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Ready-To-Use Lipid-Based Nanoparticles Solution as an Innovative Alternative to LNPs for the Delivery of RNA Therapeutics

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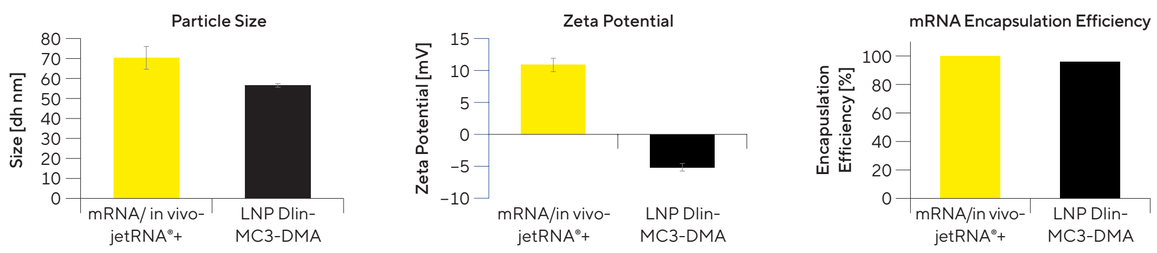
1. Introduction

Ionizable lipid nanoparticles (LNPs) have been widely used for in vivo delivery of RNA therapeutics with the particularity that they predominantly end up targeting the liver. The current challenge is the use of commercially available lipids to achieve specific formulations that can ensure wider biodistribution of the RNA once delivered to target different organs and cell types. mRNA/in vivo-jetRNA®+ nanoparticles and LNPs were characterized by DLS to assess size and zeta potential and mRNA encapsulation efficiency was assessed using the RiboGreen assay. Here we demonstrate how our ready-to-use in vivo-jetRNA®+ transfection reagent can efficiently deliver mRNA to different organs. Firstly, we evaluated in vivo biodistribution using both systemic and local administration routes (intravenous, intraperitoneal and intramuscular). Secondly, we performed an in vivo toxicity study in mouse post-delivery of mRNA-in vivo-jetRNA®+ nanoparticles.
In addition to biodistribution, efficacy and toxicity, we assessed the stability of mRNA encapsulated within in vivo-jetRNA®+ when stored at 4 °C or room temperature for 1 month.

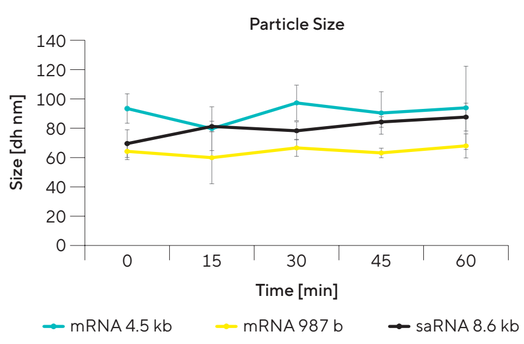
2. mRNA Nanoparticles Characterisation and Stability

Figure 1: *mRNA /in vivo-jetRNA®+ Nanoparticles are Positively Charged and Encapsulate 100% of mRNA*



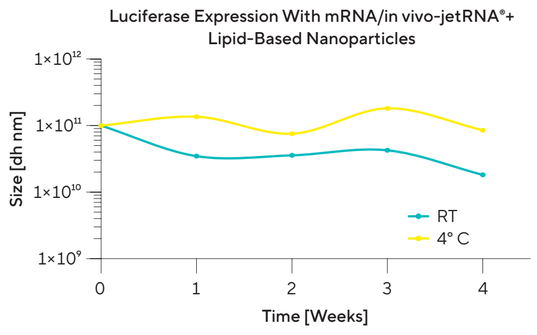
Note. Size and zeta potential of lipid-based nanoparticles with mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer at 50 ng/µL after 1 hr of complexation or ionizable LNPs at 250 ng/µL were measured by dynamic light scattering (DLS). Encapsulation efficiency was assessed by the RiboGreen assay.

