Journal of Experimental Biology and Agricultural Sciences, August - 2022; Volume – 10(4) page 737 – 742

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Potential COVID -19 Therapeutics in Clinical Trials – A Brief Review

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Received – November 01, 2021; Revision – January 14, 2022; Accepted – March 28, 2022
Available Online – August 30, 2022

DOI: http://dx.doi.org/10.18006/2022.10(4).737.742

KEYWORDS

COVID-19

Clinical trials

Anti-viral

Vaccines

ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS – CoV2), the causative viral pathogen of the COVID-19 pandemic belongs to the family of Coronaviruses which are positive single stranded RNA viruses. The scientific fraternity has developed and developing various types of vaccines for prevention against COVID-19, such as inactivated virus vaccines, mRNA vaccines, replicating vector protein subunit vaccines, etc., Out of which ten vaccines namely Novovax, Covovax (protein subunit vaccines), Pfizer BNT16b2, Moderna mRNA 1273 (mRNA vaccines), Johnson & Johnson Ad26, Cov2.S, Astrazeneca AZD1222, Covishield (non-replicating viral vector vaccines), Covaxin, Sinopharm BBIBP-CorV, CoronoVac (inactivated vaccines) have been approved for clinical use by WHO. There is an urgent need for SARS-CoV2 specific therapeutics for the treatment of COVID-19 as there is the emergence of various variants such as Alpha, Beta, Gamma, Delta, Omicron, etc. The emergence of variants that possesses immune evading property and spike protein mutation have increased infectivity and more pathogenicity which impelled the need to develop various therapeutics for the treatment of COVID-19.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

This review compiles the information about potential antiviral candidates in preclinical trials intended for the treatment of COVID-19. The clinical development of such antivirals will be very crucial for the treatment of COVID-19 and also to curb the spread as the present scenario depends on the development of effective prophylactic vaccines.

1 Introduction

The outbreak of COVID-19 was caused by the severe acute respiratory syndrome coronavirus 2 (SARS – CoV2), which was initially referred to as novel coronavirus 2 or nCoV2 and was first reported in Wuhan, China in December 2019 (Narayanan et al. 2021). Soon the virus was spread worldwide after which the World Health Organisation (WHO) declared it a pandemic in March 2020 (Jebril 2020). Globally, as of 4 July 2022, the total numbers of confirmed cases were 546,357,444 including 6,336,415 deaths as per World Health Organisation (World Health Organization 2022a). Thanks to the continuing research about the coronaviruses and collaboration among worldwide scientists for very quick sequencing of the SARS-CoV2 genome. This whole genome sequencing was carried out by various organizations worldwide such as NCBI, GISAID, and Gen Bank and the collaboration of these organizations was very useful to know the details about the pathogen, which enabled us to devise protective measures and also research and develop therapeutics to curb the infection (NCBI Resources 2022). This review provides an insight about the current status, preclinical research, and clinical trial progress of the potential candidates for the treatment of COVID-19.

2 Entry & Multiplication of SARS–CoV2 into the Host Cells

Coronaviruses reported in 1966 for the first time by Tyrell and Bynoe are positive single stranded RNA viruses that are known to infect animals and humans (Velavan and Meyer 2020). These viruses belong to the order of *Nidovirales*, suborder *Coronavirineae*, and to the family *Coronaviridae*. The family *Coronaviridae* is further subdivided into *Orthocoronavirinae* subfamily. There are four genera amongst this *Orthocoronavirinae* subfamily viz., alpha coronavirus, beta coronavirus, gamma coronavirus and delta coronavirus. Among these genera, alpha and beta coronavirus are known to infect humans and animals. The pathogenic viruses which are known to infect humans and animals including the severe acute respiratory syndrome coronavirus 2 (SARS – CoV2) and also a causative viral pathogen of the current pandemic belong to the genus beta coronavirus. Severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome (MERS- CoV) are the pathogens from this genus that caused an outbreak in the past. SARS – CoV2 possesses a genome 80% identical to SARS-CoV and approximately 50% identical to MERS- CoV. These pathogenic human coronaviruses (HCoVs) are known to infect the respiratory tract which may develop into life-threatening respiratory tract infections (V'kovski et al. 2021; Narayanan et al. 2022).

