Sudarshan Publications

Available online at https://s-epub.in/index.php/trp/index

Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2024.v16.i01.pp11-20

LIPID NANOPARTICLES AS CARRIERS FOR MRNA VACCINES: COMPOSITION, STABILITY AND MANUFACTURING

VALENTINA PELLEGRINI[1]*, DIMITRIOS VLACHOS[2], GONCALO CORREIA[3]

Affiliation [1]Department of Industrial Pharmacy, Cilento Institute of Pharmaceutical Sciences, Salerno, Italy
[2]Department of Industrial Pharmacy, Macedonia Plain Institute of Pharmacy, Serres, Greece
[3]Department of Industrial Pharmacy, Douro Valley Institute of Pharmaceutical Sciences, Vila Real, Portugal

*Corresponding author: Valentina Pellegrini, v.pellegrini@cips-sa.it
ORCID iD: Valentina Pellegrini 0000-0001-0616-4151; Dimitrios Vlachos 0000-0001-0892-4791; Goncalo Correia 0000-0001-0791-6757

Article info

Received date:02-08-2023
Accepted date:16-11-2023
Published date:27-03-2024
ISSN;0975-8216

Keywords:

Lipid nanoparticles, mRNA vaccines, Ionizable lipids, Formulation stability, Manufacturing

Abstract

Lipid nanoparticles (LNPs) made possible the first licensed mRNA vaccines by protecting the fragile message and delivering it into cells. This narrative review summarises published work on their composition, structure, stability and manufacture, and reports no new data. It describes the roles of the ionizable lipid, phospholipid, cholesterol and PEG-lipid, the chemical and physical routes by which the particles lose activity, and the rapid-mixing and downstream processes used to produce them at scale. Safety findings, including reactogenicity and rare hypersensitivity, are discussed. Much of the mechanistic evidence comes from siRNA and animal work, and key manufacturing data remain proprietary, which limits what can be concluded about future products.

1. INTRODUCTION

For most of the twentieth century, messenger RNA looked like an unpromising medicine. It is large, negatively charged, easily degraded by ubiquitous nucleases and recognised by the innate immune system as a sign of infection. The idea that cells could be told to make a therapeutic or antigenic protein by supplying the message was nevertheless clear from the early 1990s, and the long path to clinical success is well told in several histories of the field [1, 2]. By 2020 the main obstacles had been overcome by two advances working together: chemical modification of the nucleosides in the transcript, and packaging of the transcript in lipid nanoparticles (LNPs) that protect it and

carry it into cells [3, 4].

The first two licensed mRNA vaccines against SARS-CoV-2, BNT162b2 and mRNA-1273, are both lipid nanoparticle formulations. Their efficacy in large trials, at roughly 91 to 93% against symptomatic disease in the extended follow-up of the pivotal studies, turned a delivery technology that had been in development for two decades into a product used by billions of people [5, 6]. A feature of these products that attracted attention from pharmaceutical scientists was how much depended on the formulation. The mRNA sequence is a template, while the lipids determine whether it reaches the cytosol, how long it lasts on the shelf and what side effects the recipient notices [4, 7, 8].

This review describes the published work on LNPs as carriers for mRNA vaccines. It covers the components and their functions, the way particles are assembled and enter cells, the chemical and physical stability problems that created the cold chain, the manufacturing processes used to make them at scale, and what is known about their safety. It is a narrative synthesis and reports no new experimental data. Where possible, claims are attached to primary studies and reviews, but much of what is known about LNPs comes from work on small interfering RNA, and the review points out where that knowledge has been carried over to mRNA without direct confirmation [9, 10].

2. FROM NAKED MRNA TO LIPID DELIVERY

Naked mRNA injected into tissue is taken up poorly and degraded rapidly, so protein expression is brief and weak. In vitro-transcribed mRNA also activates Toll-like receptors and other pattern-recognition receptors, causing inflammation and shutting down translation. Karikó and colleagues showed that replacing uridine with modified nucleosides such as pseudouridine reduced activation of these receptors, and that the resulting transcripts were translated more efficiently and were more stable in cells [11, 12]. The principle was adopted in both licensed vaccines, which use N1-methylpseudouridine. Chemical modification and sequence optimisation therefore solve the immunological problem at the level of the molecule, leaving delivery as a problem for the formulation [1, 3, 12].

