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Review Article

While we wait for a vaccine against SARS-CoV-2, why not think about available drugs?

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Summary

Efforts to develop a specific vaccine against SARS-Cov-2, the causative agent of the coronavirus disease 2019 (COVID-19), have just begun trial phase 1, but full validation of this and other current developments is likely to take many more months to reach completion. The ongoing pandemic constitutes a major health burden of world proportions that is also having a devastating impact on whole economies worldwide, the knock-on effects of which could be catastrophic especially in poorer countries. Alternative measures to ameliorate the impact and hamper or minimally slow down disease progression are urgently called for. This review discusses past and currently evolving data on the etiological agent of the current pandemic, SARS-CoV-2, and its host cell receptors with a view to disclosing alternative palliative or therapeutic approaches. Firstly, SARS-CoV-2 exhibits marked tropism for cells that harbor the membrane-bound metalloprotease angiotensin-converting enzyme 2 (ACE2) at their plasmalemma, predominantly in cells lining the oral cavity, upper respiratory tract, and bronchoalveolar cells, making these epithelial mucosae the most likely viral receptor cell targets. Secondly, the crystal structures of several coronavirus spike proteins in complex with their cell host target receptors, and of SARS-Cov-2 in complex with an inhibitor, are known at atomic resolution

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through X-ray diffraction and cryo-electron microscopy studies. Thirdly, viral entry of other viruses has been successfully blocked by inhibiting viral endogenous proteases or clathrin/dynamin-dependent endocytosis, the same internalization pathway followed by ACE2 and some viruses. Fourthly, the target cell-surface receptor molecules and SARS-CoV-2 possess other putative sites for drugs potentially modulating receptor activity or virus processing. A multi-pronged pharmacological approach attacking more than one flank of the viral-receptor interactions is worth considering as a front-line strategy.

Keywords: Coronavirus / COVID-19 / SARS-CoV-2 / design drugs / ACE2 / prophylaxis /

Introduction

Human coronaviruses (HCoVs) were discovered in 1965 in patients with the common flu and coined B814, with the prefix “corona” subsequently added in reference to their relatively large spikes (or spines) resembling a crown (Li 2013). HCoVs belong to the family of *Coronaviridae* enveloped viruses that harbor between 26 and 32 kilobases of single-stranded positive-sense RNA, the largest so far observed for an RNA virus (Su et al. 2016; Li 2013). CoVs infect a wide spectrum of avian and mammalian species. Seven CoVs are currently known. Members of the first group (HCoV-OC43, HCoV-293, HCoV-NL63 and HKU1-CoV) circulate in humans and generally cause mild, self-limiting respiratory diseases. HCoVs in the second group are more pathogenic and share the tropism for epithelial cells containing membrane bound proteases; they are the etiological agents responsible for the severe acute respiratory syndrome (SARS-CoV), Middle East respiratory syndrome (MERS-CoV), and the ongoing outbreak of CoV disease (COVID-19) originating in the city of Wuhan, Hubei province in China. The etiological agent, SARS-CoV-2, is a beta-CoV that has been isolated from human bronchoalveolar epithelium of infected patients (Zhou et al. 2020b). Full-genome sequencing of SARS-CoV-2 showed that it has 79.5% sequence

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identity with SARS-CoV and is 96% identical to the Chinese bat CoV, BatCoV RaTG13 (Zhou et al. 2020b). Initial reports on the epidemiology of the evolving COVID-19 disease pointed to a peculiar age distribution, which unlike other viral diseases, largely spares children and adolescents below the age of 15 from the clinically manifest forms (Li et al. 2020a; Wu and McGoogan 2020; del Rio and Malani 2020; Young et al. 2020). The adult severe forms of the disease share both the potential of evolving to fatal outcomes and the lack of specific therapeutic strategies against them.

CoVs are related zoonotically and share common phylogeny, structural and pathogenic properties but show differences in tropism, cell-surface receptors, mechanisms for entry into cells, etiopathology, clinical presentation and epidemiological characteristics. In humans, CoV infections predominantly affect the respiratory and gastrointestinal tracts. The potential emergence of a SARS-like cluster of a circulating bat CoV, SHCO14-CoV, with experimentally demonstrated human cell infective capacity and high pathogenicity, was already reported in 2015 (Menachery et al. 2015). The authors warned of the potential risk of SARS-CoV re-emergence. Analysis of evolutionary, genetic and pathogenic aspects of CoVs reiterated the warning (Su et al. 2016): “considering the high frequency of recombination of these viruses, with unpredictable changes in virulence, and with multiple viral species hosted by different animals that are likely to interact with each other, it is not a matter of if, but of when, a new CoV would emerge and cause a new outbreak of human disease”. The current, perhaps inevitable outbreak of COVID-19 attests to the correctness of these predictions.

Infection: Viral recognition and entry into target cells

Coronavirus possesses a few structural proteins (nucleocapsid, spike, envelope and membrane) and various other non-structural proteins, one of which, the surface spike S glycoprotein, plays the key role in addressing and infecting the cell containing target membrane-bound receptors. Virion entry is a multi-step process involving attachment to the cell surface, receptor engagement, protease processing and

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membrane fusion. The SARS-CoV enters human epithelial cells in vitro through the apical surface, and viruses replicated in these cells are also released via the apical plasmalemma. This correlates with the cellular distribution of its main molecular target at the plasmalemma, the angiotensin-converting enzyme 2 (ACE2), which in well-differentiated epithelial cells is more abundantly expressed at the apical surface than in the basolateral membranes and is used by SARS-CoV to more readily infect such differentiated cells (Jia et al. 2005). Based on this evidence, and the phylogenetic kindredness among CoVs, it was highly likely that SARS-CoV-2 would also follow the same entry route, as recently demonstrated (Zhou et al. 2020b).

It has long been known that the viral spike S glycoprotein is critical for host range and tropism (Supekar et al. 2004; Li et al. 2005). The S protein is a trimer, consisting of three S1-S2 subunit heterodimers. During viral infection, the trimer is cleaved into its S1 and S2 subunits. The S1 N-terminal domain contains the receptor binding domain (RBD), which can bind a variety of targets including polypeptide segments of proteolytic enzymes or carbohydrate moieties. The latter include neuraminic acid or heparan sulphate. In mouse hepatitis virus (MHV) beta-CoV the S1 N-term domain is recognized by cell adhesion carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Kubo et al. 1994). Another zinc metalloprotease enzyme, aminopeptidase N (APN, CD13), acts as a viral-recognition protein for human H229E-CoV, transmissible gastroenteritis virus, porcine epidemic diarrhea, and feline infectious peritonitis virus; and the dipeptidylpeptidase 4 (DPP4) at the apical surface of unciliated bronchia epithelial cells performs this function for MERS-CoV, a member of the beta-CoV genus which is not recognized by the ACE2 receptor, and which has a remarkable evolutionary ability to adapt to species variations in its DPP4 cell-surface target by modifying the S protein surface charge (Letko et al. 2018). In addition to DPP4, MERS-CoV can infect human pulmonary epithelial cells through highly specific but low affinity interactions with sialic acid residues present in host cell-surface glycoproteins (Li et al. 2017a) using a different region of its spike protein S

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(Park et al. 2019).

To identify the putative receptor of SARS-CoV-2, sequence and phylogenetic analyses of other CoVs were carried out together with a molecular modeling exercise on the S protein of the SARS-CoV-2 virus. The results of these in silico studies led Xu and coworkers to formulate the hypothesis that ACE2 was the SARS-CoV-2 cell host receptor (Xu et al. 2020b). This contention was followed soon after by the experimental demonstration that ACE2 was indeed the cellular target of SARS-CoV-2 (Zhou et al. 2020b). Remarkably, the S protein of HNL63-CoV, an alpha-CoV, is recognized by ACE2 (Li et al. 2005). It is apparent that CoVs exhibit predominant binding tropisms towards membrane-bound proteases, but such tropisms are by no means absolute.

Spike S protein-mediated binding of CoVs to the surface of the host cells and fusion to the cell-surface membrane appears to be a conserved, shared mechanism. It is hypothesized, however, that the two mechanisms may have evolved separately (Li 2016): ancestral CoVs would have initially harbored a primordial spike with S2 domains only, functionally inefficient because the virus had to randomly diffuse to reach target cells: membrane fusion eventually occurred in a receptor-independent manner. The spike would have subsequently evolved to acquire a galectin-like S1 N-term domain through gene capture, thus enhancing CoV efficiency in infecting cells. A third evolutionary stage is purported to be the appearance of an S1 C-term domain through gene duplication of the S1 N-term domain (Li 2016). A comprehensive review on the binding-proteolytic activation-fusion mechanism of CoVs entry (Millet and Whittaker 2015) emphasizes that the proteolytic activation step is the critical one for the fusion of S1 to occur, since it allows for controlled release of the fusion peptide into the target plasmalemma.

