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Review

Messenger ribonucleic acid vaccines against infectious diseases: current concepts and future prospects

Darin K Edwards and Andrea Carfi

Over the past two decades, scientific and technological advancements have revealed messenger ribonucleic acid (mRNA)-based vaccines as a well-tolerated and effective platform to combat infectious disease. The potential of mRNA-based vaccines was epitomized during the severe acute respiratory syndrome coronavirus 2 pandemic, wherein mRNA-based vaccines were rapidly developed and found highly efficacious with an acceptable safety profile. These properties together with the capability to quickly address pathogens of pandemic potential, pathogens with complex antigens, and multiple pathogens within a single vaccine have revitalized the field, and multiple mRNA-based vaccines have now entered clinical development. This review summarizes current mRNA-based vaccine technology, perspectives on ongoing clinical studies, and future prospects for the field.

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Introduction

Vaccination remains one of the most effective strategies to combat pathogens and avert public health crises worldwide. Over the last two centuries since the development and widespread use of the smallpox vaccine, vaccine technologies have remarkably progressed due to continued advancements in our understanding of vaccine science [1,2]. These new technologies may address existing hard-to-target pathogens as well as novel infectious diseases that pose additional threats to humanity.

One such technological advancement in the vaccine field is the use of messenger ribonucleic acid (mRNA), which has historically faced challenges, including instability, reactogenicity, inefficient delivery and translation, and poor immunogenicity. The recent development of two well-tolerated and highly efficacious mRNA-based vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has highlighted the developmental progress of this technology and its potential to combat multiple infectious diseases. With more than two billion total doses of the two authorized SARS-CoV-2 mRNA vaccines administered worldwide (mRNA-1273 [Spikevax; Moderna, Inc., Cambridge, MA] or BNT162b2 [Comirnaty; BioNTech, Mainz, Germany; Pfizer Inc., New York, NY]) [3], the mRNA vaccine platform has demonstrated the ability for the expeditious and scalable development of well-tolerated and efficacious vaccines.

The global success of the mRNA-based SARS-CoV-2 vaccines has further reinvigorated interest in the mRNA platform and accelerated the development of mRNA vaccines against additional infectious-disease targets. The mRNA platform affords several advantages, including generation of difficult-to-manufacture multiprotein complexes, concomitant expression of multiple antigens (e.g. combination vaccines against multiple pathogens), and precise protein engineering enabling expression of structurally stabilized versions, virus-like particles, or multicopy antigen presentation. Further, the manufacture of mRNA-based vaccines follows well-defined and consistent processes, using similar reagents, regardless of the antigen encoded by the mRNA, which simplifies the production, scale-up, quality control, and overall development timelines. Finally, mRNA vaccines deliver a 'digital code' of the antigen without a need for protein purification or pathogen inactivation, which is particularly advantageous when a rapid response is essential to tackle outbreaks or pandemic situations [4]. Here, we summarize key features of mRNA-based vaccines against infectious disease, our current understanding of their mode of action, recent findings from ongoing clinical studies, and future prospects for the field.

Basic concepts of mRNA vaccine technology

Endogenously, mRNA acts as a blueprint for protein synthesis, first transcribed from genomic DNA in the nucleus and then transported to ribosomes in the cytoplasm for translation into proteins. In eukaryotes, mRNA follows a basic (5′–3′) structure, consisting of a 5′ cap, a 5′ untranslated region (UTR), an open reading frame (ORF) encoding the protein, a 3′ UTR, and a poly(A) tail. Each of these structural components are critical in regulating mRNA translation and stability, ultimately impacting protein expression and biological activity [5–9].

Owing to the length and structural characteristics of mRNA, its production and characterization are different than that of other RNA therapeutics, such as small-interfering RNA (siRNA) consisting of short double-stranded RNA (dsRNA). Production of mRNA is currently accomplished through *in vitro* transcription (IVT), which utilizes linearized DNA as template, an RNA polymerase, and nucleotide triphosphates in a buffered environment [10,11]. Purified IVT mRNA ultimately resembles fully processed, mature, endogenous mRNA molecules present in the cytoplasm of eukaryotic cells.

