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Review Article

mRNA-based COVID-19 vaccines: a new age

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ABSTRACT

The development of vaccines based on mRNA technology involves more than a decade of hard work and important advances. Many clinical trials are underway to test these vaccines for the treatment and prevention of infections and diseases, such as cancer, cytomegalovirus, Ebola, hepatitis C virus, human immunodeficiency virus, influenza, malaria, rabies, and Zika. However, after the COVID-19 pandemic in 2020, it played a leading role in an important race to develop therapeutic strategies, mainly a vaccine, against the disease. mRNA technology allows the quick and safe creation of vaccines and large scale production. There are currently mRNA vaccines against COVID-19 (Pfizer-BioNtech® and Moderna®) that have received the emergency use authorization of regulatory entities, including the FDA in the USA, the EMA in Europe, and many others, in the process of obtaining clinical data so that they are available in a short time. On the other hand, phase 3 clinical trials continue their course. In preliminary analyses, remarkably high levels of efficacy have been reported, reaching around 95% effectiveness against mild-moderate disease and up to 100% against severe disease and death. The various clinical trials show a robust safety profile, equal to or better than that of many commonly used vaccines, although they are not free of adverse events. Despite this, there are still significant technical challenges and doubts due to the lack of long-term information. mRNA vaccines represent a new era in vaccination and one of the most important advances in health, science, and technology in recent times. In this review, we will show the basic principles of mRNA vaccines and focus on the vaccines used against COVID-19. Scientific evidence shows that mRNA vaccines are one of the best options not only as a defense against the SARS-CoV-2 pandemic but also as a novel technology against various diseases.

Key words: mRNA vaccines; CVnCoV; mRNA-1273; BNT162.

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RESUMEN

El desarrollo de vacunas basadas en la tecnología de ARNm tiene más de una década de arduo trabajo e importantes avances; varios estudios clínicos se llevan a cabo para probar estas vacunas en el tratamiento y prevención de infecciones y enfermedades como cáncer, citomegalovirus, ébola, virus de hepatitis C, virus de inmunodeficiencia humana, influenza, malaria, rabia y Zika. Sin embargo, no fue hasta la pandemia de COVID-19 en 2020 que tomó un rol protagónico en una importante carrera para desarrollar estrategias terapéuticas contra la enfermedad, principalmente una vacuna. La tecnología de ARNm permite la generación de vacunas de manera rápida y segura, con la posibilidad de escalar la producción a grandes niveles. En la actualidad, ya contamos con vacunas de ARNm contra COVID-19 (Pfizer-BioNtech® y Moderna®) que cuentan con el registro de emergencia de entidades reguladoras, entre ellas la FDA en EUA y la EMA en Europa, y otras tantas en proceso de obtención de datos clínicos que permitirán su disponibilidad en poco tiempo. Por otra parte, los ensayos clínicos de fase 3 siguen su curso. Los análisis preliminares registran niveles de eficacia notablemente altos: en torno a 95% contra la enfermedad leve-moderada y hasta 100% contra la enfermedad grave, incluida la muerte. Los distintos ensayos clínicos muestran un perfil de seguridad sólido, igual o mayor que el de muchas vacunas de uso común, aunque las vacunas no están exentas de eventos adversos. A pesar de lo anterior, existen importantes retos técnicos y dudas debido a la falta de información a largo plazo. Las vacunas de ARNm representan una nueva era en la vacunación y uno de los avances más importantes en salud, ciencia y tecnología en los últimos tiempos. En esta revisión mostraremos los principios básicos de las vacunas de ARNm y nos centraremos en las vacunas utilizadas contra la COVID-19. La evidencia científica demuestra que las vacunas de ARNm son una de las mejores opciones, no solo para combatir la pandemia de SARS-CoV-2 sino como una tecnología novedosa contra diversas enfermedades.

Palabras clave: vacunas ARNm; CVnCoV; mRNA-1273; BNT162.

INTRODUCTION

Vaccines are one of the most important elements in public health, characterized by being able to provide protection against a wide range of diseases, particularly those that are infectious. The basic principle of all vaccines is the generation of immunological memory against a specific microorganism. Vaccines contain molecules of the microorganism against which immunity is to be created. Upon administration, they allow the person to generate the immune response without the need for natural infection. The success of vaccines as a disease prevention and control strategy is extraordinary. Since their use as instruments of public health, they have prevented the infection and death of millions of people for generations around the planet.[1]

The SARS-CoV-2 virus causing COVID-19 (coronavirus disease) was declared a pandemic by the WHO in 2020, and one of the first responses was the urgent need for an effective and safe vaccine against this disease.[2] Although there are strategies that reduce the rate of COVID-19 infections, such as physical distancing, hand washing, and the use of face masks, these measures are not totally effective and, in general terms, are difficult to implement strictly enough as to control the pandemic.[1,2] Currently, there are no widely available therapies to effectively control the disease. In general, we still use treatments focused on symptom control. Large numbers of therapies and drugs are currently being tested in clinical trials; however, they are still far from being widely available.[2]

With public health measures being limited and difficult to implement and without any highly effective treatment against COVID-19, humanity's great bet is on the development and implementation of vaccines. Vaccine development has historically been a challenging journey that takes many years, even decades, and does not always culminate in highly efficient products. Perhaps the biggest effort in terms of knowledge and development in biotechnology during the pandemic has been the development of a new group of vaccines based on synthetic ribonucleic acid (RNA) that contains the genetic information encoding proteins of the SARS-CoV-2 virus.[3] Although not a novel development, these vaccines had never been applied nor produced at a large scale. In this review, we will analyze how RNA-based vaccines work, their application in the fight against COVID-19, and the perspectives of this new generation of vaccines. mRNA vaccine technology is one of the most promising in the healthcare field. We are at the beginning of an exponential growth in their use and applications, which makes them one of the most relevant topics in the scientific community.