Lipid-based delivery of nucleic acids has a longer history than mRNA vaccines. Cationic liposomes were used for transfection from the 1980s, and the move to ionizable lipids was driven by work on siRNA, culminating in patisiran, the first approved RNA interference therapy, which is formulated as an LNP [10, 13, 14]. Ionizable lipids carry a charge at low pH, which allows efficient encapsulation of the negatively charged nucleic acid during manufacture, and are close to neutral at physiological pH, which reduces toxicity and non-specific interactions in the circulation [13, 14]. The same design logic was then adapted for mRNA, which is a much larger molecule than siRNA and needs different lipid ratios and structures [7, 9, 10].

The route of administration influences the formulation. For hepatic siRNA, LNPs were designed for intravenous injection and uptake by hepatocytes through apolipoprotein E adsorption, whereas vaccines are injected into muscle and need to reach antigen-presenting cells in the draining lymph nodes [7, 10]. Pardi and colleagues compared several routes in mice and found that intramuscular and intradermal injection of LNP-formulated nucleoside-modified mRNA gave prolonged protein expression at the site, with a measurable fraction reaching the liver [15]. These data help explain why optimising a vaccine LNP for intramuscular delivery, as Hassett and colleagues did, led to lipids that differ from those best for the liver [15, 16].

Some of the early confusion in the field came from reading results across platforms. Lipid structures optimised for siRNA in the liver did not always perform in the same way with mRNA, and in vitro transfection results often failed to predict in vivo potency. Several groups therefore moved to design-of-experiments approaches and screening in animals to optimise lipid ratios, which sped up development and exposed interactions between components that single-variable studies had missed [16, 17]. The quality-by-design literature encourages this kind of structured, risk-based approach,

in which interactions between formulation and process variables are studied deliberately and not left to chance, and it provides a useful vocabulary for the quality attributes discussed later [18].

3. COMPOSITION OF THE LIPID NANOPARTICLE

Four lipid classes make up the particle, and each has a defined job. The ionizable lipid is the functional centre, and the licensed vaccines use proprietary compounds, namely ALC-0315 in BNT162b2 and SM-102 in mRNA-1273. A phospholipid, usually distearoylphosphatidylcholine (DSPC), contributes to the structure. Cholesterol fills spaces between lipids and affects membrane rigidity. A PEG-lipid on the surface controls particle size and reduces aggregation [9, 19]. The molar proportions used in many formulations are close to 50% ionizable lipid, 10% phospholipid, 38.5% cholesterol and 1.5% PEG-lipid, a ratio derived from siRNA work with DLin-MC3-DMA, though vaccine formulations vary around it [14, 19]. The principal components are summarised in table 1, and the entries are grouped in fig. 1.

Ionizable lipid chemistry has had the largest effect on potency. The apparent pKa of the lipid in the particle, typically between 6 and 7, determines how much of the lipid is protonated at endosomal pH and how much remains neutral in the blood [13, 14]. Semple and colleagues tested a series of cationic lipids and found that potency for siRNA delivery rose as the pKa and tail structure were tuned, and Jayaraman and colleagues defined an optimum pKa range of roughly 6.2 to 6.5 for hepatic delivery [13, 14]. For intramuscular mRNA vaccines, lipids with branched tails and biodegradable ester linkages improved expression and tolerability in animal studies [16, 20]. These studies established design principles, though the precise rules differ between targets [16, 19, 20].

Helper lipids matter more than their small share might suggest. DSPC, with its saturated tails and high phase-transition temperature, gives a stable, ordered shell, whereas unsaturated phospholipids such as DOPE favour non-bilayer structures that may assist membrane fusion and endosomal escape [21]. Simulations and biophysical measurements of ionizable lipids mixed with phosphatidylcholine show that the charge state of the amino lipid changes how it packs with the phospholipid, which may explain why a given phospholipid works well with one ionizable lipid and badly with another [22]. Choice of helper lipid is therefore partly an empirical matter, tied to the ionizable lipid and the cargo [19, 21, 22].