As mRNA and certain IVT by products are inherently immunostimulatory, different approaches to mRNA chemistry and manufacturing have been developed to modulate innate immune responses (Figure 1) [12,13].

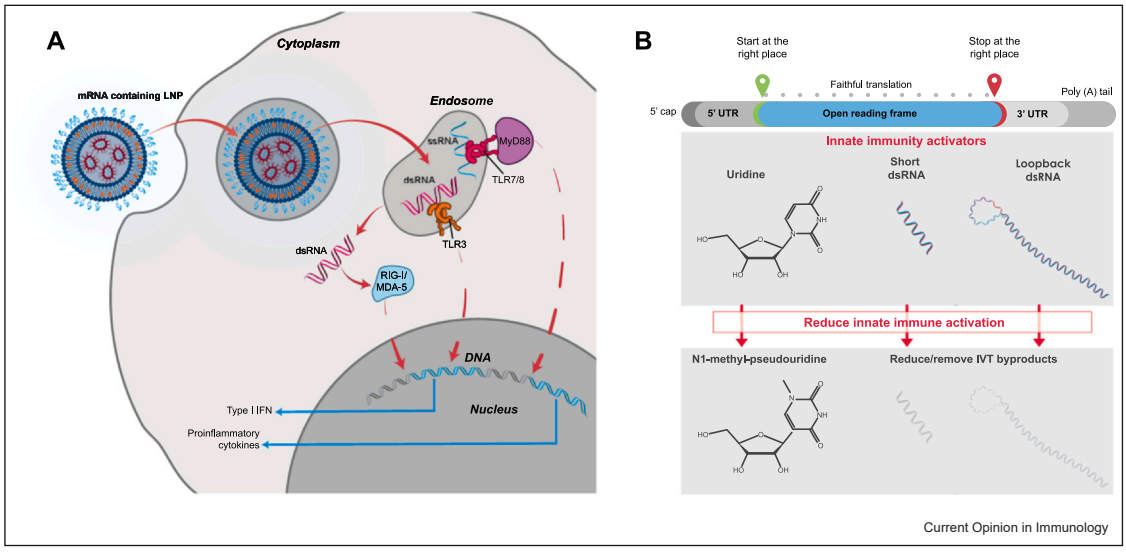
Notably, mRNAs contained within mRNA-1273 and BNT162b2 replace the uridine nucleotide with a modified uridine (N1-methyl-pseudouridine [N1-methyl-Ψ]) to minimize innate immune activation through Toll-like receptor (TLR)7/8 signaling [12]. In contrast, other mRNA-based vaccine platforms have focused on unmodified, sequence-engineered mRNA. For example, CureVac uses unmodified mRNA for their SARS-CoV-2 vaccine with a codon-optimized ORF, containing enriched guanine and cytosine content, and sequence-engineered 5′ and 3′ UTRs [13,14].

Of note, mRNA does not integrate with the cellular genome and is quickly degraded by endogenous processes after injection, which decrease risks of metabolite-induced toxicity [15]. However, to function, mRNA must overcome certain physiological barriers for efficient delivery to cells and subsequent translation. In this regard, multiple delivery techniques have been developed to protect mRNA as well as to facilitate its cellular entry and delivery into the cytoplasm where translation occurs.

