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CURRENT UPDATES ON THERAPEUTIC AND VACCINE APPROACHES FOR COVID-19 DISEASE

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INTRODUCTION

Several infectious viruses are rapidly emerging and evolving for the last two decades, because of their rapid mechanism of mutation (Zolnikova *et al.* 2018). The viruses associated with respiratory infections are especially considered as major etiological agents of death in both developing and developed countries. It has been reported that acute respiratory infections such as influenza, pneumonia, adenovirus, respiratory syncytial virus and enterovirus infections are responsible for millions of deaths globally (Olaimat *et al.* 2020).

Coronaviruses are most commonly involved in causing respiratory, neurologic and gastrointestinal (GI) disorders (Jiang *et al.* 2020). These viruses are called so because of their crown-shaped structure attached with long surface spikes (Zu *et al.* 2020). These are highly diverse enveloped viruses with single strand, large (25-32 kb), and positive sense RNA genome (Zhu *et al.* 2020). The host organisms of coronaviruses include humans and several other vertebrates like bats, camels, mice, cats, masked palm civets and dogs (Jiang *et al.* 2020). Among these infectious coronaviruses, SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 are highly pathogenic and are linked with serious infections and mortalities in human beings (Lu *et al.* 2020; Meo *et al.* 2020). SARS-CoV-1 was first reported in 2002 in China and an outbreak of severe acute respiratory syndrome (SARS) appeared across the world, involving 37 countries with 10.87% causalities (Zhai *et al.* 2005). Later, MERS-CoV was first identified in 2012 in Saudi Arabia (Jiang *et al.* 2020; Rodriguez-Morales *et al.* 2020), causing 30% or more mortality in Middle East countries (Luo and Gao 2020). Recently, severe acute respiratory syndrome coronavirus-2 (previously known as 2019 novel coronavirus, 2019-nCoV) rapidly emerged from an epidemic in Wuhan city of China in December, 2019 to Public Health Emergency of International Concern on 30th January, 2020 and a Global Pandemic on March 11, 2020. The virus is described as successor to SARS CoV-1, the strain that caused an outbreak on SARS in 2002-2004 (Gorbalenya *et al.* 2020). Moreover, it is more transmissible and contagious than both SARS-CoV-1 and MERS-CoV. It is an enveloped, positive sense, single stranded RNA virus with a helical nucleocapsid. At the time of writing this review (17th March, 2020), a total of 121,423,007 confirmed cases of COVID-19 have been reported from around the globe, out of which 2,684,813 people have died (Worldometer 2020). It is worth mentioning that actual positive cases are much higher than these numbers, the reasons being subclinical infections, under-reporting and lack of proper diagnostic facilities in some parts of the world (Krantz and Srinivasa Rao 2020). The scale of humanitarian and economic impact drives the exploration of conventional and novel strategies for effective disease control.

Treatment Strategies

Potential non-vaccine therapy, including protease inhibitors, monoclonal antibodies and antiviral drugs is being developed or clinically tested cases. One example of antiviral drug is remdesivir, which is a nucleoside analogue prodrug under clinical investigation. Some combinations have also been shown to be of superior value to the drugs used separately. A combination thereof, is interferon beta-1b, oral ribavirin (nucleoside analogue) and oral lopinavir and ritonavir (protease inhibitors) (Wang *et al.* 2020a). Several therapeutic agents undergoing different phases of clinical trial have been considered and summarized in Table 1.

Anti-Malarial Drugs

Chloroquine and Hydroxychloroquine: Chloroquine has been considered as a drug of choice for malaria treatment and various immune disorders like rheumatoid arthritis for many years (Rynes 1997). Its anti-COVID-19 activity was found to be very low, with EC50 of 1.13 µM (Wang *et al.* 2020b). Due to these appropriate *in vitro* results of low EC50 and higher cytotoxic concentrations (CC50), chloroquine has been introduced into human clinical studies. Chloroquine was administered to COVID-19 patients in more than 10 hospitals in China. The results were promising in terms of decreasing viral load, controlling the exacerbation of pneumonia and reducing the course of disease (Gao *et al.* 2020a). Chloroquine actually inhibits the fusion and entry of the virus into the host cells and reduces the production of cytokines (Rabi *et al.* 2020). The potential effect of chloroquine against SARS-CoV-2 infection was identified in a report received during the outbreak in China (Gao *et al.* 2020a). Prior to the outbreak of COVID-19, many *in vitro* studies on chloroquine revealed its ability to inhibit viral replication of another coronavirus (SARS-CoV) responsible for causing severe acute respiratory syndrome (Keyaerts *et al.* 2004; Vincent *et al.* 2005).

Table 1: Summary of potential therapeutic candidates undergoing clinical trials for COVID -19

Drug	Trial Registration No.	Phase	Action	Posology	Reference
Antiviral drugs					
Remdesivir	NCT04292899	III	Inhibits RdRp polymerase inhibiting RNA synthesis	200 mg, OD, IV for 1 day followed by 100 mg, OD, IV for next 4-9 days	(Goldman *et al.* 2020; Sheahan *et al.* 2020)
Lopinavir + Ritonavir	ChiCTR2000029539 NCT04455958	II	Lopinavir: Inhibits 3CL protease activity, Blockage of protein processing Ritonavir: same as lopinavir	Lopinavir: 400 mg oral Ritonavir: 100 mg BD oral for 14 days	(Cao *et al.* 2020)
Favipiravir	ChiCTR2000030254 NCT04600999	Completed and recommended	Antiviral, RNA-dependent RNA polymerase inhibitor	1600 mg Oral BD for day 1, followed by 600 mg oral BD till the trial end	(Agrawal *et al.* 2020)
Ivermectin + Nitazoxanide	NCT04360356	II	Ivermectin: Antiviral/ Antiparasitic inhibits viral replication and assembly of new virions. Nitazoxanide: Antiviral, Antiparasitic, interferes with 3CL protease activity	Ivermectin 200 mcg/kg OD oral, empty stomach + Nitazoxanide 500 mg BD oral during meal for 6 days	(Patra *et al.* 2018)
Darunavir/ Cobicistat	NCT04252274	III	800mg/150 mg –one tablet oral for 5days	Protease inhibitor Darunavir: Cytochrome P-450 CYP3A Inhibitors Cobicistat: Cytochrome P-450 Enzyme Inhibitors	(Chen *et al.* 2020)
Arbidol (Umifenovir)	NCT04260594	IV	14-20 days course of 2 tablets TID	hinders trimerization of viral spike glycoprotein and inhibits host cell adhesion.	(Gao *et al.* 2020c)
Ribavirin + Interferon beta-1B	NCT04494399	II	S/C injection of interferon beta-1B 16 million IU for 5 days 400 mg BD oral for 5 days	Ribavirin: inhibit capping of viral transcripts and viral polymerase Interferon beta-1B: Enhance activity of suppressor T cell and reduce proinflammatory cytokines	(Brzoska *et al.* 2020; Rahmani *et al.* 2020)
Oseltamivir	NCT04516915	II	Oseltamivir alone 75mg twice daily for 14 days in hospitalized pts. Or IMU-838, 22.5mg BD for 14 days +75mg BD for 14 days	Oseltamivir: inhibits the neuraminidase enzyme, expressed on surface of virus. This enzyme facilitates release of virus from the infected cells and aids in movement of virus in the respiratory tract.	(Tan *et al.* 2020)
Antimalarial drugs					
Chloroquine	NCT04353336 NCT04286503	II	500 mg/dose orally BID for 7-14 days	Increase the endosomal pH and changes ACE-2 glycosylation, interfere with interaction of virus and receptor.	(Fantini *et al.* 2020; Snawerdt *et al.* 2020)
Hydroxy-chloroquine	NCT04261517 NCT04328272	III	400 mg OD for 5 days	Enhance endosomal pH and changes glycosylation of ACE-2, interfere with interaction of virus and receptor.	(Hashem *et al.* 2020)
Anti-interleukin drugs					
Tocilizumab	NCT04317092 NCT04320615 NCT04356937	II III III	1 IV infusion, dose 8 mg/kg, up to max dose of 800 mg. 1 additional dose could be administered if clinical condition worsen with no improvement.	monoclonal antibody which competitively impedes the binding of interleukin-6 to its receptor.	(Gotera 2020; Sahebnasagh *et al.* 2020; Ucciferri *et al.* 2020)
Sarilumab	NCT04315298 NCT04327388	II III	Single dose IV on day 1 and second dose may be given after 24-48 hrs of first dose.	Human recombinant monoclonal antibody which blocks IL-6 receptors, inhibiting IL-6–mediated signaling.	(Fala 2018)

