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Review

An update review of globally reported SARS-CoV-2 vaccines in preclinical and clinical stages

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the rapidly spreading pandemic COVID-19 in the world. As an effective therapeutic strategy is not introduced yet and the rapid genetic variations in the virus, there is an emerging necessity to design, evaluate and apply effective new vaccines. An acceptable vaccine must elicit both humoral and cellular immune responses, must have the least side effects and the storage and transport systems should be available and affordable for all countries. These vaccines can be classified into different types: inactivated vaccines, live-attenuated virus vaccines, subunit vaccines, virus-like particles (VLPs), nucleic acid-based vaccines (DNA and RNA) and recombinant vector-based vaccines (replicating and non-replicating viral vector). According to the latest update of the WHO report on April 2nd, 2021, at least 85 vaccine candidates were being studied in clinical trial phases and 184 candidate vaccines were being evaluated in pre-clinical stages. In addition, studies have shown that other vaccines, including the Bacillus Calmette-Guérin (BCG) vaccine and the Plant-derived vaccine, may play a role in controlling pandemic COVID-19. Herein, we reviewed the different types of COVID-19 candidate vaccines that are currently being evaluated in preclinical and clinical trial phases along with advantages, disadvantages or adverse reactions, if any.

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 $\geq$ 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

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storm such as monoclonal antibody against specific receptor like interleukin receptor (e.g. tocilizumab and pegylated interferon α-2a and −2b) are currently prescribed for the patients. Inhibitors of RdRP, reverse transcriptase and protease of SARS- CoV-2 and antiviral antibiotics (e.g. favipiravir and remdesivir etc.) usage are still in doubt and their exact effects on the process of the infection are still unclear [7,8].

However, this pandemic is not going to be stopped unless access to effective and safe vaccines becomes available. An ideal coronavirus vaccine must elicit strong humoral and cellular immune responses, it must have easy storage and transport requirement which must be available and affordable for all the countries, especially lower- middle-income ones [9].

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 evaluates 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 (pp1a, pp1b) 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

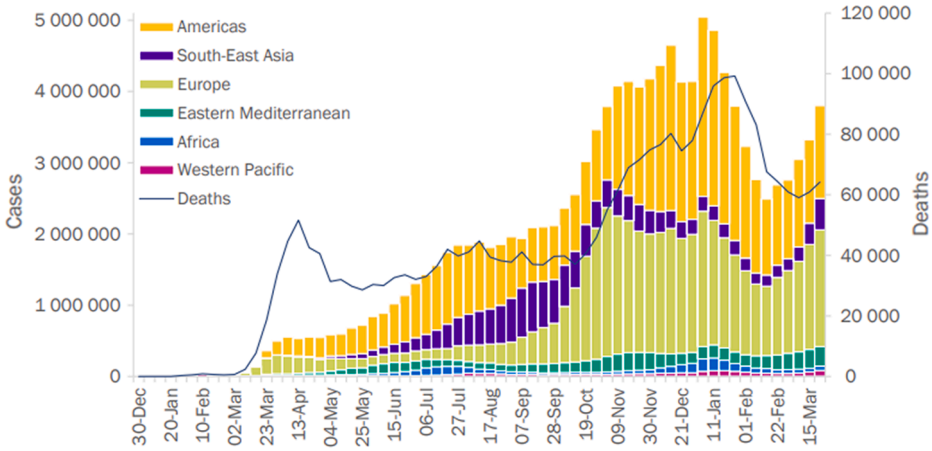

Fig. 1. COVID-19 case report and global death rate according to latest update of WHO on 28 March 2021.

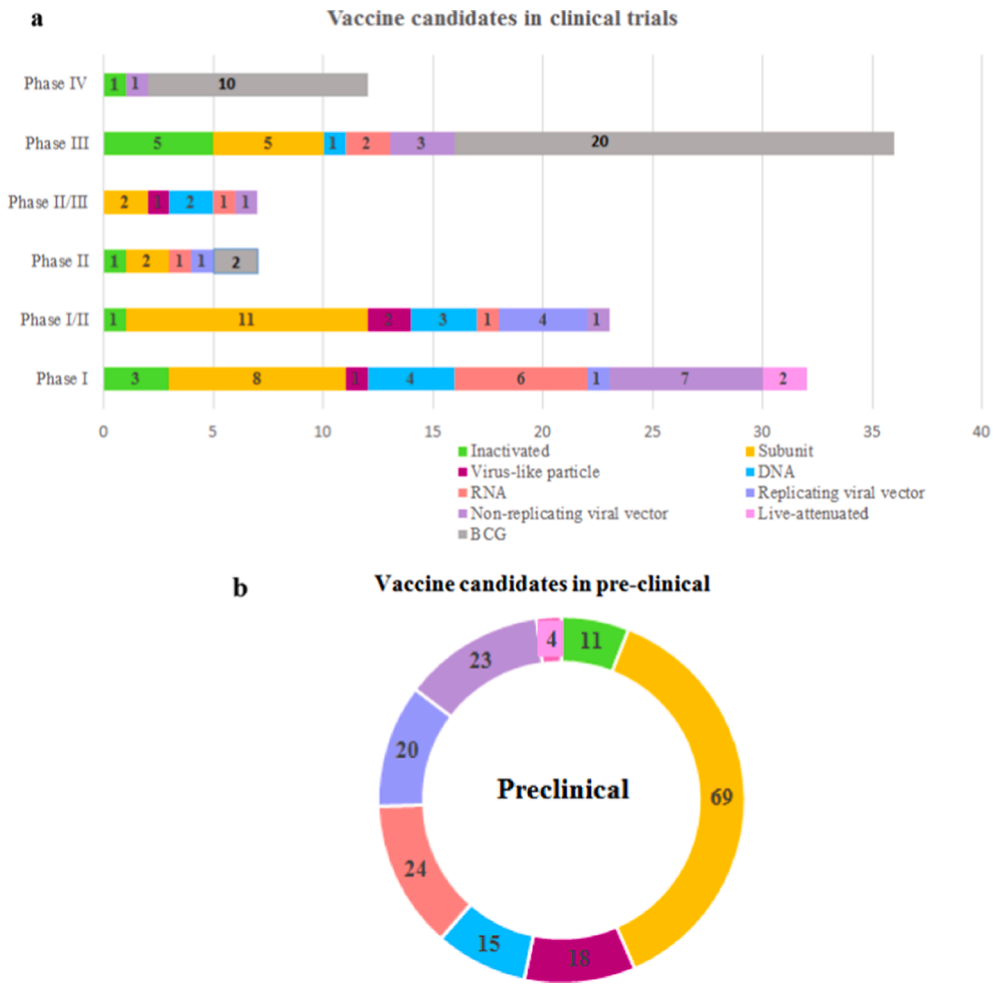

Fig. 2. Landscape of COVID-19 vaccine development. Vaccines candidates in the clinical trial (a) and preclinical (b) phase based on the last update of the WHO report on April 2nd, 2021. Also, based on ClinicalTrials.gov (https://www.clinicaltrials.gov/) and EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/) databases, different phases of BCG vaccination against COVID-19 were investigated.

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

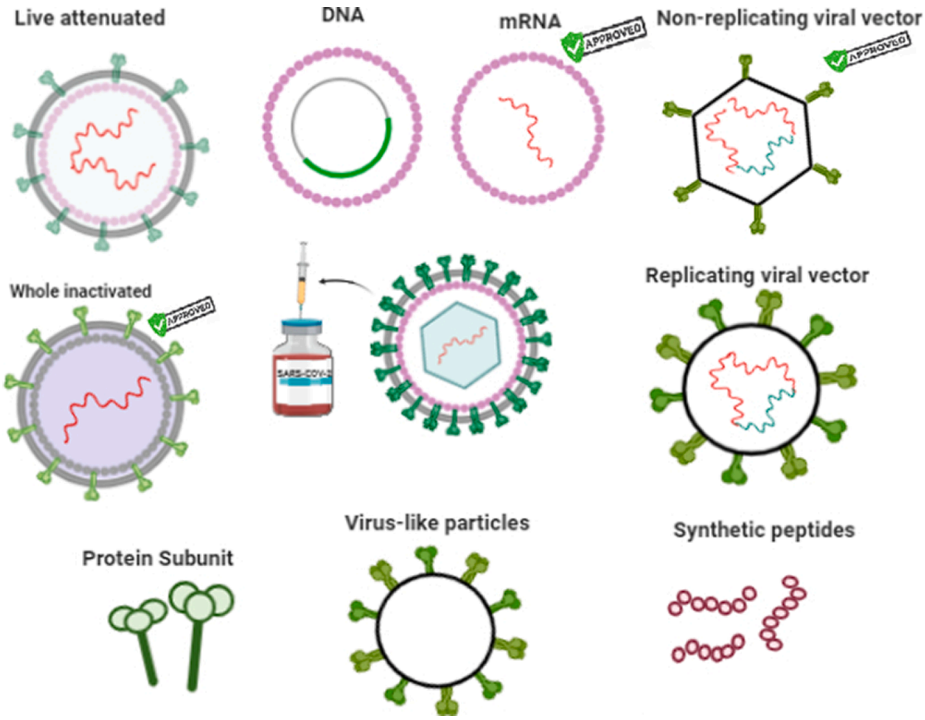

Fig. 3. Vaccine platform against SARS-CoV-2. (left to right) Live attenuated vaccines that inactivate by a series of passages in which the virus loses its pathogenicity but does not its proliferative potential so can stimulate the immune response. Nucleic acid-based vaccines (DNA and RNA) which harbor plasmid DNA and RNA sequence that encoding the specific antigen. These vaccines can be loaded on carriers (like liposomes, etc.). Viral vectors (non-replicating and replicating viral vector) vaccines; non-replicating viral vector vaccines (adenovirus) and replicating viral vector vaccines (influenza, VSV, measles, paramyxovirus and yellow fever virus) may encode for S protein or N protein of SARS-CoV-2. Inactivated virus (Whole and Protein or peptide subunit) vaccines; Whole virus vaccines can be attenuated or inactivated form of SARS-CoV-2 virion. Protein or peptide subunit vaccines which were constructed in recombinant way, composed of at least 50 specific antigen proteins plus adjuvants for strengthening the immune response. Nanoparticle-based vaccines like VLPs (virus like particles), lipids material and metals used for carrying antigens which effect APCs functions. Figure from Biorender.com.

and TNF-α affect immune cells and endothelial cells to release of 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, IFN-γ, interferon gamma-induced protein 10 (IP-10), and monocyte chemoattractant 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 I 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 microclotts 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-I which is happening in two ways. IFN-I 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. NKs 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 exhausted 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 clarifying 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].

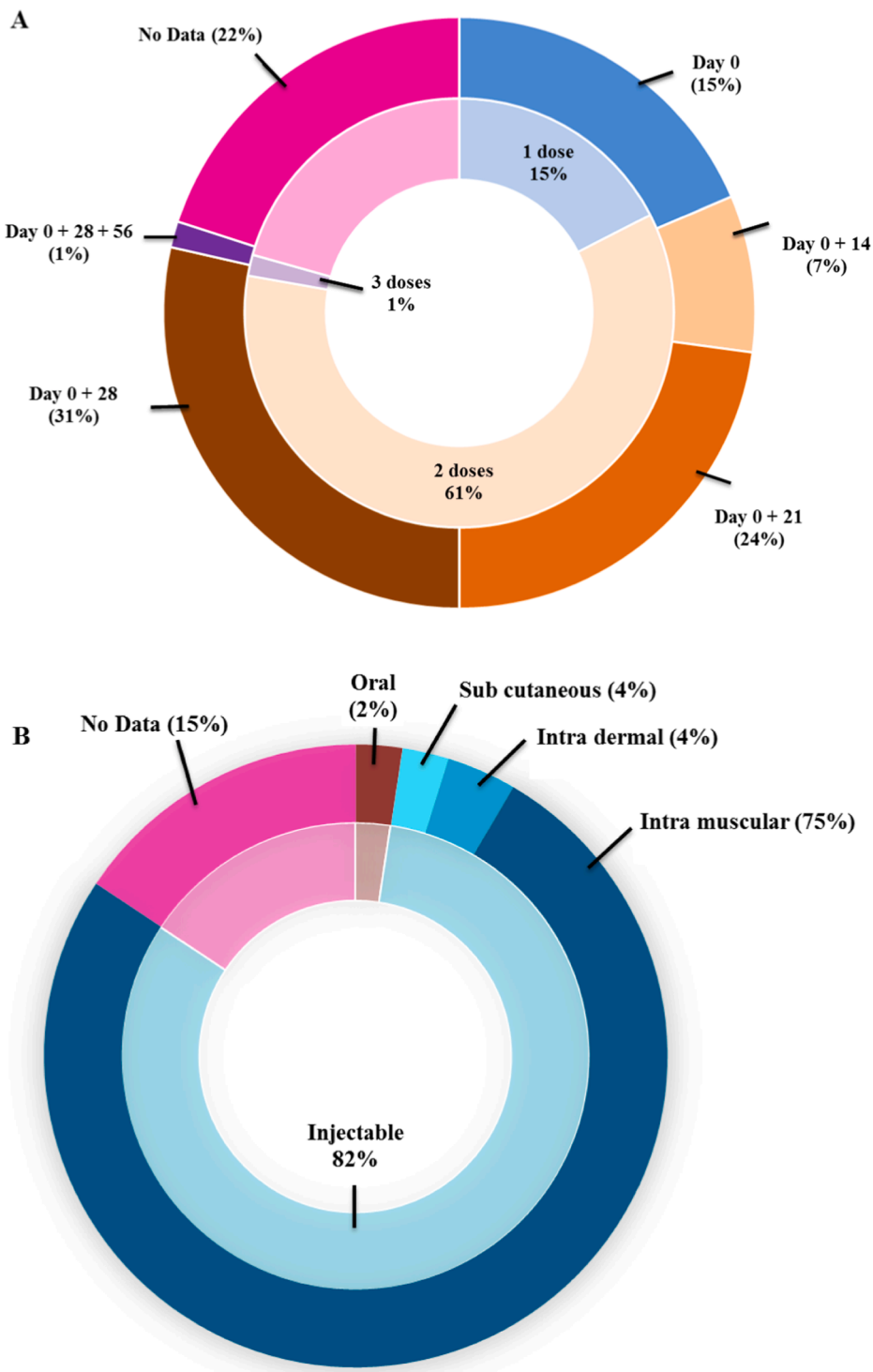

Fig. 4. Dosage (A) and Route (B) of administration of COVID-19 candidates vaccine in different clinical phase.

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 co-ordinate 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.

