SciencePG
Science Publishing Group

Review Article

An Insight into ACE2 Expression Associated Complexities of COVID-19 and the Possible Vaccine Strategies to Control Viral Entry into Host Cells

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Abstract: The first detected COVID-19 virus in China in late December 2019, has now spread to over 200 countries and territories across the globe. As it is a novel virus, in course of time, new symptoms and new complexities in patients have arisen, which demand immediate attention. This ambiguous, fast mutating nature and strain variations of SARS CoV2 have hindered the way of vaccine development. However, recognizing this new virus as a member of coronavidae family and its similarity with previously prevailed SARS-Cov virus has advantages in order for its further characterization and identification of the route of entry. Hence, this review aims at providing an overview of the viral entry pathway and explaining the reasons behind the vulnerability of an individual based on his/her age, sex, weight, other existing diseases and genetic make-up. It will also try to explicate various newly emerged symptoms of COVID-19 from the perspective of cytokinin storm theory. Furthermore, it summarizes all the therapeutic strategies based on preventing virus entry, some of which are already developed or under trial or yet confined in theory using currently published literature, scientific reports and research articles about SARS-CoV2. This review will help better understand the COVID-19 complicacies with latest concepts on therapeutic strategies against SARS-CoV2.

Keywords: SARS-CoV, COVID-19, ACE2, Viral Entry Pathway, Vaccine Strategy

1. Introduction

Since the dawn of human civilization, human race has faced numerous recurrent attacks by pathogens and diseases, some of which have been completely eliminated, however, some are still persisting. From the earliest recorded history of the pandemic in 430 B.C, the world has encountered Justinian plague, the Black Death, Cholera pandemic, Spanish flu and so on. Among them, various members of the Coronaviridae family frequently appeared in the human population and they usually caused mild respiratory diseases [1]. The emergence of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), a member of this family was first reported in 2002, in Guangdong province, China, and subsequently, twenty-six countries were affected. Approximately 8,096 cases were reported worldwide, and it causes 774 deaths (~10% case mortality rate). WHO reported that, the last known human to human transmission thread was cut off in July, 2003. Several predictable methods like travel restrictions and separating patient, home isolation was seemed to work very well in that period [1].

Again in 2012, another member of the Coronaviridae family, known as the Middle East respiratory syndrome coronavirus (MERS-CoV) was first spotted in Middle

Eastern country, Saudi Arabia. Since 2012, 27 countries have been affected by MERS, and about 2,500 cases have been reported by January 2020 with ~35% mortality rate [2].

In December 2019, the world encountered the appearance of a new infectious respiratory disease in Wuhan, Hubei province, China [1-4]. In February 2020, WHO named the disease as coronavirus disease 2019 (COVID-19) and in March 2020 declared it as a pandemic [4]. As reported by World meter [4] (https://www.worldometers.info/coronavirus/?utm_campaign =homeAdUOA?Si), the no of death to date is 2,412,826.

During the period of SARS-CoV, due to the nature of rapid transmission and severity, scientists put great effort to identify the characterization and mechanism of transmission of the virus. Within a very short time, it was being identified as a positive-stranded RNA virus of the coronavirus family with projected spikes on the envelope and its genome was also sequenced [5, 6]. Several studies had been conducted during that time in order to identify the receptor for SARS-Cov, which finally revealed receptor-angiotensin converting enzyme II (ACE2) as a functional receptor for the SARS virus and helped to better understand the mechanisms of SARS-CoV entry within host cell [7, 8].

After 18 years, in 2019, when the new infectious virus arises, now known as COVID-19, scientists find out along with 76.5% similarities in amino acid sequences of this newly identified virus and previously identified SARS-CoV, that they also have resemblance with homologous structures of their spike proteins [9-11]. This illustration was very crucial as it leads the researchers to predict a strong binding affinity between the spike proteins of SARS COV-2 and ACE-2. To verify this prediction, Zhou et al., carried on a study using both HeLa cells producing and not producing ACE2 proteins from not only humans and mice but also other animals including Chinese horseshoe bats and pigs to observe the virus infectivity [12]. They showed that COVID-19 used ACE2 proteins as an entry receptor to enter into cells expressing ACE2, except mouse ACE-2. Thus, they confirmed that the newly emerged COVID-19 uses the same ACE2 for their cellular entry as SARS-CoV did. Furthermore, SARS-CoV-2 was not found to utilize aminopeptidase N (APN) or dipeptidyl peptidase 4 (DPP4) as receptor for invasion; those are used by other coronaviruses [12].

These studies have opened up a new horizon to the scientists. Now several studies and trials are going on to discover the most efficient drug or vaccine to prevent the viral entry in the human body. This review aspires present the overall function, characterization of ACE-2 and will try to explain the causes of different complexities arisen into Covid-19 patients. It will also emphasize on various therapeutic strategies including some drugs that are already available in the market, some are under trial while some are being considered in new theoretical approaches.

