Novel Lipid Nanoparticle Delivery Reagent and Rapid Manufacturing Workflow to Accelerate Preclinical Development of RNA Vaccines

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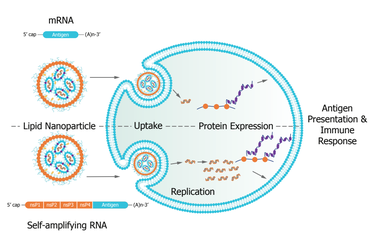
Purpose

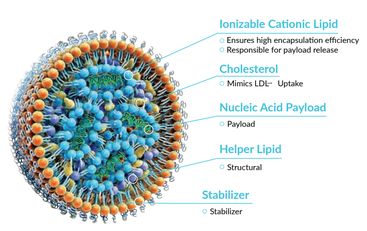
- The biggest vaccine campaign in history is ongoing against COVID-19, with RNA vaccines playing a key role.
- Due to the rapid degradation of RNA and low cellular uptake, an essential element of RNA vaccines are lipid nanoparticle (LNP) delivery systems.
- Limited access to ionizable lipids and LNP compositions, and the difficulty in scale-up production of RNA-LNPs remain challenges in the field.
- In this study, we aim to highlight that commercially available LNP reagent mix, GenVoy-ILM, is an accessible and easy-to-use LNP formulation that allows for rapid preclinical development of RNA vaccines.

Genetic Medicine Toolkit

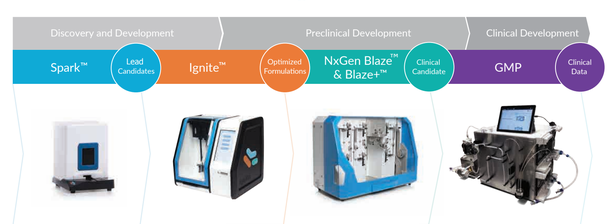
Genetic Payload Platform



GenVoy™ Delivery Platform



NanoAssemblr® Manufacturing Platform



Objectives

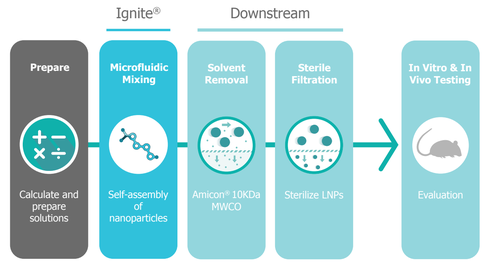
Produce lead RNA LNP candidates → RNA LNPs encapsulating different mRNA and saRNA constructs were prepared using GenVoy-ILM™ and a NanoAssemblr® Ignite™ and characterised.

Determine *in vitro* activity → In vitro toxicity and protein expression were assessed in HEK293 cells.

Determine *in vivo* activity → In vivo distribution and protein expression were assessed by bioimaging; immunogenicity studies were performed using a SARS-CoV-2 encoded saRNA.

Methods

RNA-LNP production: GenVoy-ILM™ or a MC3 benchmark lipid mix were formulated with RNA into LNPs on the NanoAssemblr® Ignite with NxGen™ microfluidics technology. The MC3 formulation was selected as LNP benchmark based on it's similarity to existing mRNA LNP vaccines.



RNA Types:
Cleancap® FLuc mRNA (5moU) - 1929 nucleotides
Cleancap® EGFP mRNA - 996 nucleotides
PNI FLuc saRNA - 8900 nucleotides
PNI EGFP saRNA - 8463 nucleotides
PNI nCoV saRNA - 11560 nucleotides

Example mRNA LNP vaccine composition:
Moderna Inc spikevax® (mRNA-1273)
SM-102:DSPC:Chol:DMG-PEG (50:10:38.5:1.5)

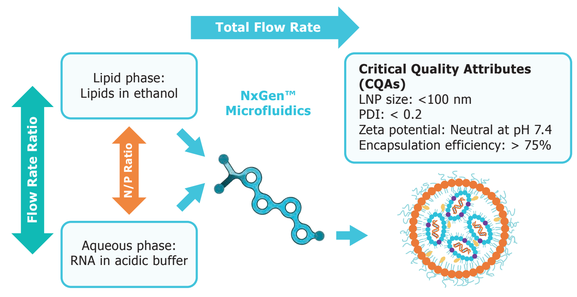
Downstream formulation processing: LNPs were diluted 40X with PBS (pH 7.4) and re-concentrated by centrifugal ultrafiltration using Amicon® Ultra-15 filter units (10kDa MWCO).

