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Review Article

A REVIEW ON PHARMACOLOGICAL TARGETS FOR TREATMENT OF COVID-19 INFECTION

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ABSTRACT

The sudden outbreak SARS-CoV-2 in Wuhan, China, which rapidly grew into a global pandemic, marked the third introduction of a virulent coronavirus into the human society, affecting not only the healthcare system, but also the global economy. In this article, we present a succinct overview of the epidemiology, pathophysiology, and targets of SARS-CoV-2. In the past 14 years, the onset of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have thrust HCoVs into spotlight of the research community due to their high pathogenicity in humans. The study of Human coronavirus (HCoV) host interactions has contributed extensively to our understanding of HCoV pathogenesis. We systematize the current clinical trials that have been rapidly initiated after the outbreak of COVID-19 pandemic.

Keywords: SARS-CoV-2, Human coronavirus, Clinical trial

1. INTRODUCTION

Coronaviruses are a family of enveloped viruses with positive sense, non-segmented, single stranded RNA genomes. The subgroups of coronaviruses family are alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. The ICTV named the virus as SARS-CoV-2. Subsequently, a group of virologists in China proposed renaming SARS-CoV-2 as human coronavirus 2019 (HCoV-19), considering that such a name would recognise the virus from SARS-CoV and keep it steady with the WHO name of the disease it causes, COVID-19 [1]. SARS-CoV-2 was reported to be a member of the β group of coronaviruses. Total six HCoVs are identified namely HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoV. Except SARS-CoV AND MERS-CoV other scan cause life-threatening pneumonia and bronchiolitis especially in elderly, children and immune-compromised patients. They also have capability to cause enteric and neurological diseases [2, 3]. In December 2019, SARS-CoV-2 spread exceptionally rapid in China and after that then to the numerous many other countries, causing coronavirus disease-19 (COVID-19). COVID-19 is the third-known zoonotic disease from coronavirus after SARS and MERS [4]. The clinical prospects of COVID-19 mainly include fever, cough and pneumonia [5].

2. EPIDEMIOLOGY

On 29 December 2019, the primary four cases of an acute respiratory disorder of unknown etiological were noted in Wuhan city, China among people connected to a nearby seafood market [6]. Research is underway to understand more about transmissibility, severity, and other features associated with COVID-19 [7]. It appears that most of the early cases had some sort of contact history with the original seafood market. Soon, an auxiliary source of infection was found to be human to human transmission by means of close contact. There was an increment of infected individuals with no history of exposure of wildlife or visiting Wuhan, and multiple cases of infection were recognized among medical professional [8, 9]. It became clear that the COVID-19 infection occurs through the exposure to the virus, and both the immunosuppressed and normal population appear susceptible. Some studies have reported an age distribution of adult patients between 25 and 89 years old. Most adult patient were between 35 and 55 years, and there were fewer identified cases among children and infants [10]. In ponder on early transmission dynamics of the virus reported the median age of patients to be 59 years, extending from 15 to 89 years, with the large part (59%) being male. It was suggested that the population most at risk be people with poor immune function such as older people and those with renal and hepatic function [6]. In India 23,452 and 1,752 with new cases were

confirmed past 24 hours 25 April 2020. Studies indicated the spread of COVID-19 are relatively quick and reported that it had spread to several other countries after outbreak in China. On 14 April 2020 there were 117021 deaths reported globally [11]. Total confirmed cases were reported in the following countries outside of China: Australia (6366), Canada (24786), France (97050), Finland (3064), Germany (125098), Italy (159516), Japan (7645), Nepal (16), Malaysia (4817), Philippines (4932), Republic of Korea (10564), Thailand (2613), United Arab Emirates (4521), United States of America (553822).

3. PATHOPHYSIOLOGY

There is numerous likeness of SARS-CoV-2 with the initial SARS-CoV. Using computer modeling, found that the spike proteins of SARS-CoV-2 and SARS-CoV have almost exact 3-D structures in the receptor-binding domain that retain van der Waals forces[12]. SARS-CoV spike protein has a strong binding affinity to human ACE2, based on biochemical interaction studies and crystal structure analysis [13]. Advance investigation indeed proposed that SARS-CoV-2 recognizes human ACE2 more effectively than SARS-CoV increasing the ability of SARS-CoV-2 to transmit from individual to individual [14]. Hence, the SARS-CoV-2 spike protein was anticipated to moreover have a strong binding affinity to human ACE2.The S1 sphere of all characterized CoV, including SARS-CoV, mediates the initial high affinity interaction with the cellular receptor [15]. The S proteins of CoV are major targets for neutralizing Abs and frame the characteristic corona of large and particular spikes on the viral envelopes [16]. Although the entrance of SARS-CoV into the host cell has demonstrated to be through the binding of S protein to ACE2, the immune response induced by SARS-CoV remains under characterized. M protein aid shape the virion particles and binding to nucleocapsid, E-protein show a role in the assembly and release of particles while N-protein aids with the binding of the genome to a replication transcription complex which is requisite for the replication of genomic material. ACE2 is a functional SARS-CoV receptor in vitro and in vivo [17]. It is required for host cell passage and ensuring viral replication. Over expression of human ACE2 upgraded infection seriousness in a mouse model of SARS-CoV infection, demonstrating that viral passage into cells is a critical step [18]; injecting SARS-CoV spike into mice worsened lung damage. Critically, this damage was weakened by blocking the RAAS pathway and depended on ACE2 expression [19]. Hence, for SARS-CoV pathogenesis, ACE2 is not only the entry receptor of the virus but moreover protects from lung damage (Figure 1).

