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Shweta Jain
Sir Madan Lal Institute of Pharmacy, Etawah (U.P.) India;

Pankaj Kumar Jain
Community Medicine, Uttar Pradesh University of Medical Sciences, Saifai, Etawah (U.P.) India

Ramakant Yadav
Department of Neurology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah (U.P.) India

Surendra Kumar Jain
Sagar Institute of Research and Technology–Pharmacy,Bhopal (M.P.) India

Ankur Vaidya
Pharmacy College Saifai, Uttar Pradesh University of Medical Sciences, Saifai, Etawah (U.P.) India

Correspondence

Ankur Vaidya
Pharmacy College Saifai, Uttar Pradesh University of Medical Sciences, Saifai, Etawah (U.P.) India

E mail:
ankur_vaidya2000@yahoo.co.in

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COVID-19 pandemic: The deadly respiratory disease of 21st century

Shweta Jain, Pankaj Kumar Jain, Ramakant Yadav, Surendra Kumar Jain, Ankur Vaidya

ABSTRACT

The sudden outbreak of 2019 novel coronavirus (2019-nCoV) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated from Wuhan, China. SARS-CoV-2 causes severe respiratory illness and becomes a major threat for humanity. Recently the entire scientist, researchers and physicians all over the countries focused to find the treatment of this pandemic disease. Numerous drugs and or vaccines have been trialed for prevention and treatment against 2019-nCoV but no therapy has been shown effective to date. Currently, numerous vaccines are under clinical investigation and mRNA-1273 vaccine (LNP-encapsulated mRNA vaccine encoding S protein) from Moderna is ahead. Although chloroquine, hydroxychloroquine, remdesivir and many other drugs had recommended against SARS-CoV-2, but still they are not the guarantee treatment of COVID-19. Recently, India, America, Russia and China introduced vaccines against COVID-19 in the market, however assurance of their 100% effectiveness are doubtful. The speed of daily new cases threatens the world and urges the scientist to crack this pandemic condition.

KEYWORDS 2019-nCoV; Chloroquine; COVID-19; Moderna; Respiratory disease; Remdesivir

1. INTRODUCTION

Humans have faced lots of virus epidemics since ancient era. Smallpox and measles viruses are among the oldest that infect humans. The first virus epidemic wiped out was reported in China about 5000 years ago. At the early age of civilization the first epidemic virus infection was reported in 1720 Plague attack, while exact after 100 years in the 1820 Cholera attacked and in the 1920 Spanish flu.The history seems to repeat again, exactly after 100 years deadly virus named Coronavirus attacked China, till now millions of people got infected and thousands of people were died with this disease. Scientist identified and reported 12 worst killer viruses. These include Ebola, Rabies, HIV, Smallpox, Hantavirus, Influenza, Dengue, MERS-CoV, Rotavirus, SARS-CoV and SARS-CoV-2. Although few of these virus infections have suitable treatment including vaccines and antiviral drugs, yet for some infection treatment is still under investigation.[1-4]

Coronaviruses are a group of related RNA viruses, which may cause respiratory tract infections from mild illness (usually caused by rhinoviruses) to lethal illness (caused by MERS, SARS and COVID-19). Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or 2019 novel coronavirus (2019-nCoV). SARS-CoV-2 primarily infects the lungs and causes severe respiratory illness in the individuals. In severe cases COVID-19 causes' death due to Acute Respiratory Distress Syndrome (ARDS) and pneumonia. It is important to remember that it does not lead to ARDS and pneumonia in all the cases, which is an occurrence in most severe cases.[5]

Coronaviruses (CoVs), a member of subfamily *Orthocoronavirinae*. Furthermore, subfamily *Orthocoronavirinae*has four genera including α-CoV (alpha-coronavirus), β-CoV (beta-coronavirus), γ-CoV (gamma-coronavirus) and δ-CoV (delta-coronavirus). Mammals are usually infected by α- and β-CoV genera, birds are infected by γ-and δ-CoVs. Ramaiah and Arumugaswami reported that this human pathogen SARS-CoV-2 is a member of the beta-coronavirus (β-CoV) genus. β-CoV is believed to evolve from a bat-CoV, carrying 30 kilo base of single positive-sense RNA genome.[6] Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) are the recent outbreaks caused by β-CoVs. Both SARS and MERS epidemics were reported in the China and Saudi Arabia in year 2002 and 2012 respectively and then globally spread. Alike of SARS and MERS, SARS-CoV-2 virus also belongs to the B lineage of the β-CoVs.

COVID-19 pandemic, also known as the coronavirus pandemic, originated in the city of Wuhan, Hubei Province, Central China, in December 2019 and the World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) on 30 January, and a pandemic on 11 March 2020. Since 18 July 2020, approximately 14,213,678 cases of COVID-19 have been reported in over 222 countries, ensuing more than 634,995 deaths. About 8,493,874 people have recovered.

2. STRUCTURE OF SARS-COV-2

COVID-19 is a spherical or pleomorphic cloak, positive-sense, single-stranded RNA with nucleoprotein within a capsid encompassed of matrixprotein. It is the largest genome (size:26 kb-32 kb) of known RNA viruses. The SARS-CoV-2 genome encodes a large non-structure polyprotein (ORF1a/b). Formed non-structure polyprotein further proteolytically cleaved into 15/16 proteins, including 4 structural proteins and 5 accessory proteins (ORF3a, ORF6, ORF7, ORF8 and ORF9) (Fig. 1). The structural proteins are essential for the SARS-CoV-2 assembly and infection includes spike (S) surface glycoprotein, membrane (M) protein (most abundant glycoprotein), envelope (E) protein and nucleocapsid (N) protein. The genes for all four structural proteins occur in the 5'-3' order. Membrane glycoprotein spans three times, resides NH2-terminal outside, while COOH terminus inside the virion. Without entails an S protein, M formulates virus particles.[7] Envelope (E) and Nucleocapsid (N) proteins are usually conserved, while Spike (S) and Membrane (M) get widely mutational changess.

Spike protein has similar genomic sequence of fish Myripristis murdjan and contains 39 nucleotide sequence (5'-aAT GGT GTT GAA GGT TTT AAT TGT TAC TTT CCT TTA CAA Tca-3'), responsible for it's binding to host cells and cleaved by host proteases into an membrane-bound C-terminal S2 region and a N-terminal S1 subunit. This S1 subunit act as a molecular chaperone, contributing to stabilize S2 in the prefusion state by reducing its tendency to transition to the post fusion conformation. The receptor-binding do-main (RBD) of S1 subunit endures hinge-like conformational arrangements, which rapidly expose or hide the determinants of receptor binding. These hides or expose states are termed as "down" (i.e. receptor-inaccessible state, more stable) and "up" (i.e. receptor-accessible state, less stable) conformation respectively. These indispensable functions of S protein make it a suitable target for antibody-mediated neutralization.[8]

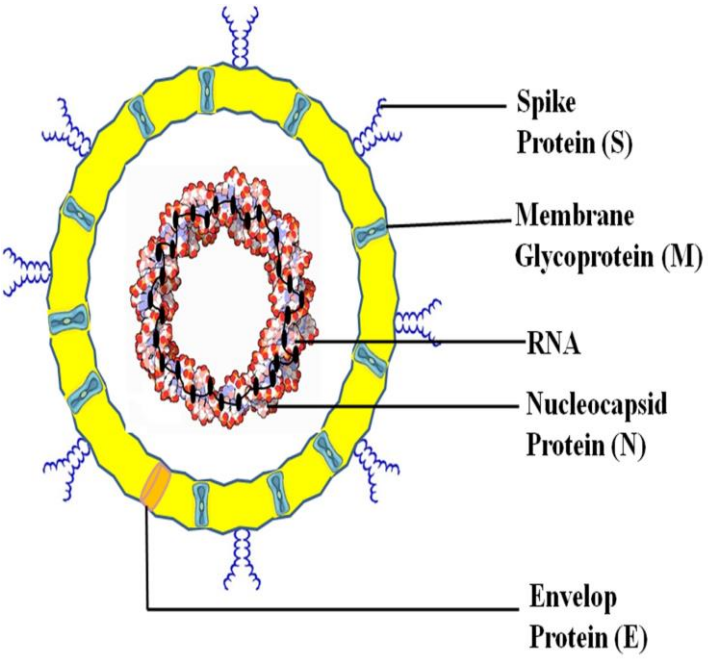

Fig. 1 Structure of SARS-CoV-2 virus representing RNA genome, surrounded by S, M, N and E proteins.

