

The 60-year evolution of lipid nanoparticles for nucleic acid delivery

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Abstract

Delivery of genetic information to the interior of target cells *in vivo* has been a major challenge facing gene therapies. This barrier is now being overcome, owing in part to dramatic advances made by lipid-based systems that have led to lipid nanoparticles (LNPs) that enable delivery of nucleic acid-based vaccines and therapeutics. Examples include the clinically approved COVID-19 LNP mRNA vaccines and Onpattro (patisiran), an LNP small interfering RNA therapeutic to treat transthyretin-induced amyloidosis (hATTR). In addition, a host of promising LNP-enabled vaccines and gene therapies are in clinical development. Here, we trace this success to two streams of research conducted over the past 60 years: the discovery of the transfection properties of lipoplexes composed of positively charged cationic lipids complexed with nucleic acid cargos and the development of lipid nanoparticles using ionizable cationic lipids. The fundamental insights gained from these two streams of research offer potential delivery solutions for most forms of gene therapies.

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Introduction

The grand vision of gene therapy (defined here as the use of genetic material to treat or prevent disease)¹ had humble beginnings rooted in basic science. In the late 1800s, Pasteur and Koch first proposed 'germ theory' postulating that microorganisms composed of proteins are the primary causative agents of infectious disease². In 1958, following the discovery of DNA double helix structure, Francis Crick formulated the central dogma of molecular biology in a paper entitled "On protein synthesis", stating that genetic information flows from DNA to RNA and then to protein³. This understanding was supported by experiments showing that purified poliovirus RNA, stripped of the envelope and capsid proteins, was infectious in HeLa cells⁴. Sceptics suggested that the low levels of infectious virus observed could have resulted from contaminating virus in the purified RNA⁵. This spurred the development of improved 'transfection' methods to enhance functional delivery of polynucleotides into cells in tissue culture. Early observations by Alexander and colleagues showed that the low infectivity of viral RNA could be enhanced in the presence of high salt concentrations⁶. Alternative, less-toxic transfection treatments using basic proteins⁷ and later DEAE dextran⁸ enhanced infectivity by about 100-fold and 1,000-fold, respectively. In 1973, it was discovered that a further 10-fold improvement in infectivity of purified viral DNA could be obtained with a calcium-phosphate-DNA co-precipitation procedure⁹, leading to transfection experiments using DNA isolated from the herpes simplex virus that provided further experimental support for the central dogma¹⁰.

The modern biotechnology industry began in the 1970s with the discovery of techniques for taking individual genes from cells and splicing them into plasmids that could be propagated in bacteria. Techniques for synthesizing mRNA were also developed^{11,12}. Transfection technology and the emerging recombinant DNA technology converged when calcium phosphate and DEAE dextran-mediated transfection methodology was applied to the delivery and expression of recombinant plasmids in cultured mammalian cells¹³⁻¹⁹. These advances in genetic engineering led Friedmann to propose making therapeutic recombinant viruses by replacing the genes that make the virus pathogenic and adding a beneficial therapeutic gene. The 1972 paper entitled "Gene therapy for human genetic disease?" is often cited as an early statement of the potential therapeutic applications of introducing functional genes into mammalian cells using viral vectors²⁰. This unleashed enormous efforts to develop approaches that enable delivery of genetic information to the interior of target cells to practice gene therapy, with the intention of introducing or correcting genes within human cells to treat diseases.

Initial demonstrations of functional *in vivo* gene delivery showed that injection of 'naked' plasmids containing viral genomes cloned into eukaryotic expression vectors could lead to infection. In 1979, polyomavirus plasmid DNA (pDNA) was demonstrated to be infectious in mice and hamsters following parenteral administration²¹. Similar outcomes were later reported in mice injected in various tissues with plasmid containing polyoma virus²². In 1982, it was reported that plasmids expressing infectious hepatitis B virus replicated in primates²³. Plasmids containing ground squirrel hepatitis virus DNA could establish infection that mimicked infection by intact virus²⁴. In 1986, a recombinant plasmid encoding chloramphenicol acetyltransferase under control of eukaryotic promoters was used to demonstrate chloramphenicol acetyltransferase activity in the liver and spleen of rats following intraperitoneal administration, establishing the potential for production of therapeutic proteins²⁵. This was followed by a demonstration that the effects of the Rous sarcoma virus,

which induces tumours upon administration in chickens, could be mimicked by a eukaryotic expression plasmid encoding the *v-src* gene, resulting in identical tumours²⁶. Some animals with regressed tumours developed protective antitumour immune responses, foreshadowing nucleic acid vaccines²⁷. However, the clinical use of naked plasmid gene delivery approaches has been limited, owing to inefficient transfection and concerns related to random spontaneous integration of DNA into the human genome among other problems²⁸.

Parallel efforts to use viral vectors to enable gene therapies were frequently linked to an immune response, which often produced antibodies against the protein product²⁹ and the vector itself. Although problematic for somatic gene therapy, this observation led to the development of vaccines based on viral vectors. Recombinant viruses can be engineered to express vaccine antigens instead of pathogenic genes, thereby stimulating an immune response against the target antigens. In 1984, a recombinant vaccinia virus expressing the rabies virus glycoprotein was developed³⁰, leading to a variety of recombinant virus vaccines³¹. Efforts to develop vaccines using non-viral approaches began in 1989 using a plasmid to express the HIV protein gp120 (ref. 32). Animals injected intramuscularly with this plasmid developed anti-HIV gp120 antibodies and cellular immunity. A subsequent collaboration between Vical and Merck demonstrated that DNA vaccination could induce a protective immune response against influenza³³. This development led to the understanding that nucleic acid vaccines can be administered repeatedly for different proteins, offering protection against various diseases without the vector-related immune response.

Nucleic acid vaccines have important advantages compared to classic vaccines. There are two pathways for antigen processing³⁴⁻³⁶. Exogenous proteins, such as those present in conventional vaccine formulations, are captured by specialized antigen-presenting cells (APCs) including B cells, macrophages, and dendritic cells. These proteins are then processed and presented on MHC Class II molecules, leading to the activation of CD4⁺ helper T cells and the stimulation of humoral immunity. By contrast, proteins synthesized within a cell are broken down and presented on MHC class I molecules, a separate process that activates CD8⁺ cytotoxic T cells, thus stimulating cellular immunity and enhancing the cytotoxic response to viral proteins. This provides potential control over chronic or latent viral infections and cancer. Consequently, nucleic acid vaccination can be effective not only for disease prevention but also for therapeutic interventions^{32,33,37-49}.

The large majority of gene therapies in development today use viral delivery systems such as adeno-associated virus (AAV) vectors⁵⁰. Although considerable progress has been made, issues related to limited genetic capacity, immunogenicity and manufacturing impede progress for viral vectors⁵¹. Further, although issues regarding random integration are reduced for AAV delivery approaches as compared to other viral vectors, concerns remain⁵². Here, we contend that non-viral lipid-based delivery systems such as lipid nanoparticles (LNP), which are now playing an increasing part in enabling nucleic acid-based vaccines and therapeutics, may well become dominant owing to advantages related to safety, tolerability, ability to re-dose, large genetic cargo, ease of design and straightforward manufacturing processes to name but a few. These delivery systems have evolved from two related streams of research. The first involves the discovery of the transfection properties of lipoplexes formed by incubation of small lipid vesicles containing permanently positively charged cationic lipids with nucleic acid polymers. The second research area concerns the development of LNP technology using ionizable cationic lipids that enable encapsulation in acidic environments, wherein the ionizable lipid is positively

charged but adopts a neutral, hydrophobic oil droplet core in the LNP at physiological pH values. The development of LNP technology for nucleic acid delivery is based on insights gained from basic studies on the physical properties and functional roles of lipids in membranes, as well as the enormous efforts made to develop liposomes for delivery of small-molecule drugs, such as anticancer drugs.

In this Perspective article, we trace the evolution of these systems over the past 60 years and suggest that lipid nanoparticle-based systems for nucleic acid delivery, in combination with advances in molecular biology, will enable the practice of most forms of gene therapy.

