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Current Developments and Challenges of mRNA Vaccines

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mRNA vaccines, infectious diseases, cancer vaccines, lipid nanoparticle, LNP, self-adjuvanting effect

Abstract

mRNA vaccines have brought about a great revolution in the vaccine fields owing to their simplicity and adaptability in antigen design, potential to induce both humoral and cell-mediated immune responses and demonstrated high efficacy, and rapid and low-cost production by using the same manufacturing platform for different mRNA vaccines. Multiple mRNA vaccines have been investigated for both infectious diseases and cancers, showing significant superiority to other types of vaccines. Although great success of mRNA vaccines has been achieved in the control of the coronavirus disease 2019 pandemic, there are still multiple challenges for the future development of mRNA vaccines. In this review, the most recent developments of mRNA vaccines against both infectious diseases and cancers are summarized for an overview of this field. Moreover, the challenges are also discussed on the basis of these developments.

Contents

1. INTRODUCTION

mRNA vaccines have opened a new era for vaccine development, attributed mostly to the success of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine established in 2020. Two mRNA vaccines, mRNA-1273 and BNT162b2, were approved by the US Food and Drug Administration (FDA) and have since been administered to several hundred million people (1). These vaccines have shown great success as they continue to exhibit an excellent protection efficacy of more than 90% (1). Moreover, mRNA vaccines also have great potential in cancer immunotherapy, owing to their ability to induce both robust humoral and cellular responses (2). Humoral immune responses are mediated by antibodies, which can neutralize viruses and toxins and opsonize bacteria and viruses for more efficient clearance by phagocytic cells. Cellular immune responses are mediated by T cells, where $CD8^+$ T cells can eliminate pathogens by killing infected cells or tumor cells. While antibodies are critical for preventing infection, T cell responses are necessary for rapid recovery following infection. In addition, cellular immunity plays a major role in mediating the therapeutic efficacy of cancer vaccines. To date, most mRNA-based cancer vaccines are still in phase I/II development, and there is no clinically approved therapeutic mRNA cancer vaccine yet.

A typical mRNA vaccine consists of two major components: mRNA and its delivery system, both of which are required for the expression of encoded antigens and the induction of adaptive immunity in the human body (**Figure 1**). First, mRNA encoding desired proteins or polypeptides provides the molecular basis for inducing specific immunity against a pathogen or cancer. For infectious disease vaccines, the antigens encoded by mRNA are usually the surface proteins of pathogens. For example, in the mRNA-1273 and BNT162b2 vaccines, the mRNA encodes the spike (S) protein of SARS-CoV-2. Some of the induced antibodies can prevent infection by inhibiting the S protein from binding to the host cellular receptor ACE2 during infection, whereas other antibodies can opsonize the viruses and therefore promote their rapid removal. For cancer vaccines, the antigens encoded by mRNA are usually the overexpressed or specific mutated proteins or neoepitopes that are presented by major histocompatibility complex (MHC) molecules. Technically, vaccine mRNA is usually manufactured by in vitro transcription (IVT) from a DNA template with T7 RNA polymerase (3). mRNA also requires optimization of the 5′ cap, 3′ and 5′ untranslated regions, nucleotide modifications, and poly(A) tail for efficient translation. In addition, a purification by high-performance liquid chromatography (HPLC) is performed to yield high-purity mRNA for vaccine application. Second, delivery systems are also

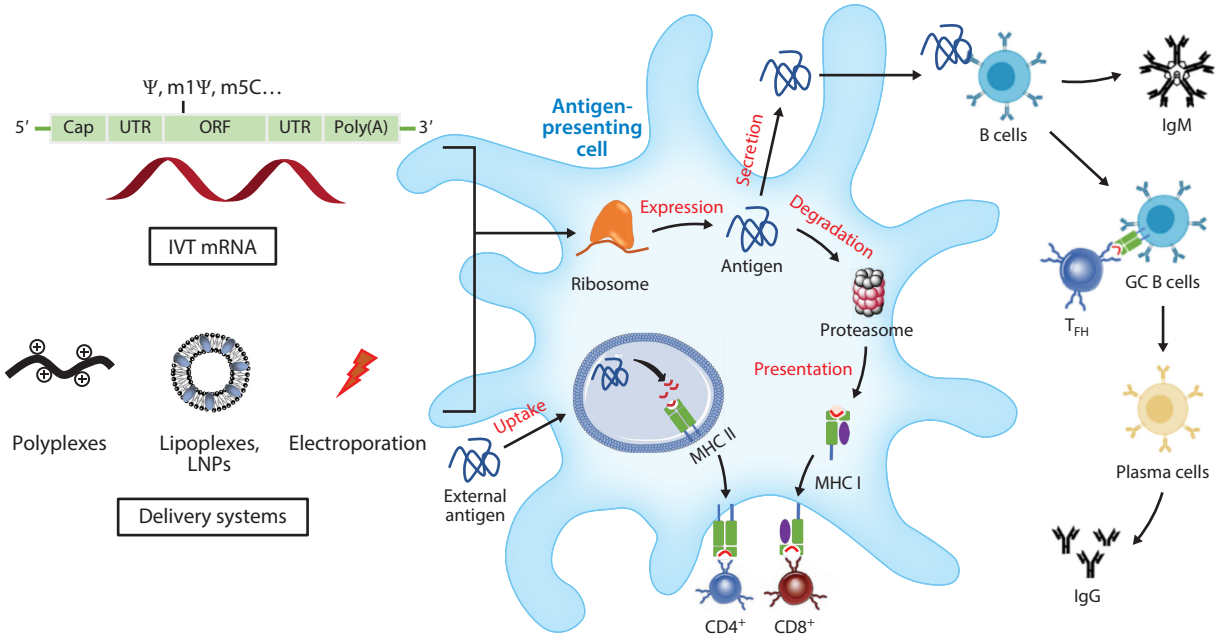

Figure 1

General mechanism of mRNA vaccines. Activation of both $CD4^+$ and $CD8^+$ T cells by presentation of antigens in two different MHC pathways is shown. Abbreviations: GC, germinal center; IgG, immunoglobulin G; IgM, immunoglobulin M; IVT, in vitro transcription; LNP, lipid nanoparticle; MHC, major histocompatibility complex; ORF, open reading frame; T_{FH}, T follicular helper cells; UTR, untranslated region.

an important component of mRNA vaccines for clinical application in the human body (4). The delivery systems not only protect the mRNA from extracellular degradation but also facilitate mRNA entry into the cytosol of antigen-presenting cells (APCs) and possibly the expression of encoded antigens. Great efforts have been put forth in optimizing both mRNA and its delivery systems, and these efforts have brought the mRNA vaccine to various clinical applications. There are various types of delivery systems based on lipids, polymers, and exosomes for in vivo mRNA delivery. Lipid nanoparticles (LNPs) are the most developed carrier for in vivo administration of mRNA and have been applied in the SARS-CoV-2 mRNA vaccine. LNPs are composed of ionizable/cationic lipid, helper lipid, cholesterol, and polyethylene glycol (PEG)-conjugated lipid. Ionizable/cationic lipid is the major active component and determines the delivery and expression efficacy of mRNA vaccines. Helper lipid and cholesterol both contribute to the stability and also benefit the membrane fusion of LNPs. Last but not least, due to the stealth effect, PEG-modified lipid is necessary to increase stability for in vivo applications. Polymers and exosomes are also potential delivery systems for mRNA, although no clinical applications have been developed yet.

mRNA vaccines have many specific advantages compared with other vaccines. First, compared with inactivated pathogens, protein subunits, and peptide vaccines, which predominantly stimulate antibody responses, mRNA vaccines induce both antibody and $CD8^+$ T cell responses due to the expression of encoded antigens in host APCs. Second, mRNA vaccines can be manufactured more rapidly (5). Since mRNA can theoretically encode any kind of antigen, the production of mRNA vaccines against different targets can be done with minimal adaptation to processing and formulation. The rapid manufacturing is quite important for the control of rapidly emerging pandemics and protection from variants of concern. Third, the self-adjuvanting effect of both

mRNA and its delivery systems can promote robust and long-lasting adaptive immune responses, in contrast to protein or peptide-based vaccines that usually require the addition of adjuvants (3). Fourth, compared with DNA vaccines, the expression of antigens encoded in mRNA vaccines is quicker and more efficient since the mRNA can be functional in cytoplasm while DNA needs to get into the nucleus and be transcribed before proteins can be made (6). Furthermore, integration of mRNA into the host DNA genome is much less likely compared with DNA vaccines. Finally, the transient activity of mRNA allows it to be completely cleared after the expression of enough antigens, potentially lowering the burden to the host homeostasis.

