Review

SARS-CoV-2/COVID-19 and Advances in Developing Potential Therapeutics and Vaccines to Counter this Emerging Pandemic Virus – A Review

Ali A. Rabaan,1 Shamsah H. Al-Ahmed,2 Ranjit Sah,3 Ruchi Tiwari,4 Mohd. Iqbal Yatoo,5 Shailesh Kumar Patel,6 Mamta Pathak,6 Yashpal Singh Malik,7 Kuldeep Dhama,6*, Karam Pal Singh6, D. Katterine Bonilla-Aldana,8,9 Shafiul Haque,10 Alfonso J. Rodriguez-Morales.9,11

1Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia.
2Specialty Paediatric Medicine, Qatif Central Hospital, Qatif, Saudi Arabia.
3Department of Microbiology, Tribhuvan University Teaching Hospital, Institute of Medicine, Kathmandu, Nepal.
4Department of Veterinary Microbiology and Immunology, College of Veterinary Sciences, UP Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigsany Vishwavidyalay Evum Go-Anusandhan Sansthan (DUVASU), Mathura 281001, India.
5Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 190025 Srinagar, Jammu and Kashmir, India.
6Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar 243 122, Bareilly, Uttar Pradesh, India.
7Division of Biological Standardization, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly- 243 122, Uttar Pradesh, India.
8Semillero de Zoonosis, Grupo de Investigación BIOECOS, Fundación Universitaria Autónoma de las Américas, Sede Pereira, Pereira, Risaralda, Colombia;
9Public Health and Infection Research Group, Faculty of Health Sciences, Universidad Tecnologica de Pereira, Pereira, Colombia
10Research and Scientific Studies Unit, College of Nursing & Allied Health Sciences, Jazan University, Jazan, Saudi Arabia
11Grupo de Investigacion Biomedicina, Faculty of Medicine, Fundacion Universitaria Autonoma de las Americas, Pereira, Risaralda, Colombia

Running Title: COVID-19 Threats, Therapeutics and Vaccines

Corresponding Author(s):
Kuldeep Dhama, PhD (kdhama@rediffmail.com), Division of Pathology, ICAR –Indian Veterinary Research Institute, Izatnagar, Bareilly, India.
Alfonso J Rodriguez-Morales, HonDSc (arodriguezm@utp.edu.co), Public Health and Infection Research Group, Faculty of Health Sciences, Universidad Tecnologica de Pereira, Pereira, Colombia.

Abstract length: 288 words
Figures and tables: 4 (2 figures and 2 tables)
Manuscript length: 12000 words
ABSTRACT
A novel coronavirus (SARS-CoV-2), causing an emerging coronavirus disease (COVID-19), first detected in Wuhan City, Hubei Province, China has resulted in an outbreak in China which has taken a catastrophic turn with high toll rates in China and subsequently spreading across the globe. The rapid spread of this virus to more than 175 countries while affecting nearly 500,000 persons and causing more than 22,000 human deaths, it has resulted in a pandemic situation in the world. The SARS-CoV-2 virus belongs to the genus Betacoronavirus, like MERS-CoV and SARS-CoV, all of which originated in bats. It is highly contagious, causing symptoms like fever, dyspnea, asthenia and pneumonia, thrombocytopenia and the severely infected patients succumb to the disease. Coronaviruses (CoVs) among all known RNA viruses have the largest genomes ranging from 26 to 32 kb in length. Extensive research has been conducted to understand the molecular basis of the SARS-CoV-2 infection and evolution, develop effective therapeutics, antiviral drugs and vaccines, and to design rapid and confirmatory viral diagnostics as well as adopt appropriate prevention and control strategies. Till date, no clinically proclaimed, proven therapeutic antibodies or specific drugs and therapeutics, and vaccines have turned up. Several molecular diagnostic tests such as Real Time-PCR, isothermal loop-mediated amplification of coronavirus (i-LACO), full genome analysis by next-generation sequencing (NGS), multiplex nucleic acid amplification, and microarray-based assays are in use currently for the laboratory confirmation of this CoV infection. In this review article, we describe the basic molecular organization and phylogenetic analysis of the coronaviruses, including the SARS-CoV-2, and recent advances in diagnosis and vaccine development in brief and focusing mainly on developing potential therapeutic options that can be explored to manage this pandemic virus infection, which would help in valid countering of COVID-19.

Keywords: SARS-CoV-2, COVID-19, immunotherapeutics, therapeutics, vaccines

1. Introduction
Since December 2019, a pneumonia-like Coronavirus emerged in Wuhan, China. Within a few weeks, a novel coronavirus was given a nomenclature as 2019 novel coronavirus (2019-nCoV) by the World Health Organization (WHO) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by International Committee on Taxonomy of Viruses (ICTV) (Gorbalenya et al., 2020), causing the disease termed as an emerging coronavirus disease (COVID-19). Since then, this virus has spread to every populous continent and infected thousands around the world - through the vast majority of confirmed cases, and fatalities fall within China and spreading to Italy, Iran, South Korea, France, Spain, USA, Japan, Spain, and many others. COVID-19 has now spread to 175 countries with nearly 500,000 confirmed cases and 22,000 human deaths, and most recently (on 11th March 2020) WHO proposed it as a pandemic situation worldwide (WHO, 2020a; Yang et al., 2020a). Unlike other coronavirus epidemics like SARS and MERS, the transmission rate of COVID-19 is much higher with spreading of the virus infection to an average of two to three individuals from every infected individual (Gates et al., 2020).
Travellers have been mainly implicated in giving wings to SARS-CoV-2 for its worldwide spread (Rodriguez-Morales et al., 2020a; Wilson and Chen, 2020). The new strain of the virus that originated in Wuhan, Hubei province of China is spreading rapidly and crossing borders. Most of the affected patients in China worked at or lived in Huanan seafood wholesale market, where live animals like snakes, bats, birds, marmots, and other wildlife farm animals were sold (Ji et al., 2020). Wuhan is now considered a hotspot for emerging infectious disease. The virus did not match with the existing known viruses. Several cases of pneumonia were reported from 31st December 2019 with unknown etiology in Wuhan, China (Chen et al., 2020a). The SARS-CoV-2 virus belongs to the family of viruses that include the common cold, and viruses of SARS and MERS (Vijayanand et al., 2004; Ramadan and Shaib, 2019; Peeri et al., 2020). It affects humans leading to respiratory infection and animals including cattle, camels, swine, cats, horses, rabbits, birds, bats, dogs, ferrets, rodents, snake, mink, and causes multiple system infection (Dhama et al., 2020a). Infected patients develop a high fever, dyspnea, pneumonia, chest pain, dry cough, myalgia and diarrhoea as clinical signs of the disease (Bassetti et al., 2020; Huang et al., 2020).

Structures of many individual proteins of SARS, MERS, and related coronaviruses, as well as their biological interactions with other viral and host proteins, have been explored along with the experimental testing of anti-viral properties of small compounds (Gui et al., 2017; Jia et al., 2019; Galasiti Kankanamalage et al., 2018). Till date, there are still not proclaimed clinically proven therapeutic antibodies, drugs and vaccines specific for coronaviruses, which makes it tougher to tackle SARS-CoV-2 caused diseases. This article, in brief, describes the molecular organization and phylogenetic analysis of the coronaviruses including the SARS-CoV-2 highlights a few advances in diagnosis and vaccine development and emphasizes in details on the different potential therapeutic options that could be pursued for therapy despite limited knowledge of the biology of SARS-CoV-2 such as neutralizing antibodies, oligonucleotides, passive antibody transfer and drug repurposing, anti-viral proteases, blocking Coronavirus receptors like an angiotensin-converting enzyme (ACE2), combination therapy, which can bring a revolutionary change to curb the SARS-CoV-2 / COVID-19 in the coming future.

2. Coronaviruses
Coronaviruses, members of the family Coronaviridae are enveloped, positive-stranded RNA viruses which appear to demonstrate spikes of glycoproteins protruding from their viral envelopes, thus exhibit a corona or halo-like appearance (Masters and Perlman, 2013; Cui et al., 2019). Earlier coronaviruses were found to be the causative agents for a broad spectrum of respiratory and gastrointestinal diseases in both wild and domestic animals, including birds, pigs, rodents, among others (Dhama et al., 2014, 2020a). Previous studies have found that six strains of CoVs are capable of infecting human, among which four strains circulate yearly to cause the common cold, and other two strains which are the source for severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS-CoV), respectively (Perlman and Netland, 2009; Dhama et al., 2014, 2020a; Cui et al., 2019; Ramadan and Shaib, 2019; Liu et al., 2020a).
Coronaviruses, among all known RNA viruses, have the largest genomes ranging from 26 to 32 kb in length (Regenmortel et al., 2000; Schoeman and Fielding, 2019). In addition to encoding structural proteins, a significant chunk of the coronavirus genome is transcribed and translated into a polypeptide, which encodes proteins essential for viral replication and gene expression (Lai and Holmes, 2001). The ~306 aa long main protease (Mpro), which is a highly conserved sequence, is a crucial enzyme for coronavirus replication (Lai and Holmes, 2001). Mpro has been utilized for developing anti-coronaviral drugs (Xue et al., 2008; Anand et al., 2003). Nevertheless, in the past two decades, a massive amount of research has been done to understand the replication, molecular basis of the coronavirus infection and evolution, develop effective therapy in forms of both vaccines and antiviral drugs, and propose efficient measures for viral detection and prevention (Perlman and Netland, 2009; Cotten et al., 2013; Li et al., 2016; Walls et al., 2016; Millet and Whittaker, 2018; Song et al., 2018; Chen et al., 2020b; Dhama et al., 2020a).