Figure 2: *in vivo-jetRNA®+ is Suitable for Different Sizes of mRNA and saRNA*



Note. Size of mRNA/in vivo-jetRNA®+ nanoparticles using 50 µg of mRNA/mL with a small size mRNA (978 b), a medium size mRNA (4.5 kb) or a self-amplifying RNA (8.6 kb) after 15, 30, 45 and 60 min of complexation was measured by DLS.

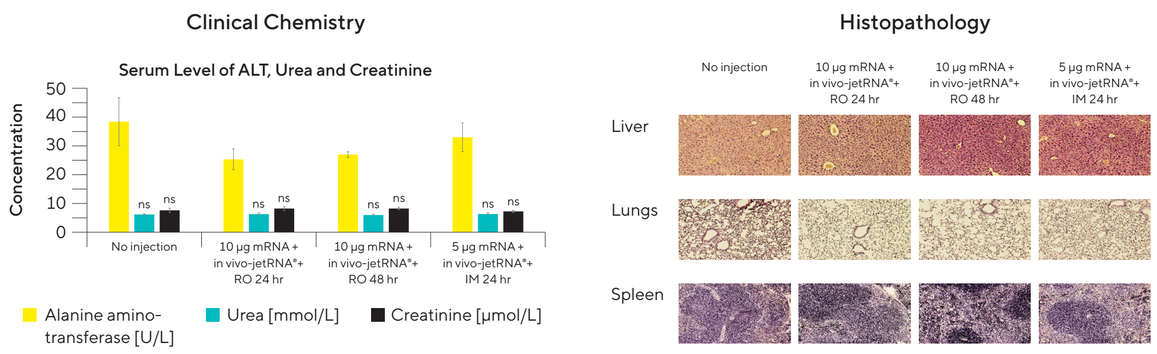
Figure 3: *mRNA/in vivo-jetRNA®+ Nanoparticles Retain Their Transfection Efficacy for up to 1 Month When Stored at 4° C*



Note. Caco-2 cells were transfected with lipid-based nanoparticles, formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer, stored at RT or 4° C for up to 4 weeks. 500 ng of mRNA encoding luciferase were used for 40,000 cells. Luciferase was assessed 24 hr after transfection.

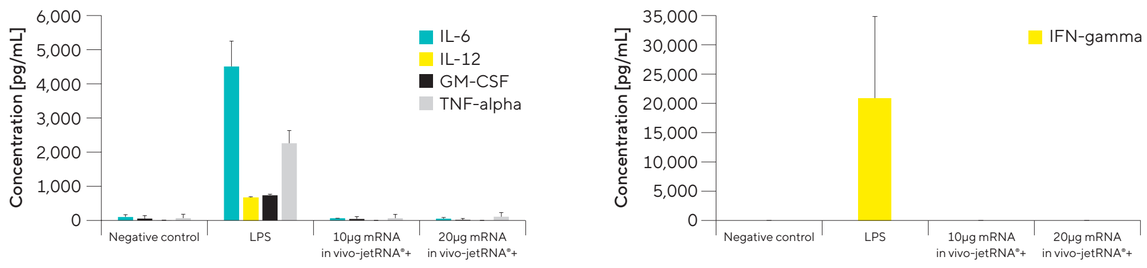
3. Safe mRNA Delivery for Systemic and Local Administration

Figure 4: *Both IV and IM Injections of mRNA/in vivo-jetRNA®+ Nanoparticles Maintain Healthy Animals*



Note. mRNA was injected into mice using in vivo-jetRNA®+ through retro-orbital (RO) injection or intramuscular (IM) injection. Complexes were formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer using 10 µg mRNA for RO or 5 µg mRNA for IM (n = 8 for each batch, 4 males and 4 females). Animals were subject to blood collection for clinical chemistry and hematology measurement (H&E stained, 40x). The following parameters were measured on plasma: alanine aminotransferase (ALT) representing the liver function and creatinine and urea representing the kidney functions. Statistical significance was analyzed by a one-way Anova analysis followed by Dunnett's multiple comparisons test with "no injection" group (ns = not significant).

