Development of mRNA-LNP for Cancer Vaccine

Fan Yan, Thomas Chamberlain, Jennifer Moon, Reihaneh Hosseinzadeh, Fransisca Sunarja, Polina Blagojevic, Kyle Stephenson, Paulo Lin, Steve Arns, Ying Tam, Ghania Chikh

Acuitas Therapeutics, Vancouver, BC, Canada

Abstract

Introduction and Objectives

Acuitas' mRNA lipid nanoparticle (LNP) technology has been clinically validated as demonstrated by Alnylam's therapeutic for hereditary ATTR (hATTR) amyloidosis, ONPATTRO®, and Pfizer-BioNTech' vaccine, COMIRNATY®, which has protected billions of people from COVID-19 in more than 180 countries. The success of the Pfizer-BioNTech COMIRNATY vaccine suggests that mRNA-LNP may not only be effective against infectious diseases, but also potentially as an anti-cancer vaccine. With continued advancements in genomic sequencing and immune modulation, mRNA-LNP cancer vaccines hold the potential to provide targeted, effective, personalized cancer treatments with less toxicity than current therapies. As clinical trials progress and more data become available, mRNA-based vaccines could revolutionize cancer therapy by overcoming limitations of current treatments and offering new hope for patients with a variety of malignancies. To create effective and innovative LNP-based cancer vaccines, Acuitas has been advancing its LNP technology through the integration of ionizable lipid screening.

Materials & Methods

From our comprehensive custom-made library of over 1,500 ionizable lipids, we have rationally selected a panel of 20 lipids to assess their relative activity as mRNA-LNP cancer vaccines. The LNP were formulated with nucleoside-modified mRNA incorporating N1-methylpseudouridine-encoding OVA as a model tumour antigen and injected intramuscularly (IM) to mice in a prime-boost schedule. Subsequent studies assessed the activity of modified and unmodified mRNA side-by-side, given that the latter is mostly used in current cancer vaccine trials. Furthermore, ALC-315™ LNP activity was compared to lipoplexes [1], an alternative mRNA cancer vaccine format in clinical development. Antigen-specific adaptive immune responses, including T cell responses and their poly-functionality, serum antibody titers and IgG isotype profiles were assessed.

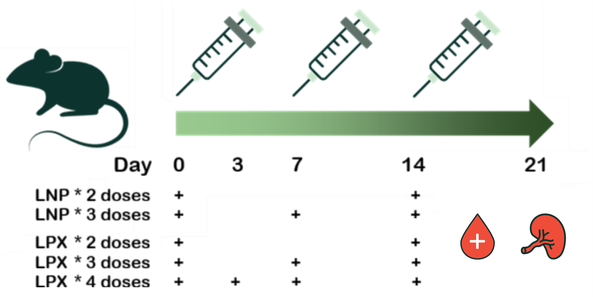
Results

Potent antigen-specific cellular responses were observed with several screened lipids including ALC-315™, the ionizable lipid used in COMIRNATY. The most active lipids induced a Th1-biased immune response, as indicated by IgG isotype profile, which is known to be favorable in the context of therapeutic cancer vaccines. Compared to a modified mRNA antigen payload, vaccination with LNP-encapsulated unmodified mRNA resulted in significantly higher functional antigen-specific CD8 responses, as indicated by MHC tetramer and intracellular cytokine staining. Importantly, mRNA in ALC-315™-based LNP induced equivalent or superior cellular immunity compared to intravenously (IV) administered mRNA-lipoplexes, despite the mRNA-lipoplexes being administered at 4x higher doses and with more boosts than IM administered LNP. Finally, LNP induced higher poly-functional T cells compared to mRNA-lipoplexes. Serum antibody measurement indicated that both ALC-315™ and lipoplex induced Th1-biased immune response, although LNP maintained a more balanced Th1/Th2 ratio than lipoplex.

Conclusion

In addition to existing clinical evidence on efficacy and safety of COMIRNATY as an infectious disease vaccine, our data demonstrated that Acuitas' LNP are also a robust mRNA delivery technology for advancing cancer vaccine development. Future studies will assess LNP effectiveness with syngeneic neoantigens to demonstrate ability to induce effective anti-tumor immune responses capable of irradicating tumors.

