Safety, Efficacy and Biodistribution of LNP-formulated mRNA

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Abstract

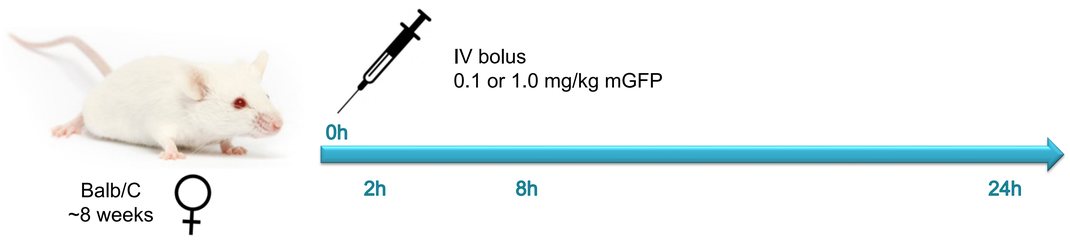
Effective delivery of therapeutic mRNA offers the potential to trigger expression of disease relevant proteins and thus opens new routes for multiple therapeutic applications. Development of mRNA therapeutics requires an in depth understanding of activity, biodistribution and safety properties of this novel therapeutic modality in combination with the selected delivery technology. Here we describe the analysis of safety aspects together with efficacy and PK/BD characterization of an LNP-formulated mRNA in mice.
GFP-mRNA (TriLink) was formulated in two different Lipid-Nano-Particles (LNPs) and administered systemically at two dose levels (1 mg/kg and 0.1 mg/kg) as a systemic bolus to Balb/C mice. Plasma and tissue samples were collected for further analysis 2, 6, and 24 hours after administration.
GFP-mRNA was quantified from plasma and tissue samples using Quantigene 2.0 Reagent Assay (Affymetrix). mRNA quantitation was linear over approx. 3 orders of magnitude, ranging from an ULOQ of 10 pg down to an LLOQ of 15 fg per well with maximally 2 mg of tissue per well. In samples from liver, kidney, and spleen significant mRNA-amounts were detected at both dose levels and for both LNP-formulations at all time points. In addition, a differing distribution of GFP-mRNA formulated with LNP1 or LNP2 was visualized in liver tissue by in situ hybridization.
For quantification of GFP protein expression we established a U-Plex assay (MSD) which has a broader linear range when compared to commercially available ELISAs. GFP protein assay displayed a robust detection between 10,000 and 15 pg GFP/ml. In liver samples of animals treated at both dose levels and at all time points GFP protein expression was detected in significant quantity.
To make the quantification analyses complete, the main lipid component of the LNP was quantified in plama and liver by LC/MS.
Finally, as a first safety assessment, we measured ALT and AST from plasma together with a panel of cytokines using the MSD multiplex platform.
Taken together the analyses described allow a careful characterization of efficacy, safety and PK/BD of mRNA therapeutics and can advance the preclinical development of this therapeutic modality.

Aim

To provide a full safety, efficacy and PK/PD analysis platform for mRNA compounds delivered by LNPs.

