mRNA vaccines for infectious diseases: principles, delivery and clinical translation

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Abstract | Over the past several decades, messenger RNA (mRNA) vaccines have progressed from a scepticism-inducing idea to clinical reality. In 2020, the COVID-19 pandemic catalysed the most rapid vaccine development in history, with mRNA vaccines at the forefront of those efforts. Although it is now clear that mRNA vaccines can rapidly and safely protect patients from infectious disease, additional research is required to optimize mRNA design, intracellular delivery and applications beyond SARS-CoV-2 prophylaxis. In this Review, we describe the technologies that underlie mRNA vaccines, with an emphasis on lipid nanoparticles and other non-viral delivery vehicles. We also overview the pipeline of mRNA vaccines against various infectious disease pathogens and discuss key questions for the future application of this breakthrough vaccine platform.

Vaccination is the most effective public health intervention for preventing the spread of infectious diseases. Successful vaccination campaigns eradicated life-threatening diseases such as smallpox and nearly eradicated polio[1], and the World Health Organization estimates that vaccines prevent 2–3 million deaths every year from tetanus, pertussis, influenza and measles (see Related links). Despite their evident success, however, conventional vaccines do not effectively tackle pathogens such as the malaria parasite *Plasmodium falciparum*, hepatitis C and human immunodeficiency virus (HIV), which evade immune surveillance[2]. Furthermore, they require regular modification to address rapidly mutating pathogens, such as the influenza virus.

Nucleic acid vaccines based on mRNA were conceived more than three decades ago in the hope of generating safe and versatile vaccines that are easy to produce[3,4]. In principle, mRNA vaccines have several advantages over conventional vaccines. Unlike some viral vaccines, mRNA does not integrate into the genome, obviating concerns about insertional mutagenesis[5]. mRNA vaccines can be manufactured in a cell-free manner, allowing rapid, scalable and cost-effective production. For example, a 5 litre bioreactor can produce almost a million mRNA vaccine doses in a single reaction[6]. Moreover, a single mRNA vaccine can encode multiple antigens, strengthening the immune response against resilient pathogens[7] and enabling the targeting of multiple microbes or viral variants with a single formulation[8]. Initially, however, mRNA was not pursued as a therapeutic because of concerns about its stability, poor efficacy and excessive immunostimulation. Fortunately, a few tenacious researchers and companies persisted. And over the past decade, by determining mRNA pharmacology, developing effective delivery vehicles and controlling mRNA immunogenicity, interest in clinical applications of mRNA has renewed[4].

During the SARS-CoV-2 pandemic, mRNA-based vaccines were shown to be highly effective against SARS-CoV-2 and were developed and administered at unprecedented speed to millions of people globally to combat COVID-19 (BOX 1). These vaccines, initially developed by Pfizer–BioNTech and Moderna, have validated the platform and stimulated substantial interest in the application of mRNA for both prophylactic and therapeutic indications.

In this Review, we describe the technical basis of mRNA vaccines, including mRNA design and synthesis, as well as the enabling delivery technologies. The latter topic is a major focus of ongoing efforts for optimization of the platform and is thus emphasized here. We overview progress in developing mRNA vaccines for a wide range of infectious diseases such as influenza, Zika and respiratory syncytial virus (RSV), and discuss key issues facing the future of the platform, including safety, duration of response, application in specific populations and achieving global vaccine access.

Principles of mRNA design and synthesis

mRNA vaccines comprise synthetic mRNA molecules that direct the production of the antigen that will generate an immune response. In vitro-transcribed (IVT)

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Box 1 | **Developing mRNA vaccines at warp speed**

The sequencing of the SARS-CoV-2 genome in January 2020 kickstarted a race to develop potent vaccines against the novel coronavirus. At that time, it would have been optimistic to expect results within a couple of years. For example, MERS-CoV and SARS-CoV vaccines required about 2 years of development before entering phase I trials[259] and have yet to be approved. Moreover, the mumps vaccine, which was then the fastest developed vaccine, required 4 years from conception to approval (see Related links). The Moderna and Pfizer–BioNTech vaccines, in contrast, were approved 11 months after initiation, 8 months of which were spent in clinical trials. Their lightning-fast development was facilitated by three factors: decades of previous research, large financial investments and the inordinate devotion of scientists, engineers, managers, medical personnel and regulatory officials working in concert and around the clock.

SARS-CoV-2 vaccines capitalized upon many years of research on other coronaviruses, small interfering RNA (siRNA) delivery systems and cancer vaccines. For example, research on MERS-CoV and SARS-CoV suggested that the antigen should be the spike protein and that its amino acid sequence should be slightly modified to lock the protein in its prefusion conformation, maximizing its immunogenicity[127]. These studies enabled Moderna to generate the mRNA-1273 sequence in 2 days and start phase I trials in 66 days[127].

A colossal investment was also required for each vaccine candidate, with Pfizer–BioNTech and Moderna receiving $1.95 billion and $2.48 billion, respectively, from the US government through its Operation Warp Speed initiative (see Related links). Finally, none of this would have been possible without the extraordinary effort of the scientific and medical communities, the willingness of BioNTech, Pfizer and Moderna to take risks, and the flexibility of regulatory agencies to allow the simultaneous conduct of phase I, II and III trials.

mRNA mimics the structure of endogenous mRNA, with five sections, from 5′ to 3′: 5′ cap, 5′ untranslated region (UTR), an open reading frame that encodes the antigen, 3′ UTR and a poly(A) tail (FIG. 1). A variation on IVT mRNA, self-amplifying mRNA, also contains replicase genes that encode RNA-dependent RNA polymerase. The virus-derived polymerase amplifies mRNA transcripts intracellularly, enabling the expression of large amounts of antigen with reduced mRNA doses[9].

The 5′ cap structure, like that of natural eukaryotic mRNAs, contains a 7-methylguanosine nucleoside linked through a triphosphate bridge to the 5′ end of mRNA[10]. As in mammals, the first or second nucleotide from the 5′ end is methylated on the 2′ hydroxyl of the ribose (2′-*O*-methylation), which prevents recognition by cytosolic sensors of viral RNA[10], and hence prevents unintended immune responses. Further, the 5′ cap protects the mRNA sterically from degradation by exonucleases, and it works synergistically with the poly(A) tail at the 3′ end, poly(A) binding proteins and translation initiation factor proteins to circularize mRNA and recruit ribosomes for initiating translation[10,11]. The length of the poly(A) tail indirectly regulates both mRNA translation and half-life. A sufficiently long tail (100–150 bp) is necessary to interact with poly(A) binding proteins that form complexes necessary for initiating translation[11,12] and protecting the cap from degradation by decapping enzymes[13].

The 5′ and 3′ UTRs flanking the coding region regulate mRNA translation, half-life and subcellular localization[10,14]. Naturally occurring UTRs from highly expressed genes, such as the α- and β-globin genes, are preferred for synthetic mRNAs[15]. However, because UTR performance can vary by cell type, alternative UTR sequences may be used that have been optimized for the desired application and intended cell target[16–18]. These engineered UTR sequences minimize mRNA degradation by excluding miRNA-binding sites[11] and AU-rich regions in the 3′ UTR[19]. Furthermore, they minimize regions that prevent ribosomes from scanning the mRNA transcript, such as sequences with secondary and tertiary structure (for example, hairpins) in the 5′ UTR[20].

The open reading frame of the mRNA vaccine is the most crucial component because it contains the coding sequence that is translated into protein. Although the open reading frame is not as malleable as the non-coding regions, it can be optimized to increase translation without altering the protein sequence by replacing rarely used codons with more frequently occurring codons that encode the same amino acid residue. For instance, the biopharmaceutical company CureVac AG discovered that human mRNA codons rarely have A or U at the third position and patented a strategy that replaces A or U at the third position in the open reading frame with G or C[21]. CureVac used this optimization strategy for its SARS-CoV-2 candidate CVnCoV, which is now in phase III trials (see the mRNA sequence in Related links). Although replacement of rare codons is an attractive optimization strategy, it must be used judiciously. This is because, in the case of some proteins, the slower translation rate of rare codons is necessary for proper protein folding[22].

To maximize translation, the mRNA sequence typically incorporates modified nucleosides, such as pseudouridine, N1-methylpseudouridine or other nucleoside analogues[23]. Because all native mRNAs include modified nucleosides, the immune system has evolved to recognize unmodified single-stranded RNA, which is a hallmark of viral infection. Specifically, unmodified mRNA is recognized by pattern recognition receptors, such as Toll-like receptor 3 (TLR3), TLR7 and TLR8, and the retinoic acid-inducible gene I (RIGI) receptor. TLR7 and TLR8 receptors bind to guanosine- or uridine-rich regions in mRNA and trigger the production of type I interferons, such as IFNα, that can block mRNA translation[24]. The use of modified nucleosides, particularly modified uridine, prevents recognition by pattern recognition receptors, enabling sufficient levels of translation to produce prophylactic amounts of protein[5]. Both the Moderna and Pfizer–BioNTech SARS-CoV-2 vaccines, which produced >94% efficacy in phase III clinical trials[25], contain nucleoside-modified mRNAs. Another strategy to avoid detection by pattern recognition receptors, pioneered by CureVac, uses sequence engineering and codon optimization to deplete uridines by boosting the GC content of the vaccine mRNA[26].

In addition to improvements to the mRNA sequence, significant advances have also been made to streamline mRNA production. Clinically used synthetic mRNA is transcribed in vitro from a DNA plasmid by using the bacteriophage RNA polymerase T7 (T3 and SP6 polymerases can also be used). It is co-transcriptionally capped (CleanCap, developed by TriLink BioTechnologies) with a 2′-*O*-methylated cap[27,28] and purified to remove double-stranded RNA (dsRNA) contaminants, reactants and incomplete transcripts. Other methods add the cap with a post-transcriptional

Pattern recognition receptors
A family of membrane-bound and cytoplasmic proteins that are expressed by innate immune cells to detect microbial components, including bacterial carbohydrates such as lipopolysaccharide and mannose, viral and bacterial nucleic acids, bacterial peptides, peptidoglycans and lipoproteins.

Nucleoside-modified
Chemically modified nucleosides have been introduced into in vitro-transcribed mRNA to reduce immunogenicity and increase translation.

reaction using capping and 2′-*O*-methyltransferase enzymes derived from the vaccinia virus. The poly(A) tail is encoded in the DNA template, which eliminates reaction steps and reduces overall production time and material loss[29].

Incorporating the poly(A) tail in the DNA plasmid also overcomes the tail length variability that arises from enzymatic polyadenylation using poly(A) polymerase. Poly(A) tails of >100 bp are optimal for therapeutic mRNAs; however, the DNA sequences that encode these long poly(A) stretches can destabilize the DNA plasmids used for transcription. A solution to overcome this stability issue is to include a short UGC linker in the poly(A) tail[30,31]. The Pfizer–BioNTech vaccine BNT162b2 against SARS-CoV-2 uses this strategy and contains a 10 bp UGC linker to produce the sequence A_{30}(10 bp UGC linker)A_{70} (see the mRNA sequence in Related links). Together, these innovations have overcome significant manufacturing bottlenecks and facilitated the development of a simple, cost-effective and scalable one-step mRNA synthesis process.