Table 1: Selected published findings on lipid nanoparticle components, stability and manufacture

Component or process	Reported role or finding	Ref.
Ionizable lipid apparent pKa 6 to 7	Neutral in blood, protonated in endosomes; tuning pKa strongly alters in vivo potency	[10, 13, 14]
Branched-tail lipids intramuscular dosing	Optimised structures improved expression and immunogenicity of mRNA vaccines in animals	[16, 17]
Biodegradable linkers	Ester groups allow faster clearance of the lipid and may help tolerability	[20]
Helper phospholipid DSPC or DOPE	Supports particle structure; choice influences membrane fusion and delivery	[21, 22]
Cholesterol and analogues	Fills gaps between lipids; some analogues change particle shape and improve intracellular delivery	[19, 23]
PEG-lipid	Limits size and aggregation; may reduce uptake and raise anti-PEG antibody concerns	[24, 25, 26]
Particle adjuvant effect	The lipid component itself is inflammatory in mice and supports immune responses	[27]
Chemical degradation	Reactive lipid impurities can form adducts with mRNA and block translation	[28]
Frozen storage	Freezing without cryoprotectant increases particle size; sucrose protects activity	[29, 30]
Lyophilisation	Sugar excipients enabled dry storage at warmer temperatures in preclinical studies	[30, 31]

Component or process	Reported role or finding	Ref.
Rapid and microfluidic mixing	Controlled mixing gives small, uniform particles and a route to scalable production	[32, 33, 34]

Cholesterol is easy to overlook, but it has a clear effect on stability and delivery. It occupies gaps in the lipid layers, adjusts fluidity and limits the leakage of cargo. Patel and colleagues found that replacing cholesterol with certain naturally occurring analogues, such as β-sitosterol, produced polyhedral particle shapes and improved intracellular mRNA delivery in cell and animal models, probably by changing how particles interact with endosomal membranes [23]. The result is notable because it shows that a component long treated as a passive filler can be a tuning parameter. Whether such analogues would meet the regulatory expectations for a vaccine excipient is a separate question [19, 23].

PEG-lipids have a dual role. They sit on the particle surface during formation, where they limit growth and give a narrow size distribution, and they provide a steric layer that prevents aggregation on storage [24]. The drawback is that the PEG layer can reduce cellular uptake and endosomal escape, which led to the use of short-chain lipids that leave the particle surface after injection [19, 24]. Anti-PEG antibodies have been described in some people, and PEG has been proposed as one possible trigger of the rare immediate allergic reactions to mRNA vaccines, although the mechanism has not been proved [25, 26]. The amount of PEG-lipid in a formulation is therefore a trade-off between stability and delivery [24, 25].

4. STRUCTURE AND CELLULAR DELIVERY

The internal structure of LNPs differs from that of classical liposomes. Cryo-electron microscopy of siRNA particles revealed an electron-dense core in which the nucleic acid is associated with ionizable lipid in inverted micelles, surrounded by a lipid monolayer that carries the PEG-lipid [35]. mRNA particles have a similar overall arrangement but a larger and more heterogeneous interior, with some water and some less ordered regions, and structural studies of the licensed vaccines indicate that the particles are not perfectly uniform in size or in the number of mRNA molecules they carry [36]. This heterogeneity is a quality attribute that regulators and manufacturers must measure, because it could influence potency [35, 36].

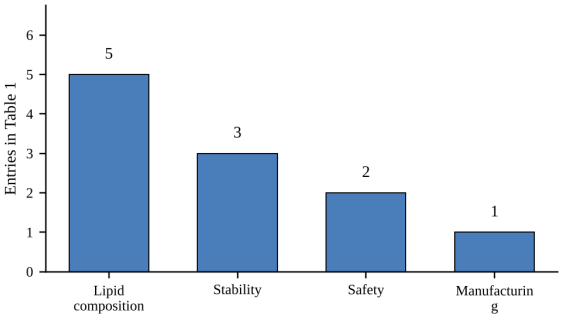


Fig. 1: Number of entries in table 1 by theme

After intramuscular injection, the particles are taken up by cells at the injection site and in lymph nodes, mainly by endocytosis. Inside the maturing endosome, the pH falls and the ionizable lipid becomes positively charged. It then interacts with anionic lipids of the endosomal membrane, a process thought to destabilise the membrane and let the mRNA enter the cytosol [13, 22]. Imaging work with siRNA LNPs indicates that endosomal escape is inefficient, with only a small percentage of the cargo reaching the cytosol, and that much of it is lost through recycling of endosomes to the cell surface [37, 38]. Similar quantitative data for mRNA vaccines are scarce [37, 38].

