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1. Introduction

The recent pandemic of coronavirus disease 19 (COVID-19) caused by coronavirus is spreading in the world very rapidly. Coronavirus is a single-stranded RNA virus with a ~27- to 32-kb size positive sense genome that encodes 27 proteins including spike (S), envelope (E), nucleocapsid (N) and glycoprotein (M) as structural proteins, RNA-dependent RNA polymerase (RdRP) and protease as non-structural proteins, as well as hemagglutinin (HE) as viral envelope glycoproteins [1].

The infection first originated in Wuhan, China in December 2019 and spread to almost all countries of the world with a high spreading potential. According to World Health Organization (WHO) report [2], COVID-19 has infected more than 100 million (123,419,065) people worldwide and caused 2.7 million deaths (Fig. 1). This pandemic as a zoonotic breakout was transmitted to human and spread among humans by close contacts and respiratory droplets which formed by sneezing, coughing and even talking, as well as contact with surfaces that were previously touched and infected by patients suffering COVID-19 [3]. Disease is characterized by fever (98%), dry cough (75%) and tiredness (45%) as the most common symptoms as well as diarrhea, loss of taste or smell and rash on skin as less common symptoms (20–30%). Moreover, uncommon serious complication including Guillain-Barre syndrome (GBS) associated COVID-19 as neuropathy complication [4] and cardiovascular disorders [5] was reported which can be consider life-threatening for patients. However according to the WHO report, serious symptoms including difficulty in breathing, chest pain and loss of speech or movement [6]. Any chronic pulmonary diseases, hypertension and cardiovascular diseases, obesity (body mass index ≥ 30), type 2 diabetes mellitus and immunocompromised state are considered as risk factors [7].

Currently, there is no definite treatment for COVID-19 and only supportive methods including oxygen therapy, plasma (or antibody) therapy, antipyretic and anti-inflammatory drugs that prevent cytokine
According to the last update of the WHO report on April 2nd, 2021, there are 85 vaccine candidates in clinical trial phase of study and 184 candidate vaccines are being evaluated in pre-clinical stages (Fig. 2). These vaccines can be classified into six different types (Fig. 3): inactivated vaccines, live-attenuated virus vaccines as a combination of both protein and gene-based vaccines, subunit vaccines and VLPs as protein-based vaccines, nucleic acid-based vaccines (DNA and RNA) and recombinant vector-based vaccines (replicating and non-replicating viral vector) as gene delivery based vaccines [8,10].

Immunogenic candidates are several viral structural proteins (spike protein) [11] and non-structural proteins (RdRP and protease) [12,13], however, spike protein is paid more attention in designing vaccines [14]. This structural protein which was encoded in the 3’ end of the SARS-CoV-2 RNA sequence, was targeted in vaccines to be detected by the host immune system [15,16]. Spike protein, as a fusion protein, helps to attach and uptake of coronavirus by host cells, specially lung alveolar epithelial cells following interaction between angiotensin-converting enzyme 2 (ACE2) receptor on cells surface and receptor-binding domain (RBD) of spike on SARS-CoV-2 [9,17,18].

Some of the vaccine candidates have effective results against the new coronavirus in primates [14]. Moreover, the production of neutralizing antibodies has been detected following different clinical trials in humans [12,19–21]. Some developers including Sinopharm, Sinovac, Pfizer-BioNTech, Moderna, Oxford-AstraZeneca, Johnson & Johnson, Bharat Biotech (Covaxin) and Sputnik V (Gamaleya Research Institute) are COVID-19 vaccines that seems to be effective [22]. This review article was written based on the latest update of candidate vaccines against COVID-19 and aimed to summarize each vaccine with their last status and characteristics.

2. Dosage and route of administration of COVID-19 candidate vaccines

According to the last update of WHO (April 2nd, 2021) [23] COVID-19 vaccines may be administrated in three program administration doses. Fig. 4-A shows that 13 (15%) of vaccines are administrated in a single dose (blue). Double-dose vaccines (light to dark brown) are 52 (61%) and are given on two different days at regular intervals, for instance, on the day 0 and day 14 (7%), day 0 and day 21 (24%) as well as day 0 and day 28 (31%). Moreover, only one vaccine candidate is administrated in three-doses in days 0, 28 and 56.

Vaccine administration categorized into two oral-based and injectable routes, while injective vaccines can be administrated subcutaneous (SC), intradermal (ID) and intramuscular (IM) routs. Among injectable vaccines, 64 (75%) are IM, 3 (4%) are SC and 3 (4%) are ID administrated (Fig. 4-B).

3. Innate immunity

Both innate and adaptive immunity are involved in identifying and eliminating invading pathogens but, non-optimal and uncontrolled response contributes disease progression. Therefore, understanding the mechanism and changes of immune responses will help to understanding the disease and treatment approaches [24].

SARS-CoV-2 disease occurs following a lung infection through a person-to-person transmission. Development of disease and outcome of infection is affected by the interaction between the virus and the immune system [25].

SARS-CoV-2 genomic structure contains 14 open reading frames (ORFs). Non-structural proteins (NSPs 1–16) are encoded by ORF1a and ORF1b, located at the 5’-untranslated region (5’-UTR), encodes two polyproteins (p1a, p1b) that are processed by two virally encoded cysteine proteases such as the papain-like protease (PLpro) and a 3C-like protease (3Clpro). Pp1a, pp1b imply in genome transcription and replication with forming a replication-transcription complex (RTC). On the other hand, RTC synthesize a nested set of subgenomic RNAs (sgRNAs). Structural proteins are encoded by other ORFs located at the 3’-UTR [1,24,26]. In addition to the aforementioned structures, there are 8 accessory proteins that are species-specific (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b and ORF14) [27].

Spike proteins are enveloped-anchored proteins consisting S1 (receptor-binding) and S2 (membrane fusion) subunits (Fig. 4). ACE2 act as the receptor for RBD of S1 subunit [28]. ACE2 as carboxypeptidases is expressed in various cell membranes, especially lung cells and enterocytes or may be found in circulatory form. It has been reported that SARS-CoV-2 interacts with renin angiotensin (Ang) aldosterone system via ACE2 enzyme. ACE2 in renin angiotensin system (RAS) enzymatic cascade mediate conversion of Ang 1 to Ang 1–7. Ang 1–7 exert the effects via Mas receptor (MasR). The ACE2/ Ang 1–7/ MasR pathway causes increases in plasma Ang-(1–7) levels and increases in ACE2 expression. Thus, ACE2 facilitate entering of SARS-CoV-2 and establishes a relationship between expression of ACE2 receptors and
susceptibility to COVID-19 disease [29,30].

Interaction of host proteases such as transmembrane protease serine 2 (TMPRSS2) and cathepsin with S protein and ACE2 facilitate entering the virus to the target cell [29]. These proteases can cleave a fusion site at the boundary between S1 and S2 subunits leading to fusion of viral and host membranes and release of viral genome into host cell (Fig. 5) [28,31]. In addition to ACE2, heparan sulfate (HS) is needed as a co-receptor on the surface of host cells for S protein binding to cells. Ectodomain of the S protein interacts with HS through RBD. The HS makes structural changes in RBD and converts it from inactive to active form and facilitates ACE2-binding. Indeed, HS primes the S protein for ACE2 interaction and it promotes viral entry [32,33]. This process can be targeted by therapeutic approaches due to interference with virus binding [32]. Host factors like valosin-containing protein (VCP) imply in entry of virus by releasing virus form endosomes [34]. Overall, virus enters to the host cells via endocytosis or fusion of its envelope with host cell membranes. Viral genome is released in host cell cytoplasm following uncoating [35]. Virus uses host translational system to make its protein, and can also shutdown host mRNA translation and inhibition of innate immune response by binding of NSP1 to the 40S ribosomal subunit [36].

Pattern recognition receptors (PRRs) known as innate immune receptors consists of: i. Toll like receptors (TLRs) especially TLR3, TLR7 and TLR8 as endosomal receptors and ii. Melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I), which are cytosolic sensors that can detect conserved pathogen-associated molecular patterns (PAMPs) like viral RNA molecules and iii. Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) which detect cytoplasmic DNA following DNA damage in viral infection [29,37]. Activation of PRRs leads to expression of transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interferon (IFN) regulatory factor 3 (IRF3), IRF7 and subsequently activation of pro-inflammatory cytokines [29]. TLR-3 contribute activation of NF-κB and IRF3 via TRIF (TIR-domain-containing adapter-inducing interferon-β) and production of type I interferons (IFN alpha and beta), inflammatory cytokines (IL-6, TNF), and IFN-gamma [38]. Interferon signaling, in turn, is involved in boosting the immune system through JAK-STAT pathway [39]. TLR7 and TLR8 are other receptors that recognize single stranded RNA molecules and activate the MyD88 pathway, and subsequent activation of signaling pathways such as mitogen-activated protein kinase (MAPK) and NF-κB leading to increase expression of IL-1β, IL-6, IL-12, TNF-α and IFNα [29]. MDA5 and RIG-I are sensors that sense viral RNA molecules [40]. They induce signal transduction via a common adaptor naming Mitochondrial Antiviral-Signaling protein (MAVS), on the mitochondrial outer membrane which lead to IFN-I production [27] but SARS-CoV-2 can suppresses IFN-I responses by binding of ORF9b to MAVS [41].

Cytokine storm is reported to be a common feature of severe cases in COVID-19. Following the described processes, activation of innate and adaptive immune cells such as macrophages, Natural killer (NK) cells, and gamma-delta T (γδ T) cells promote cytokine storming [42]. IFN-γ
and TNF-α affect immune cells and endothelial cells to release pro-inflammatory cytokines like IL-6 and IL-1β, which recruit neutrophils and T lymphocytes [42,43] (Fig. 6).

Neutrophils are the first presented leukocytes in infected sites [44] and neutrophils infiltration in pulmonary capillaries and alveolar space is taking place in severe COVID-19. The neutrophilia in COVID-19 may be related to cytokine storms [45]. Neutrophils can phagocyte pathogens or form neutrophil extracellular traps (NETs) to remove them [44]. NETs are the main effector mechanism following activation of neutrophils which have a dual function: they may kill microorganisms, and they can damage host tissues of COVID-19 patients, as increased level of NETs in plasma and lung tissue is reported in the patients. COVID-19 trigger the release of NETs to promote lung apoptosis [46] and, on the other hand, they enhance thrombi formation and cytokine production. In this regard, inflammation lead to widespread thrombus formation and disseminated intravascular coagulation [47].

In accordance with described findings, increased levels of IL-1, INF-γ, interferon gamma-induced protein 10 (IP-10), and monocyte chemo-attractant protein 1 (MCP-1) are detectable and stimulate the T-helper type 1 (Th1) cell activation [48]. Higher levels of these cytokines and CXCL10, CCL2, and TNF-α correlate with severity of disease. Also, high serum levels of granulocyte colony-stimulating factor, IP-10, TNF-α, MCP-1, and macrophage inflammatory protein 1A were reported in patients admitted to the intensive care units [49].

On the other hand, high circulating levels of cytokines can activate macrophages [49]. Two types of macrophages reside in lungs including alveolar macrophages (M1) and interstitial macrophages (M2). M1 macrophages are activated by PAMPs and enhanced recruitment of immune cells to lungs. M2 macrophages have opposite effect by inducing the release of anti-inflammatory cytokine [50]. ACE2, furin and TMPRSS2 are expressed on macrophages. SARS-CoV-2 can infect macrophages and dendritic cells (DCs) and drive invasion in lungs [51]. It is not clear exactly what effect SARS-CoV-2 has on the macrophage phenotype, but impaired or delayed Type 1 IFN signaling has been demonstrated. It was noted that ORF6 and ORF8 inhibit the type 1 interferon signaling [52].

Dysregulated macrophage responses can be harmful for host. Aberrant activation of macrophages contributes coagulation in severe form of disease. In this regard, decrease in platelet count and increase in D-dimers, as fibrin degradation products, and presence of microclots in organs such as lungs, lower limbs, brain, heart, liver and kidneys were described in patients with COVID-19 [53].

DCs interplay between innate and adaptive immunity. There are three subset of DCs including type 1, type 2 myeloid/conventional DCs (cDC1/2 in mice) and plasmacytoid DC (pDCs). pDCs are distributed in the respiratory tract [53] and expression of ACE2 on dendritic cells allows the virus to infect these cells. Interaction between CD147 and spike protein is also a new route of infection [25]. DC-specific molecular nonintegrin-3-adherent (DC-SIGN, CD209) is a type of innate immunity receptors that is expressed on DCs and to a less extent on B-cells and macrophages. DC-SIGNs interact with mannose residue on pathogens and fucosylated residues on endothelial, epithelial and myeloid cells. Decrease in expression of DC-SIGN accompanied with lower risk of severe infection. Interaction of COVID-19 and DC-SIGN may contribute virus escaping [53].

Following activation of pDCs, they exert antiviral activities like production of IFN-α which is happening in two ways. IFN-α promote macrophages to remove virus from circulatory system or may prime adaptive immunity by persevering cDC integrity [54].

The other effectors of innate and adaptive immunity are NK cells. NK cells can directly cause cell lysis, and indirectly, they cause inflammatory cytokine production such as IFN-γ and TNF-α, antibody-dependent cell cytotoxicity (ADCC) and interact with TLR ligands [55,56]. Major histocompatibility complex class I (MHC I) molecules as ligand for NK receptors such as killer cell immunoglobulin-like receptors (KIRs) (KLRG1, and TIGIT) and the lectin-like CD94-NKG2A prevent killing of self-cells that express MHC-I [57]. The function of NK cells in COVID-19 is enhanced with increased expression of NKG2A. In this regard, production of IFN-γ, IL-2, granzyme B, and TNF-α are reduced [41].

