Ready-to-use liposome-based transfection reagent is an innovative alternative to LNPs for the delivery of RNA therapeutics

Claire Guéguen, Malik Hellal, Mélodie Seiler, Thibaut Benchimol, Morgane Ziesel, Maeva Martin, Kassandra Renaud, Patrick Erbacher

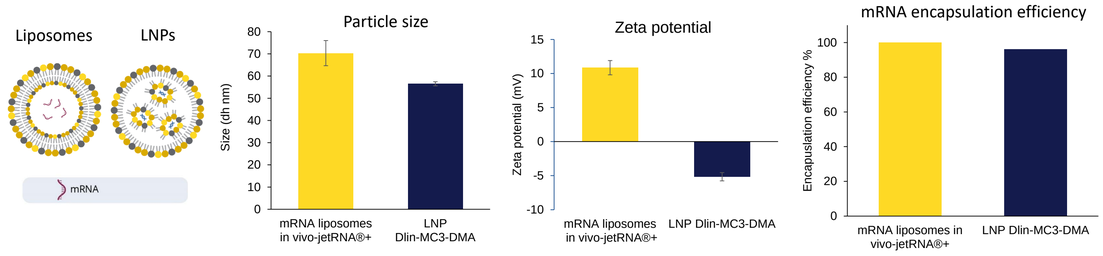
Polyplus-transfection®, Vectura, 75 rue Marguerite Perey, 67400 Illkirch, France

Abstract

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. Liposomes 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 cell lines and organs. Firstly, we evaluated *in vivo* biodistribution using both systemic and local administration routes (respectively intravenous and intramuscular). Secondly, we performed an *in vivo* toxicity study in mouse post-delivery of mRNA- *in vivo*-jetRNA®+ liposomes. In addition to biodistribution, efficacy and toxicity, we assessed the stability of mRNA encapsulated within *in vivo*-jetRNA®+ liposomes by comparing efficacy of complexes following their storage at 4°C for at least 1 month to freshly prepared complexes.

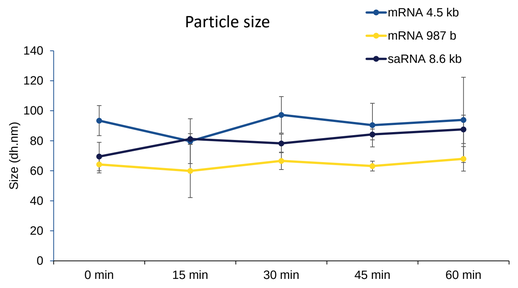
mRNA liposomes characterisation and stability

mRNA liposomes with *in vivo*-jetRNA®+ are positively charged and encapsulate 100% of mRNA



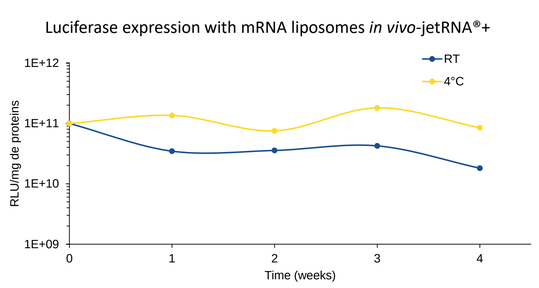
Size and zeta potential of liposomes with mRNA/*in vivo*-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer at 50 ng/µL after 1h of complexation or ionizable LNPs at 250 ng/µL were measured by dynamic light scattering (DLS). Encapsulation efficiency was assessed by the RiboGreen assay.