Immune activation by the particle itself is a distinctive and probably useful feature of the platform. Preclinical studies suggest that LNPs containing ionizable lipids are inflammatory, stimulating interleukin-6 and other cytokines and acting as an adjuvant that supports T follicular helper cell and antibody responses [27]. This helps to explain why mRNA vaccines elicit strong responses without a separate adjuvant. It also raises the possibility that reactogenicity, meaning the sore arm, fever and malaise many recipients report, is linked to lipid components as much as to the mRNA [8, 27]. Reducing inflammation while keeping adjuvant activity is a design aim that remains unmet [8, 19, 27].

Cargo size and design interact with the carrier. A vaccine transcript encoding a full-length viral spike protein is far longer

than the siRNA duplexes on which LNP technology was first developed, and long, flexible mRNA chains condense differently with ionizable lipid, so the lipid to RNA ratio that works for siRNA cannot simply be copied [7, 35]. The untranslated regions, the cap structure and the poly(A) tail affect translation and stability of the mRNA but do not alter how it packs in the particle, to a first approximation, while secondary structure and length do have some effect on encapsulation [1, 36]. Developers therefore often re-optimise the lipid ratios for each new antigen or payload [16, 17].

Distribution beyond the injection site has been examined mainly in animals. After intramuscular injection, a fraction of particles reaches the liver through the lymphatics and bloodstream, and measurable expression in liver has been reported in mice [15]. Apolipoprotein E binding probably contributes, as it does for intravenous LNPs [10]. For a vaccine, hepatic expression is largely incidental, but it has implications for the safety assessment of lipids that are metabolised in the liver, and toxicology studies of new lipids usually look at liver enzymes and histology for this reason. The rate at which lipids are cleared, and whether they accumulate on repeat dosing, has driven interest in biodegradable lipids with ester linkages [15, 20].

5. STABILITY AND STORAGE

Stability requirements helped shape the early rollout of mRNA vaccines. The first product was shipped and stored at about −80 to −60 °C, while the second was held at −20 °C, and thawed vials had short in-use times at refrigerator temperature [29, 36]. The cold chain was a consequence of the chemistry of two molecules, mRNA that is prone to hydrolysis and lipids that are prone to oxidation and hydrolysis, and of the physical vulnerability of a colloid in aqueous suspension. Crommelin and colleagues argued that the stability limits were not an inherent defect of the platform but a reflection of speed of development, and that improved formulations could be expected to relax them [29].

Chemical degradation of the mRNA is the most obvious pathway. The phosphodiester backbone can be cleaved by hydrolysis, which is faster at higher temperature and in the presence of divalent cations, and a single break can stop translation. Inside the particle, mRNA is protected from nucleases, but not from reactions with the lipids themselves. Packer and colleagues found that trace amounts of reactive impurities formed during manufacture or storage of ionizable lipids, notably aldehydes derived from oxidation, can bind covalently to the mRNA and form adducts that block translation [28]. This points to the quality of the lipid raw material as a determinant of the shelf life of the finished product [28, 36].

Physical instability is the second concern. Particles may fuse or aggregate during storage, or when frozen and thawed, which changes size distribution and may compromise potency or injection safety [30, 36]. Ball and colleagues examined LNPs stored at different temperatures and in different buffers and found that freezing without a cryoprotectant increased particle size, whereas storage at refrigerator temperature preserved particle properties for months. They also showed that lyophilisation in the presence of sugars such as sucrose and trehalose retained both size and activity [30]. Zhao and colleagues reported that lipid-like nanoparticles carrying mRNA maintained activity when stored under suitable conditions with cryoprotectants for extended periods [31].

