Journal of Research in Pharmacy

Review Article

www.jrespharm.com

Review: mRNA delivery systems in vaccinations and COVID-19

Nada WALWEEL[1,2], Aybuke Ulku KUTLU[1,2], Ummugulsum YILMAZ[1,2], Zakarya AL-SHAEBI[1,2], Jalil CHARMI[3], Omer AYDIN [1,2,3,4] *

1 Department of Biomedical Engineering, Erciyes University, 38039, Kayseri, Turkey.
2 NanoThera Lab, ERFARMA-Drug Application and Research Center, Erciyes University, 38280, Kayseri, Turkey.
3 ERNAM-Nanotechnology Research and Application Center, Erciyes University, 38039, Kayseri, Turkey.
4 ERKAM-Clinical Engineering Research and Implementation Center, Erciyes University, Kayseri 38030, Turkey.
* Corresponding Author. E-mail: biomer@umich.edu; omeraydin@erciyes.edu.tr (O.A.); Tel. +90-352-207-6666 / Ext: 32984.

Received: 19 March 2022 / Revised: 05 May 2022 / Accepted: 05 May 2022

ABSTRACT: mRNA vaccines open promising avenues for overcoming a variety of diseases due to their high therapeutic utilities, rapid growth capacities, and safe administration potentials. With the emergence of COVID-19, the use of mRNA vaccines has become even more widespread and far-reaching. However, for mRNA to be delivered to target cells and tissues, several obstacles must be overcome. For instance, naked mRNAs get easily and hastily degraded by ribonucleases in tissues and the bloodstream, and since mRNAs are large and polyanionic molecules, they cannot passively diffuse across cell membranes. Even though mRNAs are internalized by APCs, they must be able to reach the cytoplasm and escape endo-lysosomal traffic. Therefore, distinctive transport systems for efficient encapsulation of mRNAs using nanocarrier systems are required to ensure their delivery to cells' cytoplasm. At this point, non-viral gene delivery systems such as polymers and lipids come to the fore, in which they can overcome the biological barriers and provide efficient delivery of mRNAs. Recently, mRNA vaccines have been used as a powerful weapon against COVID-19 pandemic which has affected the whole world since December 2019. This was clear by the emergence of Pfizer-BioNTech and Moderna vaccines, which offered mRNA vaccines with auspicious treatment abilities. A variety of carrying candidates have been utilized in such vaccines as polymers, metal nanoparticles, as well as LNPs, which each comes with its cons and pros in delivering mRNA. All of these mentioned points will be clarified and discussed in detail in this review paper.

KEYWORDS: SARS-CoV-2; COVID-19 vaccines; nanodelivery systems; mRNA delivery.

1. INTRODUCTION

1.1. Overview of Vaccines

Vaccines are designed to elicit a strong immune response to an antigen and give long-term protection against a specific disease. In 1796, the first vaccine against cowpox emerged as a first trial by Edward Jenner [1]. Since then, vaccination has been regarded as an effective and safe technique for the protection of infectious diseases, with more than thirty infectious diseases being conserved [2–4]. Each vaccine platform has its own set of benefits and drawbacks. In general, speed and flexibility of manufacture, safety, and reactogenicity, the profile of humoral and cellular immunogenicity, duration of immunity, scale and cost of manufacturing, vaccine stability, and cold chain needs are all important considerations when developing vaccines [5].

The terms antigen (Ag), adjuvant, and carrier system are all intertwined in the term vaccine. Antigen is a foreign molecule that activates the immune system when it binds to host cells. Due to the insufficient immune response provided when merely using the antigen in the vaccination, the adjuvant is a chemical that improves the body's immune reaction to that antigen in vaccines. The carrier system is a necessary component of vaccine production for the vaccine to reach the target cells and pass through the various bio-barriers, as depicted in **Figure 1**.

How to cite this article: Walweel N, Kutlu A.U, Yilmaz U, Al-Shaebi Z, Charmi J, Aydin O. mRNA delivery systems in vaccinations and COVID-19. J Res Pharm. 2022; 25(6): 1084-1101.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.205

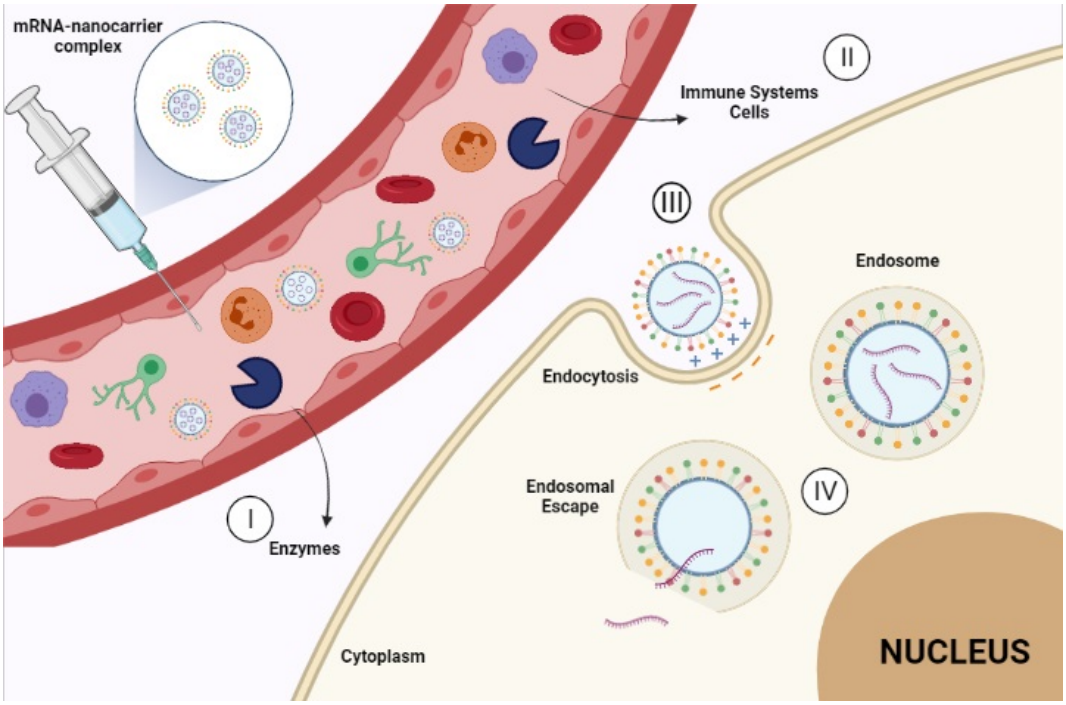

Figure 1. Intracellular barriers for mRNA delivery: (I) The sensitive structure of mRNA makes it vulnerable to disruption by the body enzymes. (II) mRNA could be perceived as a foreign substance by immune cells and consequently promote undesired immune responses. (III) mRNA entrance into cells could not happen passively, since the cell membrane also possesses a negative charge. (IV) mRNA endosomal escape should be facilitated to prevent its degradation by the enzymes in the endocytosis pathway. (This figure was created using BioRender)

Viral vectors, proteinaceous NPs, and synthetic NPs such as liposomes [6, 7], gold NPs [8, 9], polymers [10, 11], dendrimers [12], and LNPs [13, 14] have been employed as carriers in this line, as illustrated in **Figure 2**.

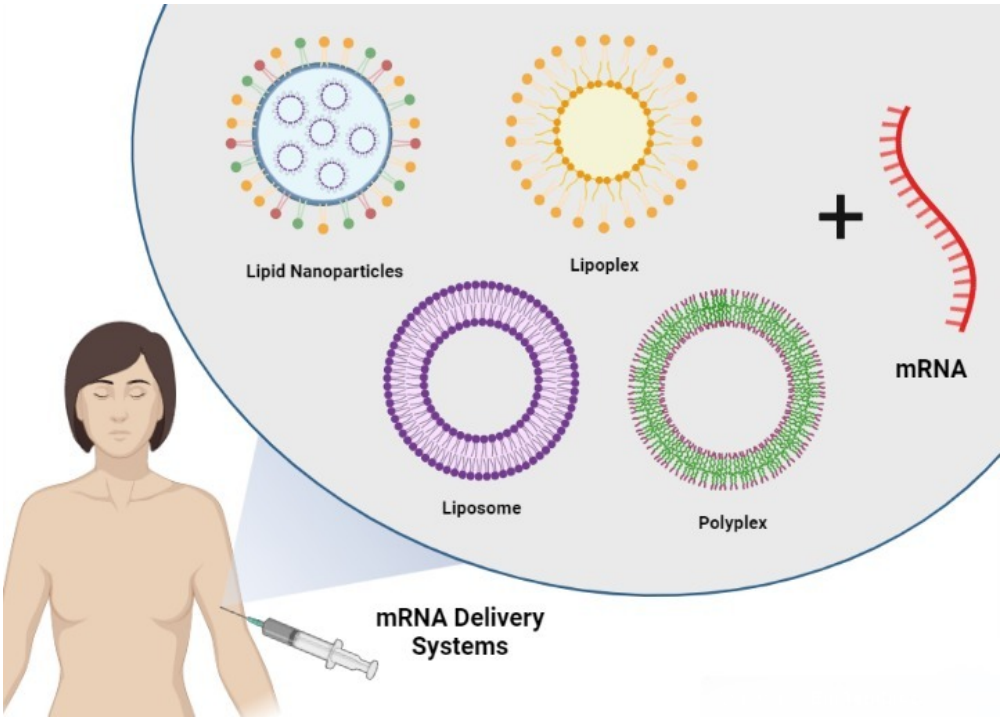

Figure 2. The most used mRNA delivery systems. (This figure was created using BioRender)

1.1.1. Inactivated vaccines and live-attenuated vaccines

The most widely used standard platforms have been inactivated and live-attenuated vaccines, which are obtained by killing or lowering the virus's danger site, allowing them to lose pathogenicity while retaining antigenicity. These vaccines do not require nanocarrier systems to achieve an effective vaccination because they have unique structures that allow them to accumulate into desired organs. However, live-attenuated vaccines have a distinct odor of danger due to their viral structures, whereas inactivated vaccines frequently require at least two vaccinations with fairly large dose amounts to manifest their effects [15].