Liposomes and lipoplexes

Early studies

In 1964 (see Fig. 1 timeline), it was discovered^{53–55} that dispersions of oolecithin (egg yolk phosphatidylcholine) in aqueous media gave rise to multilamellar systems consisting of concentric lipid bilayers encapsulating part of the aqueous medium, supporting the proposal that a fundamental role of lipids in biological membranes was to provide the permeability barrier between internal and external environments. This observation also initiated intensive efforts to characterize the biophysical properties of lipids and their relation to functional roles in membranes. These efforts began with developing ways to generate well-defined liposomal systems that could be used to investigate the molecular dynamics, lateral mobility, permeability, phase transition, fusogenicity and other properties of lipid membrane systems. Note that the term liposome⁵⁶ is generally accepted to connote an aqueous dispersion of nano-sized or micro-sized particles composed of lipids organized in a bilayer structure. A variety of formulation approaches were developed, including sonication to produce small bilayer vesicles

in 1969 (ref. 57), dissolving lipids in ethanol and dispersing in water to produce very small vesicular systems in 1973 (ref. 58), dissolving lipids in ether and injecting into aqueous media to produce unilamellar systems with larger trapped volumes⁵⁹, dissolving the lipids in detergent followed by dialysis⁶⁰ to again form small vesicles, fusing negatively charged liposomes in the presence of Ca²⁺ and then generating large liposomes by addition of EDTA⁶¹, and, then in 1979, extrusion of multilamellar systems through polycarbonate filters to form unilamellar liposomal systems of various sizes^{62,63}.

Substantial effort was invested in generating liposomal systems that contained nucleic acid cargo with the intent of delivering nucleic acid-based drugs into cells. These efforts began with the observation in 1977 that mRNA could be encapsulated in liposomes produced by the ether formulation technique⁶⁴ and the demonstrations in 1978 that liposomally encapsulated mRNA coding for globin could induce expression of globin following incubation with cells *in vitro*^{65,66}. It was also shown that negatively charged liposomes prepared by the Ca²⁺–EDTA procedure could be used to deliver SV40 DNA into cells⁶⁷. In 1982, *in vivo* gene delivery and expression using non-infectious recombinant plasmid DNA was demonstrated, showing that liposomally encapsulated pDNA containing the rat pre-proinsulin gene could be expressed *in vivo* after intravenous administration^{68,69}. The liposomal delivery system used contained PC, the negatively charged lipid phosphatidylserine (PS) and cholesterol, and used the ether formulation process⁵⁹. Although promising, these studies suffered from poor encapsulation efficiencies, low levels of expression and an inability to scale the formulation processes. For example, the trapped volume of liposomes prepared in aqueous media is only a small proportion of the total aqueous volume⁷⁰, so DNA and mRNA encapsulation efficiency

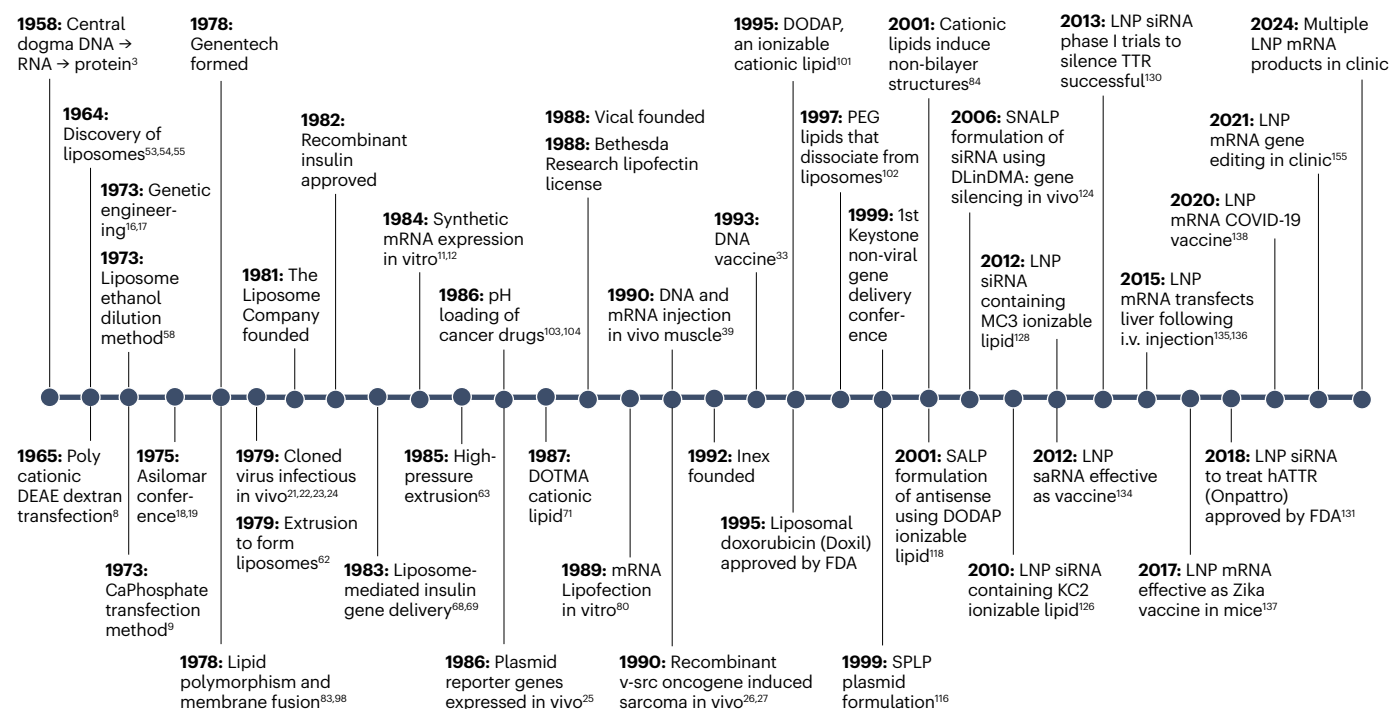


Fig. 1 | Timeline of events leading to LNP-enabled RNA vaccines and therapeutics. These events span over 60 years, are somewhat subjective and reflect the experience of the authors. DEAE, diethylaminoethyl; *i.v.*, intravenous; LNP, lipid nanoparticle; PEG, polyethylene glycol; siRNA, small interfering RNA; SPLP, stabilized plasmid lipid particle; SALP, stabilized antisense lipid particle; SNALP, stabilized nucleic acid lipid particle.

by 'passive' encapsulation processes was low. Furthermore, liposomes with a negatively charged surface repel negatively charged nucleic acids, also inhibiting encapsulation.

Liposomes containing cationic lipids

In 1987, Felgner (at Syntex) hypothesized that positively charged liposomes could provide a way to enhance the encapsulation efficiency of negatively charged nucleic acid polymers in lipid-based systems^{5,71} (Box 1). There are no positively charged bilayer-forming lipids in nature and, at that time, no synthetic positively charged bilayer-forming lipids existed. Drawing on design principles derived from liposome research, a series of cationic lipid molecules were synthesized. A lead example was *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA)⁷¹. When DOTMA is mixed with equimolar levels of 'helper' lipids such as dioleoyl-PC (DOPC) or dioleoylphosphatidylethanolamine (DOPE), dispersed in aqueous media and sonicated,

this leads to the generation of very small, stable, positively charged liposomes. When these liposomes are mixed with pDNA, 'lipoplexes'⁷² of varying sizes and morphology can be formed⁷³. It is of note that the lipoplex structure is sensitive to the phase preferences of the helper lipid used. The helper lipid DOPC adopts a stable bilayer phase when dispersed in aqueous media⁷⁴, whereas DOPE adopts the hexagonal H_{II} phase consisting of hexagonally packed tubes with an aqueous core^{75,76}. When the cationic liposomes contain DOPE, low-angle X-ray scattering and electron microscopy suggest nucleic acid is contained within the aqueous tubes formed by lipids in a disordered hexagonal H_{II} phase⁷⁷ (Fig. 2). Alternatively, when the helper lipid is DOPC, the nucleic acid appears to be sandwiched between concentric lipid bilayers^{37,72,77–79}.

The lipoplex represented a significant encapsulation advance over previous transfection protocols⁷¹ and a potential starting point for constructing transfection competent nanoparticles through rational

Box 1 | Roles of academia and industry in the evolution of LNPs

The journey to develop lipid nanoparticle (LNP) nucleic acid delivery systems spans six decades. This long timeline reflects enormous research and development efforts, both in academia and industry. In 1988, Felgner co-founded and led research efforts at Vical, and in 1992, Cullis co-founded and led research efforts at Inex Pharmaceuticals, resulting in an investment of more than US\$200M in basic research, manufacturing and clinical development that supported the development of nucleic acid-based therapeutics. This investment was complemented by the founding of CureVac in 2000, BioNTech in 2008 and Moderna in 2010, leading to >US\$2B of investment to achieve commercial products. In 2021, the combined market capitalization of biotechnology companies developing LNP mRNA vaccines and therapeutics was in excess of US\$100B. Academic investment through agencies such as the National Institutes of Health (NIH) and the Canadian Institutes for Health Research (CIHR) played a crucial part in very early discovery efforts and continues to have roles in technology development; however, the scale of academic efforts now pales in comparison with efforts in industry. A complex interplay between academia and industry led to the LNP systems that are now enabling a variety of vaccines and gene therapies.

