Cell Transplantation
Volume 31: 1–14

DOI: 10.1177/09636897221090259
journals.sagepub.com/home/cll
SAGE

Modified mRNA-Based Vaccines Against Coronavirus Disease 2019

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Abstract

The pandemic of coronavirus disease 2019 (COVID-19) continuously causes deaths worldwide, representing a considerable challenge to health care and economic systems with a new precedent in human history. Many therapeutic medicines primarily focused on preventing severe organ damage and complications, which can be fatal in some confirmed cases. The synthesized modified mRNA (modRNA) represents a nonviral, integration-free, zero-footprint, efficient, and safe strategy for vaccine discovery. modRNA-based technology has facilitated the rapid development of the first COVID-19 vaccines due to its cost- and time-saving properties, thus initiating a new era of prophylactic vaccines against infectious diseases. Recently, COVID-19 modRNA vaccines were approved, and a large-scale vaccination campaign began worldwide. To date, results suggest that the modRNA vaccines are highly effective against virus infection, which causes COVID-19. Although short-term studies have reported that their safety is acceptable, long-term safety and protective immunity remain unclear. In this review, we describe two major approved modRNA vaccines and discuss their potential myocarditis complications.

Keywords

modified mRNA, modRNA vaccine, SARS-CoV-2, COVID-19, myocarditis

Introduction

Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome (SARS) found in December 2019, which is caused by a novel RNA coronavirus [severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)][1–4]. The World Health Organization (WHO) declared that SARS-CoV-2 has led to a pandemic worldwide in 2020 and then caused dramatic mortality and morbidity[5–7]. It has resulted in a major medical burden and economic crisis. As of today, over 237 million confirmed cases have been identified worldwide, and the global death toll has exceeded 4.8 million (WHO update on October 13, 2021).

SARS-CoV-2 belongs to betacoronavirus and is an RNA virus including single positive-sense RNA and envelope[8–11]. The virus particle morphology displays an oval or circle, and the diameter ranges between 80 and 120 nm. The genome contains 26 to 32 kb and has 80% similarity with SARS-CoV. Thus, it is like other coronaviruses such as SARS-CoV. Its viral structure is majorly composed of the nucleocapsid (N), envelope (E), membrane (M), and spike (S) glycoproteins[9]. The nucleocapsid protein combines with the single positive-stranded RNA[12]. E, M, and S proteins are embedded in the host cell membrane–derived viral envelope. The crown property of viral particles is defined by the spike glycoprotein[9]. The virus uses the receptor-binding domain (RBD) of spike glycoprotein to interact with the angiotensin-converting enzyme 2 (ACE2) receptor expressed on the host cell surface for virus entry. The ACE2 is expressed in gastrointestinal, pulmonary, hepatic, and human renal cells; thus, these organs are primarily infected by SARS-CoV-2 virion[13–15]. The spike glycoprotein is composed of S1 and S2 subunit. The S1 subunit has RBD for ACE2 interaction and S2 contributes to host membrane fusion and endocytosis[16–20].

The replication life cycle of SARS-CoV-2 includes virus entry, replication, assembly, and release, which are described in the following four steps. (1) The virus uses two ways to attach the host cells, such as in an endosome or cell membrane fusion manner. Spike glycoproteins of SARS-CoV-2 first interact with vimentin or heparan sulfate located on the host cell surface to attach the cell membrane and then interact with ACE2 receptor[14,21–23]. The host cellular serine protease TMPRSS2 cleaves spike protein into S1 and S2 domains,

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Submitted: November 10, 2021. Revised: February 26, 2022. Accepted: March 2, 2022.

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which further initiates the membrane fusion occurrence between the viral membrane and host cell membrane. The host's antiviral immunity is less to be activated by the virus's entry way of membrane fusion, which is more effective for viral replication[24]. (2) Viral replication occurs after entry; genomic RNA of coronavirus is released into host cytoplasm and then polyproteins are translated. Nonstructural proteins (NSPs) and structural proteins (S, E, and M) are encoded for viral RNA synthesis and viral assembly, respectively. In addition, the host shutoff factor, Nsp1, is first encoded and interferes with host translation and degrades host mRNA, which further influences host immune responses[25]. Structural proteins interact with the endoplasmic reticulum (ER). The viral RNA replication occurs in the ER-formed double-membrane vesicles (DMVs) and escapes from host immunity. (3) Virion assembly: Viral RNA leaves DMVs through pores created by Nsp3 protein and then interacts with nucleocapsid proteins to form genomic RNA. They are further assembled with viral precursors that are delivered to the cell surface through transport vesicles. (4) Virion release: Virions use the exocytosis mechanism to release from the infected cell and then infect other host cells again. SARS-CoV-2 has a feature that is different from other coronaviruses, namely, a second cleavage site in the spike glycoprotein. The furin cleavage site for proteolytic cleavage is considered to assist the virus to enter the host cell. Some mutations in this site are features for SARS-CoV-2 variants, such as Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2)[25].