2. Function and Characteristics of ACE2

The Renin-Angiotensin-Aldosterone System (RAAS) is a multi-organ endocrine system for blood pressure and fluid, electrolyte level regulation, which comprised of the renin, angiotensin II and aldosterone hormones. In the first stage of the RAAS, reduced blood pressure activates renal juxtaglomerular apparatus to cleave secreted prorenin to convert it into Renin. Renin acts on, Angiotensinogen protein, which is continuously produced in the liver to form angiotensin I. Upon cleaving off two amino acids Angiotensin I is further converted to angiotensin II. This reaction is catalyzed by an enzyme angiotensin converting enzyme (ACE), present in vascular endothelium, particularly of the lungs [13]. Receptors of Angiotensin II are present throughout the body. It can exert it's function by binding to G-protein coupled receptors, namely the AT1 and AT2 receptors, among them AT1 receptor participate in most of the activities. Major functions of Angiotensin II include vasoconstriction, potassium excretion and renal sodium reabsorption, elevation of blood pressure, aldosterone synthesis, and induction of inflammatory pathway and activation of the pro-fibrotic system [14, 15].

On the other hand, ACE2 is an 805 amino acid long zinc metallopeptidase. ACE2 gene is 40 kb in length, located on chromosome Xp22. Even though ACE is positioned at a different location, chromosome 17 but ACE and ACE2 share similarities of 18 exons and 40% identical sequence [16].

ACE2 converts Angiotensin II to angiotensin (1-7), that function in vasodilating, anti-inflammatory and anti-fibrotic effects through mas oncogene product (MAS) receptor. Furthermore, ACE2 cleaves Angiotensin I into angiotensin (1-9), and then ACE cleaves angiotensin (1-9) into angiotensin (1-7). Hence, ACE2 not only decreases Ang II concentrations but also increases Ang (1-7), which ultimately, offsets the effects of Ang II [17].

Thus, the consequences of RAAS activation depend on the balance between ACE and ACE2, and therefore, coordinate between vasoconstriction, pro-inflammatory; and vasodilating, anti-inflammatory pathways [18].

ACE2 is predominantly expressed in the cell membrane of myocytes and endothelium of vessels of gallbladder, stomach, colon, small intestine, heart, kidney, and testis. Abnormal expression of ACE or ACE2 is linked with progression of several diseases, for instance, ACE2 deficiency results in systolic hypertension, along with hyper stimulation of RAS which further causes cardiac injury and lung injury. In contrast, renal diseases display increased ACE2 expression and ACE2 deficiency could deteriorate the Acute respiratory distress syndrome (ARDS) [15, 19].

3. ACE2 as a Gateway to SARS

Several members of the Coronaviridae family have been found circulating in the human population and primarily shows flu-like symptoms- fever, cough, mild respiratory distress [20]. All the Coronaviruses are a member of the Coronaviridae family, order Nidovirales. Upon analyzing the phylogenic association and genomic structure, they are divided into Alphacoronavirus, Betacoronavirus,

Gammacoronavirus and Deltacoronavirus genera. They have differences in their target host, specifically, the gammacoronaviruses and deltacoronaviruses mainly infect birds, but can infect mammals as well [21]. On the contrary, Alphacoronaviruses and betacoronaviruses can cause severe disease in animals, for example, porcine enteric diarrhoea virus (PEDV), swine acute diarrhoea syndrome coronavirus (SADS-CoV) etc. Along with that, they can cause both severe respiratory distress (SARS-CoV, MERS-CoV and SARS-CoV2) and mild respiratory disease (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1) in humans [22].

The trimetric S-protein of coronaviruses is a crown-shaped class I viral membrane fusion protein that sits throughout the surface of all CoVs and projects outwards to give a 'corona' (a part of the body resembling or likened to a crown)-like appearance to the virus. The virus uses these spikes to initiate primary high-affinity binding to the appropriate receptor on the surface of target cells. The ectodomain comprised of three S1 subunit heads, which participate in host receptor-binding and a trimeric S2 subunit stalk, that fuses viral and host membranes and facilitate viral genomes to invade into the host cells [19]. S1 subunit has two receptor-binding domains (RBDs), the N-terminal domain mainly binds sugar receptors, while the C-terminal domain appears to bind protein receptors (e.g., APN, ACE2, and DDP4) [10, 23].

Despite having non-identical RBDs, SARS-CoV, SARS-CoV-2 and HCoV-NL63 have been seen to enter into a human through ACE2 [19]. Their slightly different binding pattern suggests that HCoVNL63 and SARSCoV may have evolutionary acquired the ability to bind the same ACE2 receptor through, three discontinuous beta-loops and continuous subdomain, respectively [24]. Again in spite of having 76.5% identical amino acid sequences SARS-CoV-2 spike glycoprotein illustrate 10- to 20-fold higher affinity toward ACE2 receptor than that of SARS-CoV [25] and indicating that SARS-CoV-2 is highly contagious compared to other CoVs.

