INTRODUCTION

The SARS (Severe Acute Respiratory Syndrome) prevailed as a regional animal health threat (especially in bats) in the year 2002-2003 (1). The disease reported its first human incidence in November 2002 in Southern Guangdong province of China. The pandemic has not just led to a potential life threat but has also prevailed its effects in the sectors of global economy and welfare.

Nature and Structure

Human beta corona viruses have novel pathogenesis with differences in phenotype and genotype. The term “coronaviruses” arose from their crown-like appearance when imaged, the Latin for crown being corona. SARS-CoV2 contains spherical or pleomorphic enveloped particles containing 4 main structural proteins. The genome structure consists of two long polypeptides and sixteen non-structural proteins. These proteins are responsible for the genotypic maintenance and virus replication/transmission: (Figure 1)(3)

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Pathogenicity

The Type I transmembrane spike (S) glycoprotein consists of two distinct functional domains S1 and S2, which mediate the virus for host cell entry. The S1 domain contains the angiotensin-converting enzyme-2 receptor-binding domain and the S2 domain facilitates adhesion between cell and virus membrane, required for cellular fusion. S proteins are enzymatically modified, exposing the fusion site for cellular adhesion by S protein priming mechanism. It is achieved through cleavage of cellular proteases S1 and S2, mediated by protein convertase namely Furin, significantly expressed in the lungs. Though the S protein cleavage site is less observed in SARS-CoV-2, it utilizes this system to convert its surface proteins with similar genomic sequence for proliferation (6). The Notch signaling pathway is a major regulator of several biological processes mediating viral infections. ADAM17, a metalloprotease significantly expressed in the lungs and heart, is involved in the shedding of surface proteins such as ACE2. ACE2 removes the terminal amino acid of angiotensin II catalytically counterbalancing ACE and Ang II actions thereby producing a “beneficial” downstream peptides such as Ang1-7 and negatively regulating the RAS (7). Furin and ADAM17 are intertwined with Notch, and thus targeting Notch could represent an alternative approach to inhibit furin and upregulate ADAM17 (10). As per a recent study, the SARS-S engages angiotensin-converting enzyme 2 (ACE2) as the entry receptor and employs the cellular serine protease TMPRSS2 for activation of S protein priming (8,9). Host cell entry is manifested by 2 pathways that is Endocytosis and Proteolytic cleavage at the ACE2 receptor. During Endocytosis spike proteins are activated by the host depending cysteine protease cathepsin L resulting in conformational change leading to attachment. Followed by proteolytic cleavage that is S-protein priming activated by TMPRSS2 serine protease. Recent studies have found that the modified S protein of SARS-CoV-2 has a significantly greater affinity for ACE2 receptor and this increase in affinity is the reason for its rapid transmission. A study on the SARS-S/ACE2 interface at the molecular level was determinant that SARS-CoV& SARS-CoV-2 share around 76% amino acid similarity (11). However, it is still hypothetical whether SARS-CoV-2 like SARS-CoV employs ACE2-TMPRSS2 pathway for host cell entry (Figure 2).

Pathophysiology

In a detailed perspective, the probable events occurring at cellular level in the areas of respiratory tract are postulated under three phases (Figure 3)

Phase-I: Asymptomatic phase – The SARS-CoV and SARS-CoV-2 bind to the epithelial cells in the nasal cavity via ACE-2 receptor. The single cell RNA indicates low level of ACE-2 expression in the conducting airway, but triggers local propagation of virus and limited innate immune response. This stage is the initial 1-2 days of infection and can be detected by nasal swab (low viral burden).

Phase-II: Symptomatic phase – The infectivity propagates and progresses down the respiratory tract into the conducting airways thereby triggering innate immune response. The disease is clinically manifested at this stage due to early markers of the innate immune system (nasal swab, sputum, throat swab). The level of early markers such as cytokines (IL1, IL6, TNF-α, CXCL10) maybe predictive of the subsequent clinical course to be initiated. CXCL10 is a major interferon responsive gene that is an excellent predictive disease marker in the alveolar Type-II pneumocyte response to COVID-19. At this stage 80% of the infected patients start showing symptoms (mild-moderate) in the upper and conducting airways in about 5-7 days.