The genome length of SARS –CoV2 is found to be approximately in the range of 30Kb with twelve open reading frames (ORFs) encoding for the non-structural proteins, structural proteins, and accessory proteins (Chan et al. 2020). The ORF 1a and ORF 1b replicase genes encode for the polyproteins which are cleaved into sixteen non-structural proteins (nsp1-nsp16). Remaining ORFs encode for the structural proteins namely Spike protein (S), an Envelope protein (E), Membrane protein (M), and Nucleocapsid (N) as well as the accessory proteins (Rahimi et al. 2021). The pathology of COVID-19 initiates with the entry of SARS–CoV2 in the respiratory tract through human angiotensin-converting enzyme 2 (hACE2) which is the primary receptor. This metallocarboxyl peptidase enzyme cleaves the peptides of the renin-angiotensin system which is found in the lungs, especially in type 2 alveolar cells, kidneys, and gastrointestinal system (Batlle et al. 2020). The hACE2 is the receptor site for Spike protein (S) of the SARS-CoV2 pathogen. Both the Spike protein (S) and the hACE2 are heavily glycosylated and possess O-linked glycans (Yang et al. 2020). Spike protein comprises two functional units namely S1 and S2 which are responsible for binding and fusion respectively. The receptor binding domain (RBD) of the spike protein binds with the host hACE2 cell after which the spike protein furin site is cleaved by transmembrane serine protease 2 (TMPRSS2) and the cell surface protein is expressed in the endothelial cells of the respiratory tract. The S1 site is responsible for stabilizing the membrane-anchored S2 subunit which contains the fusion machinery for the fusion of the viral membranes with the host cell (Walls et al. 2020).

Once the virus entered into the host cell, the genome of SARS-CoV2 initiates the viral RNA synthesis by utilizing the host cell machinery. Open reading frame 1a synthesizes the polyproteins pp1 a/ b which is cleaved into non-structural proteins that happens in the host cell cytosol. As mentioned earlier these non-structural proteins are involved in the translation machinery as the replicase and in various processes (Rohaim et al. 2021). The replicases generate the subgenomic mRNAs encode for the four structural proteins viz., spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins as well as the accessory proteins which take part in the assembly and transport of infective viral particles (Sicari et al. 2020; Kumar et al. 2021). The translated viral proteins are translocated in the endoplasmic reticulum of the host cell which is facilitated by the Golgi intermediate compartment

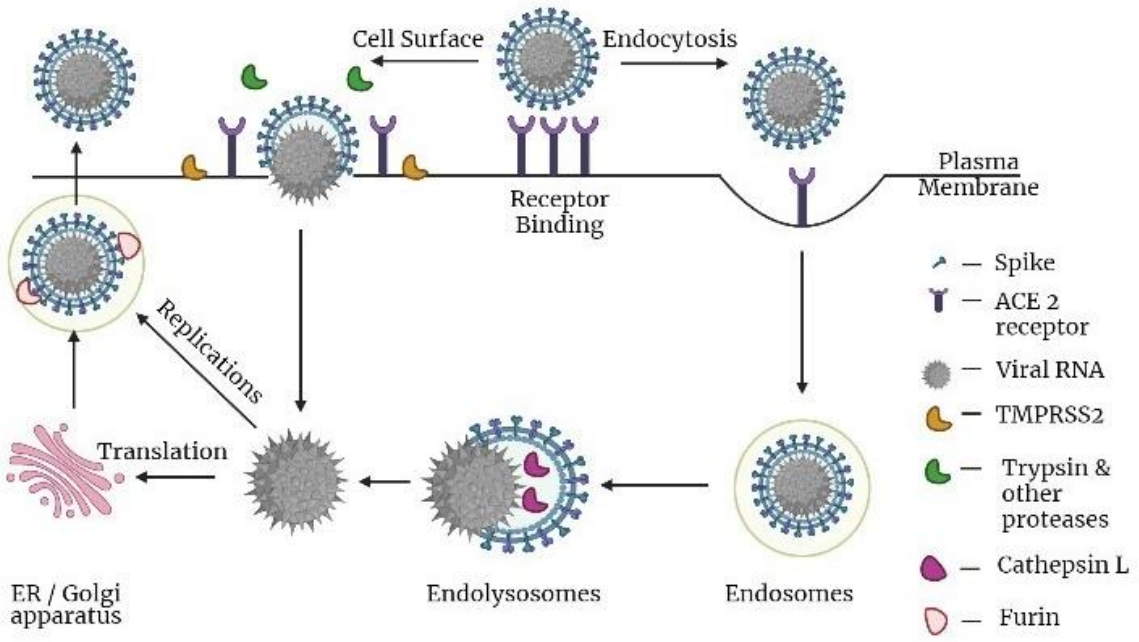

Figure 1 Entry of SARS-CoV-2 into the host cell (ACE2, angiotensin converting enzyme 2, TMPRSS2 – Trans Membrane Protein Serine Protease 2, ER, Endoplasmic reticulum, SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2)

(ERGIC) of the SARS-CoV2 for glycosylation, folding, and assembling of virus budding. The fully assembled virion is then released by exocytosis (Kadam et al. 2021). Figure 1 depicts the entry of the virus into the host cells.

