Advances in Clinically Viable LNP Compositions for mRNA Delivery

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1 Abstract

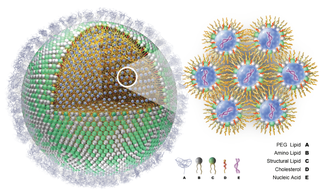
Arbutus' Lipid Nanoparticle (LNP) platform is the leading nucleic acid delivery technology platform enabling a number of early and late stage clinical trials. They are designed to deliver therapeutic nucleic acids (NA) to sites of disease, and have been used to target both viral and endogenous gene targets. We continuously seek to broaden the Therapeutic Index (TI) of the LNP platform, from both potency and tolerability perspectives.

It is important to recognize that nucleic acid drugs can stimulate cytokine release that may be accentuated by the delivery vehicle. This is sporadically observed with siRNA-LNP products in the clinic and can be managed with steroid premedication. It would be advantageous to address these drug properties at the compositional level in advance. This is particularly true for mRNA payloads, that preclinical data suggests are more likely to provoke inflammatory responses than smaller oligonucleotides. mRNA payloads have garnered increasing interest for a range of therapeutic strategies, and their safe and successful formulation demands appropriate attention.

Here we test new formulation strategies to address the challenge of immune stimulation. Initial activity/tolerability screens are run with siRNA payloads in murine models, with compositions of interest advanced to porcine and primate models. We found good corroboration between these models, with a similar hierarchy of the compositions' tendency to provoke an inflammatory response. By using an siRNA targeting the endogenous gene target transthyretin (TTR), the NHP model helped identify a lead LNP composition that was significantly more potent than those currently in the clinic.

These strategies were equally applicable to LNP bearing mRNA payloads. We further demonstrate that the method/extent of purification of the mRNA payload, prior to formulating, plays a significant role.

2 Arbutus' Lipid Nanoparticles in the Clinic



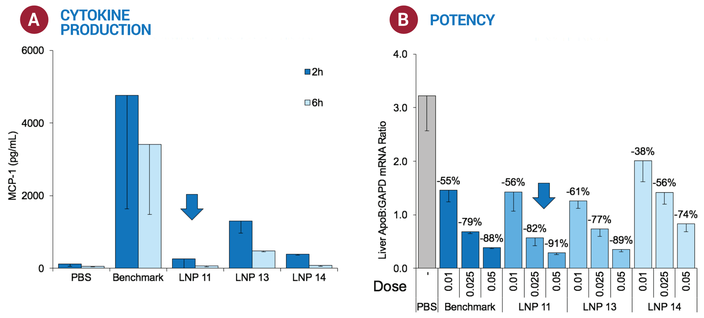
- LNP are comprised of neutral, cationic and PEG-lipids in compositions designed to confer desired pharmacokinetic and pharmacodynamic properties to their NA payloads.
- LNP protect the NA against nuclease degradation in the blood, and enable effective delivery to the cytoplasm of target cells.

- 10 LNP drug products have entered clinical trials
- Over 250 patients treated, some for over 1 year duration (TKM-PLK1 and ALN-TTR02).
- Potent, long lasting effects after a single dose (siRNA)
- Growing body of clinical safety data and understanding of key hurdles for LNP development

Representative Clinical Activity

Product	Company	Phase	Indication	Comments
Patisiran (ALN-TTR02)	Alnylam	3	Amyloidosis	• Safely dosed for up to 25 months in some patients • Efficacy of up to 94% TTR knockdown with physiological effect • NDA submission anticipated in 2017
TKM-PLK1	Arbutus	2	Oncology	• Safely dosed for up to 18 months • Evidence of anti-tumor activity based on a decrease in tumor size
TKM-HBV	Arbutus	2	Hepatitis B	• Currently in Phase IIa trial in HBV patients • Activity data expected 2H 2016
TKM-Ebola	Arbutus	1	Ebola Infection	• Lyophilized formulation
DCR-PH1	Dicerna	1	Primary Hyperoxaluria	• Dicer Substrate Payload

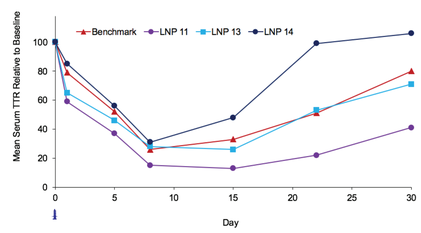
3 Murine Primary Screens Identify Compositions of Interest