The major transmission mode of SARS-CoV-2 is human to human via the inhalation of droplets from the cough or sneeze of confirmed cases, as well as by direct contact with a contaminated surface[26]. SARS-CoV-2 is similar to SARS-CoV and causes respiratory infections such as pneumonia, dyspnea, cough, fever, and acute respiratory distress syndrome[27]. In some older confirmed patients with chronic diseases, the virus infection may cause severe complications and organ damage, ultimately resulting in death[28,29]. Unfortunately, the virus causes the most cases in its asymptomatic course, in which those infected with the virus can pass it on without knowing they are infected[30]. The most common syndromes include the elevation of body temperature, cough, fatigue, and loss of sense of smell[31]. A report showed various syndromes in different proportions collected from confirmed patients, such as 87.9% with fever, 67.7% with cough, 38.1% with fatigue, 33.4% with the accumulation of phlegm, 18.6% with dyspnea, 14.8% with muscle ache, 13.9% with a sore throat, 13.6% with headache, 11.4% with chills, 5% with nausea and vomiting, 4.8% with stuffy nose, and 3.7% with diarrhea[32].

COVID-19

Currently, promising therapeutic treatments against COVID-19 have been tested widely based on their effective antiviral treatment of MERS-CoV (Middle East respiratory syndrome coronavirus) and SARS-CoV such as antiviral, anticoagulant, and anti-inflammatory indications. Remdesivir and Lopinavir were found to have antiviral efficacy against COVID-19 through the termination of viral RNA transcription and inhibition of 3CLpro enzyme activity, respectively[33–37]. Remdesivir only or co-administration with interferon-β or chloroquine was reported as being highly effective against COVID-19[38–41]. Studies suggest that excessive inflammation plays a role in COVID-19 complications. The hyperinflammatory immune responses caused by cytokine storm are demonstrated to be suppressed by many immunomodulators such as tocilizumab, colchicine, eculizumab, and dexamethasone[42–45]. Both case reports and retrospective observational cohort studies reported that treatment with a monoclonal antibody tocilizumab against interleukin (IL)-6 receptor improved pneumonia in severe confirmed cases[46]. An oral anti-inflammatory drug colchicine can treat pericarditis, gout, and coronary disease, and further prevent complications of COVID-19 confirmed patients[47]. Eculizumab is a monoclonal antibody as a complement inhibitor binding to the complement protein C5 with high affinity, thus suppressing the C5 cleavage and activation. COVID-19 patients who had severe pneumonia or acute respiratory distress syndrome successfully recovered after treatment with eculizumab[48]. The main ingredient of dexamethasone, dexamethasone sodium phosphate, is a synthetic adrenal cortex hormone with a powerful anti-inflammatory effect. It is used to treat adrenal cortex hormone deficiency and various organ inflammations. In COVID-19 patients, dexamethasone administration caused lower mortality in those who received oxygen alone or invasive mechanical ventilation, whereas those taking no respiratory support did not see the observation[49]. The inhibition of viral entry into host cells is achieved by authorized monoclonal antibodies such as Bamlanivimab, Imdevimab, and Casirivimab, or by the suppression of kinase signaling-associated pathways such as Imatinib and Rapamycin, has been used in confirmed cases with severe complications and hospitalization[33,37,50–53]. Recently, the emergency use authorization (EUA) of COVID-19 modified mRNA (modRNA) vaccines has been successively authorized, displaying the most powerful option to impede the COVID-19 pandemic. On August 23, 2021, the US Food and Drug Administration (FDA) approved the full use of the Pfizer/BioNTech (BNT) vaccine against SARS-CoV-2 infection, making it the world's first fully licensed COVID-19 vaccine.

COVID-19 modRNA Vaccines

modRNA Vaccine Development

The vaccine is a biological agent that provides protective immunity against specific infectious diseases. Conventional vaccines can be produced from killed microorganisms, their toxins, or their surface proteins to induce specific immunity

in a host. Vaccination enables the body to acquire protective immunity to eliminate the microorganisms related to the antigen that may be encountered in the future. The conventional approach involves typical antigen identification, beginning with the culture of pathogenic microorganisms in the laboratory, dissecting their component proteins, and then analyzing these proteins *in vitro* and *in vivo*, finally resulting in the characterization of target proteins that exhibit requisite protective immune responses. However, many pathogenic proteins are only transiently expressed in the infection process. Some pathogenic proteins are difficult to express *in vitro* and it is thus challenging to acquire sufficient quantities for vaccine production. To overcome these challenges, bioinformatics was first applied and used in vaccinology against Serogroup B meningococcus[54] by Masignani et al.[55] called reverse vaccinology. It uses the information of genomic sequences to find target antigens for new potential vaccines. A whole genome of pathogens can be screened using bioinformatics methods to search effective target antigens by a computational method[56]. The screened gene may feature potential antigenicity or code for critical proteins with immune cell epitopes, signal peptides, and extracellular localization[57]. A good vaccine target antigen such as outer membrane proteins can be selected and designed from these genes. Once the target antigen is selected, it can be expressed *in vitro* and examined in animal models treated with infection[58]. The use of bioinformatics to find vaccine target antigens is quick and efficient. Conventional approaches may need decades to decipher pathogenic antigens, immunity, and diseases. However, *in silico* methods can be implemented easily and quickly, allowing one to identify target antigens for analysis in only a few years[59]. Many programs make reverse vaccinology information more accessible. Vaxign (Vaccine Design) was created in 2008, a comprehensive program including vaccine target antigen prediction and analysis system according to the principle of reverse vaccinology. It was demonstrated to be efficient and accurate for predicting target antigen and completely public access use[60]. Another software, RANKPEP, is also used for predicting peptide binding. PSSMs (position-specific scoring matrices) is used in both Vaxign and RANKPEP for analyzing sequence alignments and protein sequences[61]. In addition, NERVE program is associated with the new data processing. Although it does not contain whole target antigen predictions and needs download, it can assist saving time. Computer-assisted bioinformatics projects are becoming very popular when they aid to select candidate vaccine antigens[62]. Therefore, the steps to develop a new vaccine include acquiring a pathogen genomic sequence, target antigen selection using computer analysis, examination of antigen-elicited protective immunity in animal models with pathogen infection, evaluation of safety and protective immunity in clinical trials, and, ultimately, FDA approval.