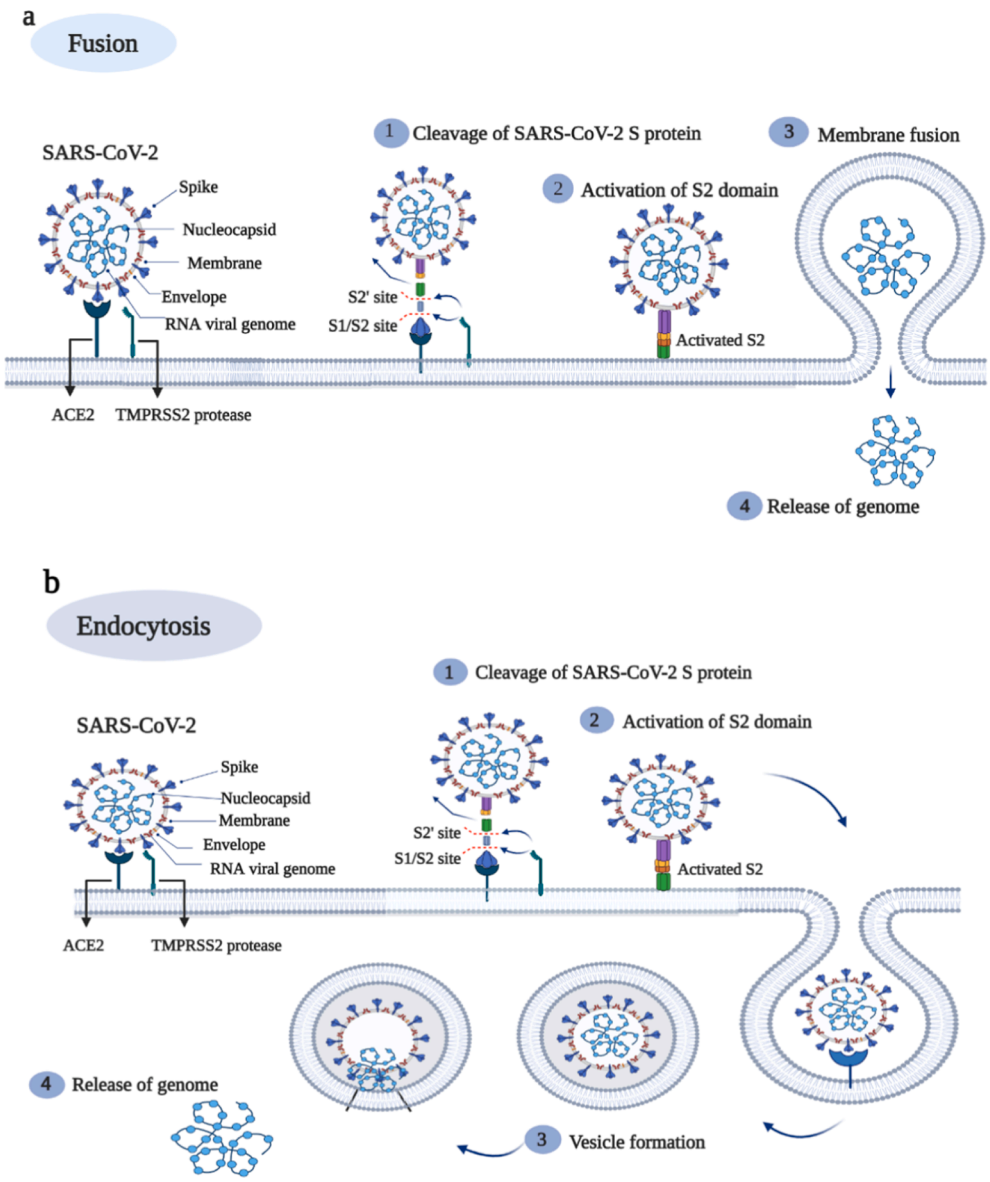

Fig. 5. Interaction between S1 in RBD domain of spike protein and ACE2 on cell membranes as well as cathepsin and TMPRSS2 (protease) furin cleavage activity on spike protein, resulted in a) fusion, genome release (virion uncoating) and endocytosis b) related entrance of SARS-CoV-2 into the lung cells, etc. Figure from Biorender.com.

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].

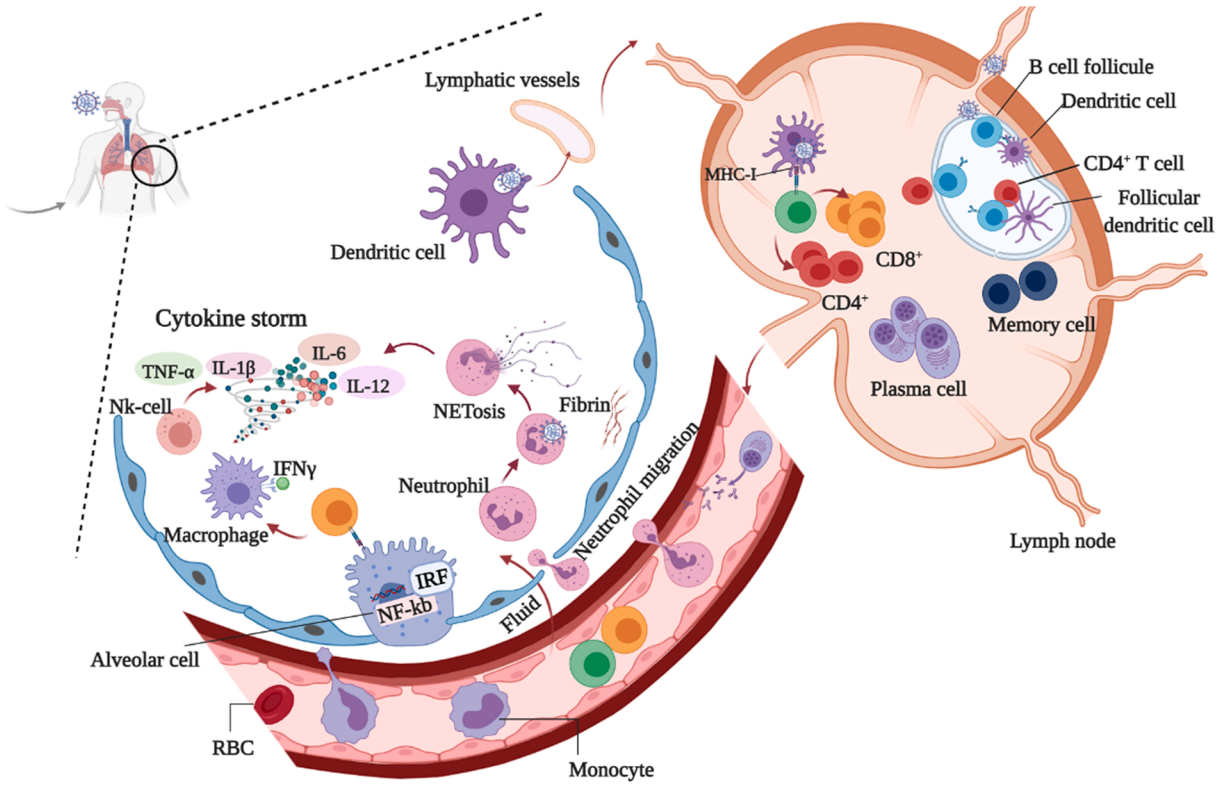

Fig. 6. Immunopathogenesis of COVID-19 infection. SARS-CoV-2 enters aerosols by alveolar cells uptaking. Alveolar cells recruit monocytes and lymphocytes. The influx of macrophages leads to hyper production of inflammatory cytokines, called cytokine storming. On the other hand, cytokines recruit neutrophils. Neutrophils also imply in cytokines storm, phagocyte pathogen and make NETs. DCs present virus antigen to T lymphocytes and they can differentiate to $CD4^+$ and $CD8^+$ T cells and lymphocytes circulate between the blood and the lymphatic system. 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]. A prospective cohort study followed two groups of patients for about one month, one with severe symptoms and the other mild or moderate form. The result of study on IgM and IgG titers was associated with a significant higher titer in severe patients than patients who didn't have severe disease. The study concluded that intensity of antibody response correlates with clearance and severity of disease. It is suggested that strong antibody response might be associated with low rate of clearance and more severe infection.

Severity of disease varies in different countries and may be due to the pre-existing of humoral immunity in exposed population with other corona viruses before COVID-19 pandemic [83,84]. A cohort study compared plasma samples before COVID-19 pandemic in two populations. The study reported that exposed population to HCoVs (SARS, MERS, HCoV-OC43, HCoV-HKU-1, HCoV-NL63, and HCoV-229E) has low incidence of infection due to the cross reactivity against SARS-CoV-2 [84]. According to a study on 135 sera from healthy subjects, 23% of volunteers reacted to SARS-CoV-2N-antigen and suggested presence of antibody against N-antigen. It attributed cross reactivity to similarity of N proteins between coronaviruses [83]. Diphtheria, tetanus, and pertussis (DTP) vaccination vaccination elicit cross reactivity against SARS-CoV-2. This could be a possible reason for protection of children against COVID-19 [85].

A peripheral blood signature, in addition to the changes mentioned earlier that occur in humoral and cellular immune responses, has been reported in studies that can vary between COVID-19 patients and recently recovered seropositive individuals therefore it can help in understanding and managing the disease. In COVID-19 patients, upregulation of IL-6, IL-8, IL-10, chemokine IP-10, cycling T cells expressing exhaustion markers (PD-1 and HAVcr-2), depletion in αβT cells and γδT cells and changes in B cell compartment noted [86].

Generally, T cell responses occur in COVID-19 patients in 1–2 week after the onset and they are dependent to the severity of disease. According to the reports, knowledge about cellular protection needs to be more investigated but it was reported that neutralizing antibodies and Th1 cells will probably be optimal [87].

5. Effective drugs against SARS-CoV-2

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

[90], it is suggested to focus on drug repositioning strategies. These strategies assess the existing antivirals for the treatment of the SARS-CoV-2 infection [91]. Peng et al [91] introduced ten potential antiviral drugs against COVID-19 by molecular docking techniques. They analyzed the binding affinity of selected drugs to viral spike protein and human ACE2. Tacrolimus (FK506) had the best affinity in attaching to both spike protein and ACE2 receptor (-11.06 and −10.1 kcal/mol, respectively). Also, their results showed that ribavirin, remdesivir and chloroquine had high affinities to ACE2 (-6.39, −7.4 and −6.29 kcal/mol, respectively).

Here, we review the potential drugs for the treatment of COVID-19. Some of these drugs are used in the clinics and some are still under assessment in clinical trials.

5.1. Remdesivir

Remdesivir (GS-5734) is the only FDA-approved antiviral drug against COVID-19 and inhibits virus's RdRp [92]. Animal studies [93] and human lung cell culture evaluation [94] have shown that Remdesivir has a significant effect on reducing viral load and clinical symptoms. Remdesivir emergency prescriptions were accepted in Taiwan, USA and Japan for patients who suffer from severe COVID-19 symptoms [88].

5.2. Hydroxychloroquine

Hydroxychloroquine (HCQ) and chloroquine (CQ) that already used to prevent malaria and auto-immune disease (e.g. rheumatoid arthritis), assuming to be effective against COVID-19 infection. These drugs have no significant effects on COVID-19 disease recovery and mortality rate [95] and simultaneously have potential to cause serious side effects [96]. Although in vitro studies have confirmed the antiviral activity of HCQ by the reduction in the proliferation of viral particles [97]. So, there's still controversy on the effectiveness of HCQ and CQ for COVID-19 patients. Borba does not recommend the higher dose of HCQ in patients due to safety risk problems [96], as the same time, Li et al consider HCQ has therapeutic potential for COVID-19 patients because of the antiviral and anti-inflammatory characteristic [98].

5.3. Ribavirin

Ribavirin is an inhibitor of RNA virus replication by several mechanisms including inhibition of the RdRp (8), is an effective treatment in COVID-19 cases (5). However, previous studies have indicated that ribavirin has no significant effect on clinical parameters and mortality rate in sever MERS infections, therefore cannot be used as a treatment for COVID-19 infections [99,100].

5.4. Favipiravir

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazine carboxamide) inhibits RdRp. First it was approved against influenza infections in Japan [88]. T-705 is a pro-drug that is activated through ribosylation and phosphorylation modification [90]. The mechanism of action is incorporation into the nascent viral RNA and chain termination induces mutagenesis. T-705 has cytopathic effect that reduces viral RNA and is effective against the COVID-19 infection [91]. Adverse drug events (ADEs) like abnormalities of hematological indexes and liver transaminases and serum uric acid level have limited the use of this drug [89,101].

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 immunomodulatory 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 patients, 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. Lopinavir 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 production 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 chemicals 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 treatment, gamma irradiation and zinc-finger reactive treatment, which usually do less damage to the pathogenic antigen structure are developed [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 Biotec 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
CoronaVac	Sinovac	Brazil	Not yet recruiting	2	0, 14 days	IM	≥18	1,200	Phase IV (NCT04756830)
		Brazil	Enrolling by invitation	2	0, 14 days	IM	≥18	30,000	Phase IV (NCT04747821)
		Hong Kong	Not yet recruiting	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	18–59	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	≥60	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 (ChiCTR2000039000)
		China	Not yet recruiting	Up to 3	–	IM	≥6	1,264	Phase I/II (ChiCTR2000031809)
BBIBP-CorV vaccine	Beijing Institute of Biological Products (BIBP)/ 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	3,000	Phase III (NCT04560881)
		China	Recruiting	Up to 3	–	IM	≥3	2,128	Phase I/II (ChiCTR2000032459)
COVAXIN	Bharat Biotech	India	Recruiting	2	0, 14 days	IM	≥18	25,800	Phase III (NCT04641481)
		India	Active, not recruiting	2	0, 14 days	IM	12–65	755	Phase I/II (NCT04471519)
		India	Recruiting	2	0, 14 days	IM	12–65	124	Phase I/II (CTRI/2020/09/027674)
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	≥60	471	Phase I/II (NCT04470609)
QazCovid-in®	Research Institute for Biological Safety Problems (RIBSP), Rep of Kazakhstan	Kazakhstan	Enrolling by invitation	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	Active, not recruiting	1, 2, 3	ND	IM	≥18	1,000	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 (NCT04691947)
	Shifa Pharmed Industrial Co	Iran	Recruitment complete	2	0, 14 days	IM	18–50	56	Phase I (IRCT20201202049567N1)

(*continued on next page*)

Table 1 (*continued*)

Vaccine	Sponsor	Location	Status	Doses	Timing of doses	Route	Age	Enrollment	Clinical Phase
COVID-19 inactivated vaccine		Iran	recruiting	2	0, 14 days	IM	51–75	32	Phase I (IRCT20201202049567N2)
MIVAC	Organization of Defensive Innovation and Research	Iran	recruiting	2	Day 0 + 14 ± 21	IM	18–55	135	Phase I (IRCT20210206050259N1)

