SARS-CoV-2: Targeted managements and vaccine development

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ABSTRACT

Infection with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) results in diverse outcomes. The symptoms appear to be more severe in males older than 65 and people with underlying health conditions; approximately one in five individuals could be at risk worldwide. The virus's sequence was rapidly established days after the first cases were reported and identified an RNA virus from the Coronaviridae family closely related to a Betacoronavirus virus found in bats in China. SARS-CoV-2 is the seventh coronavirus known to infect humans, and with the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), the only ones to cause severe diseases. Lessons from these two previous outbreaks guided the identification of critical therapeutic targets such as the spike viral proteins promoting the virus's cellular entry through the angiotensin-converting enzyme 2 (ACE2) receptor expressed on the surface of multiple types of eukaryotic cells. Although several therapeutic agents are currently evaluated, none seems to provide a clear path for a cure. Also, various types of vaccines are developed in record time to address the urgency of efficient SARS-CoV-2 prevention. Currently, 58 vaccines are evaluated in clinical trials, including 11 in phase III, and 3 of them reported efficacy above 90 %. The results so far from the clinical trials suggest the availability of multiple effective vaccines within months.

1. Introduction

Nearly a year since the first report of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) cases in the Wuhan province in China, over 57 million cases have been diagnosed, and over 1.37 million have died worldwide as of November 22, 2020 [1]. Some countries have managed to control the disease's spread by acting early, promoting contact tracing, encouraging self-distancing, and asking people to wear a mask in public. However, the last few weeks have seen the emergence of a second wave of hospitalization in the United States, Brazil, Argentina, and a few European countries. The sharp increase in Europe forced some states to implement a second partial lockdown. Other parts of the world, such as Iran, Sri Lanka, Philippines, continue to observe a steady increase in the number of new cases, while others continue to register the same numbers or a decrease in reported cases and COVID-19

associated deaths [1]. It is now clear that the disease will only be contained when an efficient therapy or vaccine will become available. This review gives a brief overview of the characteristics of SARS-CoV-2, the pathophysiology of the infection, the treatments currently tested, and vaccines developed.

2. SARS-CoV-2 origin

SARS-CoV-2 belongs to the *Betacoronavirus* genus, a group of related RNA viruses that can cause fatal respiratory tract infections. This genus includes the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS, and SARS-CoV-2 [2]. Only four common mildly pathogenic coronaviruses are endemic to humans, including HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E [3]. Both MERS and SARS-CoV are zoonotic pathogens

transmitted to humans from intermediate hosts such as dromedary camels and civet cats, respectively [4,5]. The origin of SARS-CoV-2 is still debated, but the most closely related viruses originate from bats such as the RaTG13 virus, sharing ~ 96 % sequence homology, and isolated from the *Rhinolophus affinis* (*i.e.*, intermediate horseshoe) bat [6]. Also, bats are the likely reservoir hosts of SARS-CoV-2; it is also imaginable that other mammalian species in close contact with the human population acted as intermediaries or amplifiers and prompted the zoonotic transfers [7]. Another plausible theory for the emergence of this virus is the asymptomatic natural selection of the virus in the human population following an initial zoonotic transmission and the acquisition of the critical mutations that improved its compatibility with humans [8]. The likelihood of these scenarios will require extensive sampling of wildlife and human populations in Wuhan, China.

3. SARS-CoV-2 genome

Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome packaged by nucleocapsid phosphoproteins [9]. Coronaviruses are one of the largest RNA viruses (26−32 kb); the SARS-CoV-2 genome varies from 29.8–29.9 kb and shares 79.6 and 50 % homology with SARS-CoV and MERS, respectively [10]. The SARS-CoV genome comprises 14 open reading frames (ORFs) encoding 27 proteins [11]. The first ORF (ORF1a and ORF1b) represents nearly 70 % of the virus genome and encompassed 15 nonstructural proteins (nsps) (Fig. 1A) [11]. The remaining ORFs in the 3'-terminal region encoded for four structural proteins, namely membrane (M), envelope (E), spike (S) and nucleocapsid (N) proteins (Fig. 1B) and eight accessories proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and ORF14) (Fig. 1A) [11]. The S protein is composed of two subunits; S_1 holds the receptor-binding
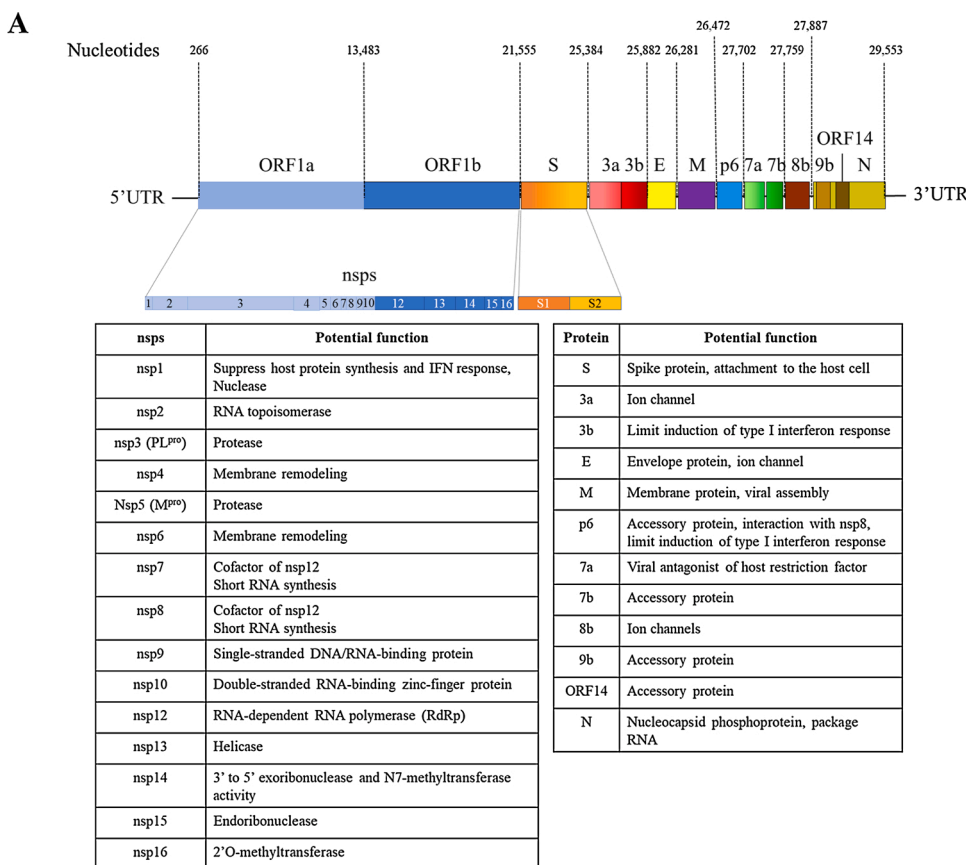
Fig. 1. Genome organization, protein function, and structure of SARS-CoV-2. (A) Genome organization and protein function of SARS-CoV-2. ORF1a and 1b at the 5′ untranslated region are coding for polyproteins (pp1a/ab) cleaved in several nonstructural proteins (nsps) required for virus replication, followed by structural proteins for spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. At the 3′ terminus, accessory genes (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) are located. The putative functions of these proteins are mentioned in the tables. (B) Structure of the SARS-CoV-2. The viral structural proteins S, M, E, and N are embedded in the membrane, while N proteins package the RNA. IFN, interferon; nsps, nonstructural proteins; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2, RdRp, RNA-dependent RNA polymerase. Adapted from [3, 170–174].

domain (RBD), which binds to a receptor on the host cell's surface, while S$_2$ is needed for the fusion of the virus with the cellular membrane host [12]. In the inner side of the envelope, the nucleocapsid (N) proteins are bound to the positive-sense single-stranded RNA genome [13]. SARS-CoV-2 is characterized by a low mutation rate (6.7 mutations per sample) when comparing to a reference genome from Wuhan (NC_045512.2) [14,15]. The phylogenic analysis of the genomic RNA sequences shared by the public database of the Global Initiative on Sharing All Influenza Data (GISAID) (https://www.gisaid.org/) grouped SARS-CoV-2 into several distinctive viral clades characterized by specific mutations. Each clade clusters related sequences that refer to a common ancestor. For example, the substitution of aspartic acid by glycine at position 614 (D614 G) in the spike protein is the most frequently sequenced in a clade observed predominantly in Europe [15]. However, no experimental evidence demonstrated a biological difference acquired by the mutations, suggesting that a single strain of the SARS-CoV-2 virus is currently present.