mRNA liposomes with *in vivo*-jetRNA®+ are stable with different RNA sizes



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

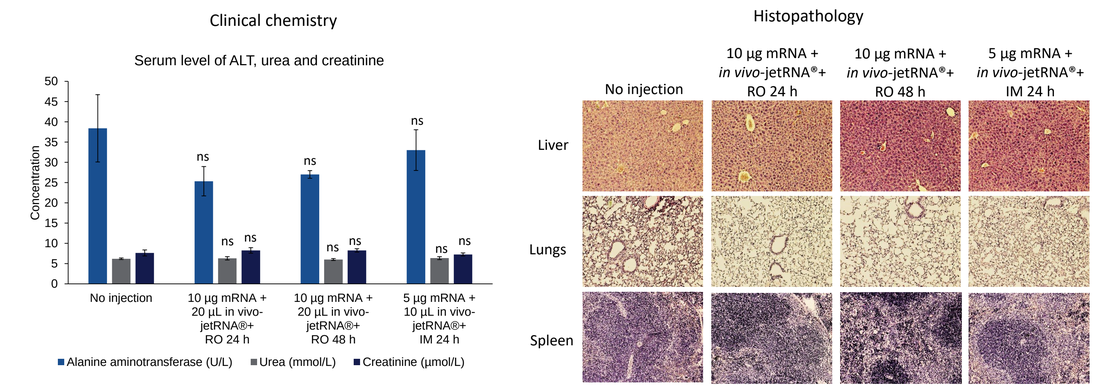
mRNA liposomes with *in vivo*-jetRNA®+ are stable up to 1 month at 4°C



Caco-2 cells were transfected with liposomes, 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 h after transfection.

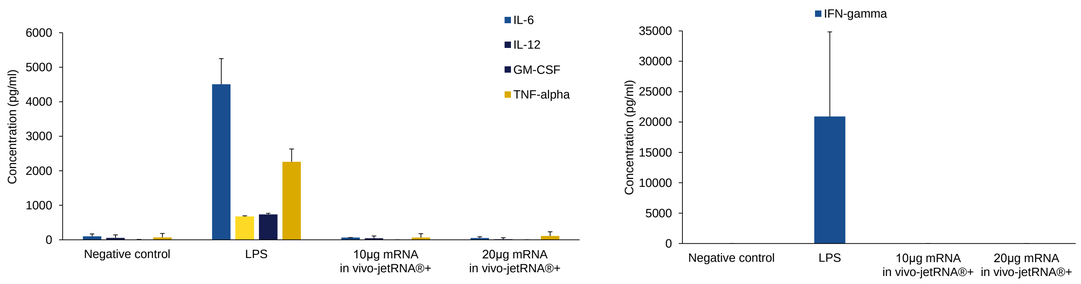
Safe mRNA delivery for systemic administration

Both IV and IM injections of mRNA liposomes maintain healthy animals



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).

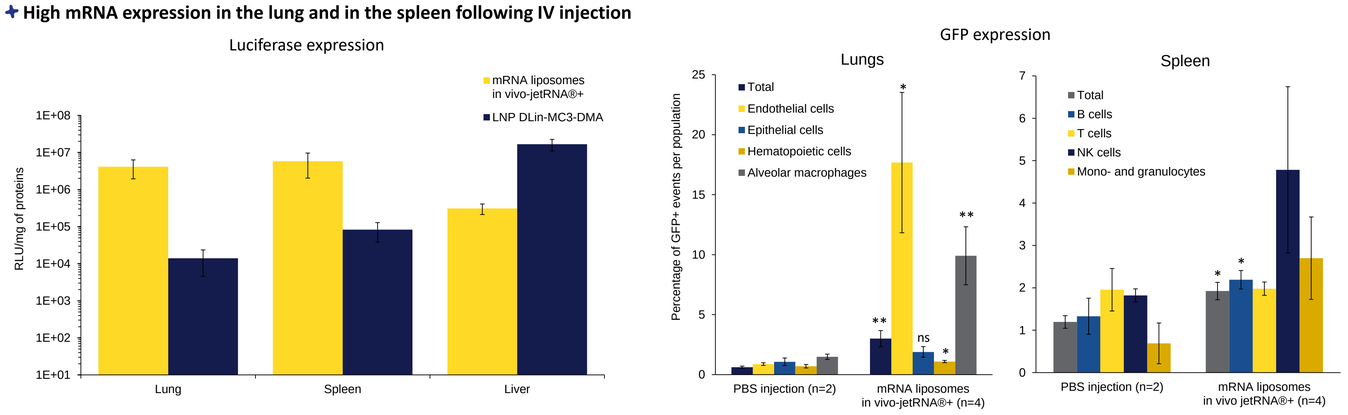
IV injection of mRNA liposomes triggers no pro-inflammatory cytokine expression



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.

