The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs

The regulatory approval of Onpattro, a lipid nanoparticle-based short interfering RNA drug for the treatment of polyneuropathies induced by hereditary transthyretin amyloidosis, paves the way for clinical development of many nucleic acid-based therapies enabled by nanoparticle delivery.

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Nanomedicines resulting from the application of nanotechnology to medicine are having an increasing impact on the treatment of disease. This applies particularly to nanomedicines using lipid nanoparticle (LNP) drug delivery systems as there are now more than ten US Food and Drug Administration (FDA) approved pharmaceuticals employing LNPs to deliver drugs to disease sites (Table 1). Most of these nanomedicines are formulations of cancer drugs that offer the benefits of reduced toxicity and/or enhanced efficacy compared to the 'free' drug[1]. Due to the clinical success of LNP-based drug delivery systems, we now have a good understanding of the requirements for successful clinical translation of LNP systems for delivery of small molecules. Translational criteria include a size range of 100 nm or less, highly efficient encapsulation techniques, a low surface charge, robust, scalable manufacturing processes and adequate product stability[2].

It is of great interest to extend LNP technology to delivery of nucleic acid-based drugs, such as short interfering RNA (siRNA), messenger RNA (mRNA) and gene editing constructs. Unmodified nucleic acid-based drugs face particular delivery problems, because they are readily broken down in biological fluids, do not accumulate in target tissues and cannot penetrate into target cells even if they get to the desired tissues. Unfortunately, many of the techniques developed for generating clinically viable LNP formulations of small molecule drugs cannot be applied to nucleic acid polymers owing to their large size and negative charge. Further, LNP formulations of small molecule drugs have only to release drug cargo after arrival in the target tissue; by contrast, LNP formulations of nucleic acid-based drugs must also facilitate intracellular delivery of these macromolecules into target cells.

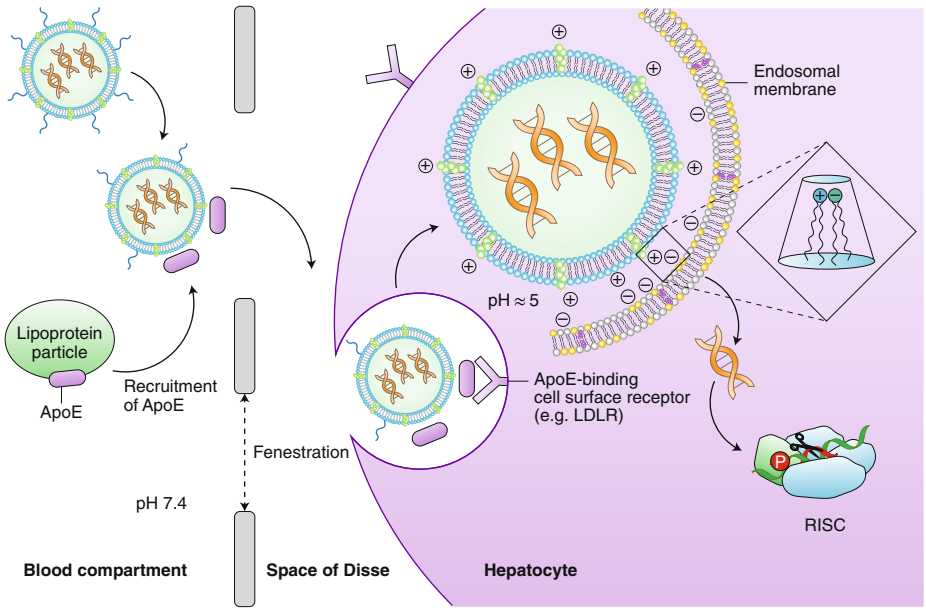

Fig. 1 | Integrated model of lipid nanoparticle (LNP)-mediated delivery of siRNA to hepatocytes in vivo. Key steps include the dissociation of PEG-lipids from the particle surface, recruitment of endogenous ApoE to the LNP surface, trafficking of LNPs through fenestrated endothelium and binding to low density lipoprotein receptors and other ApoE-binding receptors on hepatocytes, internalization of LNPs via endocytosis, protonation of the ionizable lipid due to the low pH in the endosome, interaction of the protonated ionizable lipid with negatively charged endogenous lipids, which results in the destabilization of the endosomal membrane, and release of siRNA into the cytoplasm, where it can engage with the RNAi machinery. RISC, RNA-induced silencing complex. LDLR, low density lipoprotein receptor.

