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The landscape of mRNA nanomedicine

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Messenger RNA (mRNA) is an emerging class of therapeutic agent for the prevention and treatment of a wide range of diseases. The recent success of the two highly efficacious mRNA vaccines produced by Moderna and Pfizer–BioNTech to protect against COVID-19 highlights the huge potential of mRNA technology for revolutionizing life science and medical research. Challenges related to mRNA stability and immunogenicity, as well as in vivo delivery and the ability to cross multiple biological barriers, have been largely addressed by recent progress in mRNA engineering and delivery. In this Review, we present the latest advances and innovations in the growing field of mRNA nanomedicine, in the context of ongoing clinical translation and future directions to improve clinical efficacy.

Messenger RNA (mRNA) is a transient carrier that transfers genetic information from DNA to ribosomes, where that information can be translated into proteins[1]. By delivering mRNAs that express antigens of infectious diseases or cancers, gene-editing components, or disease-related therapeutic proteins, various clinical applications—including vaccines and gene-editing and protein therapies—can be achieved[2,3,4]. In 1976, it was shown for the first time that nucleic acids could be encapsulated and delivered by tiny particles, in this case composed of polymers[5]. Although initially ridiculed by the scientific community[6], 2 years later, exogenous nucleic acids (this time in the form of mRNAs) were delivered by liposomes and reported to be able to produce proteins in human and mouse cells[7,8]. Since then, mRNAs have demonstrated therapeutic efficacy in various preclinical studies, laying the foundation for establishing mRNA as a drug and a vaccine[9,10].

Nevertheless, the path to the successful application of mRNA as a drug was not straightforward. Initially, the instability, immunogenicity and high production cost of mRNA substantially dampened the enthusiasm of companies and the scientific community for investing resources. Encouragingly, these issues have been gradually addressed by the rapid development of mRNA engineering technologies, including chemical modification[11], sequence optimization[12,13] and purification[14], which drew on the work of numerous researchers over several decades[15] (Box 1). These advances laid the foundation for the therapeutic use of mRNA, but clinical translation requires expression in target cells or tissues in vivo. Taking advantage of progress in drug delivery systems[6], a wide range of materials[16,17]—such as lipid nanoparticles (LNPs)[18], polymeric nanoparticles[19,20] and lipid–polymer hybrid nanoparticles[21–23]—have been developed for in vivo delivery, with the goal of protecting mRNA from rapid degradation by ubiquitous RNases and helping it cross multiple biological barriers[9,10,24]. Although such technological progress has enabled many preclinical and clinical studies of mRNA drugs over the past two decades, no mRNA nanomedicines (mRNA vaccines or therapies) had been approved by any regulatory authority until very recently, in 2021.

Since then, successes in the development of the two coronavirus 2019 (COVID-19) mRNA vaccines (Moderna mRNA-1273 and Pfizer/BioNTech BNT162b2)[25–29] has fueled a renewed and intense research interest in mRNA engineering and delivery, offering the promise of clinical translation of various mRNA-based therapies. Here, we provide an overview of the field of mRNA nanomedicines. We discuss the technical challenges of mRNA-based therapies and link these to biological mechanisms and clinical outcomes. In addition, we highlight the recent innovations and advances in mRNA engineering and delivery methods that have expedited the clinical translation of mRNA therapies for various disorders, including infectious diseases, cancers and inherited diseases.

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BOX 1

Key advances in the development of mRNA therapeutics

1961
- Discovery of mRNA[215]

1976
- First in vivo nucleic acid delivery by polymeric particles[5]

1978
- Delivery of mRNA to human and mouse cells by liposomes[7,8]

1995
- First mRNA-based cancer vaccine evaluated in mice[216]

2005
- Kariko, Weissman and colleagues[11] reported for the first time that nucleoside modifications substantially reduced TLR signaling in response to mRNAs

2008–2012
- Kariko, Weissman and colleagues further demonstrated that nucleoside modifications could limit PKR[38] and 2′-5′-oligoadenylate synthetase activation, promote resistance to cleavage by RNase L[51] and eventually enhance the translational capacity and stability of mRNA[49,50].

2017
- First clinical trial of personalized mRNA-based cancer vaccine[217]

2020
- Two COVID-19 mRNA vaccines (mRNA-1273 and BNT162b2) received emergency use authorization in the United States

2021
- Adjuvant activity of LNPs in COVID-19 mRNA vaccines was identified[193]

Challenges regarding the clinical use of mRNA

The clinical use of mRNA for therapeutic purposes requires sufficient mRNA translation in the cells of interest without causing unwanted immune responses. However, achieving this goal requires overcoming several barriers involving mRNA synthesis and delivery within extracellular and intracellular contexts[9,24]. Therapeutic mRNA is synthesized via in vitro transcription (IVT) in a cell-free system using a linear DNA template generated from a plasmid or PCR and RNA polymerase T7, T3 or SP6 (ref. [30]) (Fig. 1a). mRNA is then purified using conventional laboratory-scale purification methods for nucleic acids (for example, precipitation in ethanol). However, these methods often fail to remove impurities such as double-stranded RNA and RNA fragments, which reduce the therapeutic efficacy and cause undesired biological responses in clinical use[31]. Upon local or systemic administration, mRNA can be rapidly degraded by the abundant nucleases in the extracellular space, removed by macrophage phagocytosis or cleared by renal filtration[24,32,33] (Fig. 1b). In the meantime, mRNA is a large, very negatively charged, single-stranded polynucleotide that is difficult to pass through negatively charged cell membranes. In fact, only 0.01% of extravasated mRNAs from blood vessels can enter target cells[2], where most of the mRNAs are trapped in endosomes and degraded thereafter (Fig. 1c). Eventually, a fraction of the internalized mRNAs escape from endosomes and reach ribosomes for therapeutic protein translation.

The immunostimulatory potential of exogenous mRNA is another major hurdle to clinical translation[15,18] (Fig. 1c). Exogenous mRNA can be sensed by pattern recognition receptors (PRRs) that play a crucial role in responding to viral RNAs, inducing immune stimulation[34]. Upon endocytosis, mRNAs can be detected by endosomal sensors called Toll-like receptors (TLRs)[35], the main family of PRRs expressed primarily but not exclusively by immune cells. mRNAs that escape endosomes can be sensed by several cytosolic PRRs. Stimulation of these PRRs eventually results in the production of type I interferons (IFNs) and other proinflammatory cytokines[36]. These secreted IFNs bind to their receptors on the stimulated cell and adjacent cells—activating the JAK–STAT pathway, which triggers the transcription of over 300 IFN-stimulated genes. Among these, IFN-inducible protein kinase RNA (PKR) can suppress activity of the translation initiator initiation factor 2, leading to inhibition of mRNA translation[37,38], and the 2′-5′-oligoadenylate synthetases[39] and RNA-specific adenosine deaminase[40] can reduce the stability of mRNAs. Because all of these challenges have substantially limited the clinical use of mRNA, advances in mRNA-related technologies are needed to address these challenges before the full therapeutic potential of mRNAs can be unleashed.

Design of mRNAs and their delivery vehicles

Rapid advances in the field of mRNA engineering and non-viral mRNA delivery have provided various solutions to challenges regarding the clinical use of mRNA. For example, issues with mRNA translatability, stability and immunostimulation can be solved by introducing innovative mRNA designs. Moreover, mRNA delivery vehicles can address at least some of the challenges of mRNA delivery. The rational design of mRNA delivery vehicles requires that they protect mRNAs from degradation by nucleases, cross various biological barriers and efficiently deliver mRNAs into the cytoplasm for robust protein expression.