Bevacizumab	NCT04275414	II	Bevacizumab 500mg + normal saline (NS) 100ml, ivdrip ≥ 90min	It inhibits angiogenesis by targeting and blocking vascular endothelial growth factor A (VEGF-A).	(Pang *et al.* 2021)
Anakinra	NCT04341584	II	At day 1, day 2 and day 3; Two IV infusions (200 mg) / day At Day 4, two IV infusions (100mg)/ day. At day 5, one IV infusion (100 mg) /day.	Interleukin 1 receptor antagonist, inhibits the production of inflammatory cytokines	(King *et al.* 2020)
Janus Kinase inhibitors					
Ruxolitinib	NCT04348071 NCT04338958	III II	2 x 10mg with defined response adapted dose increase to 2 x 20mg for 7 days	Inhibit competitively ATP-binding catalytic site of the kinase domain. Inhibit of the JAK-STAT pathway	(Yeleswaram *et al.* 2020)
Baricitinib	NCT04373044	II	Daily dose by mouth for 14 days in combination with antivirals	reversibly inhibits JAK1 and JAK2 , block phosphorylation and activation of signal transducers and activators of transcription (STATs)	(Favalli *et al.* 2020)
Convalescent anti-SARS-CoV-2 plasma	NCT04345289	III	Reported dosage varied depending upon the amount of transfused plasma and antibody titer	Binding of the transfused antibodies to the pathogenic organism, leading to phagocytosis, cellular cytotoxicity, or direct pathogen neutralization	(Bloch *et al.* 2020)

Hydroxychloroquine is a chloroquine derived drug and differs only by one hydroxyl group. It is considered to be an appropriate alternative to chloroquine with less toxic effects for the treatment of COVID-19 and is undergoing phase III clinical trial (NCT04261517) (Effectiveness of Hydroxychloroquine in Covid-19 Patients - Full Text View - ClinicalTrials.gov, n.d.). In a previous study, hydroxychloroquine was found to have more potent antiviral activity *in vitro* than that of chloroquine (Yao *et al.* 2020). Three mechanisms have been proposed for the antiviral activity of hydroxychloroquine and chloroquine. The first mechanism involves the interference of the drug with terminal glycosylation of the cellular receptor ACE2, hence inhibiting virus-receptor binding; the second mechanism involves the increase in pH induced by the drug, leading to acid cellular organelles, hindering endocytosis of virion transport and potentially affecting post-translational modification of newly synthesized molecules of virus; and third mechanism comprises the drug induced disturbance in the process of assembling of virion and viral protein molecules synthesis (Savarino *et al.* 2003; Cortegiani *et al.* 2020).

A clinical trial was performed in France with 36 confirmed COVID-19 patients. The end point was the absence or presence of virus within six days from inclusion in the protocol. The results showed a significant decrease or absence of viral load and this effect was summed up by azithromycin. An important point to be noted is that the patients included in this study ranged from asymptomatic to those with pneumonia and none was critically ill (Gautret *et al.* 2020).

Despite the lack of strong and reliable evidence of efficacy, due to the pressure exerted by COVID-19 worldwide, many health authorities have implemented official guidelines for the use of hydroxychloroquine and chloroquine for the treatment of COVID-19 patients (Liqian and Zheng 2020). Both hydroxychloroquine and chloroquine have been in clinical use for many years, and the safety profile of both the drugs is well established (Devaux *et al.* 2020). Reported side effects include gastrointestinal upset with hydroxychloroquine and retinal toxicity with long term use of both drugs (Mavrikakis *et al.* 1996; Srinivasa *et al.* 2017). In addition, heart arrhythmia and cardiomyopathy have also been reported (Costedoat-Chalumeau *et al.* 2007; Pieroni *et al.* 2011; Sabato *et al.* 2017).

Antiviral Drugs

Favipiravir: Favipiravir is a protease inhibitor drug, and was used for the first time in Wuhan, China against the SARS-CoV-2 pandemic (Agrawal *et al.* 2020). It is a purine nucleoside analogue and is included in class of pyrazines. This drug was found to demonstrate *in-vitro* antiviral activity against SARS-CoV2 with relatively high concentrations (EC_{50}: 61.88 µM) compared to remdesivir and chloroquine (Coomes and Haghbayan 2020). Another study comprising non-randomized clinical trial on favipiravir showed that the drug significantly decreased the treatment duration of SAR-CoV-2 infection and showed better therapeutic results than lopinavir/ritonavir (Cai *et al.* 2020). Its protein binding is 54%, bioavailability ~94% and small apparent volume of distribution is 10–20 L. After a single dose, it reaches Cmax within 2 hours and due to rapid renal eradication, it has a low half-life of 2.5-5.0 hours (Agrawal *et al.* 2020).

Lopinavir/Ritonavir

Lopinavir (LPV) is an antiviral agent most commonly used in combination with ritonavir for HIV disease treatment

(Meini *et al.* 2020). Lopinavir is a protease inhibitor drug that is used for the treatment of SARS-CoV-2 and suppresses 3-chymotrypsin-like protease. Lopinavir/ Ritonavir shows no statistical difference in fatality rates and no reduction in duration for medical development (Giovane *et al.* 2020).

Ivermectin/Nitazoxanide

Ivermectin is an FDA-approved broad spectrum anti-parasitic agent that also shows anti-viral activity. The causative agent of COVID-19 epidemic, SARS-CoV-2, is a single stranded positive sense RNA virus that shows severe acute respiratory syndrome (Caly *et al.* 2020). Nitazoxanide is a broad-spectrum antiparasitic drug that shows antiviral activity against influenza, hepatitis B&C, and coronaviruses. It suppresses SARS-CoV-2 reproduction at low micromolar concentrations in Vero CCL81 units and is available in oral form (Rocco *et al.* 2020).

Darunavir/Cobicistat

Darunavir is a protease inhibitor that is used for the treatment of HIV-1 disease. It is highly compatible with bone and renal profile development but has fewer positive effects on different lipoids (Deeks 2018). Cobicistat is another agent used to enhance the plasma levels of darunavir among other antiretroviral drugs and a specific cytochrome P450 3A inhibitor (Kakuda *et al.* 2015).

Oseltamivir

Oseltamivir is an FDA-approved neuraminidase inhibitor with an antiviral activity against influenza A and B, and pneumonia caused by severe acute respiratory syndrome coronavirus (SARS-CoV) (Tan *et al.* 2020). It was not effective in the treatment of COVID-19 disease with hypoxia or dyspnea in Wuhan because COVID-19 pneumonia developed resistance at the onset of therapy (Chiba 2020).

