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Simplifying Progress

Novel Generation of Cationic LNP Offer New Possibilities in the Delivery of RNA Therapeutics

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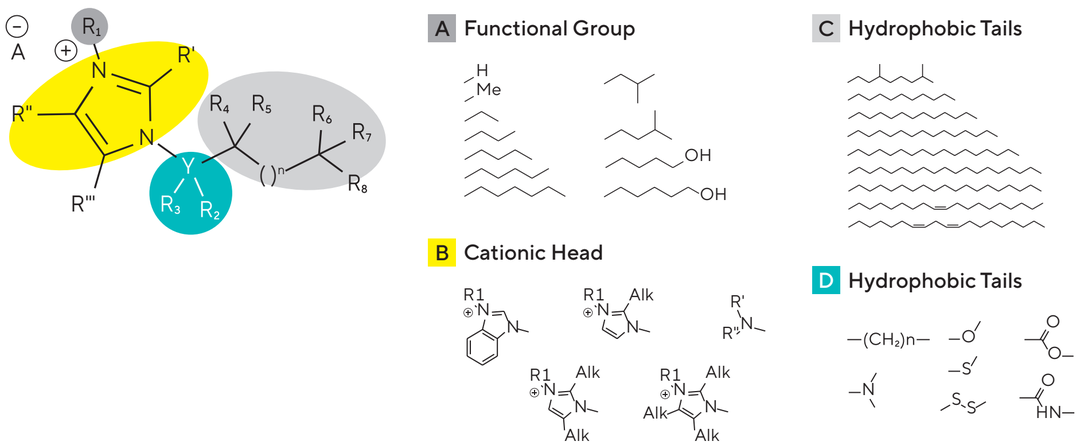
1. Introduction

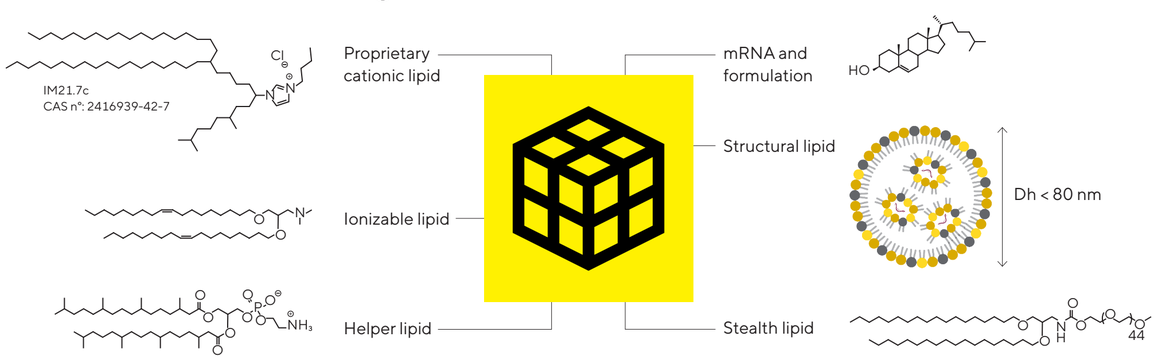
Lipid nanoparticles (LNPs) electric charge is known to control in vivo distribution and expression of mRNA-LNPs for intravascular (IV) administration. Whereas neutral LNPs predominantly end up targeting the liver, reducing the amount of cationic lipid in mRNA-LNPs creates a negative LNP, due to an excess of anionic mRNA, that targeted the spleen after IV injection, versus positively charged LNPs targeting the lung. A novel family of permanent cationic lipids has been developed to answer the next challenges in the development of RNA therapeutics. The combination of an imidazolium polar head and an accentuated molecular cone-shape through improved lipid tail branching, increases formulation stability and facilitates endosomal release, respectively. Due to these properties, cationic LNP (cLNP) have shown promising results in term of stability, potency, in vivo distribution and targeting.

2. Library of Cationic or Ionisable Lipids

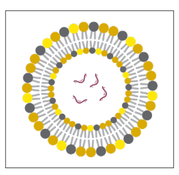


Note. Innovative cationic or ionizable lipids have been synthetized following various modifications listed above. Several convergent synthetic routes have been optimized to generate lipids at multigram scale.

3. Formulation Design

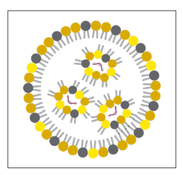


4. Liposomes vs. LNPs



mRNA Liposomes

- Off the shelf product
- Pipette mixing liposomes:
  - jetMESSENGER®
  - in vivo-jetRNA®+
  - + mRNA