Phase-III: Progressive-Terminal phase – The SARS-CoV2 reaches the alveolar Type-II pneumocytes and propagates within them, cells replicating and releasing viral particles into the adjacent cells. The Type-II pneumocyte cells are not preferentially infected much as Type-II cells, as they are the precursors for Type-I pneumocyte cells. This stage is manifested by few multi-nucleated giant cells, diffuse alveolar damage with fibrin rich hyaline membrane and epithelial damage triggering to apoptosis (vigorouse innate immune response & epithelial regeneration). The mucociliary clearance is reduced leading to blockage of gas exchange in the alveoli leading to hypoxemia in around 10-14 days (13) and tend to develop Pulmonary Infiltrate, Ground Glass Infiltrate, Pneumoniitis, and progression to ARDS. The triggered immune response leads to an increased concentration of highly pro-inflammatory cytokines. The most recent clinical studies in China reported that IL-6 is one of the most important cytokines involved in COVID-19-induced cytokine storms. However, in severely affected patients, IL-6 is moderately increased (25.2 pg/mL) compared to typical levels in cytokine release a syndrome (more than 1600 pg/mL in cytokine release a syndrome). Hence explains why no serious vasoplegic shocks are observed. The cytokine storm in COVID19 patients is characterized by hyper innate immune response and activation of Th cell-mediated immunity, mediated by overproduction of pro-inflammatory cytokines progressive to cardiovascular collapse, multiple organ dysfunction, and death rapidly (41).

Risk Factors: Individuals with age group >65 years or with prior chronic medical conditions such as Hypertension, Diabetes with HbA1c level >7.5%, severe Obesity, lung disease, heart disease, chronic kidney or liver disease, immunocompromised patients, chemotherapy or immunotherapy undergoing patients, post-solid organ transplantation, HIV with CD4 cell count < 200 copies/mm³ are at higher risk to develop worsened symptoms.

Clinical risk factors: Respiratory rate >24 breaths/mm, Oxygen saturation <94% on room air and <90% in hypoxemia, D-dimer level >1µg/mL in lung disease are at higher risk to mortality (13).
Evidence Based Pharmacotherapy

Based on the review and evaluation of various studies conducted, the therapeutic agents targeting the viral proliferation only are postulated under 2 categories by the authors as the following:

1. Agents with clinical evidence of therapeutic effects
2. Agents with No reported evidence of therapeutic effects

Agents with clinical evidence of therapeutic effects:
Anti-viral Agents

Remdesivir

Interestingly this antiviral drug, Remdesivir, or GS-5734, is an adenosine triphosphate analog which was initially discovered in 2016 for the Ebola virus. In 2017, its inhibitory action against the coronavirus family was also demonstrated and is now being studied clinically whether it has any activity against the SARS-CoV-2. Remdesivir is a nucleoside analog that inhibits the RNA polymerase activity by incorporating into RNA (additional nucleotides cannot be added, hence terminating the RNA transcription) (14). As the only route of administration for Remdesivir is intravenous, the clinical studies are limited to be used in the hospital settings mostly (15). The ongoing clinical trials (Figure 4) are using a therapeutic regimen of 200mg once daily on the first day, followed by 100mg once daily for next 9 days in SARS-CoV-2 (Gilead Sciences Inc.) (14, 43).

Favipiravir

This agent however approved by the U.S. FDA for therapeutic use in resistant cases of influenza, has been used in China to treat patients with COVID-19. It is a modified pyrazine analog that targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes. As per a recent open-label, non-randomized clinical trial comparing Favipiravir with LPV/RTV concluded that Favipiravir reduces fever, duration of viral shedding and rapidly improved status in chest computed tomography (CT) findings (42).

