Global Vaccine and Immunization Research Forum

Nucleoprotein Antigen Adjuvanted with Liposomal Cholesterol-linked TLR7/8 Agonist Showed Protective Immunity in Influenza Mouse Model

Chul Kim, MD, Ph.D
Progeneer Inc.

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Disclaimer

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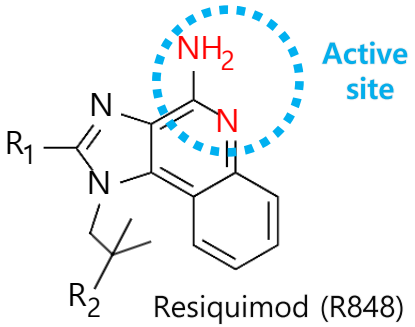
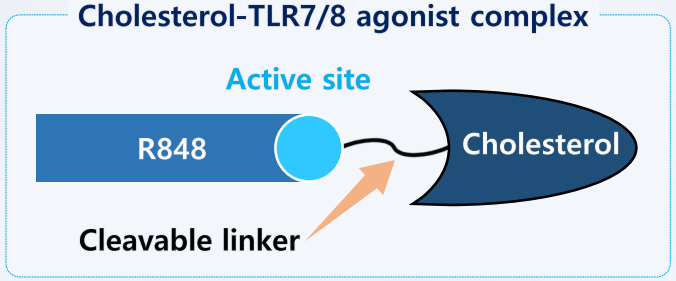
ProLNG-001 Is a Novel TLR7/8 Agonistic Adjuvant Candidate

- ProLNG-001 is a liposomal adjuvant that contains key molecules, ProLNG-S.
- ProLNG-S timely activates toll-like receptors (TLRs) 7/8, hence reduces reactogenicity.
- As ProLNG-S is cholesterol derivatives, it can be easily formulated by liposome or lipid nanoparticle (LNP).

ProLNG Key Characteristics

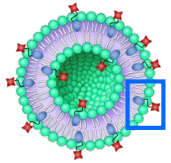


Resiquimod (R848)
-TLR7/8 agonist

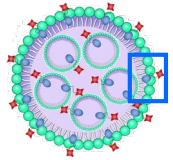


- Capping the active site of TLR7/8 agonist minimized systemic toxicity and prolong the immune response

ProLNG Formulation



ProLNG in Liposome

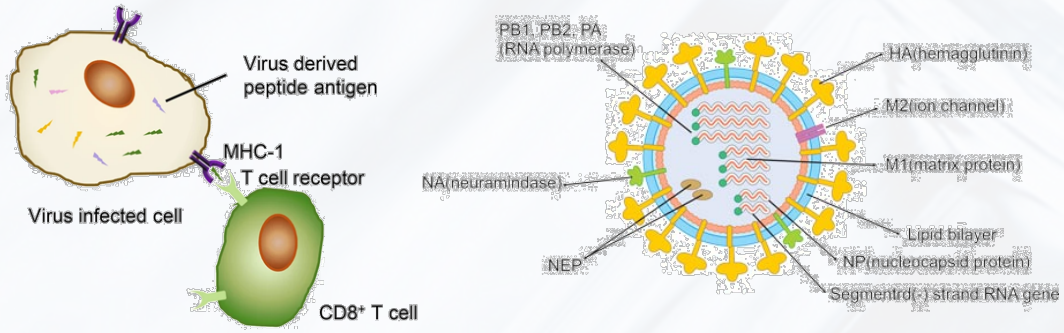


ProLNG in LNP

- Liposome/LNP formulation
- Increased T cell immune response compared to R848
- Sustainability and insignificant toxicity based on prolonged mechanism

Needs of Developing Flu Vaccine by Utilizing Highly Conserved Protein

- Influenza viruses cause death in several hundred thousand people globally each year.
- Currently commercialized vaccines use hemagglutinin as main antigen, which is highly variable among influenza strains as well as vulnerable to morphological changes.
- Highly conserved internal proteins, such as nucleoprotein (NP), are good candidates for universal influenza vaccine but require strong CD8 + T cell responses.
- ProLNG-001 with NP antigen was tested in influenza challenge mouse model.