Delivery vehicles for mRNA vaccines

Because mRNA is large (10^4–10^6 Da) and negatively charged, it cannot pass through the anionic lipid bilayer of cell membranes. Moreover, inside the body, it is engulfed by cells of the innate immune system and degraded by nucleases. Various techniques, including

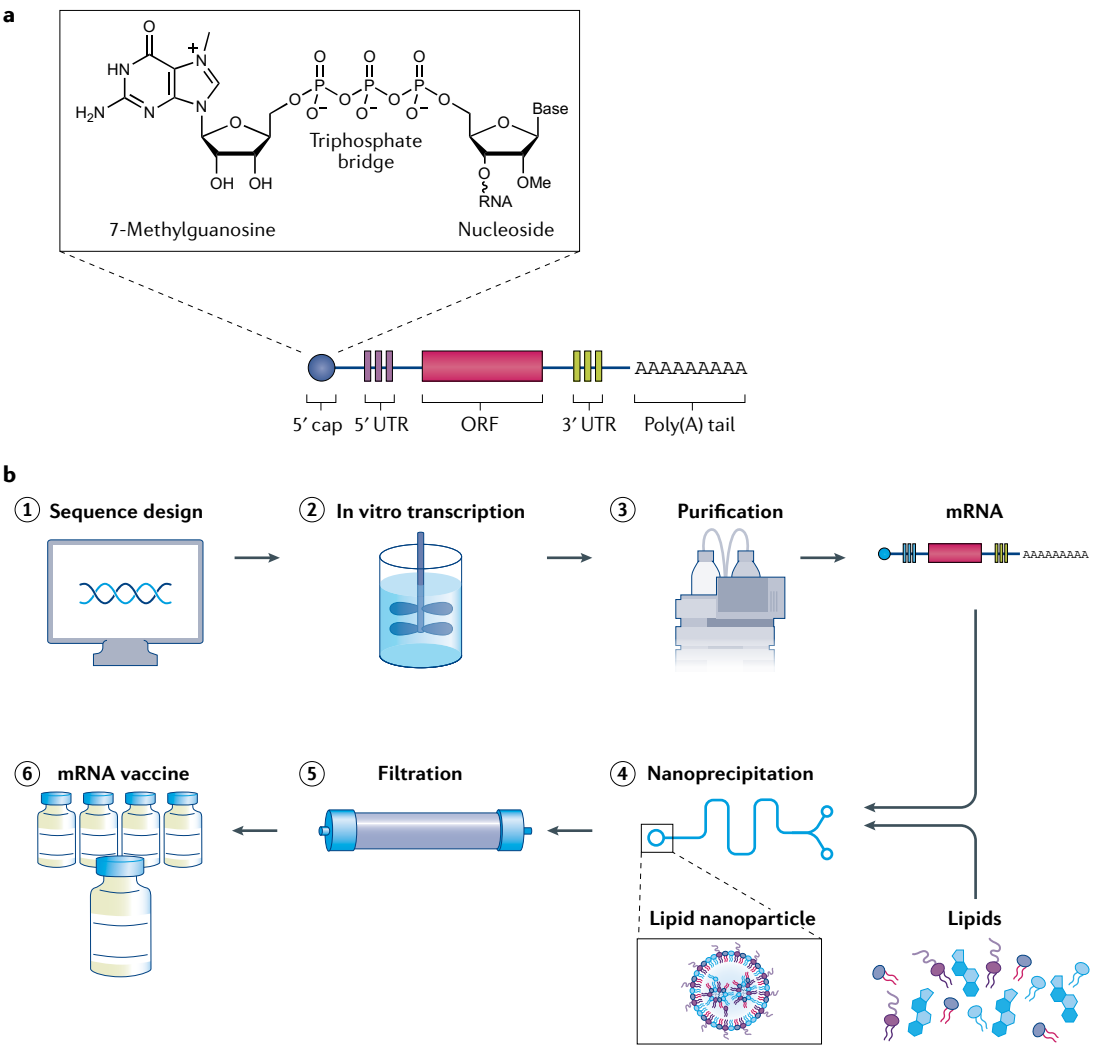
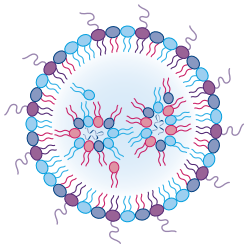
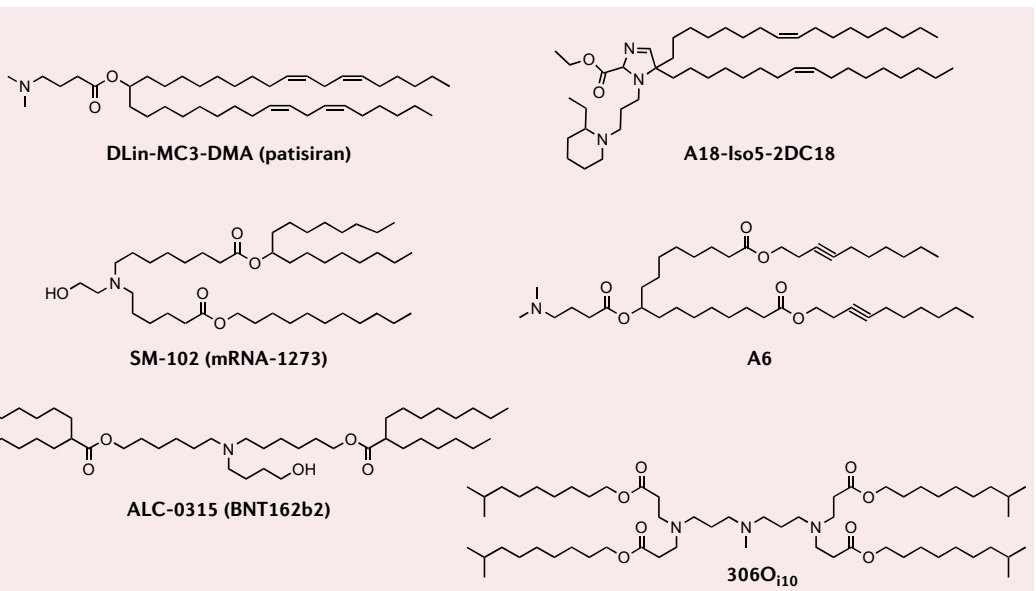


Fig. 1 | **IVT mRNA is formulated into lipid nanoparticle vaccines using a cell-free production pipeline. a** | In vitro-transcribed (IVT) mRNA contains five structural elements: a 5′ cap containing 7-methylguanosine linked through a triphosphate bridge to a 2′-*O*-methylated nucleoside, flanking 5′ and 3′ untranslated regions (UTRs), an open reading frame (ORF) and a poly(A) tail. **b** | The mRNA is synthetically produced and formulated into vaccines. (1) Once the genome of a pathogen has been sequenced, a sequence for the target antigen is designed and inserted into a plasmid DNA construct. (2) Plasmid DNA is transcribed into mRNA by bacteriophage polymerases in vitro and (3) mRNA transcripts are purified by high performance liquid chromatography (HPLC) to remove contaminants and reactants. (4) Purified mRNA is mixed with lipids in a microfluidic mixer to form lipid nanoparticles. Rapid mixing causes the lipids to encapsulate mRNA instantaneously and precipitate as self-assembled nanoparticles. (5) The nanoparticle solution is dialysed or filtered to remove non-aqueous solvents and any unencapsulated mRNA and (6) the filtered mRNA vaccine solution is stored in sterilized vials.

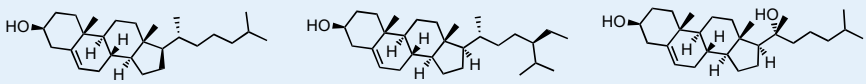
a Lipid nanoparticle



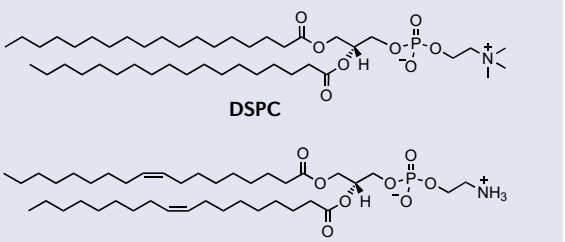
Ionizable lipid



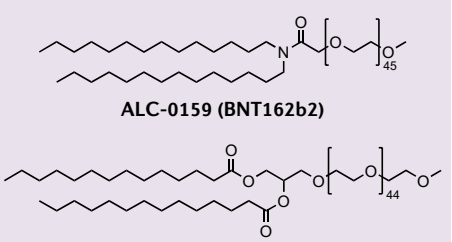
Cholesterol variants



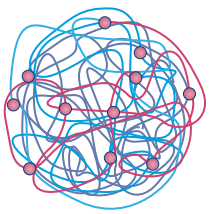
Helper lipid

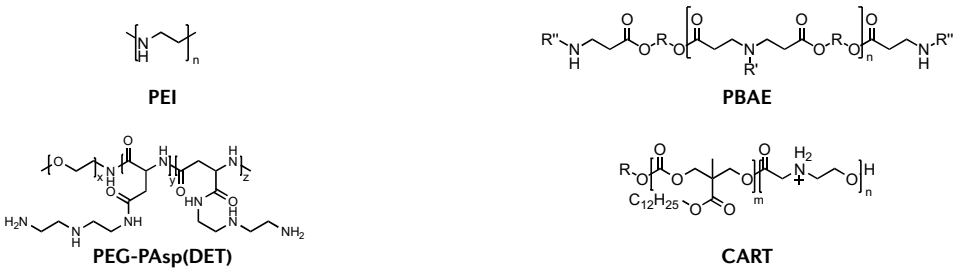


PEGylated lipid

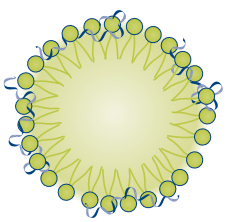


b Polymeric nanoparticle





c Cationic nanoemulsion



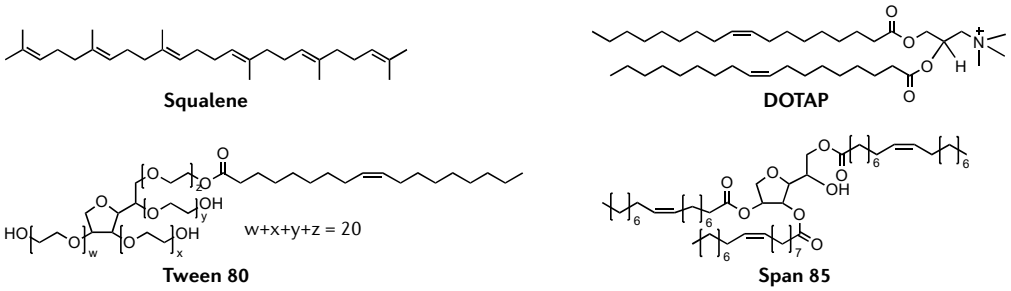


Fig. 2 | **All mRNA delivery vehicles contain cationic or ionizable molecules.** **a** | Lipid nanoparticles encapsulate mRNA in their core. They consist of four components: ionizable lipids, such as DLin-MC3-DMA[42], SM-102 (REF.[53]), ALC-0315 (REF.[54]), A18-Iso5-2DC18 (REF.[60]), A6 (REF.[57]) and 306O_{i10} (REF.[46]); cholesterol or its variants, β-sitosterol[64] and 20α-hydroxycholesterol[63]; helper lipids, such as DSPC[70] and DOPE[69]; and PEGylated lipids, such as ALC-0159 (REF.[32]) and PEG-DMG[32]. **b** | Polymers, such as PEI[164], PBAE[91], PEG-PAsp(DET)[94] and CART[99] form polymer–mRNA complexes. **c** | Cationic nanoemulsions contain a squalene core surrounded by an outer shell made of cationic lipid (for example, DOTAP) and surfactants, such as Tween 80 and Span 85. The mRNA adsorbs to the surface via electrostatic binding[109].

electroporation, gene guns and ex vivo transfection can intracellularly deliver mRNA in a dish[23]. In vivo application, however, requires the use of mRNA delivery vehicles that transfect immune cells without causing toxicity or unwanted immunogenicity (FIG. 2). Fortunately, a number of innovative materials-based solutions have been developed for this purpose.

Lipid-based nanoparticles

Lipid-based nanoparticles are the most clinically advanced of the mRNA delivery vehicles. All SARS-CoV-2 mRNA vaccines in development or approved for clinical use as of June 2021 employ lipid nanoparticles (LNPs). LNPs offer numerous benefits for mRNA delivery, including ease of formulation, modularity, biocompatibility and large mRNA payload capacity. Aside from the RNA drug, LNPs typically include four components (BOX 2), each of which is described below: an ionizable lipid, cholesterol, a helper phospholipid and a PEGylated lipid, which together encapsulate and protect the fragile mRNA core[32].

The cationic lipid DOTMA and its synthetic analogue DOTAP were the first lipids to deliver mRNA in 1989 (REF.[33]). Their positively charged amines facilitate the encapsulation of negatively charged RNA. Many other cationic lipids have been used for RNA delivery since then, including the commercially successful Lipofectamine[34]. Unfortunately, although cationic lipids are highly effective at mRNA delivery, they also trigger toxic pro-apoptotic and pro-inflammatory responses[35,36].

Ionizable lipids were developed to overcome these safety issues. These lipids are neutral when injected into the bloodstream at physiological pH, which improves their safety and extends circulation time compared with cationic lipids[37]. Ionizable lipids are formulated with mRNA into nanoparticles in acidic buffer so that the lipids are positively charged and attract the RNA cargo. Moreover, they are positively charged in the acidic environment of the endosomes, which promotes their fusion with the endosomal membrane, releasing them into the cytoplasm[38,39].

