Review Article

An Overview of the Approaches for the Pharmacotherapy of Pandemic Causing Coronaviruses of the 21st Century

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Prajapati *et al.*: Overview of Pharmacotherapeutics of Coronaviruses

The 21st century has witnessed 3 major pandemics caused by coronaviruses namely, severe acute respiratory syndrome, Middle East respiratory syndrome and coronavirus disease-19. Though previously considered non-pathogenic in humans, these viruses have found a way to infect the human population. The viruses have traversed various intermediate host pools from their natural reservoirs before spilling out in humans. From severe acute respiratory syndrome in 2003 to Middle East respiratory syndrome in 2012 to coronavirus disease-19 in 2019, clinical research, safety and efficacy studies performed during each outbreak have led to new developments in the pharmacotherapeutics of human coronavirus infections. Severe acute respiratory syndrome was initially identified as a disease-causing pneumonia and hence was treated by antibiotics followed by corticosteroids for acute respiratory distress syndrome. After detecting the viral origin of the disease, the use of antivirals like ribavirin, ritonavir/lopinavir, Type I Interferons and their combinations started to gain momentum. Middle East respiratory syndrome led to repositioning of therapies employed in severe acute respiratory syndrome along with introduction of new agents like mycophenolic acid, cyclosporine and remdesivir. The emergence of novel coronavirus causing coronavirus disease-19 has led to repurposing of molecules like chloroquine, remdesivir and favipiravir for containing the virus. In addition, the effectiveness of plasma therapy and antibody treatment has been investigated through small scale observational studies.

Key words: Pharmacotherapeutics, coronavirus, severe acute respiratory syndrome, Middle East respiratory syndrome, coronavirus disease-19

Coronavirus (CoV) belong to the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales[1]. These enveloped viruses contain unusually large single-stranded positive Ribonucleic Acid (RNA) as their genome (27 to 32 kb)[2,3]. Out of the four genera in this subfamily, alpha-CoVs and beta-CoVs infect mammals only (Table 1). In humans, 229E, OC43, NL63 and HKU1 strains of Human CoV (HCoV) are prevalent causing upper respiratory tract and gastrointestinal tract infections in immunocompetent individuals. The infections may vary from mild conditions like common cold to severe manifestations like bronchitis and pneumonia[4].

In 1965, the first occurrence of HCoV was detected in the nasal discharge of a patient[4]. Before the beginning of the 21st century, CoVs were abundantly circulating and vastly studied in veterinary medicine because of their reduced impact on human health. However, the advent of the 21st century witnessed two highly pathogenic incidences of HCoVs namely, Severe Acute Respiratory Syndrome (SARS) in 2003 and Middle East Respiratory Syndrome (MERS) in 2012. Both these strains led to worldwide epidemic situations with notably severe morbidities and mortalities associated with their spread[5,6]. Though causative agents of SARS and MERS dwell mainly in zoonotic reservoirs, occasional spill-over *via* intermediate hosts has occurred in susceptible human populations[4]. HCoVs are likely to have originated in bats and rodents, which are considered as the natural hosts. Human transmission of some viruses has been proven through intermediate hosts like camels, cows, civets and pigs[1].

CoVs are composed of different viral proteins (fig. 1), the first one being Spike (S), a type I transmembrane protein found on the virus surface,

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which gives the virus a distinctive 'corona' or crown like appearance (hence the name); a Membrane (M) protein, a protein which contains triple-spanning transmembrane region with its N terminal ending in exterior region and long C terminal ending in interior; a small membrane Envelope protein (E), a highly hydrophobic enigmatic protein which plays a role in virion assembly and finally, a Nucleocapsid (N) protein which undergoes multimerization and packages the viral genome into a ribonucleoprotein complex and is crucial for the virus assembly[2,6,7]. It is estimated that the mutation rate in CoV is higher compared to other single-stranded RNA (ssRNA) viruses and its average substitution rate is about ~10^{-4} substitutions per year per site. These substitutions are the reason for wider host domain of CoVs and their transmission among various species[4].

Coronavirus Disease-19 (COVID-19), the recently emerged CoV disease caused by SARS-CoV-2 is phylogenetically related to SARS-like bat CoV. Though not proven yet, SARS-CoV-2 may have enhanced human-to-human transmission rate as compared to other HCoVs[8]. In this review, we discuss the origin and epidemiology with descriptive information on already used and potential therapeutic approaches to treat SARS (2003), MERS (2012) and COVID-19 infections.