Manufacturing Parameters - Ignite settings:
Total Flow Rate: 20 mL/min
Flow Rate Ratio (Aqueous:Organic): 3:1
N/P ratio: 6

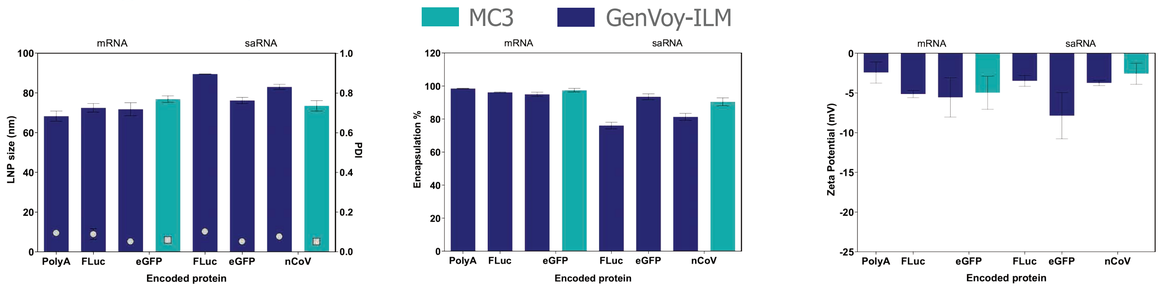
Lipid Compositions in this study:
GenVoy-ILM - IL:DSPC:Chol:Stabilizer (50:10:37.5:2.5)
MC3 - Dlin-MC3-DMA:DSPC:Chol:DMG-PEG (50:10:38.5:1.5)

RNA-LNP characterization and in vitro activity: RNA-LNP size, PDI and zeta potential were determined using DLS and ELS (Malvern Zetasizer Ultra). The encapsulation efficiency (EE%) of the RNA was determined using Ribogreen™ reagent. In vitro expression/potency and viability were determined by flow cytometry (Attune NxT). HEK293 cells were treated with eGFP mRNA and saRNA LNPs in the presence of ApoE (1µg/mL) and fluorescence was determined.

In vivo expression and immunogenicity: Female BALB/c mice (n=5) were injected IM with LNPs containing FLuc mRNA (5µg/leg) or saRNA (1µg/leg) and expression was determined using fluorescence and luminescence imaging (IVIS) over 28 days. D-luciferin (150mg/kg) was injected IP 15-20 minutes before luminescence imaging. To determine the immunogenicity of the saRNA-LNPs, female BALB/c mice (n=5) were immunized by IM injection on day 0 with LNPs encapsulating 1µg nCoV saRNA and boosted at day 28. IgG levels in serum on day 27 and day 42 were measured by ELISA.