Ribavirin/Interferone beta-1B

Ribavirin is a non-interferon agent with broad spectrum of antiviral activity against extensive RNA and DNA viruses throughout the epidemic of severe acute respiratory syndrome and Middle East respiratory syndrome (Wang *et al.* 2020c). Interferons (IFNs) have a resistance against viral diseases, such as a constituent of intrinsic immunity and also against Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV. While IFN α is less commonly used than IFN β, and also essential for the treatment of MERS-CoV, IFN β is considered to be an effective agent for the treatment of SARS-CoV-2 (Rahmani *et al.* 2020).

Arbidol (Umifenovir)

Arbidol (ARB) is also called as Umifenovir, and is used for the treatment of infections caused by certain viruses, including hepatitis C virus (HCV) and influenza A and B viruses. ARB may suppress COVID-19 disease by intrusive relief of SARS-CoV-2 from receptor-mediated follicles (Nojomi *et al.* 2020). It may also show a suppressive effect on a wide range of diseases for example RNA viruses. However, there is an inadequate indication to promote the use of this medicine for improvement of infected persons with COVID-19 (Teimury and Khaledi 2020).

Given all the benefits of therapeutic management of disease, the need for prophylactic management does not diminish. A safe vaccine has yet to be developed and evaluated to provide effective strategy for controlling the pandemic. Therefore, there is an ultimate need to vaccinate the entire population against SARS-CoV-2.

Convalescent Plasma Therapy

Currently, convalescent plasma has been approved by the FDA under Emergency IND (eIND) for severe and life-threatening condition of COVID-19. Clinical trials are underway, as the convalescent plasma contains antibodies donated by confirmed COVID-19 recovered patients, and its administration may theoretically assist to clear virus. Convalescent plasma has already been administered against viral infections of MERS, H1N1 influenza and SARS (Soo *et al.* 2004; Arabi *et al.* 2015).

Limited data are available regarding the efficacy of convalescent plasma against SARS-CoV-2. In a case study, the clinical symptoms and status was improved with the administration of convalescent plasma to 5 severely ill COVID-19 patients (Shen *et al.* 2020). In another study, 10 critically ill COVID-19 patients showed improvement in laboratory reports within 3 days and lung lesions within 7 days following administration of convalescent plasma (Duan *et al.* 2020). However, further clinical studies are needed to determine the advantages and limitations of current therapeutic protocol. Many clinical trials are going on and mentioned on clinicaltrials.gov (NCT04333251, NCT04323800, and NCT04345523). Due to scarcity of clinical trial data, NIH neither recommend for nor against convalescent plasma administration (COVID-19 Treatment Guidelines 2020).

Adjunctive Treatments

Multiple cohort studies in patients with COVID-19 showed that most patients died due to increased interleukin-6 (IL-6) levels and serum ferritin levels. Moreover, in another cohort study, the ICU patients were observed with higher serum levels of IL-2, IL-7, interferon-γ inducible protein 10 (IP10), tumor necrosis factor α (TNF-α), granulocyte colony-stimulating factor, monocyte chemoattractant protein 1 (MCP1) and macrophage inflammatory protein 1-α (MIP1A) (Grasselli *et al.* 2020; Ruan *et al.* 2020; Zhou *et al.* 2020a). These hyperinflammatory reactions in patients with COVID-19 suggested a cytokine release syndrome (CRS) (Chiang et al. 2008; Ramos-Casals et al. 2014; Huang et al. 2020; Mehta et al. 2020). Cytokine release syndrome in the most critical cases leads to intravascular coagulation, circulatory shock, and multiorgan failure

(Shimabukuro-Vornhagen et al. 2018). These observations have sparked the importance of immunosuppression therapy, including anticytokine agents, corticosteroids and immune-modulatory agents. These active agents not only assist to reduce mortality rate but also improve the clinical outcomes.

Corticosteroids

A classical immunosuppressive drug, methylprednisolone, has been used against the progress of pneumonia and proved to be effective for the treatment of acute respiratory distress syndrome (ARDs). Patients with COVID-19 pneumonia have been observed to develop acute respiratory failure (Huang *et al.* 2020). Methylprednisolone is, therefore considered to be a candidate drug for use in critically ill cases. In a clinical study with 201 COVID-19 patients having ARD, administration of methylprednisolone (1–2 mg/kg/daily IV dose for a week) reduced the mortality rate (Wu *et al.* 2020). In another study with 46 severely ill COVID-19 patients, early administration of low-dose of methylprednisolone improved the clinical status and shortened the disease course (Wang *et al.* 2020d). Another short and limited study with 15 patients and no control group indicated the usefulness of low dose corticosteroids treatment in severe COVID-19 pneumonia patients (Zhou *et al.* 2020).

A fluorinated corticosteroid, dexamethasone has also been considered earlier to be used in the treatment of ARD. In a clinical trial, it was found that the use of dexamethasone in patients with moderate to severe ARDS significantly reduced the duration of mechanical ventilation, ICU mortality and all-cause mortality at 60 days (Villar *et al.* 2020). However, the use of corticosteroids in ARDS due to COVID-19 remains more complicated and controversial than historical ARDS literature might suggest. Clinical trials are currently going on or are expected to carry out to investigate the role of hydrocortisone or methylprednisolone in COVID 19 with severe hypoxia and ARDS, either administered alone or in combination with anti-interleukin agents and can be seen on clinicaltrials.gov (NCT04244591, NCT04330638, NCT04348305 and NCT04323592).

Interleukin-6 Receptor Antagonists

The use of Interleukin-6 receptor antagonist is currently suggested only in the context of a clinical trial (Alhazzani *et al.* 2020).

Tocilizumab

Tocilizumab is a humanized monoclonal antibody which blocks both soluble and membrane-bound IL-6 receptors. Interleukin-6 is the major component of cytokine release syndrome and hyperinflammatory response.

Tocilizumab was recently approved by FDA for CRS (Sanders *et al.* 2020). Many clinical studies are going on by using several dosing strategies on hospitalized patients with either critically ill condition or non-critically ill patient populations, which can be found on clinicaltrials.gov (NCT04345445, NCT04332094, NCT04331795, NCT04332913, NCT04346355, NCT04339712, NCT04320615, NCT04335071, NCT04322773, NCT04317092, NCT04310228, NCT04306705, NCT04335305, NCT04315480, NCT04330638, NCT02735707, NCT04331808, NCT04347031 and NCT04349410).

Sarilumab

It is another IL-6 receptor inhibitor. Theoretically, based on the mechanism of action, it would be considered beneficial in the COVID-19 related CRS (Stebbing *et al.* 2020). Several clinical trials are conducted or still ongoing and can be found on clinicaltrials.gov (NCT04321993, NCT04341870, NCT04327388, NCT04345289, NCT04324073, NCT04315298 and NCT02735707).

Anakinra

It is an antagonist of the interleukin-1 (IL-1) receptor. This potent drug has a theoretical place in the treatment due to increased IL-1 levels in COVID-19 and therefore may cause a significant reduction in cytokine storm (Mehta *et al.* 2020). Anakinra has also been administered for the treatment of hemophagocytic lymphohistiocytosis and CAR-T–associated CRS. Several clinical trials are going on to indicate the use of anakinra alone or in combination with other therapeutic agents. These can be found on clinicaltrials.gov (NCT04324021, NCT04330638, NCT04339712 and NCT04341584).