Lyophilisation is attractive because it could remove the need for deep freezing, but it brings its own difficulties. The freeze-drying cycle exposes particles to freezing stress and dehydration, and the process must be optimised for each formulation [30, 31]. The residual moisture in the dried cake, the choice and amount of bulking sugar and the method of reconstitution all affect the outcome, and the physical state of the lipids at storage temperature matters for long-term stability [29, 30]. Published demonstrations of room-temperature stability are mostly preclinical, so the data are still insufficient to support licensing at the scale of a global vaccine programme [29, 30, 31].

A stability programme for an LNP vaccine follows the general principles of ICH guidance, but adds tests that are specific to the dosage form. In addition to assay and degradation products, the programme tracks mRNA integrity, encapsulation efficiency, particle size and polydispersity, lipid content and lipid-related impurities, pH and sub-visible particles [18, 39]. Accelerated and stress conditions are used to identify degradation routes, and real-time studies set the shelf life. The multiparameter nature of the programme means that a formulation can fail for physical rather than chemical reasons, and the choice of stability-indicating methods is a significant development decision [18, 36, 39].

Buffer composition, pH and ionic strength are further variables. Tris and phosphate buffers behave differently on freezing, and the pH of a frozen sample can shift, potentially harming either the RNA or the lipids [29, 30]. Sucrose is widely used as a cryoprotectant in frozen LNP products, and changes in formulation after the first generation of vaccines have reportedly improved the tolerance of the product to refrigeration and freezing [29, 31]. Studies in this area are often reported in the industry or regulatory literature rather than in the open journals, which limits what can be reviewed here, and readers should treat the figures quoted for shelf life as dependent on the exact product and presentation [29, 36].

6. MANUFACTURING PROCESSES

Making an LNP vaccine involves three stages that are normally run separately, namely production of the mRNA drug substance, formation of the particles, and fill-finish. The mRNA is made by in vitro transcription from a DNA template with a bacteriophage RNA polymerase, then capped, purified and sometimes polyadenylated enzymatically [40, 41]. Each step has its own control strategy. The step that most distinguishes the product from conventional biologics is LNP formation, because the quality attributes of the particles are decided by how the components are brought together, not just by what they are [32, 40].

Early LNP processes mixed lipids and nucleic acids by simple rapid injection of a lipid solution in ethanol into an aqueous buffer, producing particles that varied from batch to batch. Controlled rapid mixing replaced this approach. In a T-junction or impingement mixer, a stream of lipids in ethanol meets a stream of mRNA in acidic aqueous buffer at high velocity, causing the ethanol concentration to fall quickly and the lipids to self-assemble around the nucleic acid [32, 34]. Jeffs and colleagues described such an extrusion-free method for encapsulating plasmid DNA, and later work applied the principle to RNA [34]. The result is more uniform particles than by bulk mixing [32, 34].

Microfluidic mixers allow even finer control. Belliveau and colleagues used a staggered herringbone micromixer to produce siRNA LNPs with narrow size distributions, and showed that particle size could be tuned by changing flow rate ratio and total flow rate [33]. The same type of device is widely used in laboratories for screening, and scaled-up versions are used for clinical manufacture. Evers and colleagues compared mixing devices and noted that the geometry, flow rate and ethanol fraction affect particle size and polydispersity, so that a process change can be as consequential as a composition change [32]. Microfluidic chips suit small volumes but scale-out requires many parallel channels or a different mixer design [32, 33].

Downstream processing removes the ethanol and adjusts the buffer. Formed particles are initially in a mixture of ethanol and acidic buffer, which is unsuitable for injection, so they are diluted, neutralised and then concentrated and diafiltered, usually by tangential flow filtration, into a neutral storage buffer containing the cryoprotectant [32, 36]. This step raises the pH above the pKa of the ionizable lipid, so the particle surface becomes neutral and the mRNA is held in the core. The product is then passed through a sterilising-grade filter, which is a delicate step because particles near 100 nm can foul membranes and the filter must pass them without loss [36, 40]. Filling in aseptic conditions follows.