mRNA–lipid nanoparticle vaccine delivery and mechanism of action

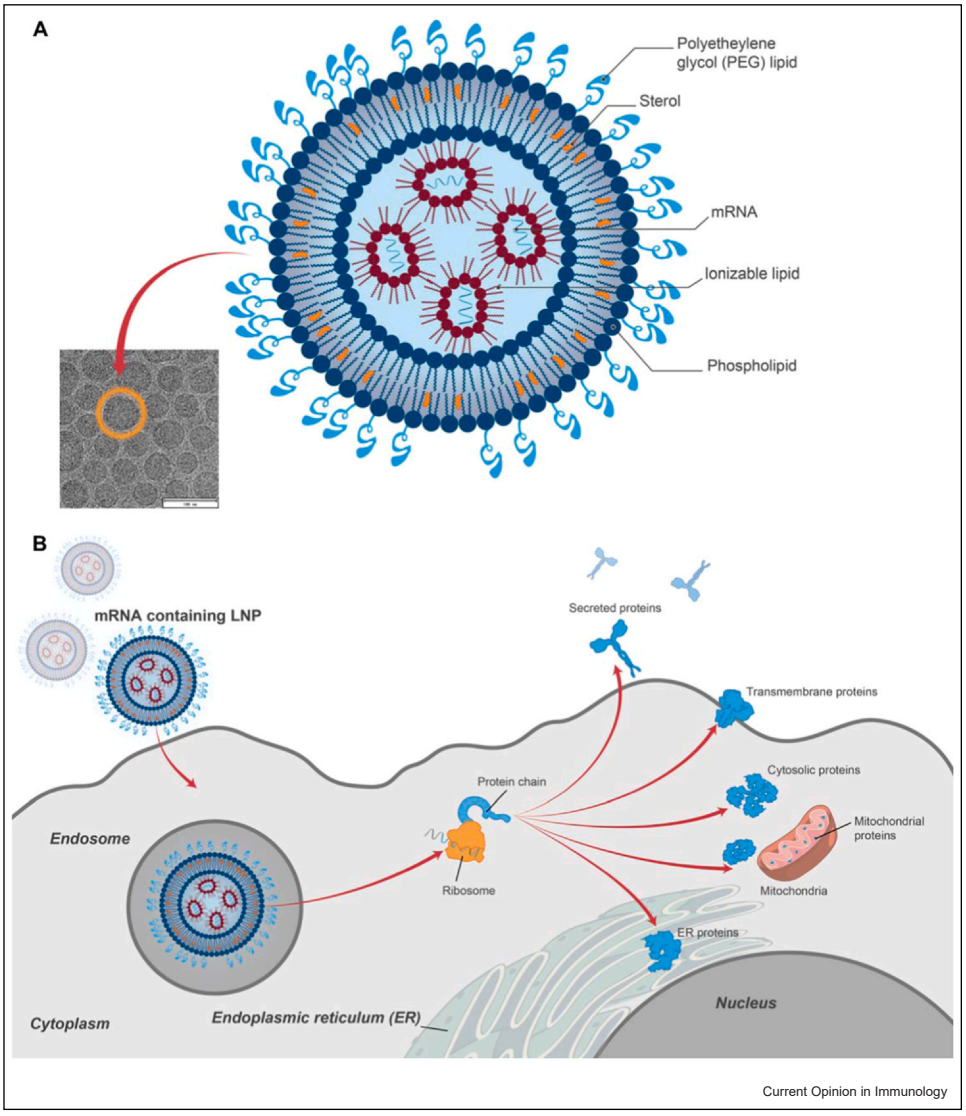
One of the leading delivery vehicles for mRNA-based vaccines is lipid nanoparticles (LNPs), approximately 100 nm in diameter and often composed of four components that together encapsulate and protect the mRNA: an ionizable lipid, a sterol, a lipid-linked

Figure 1



Technological approaches to reduce innate immune activation by mRNA-based vaccines. **(a)** After entering the cell, IVT mRNA and certain IVT-related impurities (such as dsRNA and ssRNA fragments) can be recognized by a variety of innate immune receptors, including endosomal TLR3 or TLR7/8 and cytosolic sensors MDA5 or RIG-I, ultimately resulting in robust type-1 interferon signaling and proinflammatory cytokine induction. **(b)** Manufacture of IVT mRNA has been steadily advanced to limit innate immune activation, including by replacing uridine-5′-triphosphate with N1-methyl-pseudouridine-5′-triphosphate within the IVT mRNA, and removing or reducing the amount of short dsRNA and loopback dsRNA produced during the IVT process. MDA5, melanoma differentiation-associated protein 5; RIG-1, retinoic acid-inducible gene I; ssRNA, single-strand RNA.

