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Unlocking the promise of mRNA therapeutics

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The extraordinary success of mRNA vaccines against coronavirus disease 2019 (COVID-19) has renewed interest in mRNA as a means of delivering therapeutic proteins. Early clinical trials of mRNA therapeutics include studies of paracrine vascular endothelial growth factor (VEGF) mRNA for heart failure and of CRISPR–Cas9 mRNA for a congenital liver-specific storage disease. However, a series of challenges remains to be addressed before mRNA can be established as a general therapeutic modality with broad relevance to both rare and common diseases. An array of new technologies is being developed to surmount these challenges, including approaches to optimize mRNA cargos, lipid carriers with inherent tissue tropism and in vivo percutaneous delivery systems. The judicious integration of these advances may unlock the promise of biologically targeted mRNA therapeutics, beyond vaccines and other immunostimulatory agents, for the treatment of diverse clinical indications.

The rapid design and development of two COVID-19 mRNA vaccines marked the advent of a new biotechnology platform for immunization against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, potentially, a wide spectrum of microbial pathogens and cancers[1–4]. The remarkably short timeframe from target identification to phase 1 clinical studies—and the convincing safety profile of mRNA vaccines after billions of administered doses—underscore the potential of a new generation of mRNA therapeutics that lies beyond vaccines and other agents that rely on the ability of mRNA and lipid nanoparticles (LNPs) to stimulate immune responses.

The pathway for the development of mRNA therapeutics presents additional challenges compared to those of mRNA vaccines (Fig. 1). Immunization requires only a minimal amount of protein production, as the immune system can markedly amplify the antigenic signal through cell-mediated and antibody-mediated immunity. In contrast, mRNA therapeutics require as much as a 1,000-fold-higher level of protein to reach a therapeutic threshold (Supplementary Table 1). In many cases, it will be necessary for mRNA therapeutics to engage a particular target pathway, cell, tissue or organ. This requirement places greater importance on the efficiency of uptake at the target cell, which drives the duration and level of expression. The tissue bioavailability, circulatory half-life and efficiency of the lipid-based carrier to deliver to the tissue of interest can be strictly rate limiting. Aside from the liver, which is readily targeted by intravenous (i.v.) delivery, efficient delivery to solid organs remains challenging. Another major hurdle is repeated dosing, which is often required in the treatment of chronic diseases. Even with optimized mRNA chemical modifications and advanced LNPs, chronic dosing eventually activates innate immunity, with concomitant attenuation of therapeutic protein expression[5,6]. Despite these remaining challenges, a host of emerging technologies is under development to systematically address them[2,7–11].

This review surveys the most promising of these new technologies. The first section discusses approaches for designing and purifying the mRNA cargo to enhance the duration and amplitude of protein production in vivo. These approaches include advances in the design of the primary chemical structure of the mRNA, novel forms of circular and self-amplifying mRNA and improved purification strategies. The second section explores improved mRNA packaging systems to enhance delivery of mRNA cargo, including ionizable LNPs, cells and cell-based extracellular vesicles. The third section discusses emerging approaches for targeting mRNA therapeutics to specific tissues, such as percutaneous catheters for delivery to the heart, pancreas and kidney, and the engineering of packaging systems with tissue-specific tropism. The fourth section considers strategies for allowing repeated dosing

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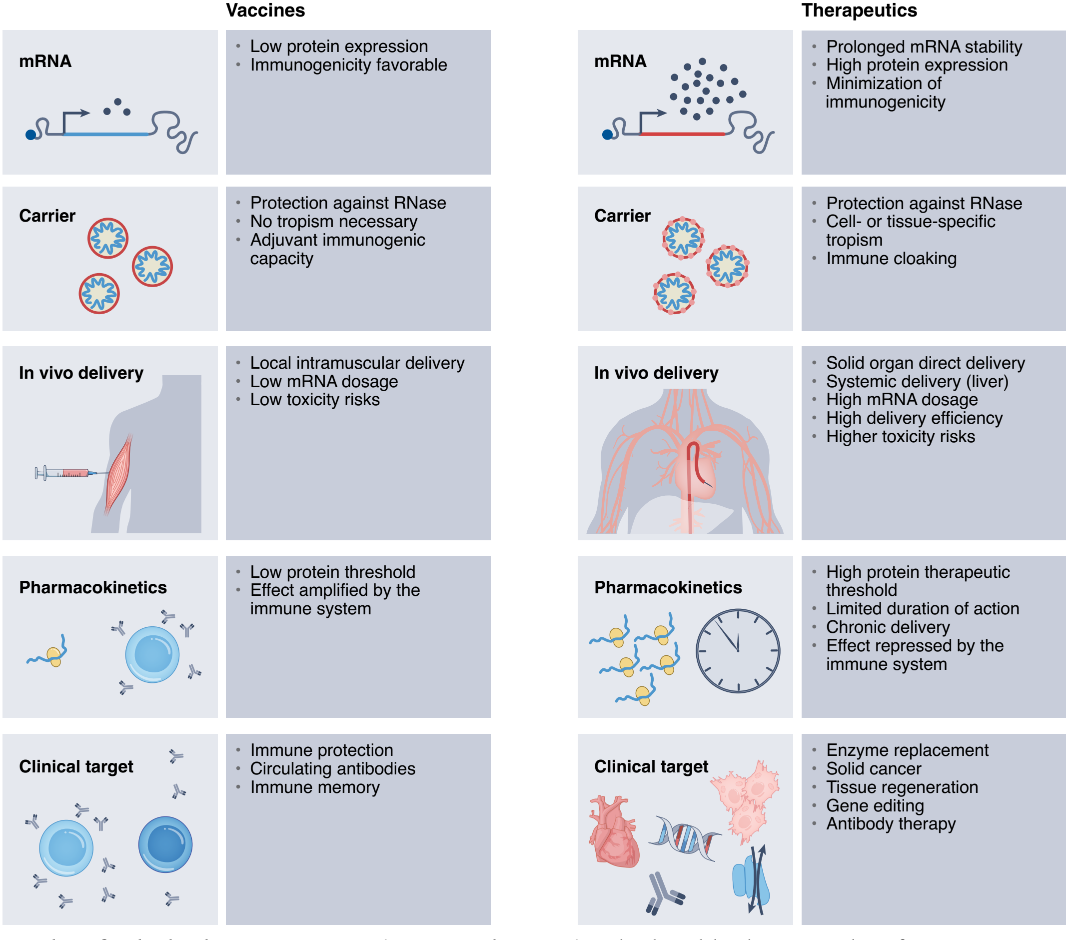

Fig. 1 | Comparative roadmap for the development mRNA vaccines versus therapeutics. The clinical development pathway for mRNA vaccines and therapeutics differs in several important respects.

for the treatment of chronic conditions. The fifth section provides a comprehensive table and summary of current clinical trends in mRNA therapeutics. Finally, the sixth section considers the scope of mRNA therapeutics and guiding principles for near-term and longer-term clinical development of this novel therapeutic modality.

