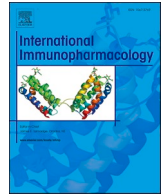




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Review

Essential considerations during vaccine design against COVID-19 and review of pioneering vaccine candidate platforms

Maryam Bayat^a, Yahya Asemani^{b,*}, Sajad Najafi^c^a Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran^b Department of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran^c Student Research Committee, Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:

COVID-19

Vaccine

Design

SARS-CoV2

Adjuvant

ABSTRACT

The calamity of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), COVID-19, is still a global human tragedy. To date, no specific antiviral drug or therapy has been able to break the widespread of SARS-CoV2. It has been generally believed that stimulating protective immunity via universal vaccination is the individual strategy to manage this pandemic. Achieving an effective COVID-19 vaccine requires attention to the immunological and non-immunological standpoints mentioned in this article. Here, we try to introduce the considerable immunological aspects, potential antigen targets, appropriate adjuvants as well as key points in the various stages of COVID-19 vaccine development. Also, the principal features of the preclinical and clinical studies of pioneering COVID-19 vaccine candidates were pointed out by reviewing the available information. Finally, we discuss the key challenges in the successful design of the COVID-19 vaccine and address the most fundamental strengths and weaknesses of common vaccine platforms.

1. Introduction

A new coronavirus pandemic broke out from Wuhan, China in December 2019 and is still spreading across the globe. The viral causative agent of this infectious calamity was named SARS-CoV2, and the resulting disease is known as coronavirus disease 2019 (COVID-19) [1]. On February 11, 2020, the World Health Organization (WHO) officially declared COVID-19 as a global pandemic [2]. The SARS-CoV2 is the seventh member of the zoonotic family *Coronaviridae*, genus *Betacoronavirus*, and shows a striking resemblance to the other previously identified members, SARS-CoV and middle east respiratory syndrome coronavirus (MERS-CoV), of this family [3]. All three viruses originate from bats and after spending parts of their life cycle in the intermediate hosts (camels for MERS-CoV, civets for SARS-CoV, and likely scaly anteaters for SARS-CoV2) are transmitted to humans and cause lethal diseases [4]. The coronaviruses are generally spherical crown-like structures under electron microscopy with an approximate diameter of 125 nm [5]. The whole body consists of a single-stranded positive-sense RNA genome entrapping by a helical nucleocapsid (N) and a borrowed envelope that embrace momentous membrane (M), envelope (E), and especially spike (S) proteins and coverages the remnants [6]. All

coronaviruses exploit the host angiotensin-converting enzyme 2 (ACE2) receptor for cellular entry with the help of their S protein except that the SARS-CoV2 tendency to the ACE2 has greatly increased and entailed higher infectivity [7,8].

2. The exigency of COVID-19 vaccine

Since the outbreak of SARS-CoV2 infection, there has been a general mobilization of governments, organizations, and research institutes to achieve effective treatment against the contagious disease. Existing drugs and treatment strategies save patients' lives to some extent, but our main need is to achieve a successful drug that can be at least 95% operative against this pandemic. For setting up the arrant chaos, we have no choice unless to obtain an immunogenic, safe, and cost-effective vaccine that covers a wide range of people all around the world as soon as possible. Besides, given the human history of dealing with infectious diseases such as mumps, measles, Spanish flu, and SARS, general vaccination is the only way to get rid of the COVID-19 pandemic and achieve herd immunity that subsequently reduces the economic, social, and psychological pressures on human society. The implementation of effective vaccine strategies contains several aspects that should be

* Corresponding author at: Department of Immunology, Medical School, Shahid Beheshti University of Medical Sciences, P.O. Box: 1985717443, Tehran, Iran.
E-mail address: yahya.asemani.ya@gmail.com (Y. Asemani).

carefully considered. Therefore, in the current paper, we intend to explain the immunological and non-immunological characteristics of COVID-19 vaccine design, review the available data from related pre-clinical and clinical trials and assess the advantages and disadvantages of pioneering COVID-19 vaccine platforms.

3. Immune arms against COVID-19

3.1. Natural immunity

The SARS-CoV2 like the other member of the coronavirus family is habitually reluctant to stimulate innate immune cells such as dendritic cells and hamper the antiviral type I and III interferon responses [9]. So, thwarting the innate immune responses by SARS-CoV2 leads to the prolongation of the incubation period and smooth transmission of the pathogenic agent without clinical symptoms [10]. Besides, the eruptive replication of the SARS-CoV2 in the early stages of the disease confirms the aberrant or failed innate immune responses and is considered as a foundation for ensuing cytokine storm complications, especially in severe COVID-19 patients [11]. So, the infected patients experienced elevated circulatory levels of inflammatory cytokines and chemokines, enhanced overactivated blood monocytes and neutrophils [12,13], and M1 macrophages accumulation in the lung derived from CD14⁺/CD16⁺ proinflammatory monocytes [14]. Also, the lack of early restriction of SARS-CoV2 replication in the airways by innate immunity leads to viral overload and resultant hyperinflammatory syndromes including acute respiratory distress syndrome (ARDS) [9].

3.2. Acquired immunity

3.2.1. T cell-mediated responses

Progressive evidence proposed that both antibody and cell-mediated arms of adaptive immunity are the prerequisites to defeat SARS-CoV2 infection. Meanwhile, the CD4⁺ T lymphocytes play determinant roles in affordable antibody response and effective CD8⁺ T cell cytotoxicity [15]. Analysis of peripheral blood T cell populations of recovered individuals from COVID-19 infection showed that all the patients had specific CD4⁺ T helper (Th) cells for the SARS-CoV2 S protein, while only 70% of blood samples contained the S protein-specific CD8⁺ cytotoxic T cells [16]. Preclinical studies indicated that the magnitude of specific T lymphocytes especially in the lung is associated with better prophylaxis of COVID-19 patients [17]. Histopathological evidence proposed that respiratory mucosal vaccination could induce such lung resident memory T cell responses compared to injectable vaccines and accompanied by the reinforced defense against SARS infection [18,19]. The phenotype of Th cells is also affected by the vaccination route, so that severe lung manifestations ensuing SARS-CoV infection were associated with Th2 phenotype dominance in parenterally vaccinated individuals while switching to Th1 responses by mucosal vaccination led to less severe SARS infection [9,20,21]. So, inducing acceptable Th1 responses particularly in the tissue-resident T cell populations should be considered in COVID-19 vaccination. Altogether, it seems that priming T cell-mediated antiviral responses is more reliable than induction of antibody secretion to achieve effective immunization in the elderly, forcefully protect the coverage of T cell response in designing the COVID-19 vaccine [10,22]. On the other hand, some evidence showed that in 35% of healthy individuals with no history of SARS-CoV2 infection, CD4⁺ T cells potentially recognized the SARS-CoV2 spike protein. Besides, CD4⁺ helper T cells can identify other SARS-CoV2 proteins in 40 to 60% of people not experience the COVID-19 infection [23,24]. These findings suggest that there is a cross-activity between SARS-CoV2-specific CD4⁺ T cells and CD4⁺ T cells related to other members of human and animal beta coronaviruses [25]. Therefore, in a society, there are different degrees of pre-existing immunity that may explain the range of susceptibility of individuals to COVID-19 infection. Surprisingly, the cross-reactive CD4⁺ T cells primarily

recognize the S2 subunit of the SARS-CoV2 spike protein. Also, CD4⁺ T cells-derived from COVID-19 patients make vigorous cross-reactivity with the S2 domain of the human OC43 and 229E coronaviruses' spike protein [24]. Since cross-reactive T cells realize both structural and non-structural viral epitopes [24,26], likely the vastness of induced responses of such cross-reactive T-cells by recombinant protein and viral vector-based vaccines compared to multivalent COVID-19 vaccines are dissimilar. So, the efficacy of killed or even live attenuated COVID-19 vaccine candidates possibly impaired due to the presence of pre-existing cross-reactive immunity. So, in terms of anti-coronavirus cross-reactive immunity, determining the status of participants in clinical trials is imperative.

3.2.2. Antibody responses

The 2 weeks after the onset of clinical symptoms, most of the COVID-19 infected patients indicate high titers of IgM and IgG antibodies [27]. Laboratory findings exhibited that the convalescent plasma of the recovered individuals contains high volumes of neutralizing antibodies [25], indicative of CD4⁺ T cell response involvement [28], which has the potential to be appraised as passive immunotherapy to improve the condition of critically ill patients. It was also found that the extent of neutralizing antibodies has a direct relationship with the severity of the COVID-19 infection [29]. More analysis revealed that the SARS-CoV2 S protein is the most target of such neutralizing antibodies, which is contained the S1 and S2 subunits. The S2 is in the proximity of the viral membrane and participates in cellular fusion while the S1 organizes farther away containing the receptor-binding domain (RBD) and attaches to the cognate host ACE2 receptor [30]. Neutralizing antibodies in COVID-19 patients pursue two main goals: restraining the S protein-ACE2 interaction by targeting the RBD domain, and blocking membrane fusion by binding to other regions of the S1 and S2 compartments [31,32]. Also, the IgG2a antibodies against the N portion, as the most frequent coronavirus protein, have been observed in the sera of COVID-19 patients with potential Fc-mediated viral clearance instead of direct neutralization [33]. Unbelievably, several studies discovered the earlier peak of the anti-S protein IgA response before emerging the IgM, although the underlying mechanisms are unknown [34]. Previous results showed that more than 90% of healthy adults are seropositive for the IgG against four common human coronaviruses (229E, NL63, OC43 and, HKU1) [35]. Such antibodies, like the antibody responses to SARS-CoV and SARS-CoV2, largely disappear within a few months. Therefore, T cell responses are likely to be more effective than antibody titers in inhibiting coronaviruses re-infection [35].

4. Immunological standpoints

To achieve an effective vaccine for COVID-19, the following should be considered around the immune responses and SARS-CoV2 infection.

4.1. Genetic alterations

Learn about the SARS-CoV2 mutation rate and presenting escape mutant variants is necessary. It has been shown that every SARS-CoV2 virion has the potential to carry mutations but the speed is slow and the mutants indicate similar sequences to their ancestors [36]. Abdullahi et al. found that various SARS-CoV2 proteins, both structural and non-structural, such as NSP (non-structural protein)2 and NSP3, RNA-dependent RNA polymerase and, S protein are constantly undergoing significant mutations. Their studies showed that these genetic changes are more pronounced in S protein [37]. Also, Dorp et al identified 198 sites in the whole genome of SARS-CoV2 with recurrent and non-aligned mutations that 80% made non-synonymous amino acid alterations at the translation level. These recurrent mutations with more than 15 events were more protruding in the coding regions of the S, NSP13, NSP6 and, NSP11 proteins, give the idea of being more affected during evolution with the novel human host [38]. Therefore, attention to genetic

alteration in SARS-CoV2 structure plays an important role in providing superior candidate antigens in the design of competent vaccine candidates or other antiviral drugs.

4.2. Efficient immune responses

Both B and T cell responses are elicited against SARS-CoV2 infection [39]. Also, the IgM and IgG antibodies appear just about 10 days of infection and nearly all the infected individuals become seroconversion after 21 days. The dominant of the secreted antibodies recognize the N and S proteins of the SARS-CoV2 with prominent neutralizing activities [40]. Accordingly, multiple vaccine candidates are studying in sub-extended clinical trials and researchers should explore the potency and quiddity of respective immune responses for mentioned antigens.

4.3. Chance of re-infection

The main conundrum is whether primary COVID-19 infection prevents the second infection and how long the patient is immune. Unfortunately, affliction to COVID-19 does not appear to prevent further infection, especially for a long time. This finding most likely reduces the success of vaccine candidates. Furthermore, studies suggest that there are genetically distinct strains of the SARS-CoV2 in communities where infection with one does not confer full immunity against the others. The finding was made in a case study by Tillett et al. that the infection occurred with two genetically distinct SARS-CoV2 strains which did not appear to have occurred naturally during evolutionary mechanisms in the human host shortly [41].