Figure 2



Current LNP technology in mRNA-based vaccines. **(a)** LNPs consist of an ionizable lipid, sterol, phospholipid, and a PEG lipid that together encapsulate and protect the mRNA core. Each lipid component has critical roles in LNP function. **(b)** The LNP-delivery system has been engineered to enable efficient cellular delivery of mRNA to cells and overcome certain physiological barriers, including protecting mRNA from degradation, uptake, and facilitating endosomal escape for subsequent translation of the encoded antigen(s).

polyethylene glycol (PEG), and a phospholipid [16] (Figure 2). Integral to the function of LNPs is the ionizable amino lipid, which promotes mRNA encapsulation and endosomal escape for cytoplasmic release. While early work demonstrated the utility of positively-charged amino lipids in delivery systems for nucleic acids, tolerability challenges limited their application. Ionizable lipids that remain neutral at physiological pH were developed to overcome these initial hurdles, including Dlin-MC3-DMA (MC3) that is the ionizable component of the first FDA-approved RNA therapeutic formulated with LNP (Onpattro, an siRNA therapeutic); however, further development led to more biodegradable LNPs with improved efficiency and tolerability, including the ionizable lipids used in both mRNA-1273 (SM-102) and BNT162b2 (ALC-0315) [16–18]. Beyond the ionizable lipid, the remaining three lipid components also play key roles in LNP function: cholesterol is a structural component that aids in stabilizing the LNP, PEGylated lipids extend the half-life of the LNP, and distearoylphosphatidylcholine (DSPC), which modulates LNP bilayer fluidity [16].

After intramuscular administration, mRNA–LNPs are endocytosed by cells at the injection site and within the draining lymph nodes. Immune cells, specifically

antigen-presenting cells (i.e. dendritic cells, monocytes, and subcapsular sinus macrophages), are the predominant populations that endocytose the mRNA-LNP, with the mRNA subsequently translated to produce the encoded protein antigen (Figure 2) [19]. Intracellular expression and processing of this antigen allows for efficient presentation on major histocompatibility complex class I and II proteins, inducing strong and persistent CD4+ and CD8+ T-cell responses, while expression of secreted or cell surface-anchored antigens engages B-cell receptors to activate B cells and antigen-specific antibody production. Follicular T-helper cell responses have also been demonstrated after mRNA vaccination, which are critical to the development of potent and durable neutralizing-antibody responses [20–23]. Notably, some LNP-delivery vehicles have been shown to have a role in inflammation and immune stimulation in preclinical mouse models [24,25]. However, advancements in LNP technology have further optimized antigen expression, tolerability, and immunogenicity for mRNA vaccines, including those against SARS-CoV-2 [16–18].

Clinical and real-world studies of mRNA-1273 and BNT162b2 against SARS-CoV-2

Decades of research to optimize the mRNA platform and its potential to enable rapid vaccine development was put to the test in response to the emergence of SARS-CoV-2 and the ensuing COVID-19 pandemic. Two SARS-CoV-2 vaccines (mRNA-1273 and BNT162b2) were expeditiously developed and comprehensively characterized in preclinical and clinical evaluations. Initial studies in adults demonstrated the safety and tolerability profiles of these mRNA vaccines, the SARS-CoV-2 antigen-specific immune profile, and the high degree of efficacy against symptomatic and severe COVID-19 disease [26–29]. Results from these trials have led to vaccine licensure in the United States and multiple countries throughout the world. Multiple clinical trials are still ongoing in the United States and other global regions, including those to support use in different age populations and at-risk groups [30,31]. In addition, modified COVID-19 mRNA vaccines that match key variants of concern are under investigation as booster vaccinations in multiple nonclinical and clinical studies [32–34].

