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

Sidra Altaf 1 and Humaira Muzaffar 2

1 Department of Pharmacy, University of Agriculture, Faisalabad; 2 Department of Physiology, GC University, Faisalabad, Pakistan

*Corresponding author: sidraaltaf8@yahoo.com

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 (Zhu 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-Moraes 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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trial Registration No.</th>
<th>Phase</th>
<th>Action</th>
<th>Posology</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Antiviral drugs</strong></td>
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<tr>
<td>Remdesivir</td>
<td>NCT04292899</td>
<td>III</td>
<td>Inhibits RdRp polymerase inhibiting RNA synthesis</td>
<td>200 mg, OD, IV for 1 day followed by 100 mg, OD, IV for next 4-9 days</td>
<td>(Goldman et al. 2020; Sheahan et al. 2020)</td>
</tr>
<tr>
<td>Lopinavir + Ritonavir</td>
<td>ChiCTR2000029539</td>
<td>II</td>
<td>Lopinavir: Inhibits 3CL protease activity. Blockage of protein processing</td>
<td>Ritonavir: 400 mg oral followed by 600 mg oral BD oral for 14 days</td>
<td>(Cao et al. 2020)</td>
</tr>
<tr>
<td>Favipiravir</td>
<td>ChiCTR2000030254</td>
<td>Completed and recommended</td>
<td>Antiviral, RNA-dependent RNA polymerase inhibitor</td>
<td>1600 mg Oral BD for day 1, followed by 600 mg oral BD till the trial end</td>
<td>(Agrawal et al. 2020)</td>
</tr>
<tr>
<td>Ivermectin + Nitazoxanide</td>
<td>NCT04360356</td>
<td>II</td>
<td>Ivermectin: Antiviral/Antiparasitic inhibits viral replication and assembly of new virions. Nitazoxanide: Antiviral, Antiparasitic, interferes with 3CL protease activity</td>
<td>Ivermectin 200 mcg/kg OD oral, empty stomach + Nitazoxanide 500 mg BD oral during meal for 6 days</td>
<td>(Patra et al. 2018)</td>
</tr>
<tr>
<td>Darunavir/ Cobicistat</td>
<td>NCT04252274</td>
<td>III</td>
<td>Protease inhibitor Darunavir: Cobicistat: Cytochrome P-450 CYP3A Inhibitors</td>
<td>800mg/150 mg – one tablet oral for 5 days</td>
<td>(Chen et al. 2020)</td>
</tr>
<tr>
<td>Arbidol (Umifenovir)</td>
<td>NCT04260594</td>
<td>IV</td>
<td>14-20 days course of 2 tablets TID</td>
<td></td>
<td>(Gao et al. 2020)</td>
</tr>
<tr>
<td>Ribavirin + Interferon beta-1B</td>
<td>NCT04494399</td>
<td>II</td>
<td>S/C injection of interferon beta-1B 16 million IU for 5 days</td>
<td>Ribavirin: inhibit capping of viral transcripts and viral polymerase Interferon beta-1B: Enhance activity of suppressor T cell and reduce proinflammatory cytokines</td>
<td>(Brzoska et al. 2020; Rahmani et al. 2020)</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>NCT04516915</td>
<td>II</td>
<td>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</td>
<td>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.</td>
<td>(Tan et al. 2020)</td>
</tr>
<tr>
<td><strong>Antimalarial drugs</strong></td>
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<tr>
<td>Chloroquine</td>
<td>NCT04255336 / NCT04286503</td>
<td>II</td>
<td>500 mg/dose orally BD for 7-14 days</td>