4.4. Immunity period

It should be noted that to achieve a successful vaccination, the development of associated antibody and cellular immune responses against SARS-CoV2 should be sustained for a long time. The SARS-CoV2 spends a limited time in our communities, and it is too early to comment on the longevity of induced protective immune responses with high certainty. Although, it is possible to somewhat predict the quality and longevity of antibody and T cell responses to the COVID-19 vaccine candidates by inspiration from vaccine studies for two closely related coronaviruses, MERS and SARS, which have provided promising long-lasting protective immune responses [42,43].

4.5. Disease enhancement phenomenon

The biggest challenge is that not only the designed vaccine award immunity against the desired infectious agent but also aggravates the course of the disease and enhanced mortality [43,44]. Disease enhancement or antibody-dependent enhancement (ADE) is such a wrecking process mediated by non-neutralizing antibody responses against vaccine candidates. This phenomenon aborts the vaccine project by vitiating the elementary vaccination goal and making the disease worse. Indeed, the ADE is mediated by Fc receptor or complement coated cells that following antibody attachment, reverted immune responses from Th1 (interleukin (IL)-2, tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ)) toward Th2 responses (IL-10, IL-6, prostaglandin (PGE2), IFN- α) and blocking signal transducer and activator of transcription (STAT) signaling pathways leading to unbridled viral replication [36]. So, regarding the ADE as the major bane of successful vaccination, maximum efforts should be done to identify efficient immunodominant epitopes and prevent the development of dysfunctional antibodies responsible for the disease exacerbation.

5. The main steps of vaccine design

Vaccine design generally involves going through three stages of appropriate antigen prediction, vaccine platform determination, and the

suitable vaccination route along with effective regimen [9]. The immunostimulatory strength of the viral antigen, the necessity for adjuvant, and the nature of the primed protective immune responses depend primarily on the vaccine platform. These features also influence the competency of a vaccine candidate for a special route of administration and the need for a booster dose to establishing a durable protective immune response. Moreover, certain types of vaccines such as live attenuated or mucosal vaccination should be presented with more pedant safety analysis.

5.1. Antigen prediction

The S, E, M, and N are the main structural proteins of the coronavirus virions that the N proteins encompass a long RNA genome while the remnant immerses in lipid bilayer viral envelope. Based on previous experience with the SARS vaccine, it has been shown that only antibodies against various epitopes of the S protein neutralized the viral particles [45]. Therefore, the new SARS-CoV2 vaccines have focused at least on priming immune responses to some parts of the S protein especially the S1 subunit and the RBD domain. However, emerging non-neutralizing antibodies against all the structural and non-structural proteins and the subsequent disruptive ADE phenomenon saberrattling as the main obstacle to effective vaccination. So, considering the most constitutive as well as conserved viral proteins such as RNA polymerase [46] in vaccine design will provide a more confidential vaccine candidate and probably relieve us a lot of worries about possible future coronavirus infections as well.

5.1.1. Reverse vaccinology model

In recent years, vaccine design has undergone extensive evolutions due to reverse vaccinology (RV). In this regard, the desired pathogen genome is first evaluated by bioinformatic analysis and then potential vaccine candidates are identified [47]. Vaxign is the first web-based system that applies the RV algorithm to effectively offer vaccine candidates for various microbial pathogens. Recently Ong et al. have achieved a new learning method namely the Vaxign-ML machine to enhance the resolution of candidate prediction [47]. Using Vaxign RV and then Vaxign-ML systems, they first predicted 6 adhesion protein candidates including S protein and 5 non-structural nsp3, 3CL-pro, nsp8, nsp9, and nsp10 proteins for the development of the COVID-19 vaccine. Contrary to previous researches around the COVID-19 vaccine design that focused on the S protein, it was the first time that the nsp3 and nsp8 were also announced as alternative candidates with significant antigenicity scores. Therefore, it seems that the solution to fight against COVID-19 infection is to use a cocktail vaccine that includes a set of candidates (nsp3, nsp8, and S proteins) instead of a given antigen (S protein) to elicit a significant protective immunity [48].

A similar study according to the *in-silico* RV strategy tried to render a multi-epitope vaccine candidate against SARS-CoV2 infection and evaluated its biological activities by computational methods. They examined three antigens (ORF3a, N and, M proteins) with the help of bioinformatics tools to find potential B-T lymphocyte-stimulating epitopes. Eventually, specific domains of the M or NOM protein contained highly scored B and T epitopes were introduced as the main vaccine candidate that established stable conjugates with Toll-like receptor (TLR) 4 and HLA-A-11:01 receptors using the imagery molecular dynamics and docking studies [49]. Therefore, RV seems to guide further research to more rapid access to immunogenic antigen cocktails in the design of the COVID-19 vaccine. However, the conjunctive application of RV, immunoinformatics, and other computational approaches may lead to achieving potential vaccine candidates more meticulously. Immunoinformatics or computational immunology employs epitope prediction tools, population coverage, and molecular docking analysis to confer conserved B and T cell peptide epitopes of pathogens with considerable immunogenicity [50]. In this regard, a current study by Sarkar et al tried to suggest epitope-based subunit vaccines relying on

immunoinformatics as well as RV technology. Initially, they engineered three vaccine constructs by continual computational experimentation, that one construct candidate showing the most restrictive effects against SARS-CoV2 using molecular docking. Eventually, the desired structure was evaluated to determine efficiency and sustainability in the biological environment, as well as to achieve the most effective strategy for mass production through codon adaptation and molecular dynamics simulation [51]. Besides, two similar but discrete studies succeed to introduce novel epitope-based peptide vaccine candidates against COVID-19. After identifying potential epitopes by epitope prediction databases like Immune Epitopes Database (IEDB), their docking to toll-like receptor (TLR)-4, 5, 7, and 8 were checked by online integrative platforms to determine their ability to induce downstream inflammatory and antiviral signaling. Moreover, the immunogenic profile of peptide candidates was analyzed by immunoinformatics [51,52]. Unlike the most common present researches that insist on the S glycoprotein, a bioinformatics and immunoinformatics study focused on immunological aspects of N protein, as highly expressed and potential immunogenic SARS-CoV2 protein, and anticipated three immunodominant sequences including N₂₂₉₋₂₆₉, N₃₄₉₋₃₉₉, and N₄₀₅₋₄₁₉ that contain linear B-cell and MHC class II epitopes. The built final construct by bioinformatics was affirmed for early SARS-CoV2 diagnosis through an antigen-capture system [53].

5.2. Vaccine platforms

Mostly, designed vaccines are divided into 6 categories based on their platform including inactivated or killed, live attenuated, DNA or RNA, protein subunit, engineered viral vector, and virus-like particle (VLP). From a more generalized perspective, vaccines require two basic components: the antigen that is either provided by the vaccine or produced by the expression system of vaccinated individual, and the non-specific innate immune stimuli, which are mainly provided by alarmins such as damage-associated molecular pattern (DAMP) or pathogen-associated molecular pattern (PAMP) molecules. The live attenuated vaccines are the only platform that delivers both necessities intrinsically, while the other non-viral vaccine platforms require artificial alert molecules commonly known as adjuvants. Furthermore, the non-viral platforms depend primarily on multiple booster doses to provide desirable protection whereas the live attenuated vaccines usually make immunity after a single dose of administration [9]. Like the non-viral vaccines, the inactivated or killed platforms sometimes require adjuvant and multiple administrations for effective immunization [54].

5.2.1. Potential adjuvants

One of the salient features of an effective vaccine is the induction of protective antibody responses using the minimum dose of the antigen so that it has the least requirement for repeated administration and assistance of immunostimulatory agents. In this way, many governments and even low-income countries globally will be able to order the new vaccine in a short time, since the cost of vaccine development will be affordable. Considering a suitable adjuvant in preparing the SARS-CoV2 vaccine is recommended to achieve this grand affair [55]. So, adjuvants that stimulate remarkable antibody as well as cellular immune responses with approved safety and efficacy, such as rOv-ASP-1, CoVaccine HT™, Matrix-M™, delta inulin, MF59®, and AS03 maybe be useful in accelerated vaccine candidate registration containing recombinant RBD or complete S proteins. Among the mentioned adjuvants, AS03, MF59®, and also CpG 1018 have already received the necessary approvals for use in human vaccines, while the rest have shown promising results in clinical and pre-clinical trials. Protollin is the other novel adjuvant that stimulates general and mucosal immunity against respiratory viral infections and should be considered in SARS-CoV2 vaccine researches. Previous reports have indicated antibody-mediated disease exacerbation following the use of inactivated SARS-CoV and MERS-CoV vaccines with or without adjuvants. To date, however, there have been no similar

reports of inactivated SARS-CoV2 vaccines and administering related vaccines with alum adjuvant in *rhesus macaque* host induced notable responses without disease aggravation. Nevertheless, Th1-assisted adjuvants can be used to solve this possible problem [47,55].

5.3. Route of administration and regimen

Indicating the most operative application route and suitable regimen is the third pillar of an effective vaccination [56]. These are more prominent for mucosal infectious agents like the current SARS-CoV2 and those pathogens that require priming innate as well as cellular and antibody immune responses for full protection [57]. The best period to control and clear SARS-CoV2 infection is within the first 2 to 12 days after infection when the person has no clinical symptoms and essential immune components should be placed in the lung mucosa before the viral entry [10]. In this regard, one of the effective variables is the route of vaccination [56]. For instance, intramuscular injection of influenza or measles vaccines mainly induces protective IgG responses that willingly appear in respiratory mucosa, but had no considerable effects on lung mucosal immunity, including the specific IgA secretion and stimulation of tissue-resident memory T cells [58]. Conversely, respiratory mucosal vaccination led to acceptable mucosal antibody responses, priming lung resident memory T cells and inducing trained immunity in macrophages [59,60]. The pulmonary administration is not a preferred route for the killed, nucleic acid, and subunit vaccines since the use of potential adjuvants and re-boost doses are inevitable for such platforms [9]. In contrast, viral vector-based vaccines especially those applying adenovirus vectors like serotype 5 of human adenovirus or adenovirus obtained from a chimpanzee host are suitable candidates for respiratory mucosal vaccination [61]. However, most common human vaccines as well as low immunogenic viral vectors such as adenovirus serotype 26 requires repeated similar administration for effective primed immunity. It is not yet clear which vaccination strategy is to be used to combat the COVID-19 pandemic and how long this strategy will last in recipient bodies, but it may be necessary to use the same or different vaccination regimen for repeated injections to reinforcing protection, such as chimpanzee-derived adenovirus (ChAd) [9]. The route of administration may also change in subsequently repeated vaccinations.

6. Stages of vaccine advancement

Unveiling a new vaccine product contains strict Research and Development (R&D) procedures that the manufacturer should be fully committed to implementing before obtaining a marketing license [36]. Also, the United States Food and Drug Administration (US FDA), WHO, European Medicines Agency (EMA), and the national authorities have enacted scrupulous regulations regarding the accurate clinical evaluation of vaccine development [62,63]. The reason for such strict regulations in the development of a new vaccine compared to other drug compounds is the potential for mass and global production and prescription for a wide range of healthy people, including pregnant women, the elderly, and the young population. Briefly, clinical trial testing of vaccine products is generally divided into four step-by-step phases including Exploratory trials, Preclinical, Clinical, and Post-marketing stages that will normally proceed over many years. Also, the clinical trial study containing three consecutive stages (I, II, and III) that the legal permissions including “Clinical Trial Authorization” before phase I to enter human experiments and the “Biological License Application Approvals” for vaccine marketing after the completion of phase III are required respectively (Table 1) [63].

7. Pioneers of the COVID-19 vaccine program

Until February 9, 2021, 63 vaccine candidates to fight against SARS-CoV2 infection have entered clinical trials, while 179 candidates are going through preclinical developments [64] (Fig. 1). Among the

Table 1
Major characteristics of vaccine development processes.