1.1.2. Viral vector vaccines

Viral vectors refer to the employment of a full virus or bacterium to strengthen the immune system. The viral gene of interest (GOI) is encoded into a variety of vectors, the most common of which are adenovirus (Ad) and vesicular stomatitis virus (VSV) [5]. Both of these viral vectors are frequently used due to their remarkable capabilities, including innate adjuvant qualities and scalability, high transduction efficiency, high transgenic expression, and a wide range of virus tropism [5, 16]. Furthermore, professional antigen-presenting cells (APCs), particularly dendritic cells (DCs), have been reported to be infected by these viruses [17]. However, these vectors have downsides, such as the necessity for more difficult production techniques, the risk of genomic integration, and the possibility of pre-existing vector immunity dampening their response [18].

Recently, Janssen Pharmaceuticals developed a vaccine for coronavirus disease of 2019 (COVID-19), which has been previously used to prevent Ebola virus infection [19]. Noticing that the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) have both recommended the use of the Janssen vaccine in a short period [20]. In a similar vein, the University of Oxford created an adenovirus vector vaccine against COVID-19 called AstraZeneca, which has also been authorized [5, 21].

1.1.3. *Protein subunit vaccines*

Protein subunit is based on the synthesis of at least one chain of peptides or recombinant antigenic proteins that have already been made in heterologous host cells [19, 22]. When compared to other types of vaccines, this one appears to be the safest because no infectious agent is utilized in the manufacturing process [23]. However, because this form of vaccination has low immunogenicity relative to other varieties, it frequently requires the addition of an adjuvant to boost its immunogenicity [24].

Regarding COVID-19, the candidate subunit NVX-CoV2373 vaccine, which includes full-length SARS-CoV-2 spike glycoproteins and Matrix-M1 adjuvant, is both safe and capable of eliciting immune responses that are higher than those seen in COVID-19 convalescent serum [25]. This vaccine is currently in phase III after generating positive results in the previous phases.

1.1.4. *Nucleic acids vaccines*

Nucleic acid-based vaccines have been introduced as promising alternatives to elicit satisfying and pleasing immune responses. Nucleic acid-based vaccines depend on the use of a section of the genetic material that provides the instructions for specific proteins, instead of using the whole microbe or virus [26].

Although messenger ribonucleic acid (mRNA) was discovered in 1961, it was first used in gene-based vaccination studies in 1990 [27]. In mRNA vaccines, the cargo (mRNA) gets translated into the infected cells, resulting in the production of virus protein. Then, the produced protein inflows into intercellular and bloody fluids where it gets identified as an external antigen and thereby stimulates the immune system, especially dendrimer cells, to make antibodies against the specific protein. Although the stability of mRNA is minimum comparing it with DNA molecules [27], mRNA- based vaccines have gained considerable attention over the past decade, especially with the emergence of COVID-19 outbreak.

DNA-based vaccines must penetrate the cell nucleus to get the carried gene translated into a virus protein, which is seen to be problematic since reaching the nucleus is not an easy process. While using mRNA vaccines, entering the cell cytosol would be enough to produce the desired antigen.

When comparing these vaccines with protein subunit vaccines, their advantages become so evident. As there are many problems with making and transmitting antigens to the body, such as the need to obtain highly precise structures to be used in protein vaccines. During antigen production in the cell, changes occur after the mRNA translation process that leads to producing the spatial structure required to accurately identify and provide sufficient immunity for vaccine efficacy. In protein subunit vaccines, one of the problems faced is the exact creation of these spatial structures in large quantities by the cells in the laboratory. While in mRNA vaccines case, the cells of the body act as the best antigen producer (preserving the most accurate spatial structures with sufficient quantities) [28]. Besides, many other features of mRNA vaccines made their use preferable comparing them with the other types of vaccines, such as the possibility of scalable production within a very short time, the rapid manufacturing for clinical-scale [29], and sensible storing temperatures. For example, Moderna COVID-19 vaccine (mRNA-1273) can be stored at -20 C for up to 6 months. Moreover, mRNA is considered to be non-infectious since it does not get integrated into the genome itself. It has also been manifested that the production rate of mRNA vaccines is much faster than the other pre-mentioned conventional vaccines. Nevertheless, mRNA vaccines show a stronger immune response when compared with the other vaccine types [30]. Since living cells are not involved in mRNA vaccines, their ease of production allows scientists to produce specific mRNAs for targeted areas, which made them seen as promising tools for treating a variety of diseases besides COVID-19.

The developed mRNA vaccines against many viral pathogens have been proven to be efficacious in preclinical studies, such as in influenza, Zika virus, Rabies virus, Ebola virus, human immunodeficiency virus (HIV), and recently COVID-19 [31]. The studies and trials on utilizing mRNA in producing vaccines have been started in the early 1990s. For example, Martinon et al. [32] have demonstrated that liposomes carrying mRNA encoding influenza virus nucleoprotein produced virus-specific cytotoxic T lymphocytes and pioneered the use of these mRNAs to deliver antigenic information enabling the synthesis of the protein of interest. In another study [33], it was revealed that mRNA application encoding vasopressin in the hypothalamus can create a physiological response in rats. Currently, researchers are working on developing three different mRNA vaccines that are aimed to be used for the treatment of different diseases such as flu vaccines, malaria vaccines, and cancer vaccines. It seems that the use of nanoparticle-based flu vaccines will be soon realized, especially with the tremendous efforts of Moderna, wherein many influenza vaccine development programs

are being run such as flu vaccine (mRNA-1010, mRNA-1020, mRNA-1030), HIV vaccine (mRNA-1644 & mRNA-1574) and Nipah virus (NiV) Vaccine (mRNA-1215).

Even though mRNA-based vaccines are among the most effective vaccines, certain limitations and constraints stand in the way of their widespread use. The large size of mRNA and its high negative charge makes the mRNA-based vaccines suffer from low penetration into cell membranes. Besides that, it should be noted that the in vitro half-life of mRNA could reach up to 50 hours, whereas its in vivo half-life is pretty much lesser; ranging from 7-30 hours [34, 35]. Nevertheless, mRNA-based vaccines could stimulate potent pathogen-specific humoral and cell-mediated immune responses. For instance, retinoic acid-like receptors (RLRs) and complication receptors cause innate immunogenicity [36].

In the next section, the main delivery systems that have been developed to overcome the mRNA-based vaccines challenges including mRNA's extremely large size, charge, instability and high susceptibility to enzymatic degradation are going to be clarified.

2. mRNA DELIVERY SYSTEMS

mRNAs are single-stranded nucleic acids that are made up of a few hundred to thousands of nucleotides (300 Da-5000 kDa) [34, 37]. Besides their variation in size, they also vary dramatically in structure with high susceptibility to nuclease degradation [37]. Currently, mRNAs have garnered tremendous attention as therapeutic agents and drug classes for vaccination [26], protein replacement therapy [38], cancer immunotherapy [39], and treatment of genetic diseases [29]. However, in order to achieve these therapeutic targets, mRNAs should be delivered into cells safely and effectively. Naked mRNAs degrade rapidly in the circulation [40] and their large macromolecular sizes and high negative charges limit their penetration across the cell membrane. Nevertheless, they possess a high affinity for promoting undesired immune responses [38]. For this purpose, a variety of viral-based delivery systems [41, 42] and mechanical-derived methods like electroporation [43–45] have been designed to deliver mRNAs but suffered from a lack of safety and efficiency. Currently, nanoparticle-based delivery systems, mostly consisting of lipid and polymer-based materials, offer promising opportunities to address the many challenges in this field [44, 46, 47].

2.1. Lipid Nanoparticles (LNPs) Delivery Systems

LNPs are the most extensively utilized non-viral gene delivery systems clinically, due to their advantages over other gene delivery systems, such as high gene therapeutic encapsulation capability, transfection efficiency, improved penetration, reduced cytotoxicity, and immunogenicity [48]. Moreover, the ease of large-scale production of lipid nanoparticles, the biocompatible and biodegradable nature of the materials, as well as the possibility of controlled and modified drug release make them preferable [49]. All of which, make them seen as ideal candidates for mRNA delivery.