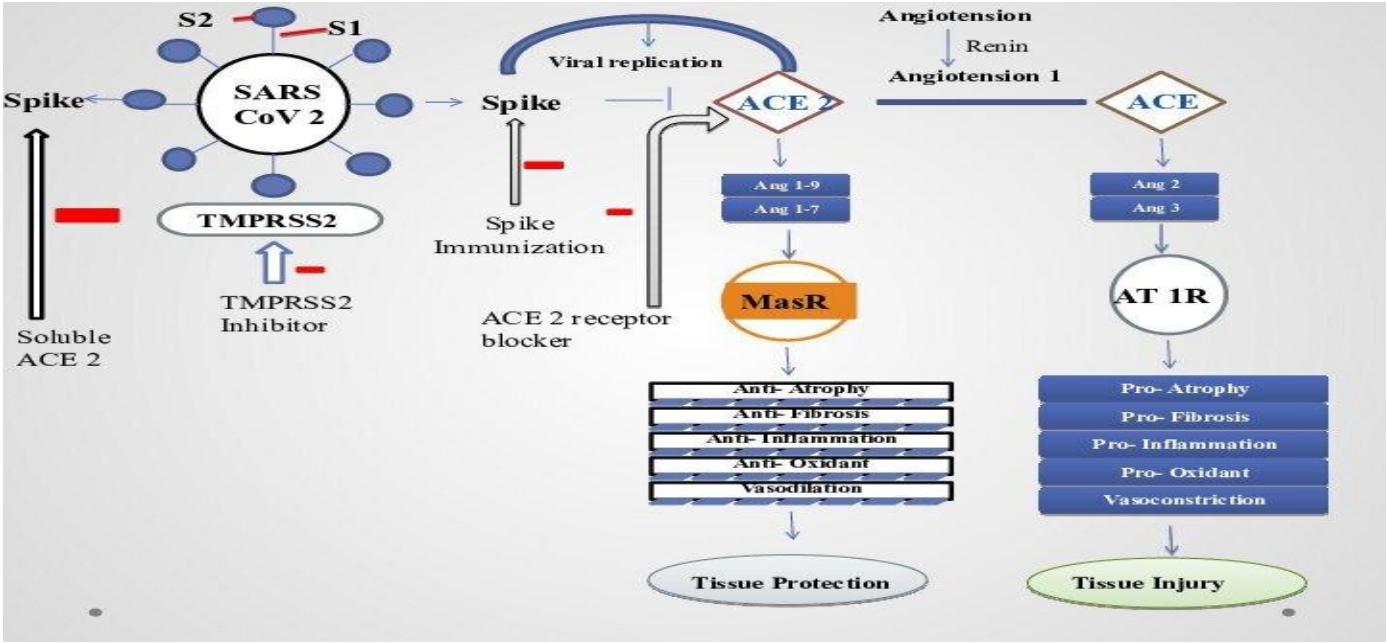

Fig. 1: SARS-CoV-2 use the ACE2 receptor for cell entry has vital suggestion for understanding transmissibility and pathogenesis. SARS-CoV-2 leads to downregulation of the ACE2 receptor, but not ACE, through binding of the S1 with ACE2. This leads to viral entry and replication, as well as extreme lung injury.

The ACE2-expressing ACE 2 have high levels of numerous viral process-related genes, including regulatory genes for viral processes, viral life cycle, viral assembly, and viral genome replication [20], proposing that the ACE2-expressing ACE 2 facilitate corona viral replication within the lung. ACE2 receptor is additionally found in numerous extra-pulmonary tissues counting heart, kidney, endothelium, and intestine. IL-8 has been appeared to be raised in blood and alveolar spaces [21], and shows a positive relationship with the number of poly-morphonuclear cells in broncho-alveolar liquid of patients with pneumonia and ARDS [22]. The rises of IL-8 and cytokines have been found within the plasma of SARS-CoV-infected patients [17] and can initiate the hyper-innate inflammatory reaction due to the SARS-CoV invasion of the respiratory tract. SARS-CoV 2 without a doubt infects the human gut epithelium has important implications for fecal–oral transmission and containment of viral. ACE2 tissue conveyance in other organs may brief the multi-organ dysfunction observed in patients [23].

4. TARGETS

4.1. Autophagy and the endocytic pathway

CoV actuates autophagy and either the ATG proteins are induced within the infection and replication of CoVs. The primary report illustrating the association of autophagy in viral replication was based on MHV [24], in which several important observations are done. First, MHV actuates the formation of double-membrane vesicles (DMVs), with likeness to autophagosome, a hallmark of autophagy. Second, the viral replication complex at DMVs co-localized with the autophagy proteins, LC3 and ATG12. Third and more vitally, MHV replication was impended in ATG5 knockout stem cells. Accordingly, the authors achieve that autophagy is involved in the forming of DMV as well as in the replication of MHV [24]. In a follow-up considers, the same group also inspected the SARS-CoVs and found comparative co-localization of the key viral replication proteins with endogenous LC3, a protein marker for autophagosome [25], proposing a similar function of autophagy in the replication of SARS-CoVs. There are two subunits of S protein have distinct functions: S1 is capable for receptor-binding, while S2 is basically for membrane fusion and both are essential for viral entry by means of the endocytic pathway and infection into the host cells. The significance of endosome-lysosome in CoVs was from an morphological considers in which two CoVs (IBV and Porcine Epidemic Diarrhea Virus (PEDV)) were found to build up in the lysosomes of cells after infection [26], demonstrating that the conceivable functional suggestion of lysosome in CoVs.

Distant CoVs including MHV, SARS-CoV and MERS-CoV have been steadily illustrated to engage the endocytic pathway as the main mechanism for viral entry into variety types of host cells (Figure: - 2). Among them, clathrin-dependent endocytosis and cathepsin mediated S protein cleavage are two critical steps for the viral entry and infection. In real, this mechanism is additionally applicable to numerous other CoVs such as IBV. The role of the endocytic pathway for viral entry, there are discrepancies of the exact mechanisms among the role of the endocytic pathway for viral entry, there are discrepancies of the exact mechanisms among instance, Found that SARS-CoVs engage clathrin and caveolae-independent endocytic pathway as the key component for viral entry [27], which is conflicting with a prior report in which SARS-CoV entry into HepG2 cells is generally mediated by the clathrin-dependent pathway [28].

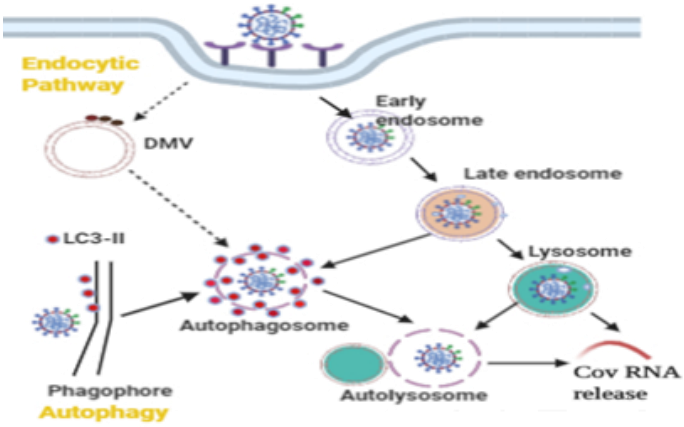

Fig. 2: Entry of CoVs into host cells is basically mediated by endocytic pathway interim the autophagy has also been involved in the viral replication in the cells a process partly associated to the formation of DMV in the host cells.

4.2. Molecular Mechanisms in Apoptosis

Apoptosis is a process of programmed cell death that is tightly regulated and anti-inflammatory. Till now, two main mechanisms of apoptosis have been established the extrinsic and intrinsic pathways. The extrinsic pathway is started by the binding of Fas ligand and TRAIL to death receptors from the TNF super-family [29]. These death receptors then enroll various death adapter proteins, such

as FADD [30], and initiator procaspases 8 and 10 to make the DISC [31]. SARS-CoV initiated apoptosis was appeared to be caspase reliant and might be hindered by over expression of Bcl2 [32, 33]. Apoptosis can also be initiated by less pathogenic strains of HCoVs, as substantiated by microarray data appearing noteworthy changes in pro-apoptotic and anti-apoptotic gene expression of Bcl2 family members amid HCoV-229E infection [34]. Although caspases 3 and 9 were enacted in HCoV-OC43-infected murine and human neuronal cells [35], expansion of pan-caspase blocker Z-VAD-FMK and the caspase-9 blocker Z-LEHD-FMK did not influence the viability of these infected neuronal cells, demonstrating that programmed cell death actuated by HCoV-OC43 could be caspase-independent. This highlights the possibility of a non-classical programmed cell death mechanism actuated in HCoV infection. Apoptotic mechanisms amid HCoV infection are likely to be controlled by viral proteins, although this has only generally been examined in SARS-CoV. Expression of SARS-CoV E protein and 7a protein progressed mitochondrial intervenes apoptosis by cloister the anti-apoptotic Bcl-XL to the ER membranes [36]. SARS-CoV protein is additionally profound pro-apoptotic and intervenes enactment of both caspases 8 and 9 [37]. Furthermore, HCoV-OC43 wild type S protein has been appeared to actuate which may lead to apoptosis. A genetic reshufflingHCoV-OC43 harboring point change at its S polypeptide initiated stronger caspase 3 enactment and nuclear dissolution than the non-mutated virus [38]. It is curiously to sign that the location of SARS-CoV proteins which are in nucleus (Fig. 3) is related with initiation of apoptosis [39]. This finding opens up to novel points of view of the connected between subcellular localization of viral proteins and caspase actuation as a mode of apoptosis regulation by HCoVs.