3. Taxonomy and Classification
ORDER: Nidovirales
FAMILY: Coronaviridae
SUB FAMILY: Coronavirinae
GENERA: Betacoronavirus

The coronavirus belongs to the Coronaviridae family, Nidovirales order (Huang., 2020). The International Committee on Taxonomy of Viruses (ICTV) had broadly divided Coronaviruses into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (van Boheemen et al., 2012). There have been outbreaks of human coronavirus (HCoVs) in the past: HCoV-229E and HCoV-OC43 outbreak in the late sixties, HCoV-NL63 in 2004, and HCoV-HUK1 in 2005. There are another two types of betacoronaviruses (zoonotic pathogens) that resulted in outbreaks in the past: SARS-CoV outbreak in Guangdong, southern China in November 2002 infected 8422 patients across the world over 29 countries (Chen et al., 2020b, Lu et al., 2020a) and MERS-CoV outbreak in Saudi Arabia in 2012 infected 2,260 cases that resulted in 803 deaths over 27 countries (Geller et al., 2012; Ramadan and Shaib, 2019). Apart from that, another coronavirus emerged in 2017, known as the swine acute diarrhoea syndrome (SADS) coronavirus (Markotter et al., 2019). Table 1 describes the broad classification of the coronavirus.

4. Phylogenetic analysis and Genomic organization
Recent phylogenetic analysis has revealed that the new virus spreading in China is a version of SARS-CoV. There are two significant similarities between SARS-CoV-2 (nCoV-2019) and SARS-CoV: they share nearly 80% of their genetic codes, and both are originated in bats (Malik et al., 2020; Zhu et al. 2020). The first study to analyze the viral genome analysis was conducted in the Wuhan Institute of Virology. Samples from seven patients initially reported cases of severe pneumonia were used for the analysis. It was found that the new virus genetic sequences were 79.5% similar to SARS-CoV (https://www.businessinsider.in/science).
The genome of CoVs consists, a single-stranded, positive-sense RNA of around 29.8 kb nucleotides in length with a 5’-cap structure and 3’-polyA tail. The polyprotein 1a/1ab (pp1a/pp1ab) is directly translated using genomic RNA as a template, which encodes nonstructural proteins (nsps) and forms the replication-transcription complex (RTC). Also, a nested set of subgenomic RNAs (sgRNAs) are produced by replication-transcription complex in a discontinuous manner of transcription. It has common 5’-leader sequences and 3’-terminal sequences. Transcription termination and acquisition of leader RNA happens at transcription regulatory sequences, which are located between open reading frames (ORFs). These sgRNAs (minus-strand) acts as the templates for the synthesis of subgenomic mRNAs (Perlman and Netland, 2009; Chen et al., 2020b; Dhama et al., 2020a).

A typical CoV’s genome and subgenome contain at least six ORFs. The first ORF (ORF1a/b), contains two-third of the whole length genome and encodes 16 nsps (nsp1-16). A-1 frameshift between ORF1b and ORF1a, leads to the synthesis of two polypeptides: pp1ab and pp1a. These are processed by chymotrypsin-like protease or two papain-like protease or main protease and one into 16 nsps. Rests of the ORFs of the genome encode four major structural proteins: membrane (M), spike (S), nucleocapsid (N) and envelope (E) proteins. Other than these proteins, different CoVs encode specific accessory proteins and structural proteins, such as 3a/b protein, HE protein and 4a/b protein (Perlman and Netland, 2009; Chen et al., 2020b; Dhama et al., 2020a).

The genomic sequence alignment among different CoVs shows 58% identity on the non-structural protein-coding region, 43% identity on the structural protein-coding region, with 54% at the whole genome level which suggests the non-structural proteins are more conserved when compared to the structural proteins and structural proteins are more diverse when in need of adaptation for new hosts. However, the CoV genome is extensive, with ~30 kb in length and is considered as the largest known RNA viruses. Such a large genome of CoVs is maintained by the unique features of the CoV RTC, which encodes for many RNA processing enzymes. The 3’-5’ exoribonuclease is one such kind of RNA processing enzyme and is unique in CoVs among different RNA viruses, also believed for providing a proofreading function to the RTC. Sequence analysis depicts that the SARS-CoV-2 has a typical genome structure same as of CoV and mainly belongs to the Betacoronaviruses cluster, which includes Bat-SL ZXC21, Bat-SARS-like (SL)-ZC45, SARS-CoV, and MERS-CoV (Chen et al., 2020b; Dhama et al., 2020a). The glycoprotein spike surface plays an essential role in receptor binding and determines host tropism (Zhu et al., 2018). These proteins of SARS-CoV and MERS-CoV bind via different receptor-binding domains (RBDs) to various host receptors. SARS-CoV utilizes angiotensin-converting enzyme 2 (ACE2) as the primary receptor and MERS-CoV utilizes dipeptidyl peptidase 4 (DPP4, also known as CD26) as the primary receptor. Initial analysis depicted that SARS-CoV-2 has a quite close phylogenetic association with SARS-like bat coronaviruses. (Malik et al., 2020; Zhou et al., 2020)

Comparison of genomes of SARS-CoV-2, SARS-CoV and MERS-CoV strains suggested that they are almost identical and possess only five nucleotide differences in the genome. The
SARS-CoV-2 genome annotation shows that they possess 14 ORFs encoding for 27 different proteins. The orf1a and orf1ab genes of the genome, respectively encode the pp1a and pp1ab proteins. They together possess 15 nsps, from nsp1 to nsp10 and nsp12 to nsp16. The genome also contains eight accessory proteins and four structural proteins (S, E, M, and N). The SARS-CoV-2 is quite similar to that of SARS-CoV, at the amino acid level. Also, there are some differences. For example, the 8b protein is 84 amino acids in SARS-CoV, whereas in 2019-nCoV, 8b protein has 121 amino acids; the 8a protein is present in SARS-CoV but absent in SARS-CoV-2 (Chen et al., 2020b; Dhama et al., 2020b).

An observation of the amino acid substitutions in different proteins occurs among them, and this sheds light into how SARS-CoV-2 differs from SARS-CoVs structurally and functionally. In total, 380 amino acid substitutions were there between the SARS-CoV-2 amino acid sequences and the SARS and SARS-like viruses. However, no amino acid substitutions were found in nonstructural protein 7 (nsp7), envelope, nsp13, matrix, or accessory proteins 8b and p6. Nevertheless, due to minimal knowledge about this novel virus, it is quite hard to give reasonable explanations for the amino acid substitutions between the SARS-CoV-2 and SARS or SARS-like CoVs and also whether these differences could affect the host transmission property of the SARS-CoV-2 when compared to SARS-CoV needs future investigation (Wu et al., 2020a).

SARS-CoV-2 became the seventh member of the human coronaviruses (HCoVs) identified as HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV (which causes severe acute respiratory syndrome), MERS-CoV (Middle East respiratory syndrome), and now SARS-CoV-2 (Rhode, 2020).

5. **The emergence of Coronavirus (SARS-CoV, MERS CoV and SARS-CoV-2)**

The Coronaviruses were known to cause only mild respiratory disease in humans; with severe infections in the immunocompromised cases and young kids mainly acting as an asymptomatic carrier as disease symptoms are not much prominent in young ones (Shen et al., 2020). Coronaviruses have the highest known frequency of recombination of any positive-strand RNA virus (Rohde, 2020). Earlier reports suggested the emergence of SARS-CoV by recombination between bat SARS related coronaviruses (SARSt-CoVs) followed by mutations in civets before its spillover. Similarly, the MERS-CoV also circulated and attained mutations for around 30 years in camels before the MERS pandemic happened (Muller et al., 2014; Cui et al., 2019), suggesting the adaptation of the viruses to the environment and different host before their spillover to humans (Ellwanger and Chies, 2018). Coronaviruses also have an essential replication process, which involves a 2-step replication mechanism. Many RNA virus genomes contain a single open reading frame (ORF), but coronaviruses can contain up to 10 separate ORFs except for SARS-CoV-2, which has single intact ORF (Rohde, 2020). That can lead to the emergence of CoV at times.