Pathophysiological and epidemiological studies must be considered to clarify pathogenesis of COVID-19. Innate immune acts as the first defense line against the virus [45]. Hyper-activation of innate immune responses and aberrant cytokine production are associated with higher morbidity and mortality in COVID-19 infection. Regulation and control of cytokine production can be important in treating and reducing mortality rates [58].
4. Adaptive immunity

Adaptive immunity is triggered through activation of T lymphocytes by a mechanism known as antigen presentation by Antigen Presenting Cells (APCs) [59]. APCs present structural (spike and nucleo-capsid) and non-structural (ORF3a and ORF7) protein antigens of SARS-CoV-2 and prime activation of T cells in mediastinal and cervical lymph nodes [60,61]. However, defective migration of DCs is reported which can’t prime T cells and lead to presence of fewer specific T cells in lungs [61]. SARS-CoV-2 virus activate both CD8+ and CD4+ T cells [62]. Antigen specific T cells can lyse infected cells, produce various cytokines to coordinate the immune system and trigger the response of antibody-producing cells [63,64]. Although early T cells response imply in reducing the disease severity and clearance after infection, disordered activation of T cells in severe form of disease is reported [65].

In acute form of the disease significant lymphopenia, drastic reduce in CD4+ and particularly CD8+ T cells can impair cellular immunity [63,66]. This reduction is associated with viral damage and inflammatory conditions which is important in exacerbating the infection [67]. Various mechanisms have been suggested for reduction of lymphocytes.

Fig. 4. Dosage (A) and Route (B) of administration of COVID-19 candidates vaccine in different clinical phase.
Apoptosis is a possible cause of lymphopenia after activation of the p53 signaling pathway and increased expression of FAS, TRAIL or caspase 3 as pro-apoptotic molecules [68,69]. A flow cytometry analysis indicated an increased in expression of FAS in circulating CD4+ and CD8+ T cells in COVID-19 patients [70]. The decline in lymphocytes may be due to pyroptosis following activation of NLR family pyrin domain containing 3 (NLRP3) inflammasome and caspase 1 [71]. In addition, other studies have claimed that lymphopenia is due to increased expression of CXCL10 (IP-10) and CCL2 (MCP-1) and increased levels of interleukin 6 which imply in suppressing the development of hematopoietic progenitor cells and high expression of ACE2 on hematopoietic stem cells [72].

During prolonged COVID-19 infection, exhaustion of NK and CD8+ T cells lead to impaired function and correlate with severity of disease. Expression of programmed cell death protein 1 (PD-1) and T cell immunoglobulin mucin-3 (Tim-3) and higher level of T-cell immunoreceptor with Ig and ITIM domains (TIGIT) as exhaustion factors is reported in severe disease [73,74]. Also, an increase in NKG2A expression related to NK and CD8+ T cells is demonstrated in COVID-19 patients. The number of CD8+ T cells and NK cells in recovering patients is restored by reducing CD94-NKG2A expression [41]. Thus, impaired T cells function may contribute in more severe infection [75]. Although a study demonstrated that circulating SARS-CoV-2-specific CD4+ and CD8+ T cells in 100% and 70%, respectively of recovered patients [64].

Upon viral infection, CD4+ T cells differentiate into Th1 and T follicular helper cells (Tfh) [76]. Th1 characterized by IFN-γ secretion, which is a robust reaction against the structural spike glycoprotein, the membrane protein and the nucleocapsid protein. CD4+ T cell responses also targeted nsp3, nsp4, and ORF8 proteins. The SARS-CoV-2-specific CD8+ cells produced IFN-γ and TNF-α, shows the tendency of the response to Th1 [64]. Zheng and colleagues noted a decreased production of IFN-γ in severe patient compared with moderate disease [74]. In this regard, it was noted that levels of IFN-γ and TNF-α in CD4+ T cells are lower in severe form compared with mild infection [74]. An evaluation on T cells responses in acute and convalescence phase revealed cytotoxic and memory phenotypes respectively. CD4+ T cells and CD8+ T cells expressing IFNγ, IL-2, and TNF-α were detectable in recovered patients from mild disease who don’t have detectable antibody responses [77].

Fig. 5. Interaction between S1 in RBD domain of spike protein and ACE2 on cell membranes as well as cathepsin and TMPRSS2 (protease) furin cleavage activity on spike protein, resulted in a) fusion, genome release (virion uncoating) and endocytosis b) related entrance of SARS-CoV-2 into the lung cells, etc. Figure from Biorender.com.
Tfh cells, as a subset of CD4+ T cells, express CXCR5 chemokine receptor which migrate to B cell follicles in response to chemokine CXCL13 [76]. They can regulate B cells response to produce long-lived memory B cells and plasma cell and consequently, antibodies [63]. Increase in Tfh cells and germinal center B (GCB) cells is reported in mild or moderate forms, depending on the severity of disease [66]. Antibodies neutralize the virus and host interaction by blocking spike protein at S1-RBD, S1- N-terminal domain (NTD) and S2 region [78]. In addition to S protein, viral nucleocapsid protein and nonstructural viral proteins such as viral cysteine-like protease associate with high titer of IgG, IgM and IgA antibody responses [79]. IgM and IgA secretion start by day 5–7 after presentation of symptoms and decrease after 28 days after the first onset. IgG appears in 7 to 10 days after onset of symptoms and will reach to its maximum level by the day 49. Maximum level of memory cells appear in day 14 but low levels are traceable in 100 days [80]. A longitudinal study with comparing kinetics of IgM-Ab and IgA-Ab showed an increase level of antibodies at 6–8 days from the onset of symptoms and IgA reaches to the maximum level at 20–22 days, while IgM-Ab reaches its highest level in 10–12 days [81]. Another longitudinal study evaluated anti-SARS-CoV-2 RBD serum IgG concentrations in person with mild disease and it indicated rapid failure in humoral immunity which raises concerns about the importance of humoral immunity in mild form of COVID-19 [82]. Development and production of anti COVID-19 drugs like remdesivir would decrease the duration and severity of COVID-19 infection [88,89]. Since there’s a limited time for designing new antiviral drugs...
and human lung cell culture evaluation [94] have shown that Remde
against COVID-19 and inhibits virus assuming to be effective against COVID-19 infection. These drugs have
19 patients. Borba does not recommend the higher dose of HCQ in pa
there ‘HCQ by the reduction in the proliferation of viral particles [97] . So,
[96] . Although in vitro studies have confirmed the antiviral activity of
5.5. Azithromycin
Azithromycin is considered as a therapeutic agent for the treatment
of COVID-19 infection by interfering in the attachment of the viral spike protein and respiratory cell’s ACE2 [100,102]. Furthermore, it has indirect effects on COVID-19 with anti-inflammatory and immuno-modulatory properties (8). Although it should also be used with more caution due to its side effects especially on the heart and liver organs [92].

5.6. Lopinavir/Ritonavir
A combination of lopinavir/ritonavir already was used for HIV pa
tients, that recently was evaluated against COVID-19 infection. 3-
chymotrypsin like protease (3CLpro) is an important protein for COVID-19 replication which is impaired by lopinavir/ritonavir. Lopi-
navir is a protease inhibitor and ritonavir improves antiviral activity by enhancement the lopinavir exposure [88]. Cao B et al reported no benefits following treatment by lopinavir/ritonavir [99] which was confirmed by the another study conducted by PW Horby et al which they declared no reduction in mortality rate or even exacerbating disease [103].

6. Major COVID-19 vaccine candidates

6.1. Inactivated vaccines
Inactivation of pathogens is a method of vaccine design and pro-
duction against several pathogens like polio, influenza, rabies, and Japanese encephalitis viruses (JEV) [104]. There are many different approaches to make these vaccines, including radiation, heat or chem-
icals which kill or inactivate the pathogen of interest. Chemicals like formaldehyde or β-propiolactone (BPL) are widely used for this aim. These chemicals destroy the viral ability to reproduce, but maintain the normal antigenic structure of the virus, which triggers the immune response [105]. Recently, new methods like hydrogen peroxide treat-
ment, gamma irradiation and zinc-finger reactive treatment, which usually do less damage to the pathogenic antigen structure are devel-
oped [106]. Inactivated vaccines stimulate helper T cells through MHC-
II pathway results in activation of humoral immune responses. But these vaccines do not usually stimulate cytotoxic T cells via the MHC-I pathway significantly and therefore lack a cellular immune response [105,107]. Killed vaccines do re-change to the pathogenic form (unlike live attenuated vaccines), they are very easy to maintain and usually do not require refrigeration, cheaper and available to developing countries. Disadvantages of these vaccines have a weaker immune response (compared to live attenuated vaccines) so they require multiple booster doses. Other disadvantages include antigen restructuring, which can lead to allergic reactions [105,106]. According to the latest update, the international clinical trial of the inactivated vaccine (Phase III) in the United Arab Emirates has been started by Sinopharm China National Biotech Group (CNBG) [108].
There are at least 22 inactivated vaccine candidates for COVID-19, of which 11 are in clinical (Table 1) and 11 are in preclinical evaluation (Table 1a, supplement).

6.2. Inactivated candidate vaccine in preclinical evaluation

6.2.1. IVAC/ Dynavax and GPO/ Dynavax and Institute Butantan/ Dynavax
The Institute of Vaccines and Medical Biologicals (IVAC; Vietnam), the Government Pharmaceutical Organization (GPO; Thailand) and the Butantan Institute (Brazil) presented the inactive vaccine candidate separately in collaboration with Dynavax (USA) /PATH. The vaccine is based on the Newcastle disease virus (NDV) vector, which expresses pre-fusion or wild-type spike protein. In fact, the NDV vector contains a combination of the transmembrane and cytoplasmic domains of NDV fusion protein (F) with SARS-CoV-2 spike; hence it is also called S-F chimera NDV vector vaccines. By injecting beta-propiolactone (BPL) inactivated NDV-S vaccine into mice, it was observed that this vaccine can elicit a strong immune response that has a
### Table 1

Inactivated COVID-19 vaccines in clinical trial phases.

| Vaccine       | Sponsor                                      | Location                        | Status                              | Doses | Timing of doses | Route | Age  | Enrollment | Clinical Phase (ID)                   |
|---------------|----------------------------------------------|---------------------------------|-------------------------------------|-------|-----------------|-------|------|------------|---------------------------------------|
| CoronaVac     | Sinovac                                      | Brazil                           | Not yet recruiting                  | 2     | 0, 14 days      | IM    | ≥18  | 1,200      | Phase IV (NCT04756830)                |
|               |                                              | Hong Kong                        | Not yet recruiting by invitation    | 2     | 0, 14 days      | IM    | ≥18  | 900        | Phase IV (NCT04775069)                |
|               |                                              | Brazil                           | Recruiting                          | 2     | 0, 14 days      | IM    | 18-49| 10,156     | Phase IV (NCT04789356)                |
|               |                                              | Brazil                           | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 2,067      | Phase IV (NCT04754698)                |
|               |                                              | Brazil                           | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 13,060     | Phase III (NCT04456595)               |
|               |                                              | Turkey                           | Recruiting                          | 2     | 0, 14 days      | IM    | 18-59| 13,000     | Phase III (NCT04582344)               |
|               |                                              | Indonesia                        | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 1,620      | Phase III (NCT04508075)               |
|               |                                              | China                            | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 1,040      | Phase III (NCT04617483)               |
|               |                                              | Chile                            | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 2,300      | Phase III (NCT04651790)               |
|               |                                              | China, Hong Kong                 | Not yet recruiting                  | 2     | 0, 14 days      | IM    | 11–100| 900        | Phase III (NCT04800133)               |
|               |                                              | China                            | Active, not recruiting              | 2     | 0, 14 days      | IM    | 18-59| 744        | Phase I/II (NCT04352608)              |
|               |                                              | China                            | Recruiting                          | 2     | 0, 14 days      | IM    | 3-17 | 552        | Phase I/II (NCT04551547)              |
|               |                                              | China                            | Active, not recruiting              | 2     | 0, 14 days      | IM    | ≥6   | 422        | Phase I/II (NCT04383574)              |
| WIBP vaccine  | Wuhan Institute of Biological Products (WIBP)/Sinopharm/China National Biotec Group Co | Bahrain, Jordan, Egypt, UAE       | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 45,000     | Phase III (NCT04510207)               |
|               |                                              | Peru                             | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 600        | Phase III (NCT04612972)               |
|               |                                              | Morocco                          | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 600        | Phase III (NCT04612972)               |
|               |                                              | China                            | Not yet recruiting                  |       | Up to 3 days    | IM    | ≥6   | 1,264      | Phase I/II (NCT04551547)              |
| BBIBP-CovV vaccine | Beijing Institute of Biological Products (BBIBP)/Sinopharm/China National Biotec Group Co | Bahrain, Jordan, Egypt, UAE       | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 45,000     | Phase III (NCT04510207)               |
|               |                                              | Peru                             | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 600        | Phase III (NCT04612972)               |
|               |                                              | Argentina                        | Active, not recruiting              | 2     | 0, 21 days      | IM    | 18-85| 744        | Phase I/II (NCT04352608)              |
|               |                                              | China                            | Recruiting                          |       | Up to 3 days    | IM    | ≥3   | 2,128      | Phase I/II (NCT04715159)              |
| COVAXIN       | Bharat Biotech                               | India                            | Recruiting                          | 2     | 0, 14 days      | IM    | ≥18  | 25,800     | Phase III (NCT0461481)                |
|               |                                              | India                            | Active, not recruiting              | 2     | 0, 14 days      | IM    | 12-65| 755        | Phase I/II (NCT04471579)              |
|               |                                              | India                            | Recruiting                          | 2     | 0, 14 days      | IM    | 12-65| 124        | Phase I/II (NCT04471579)              |
| CAMS vaccine  | Institute of Medical Biology (IMB), Chinese Academy of Medical Sciences (CAMS) | Brazil, Malaysia                 | Not yet recruiting                  | 2     | 0, 28 days      | IM    | ≥18  | 34,020     | Phase III (NCT04659239)               |
|               |                                              | China                            | Recruiting                          | 2     | 0, 28 days      | IM    | 18-59| 942        | Phase I/II (NCT04412538)              |
|               |                                              | China                            | Enrolling by invitation             | 2     | 0, 28 days      | IM    | ≥6   | 471        | Phase I/II (NCT04470609)              |
| QanCovid-in®  | Research Institute for Biological Safety Problems (RIBSP), Rep of Kazakhstan | Kazakhstan                        | Recruiting                          | 2     | 0, 21 days      | IM    | ≥18  | 3000       | Phase III (NCT04691908)               |
|               |                                              | Kazakhstan                       | Active, not recruiting              | 2     | 0, 21 days      | IM    | ≥18  | 244        | Phase I/II (NCT04530357)              |
| Beijing Minhai vaccine | Shenzhen Kangtai Biological Products Co., Ltd./Beijing Minhai Biotechnology Co., Ltd. | China, Jiangsu                    | Recruiting                          | 1, 2, 3| ND           | IM    | ≥18  | 1000       | Phase II (NCT04756323)                |
|               |                                              | China, Jiangsu                   | Active, not recruiting              | 1, 2, 3| ND           | IM    | ≥18  | 180        | Phase I (NCT04758273)                 |
| VLA2001       | Valneva, National Institute for Health Research, United Kingdom | United Kingdom                    | Recruiting                          | 2     | 0, 21 days      | IM    | 18-55| 150        | Phase I/II (NCT04671017)              |
| ERUCOV-VAC    | Erciyes University                           | Turkey                           | Recruiting                          | 2     | 0, 21 days      | IM    | 18-55| 44         | Phase I (IRCT20200120049567N1)       |
|               |                                              | Shifa Pharmed Industrial Co      | Recruitment complete               | 2     | 0, 14 days      | IM    | ≥18  | 56         | Phase I (IRCT20200120049567N1)       | (continued on next page)
high titer of S-specific antibodies [109,110].