Scale and supply have been a practical limit as much as a scientific one. Rosa and colleagues reviewed the process steps and

found that capping, purification of the transcript and LNP formation were bottlenecks, with enzymes, specialised lipids and single-use consumables in short supply during the pandemic [40]. Kis and colleagues modelled the resources needed to meet global demand and concluded that the doses of mRNA needed were small in mass terms but that the lipids, the process equipment and the trained staff would restrict how fast capacity could grow [41]. Because several of the lipids used are proprietary and made by few suppliers, security of supply of raw materials has been a significant concern [40, 41].

Lipid synthesis is its own manufacturing problem. Ionizable lipids are made by multi-step organic synthesis, and the final material has to be purified to a high standard because trace impurities, including aldehydes and N-oxide species, have been linked to loss of mRNA activity [28]. Kis and colleagues noted that the lipid supply chain was less developed than that for the mRNA, which had been scaled up for vaccines only recently, and that GMP-grade production of novel lipids needed new capacity [41]. Suppliers must document starting materials, control residual solvents and show batch-to-batch consistency, which fits the control-strategy approach described in the quality-by-design literature [18, 41].

Quality by design provides a framework for linking material attributes and process parameters to product quality. For LNPs the critical quality attributes include particle size and polydispersity, mRNA content and integrity, encapsulation, lipid identity and content, impurities and potency, and the critical process parameters include flow rate ratio, mixing speed, temperature, buffer pH and N/P ratio [18, 32]. The quality-by-design framework expects that the relationship between the two sets is understood and that a design space is defined [18]. For a newer technology, building this knowledge is slow, and a good deal of it has so far been generated within companies [18, 40].

Analytical methods for the finished product deserve attention too. Dynamic light scattering gives size and polydispersity, but it does not resolve mixed populations well, and techniques such as field-flow fractionation, cryo-electron microscopy and multi-angle light scattering give more detail [32, 36]. Encapsulation is commonly measured with a fluorescent dye that binds free RNA, mRNA integrity by capillary electrophoresis, and lipid content by liquid chromatography with a charged-aerosol or mass-spectrometric detector [28, 36]. Each method adds cost and complexity, and harmonised reference materials for LNP characterisation are still lacking, which makes comparison between laboratories and between products difficult [36, 40].

7. SAFETY AND QUALITY CONSIDERATIONS

Clinical trials of the two licensed vaccines found that local reactions and systemic symptoms such as fatigue, headache and fever were common and usually short-lived, and that serious adverse events were uncommon and balanced between vaccine and placebo groups [5, 6]. Reactogenicity is partly a consequence of the lipid particle's own adjuvant activity, as preclinical studies suggest [27]. Dose, age and prior infection modify the pattern, and later lipid or process changes may alter it further. Because the particle and the mRNA contribute differently, safety evaluation of a new LNP vaccine needs to test the formulation, and not only the sequence [8, 27].

Immediate allergic reactions to mRNA vaccines are rare but have received much scrutiny. Reports early in the vaccination campaigns described anaphylaxis at rates of a few per million doses, mostly in people with a history of allergy [26]. PEG, or a PEG-lipid, has been suggested as the culprit because anti-PEG antibodies exist in the population and complement activation can follow exposure to PEGylated nanoparticles, but confirmatory testing has not been conclusive and other components have not been excluded [25, 26]. Observation periods after vaccination, readiness to treat anaphylaxis and allergy assessment for those who have had a reaction are the pragmatic measures recommended [26].

Lipid raw materials are novel excipients with a limited toxicological history, since the ionizable lipids and PEG-lipids in current

vaccines had not previously been used in marketed medicines in large populations, apart from siRNA products given in small groups of patients [10, 20]. Their purity matters for both safety and stability, as impurities from synthesis can react with the mRNA [28]. Biodegradable lipids, in which ester bonds allow hydrolysis and rapid clearance, are intended to reduce long-term exposure, and animal studies have shown faster elimination than for earlier lipids [20]. Specifications for the lipids, including limits on reactive impurities, are an important part of the control strategy [18, 28].