Enhancing protein yield

The inherent immunogenicity of mRNA, although enhancing its efficacy as a vaccine, hinders its use as a therapeutic, which requires a much higher level of protein expression (Fig. 1). Mouse models in applications such as enzyme replacement, localized regenerative therapeutics and oncology typically require a 50–1,000-fold-higher mRNA dosage as compared to mRNA vaccines (Supplementary Table 1)[7,9–31]. The need for high levels of protein expression has led to multiple strategies for optimizing the mRNA cargo to minimize innate immune responses, enhance mRNA stability and maximize translation (Fig. 2). However, for any given indication, the properties of the mRNA cargo must be considered in relation to the efficiency of the delivery system—for example, direct versus systemic injection—and the modality of action of the protein of interest.

mRNA cargo

Each component of an individual mRNA—the cap, 5′ and 3′ untranslated regions (UTRs), open reading frame (ORF) and polyadenylated (poly(A)) tail—can be optimized to enhance protein expression (Fig. 2). 5′ cap analogs and 3′ poly(A) have been designed to maximize mRNA stability and translational efficiency through exonuclease protection and enhanced catalysis to the ribosomal complex[32–36]. Optimization of the poly(A) tail length (100–300 nucleotides) has proven critical in balancing the synthetic capability of a given mRNA[34,36]. Similarly, improved 5′ cap analogs not only increase translational capacity but also enhance capping efficiency, from 70% to 95%, greatly improving the in vitro transcription process[32,37]. The composition of the 3′ and 5′ UTRs can also be customized for the target cell of interest, increasing the efficiency and tissue specificity of translation[35,38,39].

At present, most mRNA products contain a synthetic UTR sequence from α-globin or β-globin[38–40], but UTR optimization can further improve protein expression by a few fold[41,42]. Careful screening and customization to the target of interest could conceivably offer a wide range of improvements in future UTR sequences, allowing each mRNA to be tailored to the targeted cell and disease-induced microenvironment to maximize protein synthesis per mRNA transcript[41–44].

Perhaps the most critical advances in mRNA vaccines and therapeutics lie in the discovery that the inclusion of chemically modified nucleosides, particularly in uridine moieties, can markedly increase protein expression after in vitro or in vivo transfection. The chemical modifications of most new RNA formulations to date have been central in intellectual property claims[45,46]. Thus far, over 130 different naturally occurring chemical modifications of RNA have been reported[47,48]. The interest in methylpseudouridine and other modified nucleosides centers on their capacity to greatly reduce (up to 100-fold) detection by the Toll-like receptors of the innate immune system, resulting in an increase in protein expression in vivo compared to unmodified mRNA[40,49–53]. Combinations of different types of chemical modifications, carriers,

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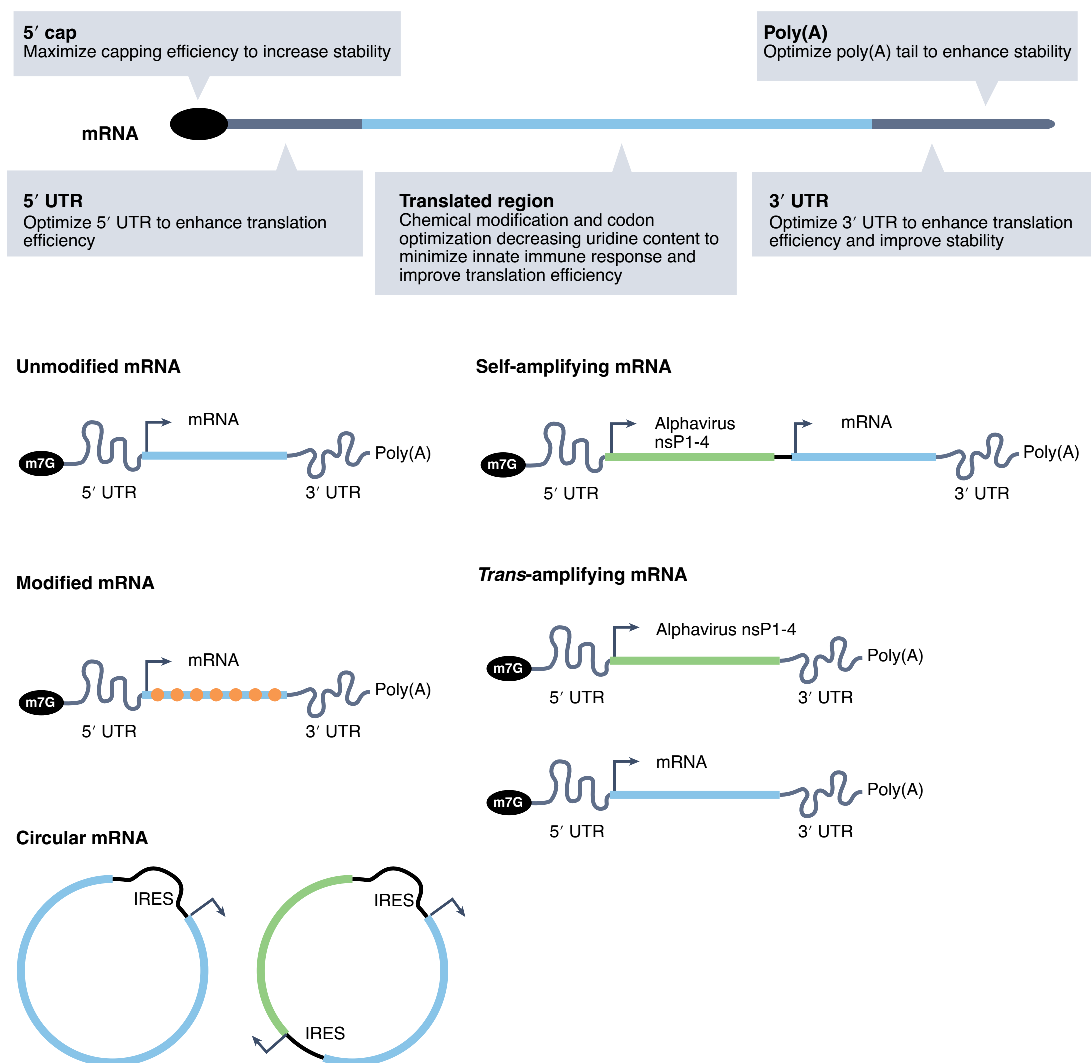

Fig. 2 | Modifications of mRNA to increase protein expression efficiency. Schematic drawing of different modifications of mRNA that are currently used in clinical application or are being investigated to increase protein expression efficiency.

methods of in vivo delivery and mRNA purity reveal a surprisingly diverse set of effects, suggesting that there may be additional room for optimization[37,40,49,50,54,55]. Furthermore, the properties and effects of many other RNA chemical modifications remain to be explored[48]. In addition to chemical modifications, further improvements may be possible by shifting from total to partial nucleoside substitution, as naturally synthesized mammalian mRNAs are typically only partially and heterogeneously chemically modified[47,52,54].

An mRNA's uridine content alone has a large influence on the activation of innate immunity. Using this insight, clinically effective unmodified mRNA vaccines have been generated that display immune-cloaking effects and enhanced translational efficiency in vivo similar to those of chemically modified mRNA vaccines[37,54]. Molecular understanding of optimal codon compositions is likely to be captured in codon-optimization algorithms that facilitate the generation of clinically effective, unmodified therapeutic mRNAs in the near future[56–58].

