Review

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An evidence-based systematic review on emerging therapeutic and preventive strategies to treat novel coronavirus (SARS-CoV-2) during an outbreak scenario

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Abstract: A novel coronavirus infection coronavirus disease 2019 (COVID-19) emerged from Wuhan, Hubei Province of China, in December 2019 caused by SARS-CoV-2 is believed to be originated from bats in the local wet markets. Later, animal to human and human-to-human transmission of the virus began and resulting in widespread respiratory illness worldwide to around more than 180 countries. The World Health Organization declared this disease as a pandemic in March 2020. There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. Nevertheless, few broad-spectrum antiviral drugs have been studied against COVID-19 in clinical trials with clinical recovery. In the current review, we summarize the morphology and pathogenesis of COVID-19 infection. A strong rational groundwork was made keeping the focus on current development of therapeutic agents and vaccines for SARS-CoV-2. Among the proposed therapeutic regimen, hydroxychloroquine, chloroquine, remdisevir, azithromycin, toclizumab and cromostat mesylate have shown promising results, and limited benefit was seen with lopinavir–ritonavir treatment in hospitalized adult patients with severe COVID-19. Early development of SARS-CoV-2 vaccine started based on the full-length genome analysis of severe acute respiratory syndrome coronavirus. Several subunit vaccines, peptides, nucleic acids, plant-derived, recombinant vaccines are under pipeline. This article concludes and highlights ongoing advances in drug repurposing, therapeutics and vaccines to counter COVID-19, which collectively could enable efforts to halt the pandemic virus infection.

Keywords: antiviral drugs; coronavirus; SARS CoV-2; vaccines.

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Introduction

In December 2019, a novel coronavirus (CoV) infection emerged from Wuhan, Hubei Province of China, has spread to many countries worldwide [1, 2]. It is believed to be originated from bats, linked initially to animal-to-human transmission in local wet markets. Subsequently, human-to-human transmission of the virus commenced, resulting in widespread respiratory illness worldwide to around 183 countries [1, 2]. On February 11, 2020, the World Health Organization (WHO) named the virus 2019-nCoV (SARS-CoV-2), and the syndrome was named coronavirus disease 2019 (COVID-19), and on March 11, 2020, the WHO declared this disease pandemic as a global health emergency [3, 4]. The ongoing spread of this virus could potentially bring major challenges to worldwide health systems and consequences on the global economy and financial market if not controlled effectively [5].

As a part of the response to this outbreak, researchers from many nations are focusing their efforts on finding possible therapeutic options to save lives and to implement appropriate preventive and control strategies [6, 7]. Currently registered clinical trials under the WHO are evaluating a wide variety of interventions for SARS-CoV-2 and include mainly repurposing agents already known. A team of researchers reported recently to the Food and Drug Administration (FDA) that approximately 70 drugs and experimental compounds may be effective in treating the CoV [7].

In this review article, a literature search was performed using PubMed and Google Scholar to identify relevant English-language articles published on CoV infections. We have outlined different potential treatment options that

could be pursued as a therapy for 2019-nCoV-2 virus, keeping the focus on agents that could be rapidly tested in patients today and broadly effective in spite of limited knowledge about virology of SARS-CoV-2. The information included in this report provides a strong intellectual groundwork for the ongoing development of therapeutic agents and vaccines.

Virology and protein targets of SARS-CoV-2

CoV is an enveloped virus with a positive sense single-stranded ribonucleic acid (RNA) genome (26e32 kb) which causes illness in humans and animals [8]. There are four classes of CoVs designated as alpha, beta, gamma and delta. The beta CoV is named after the crown-like spikes that its genome encodes several structural proteins, including the glycosylated spike (S) protein that functions as a major inducer of host immune responses [9, 10]. The viral genome also encodes nonstructural proteins which can be potential drug targets including RNA-dependent RNA polymerase (RdRp), CoV main protease (3CLpro) and papain-like protease (PLpro) [11, 12]. The beta CoV class includes severe acute respiratory syndrome (SARS) and severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS) and Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2, the COVID-19 causative agent [8, 9]. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 attacks the lower respiratory system to cause viral pneumonia, but it may also affect the kidney, gastrointestinal system, heart, liver and central nervous system leading to multiple organ failure [9].

Entering a vulnerable cell (fusion and endocytosis)

Several reports confirmed that the first step of the human COVID-19 pathogenesis is that the virus specifically recognizes the angiotensin converting enzyme 2 (ACE2) receptor by its spike protein, and it is well known that the ACE2 receptor is widely distributed on the human cell surface, especially the alveolar type II cells and capillary endothelium [13, 14]. Following receptor binding, a host type 2 transmembrane serine protease (TMPRSS2) facilitates cell entry of the virus via the S protein [14].

Releasing viral RNA and translation

After CoV enters the cell, it releases a snippet of genetic material called RNA which is read by the host cell. Infected cells begin to churn out new spikes and other proteins by viral proteinases 3CLpro and PLpro in making of more copies of the CoV [14, 15].