<td>Increase the endosomal pH and changes ACE-2 glycosylation, interfere with interaction of virus and receptor.</td>
<td>(Fantini et al. 2020; Snawerdt et al. 2020)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>NCT04265917 / NCT04328272</td>
<td>III</td>
<td>400 mg OD for 5 days</td>
<td>Enhance endosomal pH and changes glycosylation of ACE-2, interfere with interaction of virus and receptor.</td>
<td>(Hashem et al. 2020)</td>
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<tr>
<td><strong>Anti-interleukin drugs</strong></td>
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<tr>
<td>Tocilizumab</td>
<td>NCT0437092 / NCT04320605 / NCT04356937</td>
<td>II</td>
<td>1 IV infusion, dose 8 mg/kg, up to max dose of 800 mg. 1 additional dose could be administered if clinical condition worsens with no improvement.</td>
<td>monoclonal antibody which competitively impedes the binding of interleukin-6 to its receptor.</td>
<td>(Gotera 2020; Sahebnasagh et al. 2020; Ucciferri et al. 2020)</td>
</tr>
<tr>
<td>Sarilumab</td>
<td>NCT04315298 / NCT04327388</td>
<td>II</td>
<td>Single dose IV on day 1 and second dose may be given after 24-48 hrs of first dose.</td>
<td>Human recombinant monoclonal antibody which blocks IL-6 receptors, inhibiting IL-6–mediated signaling.</td>
<td>(Fala 2018)</td>
</tr>
<tr>
<td>Drug/Combination</td>
<td>Phase</td>
<td>Details</td>
<td>Description</td>
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<tr>
<td>Bevacizumab NCT04275414</td>
<td>II</td>
<td>Bevacizumab 500mg + normal saline (NS) 100ml, ivdrip ≥ 90min</td>
<td>It inhibits angiogenesis by targeting and blocking vascular endothelial growth factor A (VEGF-A). (Pang et al. 2021)</td>
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<tr>
<td>Anakinra NCT04341584</td>
<td>II</td>
<td>At day 1, day 2 and day 3; Two IV infusions (200 mg) / day At Day 4, two IV infusions (100mg)/ day.</td>
<td>Interleukin 1 receptor antagonist, inhibits the production of inflammatory cytokines (King et al. 2020)</td>
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<tr>
<td>Janus Kinase inhibitors</td>
<td></td>
<td>2 x 10mg with defined response adapted dose increase to 2 x 20mg for 7 days</td>
<td>Inhibit competitively ATP-binding catalytic site of the kinase domain. Inhibit of the JAK-STAT pathway reversibly inhibits JAK1 and JAK2, block phosphorylation and activation of signal transducers and activators of transcription (STATs) (Favalli et al. 2020)</td>
<td></td>
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</tr>
<tr>
<td>Ruxolitinib NCT04348071</td>
<td>III</td>
<td>Daily dose by mouth for 14 days in combination with antivirals</td>
<td>Binding of the transfused antibodies to the pathogenic organism, leading to phagocytosis, cellular cytotoxicity, or direct pathogen neutralization (Bloch et al. 2020)</td>
<td></td>
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</tr>
<tr>
<td>Convalessent anti-SARS-CoV-2 plasma NCT04345289</td>
<td>III</td>
<td>Reported dosage varied depending upon the amount of transfused plasma and antibody titer</td>
<td>(Costedoat 2020)</td>
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</table>