4. Life cycle of the virus

Viruses are obligate intracellular parasites and the SARS-CoV-2 life cycle debuts when the transmembrane S structural glycoprotein binds to its receptor, angiotensin-converting enzyme 2 (ACE2), on the host cell [16]. ACE2 was also identified as a functional receptor for SARS-CoV and human coronavirus NL63 [17,18]. The RBD of the S1 subunit binds to the peptide domain of ACE2; this interaction triggers the priming of S1 at the S1/S2 junction by cellular proteases such as furin or endosomal proteases and exposes a secondary site (S2') cleaved by the transmembrane serine protease 2 (TMPRSS2), which reveals the fusion peptide of the S2 subunit [12,19,20]. The cleavage modifies the conformation of the cleaved protein S irreversibly and allows the S2 subunit to insert into the host membrane and guide the fusion of the viral and cellular membranes [12]. Previous studies have reported the essential role of TMPRSS2 in the cellular entry of SARS-CoV-2, as well as SARS-CoV and MERS [21–24]. Hoffmann et al. demonstrated that camostat mesylate, an inhibitor of TMPRSS2, partially blocked the cellular entry of SARS-CoV-2 *in vitro* [12]. In addition to ACE2, CD147 was mentioned as a potential transmembrane receptor to mediate the cellular entry of SARS-CoV-2 [25]. CD147, also known as basigin or extracellular matrix metalloproteinase inducer, is a transmembrane glycoprotein, a member of the immunoglobulin superfamily, expressed on the surface of red blood cells, lymphocytes, dendritic cells (DCs), monocytes, macrophages, and many tissues [26,27]. CD147 is a known target for malaria treatment, as anti-CD147 antibodies prevent the red blood cell invasion by the protozoan Plasmodium falciparum [28], which has also been shown to facilitate the cellular entry of various viruses, including HIV-1 [29] and measles virus [30]. Wang et al. demonstrated the direct interaction, and colocalization of protein S and CD147 and the blocking of CD147 by humanized anti-CD147 antibody (Meplazumab) decreased SARS-CoV-2 replication *in vitro* [25].

The SARS-CoV-2 entry into the cells, similarly as for SARS-CoV, is achieved by receptor-mediated endocytosis [31]. The viral RNA is released in the cytoplasm, uncoated, and the translations of the ORF1a and 1b are initiated. ORFs 1a and 1b code for the polyproteins pp1a (nsp1-11) and pp1ab (nsp1-16), respectively, depending on a ribosomal (-1)-frameshift demonstrated in coronavirus 31 years ago [32]. The polyproteins processing is coordinated spatially and temporally by viral proteases such as M^{pro} (nsp5) and PL^{pro} (nsp3) into nsps involved in the replication/transcription complex [33,34]. The nsps rearranged portion of the membrane derived from the rough endoplasmic reticulum into vesicles where the viral assembly is organized [35]. Viruses are then released from the host cell by exocytosis via secretory vesicles [36].

5. Pathophysiology

The pathophysiology of SARS-CoV-2 is similar to that observed with SARS-CoV and mainly affects the respiratory system, but other organs may be involved [37–40]. The presenting symptoms are a fever with dry cough and dyspnea [38] accompanied by headaches, dizziness, muscle pain, joint pain, and fatigue with gastrointestinal symptoms, including vomiting and diarrhea [39]. A multisystem inflammatory syndrome related to SARS-CoV-2 has been reported in older children and associated with severe abdominal pain, cardiac dysfunction, and shock, bearing similarities with the Kawasaki disease [41].

Like SARS-CoV and MERS, respiratory droplets are the primary source of transmission of SARS-CoV-2 from human to human. The similarities of SARS-CoV-2 with SARS-CoV and MERS also suggest the possibility of fecal-oral transmission that remains unproven [42]. The median incubation period of SARS-CoV-2 varies from 4 to 6 days before developing symptoms [37,43–45]. Several studies reported a high viral load before or on symptom onset similarly to influenza, suggesting a high transmission potential even with mildly symptomatic cases; this appears to differ from the observation made with SARS-CoV showing a delayed peak of the viral load of several days following the symptoms' onset [46–49].

Patients infected with SARS-CoV-2 may have minimal symptoms or experience acute respiratory distress syndrome (ARDS) with uncontrolled inflammation that may lead to multiple organ failures. Most patients with mild symptoms showed ground-glass opacity on chest computed tomography (CT) on admission [37]. Similar to SARS-CoV and MERS, the severity of the symptoms is strongly associated with the patients' age that may be explained by a weaker or dysregulated immune system [37,38,50].

SARS-CoV-2 is a cytopathic virus that kills the cells and damages the tissue host as part of its replication cycle [51]. ACE2 was described as the primary receptor promoting the cellular entry of SARS-CoV-2 [16]. ACE2 is highly expressed on the alveolar epithelial type II cells' surface, critical for the lungs' gas exchange function preventing the alveoli from collapsing [52]. However, ACE2 is also present on the surface of type I pneumocytes, endothelial cells, and in extrapulmonary tissues, including heart, intestine, blood vessels, kidneys, and urinary bladder [53,54]. The alveolar epithelial type II cells were also described as a potential viral reservoir that may disseminate the virus to other organs and individuals [54,55]. The epithelial cell death following viral infection has been associated with apoptosis and a pro-inflammatory program cell death named pyroptosis, which triggers the release of pro-inflammatory cytokines, for example, interleukin-1β (IL-1β) and IL-18 (Fig. 2A-B) [56,57]. Previous studies have reported the increased level of pro-inflammatory cytokines in patients infected with SARS-CoV-2, including IL-1β, IL-6, IL-8, TNF-α, IP10, MCP-1, and RANTES [38,58] (Fig. 2C). Studies on SARS-CoV and MERS also reported the elevation of pro-inflammatory cytokines in patients' serum and their association with pulmonary inflammation and extensive lung damage (for review, see [38]). The secretion of cytokines and chemokines recruit monocytes and macrophages, which release cytokines to prime T-cell adaptive immune response. In most patients, the immune cells' recruitment will clear the virus, and inflammation will recede. However, in a few patients with a weak immune system and/or other unknown preexisting conditions, an uncontrolled inflammatory response linked to a cytokine storm, characterized by the increased plasma level of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1α, and TNF-α, has been associated with the severity of the pulmonary inflammation, and likely the leading cause of death (Fig. 2D) [38].

As described above, epithelial cells, macrophages of the alveoli, and DCs underneath the epithelium are the main components for the innate immunity to the virus [59]. Antigen presentation by DCs and macrophages will initiate the T-cell responses. SARS-CoV-2 enters these cells either by phagocytosis of apoptotic cells infected by virus-like epithelial cells [60] or via pattern recognition receptors (PRRs) [61]. The SARS-CoV-2 may also use the ACE2, expressed in macrophages and DCs [62,63]. The SARS-CoV-2 virus may bind to the dendritic cell intercellular adhesion molecules 3-grabbing non-integrin (DC-SIGN), and
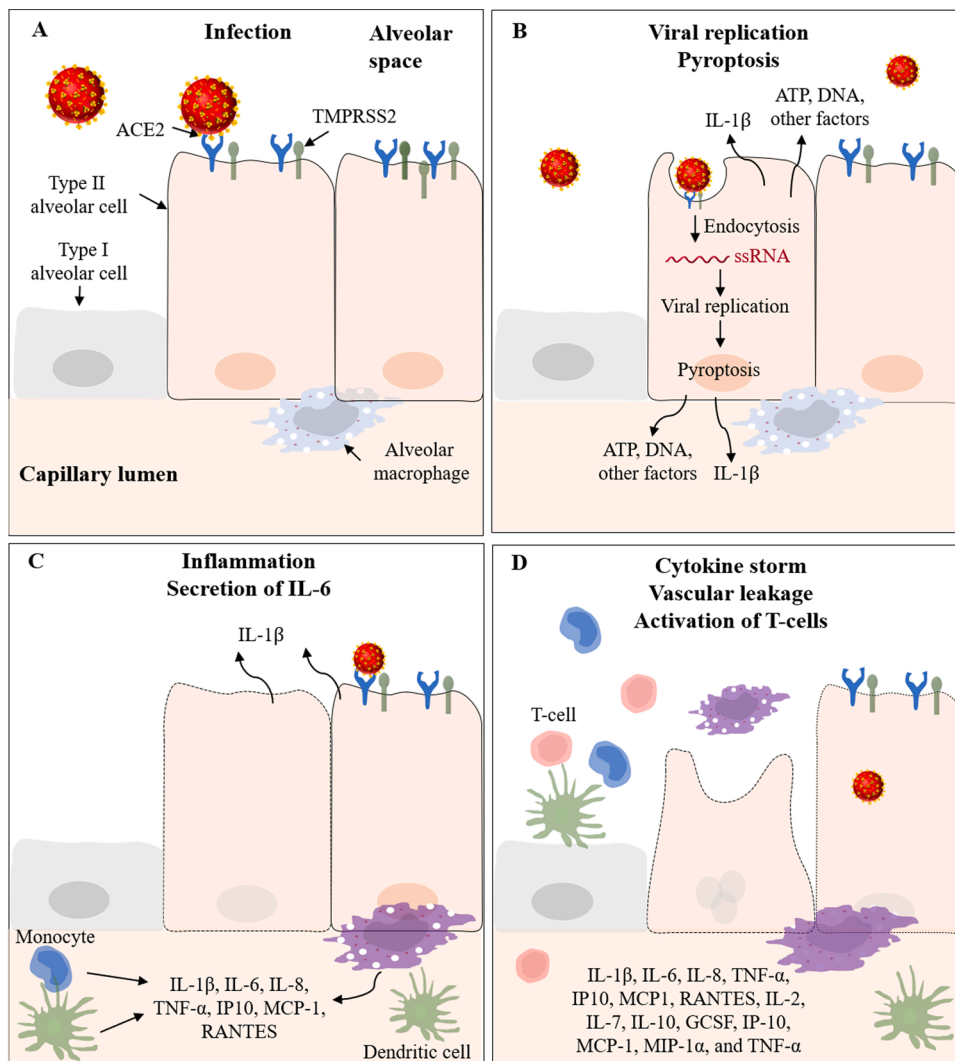
Fig. 2. Infection of the alveolar epithelial type II cells by the SARS-CoV-2. (A) SARS-CoV-2 targets cells expressing angiotensin-converting enzyme 2 (ACE2). (B) The virus is endocytosed. The transmembrane serine protease 2 (TMPRSS2) cleaved the S proteins to allow the viral RNA into the host cell cytoplasm. The replication of the virus triggers pyroptosis and the release of DNA, ATP, IL-1β, and other factors. (C) The factors are recognized by the neighboring cells and resident alveolar macrophages, promoting the secretion of pro-inflammatory cytokines and chemokines, including IL-6, IL-8, TNF-α, IP10, MCP-1, and RANTES. These factors attract monocytes and dendritic cells, amplifying the signal. (D) These signals lure T-cells and more immune cells that, in a defective immune response, trigger a cytokine storm damaging the lungs and affecting other organs. IL, interleukin; IP10, interferon-γ-inducible protein; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein 1α; GCSF, granulocyte colony-stimulating factor; TNF, tumor necrosis factor; RANTES, regulated on activation, normal T-cell expressed and secreted.