Stage	Appraisal	Approximate duration	Comment
<i>Laboratory & animal studies</i>			
Exploratory stage	Antigen identification & concept validation	2–4 years	<ul style="list-style-type: none"> • Research-intensive stage • Desired natural or synthetic antigen detection or production
Preclinical Stage	Safety & immunogenicity of vaccine candidates, starting dose determination for further studies	1–2 years	<ul style="list-style-type: none"> • Tissue- or cell-culture & animal testing • Adjuvant selection • Immunogenicity studies • Good Laboratory Practices (GLP) safety studies (<i>in vivo</i> validation) • Potency assay development • Infection challenge studies with the animals
<i>FDA approval during 30 days & subjecting to human studies</i>			
Clinical Stages Phase I	Safety & immunogenicity of vaccine candidates	<1 year	<ul style="list-style-type: none"> • Involves a small group of healthy adults (20–100 subjects) • Usually, non-blinded studies • May be using challenge model in a small part of participants • An attenuated or modified copy of pathogen applied for challenging • Evaluating local & systemic reactions • Relating dose size to the side effects
Phase II	Safety & immunogenicity, proposed doses, schedule of immunizations method of delivery, partial efficacy	2 years	<ul style="list-style-type: none"> • Randomized & well-controlled trials • Hundreds of healthy adults • May contain at risk groups • Evaluating clinical & laboratory responses (antibody response) • Determining most common short-term side effects
Phase III	Safety & efficacy	Many years	<ul style="list-style-type: none"> • Determining efficacy & safety in target population (thousands) • Determining certain rare side effects • Randomized & double-blind studies • Involving the experimental vaccine against placebo • Evaluating disease & infection prevention • Evaluating antibody or other

Table 1 (continued)

Stage	Appraisal	Approximate duration	Comment
<i>Laboratory & animal studies</i>			
Regulatory approval & licensure	Marketing authorization	In progress	<ul style="list-style-type: none"> • pathogen related immunity • Submitting a Biologics License Application to the FDA • Inspecting facilities & reviewing the manufacturer's tests for potency, safety & purity by FDA • Vaccine approval (granted for an initial 5 years)
Phase IV	Post marketing safety & efficacy	In progress	<ul style="list-style-type: none"> • Conducting after vaccine releasing • Testing safety, efficacy, & other potentials by manufacturer • Collecting data from vaccinated individuals

vaccine candidates in clinical evaluation four inactivated, two protein subunit, four adenovirus- and two mRNA-based vaccines constitute the leading candidates in the COVID-19 vaccine design scheme. Hereunto, only BNT162b2, mRNA-1273 (mRNA-based) along with BBIBP-CorV and CoronaVac (inactivated) vaccines have been an emergency or conditionally approved in some countries and the other candidates were allowed for early or limited use [65] (Table 2).

8. Live attenuated vaccines

The strategy of creating an attenuated strain of real pathogens through *in vitro* manifold passages has already been used successfully in manufacturing attenuated live viral vaccines such as measles, mumps, and rubella (MMR), oral polio vaccine (OPV), and vaccine for rotaviruses [94]. Over the processing of this vaccine's generation, the virulence genes are mutated or deleted, and thus the pathogen reproduces to a limited extent in the live host without causing serious disease. The manipulated viral particles generate long-acting antibody and cellular immune responses by replicating in the host and are therefore important for achieving herd immunity and disrupting the transmission cycle (Fig. 2) (Table 3). Similarly, several structural and non-structural genes that are not involved in viral reproduction have been nominated to create attenuated forms of zoonotic coronaviruses [95-97]. Protein E is one of the structural proteins which has been deleted to produce attenuated coronaviruses [95,96], but there have been reports of conversion to virulent strains [98]. In addition to the preferential deletion of virulence genes, another mechanism for producing attenuated phenotypes of pathogenic viruses is applying the codon deoptimization approach. In this strategy, due to the changes in the coding sequence of certain viral proteins, the *in vivo* translational speed is significantly slowed down, but the virus can continue to multiply [99,100]. However, the feasibility of this largely depends on proving the genetic irreversibility of the modified species. This is challenging especially for coronaviruses because, at least in theory, it is possible for a combination to occur between the *in vitro* attenuated and wild-type viral species, re-forming novel pathogenic strains [101]. Besides, the transport of these vaccines requires a cold chain, which limits their use over long distances. That's why only three research institutes including Indian Immunologicals Ltd and Griffith University, Turkish Mehmet Ali Aydinlar University, and Codagenix and Serum Institute of India exploited codon

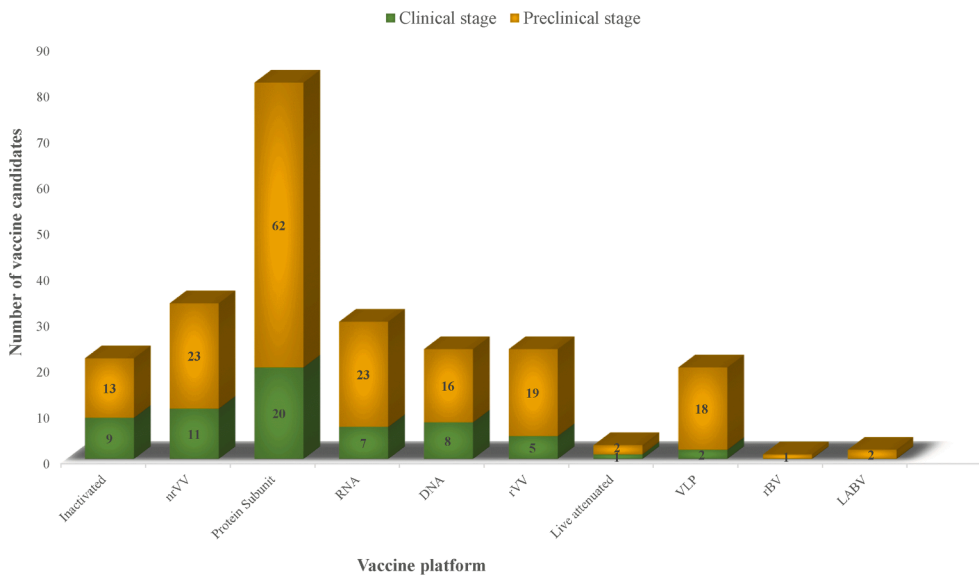


Fig. 1. Profile of vaccine candidates against severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) based on platform and clinical stage. 63 vaccine candidates are in clinical evaluation, while 179 candidates are still in the preclinical stage. The highest frequency is related to protein subunit vaccines and none of the vaccines with LABV and rBV platforms have entered the clinical experiments. nrVV, non-replicating viral vector; rVV, replicating viral vector; VLP, virus-like particles; rBV, replicating bacteria vector; LABV, live attenuated bacterial vector.

deoptimization technology to attaining SARS-CoV2 attenuated vaccine and now they pass through the preclinical stage [102]. COVI-VAC is the only first-in-human live attenuated COVID-19 vaccine that was developed in collaboration with the Serum Institute of India and Codagenix company and is currently in phase I clinical trial.

9. Inactivated vaccines

Inactivated or killed viruses by chemical or physical approaches such as heat and formaldehyde are the alternative vaccine candidates that have been utilized to combat influenza, hepatitis A virus (HAV), and polio (IPV) [103,104]. In this approach, viral particles no longer have the ability to reproduce and pathogenicity, although intact viral antigens with natural conformational structures are provided to induce antibody responses (Fig. 2) (Table 3). Unlike live attenuated vaccines, there is no safety concern about the return of pathogenic species in killed vaccines [105,106]. At present, nine progressive clinical trials along with twelve further inactivated SARS-CoV2 vaccine candidates in the preclinical stage are under investigation [64]. BBIBP-CorV is an inactivated vaccine candidate with aluminum hydroxide adjuvant against SARS-CoV2 that has been tested by Sinofarm company in China on a wide range of animal models and fortunately has shown promising results in inhumane mammals [47]. PiCoVacc is the other inactivated alum-based SARS-CoV2 vaccine by the Chinese Sinovac Biotech Ltd with confirmed preclinical outcomes that protects rhesus macaques against SARS-CoV2 complications [66]. Indeed, vaccination with PiCoVacc diminished viral RNA load and mitigates anti-S and anti-nucleocapsid antibodies-related immunopathology [66]. However, inactivated vaccines are often associated with adjuvants and require repeated doses to induce protective immune responses [9]. Besides, the use of alum adjuvant greatly limits the administration of the respiratory route of vaccines, and it is unclear how long intramuscular injections of BBIBP-CorV and PiCoVacc vaccines can provide mucosal immunity through the delivery of serum antibodies to the lungs. Moreover, the cytotoxic CD8⁺ T cell responses, which are important for coping with COVID-19, are not well stimulated after inactivated vaccines [9,47]. Similar experiences with the respiratory syncytial virus (RSV) and SARS-CoV inactivated vaccines exhibited disease exacerbation, probably due to the dominance of Th2 response and eosinophil accumulation especially in old patients [107,108]. The use of BBIBP-CorV and PiCoVacc vaccines did not worsen the pulmonary side effects in animal models over one week, although alum stimulates Th2 responses, which makes their use questionable. Therefore, the use of Th1 stimulant adjuvants such as

modified alum may alleviate the problem of worsening pulmonary complications about these vaccines [109]. CoronaVac by the Chinese Sinovac Biotech company is the other leading inactivated vaccine with alum adjuvant that has started phase III clinical trials in Indonesia, Brazil, and Turkey from July [64]. The BBV152 is a whole virion inactivated SARS-CoV2 vaccine with aluminum hydroxide gel (Algel) or a novel TLR7/8 agonist adsorbed Algel formulation that has shown promising results in the preclinical phase in mice, rats, and rabbits. Studies have shown that utilizing two different concentrations of this vaccine in all three species induced effective neutralizing antibody responses. Also, the formulation containing TLR7/8 agonist primed Th1-biased antibody responses with elevated IgG2a and increased the response of specific IFN γ -producing CD4⁺ T lymphocytes [110]. The vaccine has now entered the phase III clinical trials but is not yet allowed for limited or emergency applications. Other inactivated vaccine candidates are in the initial stages of clinical trials [64].

10. Viral vector vaccines

Engineered vectors are a novel generation of vaccines that invoke recombinant DNA technology to insert the encoding gene of pathogen antigens into the genome of bacterial or viral vectors [111]. Following vaccination, the recombinant vector sometimes multiplies in the host body and induces potent B and T cell immune responses by expression and processing of pathogen antigens (Fig. 2) (Table 3). *Escherichia coli*, adenovirus (Ad), and poxvirus are among the most widely used bacterial and viral vectors, respectively. Manufacturing of vaccines against meningococcus, hepatitis B virus (HBV), human papillomavirus (HPV), *Haemophilus influenzae* type b (Hib), and pertussis are the most common examples of vector utilization in vaccine design [36]. Viral vectors based on their ability to propagate in the host cells are divided into two main categories including non-replicating and replicating vectors. The non-replicating vectors have lost their reproductive ability by deleting a certain part of their genome, but retain the capacity of expressing a target gene. These are account for a large share of vaccine production and are primarily designed based on adenovirus as well as adeno-associated virus (AAV), Modified Vaccinia virus Ankara (MVA), influenza, parainfluenza, and Sendai viruses [72,112,113]. Most of these vectors are injected intramuscularly and induced passable specific cellular and humoral immune responses. Besides, high titers of these vectors can be achieved by the laboratory instruments [112]. On the other hand, replicating vectors compose attenuated or vaccine-type viruses that expressing the foreign antigen and proliferate somewhat in

Table 2

The pioneers in the race of putative vaccine candidates against COVID-19 according to the World Health Organization reports.