ACE and ACE2, Ying-Yang in the renin-angiotensin-aldosterone system (RAAS) and their uncertain roles as risk factors in COVID-19

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Endothelial ACE is a key metallopeptidase enzyme in the renin-angiotensin-aldosterone system (RAAS), playing a crucial role in the regulation of blood pressure and homeostasis of body fluids, and constituting an alternative target in pharmacotherapies of cardiovascular and renal diseases. Human endothelial ACE catalyzes the removal of the carboxy-terminal dipeptide from the decapeptide angiotensin I to produce angiotensin II, the active vasoconstrictor form of the hormone. Angiotensin II and aldosterone are the two key biologically active hormones of the RAAS homeostatic system (Li et al. 2017b) (Mirabito Colafella et al. 2019). ACE2 has been studied less than ACE in the RAAS field but is gaining increasing attention in relation to virus infections and the current COVID-19 pandemic. ACE2 is also a metalloprotease, initially described in a study searching for novel genes related to heart failure, and found in membrane-associated and secreted forms predominantly in endothelial cells of human heart, kidney, and testis (Donoghue et al. 2000). Endothelial ACE and ACE2 share 42% identical amino acid residues in their catalytic domain, suggesting a common ancestor (Donoghue et al. 2000).

The two enzymes act on two counterbalancing pathways of the RAAS system: upon conversion of angiotensinogen to angiotensin I by the renin system, ACE-generated angiotensin II acts on the AT_1 receptor, mediating, among other responses, catecholamine release from peripheral sympathetic neurons, vasoconstriction, mild bronchoconstriction, water and sodium retention, aldosterone synthesis, and inflammatory reactions. The counteracting RAAS pathway mediated by ACE2 hydrolyzes angiotensin II to generate angiotensin 1-9, in which case it is further processed by other proteases down to angiotensin 1-7, or directly cleaved to 1-7 by ACE2 itself. Angiotensin 1-7 targets the angiotensin II type 2 receptor (AT_2-R) and the angiotensin 1-7 Mas receptor, producing vasodilatory and natriuretic physiological actions (Mirabito Colafella et al. 2019; Li et al. 2017b). By sequestering angiotensin I, ACE2 limits substrate availability for the biosynthesis of angiotensin II by ACE, thus resulting in a vasodilatory effect and a protective role in hypertension, heart failure and other pathological entities.

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Avian influenza H5N1 virus, phylogenetically unrelated to the CoVs, appears to affect the RAAS system: infected patients exhibit higher levels of angiotensin II in serum, a sign which correlates with the severity and lethality of the disease. Furthermore, parallel studies conducted in animal models showed that disease severity correlates with the downregulation of ACE2 in lung, which can be reversed, increasing animal survival, by administration of recombinant human ACE2 (Zou et al. 2014).

Whether patients with hypertension and diabetes are at a higher risk of being infected with the SARS-CoV-2 virus is currently a matter of debate. Early epidemiological observations indicated higher morbidity and mortality among elderly Chinese COVID-19 patients with hypertension (Wu et al. 2020; Zhou et al. 2020a). However, according to a more recent analysis these initial studies did not adjust for cofounding variables, thus leaving the association between COVID-19 and hypertension an open question (Patel and Verma 2020). It has been hypothesized that therapeutically addressing the ACE2/Mas arm of the RAAS system may be problematic in patients with severe COVID-19 who also suffer from diabetes or hypertension as co-morbidities, because ACE2 expression increases in diabetic patients treated with ACE inhibitors and angiotensin II AT_1 receptor inhibitors, such that increased ACE2 expression would facilitate and worsen SARS-CoV-2 infection (Fang et al. 2020). The evidence in support of this hypothesis is still contentious.

In a cohort of 416 Chinese patients with COVID-19 about 60% had hypertension and ~20% had cardiac injury (Shi et al. 2020). The authors attributed the higher risk of mortality to the latter condition. The recent experimental demonstration that ACE2 is the target of SARS-COV-2 (Zhou et al. 2020b) has prompted the hypothesis that ACE2 is upregulated in patients treated with ACE inhibitors (ACEIs) and angiotensin II AT_1 receptor blockers (ARBs) (Wan et al. 2020). These authors also indicate that ACE2 polymorphisms associated with diabetes and hypertension might result in a higher risk for SARS-Cov-2 infection, especially in Asian populations, and suggest the use of calcium channel blockers as an alternative therapeutic for hypertension in these patients. However, there is no evidence to date that

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ACE2 is overexpressed in pulmonary tissue of COVID-19 patients. In fact, ACE2 was found to be downregulated upon infection by SARS-CoV in an experimental animal model of chemical lung injury, thus leaving angiotensin II uncleaved, aggravating the pathology (Kuba et al. 2005). Furthermore, ACE2 has been found to exert protective effects against experimentally-induced acute pulmonary damage in several models of acute respiratory distress syndrome (Imai et al. 2005). It has also been suggested that inducing increased ACE2 activity might constitute a therapeutic approach in acute lung failure (Kuba et al. 2006). In the short run, the current COVID-19 pandemic is posing challenges that require unprecedented speed to resolve, in many instances with insufficient evidence, as seems to be the case with hypertension, diabetes and other presumed co-morbidities and their pharmacological management. In the long run, it is likely that upon control of the current COVID-19 pandemic the SARS-CoV-2 will reemerge in its present or in mutated forms and become chronic (Patel and Verma 2020). If this turns out to be the case, a deeper understanding of the RAAS system in relation to coronavirus infection will be compellingly essential, as will the need to establish appropriate protocols for patients suffering cardiovascular diseases, the most common non-communicable epidemic.

Differences between ACE and ACE2

The two enzymes differ not only in structure but also in their organ localization: while both ACE and ACE2 are ubiquitously distributed in all endothelial cells in the human body, ACE2 was initially found in non-endothelial cells in heart, kidney and testis and tubular epithelium of the kidney (Donoghue et al. 2000). Subsequent work screened and found expression in oral and nasal mucosae and pulmonary alveolar and gastrointestinal epithelia, suggesting possible routes of viral entry, and also in liver, kidney, lymph nodes, thymus, spleen and brain (Hamming et al. 2004; Xu et al. 2020a). An RNA-seq profiling study designed to explore the putative presence of ACE2 in the epithelial mucosa of the

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human oral cavity found highest expression in the tongue in comparison to the gingival or the rest of the buccal mucosa (Xu et al. 2020a).

Because of its pathological and clinical implications, pulmonary distribution of ACE2 is particularly important. Alveoli are lined by two types of alveolar epithelial cells, or pneumocytes: type 1 pneumocytes (AT1) are nonreplicating, large and relatively flat cells whose main function is to regulate the O_2-Co_2 exchange. The smaller and less abundant AT2 cells are involved in the production and secretion of surfactant and can re-differentiate into AT1 under diseased conditions. SARS-CoV primarily targets ACE2 in ciliated bronchoalveolar epithelial cells and AT2 cells (Li et al. 2003; Qian et al. 2013). A recent graph-based bioinformatic data analysis recently corroborated that the highest, though not exclusive, expression of ACE2 was in a very small proportion (0.64%) of all human pulmonary cells. Of these, the vast majority (80%) corresponds to AT2 cells (Zhao et al. 2020). Interestingly, several other ACE2-related genes that facilitate viral reproduction and transmission are also highly expressed in the AT2 cells (Zhao et al. 2020). Using a novel functional viriomics approach, the receptor binding domain of lineage B (beta)-CoVs can be divided into functionally distinct clades. ACE2 was found to be the entry receptor specific for clade 1 of such lineage. When tested with this new assay, SARC-CoV-2 incorporated into cells expressing ACE2, but not other cell-surface receptors. Furthermore, several viruses exhibit compatibility with a still unknown receptor in human cells (Letko et al. 2020). ACE2 is developmentally regulated in mouse pulmonary epithelium (Wiener et al. 2007). The lack of manifest clinical symptomatology and/or severe forms of COVID-19 in children and adolescents below the age of 15 mentioned above could be related to the presence of an immature form of ACE2 in this population, although cross-immunity to SARS-CoV-2 awarded by exposure to other CoVs or other immune system-based hypothesis could be invoked.

