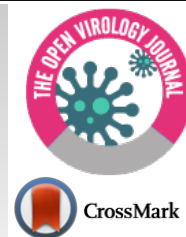




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PERSPECTIVE ARTICLE

Strategies and Challenges to Develop Therapeutic Candidates against COVID-19 Pandemic

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1. PERSPECTIVE

The terrible story of the coronavirus disease (COVID-19) needs no introduction in the present hour. It is evident that viral diseases have always appeared as a mystery and are the fatal ailments in human beings. The history justifies this fact of viral epidemics, such as SARS-CoV prevailed during 2002-2003, H1N1 during 2009, MERS-CoV in 2012 and the most dreadful COVID-19 from December 2019 to till date [1]. This ailment has emerged as a wonderstruck and put the whole world in worry and confusion. According to the latest WHO situation report-90, up to 29th April, there are 30,18,681 total confirmed cases of COVID-19 and approximately 2,07,973 people had lost their lives all over the world [2]. Coronaviruses (CoVs) are the major microorganisms causing fatal respiratory disease outbreaks worldwide. These constitute a large group of single-stranded RNA viruses and can be isolated from a wide variety of animal species [3,4]. CoVs have an appearance like a crown (*coronam* a Latin term) due to the presence of glycoprotein spikes on its envelope [5]. To date, seven human coronaviruses (HCoVs) have been identified. The common HCoVs are HCoV-OC43, and HCoV-HKU1; HCoV-229E and HCoV-NL63 (α CoVs), which are responsible for the common cold and lower extent of upper respiratory disorders. Other HCoVs are severe acute respiratory syndrome causing coronavirus (SARS-CoV), SARS-CoV-2 and the Middle East respiratory syndrome causing the coronavirus (MERS-CoV), which can cause epidemics with severe respiratory manifestations [6].

2. PATHOGENESIS OF COVID-19

The progression of COVID-19 can be divided into three

major stages: Asymptomatic stage, upper airway response stage and progression to Acute Respiratory Distress Syndrome (ARDS) [7]. The first stage is regarded as the stage after 1-2 days of the infection. In this, SARS-CoV-2 enters the nasal cavity and gets bounded to epithelial cells there with the help of the ACE2 receptor [8, 9]. When it enters inside the cell, the viral RNA gets released into the cytoplasm of the cell where translation occurs, leading to the formation of proteins and replication [10]. It starts replicating and ciliated cells are believed to be infected here [11]. It is the local propagating stage, viral loads are low, and the infection can be detected by using nasal swabs. The innate immune response at this stage is low. In the next few days, the virus starts propagating towards lower respiratory track along with conducting organs and the extent of innate immune response gets elevated. At this stage, the sputum analysis represents a virus and other markers of innate immunity. It confirms the manifestation of COVID-19 disease. The level of cytokine CXCL10 can be predicted for clinical studies [12]. The infected epithelial cells yield beta and lambda interferons [13]. CXCL10 is a gene that is highly responsive to interferons in alveolar type II cell response in the case of SARS-CoV and is also a disease marker for SARS [14 - 16]. It was evident that for about 80% of infected people, the disease was mild and limited only to the upper respiratory region. The rest of about 20% sufferers, unfortunately, go to severe stage 3 and are associated with respiratory infiltrates. The virus enters the lungs and alveolar type II cells and lies in peripheral or pleural sites [17]. A large population of viruses gets released and cells undergo apoptosis and ultimately die. The results are the development of pulmonary toxin, which invades the adjacent cells leading to secondary pathway initiation for regeneration of epithelial cells [18]. It is reported that these manifestations led to alveolar damage with the evident presence of fibrin rich membranes and giant cells [19].

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To recover at this stage, a vigorous immune response is required with extremely rapid epithelial regeneration. The elder people are at very much higher risk at this stage due to low immune response and slow epithelial regeneration. This pathogenesis is not still 100% clear and may take few more months to resolve the unclear mechanisms.

A huge number of research groups from all parts of the world are continuously working in order to develop therapeutic agents against COVID-19, but no vaccine or therapeutic

candidate has been developed to date. The rapidly expanding knowledge regarding SARS-CoV-2 virology provides a significant number of potential drug targets. These targets include spike protein (S), an envelope protein (E), membrane protein (M), protease, nucleocapsid protein, hemagglutinin esterase, helicase and several Nonstructural Proteins (NSPs) [20 - 22] which are currently being focussed for drug discovery and drug development. The drugs or vaccines which are currently under investigation or in clinical trials against COVID-19 have been depicted in (Table 1).