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/Acıbadem 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 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	United Kingdom	Recruiting	1–2	Day 0 or 0, 28 days	IN	18–30	48	Phase I (NCT04619628)
MV-014-212	Meissa Vaccines, Inc.	USA	Not yet recruiting	3	Day 0 ± 35	IN	18–69	130	Phase I (NCT04798001)

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	Australia, USA	Active, not 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	Not yet recruiting	2	0, 21 days	IM	≥18	30,000	Phase III (NCT04611802)
	UK	Recruiting	2	0, 21 days	IM	18–84	15,000	Phase III (NCT04583995)
AZLB protein subunit vaccine	China	Recruiting	2 or 3	0, 28 or 0, 28, 56 days	IM	18–59	50	Phase I (NCT04445194)
		Recruiting	2 or 3	0, 28 or 0, 28, 56 days	IM	≥60	50	Phase I/II (NCT04550351)
		Recruiting	2 or 3	0, 28 or 0, 28, 56 days	IM	18–59	900	Phase II (NCT04466085)
		Not yet recruiting	2 or 3	0, 28 or 0, 28, 56 days	IM	18	29,000	Phase III (ChiCTR2000040153)
KBP-COVID-19	USA	Not yet recruiting	2	0, 21 days	IM	18–70	180	Phase I/II (NCT04473690)
Sanofi/GSK protein subunit vaccine	USA	Not yet recruiting	2	0, 21 days	IM	≥18	34,520	Phase III (PACTR202011523101903)
Biological E Ltd BECOV	India	Recruiting	2	0, 28 days	IM	18–65	360	Phase I/II (CTRI/2020/11/029032)
Clover Biopharmaceuticals AUS Pty Ltd	Australia	Not yet recruiting	2	0, 21 days	IM	18–75	34,000	Phase II /III (NCT04672395)
Vaxine PtvLtd	Australia	Recruiting	1	0	IM	18–65	40	Phase I (NCT04453852)
Medigen MVC-COV1901	Taiwan	Recruiting	2	0, 28 days	IM	20–50	3700	Phase II (NCT04695652)
Instituto Finlay de Vacunas FINLAY-FR-1-FR-1	Cuba	Recruiting	2	0, 28 days	IM	19–80	676	Phase I/II (RPCEC00000332)
Instituto Finlay de Vacunas FINLAY-FR-1-FR-1	Cuba	Pending	2	0, 28 days	IM	19–80	44,010	Phase III (RPCEC00000354)
FBRI SRC VB EpiVacCorona	Russian Federation	Active, not recruiting	2	0, 21 days	IM	≥18	3000	Phase III (NCT04780035)
WestChina Hospital, Sichuan University	China	Not yet recruiting	2	0, 28 days	IM	18–85	12 group different size	Phase II (ChiCTR2000039994)
Tübingen CoVac-1	Germany	Not yet recruiting	1	–	SC	≥18	36	Phase I (NCT04546841)
Covaxx UB-612	Taiwan	Not yet recruiting	2	0, 28 days	IM	≥18	7320	Phase II /III (NCT04683224)
Adimmune AdimrSC-2f	Taiwan	Recruiting	2 or 3	Different days	–	20–60	70	Phase I (NCT04522089)
Nanogen Pharmaceutical Biotechnology	Vietnam	Recruiting	2	0, 21 days	IM	12–75	620	Phase I /II (NCT04683484)
Center for Genetic Engineering and Biotechnology (CIGB)	Cuba	Pending	3	0, 14, 28 or 0, 28, 56 days	IN	19–54	88	Phase I /II (RPCEC00000345)
Center for Genetic Engineering and Biotechnology (CIGB)				0, 14, 28 or 0, 28, 56 days	IM	19–54	132	Phase I /II (RPCEC00000346)
Shionogi & Co., Ltd.	Japan	Recruiting	2	0,21 days	IM	≤64	214	Phase I /II (jRCT2051200092)
University Medical Center Groningen	Netherlands	Not yet recruiting	–	–	IM/SC	18–55	130	Phase I /II (NCT04681092)
Razi Vaccine and Serum Research Institute	Iran	recruiting	3	0, 21, 51 days	IM/IN	18–55	133	Phase I) IRCT20201214049709N1)
The University of Queensland	Australia	recruiting	2	0, 28 days	IM	≥18	216	Phase I (NCT04495933)
SK Bioscience Co., Ltd. and CEPI	Republic of Korea	Recruiting	2	0, 28 days	IM	19–85	260	Phase I/II (NCT04742738)
VIDO-InterVac, University of Saskatchewan	Canada	Recruiting	2	0, 28 days	IM	≥18	108	Phase I/II (NCT04702178)
Walter Reed Army Institute of Research (WRAIR)	USA	Not yet recruiting	2	0, 21 days	IM	18–55	72	Phase I (NCT04784767)
EuBiologics Co.,Ltd	South Korea	recruiting	2	0, 21 days	IM	19–75	280	Phase I/II (NCT04783311)
SK Bioscience Co., Ltd	Republic of Korea	Active, not recruiting	2	0, 28 days	IM	19–55	50	Phase I (NCT04760743)
Jiangsu Rec-Biotechnology Co., Ltd	New Zealand	Not yet recruiting	2	0, 21 days	IM	18–80	160	Phase I (NCT04818801)

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 Recom bivax 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 in the lymph nodes. These interactions trigger second series of signals that activate T and B lymphocytes [132–134].

DNA vaccines can be administrated as naked plasmids or with carriers like liposomes or polymer-based nanoparticles. The vaccines may be injected with, electroporation or/ and gene guns, while application of adjuvants or chitosan enhance the immunogenicity of DNA vaccines. Among the aforementioned methods, electroporation is used as the method of choice in pre-clinical trial stage in animals for COVID-19 DNA [135]. However, electroporation may also be considered as a limitation for DNA vaccines [136].

During the first outbreak of SARS in China in 2002, a viral DNA vaccine containing a plasmid encoding spike protein, was developed and evaluated for immunogenicity and safety in phase I clinical trial. Three doses of SARS DNA vaccine were well-tolerated in ten adults and induced neutralizing antibody production with 100% and 20% responses of $CD4^+$ and $CD8^+$ T cells, respectively [137]. 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 it's possible 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 efficacy 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 vaccine 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 recombinant 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 proteins, 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 (mora than 1000 healthy adult volunteers). Recently, Zydus Cadila announced that they have expected to test ZyCoV-D on about 30,000

Table 4
VLP vaccines in the clinical trial phases against COVID-19.

Sponsor	Location	Status	Doses	Timing of doses	Route	Age	Enrollment	Clinical Phase
Medicago Inc/GSK/Dynavax	Canada	Recruiting	2	0, 21 days	IM	18–55 ≥18	30,918	Phase II /III (NCT04636697)
SpyBiotech + Serum Institute of India	Australia/ India	Recruiting	2	0, 28 days	IM	18–79	280	Phase I/II (ACTRN12620000817943)
VBI Vaccines Inc	Canada	Not yet recruiting	2	0, 28 days	IM	≥18	780	Phase I/II (NCT04773665)
The Scientific and Technological Research Council of Turkey	Turkey	Not yet recruiting	2	–	SC	18–59	36	Phase I (NCT 04818281)

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)
		Active, not recruiting	2	Day 0 and Day 28	ID	18 Years and older	401	Phase II /III (NCT04642638)
			2	Day 0 and Day 28	ID	18–85	640	Phase II (ChiCTR2000040146)
AnGes + Takara Bio + Osaka University	Japan	Active, not recruiting	2	Day 0 and Day 14	IM	18-older	500	Phase II /III (NCT04655625)
	Japan	Active, not recruiting	2	Day 0 and Day 14	IM	20–65	30	Phase I/II (NCT04463472)
Zydus Cadila	India	Active, not recruiting	3	0, 28 and 56 day	ID	18–55	over 1000	Phase III (CTRI/2020/07/026352)
Genexine Consortium	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	2	Day 0 and Day 28.	ID	18-over 55	36	Phase I (NCT04627675)
Entos Pharmaceuticals Inc	Canada	Not yet recruiting	2	Day 0 and Day 14		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	Not yet recruiting	2	Day 0 and Day 28	ID or 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)

people in the Phase- III trials that started since November 2020.

ZyCoV-D administration was carried out in two phass (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, msDNA vaccine and DNA group 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

chimeric SARS-CoV-2 genes and will be delivered by in vivo electroporation. 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 University 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, reactogenicity 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 technology. 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 characteristic. 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 efficacy 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 induction of neutralizing antibodies [154,155]. Smaller types of DNA vectors 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 NCT03345043), 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 immunodominant antigens that can be taken up by receptor-mediated endocytosis, 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 humoral 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 transfected 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 infection, 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. Countries 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 results 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 proprietary lipid SM-102 (heptadecan-9-yl 8 ((2 hydroxyethyl)(6 oxo 6-(undecyloxy)hexyl)amino)octanoate) and the other 3 lipids are commercially available including cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000-DMG) [164].

In Nov. 15, 2020, Data and Safety Monitoring Board (DSMB), which oversees phase III of the clinical trial, reported 94.5% efficacy of mRNA-1273 vaccine [111]. The mRNA-1273 vaccine can be stored at 2–8 °C for up to 30 days or frozen (−15 °C to −25 °C) for long-term storage [164]. Although very rare, some minor side effects are observable in some participants. Immediate systemic allergic reactions (e.g., anaphylaxis) can occur in about one per 450,000 vaccinations for vaccines that do not

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	6,750	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 (NCT04677660)
		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/ GermanySouth 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	4,000	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)
CVnCoV	CureVac AG	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 (NCT04652102)
		Peru, Panama	Recruiting	2	0, 28 days	IM	≥18	660	Phase II (NCT04515147)
		Belgium, Germany	Recruiting	2	0, 28 days	IM	18–60	284	Phase I (NCT04449276)
ARCT-021	Arcturus/Duke-NUS	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	

(*continued on next page*)

Table 6 (*continued*)

Vaccine	Sponsor	Location	Status	Doses	Timing of doses	Route	Age	Enrollment	Clinical Phase
LNP-nCoVsaRNA	Imperial College London	UK	No longer recruiting	2	–	IM	18–75	320	Phase I/II (NCT04480957)
ARCoV	Shulan (Hangzhou) Hospital + Center for Disease Control and Prevention of Guangxi Zhuang Autonomous Region	China	Not yet recruiting	–	0, 14 or 0, 28 days	IM	18–80	168	Phase I (ISRCTN17072692)
ChulaCov19	Chulalongkorn University	Thailand	Not yet recruiting	2	0, 21 days	IM	18–75	96	Phase I (ChiCTR2000034112)
PTX-COVID19-B	Providence Therapeutics	Canada, Ontario	Recruiting	2	0, 28 days	IM	18–64	60	Phase I (NCT04765436)
CoV2 SAM	GlaxoSmithKline	USA	Recruiting	–	0, 28 days	IM	18–50	40	Phase I (NCT04758962)
mRNA-1273.351	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	USA	Not yet recruiting	3	0, 28, 56 days	IM	18–99	210	Phase I (NCT04785144)
MRT5500	Sanofi Pasteur and Translate Bio	USA	Recruiting	2	0, 21 days	IM	≥18	415	Phase I/II (NCT04798027)

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 fibritin foldon trimerization domain to increase its immunogenicity through multivalent presentation, but BNT162b2 encoding prefusion 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 modRNA. 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 sever 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 comparing 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, 36 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-nCoVsaRNA

The LNP-nCoVsaRNA 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/RNACure 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 (HGCO19 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 Chimera 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.

RNA platform technology has a low dose formulation, scalable manufacturing and high stability of the vaccine [184].

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 adeno-associated virus, poxviruses, parainfluenza etc. based vectors that 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 IFNγ, 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 TNX-1800, which is a live modified horse pox virus designed to express the S protein of SARS-CoV-2, and it is based on Toxin's 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-vectored 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 (IIBR) 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; Azd1222 (ChAdOx1 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. Azd1222

Chimpanzee adenovirus vector (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

Table 7
Replicating viral vector vaccines in clinical trials.

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	Israel	Recruiting	1	ND	IM	18–85	1040	Phase I/II (NCT04608305)
Shenzhen Geno-Immune Medical Institute	China	Recruiting	3	0, 14, 28 days	SC	6 Months to 80 Years	100	Phase I)NCT04299724)
Cellid Co., Ltd.	South Korea	Recruiting	1	ND	IM	19–64	150	Phase I /II) NCT04666012)
Aivita Biomedical, Inc	USA	Not yet recruiting	1	0	IM	≥18	175	Phase I /II (NCT04386252)
Mahidol University, The Government Pharmaceutical Organization	Thailand	Not yet recruiting	2	0, 28 days	IM	18–75	460	Phase I /II) NCT04764422)

Table 8
Non-replicating viral vector vaccines in clinical trials.

Sponsor	Location	Status	Doses	Timing of doses	Route	Age	Enrollment	Clinical Phase
AstraZeneca company	Kenya	Recruiting	2	0, 28 days	IM	18–55	400	Phase I (PACTR202005681895696)
	Russia	Not yet recruiting	2	0, 29 days	IM	18–100	100	Phase II (NCT04686773)
	South Africa	Recruiting	2	0, 28 days	IM	18–65	2000	Phase I/II (PACTR202006922165132)
	UK	Recruiting	1–2	0, 28 days	IM	18–55	12,390	Phase II /III (NCT04400838)
	Brazil	Recruiting	2	0, 28 days	IM	18 and older	10,300	Phase III (ISRCTN89951424)
	Denmark	Recruiting	1–2	0, 28 days	IM	18 and older	10,000	Phase IV (NCT04760132)
Beijing Institute of Biotechnology	China	Recruiting	1	Day 0	IM	18 and 60 years	108	Phase I (ChiCTR2000030906)
	China	Active, not recruiting	1	Day 0	IM	above 18 years	500	Phase II (ChiCTR2000031781)
	Pakistan	Recruiting	1	Day 0	IM	adults aged 18 years old and above	40,000	Phase III (NCT04526990)
Gamaleya Research Institute	Moscow	Study Completion Date: August 10, 2020	2	0, 21 days	IM	18–60	38	Phase I/II (NCT04436471)
	Russia	Active, not recruiting	2	0, 21 days	IM	18–111	33,758	Phase III (NCT04530396)
by Janssen Pharmaceutical Company	US and Belgium	Active, not recruiting	1–2	0, 57 days	IM	18-Older	1085	Phase I/II (NCT04436276)
	Japan	Active, not recruiting	2	0, 57 days	IM	20-older	250	Phase III (NCT04509947)
	Netherlands	Active, not recruiting	1–2	Day 0 or Day 0 + 56	IM	18 to 55 Years		Phase II (EUCTR2020-002584–63-DE)
	Netherlands	Active, not recruiting	1	Day 1	IM	18 Years and Older	44,325	Phase III (NCT04505722)
Immunity Bio, Inc	USA	Recruiting	1–2	0, 22 days	SC or oral	18–55	60	Phase I (NCT04591717)
ReiThera Srl	Italy	Recruiting	1	Day 0	IM	18–85	90	Phase I (NCT04528641)
		Not yet recruiting	1	Day 0	IM	18 Years and older	10,300	Phase II /III (NCT04791423)
Vaxart	USA	Active, not recruiting	2	0, 28 days	Orally	18–55	35	Phase I (NCT04563702)
Universitätsklinikum Hamburg-EppendorfLudwig-Maximilians - University of Munich	Germany	Recruiting	2	0, 28 days	IM	18–55	30	Phase I (NCT04569383)
Shenzhen Geno-immune medical institute	China	Recruiting	1	Day 1	SC and IV	6 Months to 80 Years	100	Phase I/II (NCT04276896)
City of Hope Medical Center	USA	Recruiting	1–2	0, 28 days	IM	18–55	129	Phase I (NCT04639466)
Altimmune, Inc.	USA	Recruiting	1–2	Day 1	IN	18–55	180	Phase I (NCT04679909)
Bharat Biotech International Limited	India	Not yet recruiting	1–2	Day 1	IN	18–60	175	Phase I (NCT04751682)
National Institute of Allergy and Infectious Diseases (NIAID)	USA	Recruiting	2–3	Day 0 + 14 + 28 **or** Day 0 + 28 + 56 **or** Day 0 + 112	IM	18–99	130	Phase I (NCT04776317)

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 5×10^{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, a trial numbered NCT04400838 was registered and performed in UK on patients who are examined in phase II/ III. However, fewer neutralizing antibodies were produced in vitro, the vaccine was reported to be more effective [144].