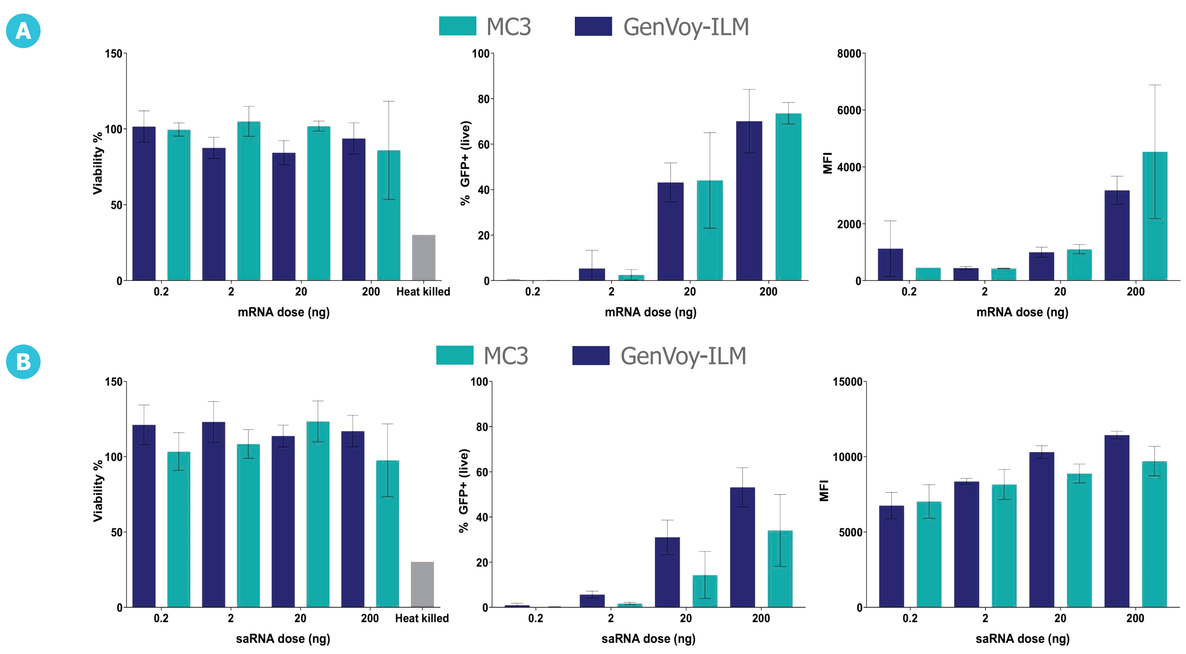


GenVoy-ILM LNPs can encapsulate both mRNA and saRNA while retaining the CQAs required for an RNA vaccine.



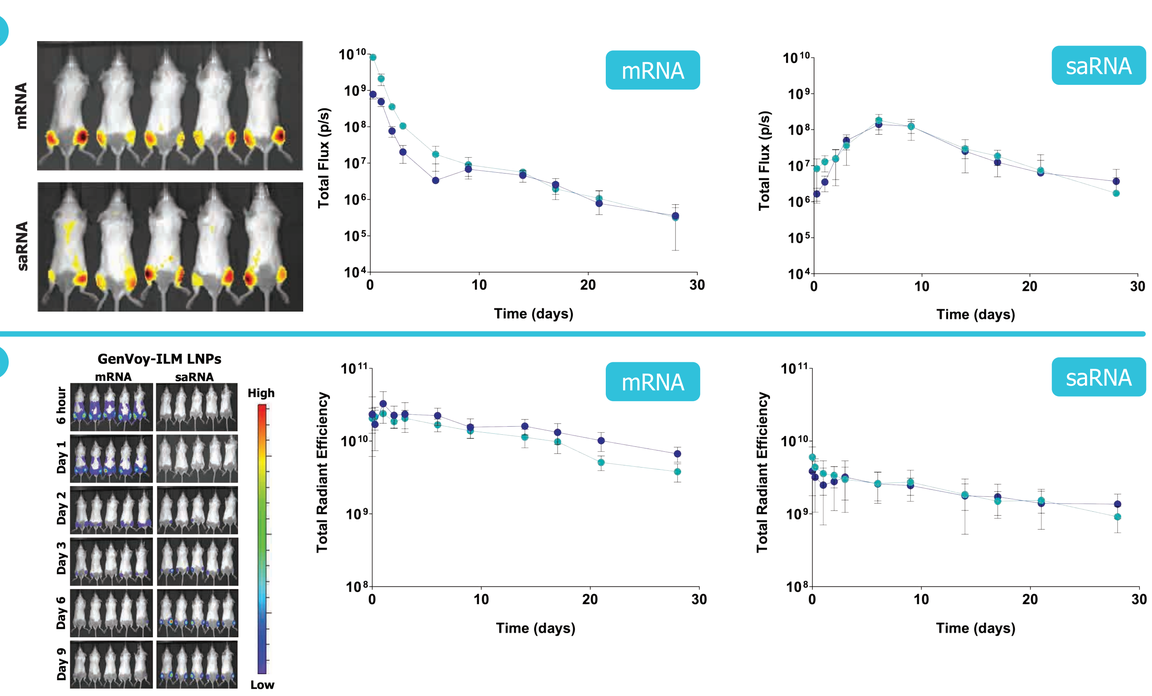
GenVoy-ILM is an Effective *In Vitro* Delivery Vehicle for mRNA and saRNA

mRNA Expression (A): Dose-response of GenVoy mRNA treated HEK293 cells showing live/dead cell proportions, transfection efficiency (GFP%(live)) and representative histogram. **saRNA expression (B):** Dose-response of GenVoy saRNA treated HEK293 cells showing live/dead cell proportions, transfection efficiency (GFP%(live)) and representative histogram. N=3 ± SD



