



Evaluation of potential anti-COVID-19 therapies

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“As with most viral diseases, preventing establishment of the infection in the host and the subsequent spread of the virus by a global program of vaccination is regarded as the most successful approach.”

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COVID-19 – the infectious disease caused by the SARS-CoV-2 virus, a member of the ssRNA coronavirus family – has given rise to over 51.5 million confirmed infections and 1,275,979 deaths worldwide, as of 12 November 2020 [1]. It is known to be related to both the SARS-CoV and MERS-CoV viruses, responsible for severe acute respiratory syndrome and Middle East respiratory syndrome, respectively [2–4]. Human-to-human transmission of COVID-19 is widely recognized to be through a respiratory mechanism [3]. The severity of the disease ranges from asymptomatic to fatal [4,5]. The main reported symptoms include fever, nonproductive cough and loss of taste and smell, with severe cases reporting acute respiratory distress, viral pneumonia and requiring intubation and mechanical ventilation [1,4,6]. To date, there is no clinically approved vaccine available, nor any antiviral drug treatment for severe cases of this disease [1,4].

Virus-encoded anti-infective targets

A number of potential drug targets expressed by members of the coronavirus family have been identified. These include the ‘Spike’ glycoprotein, a trimer that binds to ACE2 on the host cell membrane [3,4]. This allows fusion of the viral and host cell membranes and viral entry into the cell [3]. Other potential targets include the cysteine proteases – 3CLpro (also known as Mpro) and PLpro – which are essential for production of new mature virions [7,8]. Another potential target is RdRP, which is needed for replication of the viral genome [5].

Drug repurposing

In order to rapidly progress new drug therapies into clinical use against COVID-19, drug repurposing has been widely investigated [9]. This has the advantage that potential therapeutics have already been approved for use in humans [9]. Virtual screening has been widely employed to aid in the repurposing of existing drug therapies for COVID-19 [2,10]. This approach is more rapid and economical than conventional lab-based testing. A number of potential drug treatments have been identified using this process. These include remdesivir, previously used for treatment of the Ebola and hepatitis C viruses and ribavirin, previously used for treatment of respiratory syncytial virus infection, hepatitis C and some hemorrhagic fevers. Remdesivir is reported to target RdRP, whereas ribavirin has been reported to target both 3CLpro and RdRP [2,6,11]. Recently, the US FDA (MD, USA) approved the use of remdesivir for COVID-19 patients aged over 12 years [12]. However, the WHO (Geneva, Switzerland) have released unpublished data from a clinical trial suggesting remdesivir does not reduce the mortality rate, shorten hospital stays or reduce the need for ventilation in patients with severe COVID-19 [1]. Other clinical trials of drug combinations are still ongoing.

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Development of new therapeutic antiviral agents

The need to develop novel, targeted antivirals to treat SARS-CoV-2 infection is clear. However, the drug discovery and development process is likely too lengthy to address the current pandemic. A fundamental understanding of the structure–activity relationship of any potential new therapeutic for its SARS-CoV-2 target is essential. There is high amino acid sequence homology between coronaviruses. For instance, SARS-CoV-2 Spike glycoprotein and 3CLpro share 76 and 96% amino acid sequence homology, respectively, with those of SARS-CoV [2,10]. Where sequence homology is high and protein structure is similar, it is likely that inhibitors developed against SARS-CoV will be effective against SARS-CoV-2 [5,9]. If not, such compounds are likely at least to provide good leads for optimization and subsequent drug development [5,9]. Computational modeling and virtual screening may assist to quickly identify leads, backed up by lab-based functional testing and x-ray crystallography to generate novel structure–activity relationship for further lead optimization [2].

When designing assays to test potential therapeutics, it is important to consider the functional state of the target protein. For instance, 3CLpro from SARS-CoV has been found only to be active as a dimer in solution [13]. It has also been demonstrated that additional affinity tags at either the C- or N-terminus are likely to reduce its enzymatic activity [13]. Due to the high sequence homology with 3CLpro from SARS-CoV-2 these criteria should be examined when establishing suitable screening assays [2,13]. A FRET-based fluorescent substrate assay and unlabeled 3CLpro has been used to determine the IC₅₀ of potential drug-like ligands targeting SARS-CoV 3CLpro [8,13]. Similarly, isothermal titration calorimetry (ITC) has been used to determine the binding affinity of potential drugs [8].