In 1985, early studies on cationic lipids, conducted by Felgner at Syntex Research Inc., resulted in the development of the first cationic lipid reagents for mRNA and plasmid DNA (pDNA) gene transfer. The intellectual property was licensed to Bethesda Research Labs—Thermo Fisher, leading to Lipofectin and Lipofectamine, members of a family of cationic lipid transfection reagents. Syntex opted not to exploit the *in vivo* applications of cationic lipids, leading Felgner to co-found Vical Inc. in 1988. A collaboration with the University of Wisconsin enabled the discovery that injection of pDNA and mRNA into mouse skeletal muscle resulted in high protein expression levels. In a collaboration with Merck, potent antiviral immune responses were discovered to be generated following intramuscular injection of plasmids encoding viral antigens. These findings enabled the development of the class of nucleic acid vaccines.

Early studies on liposomes led by Cullis at University of British Columbia (UBC) resulted in the formation of the Canadian Liposome Company in 1986 and Inex in 1992 and ultimately led to regulatory

approval of three lipid-based formulations of small-molecule drugs, including Abelcet¹⁸³ (approved by the FDA in 1995 to treat fungal infections), Myocet¹⁸⁴ (approved by the European Medical Agency in 2000 to treat metastatic breast cancer) and Marqibo¹⁸⁵ (approved by the FDA in 2012 to treat acute lymphoblastic leukaemia). In 1996, Inex began research on lipid-based formulations of plasmid DNA and antisense oligonucleotides, contributing to the development of the stabilized plasmid lipid particle (SPLP) system¹¹⁶ for delivery of plasmid DNA and the stabilized antisense lipid particle (SALP) system¹¹⁸ for delivery of antisense oligonucleotides using the ionizable cationic lipid DODAP.

In 2000, Cullis catalysed the formation of Protiva BioTherapeutics (a spin-off from Inex) to develop gene therapies. Protiva applied the SALP technology to the delivery of small interfering (siRNA) in a collaboration with Alnylam Pharmaceuticals, developing the ionizable lipid DLinDMA¹²³ and the SNALP formulation for gene silencing¹²⁴. In 2007 Inex rebranded as Tekmira Pharmaceuticals. During the period 2006–2008, Inex–Tekmira, in collaboration with UBC and Alnylam, developed the ionizable lipid DLinKC2DMA¹²⁶ that enhanced the gene silencing potencies of LNP siRNA formulations. Following the merging of Tekmira and Protiva in 2008, Cullis and senior colleagues left Tekmira to co-found ALCana Technologies in 2009 (ALCana later rebranded as Acuitas Therapeutics). ALCana (Acuitas), in collaboration with UBC and Alnylam, discovered DLinMC3DMA (ref. 128), an ionizable lipid that further enhanced the gene silencing potency of LNP siRNA systems. Alnylam took LNP siRNA systems containing DLinMC3DMA and siRNA to silence transthyretin into the clinic, resulting in FDA approval of Onpattro (patisiran) in 2018 to treat the hereditary condition transthyretin-induced amyloidosis¹³⁰. Subsequently, Acuitas applied related LNP technology to mRNA vaccines in collaboration with the University of Pennsylvania¹³⁷. The excellent vaccine responses elicited by LNP mRNA formulations resulted in a collaboration between Acuitas and BioNTech to develop an influenza vaccine. Following the outbreak of the COVID-19 pandemic, the LNP technology of Acuitas using the ionizable cationic lipid ALC0315 was incorporated into the Pfizer–BioNTech COVID-19 mRNA vaccine called BNT162b2 (Comirnaty)¹⁷⁸.

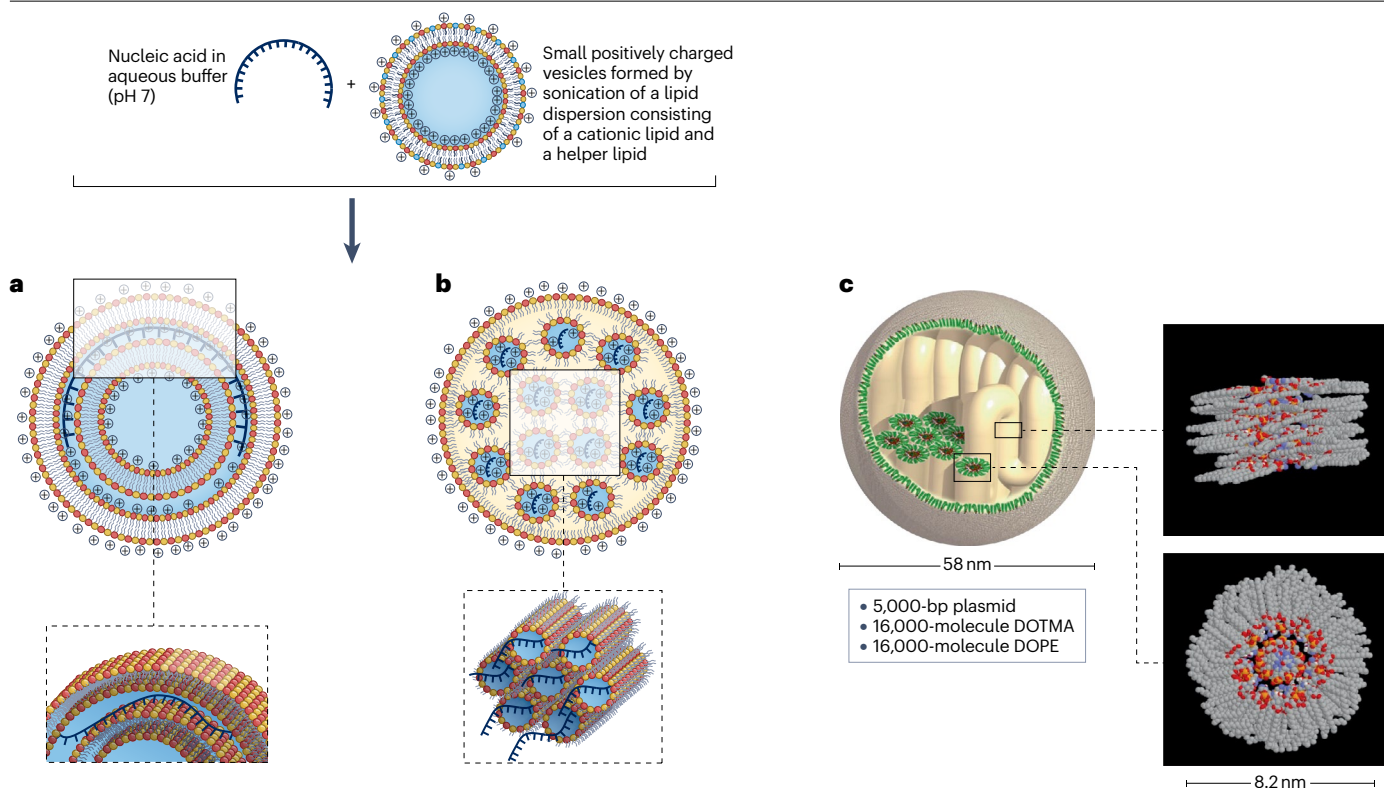


Fig. 2 | Formation and structure of a lipoplex. Nucleic acid polymers (DNA or RNA) are mixed with small (<50 nm) bilayer vesicles formed from mixtures of a cationic lipid and a helper lipid. **a**, If the helper lipid is a bilayer-forming lipid such as DOPC, then stacked bilayers are formed wherein the nucleic acid is trapped between closely apposed bilayers⁷⁸. **b**, If the helper lipid is a lipid such as DOPE that prefers non-bilayer hexagonal H_{II} phase structure, then the

nucleic acid can be contained within the aqueous channels characteristic of this phase⁷⁷. **c**, Optimal lipoplex transfection is achieved when the cationic lipid-to-nucleic acid ratio is >1 and the resulting lipoplex has a net positive charge. A space-filling model shows how a 5-kilobase pair plasmid molecule can be condensed into a disordered hexagonal array under these optimal transfection conditions^{79,82}. bp, base pair.