Although the success of the SARS-CoV-2 mRNA vaccine brings great inspiration to this field, there are still many challenges that remain for future development. Confirmed positive cases of coronavirus disease 2019 (COVID-19) have occurred even after patients have been immunized with the SARS-CoV-2 mRNA vaccine (7). The rapid decline of neutralizing antibody titers following SARS-CoV-2 mRNA vaccination suggests the need to induce sustained effective immunity. The clinical outcome of cancer vaccines is still far from effective protection. Therefore, the efficacy of mRNA vaccines should be further developed via optimization of the mRNA and its delivery systems. Additionally, the safety of mRNA vaccines raises concern due to the greater number of cases of adverse reactions compared with traditional inactivated vaccines. Moreover, issues such as storage and antigen mutations should be taken into consideration for future development of mRNA vaccines (8). In this review, the most recent developments of mRNA vaccines for infectious diseases and cancer are summarized, and the major challenges and possible solutions for further applications of mRNA vaccines are discussed.

2. RECENT DEVELOPMENTS OF mRNA VACCINES

2.1. Infectious Diseases

Since the first vaccine was used for cowpox in 1796 (9), researchers have developed vaccines for the prevention and control of many infectious diseases. Traditional vaccines, mostly inactivated pathogens, have shown great success in the prevention of more than 30 infectious diseases worldwide, even resulting in the eradication of multiple infectious diseases. However, traditional vaccines for some more challenging infectious diseases still fail to reach a high level of protection. mRNA vaccines emerged as an advanced technology for vaccines, inducing both strong humoral and cellular immune responses. Due to the rapid spread of COVID-19 worldwide, two mRNA vaccines against SARS-CoV-2, mRNA-1273 and BNT162b2, were approved by the FDA for emergency use authorization in 2021. Moreover, multiple mRNA vaccines designed for challenging viruses that induce chronic or repeated infections such as human immunodeficiency virus (HIV) have also been moved into clinical trials (**Table 1**). The exciting results of these applications underscore the importance of mRNA vaccines in the future development of vaccines against infectious diseases.

2.1.1. Emerging or reemerging infectious viruses. Emerging or reemerging infectious viruses are characterized by the emergence of new pathogens or pathogens that have reappeared after an undetectable period; these viruses are a severe threat to human health due to the suddenness and uncontrollability of the spread (10). First, due to the urgent requirements of effective vaccines for controlling emerging or reemerging viruses, the traditional strategy of vaccine development (inactivated viruses) might not be able to provide the necessary rapid progress during pandemics (11). Second, reemerging viruses such as influenza are highly variable, resulting in challenges in the development of a broadly effective vaccine (12). mRNA vaccines have the potential for rapid, inexpensive, and scalable good manufacturing practices and can be regarded as an ideal platform for developing vaccines against emerging infections with high efficacy and timeliness.

Table 1 Active clinical trials of mRNA vaccines against infectious diseases

Disease	Product name	Antigen	Delivery system	Phase(s)	NCT number(s)
COVID-19	ChulaCov19	Unknown	LNP	I	NCT04566276
	SAM-LNP-S, SAM-LNP-S-TCE	S protein plus additional SARS-CoV-2 TCEs		I	NCT04776317
	PTX-COVID19-B	Full-length S protein		I	NCT04765436
	mRNA-1283	RBD and N-terminal domain		I	NCT04813796
	mRNA-1273.351	S protein of the SARS-CoV-2 B.1.351 and P.1 variant		I	NCT04785144
	mRNA-1273.211	Combined mRNA-1273 and mRNA-1273.351		II, III	NCT04927065
	NA	RBD		III	NCT04847102
	CVnCoV	Full-length S protein		I, II, III	NCT04838847, NCT04860258, NCT04848467, NCT04652102, NCT04674189, NCT04515147
Rabies	CV7202	Rabies virus glycoprotein	LNP	I	NCT03713086
Zika virus	mRNA-1893	prM/ENV protein	LNP	II	NCT04917861
Respiratory syncytial virus	mRNA-1345	F glycoprotein	LNP	I	NCT04528719
Seasonal influenza	mRNA-1010	Unknown	LNP	I, II	NCT04956575
Cytomegalovirus	mRNA-1647	Subunits of membrane-bound pentamer complex and the full-length membrane-bound glycoprotein B	LNP	II	NCT04232280
HMPV and PIV3	mRNA-1653	Viral antigenic protein associated with HMPV and PIV3	LNP	I	NCT04144348

Abbreviations: HMPV, human metapneumovirus; LNP, lipid nanoparticle; NA, not applicable; NCT, National Clinical Trial; PIV3, parainfluenza virus type 3; prM/ENV, premembrane and envelope; RBD, receptor-binding domain; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCE, T cell epitope.

2.1.1.1. Coronaviruses. Three types of coronaviruses including SARS-CoV, SARS-CoV-2, and Middle East respiratory syndrome–related coronavirus (MERS-CoV) can cause acute respiratory diseases known as SARS, COVID-19, and MERS, respectively. Owing to their quick spread, rapid mutation, and high mortality rates, community-wide (mass) vaccination might be the only way to control the transmission and risk of these viruses (13).

The efficacy and rapid production of mRNA vaccines for infectious diseases were seen first-hand in the development of the mRNA vaccines against SARS-CoV-2. Early in January 2020, the design of the COVID-19 mRNA vaccine started in many research institutes and companies after the publication of the genomic sequences of SARS-CoV-2. The antigens encoded in the mRNA sequence included the full S protein (14–16), receptor-binding domain (17, 18), and virus-like

particles containing S, membrane (M), and envelope (E) proteins (19). Among these various proteins, the S protein became the most used. Inspired by previous experience with the MERS mRNA vaccine, most mRNA encoding the S protein contains two proline mutations at K_{986} and V_{987}. Moreover, almost all of the candidate mRNA vaccines used LNPs as the delivery system. After preclinical evaluation of the safety and efficacy of the vaccines in mice and nonhuman primates, two leading companies, Moderna and Pfizer/BioNTech, published their phase I/II clinical trial data early in July and August 2020 (20–23). The mRNA vaccines developed by these companies were proved to elicit acceptable safety profiles and robust neutralizing antibody responses in healthy adults. In December 2020, both companies reported the results of their phase III clinical trials, showing the vaccines to be roughly 95% effective (24, 25). After the evaluation of clinical results, the FDA approved the emergency use of these two vaccines, BNT162b2 and mRNA-1273, on December 11 and 18, 2020, respectively. The development of these two COVID-19 mRNA vaccines took less than one year, thus demonstrating the great potential of mRNA vaccines in combating infection from rapidly emerging viruses.

