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Engineering of the current nucleoside-modified mRNA-LNP vaccines against SARS-CoV-2

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1. Introduction

Since naked in vitro transcribed mRNA molecules were expressed in vivo after direct injection into mouse muscle, mRNA has been investigated extensively as a preventive and therapeutic modality [1–3]. Messenger RNA (mRNA) vaccines are designed to direct cells to express virtually any desired protein inside the host cells and tissues that can have a therapeutic or preventive benefit, potentially addressing a broad spectrum of diseases [3,4].

Following this approach, mRNA is synthesized in a cell-free system and manufactured within standardized and controlled conditions, allowing a comparatively fast design and a relatively inexpensive and straightforward large-scale production. The complete mRNA code, for example, for the Moderna Therapeutics vaccine, was compiled in two days, and the materials for the first clinical trials were manufactured and delivered within 45 days. On March 16, 2020, the first trial participants were vaccinated, just 66 days after the SARS-CoV-2 genome was made public on January 10, 2020 [5].

Delivery of mRNA is safer than whole viral particles, as the former is a non-infectious transient carrier of information. Furthermore, recombination among single-stranded RNA species is rarely possible, and cytosolic mRNA cannot be integrated into the host genome [6]. Moreover, mRNA exhibits self-adjuvating properties as a result of its capacity to bind to pattern-recognition receptors (PRRs) like Toll-like receptor 7 (TLR7), promoting cellular immunity [7], and at the same time provides the technological basis to deliver in a single molecule, open reading frames encoding a wide variety of antigens, modulators, and cell-signaling factors [8].

mRNA therapeutics combine safety with fine dose control and the potential for multiple administrations with a reduced risk of pre-existing or anti-vector immunity [9]. For example, upon intravenous injection into mice of a single dose of lipid nanoparticle (LNP)-encapsulated nucleoside-modified mRNAs encoding the heavy and light chains of the anti-HIV-1 neutralizing antibody VRC01, Pardi and colleagues observed high levels of functional antibody in the serum, protecting the humanized mice from HIV-1 infection. [9,10]. Thran and colleagues employed an unmodified-mRNA-LNP complex to protect from otherwise lethal challenges with rabies virus, botulinum toxin, and a B cell lymphoma through a system expressing three monoclonal antibodies [11]. Furthermore, nucleoside-modified mRNA-LNP influenza vaccines induced humoral immune responses in the recipients, with a safety profile comparable to an influenza vaccine using inactivated influenza virus [12,13]. The above are just a few representative examples illustrating the potential of mRNA vaccines to provide successful prophylactic approaches.

Eleven months after discovering the SARS-CoV-2 virus, it has been
confirmed that a mRNA vaccine for coronavirus disease 2019 (COVID-19) is effective and safely tolerated [14]. More than three decades of scientific advances into RNA biology, chemical modifications, lipid-based delivery systems, and nanotechnology have led to fast progress in the development of mRNA-based vaccines against infectious diseases and their move into clinical trials [3,4,9,15,16].

Herein, we overview molecular aspects of engineering the two lipids nanoparticle-encapsulated, nucleoside-modified mRNAs vaccines for COVID-19, highlighting the main differences between Moderna’s mRNA-1273 vaccine and the Pfizer/BioNTech (BNT162b2) vaccine in terms of the strategies followed to optimize their effectiveness and safety.

2. Relevant molecular features of the SARS-CoV2 spike protein

The SARS-CoV-2 RNA genome is introduced in the host cell using a highly glycosylated homotrimeric S protein (spike glycoprotein) to achieve fusion with target cell membranes [17,18].

The transmembrane SARS-CoV S-protein spike trimer possesses an N-terminal receptor-binding S1 subunit and a C-terminal S2 subunit. The S1 subunit is subdivided into the N-terminal domain (NTD), followed by the receptor-binding domain (RBD) and two structurally conserved...
subdomains (SD1 and SD2) [19]. Binding to the host receptor via the RBD in S1 is followed by proteolytic cleavage of the spike by host proteases into the membrane-associated S2 part and the distal S1 part, which remain associated through non-covalent interactions [20,21]. S1 binds the angiotensin-converting enzyme 2 (ACE2) on the host cell surface, while S2 mediates membrane fusion [21,22]. Proteolytic cleavage of the S protein by furin or other cellular proteases like TMPRSS2 [21] at the S1/S2 site is essential for the infection, as it separates two functions of spike [23]. For example, the furin cleavage site has been shown to be essential for efficient viral entry into human lung cells, especially in terms of cell-cell fusion, to form a syncytium to facilitate viral spread from one cell to another [24].