Design of mRNAs

IVT mRNAs are structurally similar to naturally occurring mature eukaryotic mRNAs, which consist of five major domains: a 5′ cap, a 5′ untranslated region (UTR), an open reading frame (ORF) encoding the protein of interest, a 3′ UTR and a poly(A) tail. The translation and stability of mRNAs can benefit from UTR optimization. UTR sequences from highly expressed genes, such as human β-globin[41], are widely used for mRNA synthesis since mRNAs containing these UTRs normally show high levels of translation and stability. Furthermore, improved expression of mRNAs can be achieved by identifying novel UTR sequences using high-throughput screening methods[42,43] or deep learning approaches[44]. Although many 5′ or 3′ UTRs can independently enhance mRNA translation, a rational combination of 5′ and 3′ UTRs can maximize the translation efficiency[45]. Also, poly(A) tails with lengths of 100–150 nucleotides can improve the stability of mRNAs and efficiently initiate translation by forming complexes with poly(A) binding proteins[46–48].

One of the most effective strategies to abrogate immunostimulation by IVT mRNAs is nucleoside modification. Compared with unmodified mRNAs, the incorporation of naturally occurring modified nucleosides—such as pseudouridine (ψ), 5-methylcytidine, N^6-methyladenosine, 5-methyluridine and 2-thiouridine—reduces cytokine production[11,49,50] by preventing recognition by human TLRs. Mechanistically, the improved translation and stability of ψ-incorporated mRNAs are ascribed to the decreased activation of PKR[38] and 2′-5′-oligoadenylate synthetases[51]. Instead of replacing the original nucleosides with some types of modified nucleosides, simultaneously replacing partial nucleosides with 2-thiouridine and 5-methylcytidine substantially suppresses the activation of TLRs and RIG-1 (another PRR) both in vitro and in vivo[52]. Furthermore, the

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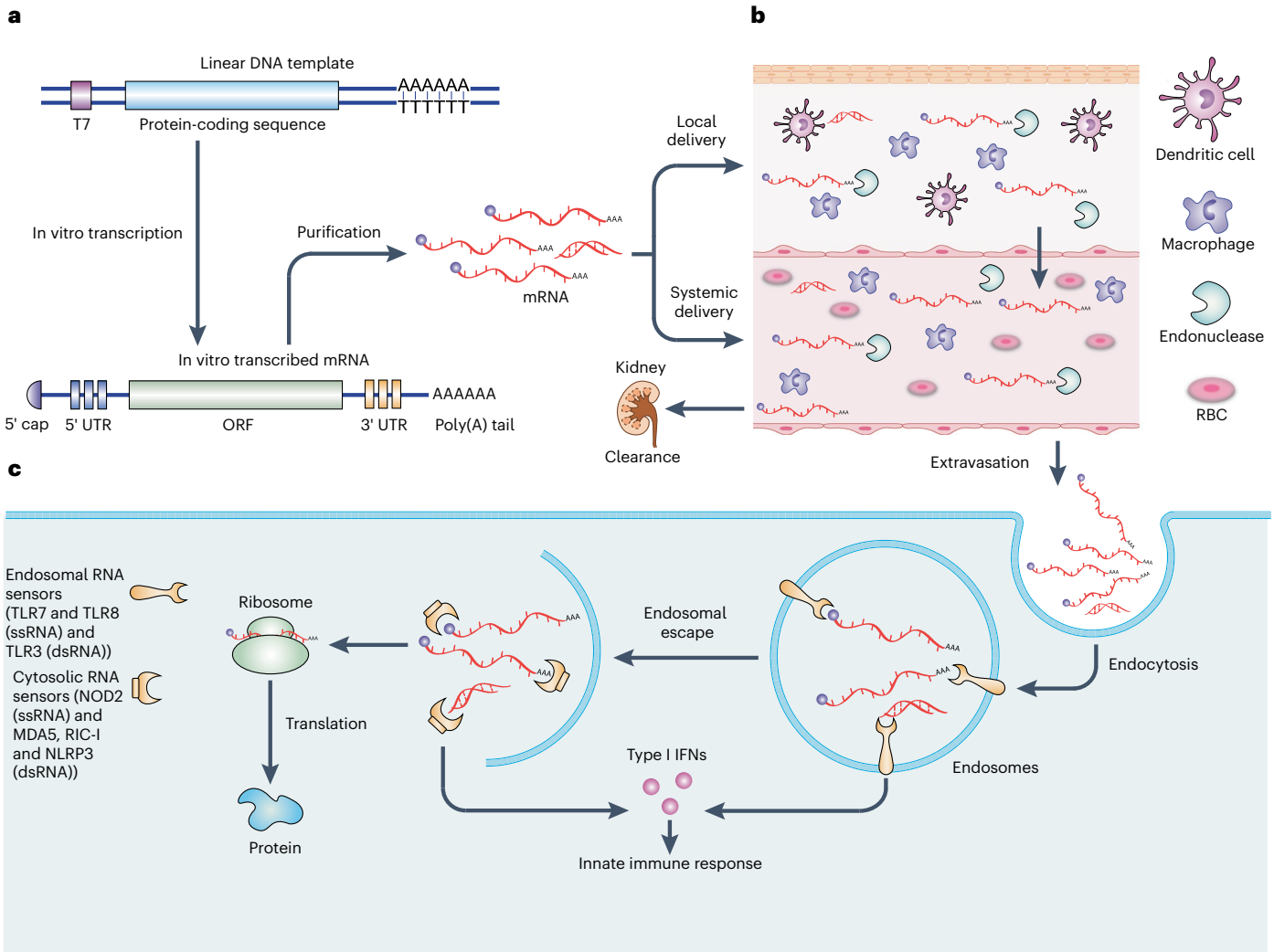

Fig. 1 | Challenges regarding the clinical use of mRNA. a, Therapeutic mRNA is synthesized in vitro using a linear DNA template and RNA polymerase (T7), followed by purification; it contains a 5′ cap, a 5′ UTR, an ORF encoding the protein of interest, a 3′ UTR and a poly(A) tail. **b**, After local or systemic delivery, mRNAs face several extracellular challenges, including rapid degradation by the abundant nucleases in the extracellular space, removal by macrophage phagocytosis and clearance by renal filtration. **c**, A fraction of extravasated mRNAs from blood vessels can be internalized by cells. Most of these internalized mRNAs are trapped in endosomes and can be detected by endosomal and cytosolic RNA sensors, which eventually reduces the translation and stability of the mRNA. An optimized 5′ cap can improve the binding efficacy of cytoplasmic mRNAs to ribosomes, eventually increasing the translation efficacy of mRNA. The endosomal escape of naked and unmodified mRNA is challenging, but can be enhanced by using mRNA carriers. Endosomal RNA sensors include TLR3 (ref. [208]), TLR7 (ref. [209]) and TLR8 (refs. [210,211]). Cytosolic RNA sensors include nucleotide-binding oligomerization domain-containing protein 2 (NOD2)[18], melanoma differentiation-associated protein 5 (MDA5)[212], retinoic acid-inducible gene-I (RIG-I)[213,214] and nucleotide-binding domain leucine-rich repeat-containing family pyrin domain-containing 3 (NLRP3)[18]. dsRNA, double-stranded RNA; RBCs, red blood cells; ssRNA, single-stranded RNA.

modified nucleoside N^1-methylpseudouridine[53] has been shown to have lower cytotoxicity and immunostimulation capacity[54] compared with ψ. It is worth noting that both mRNA vaccines from Moderna and Pfizer–BioNTech use nucleoside-modified mRNAs to avoid unintended immune responses. Another strategy to reduce immunostimulation by IVT mRNAs is enhanced mRNA purification. IVT mRNAs purified by high-performance liquid chromatography, free of double-stranded RNA contaminants, display 10- to 1,000-fold higher protein expression in primary cells than unpurified mRNA, without inducing the production of IFNs or inflammatory cytokines[14]. While high-performance liquid chromatography purification is widely used for mRNA production, a simple, fast and cost-effective cellulose-based purification method provides an alternative for the production of highly pure IVT mRNA[55].