DODAP and DODMA were the first ionizable lipids used for RNA delivery[40,41]. Efforts to enhance the efficacy of DODMA through rational design led to the creation of DLinDMA[41] and, ultimately, DLin-MC3-DMA[42]. The latter made history as the ionizable lipid in the first FDA-approved LNP formulation: the small interfering RNA (siRNA) drug, patisiran (Onpattro). In addition to potently and safely delivering siRNA, DLin-MC3-DMA has also been used for mRNA delivery[43–46].

Although the rational lipid design approach described above has been successful in certain contexts, it is relatively slow. To accelerate materials discovery, many groups in academia and industry have used combinatorial reaction schemes to synthesize large libraries of potential delivery materials for testing. This approach has generated numerous potent lipids, including C12-200 (REF.[47]), 503O_{13} (REF.[48]), 306O_{i10} (REF.[46]), OF-02 (REF.[49]), TT3 (REF.[50]), 5A2-SC8 (REFS[51,52]), SM-102 (used in the Moderna vaccine mRNA-1273 against SARS-CoV-2)[53] and ALC-0315 (used in the Pfizer–BioNTech vaccine BNT162b2)[25,54]. Also, structure–activity relationship datasets from these large screens have identified factors that predict efficacy, including lipid pK_a[48], surface charge at pH 5 (REF.[55]) and haemolytic activity at pH 5.5 (REFS[56,57]), which can serve as design guides for future studies.

In addition to the search for improved efficacy, a growing interest in improving the specificity of delivery, particularly for vaccines and immunotherapies, has spurred efforts to target immune cells. Lipids containing polycyclic adamantane tails, such as 11-A-M[58], or those containing cyclic imidazole heads, such as 93-O17S[59], have been designed to target T cells in vivo. Although the mechanism is unclear, the cyclic groups of these lipids are crucial for targeting T cells. Furthermore, the cyclic amine head group of the lipid A18-Iso5-2DC18 has been shown to strongly bind to the stimulator of interferon genes (STING) protein, resulting in dendritic cell maturation and potent antitumour immunity[60]. It has also been shown that cyclic vitamin C-derived lipids delivered antimicrobial peptide and cathepsin B mRNA to macrophage lysosomes, eliminating multidrug-resistant bacteria and protecting mice from bacteria-induced sepsis[61].

Although ionizable lipids are, arguably, the most important component of LNPs, the three other lipid components — cholesterol, helper lipid and PEGylated lipid — also promote nanoparticle formation and function. Cholesterol, a naturally occurring lipid, enhances the stability of the nanoparticles by filling gaps between lipids, and it aids fusion with the endosomal membrane during uptake into the cell[62]. Several studies have also shown improved efficacy with cholesterol analogues[63–65], which likely reduce binding to Niemann–Pick type C1, a protein that traffics cholesterol and recycles ~70% of LNPs from late endosomes back into the extracellular fluid[64,66].

Helper lipids modulate nanoparticle fluidity and enhance efficacy by promoting lipid phase transitions that aid membrane fusion with the endosome[67,68]. The choice of an optimal helper lipid depends on both the ionizable lipid material and the RNA cargo. For example, for lipidoid materials, saturated helper lipids (for example, DSPC) are best at delivering short RNAs (for example, siRNA)[58], and unsaturated lipids (for example, DOPE) are best for mRNA delivery[69,70]. DSPC, however, has been incorporated into the FDA-approved SARS-CoV-2 vaccines mRNA-1273 and BNT162b2. It is possible that DSPC outperforms DOPE for these ionizable lipids, and it is also a convenient choice because it is a component of a previously FDA-approved LNP siRNA therapy, patisiran (Onpattro). Studying helper lipids has led to the design of potent unsaturated ionizable lipids, such as A6 (REF.[57]) and 4A3-Cit[71] that enhance

Endosome
A membrane-bound organelle that engulfs nutrients and macromolecules (including delivery vehicles) and transports them inside the cell.

Box 2 | **Formulation and scale-up of lipid nanoparticles**

Lipid nanoparticles (LNPs) are formulated by precipitating lipids from an organic phase by mixing them with an aqueous phase. The lipids commonly used in mRNA vaccines — an ionizable lipid, a helper lipid, cholesterol and a PEGylated lipid — are dissolved in ethanol. Separately, mRNA is dissolved in an aqueous citrate or acetate buffer at pH 4. Mixing the two solutions protonates the ionizable lipid, causing electrostatic attraction between the protonated lipid and the anionic mRNA. This effect, when coupled with the hydrophobic interactions due to the poor aqueous solubility of the lipids, drives spontaneous self-assembly of LNPs with the mRNA encapsulated inside. LNPs are then dialysed or filtered to remove non-aqueous solvent and bring the solution to physiological pH.

Microfluidic mixers enable the formulation of small-sized nanoparticles with low dispersity and high encapsulation efficiency. Precision NanoSystems' NanoAssemblr platform has been widely employed for LNP formulation in laboratory settings[260]. These systems use cartridges that have a staggered herringbone micromixer (SHM) architecture. The structure of SHMs enables the solvents to mix within microseconds. Because this timescale is smaller than the time necessary for lipid aggregation, SHMs produce small nanoparticles of uniform size[260]. The particle size distribution can be regulated by adjusting the total flow rate and the flow rate ratio between the aqueous and ethanol streams. A total flow rate of 12 ml min^{-1} and a flow rate ratio of 3:1 is commonly used to generate small monodisperse LNPs. Multiple SHMs can also be connected in parallel to increase throughput for large-scale production[261].

Although SHMs have several advantages, their utility for good manufacturing practice (GMP) manufacturing is limited primarily by solvent incompatibility. SHMs are commonly fabricated using polydimethylsiloxane[260], which can deform the mixer architecture upon long-term exposure to organic solvents such as ethanol. Although the cartridges are replaced after several runs in the lab, this is infeasible for continuous manufacturing at GMP scale. Instead, T mixers are employed for LNP scale-up[25,135]. They are compatible with organic solvents[260], produce morphologically similar LNPs to SHMs[74] and operate at the much higher flow rates (60–80 ml min^{-1}) necessary for large-scale manufacturing[260].

vesicle fusion, and zwitterionic lipids, such as 9A1P9, that improve endosomal escape by promoting phase transition[72]. In addition to enabling membrane fusion, helper lipids also influence target organ specificity: cationic lipids redirect liver-targeted formulations to the lungs, whereas anionic lipids redirect them to the spleen[72,73].

The PEGylated lipid component of LNPs consists of polyethylene glycol (PEG) conjugated to an anchoring lipid such as DMPE or DMG. The hydrophilic PEG stabilizes the LNP, regulates nanoparticle size by limiting lipid fusion[74] and increases nanoparticle half-life by reducing nonspecific interactions with macrophages[75]. Both the molecular weight of the PEG and the length of the lipid anchor can be tuned to alter efficacy, circulation time and immune cell uptake, depending on the application. The molecular weight of the PEG can vary from 350 to 3,000 Da[76], and the lipid anchor's tail length can vary from 10 to 18 carbons[77,78]. Larger molecular weights and longer lengths tend to result in nanoparticles with longer circulation times and reduced immune cell uptake. The mRNA-1273 and BNT162b2 SARS-CoV-2 vaccines contain PEGylated lipids of 2 kDa molecular weight and 13 and 14 carbon-long saturated lipid anchors, respectively[32].

Polyplexes and polymeric nanoparticles

Although less clinically advanced than LNPs, polymers offer similar advantages to lipids and effectively deliver mRNA[79]. Cationic polymers condense nucleic acids into complexes called polyplexes that have various shapes and sizes and can be taken up into cells by endocytosis. The mechanisms by which polyplexes escape from endosomes are uncertain; one possible mechanism is that proton buffering by the polymer leads to osmotic swelling and rupture of the endosomes — the proton sponge hypothesis[80].

Polyethylenimine is the most widely studied polymer for nucleic acid delivery. Although its efficacy is excellent, its application is limited by its toxicity[81] owing to its high charge density[82]. Use of a low molecular weight form, incorporation of PEG into the formulation[83], conjugation to cyclodextrin[84–86] and disulfide linkage[87] can mitigate the toxicity of polyethylenimine. Additionally, several alternative biodegradable polymers have been developed that are less toxic. Poly(β-amino ester)s, for example, excel at mRNA delivery, especially to the lung[88–90]. Because they are easily synthesized by the Michael reaction[91], large poly(β-amino ester) libraries have been created that facilitate structure–function studies.

Similar to poly(β-amino ester)s, poly(amidoamine)s are biodegradable polymers that are synthesized by the Michael reaction and allow facile modifications to their core and periphery[52]. Poly(amidoamine)s form hyperbranched tree-like spherical dendrimers that efficiently form mRNA complexes owing to the high amine density on their periphery[82]. Although charge density is favourable for mRNA complexation, excessive charge can cause toxicity and serum aggregation[82,92]. Fortunately, these issues can be mitigated by introducing disulfide linkages or incorporating PEG in the dendrimer core[82,92].

Like the ionizable lipids in LNPs, pH-responsive polymers have also been used for mRNA delivery. Poly(aspartamide)s conjugated to ionizable aminoethylene side chains are protonated at the acidic pH inside endosomes, facilitating RNA delivery. The hydrophobicity and length of the side chain influence poly(aspartamide) protonation and delivery efficacy[93]. For example, PEGylated poly(aspartamide) with an ethylenediamine side chain delivers mRNA to liver[94], brain[95], spinal cord[96], knee joint[97] and olfactory nerves[98]. In addition to poly(aspartamide)s, pH-responsive charge-altering releasable transporters have gained attention owing to their unique mRNA delivery mechanism. Instead of protonating inside endosomes, these charge-altering releasable transporters self-degrade into neutral, non-toxic by-products at cytosolic pH, leading to rapid release of the mRNA into the cytoplasm[99,100].

Other delivery systems

In addition to lipid and polymer-based vehicles, peptides can also deliver mRNA into cells, thanks to the cationic or amphipathic amine groups (for example, arginine) in their backbone and side chains that electrostatically bind to mRNA and form nanocomplexes. For example, a fusogenic cell-penetrating peptide containing repetitive arginine-alanine-leucine-alanine (RALA) motifs changes conformation at endosomal pH, facilitating pore formation in the membrane and endosomal escape[101,102]. RALA delivers mRNA to dendritic cells (professional antigen-presenting cells of the immune system) to elicit T cell-mediated immunity[103].

Adjuvant
An additive that boosts the immunogenicity of vaccines by triggering pattern recognition receptors, leading to the secretion of cytokines and chemokines.

There is also a commercially available cell-penetrating peptide, PepFect14, that delivered mRNA to ovarian cancer cells in a mouse xenograft model[104]. Arginine-rich protamine peptides (of about 4 kDa), which are positively charged at neutral pH, can also condense mRNA and facilitate its delivery[34]. Protamine complexed with mRNA activates Toll-like receptor (TLR7, TLR8) pathways that recognize single-stranded mRNA[105]; thus, it can act as an adjuvant for vaccine or immunotherapy applications. CureVac AG is evaluating a protamine-containing delivery platform, RNActive, in clinical trials for melanoma[106], prostate cancer[107] and non-small-cell lung cancer[108].

Finally, squalene-based cationic nanoemulsions also deliver mRNA. These nanoemulsions consist of an oily squalene core stabilized by a lipid shell that adsorbs mRNA onto its surface[109]. Some squalene formulations, such as Novartis's MF59, act as adjuvants in FDA-approved influenza vaccines[110]. MF59 causes cells at the injection site to secrete chemokines, which recruits antigen-presenting cells, induces differentiation of monocytes into dendritic cells and enhances antigen uptake by antigen-presenting cells[111–113]. The mechanism by which squalene-based cationic nanoemulsions escape from endosomes to deliver mRNA into the cytoplasm remains unclear.

Progress with mRNA vaccines for infectious diseases

Vaccines for infectious diseases are currently the most advanced application for mRNA therapeutics. The majority of mRNA vaccines currently in preclinical trials and in clinical use are administered as a bolus injection into the skin, muscle or subcutaneous space, where they are taken up by immune or non-immune cells and translated into antigens that are displayed to T and B cells (FIG. 3). Both the mRNA and the delivery vehicle enhance the immunogenicity and efficacy of mRNA vaccines.