After binding the host cell, the intact ACE2 or its transmembrane domain together with the virus enter into endosomes, and subsequently, it fuses with lysosomal membranes for complete internalization [26, 27]. The membrane fusion process is facilitated by some transmembrane proteinases. For example, ADAM17 (a disintegrin and metallopeptidase domain 17), TMPRSS2 (transmembrane protease serine 2), and Tumor Necrosis Factor Alpha converting enzyme and some other proteins such as vimentin and clathrin [28]. Among these TMPRSS2 plays an indispensable role in the process of SARS-CoV-2 invasion, it cleaves and activate the viral spike-domain (S) by cleaving at the S1/S2 sites, thus expedite the process [1]. Interestingly, it is not that, this protein has come to light due to its role in SARS CoV2 entry, in 2020; but it was previously accounted for the entry of SARS-CoV in 2003 and entry of influenza H1N1 virus during influenza pandemics of 2009 and 2018 [29, 30].

4. Risk Factors Associated with COVID-19 Severity and ACE2 Expression

4.1. Genetic Factors

The ACE2 gene shows a number of polymorphisms, those have been found to correlate with susceptibility of hypertension [31, 32]. Moreover, ACE2 polymorphism is linked to stroke, diabetes mellitus, ventricular hypertrophy, and coronary artery disease in Asian populations [32]. The polymorphic variation of ACE2 gene expression and its relation to SARS-CoV-2 is not completely revealed yet. However, in vitro studies have showed an increase in infection rate with an increase in ACE2 expression [23], so it can be assumed that the expression pattern or variants may have a definite association with the severity of COVID-19. To investigate the epidemiological variation of ACE2 gene variants and its association with SARS CoV infection, Cao and his coworkers studied the expression quantitative trait loci (eQTLs) of ACE2 [33]. They selected 15 eQTL variants and further studied their allelic frequency in East Asian and European population. Their study finds a moderate variation of ACE2 expression in different population [33] and remarkably, they revealed a positive correlation between the presence of higher allelic frequency and elevated tissue expression of ACE2 in East Asian population, but no ACE2 mutant was found to resist viral binding to host cell [33]. As various studies and reviews have not identified any specific relation between ACE2 SNP (Single Nucleotide Polymorphism) and severity of COVID 19 in various ethnic groups yet, so further screening is needed [34].

4.2. Age

During ageing, immune system changes and elderly people develop immunosenescence and inflammaging, which are the major reasons for high mortality rates in old aged people [31]. Progressively loss of neutrophil mobility to migration at sites of infection to kill infected cells with aging may act as another reason [32].

Both in the process of ageing or DNA damage; several nuclear proteins, histone deacetylase SIRT1 and chromatin modifiers move to the site of genomic instability to promote DNA repair and again back to their location [33]. These relocalization cause aggregation of epigenetic changes, which may collaborate to deteriorate, immune cell function and overall cellular identity [33].

Advancing age changes in DNA methylation profiling of the transcriptional regulation site of ACE2 [35], it could explain why complications related to ACE2 expression more aggressively arise in older people. In elder patients with COVID-19, a sharp decline of NAD+ level is found [36]. This phenomenon slows down the activation of SIRT1, which subsequently hamper downregulation of cytokine production and chronic inflammation [36]. Thus, exacerbate the complexity of COVID-19 patients.

4.3. Sex

At the begging of the pandemic, it was quite visible that the older men had higher rates of morbidity and mortality due to COVID-19 compared to women [37]. Later on, several attributing factors were identified behind this male predominance in disease susceptibility. As ACE2 is established as the main gateway of SARS CoV virus entry, so some scientists indicate that increased expression of ACE2 might be a reason behind this susceptibility [38]. However, some other studies showed no significant association between gender and ACE2 expression, thus making this indication invalid [39]. As previously described, TMPRSS2 assist in the process of viral entry. Previous study by Lin B. *et al*. showed the positioning of some androgen receptor elements (ARE) in the transcription start site of the TMPRSS2 gene [40]. Thus, the androgen receptor promotes TMPRSS2 gene expression, and it increases upon exposure to androgens [41]. Higher level of androgen in man can explicate why men are more vulnerable to get infected by SARS CoV in comparison to woman and children who have comparatively less expression of the androgen receptor [42]. However, a recent study of Stopsack *et al*. showed that men and women do not show significant difference in TMPRSS2 expression in lung tissue and the amount of androgen expressed in women is enough to not make any distinction in TMPRSS2 expression of lungs [29, 43].

