Alterations in NHP Liver Gene Expression Profiles Following Intravenous Infusion of mRNA-LNP

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Abstract

Introduction

Lipid nanoparticles (LNP) have been used extensively in the development of nucleic acid-based therapeutics due to their efficacy, low immunogenicity and versatility in encapsulating various therapeutic modalities. In preclinical studies, non-human primates (NHP) are routinely used to evaluate the pharmacodynamic activity and safety of new LNP formulations and nucleic acid payloads, often with low numbers of animals and substantial inter-animal variability observed.

Methods

To evaluate the magnitude and possible sources of inter-animal variability in the pharmacodynamic response to mRNA-LNP treatment, cynomolgus monkeys were given a single 1-hour intravenous infusion of saline (n=4) or LNP encapsulating human IgG mRNA (2.0 mg/kg dose) (n=20). Blood was sampled at various time points post-dose and plasma IgG expression was assessed as a potency readout. mRNA-LNP treatment resulted in a potency distribution spanning an approximately 18-fold range of IgG concentrations (16 to 293 µg/mL), with no apparent correlation to individual pharmacokinetic profiles. To investigate changes in gene expression that may drive the magnitude of pharmacodynamic response and/or inter-animal variability, liver biopsies were collected before treatment and 2 days after dosing, and were processed for transcriptomic analysis. Strand-specific library construction for PE150 Illumina sequencing was performed following globin and ribodepletion.

Results

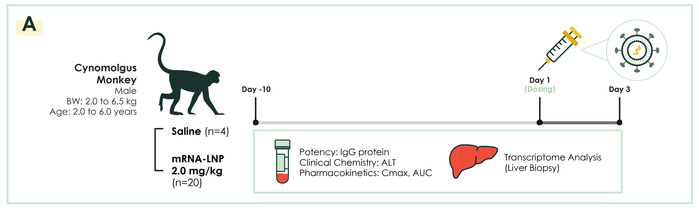
Principal component analysis resulted in two generally discernible groups, with one cluster comprising post-LNP treatment samples. Analysis of differentially expressed genes revealed 268 and 182 transcripts that were significantly upregulated and downregulated, respectively, in post-LNP treatment samples compared to saline control samples at a matched timepoint (48 h; log2FC ≥ |2| and p < 0.05). Differentially regulated genes were enriched in biological processes involved in immune/inflammatory response as assessed by functional over-representation analysis. Significantly upregulated genes include FOSL1, an AP-1 transcriptional factor subunit that negatively regulates type I interferon signaling; CCL18, a primate-specific chemokine produced by macrophages and dendritic cells that is associated with a tolerogenic response; and DHRS9, a dehydrogenase/reductase that oxidizes oxylipins, a class of immunoregulatory mediators. Upregulation of these genes suggests that pathways involved in immune modulation and preventing immune system dysregulation are activated following LNP treatment. Hierarchical clustering and investigations into the contribution of animal body weights or liver transaminase elevations did not reveal any obvious trends that correlated with pharmacodynamic response.

1 Sequencing and Bioinformatics Analysis

Library construction and sequencing were performed at the BC Genome Sciences Center using the Illumina NovaSeq platform. Bioinformatic analysis was performed by the UBC Sequencing and Bioinformatics Consortium using the genome sequence for *M. fascicularis* obtained from NCBI (GCF_012559485.2). Information on the analysis pipeline is included below:

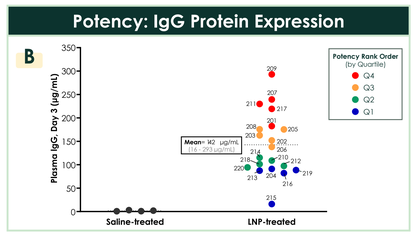
- **Read Quality Control**: FastQC (version 0.11.8); cutadapt (version 2.3); Picard (version 2.20.2)
- **Read Alignment**: STAR (version 2.20.2)
- **Read Counting**: featureCounts (version 2.0.1)
- **Differential Gene Expression Analysis**: DESeq2 (version 1.42.0)
- **Overrepresentation Analysis**: clusterProfiler (version 4.10.0)

2 mRNA-LNP treatment resulted in a broad potency distribution



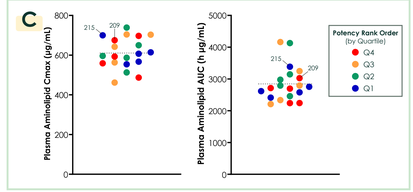
A. Male cynomolgus monkeys were administered saline (n=4) or 2.0 mg/kg IgG mRNA-LNP (n=20) via a 1-hour intravenous infusion. Blood was sampled pre-study (Day -10) and at 48 h post-dose (Day 3) to assess potency by IgG protein expression. Bulk RNA sequencing was performed using liver biopsies collected on Day -10 and Day 3 for differential gene expression analysis.