L-SIGN [64–66]. These APCs then present viral antigens to T-cells. $CD4^+$ and $CD8^+$ T-cell in the draining lymph nodes. $CD4^+$ T-helper cells induce and help B-cells to produce the virus-specific antibody. The cytotoxic $CD8^+$ T-cells can kill virally infected cells. It has been observed in patients with severe diseases a storm of pro-inflammatory cytokines, mainly IL-6, TNF-α, G-CSF in addition to the anti-inflammatory cytokine IL-10 and the chemokines MCP-1, MIP-1α, and IL-8, but the more severe disease was noted with higher levels of IL-6 [38,67–69]. In the patients never exposed to SARS-CoV-2, the T-cells' cross-reactivity was detected in 20 % of the cases, probably due to previous exposure to common cold coronaviruses [70]. Specific T-cells against the proteins S, M, and N were detected in the peripheral blood mononuclear cells from convalescent SARS-CoV-2 patients [71]. Furthermore, $CD4^+$ T-cells showed strong reactivity against M and S proteins, mostly in mild SARS-CoV-2 patients [72]. In severe SARS-CoV-2 cases, $CD4^+$ with memory phenotype and $CD8^+$ T-cells with effector phenotype were detected in the blood [70]. Further studies will be required to understand the T-cells' involvement in the progression of severe SARS-CoV-2 symptoms.

Furthermore, thrombosis and pulmonary embolism have been observed in severe SARS-CoV-2 diseases [73]. Platelets were hyperactivated, and aggregation occurred at thrombin suboptimal concentrations [74]. This may be due to the massive inflammation enhancing cytokines and increasing the liver's production of clotting factors. Furthermore, the cytokine TNF-α highly produced in SARS-CoV-2 has been implicated in promoting overexpression of tissue factor (TF) in

platelets and macrophages [75]. TF acts as a receptor and cofactor for coagulation factors VII and VIIa [76,77]. Thus, the cytokine-induced TF synthesis and release can significantly impact the coagulation responses [78]. Another mechanism noted in the pathogenesis of COVID-19 is the interaction with hemoglobin via CD147 and CD26 expressed on erythrocytes [79].

The immune response and associated molecular events to SARS-CoV-2 infection remain poorly understood; it is critical to identify these elements to direct an efficient therapeutic approach. A recent study reported that individuals previously infected with COVID-19, which triggered an antibody, was sufficient to protect most individuals from reinfection in the six months following infection; the long term protection remains to be determined [80].

6. Therapeutic approaches

According to the World Health Organization (WHO), there are no specific medicines or vaccines for SARS-CoV-2. In the early days of the pandemic, many governments worldwide have to some degree implemented measures recommended by WHO to limit the spread of the virus such as self-isolation, social distancing, hand washing, closure of schools and universities, and wearing a mask in public places [81]. These measures proved to be efficient, when followed, and saved countless lives [82].

Several medications are currently evaluated in clinical trials; 3947

clinical trials are presently listed on clinicaltrials.gov, including 490 phase-III and 104 phase IV clinical trials as of November 19, 2020. The management provided to the patients is mainly supportive and critical for at least 5% of patients developing severe symptoms and requiring hospitalization [82]. Available drugs have been repurposed for the treatment of SARS-CoV2, including lopinavir-ritonavir, remdesivir, favipiravir, arbidol, chloroquine and analogs hydroxychloroquine, and azithromycin and tested in clinical trials [83–87]. However, results were not very promising for any of them to be a definite therapy yet. Fig. 3 schematized some of the various therapeutic strategies presently assessed in various clinical trials.

6.1. Inhibitors of virus entry

6.1.1. Arbidol

Arbidol, an anti-influenza virus drug, was also efficient in inhibiting SARS-CoV-2 infection in vitro [88]. Arbidol prevents the interaction between ACE2 and the viral protein S by blocking its trimerization of the viral protein S [89]. In a small non-randomized controlled study, arbidol showed a tendency to improve recovery and decrease mortality [90]. A comparison of the safety and efficacy of arbidol or lopinavir/ritonavir to the standard of care in a phase IV interventional clinical trial (NCT04252885) showed little benefit of both treatment regimen for improving the clinical outcome of patients hospitalized with mild/moderate COVID-19 over the standard of care [91]. However, further power randomized placebo-controlled studies are required to validate these observations.

6.1.2. Recombinant soluble form of human ACE2

A similar initiative trying to block the interaction between the viral protein S and ACE2 is currently evaluated in a phase I clinical trial using a recombinant soluble form of human ACE2 (NCT00886353) based on the previous clinical trial demonstrating its safety [92]. In a case report, a 45-year-old woman infected by SARS-CoV-2 initially received hydroxychloroquine (400 mg twice daily) and anticoagulant nadroparin (4 mg once daily); as her condition worsened, she was intubated. On the 9th day after the onset of symptoms, she was given human recombinant soluble ACE2 (APN01; 0·4 mg/kg, i.v., twice daily) [93]. After 7 days, the treatment was well-tolerated, and her condition improved gradually; she was extubated on day 21. The recombinant soluble ACE2 decreased the plasma concentration of angiotensin II while promoting the increase of its metabolites' angiotensin 1–7 and angiotensin 1–5, and cleaving angiotensin I into angiotensin 1–9 [93]. Angiotensin II is elevated in lung injury and is associated with worsening conditions such as hypertension, diabetes, inflammation, and affecting the physiology of the heart, lungs, kidneys, vasculature, and liver [93]. The treatment was also associated with a decrease of pro-inflammatory cytokines (IL-6 and IL-8) and other inflammation markers (TNF-α, ferritin) [93].