Structures of CoV surface spike protein S and host cell receptor, ACE2

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The X-ray structure of human testicular ACE in complex with one of the most widely used inhibitors, lisinopril (N2 -[(S)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline) was solved at 2.0 Å resolution (Natesh et al. 2003). Knowledge of this structure had implications in subsequent studies of ACE2 and CoV structures. Before the atomic structure of ACE2 was known, an homology model was produced on the basis of the somatic ACE enzyme structure (Natesh et al. 2003) suggesting the complementarity of the viral binding domain of ACE2 and the SARS-CoV spike protein S1 (Prabakaran et al. 2004). The X-ray structure of the "proteolytically stable core" of the SARS-CoV was obtained in 2004 by Carfi and collaborators (Supekar et al. 2004). The crystallographic data of this beta-CoV constituted one of the first descriptions at atomic level. This was followed in 2005 by the more detailed X-ray diffraction study at 2.9 Å of the SARS-CoV S protein in complex with human ACE2, by Harrison and coworkers (Li et al. 2005). The crystallographic data disclosed two domains in ACE2: the core region (Supekar et al. 2004) and the RBD comprised between residues 306-527, within which a loop formed by residues 424 to 494 constitutes the receptor binding motif (RBM) in SARS-CoV. This critical region contacts the complementary surface of its host cell receptor, ACE2 (Li et al. 2005; Li 2016). The core domain consists of five-stranded antiparallel β-sheets. The RBD possesses a concave surface in the case of SARS-CoV, and a flat surface in the case of another beta-CoV, MERS-CoV, that binds to the receptor complementary surface, as described below. A recent molecular modeling exercise revealed the similarity between the geometric and physicochemical properties of the viral RBD-ACE2 interfaces of SARS-CoV and SARS-CoV-2, and with those of antibody-antigen contacts, stabilized by electrostatic interactions, suggesting similar evolutionary maturation pathways followed by the two otherwise dissimilar systems (Brielle et al. 2020).

The host cell provides the proteolytic cleaving enzymes required for the transition of the S protein from the pre-fusion to its post-fusion conformation. The proteolytic digestion unveils the S1 and S2 subunits. The S1 subunit mediates binding to the plasmalemma, whereas the S2 subunit intervenes in

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membrane fusion. Another S1 spike protein complexed with ACE2, the S1 from respiratory NL63-CoV (a group 1 coronavirus) bound to the NL63-CoV RBD, was subsequently determined by Wu and coworkers (Wu et al. 2009). NL63-CoV RBD showed 2 layers of beta-sheets, presenting 3 discontinuous receptor-binding motifs (RBMs) to bind ACE2. NL63-CoV and SARS-CoV have no structural homology in RBD cores or RBMs; yet the 2 viruses were found to recognize common ACE2 regions, leading these authors to postulate the occurrence of a "virus-binding hotspot" on ACE2. Interestingly, among group I CoVs, RBD cores are conserved but RBMs are variable, explaining how these viruses recognize different receptors (Wu et al. 2009). Along these lines, a recent phylogenetic study postulates that SARS-CoV-2 could potentially recognize ACE2 isoforms from various animal species (Wan et al. 2020).

The human beta-CoV HKU1-CoV, related to the SARS-CoV and MERS-CoV, causes mild, predominantly respiratory disease. Its structure in the pre-fusion conformation was solved in 2016 at 4.0 Å resolution using single-particle cryo-EM (Kirchdoerfer et al. 2016). At that time, the receptor for HKU1-CoV was not known, but antibodies against the C-term domain blocked infection, suggesting that this region of the virus was responsible for attaching to the target cell. The cryo-EM structural data of the S1 protein-ACE2 complex revealed that only one S1 receptor-binding domain of the trimeric S glycoprotein binds ACE2 adopting an open, protruding “up” conformation which enables it to undergo target recognition and, interestingly, keep the fusion S2 domain distant from the target. Upon binding to ACE2, the viral S protein is cleaved by host cell membrane proteases and undergoes a series of conformational transitions which release the spike fusion peptide (Kirchdoerfer et al. 2018). These authors proposed a model of how the virus progressively fused with the host cell membrane as the spike S protein was destabilized upon binding and underwent proteolytic cleavage. The conformational transitions result in an extended conformation of S2 that enables subsequent insertion of the fusion peptide into the host membrane. The model also provided some suggestions as to the possible design of a structure-based vaccine. One year later, the cryo-EM structures of SARS-CoV and MERS-CoV spike proteins became

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available, with suggestions on design neutralizing antibodies (Yuan et al. 2017). Currently other possibilities of vaccine development are underway, using e.g. the viral mRNA to elicit an immune response. The vaccine, known as mRNA-1273, has already passed animal tests and has just commenced phase 1 clinical trials https://corona.kpwashingtonresearch.org/.

At the post-fusion stage, S1-ACE2 complexes cluster together and adopt a rosette-like structure (Song et al. 2018). The structure of the S1-ACE2 complex in what is presumably the virus entry stage and the ulterior dissociation of the complex could be imaged in a cryo-EM study (Song et al. 2018). The authors hypothesized that the opening of the S1 subunit upon binding to the ACE2 receptor may promote the release of the S1-ACE2 complex and S1 monomers from the pre-fusion spike, triggering the conformational transition from pre-fusion to post-fusion conformers.

The pre-fusion conformer of the porcine epidemic diarrhea alpha-coronavirus S protein, resolved using cryo-EM at 3.1 Å, added information on the heterogeneity of conformational states that the S protein undergoes to make the RBD accessible for host cell binding to the potential receptor, porcine aminopeptidase N (Wrapp and McLellan 2019). These authors subsequently presented the 3.5 Å structure of the pre-fusion structure of the SARS-CoV-2 S protein (Figure 1) and tested the putative binding of monoclonal antibodies against the RBD of SARS-CoV, without finding any apparent antibody cross-reactivity to SARS-CoV-2 (Wrapp et al. 2020). The structure is quite similar to that of the S protein from the human beta-CoV HKU1-CoV also obtained by cryo-EM (Kirchdoerfer et al. 2016).

Another recent cryo-EM study has resolved the structure of the full-length human ACE2 with or without the RBD of the S1 spike protein of the new SARS-CoV-2, in the presence of a neutral amino acid transporter, B°AT1, which awards stability to the crystal structure, at a resolution of 2.9 Å (3.5 Å at the RBD) (Yan et al. 2020). This is the most detailed structure of ACE2 to date (Figure 2). Yet another cryo-EM contribution has recently determined the structure of the SARS-Cov-2 spike glycoprotein trimer and shown that it carries a furin cleavage site between the S1 and S2 subunits, a peculiarity that

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sets SARS-CoV and other CoVs (Wells 2020). The furin site in processed during biogenesis of the virion.

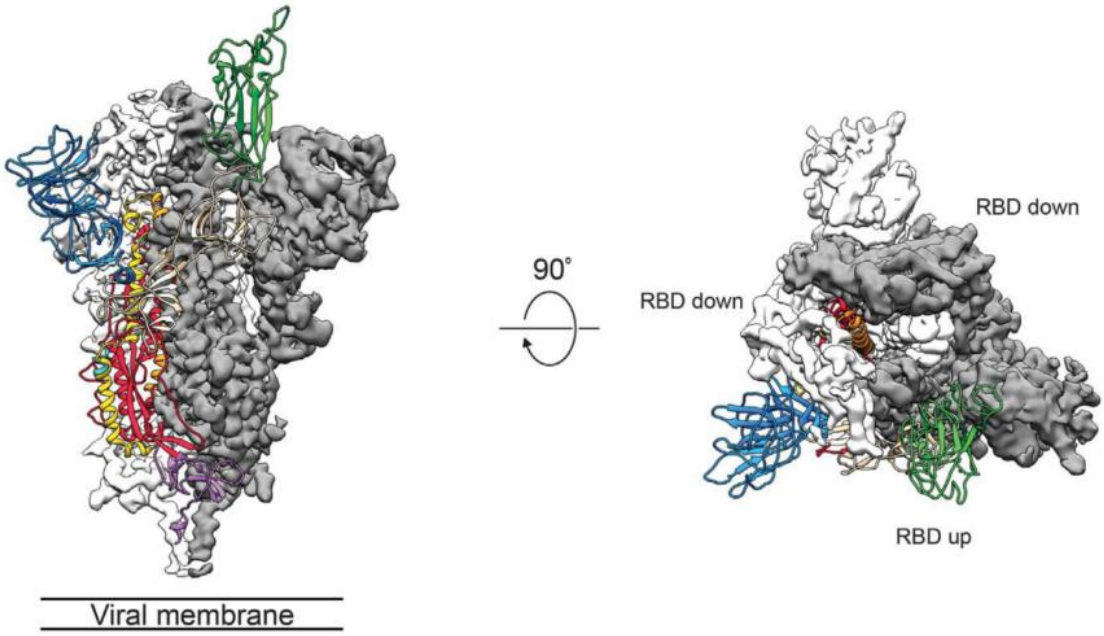

Figure 1

One viral RBD is recognized by one extracellular peptidase domain of ACE2, in a single molecule-to-molecule fashion (Figure 2), in a manner akin to the recognition of SARS-CoV S1 protein by ACE2 (Li et al. 2005; Song et al. 2018; Yan et al. 2020; Hoffmann et al. 2020; Wells 2020).