Potency: IgG Protein Expression



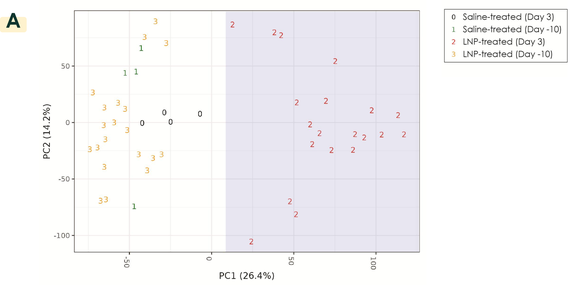
B. Plasma IgG expression was determined by ELISA at 48 h post-administration. mRNA-LNP treatment resulted in a broad distribution in potency spanning approximately 18-fold in IgG concentration (mean: 142 µg/mL; range: 16 to 293 µg/mL). Individual datapoints are color-coded based on quartile ranking (Red=Q4; Orange=Q3; Green=Q2; Blue=Q1).

Pharmacokinetics: Cmax and AUC

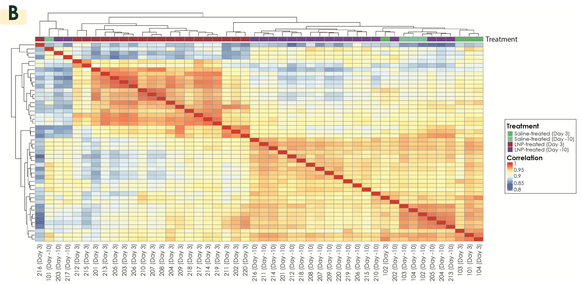


C. Potency does not appear to be driven by pharmacokinetic parameters including Cmax (left) or AUC (right). Plasma levels of the parental aminolipid in the mRNA-LNP formulation were determined by LC-MS/MS. Cmax = maximum concentration; AUC = area under curve.

3 Similarities in overall gene expression profiles were observed in mRNA-LNP-treated animals



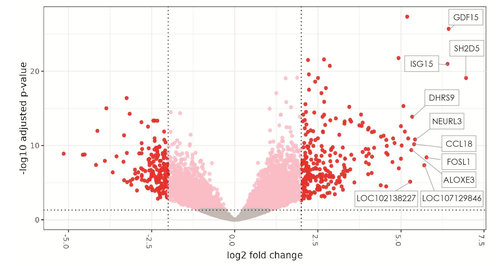
A. To determine if differential gene expression drives the magnitude of pharmacodynamic response, transcriptome analysis was performed on liver biopsies obtained pre- and post-LNP treatment. Principal component analysis, a dimensionality reduction approach, was performed and resulted in two primary clusters. One general cluster consisted of Day 3 LNP-treated samples (shaded in blue), suggesting similarities in gene expression profiles. A secondary cluster consisted of all remaining samples, including Day -10 and Day 3 saline-treated samples and Day -10 LNP-treated samples.



B. Hierarchical clustering heatmap. Pairwise correlation of gene expression across all samples is shown. General clustering of samples by treatment was observed, suggesting similarities in gene expression profiles.

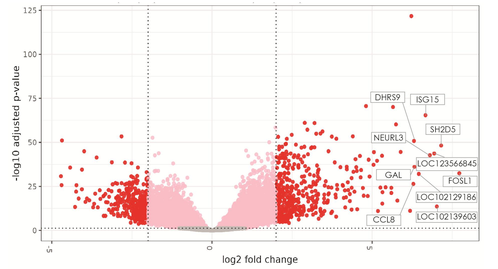
4 mRNA-LNP treatment resulted in upregulation of some genes involved in immune modulation

LNP-treated (Day 3) vs. Saline-treated (Day 3)



Gene	Gene Name	Log2 Fold Change	Adjusted p-value
SH2D5	SH2 domain containing 5	6.96	8.06E-20
GDF15	Growth differentiation factor 15	6.43	1.94E-26
ISG15	ISG15 ubiquitin like modifier	6.40	1.04E-21
FOSL1	FOS like 1, AP-1 transcription factor subunit	5.77	3.98E-09
LOC107129846	Uncharacterized LOC107129846	5.70	4.61E-08
NEURL3	Neuralized E3 ubiquitin protein ligase 3	5.42	1.53E-11
CCL18	C-C motif chemokine ligand 18	5.40	6.68E-11
DHRS9	Dehydrogenase/reductase 9	5.34	1.32E-14
ALOXE3	Arachidonate lipoxygenase 3	5.31	4.18E-10
LOC102138227	Interferon lambda-4	5.28	7.49E-06

LNP-treated (Day 3 vs. Day -10)