The aforementioned structural insights reveal the S protein as a crucial target for vaccine development, therapeutic antibody generation, and clinical diagnosis of COVID-19 [25,26]. Specifically, a vaccine targeting the S protein could prevent the spread of SARS-CoV-2 by impeding its initial activation through blockade of S protein binding to the host receptor ACE2 [17,25,26], mainly if the antibody titer against S protein is high enough to prevent the virus from being engulfed into endosomes or undergoing fusion at the host cell surface [25,27].

3. Key features for the mRNA-based vaccines engineering

mRNA vaccines constitute a subtype of nucleic acid vaccines, which is divided into two categories: self-amplifying RNA (encoding both an antigen and an RNA replicase that amplifies the recombinant mRNA) and non-replicating mRNA (encoding only the antigen) [28], which has been the model approved and employed in the current vaccines against COVID-19.

The basic structure of non-replicating mRNA closely resembles “mature” eukaryotic mRNA. Usually, it consists of an in vitro-transcribed mRNA (IVT-mRNA) is composed of the antigen-encoding mRNA and non-replicating mRNA (encoding only the antigen) [28], which has been the model approved and employed in the current vaccines against COVID-19.

After transcription, the sample contains the antigen-encoding mRNA (Fig. 1A), whose secondary structures are recognized by cell-surface receptors, and clinical diagnosis of COVID-19 [25,26]. Specifically, a vaccine targeting the S protein could prevent the spread of SARS-CoV-2 by impeding its initial activation through blockade of S protein binding to the host receptor ACE2 [17,25,26], mainly if the antibody titer against S protein is high enough to prevent the virus from being engulfed into endosomes or undergoing fusion at the host cell surface [25,27].

3.1. mRNA capping

Eukaryotic mRNAs, as well as RNA viral genomes, have a 7-methylguanosine (m7G) cap at the 5′ end of the mRNA sequence (m7GpppN structure), attached to the first RNA nucleotide through a 5′,5′-triphasphate bridge (ppp) during mRNA in vitro transcription [31,32].

The cap structure plays essential functions in mRNA translation by recruiting translation initiation factors, and different 5′ caps can be incorporated into mRNAs [30] (Fig. 1A). For example, CAP0 protects endogenous mRNA from nuclease attack and is also involved in nuclear export and translation initiation. Both CAP1 and CAP2 are two 5′ caps that contain additional methyl groups on the second or third ribonucleotide and are less immunogenic than CAP0 [32].

Cap analogs such as anti-reverse-Cap-analog [CAP-0 (N7MeGpppN) structure] or Clean Cap [CAP-1 (N7MeGpppN7MeG) structure] [33] are the two main approaches used to produce capped mRNA in in-vitro transcription (IVT) [30]. Uncapped (5′ppp or 5′pp) or inadequately capped mRNAs can be recognized by PRRs such as Retinoic acid-inducible Gene-I-like (RII-I-like) receptor [34]. However, treatment of the uncapped IVT-mRNAs with phosphatases might avoid recognition by RIG-I, where the latter recognizes the 5′ triphosphate of uncapped mRNA, thus avoiding mRNA translation [30,34].

3.2. Untranslated Regions

The 5′- and 3′-UTR elements flanking the coding sequence influence the stability and translation of mRNA. Optimization of the 5′-UTR of mRNA (Fig. 1A), whose secondary structures are recognized by cell-specific RNA binding proteins, can maximize the translational yield of mRNAs useful for therapeutics and vaccines [35]. The use of α-globin or β-globin UTRs from Xenopus laevis or human has been a standard approach in mRNA vaccine design due to their high stability [36]. However, recently, Orlandini von Niesse and colleagues, through the Systematic Evolution of Ligands by Exponential enrichment (SELEX) method, developed a cell culture-based systematic selection process to identify novel 3′-UTR motifs that could induce approximately threefold higher protein production via IVT compared to the classical human β-globin 3′-UTR used in effective mRNA vaccines [13]. Therefore, these regulatory sequences can be derived from viral or eukaryotic genes and modified through the systematic enrichment of naturally occurring RNA sequences to improve the protein synthesis efficiency of mRNA in vitro and in vivo and to significantly increase the half-life of therapeutic mRNAs [13,37].