The 5′ cap design provides another method to reduce unwanted immunological responses elicited by mRNAs. The natural eukaryotic 5′ cap (cap-0) is a 7-methylguaniosine (m7G) linked to the first nucleotide located at the 5′ end of mRNA through a 5′-5′-triphosphate bridge (m7GpppN)[56]. Cap-0 sterically inhibits the degradation of mRNAs by nucleases and initiates translation via binding to eukaryotic translation initiation factor 4E[56]. Compared with cap-0, two additional 5′ caps (cap-1 and cap-2), bearing an additional methyl group on the 2′ hydroxyl of the ribose from the first and second nucleotide, are more widely used in mRNA synthesis due to their lower immunostimulatory potential[57]. Currently, mRNAs with cap-1 structure can be conveniently manufactured using a co-transcriptional capping method (https://www.trilinkbiotech.com/cleancap), exhibiting minimal immunostimulation and satisfying translation efficiency. In addition, the stability and translation of mRNAs can be simultaneously enhanced by a computational experimental platform[58], while translation and immunostimulation can be modulated by chemo-enzymatic modifications[59,60].

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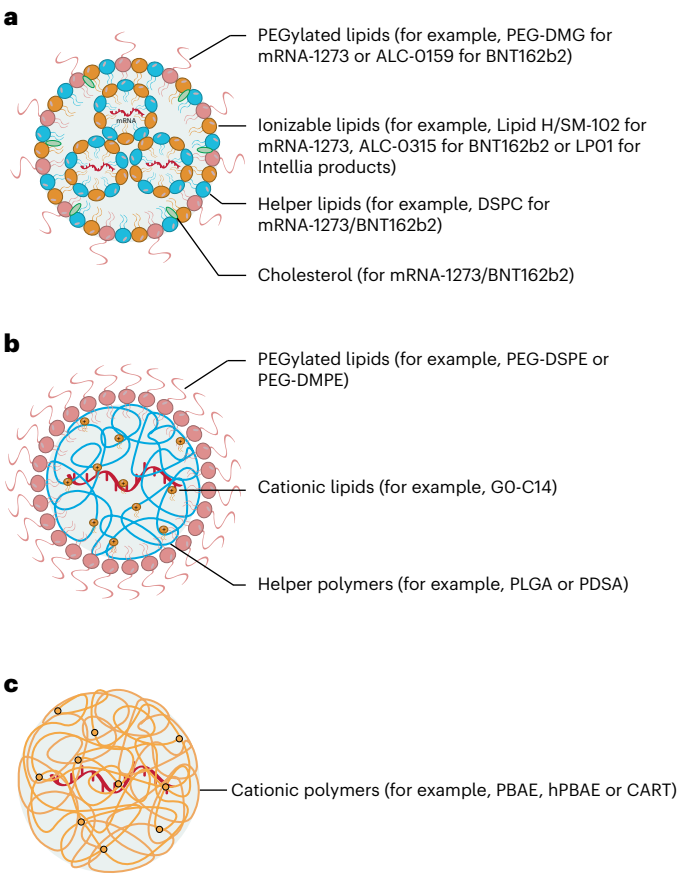

Fig. 2 | mRNA delivery vehicles. a, LNPs often consist of four basic components: a PEGylated lipid, a helper lipid, cholesterol and a cationic or ionizable lipid. The ionizable lipids largely determine the functions and efficacy of the LNPs. DSPC, distearoylphosphatidylcholine. PEG-DMG, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol. **b**, Lipid–polymer hybrid nanoparticles normally contain a cationic or ionizable lipid, a PEGylated lipid and a helper polymer. In some cases, the cationic or ionizable lipid and the helper polymer are replaced by a helper lipid and a cationic polymer. PDSA, poly(disulfide amide). PEG-DSPE, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol); PEG-DMPE, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol). **c**, Polymeric nanoparticles consist simply of cationic polymers.

Messenger RNA delivery vehicles

The rapid clinical translation of mRNA-based vaccines or therapies has benefited from the development of delivery vehicles to protect and deliver the highly unstable mRNA molecules. Currently, the major mRNA delivery systems are lipid-based nanoparticles, polymer-based nanoparticles and lipid–polymer hybrid nanoparticles.

Lipid-based nanoparticles. Lipid-based nanoparticles are the most intensively studied and clinically advanced vehicles for mRNA delivery[3,9,61]. The most widely used are cationic and ionizable LNPs[62], which typically contain a cationic or ionizable lipid, cholesterol, a helper phospholipid and a PEGylated lipid (Fig. 2a). Cationic lipids, such as DOTMA[63] or DOTAP[64], bearing quaternary ammonium groups, remain positively charged in a pH-independent manner. This cationic environment allows efficient condensation of negatively charged mRNAs, making cationic lipid-based systems the most widely used systems for mRNA delivery in early clinical studies[65]. Although cationic lipid-based nanoparticles encoding shared tumor antigens or disease-related autoantigens have shown promise in cancer immunotherapy[66] and the treatment of experimental autoimmune encephalomyelitis[67], their potential cytotoxicity[68] and relatively short blood circulation time[69] have impeded their clinical translation. To address these issues, lipid-PEGs were employed and a variety of novel ionizable lipids were developed. Unlike cationic lipids, which possess permanent positive charges, ionizable lipids remain neutral at physiological pH but can be protonated at acidic pH[70]. The neutrality of ionizable lipids in physiological fluids reduces the toxicity and, to some extent, increases the circulation half-life of ionizable LNPs. In addition, the protonation of ionizable lipids at acidic pH not only allows convenient condensation and encapsulation of mRNAs in acidic buffer, but also facilitates the escape of mRNAs from the acidic endosomes[48]. The PEG layer introduced by lipid-PEGs largely improves the circulation half-life of ionizable lipid-based nanoparticles and reduces nanoparticle aggregation, as well as reducing unfavorable interactions with serum proteins[71].