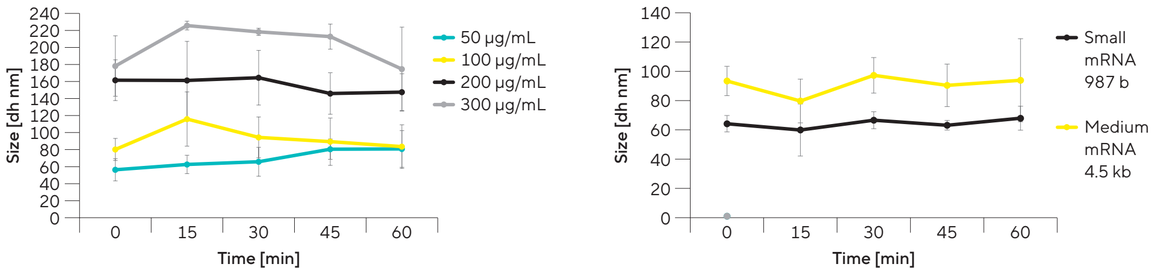


mRNA-LNP

- Made with microfluidic technology (NanoAssemblr)
- by mixing lipids including our proprietary cationic lipid (Patent n° EP18306417)

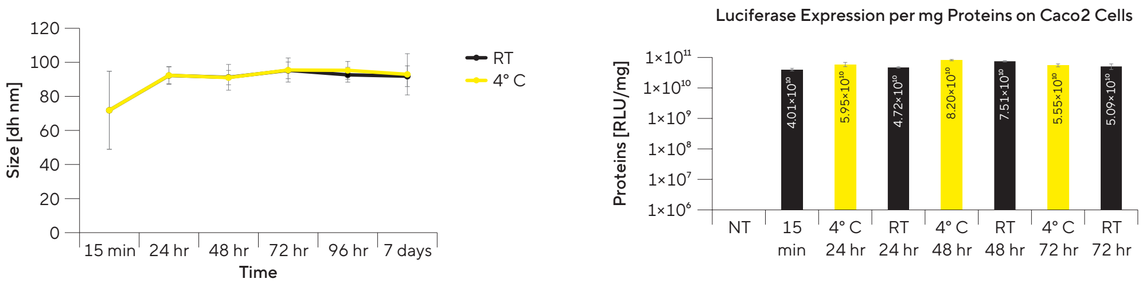
5. Liposomes Are Stable at High mRNA Concentration up to 7 Days

Figure 1: *Liposomes With in vivo-jetRNA®+ Are Stable With up to 300 µg of mRNA/mL With Different Sized mRNA*



Note. Size of liposomes with in vivo-jetRNA®+ at 50, 100, 200 or 300 µg of mRNA/mL with a small mRNA (1929 b) or at 50 µg of mRNA/mL with a small size mRNA (978 b) and a medium size mRNA (4.5 kb) after 15, 30, 45 and 60 min of complexation were measured by dynamic light scattering (DLS).

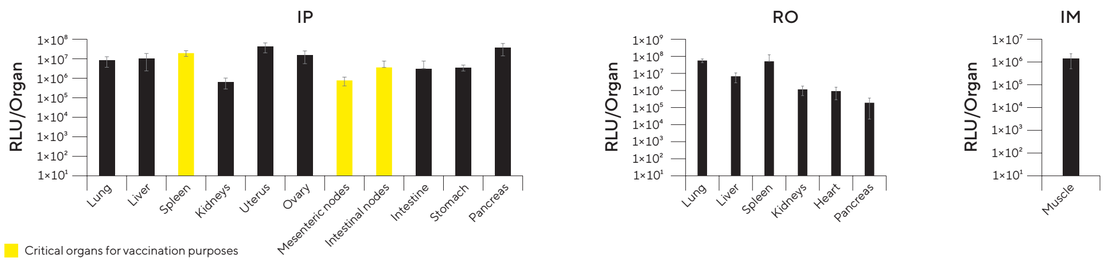
Figure 2: *Liposomes With in vivo-jetRNA®+ Are Stable up to 7 Days*



Note. Size of liposomes with in vivo-jetRNA®+ at 50 µg/mL after 15 min, 24, 48, 72 or 96 hours or 7 days of complexation were measured by DLS. Caco2 cells were transfected with liposomes formed with a mRNA/in vivo-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer. 500 ng of mRNA encoding luciferase were used for 40,000 Caco2 cells. Luciferase was assessed 24 hr post-transfection.

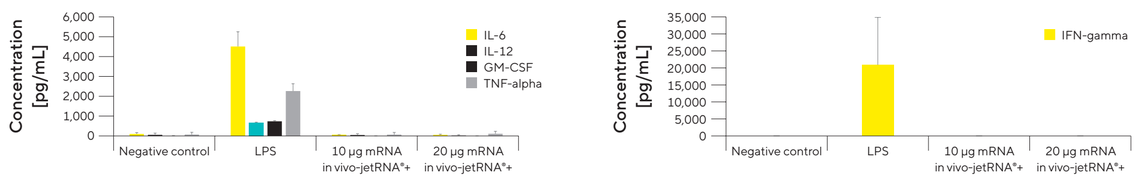
6. Liposomes: Efficient and Safe mRNA Delivery for Local and Systemic Administration

Figure 3: *in vivo Efficacy and Biodistribution*



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, 10 µg mRNA for intravenous injection (retro-orbital injection – RO) or 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 24 hr post-injection.