4.4. Obesity

Obesity is another pivotal independent risk factor behind the severity of COVID-19. The role of obesity as a discrete risk factor becomes apparent, when a study was undertaken COVID-19 patients under 60 years old, revealed that Obese class I patients with (BMI 30 - 34 kg/m^2) and patients at the highest margin of Obese class I >35 kg/m^2 tend to be 1.8 and 3.6 folds more likely to get admitted in hospital with a critical condition, respectively [44]. However, patients, those were overweight but not obese (BMI <30 kg/m^2) showed a lower frequency in comparison to the above-mentioned groups [44]. Previous researches showed that obesity is associated with the increased presence of the anti-inflammatory circulating CD4+ T and T-regulatory cells, indicating a pro-inflammatory state with reduced ability to combat the infection, that was needed to control the viral spread [45]. Due to enhanced macrophage infiltration into adipose tissue, immune cells, e.g. Monocytes, macrophages and T-lymphocytes produce an increasing amount of cytokines and chemokines [46]. In obese people, adipose tissues secrete, TNFa, IL-6, TGF- ß (transforming growth factor b), or acute phase proteins, thus increases the overall accumulation of inflammatory cytokines [46-48]. This excessive production of pro-inflammatory cytokine is a prominent characteristic of ARDS [49]. On the other hand, leptin (adipokines) from adipose tissue drives these pro-inflammatory effects and leptin availability is positively correlated to the elevated Ang II level and decrease in ACE2 expression and activity [50, 51].

4.5. Comorbidity

SARS-CoV-2 have been evident to infect people from all sex, age group and races. However, people with comorbidities, for example, chronic respiratory distress, diabetes, malignancy, hypertension and cardiovascular diseases are found to be at increased risk of developing severe and critical forms of COVID-19 than healthy individuals [52].

With the surge of COVID-19, scientists have evident that, this virus is threatening particularly for the patients with heart disease and related detrimental health conditions, especially hypertension. Although previously, it was unambiguous if hypertension act as a risk factor for COVID-19 or not. However, newly conducted several studies and emerging data suggested that patients with elevated blood pressure have a two-fold higher risk of dying from the COVID-19 compared to patients with normal blood pressure [33]. A study with available data, in Italy showed 509 of 1043 COVID-19 patients had hypertension [53]. As ageing and age-related weak immune system induce high blood pressure, so studies have reported that hypertension increased the odds ratio (OR) of death in COVID-19 patients and it is more frequent in older people. Furthermore, studies found that older people have a higher mortality rate than younger people [54]. High blood pressure can further elevate the complicacy by damaging the arteries and prompt atherosclerotic plaques breaking thus, leading to serious complications like heart failure, heart attack and stroke [55].

Cardiovascular diseases are considered as a predominant risk factor of COVID-19, myocardial injury is found to be related to myocarditis and/or ischaemia [56]. A study by Monteil V. et al., focusing on human recombinant soluble ACE2 (hrsACE2) mediated therapeutic strategy, showed that SARS-CoV-2 can directly infect engineered human blood vessel organoids [57]. Other proposed mechanisms include a cytokine storm triggered by an imbalanced response of type 1 and type 2 T-helper cells, sympathetic hyperactivity, low oxygen supply due to lungs injury and hypoxemic myocardial cell damage caused by respiratory dysfunction [58]. Myocarditis is another potential cardiovascular manifestation of COVID-19. Binding of SARS-CoV-2 to the ACE2 receptors present on myocardium causes down-regulation of the ACE2 receptors, resulting in unimpeded angiotensin II accumulation, which further leads to adverse myocardial remodelling [59]. A plausible mechanism can be, migration of CD8+ T lymphocyte to the cardiomyocytes and causing myocardial inflammation. Proinflammatory cytokines accelerate lymphocyte activation and promote further cytokine production, thus causes a positive feedback loop of immune activation and tissue inflammation [60].

COVID-19 patients may have increased risk of developing venous thromboembolism (VTE) [58, 61]. Virus induced acute inflammatory response and infection-mediated vasculitis may trigger this hypercoagulation process. In addition to it, depletion of the coagulation and fibrinolytic

system by IL-6 and tumor necrosis factor-a leads to both bleeding and thrombosis, thus further worsen the situation [62]. Some critically ill COVID-19 hospitalized patients are found to have such altered coagulation patterns with remarkable elevation in D-dimmer and fibrin degradation products in the blood [63]. Significance of elevated D-dimer levels (> 1 g/L) as an independent predictor of in-hospital death became more apparent from a study of China, where 71.4% novel coronavirus pneumonia (NCP) patients with disseminated intravascular coagulation did not survive [58, 63].

An epidemiological study in China, conducted on 72,314 patients with COVID-19 showed that diabetic patients have three times higher mortality rate than other COVID-19 patients [64]. There are several mechanisms that act to increase this severity rate. When SARS CoV2 bind to the ACE-2 receptor, it activates a proprotein convertase Furin, which is a membrane-bound protease expressed abundantly in diabetic patients. This protein facilitates the entry of the virus into host cells by reducing viral dependency on other protease enzymes [65]. Moreover, in vitro study revealed that SARS-CoV can even attack and damage pancreatic islands through their entry by ACE-2 receptor, causing a deficiency in insulin production [66]. This can probably cause hyperglycemia in patients with SARS-CoV and aggravate the situation of diabetic patients.