6.1.3. Chloroquine and analog hydroxychloroquine

Chloroquine and analogs hydroxychloroquine act as viral entry inhibitors; by increasing endosomal pH required for virus/cell fusion and interfering with the cellular receptors' glycosylation for SARS-CoV-2 (ACE-2) [94]. In contrast to the early reports of poorly controlled studies, chloroquine and analogs hydroxychloroquine failed to demonstrate any benefit for treating this disease (for review, see [95]). The interim report of WHO solidarity trial involving 11,266 hospitalized COVID-19 patients involving 405 hospitals in 30 countries assessed the efficacy of four repurposed antiviral drugs, including remdesivir (intravenous, day 0: 200 mg, day 1 to 9: 100 mg), hydroxychloroquine (oral, 800 mg twice over 6 h, then 400 mg twice daily for 10 days), lopinavir/ritonavir (oral, 400 mg lopinavir +1 g ritonavir twice daily for 14 days), and interferon-β1a (subcutaneous, 3 doses 44 µg over 6 days or intravenous., 10 µg once daily over 6 days) (WHO registration: ISRCTN83971151, NCT04315948). Overall, most patients hospitalized were aged between 50–69 years (45 %) and had underlying conditions including diabetes (25 %), heart disease (21 %), chronic lung disease (6%), asthma (5%), and chronic liver disease (1%) [96]. The report
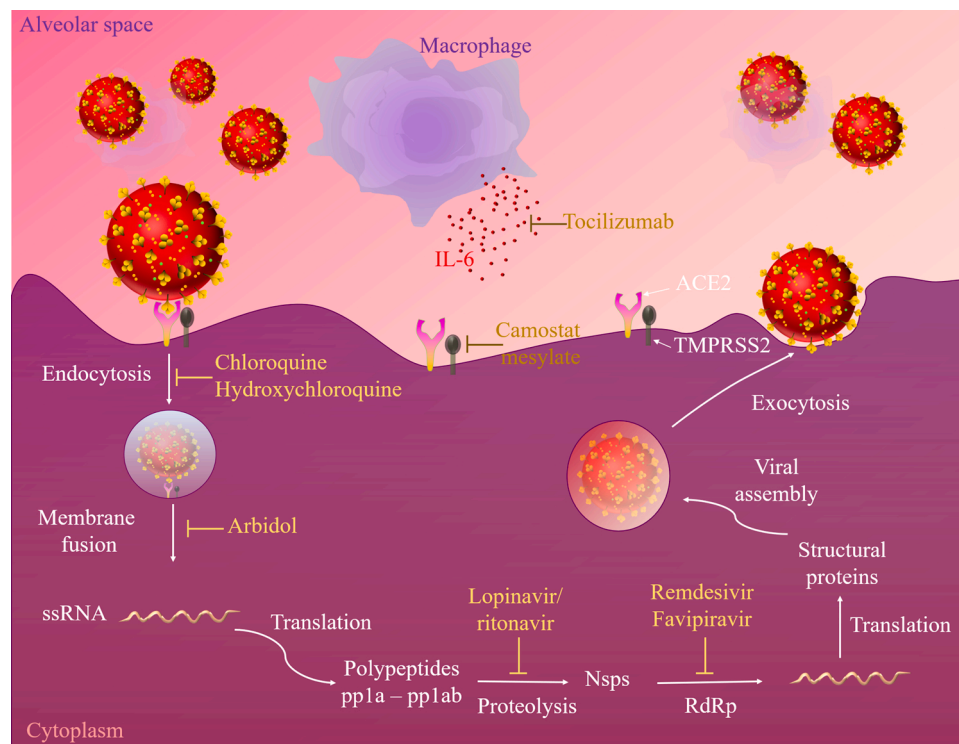
Fig. 3. Therapeutics trialed for the treatment of SARS-CoV-2. Repurposed and investigational drugs were assessed in various clinical trials, either as single-drug treatment or combinations. ACE2: angiotensin-converting enzyme 2; TMPRSS2, transmembrane serine protease 2; nsps, nonstructural proteins; pp1a; polyprotein 1a; pp1ab; polyprotein 1ab; RdRp, RNA-dependent RNA polymerase; IL-6, interleukin-6.

concluded that remdesivir, hydroxychloroquine, lopinavir/ritonavir, and interferon-β1a had little to no effect on COVID-19 patients as measured by the overall death, placement under ventilation, or duration of hospitalization [96].

6.2. Inhibitor of proteolysis

Lopinavir-ritonavir, a combination medication used for HIV/AIDS treatment, is a protease inhibitor that inhibits viral protein synthesis, potentially targeting the SARS-CoV-2 viral protease M^{pro} essential for the replication. Lopinavir-ritonavir also demonstrated efficacy *in vitro*, inhibiting SARS-CoV-2 replication [97]. The combination failed to procure any benefit beyond standard care in clinical trials [98,99].

6.3. Inhibitors of the viral RNA-dependent RNA polymerase

Remdesivir (adenosine analog prodrug) and Favipiravir (guanine analog prodrug) act as inhibitors of the viral RNA-dependent RNA polymerase (RdRp), demonstrated potent antiviral activity *in vitro* [94,100, 101].

6.3.1. Favipiravir

Favipiravir was approved for the treatment of SARS-CoV-2 in Russia and India and currently assessed in many countries. The drug is given orally and improves the patients' conditions in the clinic compared to the standard of care but no significant difference in viral clearance, the requirement for oxygen support, and side effect profile [102]. In an open-label clinical trial (ChiCTR2000029600), COVID-19 patients were treated with favipiravir (1600 mg twice daily on Day 1 and 600 mg twice daily on days 2–14, orally) or lopinavir-ritonavir (400 mg/100 mg twice daily orally). All patients received interferon (IFN)-α by aerosol inhalation (5×10^6 international unit twice daily [103]. The favipiravir group had an early viral clearance (median 4 days) compared to patients treated with lopinavir-ritonavir (median 11 days); the chest CT findings were improved in the favipiravir group at day 14 (91.4 % vs. 62,2 %) [103]. The patients experienced a lower incidence of side effects in the favipiravir group (11.4 % vs. 55.6 %) [103]. Another clinical trial (ChiCTR2000030254) compared favipiravir treatment (1600 mg twice a day on the first day followed by 600 mg twice a day for 10 days) to arbidol (200 mg three times per day for 10 days) [104]. Clinically confirmed patients and not the virally confirmed ones were allocated randomly to each group; only 46.5 and 38.3 % of patients were nucleic acid positive at enrollment in the favipiravir and arbidol group, respectively [104]. The clinical recovery rate and auxiliary oxygen therapy or noninvasive mechanical ventilation did not significantly differ between the two groups; favipiravir tended to relieve adverse effects more rapidly [104]. Favipiravir is currently evaluated in 17 phase-III clinical trials. The data obtained for favipiravir in clinical trials are encouraging, but the completion of large, randomized double-blinded, placebo-controlled clinical trials will provide unbiased data.

6.3.2. Remdesivir

Remdesivir results in clinical trials were conflicting. Remdesivir failed to demonstrate any statistically significant clinical benefits for severe SARS-CoV-2 patients in a randomized, double-blind, placebo-controlled, multicentre trial in Wuhan, China, but underpowered (clinical trial: NCT04257656) [105]. In a double-blind, randomized, placebo-controlled trial sponsored by the US National Institute of Allergy and Infectious Diseases (NIAID) (NCT04280705), patients received 200 mg on day 1, followed by 100 mg daily for up to 9 days given intravenously. Remdesivir was shown to improve the recovery time from 15 to 10 days for COVID-19 patients (rate ratio for recovery, 1.29 [95 % CI, 1.12–1.49]) [106]. Patients had preexisting conditions such as type 2 diabetes (30.6 %), hypertension (50.7 %), or obesity (45.6 %) [106]. However, no significant differences in survival were observed;

mortality was decreased by 3.8 % in the remdesivir versus placebo groups (hazard ratio, 0.73; 95 % CI, 0.52–1.03) [106]. The Food and Drug Administration (FDA) approved remdesivir on October 22nd, 2020. More recently, following the WHO SOLIDARITY trial results, the WHO suggested against the use of Remdesivir for hospitalized patients with Covid-19 regardless of the severity [107].

6.3.3. Avifavir

More recently, avifavir, introduced as the first favipiravir-based drug, was approved for the treatment of SARS-COV-2 in Russia. An interim report of a phase II/III clinical trial of 60 hospitalized patients with mild COVID-19 symptoms randomized equally in three groups receiving avifavir 1600 mg twice on day 1 followed by 600 mg twice on days 2–14, or avifavir 1800 mg twice on day 1 followed by 800 mg twice on days 2–14, or standard of care as a control group [108]. Both avifavir dosages had a similar effect on viral clearance on day 5, which was achieved in 25/40 patients (62.5 %) compared to 6/20 patients treated by the standard of care [108]. Despite the small sample size, avifavir was granted fast-track marketing authorization by the Russian Ministry of Health [108].

6.4. Alternative strategies

6.4.1. Targeting host proteins and immune response modulators

Alternative strategies targeting host proteins and immune response modulators are currently developed. Camostat mesylate and nafamostat mesylate target the TMPRSS2, an essential serine protease critical for the cellular entry of SARS-CoV-2 into the lung cells [12,109]. Both compounds are approved in Japan to treat pancreatitis and are currently assessed in several clinical trials for the treatment of SARS-CoV-2. Immunomodulators as Nitazoxanide were also used for interference with host-regulated pathways involved in viral replication, amplifying cytoplasmic RNA sensing, and type I IFN pathways [101].

6.4.2. Targeting inflammation and coagulation

In severe cases of SARS-CoV-2, anti-inflammatory and anti-Coagulative agents have been proposed and implemented because of the profound inflammatory response and the thrombotic phenomena described in the lungs of patients with severe SARS-COV-2, including administration of low-molecular-weight heparin as a standard measure in hospitalized patients with SARS-CoV-2 [110].