5.1. **SARS-CoV**

SARS-CoV emerged first in the Guangdong Province of China; and spread internationally to 37 different countries. Noteworthy outbreak sites included China, Hong Kong, Taiwan,
Singapore, and Vietnam. However, outside the Southeast Asia area, Canada was also significantly affected. WHO reported 8,437 SARS cases and 813 deaths between the period of November 2002 to July 2003. Transmission is presumed to be from Chinese horseshoe bats harbouring SARS-like viruses directly to humans or via intermediate hosts, i.e. palm civets or raccoon dogs in the live-animal markets of China. The employment of control measures inclusive of quarantine and air travel blockade, ultimately resulted in the disappearance of SARS-CoV in 2003, with no human infections reported ever since (Vijayanand et al., 2004; WHO, 2020b).

5.2. MERS-CoV

MERS-CoV was first identified in the Kingdom of Saudi Arabia in 2012. Cases have been geographically restricted to the Arabian Peninsula. However, a smaller number of cases were also found in Europe, i.e. in people who had travelled to the Arabian Peninsula or had been in contact with people who had. Individual cases and small clusters continue to be reported in that region. MERS-CoV is thought to be transmitted from camels to humans, with the possibility that at some point bats infected camels. MERS-CoV is thought to have emerged from bats or other small animals, and have infected humans via dromedary camels acting as intermediate hosts (Ramadan and Shaib, 2019; WHO, 2020c).

5.3. SARS-CoV-2

A novel (new) coronavirus (SARS-CoV-2) that was first detected in Wuhan City, Hubei Province, China is posing a significant threat now by creating a pandemic situation of the COVID-19 (WHO, 2020d; Yang et al., 2020a). The first case was detected in December 2019, and presently nearly 500,000 confirmed cases detected (WHO, 2020a). The disease terrorized over 175 countries/territories/areas with 22,000 deaths which is probably not the last figure to be known (WHO, 2020a). In early March 2020, the pandemic started to subside in China (Flahault, 2020) but started to haunt Europe and the United States. In this context, other than China, the major blow was felt by Italy, Spain, Iran and France with 7500, 3,650, and 2077 deaths, respectively but the pain of countries like France (1,330 deaths), USA (944 deaths), UK (465 deaths), Republic of South Korea (126 deaths), Netherlands (356 deaths) and Germany (206 deaths) could not be overlooked.

However, only a few countries are left beyond the reach of this unfortunate pandemic, but their possibility of remaining untouched by this pathetic virus is meagre because within a short period the virus emerged in many new countries including India where two individual recently got victimized. Now the number of new confirmed cases and deaths are shooting day by day with no hope of sudden halt leaving the global population in a stage of physiological, psychological, and socioeconomic stress. In a study, the psychological stress of general public along with nurses involved in the treatment of COVID-19 patients was evaluated based on vicarious traumatization scores and revealed that the scores were found significantly higher for the general public than nurses (Li et al., 2020a).

The origin of SARS-CoV-2 was postulated to be from bats, considering them the natural reservoirs of the virus (Chan et al., 2020a). The search of probable intermediate hosts is going
on keeping the main focus on animal species available for sale in Huanan seafood market. Recently, a study suggested the pangolins to serve as a natural reservoir host of SARS-CoV-2-like coronaviruses (Pangolin-CoV) based on 91.02% sequence similarities at the whole-genome level between pangolin-CoV and SARS-CoV-2. Additionally, the S1 protein of pangolin coronavirus was reported to be more closer to SARS-CoV-2 than bat coronavirus (RaTG13) (Zhang et al., 2020a). Initially, the disease was thought to be spreading via seafood consumption, but later contact transmission of SARS-CoV-2 among humans was confirmed. In nearby places like clinics and hospitals, CoV can be transmitted through the air if people remain there for a long duration of time, suggesting the risk of aerosol transmission (Cascella et al., 2020).

The emergence of SARS-CoV in 2002 established CoVs as capable of causing severe disease in humans. This ability was once again demonstrated by the emergence of a second severe CoV a decade later; the MERS-CoV in 2012. The MERS-CoV and SARS-CoV emergence are believed to be the result of spill-over of bat-adapted CoVs into an intermediate host. They are transmitted from person to person through respiratory droplets and close contact (https://www.cdc.gov/coronavirus/types.html). The virus is believed to be transmitted to patients living in or working at areas in proximity to wholesale seafood markets and places where live animals like snakes, bats, birds, marmots, and other wild or farm animals were sold (Ji et al., 2020). Rarely animal coronaviruses infect humans severely except for SARS-CoV, MERS-CoV and now SARS-CoV-2. Seldom coronaviruses cause lower respiratory infection or pneumonia but SARS-CoV-2 causes. Coronaviruses have seasonal pattern usually affecting in winter, however, can infect out of season also (Rohde, 2020).

6. COVID-19 Clinical Pathology

The coronavirus claimed its first life on January 10th, 2019, and since then the death toll has climbed at an alarming and accelerating rate. The virus seems lethal, causing severe acute respiratory symptoms, including fever, dyspnea, asthenia and pneumonia, thrombocytopenia, and increased C-reactive protein and lactate dehydrogenase levels among people in Wuhan, China (Zhu et al., 2020; Lu et al., 2020b). As per reports, mild COVID-19 cases revealed higher levels of pro-inflammatory cytokines and chemokines like IFN-α, IL-1β, MCP-1 and IP-10 whereas individuals with severe COVID-19 had upregulation of IP-10, IL-8, IL-10, TNF-α, G-CSF, MCP-1 and MIP-1A (Huang et al., 2020; Qin et al., 2020) resulting into cytokine storm syndrome followed by severe pulmonary damage and death due to respiratory failure. Additionally, all the blood cells except neutrophils were reported to be decreased with fall in lymphocytes subsets like T cells, B cells and NK cells in severe COVID-19 cases (Qin et al., 2020). Chest radiographs show invasive lesions in both lungs with flaws of a variable degree in the lungs. Additionally, bilateral multilobular subsegmental consolidation, ground-glass opacity with many mottling was also reported in the COVID-19 patients (Bassetti et al., 2020; Huang et al., 2020). Recently, myalgia and fatigue are found associated with rhabdomyolysis in a COVID-19 patient in Wuhan, China suggesting the need of rapid clinical diagnosis followed by favourable hydration treatment to reduce the risk of severe outcomes as a result of rhabdomyolysis (Jin and Tong, 2020).
Additionally, COVID-19 patients may also manifest neurological signs such as headache, nausea and sometimes vomiting, but even dysgeusia and anosmia. Moreover, SARS-CoV infection has been reported in nervous tissue of experimental animals and patients with heavy involvement of brainstem. In this context, acute respiratory failure in COVID-19 patients suggests the probable invasion of the brain by SARS-CoV-2 (Li et al., 2020b). Recently, a study supported the neurotropic potential of the SARS-CoV-2 virus as 36.4% of involved COVID-19 patients manifested neurological signs (Mao et al., 2020). Those who developed severe pneumonia, pulmonary oedema, hypoxemic respiratory failure, gastrointestinal infection, multiple system failure or Acute Respiratory Distress Syndrome (ARDS) succumb to the disease. The threat is still looming high on the event of this deadly virus created a pandemic situation (Gralinski and Menachery, 2020; Greenland et al., 2020; Guo et al., 2020; WHO, 2020a; Yang et al., 2020a).

7. COVID-19 Diagnostics
Several molecular diagnostics tests such as Real Time-PCR for genes encoding the internal RNA-dependent RNA polymerase and Spike's receptor binding domain, full genome analysis by next-generation sequencing (NGS), isothermal loop-mediated amplification of coronavirus (i-LACO), multiplex nucleic acid amplification, and microarray-based assays are in use currently for the laboratory confirmation of coronavirus infection (Chan et al., 2020b; Yu et al., 2020a; Zhang et al., 2020b). Diagnostic tests for SARS-CoV-2/COVID-19 cases include viral genome sequencing, RT-PCR, real-time RT-PCR (rRT-qPCR), POCT/bedside testing, reverse transcriptional loop-mediated isothermal amplification (RT-LAMP), serological assays (enzyme-linked immunoassay, ELISA), computed tomography technique (CT) imaging, X-Ray (Corman et al. 2012; Dhama et al. 2020a; Huang et al. 2020; Xu et al. 2020; Zhang et al. 2020b; Yu et al. 2020a). A reverse real-time PCR assay (rRT-PCR) is required for useful and timely screening of COVID-19 patients, which can be carried out in clinical samples like fibre bronchoscope brush biopsies, bronchoalveolar lavage, nasal swabs, pharyngeal swabs, sputum, blood and faeces (Wang et al., 2020a). An interactive web-based dashboard for monitoring COVID-19 in real-time mode has also been reported (Dong et al. 2020). A fluorescence-based quantitative PCR assay based on SARS-CoV-2 N and ORF1ab regions has been developed (Wang et al. 2020b). Moreover, testing for COVID-19 requires travelling to a clinical setting that could lead to increased risk of disease transmission hence a rapid, cheap, user-friendly and sensitive diagnostic tool must be developed for use by ordinary people in their homes (Yang et al., 2020b). In this context, a potential RNA-based POCT diagnostic device which combines a LAMP assay technology and a paper-based POCT was described as a home-based highly accessible and sensitive COVID-19 diagnostic tool with the additional advantage of smartphone integration enabling the individuals to record and share the results with healthcare workers and subsequent clinical care (Yang et al., 2020b).