design, such as the incorporation of targeting elements (for example, cell surface receptor ligands or antibodies) to enhance affinity for target cells. Surprisingly, however, lipoplexes could efficiently transfect pDNA and mRNA into cultured cells without adding additional functional groups^{71,80,81}. This led to the synthesis and screening of numerous cationic lipid molecules and formulations thereof to optimize in vitro transfection activity⁸². Studies have revealed that optimized lipoplexes could deliver up to a million plasmids per cell, resulting in up to 300 plasmids in the nuclear fraction⁵. Interestingly, the transfection efficiency was improved by incorporation of equimolar (with respect to the cationic lipid) levels of the H_{II} phase preferring lipid DOPE in the cationic liposomes. This is consistent with the role of ‘non-bilayer’ lipids such as DOPE in membrane fusion events⁸³, and probably related to enhancing fusion events between lipoplexes and endosomal membranes, leading to cytoplasmic delivery of the nucleic acid cargo⁸⁴. Conversely, inclusion of the bilayer-preferring lipid DOPC inhibited transfection^{71,82,85–87}. Today, DOTMA–DOPE transfection reagents have widespread use in molecular biology laboratories and have established the utility of non-viral lipid-based systems as a leading technology in efforts to practice gene therapies^{82,88–94}.

The development of in vivo gene delivery applications of cationic lipid formulations was pursued by Vical (Box 1). Initial studies have characterized gene expression from lipoplex DNA and mRNA after

direct injection into mouse tissue including muscle^{32,39}. High gene expression levels in skeletal muscle were observed. Remarkably, even the ‘naked DNA’ control group without cationic lipids exhibited gene expression levels on par with those containing cationic lipids. This unexpected finding established Vical as a leader in the field of naked DNA gene therapy and vaccines^{32,37–39}. The substantial transfection levels observed in the absence of cationic lipids were later attributed to pressure-induced effects that enable macromolecules to pass through temporary disruptions in muscle cell membranes^{95–97}.

Development of lipid nanoparticle delivery systems

Early studies

The evolution of lipid nanoparticle (LNP) systems that contain a hydrophobic core of neutral ionizable lipid and polar regions containing nucleic acid cargo was built on an understanding of bilayer liposomal systems, but also relied heavily on basic research studies on lipid polymorphism and lipid asymmetry, as well as experience gained from developing liposomal formulations of anticancer drugs. Lipid polymorphism refers to the intriguing finding that a high proportion of the lipids in biological membranes will preferentially adopt non-bilayer structures such as the hexagonal H_{II} phase when placed in an aqueous medium⁸³. The reasons why lipids adopt such

structures and the functional roles they have in membrane-mediated phenomena became a major topic of membrane research. In 1978, Cullis and Hope proposed that non-bilayer lipids have a direct role in providing the intermediary structures required for membrane fusion⁹⁸. The reason lipids adopt non-bilayer structures was suggested to arise from their 'shape' properties, wherein lipids preferring a bilayer structure have a cylindrical shape, whereas lipids that adopt structures such as the H_{II} phase exhibit a cone shape where the area subtended by the acyl chains is somewhat larger than the area subtended by the headgroup⁸³. In 2001, it was found that mixing cationic lipids with the negatively charged bilayer-forming lipids found in biological membranes led to formation of H_{II} phase structures⁸⁴. This finding is consistent with the ability of cationic lipids to disrupt bilayer structures in biological membranes to achieve intracellular delivery of nucleic acid cargos, and was attributed to induction of pronounced cone shape lipid structures resulting from electrostatic interactions between the positively and negatively charged headgroups⁸⁴. Such concepts proved useful for the rational design of ionizable cationic lipids.

Studies on lipid asymmetry also gave rise to insights and tools essential to the development of LNP nucleic acid delivery systems. These studies, which were initiated to determine the consequences of the asymmetric transbilayer distributions of lipids observed in biological membranes⁹⁹, began in 1987 and were initially directed towards showing that pH gradients could modulate the transbilayer distributions of weak-base and weak-acid lipids in lipid vesicles¹⁰⁰. Subsequent work focused on understanding factors affecting membrane fusion between bilayer vesicle systems. In 1994, it was shown that inclusion of the weak-base ionizable cationic lipid ALI (1,2-dioleoyl-3-dimethylammonium propane, later called DODAP) resulted in generation of lipid asymmetry in unilamellar vesicles in response to pH gradients and could be used to modulate membrane fusion depending on its location on the inner or outer monolayer¹⁰¹. Subsequent work focused on the effect of PEG lipids on membrane fusion of phosphatidylethanolamine (PE)–PS vesicles induced by Ca^{2+} and showed that fusion was inhibited by the presence of a PEG lipid coating. However, if a short-chain (C_{14}) version of the PEG lipid was used, then it could dissociate from the PE–PS vesicle allowing fusion to proceed¹⁰². Both ionizable cationic lipids and PEG lipids that dissociate from lipid nanoparticles later proved essential for the development of effective LNP siRNA and mRNA delivery systems.

Liposomal delivery of small-molecule drugs

Insight gained from the development of liposomal systems for delivery of small-molecule drugs has provided considerable guidance for the design of LNPs for gene therapy. Studies aimed at developing such systems to more accurately deliver small molecules (particularly cancer drugs) to disease sites began in earnest in 1986 with the development of scalable procedures for making and loading liposomes with drugs. The extrusion technique for formulating liposomal systems with a diameter of 100 nm or less went through two stages – first, the demonstration that forcing large multilamellar liposomes sequentially through polycarbonate filters with defined pore size could produce smaller bilayer systems⁶² and the subsequent demonstration⁶³ in 1985 that this process was amenable to scaleup by using high pressures to rapidly extrude the multilamellar systems directly through polycarbonate filters with a pore size of 100 nm or less. The following year, the readily scalable pH 'remote loading' technique for loading weak-base drugs^{103,104} into liposomes was discovered, followed by the

development of a popular version of the pH loading technique relying on encapsulation of ammonium sulphate¹⁰⁵.

The advent of scalable processes for both formulating and loading liposomal systems, coupled with the observations that a PEG coating endowed liposomal systems with long circulation half-lives¹⁰⁶ that enabled preferential distribution to tumour sites¹⁰⁷, triggered intense efforts to develop liposomal formulations of anticancer drugs with clinical applications. As noted in many reviews¹⁰⁸, this has led to more than ten liposomal products that have been approved by the US Food and Drug Administration (FDA), the European Medical Agency (EMA) and many other jurisdictions worldwide. These systems primarily contain anticancer drugs and have the benefits of reduced toxicity and enhanced efficacy compared to administration of the 'free' form of the drug. The main lessons learned from this enormous body of work is that to achieve these benefits, the liposomal systems must be small (<100 nm diameter), long circulating to reach extra-hepatic tissues (circulation half-life of 10 h or more in mice, leading to half-lives in excess of 24 h in the clinic^{109,110}), and composed of lipids such as hydrogenated soy PC, distearoyl PC (DSPC) or sphingomyelin¹¹¹ that promote long circulation half-lives and have optimized retention-payout properties for encapsulated drugs in vivo. Further, the encapsulation process must allow trapping efficiencies approaching 100% with high drug-to-lipid ratios.

Stabilized plasmid lipid particles

In the mid-1990s, collaborative efforts conducted at Inex Pharmaceuticals and UBC (Box 1) tackled the problem of developing lipid-based formulations of nucleic acid delivery systems that could exhibit the long circulation half-lives required to access sites of disease such as tumours following intravenous (i.v.) injection. Lipid-based systems containing permanently charged cationic lipids were not ideal for such in vivo applications as positively charged liposomes activate complement^{112,113} and are rapidly cleared from the circulation following i.v. administration¹¹⁴. Experience with liposomes for delivery of small-molecule drugs had established that liposomes with little or no surface charge can exhibit extended circulation half-lives¹¹⁵. Thus, efforts were made to develop lipid-based systems that either used very small amounts of permanently cationic lipids to entrap nucleic acid polymers, or to develop new entrapment procedures that allowed production of a net neutral delivery system at physiological pH values. In 1999, the first such system was developed, with the demonstration that pDNA could be encapsulated into lipid-based systems called stabilized plasmid lipid particles (SPLP) containing PC or PE/cholesterol/dioleoyldimethylammonium chloride (DODAC)/PEG lipid¹¹⁶, using small amounts of the cationic lipid DODAC (~6 mol%) in a detergent dialysis procedure. The presence of the PEG lipid was necessary to prevent aggregation during formulation. Subsequent studies have shown that intravenously delivered SPLP exhibited dramatically longer circulation lifetimes and reduced toxicity compared to complexes and could transfect distal tumours¹¹⁷. Notably, it was found that formulations of SPLP containing short-chain (C_{14}) PEG lipids that can rapidly dissociate from lipid-based systems could modulate their transfection properties¹¹⁷. The in vitro transfection potency of SPLP formulated with PEG-Cer (C_{14}) was significantly better than SPLP formulated with PEG-Cer (C_{20}), a feature attributed to the ability of the PEG-Cer (C_{14}) to dissociate from the SPLP in the presence of acceptor membranes or vesicles¹¹⁶. A fundamental difficulty remained, however, in that the detergent dialysis formulation process was not scalable.