Table 1. Drugs under investigation against COVID-19.

Therapeutic Class	Therapeutic Agent	Description	Current Status	Reference
Interleukin Antagonist	Anakinra	Interleukin-I (IL-1) receptor antagonist with an intent to end cytokine invasion	Under clinical trial (NCT04330638)	[23]
Antibody	Baricitinib	Janus Kinase (JAK) inhibitor, recommended through an artificial intelligence approach	Clinical data not available	[24]
Antibody	Bevacizumab	A recombinant humanized antibody which prevents the association of vascular endothelial growth factor with its receptors which is official in the USA against multiple cancers	Under evaluation in a clinical trial in China against COVID-19 (NCT04275414)	[25]
Antibiotic	Brilacidin	A defensive peptidomimetic under development by Innovation Pharmaceuticals	Under evaluation against SARS-CoV-2	[26]
Anti-Malarial	Chloroquine	Elevates endosomal pH and interferes with ACE2 glycosylation	Under evaluation in a clinical trial (NCT04344951) against COVID-19	[27]
Antiviral	Darunavir	HIV-protease inhibitor	Under evaluation in a clinical trial (NCT04252274), still, no clinical human data is reported in support of its use against COVID-19	[28]
Antibody	Eculizumab	Humanized monoclonal IgG antibody, responsible for the prevention of formation of membrane attack complex by binding to complement protein C5	Under evaluation in a clinical trial (NCT04288713) for COVID-19	[29]
Antiviral	Favipiravir	RNA dependent RNA polymerase (RdRp) inhibitor with wide anti-viral activity	Under evaluation in a clinical trial (NCT04273763) against COVID-19	[30]
Antiviral	Galidesivir	Nucleoside RNA polymerase inhibitor against Ebola virus	Currently under evaluation by Biocryst Pharma	[31]
Antiviral	Griffithsin	An algae derivative as a potent HIV entry inhibitor	Reported active against SARS-CoV-1 and under evaluation against SARS-CoV-2	[32]
Antiviral	Lopinavir	Viral proteases inhibitor (3CLpro or PLpro)	The repurposed drug, currently under clinical trials (NCT04307693)	[33]
Antiviral	Nelfinavir	HIV-1 protease inhibitor	Homology modelling <i>in silico</i> activity is reported but no clinical data available	[34]
Anthelmintic	Niclosamide	Kills tapeworms	Have <i>in vitro</i> activity against SARS-CoV-1, under investigation against SARS-CoV-2	[35]
Antiviral	Remdesivir	RdRp inhibitor and blocks viral replication	Under evaluation against SARS-CoV-2 in clinical trials (NCT04302766, NCT04292899, NCT04292730, NCT04280705)	[36 - 38]
Antiviral	Sofosbuvir	Antiviral against Hepatitis C	Reported <i>in vitro</i> activity against SARS-CoV-1, under investigation against SARS-CoV-2	[39]
Anti-oxidant	Vitamin C	Anti-oxidant	A clinical trial is going on in China for COVID-19 (NCT04264533)	[40]
Antiviral	Umifenovir	Antiviral against Influenza	Evaluated in Chinese clinical trials (200mg orally thrice a day for no more than 10 days) for COVID-19 revealed potent <i>in vitro</i> activity	[41]

(Table 1) contd....

Therapeutic Class	Therapeutic Agent	Description	Current Status	Reference
Herb	XueBijing	Chinese herbal extract	Administration of this extract has remarkably reduced the mortality rate due to COVID-19 in China, administered as 100ml intra venous infusion twice a day	[42]
Interleukin Antagonist	Tocilizumab	Interleukin-6 (IL-6) receptor inhibitor humanized monoclonal antibody which can restore T Cell counts	Under evaluation in a clinical trial (NCT04317092) against COVID-19	[43, 44]

Various treatment strategies to treat and manage the SARS-CoV-2 infection have been summarized in the following sections.