In addition to the amplitude of protein expression, a key limitation of mRNA therapeutics for chronic diseases is the relatively short duration of protein production, which necessitates repeated administration. In parallel to the above-mentioned mRNA structural optimizations, which tackle immune stimulation and protein expression level, several approaches in development aim to enhance the duration of protein expression (Fig. 2). Self-amplifying mRNAs (saRNAs) use the self-replication basis of an RNA alphavirus, which can amplify RNA transcripts in the cytoplasm, but replace the viral structural coded genes with the gene of interest[59–62]. Because saRNA transcript replication extends expression kinetics, it would be favorable for enzyme replacement therapy by decreasing the frequency of delivery[60]. This approach also increases protein expression, requiring ~10-fold less RNA for a similar amplitude of protein expression compared to linear modified mRNA, and is now being tested as an in vivo, scalable process for vaccine production[60–62]. Additionally, saRNAs can be delivered as two separate transcripts (trans-amplifying mRNA), which helps reduce the mRNAs' overall size (Fig. 2)[63,64].

Another alternative to linear mRNA is circular mRNA (circRNA) (Fig. 2). The back-folding of the RNA's loose ends during processing shields circRNA from exonuclease activity, which extends the RNA lifespan by two-fold in transfected HEK293 cells[65–67]. This extended longevity increases the total protein yield without increasing the amplitude of protein expression compared to linear modified mRNA[65–67]. Importantly, circRNA circumvents the need for costly 5′ capping and cumbersome 3′ poly(A) tail by introducing internal ribosomal entry site (IRES) sequences[66,67]. Moreover, circularization strongly reduces RIG-1 and Toll-like receptor recognition without using chemical substitution[65,66]. Inversely, total replacement of uridine by methylpseudouridine completely abrogated the translation of circRNA[66].

RNA purification

Standardization of mRNA quality is crucial when comparing publications and preclinical data in vitro and in vivo. Many studies show conflicting results regarding protein expression capacity or the level of immune stimulation with a variety of modified, unmodified, purified and unpurified mRNAs[37,40,49,50,54,55]. Most aspects of mRNA in vitro synthesis can give rise to varying proportions of unwanted side products, such as double-stranded RNA (dsRNA), uncapped mRNA or mRNA fragments. These side products can strongly interfere with mRNA translation, activate innate immunity or lead to overestimation of the total functional mRNA cargo[53,55,68]. High-performance liquid chromatography (HPLC) is often used for size purification of mRNA products. Other systems, such as cellulose purification, anion exchangers or hydrogen bonding, have been developed with a similar purpose[53,68,69]. Usage of chemically modified nucleosides or optimization of the nucleoside composition mix has been shown to reduce >3-fold dsRNA byproducts[53,55]. Notably, purification of either unmodified or modified mRNA increased expression of human EPO ≥400% and 30%, respectively, in mice, emphasizing the importance of purification[53,55].

Packaging systems

The inherent lability of mRNA requires a packaging/delivery system to protect it against degradation by nucleases and to allow efficient cellular uptake, intracellular release and translation into protein (Fig. 3a). Most of the mRNA therapeutics under development rely on LNPs, which were initially reported over six decades ago[70]. LNPs have since undergone numerous alterations and advancements, culminating in their first clinical use for the delivery of small interfering RNA (siRNA)[71,72]. Meanwhile, packaging systems based on cells, extracellular vesicles and biomimetic vesicles are being developed and validated in preclinical studies as alternative approaches.

Lipid-based packaging

Current versions of RNA-loaded LNPs are advanced derivatives of the phospholipid-based liposomes first generated in the 1960s[70]. Today, LNPs are composed of four key components: structural lipids, cholesterol, ionizable cationic lipids and stealth lipids (Fig. 3b). Structural lipids are the fundamental scaffold of LNPs and are mainly neutrally charged phospholipids. The addition of cholesterol at various ratios stabilizes the LNP structure and enables modulation of its properties, such as membrane fluidity, elasticity and permeability[73–75]. Positively charged cationic lipids are needed for the loading of negatively charged nucleic acids into LNPs[76,77]. However, they have considerable drawbacks as well. Cationic lipids induce cytotoxicity, opsonization with plasma proteins and low transfection efficiencies due to rapid splenic and hepatic clearance[78–81]. Therefore, intensive efforts were made to modulate their physiochemical properties, which resulted in the discovery of pH-sensitive ionizable cationic lipids that can substantially reduce LNP immunogenicity. Ionizable cationic lipids are neutral in charge in the circulation, which cloaks them from cellular or molecular recognition. After cellular uptake by the endosomal pathway, they become ionized and fuse with the endosomal membrane, releasing the mRNA cargo into the cytoplasm for subsequent translation[77,82,83] (Fig. 3a). Various ionizable lipids have been developed, such as the DLin-MC3-DMA (MC3) lipid reported in 2012 (refs. [84–87]). MC3-composed LNPs showed a functional ED_{50} ~20-fold lower in mice and non-human primates than the previous gold-standard ionizable lipid KC2. These improvements in efficacy contributed to the first clinical delivery and regulatory approval of the siRNA Onpattro in 2018 (refs. [71,72]). Current authorized COVID-19 mRNA vaccines use MC3 analogs (Moderna's SM-102 and Pfizer's ALC-0315) with improvements in the lipid's toxicity and biodegradability profile[87,88].

Stealth lipids, mainly polyethylene glycol (PEG) polymer–conjugated lipids, have been added to the composition of LNPs to reduce immunogenicity. PEGs are broadly used to augment the colloidal stability of nanoparticles in fluids[89–92] and are physiologically inert because they cloak potential epitopes. These capacities to reduce aggregation and opsonization also improve the immunogenicity and in vivo retention of PEGylated LNPs, enhancing safety and efficacy[89,93,94]. Interestingly, incorporation of PEGylated lipids facilitates the manufacturing of small, homogeneous LNPs, typically 50–100 nm in diameter, which makes them less likely to activate the immune system[86,93,94]. On the other hand, growing concerns regarding PEG hypersensitivity may limit the utility of PEGylated lipids for therapies requiring chronic administration[89,95,96]. Current research focuses on further optimization of PEGylated lipids or the development of different stealth lipids, such as polysarcosine-conjugated lipids[97].

Cell-based packaging

An alternative to LNPs is the use of biological delivery vehicles such as cells. Rather than delivering an mRNA cargo into targeted cells, this approach harnesses the cellular paracrine function to directly deliver proteins synthesized from mRNA introduced into cells ex vivo. It offers many advantages compared to synthetic LNPs, including biocompatibility, extended longevity in circulation and endogenous intracellular/intercellular signaling[98,99] (Fig. 3c). Cells have been used as drug carriers to deliver enzymes, therapeutic drugs or lipid particles to targeted sites. A wide range of customization is possible by introducing mRNA into a variety of available cell types (for example, immune cells, blood cells and mesenchymal cells) and through further genetic engineering[98–102]. This approach could also be combined with existing cellular therapies to accentuate desired therapeutic or kinetic effects. However, cell-based delivery of mRNA therapeutics may be limited by the same caveats that apply to cell therapies in general, such as donor haplotype compatibility, homogeneous production, cumbersome quality control and a restricted intracellular delivery capacity[103] (Fig. 3c).