Recent clinical results show that both BNT162b2 and mRNA-1273 exhibit broad protection among working adults, elderly individuals, and immune-compromised people (26–33). A single dose of the mRNA vaccine elicited an antibody response and contributed to the reduction in the spread of COVID-19 (34–36). However, in some patients with underlying conditions, the antibody response and efficacy of the mRNA vaccine was reported to be impaired after either the first or second injection (37–46). In patients who had previously been confirmed positive for SARS-CoV-2, both the mRNA-1273 and BNT162b2 vaccines induced strong immune responses after the first dose (47–50). This immune response was similar to or even exceeded the immune response in noninfected individuals who had received two doses of the vaccine. This outcome suggests that a single dose of the vaccine may be enough to obtain a high level of antibody titers in the seropositive individuals (47, 51–53). Furthermore, the emerged P.1 and B.1.351 variants showed strong resistance to neutralization by antibodies from BNT162b2- and mRNA-1273-immunized individuals, as discussed in Section 3.1 (48, 49). Although these vaccines have shown some success in slowing the spread of the virus, the vaccines are still far from providing full protection against SARS-CoV-2 infection owing to the rapid emergence of virus variants. As it was the first time for the broad application of an mRNA vaccine, the safety and efficacy still need to be carefully evaluated on the basis of clinical data from large studies; this is an important step for further vaccine development. All these clinical outcomes have enriched our knowledge about mRNA vaccines and helped us understand how to further improve their efficacy.

2.1.1.2. Flaviviruses. Flaviviruses are vector-borne RNA viruses, which can emerge unexpectedly and potentially cause many severe diseases in humans. Multiple flaviviruses have become established or have emerged as threats to public health including dengue virus (DENV), West Nile virus, Japanese encephalitis virus, yellow fever virus, and Zika virus (ZIKV) (50). The development of flavivirus vaccines is usually complicated by the potential of antibody-dependent enhancement (ADE) (54). Traditional flavivirus vaccines are mainly based on live attenuated viruses with a high risk of ADE. Recently, mRNA vaccines were investigated as the next generation of vaccines for flavivirus infectious diseases (55).

DENV is the most contagious and life-threatening flavivirus, causing roughly 390 million infections annually in endemic regions. Among the cases, approximately 0.5 to 1 million infections develop into severe diseases that could be fatal (56). More importantly, severe cases show significant association with secondary heterologous infections due to the ADE, leading to more severe disease symptoms (57). To date, there is no specific antiviral, and although a tetravalent live attenuated vaccine has been approved in some countries, its efficacy is suboptimal, making

DENV infection a major public health threat in tropical and subtropical countries (56). ADE is regarded as the most serious problem in the development of a DENV vaccine. For example, the production of poorly neutralizing but enhancing antibodies by CYD-TDV (Dengvaxia®), a tetravalent live attenuated vaccine, increased the risk of hospitalization of seronegative recipients more than that of seropositive individuals (58). The earliest study of an mRNA vaccine against DENV was published in 2019 using the mRNA encoding immunodominant T cell epitopes of DENV1 and LNPs as the delivery system (59). The T cell–based mRNA vaccine showed robust T cell responses and protection in human leukocyte antigen (HLA) class I transgenic mice. Recently, another mRNA vaccine against DENV encoding the premembrane and envelope (prM/ENV) structural proteins of DENV1 showed strong neutralizing antibody responses and elicited excellent protection in both immunocompromised AG129 mice and wild-type C57BL/6 mice (60). Additionally, vaccination with both the wild-type and mutant constructs significantly reduced heterologous ADE of DENV2 in K562 cells.

ZIKV is a neurotropic virus that was first discovered in 1947 in the Zika Forest of Uganda and that spread to the Western Hemisphere in 2013, leading to a global pandemic. In 2017, two groups reported that prM/ENV-coding mRNA vaccines encapsulated in LNPs elicited high titers of neutralizing antibodies that protected against ZIKV infection (61, 62). Owing to the shared 54–59% amino acid identity in the viral E protein of DENV, the ZIKV vaccines have the possibility of induction of ADE to DENV (63). Fortunately, antibodies induced by the fusion loop mutant ZIKV vaccines resulted in less ADE of DENV infection in cells and in AG129 mice (61). The other mRNA vaccine (62) showed less effective neutralizing antibodies but generated higher protection against ZIKV challenge in nonhuman primates after a low dose (30 μg) of vaccination. Recently, self-amplifying RNA (saRNA) has been applied in the development of ZIKV vaccines (64–66). Multiple delivery systems such as electroporation, nanoemulsion, and LNPs were used in these saRNA-based ZIKA vaccines, which show different efficacies at low doses. Compared with conventional mRNA-based vaccines, the saRNA-based vaccines can elicit neutralizing antibodies to some extent in mice, even at a low dose of 10 ng of mRNA per injection when formulated in LNPs (66). Moreover, the efficacy could be further improved by optimization of the LNP formulations.

The ideal flavivirus vaccines should elicit antibodies with both high quantity and high quality to avoid the potential of ADE. On the one hand, both mRNA and saRNA vaccines encoding subunit proteins of flaviviruses show the ability to generate extraordinarily high titers of neutralizing antibodies. On the other hand, the mutation of encoded virus proteins shows a significant increase in neutralization antibody titers and a reduction in ADE (60, 61). On the basis of these results, mRNA vaccines hold great potential for the development of next-generation flavivirus vaccines.

2.1.1.3. Influenza viruses. Influenza viruses (which cause an infectious disease commonly known as the flu) remain a seasonal threat to human health, with flu symptoms ranging from mild to severe and even being fatal. Due to the rapid viral mutation, the influenza virus vaccine is usually multivalent and needs to be updated every flu season (67). However, traditional seasonal influenza virus vaccines that generate hemagglutinin (HA)- and neuraminidase (NA)-specific neutralizing antibodies still fail to elicit either strong or broad protection against potentially pandemic influenza viruses, with efficacy as low as 10% and generally not exceeding 60% (68). mRNA vaccines not only can induce strong immune responses but also can be rapidly adjusted to target new variant antigens, showing great potential for the development of effective influenza virus vaccines. Moderna has developed two mRNA vaccines against the H10N8 and H7N9 influenza viruses, which have fatality rates as high as ∼30% (69). The two mRNA vaccines encoded the HA of H10N8 and H7N9 and utilized LNPs as the delivery system. A single dose of the two vaccines generated potent neutralizing antibody titers in mice, ferrets, and cynomolgus

monkeys. However, phase I clinical trials demonstrated that the two mRNA vaccines had favorable safety but low antibody response of only 10-fold increase of titer compared with placebo, even when the mRNA dose reached 100 μg per dose (70). Other influenza mRNA vaccines also have not achieved robust antibody responses (71). The low immunogenicity of HA protein might contribute to the modest antibody and T cell response of HA-encoding vaccines (72).

Apart from HA-encoding vaccines, a multitargeting mRNA vaccine encoding a combination of conserved influenza virus antigens including the HA stalk domain, NA, matrix-2 ion channel (M2), and nucleoprotein (NP) was reported (73). Although the HA head region shows substantial variability, the HA stalk remains conserved across many influenza virus subtypes. Similarly, the NA head, M2, and NP antigens all have a high degree of conservation. Thus, mRNAs encoding these conserved regions could have high potential for development of a universal influenza vaccine. The combined administration of multiple antigens does not change the magnitude of humoral immune responses compared with a single antigen delivered alone, suggesting that combined antigens could be applied in a single administration. Notably, a single dose of the combination vaccine successfully protected mice from infection with seasonal influenza virus by heterologous challenge with H1N1 strains as well as H5N8 and cH6/1N5 strains. Moreover, a vaccine dose as low as 0.05 μg still elicits complete protection from mortality, suggesting the potential high efficacy of this mRNA-based vaccine.