Methods & Results

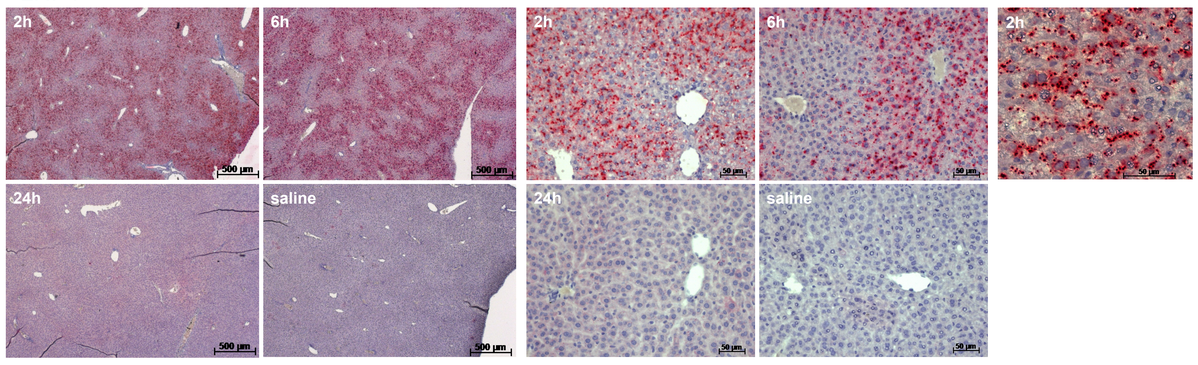
Experimental Outline



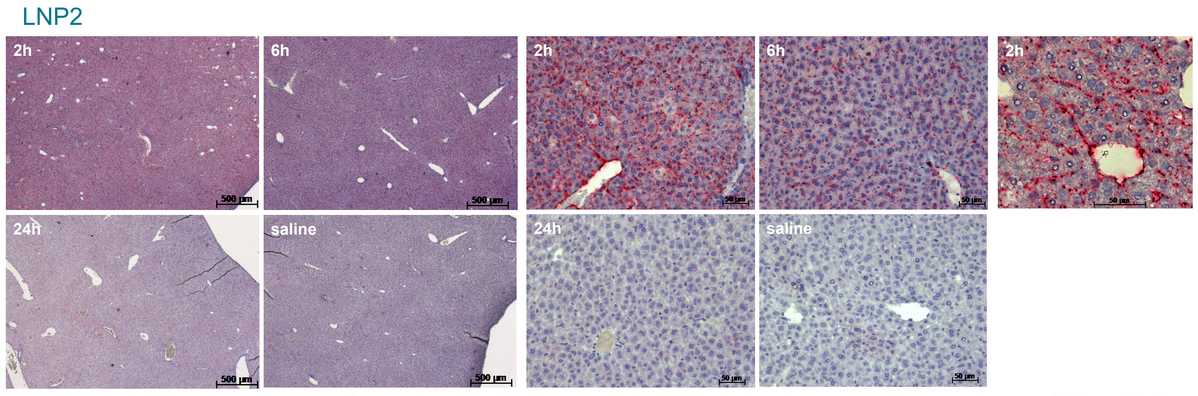
- GFP-mRNA (mGFP) from TriLink was formulated with two different LNPs and administered in two doses
- Animals were sacrificed at 3 different time points post administration
- Harvest of:
  - blood for plasma: mGFP quantification & cytokine 10-plex
  - Liver: mGFP quantitation & cationic lipid quantification & GFP-ELISA
  - Spleen: mGFP quantitation & GFP-ELISA
  - Kidney: mGFP quantitation & GFP-ELISA

GFP-mRNA visualization by in situ Hybridization

LNP1



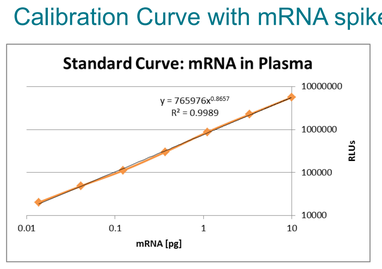
LNP2



In situ hybridization was performed with RNAscope (acd-Bio) in liver paraffin sections. GFP-mRNA formulated in LNP1 was distributed in a pattern concentrated around vessels whereas GFP-mRNA formulated in LNP2 was more evenly spread. At high magnification an accumulation of a significant portion of LNP2-mRNA in vessels and sinusoids was observed.