The development of ionizable lipid-based nanoparticles for mRNA delivery has, to a great extent, benefited from studies over decades on ionizable lipid-based nanoparticles for the delivery of small interfering RNAs (siRNAs), which enable targeted silencing of endogenous mRNAs. For instance, DLin-MC3-DMA (MC3)[72,73] is an ionizable lipid designed for the first US Food and Drug Administration–approved siRNA drug patisiran[74], which treats hereditary transthyretin-mediated amyloidosis. By optimizing the parameters of these formulations, such as the ratio of RNA to total lipid and the ratio of the aqueous solution to organic solvent (ethanol), MC3-based LNPs have been used in the development of various mRNA-based therapies, including vaccines against Zika virus[75–77], human immunodeficiency virus[78] and Lyme disease[79], as well as treatments for cystic fibrosis[80] and lymphedema[81]. One limitation of MC3 is its poor degradability, which can result in toxicity issues when repeated dosing is required. To address this challenge, as well as further increase the efficacy of MC3, Moderna has developed a biodegradable lipid named lipid 5 (ref. [82]), which contains primary esters and more branching tails than MC3. Indeed, repeated systemic and local administration of lipid 5-based mRNA LNPs has alleviated acute intermittent porphyria[83] and achieved durable anticancer immunity[84] in animal models without any obvious toxicity.

In addition to MC3, several other ionizable lipids initially developed for siRNA delivery, such as cKK-E12 (refs. [85,86]) and C12-200 (ref. [87]), have also been reformulated to deliver mRNAs to the liver for gene editing and protein replacement. However, delivery of therapeutic mRNAs to non-liver tissues by these LNPs is difficult owing to their selective accumulation in livers—a phenomenon probably determined by the ionizable properties of these lipids. Meanwhile, obvious liver toxicity has been observed when cKK-E12-based LNPs have been administered at an mRNA dose of 2.25 mg kg^{-1} or higher[88]. Intensive exploration of the new generation of ionizable lipids eventually led to the creation of lipid H/SM-102 (refs. [89,90]) and lipid ALC-0315 (ref. [91]), resulting in the rapid development of the two effective COVID-19 mRNA vaccines. The favorable safety profiles of these vaccines are probably attributed to the biodegradability of the lipids. In addition, Intellia has achieved robust and persistent in vivo gene editing in animal models using another biodegradable lipid (LP01)-based LNP carrying Cas9 mRNA and guide RNA[92]. Compared with non-biodegradable lipids, the biodegradable LP01 has less liver bioaccumulation and fewer safety risks. Although the ionizable lipids undoubtedly play a key role in the activity of LNPs, other components are also important. Helper lipids can promote endosomal escape of LNPs by adjusting their fluidity, consequently enhancing their efficacy[93], while cholesterol plays an important role in LNP stability.

Lipid–polymer hybrid nanoparticles. Another type of nanoparticle is the lipid–polymer hybrid nanoparticle, which normally includes an ionizable (or sometimes cationic) lipid, a hydrophobic polymer and a PEGylated lipid[22,94,95] (Fig. 2b). These nanoparticles have been used to efficiently restore the tumor suppressor PTEN and suppress tumor growth in multiple mouse models of prostate cancer[22]. In this platform,

a hydrophobic helper polymer poly(lactic-co-glycolic acid) (PLGA) replaces the helper lipid and cholesterol of LNPs. Notably, this platform exhibits excellent serum stability[96], which can probably be ascribed to the strong hydrophobic interaction between the polymer and lipids, making it ideal for systemic delivery of therapeutic mRNAs to tumors. Replacing the helper polymer PLGA with a redox-responsive polymer poly(disulfide amide) has resulted in a redox-responsive platform for systemic delivery of the tumor suppressor *p53* mRNA, achieving efficient tumor suppression in preclinical models[21]. The PEGylated lipid PEG-DMPE has also been added to improve the efficacy of the platform. Moreover, by simply changing or functionalizing the terminals of lipid-PEGs coated on the surface of the polymeric core, local delivery or organ-specific delivery could easily be achieved. For example, by using DSPE-PEG-NH_2 or DSPE-PEG-SH, mucoadhesive mRNA nanoparticles can be generated for intravesical delivery of mRNAs to upregulate desirable proteins in mouse bladder tissues in situ[23], and could also be used to upregulate target protein for various other applications within mucosal organs in situ. Other lipid–polymer hybrid nanoparticles have also been recently developed for effective mRNA delivery and tested in preclinical models[97–100].

Polymer-based nanoparticles. Polymeric nanoparticles consist simply of cationic polymers (Fig. 2c). Early studies focused on the use of polyethylenimine or poly-L-lysine for nucleic acid delivery. However, the notable toxicity of polyethylenimine and poly-L-lysine (these highly positively charged polymers can easily interact with negatively charged cellular components, inhibiting normal cellular process) has limited their application in mRNA delivery[101]. To address this issue, a series of biodegradable poly(β-amino esters) (PBAEs)[102,103] have been synthesized. For example, PBAE-based nanoparticles have been used for in vivo delivery of functional mRNAs to circulating T cells[104] and multiple tissues[105]. Furthermore, a hyperbranched PBAE (hPBAE) has been prepared for direct delivery of mRNAs to the lung via inhalation[19]. Another promising polymer for mRNA delivery is the charge-altering releasable transporter (CART)[33,106]. Unlike traditional cationic polymers, CARTs can release their mRNA cargo in the cytoplasm through a unique mechanism. The initial positive charges of the oligo(α-amino ester) can effectively condense and encapsulate mRNA and deliver it to the cells, through which the CARTs undergo a degradative, charge-neutralizing intramolecular rearrangement, leading to the rapid release of functional mRNAs. This unique characteristic has resulted in the use of CARTs for in vivo delivery of mRNA to lymphocytes[107], which could enable treatment strategies for a wide variety of diseases—for example, effectively stimulating immune responses against tumors[108] and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[109].

Promising recent innovations

Innovations in mRNA engineering and delivery have been fueled by the successful development of the COVID-19 mRNA vaccines. These new technologies may potentially produce next-generation mRNAs with greater stability and more robust expression, which could enable the application of mRNAs to the fields of protein therapy and gene editing (usually requiring higher and sustained mRNA expression than mRNA vaccines). In addition, innovations in mRNA delivery can greatly boost the in vivo delivery efficiency of mRNAs in various applications. These innovations should further promote the clinical translation of different mRNA therapies.

Innovations in mRNA engineering

Self-amplifying mRNA. Self-amplifying mRNA contains an alphavirus-based replicon that can amplify the expression of encoded proteins, and therefore requires a much lower dosage than conventional mRNAs in most applications[110,111]. The incorporation of additional replicon genes makes the size of self-amplifying mRNAs larger than conventional mRNAs. Thus, the formulations used for conventional mRNAs may need to be further optimized for the larger-size self-amplifying mRNAs[111,112]. While nucleoside modification is widely used in currently approved or currently investigated mRNA-based therapies, self-amplifying mRNAs cannot contain these modifications because they can interfere with the self-amplification process[113]. Self-amplifying mRNA-based SARS-CoV-2 vaccines have already demonstrated their ability to induce high neutralizing antibody titers in animals[114] and several candidates are currently being tested in clinical trials[48]. These have the potential to be used at lower doses (1–10 μg) than conventional mRNAs (30–100 μg) in COVID-19 vaccines[113].

