High-throughput *in vivo* screening of extra-hepatic lipid nanoparticles enabled by machine learning design of novel chemistry

Scher, Hanna; Chang, Leslie; LaRue, Mark; Singh, Shreya; Srey, Metta; Hamilton, Greg; Mankoo, Parminder; Sarisozen, Can; Deck, Melissa

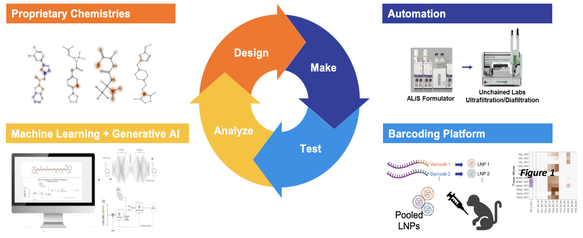
LiberateBio

Abstract

The promise of nucleic acid-based therapeutics is immense for treating a wide range of diseases. The challenge lies in the ability to deliver these therapies to their intended sites in body, ideally specifically and efficiently. Lipid nanoparticles (LNPs) have emerged as a powerful tool for delivery due to their ability to encapsulate and protect nucleic acids and facilitate their entry into cells. With the approval of ONPATTRO and COVID mRNA vaccines, most development is limited to liver indications and vaccines. Most LNPs accumulate in the liver when administered systemically with no or limited exposure in other tissues and organs. Liberate Bio is hoping to realize the full potential of nucleic acid medicines by building nanoparticle chemistries using novel cationic ionizable lipids to unlock delivery to extrahepatic tissues. We have developed a high-throughput pooled biodistribution assay focused on non-human primates enabling us to screen as many as 96 formulations simultaneously in a single dosing. This has enabled us to generate large amounts of high-quality data in a short timeframe to supply the curated data requirements for improved Machine Learning models.

Objectives

To establish a high-throughput platform to nominate, generate, evaluate and analyze novel lipid chemistries for delivery to extrahepatic tissues in a non-human primate.



Methods

Individual LNPs encapsulating unique barcoded mRNAs are manufactured by an optimized formulation platform for comparable critical process attributes regardless of the scale (Figure 2, A). All LNPs with unique ionizable lipids are characterized individually upon formulation, and a summary of colloidal attributes is given in Figure 2, B. Particle size and PDI of more than 200 unique LNPs with attributes suitable to be dosed in pooled NHP screenings show a good range of CQA representation, with monomodal size distributions. LNPs are pooled based on multiple parameters for NHP screens, and pools are characterized prior to dosing to ensure colloidal stability and safety of the dosing materials. Figure 2, C shows seven different NHP pool characterization parameters, which cover a representative range of individual LNPs. Each data point in Figure 2, C indicates a dosing pool with 25-100 LNPs.

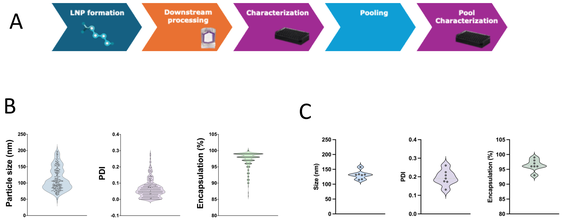


Figure 2

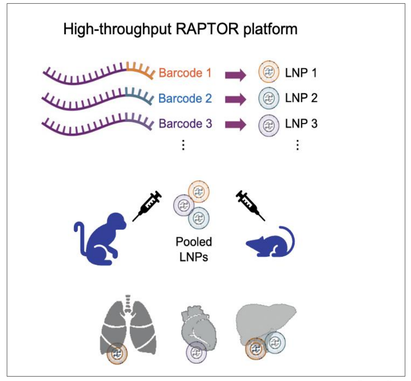


Figure 3

Pooled formulations described are then dosed in both non-human primates and mice. At terminal collection, many punches from multiple organs are collected and stored to preserve RNA and protein integrity. Tissues are homogenized and relevant molecules (RNA or Protein) extracted. Barcode enrichment and subsequent library amplification were conducted and libraries sequenced using standard NGS or Quantum-Si Platinum® Next-Gen Protein Sequencing ™ (NGPS™) platforms.

For RNA barcode detection, extensive bioinformatics pipeline optimization was conducted including UMI length, Hamming distance and QC of barcode counting. Barcode counts were normalized to pooled dosing formulation input, to account for difference in RNA amounts. Within a sequencing run, normalized barcode counts were then evaluated for fold change to quantified MC3 barcode counts on a per organ basis.

Similarly for NGPS, in silico (synthetic peptides) pools were sequenced and used for normalization of study sequencing. For NGPS, both enrichment and library protocols were developed to increase sensitivity and reproducibility of sequencing.

