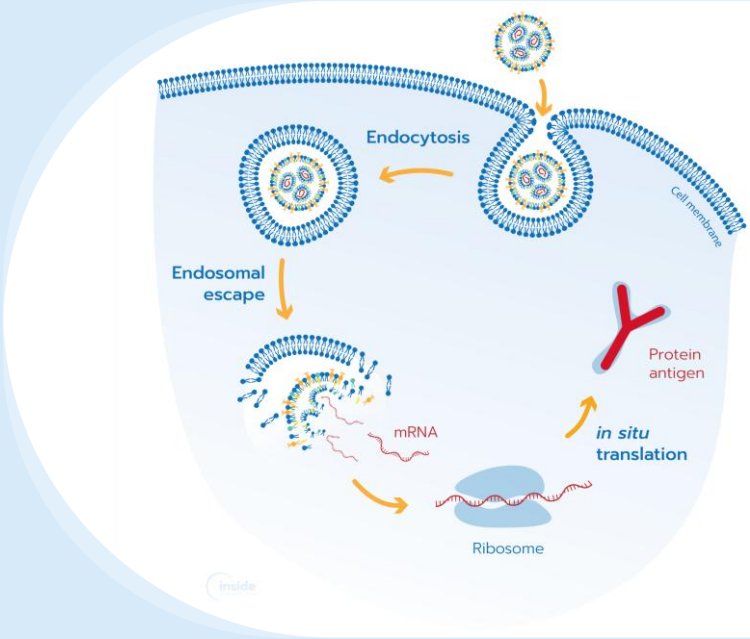
Application note Short summary

In vivo Evaluation of mRNA-LNP Vaccines Formulated Using TAMARA

The growing adoption of **mRNA-based vaccines** is reshaping strategies to combat infectious diseases. These technologies enable rapid antigen design and transient protein expression, providing a flexible platform to address both emerging and established pathogens.

Lipid nanoparticles (LNPs) have become the leading non-viral platform for RNA delivery, protecting mRNA from degradation while promoting cellular uptake and intracellular release. Their clinical success, notably in mRNA COVID-19 vaccines, has demonstrated their ability to enable efficient in vivo delivery, with modular compositions allowing fine control over biological performance.

In this study, **mRNA-LNPs formulated using TAMARA were evaluated in vivo** following intramuscular (IM) administration in mice. Antigen expression and biodistribution were monitored using bioluminescence imaging, while ELISA and ELISpot assays were performed to characterize the induced humoral and cellular immune responses.



The findings highlight the ability of mRNA-LNP formulations to drive effective antigen expression in vivo and trigger both antibody-mediated and cellular immune responses, reinforcing their potential as a versatile platform for vaccine development.

This work was carried out by Csaba Bajusz at the HUN-REN Biological Research Centre, Szeged, and supported by Sezen Gul from Inside Therapeutics.

In vivo expression & biodistribution of mRNA-LNPs

mRNA-LNPs (5 µg, ALC-0315 based formulation) were administered intramuscularly in BALB/c mice, and luciferase (Luc) expression was monitored over time using **IVIS imaging**.

Key findings

- **Strong and sustained signal at the injection site, indicating efficient local expression.**
- Tagged Luc construct showed slightly lower expression compared to wild-type, while remaining robustly expressed.
- The liver signal exhibited a rapid decline over time compared to the injection site, indicating transient systemic exposure.

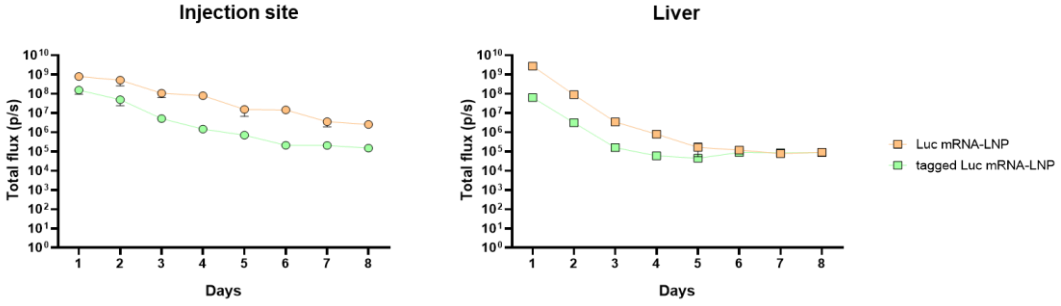
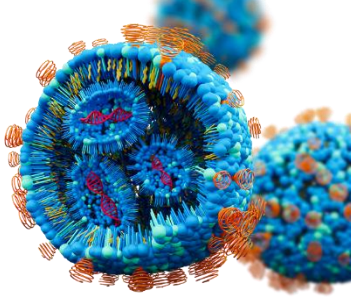


Figure 1 : Bioluminescent signal measured over time at the injection site (left) and in the liver (right) following IM administration of Luc mRNA-LNPs (wild-type, orange; tagged construct, green) in BALB/c mice.