General principles of mRNA vaccines

Generally speaking, classic vaccines are based on various strategies that allow our body to be in contact with elements of the virus and mount an immune response. The main strategies are attenuated virus, killed virus, subunits, and mRNA.[1,3,4] There are multiple reviews detailing the main strategies in the creation of vaccines. In this work we will focus on mRNA vaccines, which are based on synthetic mRNA sequences that encode virus proteins. The encoded protein in SARS-CoV-2 is Spike, one of the most important proteins in the functioning of the virus and essential to cell infection. It has also been found that the human body is naturally capable of generating antibodies (Abs) and immune memory against this protein.[4,5,6] Genomic and molecular biology technology have made it possible to sequence SARS-CoV-2 within a few weeks after its isolation, and hundreds of complete genomes of the virus are currently being sequenced every day around the world. This enormous sequencing capacity allows us to rapidly generate new mRNA sequences that could be more effective or even adapt vaccines to new pathogens.[6]

The generation of synthetic mRNA sequences is relatively simple; however, there is still a huge area of development related to stability and scalability in synthesis. Although mRNA must be degraded within a reasonable time after entering the target cell, degradation should also occur after generating sufficient virus protein.[4] On the other hand, the low stability of mRNA poses logistical problems, since these vaccines normally require cold chains that can reach –70°C, which greatly hinders distribution and conservation on a large scale. Scalability is an important issue, as it offers substantial advantages by not relying on living cells or the management of active viruses, as many other vaccines do. Still, few vaccine production facilities currently have the technology to generate the necessary elements for mRNA vaccines.[4,6] A general process of mRNA vaccine development is shown in Figure 1.

There are key elements to ensure the optimum stability of the vaccine. The 5 'and 3' UTRs (untranslated regions) have an influence on both the stability and the translation of mRNA. Basic elements such as cap 5' and a poly (A) tail at the 3' end are basic to ensure an adequate half-life of the molecule. The codons used are also important, mainly in translation. In general, the aim is to use the most common codons in human cells, which allows for a more efficient translation. However, it must also be considered that the change of codons can influence the processivity and precision of the ribosome. Codon usage remains an area of development in mRNA vaccines.[3,4,7]

Chemical modifications are a common practice to increase nucleotide resistance against degradation or translation rate. Some studies show that the change of nucleotides in pseudouridine and 5-methylcysteine increases translation and decreases RNA immunogenicity. Modifying the structure

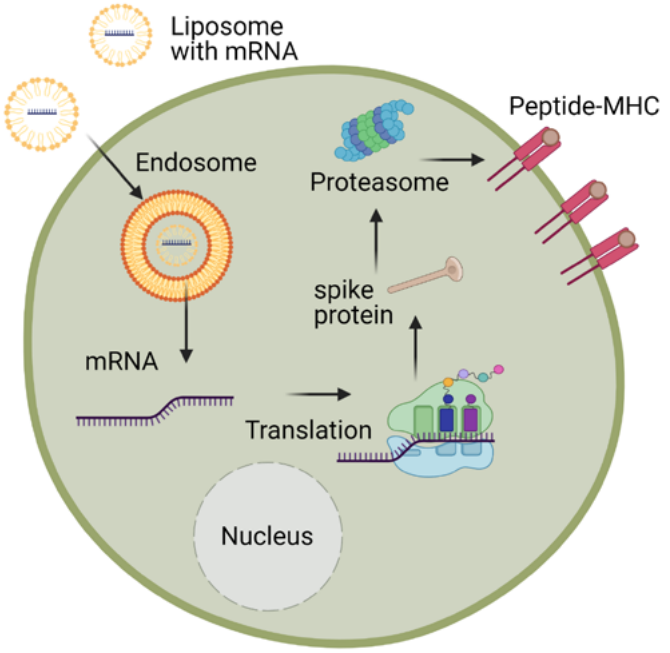

FIGURE 1. General process of mRNA vaccines.
Lipid particles are endocytosed. Once inside the cell, the mRNA contained in the particles is released into the cytoplasm. The mRNA particles are translated by the cellular ribosomes, expressing the S protein of the SARS-CoV-2 virus. Enzymes of the transcriptional machinery of the virus are also expressed in self-amplification mRNA vaccines. The viral S protein is processed by the proteosome and exposed to immune system cells by the major histocompatibility complex (MHC). Created in BioRender.com

of the 5'cap (usually a cap 1) is another strategy; the anti-reverse cap analogue and phosphorothioate derivatives have been shown to improve transcription by adding the cap only in the forward orientation. Once the features and design of the mRNA are completed, the evaluation of possible structural modifications must be done. The formation of certain secondary structures and double-stranded RNA (dsRNA) can significantly decrease translation rates and increase the immunogenicity of the mRNA at the same time. On the other hand, to avoid contamination with other molecules or even with unwanted secondary structures, purification methods such as fast protein liquid chromatography (FPLC) or high-performance liquid chromatography (HPLC) must be used.[7]

In general, we can distinguish two types of mRNA structures used for vaccines: conventional mRNA and self-amplification mRNA. The first are based on the virus sequence of positive-sense single-stranded RNA. They are composed of the sequence of the antigen of interest flanked by regulatory regions (5´ and 3´ UTR), a 5´cap structure and a poly (A) tail, a structure relatively simple and small. In self-amplifying RNAs the nucleic acid molecule contains the antigen of interest and the information of the transcriptional machinery of the