In 2019, our group reported the successful intramuscular delivery of VEGF protein by injecting skin fibroblasts pre-loaded with modified mRNA encoding VEGF into mice[104]. The treatment significantly decreased tissue necrosis by increasing vascular density in the murine ischemic limb. In a follow-up study, we further examined the therapeutic potential of this approach by delivering rat bone marrow–derived mesenchymal stem cells (MSCs) pre-loaded with modified mRNAs encoding VEGF and BMP-2 in a mouse model of skull defect[102]. Treated animals showed improved osteogenesis and vasculogenesis, resulting in skull healing[102]. Others have shown similar successful delivery of mRNAs or protein cargos using MSCs, neutrophils, monocytes and erythrocytes[98–101,103].

Extracellular vesicle–based packaging

Another novel approach uses extracellular vesicles (EVs) as a delivery vehicle. EVs encompass a heterogeneous group of extracellular bilayer membrane vesicles produced by most, if not all, cell types[105]. The characteristics and biogenesis of EVs have been extensively discussed elsewhere[106–108]. In mammalians, there are three major types of EVs based on their size and intracellular origins[106,107]. Exosomes (50–150 nm), which are the most studied and characterized EVs, shuttle and deliver their cargo between cells through endocytosis and exocytosis. After uptake by recipient cells, exosomes are processed by the endosome similar to LNPs. However, exosomes are further processed intracellularly for 'sorting' and 'exchanging' before being degraded by the lysosome or shuttled toward other cells. Microvesicles (50–500 nm) are produced at a low rate through plasma membrane budding and apoptotic bodies (>1 µm), which are a specific feature of apoptotic cells.

Studies suggest that EVs play a crucial role in homotypic and heterotypic intercellular communications throughout the body[107]. They can carry and deliver a variety of cargos, ranging from metabolites, short nucleic acids and amino acids to full-length mRNAs and proteins[106,109–111]. As natural vesicles, EVs possess multiple advantages in terms of drug delivery, such as biocompatibility and hypoimmunogenicity[106]

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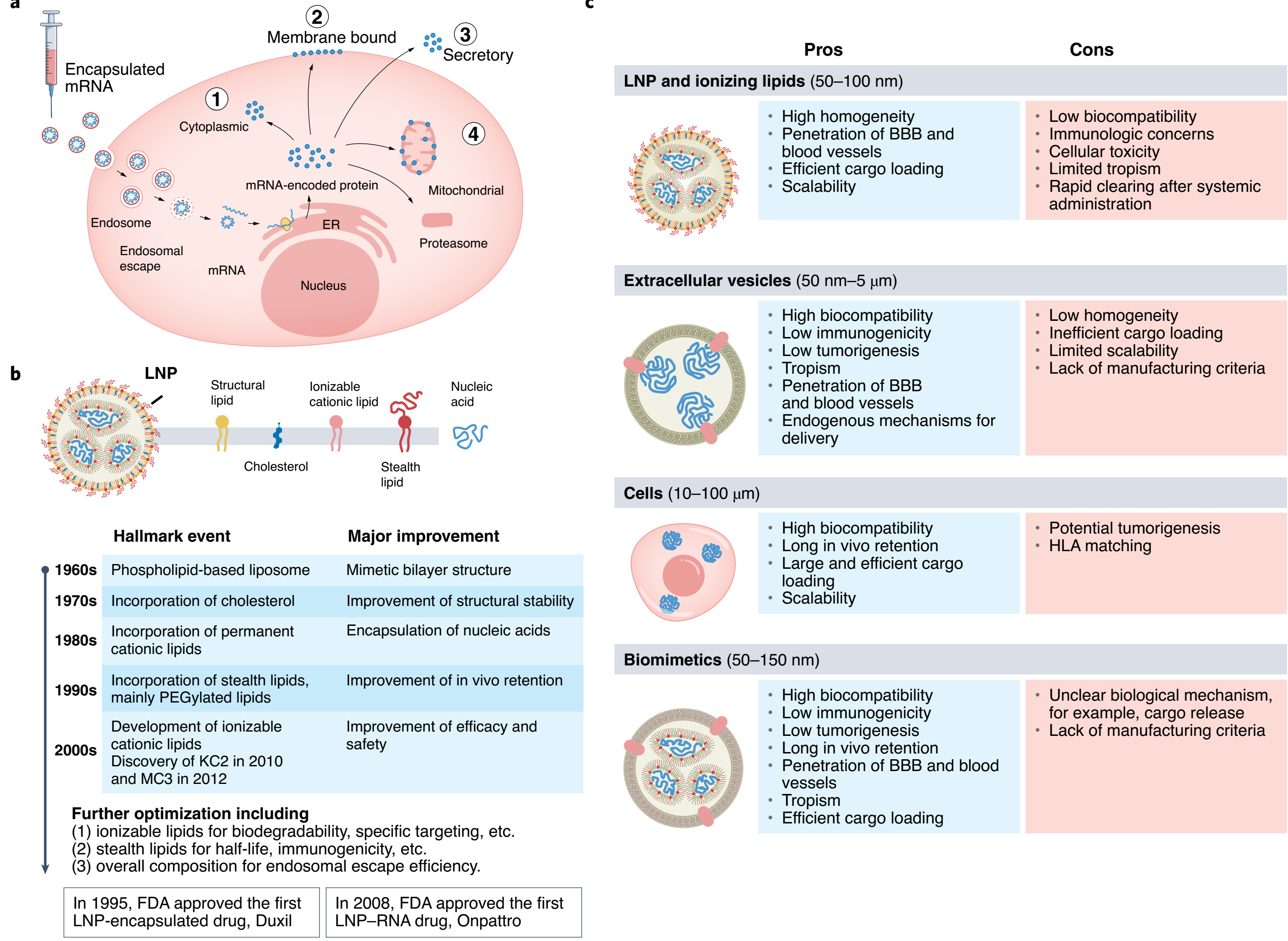

Fig. 3 | Modular delivery systems for mRNA. a, Schematic drawing of the intracellular delivery of mRNA and translation into protein. **b**, Timeline development and milestone improvement of LNPs. **c**, Pros and cons of advanced carriers. ER, endoplasmic reticulum; HLA, human leukocyte antigen.

(Fig. 3c). At present, the function and applications of EVs and, in particular, exosomes are being intensively explored in diagnosis, prognosis and therapeutics for oncology and cardiovascular diseases, with a strong focus on the EVs' immunomodulatory and cargo delivery properties[106,112,113].

Similarly to cell-based delivery systems, the cellular source from which EVs are derived is crucial for their potential application. It is thought that EVs can 'inherit' properties from their parental cells. For example, EVs derived from blood cells conserved their capacity to penetrate the blood–brain barrier (BBB) after systemic administration[109,114,115]. Likewise, EVs derived from MSCs possessed similar anti-inflammatory and paracrine properties[116,117]. Furthermore, preclinical studies suggest that EVs derived from a variety of cell types do not induce toxicity and are well-tolerated after repeated dosing[118]. Therefore, EVs might not only act as an inert vehicle but can also potentially be engineered for specific delivery and repeated dosing[119,120]. Fundamental challenges revolve around the characterization, isolation and purification of homogeneous EVs, as typical biomarkers still need to be identified and standardized. Different isolation methods have been comprehensively examined[113,121–123]. An additional challenge is efficient loading of EVs. Strategies typically involve post-loading of the cargo directly with isolated EVs via conventional methods (for example, electroporation, sonication, extrusion or freeze–thaw cycles) or pre-loading the desired cargo into the parental cells before EV isolation[124].