IL-6 is an inflammatory cytokine targeted in neutralizing therapies in several inflammatory diseases [111]. Increased serum concentrations of IL-6 were noted during severe infections with Coronavirus SARS-CoV-2, suggesting possible implications for anti-IL-6 therapy in SARS-CoV-2 disease [112]. Tocilizumab, an inhibitor of IL-6, might reduce the risk of invasive mechanical ventilation or death in patients with severe SARS-CoV-2 [113]. Tocilizumab is currently registered in 71 clinical trials for the treatment of SARS-CoV-2, a randomized double-blinded phase III clinical trial (NCT04320615) compared tocilizumab (8 mg/kg, i.v.) to the standard of care in severe COVID-19 patients [114]. The outcome of the treatment was measured at day 28; also, tocilizumab did not improve the clinical status or mortality when compared to the standard of care; it decreased the stay in the intensive care unit by 5.8 days (9.8 days) and shorter the discharge time by 8 days (20 days) [114]. Additional clinical trials will be necessary to determine the advantage of tocilizumab on the duration of the hospitalization.

6.4.3. Immunomodulation by glucocorticoids

Glucocorticoids are also assessed in several clinical trials; their immunosuppressive effect requires careful consideration. It may influence the viral clearance, while their anti-inflammatory effect may limit the tissue damage induced by the cytokine storm and favor their use in severe COVID-19 cases [115]. A randomized, controlled clinical trial in the United Kingdom using dexamethasone in severely ill patients reduced deaths by about one-third of patients on ventilators [116,117].

The randomized evaluation of the COVID-19 therapy (RECOVERY) trial (NCT04381936) demonstrated that a dose of dexamethasone (6 mg) once daily for up to 10 days reduced the mortality of patients under respiratory assistance but may be harmful to patients who do not require oxygen support [118]. The WHO has recently recommended the use of corticosteroids to treat hospitalized patients with severe and critical Covid-19 [107]. Additional glucocorticoids were evaluated in clinical trials, including methylprednisolone (NCT04343729, 2020-001934-37, and IRCT20200404046947N1), hydrocortisone (NCT02735707, NCT02517489). Methylprednisolone Both clinical trials assessing hydrocortisone were stopped earlier for failing to meet the prespecified criteria for statistical superiority [119,120].

6.4.4. Passive immunotherapy and neutralizing antibodies

Passive immunotherapy using plasma from recovering patients was considered a promising option in the treatment of SARS-CoV-2 infections. The WHO and the FDA have permitted plasma therapy for severe conditions upon failure of trial medications. Several clinical trials are underway to test the effectiveness of hyperimmune plasma [121]. A recent study demonstrated that antibodies in the serum of patients infected with SARS-CoV effectively neutralized SARS-CoV-2 *in vitro* [122]. The administration of convalescent plasma to 5,000 COVID-19 hospitalized patients demonstrated to be safe as the occurrence of serious adverse events was low and likely to be beneficial when given at an early stage of the development of COVID-19 [123].

LY-CoV555 (bamlanivimab, LY3819253), a neutralizing IgG1 monoclonal antibody that binds with high affinity to the SARS-CoV-2 spike protein, was isolated from some of the first patients in North America [124]. In a phase II clinical trial (NCT04427501) sponsored by Eli Lilly and Company, patients received a single dose of either LY-CoV5555 at 700, 2,800, or 7,000 mg or placebo. Only the single intravenous infusion of LY-CoV555 (2,800 mg) appeared to accelerate the viral load's natural decline [124]. The LY-CoV555 treated patients had fewer hospitalizations than the placebo group, 1.6 vs. 6.3 %, respectively, and 4.2 vs. 14.6 % when considering high-risk patients, respectively [124]. Eli Lilly has been granted emergency use on November 10, 2020, by the FDA, to treat mild to moderate Covid-19 adults and pediatric patients older than 12 years of age, weighing 40 kg, and at risk of developing severe Covid-19 and /or hospitalization.

AZD7442 is a combination of two long-acting antibodies derived from convalescent patients after SARS-CoV-2 infection. The antibodies (COV2-2196 and COV2-2130), isolated by the Vanderbilt University Medical Center, recognized non-overlapping sites of the viral spike protein and were shown to neutralize the SARS-CoV-2 infection synergistically in two murine models [125]. These antibodies were licensed by AstraZeneca in June 2020 and modified to extend their half-life for 6–12 months and reduce the Fc receptor binding. AZD7442 entered phase I clinical trial (NCT04507256) in August 2020. AstraZeneca started two phase III clinical trials in November 2020, Storm chaser (NCT04625972, 1125 participants) and Provent (NCT04625725, 5,000 participants). The Provent trial will evaluate the safety and efficacy for the prevention of SARS-CoV-2 infection for up to 12 months following a single injection, while the Storm Chaser trial will evaluate post-exposure prophylaxis and pre-emptive treatment following a single injection. Recently, the US government secured 100,000 doses of AZD7442.

REGN-COV2, manufactured by Regeneron Pharmaceuticals, is a cocktail of two neutralizing synthetic antibodies, casirivimab (REGN10933) and imdevimab (REGN10987), targeting non-overlapping epitopes of the viral spike protein. On November 21st, 2020, REGN-COV2 received an emergency use authorization by the FDA to treat mild to moderate COVID-19 adults and pediatric patients older than 12 years of age, weighing 40 kg, and at high risk of developing severe COVID-19. This emergency use authorization includes subjects who are 65 years or older or who have chronic medical conditions. In a preclinical study, REGN-COV2 reduced the viral load in upper and lower airways when given as a prophylactic or as a treatment in rhesus

macaques [126]. In an animal model of severe COVID-19, REGN-COV2 decreased the viral lung titers and evidence of pneumonia [126]. Regeneron Pharmaceuticals announced preliminary data from 275 patients in a phase I/II/III trial (NCT04425629, NCT04426695, and NCT04452318), showing that REGN-COV2 reduced viral load, alleviate symptoms in non-hospitalized patients infected with COVID-19, and decreased patient medical visits (https://investor.regeneron.com/press-releases). For patients at high risk for disease progression, hospitalizations, and emergency room visits occurred in 3 % of the REGN-COV2 patients compared to 9 % of the placebo-treated patients (https://www.fda.gov/news-events/fda-newsroom/press-announcements).

Additional neutralizing antibodies are currently evaluated in multiple phase I clinical trials, including VIR-7831, 47D11, CT-P59, ALVR109, STI-1499, IVIG, and COVID-HIG (for review see [127].

7. Vaccine strategy

The genetic drifting of SARS-CoV-2 has been extremely slow [128], increasing the prospect of designing vaccines that will confer immunity to the global population against all viral clades. Although very few efficient vaccines are available for RNA viruses, in many cases and for certain technologic restrictions and economic issues, these vaccines are barely used, and many of them are not licensed. Vaccines against SARS-CoV were developed following the SARS outbreak in 2002–2003, and only two were assessed in phase I clinical trials using a recombinant DNA vaccine (NCT00099463) [129]or an inactivated SARS-CoV vaccine [130]. As contact tracing and quarantine put an end to the transmission, no further SARS-CoV vaccine development was undertaken [131]. The outbreak of MERS in 2012 led to the development of several vaccines currently evaluated or recruiting in phase I clinical trials. The development of a vaccine is usually a lengthy process that could take over 15 years. Since the SARS-CoV-2 pandemic started, scientists worldwide are combining efforts, sharing data, and establishing collaboration to speed up a vaccine's development in just a few months. Several governments, foundations, and companies have pledged partnerships and funding to accelerate the research for a vaccine, including the Accelerating COVID-19 Therapeutic Interventions and Vaccine [132]. Preclinical studies on SARS-CoV and MERS informed the best vaccine development strategies and identified key protein targets. The candidate vaccines' preclinical toxicology is accomplished in just a few months, followed by clinical trials with overlapping phases. Some manufacturers have already started the vaccine's large-scale production to face the Brobdingnagian demand before completing phase III. Before developing a phase III clinical trial, Russia and China have granted early or limited approval, respectively. Cansino Biologics vaccine (Ad5-nCoV) is limited to the Chinese military use, and the Gamaleya Research Institute vaccine (Gam-COVID-Vac) in Russia was licensed and rebranded as Sputnik V in August 2020 without a phase III clinical trial. Currently, 261 vaccines are tested; most of the vaccines are in the preclinical phase of development, while 11 vaccines have entered phase III clinical trials (Fig. 4 and Table 1) [133]. Various platforms have been exploited for the development of SARS-CoV-2 vaccines (Fig. 4). Classical platforms include the whole inactivated virus, live attenuated virus, protein subunit, and virus-like particle and next-generation platforms where few vaccines have been licensed and include viral vector, antigen-presenting cells, DNA, and RNA.