By the end of 2019, 15 mRNA vaccine candidates against infectious diseases had entered clinical trials, with none in phase III trials[79,114] (TABLE 1). At that time, it was thought that it would be at least another 5–6 years

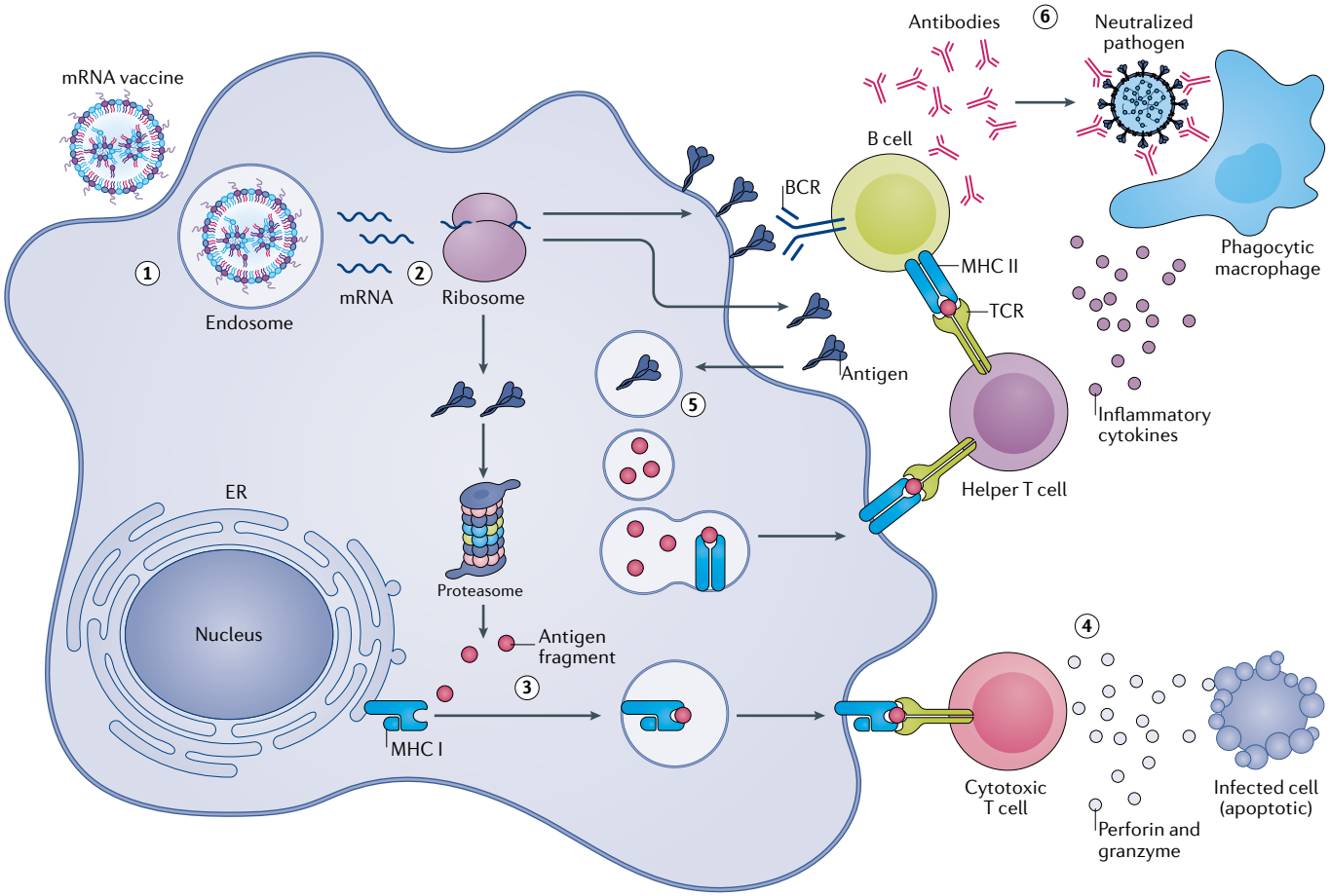


Fig. 3 | **Messenger RNA vaccines elicit immunity through transfection of antigen-presenting cells.** (1) Injected mRNA vaccines are endocytosed by antigen-presenting cells. (2) After escaping the endosome and entering the cytosol, mRNA is translated into protein by the ribosome. The translated antigenic protein can stimulate the immune system in several ways. (3) Intracellular antigen is broken down into smaller fragments by the proteasome complex, and the fragments are displayed on the cell surface to cytotoxic T cells by major histocompatibility complex (MHC) class I proteins. (4) Activated cytotoxic T cells kill infected cells by secreting cytolytic molecules, such as perforin and granzyme. (5) Additionally, secreted antigens can be taken up by cells, degraded inside endosomes and presented on the cell surface to helper T cells by MHC class II proteins. (6) Helper T cells facilitate the clearance of circulating pathogens by stimulating B cells to produce neutralizing antibodies, and by activating phagocytes, such as macrophages, through inflammatory cytokines. BCR, B cell receptor; ER, endoplasmic reticulum; TCR, T cell receptor.

Table 1 | **Clinical trials of mRNA vaccines against infectious diseases beyond COVID-19**

Funding source	Name	Target	Vaccine type	Route of administration	Clinical trial phase	Clinical trial identifier
Moderna	mRNA-1647	CMV	Nucleoside-modified mRNA–LNP	Intramuscular	Phase II	NCT04232280, NCT03382405
Moderna	mRNA-1443	CMV	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03382405
Moderna	mRNA-1893	Zika	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT04064905
Moderna	mRNA-1325	Zika	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03014089
Moderna	mRNA-1653	hMPV/PIV3	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT04144348, NCT03392389
Moderna	mRNA-1345	RSV	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT04528719
Moderna, Merck	mRNA-1777 (V171)	RSV	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	Unregistered
Moderna, Merck	mRNA-1172 (V172)	RSV	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	Unregistered
Moderna	mRNA-1851 (VAL-339851)	Influenza A (H7N9)	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03345043
Moderna	mRNA-1440 (VAL-506440)	Influenza A (H10N8)	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03076385
Moderna	mRNA-1010	Influenza A (H1N1, H3N2), influenza B (Yamagata lineage, Victoria lineage)	Unknown	Intramuscular	Phase I/II	NCT04956575
Translate Bio, Sanofi	MRT5400	Influenza A (H3N2)	Unknown	Intramuscular	Phase I	Unregistered
Translate Bio, Sanofi	MRT5401	Influenza A (H3N2)	Unknown	Intramuscular	Phase I	Unregistered
Moderna	mRNA-1944	Chikungunya	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03829384
Moderna	mRNA-1388 (VAL-181388)	Chikungunya	Nucleoside-modified mRNA–LNP	Intramuscular	Phase I	NCT03325075
CureVac	CV7201	Rabies	Unmodified mRNA complexed in RNActive	Intradermal, intramuscular	Phase I	NCT02241135
CureVac	CV7202	Rabies	Unmodified mRNA–LNP	Intramuscular	Phase I	NCT03713086
GSK	GSK3903133A	Rabies	Self-amplifying mRNA in cationic nanoemulsion	Intramuscular	Phase I	NCT04062669

CMV, cytomegalovirus; GSK, GlaxoSmithKline; HIV, human immunodeficiency virus; hMPV, human metapneumovirus; LNP, lipid nanoparticle; PIV3, parainfluenza virus type 3; RSV, respiratory syncytial virus.

before an mRNA vaccine would obtain regulatory approval. These expectations were upended when the COVID-19 pandemic overtook the world in early 2020. Over the ensuing months, mRNA vaccine development, manufacturing and deployment were put to the ultimate test.

SARS-CoV-2

At the start of 2021, a year after the first COVID-19 case was reported in China[115], SARS-CoV-2 had infected more than 150 million people and claimed more than three million lives worldwide (see Related links). Although most SARS-CoV-2 infections are not life-threatening in younger people without pre-existing medical conditions, severe infection can trigger an unchecked immune response in the lungs that destroys epithelia and alveoli. This damage can cause pulmonary oedema, dangerous increases in vascular permeability and death[116,117].

Most SARS-CoV-2 vaccine candidates induce an immune response to the spike protein on the virus surface (FIG. 4). The spike protein binds to its receptor on the host cell surface, angiotensin-converting enzyme 2 (REF.[118]). The attached spike protein is then cut open by the cell's transmembrane serine protease 2, which induces a conformational change that exposes the spike protein's fusion peptide and facilitates fusion with the cell or endosomal membrane[118]. Usually, the antigen encoded by the vaccine mRNA is either the full-length spike protein or the spike protein's receptor-binding domain.

As of 18 June 2021, 185 COVID-19 vaccine candidates were in preclinical development and an additional 102 had entered clinical trials (see Related links). Of those in clinical trials, 19 were mRNA vaccines (TABLE 2). On 11 December 2020, the Pfizer–BioNTech vaccine BNT162b2 received emergency authorization from the FDA and became the first mRNA drug approved for

Neutralizing antibody
An antibody that binds to pathogens and prevents them from infecting cells.

use in humans. One week later, the Moderna vaccine mRNA-1273 was also authorized for use in the USA. Ultimately, they were the first SARS-CoV-2 vaccines to be authorized in the USA, the UK, Canada and several other countries.

Pfizer and BioNTech co-developed five mRNA vaccine candidates that encode variants of the spike protein antigen[119]. The two lead candidates, BNT162b1 and BNT162b2, use LNPs that are formulated using Acuitas Therapeutics' ionizable lipid ALC-0315 and a nucleoside-modified mRNA in which all uridines are replaced by N1-methylpseudouridine to enhance mRNA translation. BNT162b1 encodes a trimerized, secreted version of the spike protein's receptor-binding domain, whereas BNT162b2 encodes the full-length SARS-CoV-2 spike glycoprotein with two proline substitutions in the S2 subunit, which lock the protein in its prefusion conformation[120,121]. In preclinical studies in rhesus macaques, two 100 µg doses of BNT162b2 delivered 21 days apart elicited neutralizing antibody titres 10.2–18.0 times higher than convalescent patient serum as well as strong $CD4^+$ and $CD8^+$ T cell responses[122]. In phase I trials of the two candidates, two 30 µg doses delivered 21 days apart induced high antibody and neutralizing titres and a robust $CD4^+$ and $CD8^+$ response with mild to moderate adverse events[27,28,123,124]. Both candidates were well tolerated and efficacious, but only the BNT162b2 vaccine advanced to phase II/III trials owing to its milder systemic and local adverse reactions[124]. In phase III trials involving 43,548 participants, BNT162b2