Assembling new copies and spreading the infection

The immune system acts as a natural harbor and helps assemble new copies of the virus which were carried to the outer edges of the cell. Before the cell finally breaks down and dies, each infected cell can release loads of copies of the virus. The viruses may infect nearby cells or end up in droplets that discharge from the lungs.

These viral lifecycle steps propose potential targets for novel drug therapy. ACE2 is an ectoenzyme that converts angiotensin II to angiotensin (1–7) at well-known sites of infection, including the lung and intestine. ACE2 acts as a potent negative regulator restraining, and overactivation of the renin-angiotensin system that may be involved in elicitation of inflammatory lung disease and further elevated pH can interfere with ACE2 glycosylation [12, 13]. Further promising drug targets which share homology with other CoVs include nonstructural proteins (3CLpro and RdRp) and a deubiquitinase, PLpro that deubiquinates certain host cell proteins, including interferon factor 3 and NF-κB, resulting in immune suppression in the host [14–16]. Table 1 summarizes the potential targets and major pharmacologic parameters of selected drugs with high target specificity and/or recognition of existing drugs that could be repurposed to treat SARS-CoV-2.

Investigational antiviral drugs

Human Immunodeficiency Virus (HIV) protease inhibitors

Lopinavir and ritonavir

These showed potential activity for other CoVs (SARS-CoV and MERS-CoV) in preclinical studies [17]. They may act by binding to M pro, a key enzyme for CoV replication, and suppress CoV activity [18, 19]. Various clinical studies done till now confirmed that there was no difference noted in the duration of viral shedding and no observed benefit with lopinavir–ritonavir treatment in hospitalized adult patients with severe COVID-19 [19, 20]. Certain adverse effects such as risk of cardiac arrhythmias (e.g., QT prolongation), risk of use in hepatic disease or hepatitis and significant drug interactions concern their use in critically ill adults with COVID-19 [19–22].

Arbidol (ARB)/umifenovir

Arbidol (ARB)/umifenovir is an indole-derivative molecule which functions as a virus–host cell fusion inhibitor

Table 1: Stages of virus replication and key proteins involved in SARS-CoV-2 infection and potential drug candidates under research.

Stage of virus replication and key proteins involved	Possible classes of selective inhibitors	Potential drug candidate for SARS-CoV-2
Cell entry, attachment and penetration S Protein: viral spike glycoprotein (a viral surface protein for binding to the host cell receptor ACE2) **TMPRSS2 (a host cell–produced protease that primes S protein to facilitate its binding to ACE2)** **Endosome/ACE2**	Soluble receptor decoys, Antireceptor antibodies, Fusion protein inhibitors	**Umifenovir** (brand name Arbidol) [22, 23] **Camostat mesylate** [43, 44] **Hydroxychloroquine/chloroquine** [45, 46]
Uncoating **Release of viral genome**	Ion channel blockers, Capsid stabilizers	-
Transcription of viral genome **Transcription of viral mRNA** Replication of viral genome **PLpro (papain-like protease)** **3CLpro (coronavirus main protease)**	Inhibitors of viral DNA polymerase, RNA polymerase, reverse transcriptase, helicase, primase or integrase	**Lopinavir** [17–19] **Ritonavir** [17–19] **Darunavir**
Translation of viral proteins **Regulatory proteins (early)** Structural proteins (late) **RdRp (RNA-dependent RNA polymerase)**	Interferons, antisense oligonucleotides, ribozymes, inhibitors of regulatory proteins	**Remdesivir** [27, 28] **Ribavirin** [31, 32] **Favipiravir** [38, 39]
Posttranslational modifications proteolytic cleavage myristoylation, glycosylation aspartyl protease	Protease inhibitors	**Nelfinavir [85] (anti-HIV drug)** **Hydroxychloroquine and chloroquine**
Assembly of virion components	Interferons, assembly protein inhibitors	**Interferons (α-1b, α-2b, β 1-a, β-1b)** [71–76]
Release **Budding, cell lysis**	Neuraminidase inhibitors, antiviral antibodies, cytotoxic lymphocytes	-

TMPRSS2: type 2 transmembrane serine protease.

targeting the S protein–ACE2 interaction to prevent viral entry into host cells [22, 23]. It is currently licensed in Russia and China for prophylaxis and treatment of influenza and other respiratory viral infections, as well as for hepatitis C virus [24]. The current dose of 200 mg orally every 8 h for influenza is being studied for COVID-19 treatment, but this lacks enough clinical experience [24–26].

Anti-Hepatitis C Virus (Anti-HCV), nucleotide inhibitors

Remdesivir is a monophosphoramidate prodrug of remdesivir triphosphate (RDV-TP), an adenosine analog that acts as an inhibitor of RdRps [27, 28]. RDV-TP competes with adenosine triphosphate for incorporation into nascent viral RNA chains [28, 29]. Once incorporated into the viral RNA, RDV-TP acts as a delayed RNA chain terminator by evading viral exoribonuclease's proofreading process [30]. In both preclinical trials and clinical studies, remdesivir has demonstrated significant activity against CoV and a high genetic barrier to resistance [30, 31]. Sofosbuvir and Ribavirin can tightly bind to the newly emerged CoV RdRp and hence contradict the function of the protein leading to viral eradication hence under investigation for COVID-19 treatment. But the dose required to inhibit viral replication is high (e.g., 1.2–2.4 g orally every 8 h) hence, limit their use only in combination with other drugs [31, 32].