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. Ad5-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. ChiCTR2000030906 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^{1o} 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 Adeno-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 18–111 age that randomized into two vaccine group by 16,501 and placebo group by 5476 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 tolerate 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 26 as vector and currently have different candidate vaccine in clinical trial stages such as NCT04436276 in phase I/II clinical trial, EUCTR2020-002584–63-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: 5e10, 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 a 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

AACOVID 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 adeno-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 possess 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 patient adoption and speed to the last dose, thermal stability, reduced chance of transmission, removes the need for cold-chain delivery and high dose of vaccine in manufacturing and transferring process, it means 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 enable 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 Fcα receptors 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 Biotecnología (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
BCG vaccines in the clinical trial phases against COVID-19.

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 (NCT04475302)
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 (NCT04384549)
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 Mdical Sciences	Iran	Recruiting	–	Intradermal	From 18 years old	500	Phase III (IRCT20200411047019N1)
Guangxi medical uniwresity	China	Prospective registration	–	–	18 Years to 80 Years	60	Phase IV (ChiCTR2000030016)
Medical Education and Drugs Department and Haffkine Institute for Training Research Testing	India	Not Yet 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 (2020-001591-15)
Radboudumc	Netherlands	Ongoing	–	Intradermal	Elderly (>=65 years)	100	Phase IV (2020-002456-21)
University of Rzeszów	Poland	Ongoing	–	Intradermal	Adults (18–64 years)	1000	Phase III (2020-002111-22)
University Medical Center	Netherlands	Ongoing	–	Intradermal	Adults (18–64 years) and Elderly (>=65 years)	1000	Phase IV (2020-000919-69)
National Korányi Institute of Pulmonology	Hungary	Ongoing	–	Intradermal	Adults (18–64 years) and Elderly (>=65 years)	1000	Phase III (2020-001783-28)
University of Southern Denmark	Denmark	Ongoing	–	Intradermal	Elderly (>=65 years)	1900	Phase III (2020-003904-15)

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 leukocytic pyrogen, upon BCG vaccination. IL-1β 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 immune and non-immune cell training can lead to effective local innate immunity and eradication of the virus before it causing 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. Analyzes showed that ORF1ab 4715L and S protein 614G variants has 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.

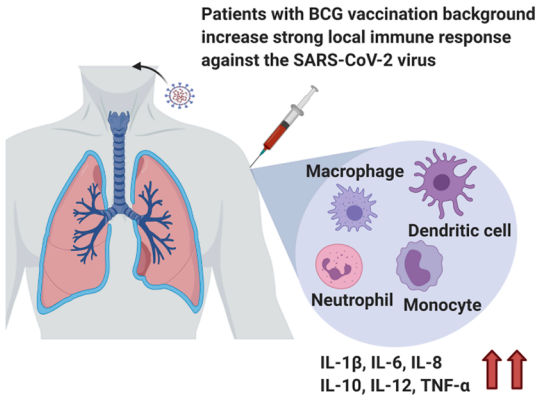

Fig. 7. How BCG vaccination may have an effect on improving host defense against SARS-CoV-2.

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 (NVCP) non-enveloped VLPs, HBsAg enveloped VLPs and alfalfa mosaic virus (AIMV) particles displaying rabies glycoprotein, whose plant hosts are *N. benthamiana*, potato and tobacco, tobacco, lettuce and spinach, respectively [242].

N. benthamiana domin 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 prefusion 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 trimmers 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 Qubec, 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 I 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 °C to 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 I clinical trial in July 2010. The study, is registered at Clintrials.gov (Clinical Trial Number: NCT00984945), 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

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 Âμg/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 Âμg/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 IFNγ, 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.

References

[1] L.A. Alanagreh, F. Alzoughool, M. Atoum, The human coronavirus disease COVID-19: its origin, characteristics, and insights, into potential drugs and its mechanisms, Pathogens 9 (5) (2020) 331.

[2] WHO, Weekly epidemiological update on COVID-19 2021. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—31-march-2021. (Accessed 30 March 2021.

[3] M. Khan, H. Khan, S. Khan, M. Nawaz, Epidemiological and clinical characteristics of coronavirus disease (COVID-19) cases at a screening clinic during the early outbreak period: a single-centre study, J. Med. Microbiol. 69 (8) (2020) 1114–1123.

[4] L.M. Trujillo Gittermann, S.N. Valenzuela Feris, A. von Oetinger Giacoman, Relation between COVID-19 and Guillain-Barré syndrome in adults. Systematic review, Neurologia (Barcelona, Spain) 35(9) (2020) 646-654.

[5] K. Matsushita, B. Marchandot, L. Jesel, P. Ohlmann, O. Morel, Impact of COVID-19 on the cardiovascular system: a review, Journal of clinical medicine 9(5) (2020) 1407.

[6] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, The Lancet 395 (10223) (2020) 497–506.

[7] A. Antonelli, G. Elia, S.M. Ferrari, R. Foddis, S. De Marco, A. Cristaudo, P. Fallahi, The COVID-19, epidemiology, clinic and prevention, Curr. Genomics 21 (3) (2020) 157.

[8] X. Chen, Z. Pan, S. Yue, F. Yu, J. Zhang, Y. Yang, R. Li, B. Liu, X. Yang, L. Gao, Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19, Signal Transduct. Target. Therapy 5 (1) (2020) 1–6.

[9] L. Dai, G.F. Gao, Viral targets for vaccines against COVID-19, Nat. Rev. Immunol. 21 (2) (2021) 73–82.

[10] B.S. Graham, Rapid COVID-19 vaccine development, Science 368 (6494) (2020) 945–946.

[11] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.-L. Hsieh, O. Abiona, B. S. Graham, J.S. McLellan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (6483) (2020) 1260–1263.

[12] Q. Gao, L. Bao, H. Mao, L. Wang, K. Xu, M. Yang, Y. Li, L. Zhu, N. Wang, Z. Lv, Development of an inactivated vaccine candidate for SARS-CoV-2, Science 369 (6499) (2020) 77–81.

[13] W. Dai, B. Zhang, X.-M. Jiang, H. Su, J. Li, Y. Zhao, X. Xie, Z. Jin, J. Peng, F. Liu, Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease, Science 368 (6497) (2020) 1331–1335.

[14] Z. Liu, W. Xu, S. Xia, C. Gu, X. Wang, Q. Wang, J. Zhou, Y. Wu, X. Cai, D. Qu, RBD-Fc-based COVID-19 vaccine candidate induces highly potent SARS-CoV-2 neutralizing antibody response, Signal Transduct. Target. Therapy 5 (1) (2020) 1–10.

[15] J. Cui, F. Li, Z.-L. Shi, Origin and evolution of pathogenic coronaviruses, Nat. Rev. Microbiol. 17 (3) (2019) 181–192.

[16] J. Yang, W. Wang, Z. Chen, S. Lu, F. Yang, Z. Bi, L. Bao, F. Mo, X. Li, Y. Huang, A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity, Nature 586 (7830) (2020) 572–577.

[17] M. Jeyanathan, S. Afkhami, F. Smaill, M.S. Miller, B.D. Lichty, Z. Xing, Immunological considerations for COVID-19 vaccine strategies, Nat. Rev. Immunol. 20 (10) (2020) 615–632.

[18] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nat. Commun. 11 (1) (2020) 1–12.

[19] P.M. Folegatti, K.J. Ewer, P.K. Aley, B. Angus, S. Becker, S. Belij-Rammerstorfer, D. Bellamy, S. Bibi, M. Bittaye, E.A. Clutterbuck, Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial, The Lancet 396 (10249) (2020) 467–478.

[20] F.-C. Zhu, X.-H. Guan, Y.-H. Li, J.-Y. Huang, T. Jiang, L.-H. Hou, J.-X. Li, B.-F. Yang, L. Wang, W.-J. Wang, Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial, The Lancet 396 (10249) (2020) 479–488.

[21] K.S. Corbett, B. Flynn, K.E. Foulds, J.R. Francica, S. Boyoglu-Barnum, A. P. Werner, B. Flach, S. O'Connell, K.W. Bock, M. Minai, Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates, N. Engl. J. Med. 383 (16) (2020) 1544–1555.

[22] C. Wang, Z. Wang, G. Wang, J.Y.-N. Lau, K. Zhang, W. Li, COVID-19 in early 2021: current status and looking forward, Signal Transduct. Target. Therapy 6 (1) (2021) 1–14.

[23] WHO, Draft landscape and tracker of COVID-19 candidate vaccines, 2021. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. (Accessed 2 April 2021.

[24] L. Perico, A. Benigni, F. Casiraghi, L.F. Ng, L. Renia, G. Remuzzi, Immunity, endothelial injury and complement-induced coagulopathy in COVID-19, Nat. Rev. Nephrol. (2020) 1–19.

[25] R.C. Borges, M.S. Hohmann, S.M. Borghi, Dendritic cells in COVID-19 immunopathogenesis: insights for a possible role in determining disease outcome, Int. Rev. Immunol. 40 (1–2) (2021) 108–125.

[26] D.E. Gordon, G.M. Jang, M. Bouhaddou, J. Xu, K. Obernier, K.M. White, M. J. O'Meara, V.V. Rezelj, J.Z. Guo, D.L. Swaney, A SARS-CoV-2 protein interaction map reveals targets for drug repurposing, Nature 583 (7816) (2020) 459–468.

[27] Y.Z. Fu, S.Y. Wang, Z.Q. Zheng, H. Yi, W.W. Li, Z.S. Xu, Y.Y. Wang, SARS-CoV-2 membrane glycoprotein M antagonizes the MAVS-mediated innate antiviral response, Cell. Mol. Immunol. 18 (3) (2021) 613–620.

[28] J. Shang, Y. Wan, C. Luo, G. Ye, Q. Geng, A. Auerbach, F. Li, Cell entry mechanisms of SARS-CoV-2, Proc. Natl. Acad. Sci. 117 (21) (2020) 11727–11734.

[29] T. Behl, I. Kaur, S. Bungau, A. Kumar, M.S. Uddin, C. Kumar, G. Pal, K. Shrivastava, G. Zengin, S. Arora, The dual impact of ACE2 in COVID-19 and ironical actions in geriatrics and pediatrics with possible therapeutic solutions, Life Sci. 118075 (2020).

[30] T.R. Rodrigues Prestes, N.P. Rocha, A.S. Miranda, A.L. Teixeira, A.C. Simoes-e-Silva, The anti-inflammatory potential of ACE2/angiotensin-(1–7)/Mas receptor axis: evidence from basic and clinical research, Curr. Drug Targets 18 (11) (2017) 1301–1313.

[31] Y. Huang, C. Yang, X.-F. Xu, W. Xu, S.-W. Liu, Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19, Acta Pharmacol. Sin. 41 (9) (2020) 1141–1149.

[32] T.M. Clausen, D.R. Sandoval, C.B. Spliid, J. Pihl, H.R. Perrett, C.D. Painter, A. Narayanan, S.A. Majowicz, E.M. Kwong, R.N. McVicar, SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2, Cell 183(4) (2020) 1043-1057. e15.

[33] M. Seyran, K. Takayama, V.N. Uversky, K. Lundstrom, G. Palù, S.P. Sherchan, D. Attrish, N. Rezaei, A.A. Aljabali, S. Ghosh, The structural basis of accelerated host cell entry by SARS-CoV-2, FEBS J. (2020).

[34] H.H. Wong, P. Kumar, F.P.L. Tay, D. Moreau, D.X. Liu, F. Bard, Genome-wide screen reveals valosin-containing protein requirement for coronavirus exit from endosomes, J. Virol. 89 (21) (2015) 11116–11128.

[35] A. Zumla, J.F. Chan, E.I. Azhar, D.S. Hui, K.-Y. Yuen, Coronaviruses—drug discovery and therapeutic options, Nat. Rev. Drug Discovery 15 (5) (2016) 327–347.