These vaccines also contain Dynavax’s CpG 1018 as an adjuvant, which is also used within the HEPLISAV-B® hepatitis vaccine and is approved by the U.S. Food and Drug Administration (FDA). The CpG 1018 adjuvant is actually a TLR9 agonist and leads to high immune stimulation [111].

6.2.2. OZG-3861 and SK-01V1

Mehmet Ali Aydinlar University (Turkey)/Acibadem Healthcare Group (Turkey) have introduced two vaccine candidates, OZG-3861 and SK-01. They are actually lyophilized vaccine candidates that have been inactivated by gamma-irradiation process. OZG-3861 version 1 contains only inactive viruses, but SK-01 version 1 has a GM-CSF adjuvant that can elicit a stronger immune response in mice [112]. Recently, administration of OZG-38.61.3 from intradermal injection in mice has been shown to reduce the concentration of viruses in causing an appropriate immune response [112].

7. Live attenuated viral vaccines

The use of live attenuated vaccines (LAVs) to induce robust and durable immunity is an old well known method of immunization against pathogens. The vaccines produced against smallpox, poliovirus, yellow fever MMR (Measles, Mumps, and Rubella) and rotavirus are based on live attenuated strains [113].

Amino acids are translated by some synonymous codons that which are being used more or less comparing to equal randomisation. This unequal frequency usage of synonymous codons is called codon bias (CB). If certain pairs of observed codons are different from the expected allocations, it is called a “codon pair bias” (CPB). These CBs can affect and increase the expression of the encoded proteins. There are several hypotheses for CB mechanisms. For example, in one hypothesis specific codons usage in the virus is matched with the codone usage in the host that leads to a higher level of viral protein expression. Of course, although this association has been observed in several prokaryotes and unicellular eukaryotes, but in multicellular eukaryotes this association is more complex due to differences of tRNA abundance in different tissues [114,115].

Deoptimization by synonymous genome recoding has created many benefits for attenuated vaccine design. Genomes of recoded viruses may contain thousands of synonymous nucleotide mutations in one or more ORF(s), which creates an attenuated strain which less unlikely change to pathogenic ancestor. In fact, recoded vaccines contain a virus protein that has a similar amino acid sequence to the wild-type, resulting in a similar immune response. Recoded viruses can increase the number of CpG and UpA RNA dinucleotides, which leads to an increase in the immune against the vaccine [114]. Of course, since synonymous recoding does not lead to specific attenuating mutations, it is possible to create a newly emerging pathogen through deoptimization strategies. The four available deoptimization strategies include Codon Deoptimization (CD), Codon-Pair Deoptimization (CPD), increasing the CpG and UpA dinucleotide, and synonymous serine and leucine codon substitutions. A CD involves recoding one or more ORFs to increase synonymous codons, in which the organism is normally low [114]. CPD also means an increase in synonymous codon-pairs that is less commonly used in the organism [116]. CpG and UpA are typically very low in the RNA genome of viruses, making them less detectable by innate immune receptors. However, CD or CPD increases the amount of CpG and UpA, which in turn increases immunogenicity and their detection by the immune system [117]. Serine and leucine codons can be nonsense mutation targets, i.e. they can lead to stop mutations by single nucleotide substitution [118].

The main advantage of live attenuated vaccines is that they are very similar to the wild-type pathogens and can elicit a strong, long-lasting immune response, and usually only one or two doses provide lifelong immunity. However, these vaccines also have disadvantages that have led to their limited use. The most important disadvantages of these vaccines is secondary mutations that can cause reversion into wild-type strains, especially in RNA viruses because the mutation rate is higher in RNA [119]. According to WHO latest report on live attenuated SARS-CoV-2 vaccines, 2 vaccine is in the clinical trial phase (Table 2) and 4 vaccines are in the pre-clinical phase. Information on preclinical vaccines is being reviewed by two live attenuated virus vaccines from Mehmet Ali Aydinlar University,Acibadem Labmed Health Services A.S. and India University of Immunology/Griffith University. Two live attenuated bacterial vectors from the Pasteur Institute of Lille (pertussis) vector and ALtraBio, TheRex are also being studied [23].

8. Subunit vaccines

Subunit vaccines are produced based on synthetic peptides or recombinant technology and are considered as a safe and reliable method. Among the subunit vaccines approved by FDA we can point to human papillomavirus (HPV), hepatitis B virus and influenza virus vaccine [120,121].

Due to fewer side effects and non-infectious nature of subunit vaccines comparing to other vaccine types (such as BCG vaccine), subunit vaccines can be used in immunocompromised patients. Disadvantages of this vaccine include slow manufacture, low immunogenicity, need for cold chain transfer and storage and adjuvants in clinical trials [78].

The S protein is a major target antigen for SARS-CoV-2 subunit vaccine candidates. But, in addition to the full-length S protein and its antigenic fragments, the S1 subunit, NTD, RBD, and the S2 subunit may also be main antigen targets for the development of subunit vaccines. S proteins have been selected because they can induce antibodies that neutralize virus infection by blocking virus binding and fusion. Subunit vaccines used either the full length or part of the S protein as the target. These vaccines are commonly expressed in eukaryotic cells using different expression systems and are being developed for delivery with different adjuvants [122,123].

Until April 2nd, 2021, 28 protein subunit vaccines have entered different stages of clinical trials (Table 3) and 69 subunit vaccines of COVID-19 were in pre-clinical evaluation (Table 3a, supplement).

9. Virus like particle (VLP)

The term VLP was first used when researchers found genetic free particles that resembled viruses with electron microscopy [124]. Usage of VLPs as vaccines date back to early nineties [125,126]. VLPs or sub viral particles are classified in subunit vaccines category, but unlike other subunit vaccines, VLPs present the structure of the intact virus. VLP vaccines require repeated immunization because, unlike live
### Table 3

Subunit vaccines in the clinical trial phases against COVID-19.

| Sponsor | Location | Status | Doses | Timing of doses | Route | Age | Enrollment | Clinical Phase |
|---------|----------|--------|-------|-----------------|-------|-----|------------|----------------|
| Novavax NVX-CoV2373 | United Kingdom | Recruiting | 2 | 0, 21 days | IM | 18-84 | 1,419 | Phase I/II (NCT04368988) |
| South Africa | Recruiting | 2 | 0, 21 days | IM | 18-84 | 4,400 | Phase II (NCT04533399) |
| USA, Mexico, Puerto Rico | Recruiting | 2 | 0, 21 days | IM | ≥18 | 30,000 | Phase III (NCT04611802) |
| AZLB protein subunit vaccine | China | Recruiting | 2 | 0, 21 days | IM | 18-84 | 15,000 | Phase III (NCT04589995) |
| Recombinant | Recruiting | 2 or 3 | 0, 28, 56 days | IM | ≥60 | 50 | Phase I (NCT04445194) |
| Novavax | India | Recruiting | 2 | 0, 28 days | IM | 18-59 | 900 | Phase II (NCT04466605) |
| KRB-COVID-19 | Not yet recruiting | 2 | 0, 21 days | IM | 18-70 | 180 | Phase I/II (NCT04747360) |
| Sanofi/GSK protein subunit vaccine | Not yet recruiting | 2 | 0, 21 days | IM | ≥18 | 34,520 | Phase III (NCT04672395) |
| Biological E Ltd BECOV | Recruiting | 2 | 0, 28 days | IM | 18-65 | 360 | Phase II /III (NCT04702178) |
| United Kingdom | Recruiting | 1 | 0 | IM | 18-84 | 40 | Phase III (NCT04533399) |
| Australia | Recruiting | 2 | 0, 28 days | IM | 20-50 | 3700 | Phase II (NCT04695652) |
| South Africa | Recruiting | 2 | 0, 28 days | IM | 19-80 | 676 | Phase II/III (RPC00000332) |
| USA | Recruiting | 2 | 0, 28 days | IM | 18-90 | 44,010 | Phase III (CHICT00000345) |
| Cuba | Pending | 2 | 0, 28 days | IM | 18-75 | 34,000 | Phase III (NCT04789035) |
| Russia | Recruiting | 2 | 0, 21 days | IM | ≥18 | 3000 | Phase III (NCT04784234) |
| Australia | Recruiting | 2 | 0, 28 days | IM | ≥18 | 7220 | Phase II /III (NCT04695652) |
| South Africa | Recruiting | 2 | 0, 21 days | IM | 18-65 | 90 | Phase I (NCT04533399) |
| Cuba | Pending | 3 | 0, 14, 28 or 0, 28, 56 days | IM | 19-54 | 88 | Phase I (NCT04664368) |
| Russia | Recruiting | 2 or 3 | 0, 28, 56 days | IM | 19-54 | 132 | Phase I /II (RPC00000346) |
| Japan | Recruiting | 2 | 0, 21 days | IM | ≤64 | 214 | Phase I/II (NCT04522089) |
| Netherlands | Recruiting | 2 | 0, 28 days | IM | 18-55 | 130 | Phase I /II (NCT04562109) |
| Iran | Recruiting | 3 | 0, 21, 51 days | IM/IN | 18-55 | 133 | Phase II (NCT04533399) |
| Australia | Recruiting | 2 | 0, 28 days | IM | ≥18 | 216 | Phase I (NCT04459933) |
| Republic of Korea | Recruiting | 2 | 0, 28 days | IM | 19-85 | 260 | Phase I (NCT04742738) |
| Canada | Recruiting | 2 | 0, 28 days | IM | ≥18 | 108 | Phase I (NCT04702178) |
| USA | Recruiting | 2 | 0, 21 days | IM | 18-55 | 72 | Phase I (NCT04784767) |
| South Korea | Recruiting | 2 | 0, 21 days | IM | 19-75 | 280 | Phase II (NCT04783311) |
| Republic of Korea | Recruiting | 2 | 0, 21 days | IM | 19-55 | 50 | Phase I (NCT04760743) |
| New Zealand | Recruiting | 2 | 0, 21 days | IM | 18-80 | 160 | Phase I (NCT04818801) |

**Table 2**

Live-attenuated COVID-19 vaccines in clinical trial phases.

| Vaccine | Sponsor | Location | Status | Doses | Timing of doses | Route | Age | Enrollment | Clinical Phase |
|---------|----------|----------|--------|-------|-----------------|-------|-----|------------|----------------|
| COVI-VAC | Codagenix/Serum Institute of India | Recruiting | 1-2 | Day 0 or 0, 28 | IM | 18-30 | 48 | Phase I (NCT04619628) |
| MV-014-212 | Meissa Vaccines, Inc. | Not yet recruiting | 3 | Day 0 ± 35 | IM | 18-69 | 130 | Phase I (NCT04798001) |
attenuated vaccines, they do not replicate inside the host [127]. These vaccine types are safe and comparatively cheap, and are not infectious because they cannot replicate due to the loss of the essential genomic component [128]. The safety and efficacy of VLP vaccines have been proven in various studies like Glaxo Smith K-line’s Engerix® (hepatitis B virus), Cervarix® (human papilloma virus), Merck and Co Inc.’s Recombivax HB® (hepatitis B virus), Gardasil® (human papillomavirus) [129,130]. Porcine circovirus (PCV) VLP based vaccines are commercially available [131].

According to the latest updated list from WHO (April 2nd, 2021), there are 4 vaccines in the clinical trial phase (Table 4) and 18 in the pre-clinical phase (Table 4a, supplement). According to Table 4, Medicago (Canada) has the most participants in phases II and III of the clinical trial of VLP-based vaccines.

10. DNA-based vaccines

The first DNA based vaccine candidate was transfected in 1990 by gene gun into a mouse skin. Later, DNA vaccines and antigen-carrying plasmids were used in different studies to stimulate humoral and cellular immune responses which led to the introduction of DNA transfection as an immunization technique. Following intradermal ID or IM administration of DNA vaccines, plasmid uptake by different cells (such as antigen presenting cells). In this situation, plasmid related genes are translating into new peptides, presented by MHCs to naive T cells, respectively [132]. Although none of the designed COVID-19 DNA based vaccine is approved, several DNA vaccines are being present in phases I, I/II, II/III and III clinical trials (Table 5) and also in the pre-clinical stage (Table 5a, supplement).

10.1. DNA candidate vaccine in clinical trial stages

10.1.1. INO-4800 and Inovio

NCT04336410 (Inovio) in phase I clinical trial, NCT04447781 (INO-4800) in phase I/II clinical trial, ChiCTR2000040146 (INO-4800) in phase II clinical trial and NCT04642638 (INO-4800) in phase II/III are different DNA plasmid vaccines developed by Inovio Pharmaceuticals that need an electroporation (EP) delivery device. INO-4800 contains pGX9501 plasmid encoding the full length spike glycoprotein of SARS-CoV-2. INO-4800 was designed by Smith and colleagues were examined in mice and guinea pigs and showed blocking ACE2 receptors by cross reaction antibodies. This reaction leads to limitation in spread of SARS-CoV-2 in lung by stimulating humoral and cellular immunity [138,139]. INO-4800 is a nucleic-acid based vaccine which is stable at room temperature for more than a year and does not require frozen state. This excellent thermostability profile beside safety and immunogenicity feature of INO-4800 enable its’ possibility to manufacturing at large scale and transport without cold chain requirements.

