Harnessing a Synthetic TLR4 Agonist for Immune-Active LNP Vaccines

Theodoros Kyriakou[1], Donald Kelemen[2] and Yvonne Perrie[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, 161 Cathedral St, Glasgow, Scotland

[2]ABITEC Corporation, 501 W. First Avenue, Columbus, OH 43215 USA

LinkedIn

Introduction

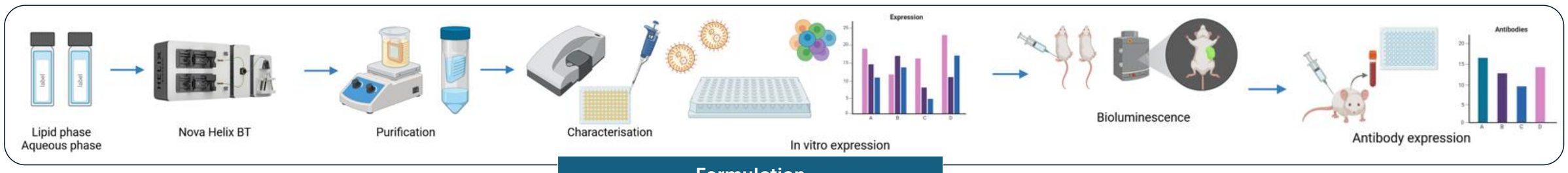
Toll-like receptor 4 (TLR4) plays a key component of the innate immune system that recognises lipopolysaccharides (LPS) from gram-negative bacteria and initiates downstream inflammatory signalling pathways. Synthetic analogues of the LPS activating moiety, (like monophosphoryl lipid A) have been developed and added as adjuvants into liposomes to enhance vaccine efficacy.

AIM ⇨ Evaluation of FP-20 and LNP incorporation as novel TLR-4 targeting adjuvant

OBJECTIVES

- Formulation of SM-102/FP-20 LNPs
- Characterisation of LNPs
- Biological effect (*in vitro/in vivo*)

Methods



Formulation

DSPC	Cholesterol	SM-102	DMG-PEG2k	FP-20
10%	38.5%	50%	1.5%	1-10%

Results & Discussion

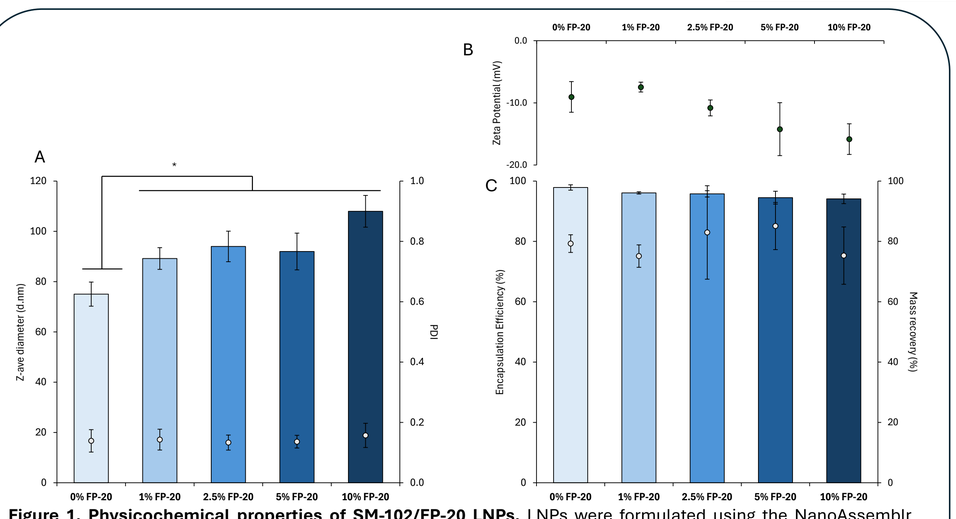


Figure 1. Physicochemical properties of SM-102/FP-20 LNPs. LNPs were formulated using the NanoAssemblr Benchtop (Mixer 0; TFR 10 mL/min; FRR 3:1) in 50 mM NaCl and 100 mM sodium acetate (pH 5.25), encapsulating mCherry mRNA. Samples were purified by either 4 h dialysis or spin column with 10 mM Tris-HCl (pH 7.4). **(A)** LNP size (bars) increased with FP-20 incorporation while maintaining monodisperse populations (PDI < 0.2, circles). **(1B)** Zeta potential remained near-neutral, with a progressive decrease upon increasing FP-20 concentration. **(C)** Encapsulation Efficiency and Mass Recovery of the FP-20 LNPs remained consistent. Data are presented as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA, with significance indicated as * ($p < 0.05$).