TABLE 1: CLASSIFICATION OF CORONAVIRUSES AND THEIR HOST SPECIES

Type of Coronavirus	Virus	Host
Alpha	229E	Human
	NL63	Human
	TGEV	Pig
	PRCoV	Pig
	FIPV	Cat
Beta	OC43	Human
	HKU1	Human
	SARS-HCoV	Human
	MHV	Mouse
	BCoV	Cow
	MERS-CoV	Human
Gamma	IBV	Chicken
	Turkey coronavirus	Turkey
Delta	BuCoV HKU11	Bird
	ThCoV HKU12	Bird
	MunCoV HKU13	Bird
	NHCoV HKU19	Bird
	PorCoV HKU15	Pig

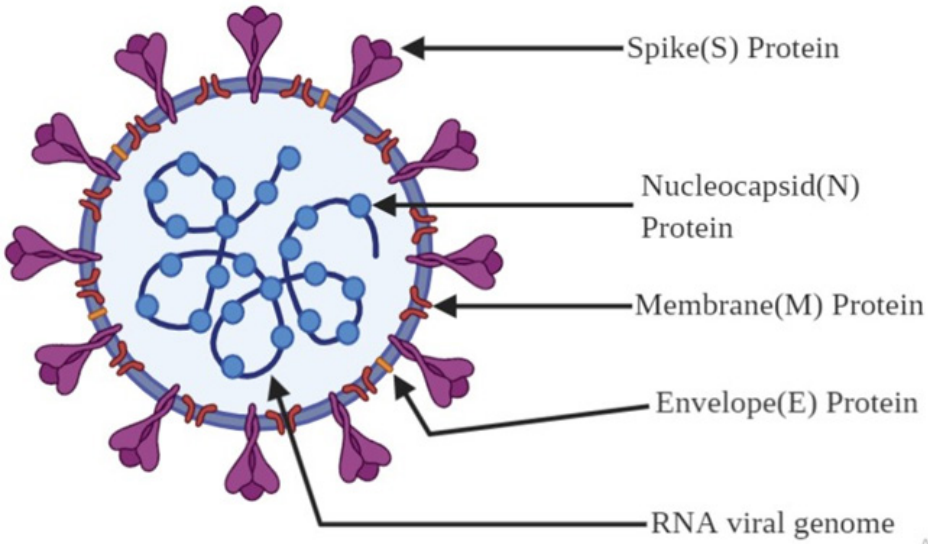

Fig. 1: The general structure of SARS-CoV-2

SARS 2003:

In November 2002, atypical pneumonia outbreaks were reported in southern China, starting with fever and mild respiratory symptoms and progressing to pneumonia in most cases. The median incubation period was found to be 4-5 d with maximum being 10 d. By February 2003, the disease had spread outside China to South-East Asia, North America and Europe[9]. On March 13, 2003, World Health Organization (WHO) declared a global alert with respect to the disease and termed it SARS. As of July 2003, 8439 cases were reported with 812 fatalities. The human chain of transmission was broken in July owing to strict isolation and tracking of infected patients which led to the receding of the first CoV pandemic. At the end of the outbreak, WHO had recorded a global case fatality ratio of 11 %[10].

In the case of SARS-CoV, it was found that masked palm civets (*Paguma larvata*), which were sold in the live animal markets of Guangdong province were the intermediate hosts. On the other hand, wild horseshoe bats (Rhinolophidae family) which were also found in the live animal markets and served in restaurants of Guangdong province, showed presence of SARS-CoV virus and thus were suggestive of a bat origin of the SARS-CoV. Evolutionary relationship between CoVs and bats showed how the virus initially spread to bats from where it diverged to civets and eventually to humans[11,12].

Initial treatment protocols for SARS:

Since SARS was unknown before 2002 and it arrived in a rather explosive manner with clustered outbreaks around the globe, it is comprehensible that there was no time to plan and conduct prospective randomized clinical trials on any pharmacotherapeutic approaches to ensure the safety and efficacy before employment for treatment[13]. To deal with initially prevalent conditions, treatment began with broad spectrum antibiotics if the case was classified as community acquired pneumonia as per guidelines of American Thoracic Society. Further, different antibiotics such as cefotaxime, levofloxacin and clarithromycin were employed for treatment. Since SARS does not respond to antibiotics, patients who were showing recovery signs with antibiotics were excluded from having SARS infection[14]. Antivirals like oseltamivir were used if patients showed possible influenza infection. Later, a combination therapy of ribavirin and low dose corticosteroids like prednisolone and hydrocortisone were used as treatment methods. Pulses of methylprednisolone were also given in response to persistence or recurrence of fever and lung opacity[15].

Antiviral regimen for SARS:

Ribavirin, a guanosine analogue, is known to have a broad spectrum of antiviral activity[16]. Though its mechanism of action is still obscure, it has been suggested to be an inhibitor of viral inosine monophosphate dehydrogenase[17]. The end state is complete disruption of RNA or DNA genome of viruses[18]. Ribavirin has been associated with hepatitis C virus, respiratory syncytial virus and various influenza virus therapies[16]. The initial experiments in Vero (African green monkey kidney) cells suggested that ribavirin played no role in inhibition of SARS-CoV replication[19,20]. However, other investigations demonstrated anti SARS-CoV activity of Ribavirin (concentration of 50 mg/ml) in foetal rhesus kidney 4 cells, although the activity tended to decrease after an incubation of 48 h[21,22]. There was no concrete clinical data to prove the effectiveness or ineffectiveness of ribavirin in SARS pharmacotherapy. Also, the observation of adverse events like dose-dependent haemolytic anaemia leading to longer hospital stay due to ribavirin therapy demotivated its use[23,24].