In recent years, much evidence has indicated the excellent ability of mRNA to elicit protective immunity against pathogen infection; this makes it as a new platform for vaccine development[63–65]. A growing interest in the utilization of modRNA-based technology to transiently express target antigens and develop prophylactic vaccines against pathogen infection. Some technological improvements, such as RNA chemistry, delivery, and stability, accelerate the maturation of modRNA vaccines. It holds great promise due to the potential of low cost and simple manufacturing processes. Moreover, modRNA vaccines have the ability to streamline the development of vaccines and help a quick response against emerging COVID-19. The concept of developing an modRNA vaccine is relatively simple compared with other vaccine systems. Once the target antigen of the pathogen SARS-CoV-2 is chosen, the target gene is synthesized and cloned into the vector. modRNA can be synthesized *in vitro* and encapsulated in the delivery system in the form of a vaccine. modRNA vaccines use host cells to directly express target antigens *in vivo*, resulting in potent humoral and cellular immunity against the SARS-CoV-2 infection. Thus, the target protein can be a secreted or membrane form protein. In addition, RNA sequences can elicit strong innate immune responses in hosts, including the production of chemokines and cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-12 that are critical factors to induce innate and adaptive immunity against a pathogen infection[66–71]. RNA molecules displayed the adjuvant effect to elicit immune responses by the recognition of specific pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs)[71,72]. Exogenous mRNA such as triphosphorylated mRNA, ssRNA, or dsRNA was demonstrated to induce interferon production through the activation of TLR 3, 7, and 8[73–76]. These TLRs initiate the expression of inflammatory cytokines such as type I interferons, TNF-α, IL-6, and IL-12[77]. mRNA delivery into cells occurs through endocytosis and results in RNA-containing endosomes in the cytoplasm. These TLR3 and TLR7/8 are expressed on endosomal membranes and recognize ssRNA, leading to the stimulation of transcription factor NF-κB and IRF-3/-7, and finally production of type I interferons and proinflammatory cytokines, respectively[78].

Recently, substantial modifications of mRNA have been investigated for their extensive applications. A modRNA-based vaccine contains 5′ cap, 5′ UTR (untranslated region), 3′ UTR, poly-A tail, and open reading frame (ORF)[79,80]. modRNA can be fully transcribed *in vitro* from a linearized DNA plasmid template by the enzymatic reaction in a cell-free system[81,82]. The first step in the manufacturing of modRNA vaccines against COVID-19 infection requires the cloning of the encoding sequence of target antigen into a plasmid template, which includes a high binding affinity of promoter sequence for the interaction with an RNA polymerase such as T7, T3, or SP6. The plasmid acts as a DNA template that transcripts an mRNA using an RNA polymerase in *in vitro* transcription (IVT). Subsequently, DNase is added to degrade the plasmid template and a novel cap analog[83–85]. As natural 5′ cap, including m7G, competes with guanosine triphosphate (GTP), it risks uncapping or becoming inactive mRNA. The natural 5′ cap was reported as a risk toward the