Lopinavir/Ritonavir (LPV/RTV)

This combination is a protease inhibitor used concomitantly with other HIV protease inhibitors initially developed for HIV-1 infection. It was approved by the FDA in 2006 as a second-generation protease inhibitor to combat resistance to standard HIV therapy. Darunavir is being studied as a possible treatment for SARS-CoV-2 due to in-vitro evidence of its potential (weak) towards SARS-CoV-2. The current clinical trials are expected to be concluded and reported by August 2020 (17).

Hydroxychloroquine sulfate & Chloroquine

HCQ and CQ are aminoquinolines used in the treatment of uncomplicated malaria, rheumatoid arthritis, chronic discoid lupus erythematosus, and systemic lupus erythematosus and in the prophylaxis of malaria in regions with CQ resistance. Hydroxychloroquine inhibits terminal glycosylation ACE2 receptor and ACE2 that is not in the glycosylated interacts weakly with the SARS-CoV-2 spike protein, further inhibiting viral entry. However another randomized study from China in patients with mild to moderate COVID-19 demonstrated no difference in recovery rates and reported a fatality in the group being treated with HCQ (18, 19).
Agents with Noreported evidence of therapeutic effects

Oseltamivir
Oseltamivir, an antiviral neuraminidase inhibitor (enzyme found on the surface of the virus, which helps in budding from the host cell, viral replication, and infectivity) used for the treatment and prophylaxis of influenza viruses A and B, pandemic H1N1 infections. As SARS-CoV-2 is not known to utilize neuraminidase in viral replication therefore is not likely to be of any therapeutic use in COVID-19 (20).

Umifenovir
Umifenovir also known as Arbidol (ARB) is an indol derivative with antiviral activity in treatment for respiratory infections. Umifenovir is a direct-acting antiviral (DAA) due to its direct virucidal effects and host-targeting agent (HTA) as it effects on one or multiple stages of viral life cycle. It is a hydrophobic molecule capable of forming aromatic stacking interactions with certain amino acid residues such as tyrosine and tryptophan, and due to interactions with aromatic residues within the viral glycoproteins. These interactions are involved in fusion and cellular recognition with the plasma membrane to interfere with clathrin-mediated exocytosis and intracellular trafficking, or directly with the viral lipid envelope (in enveloped viruses). Interactions at the plasma membrane serve to stabilize and prevent viral entry (e.g. stabilizing influenza hemagglutinin inhibits the fusion step necessary for viral entry) (21).

 Baloxavir
Baloxavir marboxil is an antiviral drug developed for the treatment of influenza A & B infections, used for treatment within 48 hours of symptom onset. The drug is a capsid-endonuclease inhibitor, has a unique mechanism of action when compared to the currently existing neuraminidase inhibitor drug class used to treat influenza infections. As per the National clinical trials database (clinicaltrials.gov), it does not include any studies of Baloxavirused against SARS-CoV-2 (22).

 Nitazoxanide
Nitazoxanide (NTZ) is a thiazolidederivative that interfereswith the survival, growth, and proliferation of several extracellular & intracellular protozoa, anaerobic and microaerophilic bacteria, helminthsand viruses. As per a recent study conclusion, NTZ suppressed viral replication by inhibiting the maturation of the viral hemagglutininesterase (HE) proteins and the viral transcription factor immediate early 2 (IE2) by activating the eukaryotic translation initiation factor 2α (an antiviral intracellular protein). There is no clinicaledvidence stating itsanimal or human use against SARS-CoV-2 for now (23,24)

Azithromycin
Azithromycin also has immunomodulatory effects apart from inhibiting protein synthesis and translation in bacteria, and has been used in chronic respiratory inflammatory diseases for this activity. In March 2020, as per a small study conducted by the French government to investigate the treatment of COVID-19 with a combination of azithromycin and hydroxychloroquine reported positive outcomes. The patients using this combination were virologically stable and cured within 6 days of therapy; however, evidence from larger studies is not yet available (25).