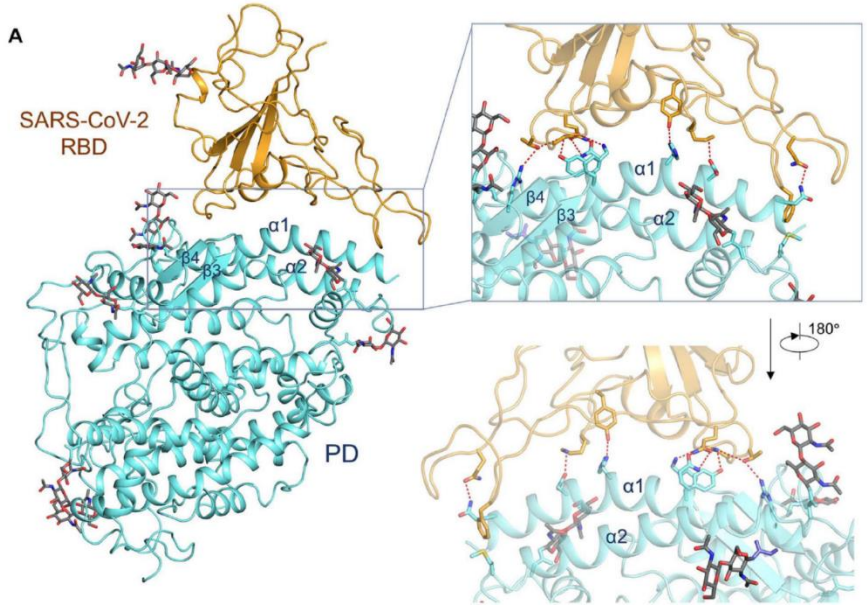

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Figure 2

The affinity of the ACE2 for SARS-CoV-2 has recently been reported to be similar (Wells 2020) or higher 10-20 times higher than that of SARS-CoV (Wrapp et al. 2020). In silico modelling of the SARS-CoV-2 protease involved in virion entry into cells has disclosed a high degree of flexibility of the protein, which not only involves the site where a known inhibitor binds, but also exposes other putative sites where enzyme blockers could bind (Wells 2020). Another molecular dynamics study comparing the differences between SARS-CoV and SARS-CoV-2 modes of binding to ACE2 suggests that the RBD of the former virus has a stronger interaction with the complementary ACE2 site (Chen et al. 2020). Based on the observation that MERS-CoV is also recognized by sialosides (Li et al. 2017a; Park et al. 2019), and that this binding involves a groove in the S protein different from the region recognized by ACE2 (Werner et al. 2019), a new predictive molecular modeling strategy, the 2D Zernike formalism, was applied to the crystal structure of SARS-CoV-2 in search for a similar domain. Indeed, a groove or pocket was found in SARS-CoV-2 that could accommodate sialic acid, raising the possibility of a second binding motif for binding to host cell receptors (Milanetti et al. 2020).

All in all, the structural information that has become available in the last few years provides a quite comprehensive picture of the virus-cell receptor complexes at atomic resolution with obvious implications for future structure-based drug and vaccine design.

Perspectives and possible avenues of intervention on targets known to date

While much of what we need to know about the viral spread, contagiousness, clinical evolution, prognosis, impact of control measures for disease mitigation and other variables of the COVID-19 disease remains terra incognita, critical examination of the literature data shows that the considerable progress accomplished in some basic aspects of SARS-CoV-2 and host receptor(s) offers hints at possible

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developments aimed at ameliorating disease progression and hopefully helping therapeutic approaches to materialize.

1. Precise specification of viral cellular targets through single-cell profiling techniques

The organ and tissue distribution of the viral receptors, as well as their expression levels, appear to be directly related to the choices of cellular targets and infection routes adopted by the viruses. An ACE2 RNA expression profile of normal human pulmonary tissue showed that ACE2 is highly expressed in AT2 human pulmonary cells, but that these constitute only a small percentage (0.64%) of the bronchoalveolar epithelial lining (Zhao et al. 2020). High expression is also found in the tongue (Xu et al. 2020a), but the oral cavity, with a reported surface area of 215 cm^2 (ref. (Collins and Dawes 1987), represents only a minute surface fraction in comparison with the upper respiratory tract and the total pulmonary alveolar area (118 ± 22 m^2 and 91 ± 18 m^2 in male and female individuals (Colebatch and Ng 1992)). The affinity of the virus for oral ACE2 receptor may be higher than that in the latter mucosae, thus providing an alternative entry point for the two SARS-CoV infections. The technologies for conducting next-generation RNA sequencing at the single-cell level (scRNA-Seq) are currently a reality. Such developments make it possible to undertake transcriptome-wide analysis of differential gene expression, differential splicing of mRNAs, and establish the tissue distribution of molecular constituents in individual cells (spatial transcriptomics, “spacialomics”) with unprecedented discriminative power that surpasses conventional antibody-based immunocytochemistry (for a review see (Stark et al. 2019). Through these approaches, selectivity of viral receptors could be narrowed down to the level of cell populations, and subpopulations within a tissue or organ with great precision, with inherent prophylactic and therapeutic consequences.

2. Exploiting our current knowledge of SARS-CoV-2 and other CoVs to inhibit virus binding to receptors

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Work on the structures of the S spike protein of CoVs and ACE2, and their complexes, has not only produced important information and ideas on the relevant epitopes for vaccine design (Supekar et al. 2004; Li et al. 2003; Imai et al. 2005; Struck et al. 2012; Song et al. 2018) but also for interventions ranging from application of recombinant ACE2 as protection against severe acute lung failure (Imai et al. 2005) to the use of ACE2 inhibitors in CoV diseases (Imai et al. 2005; Struck et al. 2012; Li 2013; Song et al. 2018). A number of ACE2 peptide inhibitors with nanomolar affinity and a non-peptide blocker with sub-nanomolar affinity, MLN-4760, have been tested on the soluble form of ACE2 (Warner et al. 2004). Current studies addressed at testing inhibitors of viral infection in vitro have reported promising results with cepharanthine, selamectin and mefloquine hydrochloride, three drugs that appear to hinder cytopathic effects of the GX_P2V virus, which is 92% homologous to SARS-CoV-2 (Fan et al. 2020).

The viral S1/S2 cleavage site is the target of another host-cell protease, the transmembrane protein TMPRSS2 (Shen et al. 2017), also a site of attack of the influenza virus. This enzyme has been shown to be blocked by camostat mesylate in combination with cathepsin inhibitors in SARS-CoV infected human HeLa cells expressing ACE2 and TMPRSS2. Camostat is a serine protease inhibitor clinically proven in oncological therapy (Kawase et al. 2012). TPPRSS2 has been pin-pointed as a potential target for the treatment of influenza virus and CoV diseases A possible line of action is to use this type of compounds as a template on which to optimize the design of more selective and/or more effective drugs.

Infection of human Vero E6 by SARS-CoV in vitro is blocked by the hexapeptide Tyr-Lys-TyrArg-Tyr-Leu (^{438}YKYRYL^{443}), an amino acid linear motif contained in the RBD of the spike protein of SARS-CoV (Figure Z). The peptide also inhibits proliferation of HCoV-NL63. Both viruses are known to target ACE2 as their cell-entry receptor (Struck et al. 2012). Using the RBD structure of the actual SARS-CoV spike protein (Li et al. 2005), modeling studies were conducted to learn about the structure of RBD-11b docked to the complementary binding domain of ACE2 (Struck et al. 2012) (Figure 4).

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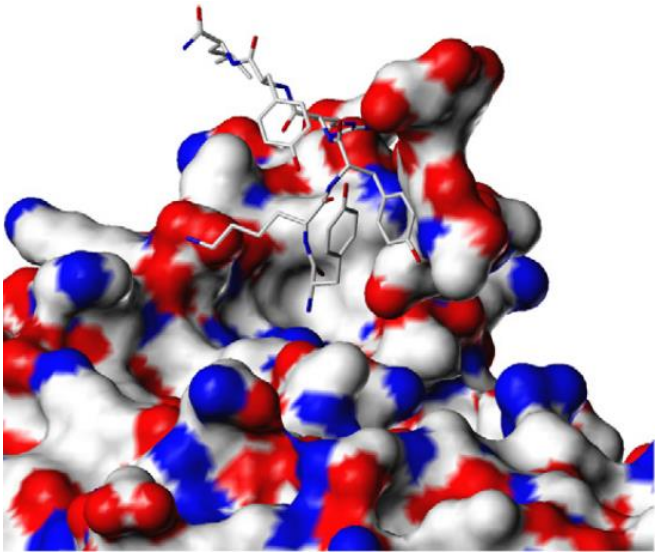

Figure 3

The tripeptide motif ^{439}KYR^{441} was found to be important for the binding stability of the peptide at the RBD site. These authors also indicate that the binding mode of RBD-11b at the ACE2 site differs from the binding mode of the hexapeptide as part of the full-length S viral protein. The differences in amino acid residues present in the RBD responsible for attachment to the cell host ACE2 (Figure 4) may account for the different affinities of SARC-CoV and SARC-CoV-2 reported by McLellan and coworkers (Wrapp et al. 2020).