Here, we describe the successful preclinical development and clinical translation of patisiran (trade name Onpattro), which is an LNP formulation of siRNA for the treatment of polyneuropathies resulting from the hereditary disease transthyretin-mediated amyloidosis (hATTR). This drug acts by inhibiting the synthesis of the transthyretin (TTR) protein in the liver. The positive results of a global phase 3 study[3] resulted in FDA approval of Onpattro in August 2018. The success of

Table 1 | LNP drugs that have received regulatory approval from the FDA or EMA

Name	Encapsulated drug	Indication	Year approved	Company
AmBisome	Amphotericin B	Fungal infections Leishmaniasis	1990 (Europe) 1997 (USA)	Gilead
Doxil/Caelyx	Doxorubicin	Kaposi's sarcoma Ovarian cancer Breast Cancer	1995 (USA) 1999 (USA) 2003 (Europe)	Johnson& Johnson
DaunoXome	Daunorubicin	Kaposi's sarcoma	1996 (Europe), 1996 (USA)	Galen
Myocet	Doxorubicin	Breast cancer	2000 (Europe)	Cephalon
Abelcet	Amphotericin B	Aspergillosis	1995 (USA)	Enzon
Amphotec	Amphotericin B	Invasive aspergillosis	1996 (USA)	Intermune
Visudyne	Verteporfin	Wet macular degeneration	2000 (USA)	QLT
Marqibo	Vincristine	Acute lymphoblastic leukemia	2012 (USA)	Spectrum Pharma
Onyvide	Irinotecan	Metastatic pancreatic cancer	2015 (USA)	Ipsen Biopharma
Vyxeos	Daunorubicin, Cytarabine	Acute lymphocytic leukemia	2017 (USA)	Jazz Pharma
Onpattro	siRNA targeting transthyretin	Transthyretin induced amyloidosis (hATTR)	2018 (USA), 2018 (Europe)	Alnylam Pharmaceuticals

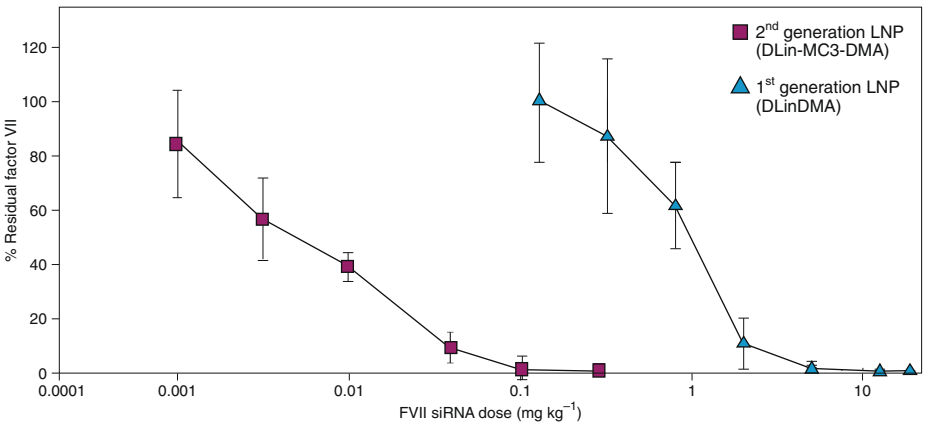

Fig. 2 | LNP siRNA systems containing 2nd generation ionizable aminolipids exhibit greatly improved potency for silencing factor VII (FVII) in the liver. The data presented shows dose-dependent silencing of FVII following i.v. injection of LNP encapsulating siRNA against FVII in a mouse model. 2nd generation LNP containing heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA) are more than two orders of magnitude more potent than 1st generation LNP containing 1,2-dilinoleyl-*N*,*N*-dimethyl-3-aminopropane (DLinDMA).

Onpattro heralds the arrival of a new class of medicines based on nucleic acid polymers. In particular, subsequent studies of closely related LNP systems containing much larger mRNA cargos indicate that LNP delivery technology can potentially enable most forms of nucleic acid-based therapies.

Preclinical development

The basic features required of an LNP siRNA system with the potential for clinical translation include efficient encapsulation of siRNA into an LNP with low surface charge, a diameter of 100 nm or less and the ability to deliver encapsulated siRNA to the cytoplasm of hepatocytes in vivo following intravenous administration. With regard to encapsulation, nucleic acid polymers can be readily associated with lipidic particles containing permanently positively charged lipids; however, such positively charged systems induce pronounced toxicity in vivo due to immune activation (activation of complement and coagulation pathways as well as cytokine stimulation) and cytotoxicity. To circumvent this problem, we developed ionizable cationic lipids that possess an amine function with an acid dissociation constant (pKa) of ~6.5 (ref. [4]). These lipids are positively charged at acidic pH values, but nearly neutral at physiological pH. Efficient encapsulation of siRNA into LNPs can then be achieved by rapid mixing of lipids in ethanol with siRNA in aqueous media at low pH (pH 4) using a readily scalable manufacturing process. These LNP systems, which have a novel 'solid core' structure[5], display low surface charge at physiological pH and are relatively non-toxic and non-immunogenic.