LNP Vaccination Schedule



ALC-315™ with Unmodified RNA Induced Strong Cellular Response

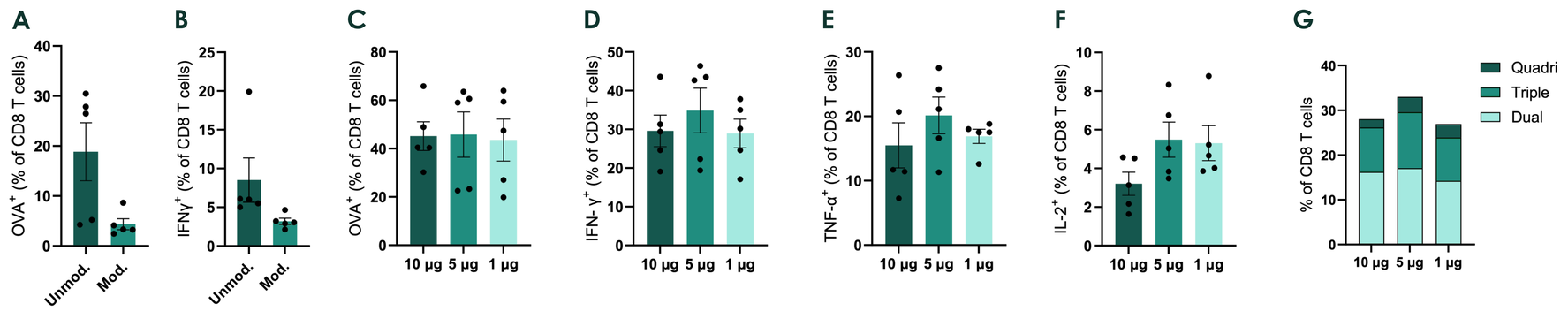


Fig. 1. Comparison of LNP-modified RNA and unmodified RNA and dose titration.
OVA-specific CD8 T cell response in spleen induced on Day 21 following IM vaccination with 2 doses of 10 µg LNP-OVA mRNA (modified or unmodified) - **A**: MHC-I dextramer positive CD8 T cells; **B**: IFN-γ secretion by splenic CD8 T cells after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining. OVA-specific CD8 T cell response in spleen induced on Day 21 following IM vaccination with 3 doses of 10, 5 or 1 µg LNP-OVA mRNA (unmodified): **C**: MHC-I dextramer positive CD8 T cells; **D-F**: IFN-γ, TNF-a, and IL-2 secretion by splenic T cells after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining. **G**: Polyfunctional CD8 T cell percentages based on quadri/triple/dual-positive staining of IFN-γ, TNF-a, CD107a and IL-2.

ALC-315™ Induced Better Cellular & Humoral Immunity than Lipoplexes (LPX)