3. REPLICATION PROCESS OF SARS-COV-2

Hoffmann and co-workers reported that SARS-CoV-2 utilizes SARS-CoV-2 receptor angiotensin converting enzyme 2 (ACE2) for access and serine protease (i.e. TMPRSS2) for S protein priming. SARS-CoV-2 attached to ACE2 by its Spike and permits COVID-19 to go inside and infect cells. ACE2 is a type I transmembrane metallocarboxy peptidase, expressed in the lung, kidney, and gastrointestinal tract, and SARS-CoV-2 could utilize ACE2 from humans, to add access into ACE2-expressing lung HeLa cells.[9] Since the SARS-CoV-2 spike binds more firmly (usually10–20-fold higher) as compared to SARS-CoV spike to human ACE2, responsible for easier spread in humans. After the entry of virus into host cell, it uncoats and replicates rapidly and

elicits a burly immune response, ensuing in cytokine storm and pulmonary tissue damage. Cytokine storm results hypercytokinaemia and abrupt produces pro-inflammatory cytokines, which is responsible for acute respiratory distress syndrome (ARDS) and multiple organ failure. Continuous RNA synthesis requires for replication of coronavirus genome. The replicase complex encompasses number of cellular proteins and 16 viral subunits. Interestingly, coronavirus replication process requires some different RNA processing enzymes that are exclusive for coronavirus and not reported in other RNA viruses. These rarely found coronaviruses RNA processing enzymes includes 3'-to-5' exoribonuclease, ADP ribose 1'-phosphatase, 2'-*O*-ribose methyltransferase and cyclic phosphodiesterase. The formed RNA genome incorporates in assembled proteins and form mature particle to further invade flanking cell (Fig. 2).

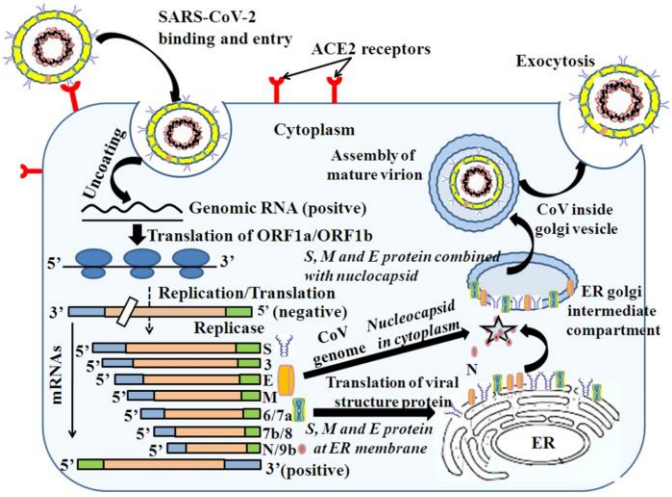

Fig. 2 Schematic diagram of replication process of SARS CoV-2 virus inside the human cell.

4. ORIGIN OF SARS-COV-2

Animals and bats have been reported as the natural cistern hosts of numerous virus and these animals/bats play a important role in transmitting numerous viruses. MERS, Ebola, SARS, Nipah, Coronavirus and many other viruses have been reported to originate through animals and or bats. Although SARS-CoV-2 is believed to originate from bats, however still confirmation requires. Very recently WHO announce that the first SARS-CoV-2 case was linked to the human seafood market in Wuhan city, China. Numerous studies suggested that bats are the natural host of SARS-CoV-2.[10] Zhou et al. reported that bats are potential host of SARS-CoV-2 possibly due to 96% identical 2019-nCoV genome to a bat coronavirus, whereas minks may be the intermediate host for SARS-CoV-2.[11] Furthermore, Lam et al. reported 85.5%-92.4% similar 2019-nCoV genome to pangolin coronavirus genomes and suggested pangolin as possible transitional host for SARS-CoV-2.[12] The above reported studies need further research to confirm that whether SARS-CoV-2 is directly transmitted from bats or by an intermediate host. Discovering the source of SARS-CoV-2 will assist to manage its spread (Fig. 3).

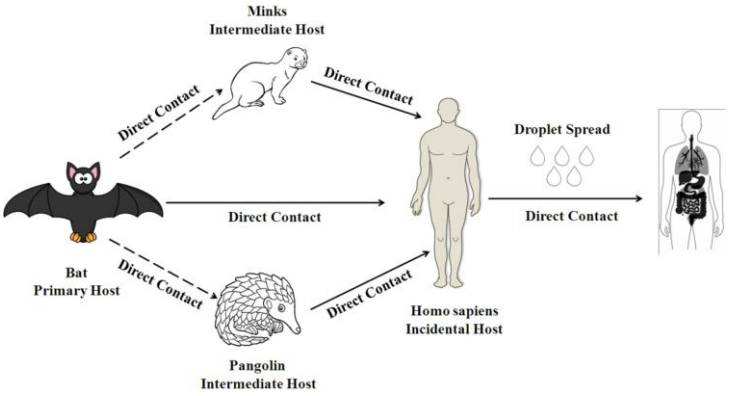

Fig. 3 Hypotheses of origin and transfer of SARS-CoV-2 virus from bat to human.

5. TRANSMISSION ROUTE OF SARS-COV-2 VIRUS

According to WHO guidelines COVID-19 can be transmitted primarily from human to human through aerosol droplets when a COVID-19 positive patient coughs, sneezes, or speaks. These droplets enter from the nose or mouth and people get infected with COVID-19. The transmission resulted from direct or indirect contact with mucous membranes in the eyes, nose or mouth. Since the SARS-CoV-2 binds with ACE2, which is overexpressed in the mucus membrane of the digestive tract, thus digestive tract is impending route of SARS-CoV-2 infection moreover to respiratory tract. Furthermore, SARS-CoV-2 can also be transmitted by touching stained objects or surfaces. However, the transmission of SARS-CoV-2 via breast milk or from pregnant women to infant has not been reported.[13]

6. CLINICAL SYMPTOMS OF SARS-COV-2 INFECTION

The symptoms of COVID-19 can range from mild to severe and seen from 2 - 14 days after exposure. Incubation period is the time between exposures to symptoms and during the incubation period, people may either symptom free or having few symptoms. Direct correlations between COVID-19 and ARDS have been reported. In severe cases of COVID-19 infection leads to ARDS and pneumonia, which may be fatal for the infected individual. ARDS causes dry cough, heavy breathing, breathing difficulties and increased heart rate. Fever, cough and tiredness are the common signs and symptoms, while difficulty in breathing or shortness of breath, headache, chills, sore throat, muscle aches, loss

of smell or taste and chest pain are the other symptoms. Nausea, vomiting and diarrhea are other less common symptoms. Researchers and clinicians suggested alarming condition for old age people, or the patients suffering from chronic diseases including diabetes, heart disease, lung disease, kidney failure or liver disease. Even immune-compromised patients also remain at higher risk of serious illness. Guan et al. (2020) studied the data of 1099 hospitalized patients with Covid-19 confirmed cases of 30 provinces of 552 hospitals in China through January 29, 2020.[14] Results showed that the fever (43.8% and 88.7% on admission and during hospitalization respectively) and cough (67.8%) were common symptoms, while diarrhea was rare (3.8%).

7. VULNERABLE POPULATION

In early December 2019, in Wuhan, China the first pneumonia case of strange origin was reported. On the basis of available data and clinical expertise, person of any age including infants, adults or older people, remains at higher risk for infection from COVID-19. Numerous clinical data reveals that the most vulnerable are the elderly or the people who are suffering from chronic disease. A U.S., virologist Lisa Gralinski, state that: "If you're over 50 or 60 and you have some other health issues and if you're unlucky enough to be exposed to this virus, it could be very bad."Around 1.3% mortality rate was reported in the U.S. recently. Recently, White House COVID-19 Taskforce projections of 100,000 to 200,000 deaths this year from COVID-19 are made with assumptions about the effectiveness of measures that are currently in place. Similar to U.S. report, China study also confirmed that only 1.0% of healthy people died from the disease, but the high mortality rate was expected (about 6%) in people with cancer, hypertension or chronic respiratory disease.[15]

8. DETECTION OF SARS-COV-2 INFECTION

Diagnostic testing to identify persons infected with SARS–CoV-2 infection is essential to control the COVID-19 pandemic. Diagnosis of COVID-19 on a massive scale becomes a key of pandemic control. However, majority of countries having limited testing capacity, hampered to control the outbreak of COVID-19. Reverse transcriptase–polymerase chain reaction (RT-PCR) and IgM and IgG enzyme-linked immunosorbent assay (ELISA) are currently available diagnostic tests for SARS-CoV-2 infections (Fig. 4).

RT-PCR is the reliable and usually used diagnostic test for COVID-19. In RT-PCR test sample has been collected from throat swab or, saliva and nasopharyngeal swabs or other upper respiratory tract specimens. Numerous RNA gene targets are used for RT-PCR. These include envelope (*env*), nucleocapsid (*N*), spike (*S*), RNA-dependent RNA polymerase. (*RdRp*), and *ORF1* genes. All genes are equally sensitive for the tests except the RdRp-SARSr (Charité) primer probe, which is less sensitive owing to mismatch in the reverse primer. Usually, in COVID-19 infected patients, in RT-PCR test, viral RNA is measured by the cycle threshold (Ct). Cycle threshold represents number of the replication cycles, which are require to produce a fluorescent signal.