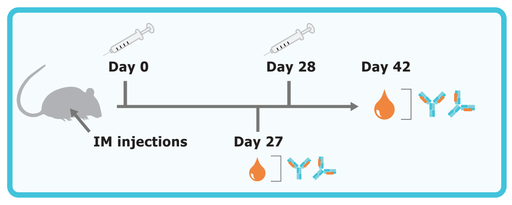
GenVoy-ILM is an Effective *In Vivo* Delivery Vehicle for mRNA and saRNA

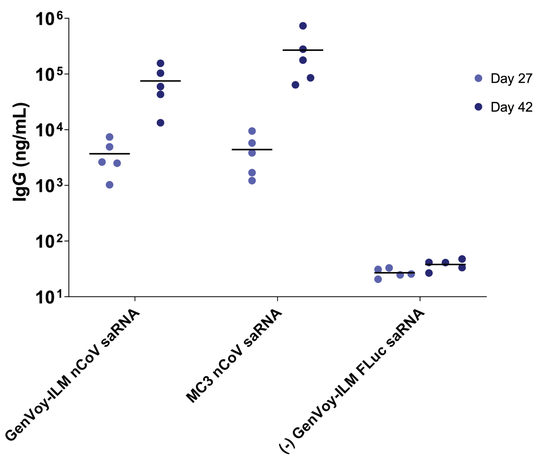
LNP Delivery (A): LNP delivery and clearance from the muscle is comparable for both GenVoy-ILM and MC3 LNPs over 28 days. **Luciferase Expression (B)**: Luciferase expression from Fluc mRNA and saRNA over 28 days, delivered using GenVoy-ILM and MC3 LNPs. N=5 ± SD

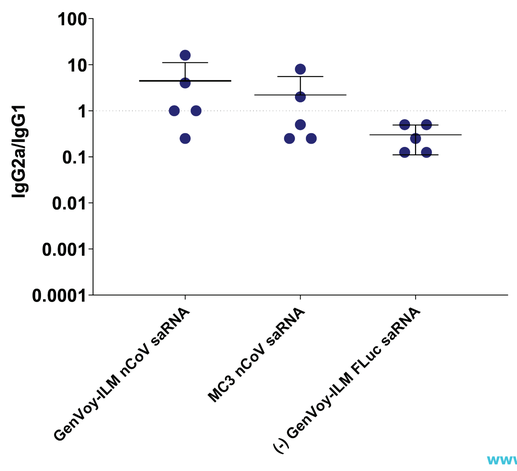


GenVoy-ILM LNPs induce an immune response against the target antigen

LNP vaccines were formulated with saRNA encoding for the full-length SARS-CoV-2 spike protein. (A) ELIZA analysis of total anti-spike serum IgG levels demonstrated that GenVoy-ILM vaccine produced antibodies against the target antigen. (B) ELISA analysis of the anti-spike IgG2a and IgG1 isotypes demonstrated that the IgG response to the GenVoy-ILM vaccine was skewed towards the IgG2a isotype which is indicative of a Th1 immune response







Conclusion

- GenVoy-ILM is a commercially available and easy-to-use LNP reagent mix that enables the rapid preclinical development of RNA vaccines.
- GenVoy-ILM LNPs can be used to encapsulate both conventional mRNA and self-amplifying RNA while retaining key critical quality attributes for an RNA vaccine.
- GenVoy-ILM LNPs are an effective in vitro and in vivo delivery vehicle for both mRNA and saRNA.
- GenVoy-ILM LNPs encapsulating SARS-CoV-2 saRNA induce an immune response in mice, which highlights its utility as a vehicle for screening RNA in preclinical development.

Acknowledgments

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