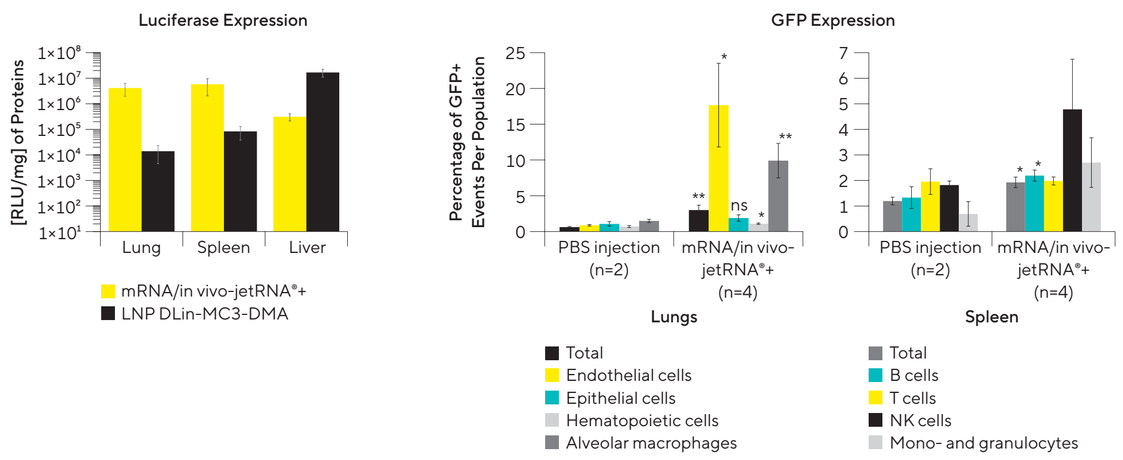
Figure 5: *IV Injection of mRNA/in vivo-jetRNA®+ Nanoparticles Triggers Low Pro-Inflammatory Cytokine Expression*



Note. mRNA complexes were formed in 200 µL of mRNA buffer using 10 or 20 µg of mRNA encoding luciferase at a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) and injected through intravenous injection (retro-orbital injection). 2 to 24 hours after injection, blood was collected and the level of IL-6, IL-12, GM-CSF, IFN-gamma and TNF-alpha was measured by ELISA (IL-6) or MACSPlex kits. As a positive control, LPS (200 µg) was administered into mice.

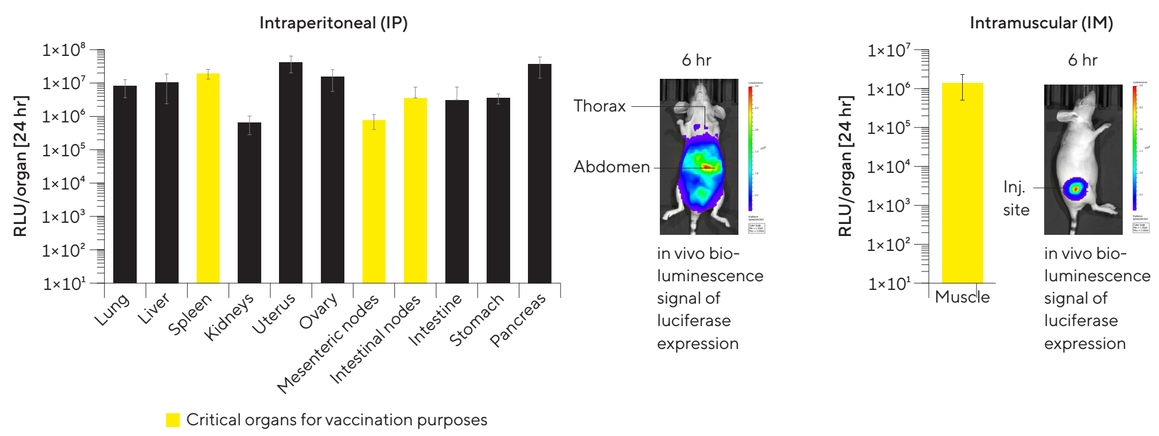
4. Efficient mRNA Delivery for Systemic and Local Administration

Figure 6: *High mRNA Expression in the Lung and in the Spleen Following IV Injection Using in vivo-jetRNA®+*



Note. mRNA was injected into mice using in vivo-jetRNA®+ through intravenous injection (retro-orbital injection). Complexes were formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer using either 10 µg for mRNA encoding luciferase or 40 µg mRNA encoding for eGFP. Luciferase expression was assessed 24 hr postinjection. GFP expression was assessed 24 hr post-injection for lung cells or 4 hr post-injection for spleen cells. Statistical significance was calculated with the unpaired student's t-test for comparing the difference between the PBS and the eGFP mRNA/in vivo-jetRNA®+ (ns = not significant, * p<0.05 and ** p<0.01).