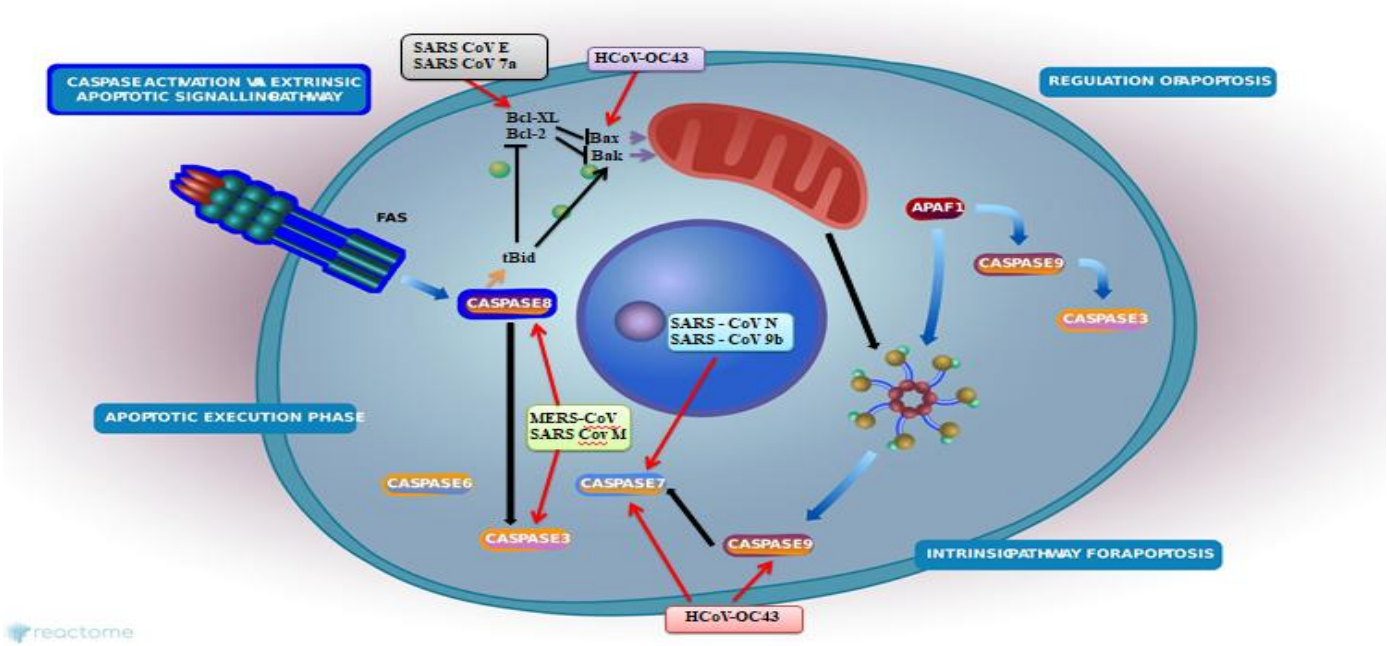

Fig. 3: Enactment of apoptosis by HCoVs. Binding of FAS receptor actuates caspase 8 enactment, which in turn enacts effector caspases 3 and 7 to stimulate apoptosis. On the other side, intrinsic pathway is coordinated by pro-apoptotic and anti-apoptotic Bcl2 family proteins, such as Bcl-XL, Bcl2, Bax and Bak to induce mitochondrial outer membrane permeabilization (MOMP). Ensuring caspase 9 enactment caused by improved MOMP stimulates caspases 3 and 7 enactment. During HCoV infection, the virus (yellow, red, grey boxes) target at numerous stages of both the extrinsic and intrinsic apoptosis signalling pathways.

4.3. Innate Immune Response

During viral infection, type I and III IFNs are quickly initiated to start the antiviral state. IFN production and the downstream signaling molecules are actually similar for the 2 types [40]. IFNs III are an initial reaction to knock down infection at the epithelial obstruction without causing immunopathology, whereas type I IFNs come into play when this first line of defence isn't adequate to control the infection [41]. The management of these 2types of IFNs is actuates when the innate immune system observe invading viruses through its PRRs (Fig. 4).