8. LIMITATIONS AND FUTURE DIRECTIONS

Several limitations apply to the literature reviewed here. A large part of the mechanistic knowledge comes from siRNA studies, rodent experiments and in vitro assays that may not predict human responses to mRNA vaccines [15, 37, 38]. Manufacturing and stability data from the companies that make the licensed products are largely proprietary and appear in the open literature in summarised form, so published work lags behind practice [29, 36]. The review is also narrative and does not weigh the studies by a formal quality score, and the evidence on stability of lyophilised products is mostly preclinical [30, 31].

Research priorities are fairly clear. Ionizable lipids that are more potent at lower doses, fully biodegradable and less inflammatory would help both tolerability and cost [19, 20, 27]. Formulations that survive refrigeration or room-temperature storage would ease distribution in low-income settings, as the cold chain debate showed [29, 30, 31]. Alternatives to PEG lipids might lessen the concern about hypersensitivity [25, 26]. Continuous manufacturing, better in-line analytics and open reference standards could lower barriers to new producers [32, 40, 41]. Improved targeting of antigen-presenting cells or specific organs is also being explored, although clinical evidence is not yet available [8, 9].

9. CONCLUSION

Lipid nanoparticles are a formulation technology whose design decisions show through in every property of an mRNA vaccine. The ionizable lipid governs encapsulation and endosomal release, the helper lipids and cholesterol shape the structure, and PEG-lipids control size and stability at some cost to uptake [9, 13, 14, 19, 24]. Stability depends on both the lipids and the mRNA, and impurities in raw materials can be as important as storage temperature [28, 29]. Manufacturing by rapid mixing is scalable but sensitive to process settings [32, 33].

For the pharmaceutical scientist the main lessons are practical. Formulation, process and analytics should be developed together within a quality-by-design framework, raw material purity should be specified tightly, and stability programmes should include tests for physical state as well as chemical integrity [18, 28, 39]. Progress in dry formulations, biodegradable lipids and alternatives to PEG could widen access and reduce side effects, but much of the evidence is preclinical, and claims should be tested in properly designed clinical and stability studies [25, 30, 31].

10. ABBREVIATIONS

LNP-Lipid nanoparticle, mRNA-Messenger RNA, siRNA-Small interfering RNA, PEG-Polyethylene glycol, DSPC-Distearoylphosphatidylcholine, DOPE-Dioleoylphosphatidylethanolamine, N/P-Ratio of ionizable lipid nitrogen to mRNA phosphate, ICH-International Council for Harmonisation, pKa-Acid dissociation constant

11. FUNDING

Nil

12. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

13. CONFLICT OF INTERESTS

Declared none

14. REFERENCES

1. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics, developing a new class of drugs. Nat Rev Drug Discov 2014;13:759–780.
2. Dolgin E. The tangled history of mRNA vaccines. Nature 2021;597:318–324.
3. Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates

immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res 2011;39:e142.