Upon evident information on ribavirin claiming that high drug concentration is required to inhibit the virus, further studies led to evaluation of other antivirals like lopinavir/ritonavir. The clinical findings suggested that patients showed mild action against the disease course and relatively fewer side effects compared to the control group[22]. Improved clinical outcomes were reported with the inclusion of lopinavir/ritonavir to a standard treatment protocol as an initial treatment for SARS. Certain preliminary and cohort studies show that inclusion of lopinavir/ritonavir along with other therapeutics lead to reduction in mortality rates and intubation as compared to control groups[25,26]. It is recommended to evaluate the efficacy of this treatment through randomised controlled trials during future epidemics[25].

As stated earlier, ribavirin is ineffective in Vero cells while Interferons (IFN) inhibit viral replication. Upon studying their combination in five different cell lines it was concluded that their combination was a potential therapy for SARS, owing to viral inhibitory

action by drastically reduced concentrations compared to single treatment. It was suggested that this combination could potentially help in suppressing SARS-CoV replication in the early phase and thus avert subsequent immunopathological damages, reduce virus shedding and thus reduce the risk of transmission[23].

IFN therapy for SARS:

The body, on exposure to any agent capable of causing infections, expresses innate defence mechanisms like Type I IFN (IFN-α/β) to control the replication of the agent[27]. Further *in vitro* studies inferred that Type I IFNs can be investigated for use against SARS-CoV[28-30]. A study with the aim of assessing the antiviral potential of recombinant IFNs α, β and γ was carried out against clinical isolates from Frankfurt and Hong Kong. Based on the results, it was concluded that IFNs inhibited the replication of SARS-CoV and IFN-β was more selective and more efficacious on Vero cells as compared to the other two. Though IFN-α inhibited SARS-CoV replication, its selectivity index was found to be 50-90 times lower than that of IFN-β. In Colorectal Adenocarcinoma (Caco2) cells, IFN-β was 5 to 10 times more effective[31]. A concentration of 1000 U/ml of IFN-β was found to inhibit the replication of the virus[32]. It was observed that when IFNs are used in combination they showed better antiviral effects. In another *in vitro* study, it was observed that IFN- γ synergized with other IFNs (IFNs-α and IFNs-β) and showed antiviral effects against both RNA and DNA viruses[33]. IFN-α, a registered drug for Hepatitis C viral infection has also been shown to inhibit SARS replication *in vitro* when tested in macaques. IFN-α was pegylated to improve its pharmacokinetic properties and the results obtained showed pegylated IFN-α to have an inhibitory action in the macaque model of infection[34]. Thus, it was inferred that type I IFNs could be a potential treatment for CoV infections and randomized clinical trials are required to further establish the clinical outcomes.

Corticosteroids for treatment of SARS:

According to non-randomized, retrospective preliminary studies of corticosteroid treatment in SARS virus, some benefits were seen in patients but optimal dosage and timing for administration was unexplored. It was demonstrated that upon early administration of corticosteroids like hydrocortisone, there was a delay in the clearance of viral load and prolongation of viraemia. This maybe a result of the immunosuppressive effects of corticosteroids in the human body[35]. Pulsed methylprednisolone has therefore been evaluated for treatment in SARS. The patients in the treatment group (pulsed methylprednisolone) had less oxygen requirement, a better radiographic outcome and less chance of requiring rescue corticosteroid therapy as compared to control (non-pulsed treatment). There were no other critical differences observed in terms of intubation, mortality rates and intensive care unit admission in the two groups[36].

A study was performed to study the effect of corticosteroids and its adverse events associated with therapy. Results obtained were not affirmative to say that the adverse events were associated with the therapy or with the comorbidities and age factor[37]. The use of corticosteroids in therapy is still controversial as there is no substantial evidence for the same. Though employment of corticosteroid therapy alone for SARS outweighs its risks as compared to its benefits, their combination with IFN-alfacon 1 has shown better oxygen saturation levels and superior treatment of pneumonia during preliminary experimentation[38].

Other potential therapeutic approaches for SARS:

During the search for finding a treatment method, it was seen that patients cured of SARS showed presence of neutralizing bodies in their sera. This led to further studies in this direction and results showed that humoral immunity and vaccines are attainable. A study was performed on the use of recombinant fusion protein (Receptor Binding Domain (RBD)-Fc) to generate immune response in rabbits. The antibodies generated against the RBD were highly effective in blocking the RBD-receptor and neutralizing the CoVs. A robust antibody response was obtained in the rabbits and the antisera extracted could completely block the SARS infection[39].

Data available on use of convalescent plasma therapy for various viral infections caused by Lassa, Ebola, Junin and Machupo virus had suggested clinical benefits[40-43]. Thus, it was postulated that antibodies from SARS patients may be used to suppress SARS. Plasma therapy was effective only in the early phase (before 14 d of onset of symptoms) for higher discharge rate of patients. Late phase administration (after 14 d of onset) of plasma led to higher mortality rates and longer hospital stay. Plasma infusion showed no

adverse reactions. However, it was seen that plasma therapy had better outcomes when patients were PCR positive and seronegative indicating that the timing of therapy was of importance[44].

A DNA vaccine as a new potential treatment option for SARs-CoV is under study. The DNA encoded by Spike (S) glycoprotein of the SARS-CoV is being used to induce immune response in mice[39]. Results have shown a robust immune response mediated by CD4 cells, CD8 cells and neutralising antibodies. The study has showed an appreciable reduction in viral replication in the lungs of mice[45].

