Superior Immunogenicity of APL-143 Ionizable Lipid in saRNA-LNP Formulations Targeting Avian Flu

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Abstract

Self-amplifying RNA (saRNA) vaccines offer a promising platform for rapid and scalable responses to emerging infectious diseases in poultry. In this study, we evaluated two Avanti ionizable lipids, APL-143 and APL-902, in comparison with two commercially available lipid nanoparticle (LNP) formulations, CL1 and GenVoy-ILM™, for their ability to deliver saRNA encoding the H5 antigen from highly pathogenic avian influenza (HPAI). LNPs were prepared using each lipid system and characterized for their ability to induce immune responses following administration.

Among the four formulations tested, LNPs incorporating Avanti APL-143 generated the strongest antigen-specific immune response. Hemagglutination inhibition (HI) titers were low across all groups; however, they were consistent with and proportional to antigen-specific ELISA values, indicating a correlated response pattern between the two assays. These results highlight APL-143 as a promising ionizable lipid candidate for saRNA delivery and support further optimization to enhance immunogenicity in poultry..

Study design

Avanti's novel ionizable lipids APL-143 (bis(2-hexyldecyl) O,O′-(3-(4-(3-hydroxypropyl)piperazin-1-yl)propane-1,2-diyl) diglutarate; Avanti Cat. No. A88143) and APL-902 (bis(2-hexyldecyl) O,O′-(3-(4-methylpiperazin-1-yl)propane-1,2-diyl) diglutarate; Avanti Cat. No. A88902) were evaluated as the ionizable lipid components of lipid nanoparticles (LNPs) formulated for poultry vaccination. Two commercial LNP mixtures—CL1 and GenVoy-ILM™ (Cytiva, Cat. Nos. 1002763 / 1002769)—were included for comparison. Each lipid formulation encapsulated a self-amplifying RNA (saRNA) vector encoding the H5 hemagglutinin gene from highly pathogenic avian influenza A virus (A/American wigeon/South Carolina/AH0195145/2021(H5N1)).

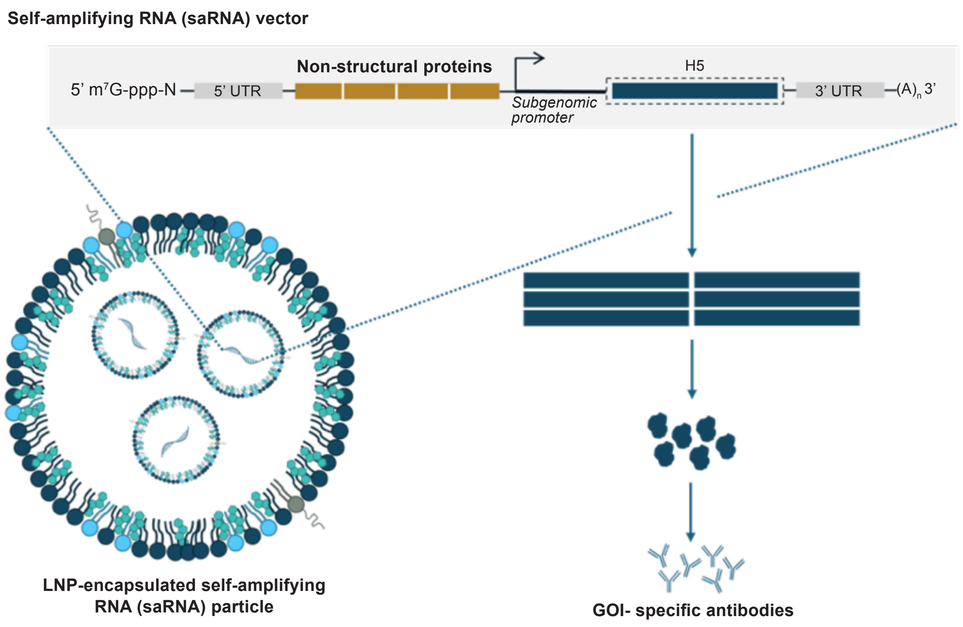

Figure 1: Lipids are used to encapsulate and deliver saRNA to cells

H5N1 represents a highly contagious and often fatal respiratory disease in domestic poultry, wild birds, swine, humans, and other mammals. LNPs incorporating APL-143, APL-902, or CL1 were prepared using the helper lipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), the stabilizing lipid cholesterol, and a PEGylated lipid. For APL-143, APL-902, and CL1 formulations, PEGylation was achieved using DMG-PEG. All formulations followed a consistent molar ratio consisting of 46% cholesterol, 40% ionizable lipid, 12.5% DSPC, and 1.5% PEG-lipid.