Janus Kinase Inhibitors

Fedratinib, baricitinib, and ruxolitinib are Janus kinase inhibitors which have been approved by FDA for the treatment of myelofibrosis and rheumatoid arthritis. Theoretically, these therapeutic agents are considered to have an effect on cytokine levels (including interferon γ), which appear to be increased in COVID-19 patients (Ramos-Casals *et al.* 2014; Huang *et al.* 2020; Mehta *et al.* 2020; Stebbing *et al.* 2020). Ruxolitinib is being investigated for both the prevention and treatment of COVID-19 and trials can be found on clinicaltrials.gov (NCT04348695, NCT04334044, NCT043 31665, NCT04348071, NCT04337359 and NCT04338958).

Other Agents of Interest

Vitamin C: Vitamin C (ascorbic acid) is an antioxidant vitamin, needed to boost the immune system (Ang *et al.* 2018). Studies have shown that several high-dose vitamin C infusions (e.g., 200 mg/kg per day through IV route, divided into 4 doses) reduced the duration of the intensive care unit (ICU) by 7.8% (Hemilä and Chalker 2019).

Vitamin C is also involved in the synthesis of endogenous catecholamines as a cofactor and maintains immune function by helping with neutrophil action and lymphocyte proliferation (Marik 2020). These properties,

along with low level of endogenous vitamin C during infection, improved interest in its clinical use in COVID-19.

Coronaviruses enhance oxidative stress that stimulates cellular malfunction and ultimately causes organ failure (Boretti and Banik 2020). However, high intravenous dose of vitamin C could have a beneficial effect by inhibiting the phenomenon of cytokines storm production due to COVID-19. Several Chinese physicians have shown positive results by using this approach for treatment of COVID-19 (Carr 2020; Cheng 2020). The successful use of high-dose intravenous vitamin C has been reported in a study on 50 moderate to severe COVID-19 patients in China. The dose range was 10-20 g/day. The oxygenation index was improved and all the patients eventually recovered and were discharged (Cheng 2020).

Probiotics

Probiotics are the living microorganisms involved in generating valuable physiological effects on the host. Several bacteria present in various fermented foods like pickle, cheese and yogurt are recognized as probiotics due to their health benefits (Kok and Hutkins 2018; Rezac *et al.* 2018). It has been suggested that human beings should consume 10^8 to 10^{10} CFU dose of probiotics on daily basis to have beneficial effects on health. Many health benefits of probiotics have been proved, including inhibition of the initiation of allergic diseases, treatment of ischemic heart disorder, decreasing blood cholesterol content, producing vitamins B, improving the bioavailability of dietary calcium, and boosting immune activity (Bustamante *et al.* 2020).

Probiotics include bacteria as well as yeast. The probiotic bacteria are *Lactobacillus acidophilus, L. brevis, L. amylovorus, L. bulgaricus, L. cellobiosus, L. curvatus, L. gallinarum, L. casei, L. crispatus, L. helveticus, L. delbrueckii spp. bulgaris, L. fermentum, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus; Lactococcus lactis; Streptococcus thermophilus, Leuconostoc mesenteroides, Pediococcus pentosaceus, P. acidilactici, Sporolactobacillus inulinus, Bifidobacterium adolescentis, B. animalis, B. bifidum, B. breve, B. essensis, B. infantis, B. laterosporum, B. thermophilum, B. longum, Propionibacterium acidipropionici, P. freudenreichii, P. jensenii, P. thoenii, Enterococcus faecium, E. fecalis, Bacillus subtilis, B. alcolophilus, B. cereus, B. coagulans, B. clausii and Escherichia coli. The* probiotic yeast include *Saccharomyces cerevisiae* and *S. boulardii* (Bron *et al.* 2012; Saad *et al.* 2013). Probiotics have been mostly considered as antibacterial agents however, anti-viral activities are also reported recently for some probiotic strains (Al Kassaa 2016).

Probiotics are involved in controlling drug-linked diarrhea, sepsis, gastrointestinal infection and respiratory tract infection (Li *et al.* 2020). An organized, randomized and controlled study on more than 5,000 infants treated with *lactobacillus plantarum* strain linked with prebiotics showed a reduction in severity of lower respiratory tract infections and sepsis (Gao *et al.* 2020). Viruses are the main causing agents for upper respiratory tract infections. The beneficial and protective effect of probiotics have been confirmed in prevention of upper respiratory tract infections. Multiple randomized controlled studies, involving the administration of probiotics to 4,230 youngsters and kids, have shown a 2-fold decrease in occurrence of respiratory tract infections and a considerable decrease in the severity of the disease in infected patients (Sencio *et al.* 2020). Furthermore, a double-blind randomized study was conducted in 523 youngsters, who received *Bifidobacterium longum* SP 07/3, *Bifidobacterium bifidum* MF 20/5 and *Lactobacillus gasseri* PA 16/8 along with some vitamins and minerals. The probiotics administration decreased the flu period and also a decrease in fever days (Pullano *et al.* 2020). A randomized, placebo controlled trial with *Lactobacillus brevis* also indicated promising results in 1,692 school kids, as the probiotic strongly reduced the risk of influenza respiratory infection (Bell *et al.* 2004). In healthy persons, many lactic acid bacteria commonly present in the upper respiratory tract are considered for probiotics (Wan *et al.* 2020). Studies have also indicated that probiotics could have a valid therapeutic and preventive contribution in the incidents of coronavirus outbreak. However, all probiotics are not involved in reducing the risk of respiratory tract infections (Turner et al. 2017). Examples of probiotics that might be useful to reduce the load of viral COVID-19 include *Lactobacillus gasseri, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium longum, Pediococcus pentosaceus, Leuconostic plantarum, L. paracasei ssp. Paracasei, L mesenteroides* (Lehtoranta et al., 2014; Zelaya *et al.* 2016) . These probiotics are involved in reducing the occurrence and severity of respiratory tract infection, as well as in boosting the immune system of the body (Zafar *et al.* 2020). A study to determine beneficial effect of *Lactobacillus coryniformis* K8 along with dietary supplements to protect healthcare workers from contracting COVID-19 was carried out and has been registered at ClinicalTrials.gov (NCT04366180) (Tahir et al. 2020).

Challenges and Progress on Vaccine Development for COVID-19

After the gene sequence of SARS CoV-2 was published on 11th January, 2020, intensive research was focused on the development of vaccines (Yadav *et al.* 2020). Some of the challenges relating to the development of a COVID-19 vaccine are as follows:

Vaccine development takes time, as the vaccine should not only be protective but also safe, because it is administered to healthy populations. The fastest development was the mumps vaccine, which took nearly 5 years (Sharma *et al.* 2020). Accelerated development involves trials to be done in smaller groups. There is considerable concern about the safety of a vaccine. If such vaccine is approved for public use globally, adverse effects may arise which might not have been observed in small groups.