ACE Inhibitors or ARBs
As per the pathogenicity, the entry by SARS-CoV-2 into the host cells tends to depend on the ACE2 receptor. The ACE inhibitors currently used, block the ACE1 receptor but not the ACE2 receptor, resulting in no clinical effects in the SARS-CoV-2 virus. Also prolonged use of ACE inhibitors & ARBs and some chronic comorbidities demonstrate to upregulate ACE2 expression. Of relevance, there is no clinical evidence indicating an increased risk of severe disease among individuals receiving either of the agents, and the agent discontinuation to down-regulation of ACE2 expression by inhibiting the PAI-1 indirectly. Therefore, no evidence to support the use or discontinuation of such agents for the treatment or prevention of COVID-19 is reported for now (26).

CONCLUSION AND SIGNIFICANCE
The COVID-19 pandemic being a new viral disease has no specific standard therapy yet. The clinical research is currently immersed to demonstrate if any of the old agents might be an effective treatment for this new disease (Figure 5). As per the above discussion, we authors conclude the following aspects for targeted drug/vaccine development:

Targeted hypothesis based agents - Aspects of the drug/vaccine development: This hypothesis-driven study intervenes the various possible approaches to find if any of the old agents might be effective in management/prevention/eradication for this new disease.

Immune Modulatory Agents
Interferon beta-1B: Interferon beta interferes to type I interferon receptors (IFNAR1 and IFNAR2c)by activating two Janus kinase (Jak1) and tyrosine kinase (Tyk2), that self-transphosphorylates and phosphorylates the receptors. The phosphorylated INFAR (Interferon Alloha receptor) receptors binds to Stat1 and Stat2 (signal transducers and activators of transcription) which combine to form a dimer and activate multiple immunomodulatory and antiviral proteins. Interferon beta binds more stably to type I interferon receptors than interferon alphaproducing better outcomes. Janus kinase inhibitors (e.g., baricitinib) (27). There is insufficient clinical data to recommend either for or against the use of convalescent plasma or hyperimmune immunoglobulin for the treatment of COVID-19.

Interleukin Inhibitors: Tocilizumab (TCZ), a monoclonal antibody against interleukin-6 (IL-6), is an alternative treatment for SARS-CoV-2 patients with a risk of cytokine storms. IL-6 is a cytokine that plays an important role in inflammatory reaction and immune response. As per amulticentre randomised controlled trial to test a monoclonal antibody against the IL-6 receptor (tocilizumab, Roche) in patients with COVID-19 pneumonia and elevated IL-6 has been conducted in China with beneficial results. In addition, other strategies have also been attempted to reduce the cytokine storm. CytoSorb is an extracorporeal cytokine adsorber that hastreated over 65 patients with severe COVID-19 in China, Italy and Germany (29). However, reported data on the effectiveness of this approach is not yet available. Interleukin-6 inhibitors (e.g.,
sarilumab, siltuximab, tocilizumab) and Interleukin-1 inhibitors (e.g., anakinra).

**Statins:** In few observational studies, it is stated that statin therapy is associated with a reduction in various cardiovascular complications and mortality in patients admitted with influenza and/or pneumonia (28). Thereby evidential that patient with viral respiratory illnesses (novel COVID-19) could derive a beneficial effect by inhibiting the PAI-1 from the continuation of the concomitant statin therapy (if any) (40).

**Notch Signaling Inhibitors**

As per a preclinical study, the Notch inhibition interfered with the immune response during viral infection using an anti-Dll1 antibody, or GSI (intranasal administration) resulting in increased mortality, defective viral clearance, and decreased IFN-γ in lungs. Notch signaling also modulates the immune response following respiratory syncytial virus (RSV) infections, which cause an increase of Jagged1 in bronchial epithelial cells and, when co-cultured with CD4+T cells, promotes Th2 differentiation. However, the reduction of Jagged1 expression promotes an increase in Th1 differentiation. Hence it is suggested that Jagged1-mediated Th2 differentiation may cause RSV-induced airway hyper-responsiveness (43). Based on these results, it is hypothesized that it is better targeting specific components of the Notch signaling (such as Dll4 or Jagged1), rather than inhibiting Notch with a GSI (30). Furin is transcriptionally induced by Notch signaling; hence it is evident to reduce Furin levels and viral proliferation. Since Notch dysregulation is significant in majority of cancers, Notch inhibitors (DAPT) that target the γ-secretase (GSI) are available and are being tested monitoring the gastrointestinal toxicity, in several Oncology Clinical trials (31).