7.1. Inactivated vaccines

Inactivated vaccines are produced *in vitro* by the inoculation of mammalian cells, usually Vero cells, certified by the WHO for vaccine production, followed by chemical inactivation with β-propiolactone [25]. Currently, 15 inactivated vaccines are assessed in various clinical trial phases, among them the BBIBP-CorV, CoronaVac, WIBP vaccine, and Covaxin have entered phase III (Table 1) (see https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape). None of these phase III clinical
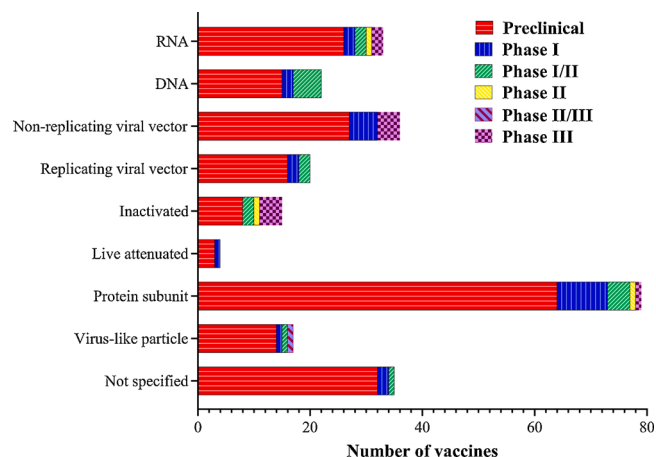
Fig. 4. Number and types of vaccines currently in the preclinical phase and the different clinical trial phases. Data were adapted from the COVID-19 vaccine tracker [133].

trials assessing inactivated vaccines has provided a preliminary report of their efficacy.

BBIBP-CorV, with aluminum hydroxide as an adjuvant, was found to be safe and induced immune response on day 42 in vaccinated adults below and over 60 years old in a randomized placebo-controlled, double-blind phase I/II clinical trial [134]. The neutralizing antibody titer (~256) was higher with a 4-μg dose and an immunization scheduled on days 1 and 21 [134]. BBIBP-CorV was approved for limited use in the United Arab Emirates in September 2020.

In phase II clinical trial, CoronaVac vaccine (3 μg) with aluminum hydroxide as an adjuvant was well tolerated, no grade 3 adverse reaction was observed. The vaccine promoted immunogenicity 28 days following a 2-doses scheduled 14 days apart [135]. The neutralizing antibody titers ranged from 23.8–65.4 when measured 28 days after a 2-doses scheduled; the titer was reduced in the older subjects [135]. The titer was also lower than the average neutralizing antibody titer (163.7) measured in convalescent patients in the same study [135]. Sinovac received emergency use approval in China to vaccinate high-risk individuals based on the phase II clinical trial. In Brazil, the phase III clinical trial was briefly stopped for 48 h following the death of a

Table 1

Vaccines in phase III clinical trials. All clinical trials are randomized, placebo controlled, and double blind, and currently recruiting except for clinical trial NCT04516746 which is not yet recruiting. All data were obtained from clinical trial.org and EU clinical trials register.

Vaccine	Platform	Sponsors	Number of Participants Age	Doses	Locations	Clinical Trial	Start Date - Estimated Completion Date (m/y)
BNT162b2	RNA	BioNTech SE Pfizer	45,000 ≥ 16 years	2 Separated by 21 days	166 locations: USA, Argentina, Brazil, South Africa, Turkey	NCT04368728	04/2020-12/2022
mRNA-1273	RNA	Moderna TX, Inc.	30,000 Adults	2 Day 1 and Day 29	100 locations: USA	NCT04470427 COVE study	07/2020-10/2022
Ad5-nCoV (5E10vp)	Non-replicating viral vector	CanSino Biologics Inc. NPO Petrovax	40,000 Adults 500 18-85 years	1 1	1 location: Pakistan 7 locations Russia	NCT04526990 NCT04540419	09/2020-01/2022 09/2020-07/2021
Gam-COVID-Vac	Non-replicating viral vector	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation	40,000 Adults	2 Day 1 and Day 21	23 locations: Russia	NCT04530396	09/2020-05/2021
Ad26.COV2.S	Non-replicating viral vector	Janssen Vaccines & Prevention B.V.	60,000 Adults	1	246 locations: USA, Argentina, Brazil, Chile, Colombia, Mexico, Peru, Philippines, and South-Africa	ENSEMBLE NCT04505722	09/2020-03/2023
Oxford ChAdOx1-S (AZD1222)	Non-replicating viral vector	University of Oxford AstraZeneca	30,000 Adults	2 Separated by 4 weeks	62 locations: USA	NCT04516746	08/2020-10/2022
WIBP vaccine BBIBP-CorV (Participants receive either WIBP vaccine or BBIBP-CorV)	Inactivated (Vero cells)	China National Biotec Group Company Limited	45,000 Adults	2 Day 1 and Day 21	6 locations: Bahrain, Egypt, Jordan, United Arab Emirates	NCT04510207	07/2020-09/2021
BBIBP-CorV	Inactivated (Vero cells)	Laboratorio Elea Phoenix S.A.	3,000 Adults	2 Day 1 and Day 21	4 locations: Argentina	NCT04560881	09/2020-12/2021
CoronaVac	Inactivated	Health Institutes of Turkey Sinovac, Butantan Institute PT Bio Farma	13,000 18–59 years 8870 Adults 1620 18–59 years	2 Separated by 14 days	24 locations: Turkey 12 locations: Brazil 1 location: Indonesia	NCT04582344 PROFISCOV NCT04456595 NCT04508075	10/2020-04/2021 07/2020-10/2021 08/2020-09/2021
Covaxin	Inactivated (Vero cells)	Bharat Biotech International limited	25,800 Adults	2 Separated by 28 days	23 locations India	CTRI/2020/11/028976	11/2020- 11/2021
NVX-CoV2373	Protein subunit	Novavax, Inc	9,000 18-84 years	2 Day 1 and Day 21	United Kingdom	2020-004123-16	09/2020-ongoing

Abbreviations: BIBP: Beijing Institute of Biological Products Co., Ltd; WIBP: Wuhan Institute of Biological Products Co., Ltd; m/y: month/ year.

participant unrelated to the vaccine injection. The efficacy of CoronaVac should be known before the end of this year.

The WIBP vaccine was assessed in overlapping phases I and II clinical trial. The vaccine was reported to be well-tolerated in an interim report when given a 5-μg dose scheduled 14 days apart; the titer was 121, 14 days after the second injection [136]. WIBP vaccine was approved for limited use in the United Arab Emirates in September 2020.

Covaxin (BBV152) (3 or 6 μg) injected with aluminum hydroxide gel or a TLR7/8 agonist absorbed gel as adjuvant demonstrated good immunogenicity in mice, rats, and rabbits [137]. The injection of Covaxin (3 μg) and TLR7/8 agonist absorbed gel in a 2-doses vaccination scheduled 14 days apart provides immune protection to macaques when challenged by SARS-CoV-2 [138]. In a 4 arms phase I clinical trial (BBV152A, BBV152B, BBV152C, and placebo), adult volunteers not older than 55 years received 2-doses scheduled 14 days apart with differences in antigen concentrations (3 or 6 μg) and 2 adjuvants (NCT04471519). In a contiguous and amended phase II, 380 healthy volunteers between the ages of 12 and 65 received 2-doses 28 days apart (NCT04471519). Bharat Biotech mentioned that the vaccine was well-tolerated, and immunogenicity of BBV152B vas superior to BBV152A, also not statistically significant in a phase I interim report. The phase II clinical trial was subsequently modified as a 2-arms double-blind, randomized trial but not controlled comparing BBV152A and BBV152B (3 μg). The phase III design was based on the phase I interim report and will evaluate the BBV152B candidate vaccine in India across 23 centers. The phase III clinical trial will involve a 2-doses scheduled 28 days apart, recruiting 25,800 volunteers, and starting in November 2020. The immunogenicity will be studied by determining the geometric mean titers by neutralizing antibodies (CTRI/2020/11/028976).

7.2. Live attenuated vaccines

The live attenuated vaccine platform was also used to develop SARS-CoV-2 vaccines. The vaccines developed by Indian Immunologicals Ltd and Griffith University, Mehmet Ali Aydinlar University, and Meissa Vaccines are all in the preclinical phase. COVI-VAC, developed by Codagenix Inc., is entering phase I clinical trial in the United Kingdom (NCT04619628). All live attenuated vaccines were generated by codon deoptimization [139–141]. The live attenuated vaccines have been developed to treat several diseases, such as tuberculosis, measles, mumps, rubella, varicella, yellow fever, and influenza [142]. The live attenuated vaccines can replicate within the host cells and be given intranasally to provide continuous antigenic stimulation, mucosal, and cell-mediated immune responses [143,144]. The route of administration of the vaccines is critical to induce a humoral and cell-mediated immune response. All the vaccines, currently in phase III clinical trials, are injected intramuscularly, which leads to a strong IgG induction but does not improve the IgA secretion in the upper respiratory tract or the protective mucosal immunity [145]. Thus, intranasal immunization may hold the advantage of protecting the upper respiratory tract, the primary port of entry of SARS-CoV-2. The live attenuated vaccine's primary concern is the risk of mutation, which may confer an increased virulence. Also, the occurrence is shallow; the evolution of the attenuated virus needs to be check regularly.