8. COVID-19 immunotherapeutics and therapeutics
SARS-CoV-2 is now growing as a potential emerging pandemic (WHO, 2020a; Yang et al., 2020a). The lessons learned from earlier SARS-CoV-2 and MERS-CoV threats, recent and past epidemics and pandemics situations of Ebola, Zika, Nipah, swine flu, avian/bird flu led
to make tremendous advances in science and research for developing suitable vaccines and therapeutics/drugs, which along with the current research advances on COVID-19 are warranted to be explored optimally (Dhama et al. 2012, 2013, 2018a, 2018b, 2020b; Munjal et al. 2017; Singh et al. 2017, 2018, 2019; Di Pierro et al. 2020; DST and Health Commission China 2020; Li et al. 2020c; Peeri et al., 2020). The novel coronavirus is spreading rapidly from human to human with a wide range of clinical symptoms like fever, cough, myalgia and fatigue with pneumonia (Zhu et al., 2020, Chan et al., 2020b; Huang et al., 2020). To overcome the symptoms of novel coronavirus pneumonia, as per the severity of patient, sedatives, analgesics, oxygen therapy, and ventilator facility should be provided (Qiu et al., 2020).

Due to the alarming situation in the city of Wuhan and other cities of China, these cities have been shut down to limit the spread of the virus. The current situation resembles the situation that happened with SARS in the 2002-2003 outbreak and the Ebola outbreak in 2014-2015 (Singh et al., 2017). During this outbreak, special quarantine rules and protocol were set up to limit and identify the patient's contact risk. There were no unique antiviral treatments available for SARS and Ebola at the time of outbreaks as situations were beyond control similar to SARS-CoV-2 outbreak. As the structure of the virus is known, so to prevent the virus entry and replication within the body of the host, various inhibitors producing hurdles at different steps are explored and tested in cell-based systems (Kilianski and Baker, 2014; Pillaiyar et al., 2020). They involve spike (S) protein inhibitors, S cleavage inhibitors, helicase and protease inhibitors, fusion core blockers, mAbs against host cell receptor, antiviral peptide targeting S2, RBD–ACE2 blockers, antiviral peptides, siRNAs, neutralizing antibodies, convalescent-phase plasma, repurposed drugs, among others (Du et al. 2009; Chan et al., 2015; Zumla et al., 2016; Dhama et al., 2020b; Lu, 2020). During the current outbreak and pandemic situation, patients require immediate treatment. The below sections will emphasize on different potential treatment options that could be pursued for therapy despite limited knowledge of the biology of SARS-CoV-2.

8.1. Developing Neutralizing antibodies

In general, the replication of coronavirus starts with the entry of S protein, which binds to the surface of the cells. This S protein fuses with the cell membrane and helps the syncytial formation and delivering of viral nucleocapsids into the cell for further replication (Tortorici et al., 2019). As per reports, neutralizing antibodies against receptor-binding domain (RBD) of S protein of SARS-CoV (Traggiai et al., 2004) and MERS-CoV (Corti et al., 2015; Chen et al., 2017; Wang et al., 2018) successfully neutralized those infections. In this context, neutralizing antibodies may prove highly useful in treating the COVID-19 as long as the SARS-CoV-2 shares the sequences in the RBD with SARS-CoV and MERS-COV (Yu et al., 2020b).

S protein was targeted for developing a neutralizing antibody therapy to combat the novel coronavirus disease (Casadevall et al., 2015). Methods such as phage or yeast display libraries which express antibody fragments could be used efficiently to identify the candidate neutralizing antibody. Traditional methods of screening such as mice or rabbits for
neutralizing antibodies would be too late during outbreaks. The only challenge is that neutralizing antibodies should be rigorously tested in animal and cell culture models to confirm that they can neutralize the SARS-CoV-2 disease infection (Shin et al., 2019; Keck et al., 2019; Dhama et al., 2020b). The alternate strategy of generating the neutralizing antibodies against S protein is to immunize large animals like sheep, goat, cow and horse and purify the polyclonal antibodies from these animals. Monoclonal antibodies can be used as potent bio-therapeutics in the form of passive immunotherapy to neutralize the SARS-CoV-2 and to control the harmful outcomes of COVID-19 (Shanmugaraj et al., 2020). These strategies may prove to be beneficial in the condition of an outbreak since they have many advantages, such as simplifying production and manufacturing. For shorter treatment strategy, this could quickly help in SARS-CoV-2 outbreak.

For the time being, immunoglobulin G has been administered in COVID-19 critical patients as therapy (Liu et al., 2020b, 2020c). FcR has a role in pulmonary inflammation; hence blocking of FcR activation can reduce inflammatory damage in COVID-19. Thus intravenous use of immunoglobulins can prove helpful in the therapy of SARS-CoV-2 induced pulmonary inflammation (Fu et al., 2020).

8.2. Oligonucleotides targeting SARS-CoV-2 RNA genome

Apart from targeting S-protein of the nCoV-2019 virus using neutralizing antibodies, targeting of viral genomes could be other option to reduce the infectivity by degrading its genome. Recently, the RNA genome of the nCoV-2019 virus has been published (Gen Bank: MN908947.3). GS-5734, a nucleotide prodrug, showed broad-spectrum anti-coronavirus activity against bat CoV, pre-pandemic bat CoV, and existing human CoV in vitro and over primary human lung epithelial cell cultures and was found promising for treating epidemic and zoonotic coronaviruses of the near future (Sheahan et al., 2017). The siRNA or antisense oligonucleotides (ASO) can be used to combat the virus by targeting its genome (Qureshi et al., 2018).

Nevertheless, there are a few challenges associated with these methods, such as conserved RNA sequence in the genome of coronaviruses is still not known. Since the conserved sequences are essential in siRNA targeting to avoid viral escape from the oligonucleotides targeting strategy. Second, the delivery of oligonucleotides (siRNA and ASO) would be very challenging. Lipid nanoparticles technology can mediate the delivery of these oligonucleotides into the lungs (Youngren-Ortiz et al., 2016). Even though they were successful in the preclinical studies in animal models (Thi et al., 2015 and 2016), siRNA candidates in viral infections like Ebola have failed in clinical trials (Dunning J et al., 2016). Oligonucleoside analogues like remdesivir, which is a broad-spectrum antiviral drug, is proposed to have beneficial effects in COVID-19 therapy (Al-Tawfiq et al. 2020; Lu, 2020). Remdesivir and chloroquine have been reported to effectively inhibit SARS-CoV-2 in vitro (Wang et al. 2020c). They were able to block virus infection at low concentration (micromolar) and showed a high selectivity index (Rothan and Byrareddy, 2020; Wang et al. 2020c).
8.3. Passive antibody transfer

One of the most effective and traditional tools used in most of the infectious outbreaks is the use of serum of patients who just recovered from the active viral infection to treat patients who contract in future (Mire et al., 2016). Patients recovered from active viral infections develop a polyclonal immune response to different antigens of SARS-CoV-2 as they neutralize active viral infections and hence convalescent-phase plasma can be used as a therapeutic alternative (Chan et al., 2015). Passive immunotherapy in the form of convalescent serum tested in MERS-CoV infected mice either as prophylactic or in therapeutic form both showed good results and supported the use of dromedary immune serum in preventing MERS-CoV infection (Zhao et al., 2015). Patients who have 100% recovery from the novel coronavirus infection, can simply donate their plasma to treat the infected patients (Mire et al., 2016; Marano et al., 2016). The same strategy of convalescent serum was used during the Ebola virus outbreak in 2014-2015 (Kraft et al., 2015). Plasma-derived from the patients who recovered from the disease has also been used as therapy (Jin et al. 2020). α-interferon atomization inhalation has been weakly recommended at a dose of 5 million U per time for adults in sterile injection water, twice a day (Jin et al. 2020). Similarly, interferon therapy has also been used; however, it aggravated pathology (Rothan and Byrareddy, 2020).