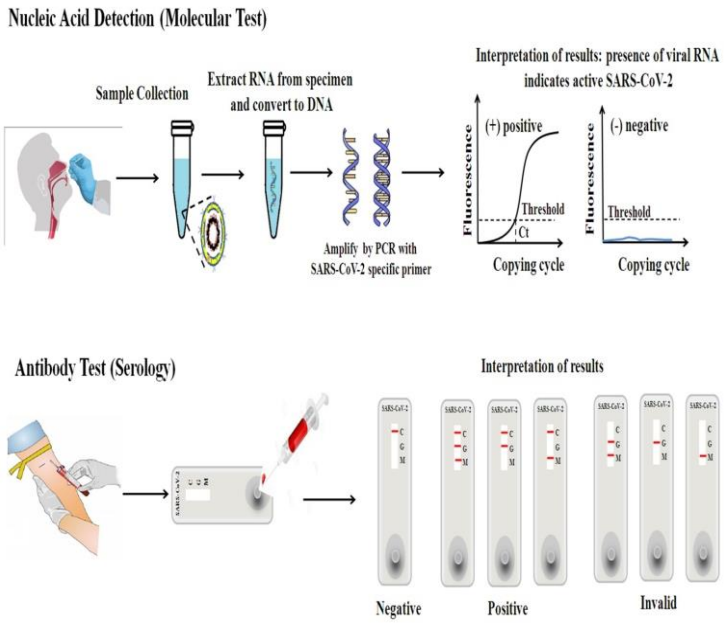

Fig. 4 Detection techniques of SARS–CoV-2 virus utilizing reverse transcriptase–polymerase chain reaction (RT-PCR) and IgM and IgG enzyme-linked immunosorbent assay (ELISA).

The lesser the value of Ct represents elevated viral RNA loads and the value of Ct<40, signifies PCR positive. However from week 3, positivity starts to decline and consequently becomes undetectable. Physician must keep in mind that RT-PCR signifies viral RNA and not the viable virus. Meanwhile, scientists have reported "positive" PCR after 2 consecutive "negative" PCRs tests performed 24 hours apart. The reason behind this paradox is still unclear. Furthermore, the different specimens respond differently for timeline of PCR positivity. PCR positivity declines more slowly in sputum as compared to nasopharyngeal swab. Wang et al. performed the testing of SARS-CoV-2 infection in 205 patients with varieties of specimens. RT-PCR positivity was reported maximum in bronchoalveolar lavage specimens, followed by sputum, then in nasal swab and least in pharyngeal swab.[16] Lower respiratory tract samples most often testing positive for the virus. Researchers also reported live virus in feces and in blood samples.

Enzyme-linked immunosorbent assay (ELISA) is another method to determine COVID-19 infection. It is based on the measuring the host immune response to SARS-CoV-2 infection. This method is based on the production of antibodies (e.g. IgM and IgG that usually seen from the fourth day after symptom) that flag or neutralise the virus. To et al. and Xiang et al. noticed that the IgM and IgG seroconversion in all 23 and 85 patients respectively between the 3rd and 4th week of clinical illness onset.[17,18] Afterwards IgM begins to decline and almost vanish by week 7, while IgG remains beyond 7 weeks. For COVID-19diagnosis, ELISA-based IgM and IgG antibody tests have 95% specificity. Guo et al. investigated the time kinetics of various antibodies produced against SARS-CoV-2, by collecting 208 plasma samples from 82 confirmed and 58 probable cases (qPCR negative, but with typical expression) and examined the host humoral response against SARS-CoV-2, with an ELISA-based assay.[19] The results demonstrated the median duration of IgM and IgA antibody detection was 5 (IQR, 3–6) days, while IgG was detected 14 (IQR, 10–18) days after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9% respectively. The positive rates of IgM antibodies in confirmed and probable cases were 75.6% and 93.1% respectively. After 5.5 days of symptom onset, the IgM ELISA showed higher detection efficiency as compared to qPCR. Furthermore, the combining of IgM ELISA assay with PCR significantly increased (98.6%) positive detection rate as compared with a single qPCR test (51.9%) for each patient.

Nowadays rapid antibody testkits for SARS-CoV-2detection are available in the marketby numerous manufactures. However, those manufacturers hide the nature of antigens used and indicate only the presence or absence of SARS-CoV-2 antibodies.

9. TREATMENT OF SARS-COV-2 INFECTION

Recently numbers of natural and synthetic drugs have been reported for symptomatic treatments.[20-29] Numerous drug design studies have been reported for search of novel compounds.[30-38] Numbers of novel drug delivery systems have been utilizes for effective drug delivery.[39-48] Several agents being used for COVID-19 are under clinical trial and few of them are available in market for sale. Instead, treatment focuses on managing symptoms as the virus runs its course. Similarly, other coronaviruses including SARS and MERS are also treated by managing symptoms. In some cases, experimental treatments are tested to see how effective they are. In most cases, doctors suggested the patient for rest, being hydrated and taking medications for symptomatic relief. Presently, researchers are working hard to find out effective treatments, and especially focus on the medicines that have already been used for the treatment of malaria and autoimmune diseases. Antiviral drugs are especially been investigated that were developed for other viruses(Fig.5).

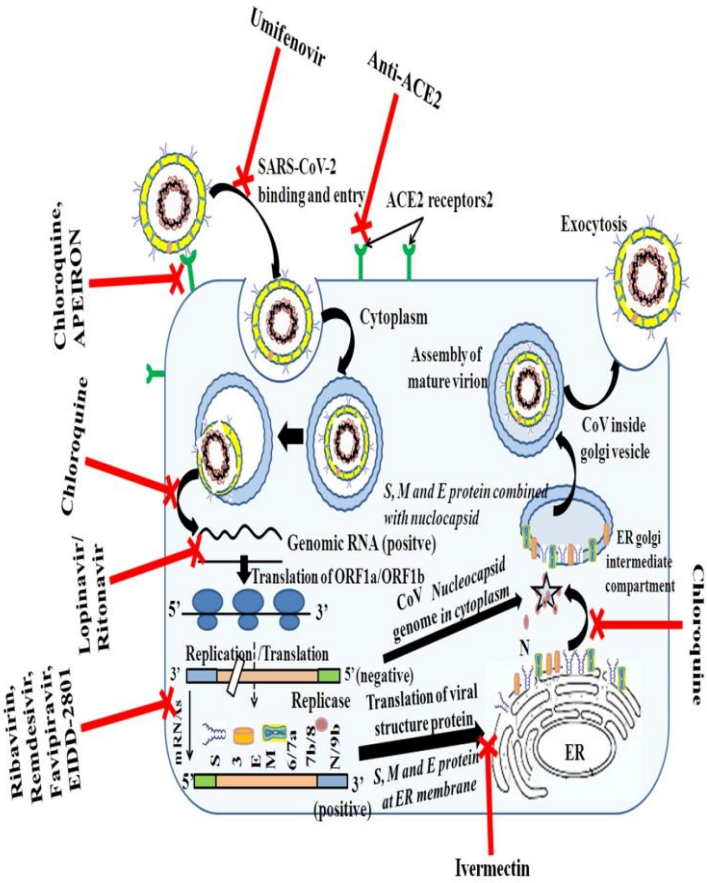

Fig. 5 Numerous antiviral drugs act on different stages of SARS-CoV-2 replication.

9.1 Antimalerial

Numerous drugs have been tested for COVID-19treatment, among which chloroquine (CLQ) and hydroxychloroquine (CLQ-OH), are the potential antimalarial drugs showed promising effects in the treatment of COVID-19 clinical studies. Chloroquine is one of the most prescribed drug in the world. The drug is supposed to exert potential antiviral effects through inhibiting nucleic acid replication and also believed as an inhibitor of endocytic pathways through elevation of endosomal pH. Both CLQ and CLQ-OH have been shown inhibit novel coronavirus SARS-CoV-2 in Vero E6 cells with an effective concentration 50% (EC 50) of 1.1 µM and 0.72 µM respectively. CLQ-OH was found to be superior over CLQ to inhibit SARS-CoV-2.[49]

In China, till date, about twenty-three clinical trials for COVID-19 treatment have been reported to examine safety and efficacy of CLQ or CLQ-OH.