A study on antiviral activities of ribavirin, 6-azauridine, pyrazofurin, Mycophenolic Acid (MPA) and glycyrrhizin were performed on two clinical isolates of CoVs (FFM-1 and FFM-2) obtained from patients with SARS. The results showed the glycyrrhizin was the most potent inhibitor of SARS-CoV and had the highest selectivity index of 67 as compared to the other entities tested[20]. However, no extensive randomized clinical trials to prove the safety and efficacy of therapeutic approaches have yet been conducted.

MERS 2012:

A decade after the first pandemic of the 21st century, another viral outbreak occurred which re-challenged therapeutic and public health interventions. A virus with very close resemblance to SARS-CoV was discovered[46]. In June 2012, a patient from Saudi Arabia was identified with SARS. Upon isolation and detection of the causative, it was found to be a novel CoV (nCoV)[47]. The virus was then named MERS-CoV because the infections were restricted to the Middle East and North Africa region with few clusters in the United States, Europe and South East Asia[48]. According to WHO data, 2494 (2102 from Saudia Arabia) cases of MERS have been detected across 27 countries around the globe and 858 patients have succumbed to it since September 2012. The case fatality ratio as of November 2019 was 34.4 %. The incubation period for symptomatic MERS is about 2-14 d.

After screening of various bats which were suspected to be the source of MERS-CoV, a bat was identified in South Africa with a very close phylogenetic relationship to MERS-CoV, though there is no concrete proof[47]. Dromedary camels have been identified as the intermediate hosts for MERS-CoV[48,49]. Data suggests that the virus has been circulating in the camels since decades before spilling over in the human population[47,50].

Initially a few clusters of MERS-CoV infections reported possible human-to-human transmission[51] which were later labelled limited to family contacts or due to nosocomial infections[48]. Unlike SARS, the MERS-CoV infections led to progressive lower respiratory tract symptoms and lymphopenia within a week after mild symptoms were seen, thus explaining a higher mortality rate.

Initial treatment protocol for MERS:

Patients found positive with MERS-CoV showed upper and lower respiratory tract infections. In many cases severe pneumonia with acute respiratory distress syndrome were also observed. Initial treatment began with broad spectrum antibiotics which were later followed by antiviral therapy. The antiviral regime and their implications were postulated from the previous experience of SARS pharmacotherapy[46]. The first line treatments possibly recommended for MERS were convalescent plasma, monoclonal antibodies and immunoglobulins in addition to standard anti-CoV antivirals[52]. It was found that repurposing of already available clinical agents was a much more effective approach[46,52,53].

Repurposing of antiviral drugs and IFNs for MERS:

There are currently no clinically proven antiviral drugs for the treatment of MERS-CoV infection. Antiviral studies reported to date have mostly been *in vitro* studies. A number of antivirals and IFNs were used as therapy options for SARS 2002-2003. An array of cohort and case studies discussed their effectiveness and ineffectiveness. Upon screening of the list of possible treatments, about 33 drugs were termed active against MERS-CoV[54].

Ribavirin, a nucleoside analogue, gets activated by the host kinases to the nucleotide (active substrate). A high concentration of ribavirin was required to inhibit the MERS-CoV replication, which was difficult to achieve *in vivo*[55,56]. *In vitro* studies examining the combination of ribavirin and IFN-α2b showed that their inhibitory concentrations dropped to ranges which could be achievable in humans. When used either prophylactically or in the early phase of treatment, clinical outcomes in patients were improved. This suggested reduced secondary transmission through reduced virus shedding[57].

A retrospective cohort study also indicated improvement in survival benefits, however, the small size of the study proved to be a limitation[58]. Recent observational studies in critically ill patients contradicted the preclinical data. There was no association derived between reduced mortality rates or faster RNA clearance upon administration of ribavirin/IFN-α combination[59]. Another retrospective cohort showed that the combination of ribavirin and IFN-α2a/IFN-β2a is ineffective for the treatment of MERS[60]. Randomized controlled trials would be helpful to determine if this combination is clinically efficacious or not. A study was performed in common marmoset models with severe disease resembling MERS in humans. The results showed that lopinavir/ritonavir improved the clinical, radiological and pathological features in the MERS-CoV infected marmosets and the treated animals had lowest viral loads[61].

Assessment of IFNs for SARS therapy indicated that Type I IFNs (α/β) can be effective therapeutic options for SARS[31]. MERS-CoV was observed to be 50-100 times more sensitive to pegylated IFN-α compared to SARS-CoV[62]. *In vitro* studies performed on infected cells showed that the virus causing lysis in the target cell were effectively inhibited by IFNs of uninfected cells proving their utility in prophylaxis or early phase after exposure[63].

In 2018, a study protocol was laid for the first randomized controlled trial investigating the combination of ritonavir/lopinavir plus IFN-β1b in laboratory confirmed MERS[64]. As an update to the trial, a statistical analysis plan has been published recently in advance of trial completion[65].