[36] M. Thoms, R. Buschauer, M. Ameismeier, L. Koepke, T. Denk, M. Hirschenberger, H. Kratzat, M. Hayn, T. Mackens-Kiani, J. Cheng, Structural basis for translational

shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2, bioRxiv (2020).
[37] T. Li, Z.J. Chen, The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence, and cancer, J. Exp. Med. 215 (5) (2018) 1287–1299.
[38] L. Onofrio, M. Caraglia, G. Facchini, V. Margherita, S.D. Placido, C. Buonerba, Toll-like receptors and COVID-19: a two-faced story with an exciting ending, Future, Science (2020).
[39] A. Yarmohammadi, M. Yarmohammadi, S. Fakhri, H. Khan, Targeting pivotal inflammatory pathways in COVID-19: A mechanistic review, Eur. J. Pharmacol. 173620 (2020).
[40] A.M. Cattelan, E. Di Meco, M. Trevenzoli, A. Frater, A. Ferrari, M. Villano, F. Gomiero, G. Carretta, L. Sasset, Clinical characteristics and laboratory biomarkers changes in COVID-19 patients requiring or not intensive or sub-intensive care: a comparative study, BMC Infect. Dis. 20 (1) (2020) 1–8.
[41] H.-W. Jiang, H.-N. Zhang, Q.-F. Meng, J. Xie, Y. Li, H. Chen, Y.-X. Zheng, X.-N. Wang, H. Qi, J. Zhang, SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70, Cell. Mol. Immunol. 17 (9) (2020) 998–1000.
[42] S.A. Vardhana, J.D. Wolchok, The many faces of the anti-COVID immune response, J. Exp. Med. 217 (6) (2020).
[43] A. Shimabukuro-Vornhagen, P. Gödel, M. Subklewe, H.J. Stemmler, H. A. Schlößer, M. Schlaak, M. Kochanek, B. Böll, M.S. von Bergwelt-Baildon, Cytokine release syndrome, Journal for Immunotherapy of, Cancer 6 (1) (2018) 56.
[44] C. Rosales, N. Demaurex, C.A. Lowell, E. Uribe-Querol, Neutrophils: their role in innate and adaptive immunity, Hindawi (2016).
[45] Y. Bai, L. Yao, T. Wei, F. Tian, D.-Y. Jin, L. Chen, M. Wang, Presumed asymptomatic carrier transmission of COVID-19, JAMA 323 (14) (2020) 1406–1407.
[46] F.P. Veras, M.C. Pontelli, C.M. Silva, J.E. Toller-Kawahisa, M. de Lima, D. C. Nascimento, A.H. Schneider, D. Caetité, L.A. Tavares, I.M. Paiva, R. Rosales, D. Colón, R. Martins, I.A. Castro, G.M. Almeida, M.I.F. Lopes, M.N. Benatti, L. P. Bonjorno, M.C. Giannini, R. Luppino-Assad, S.L. Almeida, F. Vilar, R. Santana, V.R. Bollela, M. Auxiliadora-Martins, M. Borges, C.H. Miranda, A. Pazin-Filho, L. L.P. da Silva, L.D. Cunha, D.S. Zamboni, F. Dal-Pizzol, L.O. Leiria, L. Siyuan, S. Batah, A. Fabro, T. Mauad, M. Dolhnikoff, A. Duarte-Neto, P. Saldiva, T. M. Cunha, J.C. Alves-Filho, E. Arruda, P. Louzada-Junior, R.D. Oliveira, F. Q. Cunha, SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology, J. Exp. Med. 217 (12) (2020).
[47] N. Mangalmurti, C.A. Hunter, Cytokine Storms: Understanding COVID-19, Immunity 53 (1) (2020) 19–25.
[48] J.M. Marchingo, L.V. Sinclair, A.J. Howden, D.A. Cantrell, Quantitative analysis of how Myc controls T cell proteomes and metabolic pathways during T cell activation, Elife 9 (2020), e53725.
[49] V. Castelli, A. Cimini, C. Ferri, Cytokine Storm in COVID-19: "When You Come Out of the Storm, You Won't Be the Same Person Who Walked in", Front. Immunol. 11 (2020) 2132.
[50] M. Gracia-Hernandez, E.M. Sotomayor, A. Villagra, Targeting macrophages as a therapeutic option in COVID-19, Front. Pharmacol. 11 (2020) 1659.
[51] Z. Abassi, Y. Knaney, T. Karram, S.N. Heyman, The Lung Macrophage in SARS-CoV-2 Infection: A Friend or a Foe? Front. Immunol. 11 (2020) 1312.
[52] G.W. Booz, R. Altara, A.H. Eid, Z. Wehbe, S. Fares, H. Zaraket, N.J. Habeichi, F. A. Zouein, Macrophage responses associated with COVID-19: A pharmacological perspective, Eur. J. Pharmacol. 887 (2020), 173547.
[53] M. Merad, J.C. Martin, Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages, Nat. Rev. Immunol. (2020) 1–8.
[54] N. Taefehshokr, S. Taefehshokr, N. Hemmat, B. Heit, Covid-19: Perspectives on Innate Immune Evasion, Front. Immunol. 11 (2020).
[55] K. Kärre, NK cells, MHC class I molecules and the missing self, Scand. J. Immunol. 55 (3) (2002) 221–228.
[56] L.L. Lanier, Up on the tightrope: natural killer cell activation and inhibition, Nat. Immunol. 9 (5) (2008) 495–502.
[57] C. van Eeden, L. Khan, M.S. Osman, J.W. Cohen Tervaert, Natural Killer Cell Dysfunction and Its Role in COVID-19, Int. J. Mol. Sci. 21 (17) (2020) 6351.
[58] L. Tang, Z. Yin, Y. Hu, H. Mei, Controlling Cytokine Storm Is Vital in COVID-19, Front. Immunol. 11 (2020) 3158.
[59] R. Warrington, W. Watson, H.L. Kim, F.R. Antonetti, An introduction to immunology and immunopathology, Allergy Asthma Clin. Immunol. 7 (S1) (2011) S1.
[60] B. Kalfaoglu, J. Almeida-Santos, C.A. Tye, Y. Satou, M. Ono, T-cell dysregulation in COVID-19, Biochem. Biophys. Res. Commun. (2020).
[61] E. Mortaz, P. Tabarsi, M. Varahram, G. Folkerts, I.M. Adcock, The immune response and immunopathology of COVID-19, Front. Immunol. 11 (2020).
[62] M. Zhou, D. Xu, X. Li, H. Li, M. Shan, J. Tang, M. Wang, F.-S. Wang, X. Zhu, H. Tao, Screening and identification of severe acute respiratory syndrome-associated coronavirus-specific CTL epitopes, J. Immunol. 177 (4) (2006) 2138–2145.
[63] P. O'Connell, Y.A. Aldhamen, Systemic innate and adaptive immune responses to SARS-CoV-2 as it relates to other coronaviruses, Hum. Vacc. Immunotherap. (2020) 1–12.
[64] A. Grifoni, D. Weiskopf, S.I. Ramirez, J. Mateus, J.M. Dan, C.R. Moderbacher, S. A. Rawlings, A. Sutherland, L. Premkumar, R.S. Jadi, Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals, Cell (2020).
[65] A.C. Karlsson, M. Humbert, M. Buggert, The known unknowns of T cell immunity to COVID-19, Sci. Immunol. 5 (53) (2020).
[66] X. Yang, T. Dai, X. Zhou, H. Qian, R. Guo, L. Lei, X. Zhang, D. Zhang, L. Shi, Y. Cheng, Naturally activated adaptive immunity in COVID-19 patients, J. Cell Mol. Med. 24 (21) (2020) 12457–12463.
[67] S. Kiss, N. Gede, P. Hegyi, D. Németh, M. Földi, F. Dembrovszky, B. Nagy, M. F. Juhász, K. Ocskay, N. Zádori, Early changes in laboratory parameters are predictors of mortality and ICU admission in patients with COVID-19: a systematic review and meta-analysis, Med. Microbiol. Immunol. (2020) 1–15.
[68] Y. Xiong, Y. Liu, L. Cao, D. Wang, M. Guo, A. Jiang, D. Guo, W. Hu, J. Yang, Z. Tang, Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients, Emerging Microbes Infect. 9 (1) (2020) 761–770.
[69] Z. Chen, E.J. Wherry, T cell responses in patients with COVID-19, Nat. Rev. Immunol. 20 (9) (2020) 529–536.
[70] S. Bellesi, E. Metafuni, S. Hohaus, E. Maiolo, F. Marchionni, S. D'Innocenzo, M. La Sorda, M. Ferraironi, F. Ramundo, M. Fantoni, Increased CD95 (Fas) and PD-1 expression in peripheral blood T lymphocytes in COVID-19 patients, Br. J. Haematol. 191 (2) (2020) 207–211.
[71] Y. Yang, F. Peng, R. Wang, K. Guan, T. Jiang, G. Xu, J. Sun, C. Chang, The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China, J. Autoimmunity (2020) 102434.
[72] A. Jafarzadeh, S. Jafarzadeh, P. Nozari, P. Mokhtari, M. Nemati, Lymphopenia an important immunological abnormality in patients with COVID-19: Possible mechanisms, Scand. J. Immunol. (2020), e12967.
[73] B. Diao, C. Wang, Y. Tan, X. Chen, Y. Liu, L. Ning, L. Chen, M. Li, Y. Liu, G. Wang, Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19), Front. Immunol. 11 (2020) 827.
[74] H.-Y. Zheng, M. Zhang, C.-X. Yang, N. Zhang, X.-C. Wang, X.-P. Yang, X.-Q. Dong, Y.-T. Zheng, Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients, Cell. Mol. Immunol. 17 (5) (2020) 541–543.
[75] M. Thoms, R. Buschauer, M. Ameismeier, L. Koepke, T. Denk, M. Hirschenberger, H. Kratzat, M. Hayn, T. Mackens-Kiani, J. Cheng, Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2, Science 369 (6508) (2020) 1249–1255.
[76] Q. Huang, J. Hu, J. Tang, L. Xu, L. Ye, Molecular Basis of the Differentiation and Function of Virus Specific Follicular Helper CD4+ T Cells, Front. Immunol. 10 (2019) 249.
[77] T. Sekine, A. Perez-Potti, O. Rivera-Ballesteros, K. Strålin, J.-B. Gorin, A. Olsson, S. Llewellyn-Lacey, H. Kamal, G. Bogdanovic, S. Muschiol, Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19, Cell 183(1) (2020) 158–168.e14.
[78] L.S.F. Frederiksen, Y. Zhang, C. Foged, A. Thakur, The long road toward COVID-19 herd immunity: vaccine platform technologies and mass immunization strategies, Front. Immunol. 11 (2020).
[79] P. Martinez-Fleta, A. Alfranca, I. González-Álvaro, J.M. Casasnovas, D. Fernández-Soto, G. Esteso, Y. Cáceres-Martell, S. Gardeta, S. Prat, T. Mateu-Alberoa, SARS-Cov-2 cysteine-like protease (Mpro) is immunogenic and can be detected in serum and saliva of COVID-19-seropositive individuals, (2020).
[80] D.S. Stephens, M.J. McElrath, COVID-19 and the Path to Immunity, JAMA 324 (13) (2020) 1279–1281.
[81] A. Padoan, L. Sciacovelli, D. Basso, D. Negrini, S. Zuin, C. Cosma, D. Faggian, P. Matricardi, M. Plebani, IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study, Clin. Chim. Acta (2020).
[82] F.J. Ibarrondo, J.A. Fulcher, D. Goodman-Meza, J. Elliott, C. Hofmann, M. A. Hausner, K.G. Ferbas, N.H. Tobin, G.M. Aldrovandi, O.O. Yang, Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19, N. Engl. J. Med. 383 (11) (2020) 1085–1087.
[83] A. Mveang Nzoghe, P.N. Essone, M. Leboueny, A.C. Maloupazoa Siawaya, E. C. Bongho, O. Mvoundza Ndjindji, R.M. Avome Houechenou, S.T. Agnandji, J. F. Djoba Siawaya, Evidence and implications of pre-existing humoral cross-reactive immunity to SARS-CoV-2, Immun Inflamm Dis 9 (1) (2021) 128–133.
[84] F.Y. Tso, S.J. Lidenge, P.B. Peña, A.A. Clegg, J.R. Ngowi, J. Mwaiselage, O. Ngalamika, P. Julius, J.T. West, C. Wood, High prevalence of pre-existing serological cross-reactivity against SARS-CoV-2 in sub-Sahara Africa, Int. J. Infect. Dis. (2020).
[85] P.A. Reche, Potential cross-reactive immunity to SARS-CoV-2 from common human pathogens and vaccines, Front. Immunol. 11 (2020) 2694.
[86] A.G. Laing, A. Lorenc, I.D.M. Del Barrio, A. Das, M. Fish, L. Monin, M. Muñoz-Ruiz, D.R. McKenzie, T.S. Hayday, I. Francos-Quijorna, A dynamic COVID-19 immune signature includes associations with poor prognosis, Nat. Med. 26 (10) (2020) 1623–1635.
[87] G.A. Poland, I.G. Ovsyannikova, R.B. Kennedy, SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates, The Lancet (2020).
[88] A. Awadasseid, Y. Wu, Y. Tanaka, W. Zhang, Effective drugs used to combat SARS-CoV-2 infection and the current status of vaccines, Biomed. Pharmacother. 111330 (2021).
[89] T.P. Sheahan, A.C. Sims, R.L. Graham, V.D. Menachery, L.E. Gralinski, J.B. Case, S.R. Leist, K. Pyrc, J.Y. Feng, I. Trantcheva, Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses, Sci. Transl. Med. 9 (396) (2017).
[90] H. Zhang, K.M. Saravanan, Y. Yang, M.T. Hossain, J. Li, X. Ren, Y. Pan, Y. Wei, Deep learning based drug screening for novel coronavirus 2019-nCov, Interdiscip. Sci.: Comput. Life Sci. 12 (2020) 368–376.
[91] L. Peng, X. Tian, L. Shen, M. Kuang, T. Li, G. Tian, J. Yang, L. Zhou, Identifying effective antiviral drugs against SARS-CoV-2 by drug repositioning through virus-drug association prediction, Front. Genet. 11 (2020).