Gao et al. investigated the outcomes of numbers of clinical trials conducted in China to determine safety and efficacy of CLQ or CLQ-OH in the management of COVID-19 related pneumonia in over 10 hospitals in Beijing, Chongqing, Guangzhou, Jingzhou, Ningbo, Shanghai and Wuhan.[50] Results showed that the over 100 patients, who received CLQ-phosphate merely control exacerbation of pneumonia and improved lung stipulation. Furthermore, patients were devoid of any severe adverse reactions of CLQ- phosphate. Subsequently, National Health Commission of the People's Republic of China, recommended the drug for addition in the next version of the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19. Department of Science and Technology of Guangdong Province recommended CLQ 500 mg b.d. for 10 days for COVID-19 positive patients.[51]

Gautret et al. evaluated the role of CLQ-OH at 600 mg daily on respiratory viral loads.[52] Results showed that at day 6 post-inclusion, 70% of CLQ-OH-treated patients were virologicaly cured compared with 12.5% in the control group (p=0.001). Moreover, 6 patients who were receiving the CLQ-OH were virologically cured at day 6 (100%), when they were treated in combination with azithromycin (AZT) (500 mg at day 1 followed by 250 mg per day for the next 4 days) as compared with patients treated with only CLQ-OH (57.1%) or without treatment (12.5%). This result and many other studies revealed that the combination of CLQ-OH with azithromycin an antiviral drug, improved the efficacy of CLQ-OH against other RNA-viruses. The combination of antimalarial drug with antiviral drug has the additional advantage that this combination permit low dose of chloroquine/hydroxychloroquine to omit severe side-effects, including cardiac toxicity and allow this combination as prophylactic with low dose to comorbidities who are at risk of COVID-19infection.The promising results of numerous studies, recommended CLQ at 100 mg daily or CLQ-OH at 300 mg weekly in mass prophylaxis in individuals exposed to COVID-19.

Derendorf et al. investigated the physicochemical properties of both CLQ-OH and AZT for the intracellular lysosomal space.[53] Both drugs were found to be accumulated about 50000 fold higher in the lysosomes as compared to cytosolic and extracellular concentrations. Although CLQ antiviral mechanism remains ambiguous, however, CLQ is believed to hamper the terminal glycosylation of ACE2 to act as a plasma membrane receptor for SARS-CoV-2. CLQ also block numerous steps of the coronavirus replication cycle. These facts suggested that CLQ may be a possible treatment for COVID-19 infections, but a lot of studies are still required.

Matrosovich et al. identified and reported that for binding of coronavirus to the respiratory tract, sialic-acid-containing glycoproteins and gangliosides act as primary attachment factors, besides their protein membrane receptor.[54] Very recently, Fantini et al. performed molecular modelling studies to examine the possible interaction between CLQ and sialic acids.[55] Molecular modelling studies revealed that the presence of ganglioside-binding domain (111–158) at the tip of the N-terminal domain of the SARS- CoV-2 S protein improves attachment of the virus to lipid rafts and assist contact with the ACE2 receptor. Furthermore, in the presence of CLQ or CLQ-OH, the viral S protein is no longer able to bind gangliosides and prevent the first step of the viral replication cycle. These molecular modelling results supported the use of CLQ and CLQ-OH, as initial therapy for COVID-19 positive patients.

On the basis of above mentioned studies chloroquine plays multiple mechanisms of action for antiviral therapy. These include (Fig.5):[56]

1. Chloroquine inhibits a pre-entry step of the viral cycle by interfering with viral particles binding to their cellular cell surface receptor. Chloroquine interferes with sialic acid biosynthesis, leadto impairment of binding of viruses,including human coronavirus HCoV-O43 and the orthomyxoviruses, which utilizes sialic acid moieties as receptors. The potent anti-SARS-CoV-1 effects of chloroquine *in vitro* were considered attributable to a deficit in the glycosylation of a virus cell surface receptor, the ACE2 on Vero cells.
2. Chloroquine can also impair virus replication by interfering with the pH-dependent endosome-mediated viral entry of enveloped viruses. Due to the alkalisation of endosomes, chloroquine was an effective *in vitro* treatment against Chikungunya virus when added to Vero cells prior to virus exposure. The mechanism of inhibition likely involved the prevention of endocytosis and/or rapid elevation of the endosomal pH and abrogation of virus–endosome fusion. The activation step that occurs in endosomes at acidic pH results in fusion of the viral and endosomal membranes, leading to the release of the viral SARS-CoV-1 genome into the cytosol.
3. Chloroquine- mediated inhibition of hepatitis A virus was found to be associated with uncoating and thus blocks its entire replication cycle.
4. Through reduction of cellular mitogen-activated protein (MAP) kinase activation, chloroquine may also inhibit virus replication.
5. Furthermore, chloroquine could alter the M protein maturation and interfere with virion assembly and budding.
6. 9.2 Angiotensin-Converting Enzyme Inhibitors
7. Numerous experts believe treatments targeting angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) could be aleadingapproachfor Covid-19treatment. ACE2 inhibition or reducing its activity in cell membranes probably reduces SARS-CoV-2 penetration

into cells. Nevertheless, ACE1 inhibitors do not inhibit ACE2.ARBs have similar effects to ACE inhibitors, but ACE inhibitors act by preventing the formation of angiotensin II rather than by blocking the binding of angiotensin II. Therefore, ARBs including losartan, valsartan, telmisartan, etc can be a novel therapeutic approach to block the attachment of SARS-CoV-2 to ACE2-expressing cells and ultimately its penetration into host cells. It is well documented that, ACEIs and ARBs protects the heart and kidney for patients with hypertension and diabetes. However, the use of ACEIs and ARBs will increase the expression of ACE2 andincrease patient susceptibility to viral host cell entry and propagation. Additionally, on prolong use of ACEIs might suppress the adaptive immune response, which is a key defence against viral infections. Similar effects have been noted with non-steroid anti-inflammatory drugs against adaptive immune response.[57]

8. These paradox responses of ACEI and ARBs may be accountable for the high mortality rate in patients suffering from hypertension, diabetes, heart disease and cerebrovascular disease. Patients suffering from these diseases, have a long term history to use ACEI and ARBs. Guan et al. extracted data regarding 1099 patients to showed specific comorbidities associated with increased risk of SARS-CoV-2 infection and developing into a severe or fatal case.[14] Results showed that 173 patients had severe disease, in whom the most common comorbidities were hypertension (24%), diabetes (16%), coronary heart disease (6%), and cerebrovascular disease (2%). Similarly, Zhou and co-workers reported that hypertension (30%; p=0·0008), diabetes (36%; p=0·0051), and coronary heart disease (15%; p=0·0001) were the most common comorbidities with significant effects on mortality, in an analysis of 191 patients.[58]
9. However, still no scientific evidence has been reported to claim the direct link between morbidity of COVID-19 with previously reported ACEI and ARBs treatment. The European Society of Cardiology state that "The Council on Hypertension strongly recommends that physicians and patients should continue treatment with their usual anti-hypertensive therapy because there is no clinical or scientific evidence to suggest that treatment with ACEIs or ARBs should be discontinued because of the COVID-19 infection". Furthermore, on March 17, 2020, the American College of Cardiology, the Heart Failure Society of America and the American Heart Association, jointly release the guidelines to continue ACEIs and ARBs as prescribed in the setting of COVID-19.
10. Furthermore, on March 17, 2020, the American Heart Association, the Heart Failure Society of America, and the American College of Cardiology put out a joint statement advocating for patients to continue ACEIs and ARBs as prescribed and that changes in medications in the setting of COVID-19 should be completed only after careful assessment.

9.3 Antiviral drugs

Numbers of antiviral drugs have been trial against SARS-CoV-2 infections. Some of them showed promising effects, but till date, none of antiviral drug has been approved officially for COVID-19 treatment. The detail mechanism of antiviral drugs is shown in Fig. 5.

9.3.1 Lopinavir/ Ritonavir/ Kaletra

Lopinavir and ritonavir are the two protease inhibitors, antiretroviral drugs. Ritonavir add to half-life of lopinavir by inhibiting the metabolising enzyme cytochrome P450 3A and thus the combination prefer. Kaletra is the combination of lopinavir (100 mg) and ritonavir (25 mg), approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children. Previous studies reveal the success of lopinavir/ritonavir against other coronaviruses. A molecular docking study performed by Dayer et al. reported that lopinavir significantly inhibit cronovirus proteinase.[59] The inhibitory potency of HIV 1 protease inhibitors to cronovirus proteinase was as follows: lopinavir>ritonavir>amprenavir>tipranavir> saquinavir. Chu and co-workers in year 2004 reported the success of lopinavir/ritonavir against other coronaviruses.[60] A significant reduction in risk of severe hypoxia or death in 41 SARS-CoV patients were reported who were treated with lopinavir/ritonavir and ribavirin, as compared to those 111 historical controls treated with ribavirin alone. Recently, Ye et al. proved that the lopinavir/ritonavir combination and regular adjuvant medicine against pneumoniashowed better recoveryof COVID-19 positive patients as compared to treatment with adjuvant medicine alone.[61] Study was performed on 47 admitted patients, who were divided into the test group and the control group according to whether they had been treated with lopinavir/ritonavir or not during hospitalization. The treatment group who received lopinavir/ritonavir and routine adjuvant medicine had a more evident therapeutic effect in normalize the body temperature and physiological mechanisms devoid of side effects, compared with the treatment of pneumonia-associated adjuvant drugs alone. A very recent clinical data published by Cao et al. on dated 7 may 2020 in The new England Journal of Medicine, revealed no significantly accelerated clinical improvement, reduced mortality, or diminished throat viral RNA detectability in serious SARS-CoV-2 infected patients who were receiving lopinavir–ritonavir treatment.[62] No beneficial results were reported with lopinavir–ritonavir treatment (on 99 patients) beyond standard care (on 100 patients).