*Virol Sin. 2021 Feb; 36(1): 13–24.Published online 2020 Sep 1. doi: 10.1007/s12250-020-00283-6

Influenza Virus	Protein (Antigen)	Location	Homology
A/Puerto Rico/8/1934 (H1N1) A/Hong Kong/1968 (H3N2)	Nucleoprotein	Inside	94%
	Hemagglutinin	Outside	43%

*Uniprot, Expasy

ProLNG-001 Vaccine Adjuvant Candidate Was Tested in Influenza Model

- Effectiveness of ProLNG-001 was tested with various doses and in combination with monophosphoryl lipid A (MPLA).
- Commercially approved adjuvant was included as positive control of the study.
- H1N1 NP was administered with or without adjuvant subcutaneously twice with 2 weeks interval in C57BL/6 mice, followed by virus infection after 2 weeks of 2nd vaccination.

G	Group (n=15)	Antigen	Adjuvant
1	Control	N/A	N/A
2	Antigen only	NP 10 μg	N/A
3	ProLNG-001 L	NP 10 μg	ProLNG-S 35 μg
4	ProLNG-001 M	NP 10 μg	ProLNG-S 70 μg
5	ProLNG-001 H	NP 10 μg	ProLNG-S 140 μg
6	ProLNG-001 M + MPLA combi	NP 10 μg	ProLNG-S 70 μg + MPLA 5 μg
7	MPLA + QS21 (AS01)	NP 10 μg	MPLA 5 μg + QS21 5 μg

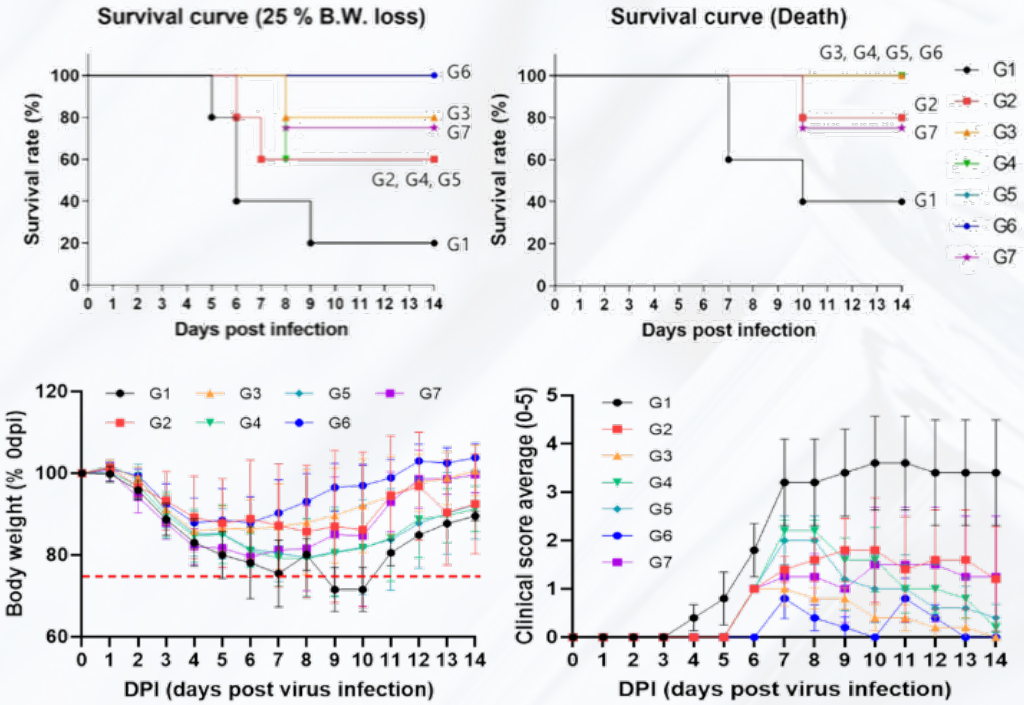
n/Group	Test items
5*	Body weight, Clinical score, Survival (0-14 DPI), Histopathology (14 DPI)
5	IgG ELISA (-14/0 DPI), Spleen (0 DPI), ELISPOT (0 DPI)
5	IgG ELISA (-21/-7/5 DPI), BAL fluid (5 DPI), Histopathology (5 DPI)