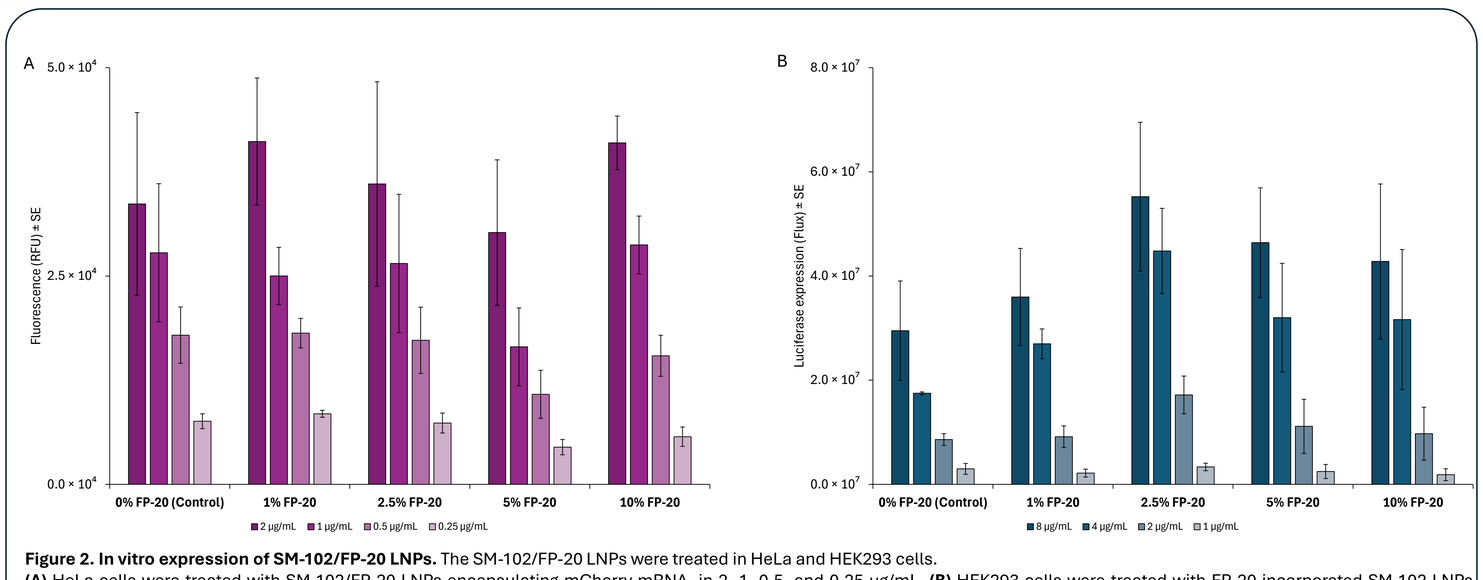


Figure 2. In vitro expression of SM-102/FP-20 LNPs. The SM-102/FP-20 LNPs were treated in HeLa and HEK293 cells. **(A)** HeLa cells were treated with SM-102/FP-20 LNPs encapsulating mCherry mRNA, in 2, 1, 0.5, and 0.25 µg/mL. **(B)** HEK293 cells were treated with FP-20 incorporated SM-102 LNPs encapsulating FLuc mRNA, in 8, 4, 2, 1 µg/mL. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA with significance indicated as * ($p < 0.05$).