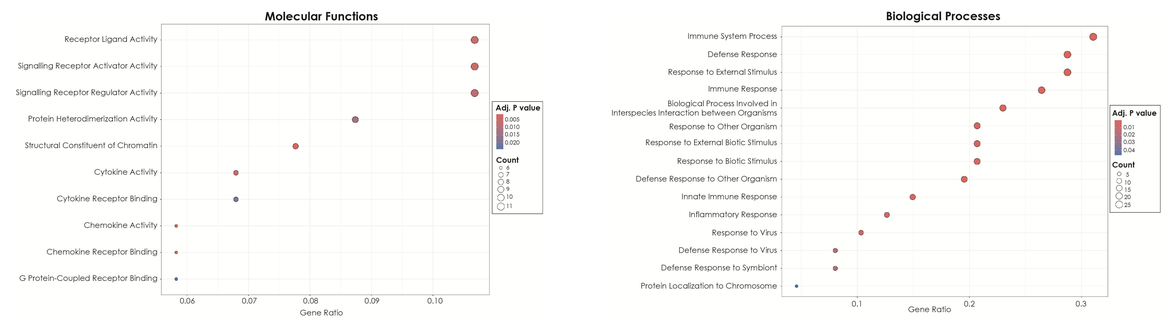


Gene	Gene Name	Log 2 Fold Change	Adjusted p-value
FOSL1	FOS like 1, AP-1 transcription factor subunit	7.72	3.41E-33
SH2D5	SH2 domain containing 5	7.15	4.74E-49
LOC102139603	Interferon lambda-1	7.02	2.33E-14
LOC123566845	Uncharacterized LOC123566845	6.94	1.91E-44
NEURL3	Neuralized E3 ubiquitin protein ligase 3	6.80	1.78E-43
ISG15	ISG15 ubiquitin like modifier	6.66	3.69E-66
LOC102129186	Retinoic acid early transcript 1E	6.45	7.83E-33
GAL	Galanin and GMAP prepropeptide	6.31	3.31E-37
DHRS9	Dehydrogenase/reductase 9	6.30	1.49E-51
CCL8	C-C motif chemokine ligand 8	6.28	3.80E-27

Differentially expressed genes (DEGs) identified in LNP-treated (Day 3) vs. saline-treated (Day 3) condition (top) and LNP-treated (Day 3 vs. Day -10) condition (bottom) are depicted in volcano plots. DEGs were filtered by adjusted p-value < 0.05 and log2 Fold Change ≥|2|. Tabulated summaries of the top 10 DEGs are provided below each volcano plot. Common genes identified in both conditions are shaded in green.

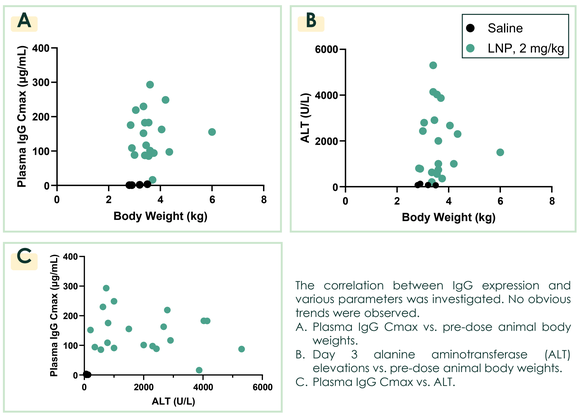
5 Over-Representation Analysis (ORA) identified gene sets that were enriched upon mRNA-LNP treatment

LNP-treated (Day 3) vs. Saline-treated (Day 3)



To control for procedure-related effects, differentially expressed genes in the Day 3 LNP-treated vs. saline-treated condition were used for ORA. Gene sets in the molecular functions and biological processes gene ontology classifications were enriched following mRNA-LNP treatment.

6 Pharmacodynamic response did not correlate with body weight or transaminase (ALT) elevation



The correlation between IgG expression and various parameters was investigated. No obvious trends were observed.

A. Plasma IgG Cmax vs. pre-dose animal body weights.
B. Day 3 alanine aminotransferase (ALT) elevations vs. pre-dose animal body weights.
C. Plasma IgG Cmax vs. ALT.

Summary

1. mRNA-LNP treatment resulted in a broad potency distribution that was not correlated with pharmacokinetic parameters (Cmax and AUC).
2. Principal component analysis and hierarchical clustering resulted in general clustering of samples based on treatment, suggesting similarities in gene expression profiles.
3. Over-representation analysis of DEGs from the Day 3 LNP-treated vs. saline-treated condition identified enrichment of gene sets in the molecular functions and biological processes gene ontology classes.
4. mRNA-LNP treatment resulted in differential upregulation of some genes involved in immune modulation, including FOSL1, CCL18 and DHRS9.
5. mRNA-LNP potency, as measured by IgG expression, does not appear to be correlated with animal body weights or ALT elevations in this study. Changes in gene expression upon LNP treatment appear to be driven by multiple factors that all together tune the pharmacodynamic response.

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