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 (Savarrino 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 (EC50: 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...
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 (IPIo), tumor necrosis factor α (TNF-α), granulocyte colony-stimulating factor, monocye 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 (NCT04244545, NCT04331004, NCT04331795, NCT04332913, NCT04340575, NCT04339712, NCT04320615, NCT04335071, NCT04327773, NCT0437092, NCT04310228, NCT04306705, NCT04335305, NCT0435480, 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 (NCT04386905, NCT04334044, NCT04331665, 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 ssp. bulgaris, L. fermentum, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus; Lactococcus lactis; Streptococcus thermophilus, Leuconostoc mesenteroides, Pediococcus pentaceaeus, 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. alcalophilus, B. cereus, B. coagulans, B. clausii and Escherichia coli. The probiotic yeast include Saccharomyces cerevisiae and S. bouardii (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 pentaceaeus, Leuconostoc plantarum, L. paracasei ssp. Paracasei, L. mesenteroides (Lehortanta 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

<table>
<thead>
<tr>
<th>Sr</th>
<th>Vaccine Name</th>
<th>Type</th>
<th>NCT Ref No.</th>
<th>Route</th>
<th>Target</th>
<th>Principal Developer</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZF2001</td>
<td>Protein subunit</td>
<td>NCT04646590</td>
<td>IM</td>
<td>RBD protein and virus neutralizing antibodies S protein</td>
<td>Anhui Zhifei Longcom Biologic Pharmacy Co, China</td>
<td>Phase III trials in China, Uzbekistan, Indonesia, Pakistan and Ecuador, 3 doses</td>
<td>(Yang et al. 2020)</td>
</tr>
<tr>
<td>2</td>
<td>ChAdOx1 nCoV-19 (AZD-1222)</td>
<td>Adenovirus vector</td>
<td>NCT04316746</td>
<td>IM</td>
<td>S protein</td>
<td>Collaboration of Oxford University and Astra Zaneca, UK</td>
<td>Under Phase III trials</td>
<td>(Jeyanathan et al. 2020; Wang et al. 2020a)</td>
</tr>
<tr>
<td>3</td>
<td>Ad5-nCoV</td>
<td>Adenovirus vector</td>
<td>NCT04341389</td>
<td>IM</td>
<td>S protein</td>
<td>CanSino Biologics, Beijing, China</td>
<td>Phase II trials, for adults aged 18 years and above, single dose</td>
<td>(Wu et al. 2020b)</td>
</tr>
<tr>
<td>4</td>
<td>mRNA-1273</td>
<td>LNP, Lipid-RNA nanoparticle</td>
<td>NCT04283461</td>
<td>IM</td>
<td>S protein</td>
<td>Moderna, NIAID, USA</td>
<td>Phase I, II, III, trials, fully synthetic, no risk of disease transmission, 2 doses, 2-year immunity</td>
<td>(Baden et al. 2020)</td>
</tr>
<tr>
<td>5</td>
<td>CoronaVac (PiCoVac)</td>
<td>Inactivated virus</td>
<td>NCT04352608</td>
<td>IM</td>
<td>Multiple surface antigens</td>
<td>Sinovac Biotech, Beijing, China</td>
<td>Phase I, II, III trials, for adults 18-59 years, 2 doses</td>
<td>(Palacios et al. 2020)</td>
</tr>
<tr>
<td>6</td>
<td>NVX-CoV2373</td>
<td>Recombinant protein nanoparticles using Matrix-M adjuvant</td>
<td>NCT04368988</td>
<td>IM</td>
<td>S protein and virus neutralization</td>
<td>Novavax, USA</td>
<td>Phase I, II trials, for adults aged 18-84 years, 2 doses</td>
<td>(Keech et al. 2020a; b)</td>
</tr>
<tr>
<td>7</td>
<td>BNT162b1</td>
<td>LNP, Lipid-RNA nanoparticle</td>
<td>NCT04523571</td>
<td>IM</td>
<td>RBD of S protein</td>
<td>BioNTech (Germany), Pfizer, Fosun (China)</td>
<td>Phase II, III trials, after Phase I, approved for emergency use in UK, USA and Singapore, 2 doses</td>