Diameters of 100 nm or less could be achieved by incorporating polyethylene glycol (PEG)-lipids that associate with the surface of the LNP. LNP size can then be regulated by adjusting the proportion of surface PEG lipid to core lipid to generate sizes over the range 20–100 nm[6]. The presence of a PEG coating on the LNP surface has the disadvantage of inhibiting interactions with target cells and thus reducing intracellular delivery. This problem was overcome by using PEG-lipids with relatively short C_{14} acyl chains. Such PEG-lipids remain associated with the particles during formulation and under storage conditions; however, in the presence of a lipid sink (for example, lipoprotein particles in plasma), the PEG-lipids can exchange out of the LNP, thereby generating an unshielded particle that can engage with target cells to enable uptake[7].

The development of LNP siRNA systems with high loading efficiencies, defined size and low surface charge satisfied the basic criteria for clinical potential; however, the potency of these systems for gene silencing in hepatocytes remained to be characterized and optimized. As the in vitro potency of an LNP nanomedicine rarely correlates with in vivo performance, we moved directly to an in vivo model to optimize gene silencing properties. LNPs containing siRNA against factor VII (FVII) were administered to mice

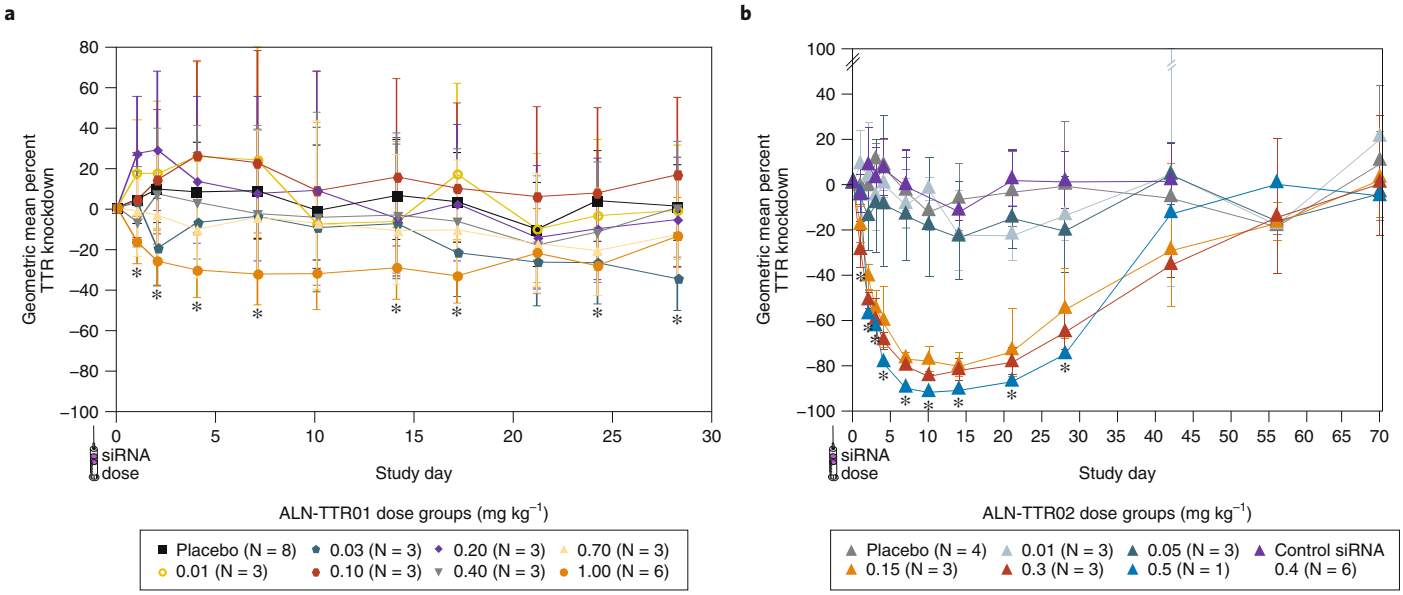

Fig. 3 | Phase I clinical trials of ALN-TTR01 and ALN-TTR02 (patisiran). a,**b**, Mean percent serum transthyretin (TTR) knockdown at the indicated time points, as compared with the baseline in groups of patients receiving either placebo or increasing doses of ALN-TTR01 (a) and in healthy subjects receiving either placebo or increasing doses of ALN-TTR02 (patisiran) (b). The error bars indicate 95% confidence intervals. Data from ref. [13].

