Lipid nanoparticles for mRNA delivery

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Abstract | Messenger RNA (mRNA) has emerged as a new category of therapeutic agent to prevent and treat various diseases. To function in vivo, mRNA requires safe, effective and stable delivery systems that protect the nucleic acid from degradation and allow cellular uptake and mRNA release. Lipid nanoparticles have successfully entered the clinic for the delivery of mRNA; in particular, lipid nanoparticle–mRNA vaccines are now in clinical use against coronavirus disease 2019 (COVID-19), which marks a milestone for mRNA therapeutics. In this Review, we discuss the design of lipid nanoparticles for mRNA delivery and examine physiological barriers and possible administration routes for lipid nanoparticle–mRNA systems. We then consider key points for the clinical translation of lipid nanoparticle–mRNA formulations, including good manufacturing practice, stability, storage and safety, and highlight preclinical and clinical studies of lipid nanoparticle–mRNA therapeutics for infectious diseases, cancer and genetic disorders. Finally, we give an outlook to future possibilities and remaining challenges for this promising technology.

Messenger RNA (mRNA), which was discovered by pioneering studies in 1947–1961 (Ref.1), is a transient intermediate between genes and proteins. By the late 1980s, investigations of mRNA structure and function resulted in the development of in vitro-transcribed (IVT) mRNA1. Since the first proof-of-concept animal study in 1990 (Ref.1), numerous strategies have been explored to ameliorate the instability and immunogenicity of IVT mRNA. Additionally, advances in drug delivery systems have expedited the preclinical development of mRNA therapeutics2–7, providing the basis for mRNA as a new class of drug (Fig. 1).

mRNA has shown therapeutic potential in a range of applications, including viral vaccines, protein replacement therapies, cancer immunotherapies, cellular reprogramming and genome editing. To achieve therapeutic effects, mRNA molecules have to reach specific target cells and produce sufficient proteins of interest. However, targeted delivery and endosomal escape remain challenging for mRNA delivery systems, highlighting the need for safe and effective mRNA delivery materials.

A variety of materials have been developed for mRNA delivery, including lipids, lipid-like materials, polymers and protein derivatives8–17. In particular, lipid nanoparticles have been thoroughly investigated and successfully entered the clinic for the delivery of small molecules18, siRNA drugs19 and mRNA20–22. Notably, two authorized coronavirus disease 2019 (COVID-19) vaccines, mRNA-1273 (REFS20,21) and BNT162b22, use lipid nanoparticles to deliver antigen mRNA. Many other lipid nanoparticle–mRNA formulations have been developed and are under clinical evaluation for the prevention and treatment of virus infections, cancer and genetic diseases23–27 (TABLES 1,2).

In this Review, we briefly overview representative lipid nanoparticles used for mRNA delivery and describe key steps in the preclinical development of lipid nanoparticle–mRNA formulations, including the overcoming of physiological barriers, different administration routes, manufacturing and safety profiles. Finally, we highlight important examples of lipid nanoparticle–mRNA formulations in clinical studies and provide future perspectives for lipid nanoparticles and mRNA therapeutics.

Development of lipids for mRNA delivery

In 1976, nucleic acids were encapsulated and delivered in polymeric particles. Later, exogenous mRNA delivery into host cells was demonstrated with liposomes23 (Fig. 1). Lipids are amphiphilic molecules that contain three domains: a polar head group, a hydrophobic tail region and a linker between the two domains. Cationic lipids, ionizable lipids and other types of lipid have been explored for mRNA delivery (Fig. 2).

Cationic lipids. Cationic lipids have a head group with permanent positive charges13,14. For example, 1,2-di-O-octadecenyl-3-trimethylammonium-propane (DOTMA), a quaternary ammonium lipid, has been applied for mRNA delivery in multiple cell types24, and was commercialized as Lipofectin in combination with 1,2-dioleoyl-sg-cyclohexyl-3-phosphoethanolamine (DOPE)24, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a biodegradable analogue of DOTMA,
also studied for mRNA delivery and is part of the commercial agent MegaFectin, together with DOPE or cholesterol. DOTMA and DOTAP have both been applied either alone or combined with other materials for mRNA delivery; for example, spleen-targeted DOTMA–mRNA lipoplexes (RNA-LPX) have been developed as systemic cancer vaccine. The same formulation has also been designed as mRNA vaccine for the treatment of autoimmune encephalomyelitis. This vaccine induces the proliferation of antigen-specific CD4+ regulatory T cells, leading to enhanced immunosuppression and a reduction of clinical symptoms in mouse models. DOTAP-based cationic nanoemulsions can deliver antigen mRNA against viral, bacterial and parasitic infections. Moreover, DOTAP–polymer hybrid nanoparticles can deliver mRNA molecules for the treatment of cancer, infections and genetic disorders. Incorporating carbonate apatite in DOTAP-based lipid nanoparticles increases the interaction between the particles and cellular membranes.

Fig. 1 | Timeline of some key milestones for mRNA and lipid nanoparticle development. COVID-19, coronavirus disease 2019; EMA, European Medicines Agency; FDA, United States Food and Drug Administration; LNP, lipid nanoparticle.
The delivery efficiency can further be improved by conjugating fibronectin to the lipid nanoparticles, which is a cellular adhesion protein accelerating the endocytic rate.

Dimethyl diocetyl ammonium bromide (DDAB), a quaternary ammonium lipid, can not only form complexes with mRNA but also stimulate innate immune responses, thereby serving as immune adjuvant for mRNA vaccines. DDAB and DOPE constitute the commercial product TransfectAce. The commercialized agent Lipofectamine is composed of DOPE and 2,3-dioleyloxy-N-[2-(sperrminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), a cationic lipid containing quaternary ammonium.