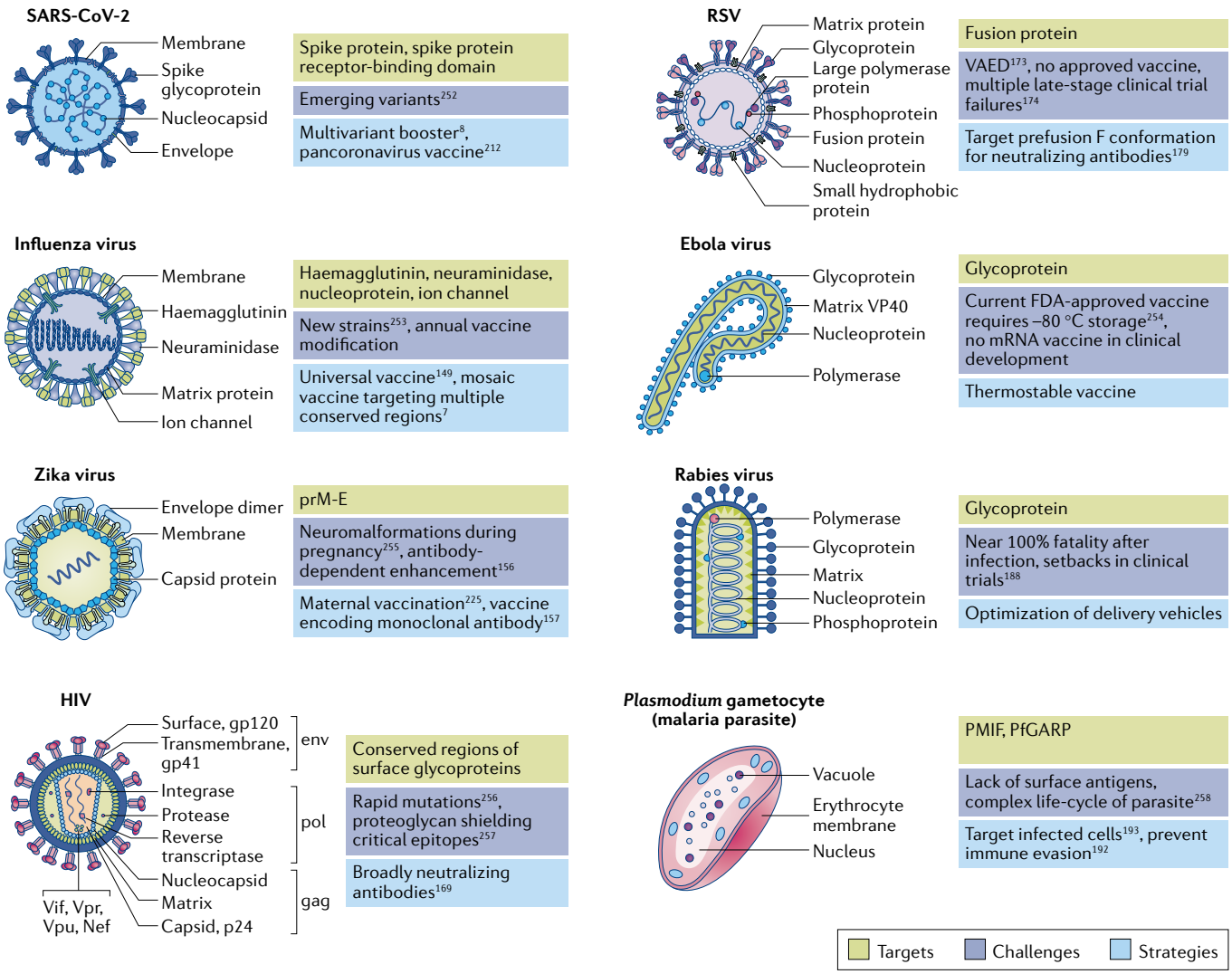


Fig. 4 | **mRNA vaccines in development protect against an array of common pathogens using disease-specific targeting strategies.** Surface proteins that enable cell entry are commonly used by mRNA vaccines to target viruses, for example, spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), haemagglutinin protein of influenza viruses, membrane and envelope protein (prM-E) of Zika virus, fusion protein of respiratory syncytial virus (RSV) and surface glycoproteins of human immunodeficiency virus (HIV), Ebola virus and rabies virus. Additionally, complex pathogens such as *Plasmodium* can be targeted using non-surface antigens such as *Plasmodium* macrophage migratory inhibiting factor (PMIF) or *Plasmodium falciparum* glutamic-acid-rich protein (PfGARP). Each pathogen poses a unique set of challenges, including high lethality, rapid mutations, immune evasion, new strains and variants[252–258]. Depending on the challenges, mRNA vaccines encoding conformation-specific proteins, conserved regions of antigens or monoclonal antibodies can be safely delivered to healthy adults, children, elderly people and pregnant people. VAED, vaccine-associated enhanced disease.

Table 2 | **Clinical trials of mRNA vaccines against SARS-CoV-2**

Name (INN)/funding source	mRNA type	Antigen	Phase	Clinical trial identifier (participants; location)	Clinical trial outcomes
BNT162b2 (Tozinameran)/ BioNTech, Pfizer	Nucleoside-modified	Transmembrane prefusion spike	Phase III	NCT04805125 (431 participants; Switzerland)	EUA in several countries; >90% efficacy in real-world conditions in USA[132] and Israel[126]; 95% overall efficacy in phase III trials[125]; 90–100% efficacy in phase III trials across subgroups defined by age, sex, race, ethnicity, baseline body mass index and the presence of coexisting conditions[125]; 1.7–4.6-fold higher neutralizing titres than convalescent serum in 18–55-year old participants, and 1.1–2.2-fold higher titres in 65–85-year old in phase I trials[124]
				NCT04816669 (610 participants; USA)	
				NCT04800133 (900 participants; Hong Kong)	
				NCT04713553 (1,530 participants; USA)	
			Phase II/III	NCT04368728 (43,998 participants; Argentina, Brazil, Germany, South Africa, Turkey, USA)	
				NCT04754594 (700 participants; Brazil, South Africa, Spain, UK, USA)	
			Phase II	ISRCTN73765130 (2,886 participants; UK)	
				NCT04894435 (1,200 participants; Canada)	
				NCT04761822 (3,400 participants; USA)	
				NCT04824638 (300 participants; France)	
				NCT04860739 (676 participants; Spain)	
				EUCTR2021-001978-37 (600 participants; Spain)	
				NCT04649021 (950 participants; China)	
				ISRCTN69254139 (820 participants; UK)	
				NCT04907331 (3,000 participants; Austria)	
				NCT04895982 (360 participants; Brazil, Germany, USA)	
			Phase I/II	EUCTR2020-001038-36, NCT04380701 (476 participants; Germany)	
				NCT04889209 (800 participants; USA)	
				NCT04588480 (160 participants; Japan)	
			Phase I	NCT04839315 (100 participants; USA)	
				NCT04816643 (4,500 participants; Finland, Poland, Spain, USA)	
mRNA-1273/Moderna, NIAID, BARDA	Nucleoside-modified	Transmembrane prefusion spike	Phase III	NCT04811664 (37,500 participants; USA)	EUA in several countries; 90% efficacy in real-world conditions in USA[132]; 94.1% overall efficacy in phase III trials[131]; dose-dependent increase in S2-P-binding antibodies and serum-neutralizing titres in phase I trials[130]
				NCT04470427 (30,420 participants; USA)	
				NCT04860297 (240 participants; USA)	
				NCT04806113 (220 participants; Canada)	
				NCT04805125 (431 participants; Switzerland)	
			Phase II/III	NCT04649151 (3,732 participants; USA)	EUA in several countries; 90% efficacy in real-world conditions in USA[132]; 94.1% overall efficacy in phase III trials[131]; dose-dependent increase in S2-P-binding antibodies and serum-neutralizing titres in phase I trials[130]
				NCT04796896 (6,975 participants; USA)	
			Phase II	ISRCTN73765130 (2,886 participants; UK)	
				NCT04847050 (120 participants; USA)	
				NCT04894435 (1,200 participants; Canada)	
				NCT04748471 (180 participants; France)	
				NCT04761822 (3,400 participants; USA)	
				NCT04405076 (660 participants; USA)	
			Phase I/II	NCT04889209 (800 participants; USA)	
			Phase I	NCT04785144 (135 participants; USA)	
				NCT04813796 (125 participants; USA)	
				NCT04839315 (100 participants; USA)	
				NCT04283461 (120 participants; USA)	

Table 2 (cont.) | **Clinical trials of mRNA vaccines against SARS-CoV-2**

Name (INN)/funding source	mRNA type	Antigen	Phase	Clinical trial identifier (participants; location)	Clinical trial outcomes
TAK-919/Takeda, Moderna	Nucleoside-modified	Transmembrane prefusion spike	Phase I/II	NCT04677660 (200 participants; Japan)	Approved in Japan based on positive interim phase I/II data (see Related links); same formulation as mRNA-1273
CVnCoV (Zorecimeran)/ CureVac	Unmodified	Transmembrane prefusion spike	Phase III	NCT04652102, EUCTR2020-003998-22 (39,693 participants; Argentina, Belgium, Colombia, Dominican Republic, Germany, Mexico, Netherlands, Panama, Peru, Spain)	47% efficacy in phase III trials (see Related links); anti-spike IgG, anti-RBD IgG and serum-neutralization titres comparable to convalescent serum[133]
				EUCTR2020-004066-19, NCT04674189 (2,360 participants; Germany)	
				NCT04860258 (1,200 participants; Belgium)	
				NCT04848467 (1,000 participants; Argentina, Colombia, Peru)	
			Phase II	ISRCTN73765130 (2,886 participants; UK)	
				NCT04515147, PER-054-20 (674 participants; Panama, Peru)	
			Phase I	NCT04449276 (280 participants; Belgium, Germany)	
ARCoV/Walvax Biotechnology, PLA	Unmodified	Secreted spike RBD	Phase III	NCT04847102 (28,000 participants; China, Mexico)	Unknown
			Phase II	ChiCTR2100041855 (420 participants; China)	
	Unmodified	Secreted spike RBD	Phase Ib	ChiCTR2000039212 (120 participants; China)	Unknown
			Phase I	ChiCTR2000034112 (168 participants; China)	
BNT162b1 (Abdavomeran)/ BioNTech, Pfizer	Nucleoside-modified	Secreted spike RBD	Phase II/III	NCT04368728 (43,998 participants; Argentina, Brazil, Germany, South Africa, Turkey, USA)	8–50-fold higher anti-RBD IgG and 1.9–4.6-fold higher neutralization titres than convalescent serum[27,123]; higher frequency of fever, fatigue and chills in participants than BNT162b2 (REF.[124])
			Phase I/II	EudraCT 2020-001038-36, NCT04380701 (476 participants; Germany)	
			Phase I	ChiCTR2000034825, NCT04523571 (144 participants; China)	
mRNA-1273.211/ Moderna	Nucleoside-modified	Transmembrane prefusion spike	Phase II	NCT04405076 (660 participants; USA)	Unknown
ARCT-021/Arcturus	Self-amplifying	Transmembrane prefusion spike	Phase II	NCT04668339 (600 participants; Singapore, USA)	Unknown
				NCT04728347 (106 participants; Singapore)	
			Phase I/II	NCT04480957 (92 participants; Singapore)	
BNT162a1/BioNTech, Pfizer	Unmodified	Secreted spike RBD	Phase I/II	EudraCT 2020-001038-36, NCT04380701 (476 participants; Germany)	Unknown
BNT162b3 (Ganulameran)/ BioNTech, Pfizer	Nucleoside-modified	Transmembrane spike RBD	Phase I/II	NCT04537949, EUCTR2020-003267-26-DE (96 participants; Germany)	Unknown
BNT162c2 (Pidacmeran)/ BioNTech, Pfizer	Self-amplifying	Transmembrane prefusion spike	Phase I/II	EudraCT 2020-001038-36, NCT04380701 (476 participants; Germany)	Unknown
MRT5500/Sanofi, Translate Bio	Unmodified	Transmembrane prefusion spike	Phase I/II	NCT04798027 (333 participants; Honduras, USA)	Unknown
LNP-nCoVsaRNA/ Imperial College London, Acuitas Therapeutics	Self-amplifying	Transmembrane prefusion spike	Phase I	ISRCTN17072692 (320 participants; UK)	Unknown
ChulaCov19/ Chulalongkorn University	Nucleoside-modified	Transmembrane spike	Phase I/II	NCT04566276 (96 participants; Thailand)	Unknown

Table 2 (cont.) | **Clinical trials of mRNA vaccines against SARS-CoV-2**

Name (INN)/funding source	mRNA type	Antigen	Phase	Clinical trial identifier (participants; location)	Clinical trial outcomes
PTX-COVID19-B/ ProvidenceTherapeutics	Nucleoside-modified	Transmembrane spike	Phase I	NCT04765436 (60 participants; Canada)	Unknown
SAM-LNP-S/Gritstone Oncology, NIAID	Self-amplifying	Transmembrane spike	Phase I	NCT04776317 (150 participants; USA)	Unknown
mRNA-1273.351/ Moderna	Nucleoside-modified	Transmembrane prefusion spike	Phase I	NCT04785144 (135 participants; USA)	Difference in serum neutralization between wild-type ancestral strain and B.1.351 reduced from 7.7-fold to 2.1-fold, 14 days after mRNA-1273.351 booster[251]
mRNA-1283/Moderna	Nucleoside-modified	Transmembrane prefusion spike	Phase I	NCT04813796 (125 participants; USA)	Unknown
CoV2 SAM [LNP]/GSK	Self-amplifying	Transmembrane spike	Phase I	NCT04758962 (10 participants; USA)	Unknown

All SARS-CoV-2 vaccine candidates in clinical trials are delivered intramuscularly. Clinical trials are regularly updated and the locations and the number of participants are subject to change. BARDA, Biomedical Advanced Research and Development Authority; EUA, emergency use authorization; GSK, GlaxoSmithKline; INN, international nonproprietary name; LNP, lipid nanoparticle; NIAID, National Institute of Allergy and Infectious Diseases; PLA, People's Liberation Army; RBD, receptor-binding domain.

showed 95% overall efficacy at preventing COVID-19 and 90–100% efficacy across subgroups defined by age, sex, race, ethnicity, baseline body mass index and coexisting conditions[125]. In a mass vaccination campaign involving 3,159,136 participants in Israel, two doses of BNT162b2 were 94% effective at preventing symptomatic COVID-19, 87% effective at preventing hospitalizations and 92% effective at preventing the onset of severe COVID-19 (REF.[126]).