**Soluble ACE2R**

ACE2 is not known whether if human recombinant soluble ACE2 (hrsACE2) blocks growth of SARS-CoV-2. As per recent study it is evident that SARS-CoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids, which can be inhibited by hrsACE2. These data demonstrate that hrsACE2 can significantly block early stages of SARS-CoV-2 infections, thus increasing the soluble ACE2 could prevent the SARS-CoV-2 proliferation (32). ACE2 solubility enhancers (e.g., PHOENIX-DX 600)

**ACE2 Receptor Blockers**

The ACE2 receptors present on the surface of lung type-2 pneumocytes act as a novel adhesion site for SARS-CoV-2 and be a potential therapeutic target for the prevention. As per prior studies, Emodin inhibits SARS entry by binding to ACE2 and also may have functions at various human protein binding studies. The ACE1 receptors catalyzes the formation of Angiotensin (Ang) II which is an active component of renin–angiotensin system (blood pressure regulation and cardioenal function) whereas, ACE2 receptor cleaves Ang II to Ang (1–7) (26) (Figure 2). ACE2 receptor could be targeted to prevent SARS-CoV-2 from entering the cells.

**Furin Inhibitors**

Inhibiting the expression of Furin could be a possible approach to prevent SARS-CoV-2 infection by inhibiting the precursor Notch signaling pathway (10). As per a study, Nano molar concentrations of a peptide designed from the avian influenza A H5N1 virus Furin cleavage sequence has reported to treat infections from several Furin-dependent pathogens such as SARS-CoV-2 (31, 38).

**ADAM17 Inhibitors**

Inhibition of ADAM17 expression reduces ACE2 shedding, whereas over expression of ADAM17 significantly increases its shedding and membrane translocation of ADAM17 leads to a reduction in plasma ACE2 protein levels leading to an increase in plasma ACE2 activity. Thus it is evident that by increasing ADAM17 levels and/or activity to enhance shedding and increase soluble/plasma ACE2 levels could block SARS-CoV-2 entry into cell (36). In a preclinical study, treatment with the chemotherapeutic agent 5-fluorouracil strongly activated ADAM17 in an animal model of colorectal cancer and non-small cell lung cancer, estradiol increased the expression and activity of ADAM17. This data indicates the higher shedding of ACE2 in women and thereby explains the reduced incidence of COVID-19 in women compared to men (34,35). Thus this hypothesis targeting the molecular pathways of virus-cell interaction could be targeted to prevent SARS-CoV-2 from entering the cells.

**TMPRSS2 Inhibitors**

As discussed in the pathogenesis SARS-CoV-2 uses ACE2 receptor for entry and the serine protease TMPRSS2 for S protein priming. The TMPRSS2 gene encodes to the serine protease family which mainly consists of a protease domain, a type II transmembrane domain, a scavenger receptor cysteine-rich domain and a receptor class A domain. The protease domain of this protein is cleaved and secreted into cell after auto-cleavage. Some corona viruses, the SARS-CoV and SARS-CoV-2 are activated by TMPRSS2 and thus be inhibited by TMPRSS2 inhibitors (37). A TMPRSS2 inhibitor approved for clinical use blocked entry and proven serine protease
inhibitor camostat mesylate, partially blocked SARS-2-S-driven entry as per a recent study and complete inhibition was attained when Camostat mesylate and an inhibitor of CatB/L were used (35). As per a preclinical study, plasminogen activator inhibitor type 1 (PAI-1) is a serine protease I inhibiting the TMPRSS2 activation. The PAI-1 regulates the various metabolic pathways and hemostasis in co-morbidities associated with COVID-19 such as Diabetes type II, cardiovascular, pulmonary, dermal, hepatic and renal dysfunctions.

Conflict of interest statement: The authors declare no conflicts of interest.

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