Table 1 | Representative clinical trials of lipid nanoparticle-mRNA vaccines against infections and cancer

| Name | Disease | Encoded antigen | Administration route | ClinicalTrials.gov identifier | Phase |
|------|---------|----------------|---------------------|-----------------------------|-------|
| mRNA-1273 | SARS-CoV-2 | Spike | i.m. | NCT04740427 | III (EUA and CMA) |
| BNT162b2 | SARS-CoV-2 | Spike | i.m. | NCT04368728 | III (EUA and CMA) |
| CVnCoV | SARS-CoV-2 | Spike | i.m. | NCT04652102 | III |
| LNP-nCoVsRNA | SARS-CoV-2 | Spike | i.m. | ISRCTN17072692 | I |
| ARCT-021 | SARS-CoV-2 | Spike | i.m. | NCT04728347 | II |
| ARCoV | SARS-CoV-2 | Receptor-binding domain | i.m. | ChiCTR2000034112 | I |
| mRNA-1440 | Influenza H1N8 | Haemagglutinin | i.m. | NCT03076385 | I |
| mRNA-1851 | Influenza H7N9 | Haemagglutinin | i.m. | NCT0345043 | I |
| mRNA-1893 | Zika virus | Pre-membrane and envelope glycoproteins | i.m. | NCT04064905 | I |
| mRNA-1345 | Respiratory syncytial virus | F glycoprotein | i.m. | NCT04528719 | I |
| mRNA-1653 | Metapneumovirus and parainfluenza virus type 3 (MPV/PIV3) | MPV and PIV F glycoproteins | i.m. | NCT03392389 | I |
| mRNA-1647 | Cytomegalovirus | Pentameric complex and B glycoprotein | i.m. | NCT04232280 | II |
| mRNA-1388 | Chikungunya virus | Chikungunya virus antigens | i.m. | NCT03325075 | I |
| CV7202 | Rabies virus | G glycoprotein | i.m. | NCT03713086 | I |

CMA, conditional marketing authorization; EUA, Emergency Use Authorization; HPV, human papillomavirus; i.d., intradermal; i.m., intramuscular; i.v., intravenous; KRAS, Kirsten rat sarcoma 2 viral oncogene homologue; MAGE-A3, melanoma antigen family A; NY-ESO-1, New York esophageal squamous cell carcinoma 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TPTE, putative tyrosine-protein phosphatase.
ammonium and spermine. Lipofectamine protocols have been optimized to deliver mRNA in diverse cell types, including alveolar cells, cardiac muscle cells and pluripotent stem cells48–50. 2-(((((3 types, including alveolar cells, cardiac muscle cells and have been optimized to deliver mRNA in diverse cell

 Ionizable lipids. Ionizable lipids are protonated at low pH, which makes them positively charged, but remain neutral at physiological pH \(^{[4,14]}\). The pH-sensitivity of ionizable lipids is beneficial for mRNA delivery in vivo, because neutral lipids have less interactions with the anionic membranes of blood cells and, thus, improve the biocompatibility of lipid nanoparticles \(^{[12,14]}\). Trapped in endosomes, in which the pH is lower than in the extracellular environment, ionizable lipids are protonated and, therefore, become positively charged, which may promote membrane destabilization and facilitate endosomal escape of the nanoparticles\(^{[5,11,14]}\). Ionizable lipids originally developed for DNA transfection, such as (2S)-2,5-bis(3-aminopropylamino)-N,N'bis(2-hydroxyethyl)-N-methylethan-1-aminium bromide (BHEM-Cholesterol) was developed by modifying the head structure of 3β-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Cholesterol) with hydroxyl groups to improve fusion with cellular membranes\(^{[41]}\). Lipid nanoparticles containing BHEM-Cholesterol have been applied to deliver mRNA encoding clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9; GM-CSF; granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; i.v., intravenous.

### Table 2 | Representative clinical trials of lipid nanoparticle–mRNA therapeutics against infections, cancer and genetic disorders

| Name       | Disease                        | Encoded protein                                         | Administration route | ClinicalTrials.gov identifier | Phase |
|------------|--------------------------------|----------------------------------------------------------|----------------------|-------------------------------|-------|
| mRNA-1944  | Chikungunya virus              | Antibody against chikungunya virus                       | i.v.                 | NCT03829384                  | I     |
| mRNA-2416  | Solid tumours                  | OX40L                                                   | Intratumour          | NCT03323398                  | II    |
| mRNA-2752  | Solid tumours                  | OX40L, IL-23 and IL-36γ                                   | Intratumour          | NCT03739931                  | I     |
| MEDI1191   | Solid tumours                  | IL-12                                                   | Intratumour          | NCT03946800                  | I     |
| SAR441000  | Solid tumours                  | IL-12sc, IL-15Sushi, IFN and GM-CSF                      | Intratumour          | NCT03871348                  | I     |
| **Genetic disorders** |                        |                                                          |                      |                               |       |
| mRNA-3704  | Methylmalonic acidemia         | Methylmalonyl-Coa mutase                                 | i.v.                 | NCT03810690                  | I/II  |
| mRNA-3927  | Propionic acidemia             | Propionyl-Coa carboxylase                                | i.v.                 | NCT04159103                  | I/II  |
| MRT5201    | Ornithine transcarbamylase deficiency | Ornithine transcarbamylase                          | i.v.                 | NCT03767270                  | I/II  |
| MRT5005    | Cystic fibrosis                | Cystic fibrosis transmembrane conductance regulator     | Inhalation           | NCT03375047                  | I/II  |
| NTLA-2001  | Transthyretin amyloidosis with polyneuropathy | CRISPR–Cas9 gene editing system                 | i.v.                 | NCT04601051                  | I     |

CoA, coenzyme A; CRISPR–Cas9, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9; GM-CSF; granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; i.v., intravenous.
can be increased by introducing ester motifs; for example, introducing ester bonds in the linker and lipidic tails of MC3 results in the lipid di((Z)-non-2-en-1-yl)-9-((4-(dimethylamino)butanoyl)oxy)heptadecanedi- oate (L319)[9], which shows better delivery efficacy and faster elimination from the liver and plasma in vivo in comparison with MC3[Ref.9]. Similarly, the biodegradable lipids heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxoethyl)amino)octanoate (Lipid 5)[9], heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate (Lipid H (SM-102))[9] and (4-(hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315)[9] have better in vivo delivery efficacy and pharmacokinetics than MC3. Of note, SM-102 and ALC-0315 are the ionizable delivery efficacies and pharmacokinetics than MC3.[10]

For example, cKK-2,5-dione (cKK- E12) have been developed to deliver mRNA molecules in vivo90–100. For example, cKK- E12-based lipid nanoparticles are applied in cancer immunotherapies94,95 and genome editing96. Replacing the lipidic chains of cKK- E12 with alkenyl amino alcohols results in 3,6-bis(4-(bis((9-bis(2-hexyldecanoate) (ALC-0315)[9] have better in vivo delivery efficacy and pharmacokinetics than MC3. Of note, SM-102 and ALC-0315 are the ionizable delivery efficacies and pharmacokinetics than MC3.[10]