3. DEVELOPMENT OF ANTIBODIES TO SARS-COV-2

It is well known that the entry of coronavirus is mediated by S protein followed by the release of viral nucleocapsid inside the cell for the purpose of replication [45]. S protein also causes syncytium formation between normal receptor-bearing cells and infected cells around them. Targeting the surface of SARS-CoV-2 with a neutralizing antibody has been looked for thoroughly by various research groups in order to provide a passive immunity [46]. The gene synthesis can be carried out in a laboratory considering the expression of S protein as an immunogen utilizing the recently launched genome sequence of SARS-CoV-2 (GenBank: MN908947.3). Traditional methods using experimental animals are also available but they are too slow as per the current situation of this pandemic. Instead, faster methods such as the utilization of phage library or yeast display library to express specific antibodies can be utilized for viral neutralization [47, 48]. The challenges in this task are rigorous testing in cell culture labs, animal models, information from other CoV species and use of a cocktail of antibodies. For quick production of antibodies, lead therapeutic candidates can be utilized for protein expression in bacteria, yeast or insect cells [49]. A much promising approach for producing neutralizing antibodies can be immunizing sheep, goat, cow or large animals with the SARS-CoV-2 proteins followed by purification of antibodies from the animals [50]. The challenge associated with the use of large animals is that there is no guarantee that they will produce neutralizing antisera or not. Another complication may be the human immune response against foreign antibodies.

4. UTILIZING OLIGONUCLEOTIDES AGAINST SARS-COV-2

The RNA genome of the SARS-CoV-2 could be targeted against COVID-19. RNA genome sequence (GenBank: MN908947.3) for this has been recently published and it can be targeted through small interfering RNAs or antisense oligonucleotides [51]. But the challenging task in this approach is to deliver oligonucleotides into the lungs. Another complication associated with this strategy is the non-availability of RNA sequence domains of SARS-CoV-2. Advancements in lipid nanoparticle-based drug delivery systems can deliver into the lungs but only up to a limited extent [52]. But if it becomes possible to deliver small RNAs or antisense oligonucleotides even up to 25% of epithelial cells, it may lead to great success for traditional gene therapy. The need of the hour is to discover the gene homology of SARS-CoV-2 and solve it for future

drug development.

5. UTILIZING RECOVERED PATIENT SERA

Another promising and simple approach is the transfer of passive antibodies from the serum of a patient recovered from COVID-19 to treat a suffering patient. These polyclonal antibodies neutralize the virus and can prevent the possibility of a new infection. The cured patients can donate their plasma, which can be transfused to the suffering patient [53, 54]. The challenging task in this approach is variability in the sera of cured and infected individuals. Although exponential prevalence of this disease may retard the scope of this therapy it still should be considered as an important strategy in the field of transfusion medicines.

6. DEVELOPMENT OF ACE2 BINDING ANTIBODIES

Administration of antibodies that can bind and block the action of ACE2 could be a significant approach to prevent the prevalence of COVID-19 infection. This strategy has been proved experimentally against SARS entry and replication [55]. Although no sequence of ACE2 genome is published, still monoclonal antibodies exist and hybridoma sequences can be cloned only in a few days. It is not concerned with the viral escape from the ACE2 binding antibody unlike the approaches against S protein [56]. The limitation of this approach is that other ACE2 receptors in different locations will also get inhibited. Also, the dose to block the ACE2 receptor in different organs of the body is not known. Another complication is turnover number of the ACE2 receptor, which will greatly influence the effect of the administered antibody. This issue can be resolved by increasing the concentration of ACE2 antagonists at the site of infection in the lungs *via* nebulization technique. Another unfortunate possibility is that direct binding to ACE2 may cause lung injury or may alter the physiology of the lungs.

7. DEVELOPMENT OF ANTIBODY LIKE MOLECULE AGAINST ACE2

Beyond developing antibodies, design and development of antibody-like therapeutic candidate against ACE2 is a much more promising approach which will directly bind and block ACE2. The validity of this approach has already been supported by literature where SARS virus was blocked from infecting cells utilizing soluble ACE2 [57]. The reported affinity of soluble ACE2 for the SARS S protein was 1.70 nM, which is comparable to the affinities of monoclonal antibodies. The life span of the circulating molecule can be increased by converting soluble ACE2 into immunoadhesin format by fusing with the immunoglobulin Fc domain (ACE2-Fc) [58].

