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Review

Potential SARS-CoV-2 vaccines: Concept, progress, and challenges

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ABSTRACT

Since September 2020, the world has had more than 28 million cases of coronavirus disease 2019 (COVID-19). Many countries are facing a second wave of the COVID-19 outbreak. A pressing need is evident for the development of a potent vaccine to control the SARS-CoV-2. Institutions and companies in many countries have announced their vaccine research programs and progress against the COVID-19. While most vaccines go through the designation and preparation stages, some of them are under evaluation for efficacy among animal models and clinical trials, and three approved vaccine candidates have been introduced for limited exploitation in Russia and China. An effective vaccine must induce a protective response of both cell-mediated and humoral immunity and should meet the safety and efficacy criteria. Although the emergence of new technologies has accelerated the development of vaccines, there are several challenges on the way, such as limited knowledge about the pathophysiology of the virus, inducing humoral or cellular immunity, immune enhancement with animal coronavirus vaccines, and lack of an appropriate animal model. In this review, we firstly discuss the immune responses against SARS-CoV-2 disease, subsequently, give an overview of several vaccine platforms for SARS-CoV-2 under clinical trials and challenges in vaccine development against this virus.

1. Introduction

New severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the current coronavirus disease 2019 (COVID-19) pandemic [1].The reproductive number (R0) estimated for SARS-CoV-2 is 2.2, which means one infected person can cause viral transmission to 2.2 other persons, thus this infection is highly transmissible with estimated 5.8-day incubation period [2]. Coronaviruses include four classes of alpha (α), beta (β), gamma (γ) and delta (δ) strains. The SARS-CoV, the SARS-CoV-2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) are in beta coronavirus class. The SARS-CoV-2genome is completely sequenced and represented similarity to MERS-CoV and SARS-CoV [3,4].

The SARS-CoV-2, like other members of Coronaviradae family, consists of an envelope surrounding a single-stranded 30-kb RNA including 14 open reading frames (ORF). Four major proteins can be found in this virus, including, nucleocapsid (N), envelope (E), membrane (M), and spike (S). The N fragment comprises T-cell epitopes [4]. The S fragment is the predominant target to synthesize the vaccine against the SARS-CoV-2, mainly because of triggering the antibodies capable of neutralizing the virus as the immune response to vaccination. The N-terminal domain of S protein sequence in the SARS-CoV-2 consists of three excess short insertions when comparing with the SARS-CoV. Moreover, the receptor-binding domain (RBD) of S fragment contains alterations in 4 out of 5 main residues [5].

Angiotensin-converting enzyme 2 (ACE2), on the cell membrane of the host, acts as a receptor for SARS-CoV-2 and SARS-CoV. The binding interaction between ACE2 and viral S protein is a central phase for triggering infection process. The primary target of SARS-CoV-2 is lower respiratory tracts, leading to pneumonia. In addition, this virus may bind to its receptor on the central nervous system (CNS), liver, kidney, gastrointestinal system and heart, resulting in multiple organ failure (MOF) [6].

Moreover, several nonstructural proteins are encoded by the viral

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genome such as PLpro (papain-like protease), RdRp (RNA-dependent RNA polymerase) and coronavirus main protease (3CLpro). The virus after entering to the host cell releases the genome as a +ssRNA, which is then translated to the proteins of the virus via utilizing the translation machinery of host cell. Subsequently, viral proteins are cleaved by PLpro and 3CLpro to form effector proteins. In addition, PLpro is a deubiquitinase capable of deubiquinating specific proteins in the host cell, such as NF-κB and interferon factor 3, leading to suppression of host immune system.

A full-length template of minus-strand RNA of the virus is synthesized using the RdRp for the replication of more viral genome [7,8]. Coronaviruses represent a high recombination rate because the replication of viral genome by RdRp result in increased rate of mutation thus, increasing the rate of homologous recombination. With respect to their high mutation rate coronaviruses are zoonotic pathogens that are capable of infecting humans and animals and result in extensive clinical symptoms, from asymptomatic features to severe symptoms result in the failure of many organs in the body [9]. Although, there is a need for months and probably years for knowing the complete characteristics of SARS-CoV-2 and its probable sources, symptoms, and host immune responses in the battle against infection. Studies are ongoing to produce the SARS-CoV-2 vaccines at high speed and large scale, mostly including DNA-based, mRNA-based, viral vectored, subunit and inactivated vaccines, as well as mainly based on S protein. However, in the way of producing a new vaccine there are so many challenges including poor success in developing human SARS/MERS vaccines, lack of appropriate animal models, limited knowledge of the SARS-CoV-2 pathophysiology, and targeting mucosal or humoral immunity [10]. The Ministry of Health of Russian Federation, on 11 August 2020, approved the vaccine Gam-COVID-Vac (Sputnik V) produced by the Gamaleya Research Institute in Moscow. Scientists have raised great concern about the safety and efficacy of this vaccine because has not yet entered Phase 3 clinical trials. It should be noted that 234 vaccine candidates were being developed as of September 2020, 38 of which in clinical trials and 33 of these in Phase I-II trials and 6 in Phase II-III trials (11).

The current review aimed to briefly overview the host immune response to SARS-CoV-2, various vaccine candidates (mainly in clinical trials) and the challenges of implementing vaccine strategies.

2. Host immune responses to COVID-19

At present the information about the host immune response to COVID-19 is very limited. According to the cumulative empirical and clinical evidence on the study coronaviruses, it is possible to predict the mechanism of host immune system to combat this virus and the viral strategy to induce these immune responses [11].

Numerous studies illustrate important changes in the innate and adaptive immunity in SARS-CoV-2 patients. Clinically, the COVID-19 infection mediated immune responses are in two stages. There is a need for specific response of adaptive immunity within the incubation period and non-severe phases to remove the virus and inhibit the disease progression to severe phases. Thus, the pathways to improve immune responses (pegylated or anti-sera IFNα) are course essential during this period [12]. Although, the first protective line to control the viral infection is a fast and well-coordinated immune response, strong inflammation of innate immunity and dysregulated protection of host adaptive immunity can develop tissue damages either at the virus entry site or at whole body. In this regard, the excessive cytokine and chemokine release, named as “cytokine storm”, is defined as uncontrolled dysregulation of immune defense in the host. Therefore, due to the main function of immune responses in SARA-CoV-2, knowing the process underlying immune dysregulation as well as the mechanisms of SARS-CoV-2 to escape from immune response help us to clinically manage the acute conditions and prevent the mild-to-severe stage transition [13]. An endogenous protective immune response can be established at the non-severe and incubation stages if the host has a suitable genetic history (e.g. HLA) and good general health eliciting a special antiviral immune response. The differences in genetic history in terms of the immune reactions against the pathogens can establish the individual alterations. However, in the impaired protective immunity, the viruses spread and the tissue is destroyed massively, particularly in organs with greater expression level of ACE2, including kidneys and intestines. Innate lung inflammation occurs in the damaged cells, predominantly due to proinflammatory granulocytes and macrophages. Therefore, during the severe stages in COVID-19 patients, the absolute number of natural killer (NK) cells, B cells, and $CD4^+$ and $CD8^+$ cells is significantly reduced in the circulation [4,14,15], and also a reduction in basophils, eosinophils and monocytes has been reported [15–17]. furthermore, most of severe COVID-19 patients showed substantially increase in proinflammatory cytokines (e.g. TNFα, CCL3, IP-10, MCP-1, GM-CSF, G-CSF, IL-17, IL-6, IL-8, IL-1β and IL-2) in the serum [17,18]. The inflammation of lungs is the major reason for the deadly respiratory diseases [14]. Suitable status of general health may therefore not be beneficial for the cases progressed to severe degree. If the lung damage develops significantly, the examinations should be directed to inhibit the inflammatory reaction and control the illness signs.

Alarmingly, some patients remain/return positive for the SARS-CoV-2 and others even relapse after discharge from hospital. This means that it may be difficult to trigger a SARS-CoV-2 virus-eliminating immune response probably in several cases, and these patients may not response to the vaccines. The survivors from non-severe condition must be checked for the presence of virus and the responses of B/T cells, especially when determining vaccine production strategies. Additionally, several coronavirus types or subtypes have been introduced. Therefore, if the vaccines that specifically target SARS-CoV-2 are found to face problems for production, the Edward Jenner approach should be considered [12].

A new infection of SARS-CoV-2 has been demonstrated in children in a recent study, associated with a remarkable inflammatory response. This condition has been known as pediatric inflammatory, multisystem syndrome temporally associated with COVID-19 (PIMS-TS). The new syndrome was temporally associated with recent exposure to SARS-CoV-2. PIMS-TS is an acute presentation of the virus in children and needs to be detected early to prevent its development and probable adverse impacts [19]. The primary signs of PIMS-TS are fever, inflammation marks (rash, oral mucosal changes, and conjunctivitis), cardiac dysfunction, and gastrointestinal symptoms. These characteristics are associated with laboratory evidence of remarkable inflammation: lymphopenia, neutrophilia, higher ferritin concentrations, and serum CRP; non-ST elevation pancarditis, and hypercoagulable state. Besides, echocardiograms usually show hyperechoic coronary arteries and left ventricular dysfunction [20]. PIMS-TS complications are systemic thrombosis and coronary artery aneurysms in nearly 13% of children in some published cohorts [21]. Approximately 2% of these children have died [22]. Retarded clearance of the SARS-CoV-2, which resulted in unchecked inflammation, is a potential mechanism for PIMS-TS [23]. Serum concentrations of proinflammatory interleukins (IL-1 beta, IL-17, IL-6, and IL-8) were very high in children with PIMS-TS. It was accompanied by monocytes and neutrophils activation [24]. However, in viral clearance, there is a handful of data on the anti-viral interferons’ function (alpha, beta, and lambda). It also has been suggested that antibody-dependent enhancement (ADE), with the host cells’ invasion amplified by serum proteases, antibody, or auto-antibody induced disease [25].

Nevertheless, PIMS-TS seem to affect only young adults and children, and ADE would be anticipated in older adults (with higher prevalence before exposure to other coronaviruses). The adult patient’s treatment with COVID-19 recovering plasma has also not been correlated with hyperinflammation. The extensive effects of intravenous immunoglobulins via Fcγ-receptors, diminishing lymphocyte apoptosis, scavenging of inflammatory mediators, and preventing hypothesizing for the PIMS-TS’ pathobiology [20].