A combinatorial library has been designed that contains lipid-like materials with different hydrophilic groups and multiple lipidic tails, highlighting the chemical diversity of ionizable lipids[96]. Many lipid-like materials, such as 1,1’-((2-(4-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200)[97], tetrakis(8-methylpyrrol) 3,3’,3”,3”’-(((methylazanediyl) bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate (306O)[98] and 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione (cKK- E12)[99], have been developed to deliver mRNA molecules in vivo90–100. For example, cKK- E12-based lipid nanoparticles are applied in cancer immunotherapies[99] and genome editing[99]. Replacing the lipidic chains of cKK- E12 with alkenyl amino alcohols results in 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), which improves mRNA delivery efficacy in vivo, compared with cKK- E12[Ref.100]. Further altering the linkage of OF-02 leads to (((3,6-dioxopiperazin-2,5-diyl)bis(butane-4,1-diyl))bis(azanetriyl))tetraakis(ethane-2,1-diyl) (9Z,9’Z,9”Z,9””Z,12Z,12’Z,12”Z,12””Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin) and (((3,6-dioxopiperazin-2,5-diyl)bis(butane-4,1-diyl))bis(azanetriyl))tetraakis(butane-4,1-diyl) (9Z,9’Z,9”Z,9””Z,12Z,12’Z,12”Z,12””Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), which allow selective delivery of mRNA into the spleen100,101. The lipid-like material N,N,N’-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3) can deliver mRNA molecules encoding human factor IX[101], CRISPR–Cas9[Ref.102], an interleukin-12 (IL-12) replicon[102] and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens[103]. Hexa(octan-3-yl)-9,9’Z,9”Z,9””Z,9””Z-((((benzene-1,3,5-tricarboxyl)tris(azanediyl))tris (propane-3,1-diy1))tris(azanetriyl))hexananoate (FTT5), which is a biodegradable analogue of TT3, further improves the in vivo delivery efficacy of mRNA encoding human factor VIII and base editing components[100]. In addition, a series of aminoglycoside-derived lipids have been synthesized as mRNA delivery materials[104–112].

Zwitterionic ionizable lipids can also be applied for mRNA delivery[96,113–116]; for example, lipids composed of a pH-switchable zwitterion and three hydrophobic tails assemble into a cone in the endosomal acidic environment, enabling membrane hexagonal transformation and allowing them to leave the endosome. Thus, lipid nanoparticle–mRNA formulations based on zwitterionic ionizable lipids can escape the endosome, leading to efficient protein expression and genome editing in vivo[114]. In addition to functioning as a delivery component, lipids can have therapeutic effects synergistic with mRNA-encoded proteins[117–119]. For example, lipids with a heterocyclic amine as head group can activate the stimulator of interferon genes (STING) signalling pathway in dendritic cells[117]. These lipids, as part of an mRNA vaccine, induce potent cytolytic T lymphocyte responses and inhibit tumour growth in mouse models[117]. Paclitaxel-conjugated lipids encapsulating tumour suppressor mRNA can be applied to integrate chemotherapy and gene therapy for triple-negative breast cancer[118].

Other types of lipid. In addition to cationic or ionizable lipids, lipid nanoparticle–mRNA formulations typically contain other lipid components, such as phospholipids (for example, phosphatidylcholine and phosphatidylethanolamine), cholesterol or polyethylene glycol (PEG)-functionalized lipids (PEG-lipids)[114,115]. These lipids can improve nanoparticle properties, such as particle stability, delivery efficacy, tolerability and biodistribution[114,115]. For example, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), a phosphatidylcholine with saturated tails, has a melting temperature of ~54 °C and a cylindrical geometry that allows DSPC molecules to form a lamellar phase, which stabilizes the structure of lipid nanoparticles[120]. DSPC has been used in the mRNA-1273 and BNT162b2 COVID-19 vaccines[121]. DOPE is a phosphatidylethanolamine with two unsaturated tails, which has a melting temperature of ~30 °C and a conical shape[120]. DOPE tends to adopt an inverted hexagonal (HII) phase, which destabilizes endosomal membranes and facilitates endosomal escape of lipid nanoparticles[122,123]. Using DNA barcode-labelled oligonucleotides, the distribution of different lipid nanoparticle formulations can be analysed in a high-throughput manner in vivo[124], for example, to quantify targeted delivery of nucleic acids in multiple tissues[122]. Based on this method, a series of phosphatidylcholines containing constrained adamantyl groups has been explored for mRNA delivery, including analysis of distribution in different cell types[122].

Cholesterol can enhance particle stability by modulating membrane integrity and rigidity[124,125]. The molecular geometry of cholesterol derivatives can further affect delivery efficacy and biodistribution of lipid nanoparticles. For example, cholesterol analogues with C-24 alkyl phytosterols increase the in vivo delivery efficacy of lipid nanoparticles[126]. Here, the length of the hydrophobic tails of the cholesterol analogues, the flexibility of sterol rings and the polarity of...
hydroxy groups impact delivery efficacy. In addition, lipid nanoparticles formulated with cholesterol derivatives adopt a polyhedral shape, and not a spherical shape, with multilamellarity and lipid partitioning. Lipid nanoparticles containing cholesterol oleate further show higher selectivity for liver endothelial cells than for hepatocytes. Moreover, oxidative modifications on the cholesterol tail enable lipid nanoparticles to accumulate more in liver endothelial cells and Kupffer cells than in hepatocytes.

PEG-lipids can have multiple effects on the properties of lipid nanoparticles. The amount of PEG-lipids can affect particle size and zeta potential. PEG-lipids can further contribute to particle stability by decreasing particle aggregation, and certain PEG modifications prolong the blood circulation time of nanoparticles by reducing clearance mediated by the kidneys and the mononuclear phagocyte system. Finally, PEG-lipids can be used to conjugate specific ligands to the particle for targeted delivery. The extent of these effects depend on the proportions and properties of the PEG-lipids (such as PEG molar mass and lipid length). For example, 1,2-dimyristoyl-rac-glycerol-3-methoxypropylene glycol-2000 (PEG$_{2000}$-DMG) and 1,2-distearoyl-rac-glycerol-3-methoxypropylene glycol-2000 (PEG$_{2000}$-DSG) are neutral PEG-lipids, and the length of their saturated alkyl chains is C14 and C18, respectively. Lipid nanoparticle-siRNA formulations containing PEG$_{2000}$-DMG have shorter circulation times and higher delivery efficacy in vivo than formulations containing PEG$_{2000}$-DSG. This difference may be attributed to the faster dissociation of PEG$_{2000}$-DMG from lipid nanoparticles, compared with PEG$_{2000}$-DSG, which may benefit cellular uptake and endosomal escape of lipid nanoparticles.