Results

Initial work was focused extensively on validating the recovery of barcodes in dose-dependent manner across lipids and tissues. Figure 4b shows a single LNP formulation dosed with three sets of barcodes in ascending doses. The subsequent barcode counts were normalized by the same dose-ascending inputs. The normalized output demonstrates equivalent recovery across a log dose range in non-human primates. Targeted deep sequencing of barcoded mRNA payloads show lipid-specific extrahepatic bioaccumulation in non-human primates (Figure 4c). Heatmap (Figure 4c) shows a pool of twenty-five novel Liberate generated lipid formulations compared to MC3 across five tissues and three biological replicates (Figure 5). Results shown as fold enrichment over MC3 in given organ. These results demonstrate liver de-targeting relative to MC3, and enrichment across different tissues – greatest enrichments are seen in spleen. A range of lipids, both extrahepatic and liver tropic, were moved forward to evaluate expression in unpooled and pooled formats.

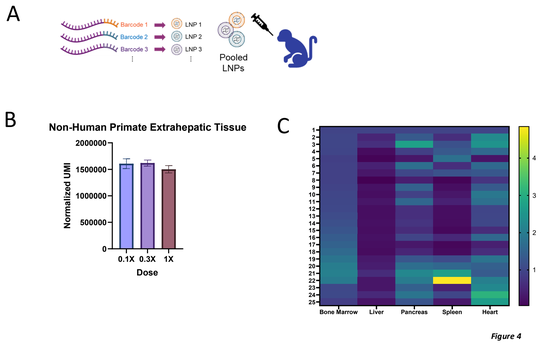


Figure 4

Biodistribution of the LNP and its cargo is the first step in a pathway to expression and potency of therapeutic nucleic acid therapy. As a subsequent screening step, we have developed an *in vivo* peptide barcoding strategy that enables sensitive and reproducible measurement of expression *in vivo*. By labeling mRNA with a nucleic acid barcode and at the same time labeling the subsequent protein product of that mRNA with a peptide barcode, Liberate can measure simultaneous biodistribution and expression information of large pools of LNP formulations. A rodent study was designed to evaluate feasibility of recovery. Figure 5c shows that when one lipid is dosed with five peptide-barcoded mRNAs in equal ratio, the recovery of the barcodes through enrichment from tissues and Quantum Si's Platinum NGPS platform for barcode identification is near expected recovery.

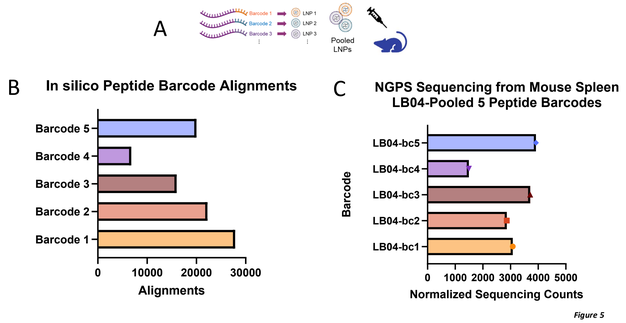


Figure 5

In a separately dosed mouse group, the five mRNA with peptide barcodes were formulated using Liberate proprietary lipid formulations with data showing ranking for expression in an unpooled format (Figure 6a). These data were used to compare to the pooled mRNA peptide recovery from a separate dosing group of animals (Figure 6b).

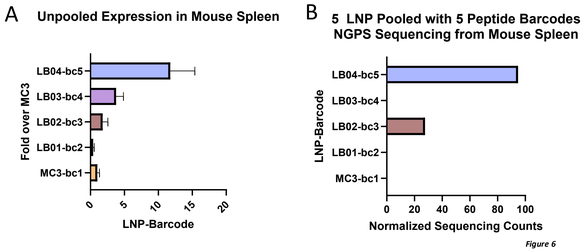


Figure 6

Conclusions

Barcode recovery of novel lipid formulations demonstrate utility of Liberate Bio's bioaccumulation in non-human primates. Liberate Bio's novel lipids and formulations show de-targeting of liver compared to the industry standard MC3 liver-tropic formulation. Additionally, these novel lipids show increased distribution to extrahepatic organs including heart, spleen and bone marrow. This assay has enabled a prioritization of interesting lipids in the most biologically relevant available model – non-human primate.

These results include the initial proof of concept data generated using mice with a pool of five LNPs which were independently evaluated individually for expression in several extrahepatic tissues. We show that this method accurately reflects the individual expression of mRNA delivered by LNPs in the spleen. These peptide barcodes were recovered utilizing the Platinum Next-Generation Protein Sequencing (NGPS) platform from Quantum-Si.

There is additional optimization required to find peptide barcodes that have similar sequencing rates to reduce requirements to normalize alignment normalization and peptide barcode drop out, as with peptide bc4. Additional work is necessary to demonstrate reproducibility of recovery across barcodes and extrahepatic organs. Liberate Bio is working closely with Quantum-Si in optimizing barcode enrichment and library prep methods to increase sensitivity, which should enable a larger pooled format.

Liberate has built a robust platform for the screening of novel LNPs in a high throughput pooled format using barcoded mRNA. We have further expanded upon that approach by integrating peptide barcoding into our screening funnel allowing us to screen rapidly for biodistribution of mRNA in organs and tissues of interest and then identify those LNPs which also demonstrate productive delivery through robust peptide sequencing. This platform results in much cheaper and quicker screening of LNPs without losing the mechanistic understanding of generating both biodistribution and expression data from pooled formulations.

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