GFP-mRNA Quantitation from Tissue Lysates

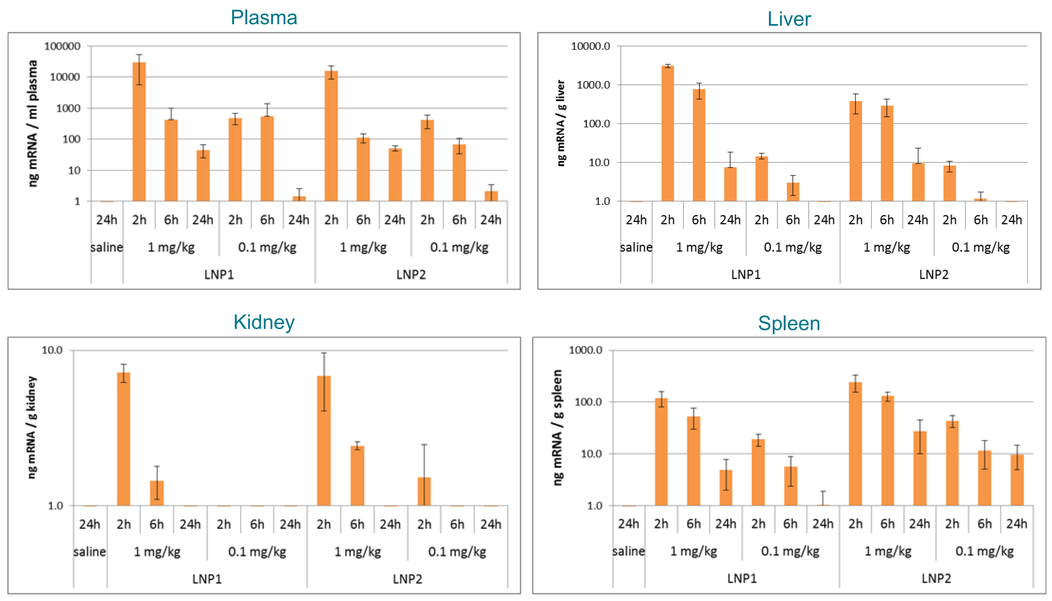
Calibration Curve with mRNA spiked into Plasma



LLOQ: 8 fg / mg of tissue or 0.5 fg / µl of plasma
ULOQ: 5 pg / mg of tissue or 0.3 pg / µl of plasma
R^2 = 0.9989

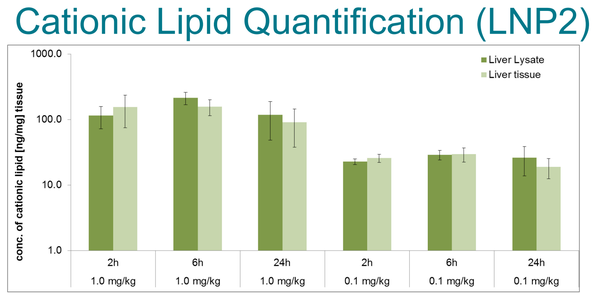
For mRNA quanititation GFP-mRNA (TriLink) spiked into lysates of respective matrices was used as a standard.

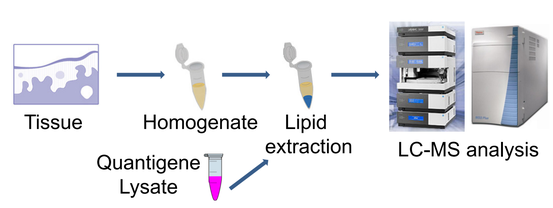
GFP-mRNA Quantitation from Different Tissues



mRNA was quantified using Quantigene 2.0 and a specific probeset to mGFP. Highest amount of mGFP was detected 2h post injection, 24h after injection mGFP was close to limit of detection.

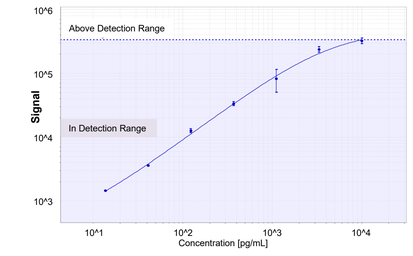
Cationic Lipid Quantification (LNP2) from Liver