Using ACE2 as SARS-CoV-2 neutralizing agent is advantageous because it can directly treat pneumonia pathophysiology. The advanced therapy suggested that the administration of recombinant ACE2 has significantly improved the acute lung injury by reducing angiotensin II levels by successive attachment of hormones to its 1a type receptor. Due to such excellent clinical abilities, recombinant ACE2 was moved to clinical trials where it revealed significant results. The limitation of ACE2-Fc therapy is that elevated extracellular levels of ACE2 in body may lead to undesirable effects. Another important outcome is that receptor targeting through an antibody can direct SARS-CoV-2 towards infecting Fc receptor-positive cells which has the capability to neutralize the antibodies. But this aspect is unclear and more clinical data is required to reveal the undiscovered mechanisms.

8. REPURPOSING OF PREVIOUSLY APPROVED ANTI-VIRAL DRUGS

Previously approved anti-HIV drugs can be repurposed for their efficacy against SARS-CoV-2 by focussing their inhibitory potentials against SARS-CoV-2 target proteins. Therefore drug repurposing is an attractive approach to the researchers, medicinal chemists, clinicians and drug developers in the present situation [59]. Drug repurposing is a technique for utilization of the therapeutic value of an existing drug by focusing on infections other than that for which it was initially proposed [60]. Several drugs have already been repurposed in order to develop a suitable therapeutic candidate against COVID-19. The repurposed drugs include Lopinavir, Ritonavir, Darunavir (against protease); Remdesivir, Ribavirin, Gallidesivir, Penciclovir (against RNA dependent RNA polymerase); Baloxavir (against endonuclease), chloroquine (against ACE2) and Baricitinib (against JAK kinase) [61]. Most of the repurposed drugs have cleared phase I trials (Table 1), have low risk of failure as well as a very small investment. This approach is able to facilitate great clinical developments at a very low cost. Drug repurposing sometimes may come into light through chance observations, but target based repurposing is based on a thorough understanding of molecular mechanisms or cellular events [62, 63]. The repurposed drug may or may not work through the same target or mechanism for which it was previously approved. Many recent reports are there where researchers have carried out drug repurposing by targeting on different-different receptors or biomolecules. Hobartner *et al.* have carried out repurposing of antiviral drug tenofovir for orthogonal RNA catalyzed labeling of RNA [64]. Sun *et al.* carried out drug repurposing of pyrimidine analogs as potent antiviral compounds against human enterovirus A71 infection with potential clinical applications [65]. Bhatia *et al.* have reported repurposing of RdRp inhibitors against SARS-CoV-2 through molecular docking tools with promising results [60]. Zhou *et al.* has carried out network based repurposing of drugs against SARS-CoV-2 and screened out 16 repurposable drugs, including melatonin, quinacrine, colchicine, mercaptopurine, irbesartan etc [66]. Another work was put forward by Singh *et al.* where they screened out a library of 123 drugs and isolated inhibitors (Raltegravir, Paritaprevir, Bictegravir and Dolutegravir) of 3CL pro and ribose methyltransferase through repurposing approach [67]. Kang *et al.* identified the SARS-

CoV-2 3C like protein inhibitory potentials of atazanavir (anti-HIV drug) by utilizing Molecule Transformer-Drug Target Interaction (MT-DTI) for repurposing [68]. In another report, Yelekci *et al.* have utilized the drug repurposing approach by targeting 3CL hydrolases and proteases of SARS-CoV-2. They screened out talampicillin, lurasidone, rubitecan and loprazolam as potential inhibitors of these enzymes through molecular modelling tools [69]. Beyond these reports, many others are available in the literature and continuous work is in progress utilizing this drug repurposing approach.

The challenge associated with drug repurposing is the inadequate efficacy of single therapeutic candidate. Another complication associated with this approach is to search and analyze the huge amount of previously reported data to make efficient and effective use against a new indication. The complex/unclear events of the pathophysiology of SARS-CoV-2 also offer a great challenge to select a candidate for repurposing.

CONCLUSION

Although there is continuous work in progress in some of the above mentioned approaches, but there is still no drug/vaccine available to treat COVID-19. Drug repurposing offers an economical and rapid strategy to discover a potential therapeutic agent in the current hectic situation. This compilation may be helpful to the researchers, drug developers and health agencies to look into the matter and work against the possible targets to develop a therapeutic candidate against COVID-19.

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CONFLICT OF INTEREST

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