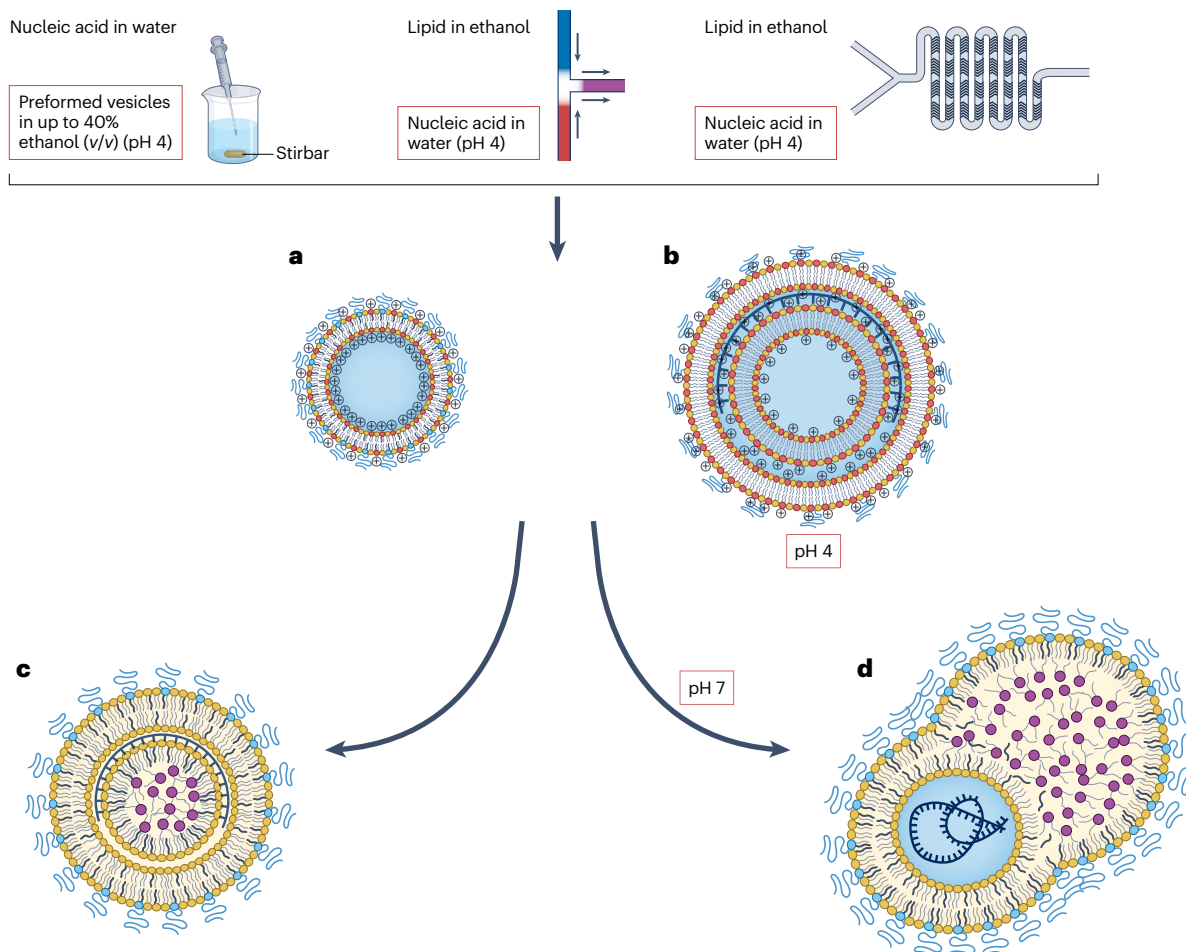


Fig. 3 | Formation of lipid nanoparticles. A variety of mixing techniques can be used to produce LNP systems containing ionizable cationic lipids, helper lipids and nucleic acid polymers. The preformed vesicle approach¹¹⁹ involves generating vesicles at pH 4 and then mixing with nucleic acid at pH 4 in the presence of up to 40% ethanol (*v/v*). Alternatively a T-tube mixer¹²² or a microfluidic mixer¹⁷⁹ can be used to mix lipids in ethanol directly with nucleic acid in a pH 4 buffer. All these approaches rely on an initial formation of small positively charged vesicles (part **a**) that interact with nucleic acid polymers (for example, siRNA, mRNA and plasmid DNA) contained within the pH 4 buffer to form complexes (part **b**). These complexes or lipoplexes are structured systems wherein the lipid–nucleic acid complex adopts organizations such as

multilamellar structures with the nucleic acid cargo contained between stacked bilayers⁷⁸. Aggregation is prevented by the presence of a small (<10 mol%) proportion of PEG lipid in the lipid mixture. On raising the pH to pH 7.4 by dialysis against PBS, the ionizable cationic lipid is converted to the neutral form and phase separates to form an amorphous oil droplet in the interior of the LNP. The morphology of the LNP is sensitive to the ionizable cationic lipid used and whether fusion is promoted by raising the pH (part **c**) or by formulating in media of high ionic strength (for example, 300 mM NaAc) at pH 4 to result in bleb structures (part **d**)¹⁸⁰. The lipid nanoparticle has a near-neutral charge at physiological pH values.

Stabilized antisense lipid particles

The next advance came in 2001 with demonstrations that antisense oligonucleotides could be encapsulated into lipid-based systems using the ionizable cationic lipids originally developed for the lipid asymmetry studies noted in section ‘Early studies’^{118,119}. Briefly, it was reasoned that if nucleic acid polymers were mixed with liposomes containing ionizable cationic lipids such as DODAP at pH values below the pK_a of the ionizable lipid, then they should become associated with those liposomes owing to the positive charge on the ionizable lipid. Previous work had established DSPC–cholesterol liposomes as robust delivery systems for small-molecule drugs¹²⁰ and that the pK_a of DODAP was 6.8 (ref. 101), thus the encapsulation properties of

DODAP–DSPC–cholesterol lipid dispersions in a pH 4 buffer were examined. PEG lipids were also included to prevent large scale aggregation/fusion of vesicles in the presence of the nucleic acids. This work went through two stages. First, it was shown that, for DODAP contents of up to 30 mol%, encapsulation efficiencies of up to 90% could be achieved when the lipid dissolved in ethanol was added to oligonucleotides in an aqueous solution of citrate buffer (pH 4) containing up to 40% by volume of ethanol, followed by an extrusion process¹¹⁸. The second stage demonstrated equally good encapsulation properties using a (potentially scalable) preformed vesicle approach (see Fig. 3), wherein oligonucleotides dissolved in aqueous media were added to an ethanolic solution (up to 40% ethanol *v/v*) of preformed vesicles formed

by dispersion of lipids in a citrate buffer (pH 4) and subsequent extrusion¹¹⁹. It was found that the extrusion step could be omitted under conditions of ‘vigorous mixing’ of the lipids in ethanol with the citrate buffer¹¹⁹. These stabilized antisense lipid particles (SALP) exhibited long circulation half-lives following i.v. administration and reduced toxicity compared to complexes¹¹⁸.