Candidate/ developer	Vaccine platform (description)	Preclinical outcomes	Clinical outcomes	Dosage/Route of administration (Timing)	Clinical stage	Ref.
CoronaVac (PiCoVacc)/ Sinovac Research & Development Co., Ltd	Inactivated (SARS-CoV2 inactivation using β -propiolactone following production in Vero cells)	Neutralizing antibody induction in mice, rat & NHP, partial-to-complete protection in macaques	Safe & immunogenic, induction of neutralizing antibodies in healthy volunteers (~90%)	2/IM (0 & 14 days)	Phase III	[47,64,66,67]
COVID-19 vaccine/ Wuhan Institute of Biological Products & Sinopharm	Inactivated (SARS-CoV2 inactivation using β -propiolactone following production in Vero cells)	Unavailable	Safe & immunogenic with low adverse reactions	2/IM (0 & 21 days)	Phase III	[64,68]
BBIBP-CorV/ Beijing Institute of Biological Products & Sinopharm	Inactivated (SARS-CoV2 inactivation using β -propiolactone following production in Vero cells)	Protection in macaques without ADE, robust neutralizing antibody responses in guinea pigs, mice, rats, rabbits & NHPs even with the lowest dose	Safe & well-tolerated, robust immune response in 100% of vaccine recipients	2/IM (0 & 21 days)	Phase III	[64,66,69,70]
AZD1222 (Covishield)/ University of Oxford & AstraZeneca	Non-Replicating Viral Vector (ChAdOx1-S)	Pneumonia prevention with intangible effects on SARS-CoV2 spread in NHP	High safety, induction of antibody & T cell responses in ~90% of cases	2/IM (0 & 28 days)	Phase III	[64,71,72]
Ad5-nCoV (Convidectia)/ CanSino Biological Inc. & Beijing Institute of Biotechnology	Non-Replicating Viral Vector (adenovirus type 5 vector carrying S protein)	Unavailable	Safe & immunogenic, induction of high RBD binding antibody in 94–100% & specific CD4 ⁺ & CD8 ⁺ T cell responses, high pre-existing anti-Ad5 immunity	1/IM	Phase III	[64,73]
Gam-COVID-Vac (Sputnik V)/ Gamaleya Research Institute	Non-Replicating Viral Vector (adeno-based (rAd26-S + rAd5-S))	Unavailable	Good safety, strong humoral & cellular immune responses (phase I & II trial but small sample), high efficacy (91.6%), immunogenicity & good tolerability in a large cohort study	2/IM (0 & 21 days)	Phase III	[64,74,75]
Ad26.COV2.S/ Janssen Pharmaceutical Companies	Non-Replicating Viral Vector (adenovirus Type 26 vector carrying S protein)	Immunogenicity & protective efficacy, detectable neutralizing antibody induction, effective viral clearance	Safe & immunogenic in younger & older adults	1/IM 2/IM (0 & 56 days)	Phase III	[64,76,77]
NVX-CoV2373/ Novavax	Protein Subunit (Full length recombinant SARS CoV-2 S protein nanoparticle vaccine adjuvanted with Matrix-M1)	Anti-spike neutralizing antibody responses in animal models	Well-tolerated & safe, high levels of antibody induction	2/IM (0 & 21 days)	Phase III	[64,78,79]
mRNA-1273/ Moderna & NIAID	RNA (novel LNP-encapsulated mRNA that encodes full-length S protein of SARS-CoV2)	Protection against SARS-CoV2 infection, induction of neutralizing antibodies & CD8 ⁺ T cells in mice models	Considerable neutralizing antibody (100%) & CD4 ⁺ T cell responses, safe but causes severe complications in high doses	2/IM (0 & 28 days)	Phase III	[64,80,81]
BNT162b2 (Tozinameran or Comirnaty)/ BioNTech, Fosun Pharma & Pfizer	RNA (codon-optimized mRNA encodes SARS-CoV2 full-length S protein encapsulated in 80 nm ionizable cationic lipid nanoparticles)	Protection in rhesus macaques and mice, high neutralizing antibody titers & Th1-biased cellular response in rhesus macaques and mice, induction of virus specific CD4 ⁺ & CD8 ⁺ T cells in macaques	Well-tolerated & highly potent, safe & effective (95%), high neutralizing antibody induction, less systemic reactivity particularly in older adults	2/IM (0 & 21 days)	Phase II/ III	[64,82,83]
COVID-19 vaccine/ Anhui Zhifei Longcom Biopharmaceutical & Institute of Microbiology & Chinese Academy of Sciences	Protein Subunit (adjuvanted recombinant protein (RBD-Dimer) expressed in CHO cells)	Unavailable	Unavailable	3/IM (0, 28 & 56 days) 2/IM (0 & 28 days)	Phase III	[64]
QazCovid-in®/ Research Institute for Biological Safety Problems & Rep of Kazakhstan	Inactivated (inactivated SARS-CoV2)	Unavailable	Unavailable	2/IM (0 & 21 days)	Phase III	[64]
CVnCoV/ Curevac AG	RNA (LNP encapsulated sequence optimized mRNA encodes for full length, pre-fusion stabilized SARS-CoV2 S protein)	Immunogenicity & protective efficacy, robust antibody & T cell responses & full lung protection in NHPs	Unavailable	2/IM (0 & 28 days)	Phase III	[64,84]
			Unavailable	2/IM (0 & 28 days)	Phase III	[64,85]

(continued on next page)

Table 2 (continued)

Candidate/ developer	Vaccine platform (description)	Preclinical outcomes	Clinical outcomes	Dosage/Route of administration (Timing)	Clinical stage	Ref.
Covaxin (BBV152 A, B, C)/ Bharat Biotech	Inactivated (whole-virion inactivated SARS-CoV2)	Protective efficacy, increasing SARS-CoV2 specific IgG & neutralizing antibodies, reducing virus replication in NHPs, pneumonia prevention without severe adverse events	Unavailable	2/IM (0 & 28 days)	Phase III	[64]
COVID-19 vaccine/ Institute of Medical Biology & Chinese Academy of Medical Sciences	Inactivated (inactivated SARS-CoV2)	Unavailable	Unavailable	2/IM (0 & 28 days)	Phase III	[64]
CoVLP/ Medicago Inc.	VLP (plant-derived VLP unadjuvanted or adjuvanted with either CpG 1018 or AS03)	Antibody response induction in mice	Unavailable	2/IM (0 & 21 days)	Phase II/ III	[64,86]
ZyCoV-D/ Zydus Cadila	DNA (plasmid DNA with mammalian expression promoters and the S gene)	Antibody response including neutralizing antibodies & T-cell immunity induction in mice, guinea pig & rabbit models	Unavailable	3/ID (0, 28 & 56 days)	Phase III	[64,87]
UB-612/ COVAXX & United Biomedical Inc	Protein Subunit (high-precision designer S1-RBD-protein containing a Th/CTL epitope peptide pool)	Unavailable	Safe & well-tolerated, induction of specific polyfunctional CD4 ⁺ /CD8 ⁺ T cell responses, specific neutralizing antibodies (100%)	2/IM (0 & 28 days)	Phase III	[64,88]
MVC-COV1901/ Medigen Vaccine Biologics, Dynavax & NIAID	Protein Subunit (S-2P adjuvanted with CpG 1018 and aluminum hydroxide)	Safe, highly immunogenic, & protective in hamsters (high neutralizing antibodies)	Unavailable	2/IM (0 & 28 days)	Phase II/ III	[64,89]
SCB-2019/ Clover Biopharmaceuticals Inc., GSK & Dynavax	Protein Subunit (S-trimer protein formulated with either AS03 or CpG/Alum adjuvants)	Virus protection, strong neutralizing immune responses in NHPs	Safe & well-tolerated, induction of robust humoral & cellular immune responses with high neutralizing activity	2/IM (0 & 28 days)	Phase II/ III	[64,90,91]
AG0301-COVID19/ AnGes, Takara Bio & Osaka University	DNA (plasmid DNA vaccine developed by using an intradermal gene transfer method expressing SARS-CoV2 S protein)	Unavailable	Unavailable	2/IM (0 & 14 days)	Phase II/ III	[64]
INO-4800/ Inovio Pharmaceuticals, International Vaccine Institute & Advaccine (Suzhou) Biopharmaceutical Co., Ltd	DNA (plasmid DNA encoding S protein with electroporation delivery mechanism)	Induction of functional antibody & T-cell responses	Immunogenic, induction of neutralizing antibodies as well as CD4 ⁺ and CD8 ⁺ T cell responses	2/ID (0 & 28 days)	Phase II/ III	[64,92,93]

Ad5, human serotype 5 adenovirus; Ad26, human serotype 26 adenovirus; ChAd, chimpanzee adenovirus; IM, intramuscular; ID, intradermal; IN, intranasal RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; LNP, lipid nanoparticle; NHP, non-Human Primates; ADE, antibody-dependent enhancement

the host cells. Animal viral vectors are more popular in this case because of limited replication in human hosts and significant innate immune induction due to species heterogeneity. Besides, mucosal administration of these xenogenic vectors will significantly stimulate mucosal immunity, which is important in combating mucosal viruses such as SARS-CoV2 [114]. Currently, two human vaccines based on viral vectors have been reported to fight Ebola and cancer maladies. This platform of the Ebola vaccine has been extensively studied and can be used as a model for other infectious diseases, while the safe anti-cancer vector vaccine induced strong T cell responses without the need for adjuvants [115,116]. Meanwhile, some viral vectors, such as Ad5 and ChAd, are preferred for use in SARS-COV2 researches because they provide acceptable protection with a single dose and demonstrate a natural tendency for the respiratory mucosa [61]. Also, this technology is available for the mass production of clinical-grade vaccines. Overall, 41 viral vector vaccine candidates against COVID-19 are under the pre-clinical stage and 16 candidates are undergoing clinical trials [64] while only 3 vaccines based on ChAdOx1, vesicular stomatitis virus (VSV), and

Ad26 viral vectors have been selected for the public-private Operation Warp Speed (OWS) partnership of the US [117]. Viral vector vaccines with attenuated or defective replication capacity against SARS-CoV2 are Ad5 or MVA-dependent and mainly express the epitopes of S protein and related RBD domain. Although the viral vectors with suitable replicative competency are more common with vaccine-type of human (influenza and measles (or zoonotic (VSV) pathogens. It is important to note that in some cases, due to previous exposure of the immune system to similar strains during a person's lifetime or prime-boost regimen, the viral vector is disarmed before any action and does not work as well as it should. This can be overcome by using animal-derived viral vectors such as ChAd or infrequent human vectors, against which the probability of previous immunity is very low or near to zero [61]. Besides, different priming and boosting vectors greatly reduce the risk of previous vector immunity. Also, some viral vectors, such as AAV, are weak stimulants of immune responses and are mostly used in human studies [114].

As of February 9, 2021, four adenovirus-based vector vaccines including Ad5-nCoV (replication-defective Ad5 containing S protein) by

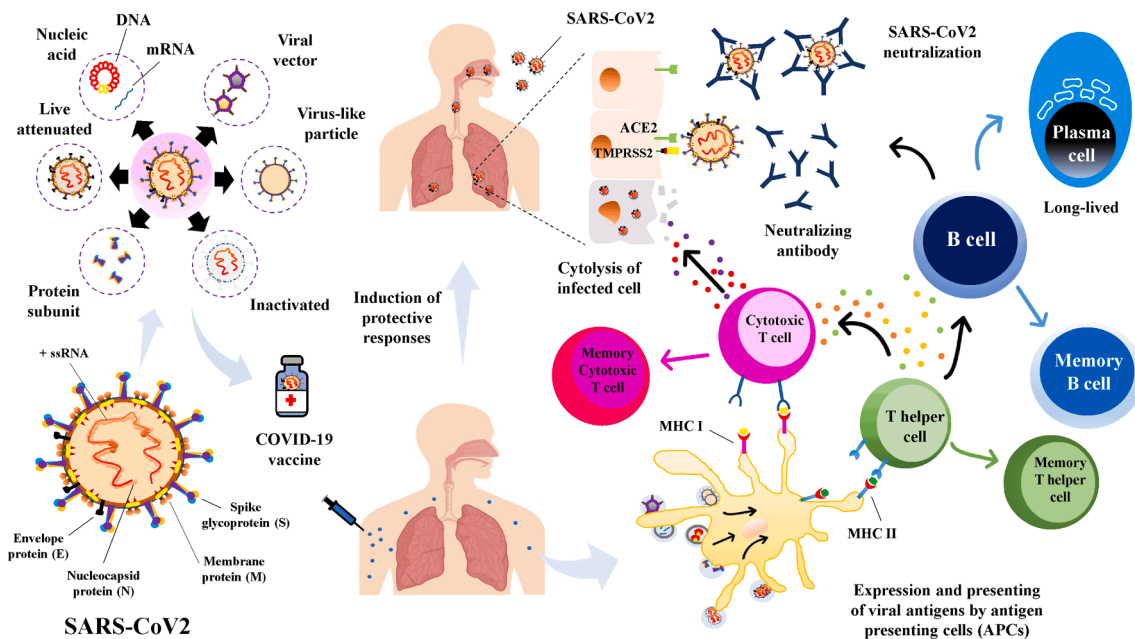


Fig. 2. Potential elicited immune responses by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) vaccine candidate platforms. The six vanguard platforms in COVID-19 vaccine design include viral vector, virus-like particle, nucleic acid (DNA or mRNA), live attenuated, inactivated, and subunit protein vaccines. Following vaccination, the viral particles or encoding genes of SARS-CoV2 proteins are harvested by tissue antigen-presenting cells (APCs), especially dendritic cells. Afterward, the engulfed viral antigens are processed and presented to CD4⁺ T helper (Th) and cytotoxic CD8⁺ T lymphocytes (CTL) by major histocompatibility complex (MHC) class II and I, respectively. Stimulation of Th cells as the conductor of the immune system leads to further induction of CTLs as well as B lymphocyte responses through various soluble and insoluble factors. Accordingly, subsequent the possible SARS-CoV2 infection via angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), the secreted neutralizing antibodies against SARS-CoV2 surface antigens primarily spike protein and CTLs neutralize the virions and remove the infected cells, respectively. Eventually, by producing memory T cells and long-lived plasma cells, the vaccinated individual becomes immune to re-infection with SARS-CoV2.