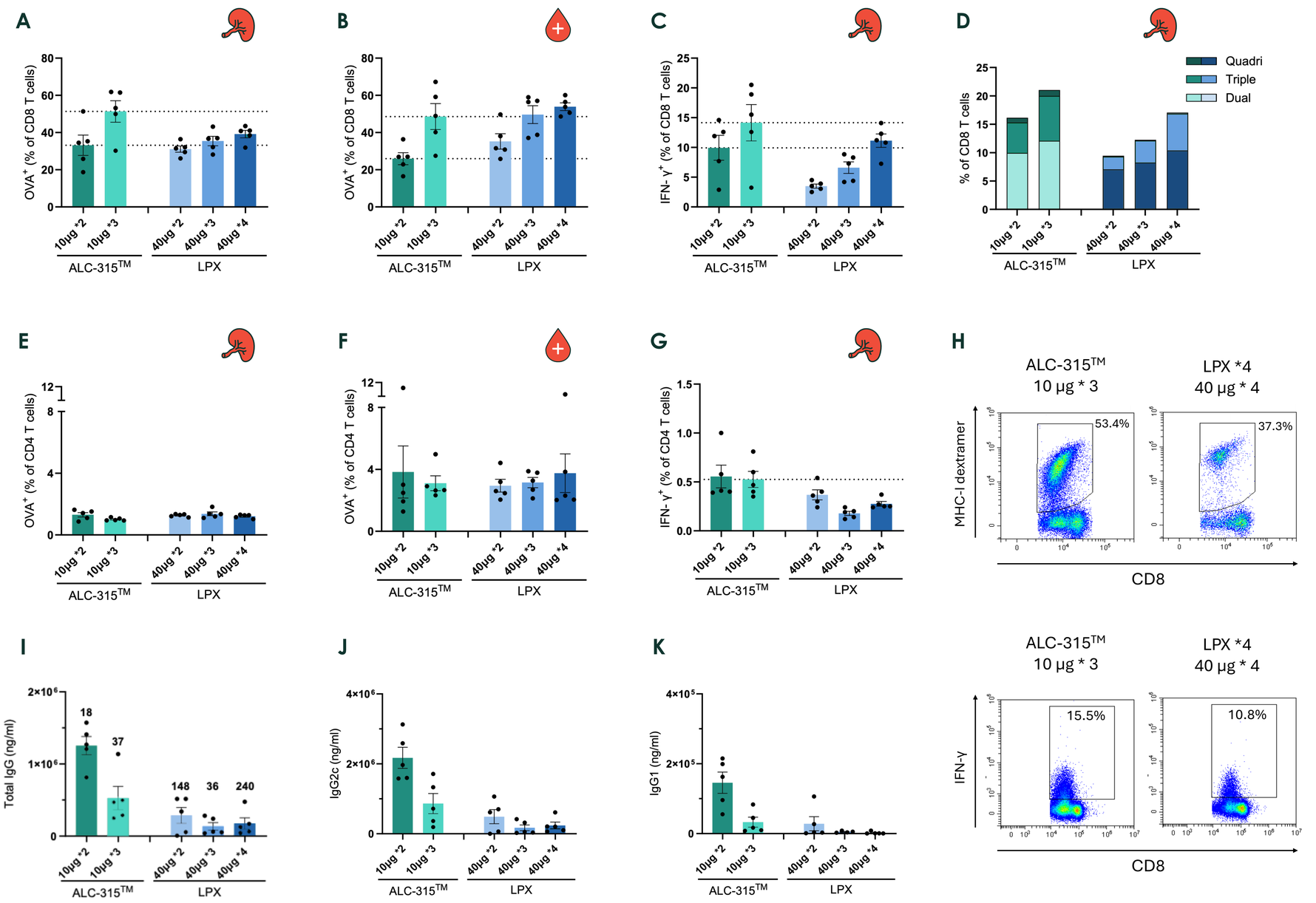


Fig. 2. Comparison of LNP and LPX.
OVA-specific T cell response induced on Day 21 following IM vaccination with 2, 3 doses of 10 µg LNP-OVA mRNA (unmodified), or 2, 3, 4 doses of 40 µg LPX-OVA mRNA (unmodified)- MHC-I dextramer positive CD8 T cells in spleen (**A, H upper panel**) and blood (**B**); IFN-γ secretion by splenic CD8 T cells after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining (**C, H lower panel**); **D**: Polyfunctional CD8 T cell percentages based on quadri/triple/dual-positive staining of IFN-γ, TNF-a, CD107a and IL-2; MHC-II tetramer positive CD4 T cells in spleen (**E**) and blood (**F**); IFN-γ secretion by splenic CD4 T cells (**G**) after *ex vivo* restimulation with 15-mer OVA peptide pool and intracellular staining; OVA specific IgG (**I**), IgG2c (**J**) and IgG1 (**K**) in plasma; IgG2c/IgG1 ratio shown as number above bars (**I**); **H** shows the flow plot of median data points within the group.