[92] G. Kokic, H.S. Hillen, D. Tegunov, C. Dienemann, F. Seitz, J. Schmitzova, L. Farnung, A. Siewert, C. Höbartner, P. Cramer, Mechanism of SARS-CoV-2 polymerase stalling by remdesivir, Nat. Commun. 12 (1) (2021) 1–7.
[93] B.N. Williamson, F. Feldmann, B. Schwarz, K. Meade-White, D.P. Porter, J. Schulz, N. Van Doremalen, I. Leighton, C.K. Yinda, L. Pérez-Pérez, Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2, Nature 585 (7824) (2020) 273–276.
[94] A.J. Pruijssers, A.S. George, A. Schäfer, S.R. Leist, L.E. Gralinksi, K.H. Dinnon III, B.L. Yount, M.L. Agostini, L.J. Stevens, J.D. Chappell, Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice, Cell reports 32 (3) (2020), 107940.
[95] J. Geleris, Y. Sun, J. Platt, J. Zucker, M. Baldwin, G. Hripcsak, A. Labella, D. K. Manson, C. Kubin, R.G. Barr, Observational study of hydroxychloroquine in hospitalized patients with Covid-19, N. Engl. J. Med. 382 (25) (2020) 2411–2418.
[96] M.G.S. Borba, F.F.A. Val, V.S. Sampaio, M.A.A. Alexandre, G.C. Melo, M. Brito, M. P.G. Mourão, J.D. Brito-Sousa, D. Baía-da-Silva, M.V.F. Guerra, Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial, JAMA Netw. Open 3(4) (2020) e208857–e208857.
[97] J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong, M. Wang, Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro, Cell Discovery 6 (1) (2020) 1–4.
[98] X. Li, Y. Wang, P. Agostinis, A. Rabson, G. Melino, E. Carafoli, Y. Shi, E. Sun, Is hydroxychloroquine beneficial for COVID-19 patients? Cell Death Dis. 11 (7) (2020) 512.
[99] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19, N. Engl. J. Med. (2020).
[100] S. Tong, Y. Su, Y. Yu, C. Wu, J. Chen, S. Wang, J. Jiang, Ribavirin therapy for severe COVID-19: a retrospective cohort study, Int. J. Antimicrob. Agents 56 (3) (2020), 106114.
[101] M. Saijo, S. Morikawa, S. Fukushi, T. Mizutani, H. Hasegawa, N. Nagata, N. Iwata, I. Kurane, Inhibitory effect of mizoribine and ribavirin on the replication of severe acute respiratory syndrome (SARS)-associated coronavirus, Antiviral Res. 66 (2–3) (2005) 159–163.
[102] A. Gislén, M. Dacke, R.H. Kröger, M. Abrahamsson, D.-E. Nilsson, E.J. Warrant, Superior underwater vision in a human population of sea gypsies, Curr. Biol. 13 (10) (2003) 833–836.
[103] P.W. Horby, M. Mafham, J.L. Bell, L. Linsell, N. Staplin, J. Emberson, A. Palfreeman, J. Raw, E. Elmahi, B. Prudon, Lopinavir–ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial, The Lancet 396 (10259) (2020) 1345–1352.
[104] F. Stauffer, T. El-Bacha, A.T. Da Poian, Advances in the development of inactivated virus vaccines, Recent Pat. Anti-Infect. Drug Discov. 1 (3) (2006) 291–296.
[105] D.K. Yadav, N. Yadav, S.M.P. Khurana, Chapter 26 - Vaccines: Present Status and Applications, in: A.S. Verma, A. Singh (Eds.), Animal Biotechnology, Academic Press, San Diego, 2014, pp. 491–508.
[106] B. Sanders, M. Koldijk, H. Schuitemaker, Inactivated Viral Vaccines, in: B. K. Nunnally, V.E. Turula, R.D. Sitrin (Eds.), Vaccine Analysis: Strategies, Principles, and Control, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, pp. 45–80.
[107] A. Mullbacher, J. Pardo, Y. Furuya, SARS-CoV-2 vaccines: Inactivation by gamma irradiation for T and B cell immunity, Pathogens 9 (11) (2020) 928.
[108] A. Awadasseid, Y. Wu, Y. Tanaka, W. Zhang, Current advances in the development of SARS-CoV-2 vaccines, Int. J. Biol. Sci. 17 (1) (2021) 8.
[109] W. Sun, S.R. Leist, S. McCroskery, Y. Liu, S. Slamanig, J. Oliva, F. Amanat, A. Schäfer, K.H. Dinnon 3rd, A. García-Sastre, F. Krammer, R.S. Baric, P. Palese, Newcastle disease virus (NDV) expressing the spike protein of SARS-CoV-2 as a live virus vaccine candidate, EBioMedicine 62 (2020), 103132.
[110] W. Sun, S. McCroskery, W.C. Liu, S.R. Leist, Y. Liu, R.A. Albrecht, S. Slamanig, J. Oliva, F. Amanat, A. Schäfer, K.H. Dinnon, 3rd, B.L. Innis, A. García-Sastre, F. Krammer, R.S. Baric, P. Palese, A Newcastle Disease Virus (NDV) Expressing a Membrane-Anchored Spike as a Cost-Effective Inactivated SARS-CoV-2 Vaccine, Vaccines 8(4) (2020) 2020.07.30.229120.
[111] dynavax, Valneva and Dynavax Announce Collaboration to Advance Vaccine Development for COVID-19, 2020. https://investors.dynavax.com/news-releases/news-release-details/valneva-and-dynavax-announce-collaboration-advance-vaccine. (Accessed 22 April 2020).
[112] G. Sir Karakus, C. Tastan, D. Dilek Kancagi, B. Yurtsever, G. Tumentemur, S. Demir, R.D. Turan, S. Abanuz, D. Cakirsoy, U. Seyis, S. Ozer, O. Elibol, M. Elek, G. Ertop, S. Arbak, M. Acikel Elmas, C. Hemsinlioglu, A.S. Kocagoz, O. Hatirnaz Ng, S. Akyoney, I. Sahin, U. Ozbek, D. Telci, F. Sahin, K. Yalcin, S. Ratip, E. Ovali, Preclinical efficacy and safety analysis of gamma-irradiated inactivated SARS-CoV-2 vaccine candidates, Sci. Rep. 11 (1) (2021) 5804.
[113] P.D. Minor, Live attenuated vaccines: Historical successes and current challenges, Virology 479 (2015) 379–392.
[114] C. Le Nouën, P.L. Collins, U.J. Buchholz, Attenuation of human respiratory viruses by synonymous genome recoding, Front. Immunol. 10 (2019) 1250.
[115] J.B. Plotkin, G. Kudla, Synonymous but not the same: the causes and consequences of codon bias, Nat. Rev. Genet. 12 (1) (2011) 32–42.
[116] N. Groenke, J. Trimpert, S. Merz, A.M. Conradie, E. Wyler, H. Zhang, O.-G. Hazapis, S. Rausch, M. Landthaler, N. Osterrieder, Mechanism of virus attenuation by codon pair deoptimization, Cell Reports 31 (4) (2020), 107586.
[117] N.J. Atkinson, J. Witteveldt, D.J. Evans, P. Simmonds, The influence of CpG and UpA dinucleotide frequencies on RNA virus replication and characterization of the innate cellular pathways underlying virus attenuation and enhanced replication, Nucleic Acids Res. 42 (7) (2014) 4527–4545.
[118] G. Moratorio, R. Henningsson, C. Barbezange, L. Carrau, A.V. Bordería, H. Blanc, S. Beaucourt, E.Z. Poirier, T. Vallet, J. Boussier, Attenuation of RNA viruses by redirecting their evolution in sequence space, Nat. Microbiol. 2 (8) (2017) 17088.
[119] P.D. Minor, Live attenuated vaccines: Historical successes and current challenges, Virology 479–480 (2015) 379–392.
[120] N. Zhang, B.-J. Zheng, L. Lu, Y. Zhou, S. Jiang, L. Du, Advancements in the development of subunit influenza vaccines, Microbes Infect. 17 (2) (2015) 123–134.
[121] N. Wang, J. Shang, S. Jiang, Lanying Du, Subunit vaccines against emerging pathogenic human coronaviruses, Front Microbiol 11 (2020) 298.
[122] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China, Jama 323 (11) (2020) 1061–1069.
[123] J. Zhang, H. Zeng, J. Gu, H. Li, L. Zheng, Q. Zou, Progress and Prospects on Vaccine Development against SARS-CoV-2, Vaccines 8 (2) (2020).
[124] P. Roingeard, Viral detection by electron microscopy: past, present and future, Biol. Cell 100 (8) (2008) 491–501.
[125] P.L. Cann, P. Coursaget, S. Iochmann, A. Touze, Self-assembly of human papillomavirus type 16 capsids by expression of the L1 protein in insect cells, FEMS Microbiol. Lett. 117 (3) (1994) 269–274.
[126] R. Kirnbauer, Papillomavirus-like particles for serology and vaccine development, Intervirology 39 (1–2) (1996) 54–61.
[127] A. Vartak, S.J. Sucheck, Recent advances in subunit vaccine carriers, Vaccines 4 (2) (2016) 12.
[128] G.R. Wi, J.Y. Hwang, M.-G. Kwon, H.J. Kim, H.A. Kang, H.-J. Kim, Protective immunity against nervous necrosis virus in convict grouper Epinephelus septemfasciatus following vaccination with virus-like particles produced in yeast Saccharomyces cerevisiae, Vet. Microbiol. 177 (1–2) (2015) 214–218.
[129] N.-C. Chen, M. Yoshimura, H.-H. Guan, T.-Y. Wang, Y. Misumi, C.-C. Lin, P. Chuankhayan, A. Nakagawa, S.I. Chan, T. Tsukihara, Crystal structures of a piscine betanodavirus: mechanisms of capsid assembly and viral infection, PLoS Pathog 11 (10) (2015), e1005203.
[130] Y.-M. Wu, C.-H. Hsu, C.-H. Wang, W. Liu, W.-H. Chang, C.-S. Lin, Role of the DxxDxD motif in the assembly and stability of betanodavirus particles, Arch. Virol. 153 (9) (2008) 1633–1642.
[131] M.O. Mohsen, L. Zha, G. Cabral-Miranda, M.F. Bachmann, Major findings and recent advances in virus–like particle (VLP)-based vaccines, Seminars in immunology, Elsevier (2017) 123–132.
[132] D. Hobernik, M. Bros, DNA Vaccines-How Far From Clinical Use? Int J Mol Sci 19 (11) (2018).
[133] M.A. Kutzler, D.B. Weiner, DNA vaccines: ready for prime time? Nat. Rev. Genet. 9 (10) (2008) 776–788.
[134] Z. Wang, P.J. Troilo, X. Wang, T.G. Griffiths, S.J. Pacchione, A.B. Barnum, L. B. Harper, C.J. Pauley, Z. Niu, L. Denisova, T.T. Follmer, G. Rizzuto, G. Ciliberto, E. Fattori, N.L. Monica, S. Manam, B.J. Ledwith, Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation, Gene Ther. 11 (8) (2004) 711–721.
[135] D.G. Ahn, H.J. Shin, M.H. Kim, S. Lee, H.S. Kim, J. Myoung, B.T. Kim, S.J. Kim, Current Status of Epidemiology, Diagnosis, Therapeutics, and Vaccines for Novel Coronavirus Disease 2019 (COVID-19), J. Microbiol. Biotechnol. 30 (3) (2020) 313–324.
[136] C. Nilsson, B. Hejdeman, K. Godoy-Ramirez, T. Tecleab, G. Scarlatti, A. Bråve, P. L. Earl, R.R. Stout, M.L. Robb, R.J. Shattock, G. Biberfeld, E. Sandström, B. Wahren, HIV-DNA Given with or without Intradermal Electroporation Is Safe and Highly Immunogenic in Healthy Swedish HIV-1 DNA/MVA Vaccinees: A Phase I Randomized Trial, PLoS ONE 10 (6) (2015), e0131748.
[137] J.E. Martin, M.K. Louder, L.A. Holman, I.J. Gordon, M.E. Enama, B.D. Larkin, C. A. Andrews, L. Vogel, R.A. Koup, M. Roederer, A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial, Vaccine 26 (50) (2008) 6338–6343.
[138] T. Smith, A. Patel, S. Ramos, D. Elwood, X. Zhu, J. Yan, E. Gary, S. Walker, K. Schultheis, M. Purwar, Z. Xu, J. Walters, P. Bhojnagarwala, M. Yang, N. Chokkalingam, P. Pezzoli, E. Parzych, E. Reuschel, A. Doan, K. Broderick, Immunogenicity of a DNA vaccine candidate for COVID-19, Nat. Commun. 11 (2020).
[139] S.O. Alturki, S.O. Alturki, J. Connors, G. Cusimano, M.A. Kutzler, A.M. Izmirly, E. K. Haddad, The 2020 Pandemic: Current SARS-CoV-2 Vaccine Development, Front Immunol 11 (2020) 1880.
[140] D. Speiser, M. Bachmann, COVID-19: Mechanisms of Vaccination and Immunity, Vaccines 8 (2020) 404.
[141] Z. Cadila, Zydus Cadila Announces Completion of Dosing in Phase I Clinical Trial of ZyCoV-D, 2020. https://zyduscadila.com/public/pdf/pressrelease/Press-Release-ZyCoV-D.pdf (Accessed 05 August 2020.
[142] T. Thacker, Zydus Cadila to test ZyCoV-D on 30,000 patients in Phase-3 trials, 2020. https://economictimes.indiatimes.com/industry/healthcare/biotech/pharmaceuticals/zydus-cadila-to-test-zycov-d-on-30000-patients-in-phase-3-trials/articleshow/79088859.cms?from=mdr (Accessed 07 November 2020.
[143] G. Montel Mendoza, S.E. Pasteris, M.C. Otero, M. Fatima Nader-Macías, Survival and beneficial properties of lactic acid bacteria from raniculture subjected to freeze-drying and storage, J. Appl. Microbiol. 116(1) (2014) 157–166.