These advances were followed by studies at Protiva Biotherapeutics (see Box 1) using a T-tube mixer originally used to formulate lipoplexes¹²¹ to perform the vigorous mixing of DSPC/cholesterol/1,2-dioleoyloxy-3-dimethylaminopropane (DODMA)/PEG lipid dissolved in ethanol with a pH 4 buffer containing pDNA¹²². The rapid T-tube mixing produces the small positively charged vesicles⁵⁸ that then associate with the negatively charged DNA, enabling an extrusion-free, more scalable approach (see Fig. 3). DODMA is a version of DODAP wherein ether links are substituted for ester links to enhance stability. These SPLP systems also exhibited long circulation half-lives and were able to transfect distal tumours following i.v. administration¹²². In 2005, to enhance the probability of fusion of the lipid carrier with the endosomal membrane following uptake into a target cell, the observation that cationic lipids combine with endogenous anionic lipid to induce the membrane disruptive H_{II} phase⁸⁴ was harnessed to design the ionizable cationic lipid 1,2-dilinoleoyloxy-*N,N*-dimethyl-3-aminopropane (DLinDMA), wherein linolenic acyl chains were incorporated to enhance the ‘cone’ shape of the lipid when in the positively charged form¹²³. Building on these advances, Protiva, in collaboration with Alnylam Pharmaceuticals, showed that siRNA designed to silence ApoB could be encapsulated into a version of SALP called stabilized nucleic acid lipid particles (SNALP)¹²⁴. Encouraging reductions in ApoB were observed in mice and non-human primates (NHPs) following i.v. administration¹²⁴, however, the potency or therapeutic index of the formulation was insufficient for clinical development¹²⁵.

Lipid nanoparticles and nucleic acid delivery

Collaborative work between UBC, Alnylam, Tekmira and subsequently Acuitas Therapeutics continued (Box 1) and, in 2010, it was reported that the gene silencing potency of LNP formulations of siRNA could be significantly improved by optimizing the headgroup of the ionizable lipid component¹²⁶. By this time formulations such as SPLP, SALP and SNALP were being described as part of a family termed LNPs¹²⁶. Note these systems could not be called liposomes as they can exhibit a nanostructured hydrophobic interior¹²⁷. Improvements in potency were again rationalized on the hypothesis that when ionizable cationic lipids become protonated in the lower pH environment of endosomes following uptake into hepatocytes, they combine with endogenous anionic lipids to engender fusion through formation of membrane disruptive non-bilayer intermediates⁸⁴, thus enabling nucleic acid cargo release into the cytoplasm (see Fig. 4). Although there is little direct evidence to support this hypothesis, it has proven to be a most productive guide for the rational design of ionizable lipids. DLinKC2DMA (2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane) was identified as a more potent ionizable lipid when incorporated into LNP containing siRNA to silence Factor VII (FVII) in the liver of rodents and NHPs following i.v. administration¹²⁶. This finding triggered an intensive campaign to identify increasingly active ionizable lipids. In 2012, DLinMC3DMA (4-(*N,N*-dimethylamino)butyric acid (dilinoleyl) methyl ester) was identified as the lead ionizable lipid and the p*K*_a of the ionizable lipids was shown to be a critical determinant of LNP activity, with the most active lipids exhibiting a p*K*_a of 6.4 (ref. 128). The following year, phase I clinical trials on healthy volunteers showed

that LNP siRNA systems containing DLinMC3DMA and siRNA to silence transthyretin in liver gave rise to rapid and robust down-regulation of transthyretin in the circulation¹²⁹. Subsequent phase III clinical trials achieved excellent results in treating the condition transthyretin-induced amyloidosis (hATTR)¹³⁰, resulting in FDA approval of Onpatro (patisiran) in 2018 (ref. 131). Onpatro has now been administered to some patients for as long as 10 years. No adverse events have been reported as owing to the cumulative effects of the high doses (0.3 mg siRNA kg⁻¹ every 3 weeks) of LNP siRNA used, with correspondingly high doses of the relatively non-biodegradable¹³² ionizable cationic lipid DLinMC3DMA.

Throughout this development process, the ratios of the lipids used in the LNP changed. Initial formulations of SPLP/SALP contained 10 mol% PEG lipid, for example^{116,118}, decreasing to 1.5 mol% for the formulation¹²⁸ that eventually was used for Onpatro. Similarly, the amount of ionizable lipid varied from 25 mol% for SALP¹¹⁸ to 40 mol% for SNALP¹²⁴ and to 50 mol% for the Onpatro formulation¹²⁸. It is interesting to note that the ‘best’ LNP composition is still a matter of contention; formulations containing 33 mol% ionizable lipid can give rise to excellent transfection properties for LNP mRNA systems both in vitro and in vivo¹³³.

Remarkably, the encapsulation processes and lipid compositions used for encapsulating and delivering siRNA could also be applied to much larger RNA molecules. This was first demonstrated in 2012 by the finding that LNP developed for siRNA delivery (containing DLinDMA) could also be used to encapsulate self-amplifying RNA (saRNA) coding for the F-protein of respiratory syncytial virus¹³⁴. When injected intramuscularly in mice, potent and protective immune responses were observed. This was followed by studies in 2015 showing that mRNA coding for erythropoietin could be encapsulated in LNP systems and that these LNP mRNA systems could transfect the liver following i.v. administration, leading to high levels of erythropoietin in the circulation in a pig model¹³⁵. A detailed mechanism of action for LNP mRNA-mediated transfection in vivo is shown in Fig. 4 and explained in Box 2. As shown, strong circumstantial evidence supports the role of the ionizable cationic lipid in inducing disruption–fusion between the LNP and the endosomal membrane following uptake. These LNP mRNA systems are able to transfect a variety of tissues (including immune cells) in vivo as further collaborative work involving Acuitas and the University of Pennsylvania revealed that LNP mRNA systems could transfect tissues via a variety of routes (i.v., intradermal (i.d.), subcutaneous (s.c.) and intramuscular (i.m.))¹³⁶. Later efforts, reported in 2017, demonstrated that LNP mRNA coding for viral protein and administered i.d. provided excellent vaccine protection against Zika virus¹³⁷. This led to a collaboration between Acuitas and BioNTech to develop an LNP mRNA influenza vaccine. In early 2020, all efforts switched to developing a vaccine for COVID-19, leading to incorporation of Acuitas’ LNP technology into the Pfizer/BioNTech COVID-19 mRNA vaccine known as Comirnaty¹³⁸.

Impact of current LNP mRNA vaccines and therapeutics. By the end of the first year of the COVID outbreak, analysis of SARS-CoV-2 seropositivity in blood specimens collected from different locations in Orange County, California, varied from 10% to 26% depending on demographics of the area where the samples were collected¹³⁹. These results predicted that it would take several years for herd immunity to reach the 80% threshold and reduce the public health consequences of the infectious disease. Following the approval of COVID-19 mRNA vaccines (Comirnaty–Pfizer–BioNTech and Spikevax–Moderna) in December 2020, the UCI Medical Center vaccinated a cohort of