3.3. Optimizing translation of IVT-mRNA

The antigen-coding sequence can be modified at specific locations and/or codon-optimized to improve the translation. IVT-mRNA can also be engineered to direct the product carrying the antigen to the desired compartment or to keep it in a soluble form as well as to be optimized to improve antigenicity or immunogenicity, either through point mutations, deletion of some segment, or deletion of putative glycosylation sites [29,38,39].

In terms of codon usage, replacing rare codons with frequently used synonymous codons can be recognized by abundant cognate tRNA in the cytosol is a recurrent strategy to increase the protein expression level of mRNAs [9,40]. ORF sequence can also be modified to obtain comparable ratios for every codon found naturally in genes encoding highly expressed proteins in human cells [30], and also G+C content optimization has been shown to increase steady-state mRNA levels in vitro and protein expression in vivo [41,42]. For example, Acuitas Therapeutics has used LNPs to deliver erythropoietin (EPO)-encoding mRNA rich in GC codons into pigs, demonstrating that it can elicit EPO-related responses with no associated immunogenicity [41].

3.4. Poly-A tail

One of the last steps of mRNA biogenesis is poly-A tail synthesis, an essential element for efficient translation. According to earlier studies, the addition of long poly-A sequences (~250 units in length) is preferable for enhancing mRNA stability. However, poly-A sequences of 120 units provide more stable IVT-mRNAs [43] and more efficient translation than shorter tails in human monocyte-derived DCs [37], while in human primary T cells, a poly-A tail longer than 300 nucleotides is more conducive to efficient translation [44].

3.5. Purification of IVT-mRNA

After transcription, the sample contains the antigen-encoding mRNA and short transcripts derived from abortive cycling during transcription initiation or incomplete transcripts derived from premature termination during the elongation process and other reaction components such as triphosphate nucleotides, salts, and enzymes [45,46]. A high-performance liquid chromatography (HPLC) step allows the separation of the intended mRNA from shorter and longer transcripts, yielding a pure single mRNA product [46,47]. Thus, implementing this purification by chromatography within a Good Manufacturing Practice (GMP) during the production process of mRNA increases the activity of mRNA preparations several-fold in terms of protein expression in vivo.
Table 1
The main differences, limitations, and optimizations suggested for Moderna’s mRNA-1273 Vaccine and the Pfizer/BioNTech (BNT162b2) vaccine.

| Feature          | mRNA-1273 | BNT162b2 | Limitations                                                                 | Optimizations needed                  |
|------------------|-----------|----------|----------------------------------------------------------------------------|----------------------------------------|
| Size (nucleotides)| 4004      | 4284     | The assembled contig includes the full coding region but could lack some sequence from the ends of the Moderna vaccine RNA[75]. | None                                   |
| Cap              | Cap 1 structure[76] | Cap1 structure | A fragment of 35-nt from 5’-UTR of the highly expressed human gene α-globin (HBA1) was incorporated. | Potential optimization of the speed of the 5’-UTR to load ribosomes onto the mRNA. |
| 5’-UTR           | Not revealed | GG-rich tract CCCCCGOGGCC was included, just upstream the Kozak consensus[77]. | The GC-rich tract and the secondary structure this propitiates in mRNA-1273 may reduce translation initiation efficiency and overall protein output[78]. | Suppression of secondary structures spanning the start codon for efficient translation initiation[77]. |
| Kozak sequence   | GCCACCAUG | GCCACCAUG | None                                                                       | Replacement of all or most synonymous codons according to usage in highly expressed genes, either ubiquitously (e.g., GGC instead of GGG), or in muscle tissues[77]. |
| Coding sequence  | GAG codon in the spike protein gene replaced all GAA codons[77]. | 14 GAA codons unchanged[77] | a) wobble more in base-pairing than U and can hybridize with A and G and, to a lesser extent, with C and U[79].<br>b) Excessive use of CGG (is not an optimal codon) in Moderna’s mRNA-1273 vaccine[77]. | a) UAA is the more efficient stop codon[80].<br>b) The optimal stop signal should be UAAA instead of UGAU/UAGU/UAAU in both mRNA vaccines[77].<br>c) Production of larger proteins with an unknown destination and with potentially deleterious effects[77]. |
| Stop codon       | Three different stop codons are used (ψGAψAAψAG)[77]. | Two consecutive UGA stop codons are used (ψGAψGA)[77]. | a) Uracil replacement by ψ increases the rate of misreading of stop codons by a near-cognate tRNAs[79].<br>b) Decrease in the number of immunogenic proteins[77].<br>c) Production of larger proteins with an unknown destination and with potentially deleterious effects[77]. | |
| 3’-UTR           | The 110-nt 3’-UTR of human β-globin gene (HBA1) placed between the last stop codon and the Poly(A) tail[77]. | The 3’-UTR comprises the human AES/TLE5 gene segment (316-nt), inserted 6-nt downstream the second stop codon, and the human mitochondrial 12S rRNA (mtRNR1) segment (139-nt), positioned downstream of the first[77]. | Decrease the number of destabilizing elements of the mRNA (e.g., reducing the number of predicted miRNAs binding sites introducing point mutations). | |
| Poly-A tail      | Not revealed | A30(GCATA/GACT)A70 30 µg | The Pfizer/BioNTech vaccine likely produces about 3.3 times as many Spike proteins as the Moderna vaccine[77]. | Increase and sustain protein expression. Decrease the amount of vaccine mRNA (µg) by increasing the mRNA translation efficiency. |
| Dosage           | 100 µg | 30 µg | | |
incorporation of modified nucleotides in the mRNA sequence, such as via the adsorption of double-stranded RNA contaminants employing simple, inexpensive, and highly scalable method for mRNA purification.