2.1. Innate immunity to infection with SARS-CoV-2

Today, limited data are available on the host innate immune responses in the patients with SARS-CoV-2. In a study on the cases (n = 99) examined in Wuhan, total neutrophils were elevated (38%), total lymphocytes were decreased (35%), the IL-6 level was increased in serum (52%) and c-reactive protein was increased (84%) [4]. Reduced lymphocytes and increased neutrophils correlate also with the seriousness of disease and death [26]. Furthermore, the ICU patients had greater serum levels of innate cytokines such as, TNFα, MIP-1A, MCP-1, and IP-10 [4]. These clinical characteristics proposed a potential role of hyper-inflammatory responses in COVID-19 pathogenesis. Efficient innate immunity against the viral infectious diseases is highly dependent on the IFN I (interferon type I) responses and their downstream cascade which eventually results in the control of viral replication and the provocation of strong adaptive immunity. The SARS-CoV has been reported to directly infect T cells and macrophages, a key feature in pathogenesis mediated by SARS-CoV, which induces delayed but increased proinflammatory chemokines and cytokines. The ACE2 is a receptor minimally expressed in T cells, monocytes and macrophages in the lungs. However the strategy of SARS-CoV2 for directly infect any immune cells is still unknown [14]. A suitable antiviral response can be created by recognizing the invasion of viruses via innate immune cells, mostly through the pathogen-associated molecular pattern, PAMP. It is known for RNA viruses, including coronavirus, that PAMPs as viral genomic RNA or dsRNA (an intermediate produced within the viral replication), are identified by cytosolic RNA sensor, TLR7 and TLR3 as well as the endosomal RNA receptors, MDA5/RIG-I. Such identification process activates the downstream signaling pathway, such as IRF3 and NF-κB transcription factors, with the nuclear translocation. Such nuclear factors trigger the expression of IFN I and some proinflammatory cytokines. These primary reactions constitute the first protective line to control the viral infection at the penetration site [27]. In turn, the IFN-I uses the IFNAR to activate the JAK-STAT pathway in which the STAT1 and STAT2 are phosphorylated by JAK1 and TYK2 kinases. After form complex of STAT/2 with IRF9, they underwent nuclear shift for starting the IFN-stimulated gene (ISG) transcription supervised by promoters-containing IFN-stimulated response element (ISRE) [11].

According to the collected data from previous coronavirus infections, the innate immunity is important in protective or destructive reactions, opening a gate for the intervention immune. Later on, active viral replication leads to the overproduction of IFN I and the release of macrophages and neutrophils as the key resources of proinflammatory cytokine. During COVID19, the SARS-CoV-2 is capable of provoking delayed IFN I and viral control loss in an early infection with related alterations in overall lymphocytes and neutrophils [4]. Several approaches may be suggested about the key role of innate immunity, including some antagonists of key antiviral agents and proinflammatory cytokines such as IFN I. In the use of IFN I as the treatment, timing of administration in a murine models of SARS-CoV infection is crucial to providing protective response [28].

2.2. Adaptive immunity to infection with SARS-CoV-2

In general, T helper type 1 (Th1) immune response plays is important for the adaptive immune response to the viral infections. The microenvironment of cytokine formed by antigen-presenting cells (APCs) directs the T cell responses. While cytotoxic T cells are necessary to kill the cells infected with virus, the Th cells organize the adaptive immunity. Humoral immunity, especially the neutralizing antibody formation, provides excellent protection through the restriction of later-phase infection and inhibits the possible recurrent infection. The epitopes of both T and B cell in SARS-CoV for the structural proteins (E, N, S and M proteins) were extensively mapped [29]. Activated Th1/Th17 cells may exacerbate the inflammation. Plasma/B cells develop specific antibodies to SARS-CoV-2, which may help to neutralize viruses. Long lasting specific IgGs and neutralizing antibodies are recorded for approximately two years after infection [30].

2.2.1. Cellular immune responses

As regards adaptive immunity, the novel SARS-CoV-2 mainly affects the counting and balance of lymphocytes. The T cell response was extensively investigated in SARS-CoV. In a cohort study in Wuhan involving 452 patients with COVID-19, the patients with severe COVID-19 reported a smaller count of total T cells (suppressor and helper T cells) [17]. Reduced regulatory T cells was observed among Th cells, with highly decrease depending on the illness intensity of patients, and in memory T cells, while the percent of naïve T cells was elevated [17]. In another study using 128 convalescent samples, CD8-positive T cell responses were prevalent higher than CD4- positive T cell reactions. In addition, specific viral T cells from the patients with severe condition appeared to be a main memory phenotype having a markedly greater number of polyfunctional CD4- positive T cells (TNFα, IFNγ, and IL-2) and CD8- positive T cells (TNFα, IFNγ, and degranulated state) when comparing with the mild-moderate patients. Potent T-cell responses significantly associated with greater neutralizing antibodies whereas higher Th2 cytokines (IL4, IL5, IL10) in the serum were identified in the fatal patients group [31]. Memory and naïve T cells are necessary for the immune system, whose equilibrium plays a pivotal role to maintain an effective defensive system. The naïve T cells activate a large and closely coordinated release of cytokines to defend against new and previously unrecognized infections, while memory T cells induce the antigen-specific immunity. Dysregulated balance in favor of naïve T cell over regulatory T cells may significantly develop the hyperinflammation [32,33].

A majority of responses (70%) induced versus structural proteins (nucleocapsid, spike, shell and membrane) was found for epitope mapping. Reportedly, the Th1 responses can effectively control the SARSCoV and MERSCoV, and probably the SARS-CoV-2. However, the critical CD8-positive T cell response must be regulated in order to prevent lung pathology. Because a majority of epitopes for both MERSCoV and SARSCoV are concentrated on the structural proteins of the virus, it is useful to map those epitopes of MERSCoV/SARSCoV with the epitopes of SARSCoV-2. Concerning the overlapped epitopes of these viruses, the convalescent serum of recovered MERS or SARS cases can be used in the passive immunization. For epitopes of T cell, it will facilitate to develop cross-reactive vaccines which will protect against all 3 human coronaviruses in future [11].

2.2.2. Humoral immune responses

As far as B cells are concerned, Wen et al found significant B cell changes exploiting single-cell RNA sequencing (scRNA-seq) for the characterization of transcriptome landscape of immune cell subtypes during the recovery stage of COVID-19. In particular, the plasma cells are elevated in peripheral blood mononuclear cells, while the naïve B cells are decreased [34]. In addition, numerous novel B cell-receptor alterations (e.g. IGHV3–23 and IGHV3–7) were identified. Moreover, isotypes have been confirmed, including IGKV3–11, IGHV3–15, and IGHV3–30, previously used for the development of virus vaccines. The highest frequencies of pairing, IGHV3–23-IGHJ4, was suggested for the recognition of monoclonal status of SARS-CoV-2 particularity [34]. In addition, the antibody seroconversion response must be importantly tracked to clinically evaluate the infections, considering the pivotal function of B cells to manage the infection. While the serum samples of patients with COVID-19 had no S1 subunit cross-binding with the SARS-CoV spike antigen, several cross-reactivity was observed in the serum specimens of patients with COVID-19 to the nucleocapsid antigens of SARS-CoV [35]. According to the findings from this study, most patients (96.8%) gained IgM or IgG seroconversion during 20 days of the symptom onset at a titer plateau during six days of seroconversion. In addition, after about 17 to 19 days of the symptom onset, all patients had positive virus-specific IgG. Rather, most patients (94.1%)

demonstrated positive virus-specific IgM after about 20 to 22 days of the symptom onset [35].

Poor and delayed responses of antibodies are related with the severe results for both types of coronavirus infections. A limited detail of SARS-CoV-2 serology was found. A pilot study reported a peak-specific IgM on the ninth day after the illness onset and a switch to IgG at the second week in one patient [4]. The serum samples of five patients with definitive COVID-19 exhibited cross-reactivity with the SARS-CoV, but not with other coronaviruses. In addition, all serum samples neutralized the COVID-19 in an in vitro plaque assay, offering a potential for successfully loading of humoral reactions [4]. However, the specific antibody titer/kinetic property associated with the severity of disease must be checked

2.3. Mechanisms involved in SARS-CoV-2 immune evasion

Most of the mechanisms rely on inhibiting innate immune responses, particularly the recognition and signaling of IFN I. The important molecules in the modulation of host immune are the viral non-structural (NS, such as NS4a, NS4b and NS15) or membrane (M) proteins. Based on the findings from the MERS-CoV patients (n = 2) with various severities, the response of IFN I in the deceased patient (poor outcome) was considerably lower when comparing with the survived patient [36]. The antigen presentation mediated by MHC class I and II was down-regulated after infection of the dendritic cells (DCs) or macrophages with MERS-CoV, which significantly decreased the activation of T-cells [37]. The response to viral infection by IFN I for MERS-CoV and SARS-CoV is suppressed. Type of IFN response induction is essential to limit the virus propagation in the host within the early disease stages. The antiviral impacts are directly caused by the IFNs, thereby limiting viral replication as well as modulating the adaptive and innate immunities. The IFNs link with their receptors expressed on different cells, including macrophages, as well as can trigger the activity of JAK/STAT signaling that forms the STAT1/2/IRF9 complex and triggers high concentrations of ISGs, including the anti-viral enzyme of RNAse L and the proinflammatory chemokine of CXCL10 [38,39]. MERS-CoV and SARS-CoV possess numerous mechanisms of evading the host antiviral immunity due to IFN Is [40], as follows:

- **Avoidance:** viruses shield themselves or their byproducts against the host. During the replication process SARS-CoV and MERS-CoV hide their intermediates (such as dsRNA) in double-membrane vesicle, DMV [41,42].
- **Suppressed induction of IFN:** the proteins of viruses inactivate the host sensor system or downstream signaling components to inhibit the expression of IFN. The nsp4a and membrane of MERS-CoV inactivate the RIG-I-mediated MDA-5 and IRF3, respectively [43,44]. In addition, the PLpro possesses deubiquitinase(DUB) potential within the cells infected with virus, as well as inactivates IRF3 in either SARS-CoV or MERS-CoV [45–47].
- **Suppressed IFN signaling:** the interferon signaling pathway is directly inhibited by the viruses. SARS-CoV nsp6 and nsp1 block STAT1/STAT2/IRF9 complex translocation and STAT1phosphorylation, respectively, inactivating the antiviral conditions in infected cell and inducing the IFN response [48,49].