CanSino Biologics, Sputnik V, or Gam-Covid-Vac (combination of Ad5 and Ad26 containing S protein) by Gamaleya Research Institute, Ad26.COV2.S (optimized Ad26 containing S protein) by Johnson & Johnson and AZD1222 (replication-deficient ChAdOx1 containing S protein) by AstraZeneca company and the University of Oxford are going through phase III clinical trials, and the Ad5-nCoV and Sputnik V have received licenses of limited and early use in China and Russia, respectively. Also, the intranasal spray of influenza vector-based-RBD vaccine, DelNS1-2019-nCoV-RBD-OPT1, as a phase II clinical trial is under investigation. Currently, an innovative COVID-19 artificial antigen-presenting cell (aAPC) vaccine was also developed by Shenzhen Geno-Immune Medical Institute using the replication-competent NHP/TYF lentiviral vector system to expressing the immunomodulatory and viral genes in modified APCs. By doing so, T cells are likely to be significantly activated, although the efficacy and safety of this vaccine in a phase I clinical trial are being investigated. Besides, a similar vaccine, named LV-SMENP-DC, is being evaluated in a phase I/II trial using non-replicating lentiviral vectors from the same company that expresses the COVID-19 SMENP mini-gene along with immunomodulatory genes in DC cells. However, other similar researches based on replication-incompetent vectors including simian adenovirus (SAV), MVA, Ad5 are in development [64].

11. Subunit vaccines

Purified viral antigen peptides such as the S protein of the SARS-CoV2 can be manufactured in various *in vitro* expression systems and applied as safe vaccine candidates. The vaccinated peptide is then processed and delivered in the context of MHC class II, and despite the weak CD8⁺ T cell induction (Fig. 2) (Table 3) [36], it provides strong stimulation to helper CD4⁺ T cells and antibody production. Therefore, the employment of adjuvants and repeated doses are recommended to stimulate as much immunity in this generation of vaccines. Subunit

vaccines are the most common platform of vaccine used to cope with COVID-19 infection, with 20 candidates in the clinical trial evaluation and 62 other vaccines in the preclinical development (44). Most of these vaccines contain all or part of the S protein, which like the SARS and MERS vaccines, induce neutralizing antibody responses [118,119]. One of the positive points of subunit vaccines is the focus of neutralizing antibody responses towards immunodominant epitopes and deflecting of ADE occurrence [120]. Nevertheless, the proteins and peptides encompassing in subunit vaccines can elicit appropriate responses when their expression, translation, and glycosylation were ensued in mammalian eukaryotic systems [121]. Besides, the protein subunit vaccines are unsuitable for mucosal vaccination and the use of unmodified alum adjuvants runs the risk of Th2 responses [107] and fueling the ADE phenomenon [122]. Based on this, 2 COVID-19 subunit vaccines produced by Novavax and GlaxoSmithKline (GSK) companies have employed Matrix-M and AS03 adjuvants to stimulate immune responses, respectively [2]. EpiVacCorona is another leading protein subunit vaccine containing aluminum hydroxide which has been in phase III of the clinical trial since November in Russia. In another effort, a recombinant new protein subunit coronavirus vaccine as the joint product of Anhui Zhifei Longcom Biopharmaceutical, Institute of Microbiology and Chinese Academy of Sciences was designed by CHO cell-expressed full-length S1-human IgG1 Fc fusion protein. Surprisingly, this candidate primed remarkable neutralizing anti-S1 antibody responses in rabbits, mice, and macaques [123]. Other candidates are passing through phase I and II clinical trials.

12. Virus-like particle (VLP) vaccines

VLPs are a group of synthetic or unprompted non-infectious viral like structures that containing prominent structural viral proteins without genetic materials (Fig. 2) (Table 3). This technology has been applying in vaccines against several viral pathogens such as HBV and HPV [124].

Table 3
Immunological characteristics as well as advantages and disadvantages of key vaccine platforms against COVID-19.

Vaccine platform	Preceding cross-reactive immunity	CD4 ⁺ T cell response	CD8 ⁺ T cell response	Neutralizing antibody response	Advantages & disadvantages	Ref.
Whole virus vaccines						
Live attenuated	Mostly cross-reactive T cells, no cross-reactive B cell	Th1	Potent induction	Potent induction	<ul style="list-style-type: none"> • Strong B & T cell responses induction following single delivery, award long-term immunity, independent to adjuvants, confer natural antigenicity • Risk for pathogenic reversion, cold chain requirement 	[9,140]
Inactivated	No cross-reactivity	Th1 or Th2 related to adjuvant system	Poor induction	Potent induction	<ul style="list-style-type: none"> • Safe & stable, no risk of pathogenic reversion, confer natural antigenicity • Poor immunogenicity, need for repeated doses, dependent to adjuvants, costly, inflammatory complications owing to adjuvant 	
Nucleic acid vaccines						
DNA-based	No cross-reactivity	Th1	Not as potent as some viral vectors	Induction	<ul style="list-style-type: none"> • Safe & heat stable, low costs, B & T cell responses induction, quick production, award long-term immunity • Relatively weaker immunity, need for repeated doses, induction, risk for insertional mutagenesis, costly, specific delivery vehicle requirement, dependent to adjuvants, unsuitable for RM delivery 	[9,140]
RNA-based	No cross-reactivity	Th1 or Th2 related to adjuvant system	depends on vaccine formulation & adjuvant system	Induction	<ul style="list-style-type: none"> • B & T cell responses induction, improving antigen presentation, ability of self-adjuvating, quick production, lower probability of adverse effect, no risk of insertional mutagenesis • Need for repeated doses, limited immunogenicity, cold chain requirement, unknown aspects of vaccine delivery & uptake, reluctance to endosomal RNA receptors resulting in faint immune induction, dependent to adjuvants 	
Replicating vector vaccine						
VSV	No cross-reactivity	Mainly Th1	Weaker induction than Ad5 & ChAd in single delivery	Induction	<ul style="list-style-type: none"> • B & T cell responses induction, long-term antigen production, potent immunogenicity with single delivery (VSV) 	[9,140]
Influenza & measles	High probability of cross-reactive B & T cells	Mainly Th1	Good induction via RM delivery	Induction (impressed by preceding cross-reactive immunity & administration route)	<ul style="list-style-type: none"> • Costly large-scale production, risk for disease emergence in incompetent hosts, disarm the vector owing to preceding cross-reactive immunity, weaker immunogenicity relative to Ad & limited human safety data (Influenza & measles), suitable for respiratory mucosal delivery (influenza) 	
Non-replicating vector vaccines						
Ad5	High probability of cross-reactive B & T cells especially in older people	Mainly Th1	Potent induction (impressed by preceding cross-reactive immunity)	Induction (impressed by preceding cross-reactive immunity)	<ul style="list-style-type: none"> • B & T cell responses induction, long-term antigen production, potent immunogenicity with single delivery (Ad5 & ChAd), suitable for respiratory mucosal delivery, established human safety data 	[9,140]
Ad26	Medium probability	Mainly Th1	Medium induction (impressed by preceding cross-reactive immunity)	Induction (impressed by preceding cross-reactive immunity)	<ul style="list-style-type: none"> • Costly large-scale production, risk for disease emergence in incompetent hosts, disarm the vector owing to preceding cross-reactive immunity, weak immunogenicity & need for repeated booster doses (Ad26) 	
ChAd	Almost no cross-reactivity	Mainly Th1	Potent induction	Induction		
Subunit vaccines						
Protein-based	No cross-reactivity	Th1 or Th2 related to adjuvant system	Poor induction	Potent induction	<ul style="list-style-type: none"> • Safe with no risk of infection, selecting highly immunogenic antigens, strong neutralizing antibody induction • Weaker induction of T cell response, decreased immune response over time, need for repeated booster doses, costly, dependent to adjuvants, unsuitable for respiratory mucosal delivery 	[9,140]
Virus-like particle (VLP) vaccines						
VLP	No cross-reactivity	Th1 or Th2 related to adjuvant system	Poor induction	Potent induction	<ul style="list-style-type: none"> • Safe with no risk of infection, strong neutralizing antibody induction, ability of self-adjuvating, cross-linking of surface B cell receptors by condensed & repetitive antigen presentation, established platform for human vaccines • Providing high yield, stable, immunogenic VLP with suitable quality is challenging, risk for a host cell-derived component, need for repeated booster doses 	[9,140]

Concerning coronavirus infections, VLPs are formed in infected eukaryotic cells by active germination and contain E, M, S, and possibly N proteins without the presence of encoding RNA genome [125]. The VLP containing S protein, like infectious viral particles, forays ACE2-expressing cells, but conversely, elicit antibody responses by cross-linking the surface B cell receptors [126]. However, the VLP vaccines, like inactivated and subunit vaccines, require adjuvants and booster doses [124]. These can either be caused by *in vivo* viral vector replication like MVA which expressed VLP crucial protein components or produced *in vitro* by VLP target cells. The well-defined efficacy of VLP-based vaccines together with the known biology and safety of coronavirus VLPs, pave the way for the mass production and Good Manufacturing Practice requirements acquisition of emerging coronavirus VLP vaccines. Of the 20 VLP-based vaccines against COVID-19, only two including CoVLP by Medicago biotechnology company and RBD SARS-CoV2 HBsAg VLP vaccine by Serum Institute of India and Accelagen Pty have arrived the clinical trials while at the rest are completing the preclinical stages [64]. CoVLP is a plant-derived candidate that mimics the wild-type virus without genetic material and involved both antibody and cell-mediated responses in preclinical testing. Currently, in a research partnership between Medicago and Dynavax, as well as Medicago and GSK with or without CPG1018 and AS03 adjuvants respectively, the safety, efficacy, and tolerability of the CoVLP vaccine are being investigated in healthy adults [127]. Also, with the aim of better stimulating antibody responses, the scientists created conjugates of the SARS-CoV2 RBD domain and HBV surface antigen, RBD SARS-CoV2 HBsAg VLP vaccine, that is undergoing phase I clinical evaluations [64]. Surprisingly, A Canadian pharmaceutical company was able to obtain the required VLP for the SARS-CoV2 vaccine using genetically manipulated plants. The results of this study were not published, but apparently, it was able to elicit significant antibody responses in mice [128].