Proprietary Novel LNP Induced Comparable Cellular Responses to ALC-315™

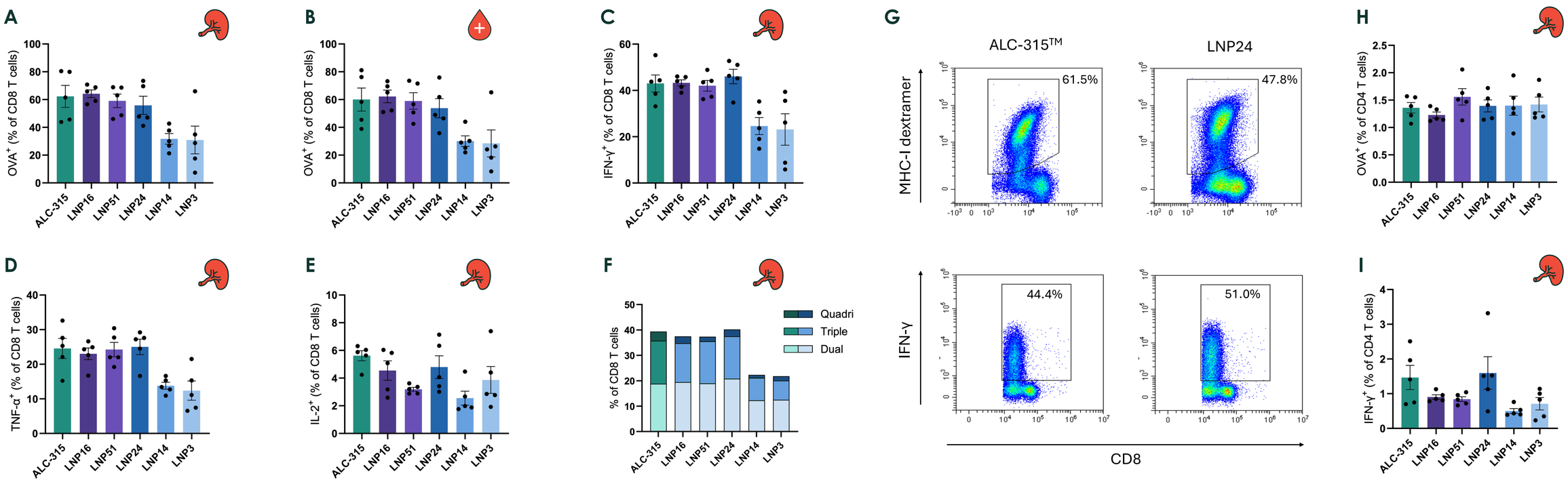


Fig. 3. Novel LNP screen.
OVA-specific T cell response induced on Day 21 following IM vaccination with 3 doses of 1 µg indicated LNP-OVA mRNA (unmodified) - MHC-I dextramer positive CD8 T cells in spleen (**A, G upper panel**) and blood (**B**); IFN-γ (**C, G lower panel**), TNF-a (**D**), and IL-2 (**E**) secretion by splenic CD8 T cells after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining; **F**: Polyfunctional CD8 T cell percentages based on quadri/triple/dual-positive staining of IFN-γ, TNF-a, CD107a and IL-2; ; **G** shows the flow plot of median data points within the group; **H**: MHC-II tetramer positive CD4 T cells in spleen; **I**: IFN-γ secretion by splenic CD4 T cells after *ex vivo* restimulation with 15-mer OVA peptide pool and intracellular staining.

Summary

1 **With unmodified mRNA, the backbone currently used in ongoing cancer vaccine clinical trials, LNP (ALC-315™) induced significantly higher CD8 T cell response compared to modified mRNA. This indicates the importance of stronger inflammatory response in enhancing APC maturation and antigen presentation for an effective and functional T cell response. ALC-315™ -based mRNA-LNP vaccine was very potent as demonstrated by maintaining strong immunogenicity at 1/10th of initially tested dose.**

2 **Compared to LPX (IV), Acuitas' LNP (IM) elicited overall better immunogenicity at only 1/4th of LPX dose and fewer vaccination occasions. This includes cellular response, as indicated by CD8 T cells functionality/polyfunctionality, and humoral response. These results provide evidence of mRNA-LNP's great potential in cancer vaccines.**

3 **In addition to ALC-315™, 3 out of the 5 screened Acuitas' proprietary LNP achieved potent and equivalent cellular response to ALC-315™. Selected LNP will be further assessed to compare potency as well as activity using syngeneic neoantigens models to demonstrate the ability of Acuitas' LNP to break tolerance and induce effective anti-tumor immune response able to irradicate tumor growth.**

References

1. Lena M. Kranz et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016 Jun 16;534(7607):396-401. doi: 10.1038/nature18300.