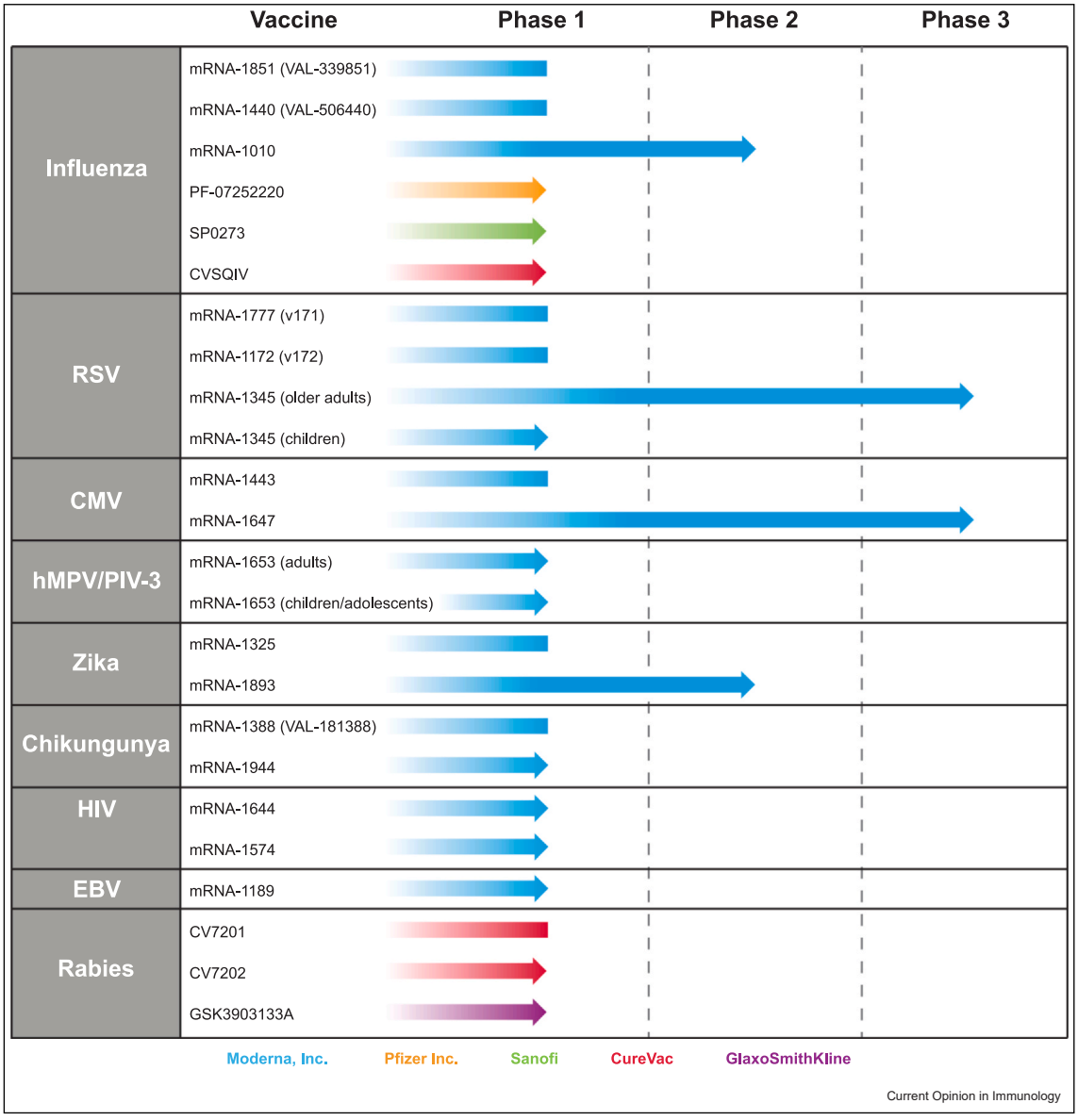
The post-authorization experience for these SARS-CoV-2 mRNA vaccines remains unprecedented, characterized by global, large-scale vaccination efforts enabling rapid and in-depth accumulation of real-world evidence on the immunity and effectiveness associated with mRNA vaccines [35–43], including in previously infected subjects (i.e. hybrid immunity) [44–47] or in heterologous booster regimens with adenoviral SARS-CoV-2 vaccines [48–50]. Assessments of mRNA vaccine-elicited immunity have shown strong and persistent germinal center reactions post vaccination, potent neutralizing antibody titers that wane over time, Fc-mediated effector antibody functions, as well as generation of cross-reactive CD4+ and CD8+ memory T-cell and B-cell responses [20,51–56]. Further, the potential impact of newly emerged SARS-CoV-2 variants on immunogenicity and vaccine-mediated protection has also been continually characterized [39,40,57–60], showing reduced and waning neutralizing antibody responses and vaccine effectiveness for certain variants, most notably the currently globally dominant omicron strain [61–64].