Other investigational drugs with potential antiviral effect

Several other antiviral drugs are being investigated, predominately those with activity against various influenza subtypes and other RNA viruses such as rintatolimod (Ampligen; toll-like receptor 3 agonist) [33], beta-D-N4-hydroxycytidine (NHC, EIDD-2801) [34], azvudine (nucleoside reverse transcriptase inhibitor) [35], danoprevir (NS3/4A HCV protease inhibitor) [36], plitidepsin (targets EF1A) [37] and favipiravir (viral RNA polymerase inhibitor) [38,39]. Chymotrypsin-like (3C-like) inhibitor, Cinanserin a serotonin receptor antagonist shown promising results as inhibitor of replication of SARS-CoV [40]. Promazine, an antipsychotic drug, has been found to exhibit a significant effect in inhibiting the replication of SARS-CoV by blocking the interaction of S protein and ACE2 [41]. Niclosamide is an anthelmintic agent that targets the viral reservoir in the gut to decrease prolonged

infection and transmission. It is under phase 2a/2b study for its promising antiviral actions [42]. Camostat mesylate, an approved drug for medical use in Japan for the treatment of chronic pancreatitis and postsurgery reflux esophagitis, has shown to prevent CoV cell entry in vitro through inhibition of the host serine protease, TMPRSS2 [43, 44]. This novel mechanism provides an additional drug target for future research.

Hydroxychloroquine and chloroquine

Hydroxychloroquine and chloroquine are commonly used antimalarial drugs and are also used to treat autoimmune conditions because of their immunomodulatory effects [45]. As inhibitors of hemepolymerase, they are recently believed to have additional antiviral activity via alkalinization of the phagolysosome, which inhibits the pH--dependent steps of viral replication [46].

The pharmacological activity of chloroquine and hydroxychloroquine was tested using SARS-CoV-2–infected Vero cells [47]. Hydroxychloroquine was found to be more potent than chloroquine *in vitro*; possible mechanisms may include inhibition of viral enzymes or processes such as viral DNA and RNA polymerase, viral protein glycosylation, virus assembly, new virus particle transport and virus release [48]. Other mechanisms may also involve ACE2 cellular receptor inhibition, acidification at the surface of the cell membrane inhibiting fusion of the virus and immunomodulation of cytokine release [49–51]. Though the studies on hydroxychloroquine for postexposure prophylaxis in healthcare workers or household contacts are underway, however, several countries have already adopted this as a measure to combat COVID-19 (Table 2) [52, 53]. Despite these promising results, this drug has several major limitations like risk of cardiac arrhythmias (e.g., QT prolongation) and retinal damage, especially with long-term use [54]. Patients with G6PD deficiency and diabetics should be cautious as it may cause significant drug interactions [54].

Hydroxychloroquine plus azithromycin

Azithromycin is a macrolide antibacterial having immunomodulatory properties in pulmonary inflammatory disorders [55]. In one study, the patients receiving combination therapy had initially lower viral loads, when compared with patients receiving hydroxychloroquine alone with similar viral burden [56]. This may be explained by their ability to downregulate inflammatory responses and reduce the excessive cytokine production associated with respiratory viral infections [57]. Other antimicrobial actions include reducing chemotaxis of Polymorphonuclear Leucocytes (PMNs) to the lungs by inhibiting cytokines (i.e., IL-8), accelerating neutrophil apoptosis, inhibition of mucus hypersecretion, decreased production of reactive oxygen species and blocking the activation of nuclear transcription factors [58–60]. However, their direct effects on viral clearance are uncertain and awaits further research.

Ivermectin is an antiparasitic agent that has shown antiviral activity against a broad range of viruses, mainly HIV1 and dengue by various mechanisms [61]. Based on the hypothesis, ivermectin may interfere with viral replication process by acting as a specific inhibitor of importin-α/β-mediated nuclear import in RNA virus, ivermectin shows synergistic activity when combined with HCQ [62, 63].

Nitric oxide (NO) is a gas with diverse biological activities and is produced from arginine by NO synthases [64]. A

Table 2: Medical management protocol for health workers and household contact of suspected/confirmed COVID-19 cases. (Indian Council of Medical Research, ICMR guidelines) [52, 53].

Category	For prophylaxis		For treatment	
	Hydroxychloroquine		Hydroxychloroquine + azithromycin	Lopinavir (200 mg)/ ritonavir (50 mg)
	Description	Dosage	Dosage	Dosage
Category 1	Asymptomatic healthcare workers involved in the care of suspected or confirmed cases of COVID-19	400 mg BD on day 1, f/by 400 mg once weekly for seven weeks	Hydroxychloroquine 200mg TID for 10 days + azithromycin 500 mg OD foe five days	2 tablets BD for 14 days or 7 days after becoming asymptomatic. (whichever is earlier)
Category 2	Asymptomatic household contact of laboratory confirmed cases of COVID-19	400 mg BD on day 1, f/by 400 mg once weekly for three weeks		

COVID-19: coronavirus disease 2019.

phase 2 study of iNO is underway in patients with COVID-19 with the goal of preventing disease progression in those with severe Acute Respiratory Distress Syndrome (ARDS), and also, NO was also found to inhibit the synthesis of viral protein and RNA synthesis [65, 66].