Circular RNA. As discussed, the stability of mRNAs can be substantially improved via nucleoside modification and optimization of coding and non-coding regions. Alternatively, improved stability can be achieved by circularization. Circular RNAs (circRNAs)—single-stranded RNAs with a closed ring structure generated through backsplicing—are a class of non-coding RNAs with potentially broad biological functions[115,116]. Recent studies have revealed that the protein-coding function of some circRNAs[117–119] holds great promise for protein translation applications. The unique closed ring structure enables higher stability of circRNAs compared with linear mRNAs, due to the lack of end motifs required for exonuclease-mediated degradation[120]. Indeed, a pioneering study showed that a circRNA constructed using a self-splicing intron exhibited robust and stable protein expression in eukaryotic cells[121]. The self-circularization of the linear RNA precursor eventually results in circRNAs containing an internal ribosomal entry site (IRES) to drive the expression of proteins[121,122] (Fig. 3a). Besides the enhanced stability, circRNAs induce far fewer undesirable immune responses than unmodified linear mRNAs since they do not activate RNA sensors such as TLRs and retinoic acid-inducible gene-I[123]. A circRNA vaccine has elicited a higher level of neutralizing antibodies than a linear mRNA vaccine, demonstrating great protective efficacy against SARS-CoV-2 and its emerging variants in mice and rhesus macaques[124]. More recently, circRNAs containing five key elements, including vector topology, 5′ and 3′ UTRs, IRESs and synthetic aptamers, were constructed[125], and simultaneous optimization of these elements resulted in higher circRNA protein yields[125]. In addition, several companies are exploring other variations on circRNAs, such as optimized IRESs (Fig. 3b).

Innovations in mRNA delivery

Novel mRNA delivery systems. While LNPs are the most clinically advanced and widely used systems for mRNA delivery, many other non-LNP systems also have great potential for mRNA delivery. The retrovirus-like protein PEG10 can selectively bind and promote the vesicular secretion of its own mRNAs. Based on this, a PEG10 virus-like particle (VLP) platform, developed by inserting genes of interest (the DNA template of mRNA) into the *Peg10* gene, has realized potent gene editing via delivering gene-editing tools into cells[126] (Fig. 3c). One major advantage of the PEG10–VLP platform is its minimal immunostimulation and toxicity, given the fact that the platform is constructed using endogenous human proteins. As discussed, the endosomal escape of mRNAs is a major challenge for mRNA delivery and the use of ionizable lipids can ameliorate this, but an alternative strategy is to directly deliver mRNAs into the cytoplasm. To this end, Entos Pharmaceuticals developed a fusogenix proteo-lipid vehicle platform[127] using low-toxicity neutral lipids and proprietary fusion-associated small transmembrane proteins[128]. The unique fusion-associated small transmembrane proteins can facilitate rapid fusion of proteo-lipid vehicle and cell membrane, enabling direct delivery of cargos (for example, mRNAs) into the cytoplasm (Fig. 3d). Similarly, a pH- and redox-responsive coacervate formed by a phase-separating peptide has achieved direct cytosolic delivery of mRNAs to cells and redox-activated release of mRNAs, bypassing classical endocytic pathways[129].

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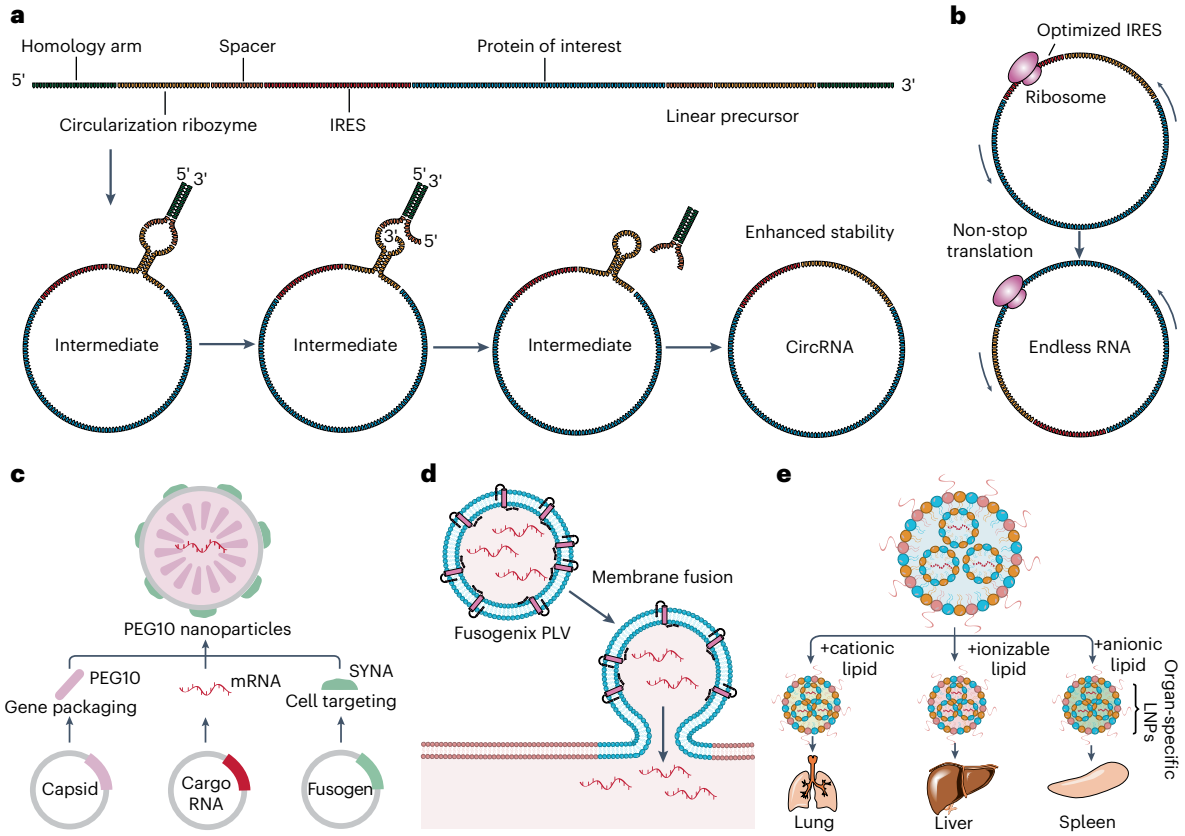

Fig. 3 | Innovations in mRNA engineering and delivery. a, Self-circularization of the linear RNA precursor is initiated by the circularization ribozyme, resulting in the RNA intermediates. The subsequent RNA splice yields the final highly stable circRNA containing an IRES for driving protein expression. **b**, An endless RNA developed by Laronde could have long-lasting protein translation by using an optimized IRES. **c**, The PEG10 virus-like particle platform or PEG10 nanoparticle consists of a PEG10 protein for mRNA packaging and fusogen syncytin A (SYNA) for cell targeting. **d**, A novel fusogenix proteo-lipid vehicle (PLV) platform comprising neutral lipids and proprietary fusion-associated small transmembrane protein enables direct cytosolic mRNA delivery. **e**, A SORT nanoparticle platform prepared by adding a supplemental SORT molecule of cationic, anionic or ionizable lipid to a conventional LNP system allows selective delivery of mRNAs to the lung, spleen or liver, respectively.

Alongside these new platforms, innovations continue to produce more potent LNPs with multiple functions, including enhanced delivery. LNPs containing heterocyclic lipids, identified using a combinatorial library, not only effectively deliver antigen mRNAs to mouse tumors, but also promote antigen-presenting cell maturation via the stimulator of interferon genes (STING) pathway, synergistically increasing the antitumor efficacy[130]. The efficacy of LNPs can be improved by introducing either unsaturated lipids[131] or alkyne lipids[88], while the modification of LNPs with a thiol group[23] or bisphosphate group[132] enables targeted delivery of mRNAs to mucus or bone. In addition, a one-component ionizable amphiphilic Janus dendrimer has enabled efficient delivery of mRNAs to different organs, holding promise for simplification of the current four-component LNP system[133,134].