**Overcoming physiological barriers**

To function in vivo, lipid nanoparticle–mRNA formulations need to overcome multiple extracellular and intracellular barriers. First, mRNA needs to be protected from nuclease degradation in physiological fluids. Second, the formulation should evade the interception by the MPS and clearance by renal glomerular filtration post systemic administration. Third, lipid nanoparticle–mRNA systems need to reach target tissues, followed by internalization by target cells.

Finally, mRNA molecules must escape endosomes to reach the cytoplasm, where translation occurs.

Lipid nanoparticle–mRNA formulations manufactured by rapid mixing exhibit a stable nanostructure, in which mRNA molecules can be encapsulated in the interior core through electrostatic interactions with the lipids. This structural feature protects mRNA molecules from nuclease degradation and increases nanoparticle stability in physiological fluids. Incorporating PEG-lipids further decreases recognition by the MPS and clearance by renal filtration. Additionally, targeted biodistribution of lipid nanoparticle–mRNA formulations can be improved by further modifying and optimizing the nanoparticle, for example, to design spleen-targeted mRNA vaccines or lung-targeted genome editing delivery systems.

Once they reach target cells, lipid nanoparticles can be internalized by multiple mechanisms, including macropinocytosis and clathrin-mediated and caveolae-mediated endocytosis. The endocytic pathway depends on the properties of the nanoparticle and the cell type. Following cellular internalization, lipid nanoparticles are usually trapped in endosomal compartments. Indeed, only a small amount of lipid nanoparticles may be able to escape from the endosome, and endosomal escape is crucial for effective mRNA delivery. Although the mechanism has not yet been fully understood, positively charged lipids may facilitate electrostatic interaction and fusion with negatively charged endosomal membranes, resulting in the leak of mRNA molecules into the cytoplasm.

Endosomal escape can be increased by optimizing the pKa values of ionizable lipids. Furthermore, the properties of lipidic tails can affect endosomal escape of lipid nanoparticles; for example, some lipids with branched tails show enhanced endosomal escape compared with their counterparts with linear tails, owing to stronger protonation at endosomal pH.

In addition, modulating the type (for example, DSPC and DOPE) and ratio of lipids may improve endosomal escape.
Administration routes

Administration routes can greatly influence organ distribution, expression kinetics and therapeutic outcomes of lipid nanoparticle–mRNA formulations. The administration route is often determined by the properties of the nanoparticles and therapeutic indications. After intravenous (i.v.) administration, many lipid nanoparticles can accumulate in the liver. The liver is inherently capable of producing secretory proteins and, therefore, i.v. administration of lipid nanoparticle–mRNA formulations can be used to produce proteins that are missing in inherited metabolic and haematological disorders, or to produce antibodies to neutralize pathogens or target cancer cells. These applications require protein translation without stimulation of an immune response, which may limit the efficiency of repeated dosing. However, i.v. administration may also lead to accumulation of lipid nanoparticles in multiple lymph nodes throughout the body, which could increase immune responses to mRNA vaccines. For example, i.v. administration of mRNA vaccines has been shown to induce stronger antigen-specific cytotoxic T cell responses compared with local injection. Broad distribution of mRNA vaccines in the body may lead to systemic adverse effects, and, thus, it may be necessary to develop lipid nanoparticles that allow targeted delivery of mRNA vaccines into tissues with abundant immune cells.

Topical administration routes have also been explored for mRNA therapeutics. Topical administration aims at achieving local therapeutic effects; for example, local injection of lipid nanoparticle–mRNA formulations enables supplementation of therapeutic proteins in specific tissues, such as heart, eyes and brain. Of note, administration of mRNA encoding vascular endothelial growth factor (VEGF) has been shown to lead to functional protein expression in the skin even in the absence of lipid nanoparticles. Indeed, mRNA delivery by direct injection into the heart muscle of patients undergoing coronary bypass surgery is currently being tested in a randomized phase II trial (NCT03370887). Moreover, lipid nanoparticle–mRNA formulations can be administered into the lungs by inhalation, for example, MRT5005 (NCT03375047).

Local administration can also prime systemic responses; for example, intradermal (i.d.), intramuscular (i.m.) and subcutaneous (s.c.) injection are commonly used for vaccination, because resident and recruited antigen-presenting cells (APCs) are present in the skin and muscle, which can internalize and process mRNA-encoded antigens. Furthermore, the vascular and lymphatic vessels of these tissues help APCs and mRNA vaccines to center the draining lymph nodes to stimulate T cell immunity. Indeed, both i.m. and i.d. administration of lipid nanoparticle–mRNA vaccines produce robust immune responses at a well-tolerated...
dose in human trials. Vaccination can also be done by intranasal administration, because APCs in the peripheral lymph nodes can readily endocytose administered lipid nanoparticle–mRNA formulations. In addition, lipid nanoparticle–mRNA formulations encoding immune stimulators can be directly delivered into tumour tissue by intratumoural injection, to boost a local pro-inflammatory environment, which leads to immune cell activation and subsequent priming of systemic anticancer responses. Finally, in utero administration of lipid nanoparticle–mRNA formulations can be applied to deliver mRNA to mouse fetuses, achieving protein expression in fetal livers, lungs and intestines.

**Considerations for clinical translation**

The properties of lipid nanoparticle–mRNA formulations need to be carefully characterized and considered for the desired application. Lipid nanoparticle–mRNA formulations may need different properties as vaccines than as therapeutics to achieve optimal therapeutic effects, for example, distinct biodistribution profiles. Vaccines need to interact with immune cells, whereas therapeutics are targeted to specific organs. Therefore, lipid nanoparticle–mRNA formulations should be designed according to biomedical demand. To translate lipid nanoparticle–mRNA systems from bench to bedside, good manufacturing practice (GMP) is crucial to ensure drug quality and therapeutic effects, in addition to considerations such as storage conditions and safety profiles.