Ionizable LNPs delivery systems; which had recently got the FDA approval of Alnylam's Onpattro [30], are comprised of multiple lipid components: Ionizable lipids; PEGylated lipids; helper lipids, and sterol lipids [50, 51]. The ionizable lipid structure is very important and plays a critical role in the delivery system [51]; since it remains neutral at a physiologically pertinent pH but picks up charge in an acidic environment. This property allows electrostatic complex formation with negatively charged mRNAs and most importantly, promotes and facilitates endosomal escape as shown in **Figure 1** [52, 53]. Due to the ionizable lipid nanoparticle's pH buffering capacity, the endosomal escape of them is presumably achieved by an effect called proton sponge; in which the acidic endosomal environment leads to the nanoparticle's protonation [54]. This protonation induces an extensive inflow of ions (H^+ and Cl^-) and water into the endosomes that subsequently leads to rupture of the endosomal membrane and the release of them.

Recently, numerous ionizable lipids are available, such as ATX-100, LP-01, OF-02, and MC3; that were used in the first FDA-approved siRNA drug, Patisiran. In addition to the ionizable lipids, Polyethylene Glycol (PEG) acts as a shield for the mRNA-loaded NPs to prevent them from being perceived as foreign substances by the immune system. Which in return extends the life of the NPs in the bloodstream and increases the nanoparticle's probability of reaching the targeted cells. Besides, PEG affects the size of the nanoparticle and prevents nanoparticle agglomeration possibility. Sterol lipids, like cholesterols, are responsible for the stabilization of the lipid bilayer, providing a degree of flexibility to the overall structure and increasing mRNA transport efficiency [55].

Recently, most of the studies focus on the use of LNPs in delivering siRNA, which makes the possibility of utilizing LNPs in encapsulating and delivering mRNA molecules questionable and doubtful, since the

differences between mRNA and siRNA are quite noticeable. It has been shown that due to the larger size of mRNA comparing it with siRNA, the structures of LNPs tend to be inverted into hexagons, which does not happen in the case of siRNA because of the multidimensional nature of the combination of RNA nucleotide structures with LNPs lipids [55]. In a recent study, many different approaches have been offered to replace some of the compounds in LNPs to solve such a problem and create the required size of mRNA [56]. The effect of these changes on LNPs characteristics such as their size, encapsulation efficiency, transfection, and internalization could be determined by placing cholesterol with β-sitosterol in the structure of LNPs. Upon further examination of Sito LNPs with cryo-TEM microscopy, researchers found that Sito LNPs had a faceted surface, compared to spherical curved Chol LNPs, which is possibly due to lipid phase separation and potentially the formation of two-dimensional lipid crystals in the LNPs membrane [56].

The advent and emergence of LNPs as delivery vehicles was a milestone in the development of mRNA vaccines because LNPs can efficiently transport mRNA both in vitro and in vivo. When injected intramuscularly, LNPs protect the mRNA intact and allow information contained in the mRNA chain to reach the cells [57]. mRNA-LNPs can be internalized and rapidly translated by APCs both at the injection site and in draining lymph nodes, enhancing the elicitation of adaptive immune responses [58]. Furthermore, LNPs have proven their ability in protecting mRNA from being degraded by nucleases [57].

Many studies have been run using LNPs as efficient carriers in delivering mRNA. For instance, Yang et al. [59] have developed nanoparticles containing ionizable lipid iBL0713 to express the desired protein in vitro and in vivo using codon-optimized mRNAs. After preparing mRNAs encoding luciferase and erythropoietin (EPO) by in vitro transcription, a PEG-coated ionizable LNP was formed. Considering the nanoparticles carrying the codon-optimized mRNA, the expression efficiency was higher than that of the non-optimized mRNA, with no toxicity observed in these constructs. At the same time, strong protein expression was obtained in vitro and in vivo without adverse effects. Oberli et al. [53] have designed an mRNA-loaded nanoparticle that can generate a strong cytotoxic T cell response, thus developing immune therapy against certain viral diseases and tumors. In this study, a lipid nanocarrier library was designed to circumvent the challenges that mRNA faces in reaching hepatocytes. These NPs have been designed in different shapes and sizes (about 50-150 nm). Among a variety of used ionizable lipids, phospholipids, cholesterols, and PEGylated lipids libraries, the most efficient lipid has been selected. Indeed, the selected lipid nanoparticle formulation had the ability to induce cytotoxic T cell response which could represent a really promising candidate for mRNA vaccines. In another study, Billingsley et al. [60] have reported a set of ionizable LNPs that are capable of achieving ex vivo mRNA delivery to human T cells in order to induce CAR expression in them. These CAR T cells recognize specific antigens on cells to be destroyed and are therefore considered a potent immunotherapeutic tool. In this study, a library of 24 ionizable lipids with a specific ratio of cholesterol, dioleoylphosphatidylethanolamine (DOPE), and lipid-anchored PEG (C14- PEG) was synthesized and then mixed with mRNA to form different LNP formulations. Seven LNP formulations were found to enhance the transfer of mRNA compared to lipofectamine, as well as a top LNP formulation (C14-4) characterized by strong transfection and low cytotoxicity. C14-4 LNPs were then selected for CAR mRNA transport into primary human T cells which induced CAR expression at levels equivalent to electroporation (EP), the current standard for mRNA transport CAR T cells, with much less cytotoxicity. Accordingly, C14-4 has been shown to be able to deliver CAR mRNA to primary T cells and generate functional CAR T cells.

Generally, in designing each delivery system, some points need to be considered, such as the size of the NPs. In general, the NPs size should be around 100 nm to be able to escape from the mononuclear phagocytic system [61]; which causes the accumulation of NPs in specific organs such as the spleen and liver [35].

Several studies have investigated the effect of varying the size of LNPs by changing the molar fraction of PEG lipids to increase the efficiency of delivery [62, 63]. For example, Arteta et al. [63] have examined the transfection efficiency of mRNA LNPs as a function of LNP size. This study investigated the uptake and protein expression of LNPs in human adipocytes and hepatocytes cell lines when administered subcutaneously and intravenously respectively. In this study, the size of the particles was controlled by varying the lipid content of PEG with smaller LNPs being produced when the amount of PEGylated lipid was high. DLin-MC3-DMA was used as the cationic lipid, while di-stearoyl phosphatidylcholine (DSPC) and cholesterol were used as helper lipids for mRNA transport. The size of mRNA LNPs was varied between ~45 and 135 nm by changing the dimyristoyl phosphatidylethanolamine-n-(methoxy(polyethylene glycol)-2000) DMPE-PEG2000 content from 3 to 0.25 mol%. It has been shown that the structural differences between the groups have affected the transfection of both cell lines. In a recent study to explore the effect of LNPs size on immunogenicity, LNPs sizes have been changed in a range of (60–200 nm) without altering their lipid composition in order to eliminate the composition effect as a variable [51]. Accordingly, it has been observed

that the small diameter LNPs were substantially less immunogenic in mice. But unfortunately, all particle sizes tested yielded a robust immune response while applied to non-human primates (NHP) [51].