<td>(Mulligan et al., 2020)</td>
</tr>
<tr>
<td>8</td>
<td>BBIBP-CorV</td>
<td>Inactivated virus</td>
<td>NCT04560881</td>
<td>IM</td>
<td>Multiple neutralizing antibodies S protein</td>
<td>Sinopharm, Beijing, China</td>
<td>Large scale Phase III trials in China and UAE, 2 doses</td>
<td>(Xia et al. 2020)</td>
</tr>
<tr>
<td>9</td>
<td>INO-4800</td>
<td>Plasmid DNA</td>
<td>NCT0462638</td>
<td>IM</td>
<td>Multiple neutralizing antibodies S protein</td>
<td>Inovio and Advaccine, China</td>
<td>Phase I, II, III trials, 2 doses with electroporation</td>
<td>(Smith et al. 2020; Tebas et al. 2020)</td>
</tr>
<tr>
<td>10</td>
<td>Inactivated SARS-CoV-2 Vaccine (Vero Cell)</td>
<td>Inactivated virus</td>
<td>NCT04477781</td>
<td>IM</td>
<td>Multiple neutralizing antibodies S protein</td>
<td>China National Biotec, China</td>
<td>Phase III trials in China, 2 doses</td>
<td>(Jeyanathan et al. 2020)</td>
</tr>
<tr>
<td>11</td>
<td>Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine</td>
<td>LNP, Lipid-sRNA nanoparticle</td>
<td>N/A</td>
<td>IM</td>
<td>Multiple neutralizing antibodies S protein</td>
<td>Imperial College Ventures, China</td>
<td>Phase I and II trials in UK, 2 doses</td>
<td>(Jeyanathan et al. 2020)</td>
</tr>
<tr>
<td>12</td>
<td>Inactivated SARS-CoV-2 Vaccine</td>
<td>Inactivated virus</td>
<td>NCT04470609</td>
<td>IM</td>
<td>Multiple neutralizing antibodies S protein</td>
<td>Qihan Li, Chinese Academy of Medical Sciences, China</td>
<td>Phase I and II trials in China, 2 doses</td>
<td>(Clinical Trials, n.d.)</td>
</tr>
<tr>
<td>13</td>
<td>CVnCoV</td>
<td>LNP, Lipid-RNA nanoparticle</td>
<td>NCT04652102</td>
<td>IM</td>
<td>S protein</td>
<td>CureVac, Germany</td>
<td>Phase III trials, for adults aged 18 years and older, 2 doses</td>
<td>(Kremersner et al. 2020)</td>
</tr>
<tr>
<td>14</td>
<td>GamCOVID-Vac-Lyo</td>
<td>Adenovirus vector, 2 viruses (rAd26, rAd5) heterologous</td>
<td>NCT04437875</td>
<td>IM</td>
<td>S protein</td>
<td>Gamaleya Research Institute of Epidemiology and Microbiology Russia</td>
<td>Phase II trials, approved for distribution in Russia, single dose and prime boost dose</td>
<td>(Logunov et al. 2020)</td>
</tr>
<tr>
<td>15</td>
<td>GX-19</td>
<td>Plasmid DNA</td>
<td>NCT04445389</td>
<td>IM</td>
<td>S protein</td>
<td>Genexine Consortium, Korea</td>
<td>Phase II trials in South Korea, 2 doses</td>
<td>(Kaur and Gupta 2020; Seo et al. 2020)</td>
</tr>
<tr>
<td>16</td>
<td>SCB-2019</td>
<td>Recombinant trimeric S protein</td>
<td>NCT04405908</td>
<td>IM</td>
<td>S protein</td>
<td>Clover Biopharmaceuticals (China), GSK (UK) and Dynavax (USA)</td>
<td>Phase I trials, 2 doses</td>
<td>(Richmond et al. 2020)</td>
</tr>
<tr>
<td>17</td>
<td>COVID-19 vaccine</td>
<td>Protein subunit</td>
<td>NCT04445921 NCT04466085</td>
<td>IM</td>
<td>Dimeric RBD</td>
<td>Anhui Zhifei Longcom Biologic Pharmacy Co, China</td>
<td>Phase I, II trials in China, 2 or 3 doses under trials (Jeyan Nathan et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>ARCoV</td>
<td>mRNA</td>
<td>N/A</td>
<td>IM</td>
<td>S protein</td>
<td>Suzhou Abogen Biosciences, Walvax Biotechnology and Academy of Military Medical Sciences, China AnGes, Inc., Japan</td>
<td>Phase I trials in China, 2 doses (Jeyan Nathan et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>AG0301-COVID19</td>
<td>Plasmid DNA</td>
<td>NCT04463472</td>
<td>IM</td>
<td>S protein</td>
<td>University of Queensland, Australia</td>