**Good manufacturing practice.** The preparation of a linearized DNA template is the initial step of GMP production of mRNA. Based on the DNA template, the mRNA is then transcribed in vitro in the presence of an RNA polymerase and ribonucleoside triphosphates. The residual DNA template is removed by DNase digestion and the mRNA molecules are capped by chemical or enzymatic methods. Finally, mRNA is purified by microbeads-based precipitation or chromatographic methods to remove the enzymes, free nucleotides, truncated nucleic acid fragments and double-stranded RNA. The purified mRNA can be dissolved in a storage buffer, filtered for sterilization and frozen for long-term storage. To increase the stability and translational efficiency of mRNA, various approaches have been explored to optimize its structural elements.

| Box 1 | Engineering mRNA molecules |
|---|---|
| mRNA normally contains five structural elements, that is, a 5’ cap, a 3’ poly(A) tail, a protein-coding sequence and 5’ and 3’ untranslated regions (UTRs). These elements are crucial for initiation, translation, termination, post-transcriptional modification and decay of mRNA. Incorporation of 5’ cap analogues allows initiation of the translation complex with the eukaryotic translation initiation factor 4E. Such 5’ cap analogues may be more resistant to decapping enzymes. The 3’ poly(A) tail is involved in interactions with the poly(A)-binding protein. Optimization of the length of the poly(A) tail and its composition can stabilize the mRNA and increase protein expression. UTRs interact with multiple RNA-binding proteins and microRNAs, and, thus, sequence engineering of 5’ and 3’ UTRs can increase the half-life and translational efficiency of mRNA. Codon optimization (for example, replacing rare codons with synonymous frequent codons) can accelerate the translation rate. Codon optimization can also form favourable secondary structures, improving translational efficiency. Incorporating chemically modified nucleosides can decrease immunogenicity and increase translation of mRNA. Circular RNA (circRNA) design can extend the duration of mRNA translation because circRNA is resistant to nuclease-mediated degradation. |

Historically, lipid nanoparticle–nucleic acid formulations were produced by thin-film hydration, reverse-phase evaporation and other methods. The sizes of lipid nanoparticles were further homogenized by extrusion techniques. Lipid nanoparticle–mRNA formulations are now commonly manufactured by rapid mixing; here, an ethanol phase (lipid components) and an aqueous phase (mRNA molecules) are mixed under specific conditions (that is, pH and flow rate). This technique allows reproducible and scalable production of lipid nanoparticle–mRNA formulations that show high encapsulation efficiency and homogeneous size distribution. mRNA is susceptible to degradation and, thus, formulation buffers should be free of any ribonuclease contaminations. Lipid nanoparticle–mRNA formulations are further purified to remove organic solvents and residual components, and the final mRNA concentration can be further increased by enrichment. The filtered and frozen lipid nanoparticle–mRNA formulations are then subject to a series of GMP standard tests, including evaluation of physical parameters (such as mRNA encapsulation, particle sizes, charges), compendial testing (such as sterility, bacterial endotoxins, particulate matter, osmolality) and other quality testing.

**Stability and storage.** Storage conditions of lipid nanoparticle–mRNA formulations are an important consideration for their clinical translation, because storage (aqueous, freezing and lyophilized storage) and the type of cryoprotectants (sucrose, trehalose or mannitol) affect the long-term stability of lipid nanoparticle–mRNA formulations. For example, the addition of 5% (w/v) sucrose or trehalose to lipid nanoparticle–mRNA formulations, stored in liquid nitrogen, allows maintenance of mRNA delivery efficacy for at least 3 months in vivo. Of note, the authorized COVID-19 mRNA vaccines are both stored in freezing conditions in the presence of sucrose. mRNA-1273 is stored at −15°C to −20°C and is directly injected after thawing, whereas BNT162b2 is stored at −60°C to −80°C and requires thawing and dilution by saline before injection. Recently, the European Medicines Agency (EMA) has approved storage of BNT162b2 at −15°C to −25°C for 2 weeks based on new stability data. Although cold-chain transportation can maintain vaccine activity, the development of lipid nanoparticle–mRNA formulations that do not require cold or frozen storage would not only decrease production and transportation costs but also expedite the process of vaccination. Therefore, it is important to investigate the factors impacting long-term storage of lipid nanoparticle–mRNA formulations.
Safety profiles. The safety profile of lipid nanoparticle–mRNA formulations correlates with the lipid components and mRNA molecules. Lipid components may activate host immune responses following systemic or local administration; for example, PEG-lipids could induce hypersensitivity reactions by stimulating the complement system. Moreover, anti-PEG antibodies could result in fast systemic clearance of subsequently administered PEGylated nanoparticles by accelerated blood clearance. The accelerated blood clearance phenomenon may change the bioavailability and biodistribution of the drug encapsulated in PEGylated nanoparticles and, thus, cause side effects. To ameliorate safety concerns, numerous natural and synthetic polymers have been investigated as alternatives to PEG, of which several are under evaluation in clinical trials. Cationic and ionizable lipids have also been reported to stimulate the secretion of pro-inflammatory cytokines and reactive oxygen species. Although the immunogenicity of these lipids has not yet been fully understood, complement system and Toll-like receptors may participate in innate immune activation. Cytotoxicity of lipid materials is also a safety concern, depending on the dose, lipid properties and cell types. In vivo application of lipid nanoparticles has been reported to induce liver and lung injuries in rodents, which may be attributed to the cytotoxicity of the materials and the induction of pro-inflammatory factors. To improve the biocompatibility of lipid nanoparticles, biodegradable lipids can be applied.

The immunogenicity of IVT mRNA is another safety concern, although eliciting cellular and humoral immunity may be advantageous for vaccination. Nevertheless, immune responses to IVT mRNA may also suppress antigen expression and negatively affect vaccine efficacy. Moreover, immune activation is undesirable for some mRNA applications, such as protein replacement therapies and genome editing. To minimize the immunogenicity of mRNA, two approaches are commonly used. Chemical modifications of specific IVT mRNA nucleotides, such as pseudouridine and N7-methylpsudesouridine, can reduce innate immune sensing of exogenous mRNA translation. Chromatographic purification can remove double-stranded RNA, an analogue of viral genome, in IVT mRNA preparations, diminishing immune activation and increasing translational efficiency. The IVT mRNA molecules used in the mRNA-1273 and BNT162b2 COVID-19 vaccines were prepared by replacing uridine with m1ψ, and their sequences were optimized to encode a stabilized pre-fusion spike protein with two pivotal proline substitutions.

Preclinical studies and clinical trials. The features of lipid nanoparticle–mRNA formulations have been thoroughly preclinically and clinically investigated, which has allowed the rapid development and clinical use of the COVID-19 lipid nanoparticle–mRNA vaccines. For example, the clinical-grade COVID-19 vaccine, mRNA-1273, was produced within a month after the SARS-CoV-2 genome sequence was available. About 2, 5 and 6 months from sequence availability, clinical trial phases I, II and III were initiated, respectively. Finally, mRNA-1273 obtained Emergency Use Authorization from the FDA and conditional marketing authorization from the EMA within a year. Many other lipid nanoparticle–mRNA formulations are in clinical trials for the treatment of infectious diseases, cancer and genetic disorders (TABLE 1). Moreover, mRNA-based cellular reprogramming, tissue regeneration and genome editing have shown therapeutic potential in preclinical studies.