Table 2: A summary of vaccine candidates undergoing clinical trials for COVID-19

Sr.	Vaccine Name	Type	NCT Ref No.	Route	Target	Principal Developer	Comments	Reference
1	ZF2001	Protein subunit	NCT04646590	IM	RBD protein and virus neutralizing antibodies	Anhui Zhifei Longcom Biologic Pharmacy Co, China	Phase III trials in China, Uzbekistan, Indonesia, Pakistan and Ecuador, 3 doses	(Yang *et al.* 2020)
2	ChAdOx1 nCov-19 (AZD-1222)	Adenovirus vector	NCT04516746	IM	S protein	Collaboration of Oxford University and Astra Zaneca, UK	Under Phase III trials	(Jeyanathan *et al.* 2020; Wang *et al.* 2020a)
3	Ad5-nCoV	Adenovirus vector	NCT04341389	IM	S protein	CanSino Biologics. Beijing, China	Phase II trials, for adults aged 18 years and above, single dose	(Wu *et al.* 2020b)
4	mRNA-1273	LNP, Lipid-mRNA nanoparticle	NCT04283461 NCT04470427 NCT04405076	IM	S protein	Moderna, NIAID, USA	Phase I, II, III, trials, fully synthetic, no risk of disease transmission, 2 doses, 2-year immunity	(Baden *et al.* 2020)
5	CoronaVac (PiCoVacc)	Inactivated virus	NCT04352608 NCT04383574 NCT04456595	IM	Multiple surface antigens	Sinovac Biotech, Beijing China	Phase I, II, III trials, for adults 18-59 years, 2 doses	(Palacios *et al.* 2020)
6	NVX-CoV2373	Recombinant protein nanoparticles using Matrix-M adjuvant	NCT04368988	IM	S protein and virus neutralization	Novavax, USA	Phase I, II trials, for adults aged 18-84 years, 2 doses	(Keech *et al.* 2020a; b)
7	BNT162b1	LNP, Lipid-mRNA nanoparticle	NCT04523571	IM	RBD of S protein	BioNTech (Germany), Pfizer, Fosun (China)	Phase II, III trials, after Phase I, approved for emergency use in UK, USA and Singapore, 2 doses	(Mulligan *et al.*, 2020)
8	BBIBP-CorV	Inactivated virus	NCT04560881	IM	Multiple neutralizing antibodies	Sinopharm, Beijing, China	Large scale Phase III trials in China and UAE, 2 doses	(Xia *et al.* 2020)
9	INO-4800	Plasmid DNA	NCT04642638 NCT04447781 NCT04336410	Intradermal	S protein	Inovio and Advaccine, China	Phase I, II, III trials, 2 doses with electroporation	(Smith *et al.* 2020; Tebas *et al.* 2020)
10	Inactivated SARS-CoV-2 Vaccine (Vero Cell)	Inactivated virus	NCT04510207	IM	Multiple neutralizing antibodies	China National Biotec, China	Phase III trials in China, 2 doses	(Jeyanathan *et al.* 2020)
11	Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine	LNP, Lipid-saRNA nanoparticle	N/A	IM	S protein	Imperial College London, UK and Morningside Ventures, China	Phase I and II trials in UK, 2 doses	(Jeyanathan *et al.* 2020)
12	Inactivated SARS-CoV-2 Vaccine	Inactivated virus	NCT04470609 NCT04412538	IM	Multiple neutralizing antibodies	Qihan Li, Chinese Academy of Medical Sciences, China	Phase I and II trials in China, 2 doses	(Clinical Trials, n.d.)
13	CVnCoV	LNP, Lipid-mRNA nanoparticle	NCT04652102	IM	S protein	CureVac, Germany	Phase III trials, for adults aged 18 years and older, 2 doses	(Kremsner *et al.* 2020)
14	GamCOVID-Vac Lyo	Adenovirus vector, 2 viruses (rAd26, rAd5) heterologous	NCT04437875 NCT04436471	IM	S protein	Gamaleya Research Institute of Epidemiology and Microbiology Russia	Phase II trials, approved for distribution in Russia, single dose and prime boost dose	(Logunov *et al.* 2020)
15	GX-19	Plasmid DNA	NCT04445389	IM	S protein	Genexine Consortium, Korea	Phase II trials in South Korea, 2 doses	(Kaur and Gupta 2020; Seo *et al.* 2020)
16	SCB-2019	Recombinant trimeric S protein	NCT04405908	IM	S protein	Clover Biopharmaceuticals (China), GSK (UK) and Dynavax (USA)	Phase I trials, 2 doses	(Richmond *et al.* 2020)

17	COVID-19 vaccine	Protein subunit	NCT04445194 NCT04466085	IM	Dimeric RBD	Anhui Zhifei Longcom Biologic Pharmacy Co, China	Phase I, II trials in China, 2 or 3 doses under trials	(Jeyanathan *et al.* 2020)
18	ARCoV	mRNA	N/A	IM	S protein	Suzhou Abogen Biosciences, Walvax Biotechnology and Academy of Military Medical Sciences, China	Phase I trials in China, 2 doses	(Jeyanathan *et al.* 2020)
19	AG0301-COVID19	Plasmid DNA	NCT04463472	IM	S protein	AnGes, Inc., Japan	Phase I, II trials in Japan, 2 doses	(Rego *et al.* 2020)
20	VIR-7831 (recombinant coronavirus-like particle covid-19 vaccine)	Plant-based virus-like particle	NCT04450004	IM	Multiple viral antigens	Medicago and Laval University, Canada	Phase I trials in Canada, for adults aged 18-55 years, 2 doses	(Craven 2020)
21	Lunar-COV19 (ARCT-021)	mRNA	NCT04480957	IM	S protein	Arcturus Therapeutics and Duke-NUS Medical School, Singapore	Phase I, II trials in Singapore, for adults aged 21-80 years, single dose	(Baviskar *et al.* 2021)
22	Covaxin (BBV152A, BBV152B and BBV152C)	Inactivated whole virion	NCT04471519	IM	Multiple viral antigens	Bharat Biotech and Indian Council for Medical Research, India	Phase I, II trials in India, 2 doses	(Rego *et al.* 2020)
23	ZyCov-D	Plasmid DNA	N/A	Intradermal	S protein	Zydus Cadila Healthcare, India	Phase I, II trials in India, 3 doses	(Jeyanathan *et al.* 2020; Kaur and Gupta 2020)
24	SARS-CoV-2 Sclamp (COVID-19) Vaccine	Protein subunit	NCT04495933	IM	Molecular clamp-stabilized S protein	University of Queensland, Australia	Phase I trials, 2 doses, development halted after unintended results in Phase I	(Normile 2020)
25	Ad26.COV2.S (Janssen COVID-19)	Adenovirus vector (Ad26)	NCT04436276	IM	S protein	Janssen, Belgium	Phase I, II trials, 2 doses	(Sadoff *et al.* 2021)
26	KBP-201 COVID-19	Protein subunit	NCT04473690	IM	RBD-based protein	Kentucky BioProcessing, USA	Phase I, II trials, 2 doses	(Mathew *et al.* 2021)
27	IAVI-Merck COVID-19	VSV vectored	N/A	IM and oral	S protein	Merck and IAVI, USA	Phase I, II, single dose	(Mahalingam *et al.* 2020)
28	COVAX19 (Monovalent Recombinant COVID19)	Protein subunit	NCT04453852	IM with Advax-SM adjuvant	S protein	Vaxine (Australia) and Medytox (South Korea)	Phase I in Australia, single dose, development halted due to lack of funds	(Jeyanathan *et al.* 2020)
29	MVC-COV1901	Protein subunit	NCT04487210	IM	S protein	Medigen Vaccine Biologics (Taiwan)	Phase I in Taiwan, 2 doses	(Mathew *et al.* 2021)
30	Covigenix VAX-001	Plasmid DNA	NCT04591184	IM	Multiple epitopes	Entos Pharmaceuticals, Canada	Phase I trials in Canada, 2 doses	(Ashraf *et al.* 2021)
31	bacTRL-Spike	Bacterial vector	NCT04334980	Oral	S protein	Symvivo Corporation, Canada	Phase I trials,	(Alturki *et al.* 2021)

There are indications that respiratory viruses are especially difficult to protect against with vaccines. This is because the mucous membranes of respiratory tract are protected by IgA antibodies, whereas vaccine response is determined taking IgG and IgM or total immunoglobulin in focus. Most vaccines are inoculated as intramuscular injection with minimal mucosal immunity or IgA secretion (Chung *et al.* 2020).