Moderna developed mRNA-1273 in collaboration with the National Institute for Allergy and Infectious Diseases at the National Institutes of Health and the Biomedical Advanced Research and Development Authority. The vaccine uses the ionizable lipid SM-102 to formulate LNPs that encapsulate an N1-methylpseudouridine-modified mRNA. The sequence encodes the SARS-CoV-2 spike protein with two proline substitutions that confer the prefusion conformation[127]. In a preclinical trial, two 1 μg doses of vaccine (a primer dose and a booster) injected into muscle induced robust virus-neutralizing activity as well as potent $CD4^+$ and $CD8^+$ T cell responses in mice[127]. It also protected the animals from upper and lower airway SARS-CoV-2 infection for at least 3 months after vaccination[127]. Rhesus macaques that received two 100 μg doses of the vaccine also developed potent humoral and cellular immune responses[128]. In comparison with serum from patients recovering from COVID-19, vaccinated macaque serum contained 15-fold higher titres of neutralizing antibodies, 348-fold more potent inhibitory activity for spike binding to the angiotensin-converting enzyme 2 and 12-fold higher virus-neutralizing activity[128]. Vaccinated macaques also elicited spike protein-specific IgG and IgA antibodies in nasal washes and bronchoalveolar lavages, suggesting that intramuscular vaccination with mRNA-1273 confers both serum and mucosal immunity[128,129]. Fortunately, these encouraging preclinical results extended to clinical trials in which mRNA-1273 was exceptionally efficacious and well tolerated[130,131]. In phase III trials involving 30,420 volunteers, two 100 μg vaccine doses were 94.1% effective at preventing the onset of COVID-19 (REF.[131]). Local pain at the site of injection was the most common side effect. After the second dose, half of the volunteers reported moderate-to-severe systemic side effects (for example, fatigue, muscle pain, joint pain) that resolved within 48 h[131]. In real-world conditions involving 3,950 healthcare personnel, first responders and other essential and frontline workers in the USA, two doses of mRNA-1273 or BNT162b2 were 90% effective against SARS-CoV-2 (REF.[132]).

Although the Pfizer–BioNTech and Moderna vaccines have demonstrated excellent efficacy and safety, their need for cold-chain storage poses logistical difficulties. mRNA-1273 can be stored at 4–8 °C for a month and 12 h at room temperature, while BNT162b2 requires storage at −60 °C. Thermostable vaccines that do not require refrigerators or freezers ease the distribution of vaccines considerably, particularly in warm countries or those without reliable cold chain storage. CureVac's candidate vaccine CVnCoV is stable for 3 months at 5 °C and 24 h at room temperature (see Related links). CVnCoV uses LNPs formulated using an ionizable lipid from Acuitas Therapeutics (potentially ALC-0315) and an unmodified mRNA sequence encoding a full-length spike protein with the two proline substitutions[133]. In phase I clinical trials, primer and booster injections of a 12 μg dose of CVnCoV administered 28 days apart generated neutralizing antibodies similar to those in plasma from patients who were convalescing from COVID-19 and was well tolerated[133]. Unfortunately, in phase IIb/III trials involving 40,000 participants and spanning ten countries, CVnCoV demonstrated 47% efficacy against COVID-19 (see Related links). Interim analysis suggests that CVnCoV's modest efficacy is attributable to emerging SARS-CoV-2 variants, as only one of the sequenced 124 cases was caused by the ancestral variant. Furthermore, 57% of the cases were caused by variants of concern: B.1.1.7, B.1.427, B.1.429, B.1.351, P.1 or B.1.617.2.

Variant
A subtype of a pathogen with a genetic sequence that differs from the reference pathogen.

Strain
A variant of the pathogen with physical properties such as transmissibility, disease severity or immune response that are different from those of the reference pathogen.

At the time of writing, CureVac, in collaboration with GSK, is developing a second-generation candidate — CV2CoV — that has been optimized to enhance translation and immunogenicity relative to CVnCoV. CV2CoV uses a 5′ UTR from the human hydroxysteroid 17-β-dehydrogenase 4 gene and a 3′ UTR from the human proteasome 20S subunit β3 gene, whereas CVnCoV used a 3′ UTR from the human α-haemoglobin gene[134]. In preclinical studies, CV2CoV showed 1.8-fold higher protein expression than CVnCoV in vitro, and elicited high titres of cross-neutralizing antibodies against the B.1.1.7, B.1.1.298 and B.1.351 variants in rats[134].

Another thermostable candidate vaccine, ARCoV, developed by the Academy of Military Science of the People's Liberation Army in China in collaboration with Walvax Biotechnology, is stable at 25 °C for a week. ARCoV encodes the receptor-binding domain of the spike protein. In preclinical studies, two 10 μg doses in mice and two 100 μg doses in cynomolgus monkeys delivered 21 days apart elicited high SARS-CoV-2-specific IgG antibodies and strong virus neutralization titres[135]. Although the reasons behind the thermostability of CVnCoV and ARCoV are unknown to the public, the mRNA secondary structure, smaller mRNA size, GC content and the lipids may be important factors.

Several promising self-amplifying mRNA vaccine candidates are also in development. The mRNAs in these vaccines encode the antigen as well as RNA replication machinery that boosts the intracellular production of mRNA transcripts. The self-amplification supports the use of vaccine doses up to 100-fold lower than those used with standard mRNA. LNP-nCoVsaRNA, developed by Imperial College London and Acuitas Therapeutics, encodes the full-length spike protein and replication machinery from the genome of Venezuelan equine encephalitis virus. In mice, 10 ng primer and booster doses injected 28 days apart into muscle yielded a robust T cell response and IgG titres similar to those in plasma from patients who had recovered from COVID-19 (REF.[136]). At the time of writing, this candidate is being evaluated in a phase I trial using a 0.1–1 μg dose-escalation protocol (ISRCTN17072692), which is the lowest RNA dose of all mRNA vaccine candidates. Another self-amplifying mRNA vaccine candidate, ARCT-021 (also known as LUNAR-COV19), was developed by Arcturus Therapeutics with their proprietary LUNAR lipid delivery vehicle and self-transcribing and replicating RNA (STARR) platform. It encodes the full-length prefusion spike protein. In transgenic mice expressing human angiotensin-converting enzyme 2, doses of 2 μg and 10 μg produced strong T cell responses, high IgG titres and protected mice from SARS-CoV-2 infection[137]. Although the ARCT-021 dose is higher than the LNP-nCoVsaRNA dose, no booster is required, ensuring that recipients are fully vaccinated after a single injection.

Influenza viruses

Influenza viruses are responsible for an estimated 290,000–650,000 deaths annually worldwide[138]. Current vaccines target the virus haemagglutinin protein (FIG. 4), which facilitates viral entry. However, the virus mutates rapidly, causing antigenic drift that requires yearly review and modification of the haemagglutinin antigen component of the vaccines. Conventional influenza vaccines, which are inactivated influenza viruses grown in chicken eggs, are subject to long production times and purification difficulties. Furthermore, the viruses mutate for optimal growth in chicken eggs, sometimes rendering them ineffective in humans. For example, loss of a glycosylation site in egg-grown vaccines was associated with poor efficacy during the 2016–2017 season[139]. There is a real need, therefore, for alternative antigen targets and production methods. Synthetic mRNAs transcribed in vitro can meet this need and ensure rapid vaccine production in the event that an entirely new influenza strain emerges. In 2013, for example, a LNP (DLinDMA)-based self-amplifying mRNA vaccine was rapidly formulated within 8 days after the H7N9 outbreak in China[140]. Unfortunately, progress to a phase I trial was impossible given the unavailability of GMP facilities for mRNA manufacturing at that time.

There has also been work towards a universal influenza vaccine that would not require yearly modification. Such a vaccine would confer immunity against several influenza strains (heterologous immunity) and subtypes (heterosubtypic immunity). In the first demonstration of an effective mRNA vaccine against influenza in 2012, three intradermal injections of 80 μg RNActive-mRNA encoding haemagglutinin from the PR8 H1N1 strain induced homologous and heterologous immunity against H1N1 and H5N1 strains, respectively, and protected the mice against a lethal viral dose (10× LD_{50})[141]. Since then, several delivery vehicles (DLinDMA, DOTAP, polyethylenimine and cationic nanoemulsions), alternative mRNA technologies (nucleoside-modified mRNAs and self-amplifying mRNAs), and alternative antigen targets have been evaluated for mRNA-based influenza vaccines[7,142–147].

Notably, the conserved stalk region of haemagglutinin, which is not prone to mutation, has recently emerged as a novel universal vaccine target[148]. A primer–booster regimen of a LNP-based mRNA vaccine (30 μg) encoding the conserved haemagglutinin stalk from the Cal09 H1N1 strain produced a stalk-specific antibody response in mice, ferrets and rabbits[149]. The broadly protective antibodies conferred homologous immunity against Cal09 H1N1, heterologous immunity against PR8 H1N1 and heterosubtypic immunity against H5N1 in mice and protected them against a lethal viral challenge[149]. Another study used LNPs to deliver a 50 ng dose of nucleoside-modified mRNA encoding three conserved influenza proteins: neuraminidase, nucleoprotein and matrix-2 ion channel protein (FIG. 4) in addition to the haemagglutinin stalk[7]. Incredibly, this minuscule mRNA dose generated broadly protective antibodies and protected mice from an extraordinarily large viral challenge (500× LD_{50}).

In two separate phase I trials in 2016, Moderna evaluated two influenza candidates. These vaccines were dosed via two intramuscular injections of LNPs encapsulating nucleoside-modified mRNA expressing full-length haemagglutinin from H10N8 and H7N9

(TABLE 1). Both produced excellent seroconversion and seroprotection[147]. Adverse effects were limited to pain at the injection site, redness, muscle pain, joint pain, headache, fatigue and chills/common-cold-like symptoms, indicating that the vaccine is safe and well tolerated[150].

Zika virus

Zika virus infection was first identified in 1947, and patients infected with Zika are often asymptomatic or experience mild symptoms such as fever, rash and muscle pain. However, Zika emerged as a global health crisis during the 2015–2016 epidemic in the Americas when the virus caused severe fetal neuromalformations and fetal death during pregnancy[151]. Fortunately, all Zika infections are caused by a single serotype, suggesting that vaccinating against an antigen from any strain could protect against all Zika strains[152]. The membrane and envelope protein (prM-E) is a common antigen choice for mRNA vaccines against Zika (FIG. 4), as neutralizing antibodies against prM-E can prevent viral fusion.

One study showed that a single 30 μg or 50 μg dose of a LNP-encapsulated nucleoside-modified prM-E mRNA protected mice and rhesus macaques, respectively, from a Zika challenge[153]. Of note, the neutralizing antibody titres generated by the mRNA vaccine were 50–100 times higher than those induced by purified inactivated virus and DNA vaccines in mice, and 50 times higher than a 1 mg DNA vaccine in macaques[153].

Importantly, poorly designed Zika virus vaccines can increase the infectiousness of the dengue virus[154]. Dengue virus is from the same viral family as Zika, and their envelope proteins share 54–59% overlapping amino acid sequence[155]. Therefore, it is possible that the envelope protein antigen encoded by a Zika vaccine spurs the production of antibodies that are cross-reactive with the dengue envelope protein. In the event of a subsequent dengue virus infection, antibody-dependent enhancement can occur in which the suboptimal anti-Zika antibodies bind to the dengue virus. This binding enhances the entry of the virus into host cells and exacerbates dengue symptoms. Moderna collaborated with Washington University School of Medicine to deliver a modified prM-E mRNA containing a mutated fusion loop epitope in the E protein. Two 10 μg doses of the LNP-encapsulated modified mRNA delivered 21 days apart protected mice from a Zika challenge and diminished the production of dengue-enhancing antibodies[156]. These encouraging preclinical results prompted a phase I trial, and interim results suggest that the vaccine — mRNA-1893 (TABLE 1) — induces 94–100% seroconversion in 10 μg and 30 μg dose groups and is well tolerated (see Related links).