Infectious diseases. Vaccines are the most effective approach to control and eradicate epidemics. The first mRNA vaccine was made of liposomes and mRNA encoding an influenza virus nucleoprotein. This vaccine, designed in 1993, was able to induce virus-specific cytotoxic T cell responses in mice. Since then, lipid nanoparticle–mRNA formulations have emerged as a potent alternative to conventional vaccine platforms, owing to their unique features. First, mRNA is a non-infective and non-integrating agent with the ability to encode a broad range of antigens. Second, mRNA vaccines can combine different antigen mRNAs; for example, six separate mRNAs have been incorporated in a cytomegalovirus vaccine, five of which encode a single pentameric antigen and one encodes a glycoprotein antigen. Finally, GMP-grade lipid nanoparticle-mRNA vaccines can be manufactured for specific antigens in a short period of time, compared with other vaccine platforms, such as recombinant proteins and inactivated vaccines. These features make lipid nanoparticle–mRNA formulations a flexible and on-demand vaccine platform to rapidly respond to emerging infectious pathogens. However, the instability and short half-life of mRNA need to be carefully considered. In addition, safety concerns and storage conditions of lipid nanoparticles need to be determined before clinical use.

To address these concerns, the engineering of mRNA molecules and the design of lipid nanoparticles have been optimized, which has contributed to the rapid development and clinical assessment of the two COVID-19 mRNA vaccines, mRNA-1273 (NCT04470427) and BNT162b2 (NCT04368728). Both vaccines use ionizable lipid nanoparticles to deliver nucleoside-modified mRNA encoding the full-length spike protein of SARS-CoV-2 (FIG. 4). Applying a prime-boost vaccination method, the vaccines induce high levels of antigen-specific antibodies and elicit robust T helper 1 cell responses. Moreover, the vaccines showed similar efficacy (~95%) in phase III clinical trials. Other COVID-19 vaccines based on lipid nanoparticle–mRNA formulations are also under evaluation in different clinical phases (TABLE 1). In preclinical studies, some vaccine candidates showed protective effects by delivering self-amplifying RNA encoding the spike protein, a cocktail of mRNAs encoding three viral proteins, modified mRNA encoding the receptor-binding domain or spike mRNA with engineered untranslated regions.

Lipid nanoparticle–mRNA vaccines are also being investigated as influenza vaccines, with some formulations in clinical trials (TABLE 1).
Haemagglutinin is an essential surface antigen of influenza viruses\textsuperscript{16,17}. The mRNA-1440 and mRNA-1851 vaccines, which are composed of lipid nanoparticles and mRNA encoding haemagglutinin from H10N8 and H7N9 influenza viruses, respectively, have completed phase I clinical studies (NCT03076385 and NCT03345043)\textsuperscript{16,17}. After i.m. prime–boost vaccination, humoral immune responses were evaluated by haemagglutination inhibition (HAI) and microneutralization (MN) assays. A 100-µg dose level induced 78.3% (HAI) and 87.0% (MN) seroconversion for H7N9 influenza viruses, respectively, and both dose levels were well tolerated.

A series of clinical trials of mRNA vaccines have been initiated against human metapneumovirus, cytomegalovirus, respiratory syncytial virus, rabies virus and chikungunya virus (Table 1). Furthermore, lipid nanoparticle–mRNA vaccines have been tested against other viruses in animal models, including human immunodeficiency virus\textsuperscript{78,144,191,192}, Powassan virus\textsuperscript{195}, Venezuelan equine encephalitis virus\textsuperscript{196}, dengue virus\textsuperscript{3} and Ebola virus\textsuperscript{97}.

Apart from viral infections, lipid nanoparticle–mRNA vaccines have been reported to induce immune protection against parasitica\textsuperscript{131} and bacteria\textsuperscript{180,186} infections. In addition, lipid nanoparticle–mRNA formulations have been applied to produce therapeutic proteins or antibodies\textsuperscript{29,74,146,155}, to edit virus genomes\textsuperscript{105} and to engineer immune cells\textsuperscript{146}. For example, a vitamin C-derived lipid nanoparticle allows mRNA delivery into primary macrophages\textsuperscript{198}. Adoptive transfer of macrophages engineered by delivering mRNA that encodes an antimicrobial peptide considerably reduced bacterial burden and increased survival in mice with multidrug-resistant bacterial sepsis\textsuperscript{199}. Furthermore, the lipid nanoparticle–mRNA formulation mRNA-1944 (NCT03829384), designed to generate anti-chikungunya virus antibody (CHKV-IgG) in vivo, is under clinical evaluation. A single i.v. injection of 0.1, 0.3 and 0.6 mg kg\textsuperscript{-1} lipid nanoparticle–mRNA formulation produced 2.0, 7.9 and 10.2 µg ml\textsuperscript{-1} CHKV-IgG 24 h post injection, respectively. The half-life of CHKV-IgG was about 69 days and the dose levels were reasonably well tolerated, including 0.3 mg kg\textsuperscript{-1} given twice a week apart.

**Cancer.** The attempt of mRNA-based cancer immunotherapies dates back to 1995, when i.m. injection of mRNA encoding carcinoembryonic antigen was shown to induce antigen-specific immune responses in mice\textsuperscript{199}. Various cancer vaccines based on lipid nanoparticle–mRNA formulations are currently in clinical trials (Table 1). For example, FixVac, which was developed based on the RNA-LPX formulation, is a systemic mRNA vaccine encoding four non-mutated antigens of melanoma\textsuperscript{196}. The interim analysis from the phase I trial (NCT02410733) showed that the metabolic activity of the spleen increased post the sixth immunization,
Circular RNA \(13,245,246\)

Circular RNA (circRNA) is a single-stranded RNA with a closed-loop structure. circRNA does not have a free 5′ cap or 3′ poly(A) tail and is, thus, unsusceptible to degradation by nucleases and more stable than linear RNA. Moreover, circRNA without a stop codon reduces the frequency of ribosome detachment from the RNA, thereby enabling continuous translation and high protein expression. Synthetic circRNA can be made by covalently linking the 3′ and 5′ ends of a linear precursor using enzymatic or chemical methods. Similar to linear mRNA, the chemical modification of specific nucleotides and chromatographic purification can minimize immunogenicity of the RNA and increase translation. Therefore, circularization can improve the stability and half-life of mRNA in a physiological environment.

indicating targeted delivery of FixVac and activation of resident immune cells\(^{200}\). After the eighth immunization, more than 75% of patients generated immune responses against at least one tumour-associated antigen, and CD8+ T cells played a major role in high-magnitude T cell responses\(^{200}\). Moreover, a combination of FixVac/anti-programmed cell death protein 1 (PD1) antibody augmented the antitumour effect of FixVac, resulting in a \(>35\%\) tumour regression rate in immune checkpoint inhibitor-experienced patients\(^{200}\). To further improve vaccine efficacy, APC uptake and T cell activation can be optimized; for example, mannose-modified lipid nanoparticle–mRNA formulations are preferentially taken up by dendritic cells\(^{160,201,202}\). The efficacy of cancer vaccines can also be boosted by co-delivery of antigen mRNA with adjuvants\(^ {160,164,203-206}\), co-stimulatory molecules\(^ {148,150,207,208}\) and immune checkpoint inhibitors\(^ {200-202,209-211}\).