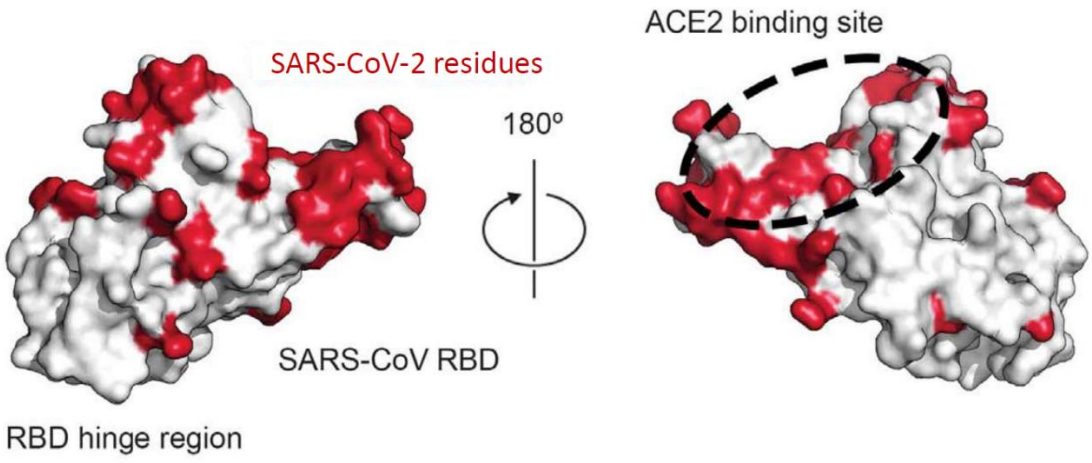

Figure 4

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3. Inhibiting virus endocytic internalization and intracellular trafficking

To enter the cells, many viruses appropriate canonical endocytic pathways used by cells under physiological conditions. One of the earliest mechanisms of CoV endocytosis studied is that of mouse hepatitis virus MHV-4; infection occurs via endocytic and non-endocytic mechanisms (Nash and Buchmeier 1997). Viral endocytic internalization operates via a clathrin-dependent, caveolin- and EPS-15-independent mechanism (Pu and Zhang 2008). Drugs that inhibit the clathrin- and dynamin-dependent endocytic pathway impede dengue virus infection of mononuclear phagocytic cells (Carro et al. 2018). Temporary inhibition of endocytosis with the anti-emetic/anti-psychotropic drug prochlorperazine is deemed to be potentially safe in humans (Chew et al. 2020). The SARS-CoV S protein is digested by cathepsin L or B in the lumen of the endocytic compartment, and on this basis the use of protease inhibitors has been explored as potential broad-spectrum anti-CoV agents, including SARS-CoV (Zhou et al. 2015). Expression of exogenous cathepsin L significantly enhances SARS-CoV but not HNL63-CoV infection via ACE2 (Huang et al. 2006). This exemplifies how two CoVs that target a common receptor molecule infect the cells using different mechanisms.

Viral endocytic mechanisms have gained recent attention partly because there are available drugs targeting these mechanisms. The relatively simple organic anti-malaria compound chloroquine (Figure 5) (see recent review in (Touret and de Lamballerie 2020), used since before mid-twentieth century, is known to moderately raise the pH of endosomes and/or lysosomes, preventing viral fusion, uncoating and further processing in the endosomal lumen and lysosome. Additional mechanisms beyond inhibition of endocytic internalization are suggested by biotechnological studies on nanoparticle uptake by macrophages (Hu et al. 2020) The possible prophylactic or therapeutic effects of chloroquine and its derivative hydroxychloroquine, also employed for autoimmune diseases (Colson et al. 2020), lupus and rheumatoid arthritis, is currently being subjected to clinical trials for COVID-19 in China and

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France (Devaux et al. 2020); critically validated results are still being awaited within the context of the SOLIDARITY program launched by the World Health Organization. Both drugs have a rather narrow margin between therapeutic and toxic effects, some of them lethal (Touret and de Lamballerie 2020). It is contended that chloroquine and derivatives not only affect the endocytic mechanisms of viral entry but may also interfere with the SARS-CoV-2 replication cycle (Devaux et al. 2020).

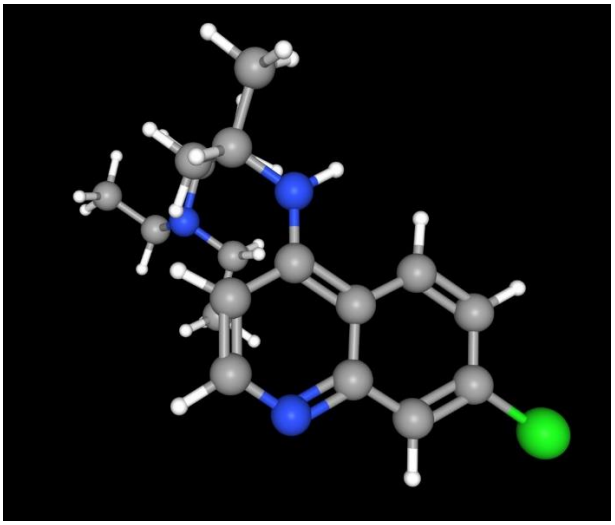

Figure 5

There is an additional component in the virion infection process: the cytoskeleton. Entry of the porcine hemagglutinating encephalomyelitis virus, a member of the CoV family, into N2 cells is facilitated by rearrangement of the cytoskeleton via the α5β1-FAK/cofilin/Rac1/cell division cycle Cdc42 pathway (Lv et al. 2019).

4. ACE2 in the context of RAAS

In addition to contemplating ACE2 as the molecule targeted by SARS-CoV-2, it is important to envisage this enzyme as a key target of therapeutic interventions based on its role in pulmonary inflammatory pathologies. Indeed, the ACE2/Ang (1–7)/Mas receptor pathway is a potential therapeutic target

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worth exploring for ameliorating allergic inflammation of the respiratory tract, respiratory airway remodeling, and airway hyperresponsiveness. Acute respiratory failure with bilateral infiltrates and hypoxemia, without hydrostatic pulmonary edema, configures a potentially severe nosological entity eventually leading to acute respiratory distress syndrome, an inflammatory condition involving increased vascular permeability and hypoxemic respiratory failure (Bellani et al. 2016). The syndrome appears to be one of the most common complications of COVID-19 (reviewed in (Tan et al. 2018). The severity of pneumonia and acute respiratory failure is positively correlated with age-dependent disequilibria of the ACE/ACE2 ratio (Schouten et al. 2016; Tan et al. 2018). Furthermore, reduced ACE2 activity has been observed in experimentally-induced acute respiratory distress syndrome. Therapeutic intervention with a protease-resistant form of angiotensin (1-7) improved all symptoms, indicating the possible association of angiotensin (1-7) deficiency with the syndrome (Wösten-van Asperen et al. 2011).

5. The SARS-CoV-2 and its endogenous proteases

Attacking the virus itself is another attractive possibility. Any of the viral proteins and the mRNA are possible targets, especially using drugs already developed for other viruses or modifications thereof. Among such already existing drugs are the first-generation anti-HIV drugs aimed at the replicase enzymatic machinery responsible for RNA replication, the nucleotide analogue drugs like remdesivir, a broad-spectrum inhibitor of RNA polymerase originally designed for the Ebola virus and congeners, and whose effectiveness against COVID-19 is currently being investigated. The anti-hepatitis C antiviral drug ribavirin in another of the potential drugs listed as candidates by the World Health Organization. Viral capping proteins are yet another possibility: SARS-CoV guanine-N7-methyl-transferase inhibitors have been screened with a yeast assay in vitro (Sun et al. 2014).

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Inhibitors of the endogenous CoV proteases active downstream of infection for the biosynthesis of polyproteins translated from the viral RNA constitute another avenue of research. SARS-CoV main protease, M^{PRO} also termed $3CL^{PRO}$, is a key enzyme needed for the cleavage of two proteins involved in viral replication. Forty unsymmetrical aromatic disulfide compounds have been synthesized and tested in vitro and found to be reversible competitive inhibitors of M^{PRO} activity, blocking viral replication. In silico docking was used to validate the in vitro assays (Wang et al. 2017). Another endogenous protease of SARS-CoV-2 is the papain-like protease (PL^{PRO}), whose atomic structure is still not known. Based on available crystallographic data of the PL^{PRO} from SARS-CoV and MERS-CoV, a recent homology modeling study found that the PL^{PRO} of SARS-CoV-2 is 97% homologous to that from a bat CoV, 80% to SARS-CoV and only 29% homologous to MERS-CoV (Stoermer 2020).

The recent availability of the co-crystal of M^{PRO} from SARS-CoV-2 with an α-ketoamide inhibitor (Zhang et al. 2020) (Figure 5) provides a structural basis on which to validate the in silico calculations and develop new virion entry blockers. A virtual screening of more than 3,000 compounds approved by the American Federal Drug Administration recently focused on the M^{PRO} of SARC-CoV-2 (Contini 2020). Protease inhibitors previously or currently used in HIV retroviral therapy, like lopinavir, indinavir and atazanavir were selected as potential candidates applicable to COVID-19 (Contini 2020; Wang 2020). The ritonavir+lopinavir combination has been used an HIV protease inhibitor. In another study the same FDA database was explored using free energy calculations, and dypyradamol was selected as the most promising drug, which according to the authors is undergoing clinical trials (Li et al. 2020b). Using the crystal structure of the viral proteases as a template, repurposing database screening combined with thermodynamics of ligand binding have highlighted the drug carfilzomib, an approved anti-cancer drug, as the best potential candidate to inhibit SARS-CoV-2 infection at the level of the proteasome, with a free energy of binding of -13.8 kcal mol^{-1}. Antibiotics like ervacycline or streptomycin were also singled out (Wang 2020). An important feature of blocking this viral protease, unlike other actors in the viral life

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cycle, is that no enzyme with such cleavage specificity is found in humans, thus minimizing the potential toxicity of inhibitors in therapeutic approaches to COVID-19.