Currently, numerous studies were reported showing no potential efficacy of lopinavir/ritonavir in the treatment of COVID-19.

9.3.2. Ribavirin

Ribavirin is a guanosine analog with antiviral activity. The earliest report of *in vitro* efficacy of five FDA-approved drugs namely ribavirin, penciclovir, nitazoxanide, nafamostat and chloroquine were tested against the first CoV-19 viral strain i.e. 2019BetaCoV/Wuhan/WIV04/20192 (WIV04), which was isolated from the lung fluid of one patient in a cohort of seven, six of whom work in proximity of the Wuhan seafood market. The report of *in vitro* direct-actinganti-viral activity against the CoV-19 established the earliest basis for clinicalguidance. Certainly, treatment with CLQ and ribavirin permits few benefits in an outbreak due to instant drug availability.Certainly, due to the low cost of CLQ over ribavirin, a CLQ phosphate multicenter trial was possible. However, similar to chloroquine prophylaxis, the government of China also recommended a 4- gram oral loading dose of ribavirin followed by 1.2 gram orally every 8 hours after CoV-19 pneumonia diagnosis. This directive was further modified to 500 mg iv BID or TID in the revised Edition 5. Recently the numbers of ribavirin in combination therapy are under clinical investigation. At present a clinical trial is continuing to determine the efficacy and safety of ribavirin in combination with interferon-alpha or lopinavir/ritonavir or with interferon-alpha and lopinavir/ritonavir in patients with coronavirus pneumonia (ChiCTR2000029387).[63] Furthermore, a combination of ribavirin, lopinavir/ritonavir and interferon beta-1b is under clinical investigation to compare with to lopinavir/ ritonavir (NCT04276688) against SARS-CoV-2 infection.[64] These studies revealed that ribavirin could be used in COVID-19 positive patients, but more studies are required.

9.3.3 Remdesivir

Remdesivir developed by Gilead Sciences, is a nucleoside analoguethat inhibits viral RNA polymerases.de Wit et al. reported the antiviral efficacy of remdesivir against SARS-CoV and MERS-CoV *in vivo*.[65] In COVID-19, remdesivir inhibits RNA polymerase (RdRp) and interfere with RNA replication. Remdesivir cannot cause an immediate stop of growing chain (i position) and allow to extend three more nucleotides down to stop the strand at (i + 3) position. Remdesivir was first used in United States for COVID-19 treatment. Remdesivir was administered through intravenous route on the evening of day 7 and on day 8the patient's clinical condition was improved without any adverse events.Due to improved oxygen saturation values to 96%, the supplemental oxygen was discontinued. The symptomatic treatment was continuing during remdesivir therapy.

Grein et al. gave remdesivir 53severe infected SARS-CoV-2 patients during the period from January 25, 2020, through March 7, 2020.[66] The remdesivir was given for 10 days, started with 200 mg intravenously administered on day 1, followed by 100 mg daily for the remaining 9 days of treatment. 36 patients (68%) showed improvement in oxygen-support. Although 25 patients (47%) were discharged and 7 were (13%) died. These results supported the further clinical investigation of remdesivir against severe Covid-19 patients.

Wang et al. also prescribed remdesivir in adults with severe COVID-19 infection.[67] Results showed that intravenous remdesivir was effectively tolerated; however no significant improvements were seen in seriously ill patients. Authors suggested to conductscrupulous study of remdesivir in patients with severe COVID-19. Recently, Wang and co-workers reported in their study that the combination of remdesivir with CLQ is efficient to control of SARS-CoV-2 infection.[68] Since both drugs are easily available and effective against diverse ailments, thus authors suggested that this combination may be beneficial against novel SARS-CoV-2 infection.

On May 1, 2020, The US FDA announced to use remdesivir for severe COVID-19 (confirmed or suspected) cases in hospitalized adults and children. Recently, US firm Gilead in talks with Indian drug companies Cipla, Hetero and Dr. Reddy's to produce remdesivir for COVID-19 treatment.

9.3.4 Favipiravir

Favipiravir is pyrazinecarboxamide derivative; inhibit RNA-dependent RNA polymerase and sold under the brand name Avigan, used to treat influenza in Japan. It is being developed and manufactured by Toyama Chemical (Fujifilm group) and was approved in year 2014 for medical use. During outbreak of Ebola virus (EBOV) in year 2014–2015 patients treated with favipiravir showed improved survival.[69] Alike of genome sequencing of the SARS-CoV-2 to SARS-CoV and MERS-CoV, favipiravir is recommended for COVID-19 treatment. Cai et al. performed clinical trial of favipiravir for 80 COVID-19 positive patients in The Third People's Hospital of Shenzhen (ChiCTR2000029600).[70] 35 patients who received favipiravir showed rapid recovery over 45 patients in the control arm (median 4 (2.5– 9) d versus 11 (8–13) d, $P < 0.001$). X-ray examinations also showed higher rate of improvement in chest imaging in the favipiravir arm compared with the control arm (91.43% vs. 62.22%). This study showed that FPV significantly perform better COVID-19 treatment in terms of disease progression and viral clearance. Furthermore, Chen et al. also reported the clinical trial result, which was conducted by the same research group to determine the efficacy of favipiravir

against COVID-19 treatment.[71] With favipiravir treatment, clinical recovery rate was increased from 55.86% to 71.43% within 7 day's. Both the fever reduction time and cough relief duration, decreased significantly after favipiravir treatment in ordinary COVID-19 positive patients and in the patients suffering from hypertension and/or diabetes. The National Medical Products Administration of China approved favipiravir as the firstanti-COVID-19 drug on March2020.

9.3.5 Umifenovir

Umifenovir is another antiviral compound developed at the Russian Research Chemical and Pharmaceutical Institute and marketed as Arbidol. Umifenovir had shown to possess antiviral activity against both DNA and RNA viruses. Umifenovir inhibits the membrane fusion between virus particles and plasma membranes, and between virus particles and the membranes of endosomes. Additionally umifenovir also reported for its immunomodulatory activity, and induce interferon and/or macrophage activation. *In vitro* studies of umifenovir, suggested antiviral activity against SARS coronavirus.[72] Recently, numerous studies reported application of Arbidol for COVID-19 treatment, but its efficacy and safety remained unclear

.

Xu and co-workers performed a retrospective cohort study against 111 novel coronavirus-infected pneumonia patients (NCP) in China, who received empirical antiviral regimens with or without Arbidol.[73] A total of 111 patients from two clinical centersin China were enrolled. Results suggested that umifenovir accelerated virologic clearance, improved focal absorption on radiologic images and reduced the need for high flow nasal catheter (HFNC) oxygen therapy in hospitalized patients. These results provided the basis for the clinical use of Arbidoland supported for further randomized controlled trials in patients with NCP. Umifenovir combination with lopinavir/ritonavir increased negative conversion rate of SARS-CoV-2 and improved chest CT scan results. However, umifenovir has not been as effective as favipiravir in clinical recovery rate and relief of fever and cough. Recently two randomized clinical trials are ongoing inChina to investigate efficacy and safety of umifenovir against COVID-19. While umifenovir combination lopinavir/ritonavir is also under clinical investigation (NCT04252885) against COVID-19.

9.3.6 Ivermectin

Ivermectin, a FDA-approved anti-parasitic agent, also possess antiviral activity. Ivermectin,has been demonstrated activity against RNA virus, including Dengue, influenza, Venezuelan equine encephalitis virus (VEEV) and West Nile Virus.[74]

Ivermectin inhibited replication of SARS-CoV-2 in *in vitro* Vero-hSLAM cells, possibly due to the inhibition of importin-α/β1-mediated nuclear import of viral proteins, as shown for other RNA viruses. However, Schmith et al. performed successful clinical trial using the approved dose of ivermectin, which showed thativermectin recommended dose was insufficient for COVID-19treatment.[75] Momekov and Momekov investigated the pharmacokinetics of ivermectin in patients used to surrogates for juxtaposition with the *in vitro* SARS-CoV-2 inhibitory findings.[76] Patri et al. hypothesized that the combination of CLQ-OH with ivermectin could act in a consequential and synergistic manner.[77] Indeed, CLQ-OH would behave as a first-level barrier by inhibiting the entry of the virus into the host cell, while ivermectin could reduce viral replication if the virus did get in, strengthening CLQ-OH antiviral effects. However, it was just a hypothesis and no *in vitro* or *in vivo* studies were conducted.