Safety and immunogenicity in specific populations such as pregnant women have been assessed, with fetal antibody transfer and antibody Fc function demonstrated [65–67]. Similar investigations have been performed in older adults, with some evidence of the impact of immunosenescence on immunogenicity [68], although vaccine effectiveness remains generally high [42,47,69]. However, real-world evidence has also indicated that vaccine effectiveness is typically lower among immunocompromised populations, particularly solid organ or stem cell transplant recipients [70]. Additionally, although post-authorization safety signals have been identified, including mild cases of myocarditis/pericarditis in younger males [71,72] and anaphylaxis [73], rates remain low and cases generally resolved. Overall, these studies have significantly contributed to the depth of our scientific knowledge on mRNA vaccines and have further advanced the field, as demonstrated by the multiple mRNA vaccines against other infectious pathogens now in clinical development.

Beyond SARS-CoV-2: clinical progress on mRNA vaccines against other pathogens

Before the clinical study of mRNA vaccines against SARS-CoV-2, mRNA-based vaccines against several other infectious disease targets had been tested in phase 1 and 2 clinical studies. The first mRNA based vaccine evaluated in a phase 1 trial was one formulated with protamine and encoding rabies virus glycoprotein (CV7201, CureVac) [74]. Though CV7201 was reasonably well-tolerated, immune responses were short-lived and induced only when the vaccine was administered with a needle-free device [74]. Two influenza vaccine candidates with a first-generation LNP formulation followed (expressing hemagglutinin from H10N8 or H7N9 strains, Moderna, Inc.) were among the first mRNA–LNP vaccine candidates to advance to clinical stage. Both vaccines were well-tolerated and raised protective immune responses [75], demonstrating that mRNA–LNP vaccines can be well-tolerated and immunogenic in humans. Subsequently, LNPs were utilized as a delivery vehicle for a second-generation rabies vaccine (CV7202, CureVac), which induced immune

Figure 3



mRNA vaccines against pathogens besides SARS-CoV-2 in clinical development. The progression and current clinical stage (phase 1, 2, or 3) of mRNA-based vaccines against infectious diseases beyond SARS-CoV-2 is shown, with blue arrows representing vaccines currently under active development and blue boxes representing vaccines not under active investigation. Two influenza vaccines by Sanofi/Translate Bio (MRT-5400 and MRT-5401) have also been under phase 1 clinical evaluation but are not included as the current status is unclear.

responses after two doses in a phase 1 trial, but showed high reactogenicity at the highest dose level (5 μg) [76].

Since this initial work, numerous mRNA-based vaccines against infectious diseases have entered clinical development and a few have advanced to late-stage clinical trials (Figure 3). Among those furthest progressed are mRNA-based vaccines against cytomegalovirus (CMV, mRNA-1647, Moderna, Inc.) and respiratory syncytial virus (RSV, mRNA-1345, Moderna, Inc.). Formulated with the proprietary LNP used for mRNA-1273, mRNA-1647 consists of six mRNAs encoding two CMV antigens (five mRNA sequences encoding for the five subunits of the pentameric complex and a single mRNA sequence encoding the full-length glycoprotein B). In a phase 1 trial, the vaccine was generally well-tolerated and three doses were immunogenic in CMV-seronegative participants, resulting in epithelial and fibroblast neutralizing antibody responses above the baseline CMV-seropositive levels,

and strongly boosted immune responses among CMV-seropositive participants [77]. The mRNA-1647 vaccine, which has recently advanced to a phase 3 efficacy study, highlights the ability of the mRNA platform to target complex antigens and multiplex antigens in a single vaccine. A phase 3 clinical trial in adults aged ≥60 years was also recently initiated for the investigational mRNA-1345 vaccine against RSV, which encodes for the F glycoprotein stabilized in the prefusion state formulated with the same proprietary LNP of mRNA-1273. Phase 1 findings in older RSV-seropositive adults (aged 65–79 years) showed that a single dose of the vaccine was well-tolerated and boosted neutralizing responses 10-fold to 14-fold above baseline [78]; the vaccine is aimed to provide protection against RSV for both older adult and pediatric populations.