INO-4800 (NCT04642638) as a randomized, placebo-controlled and multi-center trial which evaluated the safety, immunogenicity and ef- ficiency of vaccine in phase II/ III of clinical trial. This vaccine has ID route administration and perform by CELLECTRA® 2000 electroporation device. The phase II will evaluate immunogenicity and safety of the vac- cine candidate in 401 participants at two dose levels in three age groups. Potential safety and immunogenicity data which obtained from the phase II will be used to start phase III of the study in 6178 participants.

10.1.2. Osaka University/AnGes/Takara Bio

Osaka University introduced vaccines are AG0302-COVID19 (NCT04655625) in phase II/ III of clinical trial and AG0301-COVID19 (NCT04463472) in I/II phase of clinical trial. AG0301-COVID19 trial is a single-centered, non-randomised, open-label, non-controlled trial in 30 healthy volunteers, aged 20–65, using two low doses of 1.0 mg of IM AG0301-COVID19 (n = 15) and high dose group with 2.0 mg of IM AG0301-COVID19 (n = 15).

AG0302-COVID19 trial as a multi-center, randomized, double-blind and placebo controlled trial, enrolled 500 volunteers with 18 and older age which are vaccinated by 2.0 mg of AG0302-COVID19 twice in 2-week intervals, Group B with 10 volunteers are vaccinated by 2.0 mg of AG0302-COVID19 twice at 4-week intervals and 10 volunteers will receive 2.0 mg of AG0302-COVID19 vaccine by three times at 2-week intervals in Group C. This vaccines, which contains DNA plasmid and adjuvant, target spike protein of SARS-CoV-2 [140].

10.1.3. Cadila healthcare limited

Zydus Cadila designed a non-replicating, non-integrating recombi- nant DNA plasmid vaccine (ZyCoV-D) that was safe, immunogenic and well tolerated in the pre-clinical toxicity studies. Moreover, the ZyCoV-D platform showed no vector response, needs a simple manufacturing system with minimal biosafety requirements (BSL-1), improved stability and lower cold chain requirements.

After the plasmid DNA uptake by cell and translation to viral pro- teins, induction of strong immunity (including cellular and humoral) were occurred. This vaccine is able to elicit high level of neutralizing antibodies in animal studies.

ZyCoV-D vaccine was evaluated on July 15th 2020 on 1084 healthy volunteers with 18–55 years, which resulted in well toleration and safety in the Phase I clinical trial. Then, from 6th of August 2020, ZyCoV-D the evaluation in the phase II clinical trial was started in a larger population (more than 1000 healthy adult volunteers). Recently, Zydus Cadila announced that they have expected to test ZyCoV-D on about 30,000
people in the Phase-III trials that started since November 2020.

ZyCoV-D administration was carried out in two phases (I and II) in four groups that each group were injected in two ways (pharmaJet needle-free injection system and needle) and 0.1 ml dose of ZyCoV-D vaccine (1 and 2 mg) [141,142].

10.1.4. Genexine Consortium

NCT04445389 (GX-19) is a COVID-19 preventive DNA vaccine that is developed and manufactured in republic of Korea, administered by IM in two doses. GX-19 target proteins on SARS-CoV-2 which is not as specific as spike protein in INO-4800. GX-19 is being surveyed in II phase of clinical trial. Phase I of this study is designed as dose increase, single and open-labeled trial with 60 subjects will be enrolled. Phase IIa is designed as randomized, double-blind, placebo controlled and a total of 150 subjects are planned to be enrolled.

10.1.5. Symvivo

NCT04334980 (bacTRL-Spike) is a synthetic DNA plasmids vaccine which encode spike protein. BacTRL-Spike is the only vaccine is administrated orally and requires only one dose. Each dose of the bacTRL-Spike vaccine contains 1, 3 or 10 billion colony-forming-units (CFU) of live genetically modified Bifidobacterium longum, which were designed as vector for plasmids containing spike gene of SARS-CoV-2. After prescription, colonization and direct attachment of B. longum to gut epithelial cells being occurred. According to the latest update of the WHO report, bacTRL-Spike is currently under evaluated in phase I clinical trial. Non-pathogenic and safety characteristic (harmless), simple and low cost production line as well as potential to lyophilize the bacterial contents of the vaccine, which leads to greater stability result in considering LAB as bacterial vector vaccine [143].

10.1.6. CORVax12

SARS-CoV-2 Spike protein plasmid DNA vaccine which is developed by Providence Health & Services and OncoSec Medical Incorporated [144], is being studied in phase I clinical trial. This trial is open-label study to evaluate the safety profile of electroporated spike protein plasmid DNA vaccine (CORVax) with or without co-administration of electroporated TAVO™ plasmid IL-12 (or IL-12p70 plasmid as drug in intervention). In fact, this DNA vaccine composed of spike protein and IL-12 immunotherapy to induce humoral response. CORVax is prescribed to 36 healthy volunteers aged 18-50 for four weeks in a prime-boost manner [145].

Among 181 candidate vaccines in pre-clinical stage, there are about fifteen DNA-based vaccines are in survey. These pre-clinical vaccine candidates can be categorized as DNA plasmid vaccine, DNA plasmids containing glycoprotein gene, DNA with electroporation, mRNA vaccine and DNA and RNA vaccine that all of them designed to elicit immune-response against spike protein. Some recent candidate vaccines are mentioned in Table 5a.

10.1.7. DIOSynVax

Recently, the University of Cambridge is examining a vaccine in the preclinical stage that consists of synthesized gene fragments. These components can be transferred by different carrier systems. DIOSynVax seems to be important in several ways including no need to cold-chain (it is thermostable), safe and to effective at elicited of immune response in phase I and IIa trials. Therefore, there is no problem in storage and transfer of the vaccine to developing countries with low income economic status. Moreover, this vaccine can be delivered pain-free without a needle into the skin, using the PharmaJet Tropis® intradermal needle-free injection System. Late autumn of this year clinical trial of candidate vaccine was started at the National Institute for Health Research (NIHR) Southampton Clinical Research Facility [146].

10.1.8. DNA plasmid vaccine RBD&N

University of Nottingham and Nottingham Trent University are working on novel DNA-based vaccine that will target two proteins of viral nucleocapsid and spike protein. Nucleoproteins are expected to kill virus-infected cells due to stimulating the immune system following recognizing by them. Nucleoproteins have a conserve sequence among all species of coronavirus and using these compounds in vaccines may confer immune response against other members of coronaviruses [147].

10.1.9. DNA plasmid vaccine develop by Karolinska institutet

Cobra Biologics and the Karolinska Institute attempt to develop a new DNA vaccine against COVID-19 vaccine. This vaccine contains

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Table 5. DNA Vaccines in the clinical trial phases against COVID-19.

| Sponsor | Location | Status | Doses | Timing of doses | Route | Age | Enrollment | Clinical Phase |
|---------|----------|--------|-------|----------------|-------|-----|------------|---------------|
| Inovio Pharmaceuticals | Republic of Korea | Active, not recruiting | 2 | Day 0 and Day 28 | ID | 18 Years and older | 120 | Phase I (NCT04336410) |
| | | Recruiting | 2 | Day 0 and Day 28 | ID | 19-64 | 160 | Phase I/II (NCT04447781) |
| AnGes + Takara Bio + Osaka University | Japan | Active, not recruiting | 2 | Day 0 and Day 28 | ID | 18 Years and older | 401 | Phase II /III (NCT04642638) |
| | Japan | Active, not recruiting | 2 | Day 0 and Day 28 | ID | 18-85 | 640 | Phase II (ChiCTR2000040146) |
| Zydus Cadila | India | Active, not recruiting | 3 | 0, 28 and 56 day | ID | 18-55 | over 1000 | Phase III (CTRI/2020/07/026352) |
| GeneOne Life Science, Inc. | South Korea | Recruiting | 2 | Day 1 and Day 29 | IM | 18-55 | 210 | Phase I/II (NCT04445389) |
| Symvivo | Canada | Recruiting | 1 | Day 0 | Orally | 18-older | 24 | Phase I (NCT04334980) |
| Providence Health & Services | USA | Not yet recruiting | 1 | Day 0 and Day 28 | ID | 18-over 55 | 36 | Phase I (NCT04627675) |
| Entos Pharmaceuticals Inc | USA | Not yet recruiting | 2 | Day 0 and Day 14 | ID | 18-84 | 72 | Phase I (NCT04591184) |
| GeneOne Life Science, Inc. | South Korea | Recruiting | 2 | Day 0 + 56 or Day 0 + 84 | ID | 19-65 | 345 | Phase I/II (NCT04673149) |
| ‘University of Sydney, BioNet Co., Ltd Technovalia’ | Australia | Recruiting | 2 | Day 0 and Day 28 | IM | 18-75 | 150 | Phase I (NCT04742842) |
| Takis + Rottapharm Biotech | Italy | Recruiting | 2 | Day 0 and Day 28 | IM | 18 Years to 65 Years | 160 | Phase I/II (NCT04788459) |
chimeric SARS-CoV-2 genes and will be delivered by in vivo electro- 
poration. This project is able to protect against SARS-CoV-2 infection in 
animal models and led to progress to phase I clinical trial. First trial in 
humans will begin in 2021 and will take place at the Karolinska Uni-
versity Hospital [148].

10.1.10. DNA plasmid vaccine develop by BioNet-Asia 
The University of Sydney announced that they are evaluating a 
COVID-19 gene-based vaccine with electroporation transformation 
(delivered via a needle-free system) developed by BioNet Asia. This 
work which is supported by Vax4COVID is started the phase1 Human 
trial that is a multi-center, observer-blinded, dose-ranging, randomized 
and placebo-controlled trial. This project evaluates the safety, reac-
togenicity and immunogenicity of different doses of the vaccine in 
healthy participants aged 18 to 75 years old in three states. The gene 
encoding the coronavirus spike protein was chosen for the vaccine 
[149,150].

10.1.11. Evvivax 
This vaccine is a linear DNA molecule type. The clinical trial is a 
single-centered trial that enrolls 30 healthy domestic feline animals and 
follows them for six months. Intramuscular administration was carried 
out in one dose per month at 1 mg/month with electroporation tech-
nology. Since Evvivax contains only the desired gene, the incidence of 
integration is also reduced; their preparation process is described as fast, 
simple with more purity. Storage and transport is easier comparing to 
RNA vaccines [151].

DNA vaccines design and manufacture process is faster than other 
vaccines and have simple storage status due to thermostability charac-
teristic. They are able to stimulate both neutralizing response and 
cytotoxic T lymphocytes (CTL) antibody and do not associate with the 
reversion risk of vaccine (in contrast with attenuated-live vaccines) or 
integration of DNA vector into the host cell chromosome due to 
removing unnecessary genes from the vector [3,4]. There are always 
concerns about the use of DNA vaccines in humans as well as poor 
immunogenicity in clinical trials which is the main failure of DNA 
vaccine development [152]. However, strategies have emerged to 
overcome this problem such as designing novel vector and adjuvants 
besides enhanced delivery methods that resulted in DNA vaccine effi-
cacy improvement. Although dose determination affects the efficacy of 
the DNA vaccine and varies between species, there is report that about 
5–10 mg of DNA vaccine prescription of per individual led to better 
efficacy [153].

Although animal studies reported that DNA vaccines and 
nucleoprotein-based vaccines against SARS-CoV-2 led to liver damage 
and secretion of eosinophils into the lungs by inducing non-neutralizing 
antibodies against the spike protein which indicate predominance of 
Th2 response, vaccinated animals showed reduced numbers of the virus 
in the respiratory tract which is thought to be associated with the in-
duction of neutralizing antibodies [154,155]. Smaller types of DNA 
vecors like mini-DNAs induce higher immunogenicity due to the high 
level of desired gene expression. Generally, according to published 
paper on Nov 4, 2020, only the immunogenicity and safety of RNA-
based vaccines and adenovirus-vectored vaccines were reported while 
DNA-based and inactivated vaccines were considered protective in non-
humane primates [156,157].

11. RNA vaccines (mRNA vaccines) 

The RNA based vaccines are introduced in the early 1990s as the 
newest generation of vaccines. Even though no RNA vaccine is approved 
until now, but several RNA based vaccines are in phase I or II clinical 
trials. These vaccines are designed for a variety of pathogens such as 
influenza virus (NCT03076385 and NCT0345043), Chikungunya Virus 
(NCT03325075), Cytomegalovirus (NCT03382405), and combined 
human metapneumovirus and human parainfluenza virus type 3 
(NCT03392389). From November 2020, at least two new vaccines, 
including mRNA-1273 from Moderna and BNT162b2 from a BioNTech/ 
Pfizer, are anticipated for final approval by the FDA Emergency Use 
Authorization (EUA) [158].

RNA vaccines contain fragments of mRNA encoding immuno-
dominant antigens that can be taken up by receptor-mediated endocy-
tosis, preferably in APCs. Some of mRNA molecules in endosome are 
detected by PRRs, especially TLRs, and stimulate production of IFN-1 
and other pro-inflammatory cytokines, which act as “self-adjuvant”. 
Alternatively, some mRNAs escape from the endosome and enter the 
cytoplasm, bind to eIF4E proteins, and are translated into proteins by 
ribosomes. These proteins can either be presented to CTLs via the 
intracellular MHC-I pathway and induce a cellular immune response, or 
can be identified as extracellular secreted proteins and presented to the 
helper T cells and B cells through the MHC-II pathway and induce hu-
moral immune response [159,160].

Delivery mechanisms of mRNA vaccines can be classified into two 
main approaches: Ex vivo and In vivo. Ex vivo approach was used 
initially, in which the transected DCs by target mRNA is re-
administered to the patient as a cellular vaccine. Recently, the in vivo 
approach has received more attention because it acts much like a natural 
infecion, in which the target mRNA is injected directly into the host and 
is absorbed by DCs. In addition, mRNA molecules can be delivered as a 
naked molecule of mRNA or lipid-based nanoparticle (LNP) containing 
mRNA. However, LNPs have received more attention because of their 
many advantages over naked mRNA such as protecting target mRNA by 
surrounding membranes [159,161]. At least 35 RNA vaccines are being 
developed for SARS-CoV-2, of which 11 are in clinical evaluation 
(Table 6) and 24 are under preclinical evaluation (Table 6a, supplement).