2.2. Polymeric Delivery Systems

Although LNPs represent the foreground of mRNA delivery, they have been seen to be extremely selective to the liver and in a lot of cases needed to be locally administrated to reach specific target tissues [65, 66]. Moreover, both large-scale production and long-term storage stability of LNP systems are somehow questionable as the technology is still considered to be new. Thus, there is an up growing interest in designing other types of delivery systems, while hopefully trying to administer them systemically [67, 68]. In this context, different studies based on the use of polymeric materials have arisen as alternative systems to deliver mRNAs [69, 70]. For example, Juanes et al. [71] recently have presented the ability of modified polyhydrazides containing cationic and six different hydrophobic aldehydes to transport mRNA into Hek293 cells. The results of the study confirmed the potential of these polymers in complexing and releasing mRNA with high specificity and low cytotoxicity, even at low polyhydrazone concentrations. As a result of flow cytometric analysis, transfection efficiency of 42% was observed. In another study, Yang et al. [37] synthesized two series of copolymers for mRNA transport consisting of diethyl aminoethyl methacrylate (DEAEMA) or dimethylamino ethyl methacrylate (DMAEMA). The first series of polymers consisted of a mPEG chain and DMAEMA and a hydrophobic alkyl methacrylate monomer (AMA) with side chains of 2 to 12 carbons in compositions ranging from 20% to 60%. The second copolymer series consisted of DMAEMA or DEAEMA and a butyl methacrylate monomer (BMA) and was maintained at a constant composition (40%) while the total molecular weight of the amphiphilic block was varied. In the study, a high-throughput workflow (HT screening) was developed in a way that allows a variety of polymers and parameters to be tested rapidly, providing insight into structure-activity relationships in the design of carriers to improve the efficacy of RNA-based therapeutics. The HT approach of the study showed that cationic concentrations within the copolymer architecture resulted in increased cytotoxicity. Ulkoski et al. [72] developed a library of multivalent peptide-functionalized bioreducible polymers (MPBPs) as safe and efficient vector systems from a core polymer backbone, drawing inspiration from the architecture of dendrimers and branched polymers to transfer various RNA species, including mRNAs. The polymer scaffold consisted essentially of cystine and L-lysine, with cysteine guaranteeing glutathione-triggered bioreduction and L-lysine providing the points at which the peptide and other branches are placed. Three amino acids were used for the peptides: L-histidine, L-lysine, and L-tryptophan. Then, another group of branches was included: tetraethylene glycol or hydrophobic stearic acid residues to tune the hydrophilicity and hydrophobicity of the vectors. The design demonstrated high delivery efficiency and low cytotoxicity when administering different types of RNAs in a range of cell lines. Jarzębińska et al. [73] have presented a study aimed to develop efficient mRNA carriers that could be utilized in vivo for both aerosol and intravenous administration for the treatment of respiratory diseases and metabolic disorders of the liver. In the study, polyplexes were obtained by grafting oligoalkylamines onto an 8000 Da poly (acrylic acid) (PAA8k) scaffold. When complexed with chemically modified mRNA at a 20 nitrogen/phosphate (N/P) ratio, all the resulted polyplexes formed uniform monodisperse particles with hydrodynamic diameters of 65 to 236 nm and an overall positive surface charge. Polymeric carriers were then examined on murine fibroblasts (NIH 3T3) for their ability to transfect cells using chemically modified mRNA encoding firefly luciferase (mRNA-FLuc) as a reporter. Since the polymeric carrier has been designed for aerosol delivery, human alveolar type II-like cells (A549) have been transfected with polymeric particles, resulting in the highest reporter enzyme levels in the identified PAA8k-treated cells.

3. COVID-19 and mRNA VACCINES

3.1. COVID-19

In late December 2019, a sudden outburst of COVID-19 caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was aroused in Wuhan city (Hubei, China) [74] that has affected tens of millions of people in a worldwide pandemic. According to the World Health Organization (WHO) [75], until writing this paper, about 400 million people have been infected by COVID-19 and resulting in around six million deaths globally. It is doubted that the total number of reported COVID-19 infections is underestimated for there are about 80% of mild or asymptomatic cases go undetected [76]. It has been shown

that only one out of six people who suffer COVID-19 undergoes serious symptoms [77] with a variable infection fatality rate based on age and sex. Consequently, the urgent need for effective, safe, and efficient treatments to broadly and massively control the spread of the pandemic became a must. So far, the most effective and promising therapies that have emerged are antiviral drugs, vaccines, and monoclonal antibodies (mAbs). As an example, Remdesivir has been considered the first antiviral drug allowed for emergency use by the Food and Drug Administration (FDA) for the treatment of COVID-19 [78]. Nevertheless, currently, the main focus is on developing powerful vaccines to restrict the spread of the disease and eliminate the epidemic. Hence, various health organizations have developed a variety of vaccines including viral vector vaccines nucleic acid vaccines, and protein-based vaccines [79]. Among these different types of vaccines, mRNA vaccines were the first to be approved, having received "emergency approval" from the FDA and "conditional approval" from the European Medicines Agency (EMA) [29]. Likewise, on August 23, 2021, the FDA approved "Pfizer-BioNTech" as the first COVID -19 vaccine [80].

3.2. Biological Properties of SARS-CoV-2

The nomenclature of coronavirus came from the Latin word corona, which means "crown". Because these viruses possess a discriminatory morphology of a crown-like structure on their surface [74]. One of the subfamilies of coronaviruses, particularly human beta-group coronaviruses, is the severe acute respiratory syndrome (SARS) coronavirus [81]. SARS-CoV-2 belongs to Nidovirales order, Cornidovirineae suborder and Coronaviridae family which has been seen in subfamilies; Letovirinae (Alphaletovirus) and Orthocoronavirinae (Alphacoronavirus [αCoV], Betacoronavirus [βCoV], Gammacoronavirus [γCoV] and Deltacoronavirus [δCoV] [82]. Coronavirus has been firstly discovered in the 1960s among four different groups of diseases: common cold, gastrointestinal infections symptoms, and respiratory syndromes (OC43, 229E, NL63, and HKU1) [83].

The coronavirus is made up of a single-stranded RNA that possesses a size of 26 to 30 kb in length [84]. In the RNA genome of viruses, both structured and non-structured proteins are coded. The genome of SARS-CoV-2 consists of four main structural proteins; spike (S protein; comprised of S1 and S2 subunits.), membrane (M), envelope (E), and nucleocapsid (N) proteins as shown ın **Figure 3**. [85–87]. The S protein is attached to the viral membrane and forms a crown-like appearance. It plays an important role in mediating viral entry into host cells by binding to the host receptor, angiotensin-converting enzyme 2 (ACE2), via its receptor-binding domain (RBD) at the C terminus of the S1 subunit [88, 89] which subsequently leads to fusion between the viral envelope and the host cell membrane via the S2 subunit [90]. The M protein is a transmembrane glycoprotein type III that forms the virion's most rich structural protein. It mainly participates in the shape of the viral envelope and has an approximate molecular weight of 25-30 kDa [91]. The E protein usually gets expressed within infected cells during the proliferation cycle [85]. The M and E proteins act on each other to produce and release viral particles [81, 92] while N proteins, which are the only proteins that bind to the RNA genome [93], attach the genome to the replication transcription complex which is required for genomic replication.

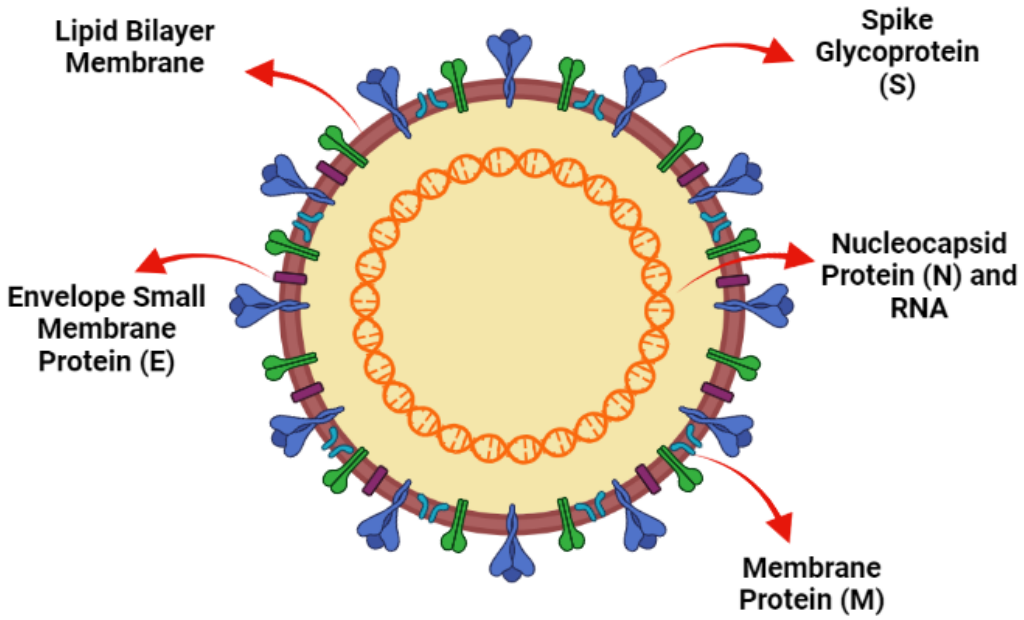

Figure 3. Representation of the structural proteins that make up SARS-CoV-2 virus (This figure was created using BioRender).

The interaction between SARS-CoV-2 and ACE2 is critical in determining the progression of SARS-CoV-2 infection [94], pinpointing that SARS-CoV-2 binds to ACE2 in the amino-terminal region. Key phases of disease progression according to the binding state between SARS-CoV-2 and ACE2 are illustrated in **Figure 4**. In the upper respiratory tract, the initial infection usually results in an asymptomatic state. Whereas the infection in the lower respiratory tract usually leads to pneumonitis (up to 90% of those who have symptoms). Unfortunately, in some cases, a severe state of disease could be reached when a disruption of the epithelial-endothelial barrier and multi-organ gets involved [94].

As demonstrated earlier, the S protein specifically binds to ACE2, which in turn mediates membrane fusion and entry into the cell [95]. Hence, suppressing the activity of the S protein could result in a blockage of the virus activity. Since then, effective strategies have emerged in the field of drug development and treatment aimed at blocking the mechanism by which the virus binds to the ACE2 receptor or blocking viral endocytosis. One of these strategies has been considered in the use of nanoparticles (NPs) for the delivery of chloroquine chemotherapeutic agents as endocytosis inhibitors to cells. Poly (lactic acid) polymeric NPs are commonly used to encapsulate chloroquine, in which the efficacy of the drug depends on how well it is delivered and taken up by the cell [85, 96]. In general, both the RNA and S proteins are the major components of the coronavirus that have been used in the development of various SARS-CoV-2 vaccine classes.