According to the above contents, SARS-CoV-2 can activate the host adaptive and innate immunities and generated long-lasting protective immunity against them. Therefore, the creation of an effective vaccine considers as a promising approach for inhibiting pandemic COVID-19. The aim of all vaccination is to expose the body to an antigen that will not cause disease but will stimulate an immune response that can suppress or kill the viruses if a person becomes infected. There are at least eight types of vaccines being tried against the SARS-CoV-2. They rely on viral parts or different viruses that we have mentioned to them at below.

3. Prioritization of COVID-19 vaccination

Since efficacious vaccines and protective medications have been introduced for COVID-19, demand is likely to outrun the supply. Thus a prioritizing strategy for vaccination is needed to reach the highest level of public health. The Joint Committee on Vaccination and Immunization's provisional advice stated that adults over 65 years old, people in shielding groups, and health workers are the priority for COVID vaccination [50].

According to an at-risk population analysis, Hassan-Smith et al. [50] suggested a draft plan for prioritizing the vaccines and protective medications. These groups include people with severe infection, such as those with non-communicable diseases (e.g., cardiovascular disease, hypertension, diabetes, and obesity) who should also be prioritized. Next, the high-risk job-related groups comprising those working in customer-facing roles, such as security and transport worker, should also be involved. Socioeconomic factors related to adverse effects in COVID-19 should also be evaluated. Moreover, a functional strategy would consider vaccination of those living in overcrowded situations or organizations such as care homes [50]. Besides, developing clinical prediction tools could be applied to notify further risk stratification [51]. Based on CDC reports, persons of any age with the hereunder conditions are at higher risk of severe illness caused by COVID-19 and should be potentially prioritized for COVID-19 vaccination: (1) cancer, (2) chronic kidney disease, (3) COPD (chronic obstructive pulmonary disease), (4) down syndrome, heart conditions, (5) immunocompromised state from a solid organ transplant, (6) obesity, (7) sickle cell disease, (8) smoking, and (9) type 2 diabetes mellitus [52].

4. Efforts for SARS-CoV-2 vaccine

4.1. Live inactivated and attenuated virus vaccines

A conventional way for viral vaccination is the use of live-attenuated vaccines or inactivated vaccines. Attenuated virus vaccines mainly induce mucosal immunity to reduce the mucosal infection of the virus.

A live influenza vaccine expressing the proteins of SARS-CoV-2 has been developed by the scientists from the University of Hong Kong. A "codon deoptimization" technology, developed by Codagenix, strives to discover the SARS-CoV-2 vaccine technologies to produce attenuated virus vaccine [53,54]. The main advantage of inactivated or attenuated vaccines is intrinsic immunogenicity and capacity to trigger the toll-like receptors (such as TLR 9, TLR 7/8 and TLR 3). Other advantages include: fast development, excellent neutralizing Ab, viral structure preservation, induction formulated with different adjuvants, excellent T/B cell response induction, and site-directed mutagenesis performed easily to improve their features. It should be noted that the live virus vaccines mostly need further experiment to ensure the safety. According to the findings from the elevated infectivity following the immunization using killed or live SARS-CoV vaccines, safety is an issue in the development of new vaccine. Moreover, these vaccines are inappropriate for sensitive individuals, including elderly people, immunocompromised individuals and infants [16,55]. The Chinese company (Sinovac Biotech) is trying to develop an inactivated vaccine named CoronaVac in the phase I, II, and III, reached emergency approval for limited use in July [56].

Table 1 contains several clinical trials on SARS-COV-2 live inactivated or attenuated vaccines.

4.2. Protein-based subunit vaccine candidates

Protein-based subunit vaccines comprise of the minimum SARS-CoV-2 structural parts capable of triggering the host protective immunity, required to be used along with molecular adjuvants to increase their immunogenicity. Subunit vaccines against the SARS-CoV depend on stimulating the anti-S protein immunity to inhibit its attachment on host ACE2 receptor [55].

Table 1
SARS-COV2 inactivated vaccines in clinical trial.

Study ID	Name of vaccine	Phase	Status of trial	Country	Company/Sponsor
NCT04352608	Coronavac, Sinovac	I/II	Active, not recruiting; Actual Primary Completion; 10th July 2020	China	Sinovac
NCT04456595	Coronavac, Sinovac	III	Recruiting; Estimated primary Completion; September 2021	Brazil	Sinovac
NCT04508075	Sinovac	III	Recruiting; Estimated Primary Completion; January 2021	Indonesia	Sinovac
ChiCTR2000031809		I/II	From 2020-04-11 To 2021-11-10	China	Wuhan Institute of Biological Products, Sinopharm
ChiCTR2000032459		I/II	From 2020-04-28 To 2021-11-28	China	Beijing Institute of Biological Products, Sinopharm
ChiCTR2000034780 NCT04510207		III	Rercruiting; Estimated Primary Completion: March 16, 2021	Abu Dhabi, Peru, Morocco and Argentina	Wuhan AND Beijing Institute of Biological Products Co
TRI/2020/07/026300 NCT04471519	BBV152	I/II	Recruiting; Estimated Primary Completion Date: June 2021	India	Bharat Biotech
NCT04470609		Ib/IIb	Enrolling by invitation Estimated Primary Completion Date: November 2020	China	Chinese Academy of Medical Sciences
ChiCTR2000032459 NCT04412538		Ia/IIa	Recruiting; Estimated primary completion date: September 2020	China	Chinese Academy of Medical Sciences

The University of Queensland is trying to achieve the viral surface proteins for presenting to immune system. Clover Biopharmaceuticals has designed and developed a trimerized protein subunit vaccine by utilizing their patented Trimer-Tag technology. However, they detected eosinophilic infiltration and enhanced infectivity while using some full-length viral S proteins. Novavax has produced a virus- like particle that express the recombinant S protein as a vaccine candidate [57,58]. Another subunit vaccine has been produced by Texas Children's Hospital Center for Vaccine Development (Baylor College of Medicine) based on the RBD from the S protein of SARS-CoV formulated on alum. The RBDs from SARS-CoV-2 and SARS-CoV bind to the same ACE2 receptor and represent over 80% similarity of amino acid sequence, so this RBD vaccine also can be developed for SARS-CoV-2. The strength of RBD vaccine is to decrease the host immunopotentiation [59,60]. In general, subunit vaccines have advantages including, excellent safety, continuous production, and capability of triggering both cell-mediated and humoral immunities. However, they require suitable molecular adjuvants and their cost-effectiveness may vary [11].

Generex is developing and producing a SARS-CoV-2 vaccine using Ii-Key technology to activate immune system. This technology utilizes synthetic peptides mimicking important viral protein fragments that are chemically attach to the 4 Ii-Key amino acids to make sure that it will effectively activate the immune system. The Ii-Key technology ensures strong $CD4^+$ T cells activation, therefore facilitates antibody production to combat the infection.

GlaxoSmithKline (GSK) in patent application WO2010063685

Table 2
SARS-COV2 subunit vaccines in clinical trial.

Study ID	Type of subunit vaccine	Phase	Status of trial	Country	Company/Sponsor
ChiCTR2000035691		I	Duration: From 2020-08-20 To 2021-12-31	China	Anhui Zhifeilongkoma Biopharmaceutical Co., Ltd. Hunan Provincial Center for Disease Control and Prevention
NCT04466085		I	Recruiting; Estimated Primary Completion Date; September 15, 2021	China	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd
NCT04445194	Adjuvanted recombinant protein (RBD Dimer)	I	Recruiting; Estimated primary completion: July 21, 2021	China	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd
NCT04368988	ARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine (SARS-CoV-2 rS)	I/II	Recruiting Estimated Primary Completion Date: December 31, 2020	Australia (multiple sites)	NVX-CoV2373, Novavax
NCT04405908	Native like Trimeric subunit Spike Protein vaccine	I/II	Recruiting; Estimated primary completion: October 20, 2020	Australia	Clover Biopharmaceuticals Clover/GSK/ Dynavax
NCT04473690	RBD-based vaccine	I/II	Not yet recruiting; Estimated completion: January 25, 2020	Not stated yet	KBP-COVID-19, Kentucky Bioprocessing, Inc
ACTRN12620000674 932p	Molecular clamp stabilized Spike protein with MF59 adjuvant	I	Recruiting; Estimated primary completion: July 1, 2021	Australia	University of Queensland/CLS/Seqirus
NCT04453852	COVAX19, Recombinant spike protein with Advax™ adjuvant	I	Recruiting; Estimated primary completion: July 1, 2021	Australia	Vaxine Pty Ltd/Medytox
NCT04487210	Protein subunit	I	Not yet recruiting; Estimated primary completion: December 31, 2021	Taiwan	MVC-COV1901, Medigen Vaccine Biologics Corp
NCT04522089	Protein subunit	I	Recruiting; Estimated primary completion: November 20, 2020	Taiwan	
NCT04527575	Protein subunit	I/II	Active, not recruiting; Estimated Primary Completion: September 1, 2020	Russia	EpiVacCorona, Federal Budgetary Research Institution State Research Center of Virology and Biotechnology "Vector
https://rpcec.sld.cu/ensayos/RPCEC00000332-Sp	Protein subunit	I/II	Estimated study completion: January 11, 2021	Cuba	Instituto Finlay de Vacunas, Cuba

describes a vaccine that contains one soluble S protein (an engineered ectodomain S protein) as an immunogen as well as an oil-in-water emulsion as an adjuvant. This vaccine is able to trigger neutralizing antibody responses as well as IgG2a or IgG2b antibody reactions in response to the SARS-CoV-2 in the animal models. Recently, GSK is working with Clover Biopharmaceuticals (Chinese firm) to produce its++ candidate vaccine for SARS-CoV-2 (see Table 2).

Table 3
SARS-COV2 viral vector vaccines in clinical trial.