Renin–angiotensin–aldosterone system (RAAS) blockade and COVID-19

As per literature search, SARS-CoV-2 is known to utilize angiotensin-converting enzyme 2 (ACE2) receptors for entry into target cells [13, 14]. It was therefore hypothesized that any agent that increases expression of ACE2 could potentially increase susceptibility to severe COVID-19 by improving viral cellular entry. However, physiologically, ACE2 converts angiotensin-II to angiotensin(1–7), which may protect against lung injury by lowering angiotensin 2 receptor binding and vasodilation [67]. There are also conflicting data regarding whether to continue or discontinue drugs that inhibit the renin–angiotensin–aldosterone system (RAAS), namely angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) in patients with COVID-19 and comorbidities such as hypertension, cardiovascular disease and diabetes which are often treated with ACEIs and ARBs [67–69]. A trial is currently under evaluation for the use of losartan in patients with COVID-19 [70].

Biologics for CoV-associated disease (immunomodulators)

Most CoV infections cause fever as the immune system response to clear the virus. In severe cases, the immune system can overreact and start attacking lung cells, making it difficult to breathe as the lungs become obstructed with fluid and dying cells which can lead to acute respiratory distress syndrome and possibly death [71].

Interferons (IFNs)

During a viral infection, the most prominent cytokines produced are interferons (IFNs) that have the ability to interfere with viral replication [72]. A vivid correlation between the innate immune response threshold and the fatality rates in COVID-19 can be found. The higher threshold of IFN-mediated immune responses can increase mortality rates in the elderly [73]. The authors concluded that the delayed IFN-related antiviral response is a possible strategy implemented by CoV to evade the immune response [71, 74]. The virus also circumvents the immune system by hiding its double-stranded RNA in vesicles, causing less IFN induction [75]. The key for success in reducing the disease fatality might be stimulation of the innate immune responses to trigger IFN production at the very early stages of the disease, which might be done through administration of agents that are able to augment IFN production. Interferon-γ combination with an interferon-I might induce synergistic benefits [76]. However, in-depth research is needed to validate it and determine the optimum dosage and timing to prevent unwanted results. Interferon–human serum albumin fusion proteins (HSA-IFNs) have significantly lengthened the plasma half-life of IFNs (e.g., from 10 h to 12 days for HSA-IFN-α2b) due to slower free IFN release into the plasma and thus may prolong the duration of action of IFN for each injection [77].

Numerous studies have indicated a “cytokine storm” with release of interleukin-6 (IL-6), IL-1, IL-12 and IL-18, along with tumor necrosis factor alpha, and other inflammatory mediators may cause severe disease in patients with COVID-19 [71, 78, 79]. IL-6 is a proinflammatory cytokine that is involved in diverse physiological processes such as T-cell activation, immunoglobulin secretion induction, hepatic acute-phase protein synthesis initiation and hematopoietic precursor cell proliferation and differentiation stimulation. The increased inflammatory response from lungs may result in increased alveolar–capillary gas exchange, making oxygenation difficult in patients with severe illness [71, 78, 79]. IL-6 inhibitors may remediate severe damage to lung tissue caused by cytokine release in patients with serious coronaviral infections [80]. Initial data of few studies suggest that tocilizumab and sarilumab, IL-6 inhibitors, may have clinical benefit as adjunctive therapy which inhibits IL-6–mediated signaling by competitively binding to both soluble and membrane-bound IL-6 receptors [81, 82]. However, the drug increases the risk of GI perforation and hepatotoxicity in patients with thrombocytopenia and neutropenia and infusion-related reactions. Leronlimab is an investigational humanized monoclonal antibody to the chemokine receptor CCR5 [83]. This drug is currently under study for its ability to enhance immune response while mitigating cytokine storm in patients with COVID-19.

RNA therapies

Virus’ RNA genome first enters a cell; it interacts with the host’s protein-making machinery, using it to make proteins that can copy RNA molecules, and these RNA-copying proteins are called as “polymerases” [84]. By blocking these RNA polymerases, the virus can no longer reproduce

that may reduce infection and can make a promising target for therapies. One such drug, remdesivir, was originally developed in the hope that it would limit Ebola virus and its relatives [27, 28]. CoV RNA encodes certain proteases that help RNA polymerase to get fully functional in order to adopt their mature configuration. Scientists have now found that HIV-1 protease inhibitor, nelfinavir, strongly inhibits the replication of the SARS-CoV, despite the fact that these viruses are unrelated [85].

Antisense oligonucleotides have also been developed to reduce the severity of SARS virus infections and to prevent or treat SARS virus–associated disease [86, 87]. Antisense oligonucleotides may also target disease-related proteins involved in the inflammatory process.