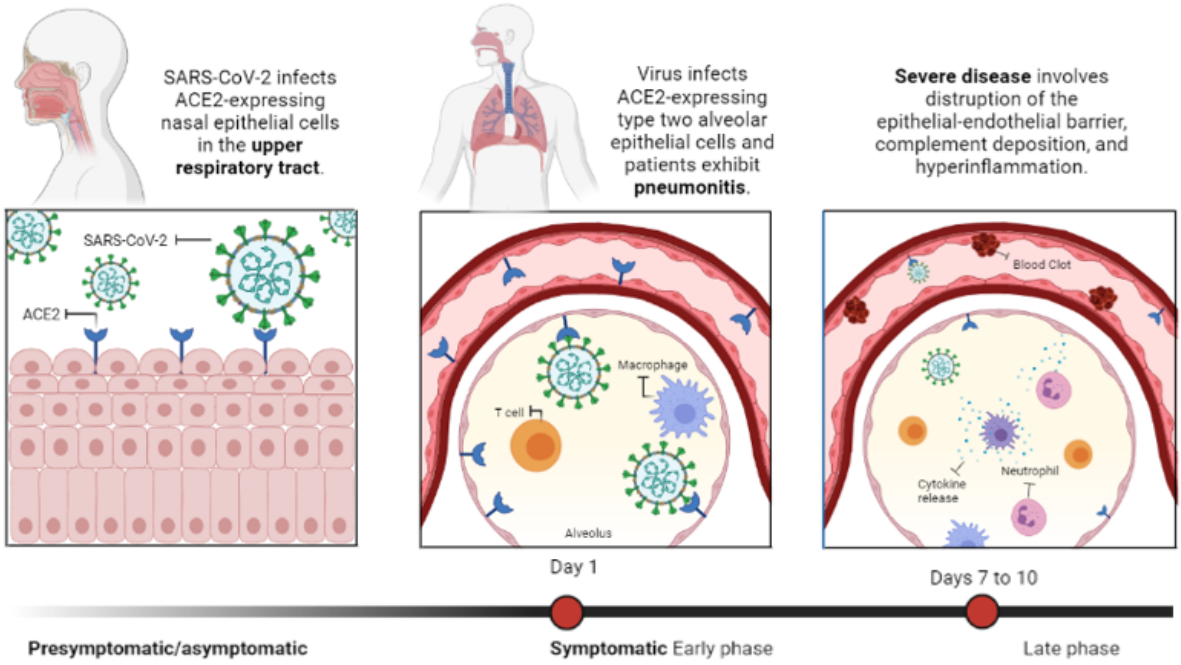

Figure 4. Key phases of disease progression [95]. (This figure was created using BioRender)

3.3. mRNA in COVID-19 Vaccination

As the novel SARS-CoV-2 virus has quickly turned into a pandemic and spread over the world [97], there is a pressing need to develop effective and safe vaccinations to combat it. Accordingly, several studies are taking place in universities, pharmaceutical, and vaccine companies in an attempt to obtain efficient vaccines to escape the pandemic under the continuous pressure of health care and economic crisis. The first clinical trial for developing a vaccine against SARS-CoV-2 began in March 2020 (NCT04283461) [98]. Among all the approaches advanced to develop an effective vaccine against SARS-CoV-2, the mRNA-based vaccine has been considered as an efficient one. As mentioned in earlier sections, using mRNA leads up to no potential risk of infection or genome integration, requires no entry to the nucleus, and can be developed very quickly and easily [99]. For example, Moderna's mRNA-1273 vaccine took only 42 days to enter Phase I clinical trials as the first mRNA vaccine against COVID -19 in the United States [58].

The working principle of the SARS-CoV-2 vaccine is the same as any vaccine. When the body encounters the virus, the immune system can recognize, fight and eliminate the virus by producing antibodies specific to the virus proteins [58]. While utilizing mRNA technology, SARS-CoV-2 proteins could be expressed in the body and accordingly relevant antibodies would be produced as a response.

In addition to a bright 30-year history of using mRNA-based technologies, utilizing mRNA vaccines reached its peak of popularity when used against COVID-19 pandemic which negatively influenced the whole world.

For instance, COVID-19 mRNA vaccine, BNT162b2, which was introduced to the market by Pfizer-BioNTech and was used under the emergency use list by WHO in January 2021, consists of an mRNA sequence encoding a disease-specific spike protein (antigen) [20].

Elia et al. [99] have synthesized several structurally distinct ionizable lipids and studied them for in vivo delivery of mRNA-LNPs in the form of LNPs for vaccine applications. This study demonstrated the development of a specific humoral and cellular response to the RBD as well as the immune system response. The LNPs were prepared by mixing the lipids and mRNA in a microfluidic mixing device. The average size of the LNPs was less than 100 nm in diameter. The results of the study have shown the possible use of the lipids obtained as advantageous candidates for the synthesis and development of COVID -19 vaccines. In this line, Zhang et al. [88] have developed a lipid nanoparticle-encapsulated mRNA (mRNA-LNP) encoding the RBD of SARS-CoV-2, called ARCoV. The synthesized ARCoV particles possessed a homogeneous morphology of solid spheres that lack an aqueous core, which is a major difference between RNA-loaded LNPs and

conventional liposomes and were characterized by a size of 88.85 nm and encapsulation efficiency of more than 95%. Two doses of ARCoV immunizations have been administrated, while the produced neutralizing antibodies have been evaluated in BALB/C mice and cynomolgus monkeys (Macaca fascicularis). The study data from the cynomolgus monkey model showed that 100 µg of ARCoV was sufficient to induce high neutralizing antibodies. Simultaneously, applying 1000 µg of ARCoV to the model resulted in no apparent adverse effects, which highlights the safety profile of the mRNA- LNPs formulation. Moreover, complete protection against SARS-CoV-2 mouse-adapted strain has been seen in mice after ARCoV mRNA-LNPs immunization. Hence, ARCoV is currently being evaluated in phase *1* clinical trials. Nevertheless, ARCoV has been manufactured in liquid formulation and thus could be stored at room temperature for at least one week (Zhang et al. 2020). This feature made ARCoV overcomes one of the most serious problems facing mRNA vaccines; the need for ultracold storage to preserve their entity, as in the storage of mRNA vaccine BNT162b2 which requires -75 ^{o}C for up to 6 months or 2 ^{o}C to 8 ^{o}C for up to 5 days to maintain its potency [58]. However, in this study, the experiments have been only applied to the adaptive mouse strain of SARS-CoV-2, which may be considered a limitation. Furthermore, the duration of neutralizing antibodies induced by ARCoV has not been determined.

Elia et al. have investigated the in vivo efficacy of an LNP-encapsulated human Fc-conjugated SARS-CoV-2 receptor-binding domain (RBD-hFc)-based mRNA vaccine containing an ionizable lipid less than 57 nm in size (RBD-hFc mRNA LNPs) [100]. In this study, the K18-hACE2 mice models have been administered intramuscularly with 5 µg RBD-hFc mRNA LNPs. In the vaccinated group, 70% of animals given a lethal dose of SARS-CoV-2 survived in comparison with the control group (unvaccinated animals). Huang and co-workers [97] have developed a new vaccine based on nucleoside-modified mRNA. The main objective of this study was to exploit the synthesized vaccine to provide long-term protection against SARS-CoV-2 using a single dose of mRNA-RBD. The nucleoside-modified mRNA, after achieving endosomal escape, has been translated into SARS-CoV-2 receptor binding domain RBD. The vaccine has been encapsulated by LNPs and contained the modified nucleoside N1-methylpseudouridine. Based on a ribogreen fluorescence assay, the encapsulation efficacy of mRNA-RBD was greater than 92%. In addition, the distinct response of LNPs in different pH environments helped in making the endosomal escape process easier. The overall size of the encapsulated vaccine was up to 78 nm. The results of the study showed that in applying a single dose of nucleoside-modified mRNA vaccine, high levels of neutralizing antibodies were maintained for at least 6.5 months, which reflects the efficient long-term protection the vaccine offers.

Among the candidate vaccines that emerged recently, a study has been published in April 2021 that intended to develop a vaccine that can get its efficacy in only one dose [101]. The vaccine, which has been named LUNAR-COV19 (ARCT-021 vaccine), targeted the full-length and unmodified SARS-CoV-2 S protein using a self-replicating mechanism. The study demonstrated the high potential of utilizing LUNAR-COV19 as a single-dose vaccine. Indeed, at this time this vaccine is in phase II. [101, 102].

RNActive® technology has developed a vaccine encapsulated with LNP called CVnCoV, which targets the full-length, pre-fusion stabilized spike protein. The uniqueness of this vaccine was manifested in its nucleotides. In which the nucleotides that made the mRNA sequences here are non-chemically modified, which enhances the expression of the protein of interest and decreases the required dose amount [103]. Curevac is another mRNA-based SARS-CoV-2 vaccine that uses nucleotides without any chemical modifications. This vaccine has also been developed to get translated into a full-length spike protein encapsulated with LNP. The vaccine has been applied to various animals and the results have shown that the vaccine elicited the neutralizing antibody titers similarly to the sera taken from patients who recovered from COVID-19 [104].