Recently, several approaches have been reported to enhance pre-loading specificity, including forward screening and targeted engineering. Candidate protein moieties were identified for efficient drug delivery in vivo through a systemic screening of moieties that participate in cargo loading of EVs[125]. By using arrestin domain-containing protein 1 (ARRDC1) to recognize an mRNA or protein of interest (for example, p53 or CRISPR–Cas9 complex), one can induce specific and efficient loading of AARDC1-mediated microvesicles (ARMMs) and successfully deliver their cargo in vitro and in vivo[111,126].

Another pre-loading approach consists of generating atypical EVs, such as virus-like particles, in mammalian cells using endogenous homologs of viral capsid genes, which enables preferential loading of the virus-like particles with mRNA containing specific motifs. Based on this technique, selective delivery systems (SEND and eVLPs) were developed to package and deliver specific RNAs in vitro and deliver CRISPR–Cas9 in vitro and in vivo[127,128].

Biomimetic packaging

Recent advances in drug delivery have highlighted the advantages of biomimetic packaging, which combines aspects of biological and synthetic particles (Fig. 3c). One such combination uses a synthetic core, with defined binding properties to encapsulate the cargo (for example, gold, silica, LNPs or polymers), that is then coated with a cellular membrane[129]. The coating alleviates the immunogenicity of the synthetic materials (for example, anti-PEG antibody), enables

tissue targeting based on the cell source and extends particle stability in the circulation. Such coatings have been implemented with membranes from various cell types, including erythrocytes[130], platelets[131], immune cells[132,133], stem cells[134], tumor cells[135,136] and MSCs[137]. Additionally, membranes from multiple cell types can be hybridized to achieve the desired properties[138–141].

A biomimetic approach that is complementary to coating consists of fusing biological (for example, EVs) and synthetic (for example, LNPs) components to form hybrid particles[142]. Such hybrid particles possess the controlled manufacturing and stable storage capacities of LNPs while retaining the biocompatibility and targeting specificity of EVs[142,143]. Although the current data are promising, this approach is at an early stage, and detailed mechanistic insights remain to be defined. An exosome/polymer hybrid was reported to be four-fold more stable in circulation and to possess enhanced storage stability and pharmacokinetic properties[144]. In another study, administration of hybrid particles composed of exosomes genetically engineered to favor immune cell recruitment and liposomes packaged with a chemotherapeutic drug efficiently inhibited tumor development in a murine carcinoma model[145]. We can expect rapid development of these novel delivery mechanisms thanks to the recent milestone success of mRNA vaccines, which highlighted both the great potential and current limitations of LNPs.

Tissue targeting

Realizing the full potential of mRNA therapeutics will require more advanced in vivo delivery systems, particularly for solid organs such as the heart, kidney, brain and lungs. The liver is the organ of choice when it comes to ease of delivery for most molecular therapies. Its fenestrated vasculature facilitates efficient homogeneous delivery and the passage of large particles. Thus, simple i.v. administration enables efficient hepatic expression of mRNA cargos with subsequent therapeutic levels of protein (Supplementary Table 1). However, targeting of most organs other than liver requires improved delivery systems, whether directly via catheters[146] or by engineering of packaging systems with appropriate tropism. Every organ has its own advantages and obstacles for efficient delivery. Therefore, specific approaches are being developed for each organ that we discuss here.

Injection, inhalation and intranasal administration

The kidney, unlike the liver, filters out large compounds and allows only small molecules to pass through. The glomerulus actively eliminates proteins above 50 kDa, and constitutive podocytes create slit diaphragms with diameters of merely 10 nm, impeding most molecular therapies delivered from the circulation to the kidney[147]. Direct subcapsular injection into the kidney's medulla or cortex can be achieved by varying the insertion depth of a needle or catheter. Efficient local delivery to the different compartments of the kidney is possible using several routes of administration[148]: (1) renal artery, targeting the glomeruli and tubular epithelium; (2) retrograde renal vein, predominantly targeting the renal tubules through the basolateral domain. Similarly to what occurs in the renal artery, increased localized pressure in the renal capillaries creates transient pores on cell membranes, resulting in nucleic acid extravasation[149]; (3) retrograde ureteral, targeting the tubular epithelium; and (4) intraparenchymal, with a few reports demonstrating the suitability of this route for treatment of renal diseases by gene therapy and oligonucleosides[150,151]. Because specific pathologies are associated with different renal compartments and cell types, drug delivery should be targeted to the cell type associated with a specific pathology.

No mRNA therapies for the kidney have yet reached the clinic, but, of the few clinical studies involving miRNA, two are for renal disease. One of the drugs targeting miR21 (RG012, lademirsen) was developed for Alport nephropathy—a genetic disorder characterized by chronic glomerulonephritis that progresses to end-stage renal disease in young adult life—and is currently undergoing a phase 2 clinical trial (HERA, NCT02855268). The other miRNA-based therapy, an antagomir-inhibiting miR17 (RGLS4326), was developed for the treatment of autosomal dominant polycystic kidney disease and is undergoing a clinical phase 1 trial (NCT04536688).

The lungs can be reached immediately via inhalation, permitting the use of lower drug dosage and, thus, reducing adverse systemic side effects. Attractive systems for pulmonary delivery enable direct, rapid and non-invasive access to the alveoli and lung parenchyma. Moreover, the airside of the lung provides a favorable environment for RNA integrity as its nuclease activity is lower than in serum[152]. Inhalation delivery also entails specific challenges. The mRNA must be highly concentrated to withstand the shear forces during aerosolization[153]. The large surface area (~100 m^2) and the presence of a protective mucosa on the surface of the lung epithelium are natural barriers for efficient mRNA delivery. Therefore, a successful pulmonary RNA therapeutic must preserve the integrity of the mRNA, penetrate through the mucosa, infiltrate the cells and release its mRNA cargo. Early clinical data from a phase 1/2 trial for cystic fibrosis (RESTORE-CF, NCT03375047) testing an mRNA encoding cystic fibrosis transmembrane conductance regulator (CFTR) delivered by inhalation (nebulization) have shown a promising safety profile for chronic mRNA delivery but failed to show any significant improvement in lung function[154–156].

The brain is both the most genetically complex organ in the body and the most difficult to treat. It is encased protectively by the skull and meninges and isolated biochemically by an extraordinary microvasculature (the BBB), composed of endothelial cells coupled by tight junctions and adherent processes[157]. The restrictive nature of the BBB presents an obstacle for drug delivery to the central nervous system (CNS). Major efforts have been made to alter or bypass the BBB for the delivery of therapeutics through direct injection into the parenchyma of the brain (intraparenchymal) or the cerebral spinal fluid (CSF). The therapeutic dosage is highly dependent on the route of administration, and it is difficult to homogeneously target the brain through a CSF injection due to the organ's size and distances between the ventricles and the cortex[158]. In contrast, the therapeutic can be delivered locally to neurons through direct intraparenchymal administration, limiting the delivery to regions neighboring the injection site. However, this represents a risky and invasive procedure requiring high technical skill, restricting widespread applicability in patients.