Neoantigens, generated by somatic mutations in cancer cells, are usually tumour-specific and have high immunogenicity\(^ {4,11}\). Neoantigens are often different between individual patients, allowing the development of personalized vaccines\(^ {4,11}\). For example, intranodal vaccination with free mRNA encoding ten neoepitopes of 13 patients with metastatic melanoma generated T cell immunity against multiple neoepitopes in all patients\(^ {200}\). Several personalized cancer vaccines using lipid nanoparticle–mRNA formulations have also entered clinical trials\(^ {210-212}\). For example, mRNA-4157 is a personalized cancer vaccine encoding up to 34 neoantigens\(^ {210,211}\). A phase I study (NCT03897881) has been performed to evaluate the immunogenicity of mRNA-4157 alone and in combination with immune checkpoint inhibitors in patients with resected and unresectable solid tumours, respectively\(^ {210,211}\). In the monotherapy group, 14 out of 16 patients remained disease-free during the study, with a median follow-up time of 8 months\(^ {210,211}\). In the combination group, the overall response rate in the cohort (human papillomavirus-negative, immune checkpoint inhibitor-naive, head and neck squamous cell carcinomas) was 50% and the median progression-free survival was 9.8 months\(^ {210,211}\). Using a similar lipid nanoparticle formulation, mRNA vaccination has also been shown to elicit specific T cell responses in patients with gastrointestinal cancer (NCT03480152)\(^ {212}\).

mRNA vaccines can further be applied to overcome insufficient stimulation of chimeric antigen receptor (CAR) T cells in the therapy of solid tumours\(^ {213}\). For example, the RNA-LPX formulation can be used to deliver mRNA encoding claudin 6 (CLDN6), a target for CAR T cell therapy in solid tumours\(^ {213}\), i.v. injection of this vaccine resulted in CLDN6 expression on splenic dendritic cells and macrophages\(^ {213}\), promoting the activation of adoptively transferred CLDN6-CAR T cells and leading to suppression of large tumours in mice at a sub-therapeutic CAR T cell dose\(^ {213}\).

Alternatively to vaccination, a pro-inflammatory tumour microenvironment can be induced by lipid nanoparticle–mRNA formulations delivering cytokines or co-stimulatory molecules\(^ {32,106,151,241,242}\) (Table 2). For example, mRNA-2416, a lipid nanoparticle encapsulating mRNA encoding human OX40L (a ligand of OX40), is in clinical evaluation for the treatment of patients with solid tumours (NCT03323398)\(^ {214}\). In this trial, mRNA-2416 was intratumourally administered every 2 weeks for up to 12 doses, with four dose levels from 1 to 8 mg\(^ {214}\). mRNA-2416 was generally well tolerated at different dose levels\(^ {214}\). Moreover, the injected lesions showed an increase in OX40L expression and enhanced T cell activation\(^ {214}\). Encouraged by these results, mRNA-2752, a lipid nanoparticle formulated with mRNA encoding human OX40L, IL-23 and IL-36γ, entered clinical evaluation (NCT03739931)\(^{215,216}\). mRNA-2752 was designed to induce a pro-inflammatory tumour microenvironment and to simultaneously strengthen T cell expansion, as well as memory responses\(^ {215,216}\). mRNA-2752 was intratumourally administered every 2 weeks for up to seven doses, alone or in combination with infusion of durvalumab\(^ {215,216}\). In the 22 patients (monotherapy: \(n = 15\); combination: \(n = 7\)), six had stable disease, one had partial responses with 52% tumour reduction and five showed tumour shrinkage in treated and/or untreated sites\(^ {215,216}\). Lipid nanoparticle–mRNA formulations have also been investigated for ex vivo engineering of CAR T cells\(^ {217}\) and for the production of antibodies, such as anti-CD20 (REF\(^ {146}\), anti-human epidermal growth factor receptor 2 (HER2)\(^ {218}\) and anti-CD3/ claudin 6 (REF\(^ {147}\)). Cancers are often accompanied by mutations in the genome and, therefore, correction of these mutations could be an effective approach for cancer therapies. For example, restoration of the tumour suppressor gene TP53 can induce tumour cell apoptosis and sensitize tumour cells to chemotherapeutics in vivo\(^ {18,218}\). Similarly, the regulation of other tumour-associated genes, such as PLK1 (REF\(^ {13}\)), Bax\(^ {219}\), Maspin\(^ {17}\), PUMA\(^ {36}\) and PTEN\(^ {220}\), can delay tumour growth in vivo.
Gene-editing components can be delivered by lipid nanoparticles to correct mutated genes in genetic disorders. mRNA-based protein replacement therapies have also emerged as a promising alternative to protein drugs, because mRNA can be translated into desired proteins with in situ post-translational modifications in host cells. Moreover, mRNA can restore different types of protein, including secretory proteins, intracellular proteins and transmembrane proteins.

Clinical trials of protein replacement therapies using lipid nanoparticle–mRNA formulations have mainly focused on inherited metabolic disorders thus far, including ornithine transcarbamylase deficiency (NCT03767270), methylmalonic acidemia (NCT03810690) and propionic acidemia (NCT04159103) (Table 2). These diseases are characterized by genetic deficiency of key enzymes, leading to an inability to process certain metabolic products. The excessive accumulation of metabolites then results in clinical symptoms and may lead to death. Therefore, the supplement of desired enzymes by lipid nanoparticle–mRNA formulations can slow down disease progression. The therapeutic potential of mRNA-based protein replacement therapies has also been tested in other metabolic disorders in preclinical studies, including hereditary tyrosinaemia type I (HTI), acute intermittent porphyria, Fabry disease, Crigler–Najjar syndrome type 1 (REF. 239), α1 antitrypsin deficiency, methylmalonic acidemia, arginase deficiency, citrin deficiency and glycogen storage disease type 1 (REF. 240). In addition, mRNA-based protein replacement therapies have been applied to haematological diseases (for example, haemophilia A, haemophilia B, thrombotic thrombocytopenic purpura) and central nervous system disorders (for example, Friedreich’s ataxia), skin diseases (for example, elastin deficiency and hearing loss in preclinical studies.