Ionizable lipid nanoparticle formation	saRNA dose	Total # animals	Gender	Species	Species
Avanti APL-902	1ug	24	Male	Chiken (Cobb 500)	1 day old
Avanti APL-143	1ug	24			
CL1	1ug	24			
GenVoy-ILM™	1ug	24			
Placebo (Saline)	0ug	24			

Table 1: Treatment groups

Five treatment groups were evaluated, each consisting of twenty-four 1-day-old male Cobb-500 chicks receiving either 1 µg saRNA formulated with APL-902, APL-143, CL1, or GenVoy-ILM™, or a saline placebo containing no saRNA.

Materials and methods

A self-amplifying RNA (saRNA) vaccine expressing the hemagglutinin (HA) gene from avian influenza H5 clade 2.3.4.4b was developed using a Venezuelan equine encephalitis virus (VEEV) TC-83 replicon backbone. The HA gene sequence, derived from strain A/American wigeon/South Carolina/AH0195145/2021 (H5N1), was codon-optimized for expression in chickens and synthesized by Integrated DNA Technologies (IDT). The optimized gene was cloned into the VEEV TC-83 plasmid and sequence-verified prior to transformation into E. coli for plasmid production. Linearized plasmid DNA served as the template for in vitro transcription, followed by enzymatic capping (Cap 1 structure) to generate the saRNA. The purified saRNA was formulated into lipid nanoparticles (LNPs) using a standardized lipid composition consisting of 40 mol% ionizable lipid, 12.5 mol% DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine; Avanti Cat. No. 730365), 46 mol% cholesterol (Avanti Cat. No. 730100), and 1.5 mol% PEG-lipid (DMG-PEG2000; Avanti Cat. No. 770151). All lipid components for Avanti-based formulations—including the ionizable lipids APL-143 (Avanti Cat. No. 857143O) and APL-902 (Avanti Cat. No. 890902O)—were sourced from Avanti Research, part of Croda Pharma. In contrast, the comparison formulations used GenVoy-ILM™, sourced from Cytiva (Cat. Nos. 1002763 and 1002769), and CL1, sourced from DC Chemicals ("Genevant CL1," Cat. No. DC59126). Formulation of saRNA-LNPs was performed using microfluidics (Precision NanoSystems NanoAssemblr Ignite+) with a 10:1 (w/w) RNA:ionizable lipid ratio, a 3:1 aqueous-to-organic flow rate ratio, and a total flow rate of 12 mL/min. Following formulation, buffer exchange into a cryoprotectant buffer was conducted prior to sterile filtration, yielding the final vaccine product. The completed LNP vaccine was stored at –80 °C until use. Physicochemical characterization was carried out by dynamic light scattering (DLS) to determine particle size and polydispersity, while encapsulation efficiency was quantified using a RiboGreen assay. Immune responses were assessed using an in-house ELISA protocol at days 21 and 28.

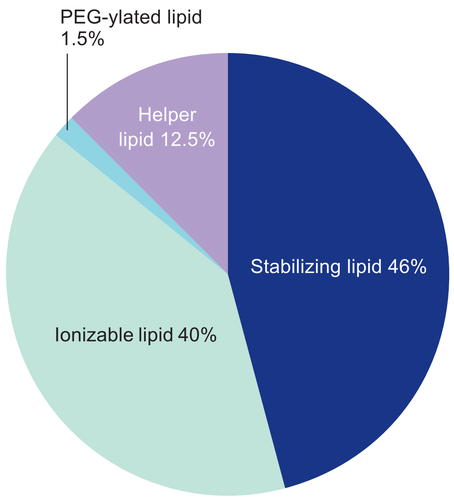

Figure 2: Lipid component percentages

Results

The LNP-saRNA formulations were transfected into BHK-21 and HEK-293T cells to observe their ability to translate and express protein in vitro via flow cytometry (Fg. 3). Flow cytometry detects levels of GOI protein expression of the lipid nanoparticles in cells using a fluorescently- labeled antibody. CL1 yielded the highest results. APL-143 and GenVoy-ILMTM produced similar results but were not nearly as high as CL1. Interestingly, APL-143 worked better in BHK-21 cells, and GenVoy-ILMTM had higher results in the HEK-293T cells. APL-902 exhibited a negligible response.