US, The United States; EMA, European Medicines Agency; UK, The United Kingdom

Comparison of the first two mRNA-based COVID-19 vaccine candidates.

Table 2

| Developers | Vaccine | Vaccine platform | Coronavirus target | Type of Candidate | Emergency use authorization | Dosage, schedule, and route of administration | Confirmed efficacy | Clinical trial registry number |
|------------|---------|------------------|--------------------|------------------|----------------------------|---------------------------------------------|-------------------|-----------------------------|
| Moderna/ NIAID | mRNA-1273 | mRNA-based therapeutics | SARS-CoV-2 Spike protein | LNP-encapsulated nucleoside-modified mRNA | US (Dec 18, 2020), Canada (Dec 23, 2020), Israel (January 4, 2021), EMA (Jan 6, 2021) | Two intramuscular injections (100 μg per dose), 28 days apart | 94.1% (measured starting from 14 days after the second dose) | NCT04470427 |
| BioNTech/ Pfizer | BNT162b2 | mRNA-based therapeutics | SARS-CoV-2 Spike protein | LNP-encapsulated nucleoside-modified mRNA | UK (Dec 2, 2020), Canada (Dec 9, 2020), US (Dec 11, 2020), EMA (Dec 21, 2021), other countries | Two intramuscular doses, 21 days apart (30 μg per dose) | 95% (measured starting from seven days after the second dose) | NCT04368728 |

US, The United States; EMA, European Medicines Agency; UK, The United Kingdom

* December 31, 2020 (Argentina, Ecuador, Chile, Panama, Mexico, Costa Rica, Kuwait, Singapore, Switzerland, South Arabia)

1 Differences in efficacy (between 94.5% and 95%) are small compared to the potential variables between the studies.
biomolecules and lipid nanoparticles (LNPs). The incorporation of chemically, naturally occurring, modified nucleosides, including modified uridine and guanosine, significantly enhances mRNA stability. These modified nucleosides contribute to increased mRNA stability and cellular delivery, offering a promising alternative to conventional mRNA vaccines. These advancements have paved the way for the development of next-generation mRNA vaccines with improved efficacy and safety profiles.
residues were replaced by 1-methyl-3′-pseudouridine modifications [91] (Fig. 1A).

The lipid nanoparticle delivery system in the trials of BioNTech is composed of the cationic lipid ALC-0315 (licensed from Acuitas Therapeutics) combined with the phospholipid 1,2-distearyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and a PEG–lipid [86] (Fig. 1B).

BNT162b2 possesses the ability to mimic the process by which natural SARS-CoV-2 viral infection occurs and confers protection against COVID-19 by the transient expression of the full-length spike antigen, once expressed on the surface of the host cells, to induce neutralizing antibody generation and cellular immune responses against it [93].

The vaccine candidate BNT162b2 was chosen as the most promising in terms of better safety profile among three others with similar technology developed by BioNTech [94,95]. Ninety to one hundred percent of vaccine efficacy was observed across subgroups defined by age, sex, race, ethnicity, baseline body-mass index, and the presence of coexisting conditions [93].