5. Bradykinin Storm- a Possible Explanation of the Clinical Symptoms of COVID-19

The COVID-19 patients show an uncontrolled inflammatory response, with the presence of a cytokine storm, which was often thought to be the result of overactivation of the lymphoid cell, but later on, it was found to be triggered by, the activation of the kallikrein - bradykinin system (KKS) and RAS [67]. Bradykinin (BK) is a protease of KKS that act as a part of inflammatory responses and induce pain after tissue injury through the induction of a BK receptor, B1 receptor. Upon activation of B1 receptors, increases calcium entry into the cell leads to elevation of intracellular free calcium level [67]. B1 receptor functions in plasma extravasation, arachidonic acid release and prostaglandin (PG) synthesis, white blood cell activation and accumulation, activation of phospholipases C and phospholipases A2 and in control of blood pressure [68, 69]. One of the enzymes of KKS is ACE, that increases available angiotensin II causes vasoconstriction and induce inflammatory cytokines production and degrade available bradykinin [15, 70].

In COVID-19, most of the enzymes that degrade BK are downregulated. Remarkably, ACE production lessens by 8-fold, in contrary, the expression of ACE2 increases by 200-fold. This imbalance leads to the overproduction of bradykinin and directing BK1-9 and BK1-8 to upregulate receptors BKB2R (207-fold) and BKB1R (2945-fold)

respectively [71]. In severe cases, the body cannot stop producing bradykinin. This phenomenon is called a "bradykinin storm".

This theory gives a clarification for the broad spectrum of COVID-19 symptoms. Excess bradykinin widens the gaps in blood vessels, which cause fluid leaking, these fluids begin to infuse into alveoli. Hence, patients have trouble breathing. When fluid leaks out from the blood vessels in the brain, patients may experience some neurological symptoms like dizziness, headaches, fogginess, and confusion. Bradykinin storm can also trigger swelling, pain, and inflammation in the body, which can result in some common COVID-19 symptoms like muscle soreness and body aches [71].

In most of our connective tissues, there is a polysaccharide present named hyaluronic acid (HA), which can hold thousand times more water than its own weight. HA is produced by hyaluronan synthesis enzyme. It is an integral membrane protein encoded by HAS1, HAS2 and HAS3 genes [72]. On the other hand, HYAL1 encodes a lysosomal hyaluronidase (Hyal-1) that degrade HA intracellularly [73] and HYAL2 gene encodes hyaluronidase (Hyal-2) enzyme, which breakdown HA extracellularly. Both of the degrading enzymes depend on CD44, a HA receptor for their function [73]. In COVID-19, HA synthesizing genes are upregulated: HAS1 (9113-fold), HAS2 (493-fold), and HAS3 (32-fold) and proteins responsible for HA degradation are downregulated: CD44 (11-fold) and HYAL2 (5-fold) [71]. This increased expression of HA in the bronchoalveolar space of the lungs can form a viscous hydrogel. When the level of HA production goes beyond the tipping point, it could significantly hamper gas exchange and cause sudden respiratory distress [71].

6. Proposed Therapeutic Strategies to Control Viral Entry

6.1. Targeting Serine Protease TMPRSS2

SARS-CoV-2 uses serine protease TMPRSS2 for S protein priming and entering into host cells. In this process, virus uses another protease, namely, cathepsin B and L (Cat B/L) as well. This endosomal cysteine proteases aid, but not pivotal to the entry process. Therefore, inhibiting both proteases may block viral entry. So, blocking TMPRSS2 can act as a potential therapeutic target for SARS-CoV2. To better understand the contribution of these proteases, Hoffmann et al., employ ammonium chloride on 293T cells (TMPRSS2) and Caco-2 cells (TMPRSS2+). Application of this water-soluble salt rises endosomal pH and subsequently blocked CatB/L activity. Ammonium chloride treatment more efficiently prevents SARS-2-S- and SARS-S-mediated entry into TMPRSS2- 293T cells, compared to TMPRSS2+ Caco-2 cells, suggesting CatB/L dependence [1]. Clinical studies have shown that TMPRSS2 inhibitor camostat mesylate, partially prevents SARS-2-S-mediated entry into Caco-2 and Vero (African green monkey, kidney) cells -TMPRSS2 cells [1, 74]. This process was completely inhibited when

TMPRSS2 inhibitor (camostat mesylate) and inhibitors of CatB/L (E-64d) were applied together [1].