[144] K.R. Emary, T. Golubchik, P.K. Aley, C.V. Ariani, B.J. Angus, S. Bibi, B. Blane, D. Bonsall, P. Cicconi, S. Charlton, Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 VOC 202012/01 (B. 1.1. 7), (2021).
[145] P.H. Services, CORVax12: SARS-CoV-2 Spike (S) Protein Plasmid DNA Vaccine Trial for COVID-19 (SARS-CoV-2) (CORVax12), 2021. https://clinicaltrials.gov/ct2/show/NCT04627675?term=DNA+vaccines+against+SARS-CoV2&cond=COVID+19+SARS-CoV2&draw=2+DNA+candidate+vaccine+in+pre-clinical+trial+stage (Accessed 15 March 2021.
[146] Cambridge, Cambridge-developed SARS-CoV-2 vaccine receives £1.9million from UK government for clinical trial, 2020. https://www.cam.ac.uk/research/news/cambridge-developed-sars-cov-2-vaccine-receives-ps19million-from-uk-government-for-clinical-trial (Accessed 26 August 2020.
[147] CanSino, CanSinoBIO's Investigational Vaccine Against COVID-19 Approved for Phase 1 Clinical Trial in China, 2020. http://www.cansinotech.com/html/1///179/180/408.html (Accessed 17 March 2020.
[148] K. Rawat, P. Kumari, L. Saha, COVID-19 vaccine: A recent update in pipeline vaccines, their design and development strategies, Eur. J. Pharmacol. 173751 (2020).
[149] D.Y. Logunov, I.V. Dolzhikova, D.V. Shcheblyakov, A.I. Tukhvatulin, O.V. Zubkova, A.S. Dzharullaeva, A.V. Kovyrshina, N.L. Lubenets, D.M. Grousova, A.S. Erokhova, A.G. Botikov, F.M. Izhaeva, O. Popova, T.A. Ozharovskaya, I.B. Esmagambetov, I.A. Favorskaya, D.I. Zrelkin, D.V. Voronina, D.N. Shcherbinin, A. S. Semikhin, Y.V. Simakova, E.A. Tokarskaya, D.A. Egorova, M.M. Shmarov, N.A. Nikitenko, V.A. Gushchin, E.A. Smolyarchuk, S.K. Zyryanov, S.V. Borisevich, B.S. Naroditsky, A.L. Gintsburg, Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia, Lancet (London, England) 397 (10275) (2021) 671–681.
[150] U.o. Sydney, University of Sydney to advance COVID-19 DNA vaccine to human trials, 2020. https://www.sydney.edu.au/news-opinion/news/2020/09/24/university-of-sydney-to-advance-covid-19-dna-vaccine-to–human-t.html. (Accessed 24 September 2020.
[151] S. BROOK, Applied DNA and Takis Biotech/Evvivax to Launch Clinical Trial for Veterinary Use of Linear COVID-19 Vaccine Candidate, 2020. https://www.evvivax.com/index.php/news/72-applied-dna-and-takis-biotech-evvivax-to-launch-clinical-trial-for-veterinary-use-of-linear-covid-19-vaccine-candidate. (Accessed 16 September 2020.
[152] J.S. Tregoning, E.S. Brown, H.M. Cheeseman, K.E. Flight, S.L. Higham, N. M. Lemm, B.F. Pierce, D.C. Stirling, Z. Wang, K.M. Pollock, Vaccines for COVID-19, Clin. Exp. Immunol. 202 (2) (2020) 162–192.
[153] J.J. Suschak, J.A. Williams, C.S. Schmaljohn, Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity, Hum. Vaccin. Immunother. 13 (12) (2017) 2837–2848.
[154] C.-T. Tseng, E. Sbrana, N. Iwata-Yoshikawa, P. Newman, T. Garron, R. Atmar, C. Peters, R. Couch, Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus, PLoS ONE 7 (2012), e35421.
[155] F. Krammer, SARS-CoV-2 vaccines in development, Nature 586 (2020).
[156] Y. Dong, T. Dai, Y. Wei, L. Zhang, M. Zheng, F. Zhou, A systematic review of SARS-CoV-2 vaccine candidates, Signal Transduct. Target. Therapy 5 (1) (2020) 1–14.
[157] P. Yuan, P. Ai, Y. Liu, Z. Ai, Y. Wang, W. Cao, X. Xia, J.C. Zheng, Safety, Tolerability, and Immunogenicity of COVID-19 Vaccines: A Systematic Review and Meta-Analysis, medRxiv (2020).
[158] R. Calandra, As Vaccines Near the Possible-Approval Line, New Challenges Await 2020. https://www.medicaldaily.com/vaccines-near-possible-approval-line-new-challenges-await-457497. (Accessed 20 November 2020.
[159] R. Verbeke, I. Lentacker, S.C. De Smedt, H. Dewitte, Three decades of messenger RNA vaccine development, Nano Today 28 (2019), 100766.
[160] S. Pascolo, Vaccination with messenger RNA (mRNA), Toll-like receptors (TLRs) and innate immunity, Springer, 2008, pp. 221–235.
[161] S. Pascolo, Messenger RNA-based vaccines, Expert Opin. Biol. Ther. 4 (8) (2004) 1285–1294.
[162] E.O.-O. Hannah Ritchie, Diana Beltekian, Edouard Mathieu, Joe Hasell, Bobbie Macdonald, Charlie Giattino, Cameron Appel and Max Roser, Coronavirus (COVID-19) Vaccinations, 2021. https://ourworldindata.org/covid-vaccinations. (Accessed 18 April 2021).
[163] L.S.o.H.T. Medicine, Equity of vaccine roll-out, 2021. https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/. (Accessed 31 March 2021).
[164] T. Zaks, Protocol mRNA-1273-P301, Amendment 3. 2020, 2020. https://www.modernatx.com/sites/default/files/mRNA-1273-P301-Protocol.pdf. (Accessed 20 August 2020.
[165] O. Zent, C. Arras-Reiter, M. Broeker, R. Hennig, Immediate allergic reactions after vaccinations–a post-marketing surveillance review, Eur. J. Pediatr. 161 (1) (2002) 21–25.
[166] S.E. Lavigne, The race for a COVID-19 vaccine, Canadian journal of dental hygiene : CJDH = Journal canadien de l'hygiene dentaire, JCHD 54 (3) (2020) 107–109.
[167] K. Karikó, H. Muramatsu, F.A. Welsh, J. Ludwig, H. Kato, S. Akira, D. Weissman, Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability, Mol. Ther. 16 (11) (2008) 1833–1840.
[168] E.E. Walsh, R.W. Frenck Jr., A.R. Falsey, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, K. Neuzil, M.J. Mulligan, R. Bailey, K.A. Swanson, P. Li, K. Koury, W. Kalina, D. Cooper, C. Fontes-Garfias, P.Y. Shi, Ö. Türeci, K.R. Tompkins, K. E. Lyke, V. Raabe, P.R. Dormitzer, K.U. Jansen, U. Şahin, W.C. Gruber, Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates, N. Engl. J. Med. 383 (25) (2020) 2439–2450.
[169] M. McCallum, A.C. Walls, J.E. Bowen, D. Corti, D. Veesler, Structure-guided covalent stabilization of coronavirus spike glycoprotein trimers in the closed conformation, Nat. Struct. Mol. Biol. 27 (10) (2020) 942–949.
[170] K. Lundstrom, The Potential of Self-amplifying RNA Vaccines for Infectious Diseases and COVID-19, Vaccine Res. 7 (1) (2020) 25–37.
[171] K. Borgers, J.-Y. Ou, P.-X. Zheng, P. Tiels, A. Van Hecke, E. Plets, G. Michielsen, N. Festjens, N. Callewaert, Y.-C. Lin, Reference genome and comparative genome analysis for the WHO reference strain for Mycobacterium bovis BCG Danish, the present tuberculosis vaccine, BMC Genomics 20 (1) (2019) 561.
[172] A.M. Lisewski, Interim estimates in null models of COVID-19 vaccine effectiveness, Int. J. Infect. Dis. 106 (2021) 169–170.
[173] E.E. Walsh, R.W. Frenck Jr, A.R. Falsey, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, K. Neuzil, M.J. Mulligan, R. Bailey, Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates, N. Engl. J. Med. (2020).
[174] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E.D. Moreira, C. Zerbini, R. Bailey, K.A. Swanson, S. Roychoudhury, K. Koury, P. Li, W.V. Kalina, D. Cooper, R.W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D.B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K.U. Jansen, W.C. Gruber, Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine, N. Engl. J. Med. (2020).
[175] S. Rauch, N. Roth, K. Schwendt, M. Fotin-Mleczek, S.O. Mueller, B. Petsch, mRNA-based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus-neutralising antibodies and mediates protection in rodents, npj Vaccines 6 (1) (2021) 57.
[176] Arcturus, Arcturus Therapeutics Announces Positive Interim ARCT-021 (LUNAR-COV19) Phase 1/2 Study Results for Both Single Shot and Prime-boost Regimens, and Up to $220 Million in Additional Financial Commitments from Singapore, 2020. https://ir.arcturusrx.com/news-releases/news-release-details/arcturus-therapeutics-announces-positive-interim-arct-021-lunar. (Accessed 9 November 2020.
[177] J. Fletcher, Clinical trial to assess the safety of a coronavirus vaccine in healthy men and women, 2020. https://www.isrctn.com/ISRCTN17072692. (Accessed 27 July 2020.
[178] Calif, Amyris and The Infectious Disease Research Institute Partner to Advance Novel RNA Vaccine Platform Beginning With COVID-19 Application, 2020. https://investors.amyris.com/2020-07-27-Amyris-and-The-Infectious-Disease-Research-Institute-Partner-to-Advance-Novel-RNA-Vaccine-Platform-Beginning-With-COVID-19-Application. (Accessed 27 July 2020.
[179] S.J.U. Fudan University, and RNACure Biopharma, Vaccine against COVID-19, 2020. https://www.genengnews.com/covid-19-candidates/fudan-university-shanghai-jiaotong-university-and-rnacure-biopharma/. (Accessed 18 May 2020.
[180] U.o. Washington, HDT-301 (also called repRNA-CoV2S), 2020. https://www.genengnews.com/covid-19-candidates/university-of-washington-uw-medicine-and-hdt-bio-hdt-301/. (Accessed 3 August 2020.
[181] C. Rademacher, Corona: vaccination without a needle?, 2020. https://www.mpg.de/14807464/0511-mpin-116799-corona-vaccination-via-the-skin. (Accessed 13 May 2020.
[182] COVID-19 Vaccine, AAVCOVID. https://masseyeandear.org/covid-19/vaccine.
[183] GeneOne, GeneOne Life Science and Houston Methodist to develop a COVID-19 vaccine, 2020. (Accessed 17 March 2020.
[184] C. Bio, Chimeron Bio Appoints CEO of Evox Therapeutics and Former CSO of Moderna, Dr. Antonin de Fougerolles to Its Scientific Advisory Board, 2020 https://www.biospace.com/article/releases/chimeron-bio-appoints-ceo-of-evox-therapeutics-and-former-cso-of-moderna-dr-antonin-de-fougerolles-to-its-scientific-advisory-board/. (Accessed 7 December 2020.
[185] T. Zaichuk, Y. Nechipurenko, A. Adzhubey, S. Onikienko, V. Chereshnev, S. Zainutdinov, G. Kochneva, S. Netesov, O. Matveeva, The Challenges of Vaccine Development against Betacoronaviruses: Antibody Dependent Enhancement and Sendai Virus as a Possible Vaccine Vector, Mol. Biol. (2020) 1–15.
[186] M. Bakhiet, S. Taurin, SARS-CoV-2: Targeted managements and vaccine development, Cytokine & growth factor reviews (2020).
[187] N.S.H. RW, Progress and prospects: immune responses to viral vectors Gene Ther. 17295304. Nayak, S., Herzog, RW (2010). Progress and prospects: immune responses to viral vectors, Gene Therapy 17 (2010) 295–304.
[188] Z.-Y. Yang, L.S. Wyatt, W.-P. Kong, Z. Moodie, B. Moss, G.J. Nabel, Overcoming immunity to a viral vaccine by DNA priming before vector boosting, J. Virol. 77 (1) (2003) 799–803.
[189] P.-J. Li, T. Jin, D.-H. Luo, T. Shen, D.-M. Mai, W.-H. Hu, H.-Y. Mo, Effect of prolonged radiotherapy treatment time on survival outcomes after intensity-modulated radiation therapy in nasopharyngeal carcinoma, PLoS ONE 10 (10) (2015), e0141332.
[190] F.-C. Zhu, Y.-H. Li, X.-H. Guan, L.-H. Hou, W.-J. Wang, J.-X. Li, S.-P. Wu, B.-S. Wang, Z. Wang, L. Wang, Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial, The Lancet (2020).
[191] S. Jain, H. Batra, P. Yadav, S. Chand, COVID-19 Vaccines Currently under Preclinical and Clinical Studies, and Associated Antiviral Immune Response, Vaccines 8 (4) (2020) 649.
[192] FDA, First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response, 2019. https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. (Accessed 19 December 2019.