All participants received two doses, 21 days apart, of either BNT162b2 or placebo, delivered into the deltoid muscle [93] (Table 2). A regimen of two-dose BNT162b2 conferred 95% protection against COVID-19 in subjects 16 years of age or older, showing a safety profile observed for other viral vaccines for about two months [93].

5. Production of the nucleoside-modified SARS-CoV-2 mRNA-LNP vaccines

Development of an mRNA vaccine is faster than inactivated vaccines, attenuated live vaccines, and subunit vaccines [96], and speed, the feasibility of manufacturing scale-up, and global access are three criteria that should be considered [97].

Globally, Pfizer/BioNTech expects to produce about 2 billion doses in 2021 (compared with the more than 50 million doses of vaccines generated during 2020 [98]). However, at the end of March of this year, they raised to 2.5 billion doses [99]. Instead, Moderna has announced that it would boost the production of its vaccines, stating that it would produce no less than 800 million doses in 2021 (compared with the 20 million doses generated by the end of 2020) and potentially triple its production in 2022 due to improvements in vaccine manufacturing methods [100].

Interestingly, Pascolo [101] has recently calculated that to produce one billion doses of prophylactic vaccines (to vaccinate ~10% of the population, 500 million people twice), Moderna will need to generate 100 kg of IVT mRNA (100 μg per dose) while BioNTech will need to produce 30 kg of IVT mRNA (30 μg per dose). Thus, the necessary volumes of in vitro transcription reactions will be at least 20,000 and 6000 L, respectively, assuming to reach a concentration of at least 5 mg/mL of mRNA at the end of the process [101].

Following practices like these, mRNA vaccines can be easily and quickly adapted to produce new vaccines against future epidemics.

6. Administration route

The in vivo local delivery of the lipid-based mRNA vaccines (subcutaneous, intramuscular, intradermal, and intranasal administration) is used to initiate the stimulatory reaction in small areas and to eliciting locally strong and long-lasting immune responses [28]. For example, it has been demonstrated that subcutaneous administration of the PEGylated LNPs facilitated the uptake by the DCs located in the lymph nodes and allowed the rapid release of mRNA vaccines [102]. Moreover, the administration route can affect the extent and quality of immune responses independently of the administered dose [103]. Subcutaneous and intramuscular administrations have been the two most frequently used injection routes for mRNA vaccination because they are less invasive and therefore do not require much training for their implementation [4,8]. However, the administration route and vaccine formulation also determine how the immune response is modulated and when the peak of antigen expression is reached [4,104].

Some recent studies highlight the importance of mucosal immune responses against SARS-CoV-2 infection [105,106]. Nevertheless, although intranasal and oral vaccinations are theoretically the most straightforward solution to elicit mucosal immunity, there are no commercial vaccines that use the pulmonary delivery route, given the additional requirement for a suitable and the resulting technical challenges in the formulation. Therefore, almost all COVID-19 vaccine candidates that have undergone clinical trials are given by injection, although they may not induce specific mucosal immunity [107].

7. Immune response evoked by both nucleoside-modified SARS-CoV-2 mRNA-LNP vaccines

After cellular internalization of LNPs, mRNA can be recognized by endosomal or cytosolic PRRs, specific for GU-rich single-stranded RNA (ssRNA), (which can be sensed by TLR-7 and –8) [108] and also by dsRNA (sensed by TLR-3) [64], in terms of the structure that the mRNA molecules could adopt. mRNA can also bind to cytosolic RNA sensors RIG-I [109] and MDA5 [64]. Upon RNA sensing, PRRs can lead to activation of the IFN-1 pathway characterized by upregulation of multiple genes, including those encoding proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-6, and IL-12, leading to antigen-presenting cell (APC) activation [104,110]. Antigen presentation on B and T cells ultimately leads to the production of the typical immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, where IgM antibodies have been shown to last up to week 12. In contrast, IgG antibodies can provide prolonged protection [97,111]. DCs represent an attractive target by mRNA vaccines, both in vivo and ex vivo, because DCs can internalize naked mRNA through various endocytic pathways [112,113] and may present whole antigens to B cells to trigger an antibody response [114].