6.2. Androgen Deprivation Therapy (ADT)

A study on 9280 COVID-19 patients in Veneto, Italy showed that males developed more severe complications (men 60% and women 40%), and have a higher death percentage (men 62% and women 38%). Among the studied sample, there were 4532 males (44%) and 118 (1.3%) were males with prostate cancer. It was observed that cancer patients receiving androgen deprivation therapy (ADT) are less likely to get infected by SARS-CoV-2 (odds ratio: 4.05; 95% CI: 1.55–10.59) [75]. This scenario can be explained by the fact that regulation of TMPRSS2 transcription is partially dependent on androgen receptor (AR). Androgen administration promotes TMPRSS2 expression in both murine and human lung epithelial cells and androgen deprivation exerts the opposite effect and results in a reduced rate of viral entry [27, 76]. This study by Montopoli et al., indicates that AR inhibitor or luteinizing hormone-releasing hormone (LHRH) agonist/ antagonists can be used not only for prostate cancer patients but also in prevention or treatment of COVID-19. However, a more recent study using data from the OAK and POPLAR trials in the Netherlands, suggests that ADT may help to reduce the severity in patients already infected with SARS CoV-2, but it is not at all a feasible treatment option for COVID-19 [77].

6.3. Blocking the ACE2 Receptor

It has been established that ACE2 receptor and some proteases (TMPRSS2) plays the major role in the entry of SARS-CoVs into host cells. This leads the researchers to discover drugs or organic components that can interfere with viral entry by blocking the ACE2 receptor to some extent. A list of such compounds is summarized in table 1.

6.4. Vaccine Targeting Spike Protein

As previously described, the trimeric S protein plays essential roles in virus pathogenesis. A receptor-binding domain of S1-protein is necessary for binding to host ACE2. Analysis of a fully recovered SARS patient revealed that their T cell-induced immune response specifically against the S2 domain, suggesting that a coronavirus vaccine can be designed to target this viral antigen [73].

A study of Chen, J. finds the presence of neutralizing antibodies against the S protein of SARS-CoV S, none of the other structural proteins including membrane (M), envelope (E), nucleocapsid (N) induces such effect [74]. In 2004, in order to discover a vaccine against SARS, a single circular plasmid DNA was designed which composed of a full cDNA sequence of SARS glycoprotein but with a deletion of last 13 carboxy-terminal amino acids. In phase I clinical trial the vaccine, VRC-SRSDNA015-00-VP was evaluated as safe. The primary responses elicited by CD4+ T cells and minor response of spike glycoprotein -specific CD8+ T-cells was also detected in the study samples. In another study, where truncated SARS S DNA vaccine along with CD8+ T cell-specific epitope N50 and CD4+ T cell-specific epitope N60 was used in BALB/c mouse model revealed its ability to significantly promote CD4+ and CD8+ T cell-mediated immune response [75, 78].

More specifically, 270 to 510 amino acids of SARS-CoV S glycoprotein representing RBD [76] or other several immunodominant sites of SARS-CoV S-protein could be chosen as a candidate for designing vaccine rather than using full-length as it may induce unwanted immune responses resulting in inflammatory and liver damage in the animal [78]. A new "ChAdOx1 vaccine" has been developed by the University of Oxford, targeting viral spike protein. They used attenuated chimpanzee adenovirus and modified it to express the SARS CoV spike protein [77].

Table 1. List of drugs/ organic compounds interfering ACE2 mediated SARS-Cov entry.

Name of the drug/ organic component	Source	Common use	Mechanism of working against Cov-19
Hydroxycholoroquine	Synthetic	Treatment of malaria	Prevent viral entry: hinder glycosylation of the host receptor, thus inhibits virus attachment to the active site of the host receptor [4, 79].
Umifenovir	Synthetic	Treatment and prophylaxis of influenza	Prevent viral entry: Interfere between viral Spike protein and host ACE2 [4]. Inhibit TMPRSS2:
Camostatmesylate,	synthetic	treatment of pancreatitis	Especially used in COVID-19 patients with dyslipidemia or hyperglycemia cell [4, 80].
Organosulfur compounds	Garlic essential oil	---	Blocks viral entry: impede interaction between host ACE2 and virus protease [4, 81].
Hesperidin, a bioflavonoid, plant pigment	Unripe citrus fruits eg. -Oranges, lemon.	---	Prevent viral entry: Lie within the connecting surface of virus RBD and host ACE2 [82].

During trial, it showed to induce T cell response within 14 days, and the presence of antibody was found within 28 days after administration [77]. The vaccine named, "Sputnik V" have been developed in Russia using similar strategy, but it involves two different types of human adenovirus, which will ensure efficient immunity-boosting after consecutive repeated vaccination within 21 days [78]. These vaccines have got market approval and many countries have started administrating these to their citizens. Another vaccine candidate against coronavirus is the Johnson & Johnson vaccine (JNJ-78436735) known as *Ad26.COV2.S* also employed the strategy to use adenovirus serotype 26 with added gene expressing SARS-CoV-2 spike protein [83, 84]. In addition to it, a new vaccine, introduced by Beijing Institute of Biological Products named *BBIBP-CorV* has recently completed clinical trial phase III. They used

coronaviruses expressing spike proteins, hence, killed using beta-propiolactone, as the vector. Their published data reported that this inactivate vector vaccine is less efficient than the above-mentioned vaccines but the storage condition is more favorable and more convenient for transportation and storage [85].