<td>Phase I, II trials in China, 2 doses (Jeyan Nathan et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>VIR-7831 (recombinant coronavirus-like particle covid-19 vaccine)</td>
<td>Plant-based virus-like particle</td>
<td>NCT04450004</td>
<td>IM</td>
<td>Multiple viral antigens</td>
<td>Medicago and Laval University, Canada</td>
<td>Phase I trials in Canada, for adults aged 18-55 years, 2 doses (Rego et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Lunar-COV19 (ART-021)</td>
<td>mRNA</td>
<td>NCT04480957</td>
<td>IM</td>
<td>S protein</td>
<td>Arcturus Therapeutics and Duke-NUS Medical School, Singapore</td>
<td>Phase I trials in Singapore, for adults aged 21-80 years, single dose (Baviskar et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Covaxin (BBV152A, BBV152B and BBV152C)</td>
<td>Inactivated whole virion</td>
<td>NCT0447519</td>
<td>IM</td>
<td>Multiple viral antigens</td>
<td>Bharat Biotech and Indian Council for Medical Research, India</td>
<td>Phase I trials in India, 2 doses (Rego et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>ZyCoV-D</td>
<td>Plasmid DNA</td>
<td>N/A</td>
<td>Intradermal</td>
<td>S protein</td>
<td>Zyduz Cadila Healthcare, India</td>
<td>Phase I, II trials in India, 3 doses (Jeyan Nathan et al. 2020; Kaur and Gupta 2020) (Normile 2020)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>SARS-CoV-2 Sclamp (COVID-19) Vaccine</td>
<td>Protein subunit</td>
<td>NCT04495933</td>
<td>IM</td>
<td>Molecular clamp-stabilized S protein</td>
<td>University of Queensland, Australia</td>
<td>Phase I trials, 2 doses, development halted after unintended results in Phase I (Sadoff et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>KB-201 COVID-19 Vaccine</td>
<td>Protein subunit</td>
<td>NCT04473690</td>
<td>IM</td>
<td>RBD-based protein</td>
<td>Kentuck BioProcessing, USA</td>
<td>Phase I, II trials, 2 doses (Mathew et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>IAVI-Merck COVID-19</td>
<td>VSV vectored</td>
<td>N/A</td>
<td>IM and oral</td>
<td>S protein</td>
<td>Merck and IAVI, USA</td>
<td>Phase I, II, single dose (Mahalingam et al. 2020) (Jeyan Nathan et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>COVAX-9 (Monovalent Recombinant COVID-19)</td>
<td>Protein subunit</td>
<td>NCT04453852</td>
<td>IM</td>
<td>S protein</td>
<td>Vaxine (Australia) and Medityco (South Korea)</td>
<td>Phase I in Australia, single dose, development halted due to lack of funds (Mathew et al. 2021) (Ashraf et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>MVC-COV191</td>
<td>Protein subunit</td>
<td>NCT04487210</td>
<td>IM</td>
<td>S protein</td>
<td>Medigen Vaccine Biologics (Taiwan)</td>
<td>Phase I in Taiwan, 2 doses (Mathew et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Covigenix VAX-001</td>
<td>Plasmid DNA</td>
<td>NCT04591884</td>
<td>IM</td>
<td>Multiple epitopes</td>
<td>Entos Pharmaceuticals, Canada</td>
<td>Phase I trials in Canada, 2 doses (Alturki et al. 2021)</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>bacTRL-Spike</td>
<td>Bacterial vector</td>
<td>NCT04334980</td>
<td>Oral</td>
<td>S protein</td>
<td>Symvivo Corporation, Canada</td>
<td>Phase I trials, (Alturki et al. 2021)</td>
<td></td>
</tr>
</tbody>
</table>

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.

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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.

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