Robust immune responses have been obtained from COVID-19 vaccines, but the responses to influenza mRNA vaccines seem to be modest. The clinical profile of influenza mRNA vaccines needs to be further evaluated in clinical trials. However, lessons can still be drawn from the present development of influenza mRNA vaccines; that is, the selection of antigen is critical in achieving the long-lasting efficacy of mRNA vaccines.

2.1.1.4. Rabies virus. Rabies disease is caused by infection with the rabies virus, leading to virtually 100% fatality in infected mammals, including humans (74). There are many safe and efficacious vaccines being developed for prevention of the rabies virus (75). The major problem with the wide administration of rabies vaccines is the unaffordable costs of traditional vaccines in developing countries (76). The low-cost manufacturing processes of mRNA vaccines might accelerate the prevention of rabies in many developing countries.

The first human study of protamine-complexed mRNA encoding the rabies virus glycoprotein (CV7201) explored various methods of injection and showed great differences in antibody response (77). The vaccine delivered by intradermal injectors (PharmaJet Tropis ID^{TM}) provided better response compared with needle-syringe injection, which limited the broad application of this vaccine owing to the requirement of specialized devices. Moreover, only 76% of participants generated an effective antibody titer higher than the World Health Organization predefined level (≥0.5 IU/mL) after vaccination with a dose of 80 μg of mRNA. In recent clinical trials, LNP-based delivery systems significantly improved the efficacy of mRNA vaccines against the rabies virus. CV7202 is an mRNA-LNP formulation that includes the same mRNA antigen from CV7201 (78, 79). With this formulation, no vaccine-related serious adverse events or withdrawals occurred. After one single injection at a dose of 1, 2, or 5 μg of mRNA, a dose-dependent antibody response could be detected in all participants. After the second injection with a 1- or 2-μg dose, all participants generated effective titers of more than 0.5 IU/mL by day 43; these titers were also on a comparable level with those vaccinated by Rabipur by day 57. A comparison of CV7201 and CV7202 showed advantages for LNPs over the protamine system and a significant improvement of the response to CV7201. A higher dose, e.g., 30 or 100 μg in SARS-CoV-2 vaccines, might provide an even better response than that provided by the current study, proving the potential for effective rabies mRNA vaccines.

Significant differences in efficacy among various mRNA formulations against HIV and rabies virus have revealed the importance of both the mRNA design and the vehicles. First, the optimization and use of multivalent antigens show many benefits in generating broad protection from different virus variants. Second, delivery systems with higher antigen expression exhibit a more robust immune response, and LNPs have proved to be the most potent and effective delivery system.

2.1.2. Chronic infectious viruses. Although mRNA vaccines exhibited preliminary success in the control of COVID-19, traditional types of vaccines such as inactivated virus and virus-based vaccines have also shown effective protection from the transmission of coronaviruses to some extent. However, for some chronic infectious viruses, such as HIV-1, hepatitis virus, and chikungunya virus, it is more difficult to obtain an effective antibody response with a therapeutic effect, because both humoral and cellular immunity are necessary to eradicate circulating virus and infected cells (80).

2.1.2.1. Human immunodeficiency virus. HIV was discovered more than 30 years ago, yet no effective vaccine has advanced to clinical application (81). The challenges in the development of HIV vaccines include the tremendous diversity of glycan shields linked to the HIV envelope (Env), conformational flexibility of the Env, and the rapid mutability of viral epitopes (82). mRNA vaccines have shown great potential for the development of an HIV vaccine due to their ability to elicit robust humoral and cellular responses. The first clinical trial of naked mRNA in humans was performed by intranodal injection of marked mRNA encoding HIVACAT T cell immunogen (HTI) and activation signals (TriMix: CD40L + CD70 + caTLR4) (HTI-TriMix), exhibiting moderate immune responses in phase I studies (83). However, no significant differences in the immunogenicity between placebo and vaccine-treated groups were observed in the phase IIa clinical trials (84). Such failure might be due to the low transfection of naked mRNA even at very high doses of 900 μg per injection. Therefore, developing a delivery system with high efficacy is necessary.

The first study of LNP-based HIV mRNA vaccines in large animals was reported in an mRNA vaccine encoding 1086C B2 ecto Env (85). The mRNA vaccine was shown to elicit high anti-gp120 IgG titers in both rabbits and rhesus macaques. The neutralization activity against tier 1 virus was detected in both species, while no neutralization activity was observed against tier 2 virus in rabbits. Only one of six rhesus macaques generated antibodies with strong neutralization to tier 2 virus, which unfortunately started to drop 4 weeks after booster immunizations. Excitingly, antibody-dependent cellular cytotoxicity in positive correlation with the vaccine efficacy was detected in most of the rabbits and rhesus macaques after two immunizations. Recently, another preclinical study of HIV-1 mRNA vaccine in nonhuman primates was also reported, with more exciting results than the previous study (86). In this study, mRNA-LNP encoding HIV-1 Env A244 gp120 elicited either the same or a superior level of polyfunctional antibodies to HIV-1 Env compared with those from protein-based vaccines in different adjuvants. As in the previous study, the antibody titers started to decrease a few days after the last boost and still remained at a relatively low level for at least 41 weeks. Although these preliminary LNP-based mRNA vaccines revealed the great potential of LNP mRNA in HIV vaccines, the immunogenicity in patients with HIV infection should be further investigated for the development of therapeutic vaccines.

Apart from direct vaccination by mRNA vaccines, several mRNA-based dendritic cell (DC) vaccines against HIV had been explored in phase I/II clinical trials and showed different levels of T cell response, as reviewed elsewhere (87). However, mRNA vaccines for HIV are still far from clinical applications. Combination with other therapeutic methods may also be necessary for a better therapeutic effect.

2.1.2.2. Hepatitis C virus. In contrast to HIV infection with no effective therapy, chronic infection by the hepatitis C virus (HCV) could be controlled and cured by direct-acting antiviral therapies. However, no effective HCV vaccines have been developed, which has hindered the global epidemic control and elimination of HCV. Additionally, possible resistance to direct-acting antiviral therapies emerged concomitantly with large-scale development and implementation. Therefore, the success of mRNA vaccines against different types of viruses provides a promising strategy for generating an effective HCV vaccine.

Few preclinical or clinical trials using mRNA-based vaccines against HCV have been reported. Sharifnia et al. (88) first reported the generation of an IVT mRNA encoding the core protein of HCV. Successful expression of the core protein was detected in monocyte-derived DCs, suggesting the possibility of generating an RNA-based vaccine against HCV. However, the in vivo immunogenicity of such mRNA was not evaluated in this work. Apart from the mRNA vaccine, the mRNA-transfected DCs were also applied for developing an HCV vaccine (89). The researchers compared the loading strategy of HCV NS5a antigen to DCs by DNA or mRNA. Interestingly, 100% of DCs expressing NS5a was observed in the cells transfected with mRNA, whereas only 10% and <1% could be found in protein-pulsed DCs and plasmid-transfected DCs, suggesting remarkably enhanced expression of antigens by mRNA. Further vaccination with both NS5a mRNA-transfected or protein-pulsed DCs resulted in significantly stronger $CD4^+$ and $CD8^+$ T cell responses and prevented infection with vaccinia virus expressing NS3/NS4/NS5 compared with the mice vaccinated with DNA-transfected DCs, NS5-enconed plasmid, or rNS5a protein with alum. These data suggest that mRNA-transfected DCs provide a safe and effective vaccination strategy against HCV.