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virus, particularly the sequence of the RNA-dependent RNA polymerase, usually of some alphavirus. The self-replicating RNAs also contain regulatory sequences which potentiate replication and translation; therefore, they are larger (9–11 kd) and more complex than conventional mRNA. Recent studies have shown advantages and disadvantages of both mRNA structures. Interestingly, it has been noted that self-replicating mRNA needs much lower doses of inoculation and promotes a prolonged and stable expression of the antigen of interest. This is why many current developments are based on self-replicating mRNA technology.[3,4]

A series of extremely important elements are considered for the inoculation and delivery of mRNA molecules. Many formulations that allow the entry of mRNA into cells and their release into the cell cytoplasm have been developed. In general terms, they are vehicles containing genetic material that are capable of fusing with the cell membrane. The most common carriers are lipid nanoparticles (LNPs), cationic lipids, polymers, and protamine sulphate. The cationic peptide protamine has been shown to protect mRNA from degradation by serum RNases. However, protamine-complexed mRNA alone demonstrated limited protein expression and efficacy in a cancer vaccine model, possibly owing to an overly tight association between protamine and mRNA. This issue was resolved by developing the RNActive vaccine platform in which protamine-formulated RNA serves only as an immune activator and not an expression vector.[8,9] In addition to the vehicle used, the inoculation site is essential to obtaining the expected therapeutic results.

Immunogenicity

The use of mRNA vaccines has several beneficial features over subunit, killed, and live attenuated ones, as well as those based on DNA.[3] mRNA vaccines strongly induce both cellular and humoral immune responses. They are relatively safe and effective because they contain only a transient carrier of information that does not interact with the host genome nor needs the whole virus.[4] As it happens, in a natural SARS-CoV-2 infection, the production of specific anti-SARS-CoV-2 Abs induced by mRNA vaccines depends on the activation of the adaptive immune response. It starts when T lymphocytes are presented a relevant peptide derived from the virus by an antigen-presenting cell. This leads to the activation of antigen-specific CD8+ (cellular immunity) and CD4+ T cells, which play a key role in the activation of B lymphocytes. The latter will eventually initiate the production of SARS-CoV-2 specific Abs targeting the SARS-CoV-2 S protein. Studies have shown that these Abs can neutralize the virus in its extracellular stage and thus inhibit the infection by SARS-CoV-2 in vitro and/or in vivo.[5,6]

Karikó et al. found that the inherent immunogenicity of mRNA can be down-modulated to further increase the safety profile.[7] Single-stranded oligoribonucleotides and their degradative products are detected by the endosomal sensors toll-like receptor 7 (TLR7) and 8 (TLR8),[10,11] resulting in type I interferon (IFN-I) production.[12] The authors found that the incorporation of N1-methyl-pseudouridine (m1Ψ) in place of uridine led to a 10-fold increase in translation over unmodified mRNA. Furthermore, they were able to show that this modification in mRNA molecules prevents the activation of TLR7, TLR8 and other innate immune sensors, reducing IFN-I signaling,[13] and undesired vaccine side-effects. For these reasons, many candidates, including the two recently licensed mRNA vaccines mRNA-1273 and BNT162b2, adopted this m1Ψ mRNA modification in their vaccine design. Nucleoside modification also partially suppresses the recognition of dsRNA species.[13]

The immunostimulatory properties of mRNA can conversely be increased by incorporating an adjuvant to increase the potency of some mRNA vaccine formats. These include traditional adjuvants as well as novel approaches that take advantage of the intrinsic immunogenicity of mRNA or its ability to encode immune-modulatory proteins. Self-replicating RNA vaccines have displayed increased immunogenicity and effectiveness after formulating the RNA in a cationic nanoemulsion based on the licensed MF59 (Novartis) adjuvant.[14] Another effective adjuvant strategy is TriMix, a combination of mRNAs encoding three immune activator proteins: CD70, CD40 ligand (CD40L), and constitutively active TLR4. Van Lint et al. found that TriMix mRNA increased the immunogenicity of naked, unmodified, unpurified mRNA in multiple cancer vaccine studies and was particularly associated with increased dendritic cell maturation and CD8+ T cell response.[15] The type of mRNA carrier and the size of the mRNA–carrier complex have also been shown to modulate the cytokine profile induced by mRNA delivery.

The application of mRNA vaccines has been restricted until recently by the instability and inefficient in vivo delivery of mRNA. Recent technological advances have overcome these issues, and multiple mRNA vaccine platforms have been developed in recent years. They have been validated in studies of immunogenicity and efficacy against infectious diseases and several types of cancer in animal models and humans. Recently, mRNA vaccines have elicited potent immunity against infectious disease targets in animal models of influenza, Zika (ZIKV), and rabies viruses, among others, using lipid-encapsulated or naked forms of sequence-optimized mRNA.[16] Highly efficient and non-toxic RNA carriers, like LNPs have been developed to allow prolonged antigen expression in vivo.[17] LNPs have become one of the most appealing and commonly used mRNA delivery tools and often consist of four components: an ionizable cationic lipid, promoting self-assembly into virus-sized particles (~100 nm) and allowing endosomal release of mRNA into the cytoplasm; lipid-linked polyethylene glycol (PEG), which increases the half-life of formulations; cholesterol, a stabilizing agent; and naturally occurring phospholipids that support lipid bilayer

structure. Geall et al. and Pardi et al. demonstrated that LNPs are potent tools for in vivo delivery of self-amplifying RNA and conventional, non-replicating mRNA, respectively.[16,17] mRNA–LNP complexes administered intradermally, intramuscularly, and subcutaneously lead to prolonged protein expression at the site of injection.[17]