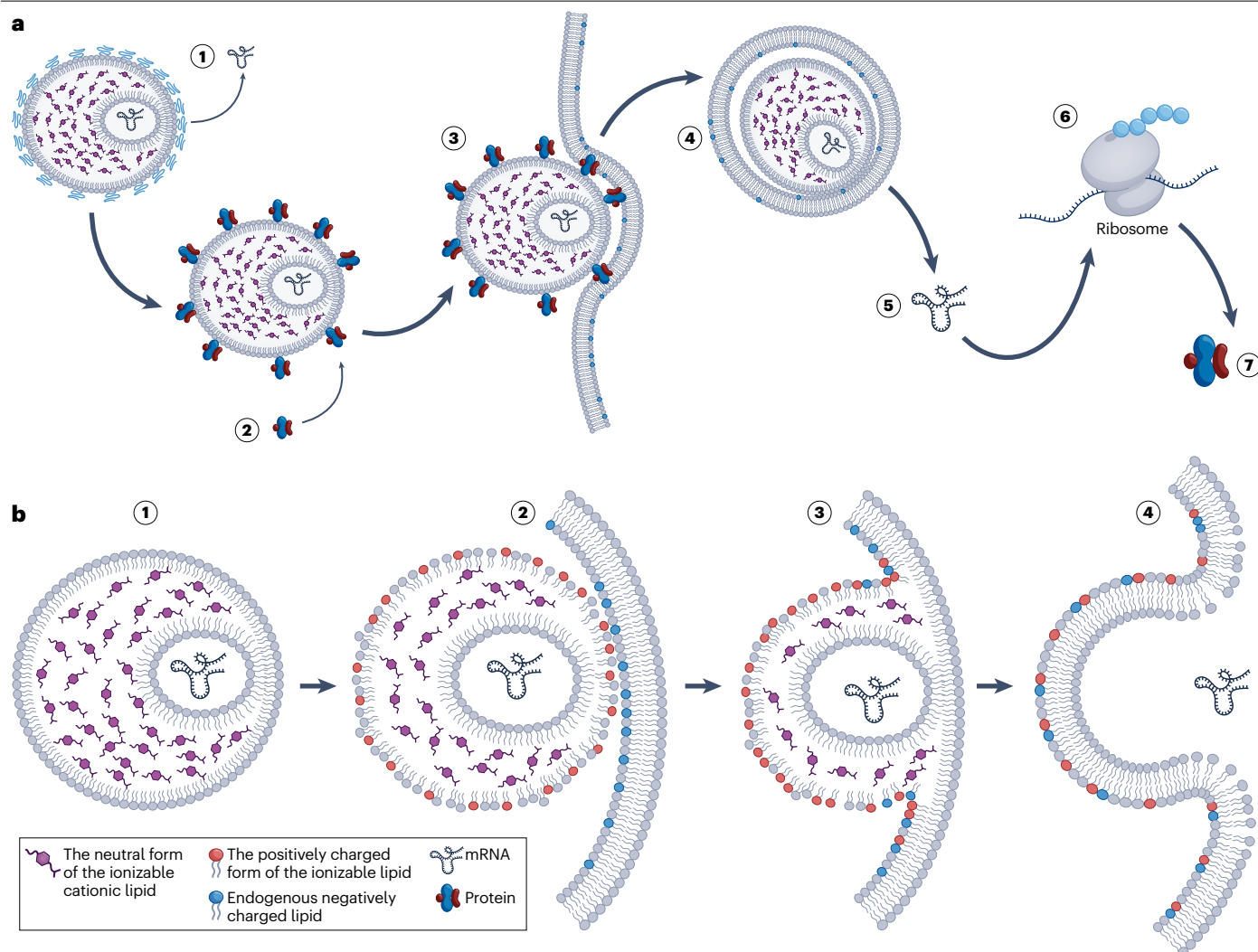


Fig. 4 | Mechanism of action of LNP mRNA delivery systems. a, An integrated model of lipid nanoparticle (LNP)-mediated delivery of mRNA to target cells in vivo. Key steps include (1) the dissociation of PEG lipids from the particle surface¹⁸¹, (2) recruitment of proteins such as endogenous ApoE¹⁸² to the LNP surface causing uptake (3) into target cells via binding to cell surface receptors such as low density lipoprotein receptors, (4) internalization of LNPs via endocytosis, (5) interaction of the protonated ionizable lipid with negatively charged endogenous lipids to cause fusion or destabilization on contact with the endosomal membrane, leading to release of mRNA into the cell cytoplasm wherein it can engage (6) with the ribosome machinery to produce (7) therapeutic protein. **b**, Detailed depiction of the proposed interaction between the lipid nanoparticle containing the mRNA and ionizable cationic lipid with endosomal membranes, leading to release of mRNA from the endosome

and subsequent transfection: (1) at neutral pH, the ionizable cationic lipid is in the neutral form and comprises an amorphous oil droplet in the LNP; (2) as the pH is lowered following uptake via endocytosis, an increasing amount of the ionizable lipid is converted to the positively charged form on arrival in the outer monolayer of lipid surrounding the LNP (note the neutral form is highly membrane permeable)¹⁰¹; (3) increasing amounts of positively charged lipids on the LNP surface leads to close association with the negatively charged inner monolayer of the endosomal membrane and hemi-fusion between the LNP membrane and the endosomal membrane; (4) hemi-fusion progresses to complete fusion and release of the mRNA into the cytoplasm. The symbol keys respectively represent (i) the neutral form of the ionizable cationic lipid, (ii) the positively charged form of the ionizable lipid, (iii) endogenous negatively charged lipid, (iv) mRNA and (v) protein.

6,000 healthcare workers. A month later, immunity in the vaccinated cohort was at 78%, rising to >95% following the boost vaccination¹³⁹. These studies have shown the spectacular immune response induced by the LNP mRNA vaccines that are continuing to be deployed today.

The world-changing impact of the LNP COVID-19 mRNA vaccines, which are estimated to have saved nearly 10 million lives in 2021

alone¹⁴⁰, is increasingly dwarfed by the potential clinical applications of other RNA-based therapeutics relying on LNP delivery systems. Many of these are enabled by the currently available LNP delivery technologies for vaccines using i.m. injection and therapeutics relying on liver (hepatocyte) transfection following i.v. administration. The intense interest in these therapeutics has led to hundreds of mRNA therapeutics (in addition to many COVID-19 vaccines) that are now in

preclinical or clinical development¹⁴¹. Over 60 vaccines and therapeutics using LNP delivery technology are now approved or currently in clinical development, excluding ongoing COVID-19 vaccine trials, with 30 having progressed beyond phase I (Table 1). Vaccine opportunities are being developed for infectious diseases ranging from herpes¹⁴² to influenza¹⁴³, to HIV¹⁴⁴ and to many forms of cancer^{145,146}. Further, using currently available LNP mRNA systems, the liver can be used as a bioreactor to secrete any protein desired into the circulation¹³⁵. Repeated injection of LNP mRNA systems to produce these secreted proteins appears possible, with equally strong expression obtained for each dose¹⁴⁷⁻¹⁴⁹. The revolutionary potential to eliminate the need for biomanufacturing plants for biologics and to produce highly personalized protein therapeutics such as mAb and protein replacement therapeutics in real time is clear.

Presently available LNP technology relying on hepatocyte transfection is being used for a broad range of diseases that can be treated using liver targets. These range from cardiovascular disease^{150,151}, liver fibrosis¹⁵² and rare diseases such as propionic acidemia¹⁵³. LNP-enabled gene editing approaches to disable pathogenic genes in the liver have considerable potential. A treatment to prevent atherosclerosis by LNP-mediated base editing of PCSK9 has been demonstrated clinically, albeit with some safety concerns¹⁵⁴, suggesting that improvement in the therapeutic index of LNP mRNA delivery may be required before widespread clinical application. The clinical potential of LNP mRNA CRISPR gene editing of transthyretin to treat hATTR amyloidosis has also been demonstrated¹⁵⁵.

Future prospects

The future of LNP-based nucleic acid therapeutics will build on advances in two rapidly progressing research areas: (1) the development of increasingly sophisticated LNP systems that enable delivery to

extrahepatic tissues following i.v. administration or direct injection into privileged organs such as the eye and brain, and (2) the progress being made in molecular biology to allow increasingly precise manipulation of delivered mRNA and genomic DNA. Given the ability of LNP systems containing cationic lipids to encapsulate and deliver essentially any size of genetic cargo, these advances can potentially be rapidly translated into therapeutics.

With regard to the development of LNPs capable of accessing a variety of tissues, recent studies have demonstrated an ability to transfect T cells¹⁵⁶, hematopoietic stem cells (HSCs) in bone marrow¹⁵⁷, endothelial cells^{158,159} and lung¹⁵⁸, heart¹⁶⁰, kidney¹⁶¹, pancreas¹⁶² and skin tissues¹⁶³ following i.v. administration. In utero, delivery results in transfection of fetal organs and tissues¹⁶⁴. The development of long-circulating LNP mRNA systems that can potentially transfect most tissues in the body following i.v. administration is possible¹³³. Direct injection of LNP RNA systems into the eye¹⁶⁵ and brain¹⁶⁶⁻¹⁶⁸ are increasingly enabling delivery to a variety of tissues in these organs. As delivery systems are perfected for each organ and associated tissues, a plethora of new therapeutic opportunities will arise. For example, transfection of HSCs in bone marrow transfection could allow new treatments for diseases ranging from sickle cell anaemia to leukaemias, lymphomas, anaemias and immuno-deficiencies, to name but a few¹⁶⁹. Alternatively, transfection of T cells in vivo could enable a host of CAR-T cell therapeutics to treat leukaemia, lymphoma and myeloma, heart disease¹⁵⁶ and diseases of ageing, such as senescence-associated disease¹⁷⁰, in addition to dramatically lowering the cost and increasing the availability of the currently available ex vivo procedure.