2.1.3. Other pathogens. Other pathogens such as bacteria, fungi, and parasites also remain as threats to public health. Though the development of effective antibiotics significantly relieves the burden of the infectious diseases brought forth by these pathogens, there is still great concern about the generation of drug resistance (90, 91). Maruggi et al. (92) reported the first application of mRNA vaccines against bacterial infections in 2017. Self-amplifying mRNA was utilized for expression of two prototype bacterial antigens and then complexed with cationic nanoemulsion. The immunization elicited significant amounts of fully functional serum antibodies in all mice and also generated consistent protection of group A and group B streptococcus infections in murine models. Recently, mRNA vaccines against malaria were also reported to have advantages over traditional vaccines in mice (93). An mRNA encoding malaria parasite, *Plasmodium falciparum* circumsporozoite protein (PfCSP), was loaded into LNPs, achieving sterile protection against infection with two *Plasmodium berghei* PfCSP transgenic parasite strains. Numerous factors were found to affect protective efficacy, such as the LNP type, mRNA dose, and interval of immunizations, providing an early assessment of the effectiveness of mRNA vaccines against malaria. These preliminary results support the idea that mRNA vaccines can be used as a potential solution for a wide range of pathogens besides viruses, due to the broad spectrum of elicited protective immune responses.

2.2. Cancers

Researchers have spent decades developing the concept of using therapeutic vaccines to treat cancer. While there is only one DC-based vaccine (sipuleucel-T) approved by the FDA for the treatment of hormone-refractory prostate cancer, the therapeutic effect was far from satisfying (94). There are several possible reasons for the modest effect of cancer vaccines, including low specificity of tumor-associated antigens (TAAs), immune escape of cancer cells, and immune suppression in the tumor microenvironment (95). Nowadays, tumor antigens applied in cancer

Table 2 Active clinical trials of mRNA vaccines against cancers

Cancer type	Product	Antigen(s)	Delivery system	Phase(s)	Combination therapy	NCT number(s)
Prostate cancer	Dendritic cell vaccine	mRNA encoding hTERT, survivin, and isolated tumor mRNA	Electroporation	I, II	NA	NCT01197625
Glioblastoma	Dendritic cell immunization	mRNA of survivin, hTERT of autologous tumor stem cells derived from tumorspheres	Electroporation	II, III	Temozolomide	NCT03548571
Non-small-cell lung cancer	BI 1361849 (CV9202)	MUC1, survivin, NY-ESO-1, 5T4, MAGE-C2, and MAGE-C1	Protamine	I/II	Durvalumab and tremelimumab	NCT03164772
Ovarian cancer	BNT115	Three ovarian cancer TAAs	LNP	I	Neoadjuvant chemotherapy	NCT04163094
	Personalized mRNA tumor vaccine	Neoantigens	Unknown	NA	NA	NCT03908671
Melanoma	mRNA-4157	Neoantigens	LNP	II	Pembrolizumab	NCT03313778, NCT03897881
	RO7198457	Neoantigens	LNP	II	Pembrolizumab	NCT03815058
Colorectal cancer	RO7198457	Neoantigens	LNP	II	NA	NCT04486378
Pancreatic cancer	RO7198457	Neoantigens	LNP	I	Atezolizumab and mFOLFIRINOX	NCT04161755

Abbreviations: hTERT, human telomerase reverse transcriptase; LNP, lipid nanoparticle; NA, not applicable; NCT, National Clinical Trial; TAA, tumor-associated antigen.

vaccines have moved from TAAs to neoantigens, showing potential therapeutic effects in clinical applications (**Table 2**). However, from the vaccine aspect, the major limitations of neoantigen-based cancer vaccines might be the low immunogenicity of neoantigens and the high cost of production (96). mRNA vaccines have emerged as an increasingly popular method for treating cancers (97). On the one hand, mRNA vaccines have advantages such as rapid development, low-cost manufacturing, and safe administration, which relieve the financial burden of neoantigen-based vaccines. On the other hand, mRNA vaccines show rapid protein expression of multiple antigens in APCs both ex vivo and in vivo, resulting in the ability to produce a robust T cell response to neoantigens.

2.2.1. Ex vivo mRNA-transfected dendritic cell cancer vaccine. Inspired by the FDA-approved DC vaccine, improving the ex vivo loading efficacy of mRNA vaccines is regarded as the low-hanging fruit in the development of next-generation cancer vaccines. As the most potent APCs, DCs can internalize, proteolyze, and present tumor antigens to $CD8^+$ and $CD4^+$ T cells via major histocompatibility complex classes I and II, respectively (98). Compared with traditional whole tumor cell, protein, or peptide-based tumor antigens, mRNA encoded antigens have the advantages of inducing a robust $CD8^+$ (and $CD4^+$) T cell response (99).

DCs are hard-to-transfect cells and are highly resistant to mRNA transfection. Though naked mRNA can be internalized into DCs by macropinocytosis, the activation of pattern recognition receptors (PRRs) in the endosome promptly induces the degradation of mRNA. As a result, only

a small fraction of mRNAs can escape from endosomes into the cytoplasm for translation into proteins. To enhance mRNA delivery into the cytosol, multiple ex vivo transfection methods have been tested. Electroporation has been the most widely adopted method for ex vivo transfection of mRNA into DCs, showing transfection rates above 90%. Because the translation of mRNA happens in the cytoplasm, a relatively weak electrical pulse is enough for effective delivery of mRNA into the cytosol with limited damage to the cells (100). Additionally, direct introduction of mRNA into the cytosol by electroporation avoids the recognition of mRNA by the innate immune system via the PRRs residing in the endosome. Other methods such as sonoporation and the use of nanoparticles are also applied in the delivery of mRNA into DCs; however, no obvious advantages could be observed when comparing these methods with electroporation for ex vivo mRNA transfection (101–103).

mRNA encoding tumor antigens can be produced directly by isolating them from autologous cancers or cancer stem cells or indirectly via IVT. To date, almost all the antigens encoded by IVT mRNAs for DC vaccines in clinical trials are TAAs, e.g., melanoma antigen gene (MAGE), gp100, and tyrosinase for melanoma (NCT01066390); prostate-specific antigen for prostate cancer (NCT01197625); carcinoembryonic antigen (CEA) for CEA-expressing cancers (NCT00228189); and so forth (104, 105). Most of these trials are still in phase I/II and none has reached phase III. Autologously isolated mRNAs might have the information of neoantigens and be able to elicit a broad T cell response. One phase III clinical trial of AGS-003, a vaccine using a patient's own amplified tumor RNA-transfected DCs, was terminated due to the lack of efficacy (NCT01582672). A neoantigen-encoded mRNA-transfected DC vaccine is expected to improve the therapeutic effect, but there is only one active clinical trial (NCT02808416) based on this approach and the progress is still unclear.