11.1. RNA candidate vaccine in clinical evaluation 
11.1.1. mRNA-1273 
The mRNA-1273 vaccine is developed by Moderna (Cambridge, MA 
02139, USA)/NIAID and its phase IV clinical trial and currently in 
anticipation of final approval by the FDA Emergency Use Authorization. 
There are currently 35 countries using the mRNA-1273 vaccine. Coun-
tries such as the USA and Israel have the highest use of the this vaccine 
[162,163]. The 18 years old and older people who have no history of 
SARS-CoV-2 infection, but are at risk of acquiring infection. Nearly 
30,000 persons have participated in phase III and received either 100 µg 
vaccine or a placebo. The dose of 100 µg was determined as the optimal 
dose based on the resuts of phase I study. Each participant should 
receive two doses of investigational product (IP) by 0.5 mL IM injection 
on day 1 and day 29. Evaluation of vaccine efficacy begins 14 days after 
the second dose. All participants must have seven scheduled clinic visits 
for blood sampling and assessment of immunogenicity on days 1, 29, 57, 
209, 394, and day 759 [164].

The carrier is a LNP which encapsulates the mRNA. The mRNA in this 
vaccine encodes full-length S protein of SARS-CoV-2, which has been 
modified in two proline residues (S-2P) to stabilize its perfusion 
conformation. The LNP consists of 4 lipids, one of which is novel pro-

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Table 6
RNA candidate vaccine in clinical evaluation.

| Vaccine          | Sponsor                                 | Location                                      | Status                  | Doses | Timing of doses | Route | Age | Enrollment | Clinical Phase   |
|------------------|-----------------------------------------|-----------------------------------------------|-------------------------|-------|----------------|-------|-----|------------|------------------|
| mRNA-1273        | Moderna/ National Institute of Allergy and Infectious Diseases (NIAID) | Denmark                               | Recruiting              | 2     | 0, 28 days     | IM    | ≥18 | 10,000     | Phase IV (NCT04760132) |
|                  |                                         | -                                            | Not yet recruiting       | 2     | 0, 28 days     | IM    | 18-100 | 60         | Phase IV (NCT04792567)  |
|                  |                                         | USA                                          | Active, not recruiting   | 2     | 0, 28 days     | IM    | ≥18 | 30,000     | Phase III (NCT04470427) |
|                  |                                         | USA                                          | Recruiting               | 2     | 0, 28 days     | IM    | 18-26 | 37,500     | Phase III (NCT04811664) |
|                  |                                         | USA                                          | Active, not recruiting   | 2     | 0, 28 days     | IM    | 12-17 | 3,000      | Phase II/III (NCT04649151) |
|                  |                                         | USA                                          | Recruiting               | 2     | 0, 28 days     | IM    | 6 Months – 11 Years | Phase II/III (NCT04796896) |
|                  |                                         | USA                                          | Active, not recruiting   | 2     | 0, 28 days     | IM    | ≥18 | 600        | Phase II (NCT04405076)  |
|                  |                                         | USA                                          | Not yet recruiting       | 2     | 0, 28 days     | IM    | 18-69 | 3,400      | Phase II (NCT04761822)  |
|                  |                                         | Japan                                         | Active, not recruiting   | 2     | 0, 28 days     | IM    | ≥20 | 200        | Phase I/II (NCT046779660) |
|                  |                                         | Japan                                         | Recruiting               | 2     | 0, 28 days     | IM    | ≥20 | 200        | Phase I/II (NCT04712110)  |
|                  |                                         | USA                                          | Active, not recruiting   | 2     | 0, 28 days     | IM    | 18-55 | 120        | Phase I (NCT04283461)  |
| BNT162 (Comirnaty) | BioNTech/Fosun Pharma/Pfizer           | Denmark                               | Recruiting              | 2     | 0, 21 days     | IM    | ≥18 | 10,000     | Phase IV (NCT04760132)  |
|                  |                                         | Sweden                                       | Recruiting               | 2     | 0, 21 days     | IM    | ≥18 | 540        | Phase IV (NCT04780659)  |
|                  |                                         | Hong Kong                                    | Not yet recruiting       | 2     | 0, 21 days     | IM    | ≥18 | 900        | Phase IV (NCT04775069)  |
|                  |                                         | USA/Argentina/Brazil/Germany/South Africa/Turkey | Active, not recruiting   | 2     | 0, 21 days     | IM    | ≥12 | 43,998     | Phase III (NCT04368728)  |
|                  |                                         | USA                                          | Recruiting               | 2     | 0, 21 days     | IM    | 12-50 | 1,530      | Phase III (NCT04713553)  |
|                  |                                         | China, Hong Kong                             | Not yet recruiting       | 2     | 0, 21 days     | IM    | 11-100 | 900       | Phase III (NCT04800133)  |
|                  |                                         | USA                                          | Recruiting               | 2     | 0, 21 days     | IM    | Child, Adult, Older Adult | Phase II/III (NCT04754594) |
|                  |                                         | China                                         | Active, not recruiting   | 2     | 0, 21 days     | IM    | 18-85 | 950        | Phase II (NCT04649021)  |
|                  |                                         | USA                                          | Not yet recruiting       | 2     | 0, 21 days     | IM    | 18-69 | 3,400      | Phase II (NCT04761822)  |
|                  |                                         | Japan                                         | Active, not recruiting   | 2     | 0, 21 days     | IM    | 20-85 | 160        | Phase I/II (NCT04588480) |
|                  |                                         | Germany                                       | Recruiting               | 2     | 0, 21 days     | IM    | 18-85 | 456        | Phase I/II (NCT04380701) |
|                  |                                         | Germany                                       | Active, not recruiting   | 2     | 0, 21 days     | IM    | 18-85 | 96         | Phase I/II (NCT04537949)  |
|                  |                                         | China                                         | Active, not recruiting   | 2     | 0, 21 days     | IM    | 18-85 | 144        | Phase I (NCT04523571)   |
|                  |                                         | Germany                                       | Not yet recruiting       | 2     | 0, 28 days     | IM    | ≥18 | 2,520      | Phase III (NCT04674189)  |
|                  |                                         | Belgium, Germany, Mexico, Netherlands, Peru, Spain | Recruiting               | 2     | 0, 28 days     | IM    | ≥18 | 36,500     | Phase II/III (NCT0452102) |
|                  |                                         | Peru                                          | Recruiting               | 2     | 0, 28 days     | IM    | ≥18 | 660        | Phase II (NCT04515147)  |
|                  |                                         | Belgium                                       | Recruiting               | 2     | 0, 28 days     | IM    | 18-60 | 284        | Phase II (NCT04449276)  |
|                  |                                         | Singapore                                    | Not yet recruiting       | –     | –              | IM    | ≥18 | 600        | Phase II (NCT04668339)  |
|                  |                                         | Singapore                                    | Recruiting               | –     | –              | IM    | 21-80 | 106        | Phase II (NCT04728347)  |
|                  |                                         | Singapore                                    | Recruiting               | –     | –              | IM    | 21-80 | 92         | Phase II (NCT04728347)  |

(continued on next page)
contain allergens such as gelatin or egg protein. Therefore, as a precaution, all participants should be monitored for at least 30 min after vaccination [164,165]. In general, injection of mRNA vaccines with SM-102 lipid formulation can cause self-limiting, local inflammatory reaction. However, it has been observed that injection of mRNA-1273 vaccine in excess of the recommended dose has led to more severe but self-limiting local inflammatory reactions. Most systemic adverse events (AVs) are mild to moderate after vaccination. Systemic adverse reactions (ARs) including fever, fatigue, chills, headache, myalgia, and arthralgia can occur, and more severe reactions such as erythema, induration, fever, headache, and nausea usually occur at higher doses. It should be noted that laboratory abnormalities, such as increase in liver functional tests and serum lipase levels, have also been observed after vaccination with similar mRNA-based vaccines, but their clinical significance is unknown. These abnormalities are without clinical symptoms or signs and usually return to normal in day one. It is not known that whether mRNA-1273, such as active vaccination or live attenuated vaccines, paradoxically increases the risk of disease [164].

11.1.2. Bnt162

The BNT162 vaccines are developed by BioNTech (Germany)/Fosun Pharma (Shanghai, China)/Pfizer (Canada). Three LNP based RNA vaccines: BNT162a1, BNT162b1, BNT162b2 and a uridine mRNA (uRNA) BNT162c2. The BNT162b2 RNA vaccine has progressed to phase III and IV trials [166,167]. There are currently 82 countries using the BNT162b2 vaccine, with Israel, the United Kingdom and the USA being the most widely used [162,163]. BNT162b1 and BNT162b2 are based on nucleoside-modified mRNA (modRNA), with BNT162b1 encoding RBD of S protein that has T4 fibrin foldon trimerization domain to increase its immunogenicity through multivalent presentation, but BNT162b2 encoding pre-fusion stabilized full-length spike protein that has been modified by two proline (2P) mutations, include K986P and V987P [168,169]. Finally, BNT162c2 is based on self-amplifying mRNA (saRNA), which can provide extensive replication which leads to a strong immune response [166,170]. BNT162b3 is also currently being studied as another candidate based on mRNA. The study consists of two parts: phase I to determine the dose and phase II/III to find the efficacy of vaccine in 43,998 volunteers. While BNT162a1 and BNT162c2 are not studied at the moment, BNT162b1 and BNT162b2 vaccines are currently in phase II/III clinical trials. Finally, BNT162b3 is in phase I/II, monitoring the effects in has 120 participants [171,172].

In this study, 2-dose was injected 21 days apart and participants were divided into three groups including phase I: 18–55 years of age, 65–85 years of age; phase II/III: ≥16 years of age. In phase I, participants in the 18–55 and 65–88 age groups receive doses of 10 μg, 20 μg, or 30 μg of the vaccine or placebo.

In Phase I, 18–55 and 65–88 years old group participants received 10 μg, 20 μg, or 30 μg doses of vaccine candidates (BNT162b1 or BNT162b2) or placebo. However, one group whose participants were 18–55 years old received only one dose of 100 μg BNT162b1.

Participants in group of 18–55 years old who received doses of 10 μg, 20 μg, or 30 μg of BNT162b1 had mild to moderate local reactions within 7 days of injection. They also had mild to moderate fever and chills, with 75% of participants receiving a second dose of 30 μg reporting a fever of 38.0 °C or higher. One month after receiving the second dose, adverse events were reported in 50% of participant volunteers who received (30 μg) of BNT162b1 while only 8% to 11.1% of participants in the placebo group reported adverse events. In elderly participants (85–65 years old) there were mild to moderate pain at the injection site in 92% after the first dose and 75% after the second dose. But systemic events were milder in this group, although many older participants experienced fatigue and headache after the first or second dose, and 33% of older participants had a fever above 38 °C. Adverse effects were seen in 17% of older participants who received 30 μg of BNT162b1. Both local and systemic reactions were more severe at dose 2 than at dose 1 [168,173].
Local reactions in the BNT162b2 vaccine were similar to BNT162b1, but none of the older participants reported redness or swelling. Systemic effects were also milder in BNT162b2 compared to BNT162b1. For example, only 17% of 18–55 year olds and 8% of 65–85 year olds had a fever more than 38.0–38.9 °C after a second dose of 30 μg BNT162b2. Severe systemic effects were reported in only a small number of young participants who received the BNT162b2 vaccine but were not seen in any other groups. Adverse effects were seen in only 25% of 18–55 year old participants who received 30 μg of BNT162b2 but were not reported in any older participants (65–85 years of age) [168,173]. In phase III, a small number of participants in both the BNT162b2 and placebo groups presented severe side effects that prevented continuing the trial. Of the BNT162b2 vaccine candidate receiving participant, four participants reported serious adverse events involving injection site-related shoulder injury, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia, and right leg paresthesia. Six participants died, two of whom received the BNT162b2 vaccine, one for arteriosclerosis and one for cardiac arrest. The other four deaths were in participants who received placebo, two due to unknown causes, one due to hemorrhagic stroke and one due to myocardial infarction. In general, none of these deaths were because of vaccine candidates [174].

Finally, based on safety and efficacy evaluation, BNT162b2 vaccine is selected as a candidate in phase II/III and its injection dose was 30 μg. This vaccine should be stored at ultra-cold (–60 °C to –80 °C) and its effectiveness has been reported to be 95% (95% credible interval, 90.3–97.6) [174].

11.1.3. CVnCoV

The CVnCoV vaccine is developed by CureVac AG (Germany) and is now in phase III clinical trial. The vaccine is based on lipid nanoparticle (LNP) containing mRNA molecules encoding full-length S protein that has been modified by two proline (2P) mutations. Injection of 2 μg of CVnCoV vaccine to Balb/c mice, induce strong humoral response with high levels of neutralizing antibodies as well as a strong CD4+ and CD8+ T cell response were induced [175].

The phase I study was carried out on 18–60 year-old participants. They received two doses of CVnCoV ranging from 2, 4, 6, 8, 12 μg per dose at 28-day intervals, while higher doses (16 and 20 μg) are being tried. Preliminary results show that no serious adverse events (SAE) and adverse events of special interest (AESI) are detectable. Generally, with increasing dose, severe or localized adverse events (AE) like injection site pain 24 h after immunization with 12 μg vaccines reported. The common solicited systemic AES is mild to moderate headache and fatigue followed by myalgia and chills, but in general, fever is very rare. Laboratory adverse effects is very rare and had no specific pattern except for transient lymphopenia, which was observed after vaccination [148].

11.1.4. Arct-021

The ARCT-021 vaccine is developed by Arcturus (Singapore)/Duke-NUS and is currently in Phase II. ARCT-021 is composed of a self-transcribing and replicating mRNA (STARR ™) that encodes for the perfusion spike protein of 2019-nCoV formulated in a LNP, in other words, a special type of delivery technology called LUNAR® lipid-mediated that increases antigen expression and vaccine efficacy at low doses. Participants receive a single dose or prime-boost regimen in the range of 1–10 μg per injection. The study in phase I/II includes 106 participants, of whom 78 participants received at least one dose, 56 participants received two doses, and 28 participants received placebo. This study consists of two parts, in phase I, single doses are injected incrementally to younger adults (21–55 years). In phase II, two more dose levels are injected into younger adults (21–55 years) and elderly (56–80 years). In general, AES were mild and no moderate or severe cases were observed, and no fevers and severe injection site reactions were observed [176].