9.3.7 APEIRON

APEIRON (APN01) is the recombinant form of the human angiotensin-converting enzyme 2 (rhACE2), and has the potential to block the infection of cells caused by the novel SARS-CoV-2 virus, and reduce lung injury. APN01 imitates the human enzyme ACE2 and blocks virus entry into the cells. The virus binds erroneously to ACE2/APN01 rather than cell surface ACE2 and no longer infects the cells. Simultaneously, APN01 blocks inflammatory reactions in the lungs and protects against acute lung injury. Recently, Austria, Denmark and Germany approved to initiate a Phase II clinical trial of APN01 to treat COVID-19.[78]

9.3.8 EIDD-2801

EIDD-2801 is an oral NHC-prodrug (β-D-N^4-hydroxycytidine-5'-isopropyl ester), showed efficacy against SARS-CoV and MERS-CoV. Preliminary studies showed that EIDD-2801 can be used as either a prophylactic or a therapeutic for SARS-CoV-2. Similar to remdesivir, EIDD-2801 works by mimicking ribonucleosides– causing debilitating errors and prevents the spread of the virus. Senior researcher and eminent professor of epidemiology at UNC-Chapel Hill Gillings School of Global Public Health, Ralph Baric state that "This new drug not only has high potential for treating COVID-19 patients, but also appears effective for the treatment of other serious coronavirus infections". EIDD-2801 reduced the viral load in mice when given as a treatment between 12 and 48 hours after infection began. Though EIDD-2801 has small therapeutic window in mice, yet researchers suggested large therapeutic window of this in humans as compared to mice.[79]

9.3.9 Interferon

Interferons (IFNs) are natural proteins, secreted by immune cells.Alfa, beta, and gamma are three classes of interferon. Interferons do not directly kill bacteria or virus, but modulate immune response against foreign substances that invade the body. Interferon alfa (2b) and beta (1a) are under clinical investigation as potential treatments for people with COVID-19 coronavirus disease. Interferon may be able to make the immune system strong by turning on quiescent parts and leading them toward the defense against SARS-CoV-2 assault. Numerous studies around the world is conducting, looking at different interferons to treat COVID-19 coronavirus. Zorzitto et al. reported that IFN-α/β are effective in inhibiting SARS-CoV replication in *in vitro*.[80] Furthermore, Haagmans et al. reported that the pegylated interferon- α protects type 1 pneumocytes against SARS coronavirus infection in monkey.[81] Thus IFN-α had proposed for COVID-19 therapy and numbers of studies have been conducted and reported. Lokugamage et al. study showed that IFN-α/β can be used as a prophylaxis against SARS-CoV-2 similar to SARS-CoV.[82] The combination of IFN-β with ribavirin, remdesivir or lopinavir/ritonavir improved pulmonary function, reduces viral loads *in vitro* in other coronaviruses. In China, vapor inhalation of 5 million U of IFN-α in combination with ribavirin twice a day for no more than 10 days has been recommend for COVID-19 treatment.[83] Recently clinical trials for IFN-α2b in combination with lopinavir/ritonavir (ChiCTR2000029387) orIFN-β1b with ribavirin and lopinavir/ritonavir (NCT04276688) have been recruited for COVID-19 treatment.[84]

Usually it has been seen that multiple antiviral combinations are more effective against viral infection as compared to single drug. Hung et al. reported phase 2 clinical trials by utilizing IFN-β1b, lopinavir–ritonavir, and ribavirin combinations against COVID-19 127 positive patients, who were hospitalized in six hospitals of Hong Kong.[85] Patients were randomly assigned (2:1) to a 14-day combination of lopinavir 400 mg and ritonavir 100 mg every 12 h, ribavirin 400 mg every 12 h, and three doses of 8 million international units of IFN-β1b on alternate days (combination group; 86 patients) or to 14 days of lopinavir 400 mg and ritonavir 100 mg every 12 h (control group; 41 patients). The combination therapy was found to be safe and efficient as compared to lopinavir–ritonavir alone. These results supported the future clinical study of a double antiviral therapy with IFN-β1b as a backbone is warranted.

10. PLASMA THERAPY

Convalescent plasma is a passive antibody therapy, refers to plasma that is collected from individuals, following resolution of infection and development of antibodies.[86] Recently, convalescent plasma has been used in the COVID-19 treatment. Initially data from China suggested clinical benefits, including radiological resolution, reduction inviral loads and improved survival. Human anti-SARS-CoV-2 plasma is the only therapeutic strategy that isimmediately available for use to prevent and treat COVID-19 (Fig. 6).

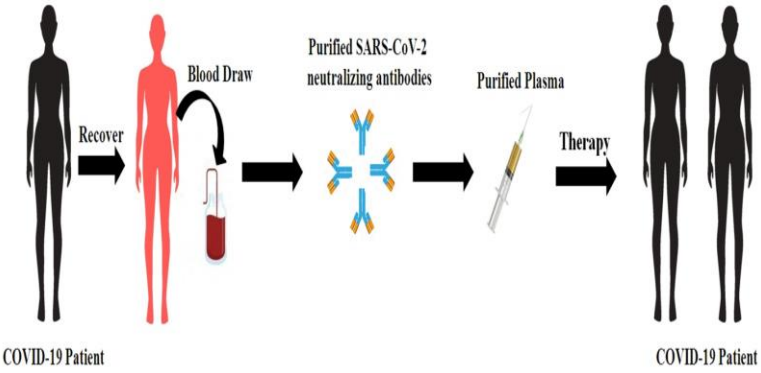

Fig. 6 Schematic diagram of plasma therapy.

Shen et al. conducted a study in Shenzhen Third People's Hospital in Shenzhen, China, in between January 20, 2020, to March 25, 2020, by transfusing convalescent plasma having SARS-CoV-2–specific antibody (IgG) to 5 critically ill patients with COVID-19 and ARDS.[87] Clinical results showed normalized body temperature within 3 days and PAO2/FIO2 increased within 12 days. Furthermore, viral load was also decreased and became negative within 12 days after the transfusion. Three patients were discharged from the hospital and 2 were in stable condition after transfusion. These results suggested for further clinical studies with large sample size and concomitant treatment modalities (e.g. ribavirin, remdesivir, corticosteroids, etc.).

Furthermore, Duan et al. also reported the results of a pilot study, conducted on 10 patients with COVID-19 positive.[88] After transfusion of convalescent plasma (200 ml), all 10 patients had improvement in common symptoms of COVID-19 within 1-3 days of transfusion, without reported any serious adverse effect. Radiological examination also demonstrated improvement in pulmonary lesions within 7 days. Similarly, high-dose intravenous immunoglobulin (IVIg) has been suggested by Cao and co-workers for COVID-19 therapy.[89] Nevertheless, while the data supported safety and potential efficacy of convalescent plasma, randomize trials are needed. On 24 March 2020, the USFDA published guideline for investigational COVID-19 convalescent plasma. Convalescent plasma infusion may aggravate hyperimmune attacks, and the optimal timing of administering convalescent plasma on COVID-19 needs to be carefully considered. In addition, the source of convalescent plasma limits its wide application, especially in countries which are in the acceleration stage and late accumulation stage of COVID-19 development. Furthermore, the transfusion-related events, involving chill, fever,

anaphylactic reactions also limits convalescent plasma use. The convalescent plasma can be potentially use until an effective treatment against COVID-19 is discovered.[90]

11. CYTOKINE INHIBITORS

Patients suffering from SARS-CoV-2 infection showed upregulation of pro-inflammatory cytokines in the blood. However, the efficacy of corticosteroids, commonly utilized antiinflammatory agents, to treat COVID-19-induced cytokine release syndrome (CRS) is controversial. Huang et al. reported increased cytokine levels in 41 inpatients.[91] Furthermore, numerous studies also confirmed increased level of erythematosus sedimentation rate (ESR), CRP, and high level of IL-6,TNFα, IL-1β, IL-8, IL2R, etc in severe COVID-19 patients (Fig. 7). SARS-CoV-2 infection drives a profound cytokine response in the host, comprising a series of mediators that are targeted in immune-mediated inflammatory diseases (IMIDs).