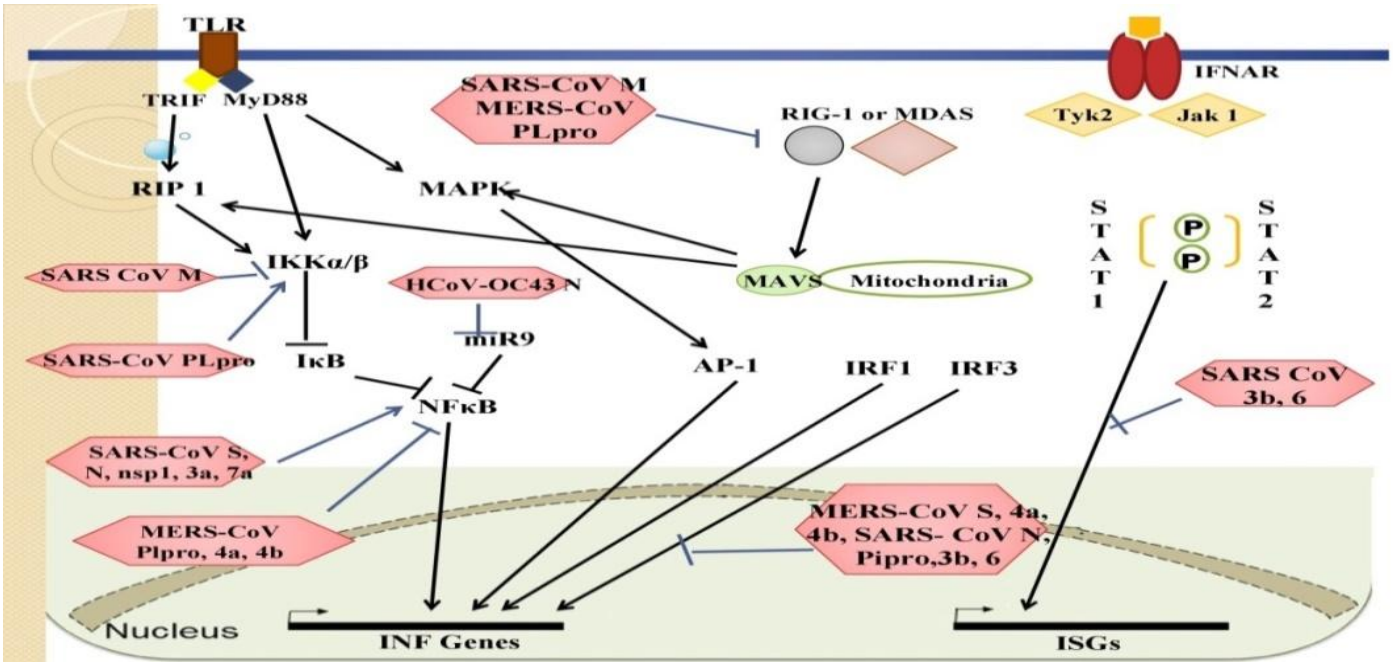

Fig. 4: HCoV viral proteins on innate immunity. During HCoV infection, PRRs such as TLRs, RIG-I and MDAS are enacted to trigger a series of signalling pathway, including NF-κB, for IFN production. These IFNs then act on IFNAR and enact the JAK-STAT signalling pathway to actuate ISGs. The pink boxes show the viral proteins that have been reported to modulate host innate immunity at numerous stages

Infection by HCoVs, particularly the supremely pathogenic SARS-CoV and MERS-CoV, is related with obstruction of IFN synthesis [42]. The potential of the infection to control type I IFN signing is a relevant hallmark for virulence [43]. Based on studies from SARS-CoV and Mouse Hepatitis Virus (MHV)-infected cells, two mechanisms have been proposed to explain the HCoV-mediated inhibition of type I IFN production [44, 45]. Firstly, CoV genomic and sub-genomic RNA replication takes place in bilayer membrane vesicles to avoid detection by PRRs [46]. Secondly, proteins encoded by the virus could obstruct with innate immune response [47]. Infection by HCoVs, particularly the profoundly causing disease SARS-CoV and MERS-CoV, is related. The potential of the virus to control type I IFN signaling is an imperative hallmark for virulence [43]. As compared to SARS-CoV and MERS-CoV, a colossal rise in type I interferon's were identified in cells contaminated with strain 229E [48, 49]. Expression of SARS-CoV M protein could suppress type I IFN production intervened by RIG-I,but not MDA5, in infected HEK293 cells [50], likely through its to begin with TMD. In any case, this hindrance was not observed when expressing the M protein of HCoV-HKU1, proposing that this activity is not sustained among all HCoV strains [51]. In another study, it was appeared that the MERS-CoV M protein seem also suppress type I IFN by inhibiting the translocation of IRF3 into the nucleus, although the precise has not yet been illustrated [52]. Furthermore, SARS-CoV Nprotein was also appeared to meddle with the activity of IRF3 [53]. The N protein of SARS-CoV likely act at the starting identification phase of viral RNA by means of its RNA binding activity, in spite of the fact that is not one or the other forms a complex with RIG-I nor MDA5 [54]. This implies that the N protein conceivably acts on other viral RNA acknowledgment procedure of the host.

4.4. Endoplasmic Reticulum(ER) Stress Response

The ER is a cellular organelle important for protein synthesis, folding, processing and post-translational modifications. In ordinary circumstances, the ER can be stacked with a very high concentration of proteins without perturbing its special luminal environment [55]. In any case, when the protein load exceeds the ER folding and preparing capacity, quick aggregation of misfolded or unfolded proteins happens inside the ER lumen. Different signaling pathways, collectively known as ER stress response or UPR, are enacted. These pathways are initiated by three ER trans-membrane sensors-protein-kinase-R (PKR)-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1

(IRE1) and activating transcriptional factor 6 (ATF6) to orchestrate the restoration of ER homeostasis by upgrading protein folding, constrict protein interpretation and upregulating genes related to protein folding, chaperoning and ER-assisted degradation (ERAD) (Fig. 5). In cases of extended and irreversible ER stress, apoptosis mechanisms are provoked [56]. During viral diseases, ER stress response is initiated. This enormous use of the ER elicit immense burden, causing the host to mount UPR as its antiviral response [57].

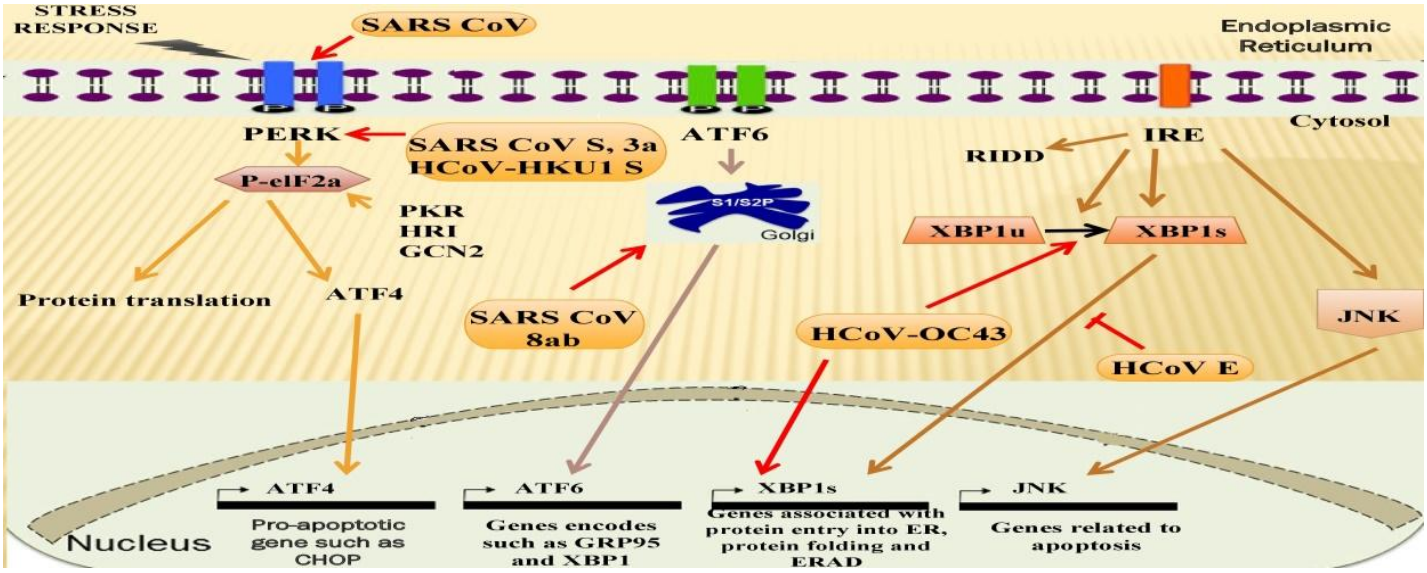

Fig. 5: During HCoV infections, the ER stress responses, which compose of 3 signaling pathways, PERK, ATF6 and IRE1, is enacted. HCoVs encode many viral proteins (orange boxes) that target the various signalling pathway of ER stress during viral infection

4.5. PERK Signalling Pathway

Enactment of PERK is induced by its separation of the luminal domain from the ER chaperone, BiP. Typically, followed by the oligomerization and autophosphorylation of PERK. In its active form, PERK phosphorylates Ser51 at the eIF2α to attenuate protein translation [58]. Phosphorylated eIF2α not as it were triggers a shutdown of global protein synthesis, but moreover upgrades the translation of ATF4 [59]. ATF4 triggers target gene expression such as GADD153, to upgrade transcription of pro-apoptotic genes. Furthermore, eIF2α can be phosphorylated by other kinases such as PKR, HRI, and GCN2. These kinases activate various downstream signalling pathways, which together form the integrated stress response [58]. PKR and eIF2α phosphorylation was identified in SARS-CoV-infected cells and hindrance of PKR using antisense peptide-conjugated phosphorodiamidatemorpholino oligomers did not influence eIF2α phosphorylation but essentially decreased SARS-CoV actuated apoptosis.