to silence the FVII gene in hepatocytes, providing a convenient assay to optimize gene silencing potency in vivo. Initial work showed that LNP siRNA systems containing the ionizable lipid 1,2-dilinoleyl-*N*,*N*-dimethyl-3-aminopropane (DLinDMA) could silence genes in hepatocytes following intravenous (i.v.) administration[8]. However, the potency and tolerability of these LNP siRNA systems was not sufficient to warrant clinical development and a search for more active formulations commenced focusing primarily on the ionizable lipid component.

A first breakthrough was reached with the development of the ionizable lipid DLinKC2DMA[9], which substantially improved the potency and tolerability of the LNP, leading to an extensive research programme aimed at achieving ever more potent ionizable cationic lipids. The pharmacodynamics, pharmacokinetics and safety of promising formulations were evaluated in rodents, and lead candidates were then tested in non-human primates (NHPs). More than 300 ionizable lipids were designed and synthesized, leading to the identification of structure–activity relationships[10]. Notably, a remarkable dependence of LNP siRNA gene silencing potency on the acid dissociation constant (pKa) of the ionizable cationic lipid was found, with an optimum around pKa ≈ 6.4. Deviation from this pKa by as little as 0.5 units could reduce potency by 100-fold or more[10]. This pKa optimum likely reflects the required balance between a low LNP surface charge to avoid rapid clearance in the circulation and a positive charge on the ionizable lipids to enable escape out of the acidic endosome following endocytosis. Positively charged lipids interact with negatively charged lipids to disrupt bilayer membranes[11], which is a probable requirement for breaking out of endosomes and thus, for cytoplasmic delivery of siRNA.

The remarkable affinity of these LNPs for the liver, in particular for hepatocytes, was found to be facilitated by the adsorption of apolipoprotein E (ApoE) on the surface of the LNPs following i.v. administration. The particle-associated ApoE acts as a highly effective targeting ligand by binding to lipoprotein receptors on the surface of hepatocytes, thereby triggering uptake into hepatocytes by endocytosis[12]. An integrated working model of LNP-mediated delivery of siRNA was then developed to describe the key steps of the LNP journey, from the site of administration to the release of the siRNA payload into the cytoplasm of hepatocytes (Fig. 1). This improved mechanistic understanding and predictability of lipid activity enabled the discovery of increasingly potent ionizable lipids, among which heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate, later termed DLinMC3DMA (or simply MC3), exhibited an improvement in potency of more than two orders of magnitude compared to the benchmark DLinDMA formulation (Fig. 2).

After confirmation of potent TTR silencing in NHPs, the MC3 formulation containing a human TTR-targeting siRNA was transitioned into preclinical development as ALN-TTR02 (later known as patisiran). Repeat-dose toxicology in rats and NHPs demonstrated a substantially improved therapeutic index compared to the first generation LNP.

Clinical development

The translation of LNP-enabled siRNA systems for the treatment of hATTR amyloidosis in humans proceeded in two stages. The first generation DLinDMA-based formulation was evaluated in a placebo-controlled phase 1 trial to determine safety and efficacy of ALN-TTR01 after administration of a single dose, ranging from 0.01 to 1 mg kg^{-1}. The study provided a number of key insights: (1) NHP studies seemed to provide a reasonable prediction of human efficacy (approximately 50% mean TTR reduction observed in NHPs at 1 mg kg^{-1}); (2) ALN-TTR01 showed an encouraging safety profile with no drug-related serious adverse events or discontinuations or mild-to-moderate infusion-related reactions in a subset of participants; and (3) a single dose of ALN-TTR01 at 1 mg kg^{-1} led to a mean reduction in serum TTR levels of 38% compared to the placebo (Fig. 3a), with one patient achieving a substantial TTR reduction of >80%. These results validated the RNAi approach, the siRNA and the LNP platform in a real patient setting for the first time. Based on these results, the MC3-based 2nd generation

LNP (ALN-TTR02, patisiran) was advanced to the clinic in a first-in-human phase 1 trial[13]. As predicted by the preclinical studies, patisiran showed improved clinical activity compared to ALN-TTR01 with rapid, robust and durable suppression of TTR levels of >80%, compared to the placebo at doses between 0.15 and 0.5 mg kg^{-1} (Fig. 3b).

The encouraging efficacy and safety profile observed in the phase 1 study paved the way for further clinical development, culminating in the randomized, double-blinded, placebo-controlled phase 3 APOLLO study[3] and finally, in the regulatory approval of Onpattro in the US and EU, with the potential for approval in other jurisdictions.