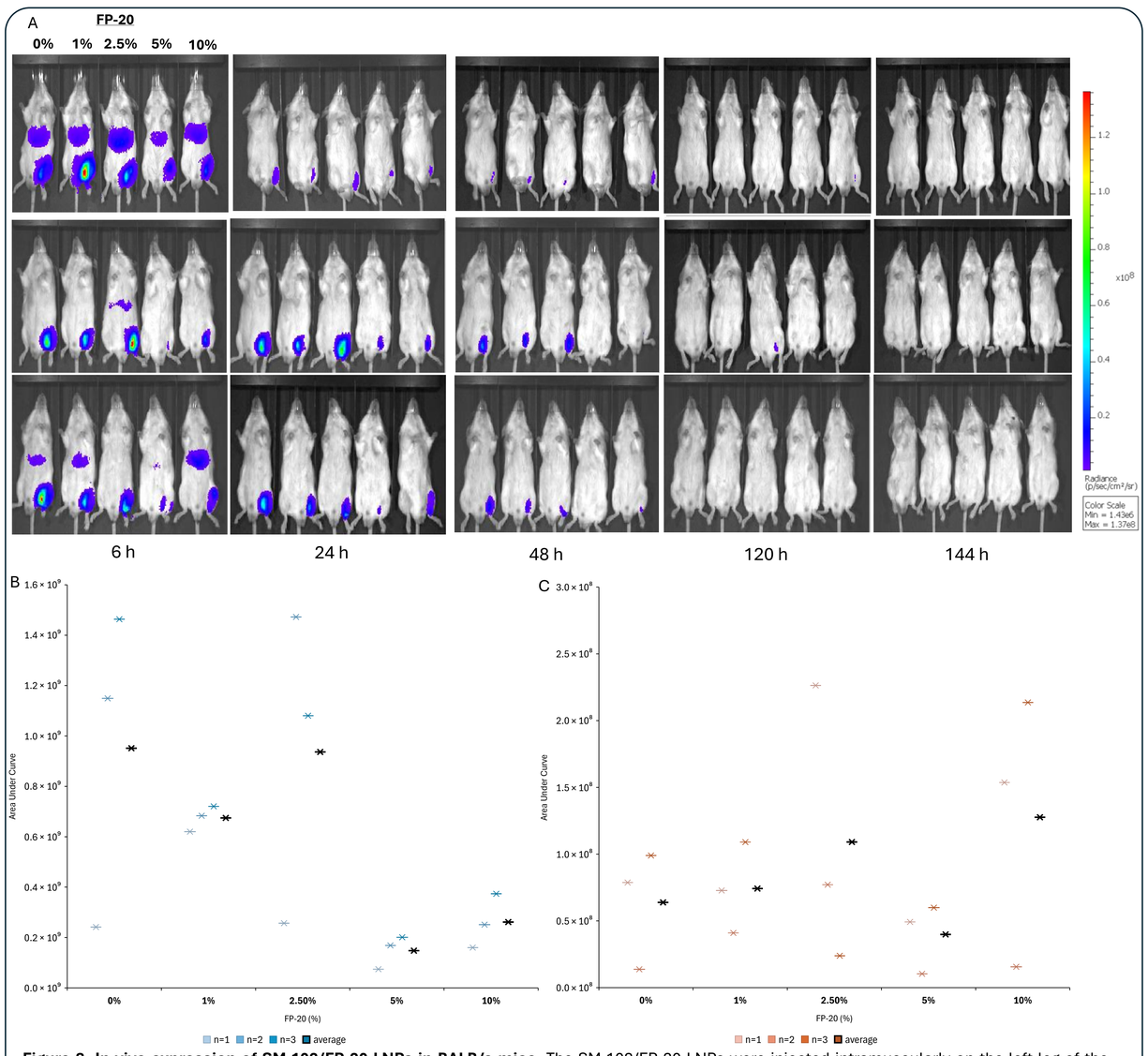


Figure 3. In vivo expression of SM-102/FP-20 LNPs in BALB/c mice. The SM-102/FP-20 LNPs were injected intramuscularly on the left leg of the BALB/c mice. The injected dose was 1 µg of FLuc mRNA. **(A)** Mice were injected subcutaneously with D-Luciferin (5 µL/g) and after 10 minutes, bioluminescence was measured using an IVIS imaging system. Expression was measured for 6 h, 24 h, 48 h, 120 h, and 144 h post-injection. Images were processed using Living Image 4.8.2. **(B)** Leg Overall Expression (AUC). Some formulations achieved higher expression (black marks), reflecting increased magnitude and/or duration of expression, yet with greater variability between animals (0%, 2.5%). Lower-expressing formulations showed more consistent responses, indicating reduced variability but limited expression. **(C)** Liver Overall Expression (AUC). Differences in AUC values were observed across formulations, highlighting variation in overall expression (black marks). Certain groups achieved elevated AUC values (2.5%, 10%) indicative of enhanced expression over time, but these were accompanied by considerable inter-animal variability, as reflected by the broad distribution of individual data points. Other formulations showed lower AUC values with more tightly grouped responses, suggesting greater consistency but reduced expression.
Data are presented as n=3. Statistical analysis was performed using a Mann-Whitney hoc test, with significance indicated as * ($p < 0.05$).