8.4. Drug repurposing using available antivirals

Drug repurposing is a promising, fast and cost-effective method that can overcome traditional de novo drug discovery and development challenges of targeting various diseases and disorders. Drug repurposing, the process of identifying new uses for the existing or candidate drugs is an effective strategy for drug discovery in multiple diseases, including infectious viral diseases. In combating the nCoV-2019 viral outbreak, use of already approved small drug molecules could inhibit the biological aspects of viral life cycle like replication, transcription, host protein interaction, boosting immunity, among others. Viral polymerase and protease inhibitors of HIV and hepatitis C virus are two potential antiviral regimens which can be repurposed against 2019-nCoV (Tsai et al., 2006; Anderson et al., 2009). During the SARS outbreak, HIV protease inhibitors like lopinavir and ritonavir had positive efficacy (Chu et al., 2004). In vitro and in vivo (over rhesus macaque) experiments suggested that combined therapeutic regimen of lopinavir/ritonavir alone or in combination with recombinant interferon-β1b (IFN-β1b) have been found useful in treating MERS-CoV infection (Falzarano et al., 2013; Chan et al., 2015; Arabi et al., 2018). Hence, these are being explored as repurposed drugs against SARS-CoV-2 to treat COVID-19 (Wang et al., 2020d). The study demonstrated that combination of remdesivir (RDV) and interferon beta (IFN β) had better anti-viral efficacy as compared to combined formula of lopinavir and ritonavir in treating MERS-CoV infections (Sheahan et al., 2020).

Since the novel coronavirus belongs to the same category of SARS, repurposing of HIV drugs against novel coronavirus could give positive efficacy. Lopinavir may have some prospects in COVID-19 therapy as has been in the SARS, and MERS therapy; however, an extensive evaluation is required (Yao et al. 2020). Efficacy of remdesivir against SARS-CoV-2 is under testing by Gilead Sciences (NASDAQ-GILD) pharmaceutical company.
National Health Commission of the People's Republic of China has advocated the inclusion of chloroquine phosphate for the cure of COVID-19 patients, in its revised guidelines for the prevention, diagnosis, and treatment of pneumonia developed due to COVID-19 infection in the vast populous country (Gao et al., 2020; Lin and Li, 2020). Future research could continue to screen currently clinically available small molecular antiviral drugs in tissue culture models to identify candidate drugs to combat the novel coronavirus infection.

The pangolin CoV and SARS-CoV-2 are more than 92% similar at amino acid levels. Thus, using pangolin coronavirus as a model three drugs namely, cepharanthine (CEP), selamectin, and mefloquine hydrochloride were observed to possess anti-SARS-CoV-2 activity with complete blocking of cell cytopathic effects (Fan et al., 2020).

Chen et al. (2020a) have used oseltamivir (75 mg), lopinavir (500 mg), ritonavir (500 mg) per os twice daily followed by ganciclovir (0.25 g) intravenously for 3–14 days in COVID-19 infected patients and they have shown safety hence can be considered as treatment options (Chen et al. 2020a). EIDD-2801 compound has proven effective against influenza viruses and can be evaluated against SARS-CoV-2 as well (Toots et al. 2019).

8.5. Anti-viral proteases

Rolf Hilgenfeld, a renowned structural biologist in the University of Lubeck, Germany has developed two compounds containing viral proteases and is intended to visit Wuhan, China at the peak of SARS-CoV-2 epidemic to test the clinical efficacy of these compounds in animals so that can use them for the treatment of COVID-19 patients suffering presently and in any future coronavirus outbreak (Cyranoski, 2020).

Anti-coronavirus protease activity was exhibited by the lopinavir (LPV), and it is proposed as a treatment option for ongoing COVID-19 infection (Yao et al., 2020). Further, a novel vinylsulfone protease inhibitor suggested treating patients suffering from the 2019-nCoV. That would help in the development of broad-spectrum anti-coronaviral agents for future epidemics (Morse et al., 2020). Recently, a breakthrough in search of antivirals came with the elucidation of SARS-CoV-2 main protease (Mpro) structure. The same could be exploited globally to design some novel drug candidates. Lately, a Deep Docking (DD) platform was used for structure-based virtual screening of nearly 1.3 billion molecules with potential of 1,000 putative ligands for SARS-CoV-2 Mpro protein (Ton et al., 2020).

8.6. Blocking Coronavirus receptors like ACE2

It is already known that ACE2 is a crucial player in the coronavirus infection by promoting cell entry (Hoffmann et al., 2020). The metallopeptidase, ACE2, has been identified as a functional receptor for SARS-CoV (Li et al., 2003) and a potent receptor for SARS-CoV-2 (Zhou et al., 2020). ACE2 is an important drug target for the treatment of cardiovascular and kidney diseases, and. Recently, Lei et al. (2020) demonstrated the potential of ACE2 based therapeutics against SARS-CoV-2, which could further be exploited alone or in combination,
and they elucidate the molecular mechanisms of their potent and broad neutralizing activity. These ACE2 fusion proteins could be used for diagnosis and as research reagents in the development of vaccines and inhibitors. ACE2 and AT1R (angiotensin receptor one blocker) molecules such as losartan as inhibitors of the renin-angiotensin system (RAS) could be a useful therapeutic option in reducing the lung inflammation and treating pneumonia in COVID-19 patients (Gurwitz, 2020; Sun et al., 2020). The virus attachment through spike glycoprotein (S) to ACE2 receptors and subsequent priming of S protein by the host cell serine protease TMPRSS2 has been exploited as therapeutic targets. In this context, the role of TMPRSS2 protease in SARS-CoV-2 replication has been reported further supporting the probable role in the development of an active therapeutic agent (Matsuyama et al., 2020). The TMPRSS2 inhibitor proved useful in blocking the virus entry and could work as a therapeutic option (Hoffmann et al., 2020).

8.7. Combination therapy
Rothan and Byrareddy (2020) have recommended the application of broad-spectrum antivirals like lopinavir/ritonavir, neuraminidase inhibitors, peptide (EK1), RNA synthesis inhibitors for the time being till specific antivirals are evaluated. Chen et al. (2020a) have used oseltamivir, lopinavir, ritonavir, ganciclovir in COVID-19 infected patients with excellent results however the combination of antiviral drugs is believed to be controversial (Jin et al. 2020). Other medications used are antibiotics (cephalosporins, quinolones, carbapenems, tigecycline against methicillin-resistant Staphylococcus aureus, linezolid, moxifloxacin or levofloxacin, azithromycin or amoxicillin and antifungal drugs), corticosteroids (prednisolone, dexamethasone), antipyretics (ibuprofen), anticoagulants (heparin) (Chen et al., 2020a; Jin et al., 2020; Rothan and Byrareddy, 2020; Zhang et al., 2020c). Huang et al. (2020) used antibiotics, methylprednisolone corticosteroid (40–120 mg per day), and oseltamivir (orally 75 mg twice daily) in COVID-19 patients along with oxygen support. However, the use of antibiotics should be taken care as patients may not always develop bacterial complications (Du et al., 2020). Other trials involved the use of lopinavir-ritonavir and interferon-α 2b in COVID-19 patients (Anonymous 2020; Habibzadeh and Stoneman 2020). National Health Commission of China recommended combination of Ribavirin and interferon-α as a treatment regimen for COVID-19 in its fifth edition; however, the efficacy of Remdisivir and Ritonavir/Lopinavir needs to be determined by randomized controlled trial (Du et al., 2020). Rigorous preclinical and clinical both kind of trials are required before commencement of commercialization of combination therapy against COVID-19 (Li et al., 2020d).

Arbidol, an effective antiviral against SARS-CoV in combination with antibiotics (moxifloxacin or levofloxacin, nemonoxacin, linezolid, azithromycin or amoxicillin), corticosteroids and oxygen therapy has been used in COVID-19 therapy (Zhang et al. 2020c). Corticosteroids have been routinely used for the treatment of Th1 and Th2 induced lung injury reported in COVID-19 (Huang et al., 2020). Conventional Chinese drugs like ShuFengJieDu and Lianhuaqingwen were also used in the treatment of COVID-19, but their efficacy needs to be determined (Lu, 2020). In contrast to this, WHO has indicated that currently no effective treatment for SARS-CoV-2 is known and use of different antibiotics, antiviral drugs, traditional Chinese drugs, corticosteroids like glucocorticoid and their
combinations are not recommended before clinical trials as their efficacy is not known and might be detrimental to COVID-19 patients (Xiao et al., 2020; WHO, 2020d). Besides, to manage the hypoxia in the COVID-19 patients' ventilation and salvage therapy is reported beneficial. An α-glucan-based mushroom extract, i.e. AHCC has been reported to be used as an immunostimulant for animals and humans affected by viral infections like the influenza virus, West Nile virus, hepatitis virus, herpes virus, papillomavirus and HIV. In this context, AHCC may be used in the prevention of COVID-19 after evaluation of its efficacy against SARS-CoV-2 (Di Pierro et al., 2020). For safe and successful treatment of severe respiratory illness in infants and children, probability of atelectasis due to invasive or non-invasive ventilation support and risks of oxygen toxicity must be taken into account (Marraro et al., 2020).