11.1.5. LNP-nCoVsSaRNA

The LNP-nCoVsSaRNA vaccine was developed by Imperial College London (United Kingdom). The vaccine is based on self-amplifying saRNA vaccine encoding the S glycoprotein of SARS-CoV-2 formulated in a LNP. The vaccine is currently in a phase I study in which two doses are given to 320 participants aged 18–75 years. The vaccine is prepared in glass vials at a concentration of 500 μg RNA per ml. The vaccine should be administered intramuscularly into the deltoid muscle [177].

11.2. RNA candidate vaccine in preclinical evaluation

11.2.1. Amyris

Amyris (USA) and The Infectious Disease Research Institute have developed a new RNA-based vaccine candidate. Instead of using shark-based squalene adjuvants, Amyris fermentation technology uses sugarcane-derived squalene adjuvants, which are less expensive and more affordable and can be produced in large quantities. It is also believed that this vaccine will be more effective than non-adjuvant RNA vaccines due to the presence of adjuvants [178].

11.2.2. Fudan University

Fudan University (China)/Shanghai JiaoTong University/RNACare Biopharma have introduced two mRNA-based vaccine candidates. One vaccine candidate can induce the host to produce VLPs with structural features and morphology similar to COVID-19. The second candidate contains the mRNA encoding the RBD of the spike protein of COVID-19 [179].

11.2.3. University of Washington/NIH/HDT Bio Corp

The University of Washington/NIH/HDT Bio Corp has nominated a vaccine called HDT-301 (also called repRNA-CoV2S). The vaccine is based on the Alphavirus-derived replicon RNA vaccine encoding the SARS-CoV-2 S protein, whose RNA replicons are surrounded by Lipid InOrganic Nanoparticles (LiONs). It is shown that HDT-301 vaccine elicits a strong immune response with IgG antibody isotypes that indicate the response of type 1 T helper cells in mice [180].

11.2.4. Max Planck Institute of Colloids and Interfaces

The Max Planck Institute of Colloids and Interfaces (Germany) is developed a vaccine candidate based on a new platform technology that specifically targets Langerhans cells called the Langerhans Cell Targeted Delivery System (LC-TDS). The main role of LC-TDS is due to the presence of a specific chemical component that can be specifically attached to Langerhans cells. This system allows the vaccines to be absorbed directly into the skin or injected into microneedles [181].

11.2.5. Gennova (HGCCO19 vaccine)

Gennova (India) is a self-replicating mRNA and its delivery system is lipid inorganic nanoparticle (LiON) as a vaccine candidate called HGCO19. This vaccine needs low injectable dose and sustained antigen release for a long time. In terms of LiON delivery system, it has adjuvant properties and also increases storage stability, reduces adverse effect and improves permeability and bioavailability [182].

11.2.6. GeneOne Life Science

GeneOne Life Science/Houston Methodist have proposed an RNA-based vaccine candidate. In addition to producing non-GMP and cGMP RNA, the company plans to stabilize and increase the delivery of RNA products [183].

11.2.7. Chimeron Bio

Chimeron Bio (USA)/George Mason University (USA) has developed a vaccine candidate based on the novel nanoparticle technology called Chimeras Encased Self Amplifying RNA (ChaESAR), which uses self-amplifying RNA and synthetic genomics to design the vaccine. This unique particle protects RNA and can be transported to various tissues.
12. Viral (replicating and non-replicating) vectors vaccines

Viral vector vaccines that categorize into the replicating or non-replicating viruses are specially designed viruses that carry the major structural genes of the vaccine candidate against SARS-CoV-2 like spike proteins [156,185]. These vaccines which are characterized by stability, strong immunogenicity and safety, carry DNA express or antigens into host cells and enable to elicit cell-mediated immunity in addition to the humoral immune responses [186]. In the design of vector based vaccines, if a vector was chosen that naturally leads to respiratory disease, this may be considered as an advantage point due to their nasal administration [156]. If a vaccine show failure during clinical trial testing, the same viral vector cannot be reused in the patient because it can enforce an immune response. Pre-existing immunity against the viral vector can render a vaccine ineffective [187]. However, pre-existing immunity can be challenged by priming with a non-viral DNA vaccine [188] or by increasing the vaccine dose or changing the administration route [189]. Replicating viral vector vaccines that designed against COVID-19 are attenuated virus strains such as measles virus, vesicular stomatitis, horse pox virus, and influenza virus [78], while non-replicating viral vector vaccines report to including adenovirus, poxviruses, parainfluenza etc. based vectors that and increased levels of IFN-γ the first fourteen days after vaccination, spike-neutralizing antibodies and clinical trial, respectively. In addition to, there are nine viral encode protein spike gene [186]. According to latest update of WHO report on 10 December 2020, there are 19 and 7 replicating viral vector vaccines against COVID-19 which are examining in pre-clinical stage and clinical trial, respectively. In addition to, there are nine viral vectored vaccines in different phase of clinical trial and eleven candidate vaccines under evaluation in pre-clinical stage that use non-replicating virus for carrying SARS-CoV-2 antigens. Most of non-replicating viral vector vaccine recipients show a diverse profile of immune responses. In the first fourteen days after vaccination, spike-neutralizing antibodies and increased levels of IFN-γ, TNF-α, and IL-2 are produced by T cell CD4+ and CD8+ stimulation [190,191]. Its note to be say that replicating viral vectors low transgenic expression and genetic toxicity, were the main disadvantage of these vaccines [190]. The newly approved Ebola vaccine is an example of a viral vector vaccine that replicates in cells [192]. Institute Pasteur in France is exploiting their measles vaccine vector technology and has developed vaccine candidates against chikungunya [193] and MERS [194]. Toxin Pharmaceuticals (New York, NY, USA) in collaboration with Southern Research (Birmingham, Alabama, USA) is developing TXN-1800, which is a live modified horse pox virus designed to express the S protein of SARS-CoV-2, and it is based on Toxins’ bio defense vaccines against small pox and monkey pox [195,196]. The International AIDS Vaccine Initiative (IAVI, NY, USA) is exploiting a recombinant vesicular stomatitis virus (r VSV) vector against COVID-19 that demonstrated efficacy of rVSV-vectorized vaccines against Simian immunodeficiency virus (SIV) in nonhuman primates (NHPs) [197] and Ebola virus in humans [198]. Replicating viral vectors low transgenic expression and genetic toxicity, were the main disadvantage of these vaccines [190]. The newly approved Ebola vaccine is an example of a viral vector vaccine that replicates in cells [192]. Until April 2nd, 2021, there are 6 replicating viral vector and 13 non-replicating viral vector vaccines in the clinical trial phase (Table 7 and 8), and 20 replicating viral vector and 23 non-replicating viral vector vaccines in the pre-clinical phase (Tables 7a and 8a, supplement).

According to Table 7, among the replicating of viral vaccines, the Israel Biological Research Institute (IBR) has the highest number of participants. Among different non- replicating viral vector vaccines which designed against COVID-19, Oxford/AstraZeneca (ChAdOx1-S nCoV-19), Gamaleya Gam-COVID-VAC/Sputnik V and Janssen Ad26.COV2.S vaccines currently are injected in 118, 24 and 2 countries, respectively. As USA and south africa are using Janssen Ad26.COV2.S vaccine, USA, United Kingdom and other European countries, Austria, India and Japan were the most countries which are using Oxford/ AstraZeneca vaccine. Moreover, Russia was the most country are using Sputnik V vaccine [162,163].

12.1. Non-replicating viral vector vaccine candidates

12.1.1. University of Oxford/AstraZeneca; Adz1222 (ChAdOx1 S nCoV-19)

AZD1222 vaccine was designed by Oxford university researchers in collaboration with AstraZeneca, UK-based global biopharmaceutical company. There are nine type of ChAdOx1 nCoV-19 in clinical trials which need two doses in 0–28 days. PACTR202005681895696 (COV004; Kenya) in phase I clinical trial, PACTR202006922165132 in phase I /II (COV005; South Africa), NCT04686773 in phase II clinical trial, NCT04400838 (COV002; UK) in phase II/III clinical trial and ISRCTN89951424 (COV003; Brazil) in phase III clinical trial were introduced and collected in Table 8a.

12.1.2. Adz1222

Chimpanzee adenovector virus (ChAdOx1) is a weakened adenovirus that is defective in its life cycle so it’s not able to cause disease in individuals especially in immune-compromised patients. ChAdOx1 has also been evaluated in more than ten different diseases and in all age groups and it is reported to be safe and potent immunogenic in all cases. ChAdOx1was engineered to contain the SARS-CoV-2 spike protein and used in novel vaccine against COVID-19, which results to strong and effective immune response following only one dose. In fact, priming the immune system is the main defense against SARS-CoV-2.

12.1.3. Clinical trials of AZD1222 vaccine

AZD1222 vaccine was designed by Oxford university researchers in collaboration with AstraZeneca, UK-based global biopharmaceutical company. Pre-clinical stage that performed by scientists at NIAID’s Rocky Mountain Laboratories (RML) in Hamilton, was done in six rhesus macaques and showed protection against SARS-CoV-2 following one

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**Table 7**

| Sponsor | Location | Status | Doses | Timing of doses | Route | Age | Enrollment | Clinical Phase |
|---------|----------|--------|-------|----------------|-------|-----|------------|----------------|
| University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy | China | Recruiting | 1 | ND | IN | ≥18 | 2 group different size | Phase II (ChiCTR2000039715) |
| Israel Institute for Biological Research/Weizmann Institute of Science/Shenzhen Geno-Immune Medical Institute | Israel | Recruiting | 1 | ND | IM | 18–85 | 1040 | Phase I/II (NCT04683005) |
| | | | | 3 | 0, 14, 28 days | SC | 6 Months to 80 Years | 100 | Phase I/II (NCT04299724) |
| Cellid Co., Ltd. | South Korea | Recruiting | 1 | ND | IM | 19–64 | 150 | Phase I/II (NCT04666012) |
| Airvita Biomedical, Inc | USA | Not yet recruiting | 1 | 0 | IM | ≥18 | 175 | Phase I/II (NCT0436252) |
| Mahidol University, The Government Pharmaceutical Organization | Thailand | Not yet recruiting | 2 | 0, 28 days | IM | 18–75 | 460 | Phase I/II (NCT04764422) |
administration of vaccine [199]. Then, phase I/II clinical trial of single dose or a 2-dose regimen of AZD1222 (COV001; UK) or MenACWY began with 1077 participants aged between 18 and 55 years in April 2020 as a randomized controlled and multicenter trial (in Oxford, Southampton, London and Bristol) [19].

Participants were randomly divided into two groups receiving a dose of $5 \times 10^{10}$ viral particles (standard dose) AZD1222 vaccine and the MenACWY vaccine group (as a licensed control vaccine) in 28-day interval. Also, another group (including 10 participants) receiving two doses of the AZD1222 vaccine was selected. All participants were monitored for seven days and blood samples were taken from them to assess for the detection of neutralizing antibodies and T cell response. This was the end of clinical trial and results were described below. The AZD1222 vaccine induced T cell and antibody responses within 14 and 28 days after vaccination, respectively. The presence of antibodies indicated immunization, which would increase even more if the next dose was given. Interestingly, among these studied groups, the last group which (the group receiving two doses of AZD1222 vaccine) showed the strongest immune response [200,201].

The results of the phase I/II clinical trial [19] led to start phase II/III trial (COV002; UK) on May 28, 2020 in high exposure volunteers like health and social care professions in nineteen places in England. The trial began with participants aged 18 to 55 years and also over 55 years old, separately, that over 55 years old participants had a shorter interval between their first and second doses. All participants received a single dose or a 2-dose regimen of AZD1222 vaccine or MenACWY. These results (rapid antibodies and T cell responses against COVID-19) caused the study to enter the phase II/III trials (COV002 (UK)) that started on May 28, 2020 in nineteen places in England with working on health and social care professions. The trial began with a study of two groups, one group receiving a booster (standard dose) after receiving the first (low dose). The second group receives two standard doses [202,203].

The phase III study was started in several sites such as UK, USA as well as Brazil (COV003) and South Africa as low-to-middle income countries. In Brazil, USA and South Africa, all participants received 2 doses of AZD1222 vaccine or saline placebo at a dose interval between less than 4 weeks to 12 weeks. Before and after administration of vaccines, blood and nasopharyngeal samples were taken. Further trials are being evaluated in the United States, Kenya, Japan and India (ChAdOx1 vaccine in India known as Covishield) with 60,000 participants. The details of each trial were mentioned in Table 8.
12.1.4. ChAdOx1 nCoV-19 (AZD1222)

In order to evaluate the efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against the new emerging variant of SARS-CoV-2 called B.1.1.7, there is no difference between rich countries and developing countries. Oxford also has stated that developing countries will receive the vaccine with no profit and will be delivered without damage. For this reason, the chief executive of AstraZeneca stated that the supply chain of the Oxford/AstraZeneca vaccine is very simple and like other vaccines already used, only a normal home freezer is needed to supply temperatures of 2–8 °C which can be stored for at least 6 months. In fact, this was one of the main advantages of the Oxford vaccine over Moderna vaccine, however little after that report Moderna announced no longer needs ultra-cold chain –80 °C for storage of vaccine and in ordinary freezer can be stored up to six months [204].

12.1.5. Storage and transport of AZD1222 vaccine

AstraZeneca is committed to Covax to distribute three billion doses of the vaccine in 92 countries at a cost of about $3 per dose. Oxford also has stated that developing countries will receive the vaccine with no profit and there is no difference between rich countries and developing countries. Given the need to more than 17 billion doses of the vaccine worldwide, there were concerns about how the vaccine would be delivered without damage. For this reason, the chief executive of AstraZeneca stated that the supply chain of the Oxford/AstraZeneca vaccine is very simple and like other vaccines already used, only a normal home freezer is needed to supply temperatures of 2–8 °C which can be stored for at least 6 months. In fact, this was one of the main advantages of the Oxford vaccine over Moderna vaccine, however little after that report Moderna announced no longer needs ultra-cold chain –80 °C for storage of vaccine and in ordinary freezer can be stored up to six months [204].