Alternatively, the neural pathways connecting the nasal mucosa and the brain provide potential routes for non-invasive drug delivery to the CNS[159,160]. The nose-to-brain pathway enables quick delivery of therapeutic agents to the CNS within minutes. Drugs with low molecular weight (<1 kDa) and high lipophilicity favor rapid intranasal uptake into the CNS but are limited by the concentrations that can be delivered to different regions of the brain and spinal cord[161,162]. The bioavailability of intranasal macromolecules can be significantly improved by formulations that include permeation enhancers. In the absence of permeation enhancers, nasal absorption declines sharply for molecular weights over 1 kDa[163,164]. Preclinical studies in rats have shown direct transport of VEGF (molecular weight: 38.2 kDa) to CNS via intranasal administration in 30 minutes[165]. Another preclinical study in mice has shown that intranasal administration of cationic liposome–encapsulated mRNA is effective for delivering therapeutics to specific brain regions (that is, cortex, striatum and midbrain)[166].

Drug delivery to the brain is particularly challenging because reaching the target site is no guarantee of success, even for lipid-soluble drugs, which can be rapidly expelled from brain endothelial cells by P-glycoprotein efflux transporters[167–169]. Mechanisms for enhanced tropism are needed to reach a specific cell type[169]. Clinically, antisense oligonucleosides (ASOs), another type of RNA therapy, have been successfully administered through direct CSF delivery to treat spinal muscular atrophy (Spinraza), which is the first FDA-approved drug for this disorder[170]. By contrast, the highly anticipated ASO treatment

for amyotrophic lateral sclerosis (ALS) (tofersen) recently missed its primary endpoints in a phase 3 trial (VALOR, NCT02623699).

Catheter delivery

Since the first successful heart catheterization in 1929 by Werner Fossmann, cardiac catheter–based therapies have become an integral part of modern cardiology, enabling efficient treatment of coronary artery disease, valvular disease and structural malformations. In addition, cardiac catheter–based delivery methods have been extensively explored in the context of gene-based and cell-based therapies[171,172]. As the heart is the central organ of the cardiovascular system, there are multiple ways to approach it intravascularly: transendocardial injections through a catheter placed in the ventricle, transepicardial injections through a catheter placed in the coronary veins, intracoronary artery infusions and retrograde coronary venous infusions (with or without blockage of the antegrade flow).

The last decade has seen great improvements in these technologies. Trans-vessel-wall microcatheters can inject cells and other therapeutic agents directly into the tissue, increasing efficacy and decreasing the risk of adverse events[173]. Preclinical studies with this endovascular device show that it can directly access target tissues, such as the heart, kidney and pancreas, without the need to seal the puncture site[146]. Re-circulation devices, which allow the agent to pass the area of interest multiple times, can enhance transduction efficiency in large animal models[174]. Selective pressure-regulated retroinfusion (SSR) with blockage of the antegrade flow is another promising approach for safe, efficient delivery of various agents, including cDNA, miRNA inhibitors and gene therapeutic agents[175].

Further improvements in catheter design aim to achieve minimal invasiveness through reducing the tubing diameter, optimizing infusion parameters to maximize distribution volume or by adding a reflux-inhibiting feature to halt backflow along the catheter's entry track[176–178]. Several future developments are to be expected with the integration of micro-electronic components that add features such as navigation and positioning through magnetic sensors or micro-cameras[179]. As these devices are adapted to the needs of mRNA-based therapeutics, they could also be combined with packaging systems that target specific cell types (see below).

Engineering packaging systems to control tropism

RNA packaging solutions have so far been designed for high delivery efficiency and low immunogenicity and cytotoxicity but with little focus on cellular specificity. Along with improved, minimally invasive localized delivery methods, new approaches for cell-type-specific delivery systems through systemic administration will be important to minimize non-specific delivery and clearance. Thus, investigation of the in vivo biodistribution of different packaging systems is critical[180]. Early LNP formulations were actively metabolized by the liver through the binding of ApoE to cholesterol molecules, facilitating their absorption by hepatocytes, similarly to the hepatic metabolism of low-density lipoprotein (LDL)[181,182]. Binding affinity to ApoE can be impaired by the addition of PEGylated lipids, disabling hepatic hoarding of the molecules[183]. Furthermore, modifying the LNPs' polarity enables selective organ targeting (SORT), with positively charged particles preferentially accumulating in the lungs, neutral particles in the liver and negative particles in the spleen[184,185]. Novel LNP candidates for tissue and cellular targeting have been uncovered via high-throughput in vivo screening and are being actively pursued[180]. Others have investigated increasing the LNPs' affinity to cellular microenvironments (pH, redox or enzymatic) or guiding them using external stimuli (heat, light, magnet or ultrasounds)[186–189]. Higher-resolution specificity can also be obtained by Anchored Secondary scFv Enabling Targeting (ASSET), which incorporates antibodies on the LNP surface[190]. For example, LNPs coated with anti-Ly6c antibodies specifically delivered RNA cargos to Ly6c$^+$ leukocytes[190]. Promising results from preclinical trials using such delivery systems illustrate their rapid adoption, including anti-PECAM1 antibody–conjugated LNPs to target pulmonary endothelium, anti-CD4 antibody–conjugated LNPs to target T cells, anti-hCD29 antibody–conjugated LNPs to target lymphoma cells and anti-CD5 antibody–conjugated LNPs to target and generate CAR-T cells in vivo[191–194].

Following an analogous strategy allows to enhance the homing capacity of cellular delivery systems through overexpression of ligands or receptors on the cell's plasma membrane. For instance, MSCs transfected with glycoprotein (for example, PSGL-1), chemokine ligands (for example, CXCL2 and CXCL5) or receptors (for example, CXCLR2 and CXCR4) coding mRNAs enabled preferential migration toward inflamed tissue[101,195]. Similarly to cells, EVs and biomimetic particles can obtain similar improvements in homing capacity as they conserve the surface characteristics of their parental cells[196,197]. EVs derived from cardiac cells showed cardiomyocyte targeting and positive therapeutic effect in a rat myocardial infarction model[196]. Macrophage membrane–coated LNPs selectively targeted tumor cells through macrophage-enriched integrins and successfully delivered antitumor drugs[132].

Chronic dosing

The ability to repeatedly deliver mRNA specifically and efficiently while maintaining high protein yields is a crucial requirement in the transition of mRNA from vaccines to therapeutics. This is vividly illustrated by enzyme replacement therapies, which rely on recombinant proteins. For example, hemophilia A and B—blood disorders caused by the lack of clotting proteins—are commonly treated by systemic injection of factor VIII or factor IX recombinant proteins, respectively, 3–7 times per week[198]. Frequent administrations are needed because of the proteins' relatively short half-life of ~12 hours. Preclinical studies in mice showed that this regimen could be replaced by a single weekly systemic injection of 0.2–0.5 mg kg^{-1} of linear modified mRNA while maintaining protein levels above a clinically relevant threshold (Supplementary Table 1)[12–14]. In an alternative approach, clinical results from DNA-based gene therapy for hemophilia using AAV vectors showed increasing protein levels in the first 2 years, after which they plateaued. Recent data suggest that a replenishment would be necessary after 5–7 years owing to immune rejection of the viral vectors[12,199]. Viral vectors have their own safety concerns, particularly in pediatric indications[12].