13. Nucleic acid-based vaccines

Novel genetic engineering techniques have facilitated the use of nucleic acids (DNA and RNA) as vaccine candidates. DNA-based vaccines are made by inserting the encoding gene of a foreign antigen into the plasmid DNA, while RNA-based vaccines are made up of mRNA expressing a microbial antigen in a lipid nanoparticle (LNP) coating. Finally, the expressed proteins are delivered to the CD8⁺ T lymphocytes with the help of MHC class I and induce robust T cell responses [36]. Although plasmid DNA has been used as valuable expression platforms for decades, RNA is one of the emerging vehicles in vaccine development (Fig. 2) [129]. Presently, 54 candidates (30 RNA-based and 24 DNA-based) vaccines of this generation have been developed against SARS-CoV2, of which only 8 DNA-based and 7 RNA-based vaccines have been licensed for clinical trials [64].

13.1. DNA-based vaccine

A DNA vaccine is a relatively novel approach that utilizes genetically manipulated DNA to produce microbial antigens. DNA plasmids are common engineered platforms for vaccine production that induced both humoral and cell-mediated immune responses. So, considering the ability of DNA vaccines to induce well-balanced antibody and cellular immune responses, opened a new window towards the use of this platform for therapeutic and preventive purposes (Table 3) [130].

Currently, a patented proposal (WO2005081716) has developed a way to better induce immune responses particularly specific CD8⁺ T cells against DNA-based vaccines for SARS infection. Accordingly, the gene encoding an endoplasmic reticulum chaperone such as calreticulin is embedded with the genes encoding at least a SARS-CoV peptide in the feature of chimeric DNA [131]. In this regard, gene gun transferring the gold-entrapped chimeric DNA encoding the calreticulin-nucleocapsid fusion gene into mice induced specific B and T cell responses against

considered N protein. Moreover, the vaccinated mice were able to significantly reduce the load of challenging vaccinia vector carrying the SARS N gene. The idea of using immunogens derived from conserved sequences of the MERS-CoV spike protein in DNA-based vaccines against MERS infection was also successful and received a patent point (WO2015081155). As expected, the use of conserved sequences as immunogens stimulated notable neutralizing especially the IgG antibodies as well as CD4⁺ and CD8⁺ cellular immunities. IL-2, TNF- α , and IFN γ were also among the cytokines that showed a corresponding increase in vaccinated animals [132]. INO-4800 is a DNA plasmid (pGX9501)-based vaccine candidate against COVID-19 expressing the full-length SARS-CoV2 S protein and developed by the US Inovio Pharmaceutical company (80). Preclinical studies in multiple animal models revealed promising immunogenicity and neutralizing antibody induction against SARS-CoV2 S protein by an INO-4800 vaccine candidate. Besides, the quality of this vaccine has been confirmed and it is currently undergoing phase II/III clinical trials. Other DNA-based vaccine candidates, including AG0301-COVID19, nCov vaccine, GX-19, Covigenix VAX-001, CORVax, and bacTRL-Spike are being evaluated for safety and effectiveness in healthy adults [64].

13.2. RNA-based vaccine

RNA vaccines providing a rapid and cell-free platform for manufacturing viral antigens using the encoding mRNA in the core of LNP covering. LNP content of such vaccines can enhance human immune responses without the need for extra adjuvants [129,133]. Also, the lipid covering easily transports the mRNA into the cytoplasm of the cells, and unlike protein subunit vaccines facilitating effective protein translation and post-translational modifications. Besides, *in vitro* transcription is employed for pathogen mRNA achievement, so there is no risk of transmitting infectious agents or microbial components. Remarkable safety and efficacy, free risk of anti-vector immune responses, prompt and cost-effective production along the possibility of repeated administration are some of the advantages of mRNA-based over other types of vaccines [134] that make them more attractive in COVID-19 vaccine researches. Generally, the conventional mRNA and the novel self-replicating and transcribing RNA (replicon) vaccines constitute the two major classes of RNA-based vaccines. In conventional strategy, the immunogenic viral protein is produced directly from the transcript included in the vaccine formulation, while replicon vaccines encode replication machinery of an alphavirus that contains the target gene. So, new RNA vaccines multiply the transcript of the viral antigen several times for a long time and attained strong elicitation of innate and adaptive immune responses. Besides, similar to live attenuated vaccines, the dose sparing phenomenon is traceable after injection of this type of RNA vaccine [135]. Another amazing feature of mRNA vaccine is the possibility of simultaneous containing of multiple mRNAs in a single dose of vaccine and applying as prophylaxis because of its ability to induce immune responses similar to natural infection (Table 3). In this regard, the mRNA vaccine produced by Moderna Company, whose patent has been issued, was able to mix mRNA encoding whole S protein, as well as S1 and S2 subunits from MERS-CoV and SARS-CoV in the context of positive charge LNPs. During the vaccination program, it was found that animals that received mRNA encoding the S2 subunit produced significantly fewer neutralizing antibodies than animals vaccinated with mRNA encoding the complete structure of the S protein. The use of mRNA encoding the full-length MERS-CoV S protein in white rabbits, in addition to a 90% reduction in viral load, produced a substantial neutralizing antibody response against MERS-CoV particles (WO2017070626). A previous patented study described that exploiting mRNA encoding ideally the S protein or S1 subunit, E and M, or N proteins would be effective in priming antigen-specific responses against MERS infection (WO2018115527). Similarly, intradermal injection of mRNA complex-entrapped in lipid capsules encoding the S protein of the MERS-CoV into mice induced specific antibody responses. Therefore,

based on the used strategies and methods in the previously registered patents for mRNA vaccines, Moderna finally unveiled the first shipment of human mRNA vaccines against COVID-19 called mRNA-1273 in the last week of February 2020. The mRNA-1273 vaccine contains the mRNA encoding a prefusion and stable conformation of SARS-CoV2 S protein that was developed in collaboration with Moderna and the National Institute of Allergy and Infectious Diseases (NIAID) and funded by the Global Coalition for Epidemic Preparedness Innovations (CEPI) partnership. BNT162 is the other anti-COVID-19 mRNA vaccine that four variants including a1, b1, b2, and c2 based on various combinations of mRNA formats in LNPs has released and received obligatory approvals from German regulators for further studies [63,132]. CVnCoV is the other lipid nanoparticle captured non-modified mRNA COVID-19 vaccine candidate that encodes full-length spike protein. Following mice and hamsters' immunization with CVnCoV, potent anti-spike neutralizing antibodies along with strong Th and cytotoxic T cell responses especially in mice models were induced. The lung tissue of vaccinated hamsters preserved incredibly after deliberate infection with the SARS-CoV2 pathogen. Also, suboptimal vaccination in hamsters not only abort viral replication but also left no adverse effects and provided substantial safety [136]. Arcturus Therapeutics incorporation discloses an innovative COVID-19 vaccine (LUNAR®-COV19 (ARCT-021)) which obtain encouraging outcomes following a single shot in lab animals. This replicon vaccine utilizes the STARR™ technology to elicit strong and protracted SARS-CoV2 spike protein expression. Mice vaccination with a single dose of ARCT-021 led to heavy neutralizing antibody responses, which gradually increased within two months after injection. Besides, robust anti-spike specific CD8⁺ T cell and Th1 responses were induced and human ACE2 transgenic mice were largely immunized against SARS-CoV2 challenge after ARCT-021 vaccination [137]. LNP-nCoVsaRNA is another self-amplifying RNA vaccine candidate against COVID-19 that was developed by Imperial College London University and has recently entered the safety phase I clinical trials. This vaccine encodes the spike protein of the SARS-CoV2 and its intramuscular injection in mice provokes specific IgG antibody and Th1 responses dose-dependently [138]. Another positive point is that the design of this vaccine will be completed in 14 days [139]. Other LNP-encapsulated vaccine candidates, including ChulaCov19 and SARS-CoV2 mRNA vaccine, are evaluating immunogenicity, tolerability, and safety in early clinical trials [64].

14. Future-oriented discussion

The world is still battling the novel SARS-CoV2 virus, and to date, various companies and research institutes have offered several treatment strategies to combat the pandemic. Given the experience of parallel coronavirus epidemics during the past decades, the only solution seems to obtain a safe and effective vaccine against COVID-19. Tireless efforts have led to the development of 242 COVID-19 vaccine candidates, of which 20 have entered the phase III large-scale efficacy human trials. Although we are still in the early stages of SARS-CoV2 identification and vaccine preparation, multiple similar vaccines especially based on advanced platforms have been extensively studied for other infectious diseases and cancers. Therefore, applying the existing knowledge available in similar researches can guide us in using the best vaccination strategy and platform. Most current researches on COVID-19 vaccine candidates have focused on intramuscular or skin administration. Based on the initial findings of the present studies and considering similar previous researches, it can be inferred that COVID-19 parenteral vaccines are most likely to protect through the induction of durable neutralizing antibodies and acceptable T cell responses. On the other hand, SARS-CoV2 is mainly transmitted through respiratory ducts and causes annoying pulmonary symptoms, so paying attention to respiratory mucosal vaccination strategies, especially in high-risk people, may lead to the initial control and clearance of the SARS-CoV2. Moreover, this mucosal vaccination strategy is needle-free and depends on a

lower dose of antigen than parenteral vaccines. However, not all vaccine platforms are safe and effective for respiratory mucosal vaccination, and providing broad-spectrum inhaler vehicles for mucosal vaccine delivery is one of the crucial limitations. As mentioned, the use of vaccine platforms that depends primarily on adjuvants to strongly stimulate especially T cell responses are costly and not suitable for respiratory mucosal administration. On the other hand, attenuated live vaccines are not recommended, especially for highly mutable viruses such as SARS-CoV2, due to the increased risk of pathogenic conversion. Viral vector vaccines are also potent stimulants of antibody and T cell responses, but sometimes their effectiveness is affected by pre-existing cross-reactive immunity. VLP-based vaccines are also the other potential candidates with established capacities in human studies. Although providing suitable VLP that covering all the expected characteristics is challenging. Nucleic acid vaccines also have a high chance of success against COVID-19, but there are obstacles such as lack of human safety data, need for the specific delivery vehicle, and depending on adjuvants. With this in mind, it seems that vaccines based on advanced platforms such as VLP, viral vector, and nucleic acid vaccines have a higher chance of success in the COVID-19 vaccine race. Given the current situation, the pattern of vaccine design and manufacturing has been greatly overstuffed and led to even preclinical and clinical evaluations running in parallel. Therefore, the provisional data from the initial analysis of vaccine studies are being available in real-time, but it does not provide valuable information regarding the durability and quality of obtained protective immunity. In many countries, the transmission rate and the new cases of the COVID-19 disease are significantly declining, and it is unclear whether the results of operating clinical trials of pioneering vaccine candidates in such volunteer countries will be reliable. Also, the separately reported efficacy of some vaccine candidates in various areas makes it a bit difficult to compare them simultaneously, and it is still too early for goal celebration in achieving a suitable efficacy and safety for COVID-19 vaccine candidates. However, given the current critical situation, the emergency application of vaccine candidates with approved preclinical potential and encouraging but limited clinical outcomes is the best solution, at least for endangered people. Inevitably, the evolving clinical trials will continue in the coming years until the longevity and quality of vaccine-induced immunity, as well as the functionality of vaccination strategies, be better understood. Therefore, until attaining a certain level of confidence in COVID-19 vaccine candidates, universal vaccination of all masses is unreasonable. It is noteworthy that, due to the existing challenges such as providing resources, formulating and distribution as well as ecumenical available different vaccine strategies and platforms, the implementation of the vaccination program will not be smooth and uniform. Hence, foundations such as COVID-19 Vaccines Global Access (COVAX) and the Coalition for Epidemic Preparedness Innovations (CEPI) have been set up to do their utmost to unite rich and low-income countries to achieve fair, transparent, and rapid access to the most effective COVID-19 vaccine candidates globally.

15. Conclusion

So far, multiple companies and research institutes globally have offered hundreds of vaccine candidates with well-established and/or just-released platforms against COVID-19. The majority are administered by intramuscular or dermal injection and, fortunately, have been able to induce acceptable humoral and T cell responses. Due to the rifle mutation of SARS-CoV2 and its major mucosal transmission, the most imperative issues are the factual longevity and efficacy of induced immune responses against SARS-CoV2 as well as stimulation of mucosal immunity, especially the response of respiratory mucosal resident T cells. The other challenge is that not all vaccine platforms are safe and effective for mucosal vaccination, and it is unclear whether current candidate vaccines can withstand future SARS-CoV2 mutants. Therefore, directing future researches to stimulate respiratory mucosal immunity and determine their efficacy against prominent mutants should

be on the agenda. On the other hand, human safety data is unavailable on some of the sophomore vaccine platforms, including RNA-based vaccines, which makes their use more cautious. Also, fair distribution of effective vaccines should be provided among all countries of the world, especially low-income regions. Therefore, until the safety and efficacy dimensions of the current vaccine candidates become clear, their widespread practice is not reasonable except for groups with high mortality and morbidity risk and in emergency cases all around the world. Otherwise, their application may be equivalent to the proverb “The cure is worse than the disease!”.