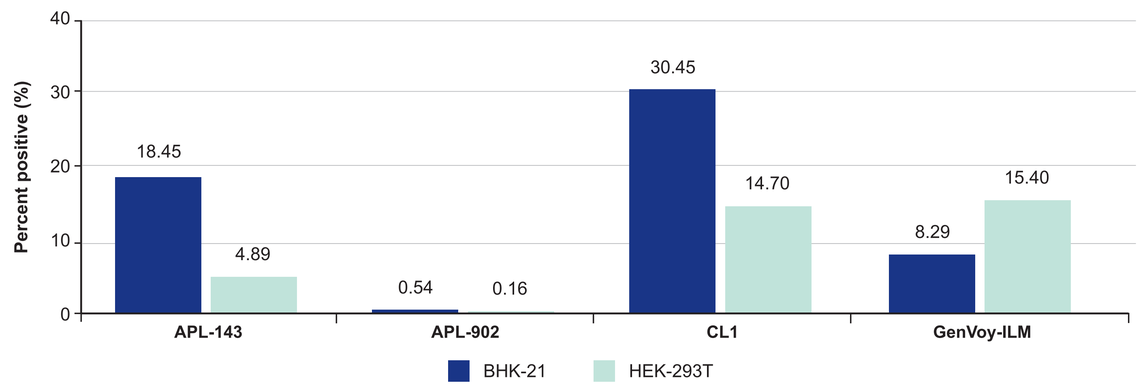

Figure 3: Flow Cytometry results of the lipids mixtures in BHK-21 and HEK-293T cell lines

The vaccine was stored at –80 °C until the time of administration. Prior to vaccination, it was thawed at room temperature and then held at 4 °C until use. Concentrated vaccine was mixed with diluent immediately before dosing. Twenty-four day-old male chicks were vaccinated intramuscularly. A 1 µg dose of saRNA was administered on day 0, followed by a 1 µg booster on day 7. Blood samples were collected on days 21 and 28 to assess antibody responses.

LNP	Hi geometric mean	Standard deviation
Avanti APL-143	2.36	3.75
CL1	1.37	2.45
GenVoy-ILM™	1.26	1.72
Avanti APL-902	1.00	0
Placebo	1.00	0

Table 2: HI Titers

The hemagglutination inhibition (HI) titers (Table 3) and enzyme-linked immunosorbent assay (ELISA) values (Table 4) showed consistent response patterns. As expected, day 28 ELISA values were higher than day 21. Among the formulations tested, APL-143 produced the strongest responses in both HI and ELISA assays, followed by CL1, then GenVoy-ILM™, and lastly APL-902. The placebo and unvaccinated controls showed no measurable response. Placebo and unvaccinated controls showed no meaningful HI or ELISA activity, confirming that the observed responses in vaccinated groups were vaccine-dependent.

	Day 21			Day 28		
	1:10	1:20	1:40	1:10	1:20	1:40
Avanti APL-143	1.29	1.00	0.55	1.71	1.37	0.67
CL1	1.07	0.79	0.55	1.27	0.94	0.67
GenVoy-ILM™	0.90	0.60	0.39	0.89	0.54	0.33
Avanti APL-902	0.54	0.38	0.27	0.63	0.43	0.29
Placebo	0.42	0.30	0.20	0.44	0.27	0.15
Avanti APL-902	0.63	0.45	0.29	0.40	0.23	0.14

Table 3: ELISA results from days 21 and 28

Conclusion

The data from this study shows promise for future vaccine development using APL-143 as ionizable lipid in poultry. A follow up study has been designed to further analyze the two highest performing lipid mixes. The study will include new vaccination schedules for the prime and boost as well as a range of RNA doses designed to generate higher antibody responses.

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