An overview of developing COVID-19 therapeutics and drugs is illustrated in Figure 1.

9. COVID-19 Vaccines
Appropriate preventive measures, as advised by WHO and CDC, must be implemented in order to delay the spread of COVID-19 so that health workers, healthcare departments, research and development wing and the government will get some time to design and test the targeted vaccines and therapeutics (Jernigan and CDC COVID-19 response team, 2020). Currently, there are no specific vaccines available against COVID-19 (Ahmed et al. 2020; Habibzadeh and Stoneman, 2020; Shang et al., 2020). Attempts are being made for the development of safe and effective prophylactic strategies (CDC, 2020; Shang et al. 2020). The earliest possibility is convalescent sera. Convalescent sera from the persons who recovered from the COVID-19 attack can be used as an immediate therapy (Casadevall and Pirofski 2020). Other sources of such sera can be genetically engineered animals, e.g. cows (Beigel et al. 2018). This serum contains immunoglobulins that can provide passive immunity. These antibodies can help in neutralization of SARS-CoV-2 or can help in antibody-dependent cellular cytotoxicity, and phagocytosis (Casadevall and Pirofski 2020).

In search of suitable vaccine against SARS-CoV-2, mRNA-based vaccine (mRNA-1273) by Moderna (NASDAQ: MRNA) and INO-4800 as a vaccine by other company such as Inovio pharmaceuticals (NASDAQ-INO) are being developed and they will soon undergo clinical trials (Dhama et al., 2020b; https://www.fool.com/investing/2020/03/12/coronavirus-vaccine-candidates-could-enter-the-cli.aspx). In this context, a joint effort is made by Oxford University and Rocky Mountain Laboratories to develop a chimpanzee adenovirus vectored vaccine (NIAID, 2020). A vaccine made by Moderna Therapeutics is sent to the NIAID for a clinical trial which is anticipated by the end of April 2020 (https://www.livescience.com/us-coronavirus-vaccine-trial-recruiting.html). Additionally, a vaccine candidate expressing S protein of SARS-CoV-2 using mRNA vaccine platform technology is expected to hit clinical trial in the coming months (NIAID, 2020). Targeting spike proteins of CoV may have a role in vaccines and therapeutics as they induce highly potent neutralizing antibodies and are involved in host receptor binding and pathogenesis (He et al., 2004; Wang et al., 2016). Targeting spike glycoprotein (S glycoprotein) in SARS-CoV-2 can be useful (Habibzadeh and Stoneman, 2020). However, multiple efforts to develop vaccines against SARS-CoV-2 have been made but not reach a commercial level and could excel only to the pre-clinical
levels until the preparation of the manuscript. Recently, WHO released a list of 35 vaccine candidates under pre-clinical level grouped into five major groups like live attenuated virus vaccines, DNA vaccines, non-replicating viral vector vaccines, replicating viral vector vaccines, and subunit vaccines (WHO, 2020e).

The other modes of vaccine development are the utilization of either the virus itself or its part for developing whole organism-based vaccines or subunit vaccines. These include attenuated or inactivated vaccines using cultured SARS-CoV-2 that can be attenuated by passaging or inactivated by physical and chemical methods such as UV light, formaldehyde, and β-propiolactone (Shang et al. 2020). However, these may have limitations of infectivity, reversion to pathogenicity, and disease-causing potential (Roper and Rehm, 2009).

Exploration of vaccine candidates of SARS-CoV-2 is essential for the development of specific vaccines (Habibzadeh and Stoneman, 2020; Shang et al., 2020) and the research and development are already being initiated (WHO, 2020e). A set of epitopes of SARS-CoV-2 have been screened, immune targeting of these epitopes can protect against this novel coronavirus and hence can provide experimental platforms for the development of vaccines (Ahmed et al. 2020). Identification of putative protective antigen peptide from SARS-CoV-2 is essential for the development of subunit vaccines (Shang et al. 2020). The timely revealing of genome sequences is proving beneficial for subunit vaccine development (Wu et al. 2020b; Chan et al. 2020a). Structural proteins of SARS-CoV-2 including envelope (E), membrane (M), nucleocapsid (N), and spike (S) are being explored as antigens for subunit vaccine development (Chan et al. 2020a; Shang et al. 2020). Ahmed et al. (2020) have explored a set of T and B cell epitopes derived from Spike (S) and nucleocapsid (N) proteins mapping identically to SARS-CoV-2, and no mutations have been noted in these epitopes in 120 genome sequences hence can serve as vaccine candidates for the development of subunit vaccines.

Recently, antigenicity, along with structure and function of spike glycoprotein, especially of linear epitope S2 subunit of SARS-CoV-2 has been evaluated (Walls et al. 2020). Neutralizing antibodies have been raised against S2 subunit of SARS-CoV-2 that cross-react and neutralize both SARS-CoV-2 and SARS-CoV (Walls et al. 2020), hence, can be explored as candidates for subunit vaccines. Subunit vaccines have limitations of low immunity, the requirement of adjuvants and sometimes inefficient protective immunity while as DNA and mRNA based vaccines are more accessible and quick to clinical trials recombinant vaccines (Shang et al. 2020). Viral-vector based vaccines can be constructed and used without adjuvant. These vaccines are possible only when the antigens having neutralizing epitopes are explored (Shang et al. 2020). Mutated SARS-CoV-2, especially with altered E protein, can be exploited as a recombinant vaccine as N protein is conserved across CoVs hence not a suitable vaccine candidate (Gralinski and Menachery 2020; Shang et al. 2020).

The vaccine based on S2 protein subunit of the spike glycoprotein (S) that helps in receptor binding and entry can have broad-spectrum antiviral effects as it is conserved in SARS-CoV-2 (Chan et al. 2020a; Gralinski and Menachery 2020; Shang et al. 2020). Targeting S1 protein
of SARS-CoV-2 can prevent virus entry and hence a strategy for controlling viral infection (Shang et al. 2020). Targeting S protein can both develop both cellular and humoral immunity by inducing neutralizing antibodies and by developing protective cellular immunity (Shang et al. 2020). Full-length S protein (Bukreyev et al. 2004), receptor-binding domain (RBD) (Song et al. 2019) of SARS-CoV have shown vaccine potential and can be explored in SARS-CoV-2 (Shang et al. 2020). Similarly, S1 subunit of spike protein in SARS-CoV-2 can be explored for antibody production hence prophylactic and therapeutic target (Chan et al. 2020a). Both aerosol or oral routes need to be explored as possible modes of administration (Shang et al. 2020).

DNA vaccines, chimeric viral vaccines, and membrane vesicle-vaccines are other probable options (Shang et al. 2020). An mRNA based vaccine by Moderna® is believed to develop antibodies against spike proteins of SARS-CoV-2, and a batch of vaccine has been delivered to the National Institute of Allergy and Infectious Diseases. This was developed 42 days after DNA sequence of SARS-CoV-2 was disclosed. Johnson and Johnson's ® is also working on the development of vaccines against COVID-19. Antibody-based treatment is being evaluated by Eli Lilly® and AbCellera®. GlaxoSmithKline® has produced a pandemic vaccine adjuvant platform that it is partnering with other institutes and companies for use in COVID-19 vaccine candidates. Vaccine candidate INO-4800 is being evaluated by Inovio Pharmaceuticals®. Recombinant DNA vaccines are being studied by Sanofi® (Forbes, 2020). https://www.forbes.com/sites/alexknapp/2020/03/13/coronavirus-drug-update-the-latest-info-on-pharmaceutical-treatments-and-vaccines/.

As per recommendations of CDC, researchers engaged in diagnosis and clinicians involved in the treatment of COVID-19 infected patients must wear all personal protective equipment including gloves, masks, goggles or face shield (Patel et al., 2020). Besides, due to short supply and unawareness of general public surgical masks are in extensive use which may only reduce the spread to some extent but not prevent the acquisition of SARS-CoV-2. Moreover, high filtration N95 masks along with PPE must be used by healthcare workers while attending patients to avoid nosocomial infection (Xiao et al., 2020).