Co-delivery of immune regulatory proteins (e.g., GM-CSF, IL-4, CD86) with tumor antigens has been broadly applied to increase the potency of DC vaccines (106). For mRNA-transfected DC vaccines, TriMix is a widely applied cocktail of mRNA encoded adjuvants (CD70, CD40L, and constitutively active TLR4) that can be transfected together with antigen-encoding mRNAs, which can increase DC activation and shift the phenotype of $CD4^+$ T cells from T regulatory cells to T helper 1–like cells (107). Recent clinical trials showed that four TAA-encoded mRNAs (MAGE-A3, MAGE-C2, tyrosinase, and gp100) in combination with TriMix-encoded mRNA as adjuvant is tolerable and improved the 1-year disease-free survival rate from 35% to 71% in stage III/IV melanoma patients (108). However, 9 out of 21 patients were still diagnosed with a nonsalvageable melanoma recurrence in the vaccine group after a median follow-up of 53 months, suggesting there is still room for further improvement.

Early clinical trials using mRNA-transfected DC vaccines showed efficacy only to a minimal extent in the treatment of late-stage melanoma (109, 110). Recent updates confirmed that the combination of an mRNA electroporated DC vaccine with ipilimumab (CTLA-4 inhibitor) elicited robust $CD8^+$ T cell response in patients with stage III or IV melanoma, especially in those patients with clinical responses (111). The upregulated therapeutic outcome indicated great potential for the combination of cancer vaccines with other treatments. Apart from melanoma, multiple clinical trials of mRNA-transfected DC vaccines are active for the treatment of various cancers including but not limited to prostate cancer, ovarian cancer, colorectal cancer, breast cancer, and glioblastoma (112).

In summary, the ex vivo delivery of mRNA to DCs is not difficult in DC vaccines, while the selection of tumor antigens and cotransfection with immune regulatory proteins seem to be the most important factors contributing to the efficacy of DC vaccines. Moreover, single treatment by DC vaccines typically shows only minimal effect; thus, the combination of vaccines with other treatments needs to be further evaluated to improve the overall therapeutic outcome.

2.2.2. Injectable mRNA cancer vaccines. Injectable mRNA cancer vaccines can produce encoded tumor antigens in the cells around the injection site or in APCs. These injectable mRNA cancer vaccines show some advantages over DC vaccines, including easy production and low cost (113). Regardless of these advantages, there are still major challenges with directly injected mRNA cancer vaccines, such as the difficulty of efficient expression in vivo, which is not a challenge in DC vaccines due to their ability to be transfected by physical methods ex vivo. Additionally, injectable mRNA cancer vaccines still face the challenges of low specificity of TAAs, immune escape, and immune suppression. Therefore, developing an effective injectable mRNA cancer vaccine may prove to be more difficult than the development of DC cancer vaccines.

Multiple delivery systems have been explored for delivery of mRNA cancer vaccines in vivo, and these delivery systems are reviewed elsewhere (3, 97). As opposed to the delivery systems for mRNA vaccines for pathogen infection, therapeutic mRNA vaccines for cancer treatments are required to generate both robust $CD8^+$ and $CD4^+$ T cell responses (114). Activation of type I interferon (IFN) proved important in developing a cytotoxic T cell response (115). Therefore, different strategies have been explored in an attempt to enhance the activation of type I IFN. On the one hand, type I IFN–related PRR agonists could be added into the formulation of delivery systems. For example, the palmitic acid–modified TLR7/8 agonist R484 was shown to increase the percentage of model antigen ovalbumin-specific $CD8^+$ T cell population (116). On the other hand, some delivery systems themselves could activate the PRRs and act as self-adjuvants. For instance, CureVac developed the RNActive® vaccine platform, which uses an mRNA/protamine complex to activate TLR7/8, which in turn can induce strong humoral and cellular responses (117). Miao et al. (118) and Luo et al. (119) discovered nanoparticles that could directly bind to stimulator of interferon genes (STING) proteins and then activate type I IFN. However, the incorporation of agonists usually resulted in the reduction of antigen expression due to the innate immune response.

Apart from enhancing immune responses by delivery systems, tumor antigens encoded in injectable mRNA cancer vaccines have already developed from TAAs to neoantigens. Neoantigens are highly specific random somatic mutations in individuals' tumor cells that are not present in normal cells and are regarded as ideal tumor antigens for cancer vaccine development (120). Traditional peptide-based vaccines are limited by their low immunogenicity and intractable physical-chemical properties, while mRNA-based vaccines are more flexible in design and also show much stronger immune responses (97). Sahin et al. (121) reported the first injectable mRNA cancer vaccine encoding neoantigens for advanced melanoma patients through intranodal injection, achieving potent T cell responses against multiple neoantigens in all patients after vaccination. Furthermore, Moderna has developed several kinds of neoantigen-encoded mRNA cancer vaccines against multiple types of cancers. Recently, preliminary results of mRNA encoding of up to 20 defined neoantigens; mutations in TP53, KRAS, or PIK3CA neoantigens; and HLA-I–predicted epitopes against gastrointestinal cancer were reported. The mRNA vaccine could induce mutation-specific T cell responses against up to nine predicted neoantigens in one patient. BioNTech has also developed a personalized mRNA cancer vaccine named RO7198457, encoding >20 neoantigens against advanced or metastatic solid tumors (122). With this vaccine, 12 of 14 patients generated T cell responses to neoantigens, while the median number of positive antigens was only two. Notably, one complete response was observed in a patient with gastric cancer, encouraging BioNTech to start more phase I and II trials (NCT04267237). Moreover, the combination of personal mRNA vaccines with other therapeutic methods was also included in the additional clinical trials (NCT03815058).

The rapid development of delivery systems and neoantigens has brought about a great revolution in mRNA cancer vaccines. Accumulating results have confirmed the great potential of individual cancer vaccines encoded by mRNA. However, the number of responsive neoantigens in clinical

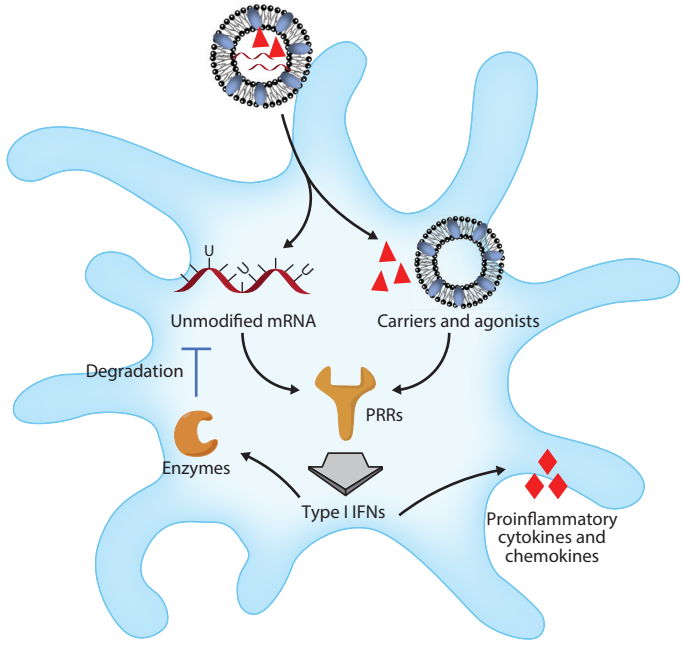

Figure 2

Balancing the antigen expression and adjuvant effect of mRNA vaccines. Both mRNA and delivery systems can alter the adjuvant property of mRNA vaccines by recognition via PRRs. The adjuvant effect can benefit the activation of adaptive immunity but hinder the expression of mRNA encoded antigens. Balancing the proinflammatory effect and antigen expression is critical to the efficacy of mRNA vaccines. Abbreviations: IFN, interferon; PRR, pattern recognition receptor.

trials is still low; thus, more accurate prediction methods should be further developed or a more in-depth investigation of the relationship between mutations and epitopes should be conducted.