interaction with the first nucleotide of reverse-orientated mRNA by a 3′ to 5′ phosphodiester interaction, and one-third of the natural 5′ cap was incorporated in the reverse orientation[86]. The natural 5′ cap-caused reverse orientation is unlikely to be recognized by eukaryotic translation initiation factor 4E (eIF4E), which dramatically influences translation efficiency. New cap analogs (P(1)-3′-O,7-dimethylguanosine-5′ P3-guanosine-5′ triphosphate) were designed, named antireverse cap analogs (ARCAs), that are not able to incorporate in the reverse orientation and skip degeneration by Dcp2 (mRNA-decaying enzymes)[83]. The incorporation of a cap analog can enhance translation efficiency and modRNA stability[65,83,87–89]. Substantial modification in mRNA was reported[90], whereas only a small subset of modified nucleotides, such as 5-methylcytidine, pseudouridine, 5-methyluridine, N6-methyladenosine, N1-methylpseudouridine, and 5-methoxyuridine, were demonstrated to enhance translation efficiency and mRNA stability[65,77,88,91–93]. In 2010, 5-methylcytidine and pseudouridine were first utilized in the generation of induced pluripotent stem cells by IVT modRNA-based reprogramming technology for the overexpression of pluripotent factors such as Oct4, SOX2, KLF4, c-Myc, and Lin-28[94]. In 2015, N1-methylpseudouridine was first reported to reduce innate immune responses through inhibiting TLR3 activation by the incorporation of modRNA[95]. In 2016, 5-methoxyuridine-modRNA was first assessed to show high protein expression and an extensive half-life[96]. In 2017, the 5-methylcytidine was reported to increase mRNA export and mRNA-binding affinity through recognition by the mRNA export adaptor ALYREF[97]. Taken together, modRNA improves the disadvantages of original IVT mRNA, such as instability and difficult delivery, and further displays some advantages, such as highly transient protein expression, stability, improved delivery, and no genomic integration[98–100]. However, it is not yet suitable for the long-term expression of proteins because of the mRNA half-life[100]. The natural poly-A tail and UTR can facilitate mRNA stability and translation efficiency; thus, the optimal length of 120 to 150 nucleotides for poly-A tail, 5′ UTR, and 3′ UTR can be designed into a plasmid template for IVT. Another method for poly-A tail addition is the encoding of poly-A tail by enzymatically adding adenine nucleotides using recombinant poly-A polymerase[65,101]. Once modRNA synthesis is complete, it needs to be purified by removing reaction components such as residual DNA template, enzymes, aberrant double-stranded transcripts, or truncated transcripts. After purification, modRNA can be stored in the desired buffer or formulated with transport materials for vaccine production. Therefore, an modRNA-based vaccine possesses the advantage of potentially saving time and costs compared with other vaccine systems.

Delivery System for modRNA Vaccine

A delivery system for the mRNA vaccine can provide protection from nuclease degradation and assistance across the cell membrane[65,102]. It also has a synergistic adjuvant effect due to the influence on immune activation[67,103]. For example, certain cationic lipid nanoparticles (LNPs) induced a systemic interferon type I response through the activation of TLR4 in mice[104]. Therefore, the mRNA transport system can aim for material developments that deliver RNA into host cells, and the delivery vesicles escape from the endosome and subsequently release RNA molecules into the cytoplasm, where protein translation begins[105,106].

LNP is the most promising and commonly used delivery system for mRNA vaccines[66,81,107–116]. LNP is composed of accurate molar ratios of cationic-ionizable amino lipids, phospholipids, cholesterols, and polyethylene glycol (PEG) lipids[117]. Cationic lipids can condense with the negative charge of mRNA at low pH. Phospholipids can increase endosomal escape and fusogenicity. Cholesterols can make vesicles more stable *in vivo* and *in vitro*. Before the use of LNP, PEG lipids can provide stabilization of the LNP formulation. In 2018, US FDA approved the first LNP-transported therapeutic RNA. A report also revealed that mRNA encapsulated with LNP enhanced the durability of *in vivo* protein expression compared with naked mRNA, and even 0.1 μg of LNP-encapsulated mRNA caused effective immune responses in mice[118]. In addition, co-treatment of LNP-formulated mRNA increases antigen-specific $CD4^+$ and $CD8^+$ T cells, even shifting their differentiation to a proinflammatory T helper (Th)1 phenotype, in spite of the produced nucleoprotein, suggesting it has an adjuvant effect[119]. Therefore, mRNA delivery by LNPs possesses synergistic inflammatory effects with the stimulation of antiviral, innate immunity, and inflammatory immune responses[67]. The formulation development makes potential mRNA vaccines that can be stockpiled and thermostable. They can treat many infectious diseases, decrease the frequency of vaccinations, and alleviate the burden on health care workers.

Pfizer/BNT162b2 modRNA Vaccines

Considering modRNA advantages, such as fast production and versatility, two modRNA vaccines against COVID-19 infection (Pfizer/BNT162b2 and Moderna-1273) have been approved for the market and are summarized in Table 1; both have been granted emergency use in many countries, especially Pfizer/BNT162b2, which recently attained full US FDA authorization. BNT and Pfizer developed one LNP-encapsulated modRNA vaccine (Pfizer/BNT162b2) encoding modified nucleosides of the spike glycoprotein S-2P of SARS-CoV-2, which is modified using two proline mutations to lock it in the prefusion conformation[120]. The first COVID-19 modRNA vaccine approved by the European Medicines Agency (EMA) is Pfizer/BNT162b2[124]. The vaccine is administered with intramuscular injection of a cycle of two doses (30 μg of modRNA for each 0.3 ml vaccine) and the interval is 21 days[125]. Preclinical studies have shown the antiviral and immunogenicity properties of Pfizer/BNT162b2 in animal models such as mice and nonhuman primates. One

Table 1. Approved COVID-19 modRNA Vaccines.