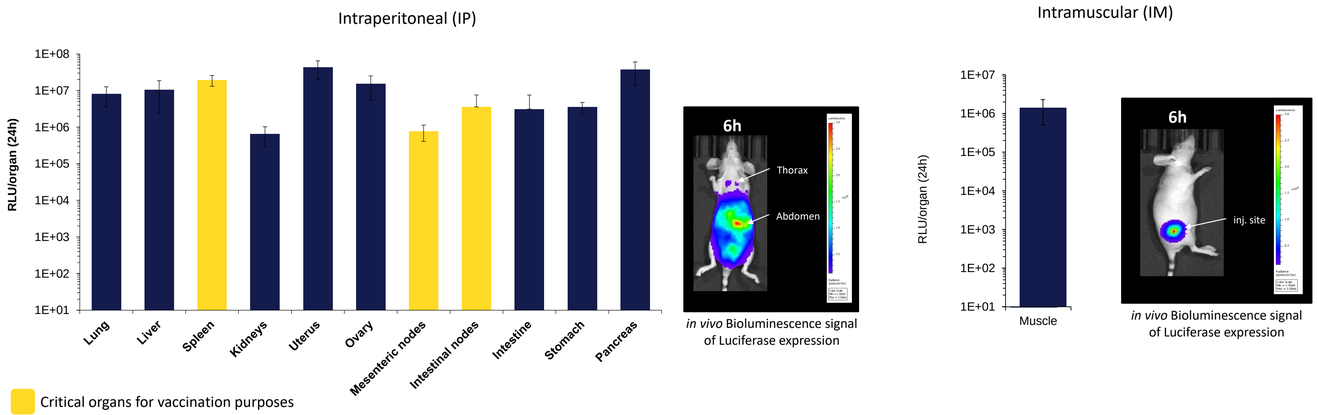
mRNA liposomes: efficient mRNA delivery for local and systemic administration

High mRNA expression in the lung and in the spleen following IV injection



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 h post-injection for lung cells or 4 h 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).

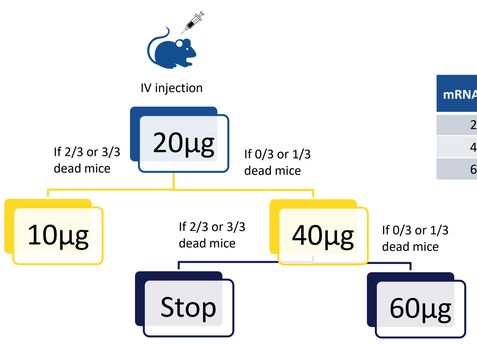
mRNA liposomes with *in vivo*-jetRNA®+ lead to efficient mRNA delivery in different organs depending on the administration routes



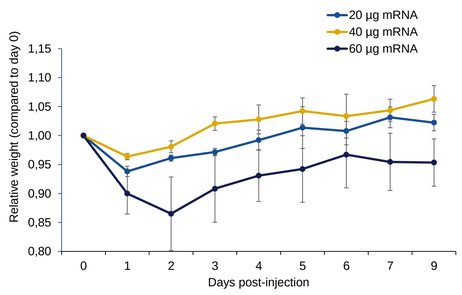
Critical organs for vaccination purposes

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 h post-injection.

LD50-like: safe mRNA delivery up to 40 µg FLuc mRNA



mRNA amount	Physical appearance	Remote behavior	Handling behavior
20 µg	Normal, good grooming	Normal	Normal
40 µg			
60 µg			



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.

Conclusion

Our novel liposome-based transfection reagent *in vivo*-jetRNA®+ is a ready-to-use alternative that requires little time nor equipment for formulation compared to current ionizable LNPs, while ensuring similar efficacy in delivery, better biodistribution to target other organs than liver, similar toxicity profile and great stability for at least one month.