The ability to combine multiple mRNA sequences into a single vaccine also has potential to target multiple pathogens or viral strains. For example, a combination vaccine against human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV) has been developed (mRNA-1653, Moderna, Inc.), with a phase 1 study in healthy adults showing the vaccine was well-tolerated and elicited functional immune responses [79]; a phase 1b study in adults and hMPV/PIV3-seropositive children was the first clinical trial for a mRNA–LNP vaccine to be initiated in a pediatric population. Further, multiple mRNA-based vaccines against influenza are currently under early clinical evaluation (Figure 3), with this novel technology potentially allowing for rapid future tackling of influenza strains of pandemic potential, multiplexing against multiple antigens to broaden immunity, and reducing vaccine production time to enable more informed decision-making on the seasonal strains to be addressed.

Future prospects and overall conclusions

As additional mRNA-based vaccine programs enter late-stage clinical trials and further research is conducted on available SARS-CoV-2 vaccines, these findings can be anticipated to prompt further technological advancements and insight into the mode of action, safety, immunological properties, and protective efficacy of mRNA-based vaccines in humans. Key among those insights should be on the duration of mRNA-based vaccine-mediated protection against disease as well as breadth of protection, particularly for rapidly mutating pathogens such as SARS-CoV-2, influenza virus, and human immunodeficiency virus. Further knowledge on the immunological properties and efficacy of mRNA-based vaccines in certain populations is also warranted, including pregnant women, infants and children, the elderly, and the immunocompromised. Safety is also paramount and will be further informed by ongoing clinical studies and real-world monitoring of safety events following mRNA-based vaccination.

Improvements to certain mRNA vaccine characteristics are also warranted, including temperature stability to enable easier vaccine handling, storage, and access to the developing world. Efforts to increase the half-life of mRNA expression through optimization of coding sequences and UTRs are also predicted to increase potency of mRNA vaccines and could result in the lowering of effective doses. For example, while an initial unmodified SARS-CoV-2 vaccine developed by CureVac (CvnCoV) only achieved 48.2% efficacy against COVID-19 [80], continuous program developments led to a second-generation candidate (CV2CoV) with improvements to the noncoding regions and increased immunogenicity and protection in nonhuman primates [14].

Beyond the conventional mRNA-based vaccines, vaccines using self-amplifying RNA, which encodes virus-derived polymerase to amplify mRNA and thereby allow for high antigen expression at a low dose level, have now also entered clinical testing [81]. Further, although still at the early phase of development, vaccines based on circular RNA, which comprises highly stable RNA in a closed-ring structure without the need for certain features of mRNA, including a 5′ cap or 3′ poly-A tail, are being evaluated in preclinical studies [82]. Additional efforts to update or modify the LNP-delivery vehicle have the potential to enable mRNA vaccine formulations that target specific cells or tissues or may further improve the tolerability or potency of the mRNA vaccine platforms. Finally, alternative delivery routes of immunization (i.e. intranasal or intradermal delivery) could further expand the applications of this vaccine technology by providing optimal protection in mucosal tissues and directly at the site of infection.

Overall, the prospect for mRNA-based vaccine platforms to combat both pervasive and emerging infectious diseases remains optimistic, with the rapid development and deployment of well-tolerated and effective mRNA-based SARS-CoV-2 vaccines paving the path forward for this contemporary and transformative technology.

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Author contributions

All authors contributed to the article conception and design, interpreting the relevant literature, drafting of the paper, and/or critically revising it for intellectual content.

Conflict of interest statement

All authors are employees of Moderna, Inc., and hold stock/stock options.

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