6.5. miRNA Mediated Downregulation of ACE2 / TMPRSS2 Expression

In the course of finding some molecules that can negatively regulate the expression of ACE2 /TMPRSS2 and subsequently impede SARS-CoV2 entry into cells, MicroRNA (miRNA) can be a good option to explore. These miRNAs are non-coding, small RNAs that bind to the target mRNA at the 3' UTR and afterwards, leads to degradation or translational downregulation of the target [86]. Applying this approach Nersisyan S, et al., found that KDM5B gene encoded lysine-specific demethylase 5B (JARID1B), can repress transcription of "hsa-let-7e / hsa-mir-125a and hsa-mir-141 / hsa-miR-200 miRNA families" [87] thus indirectly affect ACE2 / TMPRSS2 expression and hamper virus entry into the host cell. By further exploiting their findings, such miRNAs can be undertaken for trials.

6.6. Administrating free Circulating ACE-2

As we have already discussed, ACE-2 plays a crucial role for SARS-CoV-2 to enter into a cell. Administrating free soluble circulating forms of ACE2 receptor may bind with SARSCoV-2 and prevent the virus from binding to ACE2 receptors present on the cell membrane and will block viral entry within the cells. Monteil and his group have already constructed a Recombinant human angiotensin-converting enzyme 2 (rhACE2) named, APN01 for this purpose and it worked successfully to retain viral entry into cells [57]. This study leads to the discovery of the drug RhACE2 APN01 (https://clinicaltrials.gov/ct2/show/NCT04335136#moreinfo) which is now in phase 2 clinical trial by the European biotech company Apeiron Biologics [88]. Another clinical trial is going on to test if a combination drug, namely, "Recombinant Bacterial ACE2 receptors -like enzyme B38-CAP (rbACE2) plus Aerosolized 13 cis retinoic acid" (https://clinicaltrials.gov/ct2/show/NCT04382950) will work better than RhACE2. Both of these trials were supposed to finish by the end of, 2020.

6.7. Inhibition of Histone Deacetylase 2 (HDAC2)

In order to show how the ACE-2 expression is modified in lung tissue, a study by Pinto et al. identified the genes related to ACE-2 regulation [89, 90]. They find several gene expression to be associated with histone modification. In this process of epigenetic modification Demethylation (KDM5B; demethylates lysine 4 of histone H3), histone acetylation (H3K27ac) and histone methylation (H3K4me1 and H3K4me3) were found to be more prominent in this context. [90]. Again, a study of Japan, Takahashi and his co-workers illustrate that deacetylase inhibitors (HDACIs) are able to

suppress AcE-2 expression in both in vitro and in cultured epithelial cell lines. Their observation puts a light on the use of HDACIs as a drug against COVID-19. Furthermore, they had clinically showed that use of Panobinostat, an HDACIs successfully reduced the amount of ACE-2 protein production, indicating its potentiality to use as a drug to prevent SARS-Covs [91].

6.8. Gene Therapy-based Strategy

Previously, gene therapy-based approaches had shown success in treating HIV-1, similarly this strategy can be a potential blueprint to combat SARS-CoV. The basic strategy could be administrating viral protein (example; spike protein of CoV) encoding DNA/RNA into the host body. Upon protein synthesis within the cell, host's immune system boosts up and produces antibody against the mimic protein and creates immunological memory. If successfully developed theses DNA/RNA vaccines may act as the most affordable form of vaccination against viruses, those tend to mutate very rapidly. Recently, a similar strategy has been used to develop the *mRNA-1273* vaccine, a modified mRNA of SARS-CoV2 spike protein, more commonly known as Moderna vaccine. It has shown 95% efficiency during the phase III clinical trial [92]. Another vaccine "*BNT162b2*" has been developed by Pfizer and BioNTech pharmaceutical company which will produce viral spike protein upon administration into the host. This is also an mRNA vaccine and has shown nearly 95% efficiency during phase III clinical trial [93]. Both of these vaccines have been launched in the market and their administration have started.

6.9. Block Bradykinin Production

As previously described, the bradykinin storm was found to be responsible for leaky blood vessels that could lead to life-threatening respiratory complications in some COVID-19 patients. Researchers suggest that a drug named icatibant can arrest body's signal transduction to induce bradykinin production. When they administer the drug, four out of nine patients showed very impressive results within 10-35 hours without any side effect. However, the study is too small to draw any conclusion. Their study also evidence that vitamin D supplement can contribute to a hindering bradykinin storm. Furthermore, they suggest the use of corticosteroids to improve patient survival rate, as steroids are found to prevent the activation of bradykinin mediated activation phospholipase A2 [71].