Authors' contributions

The authors contributed equally to all aspects of the article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank the Shiraz and Shahid Beheshti University of Medical Sciences.

References

- [1] F. Song, et al., Emerging 2019 Novel Coronavirus (2019-nCoV) Pneumonia, *Radiology* 295 (1) (2020) 210–217.
- [2] D. Cucinotta, M. Vanelli, WHO Declares COVID-19 a Pandemic, *Acta Biomed.* 91 (1) (2020) 157–160.
- [3] B. Hu, et al., Characteristics of SARS-CoV-2 and COVID-19, *Nat. Rev. Microbiol.* 20 (2020) 1–14.
- [4] R. Singla, et al., Human animal interface of SARS-CoV-2 (COVID-19) transmission: a critical appraisal of scientific evidence, *Vet. Res. Commun.* 44 (3–4) (2020) 119–130.
- [5] M. Pal, et al., Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update, *Cureus* 12 (3) (2020) e7423 e7423.
- [6] Y.A. Malik, Properties of Coronavirus and SARS-CoV-2, *Malays. J. Pathol.* 42 (1) (2020) 3–11.
- [7] W. Ni, et al., Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19, *Crit. Care* 24 (1) (2020), 422–422.
- [8] M. Bayat, et al., An overview of some potential immunotherapeutic options against COVID-19, *Int. Immunopharmacol.* 95 (2021), 107516.
- [9] M. Jeyanathan, et al., Immunological considerations for COVID-19 vaccine strategies, *Nat. Rev. Immunol.* 20 (10) (2020) 615–632.
- [10] E. Prompetchara, et al., Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic, *Asian Pac. J. Allergy Immunol.* 38 (1) (2020) 1–9.
- [11] M. Merad, J.C. Martin, Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages, *Nat. Rev. Immunol.* 20 (6) (2020) 1–8.
- [12] Y. Zhou, et al., Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients, *Natl. Sci. Rev.* 7 (6) (2020) 998–1002.
- [13] F. Zhou, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, *Lancet* 395 (10229) (2020) 1054–1062.
- [14] D. Blanco-Melo, et al., Imbalanced host response to SARS-CoV-2 drives development of COVID-19, *Cell* 181 (5) (2020) 1036–1045.e9.
- [15] S. Gujar, et al., Repurposing CD8+ T cell immunity against SARS-CoV-2 for cancer immunotherapy: a positive aspect of the COVID-19 pandemic? *Oncoimmunology* 9 (1) (2020) 1794424.
- [16] Z. Chen, E.J. Wherry, T cell responses in patients with COVID-19, *Nat. Rev. Immunol.* 20 (9) (2020) 529–536.
- [17] C. Melenotte, et al., Immune responses during COVID-19 infection, *Oncoimmunology* 9 (1) (2020) 1807836.
- [18] M. Hellfritsch, R. Scherließ, Mucosal vaccination via the respiratory tract, *Pharmaceutics* 11 (8) (2019) 375.
- [19] J.R. Teijaro, D.L. Farber, COVID-19 vaccines: modes of immune activation and future challenges, *Nat. Rev. Immunol.* 21 (2021) 1–3.
- [20] S.C. Gilbert, T-cell-inducing vaccines—what's the future, *Immunology* 135 (1) (2012) 19–26.
- [21] S.M. Todryk, T cell memory to vaccination, *Multidisciplinary Digital Publishing Institute* (2018) 84.
- [22] R. Singh, et al., COVID-19: Current knowledge in clinical features, immunological responses, and vaccine development, *FASEB J.* 35 (3) (2021), e21409.
- [23] A. Grifoni, et al., Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals, *Cell* 181(7) (2020) 1489–1501. e15.
- [24] J. Braun, et al., SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19, *Nature* 587 (7833) (2020) 270–274.
- [25] J. Mateus, et al., Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans, *Science* 370 (6512) (2020) 89–94.
- [26] N. Le Bert, et al., SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls, *Nature* 584 (7821) (2020) 457–462.
- [27] J. Zhao, et al., Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, *Clin. Infect. Dis.* 71 (16) (2020) 2027–2034.
- [28] A. Grifoni, et al., Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals, *Cell* (2020).
- [29] J. Seow, et al., Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection, *MedRxiv* 20 (2020) 48429.
- [30] A.C. Walls, et al., Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, *Cell* 181 (2) (2020) 281–292.
- [31] S. Jiang, et al., Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses, *Trends Immunol.* 41 (5) (2020) 355–359.
- [32] J. Duan, et al., A human SARS-CoV neutralizing antibody against epitope on S2 protein, *Biochem. Biophys. Res. Commun.* 333 (1) (2005) 186–193.
- [33] K. Nakanaga, et al., Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice, *J. Virol.* 59 (1) (1986) 168–171.
- [34] H.-Q. Yu, et al., Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients, *Eur. Respir. J.* 56 (2) (2020) 2001526.
- [35] A. Sariol, S. Perlman, Lessons for COVID-19 immunity from other coronavirus infections, *Immunity* 53 (2) (2020) 248–263.
- [36] M.S. Khuroo, et al., COVID-19 Vaccines: A Race Against Time in the Middle of Death and Devastation!, *J. Clin. Exp. Hepatol.* 10 (6) (2020) 610–621.
- [37] I.N. Abdullahi, et al., Implications of SARS-CoV-2 genetic diversity and mutations on pathogenicity of the COVID-19 and biomedical interventions, *J. Taibah Univ. Med. Sci.* 15 (4) (2020) 258–264.
- [38] L. van Dorp, et al., Emergence of genomic diversity and recurrent mutations in SARS-CoV-2, *Infect. Genet. Evol.* 83 (2020), 104351.
- [39] I. Thevarajan, et al., Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19, *Nat. Med.* 26 (4) (2020) 453–455.
- [40] Y. Chen, L. Li, SARS-CoV-2: virus dynamics and host response, *Lancet Infect. Dis.* 20 (5) (2020) 515–516.
- [41] R.L. Tillett, et al., Genomic evidence for reinfection with SARS-CoV-2: a case study, *Lancet Infect. Dis.* 21 (1) (2021) 52–58.
- [42] C.Y. Yong, et al., Recent advances in the vaccine development against Middle East respiratory syndrome-coronavirus, *Front. Microbiol.* 10 (2019) 1781.
- [43] R.L. Roper, K.E. Rehm, SARS vaccines: where are we? *Expert Rev. Vaccines* 8 (7) (2009) 887–898.
- [44] L. Peeples, News Feature: Avoiding pitfalls in the pursuit of a COVID-19 vaccine, *PNAS* 117 (15) (2020) 8218–8221.
- [45] U.J. Buchholz, et al., Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity, *PNAS* 101 (26) (2004) 9804–9809.
- [46] S.F. Ahmed, et al., Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies, *Viruses* 12 (3) (2020) 254.
- [47] Q. Gao, et al., Development of an inactivated vaccine candidate for SARS-CoV-2, *Science* 369 (6499) (2020) 77.
- [48] R. Sealy, et al., Preclinical and clinical development of a multi-envelope, DNA-virus-protein (DVP) HIV-1 vaccine, *Int. Rev. Immunol.* 28 (1–2) (2009) 49–68.
- [49] M. Enayatkhani, et al., Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: an in silico study, *J. Biomol. Struct. Dyn.* 1–16 (2020).
- [50] B. Sarkar, et al., Immunoinformatics-guided designing of epitope-based subunit vaccines against the SARS Coronavirus-2 (SARS-CoV-2), *Immunobiology* 225 (3) (2020), 151955.
- [51] M. Bhattacharya, et al., Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-CoV-2): Immunoinformatics approach, *J. Med. Virol.* 92 (6) (2020) 618–631.
- [52] N. Rahman, et al., Vaccine design from the ensemble of surface glycoprotein epitopes of SARS-CoV-2: An immunoinformatics approach, *Vaccines* 8 (3) (2020) 423.
- [53] Y. Dai, et al., Immunodominant regions prediction of nucleocapsid protein for SARS-CoV-2 early diagnosis: a bioinformatics and immunoinformatics study, *Pathog. Glob. Health* 114 (8) (2020) 463–470.
- [54] S. Rauch, et al., New vaccine technologies to combat outbreak situations, *Front. Immunol.* 9 (2018) 1963.
- [55] T. Gupta, S.K. Gupta, Potential adjuvants for the development of a SARS-CoV-2 vaccine based on experimental results from similar coronaviruses, *Int. Immunopharmacol.* 86 (2020), 106717.
- [56] M. Jeyanathan, et al., New tuberculosis vaccine strategies: taking aim at unnatural immunity, *Trends Immunol.* 39 (5) (2018) 419–433.