Nitazoxanide is another broad spectrum antiviral which was found to be effective against MERS-CoV by inhibiting the expression of viral N protein[66]. In the screening performed on National Institutes of Health clinical collection library 2, nitazoxanide was the top compound which showed significant anti-CoV activity[67,68]. *In vitro* studies revealed that tizoxanide, the active metabolite of nitazoxanide, inhibited the replication of CoVs[66].

New treatment approaches for MERS:

Cyclosporine-A was found to inhibit SARS-CoV and HCoV 229E *in vitro*, by blocking functional interactions between viral proteins and cellular cyclophilins[62,69,70]. *In vitro* study also showed that this drug showed inhibitory action against MERS-CoV replication. The study was performed on Vero cells and mock-infected Huh7 cells[62]. A study employing human lung tissue cells showed that the combination of IFN-α and cyclosporine-A led to considerable abatement in virus replication in the *ex vivo* culture compared to single treatment by either of them. The increased levels of IFN stimulated genes may reduce the potential immunosuppressive effect of cyclosporine-A[71]. No large scale clinical trials have been performed to prove cyclosporine's efficacy against MERS-CoV.

MPA like ribavirin, inhibits cellular inosine monophosphate dehydrogenase and thus shows antiviral potential against a number of viruses including influenza A[72]. Although MPA showed no effect on SARS-CoV, a screening study suggested that of all the drugs being screened, MPA exhibited the inhibitory action on MERS-CoV at concentration achieved by standard clinical oral dose[73]. Because data suggested that ribavirin did not inhibit MERS-CoV replication, MPA was considered a potential drug. *In vitro* studies on various IFNs and MPA showed that MERS-CoV infections can be treated with MPA (IC_{50}=2.87 μM). The study stated that the combination of IFN-β and MPA can be a potential treatment and should be studied for MERS-infected patients[74].

A comparative study was performed to determine the therapeutic efficacy of remdesivir and combination lopinavir/ritonavir and IFN-β on MERS-CoV. Assessment performed on the mouse model showed that remdesivir was active both prophylactically and therapeutically. Remdesivir improved lung function and reduced the viral loads and severe lung pathology whereas the combination lopinavir/ritonavir and IFN-β was unable to reduce viral replication or pulmonary pathology[75]. Remdesivir was tested for treatment of MERS-CoV in a non-human primate model of rhesus macaque. Prophylactic remdesivir treatment inhibited viral replication in lung tissues thus preventing clinical disease. Clinical benefits of remdesivir therapy were seen with reduction in clinical signs, inhibition of viral replication and reduction in lung lesions. Thus, it was suggested that Remdesivir was a highly effective therapeutic against MERS-CoV and could be used prophylactically, in early phase of mild symptoms or to improve recovery in severe cases[76].

Camostat and Heptad Repeat 2 peptide were reported as MERS-CoV fusion inhibitors. *In vitro* studies

on Calu-3 cells derived from human bronchial submucosal gland suggested that Camostat inhibited the entry of viruses in these cells. But the same results were not observed in immature lung tissue[77]. Viral replication and cell-cell fusion was inhibited by HR2P[78,79]. Another study on Vero-TMPRSS2 cells showed that Camostat is capable of reducing the MERS-CoV entry in healthy cells by 15-folds[77].

Antibodies for treatment of MERS:

Passive immunotherapy through monoclonal and polyclonal antibodies serves as a superior therapeutic approach for highly pathogenic existing and newly emerging viruses[80]. According to a study report, the spike (S) protein of MERS-CoV[81] interacts with CD26 (also known as DPPIV) receptor for triggering an infection[82]. In the human body, CD26 surface protein can be found in T lymphocytes, bronchial mucosa and the brush border of proximal tubules. Laboratory developed anti-CD26 monoclonal antibodies have been shown to neutralize the MERS-CoV infection by inhibiting interactions between spike protein of virus and CD26 surface cells[83].

Another study targeted to find outcomes in a human transgenic mouse model using humanized Monoclonal Antibodies (hMS-1) against MERS-CoV. The results of pre *in vitro* studies performed suggested that they have potent ability to neutralize the MERS-CoV infection. After 3 d of hMS-1 therapy, the viral titres from the mouse lungs were significantly lower compared to the control[84].

A potent MERS-CoV neutralizing monoclonal antibody was extracted from memory B cells of an infected individual. The antibody named LCA60 interfered with the binding of MERS-CoV to the cellular receptor CD26. The study was conducted on a mouse model infected with MERS-CoV where the LCA60 was administered intranasally. Reduction of 100-1000 fold in the lung viral titres were achieved which showed that the antibody showed high efficacy both in prophylaxis and therapy[85].

Human polyclonal antibodies for MERS-CoV obtained from Transchromosomic (Tc) bovines prove to be potent *in vitro* and in mouse models. The study revealed that the antibodies produced by Tc bovine in response to spike protein nanoparticle vaccine produced antibodies which showed promising results in mice model by rapidly reducing the viral titres in lungs below the limit of detection[86].