Name	Developer	Location	Target	Modification	Route	Dose	Efficacy	Safety	References
Pfizer/BNT162b2	Pfizer and BioNTech	Germany	S-2P	Proline mutations	Intramuscular injection	A cycle of two doses (30 μg of modRNA for each 0.3 ml vaccine), 21 days apart	94.1%	The adverse events contained headache, fatigue, and pain at injection site; low frequency for severe adverse events	Walsh et al.[120] and European Medicines Agency. Comirnaty (COVID-19 mRNA vaccine nucleoside modified)[121]
Moderna-1273	Moderna	USA	S-2P	N1-methyl-pseudouridine	Intramuscular injection	A cycle of two doses (100 μg of modRNA for each 0.5 ml vaccine), 28 days apart	95%	Systemic adverse events were common following full vaccination; low frequency for severe adverse events	European Medicines Agency[122] and Vogel et al.[123]

COVID-19: coronavirus disease 2019; modRNA: modified mRNA.

dosage of Pfizer/BNT162b2 displayed increased neutralization titers against pseudovirus in a dose-dependent manner in mice. The vaccine enhanced neutralization titers against SARS-CoV-2 in rhesus macaques. The vaccine also elicited strong immune responses of $CD4^+$ Th1 and IFN-γ^+ $CD8^+$ T cells in rhesus macaques and mice. Moreover, the lungs of rhesus macaques fully got protection from SARS-CoV-2 infection[126].

In the initial stage of the vaccine discovery, a similar vaccine was simultaneously examined, Pfizer/BNT162b1, which encoded the RBD of SARS-CoV-2[120]. Pfizer/BNT162b1 is not like Pfizer/BNT162b2, that is, RBD located on the host cell membrane, instead of secreted proteins. To evaluate the difference in safety and immunity profiles between Pfizer/BNT162b2 and Pfizer/BNT162b1, phase I clinical trial was performed with dose-increasing and blind observation which contained both elderly (65–85 years of age) and young (18–55 years of age) participants in the United States[120]. The trial study demonstrated that Pfizer/BNT162b2 displayed decreased systemic severe adverse events (SAEs) compared with Pfizer/BNT162b1, especially in the older participants; 8% of the elderly administrating Pfizer/BNT162b2 only showed mild fever ranged from 38.0°C to 38.4°C. In addition, BNT162b1 and BNT162b2 elicited similar neutralizing antibody immune responses in both young and older participants, and the 50% neutralizing antibody titers (NT50) were higher than convalescent human serum[120]. Taken together, these results made the developers finally choose Pfizer/BNT162b2 with a cycle of two doses (30 μg of modRNA for each 0.3 ml vaccine) for safety and efficacy examination in phase III clinical trial.

There were 43,448 participants to be recruited in phase III clinical trial, with 21,728 administrating placebos and 21,720 administrating Pfizer/BNT162b2[121]. The study indicated that placebo and Pfizer/BNT162b2 groups showed 162 and 8 confirmed cases, respectively, in a total of 170 participants with full modRNA vaccination. Therefore, Pfizer/BNT162b2 displayed 95% effectiveness for the prevention of SARS-CoV-2 infection[121]. Moreover, one happened in the Pfizer/BNT162b2 participants and nine happened in the placebo participants in a total of 10 cases of severe COVID-19[121]. The vaccine caused adverse events such as headache, fatigue, and pain at the injection site[121]. Pfizer/BNT162b2 group showed a similar frequency of SAEs compared with the placebo group[121].

Moderna-1273 modRNA Vaccines

The second COVID-19 modRNA vaccine approved by the EMA is Moderna-1273. Moderna developed one LNP-encapsulated modRNA vaccine (Moderna-1273) encapsulated with modified nucleosides encoding the S-2P spike glycoprotein of SARS-CoV-2, which is modified by N1-methyl-pseudouridine[122,127,128]. Moderna-1273 has been approved for the prevention of COVID-19 infection in humans 18 years of age and the elderly. It is received with intramuscular injection of a cycle for two doses (100 μg modRNA formulated in SM-102 LNP for each 0.5 ml vaccine) and the interval is 28 days[129]. A report indicated the increased levels of neutralizing antibody and $CD8^+$ T cells against D614G variant and wild-type virus were found in Moderna-1273-treated mice, and mouse lung was protected from COVID-19 infection[128]. In the study of nonhuman primates, two doses of 10 or 100 μg of Moderna-1273 showed NT50 of 501 and 3,481, respectively. The group of 100 μg dose displayed protection from COVID-19 infection in lungs and nose[130].

To examine the immunity and safety profiles of Moderna-1273, a phase I clinical trial was conducted with 45 participants between 18 and 55 years of age. They were randomly treated with different doses of Moderna-1273 such as 25, 100, or 250 μg at the 4-week interval[122]. The 100 and 25 μg doses of Moderna-1273 elicited PRNT80 (80% inhibition of SARS-CoV-2 infection) titers of 654.3 and 339.7, respectively, that are higher than convalescent human serum[122]. Although recipients did not suffer from a high fever following the first dose of modRNA vaccine, 100 and 250 μg showed 40% and 57% fever ranged from 38°C to 38.9°C , respectively[122]. The safety study showed that more than 50% of participants had adverse events such as headache, pain, chill, and myalgia[122]. Phase III clinical trial of Moderna-1273 was performed at a cycle of two doses of each 100 μg, intramuscularly injected twice 28 days apart, for preventing COVID-19 infection[123]. There were 30,420

participants to be recruited; 2.2% of participants had virological or serological data consistent with previous COVID-19 at the time of enrollment. In the placebo group, 185 confirmed cases were recorded, while 11 cases were documented in the Moderna-1273 group, indicating a 94.1% efficacy for Moderna-1273 vaccination. The effectiveness of the vaccine has also been validated in the older participants (older than 65 years of age).