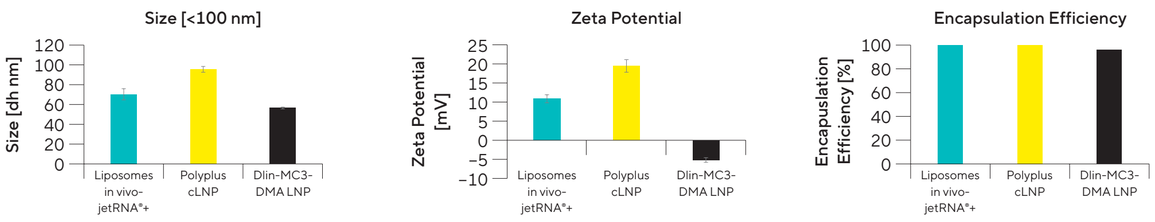
Figure 4: *No Pro-Inflammatory Cytokine Expression*



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.

7. One Cationic Lipid – Two Delivery Systems With Similar Behaviors

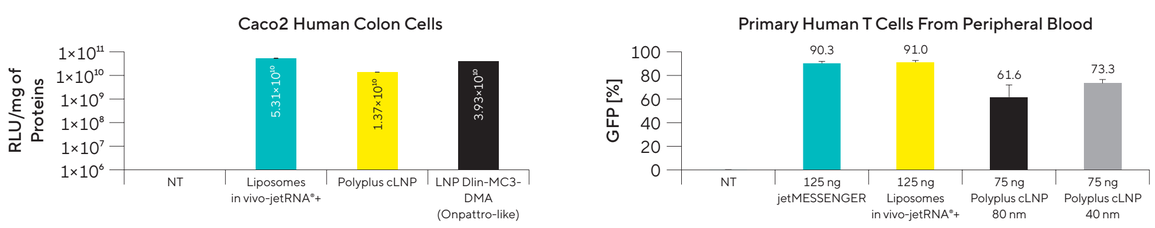
Figure 5



Note. Size and zeta potential of liposomes with in vivo-jetRNA®+ at 50 ng/µL after 1 hr of complexation or LNPs at 250 ng/µL were measured by DLS. Encapsulation efficiency was assessed by the RiboGreen assay

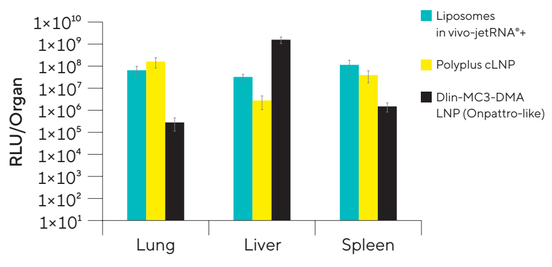
8. Cationic LNPs: Different Biodistribution Compare to Neutral LNPs

Figure 6: *in vitro Transfection Efficacy*



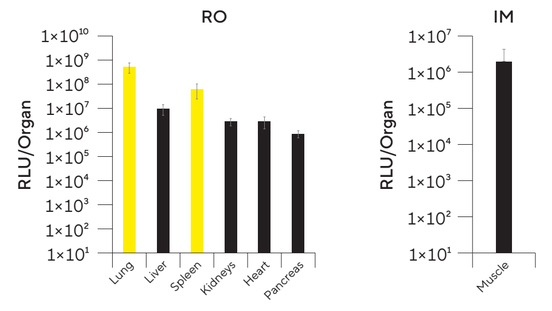
Note. Caco2 and primary human T cells were transfected with liposomes formed with a mRNA/in vivo-jetRNA®+ or jetMESSENGER® ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer or LNPs using either 500 ng or 75 and 125 ng of mRNA encoding luciferase or GFP for respectively 40,000 Caco2 cells or 187,500 T cells. Luciferase or GFP expression was assessed 24 hr or 48 hr after transfection for respectively Caco2 cells or T cells.

Figure 7: *Different Expression Pattern Profile With Polyplus Positively Charged LNP Compared to Dlin-MC3-DMA LNP*



Note. mRNA encoding luciferase was injected into mice using in vivo-jetRNA®+ or Polyplus cLNP or Dlin-MC3-DMA LNP through intravenous injection. Liposomes were formed using 10 µg of mRNA with an mRNA/in vivo-jetRNA® ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA buffer. Luciferase expression was assessed 24 hr post-injection.

Figure 8: *Polyplus cLNP Mainly Targets Lung and Spleen*



Note. mRNA encoding luciferase was injected into mice using Polyplus cLNP through different administration routes. 10 µg mRNA were injected for intravenous injection (retro-orbital injection – RO) or 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 24 hr post-injection.

9. Conclusion

A novel family of proprietary cationic and ionizable lipids solve a current limit of LNPs in being able to target different organs and cell types. The new cationic LNP formulation ensures same delivery efficacy as LNPs with ionisable lipids, while improving biodistribution to target organs other than liver.