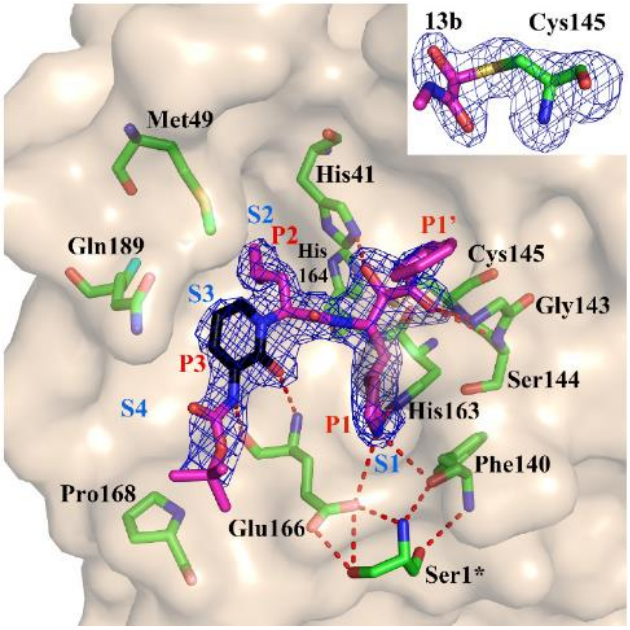

Figure 6

The diversity of candidate drugs resulting from “repurposing” database analyses, in some cases stemming from the same database, calls for alternative refining techniques. Deep learning is increasingly gaining momentum in protein structure prediction (Wardah et al. 2019; Senior et al. 2020; Singh 2020). Deep learning strategies have also impacted on the field of drug design (Dana et al. 2018). Deep learning constitutes a viable methodology which should be exploited to screen data banks of small drugs docking on cell host and viral proteases, SARS-CoV-2 surface proteins and other putative targets on the basis of structural and thermodynamic parameters to find new suitable small drug inhibitors, and explore *combinations of two or more drugs acting synergistically on different targets*.

6. Lipid metabolism in CoV-infected cells

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Plus-strand RNA virus replication, as is the case with CoVs, sequesters the lipid metabolic machinery in the infected cell, hijacking the enzymes normally involved in lipid synthesis and processing. Structurally this results in the formation of double-membrane vesicles and other abnormal membrane structures generically referred to as replicative organelles. These are the platforms for virion replication and transcription and virion morphogenesis. An inhibitor of the cytosolic phospholipase $A_2\alpha$, pyrrolidine-2, was found to reduce the formation of double-membrane vesicles in cells infected with the related human H229E CoV, impairing virion replication (Müller et al. 2018). The drug also diminished the formation of lyso-phospholipids, the products of phospholipase $A_2\alpha$ enzymatic activity that are essential for CoV replication. It would be worth exploring the lipidomics of SARS-CoV-2 to investigate whether the virus possesses any singularity in terms of composition which can be exploited in its control.

Conflict of interest

The author declares no conflict of interest

Figure legends

Figure 1. Side (left) and top (middle) views of the SARS-CoV-2 S protein prefusion structure with a single RBD in the "up" conformation obtained by cryo-EM. The two RBD down protomers are shown in either white or gray surface rendering; the RBD "up" protomer is shown in green ribbon rendering. From the cryo-EM study of ref. (Wrapp et al. 2020), held under Creative Commons license.

Figure 2. Interactions between the receptor-binding domain (RBD) of SARS-CoV-2 and its cell-surface receptor molecule, the enzyme ACE2. The latter (light blue-ribbon rendering) engages essentially a single linear motif, the α1-helix, to recognize the corresponding viral RBM (golden rendering), with contributions from the α-2-helix, as can be appreciated in the inset. From ref. (Yan et al. 2020), held under Creative Commons license.

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Figure 3. Snapshot at 3 ns of the hexapeptide inhibitor RBD-11b sitting on the peptide receptor pocket of ACE2. Color rendering is according to atom type. From the molecular dynamics study of ref. (Struck et al. 2012), with permission from Elsevier.

Figure 4. Hinge domain of the SARS-CoV receptor-binding domain (RBD, white surface rendering) (PDB 2AJF) with residues that vary in the related SARS-CoV-2 RBD shown in red. These structural differences may constitute the basis of the differences in affinity of the two viruses for the common ACE cell-host enzyme, ACE2. The binding site for "landing" of the RBD onto its target, ACE2, is better appreciated in the 180° rotated figure on the right (outlined with a black dashed line). From the cryo-EM study of ref. (Wrapp et al. 2020), held under Creative Commons license.

Figure 5. Chemical structure of chloroquine, a drug used for many decades against malaria, reenters stage as a potential anti-SARS-CoV-2 agent. Drawn using PubChem, freely accessible chemistry database.

Figure 6. Substrate-binding cleft of M^{PRO}, the endogenous protease in SARS-CoV-2, with compound 13b, a peptidomimetic α-ketoamide inhibitor of the viral enzyme (Zhang et al. 2020). Fo-Fc density is shown for the inhibitor. Atom color rendering: magenta, carbon (except in the pyridone ring, which is black), red, oxygen; blue, nitrogen, and yellow, sulfur. Light-blue symbols S1, S2, S3, S4 indicate the canonical binding pockets for moieties P1, P2, P3, P4 (red symbols) inhibitor. Red dashed lines represent H-bonds. *Inset*: Thiohemiketal resulting from the nucleophilic attack of the catalytic Cys residue on the α-carbon of the inhibitor in its Fo-Fc density (contoured at 3σ). From (Zhang et al. 2020), held under Creative Commons license.

References

Bellani, G., J. G. Laffey, T. Pham, E. Fan, L. Brochard, A. Esteban, L. Gattinoni, F. van Haren, A. Larsson, D. F. McAuley, M. Ranieri, G. Rubenfeld, B. T. Thompson, H. Wrigge, A. S. Slutsky, A. Pesenti, f. t. L. S. Investigators, and t. E. T. Group. 2016. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. *JAMA* 315 (8):788-800.

Brielle, E. S., D. Schneidman-Duhovny, and M. Linial. 2020. The SARS-CoV-2 exerts a distinctive strategy for interacting with the ACE2 human receptor.2020.2003.2010.986398.

Carro, A. C., L. E. Piccini, and E. B. Damonte. 2018. Blockade of dengue virus entry into myeloid cells by endocytic inhibitors in the presence or absence of antibodies. *PLoS Negl Trop Dis* 12 (8):e0006685.

Chen, Y., Y. Guo, Y. Pan, and Z. J. Zhao. 2020. Structure analysis of the receptor binding of 2019-nCoV. *Biochem Biophys Res Commun*.

Chew, H. Y., P. O. De Lima, J. L. Gonzalez Cruz, B. Banushi, G. Echejoh, L. Hu, S. R. Joseph, B. Lum, J. Rae, J. S. O'Donnell, L. Merida de Long, S. Okano, B. King, R. Barry, D. Moi, R. Mazzieri, R. Thomas, F. Souza-Fonseca-Guimaraes, M. Foote, A. McCluskey, P. J. Robinson, I. H. Frazer, N. A. Saunders, R. G. Parton, R. Dolcetti, K. Cuff, J. H. Martin, B. Panizza, E. Walpole, J. W. Wells, and F. Simpson. 2020. Endocytosis Inhibition in Humans to Improve Responses to ADCC-Mediating Antibodies. *Cell* 180 (5):895-914.e827.

Colebatch, H. J., and C. K. Ng. 1992. Estimating alveolar surface area during life. *Respir Physiol* 88 (1-2):163-170.

Collins, L. M., and C. Dawes. 1987. The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *J Dent Res* 66 (8):1300-1302.

Colson, P., J. M. Rolain, and D. Raoult. 2020. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. *Int J Antimicrob Agents* 55 (3):105923.

Contini, A. 2020. *Virtual Screening of an FDA Approved Drugs Database on Two COVID-19 Coronavirus Proteins*.

Dana, D., S. V. Gadhiya, L. G. St Surin, D. Li, F. Naaz, Q. Ali, L. Paka, M. A. Yamin, M. Narayan, I. D. Goldberg, and P. Narayan. 2018. Deep Learning in Drug Discovery and Medicine; Scratching the Surface. *Molecules* 23 (9).

del Rio, C., and P. N. Malani. 2020. 2019 Novel Coronavirus—Important Information for Clinicians. *JAMA*.