Study ID	Name/Type of vaccine	Phase	Status of trial	Country	Company/Sponsor
NCT04497298	Replicating Measles-vector based	I	Recruiting; Estimated primary completion: November 2020	France	Institute Pasteur/Themis/ Univ. of Pittsburg CVR/ Merck Sharp & Dohme
NCT04498247	Replicating Measles-vector based	I/II	Recruiting; Estimated primary completion: March 16, 2022	France	Merck Sharp & Dohme Corp
NCT04324606	Non-replicating Based on adenovirus vaccine vector with SARSCoV-2 spike protein (AZD1222, ChAdOx1 nCoV-19)	I/II	Active, not recruiting Estimated primary Completion: May 2021	UK	Oxford and Astra-Zeneca
NCT04400838	Non-replicating Based on adenovirus vaccine vector with SARSCoV-2 spike protein (AZD1222, ChAdOx1 nCoV-19)	II/III	Recruiting; Estimated primary completion: August 2021	UK	Oxford and Astra-Zeneca
NCT04444674 PACTR202006922165 132	Non-replicating Based on adenovirus vaccine vector with SARSCoV-2 spike protein (AZD1222, ChAdOx1 nCoV-19)	I/II	Recruiting; Estimated Primary Completion: October 2020	Multicentre study in South Africa	Oxford and Astra-Zeneca
NCT04516746	Based on adenovirus vaccine vector with SARSCoV-2 spike protein (AZD1222, ChAdOx1 nCoV-19)	III	Not yet recruiting; Estimated Primary Completion: December 2, 2020	USA	Oxford and Astra-Zeneca
ISRCTN89951424	Non-replicating Based on adenovirus vaccine vector with SARSCoV-2 spike protein	III	Ongoing Study duration May 2020 to July 2021	Brazil	Oxford and Astra-Zeneca
NCT04398147	Non-replicating Recombinant Novel Coronavirus Vaccine, Adenovirus Type 5 Vector Ad5-nCoV	I /II	Not yet recruiting; Estimated Primary Completion: December 2021	Canada	CanSino Biologics Inc. Beijing institute of biotechnology Canadian Center for Vaccinology
NCT04526990	Coronavirus Vaccine, Adenovirus Type 5 Vector Ad5-nCoV	III	Not yet recruiting; Estimated Primary Completion: December 30, 2021	Pakistan	CanSino Biologics Inc. Beijing Institute of Biotechnology
ChiCTR2000030906 NCT04313127	Non-replicating Recombinant Novel Coronavirus Vaccine, Adenovirus Type 5 Vector Ad5-nCoV	I	Active, not recruiting Estimated primary completion: Dec 30 2020	China	CanSino Biologics Inc.
NCT04341389 ChiCTR2000031781	Non-replicating Recombinant Novel Coronavirus Vaccine, Adenovirus Type 5 Vector Ad5-nCoV	II	Active, not recruiting; Estimated Primary Completion: January 31, 2021	China	Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China CanSino Biologics Inc.
2020-002835-31	Replication defective Simian Adenovirus (GRAd) encoding S, Grad-CoV2	I	Adult enrolment is expected to end in the second week of September and the first safety and immunogenicity results will be available by the second week of October. The enrolment of the elderly will end in the first week of November and the first results will arrive by the second week of December. Final safety and immunogenicity data will be available within one year of study approval.	Italy	ReiThera/LEUKOCARE/ Univercells
NCT04436471	Non-replicating vector Gam-COVID-Vac rAd26 Component, 1 vaccination (recombinant adenovirus vector) rAd5 Component, 1 vaccination (a vector based on the human adenovirus type 5)	I	Completed; Actual Primary Completion Date: August 3, 2020 Actual Study Completion Date: August 10, 2020	Russia	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation
NCT04437875	Non-replicating vector Gam-COVID-Vac Lyo, Gamaleya Adenovector virus	I/II	Completed; Actual Primary Completion Date: August 3, 2020 Actual Study Completion Date: August 10, 2020	Russia	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation
NCT04436276	Non-replicating vector Ad26.COV2-S, JnJ	I/IIa	Recruiting; Estimated Primary Completion: September 15, 2021	US and Belgium	Janssen Vaccines & Prevention B.V., Johnson & Johnson
NCT04505722	Non-replicating vector Ad26.COV2-S, JnJ	III	Not yet recruiting; Estimated Primary Completion: March 10, 2023	US, Brazil, Chile, Columbia, Mexico, Peru, Philippines, South Africa, Ukraine	Janssen Vaccines & Prevention B.V., Johnson & Johnson
NCT04509947	Non-replicating vector Ad26.COV2-S, JnJ	I	Not yet recruiting; Estimated Primary Completion: January 8, 2021	Japan	Janssen Vaccines & Prevention B.V., Johnson & Johnson

4.3. Viral-vector vaccines

Live vector vaccines contain the live viruses capable of expressing the desired antigen(s). They combine the safety of subunit vaccines and potent immunogenicity of live attenuated vaccines, with extensive application in triggering the cell-mediated immunity in the *in vivo* condition. Potent immune response induced by viral vector-based vaccines causes a high protein expression level and represents a long lasting stability. Adenovirus Type 5 (Ad5) is well established and used in immunological practice that exhibit high efficiency, and shows simple manipulation and ease of purification. However, the specific response to the vaccine can be decreased by the presence of adaptive immune response to the vector antigens. CanSino has reported that $CD4^+$ and $CD8^+$ T were activated in the recipient of vaccine. Though, the T cell or antibody response to vaccine was partially mitigated duo to pre-existing immune response to Ad5 viral vector. By contrast, Zhu et al. [61] and Folegatti et al. [62] revealed that using Ad5 vectored SARS-CoV-2 vaccines could trigger both cell-mediated and humoral immunities in the most of participants in trials. In addition, Ad26 and Ad35 are less common adenoviral serotypes that are widely utilized in vaccine development because they can induce a robust immune response without additional adjuvants and mimic the natural infection with low risk of heterologous response [63].

Houston-based Gre_ex Inc. has produced an adenovirus vector vaccine, using Gre_ex vector for COVID19. Recently, it has been entered to animal study [64]. Johnson & Johnson is a multinational company working on SARS-CoV-2 vaccine [65] utilizing Janssen's AdVac® adenoviral vector as well as generating in their PER.C6® cell line method, like their Ebola vaccine system. Tonix Pharmaceuticals developed Horsepox Virus (TNX-1800)-based SARS-CoV-2 vaccine. AdVac® is an adenoviral vector vaccine that has been produced by Johnson & Johnson. Chen Wei group developed adenovirus type-5 (Ad5)-vectored COVID-19 vaccine in human clinical trial on 16 March 2020 [61].

Another recombinant adenovirus vector vaccine (Ad5-nCoV) developed by Cansino Biologics Inc, is present in phase I clinical trial on March 2020 and recruited 108 healthy individuals in Wuhan, China [66]. Shenzhen Geno-Immune Medical Institute has developed two vaccines using lentivirus vector, LVSMENP-DC and COVID-19/aAPC. The LVSMENP DC vaccine was produced via modifying dendritic cells with lentiviruses that express immune modulatory genes and SMENP (a minigene of SARS-CoV-2). It is present in the phase I clinical trial since March 24, 2020 with 100 participants and estimated time for completion of the study is December 31, 2024 [67]. The COVID-19/aAPC vaccine produced via performing lentivirus modifications involves the immune modulatory genes and the SARS-CoV-2 minigenes to the artificial antigen presenting cells (aAPCs). It has been entered the phase I clinical trial on 15th February 2020 and estimated time for completion of the study in December 31, 2024 [65].

The AstraZeneca Company and the University of Oxford achieved a vaccine candidate AZD1222 using a chimpanzee adenovirus named ChAdOx1. The vaccine was capable of inducing antibody response as well as cellular immune response. AZD1222 began phase II/III trail in India and England and phase III in U.S, Brazil, and South Africa. On September 8, 2020, AstraZeneca stopped vaccine global trials to study one volunteer, who developed a form of inflammation called transverse myelitis as an serious adverse effect [68] (see Table 3).

4.4. Nucleic-acid vaccines

Nucleic acid platforms for COVID-19 have been developed by various major biotechs. For instance, Moderna Therapeutics and Curevac are developing RNA vaccines, while Inovio Pharmaceuticals explores a DNA-based vaccine.

4.4.1. DNA vaccines

The DNA immunization started in 1993 with promising outcomes in mouse models resulting in protective immune response against influenza. For decades, such results have not been translated into similar outcomes in humans. Recently, new modifications and formulations of nucleic acid vaccines have been developed. This approach is capable of giving the initial licensed human DNA vaccine [65]. DNA vaccines can trigger both cell-mediated and humoral immunities in animal models although, suitable immunogenic responses have not been obtained yet [69]. The advantages of DNA vaccines include: rapid and easy design, manipulation, and production and the ability to induce both B and T cells responses. However, the needs for an effective delivery system and lower immune responses compared to live vaccines are major disadvantages.

The INO-4800 as one of the DNA vaccines has been produced by the Innovation and Value Initiative (IVI), Inovio and the Korea National Institute of Health (KNIH) in collaboration with the Coalition for Epidemic Preparedness Innovations (CEPI). This vaccine is now tested for safety and immunogenicity in phase I and II clinical trial in South Korea. INO-4800 vaccine comprises of pGX9501 plasmid, which is able to express the full-length SARS-CoV-2 S protein [66]. Table 4 contains an update list of several DNA vaccines in phase I, II, and III clinical trials.

4.4.2. RNA vaccines

The mRNA vaccines possess some advantages over prophylactic vaccines such as the capability of binding some mRNAs onto a single vaccine and inducing a much stronger immune response because of their ability to mimic natural infection. On February 24, 2020, Moderna declared that the first class of mRNA-1273 versus SARS-CoV-2 has been released for human use. National Institute of Allergy and Infectious Diseases (NIAID) affiliated to the National Institutes of Health (NIH) is performing its clinical trial (within phase I) in the USA on 120 participants. The mRNA-1273 is non-replicating RNA vaccine containing a dispersion of lipid nanoparticles containing mRNA targeting the prefusion stabilized S protein and inducing the antibody response. Results from early-stage clinical study indicated that mRNA-1273 vaccine was safe and immunogenic; therefore this vaccine entered phase III clinical trial that is conducted at U.S. clinical research sites, with approximately 30,000 healthy adult participants [36]. Other mRNA vaccine candidates for SARS-CoV-2 sponsored by BioNTech and Pfizer are now within the phase II/III clinical trial in the Germany and the United States. The BNT162 vaccines comprise at least 4 experimental vaccines with different target antigens and mRNA formats. BNT162b1 and BNT162b2 are lipid nanoparticles containing modified nucleoside RNAs. BNT162b1 contains an optimized SARS-CoV-2 RBD antigen and was administered to 45 healthy individuals aged 19 to 54 and found to induce immunity and inhibit SARS-CoV-2 infection. Its side effects were dose-dependent injection site pain, headaches, and fatigue. BNT162b2 expressed an optimized SARS-CoV-2 S protein. It was entered the phase II/III clinical trial with 30,000 participants aged 18–85 in 120 countries [70,71].