Targeting the host immune system through inflammatory cytokine blockade, stem cell therapy, immune cell depletion, transfusion of convalescent plasma and artificial extracorporeal liver support may be effective for COVID-19. Recently, IL-6 blockade is a promising strategy for COVID-induced CRS. Numbers of studies reported the elevated level of IL-6 levels in COVID-19 patients and might serve as predictive biomarker for disease severity.[92] Recently, De Diego et al. demonstrated that inhibiting nuclear factor kappa-B(NF-κB), increased animal survival, with reduced IL-6 levels.[93] Schoeman and Fielding et al. reported that E protein of SARS-CoV-2 and SARS-CoV share 95% homology, thus possibility to elicit a similar immune response in both viruses.[94] Hence targeting the IL-6 may be effective for COVID-induced CRS. A humanized antiinterleukin-6-receptor (IL-6R) monoclonal antibody

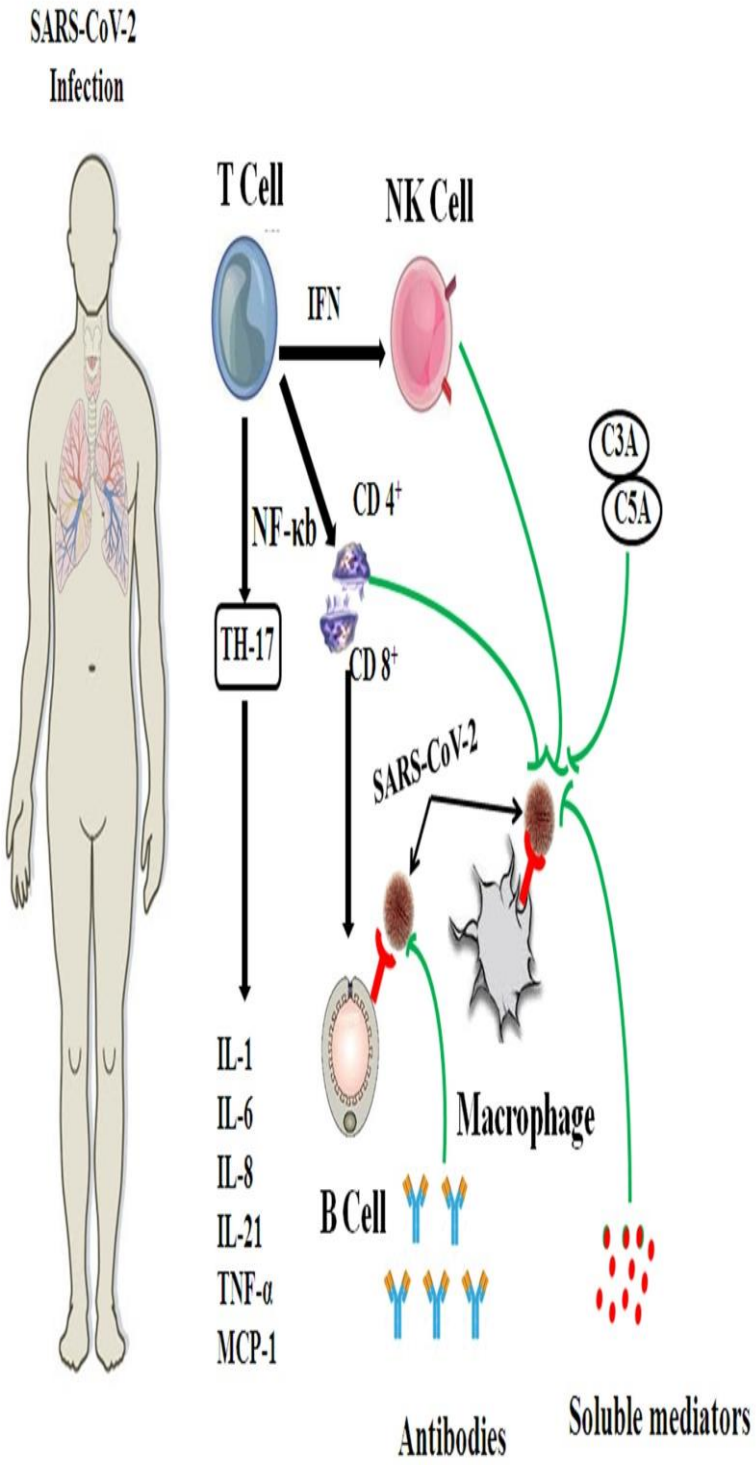

Fig. 7 Possible immune response after attack of SARS-CoV-2 virus.

tocilizumab, inhibits interleukin-6 (IL-6) was administered intravenously to test in the treatment of COVID-19 in China and Italy with encouraging results. Even to evaluate the role of tocilizumab in COVID-19 treatments, the pharmaceutical manufacture company of tocilizumab has made the drug available free of charge in Italy for COVID-19 pandemic. Michot et al. reported first observation of a COVID-19 positive patient suffered from lung disease successfully treated after two infusion with tocilizumab.[95] Xu et al. determined the efficacy of tocilizumab to improve clinical symptoms and repress the deterioration of severe COVID-19 patients.[96] The 21 COVID-19 patients were admitted in The First Affiliated Hospital of University of Science and Technology of China, given tocilizumab in addition to routine therapy between 5 and 14 February 2020.In all patients fever normalized within one day and other symptoms improved remarkably within a few days. 15 patients required less oxygen supply and 1 patient needed no oxygen therapy within 5 days treatment. Lung lesion opacity absorbed in 19 patients (90.5%) and percentage of lymphocytes in peripheral blood also returned to normal in 52.6% of patients on the fifth day after treatment. No adverse reactions were observed and all patients were discharge on average 15.1 day after giving tocilizumab. This preliminary study supported to use tocilizumab in severe and critical COVID-19 patients.

Janus kinase (JAK) inhibitors inhibit type I/II cytokine receptors, are currently being used for the treatment of COVID-19. Baricitinib is a selective JAK1/JAK2 inhibitor, showed effective treatment for ARDS in COVID-19 and decreased the virus infectivity for lung cells. Furthermore, baricitinib also interrupts the passage and intracellular assembly of SARS-CoV-2 into the target cells via disruption of AP2-associated protein kinase 1 (AAK1) signaling and also reduced the inflammation in patients with ARDS. In contrast, tofacitinib an effective oral JAK2/1/3 inhibitor does not significantly inhibit AAK1.[97] Other JAK1/2 inhibitors including ruxolitinib, memolitinib, and oclacitinib can potentially affect signaling pathways downstream of the receptors involved in COVID-19 development.Ruxolitinib is currently approved by the FDA for the treatment of patients with myeloproliferative neoplasms, under phase III clinical trial to treat patients with coronavirus disease 2019 (COVID-19)related cytokine storm.

As reported on Sept 29 2019, ten US FDA approved and four off-label indications for anti-TNF therapy, indicating that TNF is a valid target in many inflammatory diseases. TNF is important in nearly all acute inflammatory reactions and reported to present in blood and disease tissues of patients with COVID-19. Anti-TNF therapy was proposed to evaluate in patients with COVID-19 on hospital admission to prevent progression to needing intensive care support. Previously, Tobinick et al. reported that inhibition of TNF-α has the potential therapeutic modulation of SARS coronavirus infection.[98] Anti-TNF antibodies infliximab or adalimumab may be beneficial for COVID-19 treatment. However adalimumab is the only TNF-α inhibitor undergoing evaluation, in a trial registered in China (ChiCTR2000030089).[99]

12. STEM CELLS

Stem cells having the ability to generate other cells called daughter cells possess specialized functions. Mesenchymal stem cells (MSCs) are widely used in stem cellbased therapyespecially in immune-mediated inflammatory diseases. MSCs improve immunomodulatory mediated cytokines qualities andshows antiviral activity. The immunomodulatory effects of MSCs are triggered further by the activation of Toll-like receptors (TLRs) in MSCs, which is stimulated by pathogen-associated molecules.[100] MSCs are expected to survive even if they are transplanted into a patient with a confirmed COVID-19 and thus trial in the treatment of COVID-19.Leng et al. transplanted MSC in 7 patients with COVID-19 pneumonia in Beijing You An Hospital, China.[101] All 7 patients showed significantly improvements (including pulmonary function) without any adverse effects. The gene expression profile revealed MSCs were free from COVID-19 infection. Both common and severe patients were recovered and discharged within 10 days after treatment.MSC populations were entrapped in the lung after intravenous administration and showed improved lung functions and COVID-19 pneumonia. These results ensure safer and effective use of MSCs against COVID-19 positive patients and especially recommended for patients having COVID-19 pneumonia. However, the major limitation of this approach is the supplying source of clinical-grade MSCs and subsequently the speed of preparation for clinical usage that here stem cell banks can play an important role. Recently a case study was reported in China on a 65-year-old female patient diagnosed in critical condition with COVID-19. Initially patient was treated with antiviral drugs, steroids and antibodies, but no significant improvements were observed, even patients vital signs worsened and then the patient was treated with MSCs and with thymosin α1; 5×10^7 cells each three times. Remarkable improvements were reported only after second injection and patient was removed from the ventilator and able to walk. These results suggested that umbilical cord mesenchymal stem cells could be an ideal treatment option alone or in combination with other immune modulators for acute COVID-19 patients.[102] These studies suggested that the stem cell therapy and especially MSCs may be a promising strategy for COVID-19 treatment. However, the cost-effective and speed of therapeutic preparation are the two major hurdles for MSC based COVID-19therapy.Recently, China, USA, Jordon, Iran, and several other countries have begun cell-based therapy clinical studies.