Another study used a passive immunization approach and delivered an mRNA encoding neutralizing ZIKV-117 monoclonal antibodies (mAbs) using a squalene-based nanocarrier[157]. A single 40 μg dose delivered either a day before or a day after viral inoculation protected immunocompromised mice from the lethal viral challenge[157]. Using a similar approach, Moderna successfully delivered mRNA encoding the mAb CHKV-24, 4 h after chikungunya virus inoculation and protected mice from chikungunya virus-induced arthritis[158]. Interestingly, these results suggest that mRNA therapeutics encoding neutralizing mAbs may have both prophylactic and therapeutic activity. Moreover, passive immunization is an appealing approach for vaccinating immunocompromised people who cannot synthesize their own antibodies owing to an impaired immune system.

HIV

Globally, HIV currently affects 38 million people and is projected to affect up to 42 million people by 2030 (REF.[159]). In 2020 alone, there were 1.5 million new infections and 680,000 deaths (see Related links). Despite 30 years of research, no effective vaccine has been developed, primarily owing to the remarkable antigenic diversity of the HIV envelope protein and the dense 'glycan shield' that conceals crucial envelope protein epitopes[160]. Several preclinical studies have delivered mRNA vaccines encoding HIV proteins using multiple delivery vehicles, including cationic nanoemulsions[109,161], DOTAP/DOPE liposomes[162], polymers[84,163,164] and ionizable LNPs[165,166], but they have had varied success. These studies suggest that novel antigens are necessary to effectively target HIV in addition to potent delivery vehicles.

A novel vaccination strategy against HIV is to isolate broadly neutralizing mAbs from infected individuals who neutralize several HIV strains. Notably, the broadly neutralizing mAbs, VRC01, have recently gained attention thanks to their ability to neutralize 98% of HIV strains[167] and prevent transmission of antibody-sensitive strains with 75.4% efficacy[168]. In one study, a single 0.7 mg kg^{-1} intravenous injection of a LNP-encapsulated, nucleoside-modified mRNA expressing VRC01, produced similar antibody concentrations to those typically achieved by injecting a 10–20 mg kg^{-1} dose of mAb protein[169]. Importantly, a single dose protected mice from intravenous challenge with HIV-1.

Respiratory syncytial virus

Respiratory syncytial virus is the leading cause of acute lower respiratory infection globally. Annually, it is responsible for an estimated 60,000 deaths in children under the age of 5 (REF.[170]) and more than 14,000 deaths in people over 65 years of age[171]. Although the burden of RSV is well recognized, 40 years of vaccine development have not yet produced an approved RSV vaccine owing to numerous challenges. In 1968, for example, a formalin-inactivated RSV vaccine candidate caused vaccine-associated enhanced disease (VAED) in children. This response triggered excessive eosinophil and neutrophil infiltration in the lungs, resulting in severe bronchiolitis or pneumonia in 80% of the vaccinated children and two fatalities[172]. Although the exact mechanisms of VAED remain unclear, the formation of non-neutralizing antibodies and a T helper 2 (T_H2)-skewed T cell response marked by upregulation of cytokines such as IL-4, IL-5 and IL-13 have been implicated[173].

Current RSV vaccine candidates focus on targeting the highly conserved F protein, which facilitates viral fusion (FIG. 4). Although some candidates have failed

clinical trials owing to insufficient neutralizing antibody titres[174], newfound structural insights into F protein conformation have revealed that vaccinating against the prefusion conformation elicits superior neutralizing antibody response[175–178]. This discovery will hopefully improve future vaccine design. Fortunately, mRNA vaccines can be designed to encode stabilized F protein conformations by engineering the coding sequence[179]. In preclinical studies, mRNA vaccines encoding either the native RSV F protein or the stabilized prefusion conformation were successfully delivered using cationic nanoemulsions[109] and LNPs[179,180] without any observed instances of VAED.

Moderna is evaluating three single-dose vaccine candidates encoding the prefusion F protein (TABLE 1): mRNA-1172 (Merck's proprietary LNPs) and mRNA-1777 (Moderna's proprietary LNPs) for adults, as well as mRNA-1345 (Moderna's proprietary LNPs) for children (see Related links). In phase I clinical trials, mRNA-1777 elicited a robust humoral response with RSV neutralizing antibodies, a $CD4^+$ T cell response to RSV F peptides and no serious adverse events[181]. The sequence of mRNA-1345 has been further engineered and codon-optimized to enhance translation and immunogenicity relative to mRNA-1777. Interim phase I data suggest that a 100 μg dose of mRNA-1345 produces approximately eightfold higher neutralizing antibody titres than mRNA-1777 1 month after vaccination (see Related links). Ultimately, Moderna aims to integrate mRNA-1345 with its paediatric human metapneumovirus/parainfluenza virus type 3 (hMPV/PIV3) candidate mRNA-1653 and vaccinate children against three distinct pathogens with a single formulation (see Related links).

Ebola virus

Ebola virus (EBOV) was first identified in 1976 as the agent responsible for sporadic outbreaks of Ebola disease. This viral haemorrhagic fever is fatal in 50–90% of patients, depending on the strain; the 2014–2016 Ebola outbreak in West Africa claimed more than 11,000 lives[182]. In 2019, the FDA approved a recombinant vesicular stomatitis virus (VSV)-based Ebola vaccine (rVSV-EBOV). Although rVSV-EBOV is 97.5% effective at preventing Ebola transmission compared with no vaccination (see Related links), clinical trials note some safety concerns (for example, acute arthritis and skin rash at high doses)[183]. mRNA vaccines against EBOV are likely safer than this virus-based vaccine because they do not replicate inside the body. One mRNA vaccine has demonstrated efficacy in mice upon delivery of an unmodified, self-amplifying mRNA encoding the EBOV glycoprotein (FIG. 4) in a poly(amidoamine) dendrimer nanoparticle. The vaccine elicited glycoprotein-specific IgG antibodies and robust expression of IFNγ and IL-2 by $CD8^+$ and $CD4^+$ T cells, and two dosing regimens (two 4 μg doses or one 40 μg dose) protected mice from a lethal challenge[184]. Another study vaccinated guinea pigs with two 20 μg doses of a LNP-encapsulated, nucleoside-modified mRNA encoding the EBOV glycoprotein, inducing high antibody titres and protecting the animals from a lethal viral challenge[185].

Rabies virus

Rabies is a zoonotic viral disease characterized by neurological symptoms resulting in near 100% fatality. Although vaccines are approved, more than 50,000 people succumb to rabies annually[186], underscoring the need for more efficacious and affordable vaccines. To meet this need, CureVac has deployed its RNActive platform to deliver unmodified mRNA encoding the rabies virus glycoprotein in its rabies candidate, CV7201 (TABLE 1). In a preclinical study, two 80 μg vaccine doses delivered 21 days apart induced high neutralizing antibody titres and elicited antigen-specific $CD4^+$ and $CD8^+$ T cell responses in mice and pigs[187]. Resultant phase I trials examined both the route of delivery (intradermal or intramuscular) as well as the delivery device (standard needle-syringe or a needleless intradermal injector)[188]. Interestingly, although the delivery route did not affect the immune response, the delivery device did, with only the intradermal injector producing a short-lived humoral response[188]. This weak delivery efficacy, together with a high incidence of adverse events[188] indicated the need for further optimization of the delivery platform. Subsequently, CureVac used proprietary LNPs made by Acuitas Therapeutics as the delivery vehicle for their newer rabies candidate CV7202 (REF.[189]). In a preclinical study, CV7202 delivered unmodified mRNA encoding the rabies virus glycoprotein and produced strong antibody and $CD8^+$ and $CD4^+$ T cell responses[189]. In non-human primates, two 100 μg doses spaced 28 days apart were well tolerated and provoked 20-fold higher antibody titres than a commercially licensed rabies vaccine[189]. Phase I results suggest that two 1 μg doses yield high neutralizing titres, strong adaptive immune responses, and were well tolerated[190].

Plasmodium

Although the vast majority of mRNA vaccines under development are for protection from viruses, there are also efforts to prevent other infectious diseases. Malaria, which is caused by unicellular eukaryotic parasites of the genus *Plasmodium*, is at the top of that list owing to its incidence and lethality. Annually, malaria afflicts more than 200 million people and kills more than 400,000 patients worldwide (see Related links). Antimalarial vaccine production has been difficult owing to the lack of surface antigens and complex life cycle of *Plasmodium*. Fortunately, the interrogation of the body's natural immune response to *Plasmodium* infection has identified potential non-surface antigen targets.

For example, the *Plasmodium*-secreted cytokine, macrophage migrating inhibitory factor (PMIF), has been shown to prevent T cells from developing long-term memory[191]. Following this discovery, a vaccine was created from a squalene-based cationic nanoemulsion loaded with self-amplifying mRNA encoding PMIF. Two 15 μg primer–booster doses improved helper T cell development and elicited anti-*Plasmodium* IgG antibodies and memory T cell responses[192]. Moreover, adoptive transfer of T cells from vaccinated mice protected unvaccinated mice from *Plasmodium* sporozoites[192].

Another mechanistic study of malarial infection identified a protein, *Plasmodium falciparum* glutamic-acid-rich protein (PfGARP), as a potential mRNA vaccine target. PfGARP is expressed on the surface of infected erythrocytes and is recognized by antibodies from children who are relatively resistant to *P. falciparum*[193]. These antibodies bind to infected erythrocytes and induce programmed cell death. This discovery prompted the development of an mRNA vaccine comprising LNPs made using a proprietary lipid from Acuitas Therapeutics and encapsulating nucleoside-modified mRNA encoding PfGARP antigen. Three 50 µg doses were able to reduce parasitaemia in Aotus monkeys after a *P. falciparum* challenge[193].

Key issues for the field

Duration of antibody response

After vaccination, translated antigens are produced or taken up by antigen-presenting cells and transported to lymph nodes, where interactions between B cells, antigen-presenting cells and follicular helper T cells (T_{FH} cells) promote the formation of a germinal centre[167]. Within the germinal centre, B cells then proliferate, differentiate and mutate their antibody genes to produce high-affinity neutralizing antibodies against the offending antigen[167]. The germinal centre reaction and T_{FH} cell induction are crucial for a durable antibody response that will protect the patient for months or years.

To enhance the first step of this immune response process, some delivery systems target antigen-presenting cells to translate the mRNA cargo. Several promising strategies that actively target antigen-presenting cells include conjugating mAbs to LNP surfaces[194] and decorating LNP surfaces with dendritic cell-specific ligands[145,146]. Alternatively, modulation of physical properties of LNPs, such as surface charge[195], has been used to improve cancer vaccines.

Additionally, altering vaccine pharmacokinetics by prolonging the translation of antigenic mRNA has emerged as an exciting tool to enhance antibody response[196]. Extending the availability of an intact antigen improves the affinity of neutralizing antibodies by diverting the efforts of the immune system away from hidden antigen epitopes and focusing them on accessible ones[197,198]. Sustained antigen availability during the germinal centre reaction has been shown to increase antibody production by approximately tenfold[199]. One study in mice showed that LNPs encapsulating nucleoside-modified mRNA circulated for longer and induced stronger T_{FH} cell and germinal centre B cell responses than LNPs carrying unmodified mRNA[200].

In preclinical studies, mRNA vaccines have elicited potent germinal centre reactions and T_{FH} cell induction against SARS-CoV-2, HIV-1, Zika virus and influenza virus[128,200–202]. In humans, two doses of the Pfizer–BioNTech vaccine BNT162b2 induced strong germinal centre B cell responses for at least 12 weeks after immunization[203]. The antibody clones produced by the germinal centre B cells predominantly targeted the receptor-binding domain of the spike protein. Furthermore, the germinal centre responses after mRNA vaccination were superior to those seen after the seasonal influenza vaccination in humans[204].

In clinical trials, two doses of mRNA-1273 also elicited durable antibody responses over a period of 6 months. Although antibody titres declined slightly over the duration of the study, high neutralizing ability was retained across all age groups[205]. These results are promising; however, the duration of antibody response is a complex phenomenon that will vary from antigen to antigen and will require longer-term data for a comprehensive understanding.