12.1.6. Ads-nCov by CanSino

Eight types of adenovirus based vaccines which belongs to human adenovirus type 5, are produced against SARS-CoV-2 by CanSino Biological Inc./Beijing Institute of Biotechnology which currently were evaluated in clinical trials and need only one dose administration to induce humoral and cellular immune response. ChiCTR2000003096 in phase I clinical trial, ChiCTR2000031781 in phase II clinical trial, and NCT04526990 in phase III clinical trial [205].

This vaccine is able to elicit a wide range of immune responses in the majority of volunteers who received both doses 1 × 10^11 VP (high dose) and 5 × 10^10 VP (low dose). Although no serious adverse effect was observed in the trial, grade 3 adverse effect was reported following high dose administration of vaccine (1.5 × 10^11 VP). Better immunogenicity and safety were reported following the administration of lower doses of this vaccine, therefore, this dose was evaluated in further trials [148].

12.1.7. Gamaleya’s Sputnik V

Gamaleya Research Institute designed an Adenovirus-based (rAd26-S + rAd5-S) vaccine candidate (initially known as Gam-Covid-Vac) which need two IM doses. RAd26-S and rAd5-S belongs to human adenovirus types 26 and 5 which harbored full-length SARS-CoV-2 glycoprotein S.

According to latest report of WHO on April 2nd, 2021, NCT04436471 and NCT04530396 are being assessed in phase I/II and phase III trials respectively [206], in Russia, UAE, India, Argentina, Brazil, Mexico, and Venezuela. In phase I clinical trial each volunteers was administrated by both rAd26-S and rAd5-S at same time while in phase III clinical trial planned to administrate participants by rAd26-S in first day and rAd5-S as robust (or booster) dose in day 21 [148].

NCT04530396 as a heterologous recombinant adenovirus (rAd)-based vaccine or Sputnik V was evaluated in a randomized, double-blind, placebo-controlled and phase III trial which performed at 25 different sites in Russia. This trial enrolled 21,977 volunteers with 18–65 years age that randomized into two vaccine group by 16,501 and placebo group by 5,476 participants. The inclusion criteria in choosing participants defined as negative SARS-CoV-2 in PCR and IgG and IgM tests, no infectious diseases and no other vaccinations in the two weeks before enrolment. Briefly, in a prime boost strategy, 0.5 mL/dose IM of Sputnik V was given in first day (first dose; rAd26) which followed by same dose (second dose; rAd5) in day 21. After 21 days, 0.1% and 1.3% of participants in vaccine group and placebo group, respectively, showed to have COVID-19 as primary outcomes by PCR confirmation test. Grade 1 adverse effect was observed by 0.3% vaccine group and by 0.4% placebo group which were serious events but they aren’t related to vaccination. According to latest published report on February 2, 2021, Sputnik V showed to be well tolerated in individuals and have 91.6% efficacy in phase III clinical trial against COVID-19. Moreover, costing less than $10 per dose and need 2–8 °C for storage [207].

12.1.8. Janssen

Janssen Pharmaceutical Companies of Johnson & Johnson develop novel vaccine which used Adenovirus Type 5 as vector and currently have different candidate vaccine in clinical trial stages such as NCT04436276 in phase I/II clinical trial, EUCTR2020-002584-33 DE in phase II clinical trial, NCT04509947 and NCT04505722 are in phase III clinical trial (Table 8).

Participants 18 years and older in NCT04505722 trial will receive IM of Ad26.COV2. S which contain 5 × 10^10 viral particles (vp) as single dose vaccine on day 1. In NCT04509947 as another phase III clinical trial, participants will receive IM injection at 2-dose (high and low) levels. Based on reports, these vaccine candidates are stable in refrigerator, well-tolerated and induced a robust immune response [208].

12.1.9. ImmunityBio, Inc. & NantKwest Inc

This institute develop a novel non-replicating viral vector type vaccine (NCT04591717) that composed of Human Adenovirus Type 5 vector (hAd5) expressing spike and nucleocapsid. This vaccine need two doses in day 0 and 21 day and administrated by SC. This study is a phase Ib and Open-Label trial of the safety, reactogenicity, and immunogenicity of prophylactic vaccination with 2nd generation E1/E2B/E3-deleted adenoviral-COVID-19 in normal healthy volunteers [209].

12.1.10. Grad-CoV2

Grad-CoV2 (NCT04528641) which was manufactured by ReiThera/LEUKOCARE/Univercells, is a non-replicating viral vector containing replication defective simian adenovirus (GRad) encoding the full-length S protein of SARS-CoV-2. Grad-CoV2 evaluated in phase I which is an open-label, dose escalation and multicenter clinical trial that try to assess safety and immunogenicity of GRad-COV2 in 90 Italian healthy volunteers aged 18–55 years and also 65–85 years that received IM of GRad-COV2 in three dose levels three dose levels: Se10, 1e11, 2e11 vp. There will be 90 healthy volunteers who divided into 2 cohorts of age, 18–55 and 65–85 years, respectively. Another trial was registered as NCT04791423 which is a phase II /III clinical trial and have single IM dose of GRad-COV2 2 × 10^11 vp. Grad-CoV2 vaccine vector’s belongs to species C adenovirus, a virus that infects gorillas [210].

12.1.11. VXA-CoV2-1

VXA-CoV2-1 is an Ad5 adjuvanted oral vaccine which is designed by Vaxart. VXA-CoV2-1 (NCT04563702) was tested in phase I clinical trial, an Open-Label and dose-ranging trial to determine the safety and Immunogenicity of VXA-CoV2-1 expressing a SARS-CoV-2 antigen and dsRNA adjuvant. This candidate vaccine administered orally to 35 healthy adult volunteers. In pre-clinical study on hamster, protection against systemic weight loss and lung weight gain following vaccination with two oral doses of Vaxart vaccine at 0 and 4 weeks were observed which this is a key indicator of lung damage due to infection. Vaxart vaccines are designed as room temperature-stable tablets that can be stored without need to refrigeration and also eliminate the risk of needle injection injury [211,212].

12.1.12. Ludwig-Maximilians – University of Munich

This institute develop a novel viral vector vaccine contains a Modified Vaccinia Virus Ankara (MVA) vector expressing the SARS-CoV-2 S protein. MVA-SARS-2-S (NCT04569383) currently evaluates in Phase I clinical trial and the obtained data is not available. In this Open and Single-center trial, total of 30 participants will receive the following vaccine regime: 15 participants will receive IM 107 infectious units (IU)
of MVA-SARS-2-S on day 0 and 28 day. Remaining other 15 participants will receive IM 108 IU of MVA-SARS-2-S on day 0 and 28 day [213].

12.2. Non replicating viral vector vaccines in pre-clinical stages

12.2.1. Ad 5 vector for intranasal administration

University of Helsinki & University of Eastern Finland develop a non-replicating viral vector Ad 5 vector which administrate as a nasal spray and using gene transfer technology. The vaccine is based on a safe adenovirus carrier, which will contain genetic information on how to produce COVID-19 virus surface protein in humans. The administration of the vaccine as a spray into the nose and the upper respiratory tract will start the formation of antibodies against the COVID-19 virus [214].

12.2.2. Aavcovid

AAVcovid as non-replicating viral vector vaccine develop by Massachusetts Eye and Ear/Massachusetts General Hospital which is currently in pre-clinical development. This vaccine based on adenovirus-associated virus (AAV) gene transfer technology to deliver genetic sequences of the SARS-CoV-2 spike antigen and can develop an immune response against coronavirus. AAVcovid posses benefits such as adaptability, potential to elicit a beneficial immune response in people and safety. This strategy is a unique vaccination plan which has potential for a potent immunity to be induced to SARS-CoV-2 from a single injection [215].

12.2.3. Gv-mva-vlptm

GeoVax developers design GV-MVA-VLPTM vaccine by using genetic sequences of the SARS-CoV-2 which contain MVA encoded VLP. MVA is a large virus capable of carrying several vaccine antigens, expresses proteins that assemble into VLP immunogen within the person receiving the vaccine. Production of VLPs in response to vaccination with GV-MVA-VLPTM can elicit both humoral and cellular immune responses [216].

12.2.4. OraPro-COVID-19

OraPro-COVID-19 vaccine developed by Stabilitech Biopharma and uses a non-replicating viral vector to deliver the COVID-19 spike protein DNA to the mucosal cells in the GI tract which ensuring no anti-vector immune response. Because a strong anti-vector response would inhibit the re-use of the same vector for second doses, if required, and limit further vaccine candidates. In addition, OraPro is a non-integrating virus which shows enhanced safety due to additional gene deletions. OraPro-COVID-19 offers benefits such as self-administration by patients without the need for needles and healthcare stuff, broad mucosal and systemic immunity, Oral administration which led to speed of patients without the need for needles and healthcare stuff, broad mucosal immunity, and using gene transfer technology. OraPro- COVID-19 will get hundreds or even thousands more doses per manufacturing run [217].

12.2.5. Adenovirus-based + HLA-matched peptides

VALO TX made a novel viral vector vaccine by using PeptiCRAd (Peptide-coated Conditionally Replicating Adenovirus) technology which this vector express coronavirus associated spike proteins and match with HLA- peptides to enhance CD8+ T-cell immune responses. In PeptiCRAd, turning oncolytic adenoviruses into targeted specific vector vaccines were occurred without the need to generate and manufacture multiple genetically modified viruses. However, most vaccines focus on boosting the B cells response, but T-cell immune response is also very important for targeting infection in the respiratory tract. COVID-19 replicates fast and it seems that the production of neutralizing antibodies may be delayed for protection, and, on the other hand, many infected cells in the lung require cell-mediated clearance adenoviral vectors are strong inducers of T-cell mediated immune responses, therefore, it is hoped that in addition to eliciting humoral immunity in lungs, the spike proteins are also able to induce T-cell immunity [218].

If adenoviruses with specific peptides was designed to stimulate CD8+ T-cells (through MHC class 1 presentation) to activate cellular immunity and then humoral immunity, the inability of older people to stimulate the immune system will be compensate [219].

Mouth, nose and the eyes as mucosal surfaces and body’s barrier defense are the key entry for SARS-CoV-2; therefore, the respiratory and gastrointestinal tract can be an important site in antiviral response against COVID-19 [220]. IgA by competitive attachment to the RBD in the S protein and also nucleocapsid protein (NP), led to neutralizes and prevent the SARS-CoV-2 binding to epithelial cells; however, IgA has low binding affinity to NP in compared to RBD [221]. In other hand, by unknown reason IgA levels are related to oxygen and other respiratory parameters in the blood which IgA was more detected in patients with severe SARS-CoV-2 than patients with mild symptoms. In accordance with this statement, if high levels of IgA and IgG are associated with severe COVID-19 patients, it has been suggested that blocking IgA Fc receptor may reduce the cytokine storm induced by immunoglobulins function. In regard to IgA levels association with the host immune response, evaluation of IgA can be used as an indicator in the diagnosis of COVID-19 [222]. According to these findings, although impact of IgA on SARS-COV-2 need further studies, development of vaccines which administered nasally or orally can provide stronger humoral immunity response against COVID-19 in mucosal surfaces which can act effectively at the site of virus entry.

12.2.6. Mva expressing structural proteins

Modified MVA is a viral vector containing infection-related-surface proteins of the virus. MVA-COVID-19 vaccine manufactured by Centro Nacional Biotecnologia (CNB-CSIC) Spain would be safe and could be administered at all ages and all types of populations (including people with immune deficiencies). Two different vaccine candidates will be initially tested in pre-clinical studies in mice, and the best one will be ready to evaluate in clinical trial [223].

12.2.7. Recombinant deactivated rabies virus containing S1

The novel vaccine was developed by Bharat Biotech; use an existing deactivated rabies vaccine as a vector for coronavirus proteins. The reason that rabies virus used as vector is well-known of this virus for producing a strong immune response, and is approved for the all type of population including children and pregnant women. This vaccine was developed in January and currently completed preliminary tests in animal models. The vaccine showed a strong antibody response when administrate to mice [224].

12.2.8. Newcastle disease virus expressing S

Newcastle disease virus (NDV) is a live viral virus vector vaccine expressing the spike protein of SARS-CoV-2 which developed by Icahn School of Medicine at Mount Sinai. NDV vector vaccines elicit high levels of neutralizing antibodies when the vaccine is given IM in mice. Moreover, no detectable viral titer and viral antigen in the lungs were reported [109]. Most of non-replicating viral vector vaccine used adenoviruses as vector and for analyzing safety, different doses of vaccine (high, medium and low dose) must be tested in which reports indicate that high doses induce a better immune response. Vaccine recipients show a diverse profile of immune responses. In the first fourteen days after vaccination, spike-neutralizing antibodies and increased levels of IFNγ, TNF-α, and IL-2 appear, which produced by T cell CD4+ and CD8+ stimulation were measured [190,191].

13. The effect of BCG vaccination on COVID-19

The Bacillus Calmette-Guérin vaccine (BCG) is a live attenuated
strain of *Mycobacterium bovis* which has a prevention effect against tuberculosis [225]. It is shown that BCG has a very good potency of immunization and also possess effective adjuvant activity inducing cellular immunity [210,226].

BCG had multifaceted protection against tuberculosis, leprosy, and heterogeneous pathogens. Moreover, it was considered as a treatment for type-1 diabetes, many types of cancer (e.g. bladder cancer) and multiple sclerosis [227]. It has also been suggested that the BCG vaccine has clinical benefit in protection to viral pneumonia [212]. The vaccine also reduces yellow fever viraemia in volunteers in the Netherlands [225] and has been shown to protect against malaria [228], Leishmania species [213,215], *Candida albicans* [229] and influenza viruses [217].

Three strains of BCG naming BCG Danish 1331, Tokyo 172-1 and Russia BCG-1 were established as World Health Organization reference strains in 2009 and 2010 [230]. Each BCG strain has different reactivity characteristics. Among them, the BCG Danish 1331 strain is the most widely used and can also be used to improve the BCG vaccine or can be used as a “carrier” for other antigens. Genomic sequences of BCG strain are available at GenBank [171,223,224].