Gene-editing tools further provide the opportunity to correct mutated genes in genetic disorders. Gene-editing components can be delivered by lipid nanoparticle–mRNA formulations to treat genetic diseases, including HTT, hypercholesterolaemia, lipoprotein metabolism disorders and transthyretin amyloidosis, which have been demonstrated in preclinical studies. Intella Therapeutics further initiated a phase I clinical trial (NCT04601051) to study the safety, pharmacokinetics and pharmacodynamics of NTLA-2001 (lipid nanoparticles encapsulating gene-editing components) in patients with hereditary transthyretin amyloidosis.

mRNA-based protein replacement therapies are also in clinical trials for the treatment of cystic fibrosis. Patients with cystic fibrosis usually suffer from repeated airway infections and chronic respiratory problems because of the defective cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel on epithelial cells. Lipid nanoparticles encapsulating CFTR mRNA can restore chloride secretion in Cfr−/− knockout mice. Translate Bio has started a clinical trial (NCT03375047) to evaluate the safety and tolerability of nebulized lipid nanoparticle–mRNA formulations (MRT5005) in patients with cystic fibrosis. In this phase I/II study, patients received a single dose of MRT5005 at three dose levels (8, 16 and 24 mg). MRT5005 was well tolerated at the 8-mg and 16-mg dose levels, and no serious side events were observed at any dose level. The lung function was evaluated by percent predicted forced expiratory volume in 1 s (PPFEV1). According to the interim report, the three patients who received the 16-mg dose showed maximal PPFEV1 increases of 11.1%, 13.6% and 22.2%, respectively, on day 8 post nebulization. mRNA-based protein replacement therapies are also being explored for heart, liver, lung and other organ diseases.

Conclusions and future directions

Progress in mRNA technologies and lipid nanoparticle-based delivery systems has allowed the development of mRNA COVID-19 vaccines at unprecedented speed, demonstrating the clinical potential of lipid nanoparticle–mRNA formulations and providing a powerful tool against the SARS-CoV-2 pandemic. A variety of lipid nanoparticles have been explored and optimized for mRNA delivery, providing valuable information for the future design of mRNA therapeutics. Based on the lessons and experiences from clinical studies, lipid nanoparticle–mRNA formulations can be further improved.

The in vivo translation efficiency of mRNA molecules could be further increased by RNA engineering. To achieve effective translation, mRNA requires five structural elements, including the 5′ cap, 3′ poly(A) tail, protein-coding sequence and 5′ and 3′ untranslated regions (UTRs). The sequences of these elements regulate translation initiation, translation termination and post-transcriptional modification of mRNA molecules. Thus, sequence engineering of these elements could improve translation in vivo. For example, optimization of the UTRs or the coding sequences results in increased protein expression, compared with wild-type controls. In addition, circular RNA (circRNA) can be synthesized to optimize mRNA properties (BOX 2). circRNA lacks the free ends necessary for nuclease-mediated degradation and, therefore, has a longer half-life than its linear mRNA counterpart.

Moreover, the delivery efficacy of mRNA could be improved, for example, by rational design of lipids through modulation of head groups and hydrophobic tails to increase cellular uptake and endosomal escape of lipid nanoparticle–mRNA formulations. Furthermore, hybrid nanoparticles may integrate the advantages of individual components to improve mRNA delivery potency. For example, pH-responsive polymers, such as poly(β-amino ester), can be incorporated into lipid nanoparticles to facilitate endosomal escape of mRNA molecules. Polymers, such as polyethyleneimine, protamine and polyaspartamide derivatives, are already widely used for mRNA delivery.
In addition, charge-altering releasable transporters and modified dendrimers can effectively deliver mRNA molecules in vitro and in vivo. Naturally derived membrane lipids (for example, exosomes and cell membranes) can also be applied for mRNA delivery. Organ-specific and cell-specific delivery of lipids nanoparticles can be achieved by modulating the lipid structures. For example, alteration of the alkyl length of a lipid results in selective accumulation of lipid nanoparticle–mRNA formulations in the liver or spleen. Alternatively, biometric lipids can be designed to achieve organ-targeted delivery. For example, neurotransmitters are endogenous chemicals that can cross the blood–brain barrier and participate in neurotransmission. Thus, neurotransmitter-derived lipids can be used for mRNA delivery to the brain following i.v. injection. Testing and comparing the cell distribution of many different lipid nanoparticle formulations remains challenging. However, barcoded nanoparticles allow in vivo high-throughput profiling of lipid nanoparticle distribution at the cell level. For example, barcoding has been applied to study how the structure of cholesterol derivatives impact cell selectivity of lipid nanoparticles, revealing that selective accumulation in liver endothelial cells, Kupffer cells and hepatocytes depends on cholesterol structures. Such large data sets will pave the way for a more profound understanding of the relationship between lipid nanoparticle properties and biodistribution.

Finally, biodegradability and multifunctionality should be considered for the design of lipid nanoparticles. Biodegradable lipids enable fast elimination of lipid nanoparticles from plasma and tissues, improving their safety and tolerability. Notably, biodegradable lipids are part of the mRNA-1273 and BNT162b2 COVID-19 mRNA vaccines. In addition to serving as delivery component, lipids may have therapeutic effects synergistic with mRNA-encoded proteins. Such multifunctional lipid materials include self-adjuvant lipids, which boost vaccine efficacy and paclitaxel-derived lipids, which allow integration of chemotherapies and gene therapies for the treatment of cancer.

In summary, mRNA has shown great therapeutic potential in a number of clinical trials and in clinical applications. The development of next-generation lipid nanoparticles and other types of delivery material will further enable mRNA-based therapies for a broad range of diseases and improve health care in the near future.

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Author contributions
X.H., T.Z. and R.L. and Y.D. contributed to conceiving the structure and writing the literature and writing the Review. T.Z. and R.L. and Y.D. reviewed and edited the manuscript.

Competing interests
Y.D. is a scientific advisory board member of Oncorus, Inc. and serves as a consultant of Rubius Therapeutics. T.Z. is an employee of Moderna, Inc. R.L. is a scientific advisory board member of Alnylam and a founder and board member of Moderna, Inc. A list of entities with which R.L. is involved, compensated or uncompensated is provided in the supplementary information. X.H. declares no competing interests.

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