In the past, recombinant nucleic acid has not resulted in the development of a successful vaccine for human use (Han 2015). Furthermore, the dependence of DNA vaccines on an injection device or an electroporator is a potential issue.

The pre-existing immunity to adenoviruses results in reduced immune response in individuals receiving adenovirus vector-based vaccines. Single stranded RNA viruses are capable of highly efficient self-amplification of RNA in host cells. Virus mutation may result in lack of efficacy of the vaccine (Lundstrom 2020).

There is risk of vaccine-enhanced disease for inactivated virus-based vaccines (Graham 2020). Moreover, fast-tracked large-scale production of vaccine stills remains a challenge to meet the demands of pandemic.

Phases of Vaccine Trials

The first phase of vaccine development is an exploratory phase, involving identification of antigens and computational modeling of whether a vaccine can help to treat or prevent a disease. The second phase is preclinical stage, which involves testing of vaccines on cell cultures and/or animal models to check for efficacy, immunogenicity and safety. Once immunogenicity and safety are verified by animal studies, progress is made for testing on human population, first in small groups and then in large groups in three phases.

Phase I (Safety): The vaccine is administered to healthy immunocompetent human subjects in small groups. Vaccine is primarily checked for safety. Appropriate dosage adjustments are made, and immunity production is checked as secondary effect.

Phase II (Expanded safety): The vaccine is given to hundreds of people (split in small groups according to demographic features). This phase again is a test for safety, while immunization is taken as secondary effect. This phase determines dosage, interval between doses and other requirement to be accorded during Phase III trials.

Phase III (Efficacy): The vaccine is given to thousands of people to evaluate its efficacy. Vaccine efficacy (VE) is defined as percent reduction in the incidence of disease in the vaccinated group as compared to placebo. In case of low disease incidence in the population, sample size should be sufficiently large to determine reliable vaccine efficacy in the population (Mahase 2020). After successful completion of Phase III trials, vaccine can be moved for Review and Approval and then to Marketing and Post-Marketing Surveillance (Sharma *et al.* 2020). Normally, regulatory bodies must review results of clinical trials and decide whether a vaccine can be approved or rejected. Under ordinary circumstances, this can take 1-2 years but, during a pandemic, vaccine can be approved on emergency basis. After marketing, effectiveness and adverse effects of vaccine are still monitored during widespread use in general public.

Vaccines Candidates

In Table 2, vaccines undergoing different phases of clinical trials are summarized. It is pertinent to mention that live vaccines are not being attempted for human use due to safety reasons (Caddy 2020).

Conclusion

The whole world is going through the deadly challenge to deal with lethal coronavirus infection in humans. Scientists and researchers from all over the world are working day and night to discover potential preventive moieties and therapeutic agents against this deadly disease. Different protocols and strategies, such as preventing the viral binding to host cells, inhibition of viral replication, use of drugs and compounds to enhance both innate as well as passive immunity, are under consideration to treat and control COVID-19. Up till now, not a single therapeutic agent has been approved against SARS-CoV-2. Several types of vaccines and pharmacological drugs are under clinical research trials and this will take several months to years to be commercially available in the market. The major challenge of COVID-19 is the development of effective therapeutic strategies against SARS-CoV-2. Some of the antiviral drugs and adjunctive therapeutic agents have shown substantial effects *in vitro*, however there is an ultimate requirement to confirm their safety and efficacy in the clinical trials. It is expected that scientific strategies will assist in developing new, effective, cheap and safe antiviral agents against SARS-CoV-2.

REFERENCES

Agrawal U et al., 2020. Favipiravir: A new and emerging antiviral option in COVID-19. Medical Journal of Armed Forces India. 76: 370–376.

Alhazzani W et al., 2020. Surviving sepsis campaign: Guidelines on the management of critically ill adults with Coronavirus disease 2019 (COVID-19). Intensive Care Medicine 46: 854–887.

Al Kassaa I, 2016. Antiviral probiotics: A new concept in medical sciences. New Insights on Antiviral Probiotics: From Research to Applications. Springer International Publishing pp: 1–46.

Alturki SO et al., 2020. The 2020 pandemic: Current SARS-CoV-2 vaccine development. Frontiers in Immunology 11: 1880.

Ang A et al., 2018. Vitamin C and immune cell function in inflammation and cancer. Biochemical Society Transactions 46: 1147-1159.

Arabi Y et al., 2015. Feasibility, safety, clinical and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. Springerplus 4: 1–8.

Ashraf MU et al., 2021. COVID-19 vaccines (revisited) and oral-mucosal vector system as a potential vaccine platform. Vaccines 9: 171-195.

Baden LR et al., 2020. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. New England Journal of Medicine 384: 403-416.

Baviskar T et al., 2021. Deciphering vaccines for COVID-19: Where do we stand today? Immunopharmacol Immunotoxicol 43: 8-21.

Bell D et al., 2004. Animal origins of SARS coronavirus: Possible links with the international trade in small carnivores. Philosophical Transactions of the Royal Society London Series B Biological Sciences 359: 1107–1114.

Bloch EM et al., 2020. Deployment of convalescent plasma for the prevention and treatment of COVID-19. The Journal of Clinical Investigation 130: 2757-2765.

Boretti A and BK Banik, 2020. Intravenous vitamin C for reduction of cytokines storm in acute respiratory distress syndrome. Pharma Nutrition 100190.

Bron PA, et al., 2012. Emerging molecular insights into the interaction between probiotics and the host intestinal