Then, to evaluate the efficacy of vaccines, pre-clinical evaluations of SARS-CoV-2 mRNA vaccines have focused on their ability to elicit robust SARS-CoV-2-binding and neutralize Ab responses in mice. Laczkó et al. found that a single 30-µg dose of an mRNA-LNP vaccine, encoding RBD (receptor binding domain) of SARS-CoV-2, promoted high titers of SARS-CoV-2-binding immunoglobulin G (IgG) in mice, just two weeks post-immunization.[18] In every mRNA vaccine candidate (Moderna mRNA-1273, BioNTech/Pfizer BNT162b2, and CureVac CVnCoV) the production of SARS-CoV-2-specific Abs was achieved by a significantly lower dose (0.2–10 µg) than the one used in Laczkó's study. Still, a booster immunization was necessary for NAb (neutralizing antibody) generation at lower doses (1 or 2 µg).[19] These Abs neutralized the virus in vitro, as measured by SARS-CoV-2-based neutralization assays,[18, 19] and their levels were sustained for two months or more post-immunization.[20] All mRNA vaccines induce a potent germinal center response, which produces a more effective and long-lasting antigen-specific Ab response.[21]

As the wild-type SARS-CoV-2 cannot efficiently replicate in common laboratory mouse strains due to the lack of appropriate receptors to initiate viral infection, studies in non-human primates (NHPs) are necessary to determine the efficacy of vaccine candidates. Interestingly, in NHPs, the clinical candidates mRNA-1273 (10 or 100 µg) and BNT162b2 (30 or 100 µg) demonstrated a robust, dose-dependent capacity to elicit SARS-CoV-2 specific Abs after two immunizations. Also, NAb values elicited in NHPs by BNT162b2 were higher than the ones found in SARS-CoV-2 human convalescent sera. The NAb responses elicited by these candidates were combined with in vivo protection against SARS-CoV-2 challenge after the booster immunization. The viral replication found in the upper respiratory tract of the infected animals that received any of these vaccines was transient. Moreover, no viral replication was found in the lower respiratory tract of the vaccinated animals.[22]

A lot has been speculated about the development of Ab disease enhancement (ADE) by SARS-CoV-2 vaccines. This phenomenon is described as the increase in disease severity induced by a vaccine if the subject is later infected by the natural virus. It can be characterized by immunopathology and a T helper 2 cell (Th2) biased response and Ab responses with poor neutralizing activity.[23] Therefore, ADE could occur when a vaccine fails to develop NAbs because of insufficient concentration/affinity or Abs incapable of binding their antigen. Some Middle East respiratory syndrome (MERS) and SARS-CoV-1 vaccines have shown evidence of ADE in animal models, a concern with COVID-19 vaccines. The Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency Vaccines (SPEAC) issued a statement after a scientific working meeting that took place in May 2020, declaring that vaccines inducing strong NAbs and predominant Th1 responses are less likely to induce ADE. The experts also mentioned that the level of NAbs and determination of the relative ratio of binding to NAbs is key to assess the potential risk of ADE in phase 1 clinical trials of COVID-19 vaccines. They suggest to perform a longer follow-up than usual in phase 1 trials to monitor this syndrome in immunized participants.[23]

While no evidence has shown that Ab-dependent enhancement can occur in SARS-CoV-2 infection, the two mRNA vaccines discussed above induce high levels of SARS-CoV-2 binding Abs and NAbs. Therefore, any potential Ab-dependent enhancement could be ruled out.[23,24] Overall, mRNA vaccines seem to activate CD8+ T cell response. A single dose of SARS-CoV-2 mRNA in mice elicits antigen-specific CD8+ T cell responses, characterized by the production of IFN-γ, IL-2, and/or TNF.[18] Preclinical studies of the mRNA clinical candidates have shown that BNT162b2 administration in mice resulted in increased amounts of IFN-γ- and IL-2-secreting CD8+ T cells in the spleen 12 days after immunization.[25] Moderna mRNA-1273 was found to elicit a CD8- T cell response in mice but, in preclinical trials, failed to induce detectable CD8+ T cell responses in macaques, even with high doses (100 µg).[26] Interestingly, in natural SARS-CoV-2 infection in humans, the S protein has been shown to elicit a relatively modest CD8+ T cell response in some COVID-19 cases.[27] Since SARS-CoV Abs levels decrease after two months of natural infection, it has been proposed that the induction of humoral and cellular responses is necessary to generate an optimal long-lasting protective response.[28,29] However, there is no indication that the induction of cytotoxic CD8+ T cells is required for a successful protection against SARS-CoV-2 via vaccination.

Safety

One key advantage of mRNA vaccines is that their production avoids common risks associated with other vaccine platforms (live virus, viral vectors, inactivated virus, and subunit protein vaccines) since they do not require toxic chemicals or cell cultures that could be contaminated with adventitious viruses. Additionally, mRNA is manufactured in a short time, so it is unlikely to get contaminated by microorganisms. This type of vaccines does not contain a live virus, and therefore, does not carry a risk of causing disease in the vaccinated person. The mRNA of the vaccine never enters the nucleus of the cell and does not affect or interact with the person's DNA. Thus, the theoretical risks of infection or integration of the vector into host cell DNA are not a concern in vaccinated people. Probst

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et al. showed that several cell types in mouse dermis take up foreign protein-coding RNA, which can also be demonstrated in human skin. Moreover, the injected mRNA metabolically decays within a few days, making this molecule a merely transient and safe carrier of information.[30] As mRNA is a non-infectious, non-integrating platform, there is no potential risk of insertional mutagenesis. mRNA is degraded by normal cellular processes, and its in vivo half-life can be regulated using various modifications and delivery methods.[31] As mentioned before, studies over the past decade have shown that the immunostimulatory profile of mRNA can be shaped by the incorporation of pseudouridine. This prevents its recognition via PRRs, and therefore, the activation of inflammation and IFN-I production, reducing the unwanted vaccine side-effects.[31,32]

The fact that vaccines are administered to healthy individuals stablishes a strict requirement for safety. Phase 1 to 2b clinical studies are in course to test several mRNA vaccines, and they have shown that these vaccines are safe and reasonably well tolerated. Potential safety concerns that are likely to be evaluated in future preclinical and clinical studies include local and systemic inflammation, the biodistribution and persistence of expressed immunogen, stimulation of auto-reactive Abs, and potential toxic effects of any non-native nucleotides and delivery system components.