The advances in molecular biology for gene editing match the advances in LNP delivery technology. Recent years have seen the progression from zinc fingers¹⁷¹ to TALEN¹⁷², to CRISPR Cas9 (ref. 173), to base editing^{171,174} and to prime editing^{175,176}. Alternatively, epigenome

Box 2 | LNP formation and mechanism of action for nucleic acid delivery

LNP systems containing ionizable lipids are clearly able to encapsulate nucleic acids and deliver them into cells in vivo. Although many details remain to be elucidated, a broad framework describing the mechanisms of LNP assembly and intracellular delivery of nucleic acid cargos has evolved that relies on the remarkable self-assembly and polymorphic properties of membrane lipids when dispersed in water. Methods of formulating LNP RNA or DNA systems are summarized in Fig. 3 and are variants of the pre-formed vesicle approach wherein the pre-formed vesicles are formed by extrusion¹¹⁸ or by rapidly dispersing lipids in ethanol into an aqueous environment. When 'bilayer' lipids in ethanol are mixed rapidly with aqueous media, they form small bilayer vesicles⁵⁸. If the lipid dispersion contains ionizable cationic lipids that adopt a bilayer structure when in the positively charged form, as well as bilayer lipids such as DSPC and cholesterol, small positively charged bilayer vesicles form when the aqueous medium has a pH well below the pK_a of the ionizable lipid¹¹⁹. These vesicles can combine with nucleic acid polymers to form condensed 'lipoplex' structures^{90,118}. The size of these structures can be controlled by inclusion of PEG lipids^{118,119}. As the pH is raised from pH 4 to pH 7.4, the ionizable lipids adopt a neutral, deprotonated form, inducing inter-vesicle fusion and phase separation of the neutral form of the ionizable lipid into hydrophobic domains in

the interior of the LNP¹⁸⁶. The nucleic acid cargo migrates to polar regions surrounded by monolayers and bilayers largely consisting of the helper lipid¹⁸⁷. Note that if fusion is induced at pH 4 by the presence of high concentrations of buffers such as Na-citrate, production of 'bleb' structures (see Fig. 3) containing nucleic acid can be favoured¹⁸⁰.

The processes associated with in vivo delivery of nucleic acid cargos are described in Fig. 4. Following administration, the PEG lipid coating, which inhibits cell uptake¹¹⁶, dissociates from the LNP exterior¹⁸¹ to render the LNP transfection competent. This is followed by adsorption of serum proteins. In the case of LNP with the 'Onpatro' lipid composition (ionizable cationic lipid, cholesterol, PEG lipid; 50/10/38.5/1.5, mol/mol), adsorption of the lipoprotein Apo-E triggers uptake into cells, particularly hepatocytes, via the LDL and scavenger receptor pathways¹⁸². Following uptake into target cells, when the LNP is in the endosome, the ionizable lipid progressively adopts the positively charged bilayer form in the low-pH environment of the endosome. Interaction between the positively charged LNP membrane and the negatively charged endosomal membrane is then suggested to trigger fusion and cytoplasmic delivery of the nucleic acid¹⁸⁴. As an extra, unexpected, benefit, the presence of the ionizable lipid in vaccine applications can act as a very favourable adjuvant¹⁸⁸.

Table 1 | Selected LNP-enabled RNA vaccines and therapeutics that have progressed beyond phase I clinical development

Drug or vaccine name	Developer(s)	Disease indication	Therapeutic class/strategy	Highest development stage	Trial identifier
Comirnaty (tozinameran)	BioNTech, Pfizer, Acuitas	SARS-CoV-2	Viral vaccine	Approved	N/A
SpikeVax (elasomeran)	Moderna	SARS-CoV-2	Viral vaccine	Approved	N/A
Onpatro (patisiran)	Alnylam, Inex/Tekmira, Acuitas	Transthyretin amyloidosis	Gene silencing	Approved	N/A
mRNA-4157	MSD and Moderna	Melanoma	Cancer vaccine	III	NCT05933577
MK-3475	MSD and Moderna	Non-small cell lung cancer	Cancer vaccine	III	NCT06077760
mRNA-1273	GlaxoSmith-Kline	Herpes zoster	Viral vaccine	III	NCT05047770
mRNA-1647	Moderna	Cytomegalovirus	Viral vaccine	III	NCT05085366
mRNA-1345	Moderna	Respiratory syncytial virus	Viral vaccine	III	NCT05330975
mRNA-1010	Moderna	Influenza	Viral vaccine	III	NCT05415462
qIRV	Pfizer	Influenza	Viral vaccine	III	NCT05540522
ARCT-810-03	Arcturus	OTC deficiency	Protein replacement	II	NCT05526066
AZD8601	AstraZeneca	Heart failure	Protein expression	II	NCT03370887
BMS-986263	BMS	Liver cirrhosis; liver fibrosis	Gene silencing	II	NCT03420768
BMS-986263	BMS	Liver cirrhosis; NASH	Gene silencing	II	NCT04267393
mRNA-4157	Moderna	Melanoma	Cancer vaccine	II	NCT03897881
mRNA-1647	Moderna	Cytomegalovirus	Viral vaccine	II	NCT04232280
mRNA-1893	Moderna	Zika virus	Viral vaccine	II	NCT04917861
mRNA-1345	Moderna	Respiratory syncytial virus	Viral vaccine	II	NCT06097299
ND-L02-s0201	Nitto Denko	Idiopathic pulmonary fibrosis	Gene silencing	II	NCT03538301
TKM-080301	Arbutus Biopharma	Hepatocellular carcinoma	Gene silencing	I/II	NCT02191878
BNT151	BioNTech	Solid tumours (advanced)	Immunotherapy	I/II	NCT04455620
BNT142	BioNTech	Solid tumours (advanced)	Bispecific antibody	I/II	NCT05262530
mRNA-3927	Moderna	Propionic acidaemia	Protein replacement	I/II	NCT04159103
mRNA-3705	Moderna	Methylmalonic acidaemia	Protein replacement	I/II	NCT04899310
mRNA-4359	Moderna	Solid tumours (advanced)	Cancer vaccine	I/II	NCT05533697
mRNA-1468	Moderna	Herpes zoster	Viral vaccine	I/II	NCT05701800
mRNA-1608	Moderna	Herpes simplex virus type 2	Viral vaccine	I/II	NCT06033261
OTX-2002	Omega Therapeutics	Liver cancer; multiple cancers	Epigenetic modifier	I/II	NCT05497453
LNP CL-0137	Sanofi	Respiratory syncytial virus	Viral vaccine	I/II	NCT05639894
MRT5005-101	Translate Bio	Cystic fibrosis	Protein replacement	I/II	NCT03375047

Search terms used were “RNA payload, including mRNA, saRNA, siRNA, miRNA” with the delivery vehicle “lipid nanoparticle”. This yielded 435 total trials. Results of interest were filtered by removal of ongoing SARS-CoV-2 vaccine trials and redundant or withdrawn trials. This yielded a list of 59 clinical trials using RNA-based vaccines or therapeutics using LNP delivery systems and, as shown in the table, 30 have progressed beyond phase I. BMS, Bristol Myers Squibb; IAVI, International AIDS Vaccine Initiative; MSD, Merck Sharp & Dohme; NASH, non-alcoholic steatohepatitis; NCI, National Cancer Institute; NIAID, National Institute of Allergy and Infectious Disease; NSCLC, non-small-cell lung cancer; OTC, ornithine transcarbamylase.

editing using LNP delivery systems offers long-term modulation of expression in target tissues¹⁷⁷. All these advances combine increasing ease of use with improved accuracy in gene editing. It is expected that these advances will continue.

A limitation of current LNP delivery systems concerns their inability to deliver DNA to the nucleus, preventing expression in non-dividing cells. Technologies such as gene writing using mRNA to insert DNA sequences selectively may overcome such limitations¹⁷⁶. Current LNP systems do facilitate delivery of encapsulated pDNA into the cell cytoplasm, leading to significant transfection in rapidly dividing cells both in vitro and in vivo owing to the breakdown of the nuclear membrane during cell division¹⁷⁸.

Conclusion

The considerable success of LNP-based systems for the delivery of nucleic acid-based vaccines and therapeutics is ushering in a new generation of gene therapies. These systems exhibit overwhelming advantages over viral and other delivery systems in terms of genetic capacity, ability to re-dose, scalability, ease of manufacturing, lower cost, and potential for highly personalized targeted therapeutics that can be developed in a matter of weeks. The future prospects for gene therapies enabled by LNP-based delivery systems are clearly transformative.

Published online: 04 July 2024

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Acknowledgements

The authors acknowledge C. Brimacombe for the preparation of the table. P.R.C. acknowledges over 45 years of continuous funding from the Canadian Institutes for Health Research (CIHR) and its precursor, the Medical Research Council of Canada.

Competing interests

P.R.C. has financial interests in Acuitas Therapeutics, Integrated NanoTherapeutics and NanoVation Therapeutics. He is Chair of NanoVation. PLF has financial interests in Gene Therapy Systems, Antigen Discovery Inc. and SeroSight.

Additional information

Peer review information *Nature Reviews Drug Discovery* thanks James Heyes, Yizhou Dong and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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