*One mouse died in G7 after 2nd vaccination, before virus infection

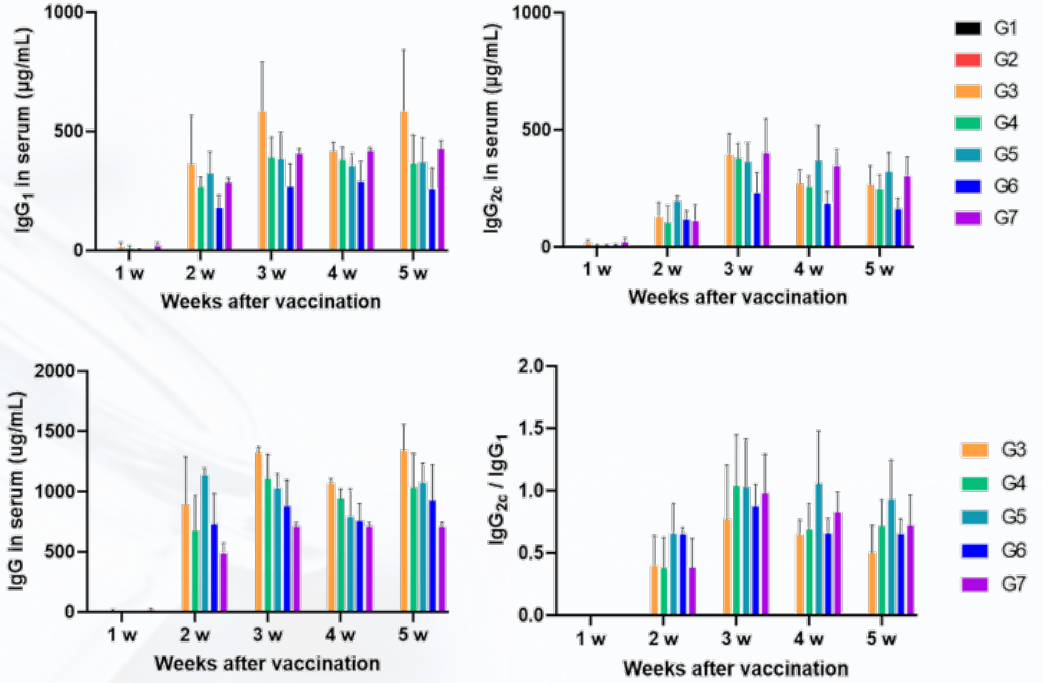
G1: Control, G2: NP, G3: ProLNG L, G4: ProLNG M, G5: ProLNG H, G6: ProLNG M + MPLA, G7: AS01

ProLNG-001 Adjuvanted Groups Showed Better Clinical Outcomes Compared to Controls

- All ProLNG-001 adjuvanted groups (G3-G6) showed promising results in terms of survival rate, body weight, and clinical score compared to the control and non-adjuvanted groups.



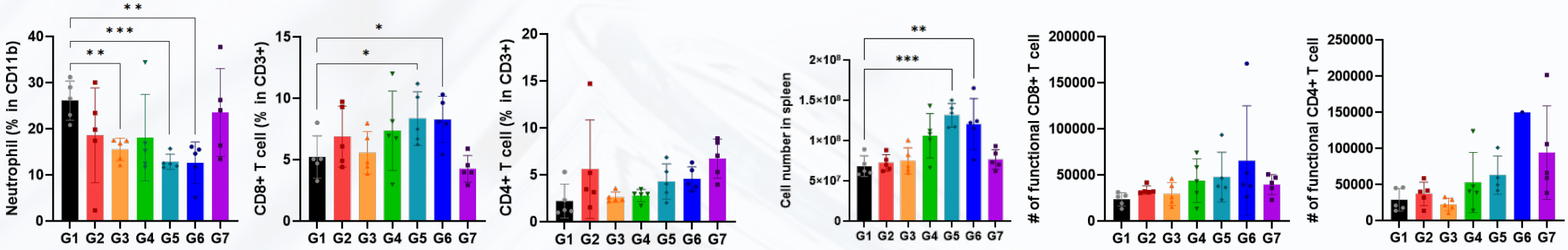
- The ProLNG-001 adjuvanted groups showed comparable antibody levels and IgG class switching with commercially approved AS01 adjuvanted group.



G1: Control, G2: NP, G3: ProLNG L, G4: ProLNG M, G5: ProLNG H, G6: ProLNG M + MPLA, G7: AS01

ProLNG-001 Affected BALF and Spleen Immune Cell Population

- BALF analysis showed that neutrophil counts were decreased in ProLNG-001 adjuvanted groups.
- CD8+ T cells were increased by ProLNG-001.
- Spleen cell numbers were increased in ProLNG-001 adjuvanted groups.
- Functional CD8+ and CD4+ T cells were also increased in those groups.



Conclusion

- The novel TLR7/8 agonists containing liposomal adjuvant, ProLNG-001, alone or in combination with MPLA showed strong protective effects in the influenza challenging mouse model when injected with NP, an influenza virus internal protein.
- ProLNG-001 also showed comparable humoral responses with commercially approved adjuvant.
- BALF and spleen analysis showed ProLNG-001 affect immune cells, especially for CD8+ T cells.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