Host-encoded therapeutic targets

ACE2, expressed on lung alveolar epithelial cells (as well as in the heart, kidney and testes) is the target for SARS-CoV-2 Spike glycoprotein binding [3]. This interaction allows SARS-CoV-2 membrane fusion with the host cell and entry of the viral genome [3]. A possible therapeutic approach has been trialed using a recombinant soluble form of human ACE2 to block Spike binding to ACE2 expressed on cell surfaces [14]. This prevents viral entry into the host cell, blocking viral replication and reducing viral burden [3,14]. Our unpublished observations using the label-free, surface plasmon resonance (SPR) technique to analyze the biomolecular interaction between ACE2 and Spike glycoprotein elegantly demonstrates this. Spike glycoprotein pre-incubated with soluble ACE2 markedly reduces Spike binding to ACE2 immobilized onto the SPR sensor surface [15].

Anti-inflammatory drugs

Treatments for COVID-19 are not limited to antivirals. They also include the use of drugs to reduce host immune responses. One documented issue is an increase in Ang II that, in addition to its cardiovascular role, acts as an inflammatory protein [16]. Ang II is degraded by ACE2 [16]. Endocytosis of the Spike-ACE2 complex, the mechanism by which SARS-CoV-2 enters cells, is thought to lead to a reduction in ACE2 availability which, in turn, contributes to an increase in Ang II. High levels of Ang II binding to AT1 lead to a signalling cascade which results in pro-inflammatory responses [16]. This in turn, may lead to acute respiratory distress [16]. It has been proposed that AT1 blockers could be used to compete with Ang II for AT1 binding sites [16]. The affinity for various AT1 blockers has been measured using radioligand binding assays [16] – another useful tool in the drug discovery process.

Other approaches to reduce the inflammatory response include treatment with corticosteroids such as dexamethasone, which reduces death by up to a third in patients on ventilators [17]. Such treatments are useful for severely affected patients to reduce side effects. However, corticosteroids have no antiviral properties and are not recommended for treatment of early stage disease [17].

Antibody-based therapies

Infusion of plasma from recovered, post-COVID-19 infection donors, containing antibodies against SARS-CoV-2 antigens, has been used prophylactically to prevent infection in high-risk individuals [18,19]. However, plasma from a single postinfection donor is only sufficient for up to three recipients. Use of recombinant monoclonal antibodies targeting the viral Spike glycoprotein and manufactured in bulk quantities overcomes the limited supply of postinfection plasma [18,19]. Clinical trials of monoclonal antibodies in patients with severe disease are ongoing, but an effective treatment has not yet been approved [1,18]. All such protein infusion therapies (antibodies and soluble ACE2) are limited by the degradation of the protein by the host and require regular retreatment to maintain protection.

Vaccination approaches

As with most viral diseases, preventing establishment of the infection in the host and the subsequent spread of the virus by a global program of vaccination is regarded as the most successful approach. While approval of an effective vaccine is awaited, many countries are implementing social distancing and the use of face coverings to control the transmission of the virus [4]. The need for an effective vaccine is clear. Over 150 vaccine trials are reported to be underway [1,4]. Most vaccines target the SARS-CoV-2 Spike glycoprotein [4]. A variety of types are currently undergoing various stages of clinical trials including protein subunit vaccines, RNA-based vaccines and replicating viral vector vaccines [4].

Pfizer (NY, USA) and BioNTech (Mainz, Germany) recently announced that their codon-optimized mRNA vaccine (BNT162b1) Phase III trial was 90% effective at protecting against COVID-19 infection [20]. However, the data have not been subjected to peer review. An effective vaccine would help to prevent the spread of the disease, allowing social distancing measures to be eased and a return toward social normality.

Conclusion

While drug repurposing has shown some limited success, it is clear that a targeted antiviral drug is required to treat patients with ongoing SARS-CoV-2 infection. A vaccine against SARS-CoV-2 is most likely to prevent infection from establishing within the host and hence the further spread of the virus, reducing mortality worldwide.

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