Antibody-directed therapy

The data indicate that the S protein is a putative target for SARS-CoV-2 antibody development as in viral infection but not the other structural proteins, M, E, and N in SARS-CoV, elicits an immune response [10]. A neutralizing antibody targeting the S protein on the surface of COVID-19 is likely the first therapy contemplated by biomedical researchers in the academia and industry, providing passive immunity to disease [88]. Certain monoclonal antibodies, for example, 80R, CR3014, F26G18, F26G19, m396, 1A9 and 4D4, which are used to target spike protein in SARS-CoV and MERS-CoV showed promising results *in vitro* and *in vivo* that could be potentially effective against SARS-CoV-2 [88–90].

Corticosteroids

Corticosteroid therapy is not recommended for viral pneumonia; however, use may be considered for patients with refractory shock or acute respiratory distress syndrome and there is no enough evidence-based data supporting its role in patients with COVID-19 [91, 92].

Convalescent plasma or immunoglobulins

Convalescent plasma is an antibody-rich product that is collected from eligible donors who have recovered from COVID-19 and FDA states its use only to confirmed patients having severe or immediately life-threatening COVID-19. It is important to determine its safety and efficacy via clinical trials before routinely administering to patients with SARS-CoV-2 [93, 94]. One possible explanation for the efficacy of convalescent plasma therapy is that the antibodies from convalescent plasma might suppress viremia [94]. However, convalescent plasma has not yet been shown to be effective in COVID-19 and needs further evaluation.

High-dose intravenous vitamin C treatment for COVID-19

The results of several meta-analyses have been established that intravenous (IV) high-dose vitamin C has significant benefit in the treatment of sepsis and septic shock, which may be because of its role as a prooxidant for immune cells but as an antioxidant for lung epithelial cells improving alveolar fluid clearance. Furthermore, vitamin C treatment known to inhibit lactate secretion produced by the activated immune cells may protect innate immunity [95, 96]. This effect may benefit patients with COVID-19 as they SARS-CoV-2 mainly affects the lower respiratory tract.

Stem cells, a therapeutic strategy

One of the concerns based on preclinical science is that the virus can infect the stem cells rendering them ineffective. It was recently brought to the light that ACE2 and umbilical cord mesenchymal stem cells (UC-MSCs) may benefit the patients with COVID-19 via immunoregulatory function. The first CoV case, treated with umbilical cord cells, was reported from China [97], leading to further to speculation and further investigations. It was known that UC-MSCs have been reported responsible of the bacterial clearance in preclinical models of sepsis, ARDS and cystic fibrosis infection. (ref) In patients with COVID-19, mesenchymal stem cell (MSC) therapy can inhibit the overactivation of the immune system and promote endogenous repair by improving the microenvironment. After entering the human body through IV infusion, part of the MSCs accumulate in the lung, which could improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis and improve lung function [98, 99]. Therefore, the fact that the transplantation of MSCs improved the outcome of patients with COVID-19 may be due to regulating inflammatory response and promoting tissue repair and regeneration which needs further evaluation and research.

Progress and prospects on vaccine development against SARS-CoV-2

Presently, the epidemic is still spreading, and there is no effective means to prevent the infection. Vaccines are the

most helpful and cost-effective means for prophylaxis and treatment of infectious diseases. Previously, lot of research has been done to develop vaccines against human CoV infections such as MERS and SARS. Conversely, till date, no licensed antiviral treatment or vaccine is available for MERS and SARS CoV infections [100, 101].

Lot of research work is directed toward the design and development of vaccines for SARS-CoV-2. Currently, those at the highest risk of acquiring COVID-19 like frontline healthcare workers, individuals aged 60 years and older or those with underlying diabetes and hypertension need vaccination [2]. Therefore, such populations might be prioritized for vaccine clinical trials. Important points to be considered while producing a vaccine against human COVID-19 are they should minimize immunopotentiation, must be suitable for stockpiling and can be given to healthcare workers, elderly and adults with underlying diabetes or hypertension [102]. Herewith, we provide a brief overview of the major candidates or vaccines under development.

COVID-19 and target product profile

Numerous approaches are adopted for the development of CoV vaccines; most of these target the surface-exposed spike (S) glycoprotein or S protein as it is the key inducer of neutralizing antibodies. The S protein molecule contains two subunits, S1 and S2. The S1 subunit has a receptor-binding domain (RBD) that interacts with its host cell receptor, angiotensin-converting enzyme 2 (ACE2), whereas the S2 subunit causes fusion between the virus and host cell membrane and then releases its viral RNA into the cytoplasm for further replication [102, 103]. Therefore, S protein–based vaccines can induce antibodies which block viral receptor binding and also virus genome uncoating in the cytoplasm. The S protein has a major role in the induction of protective immunity during infection with SARS-CoV by eliciting neutralizing-antibodies and T-cell responses [103].

The SARS-CoV-2 shares high genetic similarity with the SARS-CoV, based on the full-length genome phylogenetic analysis approximately 89% nucleotide similar to SARS-like CoVs (genus beta-CoV) found in Chinese bats [104, 105, 106]. On this basis, the early development of SARS-CoV-2 vaccine started and also suggested that the receptor of SARS-CoV-2 might be the same as that of SARS-CoV receptor (ACE2) [107].