CureVaćs mRNA vaccine (CVnCoV) candidate is comprised of mRNA nucleotides with no chemical alterations and is intended to induce balanced and effective immune system activation. This mRNA vaccine is formulated with lipid nanoparticles (LNP) and expresses the full-length S protein. The phase I and IIA clinical trial is ongoing in U.S.

LNP-nCoVsaRNA is a SARS-CoV-2 vaccine candidate developed by Imperial College of London. This mRNA vaccine is in phase I clinical trial and have been used for other diseases such as Influenza (H7N9), EBOV, LASV, MARV, and RABV.

Chinese researchers developed a vaccine candidate named ARCoV, as lipid-nanoparticle-encapsulated mRNA (mRNA-LNP) expressing the SARS-CoV-2 RBD. Immunization with ARCoV mRNA-LNPs triggers potent Th1-biased cellular response and neutralizing anti-SARS-CoV-2 antibodies in mice and primates. ARCoV is stable at an ambient temperature for minimally a week and is produced in liquid formulation, which is currently in phase I clinical trial [72].

Indeed, human DNA- and mRNA-based vaccine candidates may be

Table 4
SARS-COV2 DNA vaccines in clinical trial.

Study ID	Vaccine name	Phase	Status of trial	Country	Company/Sponsor
NCT04336410	INO-4800, Inovio	I	Active, not recruiting; Estimated Primary Completion: July 2021	USA	Inovio Pharmaceuticals
NCT04447781	INO-4800	I/IIa	Recruiting; Estimated primary completion: February 22, 2022	Not stated yet	International Vaccine Institute
NCT04527081	AG0302-COVID19, AnGes	I/II	Recruiting; Estimated Primary Completion: November 26, 2021	Japan	AnGes
JapicCTI205328	AG0301-COVID19	I/II	Recruiting Estimated primary completion date: September 26, 2020 Duration: 25.6.2020-31.7.2021	Japan	AnGes, Inc
NCT04445389	GX-19, Genexine	I/IIa	Recruiting; Estimated Primary Completion: March 17, 2021	Korea	Genexine, Inc.
CTRI/2020/07/026352	DNA plasmid vaccine, Cadila Healthcare Limited	I/II	Recruiting; Estimated Primary Completion: July 13, 2021	India	

unable to trigger a protective immunity after a single immunization because they, like recombinant and inactivated subunit protein-based vaccines, need to be administered in several times over prolonged duration to have immunogenicity [73]. Table 5 contains an update list of several RNA vaccines in clinical trials. Besides, a review of the adverse outcomes and the licensed SARS-CoV-2 vaccines' participants were summarized in Table 6.

4.5. Virus-like particle

Virus-like particle (VLP) is a nanostructure with self-assembly feature that contains structural proteins of virus. Molecular and morphological characteristics of VLP are similar to authentic viruses. Due to lack of genetic materials, VLP is not able to replicate or cause infection and does not require biosafety protection and specific laboratory settings. Thus, VLP is a suitable and safe model for viral molecular studies and vaccine design [74,75].

Xu et al revealed that the presence of M and E proteins are essential for effectively assembly and release of VLPs from the SARS-CoV-2. They suggest that SARS-CoV-2 VLPs mimic native virion particles molecularly and morphologically, which not only provides incentives for viral morphological studies but also offer a possible vaccine for SARS-CoV-2 [76].

Three SARS-CoV-2 mRNA vaccines have been designed by Lu et al that express different antigens in the vaccinated host including, RQ3011-RBD, RQ3012-Spike, and RQ3013-VLP. The RQ3011-RBD vaccine is able to encode the SARS-CoV-2 S glycoprotein RBD (residues 331–524) with a membrane-anchoring helix in C-terminal and a signal peptide in N-terminal. The RQ3012-Spike vaccine expresses the full-length S protein and the RQ3013-VLP vaccine is consist of mRNAs cocktail that expresses the S, M, and E proteins to make SARS-CoV-2 VLPs. They utilized the LNPs for packaging the mRNAs and evaluated the immunogenicity for all mRNA LNP vaccines in the BALB/c mice. Their results showed that only RQ3013-VLP stimulated both cell-mediated and humoral immunities. They found that the presence of S protein in VLPs induces a stronger immune response than when shown at the cell surface [77,78].

Zha et al designed a vaccine candidate for COVID-19 via displaying the RBD domain of SARS-CoV-2 on $CuMV_{TT}$ VLP (cucumber mosaic virus) and evaluated the immunogenicity of all vaccines in mouse models. $CuMV_{TT}$ VLPs contain one tetanus toxin-originated universal T cell epitope. Moreover, these VLPs package bacterial RNA during the

Table 5
SARS-COV2 RNA vaccines in clinical trial.

Study ID	Vaccine name	Phase	Status of trial	Country	Company/Sponsor
NCT04283461	mRNA-1273, Moderna	I	Active, not recruiting Estimated primary completion: November 2021	USA, Washington	National institute of Allergy and Infectious diseases; Moderna Therapeutics; Lonza
NCT04405076	mRNA-1273	IIa	Active, not recruiting Estimated Primary Completion Date: March 2021	USA (multiple sites)	ModernaTX, Inc. NIAID Lonza
NCT04470427	mRNA-1273	III	Recruiting Estimated Primary Completion Date: October 27, 2022	USA (multiple sites)	ModernaTX, Inc. NIAID
NCT04380701	BNT162, BioNTech mRNA vaccine	I/II/III	Recruiting; Estimated Primary Completion Date: August 2020	Germany	Pharmaceuticals GmbH and Pfizer Inc.
NCT04368728	BNT162	I/II/III	ctive, not recruiting Estimated primary Completion Date: June 28, 2021	Multicenter, Germany, USA	Biontech SE
ChiCTR2000034825	BNT162b1	I	Study duration: From 2020-07-20 to 2020-12-31	China	Jiangsu Provincial Center for Disease Prevention and Control. BioNTech RNA Pharmaceuticals GmbH. Shanghai Fosun Pharmaceutical Development, Inc
NCT04523571	BNT162b1	I	Recruiting; Estimated Primary Completion: September 2020	China	Sponsor: Biontech Collaborator: Shanghai Fosun Pharmaceutical Development Ca, Ltd
ISRCTN17072692	RNA vaccine LNP-nCoVsaRNA	I	Planned to start mid June and last for 2 months Interim results available end of August	UK	Imperial College London
NCT04449276	CVnCoV, CureVac	I	Recruiting; Estimated primary completion: August 2021	Germany	Curevac, CEPI
NCT04515147	CVnCoV, Curevac	IIa	Not yet recruiting; Estimated Primary Completion: November 9, 2021	Not stated yet	Curevac
NCT04480957	LUNAR-COV19	I/II	Recruiting; Estimated primary completion: December 2020	Singapore	Arcturus Therapeutics, Inc
ChiCTR2000034112	RNA vaccine	I	From 2020-06-25 To 2021- 12–31	China	People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech
NCT04537208	LNP-mRNA	I	Recruiting; Started on September 3, 2020 Estimated Primary Completion DateNovember 2020	U.S.	Translate Bio/Sanofi Pasteur

Table 6
The adverse effects and the participants of licensed SARS-CoV-2 vaccines.