3. CURRENT CHALLENGES OF mRNA VACCINES

3.1. Balancing Antigen Expression and Adjuvant Effect

Adjuvants are necessary in traditional vaccines; however, the role of adjuvant effects in mRNA vaccines in generating adaptive immunity is still debated. Both mRNA and delivery systems can alter the adjuvant property of mRNA vaccines, making the use of these vaccines a challenge for future applications (**Figure 2**).

IVT mRNAs can be recognized by multiple PRRs, including retinoic acid–inducible gene-I-like receptors, oligoadenylate synthetase receptors, and RNA-dependent protein kinase, and show inherent adjuvant properties. Such adjuvant effects are a double-edged sword that can be either beneficial in activating APCs for the generation of adaptive immunity or detrimental due to blocking mRNA translation. Different strategies of mRNA chemistry and manufacturing processes have been applied for reducing the innate immune activation of mRNAs, such as 5′ capping, nucleoside modification, poly(A) tail modification, and HPLC purification, which are reviewed elsewhere (123). Recently, a direct comparison of the effect of these processes on innate immunity was carried out by Moderna (124). Its findings confirm that uridine modification by either canonical uridine or N1-methyl-pseudouridine (m1Ψ) and the reduction of double-stranded RNA by HPLC purification are both necessary and sufficient for controlling the innate immune activation of IVT mRNA in multiple cell and in vivo models. Moreover, an increasing

amount of research proved that the inherent adjuvant effect could increase the adaptive immune response of mRNA vaccines. CureVac has applied optimized and chemically unmodified mRNAs in several mRNA vaccines undergoing clinical trials. Lutz et al. (79) first reported that sequence-optimized and chemically unmodified mRNA are highly immunogenic and well tolerated in nonhuman primates. Single intramuscular vaccination with LNP-mRNAs induced protective antibody titers against rabies or influenza viruses in nonhuman primates. Such technology was also applied in the CureVac COVID-19 vaccine, named CVnCoV. Unfortunately, CVnCoV showed a vaccine efficacy of only 47% against COVID-19 of any severity. Such failure might be caused by the increasing variants of the SARS-CoV-2 virus or the overstimulation of the innate response, but the cause is still under debate (125). CV2CoV, the second generation of the CVnCoV COVID-19 vaccine, elicited high levels of virus-neutralizing antibodies in rats and showed cross-neutralization of SARS-CoV-2 variants, i.e., B.1.1.7 and B.1.351 (126).

Apart from the adjuvant effect of mRNA, the delivery systems also have an adjuvant effect due to their inherent properties or coencapsulation of other immune agonists. As mentioned above, two different STING agonists were explored to enhance the therapeutic outcome of cancer vaccines (118, 119). Notably, the STING-activable LNP A18 also showed the strongest expression of mRNA at both local sites and draining lymph nodes, suggesting the coexistence of a high adjuvant effect and efficient antigen expression. Recent research reported the highly inflammatory effect of some preclinical LNPs (127). Intradermal injection of these blank LNPs induced massive neutrophil infiltration and production of various inflammatory cytokines and chemokines, e.g., IL-1β, IL-6, CCL3, and CCL4. The potent adjuvant activity might also contribute to the induction of adaptive immune responses by mRNA vaccines. However, stimulation by LNPs should be more carefully considered due to the potential toxicity and inflammation (128).

Therefore, it is a great challenge to balance efficient antigen production, sufficient adjuvant effects, and side effects of current mRNA vaccines. To achieve such balance, more efforts should be spent on the investigation of the interaction among LNPs, mRNA, and the innate immune system.

3.2. Large-Scale Manufacturing of mRNA

The manufacturing of mRNA vaccines has three main steps: synthesis, purification, and formulation; each step also contains several substeps. However, all these steps have not been developed in a continuous manufacturing process. As reported by *The New York Times*, storage and transportation among different facilities in three states is still required during the manufacturing process of BNT162b2, and it takes 60 days to produce millions of doses of the vaccine (129). In particular, for mRNA synthesis, the stored plasmid encoding the template is amplified, purified, tested, and cut into a DNA template for IVT in the facility in Chesterfield, Missouri. Then the DNA template is stored under – 20°C and shipped to the mRNA manufacturing facility in Andover, Massachusetts. The mRNA is produced, purified, tested, and frozen to under –20°C and then shipped to another Pfizer facility in Kalamazoo, Michigan. The mRNA is formulated with LNPs and packaged in vials. Finally, samples are sent back to Pfizer's Chesterfield facility and tested again. Moreover, owing to the limitations of transportation, such processes can only produce millions of doses of the vaccine, which is far from meeting the vaccination needs of 6 billion people in the world.

A continuous manufacturing process might significantly increase the efficiency of mRNA vaccine manufacturing, and such a process is already used in the chemical and pharmaceutical industry for flexible and cost-effective manufacturing (130). First, all three facilities could be coupled with a fluidic system, in which the IVT, purification, and formulation of mRNA with LNPs could be achieved by automatic processes. The avoidance of transportation among three states would significantly reduce operation time and facilitate the automation technologies, increasing both the

quality and productivity. Second, a process integrated by continuous manufacturing could also help by recycling and reusing the raw compounds, such as enzymes or NTPs. A continuous manufacturing process might be a promising strategy for large-scale manufacturing of mRNA vaccines at low cost.

3.3. Stability During Storage and Transportation

The quality of vaccines is highly sensitive to temperature, so storage and transportation under a proper temperature range from production to administration is important for their efficacy. A cold chain is usually necessary for the storage and transportation of vaccines, while the supply chain for mRNA vaccines might need to be even "colder" (131). Compared with other types of vaccines that can be stored and transported at 2–8°C for months, BNT162b2 and mRNA-1273 need to be kept under −80°C and −20°C, respectively (132). The need for such cold storage remains a challenge for mRNA vaccines. The strict temperature for storage of mRNA vaccines can be attributed to the instability of the LNP-mRNA system.

Plenty of studies have focused on the stability of mRNA molecules themselves, while the stability and storage of formulated mRNA has been rarely investigated. Freeze-drying using lyoprotectants has been shown to maintain the neutralizing antibody levels and protection of an mRNA-protamine formulation against rabies (133). There are several patents claiming successful long-term storage of mRNA-protamine formulations at room temperature after freeze-drying by adding different lyoprotectants, such as lactate, mannose, trehalose, and so forth (8). Recently, the performance of freeze-dried LNP-mRNAs with different lyoprotectants (sucrose, trehalose, and mannitol) was compared with that of nonlyophilized LNPs (134). It was identified that freeze-drying by the addition of 20% (weight by volume) sucrose or trehalose to LNPs could maintain their mRNA delivery efficiency in vitro, but these lyophilized LNPs did not show efficiency in vivo. It is speculated that the nanostructure of the LNP-mRNA might be changed during freeze-drying and reconstitution, affecting the interactions between LNPs and plasma and resulting in the loss of mRNA delivery efficiency in vivo.

To date, there is still no solution to address the necessity for such extreme cold chain storage and transportation of mRNA vaccines, which might place critical limitations on large-scale mRNA vaccine applications in the future. It is urgent to generate an overview of all the challenges for formulated mRNA stability besides the mRNAs themselves.

3.4. Safety

Another concern raised by mRNA vaccines is the relatively high occurrence of side effects compared with those caused by traditional inactivated vaccines, especially for grade 3 adverse reactions. The large-scale application of the mRNA COVID-19 vaccine provided the opportunity for an in-depth investigation of the adverse reactions of mRNA vaccines. However, the causes of some severe adverse reactions to mRNA COVID-19 vaccines are still unclear and are important to understand for further optimization of mRNA vaccines.