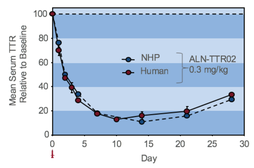


- Formulation Development screening / hit identification begins with mouse models. Example above shows 3 novel compositions with expanded TI vs benchmark (a clinical composition)
- (A) – Immune Stimulation: High doses (10 mg/kg) of LNP are necessary to consistently detect a cytokine response in mice (n = 5). Plasma samples assessed for cytokines at 2 h and 6 h. Representative cytokine results (MCP-1) reveal that all three novel compositions are less stimulatory than the benchmark control.
- (B) – Preliminary activity screening is performed in a mouse ApoB silencing model. A single IV administration of LNP is given at the doses described, and liver ApoB mRNA levels determined by QuantiGene assay 48 h post administration (n= 3, +/- s.d.).

4 Increased TI in Non Human Primates

POTENCY

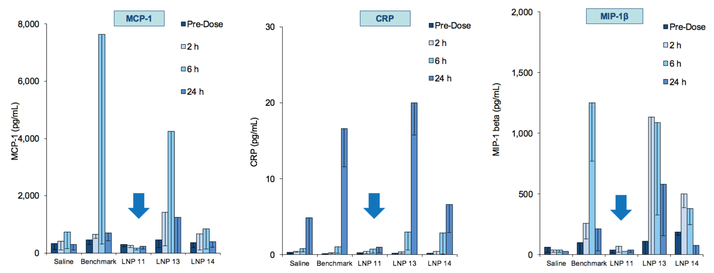




Historical Alnylam Data with Clinical Candidate ALN-TTR02 B.U. Med. Center, July 2012

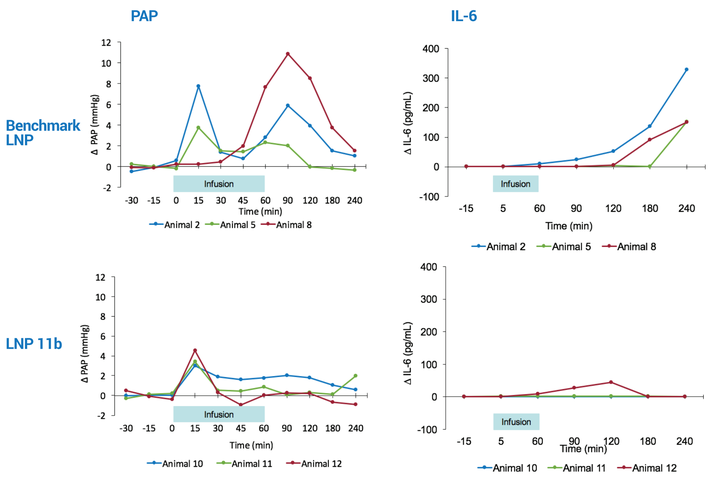
- Hit compositions are advanced to NHP studies, which have proved to be excellent predictors of LNP potency in man.
- Cynomolgus monkeys received LNP (IV) containing the same TTR siRNA sequence as ALN-TTR02 (currently in Phase 3 clinical trial). Dosed at 0.0375 mg/kg, n = 4.
- LNP 11 displays excellent knockdown, with profile similar to ALN-TTR02 at 1/8 the siRNA dose.
- As with murine model, LNP 14 is slightly less potent, but exhibits good silencing given low dose.

CYTOKINE PRODUCTION



- To evaluate immune stimulation, cynomolgus monkeys received LNP (IV) at a dose of 2 mg/kg siRNA (n=4).
- Tolerability hierarchy of compositions similar to mouse models.
- LNP 11 is particularly well tolerated, with cytokine profile similar to saline control. Given the additional improvement in potency, the increase in TI for this composition is considerable.