Exogenously delivered mRNA uses the host cell translation machinery to produce the S protein of the virus. However, antigen expression is not the final aim for assessing the effectiveness of a vaccine. The capability to induce cellular immunity (T cell activation) may better reflect whether an immune response directed against the S protein will be protective [115], being a requirement to eradicate the intracellular SARS-CoV-2 reservoir. Nevertheless, an indiscriminate immune activation can also induce mRNA degradation and reduce antigen expression [116].

It has been shown that the immune responses elicited by the mRNA-1273 vaccine increased with time and dose of the vaccine and that highly-neutralizing antibody responses were also elicited in a dose-dependent fashion in the vaccinated group [97]. However, both the mRNA-1273 and BNT162b2 vaccines induced in the inoculated participants the production of similarly high dose-dependent neutralizing antibody titers against SARS-CoV-2 [117]. Moreover, in humans, both preparations elicited S-specific CD4+ T cell responses targeting the S1 (including RBD) and S2 regions of the S glycoprotein [81]. In terms of the induction of CD8+ T cell responses, results indicate that BNT162b2 outperforms mRNA-1273. Ninety-one percent of the study participants vaccinated with BNT162b2 mounted significant S-specific CD8+ T cell responses [118], compared to low or undetectable levels in the clinical evaluation of mRNA-1273 [5,81].

Concerning this, vaccine strategies that induce strong cellular responses in addition to humoral immunity in an adequate balance present a significant advantage in the current outbreak [117,119].

8. Concluding remarks

While established conventional approaches have allowed the development of many currently available vaccines, mRNA-based vaccines have benefited significantly through breakthroughs in novel technologies based on at least three decades of scientific development, allowing them to reach the market of mass vaccination for the first time.
at relatively high speed [20].

On the other hand, whereas the majority of early work in mRNA vaccines focused on cancer therapeutics, several reports have demonstrated the potency and versatility of mRNA to protect against a wide variety of viral agents, including influenza virus [120,121], Ebola virus [121] Zika virus [56], and now SARS-CoV-2 virus [5,93].

The Pfizer-BioNTech COVID-19 and Moderna COVID-19 vaccines are two mRNA vaccine platforms that have shown good tolerability, inducing antigen-specific T and B cell immune responses with minimal differences in immunoinstrumental profiles [5,14,85,93,122]. However, many experimental approaches are needed to prove that the optimizations suggested and implemented by both developers are the most appropriate. Only until then, we could do an entirely rational critical analysis to improve the design of both vaccines, leading to their more optimized development in the future. In this regard, further research is needed to determine how different human populations respond to the mRNA vaccine components and how these nucleoside-modified mRNA molecules elicit long-lasting and robust cellular and humoral immune responses against SARS-CoV-2 in humans, in terms of differences between individuals [14,107].

The inherent structural instability of the spike protein has been an essential aspect in the design of vaccines because the loss of its native conformation may lead to the induction of antibodies with lower neutralizing activity [20,119]. Furthermore, new strategies and optimizations are required to decrease the doses employed by these mRNA vaccines and elicit more robust and extended memory responses with just one immunization [123]. Efforts to develop thermostable formulations more suitable for wide distribution and long-term storage, while preserving biological activity, have been gaining interest, specifically freeze-dried mRNA [124], lyophilized mRNA[125] as well as protamine-complexed mRNA formulations [126,127].

In order to meet global demand, mRNA vaccines can be designed and produced massively at relatively high speed, even though this relies on the synthesis of RNA and the procurement of the required supplies, like capping compounds, triphosphate nucleotides, cholesterol, and other components for the lipid nanoparticles, as well as large numbers of vials glass vials, syringes, dry ice, and cold packs for distribution.

Although several of the current COVID-19 vaccine platforms elicit neutralizing antibodies against the S protein of SARS-CoV-2, these may exhibit different protection grades among different population groups such as children, pregnant women, immunocompromised populations, and immunosenescent age groups ≥ 65 years [117,128], an aspect that deserves to be evaluated in the medium and long term.

Pfizer-BioNTech and Moderna Therapeutics plan to update their vaccines and develop booster doses to improve their efficacy against any future variants [129]. More extensive relevant human clinical experience will give us a more comprehensive insight into mRNA vaccine approaches.

CRedit authorship contribution statement

Javier T. Granados-Riveron: Conceptualization, Writing – review & editing. Guillermo Aquino-Jarquin: Conceptualization, Writing – original draft, Funding acquisition.

Conflict of interest statement

None

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