Though the outbreaks are spreading worldwide healthcare workers caring for COVID-19 patients and other close contacts, as well as immunocompromised individuals are under high risk (CDC, 2020; Rohde, 2020; Yang et al., 2020a). Surprisingly, pregnancy-associated immunosuppression is reported beneficial for the mother as far as the severity of SARS-CoV-2 infection is concerned possibly due to protection of lungs from cytokine storm brought by the immune system to counter the infectious agent (Who is getting sick…. statnews.com/2020/03/03/who-is-getting-sick-and-how-sick-a-breakdown-of coronavirus - risk-by-demographic-factors/).

Several measures were taken and still on to curb the disease, but no acclaimed antiviral or vaccines are available till date as researchers are at large, for which purpose researchers are working day and night as discussed above, and hopefully, appropriate proven vaccines and
therapeutics would be available soon (Dhama et al., 2020b; Hui et al., 2020; Lai et al., 2020; Rodriguez-Morales et al., 2020).

An overview of designing and developing COVID-19 vaccines is depicted in Figure 2.

**Patents on successful methodologies on various aspects of coronaviruses**

Global research focuses on coronavirus infections, especially the recently happened outbreaks of SARS, MERS and the most newly COVID-19. This involves exploration of diagnostics, prophylactics and therapeutics. Accordingly, patenting of successful methodologies has gained immense importance, safeguarding the interests of scientists and institutions. Various patents are being filed, and many approved on diagnostic, prophylactic and therapeutic aspects of coronaviruses and their diseases, especially SARS-CoV caused by SARS, MERS-CoV caused by MERS and the most newly SARS-CoV-2 caused COVID-19. As per one study, around 80% of patents are related to therapeutics, 35% for vaccines and 28% for diagnostics agents or methods (Liu et al. 2020c). Similarly, on MERS, more than 100 patents are published on therapeutics, and more than 50 on diagnostics and prophylactics (Liu et al. 2020c). These patents cover a range of research areas.

Table 2 provides details about the various fields and research areas for which patents have been applied and granted. These areas include developing novel, rapid, and specific diagnostics which are cheap and readily available (Liu et al. 2020c; Shenliang, 2016; Van Der Hoek 2007; Wei et al. 2016). For this purpose, exploration of novel methods or techniques is being elucidated that can be transformed into diagnostic technology. Further, identifying diagnostic markers are vital. Similarly, for the treatment purpose, novel drugs or therapeutic agents are being explored (Liu et al. 2020c). These need to be effective against coronaviruses, including SARS-CoV, MERS-CoV and SARS-CoV-2 (Chang et al., 2017; Emma et al. 2018; Liu et al., 2020c). These can be viral protease inhibitors (Emma et al. 2018), enzyme inhibitors (Kim et al. 2017), antivirals (Radtke et al. 2019; Shenliang, 2016), immunomodulators (Jordi et al. 2019), and treatment adjuvants (Adams and Lian 2020). This is the primary class which has attracted most of the patents (Liu et al. 2020c). Either a drug target is located or a direct antiviral compound, molecule or agent is established, or an indirect immunomodulator that raises the immune response against virus is identified as shown in Table 2. Vaccines which are the main backbone for the prevention strategies and are mostly lacking especially for novel coronaviruses have shown immense research prospects and are attracting a sufficient number of patents as shown in Table 2 (Liu et al. 2020c). Exploring novel vaccine candidates, targeting appropriate antigens, evaluating immune responses and transformation into a safe, effective and potent vaccine are some of the areas on which patents have been published (Bickerton et al. 2017; Sutter et al. 2016; Johnson et al. 2017). They can be univalent targeting a single pathogen (species/strain) (Brownlie et al. 2012; Sutter et al., 2016) or multivalent targeting many at a time (Johnson et al. 2017). Presently no specific therapeutics or vaccines are available against SARS-CoV-2. Since there is some degree of genomic and structural identity hence therapeutics active against SARS-CoV or MERS-CoV or other broad-spectrum antivirals (e.g. remdesivir, chloroquine) are being explored for SARS-CoV-2. Identification of specific therapeutic targets and vaccines candidates will enable developing of specific and potential drugs or vaccines that can prove useful in the prevention and control of SARS-CoV-2 (Liu et al. 2020c).
10. Conclusion and Future Perspectives
Currently, SARS-CoV-2 outbreak has taken a disastrous turn with high toll rates alone in China itself, most likely the infection is spreading across the globe. There are no licensed vaccines or therapeutic agents (i.e., antivirals and monoclonal antibodies) indicated for this coronavirus prevention or treatment. However, researchers are working to develop countermeasures. Several vaccine candidates for both SARS-CoV-2 are in early clinical trials. This review is an accumulative hub of the latest knowledge and out comings of all novel approach to tackle this deadly disease. Validated and clinically proven therapeutic measures are in great need at this hour of crisis to ensure global safety and on stopping this pandemic before it leads to distressing global outbreaks.

Under the current COVID-19 pandemic scenario the global threats are increasing, more countries are being predisposed, and new outbreaks are being reported, increasing the number of infected cases and risks to non-infected persons—this necessitates timely intervention for appropriate management of affected ones and prevention of threats to healthy ones. Non-availability of specific antivirals against COVID-19 is causing heavy tolls in infected persons. Utilization of conventional therapies as supportive treatment though helps in managing severity but is proving ineffective on a long term basis as the overall mortality is changing significantly daily. Non-specific antiviral therapy by oseltamivir, ganciclovir; antibacterial therapy by moxifloxacin, ceftriaxone, azithromycin; and glucocorticoid therapy is being improvised by adding broad-spectrum antivirals such as remdesivir, chloroquine, or lopinavir/ritonavir. Passive therapy by α-interferon atomization inhalation, immunoglobulin G therapy, or plasma therapy is proving beneficial.

However, there is a dire need for development and evaluation of specific antivirals against COVID-19. There have been attempts for exploring the applicability of already existing antivirals, potential antivirals or combination thereof along with supportive medicines; however, the focus should be on designing and adopting safe and effective modalities against SARS-CoV-2. These include developing neutralizing antibodies, oligonucleotides targeting SARS-CoV-2RNA genome, passive antibody transfer, drug repurposing using available antivirals, anti-viral proteases, blocking coronavirus receptors like ACE2, targeting spike proteins, and combination therapies.

Alternative measures like neutralizing antibodies, oligonucleotides, passive antibody transfer and drug repurposing can bring a revolutionary change until the core researchers are busy finding the specific target to curb the SARS-CoV-2 which can be time taking and still at large.

Author contributions
All the authors substantially contributed to the conception, design, analysis and interpretation of data, checking and approving the final version of the manuscript, and agree to be accountable for its contents. AAR, SHA, RS and SH initiated this review compilation; RT, SKP, MP, MIY, DKB, YSM and KD updated various sections. SKP and MP developed...
Figure and MIY developed Table of Patents. KD and AJR-M reviewed advances in therapeutics and vaccines, overviewed and edited final.

**Funding**
This compilation is a review article written, analyzed and designed by its authors and required no substantial funding to be stated.

**Acknowledgement**
None.

**Conflicts of interest**
All authors declare that there exist no commercial or financial relationships that could in any way lead to a potential conflict of interest.

**References**
38. Dhma K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, Chaicumpa W. COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing...


133. Qureshi A, Tantray VG, Kirmani AR, Ahangar AG. A review on current status of small-molecule inhibitor against MERS-CoV main protease, and preparation method and application thereof. CN105837487A.


150. Shenliang TW. 2016. High-efficiency ranilla luciferase gene expression recombinant HCoV-OC43 (human coronavirus OC43) virus and application thereof. CN105671006A.