Although all the clinical data reviewed indicate that mRNA vaccines are safe to use in humans, this is the first time that a vaccine of this type has been licensed, which raises some potential concerns. There have been rare reports of individuals experiencing anaphylaxis following immunization with COVID-19 mRNA vaccine.[33] As with all vaccines, some people can have allergic reactions to one or more of the components included in the vaccine: an adjuvant, an antibiotic used in the manufacturing process, one of the lipids enveloping the mRNA, or even a salt used as a diluent. As it stands, the offending component of this mRNA vaccine remains unclear, but anaphylaxis represents a major concern for people with a history of severe allergies.

A possible safety concern regarding these novel vaccines is that some mRNA-based vaccine platforms induce a potent IFN-I response[34] associated with inflammation. If it persists, this process becomes chronic, leading to potential autoimmunity.[35] It has been proposed that the identification of individuals at an increased risk of autoimmune reactions before mRNA vaccination could allow for reasonable precautions to be taken. Another potential safety issue is the presence of extracellular RNA during mRNA vaccination. Some studies have addressed this concern and found that extracellular RNA promoted blood coagulation and pathological thrombus formation.[36] In addition, Fischer et al. showed that extracellular naked RNA increases the permeability of tightly packed endothelial cells and may contribute to edema.[37]

The vaccine associated enhanced respiratory disease (VAERD) is a safety issue that requires further investigation regarding SARS-CoV-2 vaccines. Lung immunopathology refers to exaggerated lung inflammation after a viral infection, which may interfere with oxygenation and can lead to a worse disease than what would normally be seen after virus infection in the complete absence of vaccination. Clinical VAERD was first seen in the 1960s among human infants with RSV infection after receiving a formalin-inactivated vaccine against RSV that led to markedly worse respiratory disease as compared to non-vaccinated infants.[38] Some vaccine studies have evaluated the balance of Th1 and Th2 because VAERD has been linked with Th2-biased immune responses in children immunized with whole-inactivated virus vaccines against RSV.[39] Even some inactivated SARS-CoV vaccines have triggered VAERD in some animal models. Studies conducted with mRNA-1273 showed that the Ig subclass and T cell cytokine profile activated after immunization trigger a balanced Th1/Th2 response as compared with SARS-CoV-2 S protein adjuvanted with alum, which clearly skewed the response to Th2 profile. This suggests that mRNA vaccination avoids the Th2-biased immune response linked to VAERD. A major goal of animal studies to support SARS-CoV-2 vaccine candidates through clinical trials is to show that sub-protective responses do not cause VAERD. For the above reasons, mRNA vaccines have been considered relatively safe. Still, they must be evaluated as different mRNA modalities and delivery systems are used in humans and tested in larger patient populations for the first time.

Pfizer-BioNTech vaccine BNT162

Since scientists in China released the genetic sequence of SARS-CoV-2 in January 2020, worldwide research for a potential vaccine was triggered. Having access to global data has been critical to a fast and efficient development. On March 17th, 2020 Pfizer and the German biotech company BioNTech announced their partnership to co-develop a potential mRNA vaccine to prevent the spread of COVID-19. Pfizer stated that both companies would jointly develop BioNTech's mRNA-based vaccine candidate BNT162 to prevent COVID-19 infection, as they had already been working with their German partner to develop an RNA influenza vaccine since 2018.[40]

BioNTech has developed multiple formats and delivery formulations for its mRNA SARS-CoV-2 vaccine platform. Some of them are utilized in *Project Lightspeed*,[41] an initiative to jointly develop and test multiple COVID-19 vaccine candidates as part of a global development program.[42] BioNTech has developed and tested a total of four SARS-CoV-2 vaccine candidates, all of them using the LNP delivery formulation, and three of the mRNA formats (uRNA, modRNA, and saRNA). Two of the four vaccine candidates include a nucleoside modified mRNA (modRNA), one includes a uridine containing

mRNA (uRNA), and the fourth vaccine candidate utilizes self-amplifying mRNA (saRNA). The longer spike sequence is included in two of the vaccine candidates, and the smaller, optimized receptor binding domain (RBD) of the spike protein is included in the other two candidates. The RBD-based candidates contain the piece of the spike that is thought to be the most important for eliciting Abs that can inactivate the virus.[41]

In April, BioNTech and Pfizer received authorization from the German regulatory authority, the Paul-Ehrlich-Institut, to initiate phase 1/2 of the clinical trial for the BNT162 vaccine candidate. The companies concurrently began two phase 1/2 umbrella trials: one with the candidate BNT162b1 in Germany[43, 44] and another one with candidates BNT162b1 and BNT162b2 in the US.[44, 45] BNT162b1 encodes the SARS-CoV-2 RBD trimerized by adding a domain of T4 fibritin (foldon) to increase its immunogenicity through multivalent display. BNT162b2 encodes the SARS-CoV-2 full-length spike, modified by two proline mutations (optimized 2-proline (2P)-mutated SARS-CoV-2 full-length S glycoprotein) to lock it in the prefusion conformation and mimic the intact virus with which the elicited virus-NAbs must interact.[46, 47]