mucosa. Nature Reviews Microbial 10: 66–78.
Brzoska J, et al., 2020. Interferons in the therapy of severe Coronavirus infections: A critical analysis and recollection of a forgotten therapeutic regimen with interferon Beta. Drug Research 70: 291.
Bustamante M et al., 2020. Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. Folia Microbiologica (Praha) 65: 245-264
Caddy S, 2020. Developing a vaccine for Covid-19.
Cai Q et al., 2020. Experimental treatment with Favipiravir for COVID-19: An open-Label control study. Engineering 6: 1192–1198.
Cao B et al., 2020. A trial of Lopinavir–Ritonavir in adults hospitalized with severe Covid-19. New England Journal of Medicine 382: 1787–1799.
Caly L et al., 2020. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 *in vitro*. Antiviral Research 178: 104787.
Carr AC, 2020. A new clinical trial to test high-dose vitamin C in patients with COVID-19. Critical Care 24: 1-2.
Chen J et al., 2020. Antiviral activity and safety of Darunavir/Cobicistat for the treatment of COVID-19. Open Forum Infectious Disease 7: ofaa241.
Cheng RZ, 2020. Can early and high intravenous dose of vitamin C prevent and treat coronavirus disease 2019 (COVID-19)? Medical Drug Discovery 5: 100028.
Chiang DY et al., 2008. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Research 68: 6779–6788.
Chiba S, 2020. Effect of early Oseltamivir on COVID-19-suspected outpatients without hypoxia. Wien Klin Wochenschr 9 : 1–6.
Chung YH et al., 2020. Covid-19 vaccine frontrunners and their nanotechnology design. ACS Nano 14: 12522-12537.
Coomes EA and H Haghbayan, 2020. Favipiravir, an antiviral for COVID-19? Journal of Antimicrobial Chemotherapy 17 : dkaa171.
Cortegiani A et al., 2020. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. Journal of Critical Care 57: 279–283.
Costedoat-Chalumeau N et al., 2007. Heart conduction disorders related to antimalarials toxicity: an analysis of electrocardiograms in 85 patients treated with hydroxychloroquine for connective tissue diseases. Rheumatology 46: 808–810.
Craven J, 2020. COVID-19 vaccine tracker. Regulatory Affairs Professionals Society.
Deeks ED, 2018. Darunavir/Cobicistat/Emtricitabine/ Tenofovir Alafenamide: A Review in HIV-1 infection. Drugs 78: 1013–1024.
Devaux CA et al., 2020. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? International Journal of Antimicrobial Agents 55: 105938.
Duan K et al., 2020. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proceedings of the National Academy of Sciences USA 117: 9490–9496.
Fala L, 2018. Kevzara (Sarilumab), a New IL-6 receptor antagonist, approved for active rheumatoid arthritis. Value Based Care Rheumatology, Vol 6, No 3.
Fantini J et al., 2020. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: What molecular dynamics studies of virus-host interactions reveal. International Journal of Antimicrobial Agents 56: 106020.
Favalli ZG et al., 2020. Baricitinib for COVID-19: a suitable treatment? The Lancet Infectious Diseases. 20: 1012-1013.
Gao J et al., 2020a. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Bioscience Trends 14: 72-73.
Gao W et al., 2020c. Clinical features and efficacy of antiviral drug, Arbidol in 220 nonemergency COVID-19 patients from East-West-Lake Shelter Hospital in Wuhan: a retrospective case series. Virology Journal 17: 162.
Gautret P et al., 2020. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents 56: 105949.
Giovane RA et al., 2020. Current pharmacological modalities for management of novel coronavirus disease 2019 (COVID-19) and the rationale for their utilization: A review. Reviews in Medical Virology 30 :e2136.
Goldman JD et al., 2020. Remdesivir for 5 or 10 days in patients with severe Covid-19. New England Journal of Medicine 383: 1827–1837.
Gorbalenya AE et al., 2020. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology 5: 536-544.
Gotera C, 2020. Treatment and research lines for the patient with COVID-19. What do we have and where are we going? International Brazilian Journal of Urology 46: 125-132.
Graham BBS, 2020. Availability includes the avoidance of safety pitfalls. Science 368: 945–946.
Grasselli G et al., 2020. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. Journal of American Medical Association 323: 1574–1581.
Han S, 2015. Clinical vaccine development. Clinical and Experimental Vaccine Research 4: 46.
Hashem AM et al., 2020. Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: A narrative review. Travel Medicine and Infectious Diseases 6: 101735.
Hemilä H and E Chalker, 2019. Vitamin C can shorten the length of stay in the ICU: A meta-analysis. Nutrients 11: 708.
Huang C et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395: 497–506.

Jeyanathan M et al., 2020. Immunological considerations for COVID-19 vaccine strategies. Nature Reviews Immunology 20: 615-632.
Jiang F et al., 2020. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). Journal of General Intern Medicine 35: 1545–1549.
Kakuda TN et al., 2015. Darunavir/cobicistat once daily for the treatment of HIV. Expert Review of Anti-infective Therapy 13: 691-704.
Kaur SP and V Gupta, 2020. COVID-19 vaccine: A comprehensive status report.Virus Research 13: 198114.
Keech C et al., 2020. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. New England Journal of Medicine doi: 10.1056/ nejmoa2026920.
Keyaerts E et al., 2004. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochemical and Biophysical Research Communications 323: 264–268.
King A et al., 2020. Anakinra in COVID-19: Important considerations for clinical trials. Lancet Rheumatology 2: e379–e381.
Kok CR and Hutkins R, 2018. Yogurt and other fermented foods as sources of health-promoting bacteria. Nutrition Review 76: 4–15.
Krantz SG and Srinivasa Rao ASR, 2020. Level of underreporting including underdiagnosis before the first peak of COVID-19 in various countries: Preliminary retrospective results based on wavelets and deterministic modeling. Infection Control Hospital Epidemiology 9 : 1–3.
Kremsner P et al., 2020. Phase 1 assessment of the safety and immunogenicity of an mRNA-lipid nanoparticle vaccine candidate against SARS-CoV-2 in human volunteers. medRxiv 2020.11.09.20228551.
Lehtoranta L et al., 2014. Specific probiotics and virological findings in symptomatic conscripts attending military service in Finland. Journal of Clinical Virology 60: 276–281.
Li X et al., 2020. Molecular immune pathogenesis and diagnosis of COVID-19. Journal of Pharmaceutical Analysis 10: 102–108.
Liqian MO and Zheng P, 2020. Chloroquine phosphate: therapeutic drug for COVID-19. Journal of Southern Medical University 40: 586-594.
Logunov DY et al., 2020. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. The Lancet 396: 887-897.
Lu C et al., 2020. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet (London, England) 395: e39.
Lundstrom K, 2020. Self-amplifying RNA viruses as RNA vaccines. International Journal of Molecular Science 21: 5130.
Luo G and SJ Gao, 2020. Global health concerns stirred by emerging viral infections. Journal of Medical Virology 92: 399–400.
Mahalingam S et al., 2020. Development of vaccines for SARS-CoV-2. F1000 Research. 9: F1000 Faculty Rev-991.
Mahase E, 2020. Covid-19: Pfizer vaccine efficacy was 52% after first dose and 95% after second dose, paper shows. British Medical Journal 371: m4826.
Marik PE, 2020. Vitamin C: An essential “stress hormone” during sepsis. Journal of Thoracic Disease 12(Suppl 1): S84-S88.
Mathew S et al., 2021. Platforms exploited for SARS-CoV-2 vaccine development. Vaccines 9: 11. doi: 10.3390/vaccines9010011.
Mavrikakis M et al., 1996. Retinal toxicity in long term hydroxychloroquine treatment. Annals of the Rheumatic Diseases 55: 187–189.
Mehta P et al., 2020. COVID-19: Consider cytokine storm syndromes and immunosuppression. The Lancet 395: 1033-1034.
Meini S et al., 2020. Role of Lopinavir/Ritonavir in the treatment of Covid-19: A review of current evidence, guideline recommendations, and perspectives. Journal Clinical Medicine 9: 2050.
Meo SA et al., 2020. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. European Review for Medical and Pharmacological Sciences 24: 2012–2019.
Mulligan MJ et al., 2020. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature 586: 589–593.
Nojomi M et al., 2020. Effect of Arbidol (Umifenovir) on COVID-19: a randomized controlled trial. BMC Infectious Diseases 20: 954.
Normile D, 2020. Development of unique Australian COVID-19 vaccine halted. Science doi: 10.1126/science.abg1208.
Olaimat AN et al., 2020. The potential application of probiotics and prebiotics for the prevention and treatment of COVID-19. npj Science of Food 4: 1–7.
Palacios R et al., 2020. Double-blind, randomized, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of treating healthcare professionals with the adsorbed COVID-19 (inactivated) vaccine manufactured by Sinovac–PROFISCOV: A structured summary of a study protocol for a randomised controlled trial. Trial 21: 1-3.
Pang J et al., 2021. Efficacy and tolerability of bevacizumab in patients with severe Covid-19. Nature Communications 12: 814.
Patra JK et al., 2018. Nano based drug delivery systems: Recent developments and future prospects 10 Technology 1007 Nanotechnology 03 Chemical Sciences 0306. Journal of Nanobiotechnology 16: 71.
Pieroni M et al., 2011. Chloroquine-induced transition from dilated to restrictive cardiomyopathy. Journal of the American College of Cardiology 57: 515.
Pullano G et al., 2020. Novel coronavirus (2019-nCoV) early-stage importation risk to Europe, January 2020. Eurosurveillance 25: 2000057.
Rabi FA et al., 2020. SARS-COV-2 and coronavirus disease 2019: What we know so far. Pathogens 9: 231.
Rahmani H et al., 2020. Interferon β-1b in treatment of