In this manner, it is likely that SARS-CoV adopts a strategy to counteract against the antiviral effects of PKR, in this way empowering viral mRNA translation to continue in any case of eIFα phosphorylation. PERK was too found to be enacted amid SARS-CoV infection, conceivably through its S and 3a proteins [60]. In another consider, it was illustrated that expression of a dominant-negative PERK mutant, that inhibited PERK kinase activity, suppressed the transcriptional enactment of Grp78 and Grp94 promoters intervened by S proteins of SARS-CoV and HCoV-HKU1 [61]. However, PERK enactment is unlikely to happen in all HCoV strains. In neuronal cells lines infected with HCoV-OC43, it was appeared that eIF2α was only transitorily phosphorylated at the early phase of infection, but was in this way suppressed and returned back to its basal level of phosphorylation, comparative to the mock-infected cells [38]. By the way, the moderate and transient increment in eIF2α phosphorylation was adequate to enact ATF4 protein translation and upregulate the downstream targets of ATF4, ATF3 and GADD153. Knockdown of PKR and PERK in IBV-infected cells attenuated IBV actuated GADD153 upregulation and IBV actuated apoptosis, in spite of the fact that the viral protein replication was unaffected [62]. Upregulation of GADD153 is hypothesized to actuate pro-apoptotic protein TRIB3 and repress pro-survival ERK protein, as well as provide a false feedback to quickly

dephosphorylate eIF2α at late phase of IBV infection. Based on these findings, we hypothesize the HCoVs might use similar mechanism to balance the PKR/PERK/eIF2α pathway in infected cells. More considers could be done on HCoV infection to analyze the enactment of the PKR/PERK/eIF2α pathway at different stages of infection.

4.6. ATF6 Signalling Pathway

Enactment of ATF6 is started by separation from the ER chaperone and BiP. ATF6 then translocate into the Golgi body, where the breakdown of protein done by S1P and S2P. The handled ATF6 then relocates to the nucleus where it turns on expression of genes include an ERSE in their promoters [63]. LikeATF4, ATF6 too actuates expression of ER chaperone proteins such asGRP78, GRP94 and transcription factors CHOP and XBP1. XBP1 is basic for IRE signalling. As GRP94/78 are also target genes of ATF6, and their promoter activities were upgraded by SARS-CoV S protein, one might hypothesize that ATF6 pathway seem to be initiated by SARS-CoV S. Notably, abnormal expression of SARS-CoV S polypeptide did not influence ATF6 promoter luciferase action [64]. 8ab protein, an accessory protein of SARS-CoV, was appeared to dwell in the luminal surface of the ER surface and enact ATF6 via encouraging its proteolysis and translocation of the processed ATF6 into the nucleus [65].

4.7. IRE1 Signalling Pathway

IRE1 was first proposed to be actuated in the same mechanism as PERK, later considers proposed that the NLD of IRE1 can specifically bind unfolded proteins [66, 67]. Enactment of its RNase domain results in improper splicing of a 252-nucleotide intron from HAC1 mRNA in yeasts and a 26-nucleotide intron from XBP1 mRNA in people. Splicing of XBP1 produces an effective transcription factor, XBP1s, that actuates expression of genes related to protein entry into the ER, folding and ERAD [68]. In a negative feedback mechanism, XBP1s too advance the transcription of E3 ubiquitin ligase synoviolin to upgrade IRE1 ubiquitination [69]. The unspliced variation XBP1u consist of a nuclear boycott signal to sequester XBP1s from the nucleus, hence making XBP1u another negative response regulator of XBP1s [70]. In a partitioned mechanism, IRE1 can cleave ER-associated mRNA species through RIDD amid late phase of ER stress. It is accepted that starting XBP1/HAC1 splicing by IRE1 promotes survival but consequent enactment of RIDD upon delayed ER stress leads to cell death, thus permitting IRE1 to play dual role in apoptosis [71, 72]. Another critical enzymatic enactment of IRE1 is its kinase activity. The kinase domain of phosphorylated IRE1 initiates the TRAF2, which then enacts other kinases to eventually enacts the JNK and regulates ER stress-dependent apoptosis [73]. Previous studies have explored the aspect of IRE1-XBP1 pathway amid SARS-CoV infection. Although no increment in XBP1 splicing was noticed in SARS-CoV-infected cells [74], erasure of E protein in recombinant SARS-CoV comes about in noteworthy XBP1 splicing and higher rate of apoptosis [75]. Since IRE1 pathway is closely related to JNK actuation, it is conceivable that the JNK pathway is additionally implicated amid HCoV-OC43 infection.

5. HYPOTHESIS

We hypothesise that in mild cases, resident macrophages starting lung inflammatory responses were able to contain the virus after SARS-CoV-2 infection; both innate and adaptive immune responses were effectively set up to control the viral replication so that the patient would recover quickly. However, in extreme or basic COVID-19 cases, the integrity of the epithelial-endothelial (air-blood) barrier was seriously hindered. In expansion to epithelial cells, SARS-CoV-2 can too attack lung capillary endothelial cells, which leads to a huge amount of plasma component exudate within the alveolar cavity. In reaction to the disease of COVID-19, lung epithelial cells might produce different pro-inflammatory cytokines and chemokines. Upon this change, monocytes and neutrophils were then chemotactic to the infection site to clear these exudates with virus particles and infected cells, resulting in uncontrolled inflammation. In this process, since of the significant decrease and dysfunction of lymphocytes, the adaptive immune response cannot be successfully started. The uncontrolled virus contamination leads to more macrophage invasion and a further worsening of lung injury. In the interim, the direct attack on other organs by dispersed SARS-CoV-2, the immune pathogenesis caused by the systemic cytokine storm and the microcirculation dysfunctions together lead to viral disease. Subsequently, effective antiviral treatment and measures to modulate the innate immune response and recover the adaptive immune response are basic for breaking the vicious cycle and progressing the result of the patients.