Future prospects

The clinical development pathway followed by Onpattro paves the way for the clinical translation of LNP nanomedicines containing nucleic acid-based drugs to enable many novel therapeutics based on silencing or expressing target genes. The ethanol-dilution rapid mixing manufacturing process employing ionizable cationic lipids can be readily extended to encapsulate much larger negatively charged molecules such as mRNA[14,15] and effective transfection has been achieved in a variety of tissues in addition to the liver[16]. LNP systems containing mRNA show promise to target and use the liver as a bioreactor for the production of therapeutic proteins, such as monoclonal antibodies[17] and hormones[18] following i.v. administration. Alternatively, when administered by intradermal or intramuscular routes, LNP mRNA systems provide highly effective vaccines for infectious diseases such as the Zika virus[19] or influenza virus[20]. Finally, LNPs containing mRNA coding for programmable nucleases show considerable potential for gene editing in vivo[21,22]. Challenges remain, including achieving improved site-specific transfection as well as improving our ability to transfect extrahepatic tissues. However, the rapid advances of recent years suggest that it is just a matter of time before these challenges are overcome. ❐

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References

1. Allen, T. M. & Cullis, P. R. Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.* **65**, 36–48 (2013).
2. Cullis, P. R., Mayer, L. D., Bally, M. B., Madden, T. D. & Hope, M. J. Generating and loading of liposomal systems for drug-delivery applications. *Adv. Drug Deliv. Rev.* **3**, 267–282 (1989).
3. Adams, D. et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N. Engl. J. Med.* **379**, 11–21 (2018).
4. Semple, S. C. et al. Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: formation of novel small multilamellar vesicle structures. *Biochim. Biophys. Acta Biomembr.* **1510**, 152–166 (2001).
5. Kulkarni, J. A. et al. On the formation and morphology of lipid nanoparticles containing ionizable cationic lipids and siRNA. *ACS Nano* **12**, 4787–4795 (2018).
6. Belliveau, N. M. et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. *Mol. Ther. Nucleic Acids* **1**, e37 (2012).
7. Mui, B. L. et al. Influence of polyethylene glycol lipid desorption rates on pharmacokinetics and pharmacodynamics of siRNA lipid nanoparticles. *Mol. Ther. Nucleic Acids* **2**, e139 (2013).
8. Zimmermann, T. S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* **441**, 111–114 (2006).
9. Semple, S. C. et al. Rational design of cationic lipids for siRNA delivery. *Nat. Biotechnol.* **28**, 172–176 (2010).
10. Jayaraman, M. et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed.* **51**, 8529–8533 (2012).
11. Hafez, I. M., Maurer, N. & Cullis, P. R. On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. *Gene Ther.* **8**, 1188–1196 (2001).
12. Akinc, A. et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. *Mol. Ther.* **18**, 1357–1364 (2010).
13. Coelho, T. et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N. Engl. J. Med.* **369**, 819–829 (2013).
14. Leung, A. K. K., Tam, Y. Y. C., Chen, S., Hafez, I. M. & Cullis, P. R. Microfluidic mixing: a general method for encapsulating macromolecules in lipid nanoparticle systems. *J. Phys. Chem. B* **119**, 8698–8706 (2015).
15. Kulkarni, J. A. et al. Fusion-dependent formation of lipid nanoparticles containing macromolecular payloads. *Nanoscale* **11**, 9023–9031 (2019).
16. Pardi, N. et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J. Control. Release* **217**, 345–351 (2015).
17. Pardi, N. et al. Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. *Nat. Commun.* **8**, 14630 (2017).
18. Thess, A. et al. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. *Mol. Ther.* **23**, 1456–1464 (2015).
19. Pardi, N. et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* **543**, 248–251 (2017).
20. Pardi, N. et al. Nucleoside-modified mRNA immunization elicits influenza virus hemagglutinin stalk-specific antibodies. *Nat. Commun.* **9**, 3361 (2018).
21. Finn, J. D. et al. A S single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep.* **22**, 2227–2235 (2018).
22. Conway, A. et al. Non-viral delivery of zinc finger nuclease mrna enables highly efficient in vivo genome editing of multiple therapeutic gene targets. *Mol. Ther.* **27**, 866–877 (2019).

Competing interests

A.A., M.A.M., M.M., K.F., M.J. and S.B. are employees of Alnylam Pharmaceuticals. S.A., X.D., M.J.H., T.D.M., B.L.M., S.C.S. and Y.K.T. are employees of Acuitas. P.R.C. has financial holdings in Acuitas.