- [57] L. Moreno-Fierros, et al., Development of SARS-CoV-2 vaccines: should we focus on mucosal immunity? Taylor & Francis, 2020.
- [58] I.M. Belyakov, J.D. Ahlers, What role does the route of immunization play in the generation of protective immunity against mucosal pathogens? *J. Immunol.* 183 (11) (2009) 6883–6892.
- [59] J. Zhao, et al., Airway memory CD4+ T cells mediate protective immunity against emerging respiratory coronaviruses, *Immunity* 44 (6) (2016) 1379–1391.
- [60] Z. Xing, et al., Innate immune memory of tissue-resident macrophages and trained innate immunity: Re-vamping vaccine concept and strategies, *J. Leukoc. Biol.* 108 (3) (2020) 825–834.
- [61] S. Afkhami, et al., Methods and clinical development of adenovirus-vectored vaccines against mucosal pathogens, *Mol. Ther. Methods Clin. Dev.* 3 (2016) 16030.
- [62] W.H. Organization, Guidelines on clinical evaluation of vaccines: regulatory expectations, World Health Organization WHO Expert Committee on Biological Standardization. Fifty-second report. Geneva, World Health, Organization 26 (23) (2004) 4113–4119.
- [63] M.S. Khuroo, et al., COVID-19 vaccines: A race against time in the middle of death and devastation!, *J. Clin. Exp. Hepatol.*, 2020.
- [64] W.H. Organization, Draft landscape and tracker of COVID-19 candidate vaccines, 2020. https://cdn.who.int/media/docs/default-source/blue-print/novel-coronavirus-landscape-covid-19_09feb.xlsx?sfvrsn=4feda45_5&download=true. (Accessed February, 9, 2021).
- [65] C.J. Zimmer Carl, Wee Sui-Lee, Coronavirus Vaccine Tracker, 2020 <https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html>. (Accessed 27, December, 2020).
- [66] H. Wang, et al., Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2, *Cell* 182(3) (2020) 713–721. e9.
- [67] H.X. Lim, et al., Development of multi-epitope peptide-based vaccines against SARS-CoV-2, *Biomed. J.* (2020).
- [68] S. Xia, et al., Effect of an Inactivated Vaccine Against SARS-CoV-2 on Safety and Immunogenicity Outcomes: Interim Analysis of 2 Randomized Clinical Trials, *JAMA* 324 (10) (2020) 951–960.
- [69] W.-J. Guan, et al., Clinical characteristics of coronavirus disease 2019 in China, *N. Engl. J. Med.* 382 (18) (2020) 1708–1720.
- [70] S. Xia, et al., Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial, *Lancet Infect. Dis.* 21 (1) (2021) 39–51.
- [71] N. van Doremalen, et al., ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques, *Nature* 586 (7830) (2020) 578–582.
- [72] P.M. Folegatti, et al., Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial, *Lancet* 396 (10249) (2020) 467–478.
- [73] K. Rawat, et al., COVID-19 vaccine: A recent update in pipeline vaccines, their design and development strategies, *Eur. J. Pharmacol.* 892 (2021), 173751.
- [74] D.Y. Logunov, et al., Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia, *Lancet* 6736 (21) (2021) 234–238.
- [75] D.Y. Logunov, et al., Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia, *Lancet* 396 (10255) (2020) 887–897.
- [76] J. Sadoff, et al., Interim Results of a Phase 1–2a Trial of Ad26. COV2. S Covid-19 Vaccine, *N. Engl. J. Med.* 20 (34) (2021) 201.
- [77] L. Dai, G.F. Gao, Viral targets for vaccines against COVID-19, *Nat. Rev. Immunol.* 21 (2) (2020) 73–82.
- [78] N.i.o. health, Phase 3 trial of Novavax investigational COVID-19 vaccine opens, 2020. <https://www.nih.gov/news-events/news-releases/phase-3-trial-novavax-investigational-covid-19-vaccine-opens>.
- [79] L. Mukhopadhyay, et al., Comparison of the immunogenicity & protective efficacy of various SARS-CoV-2 vaccine candidates in non-human primates, *Indian J. Med. Res.* 153 (1) (2020) 93–114.
- [80] L.A. Jackson, et al., An mRNA vaccine against SARS-CoV-2—preliminary report, *N. Engl. J. Med.* 383 (2020) 1920–1931.
- [81] K.S. Corbett, et al., SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness, *Nature* 586 (7830) (2020) 567–571.
- [82] F.P. Polack, et al., Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine, *N. Engl. J. Med.* 383 (27) (2020) 2603–2615.
- [83] A. Cagigi, K. Loré, Immune Responses Induced by mRNA Vaccination in Mice, Monkeys and Humans, *Vaccines* 9 (1) (2021) 61.
- [84] S. Rauch, et al., mRNA vaccine CVnCoV protects non-human primates from SARS-CoV-2 challenge infection, *bioRxiv* 12 (23) (2020), 424138.
- [85] Bharatbiotech, COVAXIN™ (BBV152) Shows Demonstration of Protective Efficacy and Immunogenicity in Non-Human Primates, 2020. <https://www.bharatbiotech.com/blog/vaccines/covaxin-animal-study-results/>. (Accessed September, 11, 2020).
- [86] J. Machhi, et al., Nanocarrier Vaccines for SARS-CoV-2, *Adv. Drug Deliv. Rev.* 171 (2021) 215–239.
- [87] P. Yadav, et al., Assessment of immunogenicity and protective efficacy of ZyCoV-D DNA vaccine candidates in Rhesus macaques against SARS-CoV-2 infection, *bioRxiv* 2 (2021), 429480.
- [88] B. Wire, COVAXX's COVID-19 Vaccine, UB-612, Induced Neutralizing Antibodies in 100% of Participants During Phase 1 Clinical Trial, 2021. <https://www.businesswire.com/news/home/20210208005198/en/COVAXX%E2%80%99s-COVID-19-Vaccine-UB-612-Induced-Neutralizing-Antibodies-in-100-of-Participants-During-Phase-1-Clinical-Trial>.
- [89] C.-E. Lien, et al., CpG-adjuvanted stable prefusion SARS-CoV-2 spike protein protected hamsters from SARS-CoV-2 challenge, *bioRxiv* 1 (7) (2021), 425674.
- [90] P. Richmond, et al., Safety and immunogenicity of S-Trimer (SCB-2019), a protein subunit vaccine candidate for COVID-19 in healthy adults: a phase 1, randomised, double-blind, placebo-controlled trial, *Lancet* 6736 (21) (2021) 241–245.
- [91] Precisionvaccinations, COVID-19 S-Trimer (SCB-2019) Vaccine, 2021. <https://www.precisionvaccinations.com/vaccines/covid-19-s-trimer-scb-2019-vaccine>.
- [92] P. Tebas, et al., Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, Phase 1 clinical trial, *EClinicalMedicine* 31 (2021), 100689.
- [93] T.R. Smith, et al., Immunogenicity of a DNA vaccine candidate for COVID-19, *Nat. Commun.* 11 (1) (2020) 1–13.
- [94] S. Plotkin, History of vaccination, *PNAS* 111 (34) (2014) 12283–12287.
- [95] F. Almazán, et al., Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate, *MBio* 4 (5) (2013).
- [96] J. Netland, et al., Immunization with an attenuated severe acute respiratory syndrome coronavirus deleted in E protein protects against lethal respiratory disease, *Virology* 399 (1) (2010) 120–128.
- [97] Y. Hou, et al., Deletion of both the tyrosine-based endocytosis signal and the endoplasmic reticulum retrieval signal in the cytoplasmic tail of spike protein attenuates porcine epidemic diarrhoea virus in pigs, *J. Virol.* 93 (2) (2019) e01758–e1818.
- [98] J.M. Jimenez-Guardeño, et al., Identification of the mechanisms causing reversion to virulence in an attenuated SARS-CoV for the design of a genetically stable vaccine, *PLoS Pathog.* 11 (10) (2015), e1005215.
- [99] B.Y. Cheng, et al., Development of live-attenuated arenavirus vaccines based on codon deoptimization, *J. Virol.* 89 (7) (2015) 3523–3533.
- [100] S. Mueller, et al., A codon-pair deoptimized live-attenuated vaccine against respiratory syncytial virus is immunogenic and efficacious in non-human primates, *Vaccine* 38 (14) (2020) 2943–2948.
- [101] Y. Tao, et al., Surveillance of Bat Coronaviruses in Kenya Identifies Relatives of Human Coronaviruses NL63 and 229E and Their Recombination History, *J. Virol.* 91 (5) (2017) e01953–e2016.
- [102] W.H. Organization, Draft landscape of COVID-19 candidate vaccines. <https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>.
- [103] A.D. Murdin, et al., Inactivated poliovirus vaccine: past and present experience, *Vaccine* 14 (8) (1996) 735–746.
- [104] J.M. Wood, J.S. Robertson, From lethal virus to life-saving vaccine: developing inactivated vaccines for pandemic influenza, *Nat. Rev. Microbiol.* 2 (10) (2004) 842–847.
- [105] M.T. Ul Qamar, et al., Epitope-based peptide vaccine design and target site depiction against Middle East Respiratory Syndrome Coronavirus: an immunoinformatics study, *J. Transl. Med.* 17 (1) (2019) 362.
- [106] Y. Watanabe, et al., Site-specific glycan analysis of the SARS-CoV-2 spike, *Science* 369 (6501) (2020) 330–333.
- [107] M. Bolles, et al., A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge, *J. Virol.* 85 (23) (2011) 12201–12215.
- [108] N. Iwata-Yoshikawa, et al., Effects of Toll-like receptor stimulation on eosinophilic infiltration in lungs of BALB/c mice immunized with UV-inactivated severe acute respiratory syndrome-related coronavirus vaccine, *J. Virol.* 88 (15) (2014) 8597–8614.
- [109] G. Del Giudice, et al., Correlates of adjuvanticity: a review on adjuvants in licensed vaccines, *Seminars in Immunology*, Elsevier (2018) 14–21.
- [110] B. Ganneru, et al., Evaluation of Safety and Immunogenicity of an Adjuvanted, TH-1 Skewed, Whole Virion Inactivated SARS-CoV-2 Vaccine - BBV152, *bioRxiv* 9 (2020) 2025445.
- [111] K.J. Ewer, et al., Viral vectors as vaccine platforms: from immunogenicity to impact, *Curr. Opin. Immunol.* 41 (2016) 47–54.
- [112] F.-C. Zhu, et al., Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial, *Lancet* 396 (10249) (2020) 479–488.
- [113] S.P. Graham, et al., Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19, *NPJ vaccines* 5 (1) (2020) 1–6.
- [114] F. Krammer, SARS-CoV-2 vaccines in development, *Nature* 586 (7830) (2020) 516–527.
- [115] I.R. Humphreys, S. Sebastian, Novel viral vectors in infectious diseases, *Immunology* 153 (1) (2018) 1–9.
- [116] S.J. Draper, J.L. Heeney, Viruses as vaccine vectors for infectious diseases and cancer, *Nat. Rev. Microbiol.* 8 (1) (2010) 62–73.
- [117] J. Cohen, Top US scientists left out of White House selection of COVID-19 vaccine short list, *Science* (2020).
- [118] H. Mou, et al., The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies, *J. Virol.* 87 (16) (2013) 9379–9383.
- [119] Y. Guo, et al., Elicitation of immunity in mice after immunization with the S2 subunit of the severe acute respiratory syndrome coronavirus, *DNA Cell Biol.* 24 (8) (2005) 510–515.
- [120] J. Oscherwitz, The promise and challenge of epitope-focused vaccines, *Hum. Vaccines Immunother.* 12 (8) (2016) 2113–2116.

- [121] L. Du, et al., Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and *E. coli* cells elicits potent neutralizing antibody and protective immunity, *Virology* 393 (1) (2009) 144–150.
- [122] M.S. Diamond, T.C. Pierson, The challenges of vaccine development against a new virus during a pandemic, *Cell Host Microbe* 27 (5) (2020) 699–703.
- [123] W. Ren, et al., Recombinant SARS-CoV-2 spike S1-Fc fusion protein induced high levels of neutralizing responses in nonhuman primates, *Vaccine* 38 (35) (2020) 5653–5658.
- [124] B. Donaldson, et al., Virus-like particle vaccines: immunology and formulation for clinical translation, *Expert Rev. Vaccines* 17 (9) (2018) 833–849.
- [125] X. Lu, et al., Immune responses against severe acute respiratory syndrome coronavirus induced by virus-like particles in mice, *Immunology* 122 (4) (2007) 496–502.
- [126] A. Naskalska, et al., Novel coronavirus-like particles targeting cells lining the respiratory tract, *PLoS one* 13 (9) (2018), e0203489.
- [127] DrugBank, Coronavirus-Like Particle COVID-19 Vaccine, 2020. <https://go.drugbank.com/drugs/DB15852>.
- [128] B. Wire, Medicago announces positive results in animal trials for its vaccine candidate against COVID-19. , 2020. <https://www.stinfo.fi/tiedote/medicago-announces-positive-results-in-animal-trials-for-its-vaccine-candidate-against-covid-19?publisherId=58763726&releaseId=69881188>.
- [129] N.A. Jackson, et al., The promise of mRNA vaccines: A biotech and industrial perspective, *NPJ vaccines* 5 (1) (2020) 1–6.
- [130] B. Ferraro, et al., Clinical applications of DNA vaccines: current progress, *Clin. Infect. Dis.* 53 (3) (2011) 296–302.
- [131] L. Paladino, et al., The Role of Molecular Chaperones in Virus Infection and Implications for Understanding and Treating COVID-19, *J. Clin. Med.* 9 (11) (2020) 3518.
- [132] C. Liu, et al., Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases, ACS Publications (2020).
- [133] K.J. Hassett, et al., Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines, *Mol. Ther. Nucleic Acids* 15 (2019) 1–11.
- [134] N. Pardi, et al., mRNA vaccines—a new era in vaccinology, *Nat. Rev. Drug Discov.* 17 (4) (2018) 261.
- [135] D.H. Fuller, P. Berglund, Amplifying RNA vaccine development, *N. Engl. J. Med.* 382 (25) (2020) 2469–2471.
- [136] S. Rauch, et al., mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents, *bioRxiv* 10 (23) (2020), 351775.
- [137] R.M. de Alwis, et al., A Single Dose of Self-Transcribing and Replicating RNA Based SARS-CoV-2 Vaccine Produces Protective Adaptive Immunity In Mice, *bioRxiv* (2020).
- [138] P.F. McKay, et al., Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice, *Nat. Commun.* 11 (1) (2020) 1–7.
- [139] S. Andrew, Imperial social enterprise to accelerate low-cost COVID-19 vaccine, 2020.
- [140] A. Haque, A.B. Pant, Efforts at COVID-19 Vaccine Development: Challenges and Successes, *Vacces*. 8(4) 739.