Compared to protein drugs, the real added value of mRNA therapeutics is the capacity to synthesize high levels of intracellular proteins. This intracrine approach enables direct targeting of metabolic diseases such as Crigler–Najjar syndrome[7,200], methylmalonic acidemia[15,16], propionic acidemia[17] and cystic fibrosis[18,19,154], which are technically difficult to treat with proteins (Table 1). For example, the current treatment for propionic acidemia consists of activating ureagenesis by daily ingestion of 100–250 mg kg^{-1} of carglumic acid[17]. Although it alleviates toxic accumulation of ammonia, it does not treat the underlying metabolic defect. In contrast, the dual delivery of 0.5–2 mg kg^{-1} of hPCCA and hPCCB mRNAs once every 3 weeks showed a sustained reduction in plasma biomarkers and enzymatic activity for 3 months in a genetic knockout murine model and is now in a phase 1 clinical trial (Table 1)[17,201].

The main concern with chronic dosing is how to maintain robust protein production with each dose delivered over multiple years. Most chronic protein therapies lose efficacy with the progressive rise of antibodies against the protein or delivery vehicle. In mouse studies of mRNA packaged in PEGylated LNPs, little to no reduction in protein levels was seen after 3–6 months of chronic treatment (Supplementary Table 1)[12,15,16]. However, some studies detected anti-drug antibodies after repeated dosing, showing the need for careful selection of regimen[16,17,20].

Immune and cytotoxic responses to LNP-encapsulated mRNA have been a major concern in early clinical studies. In vivo monitoring of locally delivered empty LNPs, similar to those used in COVID-19 vaccines, showed significant inflammation caused by the ionizable lipid components[202]. PEGylated constituents are also immunogenic,

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Table 1 | Summary of different classes of potential mRNA therapeutics

Organ of interest	Type of therapy	Disease	mRNA cargo	Delivery frequency
Liver	ERT (endocrine)	Hemophilia A[a]	Factor VIII	Chronic injection
		Hemophilia B[a]	Factor IX	
		Crigler–Najjar syndrome[a]	UGT1A1	
	ERT (intracrine)	Methylmalonic acidemia[a,b]	MMUT	Chronic injection
		Propionic acidemia[a,b]	PCCA/PCCB	
		OTC deficiency[b]	OTC	
		Gycogen storage disease type 1A[b]	Glucose-6 phosphatase	
	Gene editing (intracrine)	Transthyretin amyloidosis[a,b]	Cas9/TTR	Single injection
		Heriditary angioedema[b]	Cas9/KLKB1	
Lungs	ERT (intracrine)	Cystic fibrosis[a,b]	CFTR	Chronic injection
Heart	Regenerative (paracrine)	Heart failure[a,b]	VEGF-A	Single injection
	Antibody (intracrine)	Heart failure[a]	Anti-PLN intrabody	Single injection
Cancer	Immuno-oncology (intra- or endocrine)	Solid tumors[b]	IL-23, IL-36, OX40L IFN-2b, GM-CSF, IL-15 IL-7, CLDN6, IL-12, IL-2	Single injection
	Antibody (endocrine)	Breast cancer[b]	Anti-HER2, CD40L, CD70, caTLR4	Chronic injection
		Solid tumors[b]	Anti-CLDN18.2	
Autoimmunity	Immune tolerization (endocrine)	Autoimmune disorders[b]	HSA-IL2m	Single injection
		Autoimmune encephalomyelitis[a]	$MOG_{35–55}$	Chronic injection
Multiple tissues	ERT (intra- or endocrine)	Fabry disease[a]	α-Gal A	Chronic injection
	Gene editing (intracrine)	Hereditary angioedema[b]	Cas9/KLKB1	Single injection

ERT, enzyme replacement therapy; UGT1A1, UDP glucuronosyltransferase family 1 member A1; MMUT, methylmalonyl-CoA mutase; PCCA, propionyl-CoA carboxylase-α; PCCB, propionyl-CoA carboxylase-β; OTC, ornithine transcarbamylase; TTR, transthyretin; KLKB1, kallikrein B1; CFTR, cystic fibrosis transmembrane conductance regulator; VEGF, vascular endothelial growth factor; PLN, phospholamban; IL, interleukin; OX40L, tumor necrosis factor superfamily member 4 (TNFSF4); IFN, interferon; GM-CSF, granulocyte-macrophage colony stimulating factor; CLDN, claudin; HER2, ERB-B2 receptor tyrosine kinase 2; caTLR4, constitutively active Toll-like receptor 4; HSA, human serum albumin; IL2m, interleukin-2–mutein fusion protein; $MOG_{33–55}$, myelin oligodendrocyte glycoprotein peptide; α-Gal A, α-galactosidase-A. [a]See Supplementary Table 1 for further information. [b]See Supplementary Table 2 for further information.

inducing anti-PEG IgM and IgG, which are responsible for the accelerated blood clearance and induction of complement activation-related pseudoallergies (CARPA)[5,6,89,203]. Moreover, PEG sensitivity in the general population has been increasing owing to continued exposure to industrial and commercial PEGylated products (drugs, toothpaste and skin care products)[5,89,204]. Inflammatory complications were observed with localized delivery of small mRNA–LNP dosages during COVID-19 vaccination campaigns and would be amplified with higher, chronic dosing[205]. Notably, several preclinical studies have shown liver toxicity during chronic administration of LNP-encapsulated mRNA cargos for protein replacement therapy[14,15,206,207]. Meticulously designed studies and detailed monitoring in small and larger animal models should help resolve most of these concerns.

Clinical studies

mRNA vaccines have completed successful phase 3 clinical trials and achieved international regulatory approval, whereas most mRNA therapeutics are in early clinical phase 1 studies focused largely on safety (Supplementary Table 2). Given that mRNA therapeutics could produce virtually any protein systemically or locally, a wide spectrum of potential disease indications and classes of protein is currently being examined. Classes of protein that could be delivered via mRNA include enzymatic proteins, receptors, intracellular proteins, mitochondrial membrane proteins, secreted proteins and gene-editing proteins (Table 1). To date, only two clinical studies have yielded encouraging results on both safety and efficacy signals: VEGF mRNA for heart failure and mRNA-encoded CRISPR–Cas9 for a hereditary amyloidosis.