Devaux, C. A., J. M. Rolain, P. Colson, and D. Raoult. 2020. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int J Antimicrob Agents*:105938.

Donoghue, M., F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, M. Donovan, B. Woolf, K. Robison, R. Jeyaseelan, R. E. Breitbart, and S. Acton. 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation research* 87 (5):E1-E9.

Fan, H. H., L. Q. Wang, W. L. Liu, X. P. An, Z. D. Liu, X. Q. He, L. H. Song, and Y. G. Tong. 2020. Repurposing of clinically approved drugs for treatment of coronavirus disease 2019 in a 2019-novel coronavirus (2019-nCoV) related coronavirus model. *Chin Med J (Engl)*.

Fang, L., G. Karakiulakis, and M. Roth. 2020. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *The Lancet Respiratory Medicine*.

Hamming, I., W. Timens, M. L. Bulthuis, A. T. Lely, G. Navis, and H. van Goor. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 203 (2):631-637.

Hoffmann, M., H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N. H. Wu, A. Nitsche, M. A. Müller, C. Drosten, and S. Pöhlmann. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*.

Hu, T. Y., M. Frieman, and J. Wolfram. 2020. Insights from nanomedicine into chloroquine efficacy against COVID-19. *Nature Nanotechnology*.

Huang, I. C., B. J. Bosch, F. Li, W. Li, K. H. Lee, S. Ghiran, N. Vasilieva, T. S. Dermody, S. C. Harrison, P. R. Dormitzer, M. Farzan, P. J. Rottier, and H. Choe. 2006. SARS coronavirus, but not human

coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. *J Biol Chem* 281 (6):3198-3203.

Imai, Y., K. Kuba, S. Rao, Y. Huan, F. Guo, B. Guan, P. Yang, R. Sarao, T. Wada, H. Leong-Poi, M. A. Crackower, A. Fukamizu, C.-C. Hui, L. Hein, S. Uhlig, A. S. Slutsky, C. Jiang, and J. M. Penninger. 2005. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436 (7047):112-116.

Jia, H. P., D. C. Look, L. Shi, M. Hickey, L. Pewe, J. Netland, M. Farzan, C. Wohlford-Lenane, S. Perlman, and P. B. McCray, Jr. 2005. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol* 79 (23):14614-14621.

Kawase, M., K. Shirato, L. van der Hoek, F. Taguchi, and S. Matsuyama. 2012. Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. *J Virol* 86 (12):6537-6545.

Kirchdoerfer, R., N. , C. A. Cottrell, N. Wang, J. Pallesen, H. M. Yassine, H. Turner, K. S. Corbett, B. S. Graham, J. S. Mclellan, and A. B. Ward. 2016. Pre-fusion structure of a human coronavirus spike protein. *Nature* 531 (7592):118.

Kirchdoerfer, R. N., N. Wang, J. Pallesen, D. Wrapp, H. L. Turner, C. A. Cottrell, K. S. Corbett, B. S. Graham, J. S. McLellan, and A. B. Ward. 2018. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Sci Rep* 8 (1):15701.

Kuba, K., Y. Imai, and J. M. Penninger. 2006. Angiotensin-converting enzyme 2 in lung diseases. *Curr Opin Pharmacol* 6 (3):271-276.

Kuba, K., Y. Imai, S. Rao, H. Gao, F. Guo, B. Guan, Y. Huan, P. Yang, Y. Zhang, W. Deng, L. Bao, B. Zhang, G. Liu, Z. Wang, M. Chappell, Y. Liu, D. Zheng, A. Leibbrandt, T. Wada, A. S. Slutsky, D. Liu, C. Qin, C. Jiang, and J. M. Penninger. 2005. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 11 (8):875-879.

Kubo, H., Y. K. Yamada, and F. Taguchi. 1994. Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. *Journal of virology* 68 (9):5403-5410.

Letko, M., A. Marzi, and V. Munster. 2020. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*.

Letko, M., K. Miazgowicz, R. McMinn, S. N. Seifert, I. Sola, L. Enjuanes, A. Carmody, N. van Doremalen, and V. Munster. 2018. Adaptive Evolution of MERS-CoV to Species Variation in DPP4. *Cell Rep* 24 (7):1730-1737.

Li, F. 2013. Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral research* 100 (1):246-254.

———. 2016. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annual review of virology* 3 (1):237-261.

Li, F., W. Li, M. Farzan, and S. C. Harrison. 2005. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 309 (5742):1864-1868.

Li, Q., X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, R. Ren, K. S. M. Leung, E. H. Y. Lau, J. Y. Wong, X. Xing, N. Xiang, Y. Wu, C. Li, Q. Chen, D. Li, T. Liu, J. Zhao, M. Liu, W. Tu, C. Chen, L. Jin, R. Yang, Q. Wang, S. Zhou, R. Wang, H. Liu, Y. Luo, Y. Liu, G. Shao, H. Li, Z. Tao, Y. Yang, Z. Deng, B. Liu, Z. Ma, Y. Zhang, G. Shi, T. T. Y. Lam, J. T. Wu, G. F. Gao, B. J. Cowling, B. Yang, G. M. Leung, and Z. Feng. 2020a. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. *Nature*.

Li, W., R. J. G. Hulswit, I. Widjaja, V. S. Raj, R. McBride, W. Peng, W. Widagdo, M. A. Tortorici, B. van Dieren, Y. Lang, J. W. M. van Lent, J. C. Paulson, C. A. M. de Haan, R. J. de Groot, F. J. M. van Kuppeveld, B. L. Haagmans, and B.-J. Bosch. 2017a. Identification of sialic acid-binding function

for the Middle East respiratory syndrome coronavirus spike glycoprotein. 114 (40):E8508-E8517.

Li, W., M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, T. C. Greenough, H. Choe, and M. Farzan. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426 (6965):450-454.

Li, X. C., J. Zhang, and J. L. Zhuo. 2017b. The vasoprotective axes of the renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol Res* 125 (Pt A):21-38.

Li, Z., X. Li, Y.-Y. Huang, Y. Wu, L. Zhou, R. Liu, D. Wu, L. Zhang, H. Liu, X. Xu, Y. Zhang, J. Cui, X. Wang, and H.-B. Luo. 2020b. FEP-based screening prompts drug repositioning against COVID-19.2020.2003.2023.004580.

Lv, X., Z. Li, J. Guan, S. Hu, J. Zhang, Y. Lan, K. Zhao, H. Lu, D. Song, H. He, F. Gao, and W. He. 2019. Porcine Hemagglutinating Encephalomyelitis Virus Activation of the Integrin α5β1-FAK-Cofilin Pathway Causes Cytoskeletal Rearrangement To Promote Its Invasion of N2a Cells. *J Virol* 93 (5).

Menachery, V. D., B. L. Yount, Jr., K. Debbink, S. Agnihothram, L. E. Gralinski, J. A. Plante, R. L. Graham, T. Scobey, X. Y. Ge, E. F. Donaldson, S. H. Randell, A. Lanzavecchia, W. A. Marasco, Z. L. Shi, and R. S. Baric. 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* 21 (12):1508-1513.

Milanetti, E., M. Miotto, L. Di Rienzo, M. Monti, G. Gosti, and G. Ruocco. 2020. In-Silico evidence for two receptors based strategy of SARS-CoV-2.2020.2003.2024.006197.

Millet, J. K., and G. R. Whittaker. 2015. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus research* 202:120-134.

Mirabito Colafella, K. M., D. M. Bovée, and A. H. J. Danser. 2019. The renin-angiotensin-aldosterone system and its therapeutic targets. *Experimental eye research* 186:107680-107680.

Müller, C., M. Hardt, D. Schwudke, B. W. Neuman, S. Pleschka, and J. Ziebuhr. 2018. Inhibition of Cytosolic Phospholipase A(2)α Impairs an Early Step of Coronavirus Replication in Cell Culture. *J Virol* 92 (4).

Nash, T. C., and M. J. Buchmeier. 1997. Entry of mouse hepatitis virus into cells by endosomal and nonendosomal pathways. *Virology* 233 (1):1-8.

Natesh, R., S. L. U. Schwager, E. D. Sturrock, and K. R. Acharya. 2003. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature* 421 (6922):551-554.

Park, Y. J., A. C. Walls, Z. Wang, M. M. Sauer, W. Li, M. A. Tortorici, B. J. Bosch, F. DiMaio, and D. Veesler. 2019. Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* 26 (12):1151-1157.

Patel, A. B., and A. Verma. 2020. COVID-19 and Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers: What Is the Evidence? *JAMA*.

Prabakaran, P., X. Xiao, and D. S. Dimitrov. 2004. A model of the ACE2 structure and function as a SARS-CoV receptor. *Biochem Biophys Res Commun* 314 (1):235-241.

Pu, Y., and X. Zhang. 2008. Mouse hepatitis virus type 2 enters cells through a clathrin-mediated endocytic pathway independent of Eps15. *J Virol* 82 (16):8112-8123.