Although different dose schemes were tested in the trials, all doses of BNT162b1 elicited an RBD-specific IgG response in the range of SARS-CoV-2 convalescent plasma within 21 days of the initial vaccination, with a detectable increase after the boost.[44,48] Additionally, both vaccine candidates elicited S-binding IgG Abs at comparable levels after the second immunization[45] and NAb values above the baseline. These NAbs were measured in vitro using a neutralization assay with a modified SARS-CoV-2 reporter virus, and they were detected only after the second immunization.[44,45,48] Although these data indicate that SARS-CoV-2 mRNA vaccines are effective when inducing SARS-CoV-2 IgG responses, a second dose of either mRNA vaccine formulation seems to be required to reach significant levels of NAbs. When evaluated in elderly subjects, both BNT162b1 and BNT162b2 induced antigen-specific IgG titers after the first vaccination, which were enhanced by a second immunization. The elderly population also needed a booster immunization to induce NAb production. Notably, NAb titers were overall lower in the elderly when compared to younger subjects.[45] It was observed that BNT162b2 elicited robust and durable CD4+ and CD8+ T cell responses in most of the trial subjects. The SARS-CoV-2-specific total CD4+ T cells promoted by BNT162b1 were polarized toward a Th1 functional profile, as measured by the frequency of SARS-CoV-2-specific CD4+ T cells producing IFN-γ and IL-2 but not IL-4, upon stimulation with SARS-CoV-2 peptides.[44,48]

BNT162b1 and BNT162b2 also showed to be safe when injected to adults. In the safety evaluation of these two candidates, pain and tenderness were reported as the most common adverse events. Fever, fatigue, and chills were the most frequently reported systemic adverse events. Reactogenicity was dose-dependent and more pronounced after the boost dose. It is important to mention that BNT162b2 induced less adverse events than BNT162b1, particularly in participants aged 65–85 years. After analyses of data from their phase 1/2 trials in Germany and the U.S., BioNTech and its collaborators selected BNT162b2 for use in their subsequent phase 3 studies.[44] The D614G mutation was included in BNT162b2 in phase 1/2 trials since this is the most commonly observed SARS-CoV-2 S variant in mutational analyses reported in the literature.[49] As of December 30, 2020, BNT162b2 had been authorized for emergency use in the United States. As such, mRNA vaccine administration to the public has commenced. Along with this decision also comes the issue of confounding the continuation of phase 3 studies. The individuals originally in the placebo control groups will also be vaccinated, rendering the long-term double-blinded study of SARS-CoV-2 vaccine-induced immunity impossible.

BioNTech/Pfizer have recently published data of the ongoing clinical phase 3 of BNT162b2.[50] In about 44,000 participants enrolled in the study, the two-dose immunization regimen (30-µg doses, 21 days apart) conferred a remarkable 95% protection against COVID-19. Only eight individuals developed COVID-19 while 162 cases were reported in the placebo group. BNT162b2 also proved to be safe: only 27% of the vaccinated individuals and 12% of the placebo group reported adverse events, mostly short-term mild-to-moderate local reactions.

Moderna Therapeutics vaccine mRNA-1273

It has been found that S proteins from members of the coronavirus family undergo a dramatic structural rearrangement to fuse virus and host cell membranes, promoting the delivery of the viral genome into the target cells. It was previously shown that prefusion-stabilized protein immunogens that preserve neutralization-sensitive epitopes are an effective vaccine strategy for enveloped viruses. Two proline substitutions (2P) at the apex of the central helix and heptad repeat 1 were identified and effectively stabilized MERS-CoV and SARS-CoV in the prefusion conformation. Therefore, just 24 hours after the release of the SARS-CoV-2 isolate sequence, 2P mutations were substituted into S positions aa986 and 987 to produce prefusion-stabilized SARS-CoV-2 S (S-2P) protein. Shortly after, Moderna and the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID), within the National Institutes of Health (NIH), initiated the production of mRNA-1273, an LNP–encapsulated mRNA vaccine expressing SARS-CoV-2 S-2P as a transmembrane-anchored protein with the native furin cleavage site (mRNA-1273),[51, 52] In March 2020, the FDA authorized the study of this vaccine candidate to proceed to clinical trials.[53]

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In a phase 1 study of mRNA-1273 conducted at Emory University in Atlanta, 45 participants aged 18–55 years received two immunizations (prime and boost) with 25, 100, or 250 µg of the SARS-CoV-2 mRNA vaccine, 28 days apart. The vaccine achieved NAb induction in all the participants. Two weeks after the second dose, the title of these Abs, detected by enzyme-linked immunosorbent assay (ELISA) for full S- and RBD-specific IgG, was superior to that observed in recovered COVID-19 patients. Antigen-specific IgG titers further increased after the boost viral neutralization was measured by in vitro neutralization assays with a pseudotyped lentivirus and wild-type SARS-CoV-2. NAbs reached levels in the range of convalescent serum only after the second immunization in all vaccine groups. mRNA-1273 potentially induces a durable Ab response, since participants of the 100-µg dosage group were followed up for 119 days after the initial vaccination (90 post-boost), and, despite a slight decrease, NAb levels remained significantly elevated in all participants.[54]

The NIH-led phase 1 study of mRNA-1273 completed the enrollment of the three-dose cohorts mentioned above and expanded to an additional six cohorts: three of older adults (ages 56–70) and three of elderly adults (ages 71 and above). The participants of this study were immunized twice with either 25 or 100 µg mRNA-1273, and a robust binding and neutralizing Ab response was detected after two doses.[55] It was also observed that individuals over 56 years of age developed a higher NAb response after a second immunization in response to the higher dose (100 µg).