7.3. Protein subunit vaccines

Protein subunit vaccines account for the highest number of vaccines developed (80). The majority is still in preclinical phases, 10 in phase I, 5 in phase I/II, 1 in phase II, and 1, NVX-CoV2373, currently recruiting in the United Kingdom for a phase III clinical trial. The protein subunit vaccine is based on synthetic peptides or recombinant proteins. The spike protein is the most suitable antigen to promote neutralizing antibodies; others use the spike protein's RBD domain. These recombinant proteins are produced in various systems, including insect cells

(NCT04522089), mammalian cells (CHO cells) (NCT04466085), yeast [146], or plants [147]. NVX-CoV2373 contains the recombinant full-length spike glycoprotein optimized in the established baculovirus Spodoptera frugiperda (Sf9) insect cell-expression system and matrix-M1 adjuvant [148]. The participants received two injections of NVX-CoV2373 at day 0 and day 21 in a randomized placebo control observer-blinded phase I/II clinical trial (NCT04368988). The vaccine was well-tolerated as no adverse event was reported in all treatment conditions [148]. Two-dose regimens of 5-μg and 25-μg rSARS-CoV-2 spike protein plus matrix-M1 had a similar response, and all participants had seroconversion [148]. The geometric mean fold rise was increased by 5 times with adjuvant matrix-M1 at day 21 (5.2 and 6.3 for doses of 5 and 25 μg, respectively) and by 100 times at day 35 for doses of 5 and 25 μg with adjuvant matrix-M1 (195 and 165, respectively) when compared to the single vaccination without adjuvant [148]. The matrix-M1 also stimulate a T-cell response; overall, the NVX-CoV2373 induced an immune response comparable to the Covid19 patients with significant illness [148]. The preliminary vaccine efficacy data are still pending for the NVX-CoV2373 phase III clinical trial. Novavax made a manufacturing agreement with Serum Institute of India Private Limited to manufacture 2 billion doses per year from 2021, as mentioned in a press release (ir.novavax.com/news-releases/news-release-details/novavax-announces-covid-19-vaccine-manufacturing-agreement-serum). The vaccine will only require refrigeration for storage.

7.4. Virus-like particle vaccines

Virus-like particles are non-infectious and non-replicating self-assembled nanostructures exposing key viral proteins. Currently, 16 virus-like particle vaccines are developed, including 1 vaccine in phase I/II (ACTRN12620000817943) sponsored by SpyBiotech and Serum Institute of India in Australia, and 1 vaccine (CoVLP) in phase II/III (NCT04636697) sponsored by Medicago Inc in Canada and not yet recruiting. Medicago Inc. has developed a plant-based system using tobacco plants to produce virus-like particles (https://www.medicago.com/en/pipeline/) [147]. The RBD-HBsAg-VLPs-Covid vaccine is based on the RBD domain of SARS-CoV-2 conjugated to the hepatitis B surface antigen (HBsAg) virus-like particles. The adult volunteers will receive 2 doses, 28 days apart (ACTRN12620000817943).

7.5. Replicating and non-replicating viral vectors vaccines

Replicating and non-replicating viral vectors account for 20 and 37 of the vaccines currently developed, respectively, and both stimulate humoral and cellular immune responses. The replicating vector vaccines infect cells that produce the antigen and more viral vectors that will infect more cells. These replicating vectors are usually derived from attenuated viruses or strains of viruses developed for vaccination and shuttle a gene expressing a viral protein, commonly the viral spike protein. Most of the replicating viral vectors are in the early phase of development. Only 3 of them are entering clinical trials, including DelNS1-2019-nCoV-RBD-OPT1 sponsored by the Jiangsu Provincial Center For Disease Control and Prevention and Beijing WANTAI Biological Pharmacy Enterprise Co, and assessed in a phase I clinical trial (ChiCTR2000037782) for the safety of the intranasal spray delivery of the influenza virus vector platform, carrier of the receptor-binding domain gene which could promote mucosal immunity. Merck Sharp & Dohme Corp is sponsoring two vaccines. The V590 vaccine is based on the recombinant vesicular stomatitis virus (rVSV) platform and shuttles a gene coding for the spike protein in a phase I clinical trial, not yet recruiting (NCT04569786) [149,150]. The V591 vaccine uses the attenuated measles vector platform to carry a gene coding for the spike protein and will be evaluated in a phase 1/II currently recruiting (NCT04498247) [150].

The non-replicating viral vectors account 4 vaccines currently in phase III clinical trials (Table 1). These vectors are mostly based on

recombinant human or simian adenovirus vectors. However, they can also use platforms based on the adeno-associated virus, parainfluenza virus, alphavirus, herpesvirus, and poxviruses (NYVAC, vaccinia virus Ankara (MVA), canarypox (ALVAC), or fowlpox (FPV)) [151–153]. The recombinant viral vectors are replication-deficient and produced the antigen in infected cells. All non-replicating viral vectors in phase III clinical trials are carriers of a gene coding for the viral spike protein [152,153].

The recombinant adenovirus type-5-vectored COVID-19 vaccine (Ad5-nCoV) produced by CanSino Biological Inc was evaluated in phase II clinical trial and demonstrated to be well tolerated with only mild and transients adverse effects such as fever and induced a rapid immune response. The neutralizing antibody responses to live SARS-CoV-2 had a geometric mean titer of 19.5 and 18.3 in participants receiving 1×10^{11} and 5×10^{1o} viral particles, respectively, at day 28 post-vaccination [154]. These results prompted the Chinese military to bypass phase III and restricted its approval to military personnel. Ad5-nCoV is concurrently assessed in phase III in Pakistan and Russia, phase II in China (NCT04341389, NCT04566770), phase I/II in Canada (NCT04398147), and phase I (NCT04313127, NCT04568811) in China.

The Gam-COVID-Vac developed by the Gamaleya Research Institute is based on two recombinant adenovirus vectors rAd5 and rAd26, encoding both for the viral spike protein [152]. Adult volunteers received either of the recombinant adenoviruses in a phase I (NCT04436471) and were assessed over 28 days following a single injection, while in phase II, rAd26 was given on day 0, and rAd5 on day 21 (NCT04437875) [152]. The neutralizing antibodies were only present when both vectors were injected, and the neutralizing antibody responses to live SARS-CoV-2 had a geometric mean titer of ~49 in participants receiving 1×10^{11} of both viral particles, 21 days post-vaccination [152]. The Russian Ministry of Health decided to forgo the phase III clinical trial before licensing the Gam-COVID-Vac under the trade name Sputnik V, as a reminder of the space race that took place more than 60 years ago. Sputnik V is currently evaluated in Russia in a phase III clinical trial with an enrollment target of 40,000 participants (Table 1); more than 20,000 participants already received the first dose, and over 16,000 participants received both. The first interim report analysis of the Sputnik V vaccine efficacy against SARS-CoV-2, obtained 21 days after the first injection, was reported to be 92 % based on 20 confirmed cases split between the vaccinated and placebo groups (https://sputnikvaccine.com/newsroom/pressreleases/). The ongoing phase III clinical is not reporting unexpected adverse effects. The Sputnik V is produced as a lyophilized formulation requiring refrigeration or frozen formulation (maximum −18 °C). The Russian Direct Investment Fund organizing the vaccine's manufacture plans 500 million doses per year in multiple countries.

Janssen Pharmaceutica produces another vaccine based on the recombinant adenovirus (Ad26) currently in phase III clinical trial named ENSEMBLE (Table 1) (https://www.jnj.com/media-center). The Ad26.COV2.S vaccine was evaluated in phase I/II clinical trial and demonstrated to be safe after a single injection of either 5×10^{10} or 1×10^{11} viral particles. The SARS-CoV-2 serum neutralizing antibody titers, measured on day 29, was not provided [155]. The preliminary vaccine efficacy data are still pending. Jansen Pharmeutica plans to manufacture 1 billion doses per year from 2021 and will only require refrigeration for storage.