5 Improved Tolerability in Minipigs

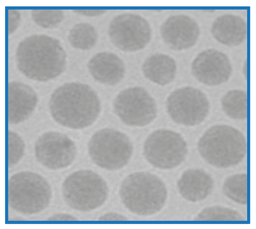


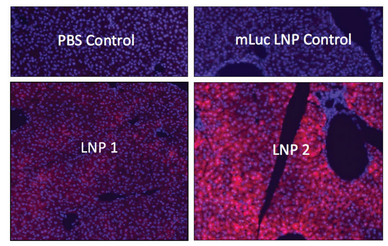
- Many approved liposomal drugs have been associated with infusion-related reactions in the clinic (e.g. Doxil (10-45%), Ambisome (10%), DaunoXome (14%)). Pigs are a particularly sensitive species, and have been put forward as an exaggerated model for the small fraction of sensitive humans in the population*.
- Hemodynamic changes (pulmonary hypertension, decreased cardiac output, increased vascular resistance (and thromboxane – a vasoconstrictor) are prominent symptoms of pig model.
- Here, LNP were administered to anesthetized Göttingen minipigs (n=3). Test article-related effects on hemodynamic parameters and inflammatory biomarkers are evaluated, following a 60 minute IV infusion of LNP at a dose of 0.3 mg/kg.
- LEFT: Compared to the Benchmark, cardiopulmonary distress is significantly less marked with LNP 11. No elevation in pulmonary arterial pressure (PAP) is observed following end of infusion.
- RIGHT: Representative cytokine data (IL-6) shows that LNP is also well tolerated with respect to cytokine response over the 4h time course of the study.

** Szebeni et al, 2012, Adv Drug Del Rev*

6 LNP with mRNA Payloads

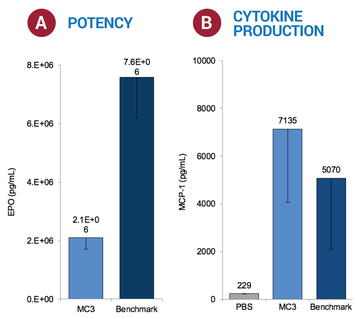
- LNP have been used to encapsulate mRNA transcripts.
- (RIGHT) Particles have a largely non-lamellar, electron dense morphology (Cryo-TEM image, right) and similar physico-chemical characteristics to those bearing oligonucleotides.





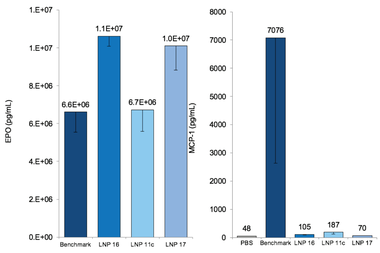
- LNP were formulated with mCherry mRNA and administered i.v. (2 mg/kg) to Balb/C mice.
- (LEFT) At 24 h, fluorescence microscopy reveals potent, uniform mRNA delivery throughout the liver. Significantly more activity (red fluorescence) is observed with LNP 2 (current benchmark) compared to LNP 1. Cell nuclei are stained blue (DAPI). No activity is observed in the negative controls.

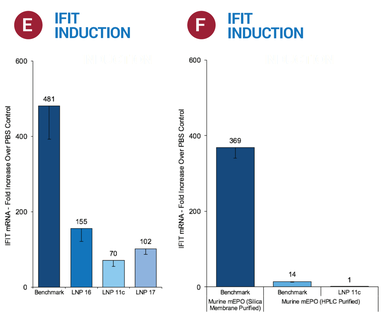
7 Novel LNP Prevent mRNA-mediated Cytokine/IFIT Response



- A murine erythropoietin (EPO) model was used to establish the TI of the benchmark LNP vs an MC3 composition similar to ALN-TTR02.
- (A) LNP bearing an EPO mRNA purified by silica spin column were administered IV (0.5 mg/kg) to mice (n = 5). The benchmark control is ~4-fold more potent than the MC3 LNP.
- (B) Plasma samples were also assessed for cytokines (MCP-1 shown). The benchmark LNP is similar to the MC3 LNP. The mRNA payload causes significantly more cytokine release than siRNA-LNP, given the dose.

- Compositions based on LNP 11 were tested in the same murine EPO model.
- (C) New compositions were up to 50% more potent than the benchmark.
- (D) Cytokine readouts show the new mRNA-LNP to be markedly better tolerated (MCP-1 shown).





- IFN-induced protein (IFIT1) mRNA is a more sensitive measure of immune stimulation. Strong IFIT1 induction in the liver can be observed even in the absence of detectable plasma IFN protein or other cytokines.
- (E) Using the above EPO mouse model (0.5 mg/kg, n = 5, t = 4h), a moderate to strong IFIT response is still observed.
- (F) Switching to an HPLC-purified mRNA payload reduces the IFIT signal significantly. This sensitive signal of immune stimulation is eliminated when using the novel composition LNP11c in conjunction with the highly purified mRNA.

8 Summary

Conclusions: Based on our clinical experience, the safe and successful translation of mRNA-LNP into man will require careful attention to both potency and immune stimulation. We have identified new lipid compositions that are significantly more potent than those currently in the clinic. More importantly, they possess an immune stimulatory profile similar to saline controls at doses far greater than those required for efficacy. This unprecedented degree of immune silence, a profile which was preserved in higher species such as pig and NHP, will be imperative in providing the necessary Therapeutic Index for mRNA therapeutics in man.