Biological membrane-based vehicles for mRNA delivery. Biological membrane-based vehicles represent another novel biocompatible platform for mRNA delivery. Distinct types of biological membrane-based systems, including cell membrane vesicles[135], bacteria-derived outer-membrane vesicles[136] and extracellular vesicles[137] (for example, exosomes[138]), have been employed for in vitro and in vivo delivery of therapeutic mRNAs. As a type of nanoscale extracellular vesicle, exosomes have been widely investigated as carriers for drug delivery[139,140]. For example, Codiak BioSciences has launched the human trial of an engineered exosome-based therapeutic, named exoSTING, for the treatment of solid tumors (NCT04592484). In one preclinical study, exosome-based mRNA vaccines induced robust immunoglobulin G and secretory IgA responses in mice, which were stronger than those induced by liposome-based vaccines[141]. Thus, biocompatible exosomes may represent a promising platform for mRNA delivery[142]. One major challenge of the clinical use of current mRNA LNPs for protein replacement therapies is the potential toxicity caused by repeated administrations during a short period. However, biological vesicles are less immunogenic and toxic than most existing platforms, making them particularly useful for repeated mRNA dosing in clinical trials.

Organ- or cell-specific mRNA delivery. Most nanoparticles preferentially accumulate in the liver after intravenous injection[88,143,144]. Thus, targeted delivery of mRNAs to non-liver tissues will considerably broaden the applications of mRNA therapies. To this end, a selective organ targeting (SORT) nanoparticle platform has been developed for tissue-specific mRNA delivery[145]. By adding a supplemental SORT molecule of cationic, anionic or ionizable lipid to the widely used four-component LNP system, selective delivery of mRNAs to mouse lung, spleen or liver (respectively) has been achieved, enabling effective CRISPR–Cas9 gene editing (Fig. 3e). A new version of the SORT nanoparticle platform containing a membrane-destabilizing ionizable phospholipid has further improved mRNA delivery efficiency[146]. A mechanistic study revealed that the binding of specific proteins to the nanoparticles' surfaces enables their selective accumulation in different tissues[147]. It is also worth noting that although development and optimization of new nanoformulations for organ-specific

delivery of mRNA via intravenous injection is meaningful, changing the routes of administration can be a more practical solution in some contexts. For instance, alternative routes of administration include intravesical delivery of mRNA nanoparticles to target bladder-specific sites[23], oral delivery of mRNA via robotic pills to target gastrointestinal tract sites[148], and inhaled delivery of mRNA nanoparticles to target lung-specific sites.

In addition to targeting organs, selective delivery of mRNAs to specific cell types allows for more precise and efficient therapies. One strategy for cell type-specific mRNA delivery is developing LNPs or polymeric nanoparticles with formulations optimized for the specific target cell type. For instance, well-optimized[149,150], biomimetic[151] and imidazole-based[152] LNPs and polymers[104,108] have been used for the targeted delivery of mRNAs to T cells for cancer immunotherapy, and selective delivery of mRNAs to leukocytes[153] has also been achieved. Another strategy is using cell-specific ligands. To enable targeted delivery of therapeutic mRNAs to Ly6c$^+$ inflammatory leukocytes in mice with inflammatory bowel disease[154], an anti-Ly6c targeting ligand was conjugated to the LNPs using a modular targeting platform named ASSET (anchored secondary single-chain variable fragment enabling targeting)[155]. One advantage of this platform is that the targeting monoclonal antibodies can be conveniently replaced according to different applications. Indeed, in another study, a ligand targeting the factor receptor epidermal growth factor receptor (EGFR) was coated to LNPs using the same ASSET platform[156]. These EGFR-LNPs selectively delivered Cas9 mRNAs and single guide RNAs (sgRNAs) to disseminated EGFR-expressing ovarian tumors in mice, suppressing tumor growth and increasing the survival rate via efficient CRISPR–Cas9 gene editing. Selective delivery of mRNAs to antigen-specific CD8$^+$ T cells[157] or CD4$^+$ T cells[158] can be achieved by conjugating antigen or CD4 antibodies to LNPs, respectively. With more research, these organ- or cell-specific mRNA delivery platforms will expand the types of diseases that can be treated by mRNA therapies.

Inhalable, intranasal or oral mRNA delivery. Inhalable delivery allows rapid and selective accumulation of mRNA drugs in the lungs, offering great promise for the treatment of lung-related diseases that have been prevalent since the current COVID-19 pandemic began. Inhaled delivery of mRNAs to the lungs was demonstrated using an hPBAE-based nanoformulation, leading to high luciferase protein expression in the lungs of mice[19]. A subsequent study using this hPBAE platform has achieved efficient Cas13a mRNA delivery to the lungs of mice and hamsters, resulting in the degradation of influenza RNA and reduction of SARS-CoV-2 replication and infection symptoms[159]. As another non-invasive administration method, intranasal delivery of vaccines can elicit mucosal immunity against respiratory pathogens, making it a promising administration method for SARS-CoV-2 vaccines[113]. Indeed, intranasal delivery of SARS-CoV-2 mRNA vaccines protected mice from SARS-CoV-2 infection, as demonstrated by the lower viral titer and less tissue damage in the lungs[160]. One mouse study suggested that immune responses induced by intranasal delivery of mRNA vaccines were lower than those given by intramuscular delivery; a potential reason for this is that the LNPs used nasally were not specifically designed for this delivery route[161]. LNP formulations targeting appropriate cell types in the upper respiratory tract may mitigate these problems.

Intramuscular injection is the major administration route for currently approved COVID-19 vaccines, but is limited by the requirement for medical or pharmaceutical staff, which may negatively impact vaccine rollout. Oral delivery provides a promising and attractive alternative for COVID-19 vaccine administration, due to its non-invasiveness, patient-friendly features and the possibility for rapid rollout. Encouragingly, an oral adenovirus type 5 SARS-CoV-2 vaccine has successfully reduced disease severity and transmission in a SARS-CoV-2-infected hamster model, leading to a phase 1 clinical trial (NCT04563702)[162]. Although oral delivery is a more challenging route for fragile mRNAs, its feasibility has been demonstrated in rodents and pigs using ingestible milli-injector capsules, holding great promise for the development of oral mRNA vaccines[163,148]. BioNTech and Matinas BioPharma recently announced an exclusive research collaboration to develop potential oral mRNA vaccines using a novel lipid nano-crystal platform[164]. This lipid nano-crystal—a stable crystalline nanoparticle containing multiple layers—is formed via the interaction of calcium and anionic phospholipids, during which active drug molecules inducing mRNAs can be loaded within the layers[165].

Translational and clinical studies of mRNA nanomedicine

The aberrant expression of proteins is characteristic of a wide range of diseases. As mRNA technology rapidly progresses, accurate manipulation of the levels of a specific protein can be easily achieved via intracellular delivery of mRNAs encoding the protein of interest (upregulation) or mRNAs encoding gene-editing components (downregulation), making mRNA nanomedicine a promising and versatile tool for the treatment of various diseases. Currently, a range of mRNA nanomedicines, including vaccines (Table 1) and protein or gene-editing therapies (Table 2), are being intensively investigated in clinical studies[166].