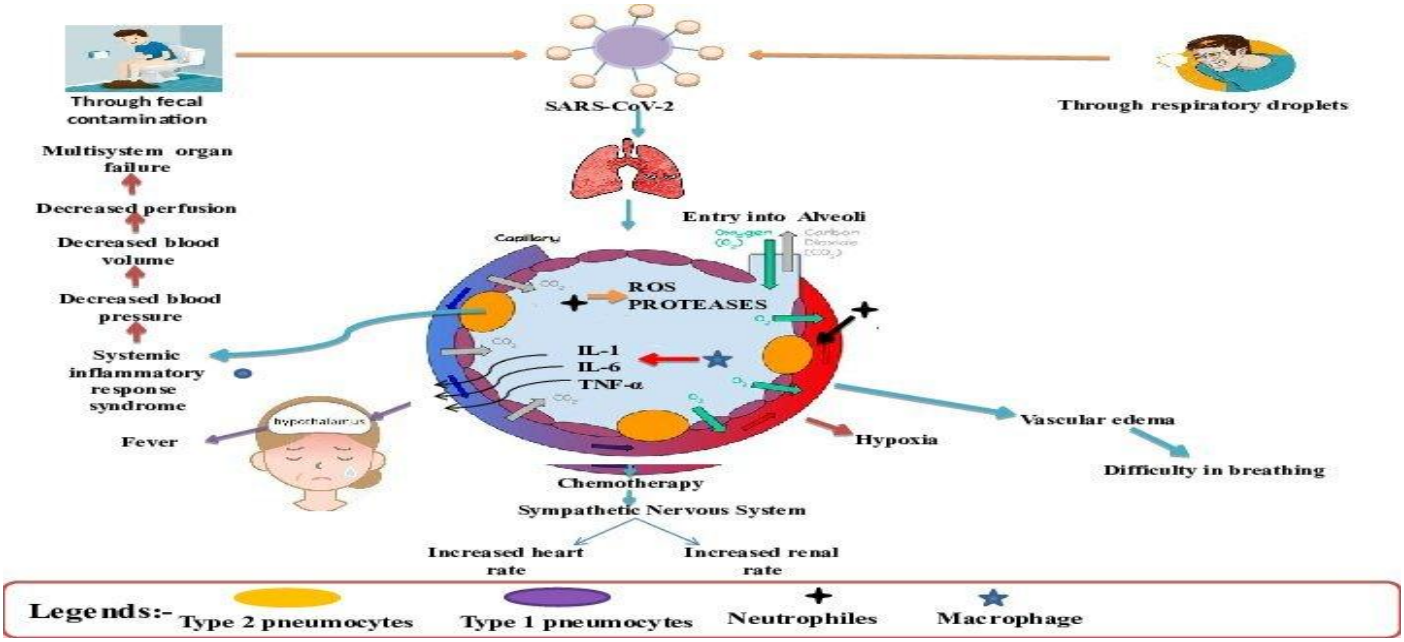

Fig. 6: Occurrence and outcome of COVID-19

6. POTENTIAL OUTCOME

6.1. Blocking Virus–Cell Membrane Fusion

6.1.1. Chloroquine, hydroxychloroquine and analogues

Chloroquine, a well-known anti-malarial medicate, is likely the most well-studied lysosomotropic agent that gathers within the acidic organelles such as endosomes and lysosomes and neutralizes their pH [76]. Chloroquine is known to block virus infection by expanding endosomal pH required for virus/cell fusion, as well as interfering with the glycosylation of cellular receptors of SARS-CoV [77]. Moreover, it was shown to specifically inhibit the replication of SARS-CoV by interfering with the glycosylation of its cellular receptor, ACE2 [78]. Recently, in vitro testing revealed its ability to effectively reduce the viral copy number of SARS-CoV-2 [79]. Therefore, a number of clinical trials have been rapidly conducted in China, which demonstrated that hydroxychloroquine was to various degrees effective in treatment of COVID-19 related pneumonia.

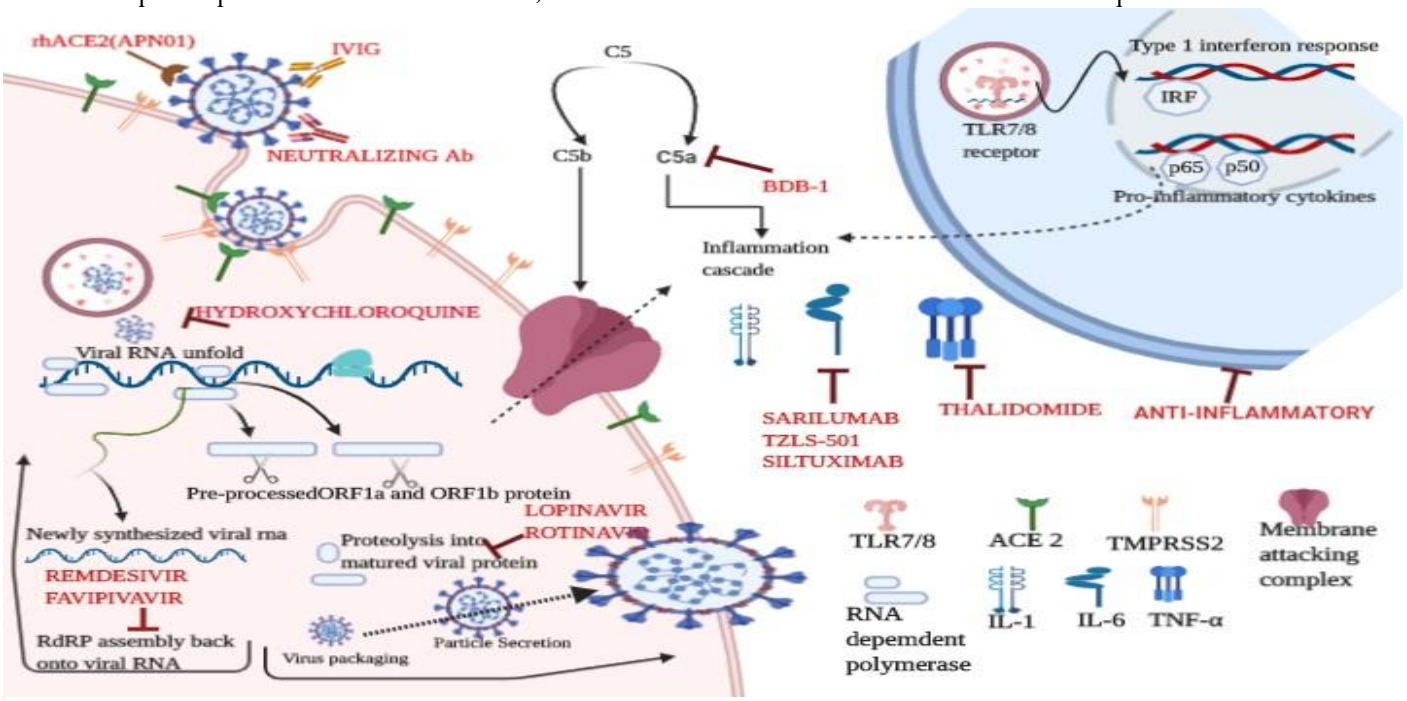

Fig. 7: Overview of therapeutic drugs undergoes clinical trial against COVID-19 in the context of host pathways and virus replication mechanisms

6.1.2. Umifenovir

Approved by Russia and China, Umifenovir is an entry inhibitor against influenza viruses and Umifenovir. Targeting hemagglutinin (HA), the major glycoprotein on the surface of influenza virus, arbidol prevents the fusion of the viral membrane with the endosome after endocytosis. Currently, it is undergoing trials as a single agent (NCT04260594, NCT04255017).