Several phase II, III and IV clinical trials are examining whether the BCG can potentiate the immune system and reduce the incidence of SARS-CoV-2 infection or reduce absenteeism among COVID-19 health care workers (Table 9). A total of 32 BCG-COVID-19 candidate vaccines, 2 vaccines in phase II, 20 vaccines in phase III and 10 vaccines in phase IV clinical trial are under review. The highest number of participants is attributed to the vaccine nominated in phase III in Australia (Murdoch Childrens Research Institute). Twelve clinical trials with live attenuated BCG-Denmark vaccine (Danish strain 1331) were performed by *M. bovis* BCG, one vaccine in Tokyo strain 172-1 and one clinical trial in Russia strain.

Table 9

| Sponsor | Location | Status | Doses | Route | Age | Enrollment | Clinical Phase |
|---------|----------|--------|-------|-------|-----|-----------|---------------|
| Murdoch Childrens Research Institute | Australia | Recruiting | 1 | Intradermal | 18 Years and older | 10,078 | Phase III (NCT04327206) |
| Texas A&M University | United States | Recruiting | 1 | Intradermal | 18 Years to 75 Years | 1800 | Phase IV (NCT04348370) |
| Ain Shams University | Egypt | Not yet recruiting | 1 | Intradermal | 18 Years and older | 900 | Phase III (NCT04350931) |
| Tuberculosis Research Centre | India | Recruiting | 1 | Intradermal | 60 Years to 80 Years | 2175 | Phase III (NCT04753902) |
| University of Campinas, Brazil | Brazil | Recruiting | 1 | Intradermal | 18 Years and older | 1000 | Phase IV (NCT04369794) |
| Harvard Medical School | United States | Not yet recruiting | 1 | Intradermal | 70 Years and older | 2100 | Phase III (NCT04534803) |
| Hospital Universitario Dr. Jose E. Gonzalez | Mexico | Active, not recruiting | 1 | Intradermal | 18 Years and older | 908 | Phase III (NCT04461379) |
| Vakzine Projekt Management GmbH | Germany | Active, not recruiting | 1 | Intradermal | ≥18 years | 59 | Phase III (NCT04387409) |
| Henry M. Jackson Foundation for the Advancement of Military Medicine | United States | Not yet recruiting | – | Intradermal | 18 Years to 64 Years | 550 | Phase III (NCT04632537) |
| Hanna Czajka, University of Rzeszow | Poland | Recruiting | 1 | Intradermal | ≥25 Years | 1000 | Phase III (NCT04648800) |
| Hellenic Institute for the Study of Sepsis | Greece | Recruiting | 1 | Intradermal | 50 Years and older | 900 | Phase IV (NCT04414267) |
| UMC Utrecht | Netherlands | Recruiting | 1 | Intradermal | 60 Years and older | 5200 | Phase IV (NCT04537663) |
| Radboud University | Netherlands | Active, not recruiting | 1 | Intracutaneous | 60 Years and older | 2014 | Phase IV (NCT04417335) |
| Assistance Publique - Hôpitaux de Paris | France | Recruiting | 1 | – | 18 Years and older | 1120 | Phase III (NCT04845494) |
| Bandim Health Project | Denmark | Recruiting | 1 | Intradermal | 65–110 years | 1900 | Phase III (NCT04542330) |
| University of Southern Denmark | Denmark | Recruiting | 1 | Intradermal | ≥18 years | 1050 | Phase IV (NCT04641858) |
| University Health Network, Toronto | Canada | Recruiting | 1 | Intradermal | ≥18 years | 3626 | Phase III (NCT04439045) |
| TASK Applied Science | South Africa | Recruiting | 1 | Intradermal | 18 Years and older | 500 | Phase III (NCT04379336) |
| Bandim Health Project | Denmark | Recruiting | 1 | Intradermal | 18 Years to 100 Years | 1293 | Phase III (NCT04373291) |
| MJM Bonten, UMC Utrecht | Netherlands | Active, not recruiting | 1 | Intracutaneous | 18 Years and older | 1500 | Phase III (NCT04328441) |
| Universidade Federal do Rio de Janeiro | Brazil | Recruiting | – | Intradermal | ≥18 years | 1000 | Phase II (NCT04659941) |
| Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences | Iran | Recruiting | – | Intradermal | From 18 years old | 500 | Phase III (IRCT20200411047019N1) |
| Guangxi medical university | China | Prospective registration | – | – | 18 Years to 80 Years | 60 | Phase IV (ChiCTR200003016) |
| Medical Education and Drugs Department and Haffkine Institute for Training Research Testing | India | Recruiting | – | IM | 18 Years to 60 Years | 60 | Phase II (CTRI/2020/05/025013) |
| Serum Institute of India Pvt Ltd | India | Not Recruiting | 1 | Intradermal | 18–99 years | 5,946 | Phase III (CTRI/2020/04/024749) |
| Indian Council of Medical Research | India | Not Recruiting | 1 | Intradermal | 18–60 years | 800 | Phase III (CTRI/2020/07/026668) |
| Radboudumc | Netherlands | Ongoing | – | Intradermal | Elderly (≥65 years) | 2000 | Phase IV (NCT04001591-15) |
| Radboudumc | Netherlands | Ongoing | – | Intradermal | Elderly (≥65 years) | 100 | Phase IV (NCT04002456-21) |
| University of Rzeszow | Poland | Ongoing | – | Intradermal | Adults (18–64 years) | 1000 | Phase III (NCT04002111-22) |
| University Medical Center | Netherlands | Ongoing | – | Intradermal | Adults (18–64 years) and Elderly (≥65 years) | 1000 | Phase IV (NCT04000919-69) |
| National Koranyi Institute of Pulmonology | Hungary | Ongoing | – | Intradermal | Adults (18–64 years) and Elderly (≥65 years) | 1000 | Phase III (NCT04001783-28) |
| University of Southern Denmark | Denmark | Ongoing | – | Intradermal | Elderly (≥65 years) | 1900 | Phase III (NCT04003904-15) |
Activation of antiviral immunity depends on a specific type of TLR signaling mechanism that is stimulated by a specific type of pathogen. Studies have shown that BCG expresses various proteins that bind to TLR and activate macrophages and dendritic cells [231]. Once activated, these cells produce pro-inflammatory cytokines. The primary cytokines stimulated by BCG include IL-2, TNF-α, and IFNγ, which are released upon the activation of CD4+ T cells [214]. There is an enhanced secretion of pro-inflammatory cytokines like IL-1β, which is also known as leukocytox pyrogen, upon BCG vaccination. IL-1p plays a crucial role in immunity against viruses [232]. Heterologous lymphocyte responses can involve the activation of CD4+ and CD8+ memory cells that are specific for non-targeted antigens. Finally, BCG is found to induce a non-specific response to non-mycobacterial infections mediated by heterologous Th1/Th17 response [233].

Innate immune memory, known as trained immunity, is one of the most recently recognized components of immune memory that has the effects of a strategy vaccine [234,235]. The most well-studied human vaccine that induces trained immunity is the BCG vaccine against tuberculosis [236]. BCG induces a general long-term boosting of innate immune mechanisms, mediated by an epigenetic, transcriptional, and functional reprogramming of innate immune cells such as monocytes, macrophages, neutrophil and dendritic cell [237]. Both innate and non-immune cell training can lead to effective local innate immunity and eradication of the virus before it causes disease or transmission to others. People with weakened immune systems are at higher risk, characterized by systemic inflammation, multiple organ dysfunction, and widespread viral replication and highly infectious. In contrast, patients with BCG vaccination background increase strong local immune response against the SARS-CoV-2 virus (Fig. 7). BCG vaccination in patients with weakened immune systems/immunodeficiency who experience COVID-19 may develop a more severe form of the disease [234].

There are studies on the effect of BCG vaccine on COVID-19, some of which are mentioned. To determine whether BCG vaccination protects against COVID-19, the results show that patients with BCG vaccination were more likely to have myalgia and less likely to need to be hospitalized [238]. Study of 12,343 SARS-CoV-2 gene sequences isolated from patients/individuals in six geographic regions compared to the SARS-CoV-2 reference sequence shows a total of 1234 mutations. Analysis showed that ORF1ab 4715L and S protein 614G variants have a positive and significant association with reduced mortality. However, in countries with vaccination program with BCG, the frequency of the S 614G variant is associated with higher mortality [239]. A study of 123 adults with COVID-19 pneumonia found that BCG vaccination status was not related to the clinical status of COVID-19 pneumonia. One of the major risk factors for severity and mortality in COVID-19 is old age [240]. The results of the Escobar et al. [241] confirmed that BCG vaccination reduces the mortality rate of COVID-19. This study requires clinical trials of BCG vaccination to confirm and establish causation between BCG vaccination and protection against COVID-19.

14. Plant-derived vaccine for COVID-19

Plant derived vaccine candidates have been proposed earlier for viruses like influenza A H1N1 HA enveloped VLPs, Norwalk virus capsid protein (NVPV) non-enveloped VLPs, HbsAg enveloped VLPs and alfalfa mosaic virus (AIMV) particles displaying rabies glycoprotein, whose plant hosts are N. benthamiana, potato, tobacco, lettuce and spinach, respectively [242].

N. benthamiana domini a unique species native to Australia, first discovered off the coast of Australia by ship surgeon Benjamin Bynoe [243]. This species belongs to the Solanaceae family and is an allotetraploid with a genome consisting of 19 chromosomes [244]. N. benthamiana can be easily transformed and manipulated by genome editing and virus-induced gene silencing (VIGS) strategies. It can also be grow in greenhouses and growth chambers [245,246]. Its weak immune system that is the result of natural genetic changes over millennia means that genetic material can be successfully hosted by the plant and is not rejected.

The Coronavirus-Like Particle COVID-19 vaccine candidate (CoVLP) is composed of recombinant spike (S) glycoprotein expressed as VLPs. S protein was changed with R667G, R668S and R670S substitutions at the S1/S2 cleavage site were modified to increase stability, and K971P and V972P substitutions were modified to stabilize the protein in the pre-fusion conformation. The signal peptide was replaced by a plant gene signal peptide, and the transmembrane domain (TM) and cytoplasmic tail (CT) of S protein were replaced by TM/CT from influenza H5 A/Indonesia/5/2005 to increase VLP assembly and germination. Self-assembled VLPs with S protein trimers are isolated from the plant matrix and subsequently purified using a process similar to that described for influenza vaccine candidates [247].

Medicago, a biomedical pharmaceutical company in Quebec, has completed the phase I clinical trial of its N. benthamiana plant-derived vaccine for COVID-19. The results of the trial demonstrated that 100 percent of subjects developed a promising antibody response after two doses of Medicago’s COVID-19 adjuvanted vaccine candidate. The adjuvant compounds used in N. benthamiana plant-derived vaccine include: AS03 adjuvant, an oil-in-water emulsion containing tocopherol and squalene, supplied by GlaxoSmithKline, and CpG 1018 adjuvant consisting of cytosine phosphoguanine (CpG) motifs, prepared by Dynavax. CoVLP vaccine and excipients were mixed immediately before administration. Both adjuvants increased the responses of IFN-γ and IL-4, but these cellular responses were more widespread in the AS03-adjuvanted groups [247].

Phase 1 clinical trial was a randomized, semi-blind study of 180 healthy subjects, male and female aged 18–55 years, in 2 intramuscular doses 21 days apart at three dose levels S protein content (3.75, 7.5 Or 15 g) alone or with adjuvant with either CpG1018 or AS03 (NCT04450004). CoVLP finished product is a liquid formulation that can be stored at 2–8 °C, easing cold chain management with existing vaccine infrastructure. According to the manufacturer (Medicago), phase II clinical trial will be administered in the United States to a population of healthy adults (18–64 years) and the elderly (over 65 years). Phase III of this study begins before the end of 2020 and is an event-based, random, blind, and placebo-controlled design that evaluates the CoVLP formulation in more than 30,000 subjects, and safety compared to placebo [247].

The N. benthamiana was introduced as a candidate for H5 VLP pandemic influenza vaccine, and Medicago Company successfully introduced its phase 1 clinical trial in July 2010. The study, is registered at Clinicaltrials.gov (Clinical Trial Number: NCT00989495), as a randomized, one-phase, adjuvant containing (Aluminium Hydroxide) in healthy adults 18–60 years of age who received 2 intramuscular doses 21 days [248]. In order to assess the immunogenicity and safety of a

**Fig. 7.** How BCG vaccination may have an effect on improving host defense against SARS-CoV-2.
quadrivalent plant-derived virus like particle as the influenza vaccine candidate- two randomized phase II clinical trials were carried out in adults 18 to 49 and 50 years old. In the phase II trial (NCT02233816), 18–49 years old subjects received 15, 30, or 60 µg/strain of a hemagglutinin-bearing quadrivalent virus-like particle (QVLP) vaccine or placebo. In the second trial (NCT02236052), ≥50 years old subjects received QVLP as high dose or placebo with additional groups of 7.5 or 15 µg/strain with alum. The results showed that QVLP vaccine caused a significant reaction of homologous and heterologous antibody responses in two higher doses. In addition, alum had little-to-no effect. The candidate vaccine also consistently elicited both homologous and heterologous antigen-specific CD4+ T cells characterized by their production of IFN-γ, IL-2 and/or TNF-α [249].

15. Conclusions

The recent pandemic, COVID-19 has resulted massive economic and social damages. Design and production of approved effective vaccines against COVID-19 is of most importance. So far, eleven vaccines are licensed for larger-scale administration due to minimal side effects, stimulating strong immunity and neutralizing antibody responses, as well as high efficacy. These vaccines include: Oxford-AstraZeneca, Pfizer-BioNTech, Moderna, Sinopharm-Beijing, Gamaleya (Sputnik V), Sinovac, Sinopharm-Wuhan, Johnson& Johnson, Bharat Biotech (Covaxin), CanSino and Vector Institute (EpiVacCorona). The equitable distribution regardless of political issues and provision of adequate doses of these vaccines, especially for developing countries, may be the next challenge for COVID-19 vaccine. These vaccines are expected to reduce mortality rate significantly.

Ethical statement

This review study was carried out with the Code of Ethics Committee No. IR. KUMS. REC.1399.1044 adopted by Kermanshah University of Medical Sciences.

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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