Humoral immune response of mRNA-LNPs

mRNA-LNPs (10 µg) encoding different antigens were administered intramuscularly in mice using a prime–boost regimen, and antigen-specific antibody titers were measured by **ELISA** 4 weeks after each administration.

Key findings

- → **mRNA constructs 1, 3, and 5 induced strong antigen-specific antibody responses, particularly after boost.**
- → Booster administration significantly increased antibody titers compared to prime alone.
- → Minimal antibody response observed in naïve and control groups, confirming specificity.

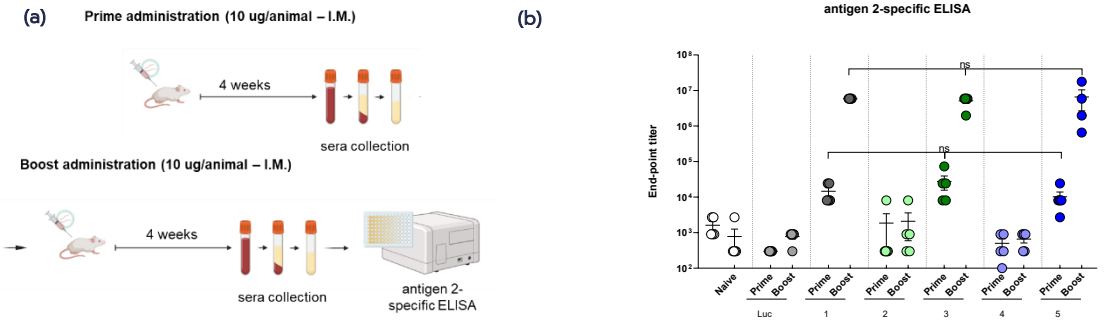


Figure 3: (a) Prime–boost immunization and sampling workflow. (b) Antigen 2-specific antibody titers measured by ELISA after prime and boost administrations of mRNA-LNPs encoding different constructs.

mRNA-LNPs generate strong antigen-specific antibody responses, significantly enhanced upon boosting.

Cellular immune response of mRNA-LNPs

mRNA-LNPs (10 µg) were administered intramuscularly in mice, and splenocytes were isolated after 12 days to assess antigen-specific cellular immune responses using **ELISpot**.

Key findings

- → **Antigen 1 mRNA-LNP induced a strong antigen-specific cellular immune response, absent in naïve and luciferase control groups.**
- → Low background in control groups and strong responses to ConA (positive control) confirm assay specificity and immune cell functionality.

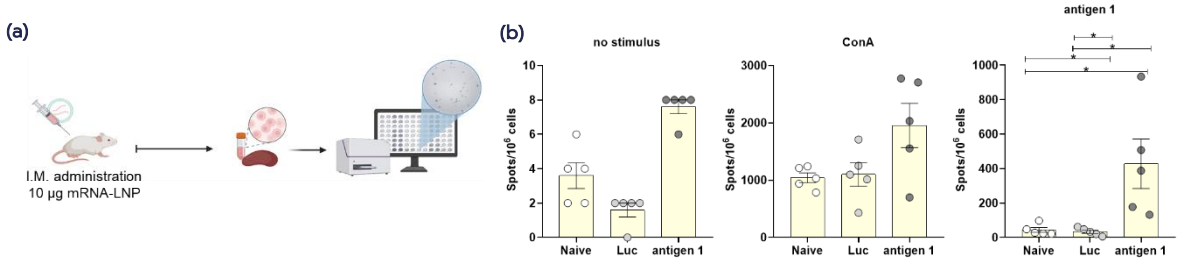


Figure 2: (a) ELISpot assay schematic. (b) Antigen-specific ELISpot responses in splenocytes after IM administration of mRNA-LNPs. Spot counts shown for naïve, luciferase control, and antigen 1 groups under no stimulation, ConA stimulation, and antigen-specific stimulation.

These results demonstrate that mRNA-LNP vaccination elicits a robust antigen-specific cellular immune response.

Conclusion

This application note highlights the **strong in vivo performance of mRNA-LNPs** across key measures relevant to vaccine efficacy. ALC-0315-based mRNA-LNPs formulated with TAMARA:

- → Enabled **efficient in vivo antigen expression**, with sustained signal at the injection site.
- → Induced **strong antigen-specific antibody responses**, significantly enhanced following booster immunization.
- → Triggered **robust antigen-specific cellular immune responses**.

mRNA-LNPs produced with TAMARA enable potent humoral and cellular immune responses in vivo, supporting effective vaccine development.

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