severe COVID-19: A randomized clinical trial. International Immunopharmacology 88: 106903.
Ramos-Casals M, et al., 2014. Adult haemophagocytic syndrome. The Lancet. Lancet Publishing Group. pp: 1503–1516.
Rego GNA et al., 2020. Current clinical trials protocols and the global effort for immunization against SARS-CoV-2. Vaccines 8: 474.
Rezac S et al., 2018. Fermented foods as a dietary source of live organisms. Frontiers in Microbiology 9: 1785.
Richmond P et al., 2020. A first-in-human evaluation of the safety and immunogenicity of SCB-2019, an adjuvanted, recombinant SARS-CoV-2 trimeric S-protein subunit vaccine for COVID-19 in healthy adults; a phase 1, randomised, double-blind, placebo-controlled trial. medRxiv 2020.12.03.20243709.
Rocco PRM etal., 2020. Early use of nitazoxanide in mild Covid-19 disease: randomised, placebo-controlled trial. European Respiratory Journal 2003725.
Rodriguez-Morales AJ et al., 2020. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Medicine and Infectious Disease 34: 101623.
Ruan Q et al., 2020. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine 46: 846-848.
Rynes RI, 1997. Antimalarial drugs in the treatment of rheumatological diseases. Rheumatology 36: 799-805.
Saad N et al., 2013. An overview of the last advances in probiotic and prebiotic field. LWT-Food Science Technology 50: 1–16.
Sabato LA et al., 2017. Restrictive cardiomyopathy associated with long-term use of hydroxychloroquine for systemic Lupus erythematosus. Journal of Pharmacy Practice 30: 571–575.
Sahebnasagh A et al., 2020. Pharmacological treatments of COVID-19. Pharmacological Reports 72: 1446–1478.
Sanders JM et al., 2020. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): A review. Journal of the American Medical Association 323: 1824-36.
Savarino A et al., 2003. Effects of chloroquine on viral infections: An old drug against today's diseases. The Lancet Infectious Diseases. 3: 722-727.
Sencio V et al., 2020. Gut dysbiosis during influenza contributes to pulmonary pneumococcal superinfection through altered short-chain fatty acid production. Cell Repair 30: 2934–2947.
Seo YB et al., 2020. Soluble Spike DNA vaccine provides long-term protective immunity against SAR-CoV-2 in mice and nonhuman primates. bioRxiv 2020.10.09.334136.
Sharma O et al., 2020. A Review of the progress and challenges of developing a vaccine for COVID-19. Frontiers in Immunology 11: 2413.
Sheahan TP et al., 2020. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nature Communications 11: 1-4.
Shen C et al., 2020. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. Journal of the American Medical Association 323: 1582–1589.
Shimabukuro-Vornhagen A et al., 2018. Cytokine release syndrome. Journal for Immunology of Cancer 6: 1-4.
Smith T etal., 2020. Rapid development of a synthetic DNA vaccine for COVID-19. doi: 10.21203/rs.3.rs-16261/v1.
Snawerdt J et al., 2020. Therapeutic options for the treatment of coronavirus disease (COVID-19). Critical Care Nursing Quarterly 43: 349–368.
Soo YOY et al., 2004. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. Clinical Microbiology and Infection 10: 676–678.
Srinivasa A et al., 2017. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: A brand-related issue? The Journal of Rheumatlogy 44: 398.
Stebbing J et al., 2020. COVID-19: Combining antiviral and anti-inflammatory treatments. The Lancet Infectious Diseases 20: 400-402.
Sadoff J et al., 2021. Interim results of a phase 1–2a trial of Ad26.COV2.S Covid-19 vaccine. New England Journal of Medicine 383: 1920-1931.
Tahir AH et al., 2020. Nutraceuticals and herbal extracts: A ray of hope for COVID-19 and related infections (Review). International Journal of Functional Nutrition 1: 6.
Tan Q 2020. Is Oseltamivir suitable for fighting against COVID-19: *In silico* assessment, *in vitro* and retrospective study. Bioorg Chem 104: Article #104257.
Tebas P et al., 2020. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, phase 1 clinical trial. EClinical Medicine 100689.
Teimury A and Khaledi EM, 2020. Current options in the treatment of Covid-19: A review. Risk Management and Healthcare Policy 13: 1999–2010.
Turner RB et al., 2017. Effect of probiotic on innate inflammatory response and viral shedding in experimental rhinovirus infection--a randomised controlled trial. Beneficial Microbes 8: 207.
Ucciferri C et al., 2020. Role of monoclonal antibody drugs in the treatment of COVID-19. World Journal of Clinical Cases 8: 4280–4285.
Villar J et al., 2020. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. The Lancet Respiratory Medicine 8: 267–276.
Vincent MJ et al., 2005. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virology Journal 2: 69.
Wan Y et al., 2020. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of Virology 94: e00127-20.
Wang J et al., 2020a. The COVID-19 vaccine race: Challenges and opportunities in vaccine formulation. AAPS PharmSciTech 21: 225.
Wang M et al., 2020b. Remdesivir and chloroquine

effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. Cell Research 30: 269–271.

Wang Y et al., 2020c. Assessment of the efficacy and safety of Ribavirin in treatment of coronavirus-related pneumonia (SARS, MERS and COVID-19). Medicine (Baltimore) 99: e22379.

Wang Y et al., 2020d. A retrospective cohort study of methylprednisolone therapy in severe patients with COVID-19 pneumonia. Signal Transduction and Targeted Therapy 5: Article # 57.

Worldometer, 2020. Coronavirus update (live): 121,423,007 cases and 2,684,813 deaths from COVID-19 virus pandemic - Worldometer.

Wu C et al., 2020. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Internal Medicine 180: 934.

Wu S et al., 2020b. A single dose of an adenovirus-vectored vaccine provides protection against SARS-CoV-2 challenge. Nature Communications 11: 1–7.

Xia S 2020. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infectious Diseases 21: 39–51.

Yadav P et al., 2020. Full-genome sequences of the first two SARS-CoV-2 viruses from India. Indian Journal of Medical Research 151: 200–209.

Yang S et al., 2020. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD 1 protein vaccine against COVID-19 in adults: Pooled analysis of two randomized, 2 double-blind, placebo-controlled, phase 1 and 2 trials 3 4. medRxiv 2020.12.20.20248602.

Yao X etal., 2020. *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clinical Infectious Diseases 71: 732–739.

Yeleswaram S et al., 2020. Inhibition of cytokine signaling by ruxolitinib and implications for COVID-19 treatment. Clinical Immunology 218: Article # 108517.

Zafar N et al., 2020. Probiotics: Helpful for the prevention of COVID-19? Biomedical Research and Therapy 7: 4086–4099.

Zelaya H, 2016. Respiratory antiviral immunity and immunobiotics: beneficial effects on inflammation-coagulation interaction during influenza virus infection. Frontiers in Immunology 7: 633.

Zhai Y et al., 2005. Insights into SARS-CoV transcription and replication from the structure of the nsp7--nsp8 hexadecamer. Nature Structural & Molecular Biology 12: 980–986.

Zhou F etal., 2020a. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet 395: 1054–1062.

Zhou W et al., 2020b. Potential benefits of precise corticosteroids therapy for severe 2019-nCoV pneumonia. Signal Transduction and Targeted Therapy 5: Article # 18.

Zhu Z et al., 2020. From SARS and MERS to COVID-19: A brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. Respiratory Research 21: 1–14.

Zolnikova O et al., 2018. Application of probiotics for acute respiratory tract infections. Italian Journal of Medicine, 12: 32-38.

Zu ZY, 2020. Coronavirus disease 2019 (COVID-19): A perspective from China. Radiology 296: 15-25.