Effectiveness of Both modRNA Vaccines Against SARS-CoV-2 Variants

Original SARS-CoV-2 belongs to an RNA virus, which utilizes genetic variation mechanisms to make them survive[131]. Unique properties of RNA virus replication contain short replication times, high mutation rates, and high yields. As of August 28, 2021, the Delta variant (B.1.617.2) of SARS-CoV-2 is the predominant variant in the United States [a Centers for Disease Control and Prevention (CDC) update on September 15, 2021]. Delta variant was reported to have increased transmissibility by more than twofold with a potential suppression in monoclonal antibody and vaccination-induced neutralization compared with original SARS-CoV-2 and first discovered in India[132]. Other variants contain Alpha (B.1.1.7), first discovered in the United Kingdom; Beta (B.1.351), first discovered in South Africa; Iota (B.1.526), first discovered in the United States; Gamma (P.1), first discovered in Japan/Brazil; Kappa (B.1.617.1) and B.1.617.3, first identified in India; and Eta (B.1.525), first identified in the United Kingdom/Nigeria. These variants possess mutations[132] that change the RBD of the spike glycoprotein and influence the effectiveness of modRNA vaccines. Alpha variant has the N501Y mutation. Beta and Gamma variants have N501Y, E484K/Q, and E417T/N mutations. Iota and Eta variants have the E484K/Q mutation. Kappa and B.1.617.3 variants have E484K/Q and L452R mutations. Delta variant has the L452R mutation.

The Alpha and Beta variants have caused the pandemic in the past, but now it is the Delta variant that is causing a worldwide pandemic. Therefore, the effectiveness of both modRNA vaccines against Alpha, Beta, and Delta variants is discussed. Reports from many countries have demonstrated the high effectiveness of both modRNA vaccination against COVID-19 infection such as against asymptomatic and symptomatic infections by original strain or variants. Studies from Qatar reported that Pfizer/BNT162b2 had high effectiveness against Alpha (90%) and Beta (75%) infections, and Moderna-1273 against Alpha (100%) and Beta (96%) infections after receiving two doses of the vaccine. Moreover, regardless of the virus strains, the effectiveness of both modRNA vaccines against critical, severe, or fatal diseases is 96% to 100%[133,134]. In three reports from Canada, one study indicated that modRNA vaccines were 79% effective against confirmed infections when Alpha and Gamma infections were the most common, while the other two studies reported that the effectiveness against Gamma and Beta was 84% and 88%, respectively[135–137]. Data from the United Kingdom have shown the effectiveness of the Pfizer/BNT162b2 modRNA vaccination against Delta-caused COVID-19 infection (79%) and symptomatic infection (88%) compared with the Alpha-caused COVID-19 infection (92%) and symptomatic infection (93%)[138,139]. Data from Canada reported that the effectiveness of Pfizer/BNT162b2 against Delta-caused symptomatic infection 7 days following the second dose was 87%, compared with 89% for the Alpha variant[136]. A study reported that the effectiveness of Pfizer/BNT162b2 against Delta-caused symptomatic infection was 54%, compared with 85% for the Moderna vaccine[140].

Myocarditis Complications

Recently, the US CDC discovered a relationship between both COVID-19 modRNA vaccines and cases of pericarditis and myocarditis[141]. Studies reported that myocarditis primarily occurred after the second dose of modRNA vaccination from Pfizer/BNT162b2 and Moderna-1273[142–153]. Myocarditis is considered a rare complication and predominantly occurs in young adult and adolescent men after COVID-19 modRNA vaccination. According to the reports of CDC, the occurrence rate of myocarditis is 12.6 cases in 1 million participants following the second dose of modRNA vaccination in 12- to 39-year-old people. In confirmed cases, patients with myocarditis almost experienced elevated cardiac troponin levels and chest pain after modRNA vaccination. Most patients had abnormal electrocardiograms with ST-segment elevation, and cardiac magnetic resonance imaging (MRI) of all tested patients showed myocarditis. These myocarditis patients did not have evidence of other viral or SARS-CoV-2 infections. The genomic profile of cardiomyopathy in one case was examined as negative, but the population of natural killer cells and the level of autoantibodies against certain autoantigens were upregulated.

In addition, the Israeli Ministry of Health reported that 148 cases had myocarditis in 10.4 million people 30 days after modRNA vaccination, most of which occurred after the second dose and most of them occurred in men aged 16 to 30[154]. Most cases needed hospitalization times of around 4 days, but they are considered mild. A study implied a possible correlation between myocarditis and modRNA vaccines in 16- to 30-year-old men[154], a stronger correlation in 16- to 19-year-old men, and a reduced correlation in older men[150,154]. The myocarditis prevalence in the 16- to 30-year-old people was 1/20,000, while the general group taking the modRNA vaccine was 1/100,000. Moreover, a study showed that 23 male soldiers were reported myocarditis in 2.8 million people who received COVID-19 modRNA vaccination according to the US Department of Defense[155]. The prevalence of adverse cardiovascular effects was lower than 0.05% in these clinical trials, which did not include myocarditis[121,123].