Major COVID-19 vaccine development programs (Table 3)

a) **Whole virus vaccines or live-attenuated or inactive whole virus vaccines:** Whole virus vaccines represent a standard approach for viral vaccinations. The whole cell antigens include all the elements of the virus, i.e. proteins, lipids, polysaccharide and nucleic acids [108]. A major advantage of whole virus vaccines is their inherent immunogenicity and ability to stimulate toll-like receptors. But live virus vaccines often require extensive testing to validate their safety. This is especially an issue for CoV vaccines, given the findings of increased infectivity following immunization with live or killed whole virus SARS CoV vaccines [109]. Following are the live attenuated vaccines under development.

- The US Healthcare giant Johnson & Johnson has started developing a vaccine against the Wuhan novel CoV SARS-CoV-2. The vaccine program is using Janssen's adenovirus-vectored vaccine and PER.C6 technologies to rapidly upscale production of the optimal candidate [110] and has planned to initiate phase 1 clinical trials by September.
- The researchers at the University of Hong Kong have developed a live influenza vaccine that expresses SARS-CoV-2 proteins [111].
- The US Codagenix, Inc. is collaborating with the Serum Institute of India, Ltd. to develop a live-attenuated vaccine against SARS-CoV-2. They have developed a "codon deoptimization" technology to synthesize "rationally designed" live-attenuated vaccines. This technology helps for the rapid generation of multiple vaccine candidates against the virus [112, 113].
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b) **Subunit vaccines:** The subunit vaccines with one or more antigens induce strong immunogenicity and efficiently stimulate the host immune system. These vaccines are safer and easier to manufacture and require the addition of adjuvants to elicit a strong protective immune

Table 3: List of various vaccine candidates for COVID-19 under development.

Type of vaccine	Vaccines under development [reference]
Whole virus vaccines: live-attenuated/inactive whole virus vaccine	– Johnson & Johnson – using adenovirus-vectored vaccine [110] – University of Hong Kong – live influenza vaccine that expresses SARS-CoV-2 protein [111] – Codagenix, Inc. in collaboration with the Serum Institute of India – live-attenuated vaccine by codon deoptimization technology. [112, 113]
Subunit vaccines	– University of Queensland – based on the "molecular clamp" technology developing a subunit vaccine against spike protein. [114] – Novavax – using recombinant protein nanoparticle technology [115, 116]. – Clover biopharmaceuticals Inc. – using their patented Trimer-Tag® technology developed a subunit vaccine. [117] – Texas Children's Hospital Center – developed SARS-CoV-2 RBD vaccine. [118, 119] – MNA-embedded SARS-CoV-2 vaccine [123]
Nucleic acid vaccines	– Inovio Pharmaceuticals /Beijing Advaccine Biotechnology Co developed a DNA vaccine "INO-4800" [125] – LineaRx and takis biotech – analyzing DNA vaccine for SARS-CoV-2 [126] – Zydus Cadila – recombinant DNA vaccine for COVID-19.[127] – Moderna Therapeutics (mRNA-1273, encoding S protein) is in phase I clinical trials. [128] – Fudan University/Shanghai Jiaotong University and Bluebird Biopharmaceutical Company – mRNA vaccine. [129] – CureVac AG – mRNA vaccine [131] – Stermirna Therapeutics [132]
Live vector vaccines	– Houston-based Greffex Inc. developed SARS-CoV-2 adenovirus vector vaccine with Greffex vector [133] – Tonix Pharmaceuticals – (TNX-1800) vaccine [134] – Johnson & Johnson – (AdVac®) vaccine [135] – University of Oxford – (ChAdOx1 nCoV-19) [136] – CanSino Biologics – (Ad5-nCoV) [137]
Synthetic peptide or epitope vaccine	– Hong Kong University of Science and Technology – peptide vaccine for COVID-19 [138]
Plant biopharmaceuticals	– Rapid response vaccines [140, 141]

COVID-19: coronavirus disease 2019; RBD: receptor-binding domain; MNA: microneedle array.

response. Subunit vaccines for SARS-CoV-2 are directed against the S-spike protein to prevent its docking with the host ACE2 receptor [109].

- The University of Queensland along with Viroclinics is synthesizing a subunit vaccine based on the "molecular clamp" technology, which locks the 'spike' protein into a shape which allows the immune system to be able to recognize and then neutralize the virus. It uses rapid response vaccine technology to develop a vaccine, and within three weeks, the candidate vaccine will be produced. This project is funded by the Coalition for Epidemic Preparedness Innovation (CEPI) [114].
- Novavax, Inc. created the COVID-19 vaccine candidates using its proprietary recombinant protein nanoparticle technology to produce antigens derived from the CoV spike (S) protein. Novavax is awarded funding from the CEPI [115]. Novavax is using its proprietary Matrix-M™ adjuvant with its COVID-19 vaccine candidate which stimulates the entry of antigen-presenting cells into the injection site and enhances antigen presentation in local lymph nodes, boosting immune responses [116]. It is assessing the efficacy in animal models to identify an optimal vaccine candidate for human testing. Phase 1 clinical testing is expected to begin in late spring of 2020 [115].
- Clover Biopharmaceuticals Inc. successfully produced 2019-nCoV subunit vaccine candidate against spike protein ("S-Trimer") via mammalian cell expression system. They detected cross-reacting antibodies from sera of multiple infected patients. Clover is the first company in the world to disclose a 2019-nCoV vaccine candidate, announced on 10th February, 2020. The vaccine development was carried out with the support of leadership teams from Chengdu Hi-Tech Park and Chengdu Clinical Center for public health in China [117].
- Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine (including University of Texas Medical Branch and New York Blood Center) has developed and tested a subunit vaccine comprising the RBD of the SARS-CoV S-protein [109,