In an interesting study, Lu et al. [105] developed three candidate mRNA vaccines (RQ3011-RBD, RQ3012-Spike, and RQ3013-VLP) for COVID -19 that can encode different forms of antigens in vaccinated hosts. In which, (I) RQ3011-RBD encodes the receptor-binding domain of the S-glycoprotein (residues 331-524) of SARS-CoV-2. (II) RQ3012 spike encodes the full-length wild-type S. (III) RQ3013-VLP: a cocktail of mRNAs encoding three structural proteins: S, M, and E, to form SARS-CoV-2 virus-like particles (VLPs). As in many studies, LNPs have been utilized to package mRNAs. The efficiency of mRNA encapsulation of all three LNP vaccine candidates was more than 98%, with an average size of 100 nm in diameter. Immunogenicity evaluation in mice showed that mice receiving the RQ3013-VLP exhibited the strongest immune response. Since the RQ3013-VLP candidate contains both M and E mRNA, M- and E-induced protein-specific immunoglobulin G was analyzed in mice receiving the RQ3013-VLP [51]. Results have shown that mice vaccinated with RQ3013-VLP had not induced M- or E-specific antibodies, while the mice received RQ3011-RBD had not shown any detectable neutralizing antibodies (NAbs). However, mice receiving the

RQ3013-VLP had prompted a 2.5-fold NAbs titer higher than mice receiving the RQ3012 spike vaccine. Yet, RQ3011-RBD (2 µg RNA/dose) has not been able to induce efficient immunogenicity in mice. Therefore, possible improvements could enhance the immunity of an RBD-encoding mRNA vaccine. The results suggested that the vaccine contains S in the secreted vesicles, as VLPs induce high immunogenicity. Therefore, these data affirmed the power of using the VLP strategy in the development of mRNA vaccines for COVID-19.

Other approaches have been applied to develop efficient vaccines, called self-amplifying RNA (saRNA). saRNA is a type of mRNA that is resistant to nuclease enzymes and could be translated into any GOI, whereas conventional mRNA-based vaccines encode only the GOI with 5′ and 3′ untranslated regions (UTRs) [106]. In a study done by Gustavo Lou and co-workers [107], an mRNA-based rabies virus glycoprotein (RVG) vaccine using the self-amplifying method has been developed. The authors studied the differences between using cationic LNPs and ionizable LNPs in vaccine delivery. They have illustrated that the cationic LNPs based on the use of different lipids represent an efficient alternative to the ionizable LNPs in delivering self-enhancing vaccines. While both cationic LNPs and ionizable LNPs elicited strong humoral and cellular-mediated immune responses in mice.

Here, it is important to note that the LUNAR-COV19 vaccine trial that was previously stated likewise relied on the utilization of self-transcribing and self-replicating mRNA technology [108]. In another study, McKay et al. [106] presented self-amplifying RNA encoding the SARS-CoV-2 spike protein, encapsulated within an LNP as a vaccine. In mouse sera, the authors discovered remarkably high and dose-dependent SARS-CoV-2 specific antibody titers, as well as robust neutralization of both pseudo-virus and wild-type virus. Additionally, the study compared the immunogenicity of the SARS-CoV-2 saRNA LNP vaccine with the immunological response of natural infection in COVID-19 recovered patients. The findings revealed that not only neutralization was proportional to the quantity of particular IgG but that the quantity produced is also larger than in recovered COVID-19 patients.

4. CONCLUSION

In addition to a bright 30-year history of using mRNA-based technologies, utilizing mRNA vaccines reached its peak of popularity when used against COVID-19 pandemic; which negatively influenced the whole world. Nevertheless, to achieve its targets, some problems need to be seriously taken to outdo, such as mRNA's high affinity in promoting undesired immunogenicity, its large size, high negative charge, and rapid degradation. mRNA delivery systems are the key to overcoming the flaws of mRNA. However, due to the limitations allied to some nanocarriers used in mRNA delivery, their mass production is still forming an obstacle in their employment in pandemic situations. Currently, nanoparticle-based delivery systems, mostly consisting of a lipid and polymer-based materials offer promising opportunities to address the many challenges in this field. The advent and emergence of LNPs as delivery vehicles specifically was a milestone in the development of mRNA vaccines in general and COVID-19 vaccines in particular. On the other hand, although mRNA vaccine studies have had a lot of success, mRNA delivery still requires more investigation.

Overall, mRNA vaccines represent an exciting new avenue for traditional vaccination methods because of their great potential, fast growth capacity, and their possible safe delivery. Therefore, with the current technological advancements, mRNA vaccines have become widely used against infectious diseases headed by COVID-19.

Acknowledgements:

Aybuke Ulku Kutlu was funded by the Council of Higher Education (YOK) 100/2000 Doctorate Scholarship Program and she recognized the support of the 120S938 numbered TUBITAK 1001 grant.

Ummugulsum Yilmaz was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) 2211/A General Domestic Doctorate Scholarship Program and the Council of Higher Education (YOK) 100/2000 Doctorate Scholarship Program.

Nada Walweel and Zakarya Al-Shaebi were funded by Türkiye Scholarships (YTB) Postgraduate Scholarship Program.

Jalil Charmi was funded by International Fellowship for Outstanding Researchers Program (TÜBİTAK 118C346).

Author contributions: The manuscript was written through the contributions of all authors. / All authors have approved the final version of the manuscript.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent). 2005; 18(1): 21-5. [CrossRef]

[2] Younger DS, Younger AP, Guttmacher S. Childhood Vaccination: Implications for Global and Domestic Public Health. Neurol Clin. 2016; 34(4): 1035-47. [CrossRef]

[3] Standaert B, Rappuoli R. Towards a more comprehensive approach for a total economic assessment of vaccines?: 1. The building blocks for a health economic assessment of vaccination. J Mark Access Health Policy. 2017; 5(1): 1335162. [CrossRef]

[4] Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Mol Ther. 2019; 27(4): 757-72. [CrossRef]

[5] Corey L, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. Science. 2020; 368(6494): 948-50. [CrossRef]

[6] Fries LF, Gordon DM, Richards RL, Egan JE, Hollingdale MR, Gross M, et al. Liposomal malaria vaccine in humans: a safe and potent adjuvant strategy. Proc Natl Acad Sci U S A. 1992; 89(1): 358-62. [CrossRef]

[7] Salomon N, Vascotto F, Selmi A, Vormehr M, Quinkhardt J, Bukur T, et al. A liposomal RNA vaccine inducing neoantigen-specific CD4(+) T cells augments the antitumor activity of local radiotherapy in mice. Oncoimmunology. 2020; 9(1): 1771925. [CrossRef]

[8] Chen X, Han W, Wang G, Zhao X. Application prospect of polysaccharides in the development of anti-novel coronavirus drugs and vaccines. Int J Biol Macromol. 2020; 164: 331-43. [CrossRef]

[9] Dykman LA. Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases. Expert Rev Vaccines. 2020; 19(5): 465-77. [CrossRef]

[10] Nevagi RJ, Skwarczynski M, Toth I. Polymers for subunit vaccine delivery. Eur Polym J. 2019; 114: 397-410. [CrossRef]

[11] Lambricht L, Peres C, Florindo H, Préat V, Vandermeulen G. Polymer-based nanoparticles as modern vaccine delivery systems. Micro Nanotechnol Vaccine Dev. 2017: 185-203. [CrossRef]

[12] Ganda IS, Zhong Q, Hali M, Albuquerque RL, Padilha FF, da Rocha SR, et al. Dendrimer-conjugated peptide vaccine enhances clearance of Chlamydia trachomatis genital infection. Int J Pharm. 2017; 527(1-2): 79-91. [CrossRef]

[13] Igyártó BZ, Jacobsen S, Ndeupen S. Future considerations for the mRNA-lipid nanoparticle vaccine platform. Curr Opin Virol. 2021; 48: 65-72. [CrossRef]

[14] Thi TTH, Suys EJ, Lee JS, Nguyen DH, Park KD, Truong NP. Lipid-based nanoparticles in the clinic and clinical trials: from cancer nanomedicine to COVID-19 vaccines. Vaccines. 2021; 9(4): 359. [CrossRef]

[15] Ji Q, Wang S, Ma J, Liu Q. A review: Progress in the development of fish Vibrio spp. vaccines. Immunol Lett. 2020; 226: 46-54. [CrossRef]

[16] Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, et al. COVID-19 vaccine development and a potential nanomaterial path forward. Nat Nanotechnol. 2020; 15(8): 646-55. [CrossRef]

[17] Larocca C, Schlom J. Viral vector–based therapeutic cancer vaccines. Cancer J. 2011; 17(5): 359. [CrossRef]