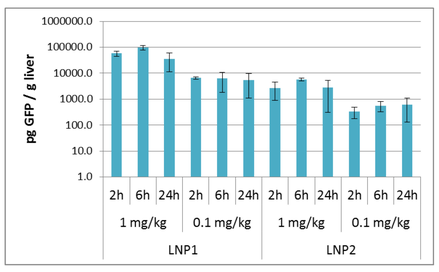


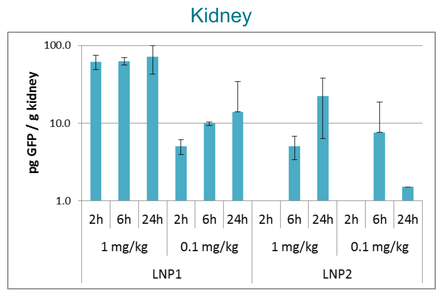


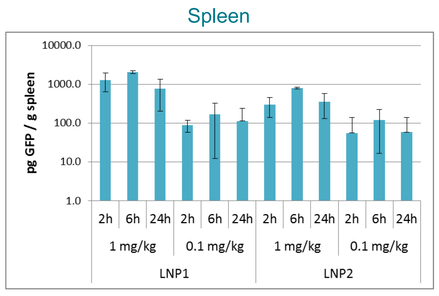
Lipid quantification was either done with direct extracts from liver tissue or with the same lysates used for mRNA quantification. Both were measured by LC/MS. Comparing the amounts of cationic lipid in liver and liver lysates, the applied detection and quantification method is suitable for both sample extraction procedures (water vs. bDNA lysis buffer). Compared with the amounts of mRNA, lipid concentrations dropped more slowly in liver.

GFP ELISA



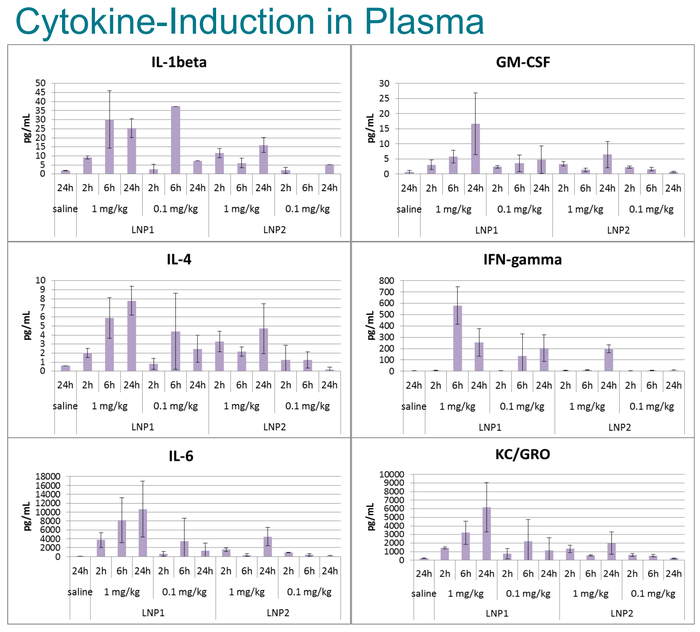






GFP ELISA was established with MSD streptavidin plates and two anti-GFP antibodies, one linked to biotin for plate binding, the other being target for a secondary antibody carrying the MSD-Sulfo-Tag.
Recombinant GFP was spiked into respective tissue lysates as a standard (4 parameter logistic fit). Background obtained with saline samples was subtracted .

Cytokine-Induction in Plasma



Ten cytokines were analyzed from mouse plasma with an MSD U-Plex panel: IL-1beta, IL-2, IL-4, IL-6, IL-10, IL-12p70, GM-CSF, IFN-gamma, KC/GRO, TNF-alpha. Compared with saline treated mice, all cytokines tested were induced in a time dependent manner. Here, only cytokines showing the strongest induction are depicted.

Axolabs' service portfolio for mRNA compounds

Analytics:
- LC/MS-based mRNA finger-printing to analyze mRNA identity.
- Poly(A)-tail characterization
- CAP-characterization.

Bioanalytics: PK and Biodistribution
- Quantitative detection of mRNA therapeutics from biological matrices (GLP & non-GLP)
- Quantitative detection of LNP components
- In situ hybridization

Biological and Pharmacological Analyses
- Safety analyses
- Cell-based assays for cell function, proliferation and toxicity (ELISA, Luminex and MDS-platforms)
- Ligand-receptor interaction and uptake studies / histology
- Flow cytometry