[193] K. Ramsauer, M. Schwameis, C. Firbas, M. Müllner, R.J. Putnak, S.J. Thomas, P. Desprès, E. Tauber, B. Jilma, F. Tangy, Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial, Lancet. Infect. Dis 15 (5) (2015) 519–527.
[194] N. Escriou, B. Callendret, V. Lorin, C. Combredet, P. Marianneau, M. Février, F. Tangy, Protection from SARS coronavirus conferred by live measles vaccine expressing the spike glycoprotein, Virology 452 (2014) 32–41.
[195] R.S. Noyce, S. Lederman, D.H. Evans, Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments, PLoS ONE 13 (1) (2018), e0188453.
[196] E. Tulman, G. Delhon, C. Afonso, Z. Lu, L. Zsak, N. Sandybaev, U. Kerembekova, V. Zaitsev, G. Kutish, D. Rock, Genome of horsepox virus, J. Virol. 80 (18) (2006) 9244–9258.
[197] D.K. Clarke, F. Nasar, S. Chong, J.E. Johnson, J.W. Coleman, M. Lee, S.E. Witko, C.S. Kotash, R. Abdullah, S. Megati, Neurovirulence and immunogenicity of attenuated recombinant vesicular stomatitis viruses in nonhuman primates, J. Virol. 88 (12) (2014) 6690–6701.
[198] A.M. Henao-Restrepo, A. Camacho, I.M. Longini, C.H. Watson, W.J. Edmunds, M. Egger, M.W. Carroll, N.E. Dean, I. Diatta, M. Doumbia, Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!), The Lancet 389 (10068) (2017) 505–518.
[199] N. van Doremalen, T. Lambe, A. Spencer, S. Belij-Rammerstorfer, J. N. Purushotham, J.R. Port, V.A. Avanzato, T. Bushmaker, A. Flaxman, M. Ulaszewska, ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques, Nature 586 (7830) (2020) 578–582.
[200] M.N. Ramasamy, A.M. Minassian, K.J. Ewer, A.L. Flaxman, P.M. Folegatti, D. R. Owens, M. Voysey, P.K. Aley, B. Angus, G. Babbage, Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial, The Lancet 396 (10267) (2020) 1979–1993.
[201] M. Voysey, S.A.C. Clemens, S.A. Madhi, L.Y. Weckx, P.M. Folegatti, P.K. Aley, B. Angus, V.L. Baillie, S.L. Barnabas, Q.E. Bhorat, Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK, The Lancet 397 (10269) (2021) 99–111.
[202] M.N. Ramasamy, A.M. Minassian, K.J. Ewer, A.L. Flaxman, P.M. Folegatti, D. R. Owens, M. Voysey, P.K. Aley, B. Angus, G. Babbage, S. Belij-Rammerstorfer, L. Berry, S. Bibi, M. Bittaye, K. Cathie, H. Chappell, S. Charlton, P. Cicconi, E. A. Clutterbuck, R. Colin-Jones, C. Dold, K.R.W. Emary, S. Fedosyuk, M. Fuskova, D. Gbesemete, C. Green, B. Hallis, M.M. Hou, D. Jenkin, C.C.D. Joe, E.J. Kelly, S. Kerridge, A.M. Lawrie, A. Lelliott, M.N. Lwin, R. Makinson, N.G. Marchevsky, Y. Mujadidi, A.P.S. Munro, M. Pacurar, E. Plested, J. Rand, T. Rawlinson, S. Rhead, H. Robinson, A.J. Ritchie, A.L. Ross-Russell, S. Saich, N. Singh, C. C. Smith, M.D. Snape, R. Song, R. Tarrant, Y. Themistocleous, K.M. Thomas, T. L. Villafana, S.C. Warren, M.E.E. Watson, A.D. Douglas, A.V.S. Hill, T. Lambe, S. C. Gilbert, S.N. Faust, A.J. Pollard, Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial, Lancet (London, England) 396 (10267) (2021) 1979–1993.
[203] M. Voysey, S.A.C. Clemens, S.A. Madhi, L.Y. Weckx, P.M. Folegatti, P.K. Aley, B. Angus, V.L. Baillie, S.L. Barnabas, Q.E. Bhorat, S. Bibi, C. Briner, P. Cicconi, A. M. Collins, R. Colin-Jones, C.L. Cutland, T.C. Darton, K. Dheda, C.J.A. Duncan, K. R.W. Emary, K.J. Ewer, L. Fairlie, S.N. Faust, S. Feng, D.M. Ferreira, A. Finn, A. L. Goodman, C.M. Green, C.A. Green, P.T. Heath, C. Hill, H. Hill, I. Hirsch, S.H. C. Hodgson, A. Izu, S. Jackson, D. Jenkin, C.C.D. Joe, S. Kerridge, A. Koen, G. Kwatra, R. Lazarus, A.M. Lawrie, A. Lelliott, V. Libri, P.J. Lillie, R. Mallory, A. V.A. Mendes, E.P. Milan, A.M. Minassian, A. McGregor, H. Morrison, Y. F. Mujadidi, A. Nana, P.J. O'Reilly, S.D. Padayachee, A. Pittella, E. Plested, K. M. Pollock, M.N. Ramasamy, S. Rhead, A.V. Schwarzbold, N. Singh, A. Smith, R. Song, M.D. Snape, E. Sprinz, R.K. Sutherland, R. Tarrant, E.C. Thomson, M. E. Török, M. Toshner, D.P.J. Turner, J. Vekemans, T.L. Villafana, M.E.E. Watson, C.J. Williams, A.D. Douglas, A.V.S. Hill, T. Lambe, S.C. Gilbert, A.J. Pollard, Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK, Lancet (London, England) 397 (10269) (2021) 99–111.
[204] Oxford, Oxford AstraZeneca vaccine to be sold to developing countries at cost price, 2020. https://www.theguardian.com/global-development/2020/nov/23/oxford-astrazeneca-results-covid-vaccine-developing-countries. (Accessed 23 November 2020.
[205] RESEARCH PROJECTS TO RESPOND TO SARS-COV2 PANDEMIC CHALLENGES AT THE CNB-CSIC. https://www.cnb.csic.es/index.php/en/research/sars-cov2-research.
[206] D.Y. Logunov, I.V. Dolzhikova, O.V. Zubkova, A.I. Tukhvatullin, D. V. Shcheblyakov, A.S. Dzharullaeva, D.M. Grousova, A.S. Erokhova, A. V. Kovyrshina, A.G. Botikov, F.M. Izhaeva, O. Popova, T.A. Ozharovskaya, I. B. Esmagambetov, I.A. Favorskaya, D.I. Zrelkin, D.V. Voronina, D.N. Shcherbinin, A.S. Semikhin, Y.V. Simakova, E.A. Tokarskaya, N.L. Lubenets, D.A. Egorova, M. M. Shmarov, N.A. Nikitenko, L.F. Morozova, E.A. Smolyarchuk, E.V. Kryukov, V. F. Babira, S.V. Borisevich, B.S. Naroditsky, A.L. Gintsburg, Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia, Lancet (London, England) 396 (10255) (2020) 887–897.
[207] D.Y. Logunov, I.V. Dolzhikova, D.V. Shcheblyakov, A.I. Tukhvatulin, O. V. Zubkova, A.S. Dzharullaeva, A.V. Kovyrshina, N.L. Lubenets, D.M. Grousova, A.S. Erokhova, Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia, The Lancet (2021).
[208] Johnson, Johnson & Johnson Initiates Second Global Phase 3 Clinical Trial of its Janssen COVID-19 Vaccine Candidate, 2020. https://www.jnj.com/johnson-johnson-initiates-second-global-phase-3-clinical-trial-of-its-janssen-covid-19-vaccine-candidate. (Accessed 15 November 2020).
[209] ImmunityBio, COVID-19 Vaccination Using a 2nd Generation (E1/E2B/E3-Deleted) Adenoviral-COVID-19 in Normal Healthy Volunteers, 2021. https://clinicaltrials.gov/ct2/show/NCT04591717. (Accessed 30 March 2021.
[210] N.M.G. de Queiroz, F.V. Marinho, M.A. Chagas, L.C. Leite, E.J. Homan, M.T. de Magalhães, S.C. Oliveira, Vaccines for COVID-19: perspectives from nucleic acid vaccines to BCG as delivery vector system, Microbes Infect. 22 (10) (2020) 515–524.
[211] G. Gasaatura, Vaxart Announces Positive Hamster Challenge Study Data for its Oral COVID-19 Vaccine, 2020. (Accessed 14 October 2020).
[212] M.-G. Hollm-Delgado, E.A. Stuart, R.E. Black, Acute lower respiratory infection among Bacille Calmette-Guérin (BCG)–vaccinated children, Pediatrics 133 (1) (2014) e73–e81.
[213] L.I. Pereira, M.L. Dorta, A.J.C. Pereira, R.P. Bastos, M.A. Oliveira, S.A. Pinto, H. Galdino Jr, W. Mayrink, W. Barcelos, V.P. Toledo, Increase of NK cells and proinflammatory monocytes are associated with the clinical improvement of diffuse cutaneous leishmaniasis after immunochemotherapy with BCG/Leishmania antigens, Am. J. Trop. Med. Hygiene 81 (3) (2009) 378–383.
[214] L. Li, D. Qiao, X. Zhang, Z. Liu, C. Wu, The immune responses of central and effector memory BCG-specific CD4+ T cells in BCG-vaccinated PPD+ donors were modulated by Treg cells, Immunobiology 216 (4) (2011) 477–484.
[215] A. Fortier, B. Mock, M. Meltzer, C. Nacy, Mycobacterium bovis BCG-induced protection against cutaneous and systemic Leishmania major infections of mice, Infect. Immun. 55 (7) (1987) 1707–1714.
[216] GeoVax Progresses in Coronavirus (COVID-19) Vaccine Development GeoVax Progresses in Coronavirus (COVID-19) Vaccine Development GeoVax Progresses in Coronavirus (COVID-19) Vaccine Development.
[217] J. Leentjens, M. Kox, R. Stokman, J. Gerretsen, D.A. Diavatopoulos, R. van Crevel, G.F. Rimmelzwaan, P. Pickkers, M.G. Netea, BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study, J. Infect. Dis. 212 (12) (2015) 1930–1938.
[218] M. Vaughan, Valo Therapeutics to Support Development of a Pan – Coronavirus Vaccine, 2020. https://www.valotx.com/article/valo-therapeutics-support-development-pan-coronavirus-vaccine. (Accessed 27th April 2020.
[219] Valo Therapeutics to Support Development of a Pan – Coronavirus Vaccine, 27th April 2020. https://www.valotx.com/article/valo-therapeutics-support-development-pan-coronavirus-vaccine.
[220] M.W. Russell, Z. Moldoveanu, P.L. Ogra, J. Mestecky, Mucosal immunity in COVID-19: A neglected but critical aspect of SARS-CoV-2 infection, Front. Immunol. 11 (2020) 3221.
[221] Y.X. Chao, O. Rötzschke, E.-K. Tan, The role of IgA in COVID-19, Brain Behav. Immun. (2020).
[222] G. Lippi, C. Mattiuzzi, Clinical value anti-SARS-COV-2 serum IgA titration in patients with COVID-19, J. Med. Virol. (2020).
[223] Y.-Q. Zheng, Y.W. Naguib, Y. Dong, Y.-C. Shi, S. Bou, Z. Cui, Applications of bacillus Calmette-Guerin and recombinant bacillus Calmette-Guerin in vaccine development and tumor immunotherapy, Expert Rev. Vacc. 14 (9) (2015) 1255–1275.
[224] N. Nieuwenhuizen, S. Kaufmann, Next-generation vaccines based on Bacille Calmette-Guérin. Front Immunol 2018; 9: 121, PUBMED, 2018.
[225] R.J. Arts, S.J. Moorlag, B. Novakovic, Y. Li, S.-Y. Wang, M. Oosting, V. Kumar, R. J. Xavier, C. Wijmenga, L.A. Joosten, BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity, Cell Host Microbe 23(1) (2018) 89–100.e5.
[226] B.R. Bloom, W.R. Jacobs Jr, New strategies for leprosy and tuberculosis and for development of bacillus Calmette-Guérin into a multivaccine vehicle, Ann. N. Y. Acad. Sci. 569 (1989) 155–173.
[227] R. Herman, L.-A. McNutt, M. Mehta, D.A. Salmon, R.A. Bednarczyk, J. Shaw, Vaccination perspectives among adolescents and their desired role in the decision-making process, Hum. Vacc. Immunotherap. 15 (7–8) (2019) 1752–1759.
[228] J. Walk, L.C.J. de Bree, W. Graumans, R. Stoter, G.-J. van Gemert, M. van de Vegte-Bolmer, K. Teelen, C.C. Hermsen, R.J. Arts, M.C. Behet, Outcomes of controlled human malaria infection after BCG vaccination, Nat. Commun. 10 (1) (2019) 1–8.
[229] J. Kleinnijenhuis, J. Quintin, F. Preijers, L.A. Joosten, D.C. Ifrim, S. Saeed, C. Jacobs, J. van Loenhout, D. de Jong, H.G. Stunnenberg, Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes, Proc. Natl. Acad. Sci. 109 (43) (2012) 17537–17542.
[230] M.M. Ho, K. Markey, P. Rigsby, J. Hockley, M.J. Corbel, Report of an International collaborative study to establish the first WHO reference reagents for BCG vaccines of three different sub-strains, Vaccine 29 (3) (2011) 512–518.
[231] S. Kumar, R. Sunagar, E. Gosselin, Bacterial protein toll-like-receptor agonists: a novel perspective on vaccine adjuvants, Front. Immunol. 10 (2019) 1144.
[232] J. Kleinnijenhuis, J. Quintin, F. Preijers, C.S. Benn, L.A. Joosten, C. Jacobs, J. Van Loenhout, R.J. Xavier, P. Aaby, J.W. Van Der Meer, Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity, J. Innate Immun. 6 (2) (2014) 152–158.

[233] H.S. Goodridge, S.S. Ahmed, N. Curtis, T.R. Kollmann, O. Levy, M.G. Netea, A. J. Pollard, R. van Crevel, C.B. Wilson, Harnessing the beneficial heterologous effects of vaccination, Nat. Rev. Immunol. 16 (6) (2016) 392–400.
[234] Y. Sohrabi, J.C. Dos Santos, M. Dorenkamp, H. Findeisen, R. Godfrey, M.G. Netea, L.A. Joosten, Trained immunity as a novel approach against COVID-19 with a focus on Bacillus Calmette-Guérin vaccine: mechanisms, challenges and perspectives, Clin. Transl. Immunol. 9 (12) (2020), e1228.
[235] M.G. Netea, E.J. Giamarellos-Bourboulis, J. Domínguez-Andrés, N. Curtis, R. van Crevel, F.L. van de Veerdonk, M. Bonten, Trained Immunity: a Tool for Reducing Susceptibility to and the Severity of SARS-CoV-2 Infection, Cell (2020).
[236] L. Bree, V. Koeken, L.A.B. Joosten, P. Aaby, C.S. Benn, A.V. Crevel, M.G. Netea, Non-specific Effects of Vaccines: Current Evidence And potential, Implic. Semin. Immunol. 2018 (2018) 35–43.
[237] M.G. Netea, L.A. Joosten, E. Latz, K.H. Mills, G. Natoli, H.G. Stunnenberg, L. A. O'Neill, R.J. Xavier, Trained immunity: a program of innate immune memory in health and disease, Science 352 (6284) (2016).
[238] C. Weng, A. Saal, W.W. Butt, N. Bica, J. Fisher, J. Tao, P. Chan, Bacillus Calmette-Guérin vaccination and clinical characteristics and outcomes of COVID-19 in Rhode Island, United States: a cohort study, Epidemiol. Infect. 148 (2020), e140.
[239] Y. Toyoshima, K. Nemoto, S. Matsumoto, Y. Nakamura, K. Kiyotani, SARS-CoV-2 genomic variations associated with mortality rate of COVID-19, J. Hum. Genet. 65 (12) (2020) 1075–1082.
[240] K. Aksu, T. Naziroğlu, P. Özkan, Factors determining COVID-19 pneumonia severity in a country with routine BCG vaccination, Clin. Exp. Immunol. 202 (2) (2020) 220–225.
[241] L.E. Escobar, A. Molina-Cruz, C. Barillas-Mury, BCG vaccine protection from severe coronavirus disease 2019 (COVID-19), Proc. Natl. Acad. Sci. 117(30) (2020) 17720–17726.
[242] Q. Chen, H. Lai, Plant-derived virus-like particles as vaccines, Hum. Vacc. Immunotherap. 9 (1) (2013) 26–49.
[243] M.M. Goodin, D. Zaitlin, R.A. Naidu, S.A. Lommel, Nicotiana benthamiana: its history and future as a model for plant–pathogen interactions, Mol. Plant Microbe Interact. 21 (8) (2008) 1015–1026.
[244] L.J. Kelly, A.R. Leitch, J.J. Clarkson, S. Knapp, M.W. Chase, Reconstructing the complex evolutionary origin of wild allopolyploid tobaccos (Nicotiana section Suaveolentes), Evolution: International Journal of Organic, Evolution 67 (1) (2013) 80–94.
[245] D. Kostoff, Relation degrees and phylesis of certain Nicotiana species determined by cytogenetic analysis, Genetica 22 (1–3) (1940) 215–230.
[246] J. Bally, H. Jung, C. Mortimer, F. Naim, J.G. Philips, R. Hellens, A. Bombarely, M. M. Goodin, P.M. Waterhouse, The Rise and Rise of Nicotiana benthamiana: A Plant for All Reasons, Annu. Rev. Phytopathol. 56 (2018) 405–426.
[247] B.J. Ward, P. Gobeil, A. Seguin, J. Atkins, I. Boulay, P.-Y. Charbonneau, M. Couture, M.-A. D'Aoust, J. Dhaliwall, C. Finkle, Phase 1 trial of a Candidate Recombinant Virus-Like Particle Vaccine for Covid-19 Disease Produced in Plants, medRxiv (2020).
[248] N. Landry, B.J. Ward, S. Trépanier, E. Montomoli, M. Dargis, G. Lapini, L.-P. Vézina, Preclinical and clinical development of plant-made virus-like particle vaccine against avian H5N1 influenza, PLoS ONE 5 (12) (2010), e15559.
[249] S. Pillet, J. Couillard, S. Trépanier, J.-F. Poulin, B. Yassine-Diab, B. Guy, B. J. Ward, N. Landry, Immunogenicity and safety of a quadrivalent plant-derived virus like particle influenza vaccine candidate—Two randomized Phase II clinical trials in 18 to 49 and≥ 50 years old adults, PLoS ONE 14 (6) (2019), e0216533.