[18] Li YD, Chi WY, Su JH, Ferrall L, Hung CF, Wu TC. Coronavirus vaccine development: from SARS and MERS to COVID-19. J Biomed Sci. 2020; 27(1): 1-23. [CrossRef]

[19] Wang M, Jiang S, Wang Y. Recent advances in the production of recombinant subunit vaccines in Pichia pastoris. Bioengineered. 2016; 7(3): 155-65. [CrossRef]

[20] Oliver SE, Gargano JW, Scobie H, Wallace M, Hadler SC, Leung J, et al. The advisory committee on immunization practices' interim recommendation for use of Janssen COVID-19 vaccine—United States, February 2021. MMWR Morb Mortal Wkly Rep. 2021; 70(9): 329. [CrossRef]

[21] Wise J. Covid-19: European countries suspend use of Oxford-AstraZeneca vaccine after reports of blood clots. BMJ; 2021. [CrossRef]

[22] Bill RM. Recombinant protein subunit vaccine synthesis in microbes: a role for yeast? J Pharm Pharmacol. 2015; 67(3): 319-28. [CrossRef]

[23] Tan M, Jiang X. Recent advancements in combination subunit vaccine development. Hum Vaccin Immunother. 2017; 13(1): 180-5. [CrossRef]

[24] VaccinesWork, What are protein subunit vaccines and how could they be used against COVID-19? https://www.gavi.org/vaccineswork/what-are-protein-subunit-vaccines-and-how-could-they-be-used-against-covid-19. (accessed April 21, 2022).

[25] Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, et al. Patel 495 N, Frieman MB, Haupt RE, Logue J, McGrath M, Weston S, Piedra PA, Desai C, Callahan K, et al. Phase 1–2 Trial 496 of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. N Engl J Med Overseas Ed. 2020; 497: 2320-32. [CrossRef]

[26] Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and challenges in the delivery of mRNA-based vaccines. Pharmaceutics. 2020; 12(2): 102. [CrossRef]

[27] Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov. 2014; 13(10): 759-80. [CrossRef]

[28] Ho W, Gao M, Li F, Li Z, Zhang XQ, Xu X. Next-Generation Vaccines: Nanoparticle-Mediated DNA and mRNA Delivery. Adv Healthc Mater. 2021; 10(8): 2001812. [CrossRef]

[29] Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. Int J Pharm. 2021; 601: 120586. [CrossRef]

[30] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov. 2018; 17(4): 261-79. [CrossRef]

[31] Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. Front Immunol. 2019: 594. [CrossRef]

[32] Martinon F, Krishnan S, Lenzen G, Magné R, Gomard E, Guillet JG, et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur J Immunol. 1993; 23(7): 1719-22. [CrossRef]

[33] Jirikowski GF, Sanna PP, Maciejewski-Lenoir D, Bloom FE. Reversal of diabetes insipidus in Brattleboro rats: intrahypothalamic injection of vasopressin mRNA. Science. 1992; 255(5047): 996-8. [CrossRef]

[34] Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: advances in technologies for therapeutic mRNA delivery. Mol Ther. 2019; 27(4): 710-28. [CrossRef]

[35] Sharova LV, Sharov AA, Nedorezov T, Piao Y, Shaik N, Ko MS. Database for mRNA half-life of 19 977 genes

obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic stem cells. \Research DNA Res. 2009; 16(1): 45-58. [CrossRef]

[36] Amor S, Fernández Blanco L, Baker D. Innate immunity during SARS-CoV-2: evasion strategies and activation trigger hypoxia and vascular damage.J Clin Exp Immunol. 2020; 202(2): 193-209. [CrossRef]

[37] Yang DC, Eldredge AC, Hickey JC, Muradyan H, Guan Z. Multivalent peptide-functionalized bioreducible polymers for cellular delivery of various RNAs. Biomacromolecules. 2020; 21(4): 1613-24. [CrossRef]

[38] Granot-Matok Y, Kon E, Dammes N, Mechtinger G, Peer D. Therapeutic mRNA delivery to leukocytes. J Control Release. 2019; 305: 165-75. [CrossRef]

[39] Vallazza B, Petri S, Poleganov MA, Eberle F, Kuhn AN, Sahin U. Recombinant messenger RNA technology and its application in cancer immunotherapy, transcript replacement therapies, pluripotent stem cell induction, and beyond. Wiley Wiley Interdiscip Rev RNA. 2015; 6(5): 471-99. [CrossRef]

[40] Shu Y, Pi F, Sharma A, Rajabi M, Haque F, Shu D, et al. Stable RNA nanoparticles as potential new generation drugs for cancer therapy. Adv Drug Deliv Rev. 2014; 66: 74-89. [CrossRef]

[41] Schaffer DV, Koerber JT, Lim K-i. Molecular engineering of viral gene delivery vehicles. Annu Rev Biomed Eng. 2008; 10: 169-94. [CrossRef]

[42] Seow Y, Wood MJ. Biological gene delivery vehicles: beyond viral vectors. Molecular Therapy. 2009; 17(5): 767-77. [CrossRef]

[43] Singh N, Liu X, Hulitt J, Jiang S, June CH, Grupp SA, et al. Nature of tumor control by permanently and transiently modified GD2 chimeric antigen receptor T cells in xenograft models of neuroblastoma. Cancer Immunol Res. 2014; 2(11): 1059-70. [CrossRef]

[44] DiTommaso T, Cole JM, Cassereau L, Buggé JA, Hanson JLS, Bridgen DT, et al. Cell engineering with microfluidic squeezing preserves functionality of primary immune cells in vivo. Proc Natl Acad Sci U S A. 2018; 115(46): E10907-E14. [CrossRef]

[45] Svoboda J, Rheingold SR, Gill SI, Grupp SA, Lacey SF, Kulikovskaya I, et al. Nonviral RNA chimeric antigen receptor–modified T cells in patients with Hodgkin lymphoma. Blood. 2018; 132(10): 1022-6. [CrossRef]

[46] Mukalel AJ, Riley RS, Zhang R, Mitchell MJ. Nanoparticles for nucleic acid delivery: Applications in cancer immunotherapy. Cancer Lett. 2019; 458: 102-12. [CrossRef]

[47] Hajj KA, Whitehead KA. Tools for translation: non-viral materials for therapeutic mRNA delivery. Nat Rev Mater. 2017; 2(10): 1-17. [CrossRef]

[48] Zhao Y, Huang L. Lipid nanoparticles for gene delivery. Adv Genet. 2014; 88: 13-36. [CrossRef]

[49] Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018; 13(4): 288. [CrossRef]

[50] Guevara ML, Persano F, Persano S. Advances in lipid nanoparticles for mRNA-based cancer immunotherapy. Front Chem. 2020: 963. [CrossRef]

[51] Hassett KJ, Higgins J, Woods A, Levy B, Xia Y, Hsiao CJ, et al. Impact of lipid nanoparticle size on mRNA vaccine immunogenicity. J Control Release. 2021; 335: 237-46. [CrossRef]

[52] Fan Y-N, Li M, Luo Y-L, Chen Q, Wang L, Zhang H-B, et al. Cationic lipid-assisted nanoparticles for delivery of mRNA cancer vaccine. Biomater Sci. 2018; 6(11): 3009-18. [CrossRef]

[53] Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, et al. Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy. Nano Lett. 2017; 17(3): 1326-35. [CrossRef]

[54] Bus T, Traeger A, Schubert US. The great escape: how cationic polyplexes overcome the endosomal barrier. J Mater Chem B. 2018; 6(43): 6904-18. [CrossRef]

[55] SG, Dowdy SF. Overcoming delivery barriers with LNPs. Nat Mater. 2021; 20(5): 575-7. [CrossRef]

[56] Eygeris Y, Patel S, Jozic A, Sahay G. Deconvoluting lipid nanoparticle structure for messenger RNA delivery. Nano Lett. 2020; 20(6): 4543-9. [CrossRef]

[57] Thomas SJ, Moreira Jr ED, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. N Engl J Med. 2021; 385(19): 1761-73. [CrossRef]

[58] Bettini E, Locci M. SARS-CoV-2 mRNA vaccines: immunological mechanism and beyond. Vaccines (Basel). 2021; 9(2): 147. [CrossRef]

[59] Yang T, Li C, Wang X, Zhao D, Zhang M, Cao H, et al. Efficient hepatic delivery and protein expression enabled by optimized mRNA and ionizable lipid nanoparticle. Bioact Mater. 2020; 5(4): 1053-61. [CrossRef]

[60] Billingsley MM, Singh N, Ravikumar P, Zhang R, June CH, Mitchell MJ. Ionizable lipid nanoparticle-mediated mRNA delivery for human CAR T cell engineering. Nano Lett. 2020; 20(3): 1578-89. [CrossRef]

[61] Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med. 2016; 375(26): 2561-9. [CrossRef]

[62] Chen S, Tam YYC, Lin PJ, Sung MM, Tam YK, Cullis PR. Influence of particle size on the in vivo potency of lipid nanoparticle formulations of siRNA. J Control Release. 2016; 235: 236-44. [CrossRef]