4. Verbeke R, Lentacker I, De Smedt SC, Dewitte H. The dawn of mRNA vaccines: the COVID-19 case. J Control Release 2021;333:511–520.
5. Thomas SJ, Moreira ED, Kitchin N, *et al.* Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. N Engl J Med 2021;385:1761–1773.
6. El Sahly HM, Baden LR, Essink B, *et al.* Efficacy of the mRNA-1273 SARS-CoV-2 vaccine at completion of blinded phase. N Engl J Med 2021;385:1774–1785.
7. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Ther Deliv 2016;7:319–334.
8. Pilkington EH, Suys EJA, Trevaskis NL, *et al.* From influenza to COVID-19: lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. Acta Biomater 2021;131:16–40.
9. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater 2021;6:1078–1094.
10. Akinc A, Maier MA, Manoharan M, *et al.* The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat Nanotechnol 2019;14:1084–1087.
11. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 2005;23:165–175.
12. Karikó K, Muramatsu H, Welsh FA, *et al.* Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 2008;16:1833–1840.
13. Semple SC, Akinc A, Chen J, *et al.* Rational design of cationic lipids for siRNA delivery. Nat Biotechnol 2010;28:172–176.
14. Jayaraman M, Ansell SM, Mui BL, *et al.* Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. Angew Chem Int Ed 2012;51:8529–8533.
15. Pardi N, Tuyishime S, Muramatsu H, *et al.* Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Release 2015;217:345–351.
16. Hassett KJ, Benenato KE, Jacquinet E, *et al.* Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. Mol Ther Nucleic Acids 2019;15:1–11.
17. Kauffman KJ, Dorkin JR, Yang JH, *et al.* Optimization of lipid nanoparticle formulations for mRNA delivery in vivo with fractional factorial and definitive screening designs. Nano Lett 2015;15:7300–7306.
18. Rathore AS. Roadmap for implementation of quality by design (QbD) for biotechnology products. Trends Biotechnol 2009;27:546–553.
19. Hald Albertsen C, Kulkarni JA, Witzigmann D, *et al.* The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev 2022;188:114416.
20. Maier MA, Jayaraman M, Matsuda S, *et al.* Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. Mol Ther 2013;21:1570–1578.
21. Cheng X, Lee RJ. The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. Adv Drug Deliv Rev 2016;99:129–137.
22. Ramezanpour M, Schmidt ML, Bodnariuc I, *et al.* Ionizable amino lipid interactions with POPC: implications for lipid nanoparticle function. Nanoscale 2019;11:14141–14146.
23. Patel S, Ashwanikumar N, Robinson E, *et al.* Naturally-occurring cholesterol analogues in lipid nanoparticles induce polymorphic shape and enhance intracellular delivery of mRNA. Nat Commun 2020;11:983.
24. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev 2016;99:28–51.
25. Kozma GT, Shimizu T, Ishida T, Szebeni J. Anti-PEG antibodies: properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals. Adv Drug Deliv Rev 2020;154–155:163–175.
26. Castells MC, Phillips EJ. Maintaining safety with SARS-CoV-2 vaccines. N Engl J Med 2021;384:643–649.
27. Ndeupen S, Qin Z, Jacobsen S, *et al.* The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. iScience 2021;24:103479.

28. Packer M, Gyawali D, Yerabolu R, Schariter J, White P. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Nat Commun 2021;12:6777.
29. Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the cold reality of mRNA vaccine stability. J Pharm Sci 2021;110:997–1001.
30. Ball RL, Bajaj P, Whitehead KA. Achieving long-term stability of lipid nanoparticles: examining the effect of pH, temperature, and lyophilization. Int J Nanomedicine 2016;12:305–315.
31. Zhao P, Hou X, Yan J, *et al.* Long-term storage of lipid-like nanoparticles for mRNA delivery. Bioact Mater 2020;5:358–363.
32. Evers MJW, Kulkarni JA, van der Meel R, Cullis PR, Vader P, Schiffelers RM. State-of-the-art design and rapid-mixing production techniques of lipid nanoparticles for nucleic acid delivery. Small Methods 2018;2:1700375.
33. Belliveau NM, Huft J, Lin PJC, *et al.* Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids 2012;1:e37.
34. Jeffs LB, Palmer LR, Ambegia EG, *et al.* A scalable, extrusion-free method for efficient liposomal encapsulation of plasmid DNA. Pharm Res 2005;22:362–372.
35. Kulkarni JA, Darjuan MM, Mercer JE, *et al.* On the formation and morphology of lipid nanoparticles containing ionizable cationic lipids and siRNA. ACS Nano 2018;12:4787–4795.
36. Schoenmaker L, Witzigmann D, Kulkarni JA, *et al.* mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. Int J Pharm 2021;601:120586.
37. Gilleron J, Querbes W, Zeigerer A, *et al.* Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. Nat Biotechnol 2013;31:638–646.
38. Sahay G, Querbes W, Alabi C, *et al.* Efficiency of siRNA delivery by lipid nanoparticles is limited by endocytic recycling. Nat Biotechnol 2013;31:653–658.
39. ICH Harmonised Tripartite Guideline. Quality of biotechnological products: stability testing of biotechnological/biological products Q5C. Geneva: ICH; 1995.
40. Rosa SS, Prazeres DMF, Azevedo AM, Marques MPC. mRNA vaccines manufacturing: challenges and bottlenecks. Vaccine 2021;39:2190–2200.
41. Kis Z, Kontoravdi C, Shattock R, Shah N. Resources, production scales and time required for producing RNA vaccines for the global pandemic demand. Vaccines 2021;9:3.