Although the myocarditis induction underlying mechanisms remain unclear, molecular mimicry between self-antigens and SARS-CoV-2 spike glycoprotein may be proposed. According to experimental reports, antibodies against viral spike glycoprotein cross-reacted with human peptide-protein such as α-myosin[156]. They may accelerate preexisting dysregulated pathways in some individuals, leading to inflammation, immune complex formation, and polyclonal B cell expansion[157]. Another possible explanation may be sex hormone differences[158–160]. Testosterone contributes to the suppression of anti-inflammatory cells[158–161] and the promotion of proinflammatory Th1 immunity[162]. Estrogen has a suppressive effect on proinflammatory cells, which results in a reduction in T-cell-regulated immunity[163]. As of June 6, 2021, there were 6,235 cases diagnosed with chest pain: 30% of those were men and 69% of those were women by the study of the VAERS database[164]. Although women had a higher prevalence of chest pain, other diagnostics such as echocardiography, laboratory biomarkers, electrocardiogram, and MRI were demonstrated as having a higher prevalence in men after COVID-19 modRNA vaccination. Thus, adult men have a higher risk for myocarditis complication after the second modRNA vaccination compared with women, which may be associated with testosterone production.

In addition, recent studies reported that the ChAdOx1 nCoV-19 COVID-19 (AstraZeneca) vaccine is prone to producing thrombocytopenia, blood clots, and antiplatelet factor 4 antibodies[165–167], whereas the effects of COVID-19 modRNA vaccines on thrombosis remain unclear. As of April 16, 2021, a study examined the frequency of SAEs associated with bleeding, blot clots, and thrombocytopenia[168]. Compared with the SAE frequency caused by the AstraZeneca vaccine, the Pfizer/BNT162b2 modRNA vaccine was related to a much lower SAE frequency in each adverse event (AE) response group. They reported 151 and 33 SAEs in respective 1 million participants with AstraZeneca and BNT vaccinations when observing AEs caused by blood clots, bleeding, and thrombocytopenia. They also reported 30 and 4 SAEs and 0.4 and 4.8 deaths in respective 1 million participants with AstraZeneca and BNT vaccinations when observing AEs caused by thrombocytopenia and venous thrombosis. These thrombotic adverse events seem not to be favored by the Pfizer/BNT162b2 modRNA vaccine.

The modRNA vaccine of COVID-19 has been used globally and supplied successfully. Although it has a few side effects on certain persons, it does not appear to elicit a full-blown fatal problem. Based on the current knowledge, it can be against many variants, despite various variants may reduce the vaccine's efficacy[169]. However, there is no doubt that the benefits from the vaccination of modRNA against COVID-19 far outweigh the risks. Current modRNA vaccines may not work if many variants are generated. In this case, the vaccine needs to be updated, as is the case with seasonal flu. COVID-19 also causes severe lung inflammation and mediates severe infections that induce high levels of proinflammatory IL-1 cytokine that can lead to death in individuals in addition to eliciting immune dysregulation. IL-1 cytokine further induces other proinflammatory cytokines and triggers the cytokine storm that also occurs in COVID-19. The modRNA vaccine directly uses human cells to produce spike protein that elicits primarily neutralizing antibodies against the body. However, certain antibodies in infected patients seem to alter spike structure, which makes it easier to bind to cells. Current studies showed harmful effects of spike protein, including TLR activation, endothelial damage, SARS-CoV-2 entry into target cells, proinflammatory cytokine release, microglia stimulation, and molecular mimicry with heat shock proteins and chaperones. Therefore, it is reasonable to consider developing treatment that prevents the deleterious effects of spike proteins by using liposomal formulations of the natural flavonoids luteolin and methoxyluteolin in addition to existing vaccines and anti-inflammatory drugs[170,171].

Conclusion

COVID-19 modRNA vaccines (Pfizer/BNT162b2 and Moderna-1273) contain modRNA, which encodes viral spike glycoprotein, in addition to buffer and salts. LPNs are used to deliver modRNA into host cells by encapsulating modRNAs as transport vesicles. Once modRNA goes into the host cells, it immediately translates the viral spike glycoprotein and elicits humoral and cellular immunity to eliminate virus infection. The IVT modRNA provides a nonviral, integration-free, zero-footprint method for expressing target antigen in host cells, and it represents a safe and efficient strategy for vaccine development. Therefore, the modRNA-based vaccine holds promise in terms of preventive vaccines against rapidly mutating SARS-CoV-2 because of its time- and cost-saving characteristics compared with other vaccine systems. A schematic overview of an modRNA-based vaccine against COVID-19 infections is shown in Fig. 1.

SARS-CoV-2 genomic sequencing has accelerated scientists' research for effective COVID-19 vaccines. Purified or isolated viral proteins of SARS-CoV-2 have been recognized as target antigens for the development of COVID-19 vaccines. However, according to the screened viral genome, RNA vaccines may be the next generation vaccines because of the rapid production of the target antigen by the RNA-based protein replacement technology. RNA vaccines are considered no genome integration in a host, whereas DNA vaccines face this issue. RNA vaccines are less prone to anti-vector immunity but not viral vector-based vaccines. Thus, they hold great promise in the rapid generation of COVID-19 vaccine. An improved mRNA-based vaccine has been validated in humans and inspires their prospects for commercialization. RNA advanced techniques such as the increase of