Vaccines against emerging viral variants

Mutations in the viral genome are common during replication. Although the majority of mutations have little or no effect on the functions of a virus, some mutations can enhance immune evasion, stymieing vaccine development. For example, rapid mutations in HIV have prevented the development of an effective vaccine for more than three decades, and the mutations in influenza viruses necessitate annual modification of vaccine formulations to target dominant strains. Novel strategies against these viruses involve delivering mRNA vaccines that target conserved regions such as the haemagglutinin stalk on influenza[149], or encodeing broadly neutralizing antibodies such as VRC01 that bind to conserved regions of the CD4 binding site on the HIV gp120 protein[206].

Emerging SARS-CoV-2 variants have also raised concerns about the cross-variant efficacy of mRNA vaccines. The B.1.351 and P.1 variants have a glutamate (E) to lysine (K) mutation at position 484 (E484K) in the receptor-binding domain of the spike protein, which promotes immune evasion[207]. Fortunately, the FDA-approved mRNA vaccines BNT162b2 and mRNA-1273 produce cross-neutralizing antibodies against B.1.351 and P.1, as well as against other variants, suggesting that they can provide protection against them. However, cross-neutralization efficacies have been significantly lower compared with the ancestral variant[208–211]. Furthermore, in phase IIb/III trials of CureVac's candidate CVnCoV, 57% of the sequenced 124 COVID-19 cases were attributed to variants of concern, which include the B.1.351 and P.1 variants (see Related links).

If these variants of concern emerge as the dominant variant over time, variant-specific mRNA boosters may be required. At the time of this writing, Moderna is evaluating the original mRNA-1273 vaccine and updated versions of the vaccine as a third dose booster — mRNA-1273.351, which encodes the spike protein from the B.1.351 variant, and mRNA-1273.211, a multivariant vaccine that is a 1:1 mix of mRNA-1273 and mRNA-1273.351 (REF.[8]).

In the long term, a pancoronavirus vaccine that offers protection from SARS-CoV-2 and future coronavirus outbreaks would be a boon. One study has already demonstrated proof of concept. Specifically, Saunders and colleagues[212] have shown that nucleoside-modified mRNA–LNP vaccines encoding the SARS-CoV-2 spike protein can elicit SARS-CoV-1 and batCoV cross-neutralizing antibodies. As with HIV and influenza, new structural insights are expected to facilitate the

discovery of conserved sites across coronaviruses, accelerating antigen discovery and vaccine design.

Safety

Overall, mRNA vaccines have promising safety profiles, with only mild or moderate adverse events occurring in clinical trials. However, there have been scattered incidents that mandate further optimization of mRNA antigens and delivery vehicle components. For example, CureVac's protamine-based rabies candidate CV7201 elicited severe adverse events in 78% of participants[188], prompting CureVac to adopt LNPs as their preferred delivery platform for their subsequent rabies candidate CV7202 (REF.[189]). As with most drugs, adverse reactions to mRNA vaccines have often increased with dose. For example, in phase I trials of CV7202, a 5 µg dose had unacceptable reactogenicity, and 1 µg was the highest dose that was well tolerated. Furthermore, in phase I trials of the Moderna influenza H10N8 vaccine, severe adverse events from the 400 µg dose stopped further evaluation of that dose[150]. Trials including lower doses up to 100 µg continued.

Anaphylactic reactions have been observed in approximately 4.7 per million anti-COVID-19 vaccinations with the Pfizer–BioNTech vaccine and 2.5 per million vaccinations with the Moderna vaccine[213], which is about two- to fourfold more than is typically seen with more traditional vaccines[214]. One hypothesis is that the allergic response is attributed to pre-existing antibodies against the PEGylated lipid used in LNPs. These antibodies are presumed to form in response to the presence of PEG in many consumer products (for example, toothpastes, shampoos and laxatives)[215]. Although PEG has long been regarded as safe, it is hypothesized to activate humoral immunity in some people in a T cell-independent manner by directly crosslinking the B cell receptor and initiating IgM production[216]. In preclinical studies, the presence of anti-PEG IgM in animal serum accelerated nanoparticle clearance and caused a complete loss of the efficacy of mRNA therapeutics[217,218]. Anti-PEG antibodies have been reported in 40% of the population, which might heighten the risk of allergic reactions in certain individuals and impede vaccine efficacy[218]. Currently, the CDC recommends that mRNA vaccines should not be given to individuals with a history of allergic response to any of the components of the Pfizer–BioNTech or Moderna vaccines (see Related links). It is clear that, as a field, we need a better understanding of how the mRNA vaccine formulations cause allergic reactions so that the formulations can be re-engineered for improved safety profiles.

Vaccination in specific populations

Most vaccines, whether traditional or mRNA, are developed with either children or healthy adults in mind. However, several populations may benefit from alternative vaccination strategies or respond to vaccination differently owing to differences in their immune system.

Maternal/neonatal vaccination. The dynamic nature of the immune system during pregnancy increases a person's susceptibility to infectious diseases, and infection can have disastrous impacts on maternal health and fetal development[219]. Cytomegalovirus infection, for example, causes complications in up to 1% of pregnancies and can lead to congenital disabilities and neurological impairment in infants[220]. Zika virus can infect cortical neurons and glial cells in the fetus, resulting in cell death, neuroinflammation and severe congenital malformations[221]. Rare in utero transmission of SARS-CoV-2 has also been reported[222,223]; its implications on maternal, fetal and neonatal health are under active investigation.

To address these challenges, maternal vaccination has emerged as a tool to improve maternal health and reduce neonatal morbidity. Maternal IgG antibodies readily cross the placenta by binding to the neonatal crystallizable fragment (Fc) receptor, enter the fetal circulation and protect the fetus from pathogens[224]. In several studies, maternal vaccination with mRNA-loaded LNPs prevented fetal Zika virus transmission in pregnant mice[225,226] and protected mouse neonates from herpesvirus[227] and group A and group B streptococci[228].

Although vertically transferred maternal antibodies can prevent neonatal infection, they can also impede the efficacy of vaccines that are administered to infants later in life. In a mouse model of H1N1 influenza infection in which influenza-specific maternal antibodies inhibited de novo vaccine responses in pups, a LNP-encapsulated, nucleoside-modified mRNA encoding haemagglutinin from the Cal09 H1N1 strain partially overcame this inhibition[229]. The mechanism remains unclear, but the authors of the report suggested that prolonged antigen availability compared with an FDA-approved inactivated virus vaccine promoted a stronger germinal centre reaction leading to robust infant immune responses in the presence of maternal antibodies.

mRNA vaccines against SARS-CoV-2 have also been shown to be immunogenic in pregnant and lactating people, and neutralizing antibodies have been detected in cord blood and human milk[230]. Preliminary data suggest that mRNA-1273 and BNT162b2 elicited similar adverse events in pregnant and non-pregnant people, and the vaccines did not increase the incidence of neonatal death or congenital anomalies[231]. However, further longitudinal studies are necessary to assess the impact of mRNA vaccines on maternal and neonatal health.

Elderly individuals. An increase in life expectancy accompanied by reduced birth rates has led to a shift in population demographics. By 2050, the proportion of the global population over 60 years old is expected to almost double from 12% to 22% (see Related links). Effective vaccines for this group are much needed, as many infectious diseases disproportionately affect the elderly. For example, 70–90% of influenza-related mortalities occur in people older than 65 years[232], and COVID-19 is reported to be 62 times more fatal in patients older than 65 than it is in younger patients[233].

Older populations are more difficult to vaccinate because ageing adversely affects both the innate and adaptive arms of the immune system. Reduced Toll-like receptor expression stalls cytokine and chemokine secretion by monocytes and macrophages and limits crosstalk with the adaptive immune system[234]. Adaptive immune

responses during infection are often inadequate owing to impaired cytokine signalling and physiological and cellular changes. These changes can include an involuted thymus[235], fewer naive B and T cells[236], diminished T cell receptor diversity[237], higher susceptibility to T cell apoptosis[238] and reduced expression of crucial receptors such as CD28 on cytotoxic $CD8^+$ T cells[239].

Fortunately, evidence is mounting that mRNA vaccines might offer robust efficacy in all age groups. For instance, in phase III trials, the Pfizer–BioNTech vaccine candidate BNT162b2 elicited >93% efficacy across all treatment groups defined by age[125]. The Moderna vaccine candidate mRNA-1273 was also highly effective, and showed 86.4% efficacy in volunteers ≥65 years old, in comparison with 95.6% efficacy in 18–65-year-old volunteers[131].

The design of delivery vehicles is important for improving vaccine efficacy in the elderly. mRNA delivery vehicles can act as inflammatory adjuvants and amplify vaccine response by enhancing antigen-presenting cell recruitment to the injection site. In a preclinical study, CureVac's RNActive delivery platform activated TLR7 and generated durable immune responses against a lethal influenza dose in 18-month-old mice[105]. Novartis's oil-in-water emulsion MF59, which has been used as an mRNA delivery vehicle, can also act as an adjuvant. MF59 amplifies the immunogenicity of influenza vaccines and has been approved for use in elderly adults[110]. In the elderly population, MF59-adjuvanted influenza vaccines enhance seroconversion and seroprotection rates compared with non-adjuvanted vaccines[240] and induce broad serological protection against strains with antigenic drift[241].

Access to vaccines

Access to vaccines is the greatest challenge in achieving widespread protection against infectious diseases, especially in low-income countries. Access is further limited by the cold storage requirements of the currently approved SARS-CoV-2 mRNA vaccines. During the 2014–2016 Ebola virus outbreak in West Africa, vaccines requiring −80 °C storage were supplied in the Democratic Republic of the Congo using portable and reusable Arktek freezers, allowing the vaccine to be administered to 400,000 people[242]. Such technologies are promising for the rapid deployment of a few million doses during new epidemics; however, vaccinating billions of people during pandemics such as COVID-19 will require thermostable vaccines. In preclinical studies, CureVac demonstrated that its sequence-optimized RABV-G vaccine against rabies could withstand exposure to temperatures ranging from −80 °C to +70 °C for several months[243]. In addition, two SARS-CoV-2 vaccine candidates are reported to be thermostable at room temperature[135] (see Related links). If these thermostable candidates show promising results in clinical trials, they can potentially simplify global access to mRNA vaccines in the near future.

Vaccine acceptance

Vaccines are effective only if they are administered. Data supporting the safety and efficacy of vaccines are profuse, and it is undeniable that vaccines have eradicated several infectious diseases in portions of the world and saved countless lives. Nevertheless, public mistrust fuelled by misinformation and anti-vaccination movements threatens the maintenance of herd immunity and puts our most vulnerable populations at risk. Declining vaccination coverage can lead to the re-emergence of life-threatening diseases. For example, measles, which was eradicated from the USA in 2000, infected more than 1,200 people in 2019 owing to poor vaccine compliance in multiple communities (see Related links). For COVID-19, information from employers and government sources has been shown to improve vaccine acceptance rates, which range from 55% to 90% around the world[244]. In the USA, excellent efficacy data from mRNA vaccine trials have increased public confidence in these vaccines; however, current acceptance rates of 56–75%[245] may be insufficient to reach at least 80–90% coverage[246], the threshold thought to be necessary for herd immunity against SARS-CoV-2. Although much of the burden of improving vaccine coverage falls on governments and public health officials, the scientific community can help by improving mRNA vaccine efficacy and safety. Enhanced efficacy will lower the acceptance rates required for herd immunity, and improved safety will stem media reports of adverse events and, thus, decrease fear of vaccination.

Outlook

Decades of progress in mRNA design and nucleic acid delivery technology, together with the discovery of novel antigen targets have made mRNA vaccines an extraordinary tool for combating emerging pandemics and existing infectious diseases. The first two mRNA vaccines, which were developed at revolutionary speed to fight SARS-CoV-2, have exceeded expectations and offer hope that the COVID-19 pandemic will end. Furthermore, these vaccines have elevated LNPs and RNA therapy from small-market products for niche diseases to a prophylactic treatment deployed successfully in large swathes of the population. The resultant abundance of positive safety and efficacy data, together with a proven path to regulatory approval, leave us optimistic that mRNA therapeutics will transform modern medicine's approach to vaccination[1,5], cancer immunotherapy[247–249], protein replacement therapy[15,23] and beyond[250].

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N.C. and K.A.W. researched data for the article. N.C. and K.A.W. wrote the article. All authors reviewed and edited the manuscript before submission.

Competing interests
K.A.W. is an inventor on the following patents related to lipid nanoparticles: US Patent 8,450,298; 8,969,353; 9,556,110; 9,872,911; 9,227,917; 9,439,968; 10,189,802; and 10,844,028. K.A.W. is bound by confidential agreements that prevent her from disclosing related consulting relationships.

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