118, 119]. The RBD in spike protein located on the outer membrane of CoV mediates receptor binding and plays a major role in virus entry into the host cell [120]. This protein is used as a potential vaccine candidate as it is the major target for neutralizing antibodies [121]. When formulated on alum, the SARS-CoV RBD vaccine elicits high levels of protective immunity on the homologous virus challenge. The RBD-based vaccine minimizes host immunopotentiation [109].

- Pasteur Institute, Johnson & Johnson and Chongqing Zhifei Biological Products Co., Ltd. also started subunit vaccine development against SARS-CoV-2 [122].
- Microneedle array (MNA)–embedded SARS-CoV-2 S1 subunit vaccine [123]: The MNA technique is a minimally invasive subcutaneous approach, with low dose requirement, low cost and less toxicity, when compared to traditional needle injection [124]. In this preclinical study, C57BL/6 female mice (five animals per group) were inoculated intracutaneously with MNAs loaded with of SARS-CoV-2-S1 or SARS-CoV-2-S1fRS09 protein or phosphate buffered saline (PBS) as a negative control. In this study, by 2 weeks after immunization with the MNA delivered SARS-CoV-2, S1 subunit vaccine showed potent antigen-specific antibody responses [123].

c) **Nucleic acid vaccines**

DNA vaccines are characteristically composed of plasmid DNA molecules that encode one or more antigens.

- Inovio Pharmaceuticals, Inc. in collaboration with Beijing Advaccine Biotechnology Co. has developed a DNA vaccine "INO-4800", which is through the preclinical studies, and also, phase 1 human testing is done to evaluate the safety and immunogenicity, and the project is being funded by the CEPI [125].
- Another DNA vaccine for SARS-CoV-2 is in preclinical studies being tested by collaboration of Applied DNA Sciences Subsidiary, LineaRx and Takis Biotech. They are working on preclinical development of a linear DNA vaccine based on polymerase chain reaction, and the vaccine constructs were produced at a scale using plasmid-based vaccine templates [126]. The initial data from preclinical animal tests showed immunogenicity and a strong antibody generation across all five vaccine candidates.
- Zydus Cadila is developing a DNA vaccine against the major viral membrane protein responsible for the cell entry of the novel CoV. Also, it is planning to develop a live-attenuated recombinant measles virus–vectored vaccine against COVID-19 [127].

mRNA vaccines have high potency, short production cycles, low-cost manufacturing and are safe for administration in comparison to the conventional vaccines [128].

- Moderna Therapeutics has developed a SARS-CoV-2 mRNA vaccine (mRNA-1273, encoding S protein) which is in phase I clinical trials on healthy volunteers [129]. This phase 1 study is being conducted by the National Institutes of Health under its own Investigational New Drug application. The safety and immunogenicity of the vaccine is being evaluated by using three dose levels of mRNA-1273 (25, 100, 250 μg) with 2 doses per schedule, given 28 days apart. In this study, 45 healthy adults will be included. Participants will be followed during 12 months after the second dose vaccination. The primary objective of this study is to evaluate the safety and reactogenicity, and the secondary objective is to estimate the immunogenicity to the SARS-CoV-2 S protein [129]. Moderna is working to begin its phase 2 clinical trials.
- Fudan University in collaboration with Shanghai Jiaotong University and Bluebird Biopharmaceutical Company is developing SARS-CoV-2 mRNA vaccine using two different strategies. The first is to use mRNA to express the SARS-CoV-2 S protein and RBD domain; the efficacy of this vaccine is now under evaluation in mice [130].
- In addition, German biopharmaceutical company CureVac AG [131], Stermirna Therapeutics [132], BDGENE Therapeutics, Guanhao Biotech, ZY Therapeutics Inc., CanSino Vaccines Biologics Inc., Baylor College of Medicine, University of Texas, Tongji university also announced their progress on mRNA vaccine development against SARS-CoV-2 [123].

d) **Live vector vaccines:** Live vector vaccines are the live viruses (the vector) that express a heterologous antigen(s), and they are broadly used to induce cellular immunity. They are characterized by combining the strong immunogenicity of live-attenuated vaccines and the safety of subunit vaccines [123]. Below mentioned are few live vector vaccines.

- Houston-based Greffex Inc. has completed the construction of SARS-CoV-2 adenovirus vector vaccine with genetically engineered Greffex Vector Platform and is planning for animal testing on approval by the USFDA [133].
- Tonix Pharmaceuticals announced research to develop a potential SARS-CoV-2 vaccine TNX-1800 (live modified horsepox virus vaccine for percutaneous administration), based on Tonix's proprietary horsepox vaccine platform [134].