Vaccines

The two effective mRNA COVID-19 vaccines were developed and rolled out at an unprecedented speed, potentially saving millions of lives and helping rebuild societies worldwide[27]. However, mRNA COVID-19 vaccines were not the first mRNA nanomedicines to enter clinical trials; many began clinical trials years earlier but their progress has been relatively slow. The success of the mRNA COVID-19 vaccines has strongly fueled the enthusiasm of both investors and researchers in mRNA nanomedicines, leading to numerous innovations and rapid progress in clinical development. While CureVac's first unmodified mRNA COVID-19 vaccine (CVnCoV) showed an unsatisfying efficacy of 48% (ref. [167]) in a phase 2/3 trial (NCT04652102), their second-generation CV2CoV displayed improved efficacy in preclinical studies[168] and is now in phase 1 clinical development (NCT05260437). Arcturus developed a self-replicating RNA-based COVID-19 vaccine that protected mice from SARS-CoV-2 infection with a single dose of 2 μg (ref. [169]); the phase 3 trial (NCT05012943) showed that their vaccine has 95% efficacy for the prevention of severe COVID-19 disease and 55% efficacy for preventing symptomatic COVID-19 disease[170]. Notably, Delta and Omicron variants were dominant during the study.

In addition to COVID-19 vaccines, the development of mRNA vaccines against many other infectious diseases has progressed in recent years. For example, Moderna's mRNA-1647 vaccine (NCT05085366; encoding cytomegalovirus pentamer complex and glycoprotein B antigens against cytomegalovirus) and mRNA-1345 vaccine (NCT05127434; encoding stabilized prefusion F glycoprotein against the respiratory syncytial virus) are being tested in phase 3 trials. More recently, Moderna's mRNA-1010 seasonal quadrivalent influenza vaccine, encoding World Health Organization-recommended strains, has entered phase 3 trials, making it the fourth mRNA vaccine from Moderna to reach phase 3 (NCT04956575)[171]. Besides infectious diseases, cancer is another major target of mRNA vaccines being intensively investigated in clinical trials. The Moderna/Merck mRNA-4157 vaccine for advanced melanoma is being investigated in a phase 2 trial (NCT03897881). This is a personalized mRNA cancer vaccine encoding up to 34 neoantigens identified and designed using next-generation sequencing and workflow automation. Similarly, BioNTech has also launched its BNT111 vaccine for melanoma, which has induced durable objective responses in checkpoint inhibitor-treated patients with melanoma in a phase 1 trial (NCT02410733)[172].

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Table 1 | Selected completed and ongoing clinical trials of mRNA vaccines

Product name	Sponsor	Immunogen	mRNA payload	Disease	Trial number	Phase	Status	Comments and references
Infectious disease								
mRNA-1273	Moderna	Stabilized S protein of wild type	Nucleoside modified	SARS-CoV-2	NCT04470427	3	Active, not recruiting	94% efficacy[25] and FDA approved
mRNA-1273.214	Moderna	Stabilized S protein of wild type and Omicron variant	Nucleoside modified	SARS-CoV-2	NCT05249829	2/3	Recruiting	Lead bivalent booster for 2022[205]
BNT162b2	BioNTech–Pfizer	Stabilized S protein of wild type	Nucleoside modified	SARS-CoV-2	NCT04368728	2/3	Recruiting	95% efficacy[26] and FDA approved
CVnCoV	CureVac	Stabilized S protein of wild type	Unmodified	SARS-CoV-2	NCT04652102	2/3	Active, not recruiting	Sequence optimized, with 48% efficacy[167]
CV2CoV	CureVac–GSK	Stabilized S protein of wild type	Unmodified	SARS-CoV-2	NCT05260437	1	Recruiting	Sequence and UTR optimized[168]
ARCT-154	Arcturus	S protein	Self-replicating	SARS-CoV-2	NCT05012943	2/3	Active, not recruiting	Low dose[169,170]
mRNA-1647	Moderna	CMV pentamer complex and glycoprotein B antigens	Nucleoside modified	CMV	NCT05085366	3	Recruiting	
mRNA-1345	Moderna	stabilized F glycoprotein	Nucleoside modified	RSV	NCT05127434	3	Recruiting	
mRNA-1010	Moderna	WHO strains	Nucleoside modified	Seasonal influenza	NCT04956575	2	Recruiting	Entering phase 3[171]
Cancer								
mRNA-4157	Moderna–Merck	Up to 34 neoantigens	Nucleoside modified	Melanoma	NCT03897881	2	Active, not recruiting	Personalized cancer vaccine
BNT111	BioNTech	Four non-mutated TAAs	Unmodified	Melanoma	NCT04526899	2	Recruiting	LPXs, with or without cemiplimab[172]
BNT113	BioNTech	Oncoproteins E6 and E7	Unmodified	HPV16+HNSCC	NCT04534205	2	Recruiting	With pembrolizumab

CMV, cytomegalovirus; FDA, US Food and Drug Administration; GSK, GlaxoSmithKline; HNSCC, head and neck squamous cell carcinoma; HPV16, human papillomavirus 16; LPXs, lipoplexes; RSV, respiratory syncytial virus; TAAs, tumor-associated antigens; WHO, World Health Organization.

Protein therapy and gene editing

mRNA-based protein and gene editing therapy has several potential clinical applications (Table 2). Chimeric antigen receptor T cell (CAR-T cell) therapy has demonstrated great efficacy in the treatment of liquid tumors[173,174], but its application to solid tumors has proven challenging—partly due to the lack of available targets. Meanwhile, standard CAR-T cell therapy requires the modification of patients' T cells outside the body, which is expensive and time consuming. To potentially address these challenges, BioNTech has identified several novel solid tumor antigens and developed a CAR-T cell therapy (BNT211) for solid tumors (NCT04503278), in which an mRNA lipoplex encoding CAR-T target antigens is administered to the patient and produces functional CAR-T cells in vivo. In addition to tumors, mRNA-based CAR-T cell therapy has also shown potential for the treatment of cardiac injuries—by generating transient antifibrotic CAR-T cells in vivo (in mice) using modified mRNAs[175].

Gene editing is another important application of mRNA nanomedicine[176–178] that enables mRNAs to downregulate the levels of a specific protein, similar to the function of siRNAs[4,94,95,179]. In primates, a single-dose treatment of LNPs loaded with mRNAs encoding a CRISPR adenine base editor achieved almost complete knockdown of PCSK9 in the liver, along with 90 and 60% decreases in blood levels of PCSK9 and low-density lipoprotein cholesterol, respectively. Surprisingly, the effect of this treatment persisted for over 8 months[180]. Intellia developed a biodegradable LP01 ionizable lipid-based system for Cas9 mRNA and sgRNA delivery, achieving >97% knockdown of serum transthyretin upon a single-dose treatment[92]. These data have led to the human trial (NCT04601051) of an LNP-based gene-editing drug (named NTLA-2001), in which an 87% reduction in the serum transthyretin protein concentration was achieved after a single dose of 0.3 mg kg^{-1} (ref. [181]). These encouraging data will strongly stimulate the clinical translation of more mRNA-based gene-editing therapies.

Challenges of current mRNA nanomedicine

Despite the success of COVID-19 vaccines, mRNA nanomedicines in development still face several challenges. Further innovations and advances are required to overcome these challenges and speed up the clinical translations of more mRNA nanomedicines.