6.1.3. Recombinant Human Angiotensin-converting Enzyme 2 (APN01)

The soluble recombinant human Angiotensin-converting Enzyme 2 (rhACE2) is expected to block the entry of SARS-CoV-2 by blocking the S protein from interacting with the cellular ACE2. Indeed, in a recent study, it was reported that rhACE2 could inhibit SARS-CoV-2 replication in cellular and embryonic stem cell-derived organoids by a factor 1,000-5,000 times [80] . It is believed that the administration of the recombinant human Angiotensin-converting Enzyme 2 (rhACE2) can decrease serum level of angiotensin II by directing the substrate away from the related enzyme, ACE. This could prevent further activation of ACE2 receptor and thereby preserve the pulmonary vascular integrity and prevent ARDS [81]. APN01, originally developed by Apeiron Biologics, has already undergone phase II trial for ARDS.

6.1.4. Teicoplanin

Teicoplanin, a glycopeptide antibiotic routinely used within the clinic to treat bacterial disease with low toxicity, had been previously reported to significantly inhibit the entry of cells by Ebola infection, SARS-CoV and MERS-CoV, via specific inhibition of the activity of cathepsin L. The efficacy of teicoplanin against SARS-CoV-2 infection was recently tested: teicoplanin was found to potently prevent the entrance of S-HIV lucpseudoviruses into the cytoplasm, with an IC50 of 1.66 μM [82].

6.1.5. EK1

Peptide OC43-HR2P, derived from the HR2 domain of human CoV OC43, has been shown to exhibit broad fusion inhibitory activity against numerous human CoVs. EK1, the optimized form of OC43-HR2P, appeared considerably appeared pan-CoV fusion inhibitory activity and pharmaceutical properties [83]. Crystal structures demonstrated that EK1 can form a steady six-helix bundle structure with both short α-HCoV and long β-HCoV HR1s, advance supporting the role of HR1 region as a viable pan-CoV target site.

6.1.6. Camostatmesylate

The *in vitro* data [84] suggested that the Japanese drug camostatmesylate, a TMPRSS2 inhibitor, might constitute a treatment option for COVID-19.

6.1.7. Baricitinib

One of the known controllers of endocytosis is the AAK1. Disturbance of AAK1 might hence hinder the entry of the virus into cells and additionally the intracellular assembly of virus particles. A high-affinity AAK1-binding medicate is the JNK inhibitor baricitinib, which too binds the cyclin G-associated kinase, another regulator of endocytosis [85].

6.2. Inhibiting the Viral Protease

6.2.1. Ivermectin

Ivermectin is an FDA-approved anti-parasitic agent which was also proven to exert antiviral activities toward both HIV and dengue virus. It can dissociate the preformed IMPα/β1 heterodimer, which is responsible for nuclear transport of viral protein cargos [86]. As nuclear transport of viral proteins is essential for the replication cycle and inhibition of the host's antiviral response, targeting the nuclear transport process may be a viable therapeutic approach toward RNA viruses [87, 88]. Recently, an in vivo study has proven Ivermectin's capability to reduce viral RNA up to 5,000-fold after 48 h of infection with SARS-CoV 2 [89].

6.2.2. Lopinavir/Ritonavir

Although coronaviruses encode a different enzymatic class of protease, the cysteine protease, theoretical evidence exists that lopinavir and ritonavir also inhibit the corona viral 3CL1pro protease [90, 91]. Lopinavir and ritonavir are used as a combination therapy for the medication and prevention of HIV/AIDS. However, they soon appeared as candidate of choice for COVID-19 therapy. The pharmacological effect of ritonavir and lopinavir on COVID-19 may be primarily due to their inhibitory effect on coronavirus endopeptidase C30, with ritonavir appearing to have stronger efficacy; the inhibitory impact of darunavir on SARS-CoV-2 and its potential pharmacological effect may be basically due to its blocking effect on papain-like viral protease [92]. Lopinavir/ritonavir combination was engaged in a clinical trial against COVID-19 in patients with serious COVID-19 (ChiCTR2000029308), no benefits of

lopinavir/ritonavir beyond standard care were observed [93].

6.3. RNA-dependent RNA polymerase inhibitors

6.3.1. Remdesivir

Remdesivir (GS-5734) is the monophosphoramidate prodrug of the C-adenosine nucleoside analogue GS-441524 [94]. It can incorporate into nascent viral RNA, and further inhibit the RNA-dependent RNA polymerase. This results in premature termination of the viral RNA chain and consequently halts the replication of the viral genome. Remdesivir was initially developed by Gilead Sciences (USA) against the Ebola virus, and has undergone clinical trial during the recent Ebola outbreak in the Democratic Republic of Congo. Although it has not been appeared to be effective against Ebola in this trial, it proved its safety for humans, which allowed it to enter clinical trials immediately in the conditions of COVID-19 emergency [95]. Importantly, it has been previously shown to exhibit antiviral activities against different coronaviruses, including SARS-CoV and MERS-CoV, in vitro and in vivo [96, 97]. In a recent in vitro study, remdesivir was also shown to inhibit SARS-CoV-2 [98].

6.3.2. Ribavirin

While *in vitro* data have not identified ribavirin as a lead candidate, a randomized clinical trial of the drug used in combination with pegylated interferon has been reported in China for COVID-19 [99] (ChiCTR2000029387). Ribavirin was demonstrated for the common medication of COVID-19 in Chinese treatment guidelines, and combination with interferon recommended [100]. However, their clinical safety and efficacy against COVID-19 were not evaluated in China.

6.3.3. Favipiravir

Similar to remdesivir, favipiravir, developed by Toyama Chemical (division of Fujifilm, Japan), functions as an inhibitor of the RNA-dependent RNA polymerase by structurally resembling the endogenous guanine [101]. In March 2020, favipiravir was approved by the National Medical Products Administration of China as the first anti-COVID-19 drug in China, as the clinical trial had demonstrated efficacy with minimal side effects.

6.4. Other candidates targeting Mpro

Determined the crystal structure of the unligandedMpro at 1.75 A resolution and used this structure to direct optimization of a series of alpha-ketoamide inhibitors [102]. The most objective of the optimization efforts was advancement of the pharmacokinetic properties of the compounds. Using a computational technique, based on the synergy of virtual screening, docking and molecular dynamics techniques, recognized lead compounds for the non-covalent inhibition of Mpro of SARS-CoV-2 [103]. Ligands were found to share a common binding pattern with aromatic moieties associated by rotatable bonds in a pseudo-linear arrangement. Molecular dynamics calculations confirmed the stability in the Mpro binding pocket of most potent binder recognised by docking, namely a chlorophenyl-pyridyl-carboxamide derivative. The Mpro arrangement, build up a 3D homology model, and screened it opposite to a therapeutic plant library consist of 32297 possible anti-viral phytochemicals/ traditional Chinese medicinal compounds [104]. These analyses revealed nine hits that may serve as possible anti-SARS-CoV-2 lead molecules for more advancement and drug development to control COVID-19.

6.5. Attenuating the Inflammatory Response

6.5.1. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) have been proven to exert anti-inflammatory function by decreasing pro-inflammatory cytokines and producing paracrine factors to repair tissues. Preclinical proofs has also shown that MSCs are able not only to restore endothelial permeability, but also reduce inflammatory infiltrate [105]. Whereas the immunomodulating impacts of MSCs have been illustrated on avian influenza viruses [106], their role in COVID-19 pneumonia is still under assessment.

