eliminate liver expression and produce >10-fold increase in tumor expression

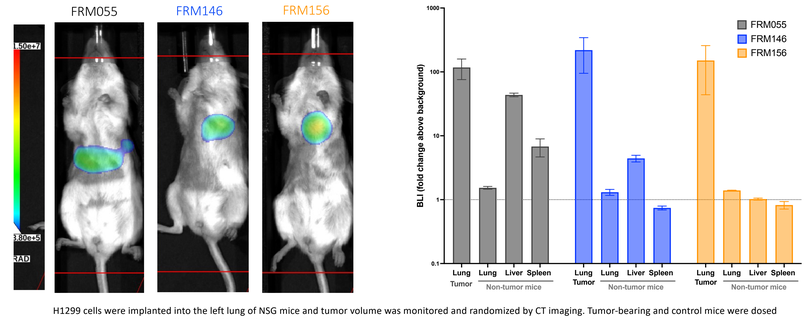
LNPs engineered for long circulation were evaluated for gene expression and biodistribution in mouse tumor models. Strikingly, we found that Earli LNPs not only produced **10-fold or greater** tumor DNA expression than patisiran-like MC3 LNPs like FRM055, but also completely abrogated liver DNA expression, resulting in a significantly increased tumor-to-liver signal-to-noise ratio (SNR).



H1299 subcutaneous (SQ) tumors in NSG mice were intravenously (i.v.) dosed with CAG-FLUC DNA-LNP at one-half of the maximum tolerated dose (1/2 MTD). After 48h, tumors and other organs were excised and *ex vivo* bioluminescence imaging (BLI) was performed to determine luciferase reporter gene expression.

Earli LNPs produce strong DNA expression in tumor-bearing but not normal lungs or liver

The ability of Earli LNPs to deliver DNA to lung tumors was evaluated in an orthotopic H1299 xenograft model. Engineered LNPs with flexible surfaces like FRM146 and FRM156 showed strong expression in tumor-bearing lungs, but almost no expression in non-tumor bearing lungs or liver.



H1299 cells were implanted into the left lung of NSG mice and tumor volume was monitored and randomized by CT imaging. Tumor-bearing and control mice were dosed intravenously (i.v.) with CAG-FLUC DNA-LNP at 1.4 mg/kg. After 48h, whole-body and *ex vivo* BLI imaging were performed to measure luciferase gene expression.

Conclusions and Next Steps

We demonstrated that comprehensive engineering of LNP composition enables extrahepatic delivery of DNA to lung tumors, with minimal expression in normal lung and liver tissues. Additional improvements in tumor transfection are currently being pursued through extensive LNP screening, including evaluation of more potent ionizable lipids. When combined with cancer-activated expression of PET reporter genes and further validation in preclinical mouse models and toxicology studies, this represents a promising non-viral delivery platform for early detection and treatment of lung cancer.