3 Current Status of COVID- 19 Therapeutics

For the prevention of COVID – 19, according to WHO there are 167 vaccines in clinical development which includes the approved vaccines in clinical use, and 198 vaccines in pre-clinical development (World Health Organization 2022b). Apart from the preventive measures and vaccination, current COVID - 19 treatments involve the treatment with FDA-approved antiviral Remdesivir (intravenous) which was approved in October 2020 (Drożdżal et al. 2020). Apart from this antiviral drug, FDA has also approved monoclonal antibodies such as bamlanivimab, casirivimab, and imdevimab which have to be administered together (U.S. Food and Drug Administration 2021; FDA 2022). Other treatment options include IV steroids, anti-clotting medications as well as interleukins as per the WHO (Tim Jewell 2021). According to the latest guidelines of the CDC, monoclonal antibodies bamlanivimab with etesevimab, casirivimab, and orimdevimab can be used for the treatment of COVID-19 (National Institutes of Health 2021). The treatment options presently involve antivirals and immune modulators for aiding the immune system. There has been continuous research for potential antivirals and monoclonal antibodies for the treatment options some of which are discussed in the next section of this review.

3.1 Molnupiravir (MK – 4482) (EIDD-2801)

Developed by Merck and Ridgeback Molnupiravir/ MK – 4482 /EIDD-2801 is an investigational, ribonucleoside analog that inhibits the replication of SARS-CoV-2 and is found to be effective against various mutant strains. The molecule Molnupiravir was researched and innovated by Drug Innovations at Emory (DRIVE), LLC, and is currently developed by Merck and Ridgeback. The oral Molnupiravir is investigated for post-exposure prophylaxis and in Phase 2/ Phase 3 trials and it has shown effective in preclinical trials. The phase 3 trials for Molnupiravir is currently undergoing in Argentina, Brazil, Canada, Chile, Colombia, Egypt, France, Germany, Guatemala, Israel, Italy, Japan, Mexico, Philippines, Poland, Russia, South Africa, Spain, Sweden, Taiwan, Ukraine, the United Kingdom and the United States (Julia Robinson 2021; Merck 2021; U.S. National Library of Medicine 2022).

3.2 AT-527

AT- 527 was researched and developed by Atea Pharmaceuticals and is an orally available double prodrug of guanosine nucleotide analog, which inhibits viral replication by interfering with viral RNA polymerase. AT – 527 is a free base of AT-511 that was proven to inhibit the viral RNA-dependent RNA polymerase (RdRp) selectively in Hepatitis C virus *in-vitro* as well as *in-vivo*. When it was investigated for potent antiviral activity against several human coronaviruses, including SARS-CoV-2, it was

found to be effective in *in-vitro* studies. The active triphosphate metabolite of AT-527 is proven to be in substantial amounts in primary human cells of the respiratory tract and may be an effective treatment option (ATEA Pharmaceuticals 2021; Good et al. 2021; U.S. National Library of Medicine 2021a).

3.3 Ritonavir/PF-07321332

Ritonavir/PF-07321332 is an investigational oral SARS-CoV-2-3CL protease inhibitor that blocks the viral protease needed for viral assembly and budding thereby arresting the viral replication in the host cell, is researched and developed by Pfizer. After an encouraging Pre-clinical trial, the Ritonavir/PF-07321332 started its Phase 1 trial in March 2021 in which it was found that it's safe and well tolerated. Currently, Ritonavir/PF-07321332 is undergoing Phase 2/3 trial EPIC-PEP (Evaluation of Protease Inhibition for COVID-19 in Post-Exposure Prophylaxis) trial after the July 2021 EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) Phase 2/3 trial (Pfizer 2021; U.S. National Library of Medicine 2021b)

3.4 Immunomodulators

Immunomodulators are substances either of biological or synthetic origin for modulating the immune system. Immunomodulators can be used to stimulate (immune stimulators), suppress (immune suppressive), or modulate (biological response modifiers such as GM-CSF) the innate and adaptive immune systems (Catanzaro et al. 2018). Immunomodulators are the potential treatment option for COVID – 19 as they can be used to stimulate an effective B and T cell-based immunity (Zhou and Ye 2021). In addition to the promising antivirals in clinical trials, there are also several immune modulators in trials, namely; AZD7442, Tocilizumab, Sarilumab, Regdanvimab, Canakinumab, Anakinra, Baricitinib, Ruxolitinib, Tofacitinib, Acalabrutinib, Imatinib, Brensocatib, Ravulizumab, Namilumab, Infliximab, Adalimumab, Otilimab, Bamlanivimab, Etesevimab, Sotrovimab, Leronlimab, Risankizumab, Lenzilumab, and IMU-838 (Timothy et al. 2020; Vincent et al. 2021).

Conclusion and future prospects

The scientific community has been on a quest for treatment options since the outbreak of the COVID-19 pandemic in which they have succeeded with the development of vaccines for prevention as well as reducing hospitalizations and mortality. The emergence of SARS-CoV2 variants does pose a greater challenge as the available vaccines even though will still prevent serious illness but may be less effective against the emerging variants as well as there may be more transmission which may lead to more serious implications worldwide. To nullify the present threat situation of variants and to curb the spread of the pandemic, we are in the need of more therapeutic options, especially for post covid-19 treatment. The potential specific antivirals against COVID-19 will not only help the scientific fraternity to treat the patients effectively but also pave the way to curb the pandemic with the available vaccines.

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