Anaphylaxis from mRNA COVID-19 vaccines in the United States is currently estimated to be 4.7 cases per million of Pfizer/BioNTech vaccine doses administered and 2.5 cases per million of Moderna vaccine doses administered, on the basis of passive spontaneous reporting methods (135). However, the incidence rate of confirmed anaphylaxis has been shown to be much larger (247 cases per million) in symptom surveys of more than 60,000 vaccinated Mass General Brigham employees (136). Though the overall risk of anaphylaxis from an mRNA COVID-19 vaccine remains extremely low, the mechanisms for this reaction might also be explored and taken into consideration for further optimization of the LNP formulation. The PEG in the LNP formulation is thought to

be a possible allergen for anaphylaxis due to approximately 72% of people having some antibodies against PEGs (137). However, there is still no direct evidence to draw a conclusion. To address this concern, some alternatives of PEGs can be involved in the formulation of LNPs and analyzed.

Due to the increasing number of cases of myocarditis and pericarditis after administration of mRNA vaccines, the FDA continues to take action to evaluate the risk (138). An in-depth evaluation of a case of presumed myocarditis after the second dose found an increase in the numbers of a specific subset of natural killer cells and increased expression of several autoantibodies compared with controls (139). As opposed to the upregulation of IL-17 in the development of conventional myocarditis, the myocarditis caused by mRNA vaccines did not show such a phenomenon, suggesting a distinct vaccine-associated immunophenotype. However, the potential mechanisms are still unclear, and more studies with a larger number of individuals are required.

Some rare cases of severe adverse events after vaccination have also been reported, such as cytokine release syndrome, cerebral venous thrombosis, and so forth (140, 141). All these severe adverse events with unclear mechanisms act as the shadow in the sun, suggesting that future clinical trials should be more careful to address these concerns.

3.5. Rapid Mutation of Targeted Antigens

Breakthrough infections of pathogens and immune escape of cancers mediated by the mutation of targeted antigens remain the most challenging issues in the development of effective vaccines (142, 143).

Infectious pathogens may establish different levels of mutation rates during transmission, especially with RNA viruses (144). The current COVID-19 pandemic has generated multiple variants of concern, such as B.1.1.7 (alpha), B.1.351 (beta), B.1.617.2 (delta), and P.1 (gamma). Notably, the delta variant took the world by storm, especially in India and the United Kingdom (145). Moderna provided a clinical update on the neutralizing activity of mRNA-1273 on 16 emerging variants (146). The results showed decreased neutralization titers ranging from 2.1- to 8-fold reductions compared with D614G. Similar reduction of neutralization titers was also observed in BNT162b2, suggesting a potential decrease in the protection effect in humans (35). Since the initial submission of this manuscript, B.1.1.529 (omicron) has rapidly replaced delta all over the world. The omicron variant has 32 mutations in the S protein compared to that of the original virus (Wuhan-hu-1), resulting in a more than 10-fold decrease in neutralization antibody titers (147). Even though a third vaccination by BNT162b2 increased the neutralization of the omicron variant (geometric mean titer, 1.11 after the second dose versus 107.6 after the third dose), the neutralization ability was still lower (by a factor of 4) than that against the delta variant. Therefore, new strategies should be applied to overcome the rapid mutation of the virus. The combined vaccination of two mixed mRNAs encoding the S proteins found in the B.1.351 and D614G lineage showed a significant increase in both the Wuhan-hu-1- and B.1.351-specific neutralization titers, but it is still questionable whether the manufacturing process of mRNA vaccines could follow the rapid emergence of virus variants (148). Another potential strategy to overcome this challenge is that the adaptive T cell–based immunity elicited by conserved epitopes might provide a path for a pan-COVID-19 vaccine that is resilient to viral drift, although this possibility still needs to be confirmed in clinical trials (149).

The mechanisms for immune escape of cancer are much more complex than are those for breakthrough infections of pathogens, as reviewed elsewhere (143). The loss or mutation of targeted antigens as well as the immunosuppressive tumor environments are considered the major reasons for the complexity. Although the discovery of neoantigens has brought cancer vaccines into a new era, the neoantigens generated from rapid mutations can also undergo

further mutations and be shaped through immunoediting mechanisms, which might reduce or even eliminate the therapeutic efficacy of vaccines based on neoantigens (150). To address this, neoantigens expressed in genes that are necessary for cancer cell survival might be ideal targets for future mRNA vaccine development. Additionally, the combinations of mRNA vaccines with agents that can reverse immunosuppression and block immune checkpoints have been shown to be more potent than single administration of vaccine therapy (151). However, not all patients are responsive to these therapies (152). Therefore, potent immune escape of mRNA cancer vaccines still remains a major concern for future development.

4. CONCLUSION AND PERSPECTIVE

mRNA vaccines show great advantages compared with other types of vaccines, owing to their induction of both humoral and cell-mediated immune responses, rapid adaptability in antigen design, and use of the same manufacturing platform for different mRNA vaccines. Multiple types of mRNA vaccines have been developed, including preventive vaccines against infectious pathogens and therapeutic vaccines against cancers. Great success has been achieved in the protection against SARS-COV-2, while the clinical outcome for mRNA cancer vaccines is far from clinical relevance. In this review, recent progress and challenges are discussed to generate a perspective for the future of mRNA vaccines.

Infectious viruses can be divided into two major types, including emerging or reemerging infectious viruses and chronic infectious viruses. mRNA vaccines have exhibited excellent protection efficacy against the recent rapidly emerged coronavirus. Owing to their easy and low-cost production, mRNA vaccines show great potential in controlling future pandemics caused by rapidly emerging viruses. However, the mutation rate of these emerging or reemerging infectious viruses is usually high, making the development of broad or seasonal mRNA vaccines a remaining challenge. Moreover, it is usually harder to generate an effective neutralizing antibody for chronic infectious viruses due to their ability to evade innate immunity. The ability to elicit both high humoral and cellular response through the use of mRNA vaccines shows great potential in protecting against and treating chronic infectious viruses. Several mRNA vaccines against HIV have been involved in clinical trials.

Therapeutic cancer vaccines have been developed for decades; however, the clinical outcome is still modest. The development of mRNA vaccines brings cancer vaccines to a new generation, mainly due to the ability to elicit robust T cell responses. mRNA cancer vaccines are still based on the discovery of more specific and immunogenic tumor antigens. Identification of neoantigens also helps researchers to develop more efficient individual cancer vaccines. Additionally, the induction of type I IFNs plays an important role in eliciting a strong T cell response with mRNA vaccines, which could benefit from the discovery of some STING-activating delivery systems. However, a successful mRNA cancer vaccine still faces the challenge of immune escape of cancer cells even when a high immune response is generated after vaccination. Therefore, mRNA cancer vaccines should also be applied in combination with other therapy strategies, such as immune agonists, cytokines, checkpoint inhibitors, and even chemotherapy.

Although great success of mRNA vaccines has been achieved, there are still multiple challenges to be addressed for future development. First, the balance between the adjuvant effect and antigen expression of mRNA vaccines should be further investigated. Second, more efforts should be spent on developing a more stable and safer mRNA vaccine. Third, due to the mutation of antigens and immunosuppression, breakthrough infections of pathogen variants and immune escape of cancer cells remain major concerns for an effective vaccine. Moreover, mRNA vaccines only play a role as a vaccine platform and are limited by the development of antigens, the knowledge

of innate and adaptive immunity, and the mechanisms of immunosuppression. Therefore, the development of mRNA vaccines should also keep up with revolutions in other fields.

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