- Johnson & Johnson in collaboration with the U.S. Biomedical Advanced Research and Development Authority to develop a vaccine against CoV has adopted the AdVac® adenoviral vector platform for vaccine development [135].
- The University of Oxford is testing their vaccine candidate (ChAdOx1 nCoV-19), an adenovirus vaccine vector against the COVID-19 spike protein. It has initiated their phase I/II randomized, placebo-controlled, multicentre clinical trial in healthy volunteers aged 18–55 years to determine the efficacy, safety and immunogenicity of the vaccine against COVID-19. The vaccine is administered via intramuscular route [136].
- CanSino Biologics has developed a adenovirus type-5 vector-based recombinant COVID-19 vaccine, Ad5-nCoV, which has completed phase I studies, and sooner, they are initiating phase 2 clinical trials in China. The phase 2 randomized study has three primary endpoints: firstly, to evaluate adverse reactions within the first 14 days of vaccination; secondly, on day 28 to check for serum levels of anti-SARS-CoV-2 neutralizing antibody and antibody against the CoV's spike protein and lastly, to follow up participants for 6 months [137].

e) **Synthetic Or peptide vaccine:** the Hong Kong University of Science and Technology has screened a set of B- and T-cell epitopes from S and N proteins of SARS-CoV; these epitopes are highly conserved in SARS-CoV-2 which guides toward the development of SARS-CoV-2 vaccines [138]. These peptide vaccines generally are prepared by chemical synthesis techniques and contain only certain fragments of intact antigens. Because of their low immunogenicity, they need additional structural modifications, delivery systems and adjuvants in the formulation [139].

f) **Plant biopharmaceuticals:** Plant expression systems for production of candidate vaccines offer advantage of affordable cost and rapid production, which aids in global vaccination programs. They produce "rapid response vaccines" as it produces more protein in a short time [140]. The plant-based biopharmaceutical production against 2019-nCoV will include the identification of potential epitopes and production of full-length viral surface proteins present in the envelope region or production of subunit vaccines expressing the immunogenic region or chimeric proteins [141].

Animal models for evaluation of SARS-CoV-2 virus

In the process of preclinical evaluation, animal models are vital to study the efficacy of vaccine candidates. Previously, by introducing hACE2 gene into the mouse genome, a SARS-CoV transgenic mouse model was developed [142]. Recently, in an animal study, they used the hACE2 transgenic mice infected with SARS-CoV-2 to evaluate the pathogenicity of the virus. In these transgenic mice infected with SARS-CoV-2, weight loss and virus replication in the lung tissue was seen. On histopathology, typical interstitial pneumonia findings were observed, and also, viral antigens were detected in the bronchial epithelial cells, alveolar epithelia and alveolar macrophages. This mouse model may facilitate the development of therapeutic drugs and vaccines against SARS-CoV-2 [143].

Cell culture systems for SARS-CoV-2

SARS-CoV-2 isolation has been attempted in Vero and the Huh-7 cells (human liver cancer cells) [144]. The monoclonal antibodies (fully human/humanized) that target both the S1-RBD and non-RBD, as well as the S2 domain of CoV, have been developed and tested in cell cultures for virus neutralizing capability, as well as in animal models for prophylactic and postexposure efficacies [145].

Bacille Calmette-Guérin vaccine (BCG) and COVID-19

Bacille Calmette-Guérin vaccine (BCG) is a live attenuated strain derived from an isolate of *Mycobacterium bovis* used broadly across the world as a vaccine for tuberculosis, with many countries, including India, Japan and China, having a universal BCG vaccination policy in infants. BCG vaccination has been shown to produce positive "heterologous" or nonspecific immune effects (trained immunity) leading to improved response against other nonmycobacterial pathogens and is proposed to be caused by metabolic and epigenetic changes leading to promotion of genetic regions encoding for proinflammatory cytokines specifically IL-1B, which has been shown to play a vital role in antiviral immunity [146, 147]. One study data suggested that BCG vaccination seems to significantly reduce mortality associated with COVID-19 [148] as this vaccination has been shown to produce broad protection against viral infections and sepsis [148]. The correlation between the beginning of universal BCG vaccination and the protection against COVID-19 needs further exploration.

Conclusion

This article concludes that, at present, neither vaccines nor direct-acting antiviral drugs are available for the

treatment of human or animal CoV infections. The drug repurposing effort summarized in this review is applicable only to adult patients and focused primarily on agents currently known to be effective against other RNA viruses including SARS-CoV, MERS-CoV, influenza, HCV and Ebola, as well as antiinflammatory drugs. The biologics for treatment of COVID-19 have potential impacts as they have shown promising results, including bioengineered and vectored antibodies, cytokines and nucleic acid–based therapies targeting virus gene expression, as well as various types of vaccines. Finally, a concerted effort to develop effective drugs and vaccines against existing and potential future CoV infections and other highly pathogenic virus outbreaks is necessary to reduce overwhelming impacts on human life and worldwide healthcare systems.

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