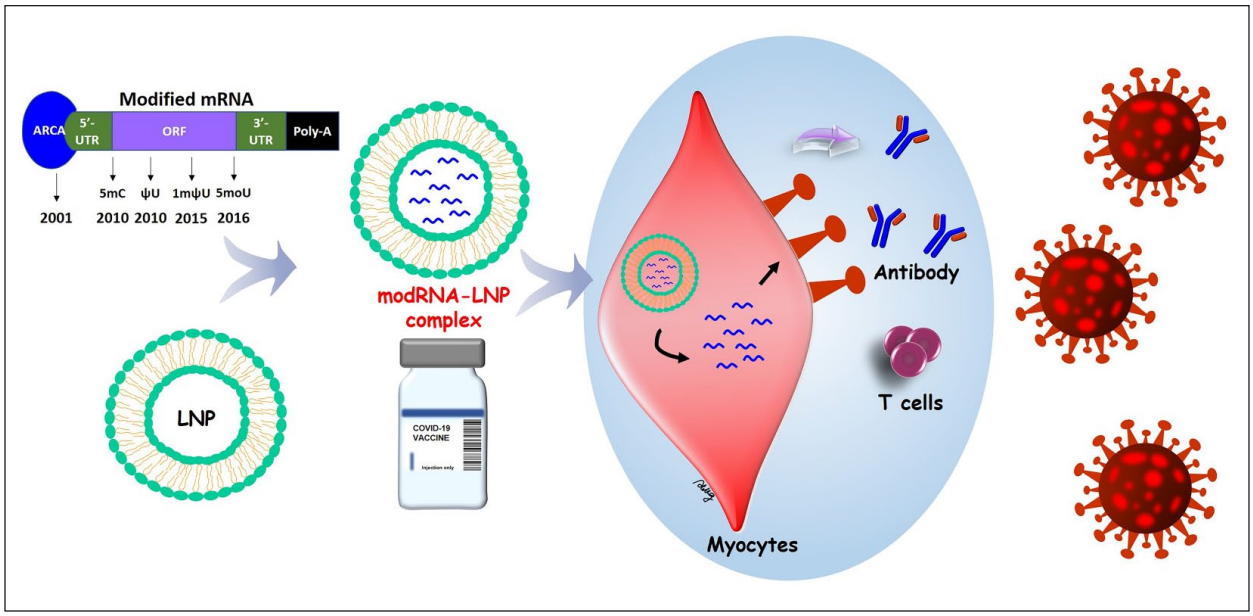

Figure 1. Schematic overview of the modRNA-based vaccine against COVID-19 infections. Based on the understanding of modified mRNA, it can be designed to synthesize *in vitro* by the robust RNA polymerase-dependent transcription from a linearized plasmid template, which can be designed to incorporate poly-A tail and UTRs, such as 5′ UTR including a Kozak sequence and 3′ UTR containing α-Globin. Natural 5′ cap can be replaced with a cap analog ARCA. Different modified nucleotides such as 5-methylcytidine (5mC), pseudouridine (ψU), N6-methyladenosine (6mA), N1-methyl-pseudouridine (1mψU), 5-methyluridine (5mU), and 5-methoxyuridine (5moU) have different functions, which can be chosen to incorporate into the mRNA for enhancing its stability and translation efficiency. DNase I could be added to digest the DNA template after modRNA synthesis. After purification, the modRNA could be diluted in the buffer and further encapsulated with LNPs to the desired vaccine. The modRNA–LNP complexes are taken up by host cells and directly produce pathogen target antigens such as the spike protein of SARS-CoV-2. Target antigens can be designed to express as secreted or membrane form, which elicit both pathogen-specific neutralizing antibodies and T cells (CD4+ and CD8+) against pathogen infection. modRNA: modified mRNA; COVID-19: coronavirus disease 2019; UTRs: untranslated regions; ARCA: antireverse cap analog; LNPs: lipid nanoparticles; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ORF: open reading frame.

mRNA stability and effective translation have inspired the growing interest in mRNA-based vaccines. On December 29, 2020, the WHO published a "Draft landscape of COVID-19 candidate vaccines," showing that seven mRNA-based vaccine candidates against SARS-CoV-2 infection were in clinical trials for different stages[172,173]. Two candidates (Pfizer/BNT162b2 and Moderna-1273) had already received EUA.

In the future, some aspects of modRNA vaccine technology still can be improved. (1) The stability and translation efficiency of modRNA can be enhanced. (2) The stability of the encapsulated modRNA vaccine at room temperature is still difficult for vaccine distribution. (3) The delivery system including materials or methods can be improved for modRNA vaccines. (4) Long-term clinical trials of modRNA vaccines are needed to better understand their extensive safety and protective immunity. (5) The production process of modRNA vaccines needs to be optimized. If modRNA vaccine can overcome these obstacles, then the future of modRNA vaccine technique is bright, and it will enter the clinic as the next generation method.

Ethical Approval

This study was approved by our institutional review board.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article, and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from Chang Gung Medical Foundation, Chang Gung Memorial Hospital, Taiwan (CMRPG1H0083 and CMRPG3M0281) and Ministry of Science and Technology, Taiwan (MOST 109-2314-B-182A-056-MY3 and MOST 110-2314-B-182A-137-MY3).

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