13. VACCINES

The genetic sequence of SARS-CoV-2 triggers intense global R&D activity to develop a vaccine against the disease. The first COVID-19 vaccine candidate entered human clinical testing with unprecedented rapidity on 16 March 2020.

Since the traditional vaccine development is a lengthy, expensive process and took many years to produce a licensed vaccine. In the COVID pandemic crisis, a vaccine quickly requires a new pandemic paradigm (Fig. 8).

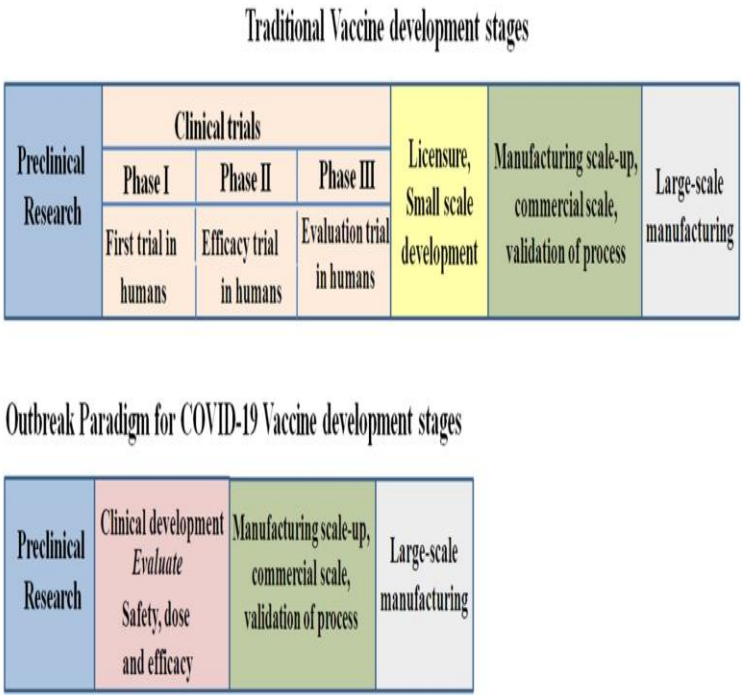

Fig. 8 Comparison of traditional vaccine development stages and outbreak paradigm for COVID-19 vaccine development stages.

Striking feature of the vaccine development for COVID-19 is the range of technology platforms being evaluated, including nucleic acid (DNA and RNA), virus- like particle, peptide, viral vector (replicating and non- replicating), recombinant protein, live attenuated virus and inactivated virus approaches. All these technology platforms have their own pro and cons.[103]

As of 8 April 2020, total 115 COVID-19 vaccine candidates were reported, of which 78 are confirmed as active and 37 are unconfirmed (development status cannot be determined from publicly available or proprietary information sources). In confirmed 78 active projects, 73 are currently at exploratory or preclinical stages. The most advanced candidates have recently moved into clinical development, including mRNA-1273 (LNP-encapsulated mRNA vaccine encoding S protein) from Moderna, Ad5-nCoV (Adenovirus type 5 vector that expresses S protein)from CanSino Biologicals, INO-4800 (DNA plasmid encoding S protein delivered by electroporation)from Inovio, and LV- SMENP- DC (DCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins; administered with antigen- specific CTLs) and pathogen- specific aAPC (aAPCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins) from Shenzhen Geno-Immune Medical Institute. Very recently, Moderna Therapeutics' Covid-19 vaccine, mRNA-1273, received Fast Track designation from the FDA. Numerous other vaccine developers have indicated plans to initiate human testing in 2020. Of the 78 confirmed active vaccine candidates, 56 (72%) are being developed by private/industry developers, with the remaining 22 (28%) of projects being led by academic, public sector and other non-profit organizations. North America is the leading country in vaccine development with 36 (46%) developers of the confirmed.

Active vaccine candidates were reported, followed with China 14 (18%), then Asia (excluding China) 14 (18%) and then in Australia and in Europe both 14 (18%) confirmed active vaccine candidates were reported (Le et al., 2020). The majority of clinical trials involving Covid-19 vaccines or treatments are showing "encouraging" results, says an analyst. Total 159 vaccines have been based on different approaches have been reported till date, out of which only 12 vaccines are under phase II clinical trials (Fig. 9).

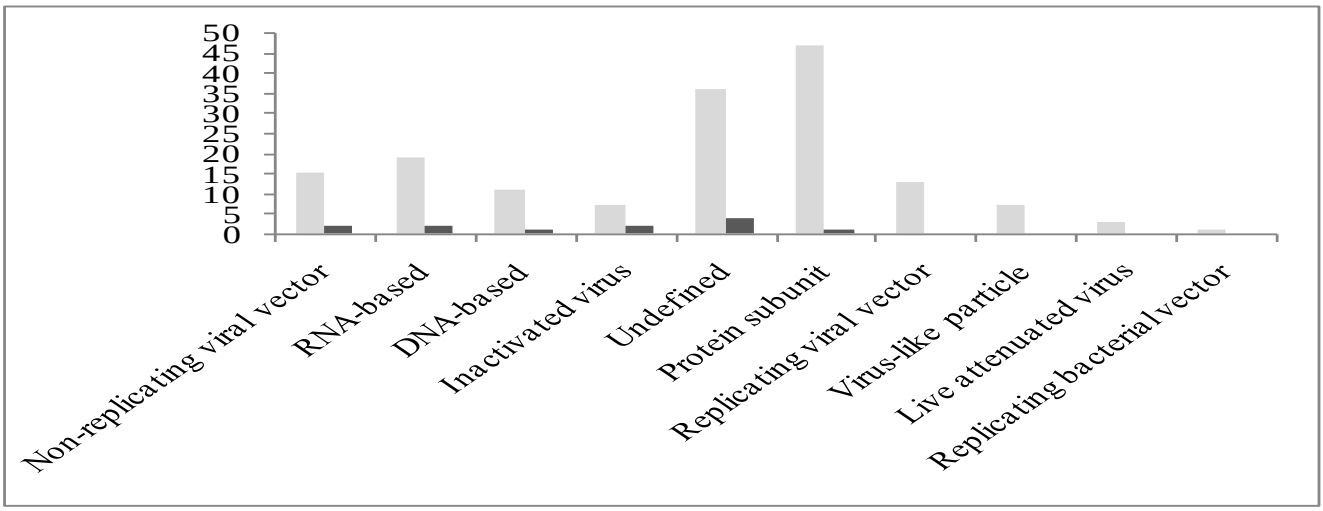

Fig. 9 Varieties of vaccines reported (green colour)

Very recently, Serum Institute of India's "Covishield" and Bharat Biotech's indigenous "Covaxin" are the two vaccines against Covid-19 that will drive the countries vaccination programme in the first phase of inoculation. In September 2020, the first batch of the 'Gam-Covid-Vac' [Sputnik V] vaccine for the prevention of the new coronavirus infection, developed by the Gamaleya National Research Center of Epidemiology and Microbiology of the Ministry of Health of Russia, has passed the necessary quality tests in the laboratories of Roszdravnadzor [medical device regulator] and has been released into civil circulation. The US Food and Drug Administration (FDA) authorized an emergency-use RNA vaccine made by Pfizer on 17 December 2020. A week after US regulators has followed with a second: another RNA vaccine, this one made by Moderna of Cambridge, Massachusetts. Two more vaccines of AstraZeneca and Johnson & Johnson are also in late-stage testing and possibly soon available in the market.

14. Current Position

Current position is very critical and day by day increasing numbers of cases worldwide. According to WHO report 106,000 new cases had been reported worldwide within 24 h in the date between 20 to 21 May 2020. Up to 21 May 2020 5,213,678 total cases have been reported, out of which 2,784,809 are active cases and 2,093,874 patients have been recovered worldwide. The total 334,995 death have been reported till date. These data are the serious threat for and under phase II clinical trials (red colour).
the world and need immediate recovery from this pandemic condition.

15. Conclusion

In the present write up, we focus on deadly respiratory diseases caused by SARS-CoV-2, its origin, replication, transmission, clinical symptoms, detection techniques and vulnerable population infected with SARS-CoV-2. Herein we elaborate the numerous therapies for COVID-19 under clinical investigation. Furthermore we also reported the worldwide data of population suffering from COVID-19 pandemic disease. On 30 January 2021, there have been 101,561,219 confirmed cases of COVID-19, including 2,196,944 deaths, reported by WHO. Although in few countries condition is more decisive due to COVID-19, while other countries showing remarkable recovery rate especially in India wherein 96.04 recovery rate was reported on 30 December 2020.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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