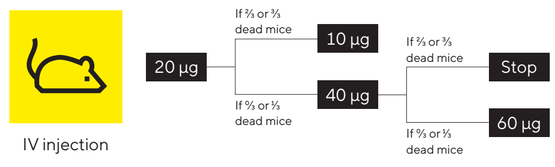
Figure 7: *mRNA/in vivo-jetRNA®+ Nanoparticles Lead to Efficient mRNA Delivery in Different Organs Depending on the Administration Routes*



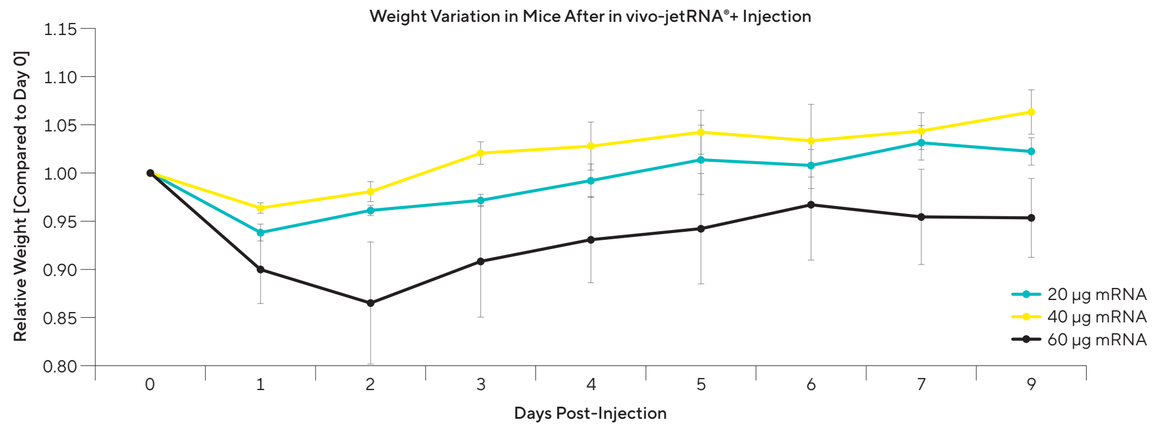
Note. mRNA encoding Luciferase was injected into mice using in vivo-jetRNA®+ through different administration routes. Complexes were formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer using either 20 µg mRNA for intraperitoneal (IP) injection or 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 24 hr post-injection.

5. LD50-Like: Safe mRNA Delivery up to 40 µg FLuc mRNA

Figure 8



mRNA Amount	Physical Appearance	Remote Behavior	Handling Behavior
20 µg	Normal, good grooming	Normal	Normal
40 µg			
60 µg			



Note. 20, 40 or 60 µg of mRNA were injected into mice using in vivo-jetRNA®+ through intravenous injection (retro-orbital injection — RO). Complexes were formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer. The body weights of mice were recorded before injection and every day of the week. Clinical observations (weight, appearance, and behavior) and scoring were made daily for 2 weeks after injection.

6. Conclusion

Our novel in vivo-jetRNA®+ lipid-based nanoparticles formulation is a ready-to-use solution that has been meticulously optimised to ensure efficient delivery of mRNA in vivo, via local or systemic injection routes, while preserving the health of the animal and its organs and being weakly immunogenic. In addition, it is stable for up to 1 month after complexation with mRNA when stored at 4 °C. in vivo-jetRNA®+ therefore lowers barriers and opens new avenues for research and development of mRNA-based therapies and vaccines.