COVID-19:

The end of 2019 witnessed some pneumonia cases of unknown etiology in Wuhan, Hubei province, China[87]. Upon genetic sequencing, the causative was found to be a nCoV with 88 % resemblance to two bat-derived SARS-like CoVs and hence it was named nCoV or SARS-CoV-2[88,89]. The SARS-CoV-2 genome has been found to be 99 % identical to CoVs from pangolins. This indicates that pangolins may act as intermediate hosts for transmission but there has been no concrete proof[90]. The onset of fever, cough and dyspnea are regarded as the most common clinical manifestations for COVID-19[91,92]. On 30th January 2020, WHO declared COVID-19 as a public health emergency of international concern[93]. The disease was declared as a pandemic by the WHO on 11th March 2020[94]. The incubation period for COVID-19 ranges from 2-14 d with the median being 5.2 d[95-97]. In contrast to MERS and SARS where transmission was reported significantly through nosocomial infections[98], SAR-CoV-2 transmission has been shown through intimate contact with infected patients and asymptomatic carriers[99]. A study showed that few asymptomatic cases were identified in close contacts and they were possible asymptomatic carriers. These carriers were confirmed positive for COVID-19 but none presented any obvious symptoms with nucleic acid screening[100]. At the time of writing this article, more than 19 million cases have been reported worldwide in about 217 countries and the number of deaths have crossed the 0.7 million mark[101]. Although there have been many advancements in the public health and therapeutics sector since the first pandemic of 2003, the world is still struggling to stop the spread of the virus. Scientists all over the world are striving to develop effective and safe treatment approaches as well as vaccines to curb the growing threat of SARS-CoV-2 to humankind.

Repurposing of drugs for treatment of COVID-19:

Repurposing of drugs plays an important role in tackling the COVID-19 outbreak. *In vitro* study on United States Food and Drug Administration (US FDA) approved antiviral drugs including ribavirin, penciclovir, nitazoxanide, nafamostat, Chloroquine (CQ), favipiravir and remdesivir was performed on clinical isolates of SARS-CoV-2. Results revealed that low-micromolar concentration of remdesivir and CQ potently blocked the virus infection with high

selectivity index. Favipiravir also showed inhibitory action against the virus infection recommending *in vivo* evaluation for further efficacy[102]. A single centered retrospective, observational study was performed using two different drug regimens. Regimen I (azithromycin, prednisolone, naproxen, and lopinavir/ritonavir) and regimen II (meropenem, levofloxacin, vancomycin, hydroxychloroquine (HCQ) and oseltamivir) study results showed that regimen I was more effective in improving clinical outcomes and shortening the patient's hospital stay[103].

CQ and HCQ are thought to block the viral entry through receptor glycosylation, proteolysis, endosomal acidification and other immunomodulatory mechanisms[104,105]. Initial study reports stated that CQ could inhibit the replication of various HCoVs[106–109]. An observational study in China employed CQ for treatment of COVID-19 patients. Preliminary reports suggest that the molecule helped in rapid decline in fever, quicker recovery time and improved Lung CTs[110]. An *in vitro* study determining the antiviral activity of HCQ against CQ on SARS-CoV-2 infected cells showed that HCQ was more potent than CQ. Further, physiology based pharmacokinetic modelling was used to find the most effective dosage regimen[111]. Among 1376 COVID-19 patients involved in an observational cohort study in New York City, none were significantly benefited or harmed through administration of HCQ for treatment[112]. A small size non-randomized trial suggested that the combination of HCQ and azithromycin decreased pulmonary viral loads in patients infected with SARS-CoV-2[113]. However, a retrospective study on HCQ and azithromycin revealed they did not show any effect on the survival outcomes or the need of mechanical ventilation, whether they were given individually or in combination[114]. Efficacy of HCQ as postexposure treatment was investigated by a randomized, double-blind placebo-controlled trial on 821 asymptomatic patients. The outcome obtained from the trial was not as expected. No significant benefit was obtained by HCQ as post exposure prophylaxis for COVID-19[115]. At the time of writing this article, 247 clinical trials were being evaluated to investigate the use of HCQ and 84 of CQ for COVID-19, with 107 and 33 respectively, in Phase 3.

Remdesivir shows broad antiviral spectrum against paramyxoviruses, pneumoviruses, and CoVs being a monophosphoramidate prodrug of an adenosine analogue. *In vitro* studies of remdesivir showed that this drug inhibited all human and animal CoVs including SARS-CoV-2[116,117]. A randomised, double-blind, placebo-controlled, multicentre trial on COVID-19 patients suggested that intravenous administration of remdesivir did not improve the time required to attain clinical improvement in comparison to placebo although remdesivir showed clinically meaningful changes in various other parameters[118]. In a randomized clinical trial funded by National Institute of Allergy and Infectious Diseases that enrolled COVID-19 infected candidates with lower respiratory tract problems, remdesivir shortened the time to recovery effectively as compared to placebo[119]. Remdesivir was approved by US FDA to be used against COVID-19 in May 2020. The European medicines agency granted approval to remdesivir in case of COVID-19 patients requiring supplemental oxygen in June 2020[120]. At the time of writing this article, 44 clinical trials were evaluating the use of remdesivir for COVID-19, with 19 of them in Phase 3 trials and 3 completed.