Vaccine	Type	Dose	Dosage	Route of administration	Overall efficiency	Number of Participants	Side effects
BNT162b2	mRNA	30 μg	2-dose series separated by 28 days	Intramuscular	52% after 1 dose; 94.6%, 7 days after second dose	**Phase I**: healthy adults, 18–55 years of age (n = 45); age 65–85 (n = 45), **Phase II/III**: healthy people and participants with but not limited to chronic, stable human immunodeficiency virus (HIV), Hepatitis C virus (HCV), or Hepatitis B virus (HBV) infections stratified into three age groups:12–15, 16–55 and greater than 55 years of age (n = 43,448).	Headache, arthralgia, myalgia, injection site pain, fatigue, chills, pyrexia (Very common), nausea, redness at injection site, injection site swelling (Common), malaise, lymphadenopathy (Uncommon), acute peripheral facial paralysis, swelling face (Rare).
mRNA-1273	mRNA	100 μg	2-dose series separated by 21 days	Intramuscular	92.1%, 14 days after 1 dose; 94.1%, 14 days after second dose	**Phase I**: healthy adults, 18–55 years of age (n = 45), or ≥ 56 years of age (n = 40). **Phase III**: adults greater than 18 years of age with no known history of SARS-CoV-2 infection (n = 30,420)	Lymphadenopathy, headache, nausea, vomiting, myalgia, arthralgia, Injection site pain, fatigue, chills, pyrexia, injection site swelling (Very common), rash, injection site erythema, Injection site urticaria, Injection site rash (Common), facial paralysis (Rare).
AZS1222 (ChAdOx1)	Viral vector	5×10^{10} Viral particles	2-dose series separated by 28 days	Intramuscular	64.1% after 1 dose; 70.4%, 14 days after second dose	**Phase I/II** (COV001): healthy adults, 18–55 years of age (n = 1077), **Phase II/III**: adults ≥ 18 years of age (n = 12,390 for UK (COV002), n = 40,000 for US, n = 10,300 for Brazil (COV003))	Vomiting, injection site induration, influenza-like illness, injection site Headache, nausea, myalgia, arthralgia (Very Common), Erythema, injection site pruritus, injection site tenderness, injection site swelling, injection site pain, injection site warmth, injection site bruising, fatigue, pyrexia, chills, malaise (Common), and Lymphadenopathy, decreased appetite, dizziness, vomiting, abdominal pain (Uncommon).
CureVac (CVnCov)	mRNA	12 μg	2-dose series separated by 28 days	Presumably i.m. injection	64.1% after 1 dose; 70.4% 14 days after second dose	**Phase I:** 250 healthy individuals aged 18 to 60 years, **Phase IIa**: 660 healthy participants in two distinct groups: older adults, ages 60 and above, and younger participants, ages 18 to 60. **Phase IIB/III:** 35,000 participants	
Ad26 SARS-CoV-2	Viral vector	5×10^{10} Viral particles	1 Dose	Injection into deltoid	85% after 28 days; 100% after 49 days	**Phase I/IIa**: healthy adults, 18–55 years of age (2 cohorts: 1a n = 377 or 1b n = 25) or greater than 65 years of age (cohort 3n = 394), **Phase III**: adults, ≥18 years of age (n = up to 60,000)	
NVX-CoV2373	Protein subunit	5 μg of protein and 50 μg of Matrix-M adjuvant	2 Doses	Injection to deltoid muscle	89.3% and 60% after 2 doses in UK and South Africa, respectively.	**Phase I**: 131 healthy adults, ages 18–59, **Phase IIa/b**: Cohort 1: healthy adults, 18–84 years of age (n = up to 4164); Cohort 2: medically stable HIV-positive (HIV +) adults, 18–64 years of age (n = 240). **Phase III**: healthy adults 18–84 years of age (n = 15,000)	
CoronaVac	Inactivated viral	3 μg with aluminum hydroxide adjuvant	2-dose series separated by 14 days	Injection	–	**Phase I**: healthy adults, 18–59 years of age (n = 144). **Phase II**: healthy adults aged 18–59 years old (n = 600). **Phase III trial**: In Brazil, ~13,000 health care, in Indonesia, enrollment consists of about 1620 healthy adults, and in Turkey, a total of about 13,000 adults	–
Gam-Covid-vac (Sputnik V)	Viral Vector	10^{11} Viral particles	2-dose series separated by 21 days	Injection to deltoid muscle	87.6%, 14 days after 1 dose; 91.1% 7 days after second dose	**Phase I/II**: healthy adults,18–60 years of age (n = 76), **Phase III**: healthy adults 18–111 years of age (stratified as 18–30, 31–40, 41–50, 50–60 and 60 +) (n = 40,000).	15% of that vaccinated report redness in the area of the vaccine shot and a slight headache that goes away within 24 h.
BBIBP-CorV	Inactivated virus	4 μg with aluminum hydroxide adjuvant	2-dose series separated by 21 days	–	–	**Phase I**: 18–59 years of age (n = 96) or ≥ 60 year of age (n = 96). **Phase II**: healthy adults, 18–59 years of age (n = 448). **Phase III**: healthy adults, 18 years and above (n = 15,000)	–

synthesis process that acts as potent adjuvants as a ligand for TLR 7/8. Via coupling of SARS-CoV-2 RBD to these VLPs, the RBD immunogenicity significantly increased and the triggered antibodies could inhibit the RBD binding to the ACE2 viral receptor [79].

A phase I clinical trial recruiting in Canada evaluates the tolerability, immunogenicity and safety of a plant-derived recombinant coronavirus-like particle. The Medicago VLP vaccine was produced by plants as bioreactors to synthesize the S protein of SARS-CoV-2. Subsequently, these proteins assemble to form VLPs that resemble the virus, without showing any of the infectious features, which are easily detected by the immune system of the host. Fig. 1 shows a schematic of several vaccine platforms designed for SARS-CoV-2, which activate the immune response of the host.

5. SARS-CoV-2 variants impact the efficacy of vaccines

In December 2020, an unforeseen rise occurred in COVID-19 cases. That was assigned to the appearance of the new SARS-CoV-2 variants 501Y.V2 (B.1.351) in South Africa and 501Y.V1 (B.1.1.7) in the UK [80,81]. In South Africa, high transmission and high herd immunity may have supported the appearance and the following spread of the variant. Both variants showed a mutation (N501Y) on the spike protein's receptor-binding domain that is suggested to participate in higher transmission [82]. It is estimated that the transmission rate is between 40 and 70% [81]. Furthermore, the 501Y.V2 variant shows two additional mutations (K417N and E484K) in the spike protein that allow a possible immune escape from antibodies [83]. Additionally, another set of mutations (N501Y, K417T, and E484K) in a new P.1 (501Y.V3) lineage has been characterized in Manaus, Brazil [84]. Also, L452R is another mutation that was observed to be increased. It was recently associated with a significant breakout in California, but health professionals stated that it's not clear if it caused more infections [85]. A critical issue is whether COVID-19 vaccines can protect against these new SARS-CoV-2 variants or not. Initial research demonstrated that individuals sera who immunized with the mRNA COVID-19 vaccines could neutralize a 501 mutation pseudo-virion, whereas it neutralizes a 501-484-417 mutant pseudo-virion to lower levels [86]. Collier et al. evaluated immune responses after vaccination with mRNA-based vaccine BNT162b22 [87]. They quantified neutralizing antibody responses after first and second immunizations with the eight amino acid mutations found in the B.1.1.7 spike protein or pseudo-viruses expressing the wild-type Spike protein. They reported that the vaccine sera displayed a wide range of neutralizing titers in the case of wild-type pseudo-viruses that were quietly reduced against the B.1.1.7 variant. This decline was also observed in sera from some recovering patients. Lowered B.1.1.7 neutralization was also reported with monoclonal antibodies targeting the RBM (5 out of 31), N-terminal domain (9 out of 10). But is not observed in RBD neutralizing mAbs binding outside the RBM. Presentation of the E484K mutation in a B.1.1.7 background to consider a newly appeared Variant of Concern (VOC 202102/02) resulted in a more remarkable loss of neutralizing function by vaccine-induced antibodies and mAbs (19 out of 31) over that obtained by the B.1.1.7 mutations alone. E484K appearance on a B.1.1.7 background provides a threat to the vaccine BNT162b [87]. Wang et al., in another study, observed that B.1.1.7 is resistant to neutralization by most mAbs to the N-terminal domain (NTD) of the spike [88]. It is also quite resistant to a few mAbs to the receptor-binding domain (RBD). However, it is not more resistant to recovered persons plasma or vaccine sera. Findings on B.1.351 are more worrying because this variant is not only resistant to neutralization by most NTD mAbs but also multiple individual mAbs to the receptor-binding motif on RBD, mainly because of an E484K mutation. Furthermore, B.1.351 is significantly more refractory to neutralization through vaccine sera (10.3–12.4-fold) and convalescent plasma (9.4-fold). Emergent variants13,14 and B.1.351 with identical spike

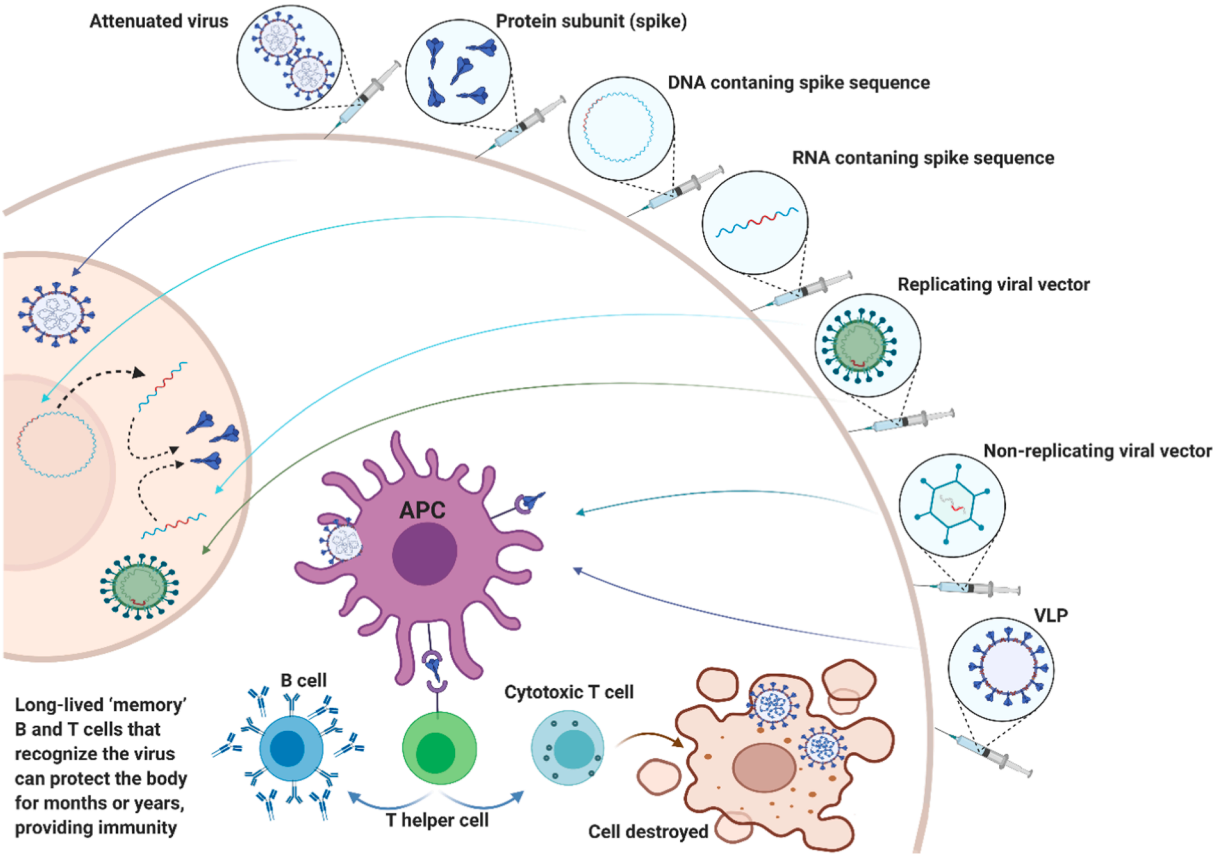

Fig. 1. Various approaches for SARS-CoV-2 vaccine designation leading to induction of host immune response.

mutations show new mAb therapy problems and warn of current vaccines' protective efficacy [88]. Besides, initial clinical trial findings of ChAdOx1 nCoV-19 suggested 74% efficacy in the UK3 but only 22% in South Africa. In contrast, NVX-CoV2373, a protein-based COVID-19 vaccine, demonstrated 89% efficacy in the UK, but in South Africa, where the 501Y.V2 variant predominates, the effectiveness was only 49% [89,90]. Similarly, efficacy differences in South Africa and the USA (57% vs. 72%) were observed for the Ad26COV2.S COVID-19 vaccine [91]. Promisingly, in South Africa, 85% protection against COVID-19 for the Ad26COV2.S vaccine has been shown. However, we are not sure about the precision estimated value released by the press [92]. A confirmed vaccine strategy that targets at risk of severe COVID-19 might be effective even in the presence of variants [93].