In phase 1 trials, mRNA-1273 elicited a measurable SARS-CoV-2-specific total CD4+ T cell response that was strongly biased towards the production of Th1 cytokines, with minimal Th2 cytokine production.[55] By contrast, the SARS-CoV-2-specific CD8+ T cell response was almost undetectable in most vaccinated individuals, even after the boost dose. These results are in line with those obtained after immunizing rhesus macaques with mRNA-1273 during preclinical trials, where no antigen-specific CD8+ T cell response was found, even with doses as high as 100 µg. It is unclear why mRNA-1273 is unable to promote effective CD8+ T cell response in larger animal models and humans, and it is a concern that needs further investigation.[56]

There were no serious adverse events reported during the phase 1 trial of mRNA-1273 that met the criteria to halt the trial. Local pain at the injection site was the most common while systemic events including fever, chills, and headache were registered with increased incidence and severity after the booster immunization and with the higher vaccine doses.[57] The same safety profile was also observed in the elderly.[55] As mentioned before, the mRNA-1273 vaccine did not show evidence of enhanced respiratory disease after infection in the short term.[58]

In October 2020, Moderna completed the enrollment of the phase 3 COVE Study, and in November. mRNA-1273 met its primary efficacy endpoint in the first interim analysis of the phase 3 COVE study. Interestingly, Moderna announced a longer shelf life for mRNA-1273 at refrigerated temperatures in that same month. In December 2020, The FDA authorized the emergency use of mRNA-1273 in individuals aged 18 years or older in the United States. The phase 3 clinical trial for Moderna vaccine mRNA-1273 is currently undergoing.[59] This trial consists of around 30,000 participants whose ages range from 18 to 85 years. All participants received a two-dose injection series of either 100 µg of mRNA-1273 or a saline placebo, separated by 28 days. The results show a 94.1% efficacy rate of mRNA-1273; only 11 COVID-19 cases were reported in the vaccine group versus 185 cases in the placebo group. The safety profile of this vaccine is also very favorable, as there are no safety issues reported.

CureVac Vaccine CVnCoV

CureVac NV (Nasdaq: CVAC) is a company of German origin with experience in the design of mRNA technology. In 2020 it began the process to create a vaccine against COVID-19 based on mRNA. The vaccine candidate called CVnCoV is a complete stabilized pre-fusion S protein-based mRNA, using LNP as a vehicle. Unlike other vaccines, CVnCoV is made exclusively of nucleotides without chemical changes based on its proprietary RNActive ® technology.[19] In October 2020, the company published its results in mice and hamsters;[19] the vaccine presented a strong humoral immune response of IgG1 and IgG2a SARS-COV-2 virus neutralizing Abs. There were IFN-γ +/TNF + CD4 + and CD8 + responses. In December 2020, the data of his study in Rhesus monkeys were released, proving the vaccine induced a robust cellular and humoral immune response, as the animals were protected against infection by SARS-CoV-2. Histological and pathological analysis as well as general evaluations show that the vaccine is safe to use in non-human primates.[19, 60]

In December 2020, the phase 2 b/3 clinical study of the CVnCoV vaccine against COVID-19 in adults began (ClinicalTrials.gov Identifier NCT04652102). This randomized, double-blind clinical trial studies 36,500 participants from various centers around the world, including Mexico. The study is designed to evaluate disease prevention in adults (18 years and older) after the application of the vaccine (12 ug) or placebo in the deltoid area on days 1 and 29 (two inoculations). The clinical trial is in progress and the first results were expected in May 2021. One of the benefits of the CVnCoV vaccine is that it can be stored for at least 3 months at temperatures between 2 and 8°C. CureVac has generated various collaborations with technology companies as Tesla and renowned pharmaceutical companies such as Bayer, Celonic, GSK, and Novartis. It aims to

ensure the large-scale production of the vaccine and generate new vaccines against various variants of the coronavirus that may be detected.

Other mRNA vaccines

There are several developments of mRNA-based vaccines from various institutions around the world; among the projects with the greatest advances are: i) self-replicating mRNA vaccine by Arcturus Therapeutics in association with the Duke-NUS School of Medicine in Singapore, and ii) the Chinese ARCoV vaccine from the Academy of Military Medical Sciences, Suzhou Abogen Biosciences and Walvax Biotechnology. Assays in mice have shown the first induces immune response and protection against SARS-CoV-2 infection. Preliminary results obtained in the clinical trial phase 1/2 showed efficacy and safety, so that a clinical trial phase 2 began in Singapore and US.[2] ARCoV presented protective effects and safety in phase 1, and phase 2 of this vaccine is currently ongoing.[2,66] There are various developments of new vaccines against COVID-19 in countries such as Japan, India, South Korea, Italy, France, and several others. Still, most of these mRNA vaccine projects are in phase 1 or 1/2 of study and, although promising, they will likely have solid results until 2022 or later (Table 1).[2,66]

DISCUSSION AND CONCLUSION

mRNA vaccines have proven their effectiveness and safety in real life. In the months after their emergency authorization by the different regulatory entities, millions of doses of Pfizer-BioNTech and Moderna vaccines have been administered in 83 and 35 countries, respectively.[61] In all studies and statistics, the protection and safety levels shown in clinical trials are favorable. Other mRNA vaccines against COVID-19 are expected to enter the market and be used massively around the world in the coming months.[2, 61] Among the concerns that still exist regarding the use of this type of vaccine, perhaps the most important is its long-term effects. Although everything indicates that it is capable of generating a protective and lasting immune response, we have yet to learn how this immune response behaves over long periods of time.