6.10. Using CRISPR/Cas13d Technology

COVID-19 has been found to acquire frequent mutations to empower itself to escape from antiviral drugs. So, it has become difficult to develop a conventional vaccine or drug against Cov-19. This challenge leads scientists to search for an option that would aim the RNA and limit its ability to reproduce. In order to target SARS-CoV-2 RNA genome. Nguyen and his coworkers proposed a new approach of using CRISPR/Cas13d technology, which employs RNase activity

of Cas13d, to knockdown specifically targeted RNA [94]. They proposed to target the replicase-transcriptase (ORF1ab) and the spike (S) of the virus [95]. They have designed 10,333 guide RNAs to target 10 protein-coding regions of virus RNA genome [96]. Both the safety profile, and presence of serotypes that are highly specific to the lungs; have made adeno-associated virus (AAV), the most suitable carrier to deliver the Cas13d effector to SARS-CoV-2

infected patients [96]. The cleavage activity of Cas13d does not depend on the protospacer flanking sequence and size of Cas13d is small enough to package up to three guide RNAs into one AAV [96]. These overall features indicate that it could be very efficient, straightforward and rapid novel approach to treat not only COVID-19 but also other RNA virus infection [96].
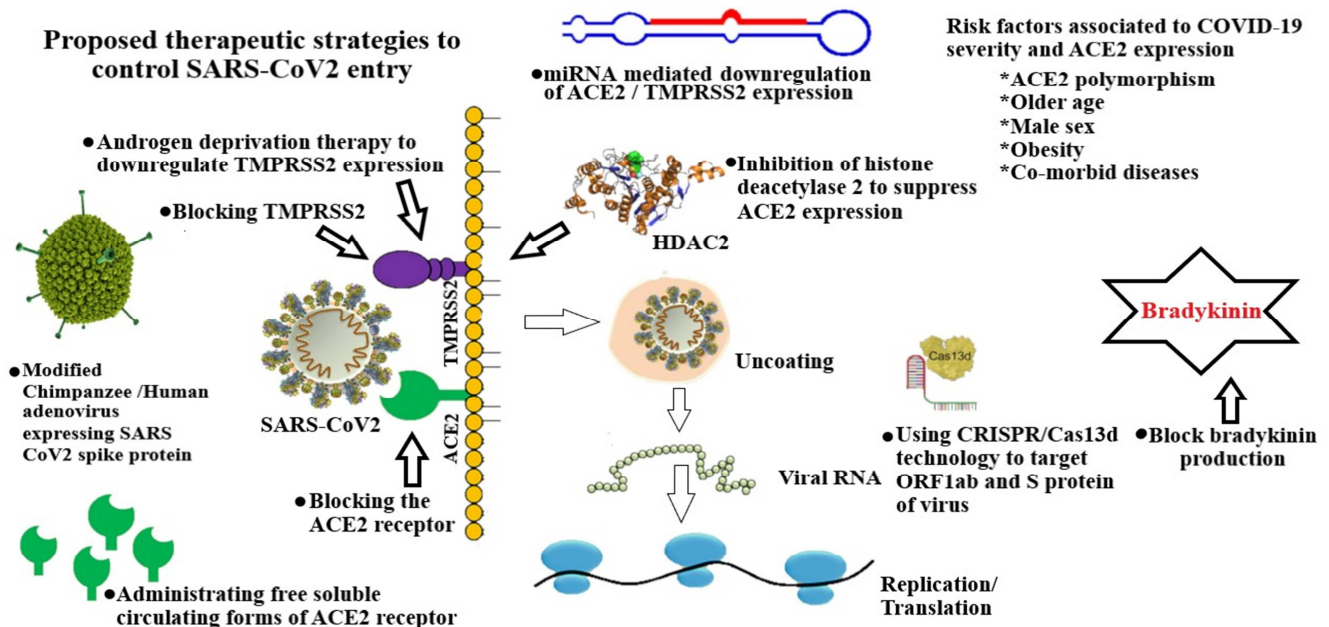

Figure 1. *Summarization of proposed vaccine strategies.*

7. Conclusion

The life losses and extreme financial losses have emphasized the instant need of a vaccine to fight against SARS-Cov2. Although several drugs, for example, ivermectin, remdesivir, dexamethason are in use during recent days to treat COVID-19 patients, they suffer from being somewhat unspecific, some have severe side effects but are providing some sort of defense. Yet, the number of deaths is increasing, and the world has reached a point, where a very efficient and specific vaccine is indispensable to save human lives. However, the high mutation rate and stereotype variation pose great challenges for the scientists to achieve it. The previous history of pandemic shows that we still do not have particular vaccines against many viruses. Hence, the doubt remains, if this time it will be successful in discovering an efficient vaccine without any side effect for SARS-Cov 2 to eradicate it from the world entirely or it will be recurring in upcoming years. Scientists worldwide are working on the ground of observed findings or theoretical basis. In recent times, some studies have uprooted possibilities of a number of strategies, and few drugs have been disapproved during the preclinical or clinical trials. It raises hope that recently, some of the vaccines have elicited successful result.

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