After a period of genetic stability, the new variants of SARS-CoV-2 caused concern since multiple new immune escape variants could be emerged in the future and resulted in a severe epidemic return, as observed in South Africa. The higher viral transmission provides a greater chance for occurring SARS-CoV-2 variants. Therefore, ending the pandemic disease is only possible when effective vaccines against new variants are administered across the world fairly. While high-income countries compete to vaccinate their people within months, they leave themselves unprotected to new variants of SARS-CoV-2 evolving in lower-income countries that vaccines could not protect them [93]. To control new variants of SARS-CoV-2, formulating new vaccines may be often required [81]. Along with the increased primary reproduction number of variants with more transmissible SARS-CoV-2, more vaccine coverage will be needed to achieve population immunity, and vaccinating children might also be critical to obtaining this coverage [93].

6. The persistence and dynamics of convalescent anti-SARS-CoV-2 antibodies and reinfection risk

A few weeks after infection with SARS-CoV-2, patients develop antibodies against viral proteins [94–97]. Some weeks after symptom initiation, most infected individuals' serum can attach to the viral spike protein and neutralize it in vitro [94,98,99]. The serum' reciprocal dilution can inhibit the viral infection up to 50% (neutralizing antibody titer at 50% inhibition [NT50]). It is generally between 100 and 200 at 3–4 weeks following the symptom occurrence [100]; however, the range of neutralizing titers is undetectable to more than 10,000 [98,99,101].

There is currently limited data on the neutralizing antibody dynamics in the months following recovery from SARS-CoV-2. In severe viral infections, antibody neutralization rises quickly after infection due to a sudden increase of short-lived antibody-secreting cells. Then diminishing this peak before reaching a stable plateau and it can be kept for years to some decades via memory B cells and long-lived plasma [102,103]. The dynamics mentioned above have been reported for many viruses, such as respiratory syncytial virus, influenza, Middle East respiratory syndrome coronavirus, seasonal human coronavirus 229E, and the SARS coronavirus 1 [104–108].

Various studies followed up antibody levels in recovered individuals from SARS-CoV-2 infection for the first few months following the symptom occurrence [94,95,98,101,109,110]. After the first three months, most of them have reported, those antibodies that target the spike protein reduced several-times from the peak [94,98,110]. This finding suggests the similar early dynamics of the antibody response to SARS-CoV-2 and other acute viral infections. In 2021, Crawford et al. [111] evaluated both the binding and neutralizing antibody levels in serial samples of plasma from 32 SARS-CoV-2–infected persons with a range of disease severity follow-up as long as 152 days after symptom initiation. They reported that, on average, neutralizing titers declined about four folds from approximately 30 to more than 90 days after symptom beginning. This reduction in neutralizing titers was accompanied with a decline in antibodies level that attaches to the spike protein and its receptor-binding domain (RBD). However, most convalesced individuals still had significant neutralizing titers at 3–4 months after symptom start [111]. In another study, Iyer, and colleagues in 2020 quantified plasma and/or serum antibody responses to the receptor-binding domain of SARS-CoV-2 'S protein in 343 North American people infected with SARS-CoV-2 (of which 93% were hospitalized) up to 122 days following symptom initiation [112]. Next, they compared the results with responses of 1548 individuals whose blood samples were taken before the pandemic. This investigation's findings were also added to rising evidence on the perseveration and decay of antibody responses after SARS-CoV-2 infection. IgA and IgM responses to RBD were short-lived, and most patients seroreverted within 2.5 months following the beginning of the illness. However, IgG antibodies were preserved at detectable levels in individuals more than 90 days after symptom start, and seroreversion was only reported in a small part of patients. These anti-RBD IgG antibodies' concentration was also highly associated with pseudo-virus NAb titers, which also showed minimal decay. Observing the persistence of IgG and neutralizing antibody responses is promising and displays the robust systemic immune memory development in patients with acute infection. These results were identical to those observed in a study on anti-RBD antibodies in 121 North American convalescent plasma donors up to 82 days from symptom onset [113] and a research work of 1,197 Icelanders who stayed seropositive by two pan-IgG SARS-CoV-2 antibody assays 120 days following the qPCR diagnosis of SARS-CoV-2 (9). These results differed from other new studies that demonstrated a more quick waning in anti-RBD titers after asymptomatic or mild SARS-CoV-2 infection [109,114].

Antibody levels declined with time, but few researchers studied the quality and nature of the memory B cells that would be needed to generate antibodies upon reinfection has not been investigated yet. Gaebler et al. [115] examined the humoral memory response in a cohort of 87 patients evaluated at 1.3 and 6.2 months following the infection with SARS-CoV-2. They reported that IgM and IgG titers against the RBD of S protein declined remarkably over this period, with IgA being less influenced. At the same time, neutralizing function in plasma was reduced by five-times in pseudotype virus assays. In contrast, the RBD-specific memory B cells' number stayed unchanged at 6.2 months following the infection. Memory B cells showed clonal turnover after 6.2 months, and their antibodies demonstrated raised potency, more somatic hypermutation, and refractory to RBD mutations, suggested the continued evolution of the humoral response. PCR and immunofluorescence analyses of intestinal biopsies took from asymptomatic patients at four months after the COVID-19 onset showed the perseverance of SARS-CoV-2 nucleic acids and immunoreactivity in the small intestine of 7 out of 14 individuals. Thus, it concluded that the memory B cell responses to SARS-CoV-2 develop between 1.3 and 6.2 months following the infection in a similar manner with antigen persistence [115]. It is not clear whether the recovered patients have a risk of reinfection with SARS-CoV-2. During an early recovery phase from initial infection, Bao et al. rechallenged the rhesus macaques with SARS-CoV-2 [116]. The monkeys readministered with the similar SARS-CoV-2 strain could not generate detectable viral spreading, clinical demonstration, and histopathological alteration. This study showed that a significant neutralizing antibody response might be involved in protecting the rhesus macaques from SARS-CoV-2 reinfection. They concluded that primary SARS-CoV-2 infection maybe protects from following reinfection [116]. However, more studies are yet needed.

Most respiratory viruses lead in immunoglobulin concentrations that persist for a few months, whereas neutralizing immunoglobulin against SARS-CoV-2 lasts only for about 40 days [117]. On the other hand, unlike seropositivity for IgG following the primary infection, positive RNA tests have been reported [118]. Nonetheless, such cases have been explained as sampling errors, silent carriers, or low commercial kits' accuracy. In some cases, the time window between the first infection and the second positive RNA test, which is approximately two months, may indicate reinfection or reactivity of a latent infection with the virus

[119].

Studies are still encouraged to develop a more efficient vaccine. Current challenges raise some concerns that vaccination may not lead to a long-term and effective immunity against SARS-CoV-2. These concerns are the existence of more than 80 genotypical variants of the SARS-CoV-2, the likelihood of reinfection, and the short-lived seropositivity for neutralizing antibodies. Moreover, Ig levels may not be associated with the risk of transmissibility of SARS-CoV-2 and viral shedding [118]. Also, the short-lasting immunity against the virus may prevent the improved homogeneity of affected individuals in a certain time frame. Thus, population immunity may not be obtained; because reinfection may happen even in the presence of neutralizing antibodies. These challenges led to the concern that abolishing the COVID-19 pandemic may not be as practical as assumed. Thus, we must rely more on transmission prevention until virus features are more characterized [120] and more effective anti-SARS-CoV-2 drugs are developed.

7. Challenges for vaccine development

In COVID-19 infection, the host immune cellular and antibody response and post infection protection are highly limited. In order to find a suitable antibody marker for protection and evaluation of vaccine efficacy, better characterization of the SARS-CoV-2 is required. There are important issues that should be considered for the development of a potent vaccine including the viral genetic changes, immune enhancement, immunosenescence in the elderly as well as decreased in antibody content over time. For testing the vaccine efficacy, suitable immunological and clinical markers required to be identified. Moreover, the cold chain storage conditions also should be taken into account to prevent the challenges like the Ebola vaccine challenge (its optimal storage temperature is under −60 °C) [10,121].

Although the S protein is targeted by multiple vaccine platforms against the SARS-CoV-2 infection, the main elements of a defensive immune response should be considered. These elements include: (1) The function of neutralizing antibodies in the host protection, specific epitopes that may targeted by neutralizing antibodies, and threshold neutralizing antibody reaction against the SARS-CoV-2. (2) The function of anti-S protein non-neutralizing antibodies attaching the infected cell membrane. (3) The function of mucosal immune response to infection or viral spread in the respiratory system. (4) The possible importance of humoral immunity against other viral ORFs to induce the immune response, mainly those located on the cell surface or secreted to extracellular environment that immune antagonist activity. (5) The importance of $CD4^+$ and $CD8^+$ T cell responses to the induction of protective immunity by a vaccine.

A major challenge with SARS-CoV-2 vaccine candidates is the lack of high-throughput animal disease models for the selection of candidate vaccines and the detailed study of vaccine immunogenicity. Evaluation of immune response and pathogenesis in various animal models including primates and non-primates is still in its early stages. Bao et al reported that the laboratory species of mouse are not prone to COVID-19 [122]. In addition, the immunogen structure, vaccine formulation, and age of vaccination can affect the immune system and the consequence of naturally occurring infection, though all of these issues about COVID-19 should be studied extensively.

8. Approaches to overcome major weak spots

Although several vaccine candidates have been developed for COVID19, there is still a considerable distance to achieve one ready for public use. However, low efficiency, immune adaptability, tolerability, and safety are the major weaknesses of current vaccine candidates. In addition, the alteration that occurs in the host during viral replication fails to resolve by most of the vaccines. All of these weak spots are major impediments for preclinical and clinical research of promising vaccine candidates. In this regard, there are some recommendations that may help to overcome these barriers.