Favipiravir is an inhibitor of RNA-dependent RNA polymerase in viral cells. It has been shown to be effective against various viruses including influenza, H1N1, avian viruses and many others[121]. *In vitro* studies revealed that favipiravir showed activity against SARS-CoV-2 but required higher drug concentration in comparison to remdesivir or CQ[102]. An open-label controlled study was performed on COVID-19 patients, in China. Those treated with favipiravir showed faster viral clearance and improved chest images than patients treated with lopinavir/ritonavir[122]. At the time of writing this article, 32 clinical trials were evaluating the use of favipiravir for COVID-19 with 14 of them in Phase 3.

The outcomes from a randomized study evaluating lopinavir/ritonavir as a therapy for hospitalized severe COVID-19 cases stated that when added to standard supportive care; it was not associated with improved clinical results, reduced pulmonary viral loads or reduced mortality[123,124]. A multicentre randomised open-label phase 2 trial on triple combination of an injectable IFN-β-1b, oral protease inhibitor (lopinavir-ritonavir), and an oral nucleoside analogue (ribavirin) was performed on COVID-19 patients. Results showed that the combination was effective in suppressing the shedding of SARS-CoV-2 when given within 7 d of symptom onset in comparison to lopinavir-ritonavir alone[125]. More clinical trials studying the efficacy of ritonavir/

lopinavir and their combinations seem necessary.

Nirmatrelvir, in combination with ritonavir, showed relatively fewer drug-related adverse reactions in addition to substantial antiviral activity against CoV. *In vitro* studies of nirmatrelvir carried out in human alveolar and bronchial epithelial cells suggested that nirmatrelvir inhibited SARS-CoV-2 viral replication. Animal studies in SARS-CoV-2 modelled mice reveal that nirmatrelvir significantly reduces viral load in the lungs of the mice. Results from the phase 2/3 combined clinical trial of nirmatrelvir plus ritonavir showed that the combination drug reduces the risk of development of severe disease among symptomatic patients by reducing the viral load. Nirmatelvir/ ritonavir is one of the first orally available therapies for COVID-19. This combination gained the emergency approval authorization by the FDA for use in individuals 12 y and older in December 2021[126-129].

A systemic screening was performed to determine the inhibitory action of several drugs by inhibiting the S glycoprotein induced cell fusion. Drugs included in study were cardiac glycosides (ouabain and digoxin), kinases inhibitors (e.g. imatinib mesylate) and anti-HIV drugs (e.g. nelfinavir mesylate) of which nelfinavir mesylate drastically inhibited Sn- and So-mediated cell fusion at micromolar concentrations[130].

SARS-CoV-2 is reported to have a significant sensitivity to IFN-α *in vitro*. IFN are suggested to decrease the viral titres by several folds. Also, studies demonstrate that SARS-CoV-2 is more sensitive to IFNs-I as compared to SARS-CoV. A study also states that IFN-I can be used as a prophylactic agent against COVID-19[131]. Few preprints showed that Type I and type III IFN show some activity against SARS-CoV-2 *in vitro* in Vero cells[132]. An uncontrolled, exploratory study was performed on hospitalised COVID-19 patients using IFN-α2b, arbidol or a combination as treatment regimen. It was observed that IFN-α2b whether given with or without arbidol caused reduction in viral shedding and reduction in virally induced inflammation by lowering the levels of CRP and IL-6, suggesting that interferon should be further investigated as therapy for COVID-19[133]. At the time of writing this article, 84 clinical trials were listed to evaluate IFN for COVID-19 treatment, of which, 17 were in Phase 3.

Antibody treatment for COVID-19:

A single canter study in Italy found that COVID-19 patients with Acute Respiratory Distress Syndrome (ARDS) characterized by hyper inflammatory syndrome showed improvement upon administration of intravenous tocilizumab (hMS-1 and Interleukin 6 inhibitor)[134]. A retrospective, observational cohort study on patients with severe COVID-19 pneumonia suggested that tocilizumab reduces the risk of invasive mechanical ventilation or death in severe patients whether administered intravenous or subcutaneous[135]. A preliminary study in China in 6 patients (2 of which were confirmed SARS-CoV positive) concluded that convalescent plasma therapy improved clinical outcomes and radiological abnormalities. The two positive patients showed elimination of virus and the other two showed increased circulating antibodies[136]. In another case, 5 patients characterized as critically ill with COVID-19 and ARDS were given convalescent plasma therapy containing neutralizing antibodies. Patients showed great response, their viral load declined within a few days of treatment and they no longer needed mechanical ventilation support[137]. The US FDA has approved the use of convalescent plasma for life threatening COVID-19 infections. Large scale multicenter randomized trials are required to confirm the safety and efficacy of plasma therapy for SARS-CoV-2. Currently 150 clinical trials on plasma therapy for COVID-19 are underway with 25 of them in Phase 3.

Vaccines for COVID-19:

Vaccine candidates for SARS and MERS demonstrated risk of direct worsening pulmonary conditions and antibody dependent exacerbation of lung disease. Therefore, testing of COVID-19 vaccines in suitable animal models and significant safety monitoring in human phase trials is challenging[138]. The S-protein of SARS-CoV-2 serves as the major target for vaccine development owing to the studies on neutralizing antibodies and their interaction with the protein[139]. An animal study on macaques for vaccine development showed a single shot vaccine inducing a robust neutralizing antibodies response and complete protection against SARS-CoV-2 infection[140].