The last non-replicating vector vaccine in phase III clinical trial, sponsored by the University of Oxford and AstraZeneca, is the ChAdOx1 nCoV-19 vaccine (AZD1222) based on the chimpanzee replication-deficient adenovirus vector (Table 1). The SARS-CoV-2 spike gene was inserted into the E1 locus of the ChAdOx1 adenovirus genome. The use of animal adenovirus may decrease the preexisting vector immunity observed using a human viral vector [154]. The safety of the ChAdOx1 nCoV-19 was assessed in phase I/II clinical trial (NCT04324606). The vaccine was administrated as a single dose and appeared safe and well-tolerated [156]. The Marburg VN assay measured the neutralizing

antibody responses to live SARS-CoV-2; 62 % of recipients (23 out of 37) had a median titer of 29 (24–32) 56 days after a single of 5×10^{10} viral particles [156]. The preliminary vaccine efficacy data for the phase III clinical trial are still pending. The phase III clinical trial was interrupted in the USA in September for 50 days after a patient developed symptoms consistent with transverse myelitis and restarted after reviewing the safety data. AstraZeneca plans to supply 400 million doses at no profit by the end of 2020 under Europe's Inclusive Vaccines Alliance (IVA) to all European Union member states. Similar agreements with the UK, USA, the Coalition for Epidemic Preparedness Innovations, and Gavi the Vaccine Alliance were made to manufacture 700 million doses. A license agreement was also completed with the Serum Institute of India to supply a billion doses for low- and middle-income countries. The vaccine will only require refrigeration for storage, facilitating its distribution worldwide.

7.6. RNA vaccines

RNA vaccines are a recent addition to the vaccine armory. The discovery that the intramuscular injection of RNA or DNA led to the expression of encoded proteins and elicited B and T cell immune response paved the way for the emergence of nucleic acid vaccines [157, 158]. Two types of RNA vaccine platforms are available, either conventional mRNA or self-amplifying mRNA [159]. The conventional mRNA is based on a simple open-reading frame flanked by untranslated regions (UTRs), a 5' cap, and a 3' poly(A) tail. The self-amplifying mRNA is based on a positive-sense single-stranded RNA virus, commonly alphaviruses. The modified viral genome comprises a gene of interest, inserted at the place of the sequences coding for structural proteins, the viral nonstructural proteins coding sequence essential for the RNA amplification, UTRs, a cap, and a poly(A) tail [159]. The modification of the mRNA increases its stability, its encapsulation into lipid nanoparticles improves the cellular delivery; the cytoplasmic localization of the translation and non-integration into the host genome are other advantages of the mRNA vaccine. Currently, 33 mRNA vaccines are developed, including 26 in the preclinical phase, 2 in phases I and I/II, 1 in phase II, and 2 in phase III, namely BNT162b2 sponsored by BioNTech SE and Pfizer (NCT04368728) and mRNA-1273 sponsored by Moderna TX and National Institute of Allergy and Infectious Diseases (NCT04470427) (Table 1). BioNTech SE and Pfizer assessed two vaccines, BNT162b1 and BNT162B2 vaccines, in a multi-phase I, II, and III clinical trial (NCT04368728). BNT162b1 contains the mRNA encoding for the spike protein's receptor-binding domain fused to a T4 fibritin-derived 'foldon' trimerization domain to increase its immunogenicity [160], while BNT162b2 contains the mRNA coding for the full-length spike protein [161]. The mRNA was optimized to improve its stability and processivity by incorporating 1-methylpseudouridine instead of uridine and encapsulated into lipid nanoparticles to decrease its immunogenicity [160]. BNT162b1 was assessed, the phase I determined the vaccine candidate and the dose [162]. In an intermediate report of the placebo-controlled, observer-blinded phase I/II, BNT162b1vaccine induced $CD4^+$, and $CD8^+$ T-cell responses in almost all participants, with TH1 helper response [160] (NCT04368728). Adults participants (18–55 years) received two doses of 1 and 50 μg of BNT162b1, 21 days apart; the geometric mean titers of SARS-CoV-2 serum neutralizing antibodies on day 43 were 0.7-fold to 3.5-fold of those of the recovered individuals for the 1 and 50 μg dose, respectively [160]. The BNT162b2 vaccine demonstrated similar SARS-CoV-2 neutralizing geometric mean titers as BNT162b1 but demonstrated less systemic adverse effects, particularly in elderly adults 65–85 years) [161]. Recently, Pfizer and BioNTech announced that the phase III clinical trial for the BNT162b2 vaccine reached all the primary efficacy endpoints. The phase III clinical trial recruited over 43,000 volunteers, of whom 41,135 have received a second dose of the vaccine candidate as of November 13, 2020, reported no serious adverse effects; only fatigue and headache were reported in 3.8 and 2% of the participants,

respectively. In a press release (www.businesswire.com/news/home/20 201118005595/en/), Pfizer and BioNTech announced that the BNT162b2 vaccine demonstrated 95 % efficacy and more than 94 % in adults over 65 beginning 28 days after the first dose based on the analysis of 170 confirmed cases of Covid-19, including 162 in the placebo group (9 severe cases) and 8 in the vaccine group (1 severe case). The vaccine was efficacious across age, gender, and ethnicities. Pfizer and BioNTech initiated a rolling submission to the European Medicines Agency (EMA) in late October and applied on November 20, 2020, for an emergency use authorization to the FDA, which could permit a limited use by the following month. This is the first SARS-CoV-2 vaccine to seek FDA approval in the US. The vaccine needs to be stored at low temperature (-60 to $-80\,^\circ$C), which may limit its diffusion in remote areas. Pfizer and BioNTech reported the production of 50 million doses in 2020 and will scale up to 1.3 billion doses in 2021.

The Moderna vaccine, mRNA-1273, which encodes the spike protein stabilized in the prefusion form [163], demonstrated to be safe in phase I clinical trial (NCT04283461). The participants received either 25, 100, or 250 µg scheduled 28 days apart; the 250-µg dose reported the most severe adverse events [164]. The median magnitude of antibody responses after the first vaccination was similar to convalescent serum samples [164]. A later study supported using the 100 µg for a phase III clinical trial [165]. The vaccine is developed in collaboration with the National Institute of Allergy and Infectious Diseases, part of the National Institutes of Health, and the Biomedical Advanced Research and Development Authority, part of the Office of the Assistant Secretary for Preparedness and Response at the U.S. Department of Health and Human Services. Moderna announced on November 16, 2020, that the mRNA-1273 met all the primary efficacy endpoint (https://investors. modernatx.com/news-releases). The phase III recruited more than 30, 000 volunteers and demonstrated safety and efficacy across all ages, gender, and ethnicities. Fatigue, myalgia, arthralgia, headache, and pain were observed in 9.7, 8.9, 5.2, 4.5, and 4.1 % of the participants, respectively, following the second injection and were all short-lived. The first interim analysis established the efficacy of the vaccine at 94.5 %, based on the analysis of 95 cases, of which 90 cases, including 11 severe cases, were in the placebo group and 5 in the vaccine group with no severe case observed. Moderna initiated a rolling submission to the European Medicines Agency (EMA) on November 17, 2020, and will apply for emergency use authorization to the FDA in the next few weeks. The mRNA-1273 vaccine will be easier to store and distribute worldwide than the BNT162b2 vaccine; the vaccine can be kept refrigerated for 30 days or frozen (-15 to $-25\,^\circ$C) for long term storage. Moderna plans to manufacture 500 million to 1 billion doses in 2021 and coordinate its distribution with U.S. Centers for Disease Control and Prevention (CDC), Operation Warp Speed, and McKesson.

7.7. DNA vaccines

The DNA vaccines are based on plasmids that can be amplified at a large scale in bacteria and expressed in eukaryotic cells. DNA vaccines have been approved for veterinary use (for review, see [166]). For human applicability, concerns have been raised on the risk of integration of DNA plasmid vaccine into the host genome or conferring to the microbiota antibiotic resistance genes. The opportunity for the integration to occur is less frequent than the occurrence of random genetic mutations [167]. In subsequent studies, no integration was detected in the genome host or antibiotic resistance acquisition by the microbiome [166]. DNA vaccines often show low immunogenicity, restricted to the injection site as delivery depends on electroporation, or a gene gun using pressurized helium to force the plasmid inside the cells [168]. The DNA vaccine plasmids for SARS-CoV-2 encode for the spike protein [169]. Currently, 22 DNA vaccines are developed; 5 are or will be evaluated in phase I/II (NCT04445389, NCT04447781, NCT04463472, NCT04527081, and CTRI/2020/07/026352) and 2 in phase I (NCT04336410 and NCT04591184).

8. Conclusion

The volume of research undertaken worldwide and its rapid transition from the bench to the clinic have demonstrated a level of urgency and collaboration never witnessed before to address individual irresponsibility and outmoded politicians. No therapeutic strategy has yet been proven to be determinant for the treatment of SARS-CoV-2. However, eleven vaccines are being evaluated in phase III clinical trials. The preliminary data demonstrated seroconversion and the presence of neutralizing antibodies to various degrees, depending on the vaccine. Three vaccines, Sputnik V, BNT162b2, and mRNA-1273, have demonstrated efficacy superior to 90 %, including in high-risk subgroups. These vaccines will be licensed soon worldwide, but enormous challenges will remain to produce sufficient doses of the vaccines and distribute them worldwide, especially in low to middle-income countries.

Authors' contributions

Moiz Bakhiet generated the idea of the review and wrote part of the manuscript. Sebastien Taurin wrote part of the manuscript and made the figures.

Declaration of Competing Interest

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

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