8.1. Engineered human mesenchymal stem cells

Studies have shown that modified mesenchymal stem cells (MSCs) expressing the SARS-CoV-2 proteins can be exploited as a new and efficient candidate for vaccines. Liu et al. introduced a novel technique against the COVID-19 based on genetically modified human MSCs (hMSCs), which is similar to the response of a small protein antigen unit, but is cleared and degraded gradually during the recognition process by the body's immune system. After several experiments on the antibody response, they achieved the injection of a collection of cocktail-like modified hMSC line. This strategy can open a new window for designing new vaccines against SARS-CoV-2 [123].

8.2. Nanocapsules

The polymeric antigen-based nanocapsules are very interstin to promote the vaccines as an antigen-adjuvant delivery complex for targeting the main cells of the immune system, especially in the liver. To overcome the previous mentioned limitations, induction of long-lasting and potent TH1-oriented immunity via single-dose vaccination as well as the production of immunogenic and safe nanocapsules is a promising approach. Nanoparticles derived from viruses are mainly attractive to novel vaccine formulations, producing strong biodegradable protein nanocapsules generated from the pathogen-specific antigen. The preparation of hydrophilic nanocontainers through water-in-oil mini-emulsions and then delivery to an aqueous solution is greatly important, as it allows hydrophilic payloads to be encapsulated efficiently in large quantities. The NS5A protein was used to produce the vaccine candidates for the hepatitis C virus. Accurately adjustable size and the surface properties of the nanocapsules, the simultaneous prescription of vaccine-antigen and vaccine -adjuvant and therefore the probability of targeting specific immune system cells, are the major benefits of synthetic particulate vaccines [124].

8.3. Autologous dendritic cell-based vaccine

The whole cell vaccines are classified into two groups of autologous and allogenic types, which are genetically modified to induce the production of chemokines, cytokines and co-stimulatory molecules reinforcing the immune stimulation. Using the immune cells extracted from the patient, specially their DCs, is another type of cellular-based vaccination. The DCs are formulated by loading the autologous DCs of patient co-treated by immunoadjuvants using nucleic material or spike antigens. Then, the DCs loaded with antigen are re-prescribed to the patient for the in induction of the immunity against viruses.

8.4. Liposomes

Liposomes are composed of biocompatible phospholipids that form spherical vesicles. They are used as either delivery vesicles or adjuvants in vaccinology [125]. Plasticity and versatility are the major strengths reported for the liposomes. It is possible to control the antigen charge, incorporation, location and size by selecting lipids and their formulation technique. Antigens linked with the surface, integrated with the lipid bilayer, or encapsulated within the liposome. The antigen site in the liposome determines the type of vaccine-induced immune responses. Encapsulated and surface-linked antigens induce T cell response, while surface-linked antigens induced the responses of B cells. The CD4 Th cell epitope inclusion induces a potent antibody reaction to the B cell antigen target. Also, the immunity on the T cell epitope is reduced via fully spatial separation of the two antigens through the liposomal bilayer, reducing the immunity on the T cell epitope. The liposomal capacity for carrying cargoes enables immunostimulatory molecules to be simultaneously transferred to the target immune cells, including cytokines or

TLR agonists, thus minimizing the systemic sensitivity to such adjuvants. The first approved liposomal vaccines were diphtheria toxin (1974). Subsequently, the liposomal vaccines of Epaxal and Inflexal V were approved for hepatitis A and influenza with human use, respectively [126–128].

8.5. Multi-epitope vaccine

According to computational studies, the engineered multi-epitope vaccine has stable structure, which induces particular immunity and thus can be a possible option for the SARS-CoV-2 vaccine.

Immunoinformatic tools have been employed to generate such SARS-CoV-2 vaccine that consists of IFN-γ, HTL and CTL epitopes capable of inducing robust immunity. It has been found that these vaccines are both antigenic and immunogenic. The simulation method of molecular dynamics (MD) has ensured the stable engineered vaccine and Molecular Docking studies verified a robust interaction between the immune receptors and the vaccine. Based on the in silico expression finding, the vaccine's expression has been verified in the bacterial host. In addition, Immune Simulation studies validated the effectiveness of the vaccine in triggering an immune response [129].

In an experimental study, the vaccine peptide platforms from the SARS-CoV-2 S protein were selected for the immunization of the mice, followed by testing the antigenic B/T-cell epitopes in all proteins encoded by SARS-CoV-2, and fabricating a new multi-epitope peptide virus vaccine. The results showed a significantly higher serum IgG level and elevated ILN CD19 cells in peptide-immunized animals in comparison with controls. Also, the density of lymphocytes secreting IFN-γ in $CD8^{+}/CD4^{+}$ cells were higher in the peptides-immunized animals when comparing with the controls. The count of splenic IFN-γ-secreting T cells was larger in the intervention group. Specific cell-mediated and humoral immunity in the animals were successfully elicited by the obtained vaccine peptides. However, there is a need for primate tests and clinical trials for the confirmation of the safety and efficacy of such vaccine peptides [130].

9. Estimating certainty of success

The accessibility to an efficient and safe COVID-19 vaccine is well-confirmed as an essential tool to control the pandemic. Therefore, the efforts and strategies are required to rapidly develop, evaluate, and create the large scale are enormous. It is necessary that as many vaccines as possible are evaluated because we cannot predict how many would be viable. To raise the success chances (due to the high attrition level during the vaccine development), all vaccine candidates should test until exclusion. World Health Organization facilitates collaboration and accelerates efforts on a scale which has not been seen before [45].

When candidate vaccines are used in human trials, they first undergo phase trials primarily to test the safety of vaccine, determine dosages and identify adverse side effects in a limited number of participants. Phase trials further analyze safety and begin investigating efficacy on bigger groups. The final phase, phase 3 trials, that few vaccines ever enter, is much larger, involving thousands of people, to confirm and evaluate the vaccine's effectiveness and to test whether there are any rare adverse effects that only appear in large groups. If a candidate for vaccine is confirmed successful in human clinical trials, the developers can seek approval by a national regulatory agency, including the U.S. Food and Drug Administration and the European Medicines Agency. The unprecedented speed and scale of the epidemic COVID-19 has forced us to make a substantial alteration in the conventional vaccine generation route that takes an average of more than 10 years, even in comparison with an accelerated 5-year period to develop the first Ebola vaccine, to produce a novel vaccine using patterns like manufacturing capacity scaling, adaptive and parallel production stages and innovative regulatory processes. Furthermore, preclinical studies of the SAR-CoV-2 vaccine candidates may require parallel clinical trials. Considering the speed imperative, the vaccines are said to be available for emergency application or such cases by early 2021 [64].

The fundamental data collection to develop and test the COVID-19 vaccines should be well defined in order to make a vaccine possible. These data include determining target antigen, correlated immune protection, immunization route, target product profile, production facility, animal models, scalability, target community and outbreak prediction.

An essential parameter in the "certainty of success" in progression of human SARS-CoV-2 vaccine is proposed infectious inoculum intensity at a personal level, and infectious force at a population level. Reducing the intensity of infectious inoculum (and infection force at population level) is predicted to prolong the incubation period, which in turn is predicted to decrease the severity of the disease, and increase the chance of anamnestic reaction when exposed to the circulating virus [35].

10. Conclusion and future perspective

The COVID-19 Humanitarian and Economic Impact Scale is a rapid assessment of next-generation vaccine production strategies via novel patterns for faster development. Very little information is currently available on the host immune response to SARS-CoV-2, although some investigations have reported certain alterations in the innate and adaptive immunity in patients with COVID-19. According to studies, a candidate for the COVID-19 vaccine should induce a strong and persistent response that includes both T cell responses and neutralizing antibodies to trigger a satisfactory protective level [131].

A prominent characteristic of COVID-19 vaccine development is the presence of various strategies such as inactivated virus methods, live attenuated virus approaches, recombinant protein, replicating and non-replicating viral vector, peptide, virus- like particle and nucleic acid vaccines [64].

The DNA/mRNA-based vaccine platforms provide a high flexibility for rapid designation and antigen manipulation. The viral vector vaccines provide a great protein expression level, prolonged stability, and a robust immunity.

There is little knowledge about the specific SARS-CoV-2 antigen (s) employed in the vaccine production. Many known candidates for vaccine development are intended to generate antibodies neutralizing ant-s, inhibiting viral attachment to ACE2 receptor on host cells. However, it is unclear how the different S proteins exploited in various vaccines interact with each other or with the genomic epidemiology of COVID-19. Previous knowledge about the development of SARS-CoV vaccines demonstrates the capabilities for various immune reinforcement impacts, which is controversial and could be useful for vaccine production.

Until the September 2020, 38 vaccines have been tested in clinical trials on humans including, 25 vaccine candidates in phase I, 14 vaccine candidates in phase II, 9 vaccine candidates in phase II, and 3 vaccine candidates approved for early or limited use. Moreover, at least 93 preclinical vaccines are under preclinical studies on animals.

Nucleic acids, protein subunits, and viral vectors are novel approaches that meet the prerequisites to overcome the challenges in vaccine development against COVID-19 and allow rapid manufacturing of vaccine. Each vaccine platform and technology Each vaccine technology has its own advantages and disadvantages associated with its manufacturing capacity, stimulating certain immune responses, and safety and efficacy for use in human. The RNA vaccine platform appears promising for developing an effective COVID-19 vaccine. At present, the mRNA vaccine from Moderna TX, Inc., is the biggest phase III trial, with 30,000 participants that is recruited in 87 centers. Considering the unprecedented scale and speed of the COVID-19 pandemic, there is a need for basic alterations from the conventional technologies of vaccine generation (from 10 to 15 years to 1–2 years) to accelerated development, evaluation and production of COVID-19 vaccine at large scale. To this end, simultaneous preclinical, clinical and scale-up fabrication steps are needed to be in parallel. However, the limited information about the

immune response of the host to the SARS-CoV-2 is a major challenge. Moreover, viral genetic changes, immune enhancement, vaccine formulation, and age of vaccine recipient are other important challenges to generate an efficient candidate of vaccine for COVID-19 that need to be studied more extensively.

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.

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