According to the latest report by the WHO, 153 vaccine candidates are currently under clinical development phase and 196 are in the preclinical stage. Four vaccines candidates AstraZeneca-SK

Bio, Serum Institute of India, Janssen and Moderna have received approval for emergency use. Pfizer/BioNTech is the only vaccine candidate that has received the full US FDA approval for the individuals 16 y and older. Table 2 lists all the vaccine candidates in Phase 4 trials currently.

According to the WHO latest report, 26 candidate vaccines are undergoing clinical evaluation while 139 are in the preclinical stage[141]. Listed in Table 2 are the top 9 vaccine candidates in development at the time of writing this overview[142,143].

Summary

Though the SARS 2002, MERS 2012 and COVID-19 causatives are not entirely identical, similar pharmacotherapeutic approaches have been evaluated for use during the emergence of these pandemics (Table 3). Introduction and employment of newer agents helped in improving the sensitivity and providing a wider range of therapy for mild to severe infection types. COVID-19 has proved to be the most explosive HCoV outbreak with enhanced human-to-human transmission rate. To combat the spread, immense efforts have been put in to evaluate previously employed approaches for repositioning of drugs along with continuous attempts to introduce effective and clinically proven pharmaceutical interventions, most importantly led by vaccines.

TABLE 2: THE TOP NINE VACCINE CANDIDATES AS PER WHO DRAFT LANDSCAPE

S. No	COVID-19 Vaccine developer/manufacturer	Vaccine platform	Type of candidate vaccine	Clinical Trial Phase
1	Sinovac Research and Development Co., Ltd	Inactivated virus	CoronaVac; inactivated SARS-CoV-2 vaccine (Vero cell)	Phase 4-NCT04756830
2	Sinopharm; China National Biotec Group Co; Wuhan Institute of Biological Products	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	Phase 4-NCT05065892
3	Sinopharm; China National Biotec Group Co; Wuhan Institute of Biological Products; Beijing Institute of Biological Products	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell), vaccine name BBIBP-CorV	Phase 4-NCT05075083
4	AstraZeneca+University of Oxford	Viral vector (non-replicating)	ChAdOx1-S-(AZD1222)	Phase 4-NCT04760132
5	CanSino Biological Inc./Beijing Institute of Biotechnology	Viral vector (non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector)	Phase 4-NCT04892459
6	Janssen Pharmaceutical	Viral vector (non-replicating)	Ad26.COV2.S	Phase 4-EUCTR2021-002327-38-NL
7	Moderna+National Institute of Allergy and Infectious Diseases (NIAID)	RNA based vaccine	mRNA-1273	Phase 4-NCT04760132
8	Pfizer/BioNTech+Fosun Pharma	RNA based vaccine	BNT162b2 (3 LNP-mRNAs)	Phase 4-NCT04760132
9	Medigen Vaccine Biologics+Dynavax-+National Institute of Allergy and	Protein subunit	MVC-COV1901 (Spike-2P protein+adjuvant CpG 1018)	Phase 4-NCT05079633

TABLE 3: SUMMARY OF TREATMENTS FOR CORONAVIRUS OUTBREAKS BASED ON *IN VITRO* AND RANDOMIZED OBSERVATIONAL STUDIES

Virus	Treatment	Level of study	Effective	Reference
SARS-CoV	Antibiotics (cefotaxime, levofloxacin and clarithromycin)	Observational study	No	[14]
	Ribavirin	Observational study	No	[23,24]
	Lopinavir/Ritonavir	Preliminary and Cohort study	Yes	[25,26]
	Interferons	*In vitro* and preclinical evaluation	Yes	[28,29,30,31,32]
	Corticosteroids	Non-randomized preliminary study	Yes	[35]

MERS-CoV	Antibiotics	Observational study	No	[46]
	Ribavirin+IFNs	Retrospective cohort study	Yes	[57,58]
	Cyclosporine	*In vitro*	Yes	[62]
	Nitazoxanide	*In vitro*	Yes	[66]
	Mycophenolic acid	*In vitro*	Yes	[73,74]
	Remdesivir	Preclinical evaluation	Yes	[75,76]
	Monoclonal and polyclonal antibodies	*In vitro* and preclinical evaluation	Yes	[80,84,86]
COVID-19	Chloroquine/Hydroxychloroquine	*In vitro* and Observational cohort study	Yes	[110,111]
	Remdesivir	*In vitro* and Randomized controlled study	Yes	[116,118,119]
	Favipiravir	*In vitro* and Controlled cohort study	Yes	[102,122]
	Lopinavir/Ritonavir	Randomized study	Yes	[123,124]
	Triple regime (Ribavirin+IFN+LPV/RTV)	Randomized multicentre study	Yes	[125]
	IFNs	Observational nosocomial study	Yes	[129]
	Tocilizumab	Retrospective observational cohort	Yes	[131]
	Plasma therapy	Preliminary study	Yes	[132,133]

Conflict of interests:

The authors declared no conflict of interests.

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