Safety

MC3 is a potent ionizable lipid used in some ongoing clinical trials. However, several preclinical studies have shown that the MC3-based LNPs were immunostimulatory and induced higher expression of proinflammatory cytokines than other LNPs in mice[87,182]. In addition, intravenous administration of MC3-based human erythropoietin (hEPO) mRNA LNPs to rats and monkeys has resulted in mild toxicological effects with an mRNA dose level of 0.3 mg kg^{-1} (ref. [183]). In this study, liver injury, variations in white blood cell counts and coagulation parameters were observed in rats, whereas reversible complement activation, splenic necrosis and depletion of lymphocytes were observed in monkeys. Notably, the toxicological effects were ameliorated when decreasing the mRNA dose to a lower therapeutic level (0.03 mg kg^{-1})[183]. Off-target effects of mRNA LNP vaccines or therapies can lead to undesired mRNA translation in various cells or organs, making them targets of killing[184].

Table 2 | Selected completed and ongoing clinical trials of mRNA nanomedicine for protein replacement and gene editing

Product name	Sponsor	Target protein	mRNA payload	Disease	Trial number	Phase	Status	Comments and references
Protein replacement								
BNT211	BioNTech	CLDN6	Unknown	Solid tumors	NCT04503278	1/2	Recruiting	CAR-T cell therapy for solid tumors
BNT141	BioNTech	Cancer antibodies	Nucleoside modified	Solid tumors	NCT04683939	1/2	Recruiting	Target CLDN18.2
ARCT-810	Arcturus	OTC	Unknown	OTC deficiency	NCT04442347	1	Recruiting	Entering phase 2 (refs. [206,207])
mRNA-6231	Moderna	IL-2 mutein	Nucleoside modified	IL-2 autoimmune disorders	NCT04916431	1	Recruiting	
Gene editing								
NTLA-2001	Intellia	TTR	Cas9 and TTR sgRNA	hATTR	NCT04601051	1	Recruiting	87% protein reduction[181]

CLDN18.2, claudin 18.2; CLDN6, claudin 6; hATTR, hereditary transthyretin amyloidosis; IL-2, interleukin-2; OTC, ornithine transcarbamylase; TTR, transthyretin.

Continued optimization of organ- or cell-specific mRNA delivery systems will help to address this issue.

Scattered reports have suggested the possibility of the integration of SARS-CoV-2 RNA[185] and mRNA from COVID-19 vaccines[186] into the genome of host cells through a LINE1-mediated retro-position mechanism. However, this conclusion has been questioned by others[187] and more meticulous studies should be conducted to validate this conclusion. If more future studies do show that engineered mRNA sequences can be integrated into the genome of host cells, specific designs can be applied to current mRNAs to inhibit their retro-transcription.

Clinical trials have shown the favorable safety profiles of the two approved COVID-19 mRNA vaccines, and most local and systemic adverse events are mild to moderate[25,26]. However, as with most other vaccines, rare cases of anaphylactic reactions have been observed[188]. In the case of mRNA vaccines, one possible reason for these anaphylactic reactions is that the PEGylated lipids in LNPs can induce allergic reactions due to pre-existing antibodies (present in up to 40% of the population)[189]. It is worth noting that the PEG dose used in current COVID-19 vaccines is much lower than that in other clinical agents and therapies that are reported to trigger rare anaphylactic reactions[189,190], and a recent study showed that a patient with pre-existing anti-PEG antibodies tolerated a COVID-19 mRNA vaccine without anaphylactic reactions being triggered[191]. Therefore, other underlying factors may be associated with these rare adverse events, and a phase 2 clinical trial (NCT04977479) has recently been launched to investigate allergic reactions to the COVID-19 mRNA vaccines.

It will be important to recognize which parts of the mRNA vaccines are responsible for these adverse events, especially in the context of treating protein deficiency and chronic diseases, where high dose or repeated dosing are required, which could further increase the risk of such events. A better understanding of these mechanisms can lead us to optimized formulations that reduce or replace the unfavorable component, thus lowering the risk of adverse events.

Adjuvanticity

Adjuvanticity can be a limitation or an advantage, depending on the context. Due to enhanced translation efficiency and stability, modified mRNAs that cannot elicit immune responses are widely used in ongoing clinical trials[11,113]. Thus, the mRNAs themselves within mRNA LNP formulations usually do not act as adjuvants. In contrast, the other part of the formulation, the LNP, is known to have adjuvanticity. Mice immunized with intramuscular injection of LNPs and 10 μg of the recombinant hemagglutinin protein immunogen have produced higher antigen-specific T follicular helper cell and germinal center B cell numbers than mice immunized with hemagglutinin protein alone, demonstrating the adjuvant activity of LNPs[192]. A recent study has also shown that the LNPs of SARS-CoV-2 mRNA vaccines could act as an adjuvant component that induces robust T follicular helper cell and humoral responses[193]. Notably, LNP has displayed stronger adjuvant potency than a widely used adjuvant, AddaVax[193]. While neither of the LNP-based COVID-19 mRNA vaccines contains any adjuvants, the strong cellular and humoral immune responses against SARS-CoV-2 elicited by these vaccines might be partially attributed to the adjuvant effect of the LNP component itself. Besides acting as an adjuvant in vaccines for infectious diseases, LNP has also potentiated the antitumor efficacy of the mRNA cancer vaccine by activating TLR4 signaling[194]. Although more and more studies have demonstrated the adjuvanticity of LNPs, how to manipulate this adjuvanticity is still challenging. To this end, the mechanism of action of LNP adjuvant and the structure–activity relationship of the lipid components should be investigated.

Conclusions and future directions

mRNA nanomedicines have already shown efficacy as vaccines for the prevention of COVID-19 and reducing the risk of hospitalization and death[3,27–29]. Encouraged by this success, more and more mRNA-based vaccines and therapies are expected to reach clinical translation. However, several crucial goals must be reached before the potential of mRNA nanomedicines is fully realized. Increasing evidence suggests that specific biological pathways may interfere with mRNA delivery or translation[195,196]. Thus, understanding how biological pathways affect in vivo mRNA delivery and translation can further improve the efficacy of mRNA drugs, while the potential toxicity and immune response raised by mRNAs and their carriers should also be carefully considered.

Ultimately, the quick and efficient implementation of mRNA nanomedicines depends largely on their stability and logistical requirements, which impact real-world implementation and rollout. Therefore, innovations that improve stability will be crucial. In recent studies, a thermostable mRNA vaccine was reported to provide protective efficacy against SARS-CoV-2 in mice[197] and entered clinical trials[198]. Moderna's next-generation COVID-19 vaccine mRNA-1283 could be stable at 2–5 °C.

New engineering advanaces will facilitate real-world applications of mRNA nanomedicines in myriad ways. For example, novel PLGA microparticles could be a promising platform for mRNA delivery with programable drug release and even enable self-boosting vaccines[199–201]. In addition, microneedle patches, which have demonstrated their safety and immunogenicity as carriers for seasonal influenza[202] and SARS-CoV-2 (refs. [203,204]) vaccines, could be a convenient and minimally invasive platform for mRNA delivery.

Since the enormous potential of mRNA nanomedicines has already been demonstrated by the unprecedented mRNA COVID-19 vaccines, we expect that continued innovation will lead to new and highly efficient mRNA-based therapies, including

vaccines for other non-COVID-19 infectious diseases, cancer immunotherapy, protein therapy, gene-editing-based therapy and potentially many others.

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Competing interests
R.L. declares the following current competing interest: Moderna. A list of all competing interests for R.L., past and present, is provided as Supplementary Table 1. The other authors declare no competing interests.

Additional information
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