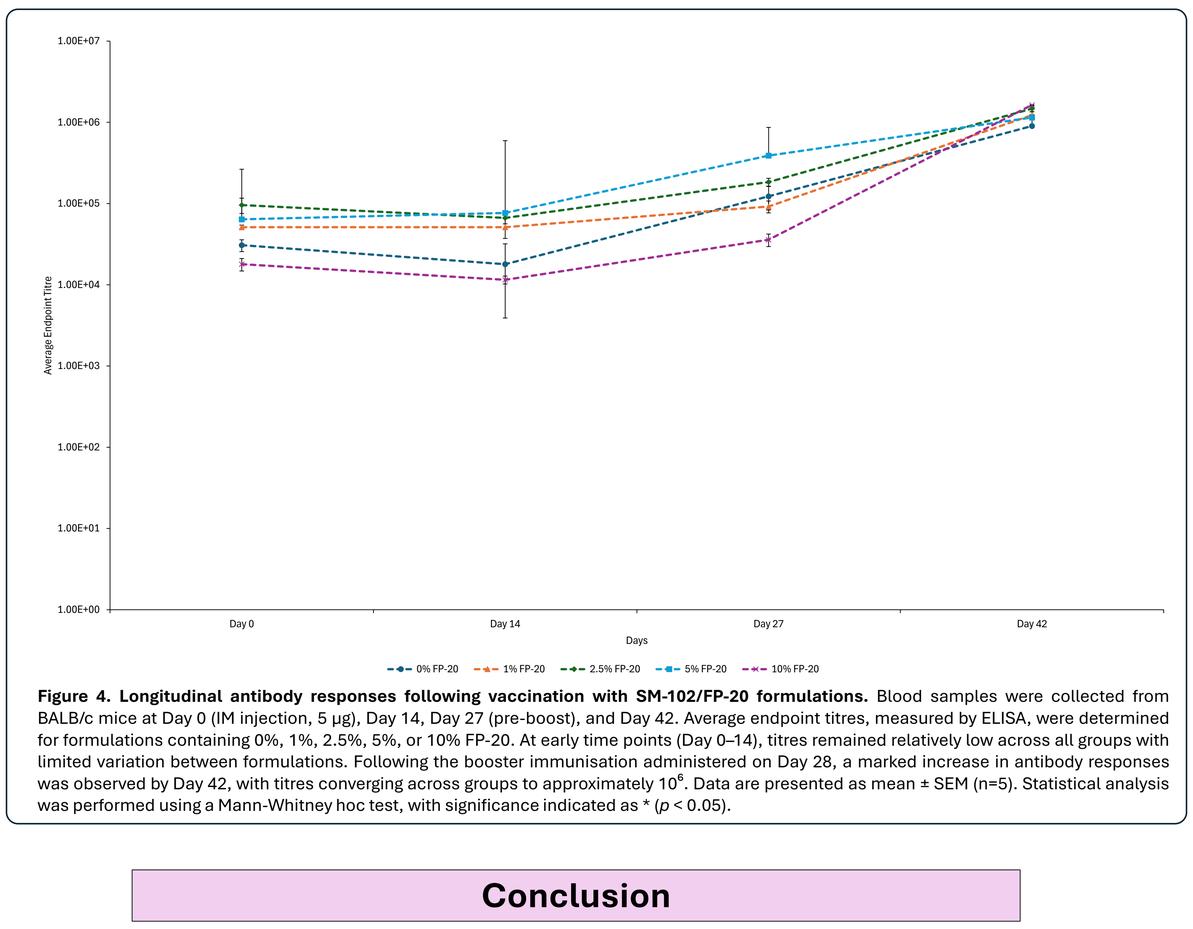


Figure 4. Longitudinal antibody responses following vaccination with SM-102/FP-20 formulations. Blood samples were collected from BALB/c mice at Day 0 (IM injection, 5 µg), Day 14, Day 27 (pre-boost), and Day 42. Average endpoint titres, measured by ELISA, were determined for formulations containing 0%, 1%, 2.5%, 5%, or 10% FP-20. At early time points (Day 0–14), titres remained relatively low across all groups with limited variation between formulations. Following the booster immunisation administered on Day 28, a marked increase in antibody responses was observed by Day 42, with titres converging across groups to approximately 10^6. Data are presented as mean ± SEM (n=5). Statistical analysis was performed using a Mann-Whitney hoc test, with significance indicated as * ($p < 0.05$).

Conclusion

- FP-20 incorporation altered SM-102 LNP properties, but maintained stability and high encapsulation
- No enhancement in *in vitro* expression vs control SM-102 LNPs
- *In vivo*: variable expression with no consistent improvement
- Antibody responses increased post-boost, but no FP-20-dependent effect observed
- Overall: limited evidence of enhanced adjuvant activity under current formulation conditions
- Potentially due to FP-20 solubility limitations
- ★ Future work: Evaluating a more soluble FP-20 compound and a broader range of ionisable lipids

Acknowledgments

This work is a collaboration funded from the ABITEC Corporation and the University of Strathclyde

ABITEC
AN ABF INGREDIENTS COMPANY

University of Strathclyde Glasgow