VEGF mRNA and heart failure

Therapeutic vasculogenesis with VEGF has been studied for decades experimentally and clinically. As a protein therapy, the utility of VEGF has been limited by the short protein half-life (<30 minutes). As a gene therapy, long-term VEGF expression tends to cause toxicity from excessive vessel permeability[208–211]. Like many paracrine factors, VEGF does not act systemically but is secreted in a pulsatile manner locally to reach target cells in a dose-dependent manner, followed by rapid degradation. A decade-long effort by our group and collaborators demonstrated robust, transient expression of VEGF for several days using chemically modified mRNA in mouse models, large animals and a first-in-human clinical study[21,22,212–216]. The transient nature of mRNA expression limits naturally the toxicity of chronic VEGF expression. Our studies included extensive optimization of the mRNA cargo to maximize protein production and used direct intramuscular injection of 'naked' modified mRNA in a citrate buffer, bypassing the need for LNPs. In a series of patients with diabetes, the treatment achieved dose-dependent VEGF protein production up to a therapeutic threshold with a single administration and transiently reversed vascular dysfunction[212]. A phase 2a trial was recently completed in cardiac patients undergoing open heart surgery, with direct VEGF mRNA intracardiac injections in hypoperfused regions, clearly establishing the safety of the procedure (Table 1)[215,23]. Although the final clinical efficacy results are pending, interim analysis of this small study documents positive trends in several independent surrogates of clinical efficacy and a significant effect ($P < 0.05$) on ejection fraction. The study underscores the potential value of a new class of paracrine

factor mRNA drugs for organ repair in general and cardiac regenerative therapeutics in particular.

mRNA-encoded CRISPR therapeutic for ATTR

A landmark clinical study has merged the fields of mRNA therapeutics and CRISPR gene editing to treat a form of hereditary amyloidosis, ATTR (Table 1)[11]. Patients with ATTR suffer from progressive amyloid deposits throughout the body. The mutant protein is exclusively produced in the liver and is released systemically, causing debilitating effects on the heart and other organs. The study documented that a single i.v. administration of LNPs encapsulating a combined cargo of Cas9 mRNA and guide RNAs resulted in cleavage of the target mutant protein gene and a remarkable >90% reduction in circulating mutant protein levels. The LNPs were designed for opsonization with native lipoproteins, facilitating preferential uptake in the liver, with few or no significant side effects noted. Because the mRNA is only transiently expressed, this approach may be safer than more conventional AAV-mediated delivery of Cas9, where expression of the nuclease persists long after the desired editing event. A recent update confirmed these promising results and demonstrated stable downregulation of circulating mutant protein without any concerning side effects 28 days after administration[217].

This study provides the first demonstration of targeted gene editing to rescue a genetic disorder in humans. The results support exploring the potential extension of CRISPR mRNA therapeutics to the myriad of genetic liver disorders involving defects in intermediary metabolism and lipoprotein diseases. Over time, advances in delivery systems may enable applications in other solid organs, such as the heart, kidney and CNS. The use of base editors for single-base-pair editing may be especially well-suited for treating rare genetic diseases. One potential challenge from the commercial and regulatory perspectives is the necessity of developing personalized Cas9-guide RNA cargos specific to the many private mutations scattered throughout disease genes.

The scope of mRNA therapeutics

Although the long-term safety and efficacy of mRNA therapeutics in large animals and clinical phase 1 trials are still under investigation, we can begin to discern the outlines of near-term clinical development from animal studies and from the handful of later-stage clinical studies to date (Supplementary Table 2). mRNA has inherent advantages in the speed of generating one or more candidate mRNAs in a cell-free format and in the ease of creating combinations of mRNAs. Although chronic systemic administration is not yet feasible even with the most advanced LNP formulations, one-time administration already appears straightforward.

Secreted proteins that are localized to specific tissues, such as tumors, are a viable opportunity today and offer 'nearest-neighbor' effects beyond the few cells that are transfected. In this decade, it is likely that multiple paracrine secreted factors, similar to VEGF, may find clinical applications via localized, tissue-specific delivery, as paracrine factors primarily act on a specific set of cells in a limited temporal window and in a pulse-like manner, avoiding systemic side effects (for example, IGF-1, VEGF, etc.). Recent studies on VEGF mRNA therapeutics are extending its potential role in wound healing, peripheral vascular physiology and bone repair with a variety of in vivo delivery systems, along with cocktails of synergistic paracrine factors[102,104,218].

Secreted or intracellular antibody therapies represent a major opportunity as mRNA facilitates delivery of large amounts of antibodies for longer time periods compared to recombinant proteins (Supplementary Table 1) and allows rapid generation of therapeutic antibody cocktails. Among intracellular proteins, notable opportunities include metabolic and mitochondrial proteins (Table 1), which are difficult to address with conventional therapies. With respect to tissue targeting, clinical studies have shown high protein expression in the liver of LNP-encapsulated mRNA after i.v. administration and uptake of naked mRNA in the intact heart via direct intra-myocardial injection. As noted above, emerging systems for direct injection of mRNA into various solid organs will likely open up new targets and clinical indications. For diseases that require tight control of the level and duration of protein expression, mRNA offers a dose-dependency that is not easily achieved with gene therapy DNA vectors, which limits toxic side effects from protein over-expression or off-target CRISPR editing.

Chronic administration of mRNA therapeutics is a longer-term goal that awaits solutions to immunogenicity concerns. Nevertheless, preclinical studies uniformly show superior protein expression profiles with mRNA compared to recombinant protein therapy (Supplementary Table 1), which has the practical advantage of allowing less frequent administration. In the shorter term, given their capacity for rapid expression of intracellular proteins, mRNA therapeutics could be adopted into emergency care to aid patients suffering from acute debilitating and life-threatening conditions, such as methylmalonic acidemia.

Beyond the broad classes of potential applications discussed above, other innovative applications can be expected. For example, our group has used mRNA for in vivo expression of intracellular antibodies (intrabodies) in the prospective treatment of heart failure and as an in vitro disease-modeling tool to dissect key regulatory mechanisms[219,24]. Others have deployed mRNA to improve engraftment of human hematopoietic stem cells by transient overexpression of CXCR40 (ref. [220]). The versatility of mRNA is likely to trigger unexplored therapeutic and investigative opportunities in the future.

Conclusion

Three decades of scientific and clinical advances coupled with a massive effort to develop mRNA COVID-19 vaccines bodes well for the future of mRNA therapeutics. As noted above, mRNA encoding any protein can be quickly made at clinical grade with a few clicks in an automated, scalable, cell-free format. In the near future, it should be possible to generate modular, scalable Good Manufacturing Practice (GMP)-level manufacturing units that can be set up in any GMP-level facility, removing the need for cold chain transport. In this regard, lyophilization of mRNA therapeutics is on the horizon and will largely obviate distribution issues that exist with current COVID-19 mRNA vaccines. As new LNP and non-LNP carriers are developed with improved side effect profiles and increased capacity, complex gene and base editing may become feasible, along with repeated administration, to foster a new approach to enzyme replacement therapy.

At the same time, it is instructive to recall a cautionary tale from the history of recombinant protein therapy. In the early days of this field, it was projected that most growth factors would become drugs. Thirty years after the cloning of VEGF, it remains to be seen if it will become a clinically valuable therapeutic. Accordingly, the future of mRNA drugs may depend on matching this 'software of life' to the biological 'hardware' of human physiological systems with increased precision, longer duration and options for chronic dosing with tolerable safety profiles. In the coming years, rapid developments in the mRNA cargo, intracellular carriers and in vivo delivery systems, coupled with deep biological and clinical insight and intuition, should offer new hope for the many patients with unmet clinical needs that cannot easily be addressed by other therapeutic modalities.

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Competing interests

K.R.C. is a past co-founder of Moderna Therapeutics and is currently a member of the board of directors of eTheRNA and SmartCella and a member of the RNA advisory board of Pfizer. E.R, R.Y and K.S.F are currently employees of SmartCella. A.G. receives consultancy fees from SmartCella.

Additional information

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