[63] Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther - Nucleic Acids. 2012; 1: e37. [CrossRef]

[64] Yanez Arteta M, Kjellman T, Bartesaghi S, Wallin S, Wu X, Kvist AJ, et al. Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles. Proc Natl Acad Sci U S A. 2018; 115(15): E3351-E60. [CrossRef]

[65] Bouchkouj N, Kasamon YL, de Claro RA, George B, Lin X, Lee S, et al. FDA approval summary: axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma. Clin Cancer Res. 2019; 25(6): 1702-8. [CrossRef]

[66] Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell Rep. 2018; 22(9): 2227-35. [CrossRef]

[67] Kowalski PS, Capasso Palmiero U, Huang Y, Rudra A, Langer R, Anderson DG. Ionizable amino-polyesters synthesized via ring opening polymerization of tertiary amino-alcohols for tissue selective mRNA delivery. Adv Mater. 2018; 30(34): 1801151. [CrossRef]

[68] Fornaguera C, Guerra-Rebollo M, Angel Lazaro M, Castells-Sala C, Meca-Cortés O, Ramos-Pérez V, et al. mRNA Delivery System for Targeting Antigen-Presenting Cells In Vivo. Adv Heal Mater. 2018; 7(17): 1800335. [CrossRef]

[69] Wahane A, Waghmode A, Kapphahn A, Dhuri K, Gupta A, Bahal R. Role of lipid-based and polymer-based non-viral vectors in nucleic acid delivery for next-generation gene therapy. Molecules. 2020; 25(12): 2866. [CrossRef]

[70] Ulkoski D, Bak A, Wilson JT, Krishnamurthy VR. Recent advances in polymeric materials for the delivery of RNA therapeutics. Expert Opin Drug Deliv. 2019; 16(11): 1149-67. [CrossRef]

[71] Juanes M, Creese O, Fernández-Trillo P, Montenegro J. Messenger RNA delivery by hydrazone-activated polymers. Medchemcomm. 2019; 10(7): 1138-44. [CrossRef]

[72] Ulkoski D, Munson MJ, Jacobson ME, Palmer CR, Carson CS, Sabirsh A, et al. High-throughput automation of endosomolytic polymers for mrna delivery. ACS Appl Bio Mater. 2021; 4(2): 1640-54. [CrossRef]

[73] Jarzębińska A, Pasewald T, Lambrecht J, Mykhaylyk O, Kümmerling L, Beck P, et al. A single methylene group in oligoalkylamine-based cationic polymers and lipids promotes enhanced mRNA delivery. Angew Chem Int Ed Engl. 2016; 55(33): 9591-5. [CrossRef]

[74] Shetti NP, Mishra A, Bukkitgar SD, Basu S, Narang J, Raghava Reddy K, et al. Conventional and nanotechnology-based sensing methods for SARS coronavirus (2019-nCoV). ACS Appl Bio Mater. 2021; 4(2): 1178-90. [CrossRef]

[75] WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/. (accessed March 15, 2022).

[76] Kobayashi T, Jung S-m, Linton NM, Kinoshita R, Hayashi K, Miyama T, et al. Communicating the risk of death from novel coronavirus disease (COVID-19). J Clin Med. 2020; 9(2): 580. [CrossRef]

[77] Anderson RM, Heesterbeek H, Klinkenberg D, Hollingsworth TD. How will country-based mitigation measures influence the course of the COVID-19 epidemic? The Lancet. 2020; 395(10228): 931-4. [CrossRef]

[78] Mei L-C, Jin Y, Wang Z, Hao G-F, Yang G-F. Web resources facilitate drug discovery in treatment of COVID-19. Drug Discov Today. 2021; 26(10): 2358-66. [CrossRef]

[79] Callaway E. The race for coronavirus vaccines: a graphical guide. Nature. 2020; 576-7. [CrossRef]

[80] FDA, FDA Approves First COVID-19 Vaccine. https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine. (accessed November 22, 2021).

[81] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol. 2015; 1282: 1-23. [CrossRef]

[82] Pal M, Berhanu G, Desalegn C, Kandi V. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): an update. Cureus. 2020; 12(3). [CrossRef]

[83] Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 2020; 27(3): 325-8. [CrossRef]

[84] Corman VM, Muth D, Niemeyer D, Drosten C. Hosts and sources of endemic human coronaviruses. Adv Virus Res. 2018; 100: 163-88. [CrossRef]

[85] Rashidzadeh H, Danafar H, Rahimi H, Mozafari F, Salehiabar M, Rahmati MA, et al. Nanotechnology against the novel coronavirus (severe acute respiratory syndrome coronavirus 2): diagnosis, treatment, therapy and future perspectives. Nanomedicine. 2021; 16(6): 497-516. [CrossRef]

[86] Rahimi H, Salehiabar M, Barsbay M, Ghaffarlou M, Kavetskyy T, Sharafi A, et al. CRISPR systems for COVID-19 diagnosis. ACS Sens. 2021; 6(4): 1430-45. [CrossRef]

[87] Su S, Wong G, Shi W, Liu J, Lai AC, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 2016; 24(6): 490-502. [CrossRef]

[88] Zhang N-N, Li X-F, Deng Y-Q, Zhao H, Huang Y-J, Yang G, et al. A thermostable mRNA vaccine against COVID-19. Cell. 2020; 182(5): 1271-83. [CrossRef]

[89] Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013; 503(7477): 535-8. [CrossRef]

[90] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020; 181(2): 271-80. [CrossRef]

[91] Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 2011; 174(1): 11-22. [CrossRef]

[92] Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2019; 16(1): 1-22. [CrossRef]

[93] de Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. Adv Virus Res. 2005; 64: 165-230. [CrossRef]

[94] Matheson NJ, Lehner PJ. How does SARS-CoV-2 cause COVID-19? Science. 2020; 369(6503): 510-1. [CrossRef]

[95] Pillay TS. Gene of the month: the 2019-nCoV/SARS-CoV-2 novel coronavirus spike protein. J Clin Pathol. 2020; 73(7): 366-9. [CrossRef]

[96] Lima TLC, Feitosa RdC, Santos-Silva D, Santos-Silva D, Maria A, Siqueira EMdS, et al. Improving encapsulation of hydrophilic chloroquine diphosphate into biodegradable nanoparticles: a promising approach against herpes virus simplex-1 infection. Pharmaceutics. 2018; 10(4): 255. [CrossRef]

[97] Huang Q, Ji K, Tian S, Wang F, Huang B, Tong Z, et al. A single-dose mRNA vaccine provides a long-term protection for hACE2 transgenic mice from SARS-CoV-2. Nat Commun. 2021; 12(1): 1-10. [CrossRef]

[98] Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020; 586(7830): 516-27. [CrossRef]

[99] Elia U, Ramishetti S, Rosenfeld R, Dammes N, Bar-Haim E, Naidu GS, et al. Design of SARS-CoV-2 hFc-conjugated receptor-binding domain mRNA vaccine delivered via lipid nanoparticles. ACS Nano. 2021; 15(6): 9627-37. [CrossRef]

[100] Elia U, Rotem S, Bar-Haim E, Ramishetti S, Naidu GS, Gur D, et al. Lipid nanoparticle RBD-hFc mRNA vaccine protects hACE2 transgenic mice against a lethal SARS-CoV-2 infection. Nano Lett. 2021; 21(11): 4774-9. [CrossRef]

[101] de Alwis R, Gan ES, Chen S, Leong YS, Tan HC, Zhang SL, et al. A single dose of self-transcribing and replicating RNA-based SARS-CoV-2 vaccine produces protective adaptive immunity in mice. Mol Ther. 2021; 29(6): 1970-83. [CrossRef]

[102] Park JW, Lagniton PN, Liu Y, Xu R-H. mRNA vaccines for COVID-19: what, why and how. Int J Biol Sci. 2021; 17(6): 1446. [CrossRef]

[103] Rauch S, Roth N, Schwendt K, Fotin-Mleczek M, Mueller SO, Petsch B. mRNA-based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus-neutralising antibodies and mediates protection in rodents. NPJ Vaccines. 2021; 6(1): 1-9. [CrossRef]

[104] Huang J, Tao G, Liu J, Cai J, Huang Z, Chen J-x. Current prevention of COVID-19: natural products and herbal medicine. Front Pharmacol. 2020; 1635. [CrossRef]

[105] Lu J, Lu G, Tan S, Xia J, Xiong H, Yu X, et al. A COVID-19 mRNA vaccine encoding SARS-CoV-2 virus-like particles induces a strong antiviral-like immune response in mice. Cell Res. 2020; 30(10): 936-9. [CrossRef]

[106] McKay PF, Hu K, Blakney AK, Samnuan K, Brown JC, Penn R, et al. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. Nat Commun. 2020; 11(1): 1-7. [CrossRef]

[107] Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: The impact of cationic lipid selection. J Control Release. 2020; 325: 370-9. [CrossRef]

[108] Huang Q, Zeng J, Yan J. COVID-19 mRNA vaccines. J Genet Genomics. 2021; 48(2): 107-14. [CrossRef]