Legends of Figures and Tables

**Figure 1.** An overview on COVID-19 therapeutics and drugs.
Figure 2. An overview on designing and developing COVID-19 vaccines.
Table 1. Classification of Coronavirus.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Viruses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphacoronavirus</td>
<td>1. Alphacoronavirus 1</td>
<td>Yang et al., 2015</td>
</tr>
<tr>
<td></td>
<td>2. Porcine epidemic diarrhoea virus (PEDV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Bat coronavirus 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. BtCoV 512</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. BtCoV-HKU8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. BtCoV-HKU2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. HCoV-NL63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8. HCoV-229E</td>
<td></td>
</tr>
<tr>
<td>Betacoronavirus</td>
<td><strong>Lineage A:</strong></td>
<td>Yang et al., 2015</td>
</tr>
<tr>
<td>(4 Lineages including</td>
<td>1. HCoV-OC43</td>
<td></td>
</tr>
<tr>
<td>A, B, C, D)</td>
<td>2. HCoV-HKU1,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Betacoronavirus 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(more commonly known as bovine coronavirus, BCoV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Murine coronavirus (MHV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Lineage B:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Severe acute respiratory syndrome-related</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SARS-CoV</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Lineage C:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Tylonycteris bat coronavirus HKU4 (BtCoV-HKU4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Pipistrellus bat coronavirus HKU5 (BtCoV-HKU5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Middle East Respiratory Syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MERS-CoV</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Lineage D:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rousettus bat coronavirus HKU9 (BtCoV-HKU9).</td>
<td></td>
</tr>
<tr>
<td>Gammacoronavirus</td>
<td>1. Avian coronavirus</td>
<td>Yang et al., 2015</td>
</tr>
<tr>
<td></td>
<td>2. Whale coronavirus SW1</td>
<td></td>
</tr>
<tr>
<td>Deltacoronavirus</td>
<td>1. HKU11</td>
<td>Yang et al., 2015</td>
</tr>
<tr>
<td></td>
<td>2. HKU12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. HKU13</td>
<td></td>
</tr>
<tr>
<td>Patent No.</td>
<td>Date</td>
<td>Basis</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>2016-07-20</td>
<td>EP3045181A1</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2007-02-15</td>
<td>JP2007502612A</td>
<td>Diagnosis, Vaccine</td>
</tr>
<tr>
<td>2012-07-05</td>
<td>ES2384445T3</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2017-10-12</td>
<td>WO2017176596A1</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2017-12-28</td>
<td>WO2017222935A1</td>
<td>Treatment and control</td>
</tr>
<tr>
<td>Date</td>
<td>Paper ID</td>
<td>Topic</td>
</tr>
<tr>
<td>------------</td>
<td>----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>2018-05-22</td>
<td>S99758858B2</td>
<td>Treatment and control</td>
</tr>
<tr>
<td>2017-08-03</td>
<td>WO2017129975A1</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2019-12-05</td>
<td>JP2019206573A</td>
<td>Immunity boosters</td>
</tr>
<tr>
<td>2020-01-02</td>
<td>US20200002370A1</td>
<td>Treatment, adjuvants</td>
</tr>
<tr>
<td>2019-11-14</td>
<td>US20190343862A1</td>
<td>Immunomodulators</td>
</tr>
<tr>
<td>Date</td>
<td>Reference</td>
<td>Section</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>2018-12-18</td>
<td>US10154985B2</td>
<td>Treatment Method</td>
</tr>
<tr>
<td>2016-11-24</td>
<td>US20160339097A1</td>
<td>Recombinant vaccine proteins</td>
</tr>
<tr>
<td>2017-01-19</td>
<td>WO2017010835A1</td>
<td>Drug/treatment Use of radotinib for prevention or treatment of viral respiratory disease</td>
</tr>
<tr>
<td>2016-06-15</td>
<td>CN105671209A</td>
<td>Diagnosis Primers, probe, kit and method for detecting bovine coronavirus</td>
</tr>
<tr>
<td>Date</td>
<td>Application Area</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2016-06-15</td>
<td>Diagnosis and treatment</td>
<td>High-efficiency ranilla luciferase gene expression recombinant HCoV-OC43 (human coronavirus OC43) virus and application thereof. The invention discloses a high-efficiency ranilla luciferase gene expression recombinant human coronavirus OC43 virus and application thereof to screening of antiviral medicines. By an overlap PCR (polymerase chain reaction) method, a ranilla luciferase gene is replaced or inserted into accessory genes (ns2 and ns12.9) to be cloned into human coronavirus OC43 full-length infectious clone pBAC-OC43FL, and four ranilla luciferase gene expression recombinant viruses, including rOC43-ns2DelRluc, rOC43-ns2FusionRluc, rOC43-ns12.9StopRluc and rOC43-ns12.9FusionRluc, are obtained respectively. The virus rOC43-ns2DelRluc is efficient in ranilla luciferase gene expression and similar to a parent virus HCoV-OC43-WT in virus growth curve, the inserted reporter gene is stable in a serial passage process, and the virus rOC43-ns2DelRluc can be successfully applied to antiviral medicine screening experiments and has an extensive application prospect in high-throughput screening of anti-coronavirus medicines and host antiviral genes.</td>
</tr>
<tr>
<td>2016-08-10</td>
<td>Prevention/treatment</td>
<td>Small-molecule inhibitor against MERS-CoV main protease, and preparation method and application thereof. The invention provides a small-molecule inhibitor against MERS-CoV main protease. The small-molecule inhibitor is designed on the basis of the crystal structure of main protease of the novel coronavirus MERS-CoV. The invention also provides a synthetic method for the small-molecule inhibitor and application of the small-molecule inhibitor in preparation of drugs used for preventing and treating MERS-CoV infections. The small-molecule inhibitor against MERS-CoV main protease can substantially inhibit the activity of main protease of the MERS coronavirus, has good inhibitory activity to main protease of coronaviruses like SARS and MHV, and presents good application prospects in preparation of drugs used for preventing or treating coronavirus infections.</td>
</tr>
<tr>
<td>2016-09-01</td>
<td>Recombinant peptides/antibodies treatment</td>
<td>Middle east respiratory syndrome coronavirus immunogens, antibodies, and their use. Methods of inducing an immune response in a subject to the Middle East respiratory syndrome coronavirus (MERS-CoV) are provided. In several embodiments, the immune response is a protective immune response that inhibits or prevents MERS-CoV infection in the subject.</td>
</tr>
<tr>
<td>Date</td>
<td>Application Code</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>2016-06-01</td>
<td>CN105624122A</td>
<td>Mutant virus as vaccine</td>
</tr>
<tr>
<td>2017-08-08</td>
<td>CN107022008A</td>
<td>Prevention and treatment</td>
</tr>
<tr>
<td>2017-08-08</td>
<td></td>
<td>Suppress polypeptide and its application of human coronary virus's infection wide spectrum</td>
</tr>
<tr>
<td>Date</td>
<td>Patent Number</td>
<td>Type</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>US9889194B2</td>
<td>Immunogenic proteins</td>
</tr>
<tr>
<td>2019-11-21</td>
<td>US20190351049A1</td>
<td>Spike protein</td>
</tr>
<tr>
<td>2019-12-05</td>
<td>US20190365925A1</td>
<td>Antigen targets</td>
</tr>
<tr>
<td>2019-09-05</td>
<td>JP2019146588A</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2019-10-17</td>
<td>US20190314497A1</td>
<td>Treatment</td>
</tr>
<tr>
<td>Date</td>
<td>Publication Number</td>
<td>Category</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>2019-03-12</td>
<td>US10226434B2</td>
<td>Treatment</td>
</tr>
<tr>
<td>2020-02-04</td>
<td>US10548971B2</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2019-05-08</td>
<td>Publication of JP6508605B2</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2019-09-10</td>
<td>US10406229B2</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2018-12-20</td>
<td>AU2015340134B2</td>
<td>Antigenic gene identification</td>
</tr>
<tr>
<td>2018-01-30</td>
<td>US9878024B2</td>
<td>Enhanced immune response</td>
</tr>
<tr>
<td>Publication Date</td>
<td>Patent Number</td>
<td>Type</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>2017-08-01</td>
<td>US9719107B2</td>
<td>Recombinant gene and protein expression</td>
</tr>
<tr>
<td>2019-06-04</td>
<td>US10307439B2</td>
<td>Drug treatment</td>
</tr>
<tr>
<td>2008-05-22</td>
<td>WO2007062656A3</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2018-01-23</td>
<td>US9872900B2</td>
<td>Vaccine</td>
</tr>
<tr>
<td>2019-05-14</td>
<td>US10280656B2</td>
<td>Treatment and immune modulators</td>
</tr>
</tbody>
</table>

Brennan et al. 2017
Blatt et al. 2019
Holst et al. 2008
Ciaramella et al. 2018
Dickey et al. 2019b
<table>
<thead>
<tr>
<th>Publication Date</th>
<th>Patent Number</th>
<th>Description</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019-10-09</td>
<td>EP3194423B1</td>
<td>Treatment antiviral</td>
<td>Method for preparing a vaccine antigen, resulting vaccine antigen and uses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The present invention relates to a method for preparing a vaccine antigen comprising a membrane protein, as well as to a vaccine antigen and a vaccine, and uses thereof.</td>
</tr>
<tr>
<td>2018-11-20</td>
<td>US10130701B2</td>
<td>Treatment/vaccine</td>
<td>Coronavirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The present invention provides a live, attenuated coronavirus comprising a variant replicase gene encoding polyproteins comprising a mutation in one or more of non-structural protein(s) (nsp)-10, nsp-14, nsp-15 or nsp-16. The coronavirus may be used as a vaccine for treating and/or preventing a disease, such as infectious bronchitis, in a subject.</td>
</tr>
</tbody>
</table>

Rosa-Calatrava et al. 2019

Bickerton et al. 2018