6.5.2. Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) has been widely applied in the field of neurology, dermatology and rheumatology. In a dose-dependent manner, IVIG exerts diverse effects on the immune system. Generally, at low doses (0.2-0.4g/kg), IVIG is used as a replacement therapy for antibody deficiencies. While at higher doses (up to 2g/kg), IVIG its immunomodulatory functions, such as suppressing inflammatory cells proliferation, inhibiting phagocytosis and interfering antibody-dependent cytotoxicity [107].

6.5.3. SARS-CoV-2-Specific Neutralizing Antibodies

The humoral immune response mediated by antibodies is vital for preventing viral disease. Therefore, the development of the specific surface epitope-targeting neutralizing antibodies is a more long-term, albeit more

specific approach to target COVID-19 [108]. AbCellera (Canada) and Eli Lilly and Company (USA) are co-developing a functional antibody that could neutralize SARS-CoV-2 in infected patients.

6.5.4. Anti-C5a Monoclonal Antibody

As complement actuation has been illustrated in acute lung injury, C5a, the bioactive molecule cleaved from C5, is responsible for the full development of tissue injury. The role of C5a includes recruitment of neutrophils and T-lymphocytes, and increasing pulmonary vascular permeability [109]. It has also been proved that anti-C5a treatment could reduce lung injury by decreasing vascular leakage and neutrophil influx into the alveolar space.

6.5.5. Blocking the Interleukin-6 Pathway

Specifically, IL-6 is a predictive factor of poor prognosis in patients with ARDS [110]. Recently it has also been reported that the elevated interleukin-6 (IL-6) is strongly associated with the need for mechanical ventilation [111]. The classical pathway of IL-6 signalling occurs through IL-6 receptors, which are expressed on neutrophils, monocytes, macrophages, and other leukocyte populations [112]. Besides binding to the membrane-bound IL-6 receptor, IL-6 can also bind to the soluble form of IL-6 receptor created by proteolytic cleavage of mIL-6R. An elevated level of circulating IL-6 is associated with a faster decline of lung elasticity and more severe bronchoalveolar inflammation. Hence, specific inhibition of IL-6-regulated signalling pathways represents a promising approach to attenuate inflammation-associated damage [113]. Sarilumab is a fully-human monoclonal antibody that inhibits the IL-6 pathway by binding and blocking the IL-6 receptor. An adaptive stage 2/3, randomized, double-blind, placebo-controlled ponder assessing the efficacy and safety of Sarilumab for hospitalized patients with COVID-19 is ongoing in the U.S [114].

6.5.6. Thalidomide

Recently, thalidomide has re-emerged as an antiangiogenic, anti-inflammatory, and anti-fibrotic agent. Through decreasing the synthesis of TNF-alpha, thalidomide has been used as a treatment for multiple inflammatory diseases, such as Crohns disease and Behcets disease [115]. In addition, preclinical studies proved that thalidomide was effective in treating H1N1-infected mice by reducing infiltration of inflammatory cells and the production of pro-inflammatory cytokines [116].

6.5.7. Methylprednisolone

Systemic glucocorticoids are currently contraindicated in SARS-CoV-2 infection, as they may prolong viral clearance. However, it is also known that the underlying pathogenesis of COVID-19 pneumonia is composed of both the direct damage caused by the virus and the excessive immune response from the host.

6.6. Vaccine

The development of vaccine represents a more long-term plan to action to prevent COVID-19 outbreaks in the future. Full-length S or S1 which contains RDB might be considered as a good vaccine antigen as it could induce neutralizing antibodies preventing host cell attachment and infection. The S antigen has been included in different types of vaccines against infections by CoVs [117]. Conserved B cell and T cell epitopes between SARS-CoV and SARS-CoV-2 were also found in the viral nucleocapsid (N) protein [118, 119].

6.6.1. mRNA-1273

In early January 2020, soon after the outbreak of COVID-19 pneumonia, the genome of SARS-CoV-2 has been sequenced. Moderna's mRNA-1273 is a synthetic strand of mRNA that encodes the prefusion-stabilized viral spike protein. After intramuscular injection to human bodies, it is expected to elicit antiviral response specifically toward the spike protein of SARS-CoV-2. Besides, unlike conventional vaccines, which are either made from inactivated pathogen or small subunits of live pathogen, synthesis of the lipid nanoparticle-encapsulated mRNA vaccine does not require the virus. Therefore, it is relatively safe and ready to be tested.

6.6.2. INO-4800

INO-4800 is a DNA vaccine candidate created by Inovio Pharmaceuticals. Like Moderna's mRNA-1273, INO-4800 is also a genetic vaccine that can be delivered to human cells and translated into proteins to elicit immune responses. Compared to conventional vaccines, genetic vaccines require lower costs of production and easier way of purification. The simple structure of nucleic acids also obviates the risk of incorrect folding, which could occur in recombinant protein-based vaccines [105, 120].

6.6.3. ChAdOx1 nCoV-19

This vaccine, created by the University of Oxford, is composed of a non-replicating adenovirus vector and the

genetic sequence of the S protein of SARS-CoV-2, and has entered a phase I/II clinical trial (NCT04324606). The non-replicating nature of adenovirus in the host makes it relatively safe in children and individuals with underlying diseases. Besides, the adenovirus-based vectors are characterized by a broad range of tissue tropism that covers both respiratory and gastrointestinal epithelium, the two main sites that express the ACE-2 receptor of SARS-CoV-2. However, the possibility of dominant immunogenicity toward the vector genes rather than the transgenes should always be considered [97].

6.6.4. Pathogen-Specific Artificial Antigen-Presenting Cells

Based on the knowledge that antigen-specific T cells are able to eradicate cancer cells as well as viral infections, generating large amounts of T cells with viral antigen specificity in a timely manner may well help us withstand the invasion of SARS-CoV-2. Efficient methods to produce enormous amounts of T cells include appropriate antigen-presenting cells that can activate effector T cells, and the differentiation and proliferation of corresponding effector, cytotoxic T cells [79].

7. CONCLUSION

In this article we present an overview of the current state of knowledge on the SARS-CoV-2 or COVID-19. In expansion to an overview of the epidemiology, targets, and hypothesis of COVID-19, we moreover combine possible therapeutic outcomes currently under study and the future point of view for the diseases. We also figure out on several signaling pathways share to the novel COVID-19 pneumonia, including its high transmissibility caused by monotonous ACE2 structure at the viral binding site. We summarize the ongoing clinical trials that have been quickly started upon the onset of the widespread emergency and are currently undergoing as for April 2020. Most of them are based on repurposing the therapeutic agents previously designed for other applications. These agents can be separated into two wide divisions, those that can specifically target the virus replication cycle, and those based on immunotherapy approaches either aimed to boost innate antiviral immune responses or alleviate damage actuated by dysregulated inflammatory responses. Whereas the immunization and therapeutic antibodies pointed to particularly target SARS-CoV-2 are moreover being tested, this solution is more long-term, as they want overall testing of their safety. We admit this may be a stimulus for more systematic way to ready ourselves in advance for any potential future pandemics.

8. REFERENCES

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