The pandemic has forced us to increase our knowledge of mRNA vaccines at an extraordinary high rate as technical, development, production, and distribution capabilities are expanded. All these advances ensure that mRNA vaccines will be one of the most widely used strategies in the control of infectious diseases in the coming years. mRNA is a safe and effective method of vaccination and offers a solution to counter the threat of emerging infectious diseases. mRNA vaccines can direct the expression of virtually any membrane-bound, soluble, or polyprotein antigens, mimicking antigen expression during natural infection. Since their effect is only transient, they are highly useful in the development of prophylactic and therapeutic alternatives. In addition, this technology potentially improves morbidity and mortality rates.[62]

mRNA vaccines have been rigorously assessed for safety, and clinical trials have shown that they provide a long-lasting immune response. They are safer for the patient since they are not produced using infectious elements as pathogen particles or inactivated pathogens. Moreover, mRNA does not integrate itself into the host genome and the RNA strand in the vaccine is degraded once the protein is made.[3] Early clinical trial results indicate that these vaccines generate a reliable immune response and are well tolerated by the vast majority of the population, with few side effects.[3]

In recent years, multiple mRNA vaccine platforms have been developed and validated in studies of immunogenicity and efficacy against infectious diseases and several types of cancer, in animal models and humans. mRNA vaccines have elicited a potent immunity against infectious diseases like influenza virus and ZIKV. In a phase 1 randomized clinical trial for mRNA vaccines against H10N8 and H7N9 influenza viruses, favorable safety and reactogenicity profiles were observed, and no serious adverse events related to the vaccine were reported.[63] Additionally, a modified mRNA vaccine protected against ZIKV and diminished the production of Abs enhancing DENV infection in cells or mice.[64] These advances have demonstrated the potential of mRNA-based vaccines. To date, clinical trials of COVID-19 mRNA-based vaccines have shown that the safety profile and efficacy rate are very favorable.[45]

mRNA vaccines can be created quickly and produced at a large scale, which reduces their cost in the long run. In addition, they are produced in a laboratory and have the potential for rapid, high-volume manufacturing with the precision and flexibility of antigen design necessary to provide both timely and effective responses to large outbreaks and epidemics. They also offer a more flexible stockpiling approach. Low-volume libraries of frozen plasmid and/or unformulated mRNA can be potentially stored for decades and then rapidly formulated and distributed as threat levels rise.[65]

Despite all the advantages of mRNA-based vaccines, there are technical challenges to overcome, the most important being storage as they need to be frozen or refrigerated due to the thermolability of the RNA. Work is ongoing to reliably produce vaccines that can be stored outside the cold chain, which will be much more suitable for use in countries with limited or no refrigeration facilities.

The global effort to create mRNA-based vaccines against COVID-19 has greatly advanced mRNA technology and increased the speed of mRNA vaccine development. This will

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Table 1. mRNA vaccines against SARS-CoV-2.

Vaccine	Manufacturer	Efficacy	Dose	Administration	Storage	Phase
Comirnaty (tozinameran or BNT162b2)	Pfizer-BioNtech and Fosun Pharma	91.30%	2 doses, 3 weeks apart	Intramuscular	Freezer storage only from –25°C to –15°C	Emergency use/Phase 4
mRNA-1273	Moderna and National Institute of Allergy and Infectious Diseases (NIAID)	90%	2 doses, 4 weeks apart	Intramuscular	30 days under refrigeration, 6 months at –20°C	Emergency use/Phase 4
CVnCoV	CureVac	Unknown	2 doses, 4 weeks apart	Intramuscular	Stable at least 3 months at 2–8°C	Phase 3
ARCT-021	Arcturus Therapeutics and the Duke-NUS School of Medicine in Singapore	Unknown	Unknown	Intramuscular	Unknown	Phase 2
ARCoV	Academy of Military Science (AMS), Suzhou Abogen Biosciences and Walvax Biotechnology	Unknown	2 doses, 2 or 4 weeks apart	Intramuscular	Unknown	Phase 3
LNP-nCoVsaRNA	Imperial College London	Unknown	2 doses	Intramuscular	Unknown	Phase 1
ChulaCov19	Chulalongkorn University	Unknown	2 doses	Intramuscular	Unknown	Phase 1
PTX-COVID19-B	Providence Therapeutics	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
CoV2 SAM (LNP)	GlaxoSmithKline	Unknown	2 doses, 30 days apart	Intramuscular	Unknown	Phase 1
mRNA-1273.351	Moderna and National Institute of Allergy and Infectious Diseases (NIAID)	Unknown	3 doses, 28 or 56 days apart	Intramuscular	Unknown	Phase 2
MRT5500	Sanofi Pasteur and Translate Bio	Unknown	2 doses, 3 weeks apart	Intramuscular	Unknown	Phase 1/2
DS-5670a	Daiichi Sankyo	Unknown	2 doses	Intramuscular	Unknown	Phase 1/2
HDT-301	SENAI CIMATEC	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
mRNA-1283	Moderna	Unknown	2 doses, 4 weeks apart	Intramuscular	Unknown	Phase 1
EXG-5003	Elixirgen Therapeutics	Unknown	1 dose	Intradermal	Unknown	Phase 1/2
mRNA COVID-19 vaccine	Shanghai East Hospital and Stemirna Therapeutics	Unknown	Unknown	Intramuscular	Unknown	Phase 1

likely benefit other health care areas, particularly oncology. There have been major efforts for several years to use mRNA technology to fight cancer, with promising results. In this way, mRNA vaccines become a new paradigm not only in vaccination but in health in general.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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