Qian, Z., E. A. Travanty, L. Oko, K. Edeen, A. Berglund, J. Wang, Y. Ito, K. V. Holmes, and R. J. Mason. 2013. Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome-coronavirus. *Am J Respir Cell Mol Biol* 48 (6):742-748.

Schouten, L. R., H. J. Helmerhorst, G. T. Wagenaar, T. Haltenhof, R. Lutter, J. J. Roelofs, J. B. van Woensel, A. H. van Kaam, A. P. Bos, M. J. Schultz, T. Walther, and R. M. Wösten-van Asperen. 2016. Age-Dependent Changes in the Pulmonary Renin-Angiotensin System Are Associated With Severity of Lung Injury in a Model of Acute Lung Injury in Rats. *Crit Care Med* 44 (12):e1226-e1235.

Senior, A. W., R. Evans, J. Jumper, J. Kirkpatrick, L. Sifre, T. Green, C. Qin, A. Žídek, A. W. R. Nelson, A. Bridgland, H. Penedones, S. Petersen, K. Simonyan, S. Crossan, P. Kohli, D. T. Jones, D. Silver, K. Kavukcuoglu, and D. Hassabis. 2020. Improved protein structure prediction using potentials from deep learning. *Nature* 577 (7792):706-710.

Shen, L. W., H. J. Mao, Y. L. Wu, Y. Tanaka, and W. Zhang. 2017. TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie* 142:1-10.

Shi, S., M. Qin, B. Shen, Y. Cai, T. Liu, F. Yang, W. Gong, X. Liu, J. Liang, Q. Zhao, H. Huang, B. Yang, and C. Huang. 2020. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA Cardiology*.

Singh, A. 2020. Deep learning 3D structures. *Nature Methods* 17 (3):249-249.

Song, W., M. Gui, X. Wang, and Y. Xiang. 2018. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. *PLoS pathogens* 14 (8):e1007236-e1007236.

Stark, R., M. Grzelak, and J. Hadfield. 2019. RNA sequencing: the teenage years. *Nat Rev Genet* 20 (11):631-656.

Stoermer, M. 2020. *Homology Models of the Papain-Like Protease PLpro from Coronavirus 2019-nCoV*.

Struck, A.-W., M. Axmann, S. Pfefferle, C. Drosten, and B. Meyer. 2012. A hexapeptide of the receptor-binding domain of SARS corona virus spike protein blocks viral entry into host cells via the human receptor ACE2. *Antiviral research* 94 (3):288-296.

Su, S., G. Wong, W. Shi, J. Liu, A. C. K. Lai, J. Zhou, W. Liu, Y. Bi, and G. F. Gao. 2016. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends in microbiology* 24 (6):490-502.

Sun, Y., Z. Wang, J. Tao, Y. Wang, A. Wu, Z. Yang, K. Wang, L. Shi, Y. Chen, and D. Guo. 2014. Yeast-based assays for the high-throughput screening of inhibitors of coronavirus RNA cap guanine-N7-methyltransferase. *Antiviral Res* 104:156-164.

Supekar, V. M., C. Bruckmann, P. Ingallinella, E. Bianchi, A. Pessi, and A. Carfí. 2004. Structure of a proteolytically resistant core from the severe acute respiratory syndrome coronavirus S2 fusion protein. *Proc Natl Acad Sci U S A* 101 (52):17958-17963.

Tan, W. S. D., W. Liao, S. Zhou, D. Mei, and W.-S. F. Wong. 2018. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. *Current opinion in pharmacology* 40:9-17.

Touret, F., and X. de Lamballerie. 2020. Of chloroquine and COVID-19. *Antiviral Res* 177:104762.

Wan, Y., J. Shang, R. Graham, R. S. Baric, and F. Li. 2020. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol* 94 (7).

Wang, J. 2020. *Fast Identification of Possible Drug Treatment of Coronavirus Disease -19 (COVID-19) Through Computational Drug Repurposing Study*.

Wang, L., B.-B. Bao, G.-Q. Song, C. Chen, X.-M. Zhang, W. Lu, Z. Wang, Y. Cai, S. Li, S. Fu, F.-H. Song, H. Yang, and J.-G. Wang. 2017. Discovery of unsymmetrical aromatic disulfides as novel inhibitors of SARS-CoV main protease: Chemical synthesis, biological evaluation, molecular docking and 3D-QSAR study. *European Journal of Medicinal Chemistry* 137:450-461.

Wardah, W., M. G. M. Khan, A. Sharma, and M. A. Rashid. 2019. Protein secondary structure prediction using neural networks and deep learning: A review. *Comput Biol Chem* 81:1-8.

Warner, F. J., A. I. Smith, N. M. Hooper, and A. J. Turner. 2004. Angiotensin-converting enzyme-2: a molecular and cellular perspective. *Cellular and molecular life sciences : CMLS* 61 (21):2704-2713.

Wells, S. A. 2020. Rigidity, normal modes and flexible motion of a SARS-CoV-2 (COVID-19) protease structure.2020.2003.2010.986190.

Werner, C. T., C. J. Williams, M. R. Fermelia, D.-T. Lin, and Y. Li. 2019. Circuit Mechanisms of Neurodegenerative Diseases: A New Frontier With Miniature Fluorescence Microscopy. 13 (1174).

Wiener, R. S., Y. X. Cao, A. Hinds, M. I. Ramirez, and M. C. Williams. 2007. Angiotensin converting enzyme 2 is primarily epithelial and is developmentally regulated in the mouse lung. *J Cell Biochem* 101 (5):1278-1291.

Wösten-van Asperen, R. M., R. Lutter, P. A. Specht, G. N. Moll, J. B. van Woensel, C. M. van der Loos, H. van Goor, J. Kamilic, S. Florquin, and A. P. Bos. 2011. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. *J Pathol* 225 (4):618-627.

Wrapp, D., and J. S. McLellan. 2019. The 3.1-Angstrom Cryo-electron Microscopy Structure of the Porcine Epidemic Diarrhea Virus Spike Protein in the Prefusion Conformation. *J Virol* 93 (23).

Wrapp, D., N. Wang, K. S. Corbett, J. A. Goldsmith, C. L. Hsieh, O. Abiona, B. S. Graham, and J. S. McLellan. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367 (6483):1260-1263.

Wu, C., X. Chen, Y. Cai, J. a. Xia, X. Zhou, S. Xu, H. Huang, L. Zhang, X. Zhou, C. Du, Y. Zhang, J. Song, S. Wang, Y. Chao, Z. Yang, J. Xu, X. Zhou, D. Chen, W. Xiong, L. Xu, F. Zhou, J. Jiang, C. Bai, J. Zheng, and Y. Song. 2020. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Internal Medicine*.

Wu, K., W. Li, G. Peng, and F. Li. 2009. Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences of the United States of America* 106 (47):19970-19974.

Wu, Z., and J. M. McGoogan. 2020. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA*.

Xu, H., L. Zhong, J. Deng, J. Peng, H. Dan, X. Zeng, T. Li, and Q. Chen. 2020a. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci* 12 (1):8.

Xu, X., P. Chen, J. Wang, J. Feng, H. Zhou, X. Li, W. Zhong, and P. Hao. 2020b. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* 63 (3):457-460.

Yan, R., Y. Zhang, Y. Li, L. Xia, Y. Guo, and Q. Zhou. 2020. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2.eabb2762.

Young, B. E., S. W. X. Ong, S. Kalimuddin, J. G. Low, S. Y. Tan, J. Loh, O.-T. Ng, K. Marimuthu, L. W. Ang, T. M. Mak, S. K. Lau, D. E. Anderson, K. S. Chan, T. Y. Tan, T. Y. Ng, L. Cui, Z. Said, L. Kurupatham, M. I.-C. Chen, M. Chan, S. Vasoo, L.-F. Wang, B. H. Tan, R. T. P. Lin, V. J. M. Lee, Y.-S. Leo, D. C. Lye, and f. t. S. N. C. O. R. Team. 2020. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA*.

Yuan, Y., D. Cao, Y. Zhang, J. Ma, J. Qi, Q. Wang, G. Lu, Y. Wu, J. Yan, Y. Shi, X. Zhang, and G. F. Gao. 2017. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nature Communications* 8 (1):15092.

Zhang, L., D. Lin, X. Sun, U. Curth, C. Drosten, L. Sauerhering, S. Becker, K. Rox, and R. Hilgenfeld. 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors.eabb3405.

Zhao, Y., Z. Zhao, Y. Wang, Y. Zhou, Y. Ma, and W. Zuo. 2020. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov.2020.2001.2026.919985.

 doi:10.20944/preprints202004.0087.v1

Zhou, F., T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen, and B. Cao. 2020a. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*.

Zhou, P., X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, H.-D. Chen, J. Chen, Y. Luo, H. Guo, R.-D. Jiang, M.-Q. Liu, Y. Chen, X.-R. Shen, X. Wang, X.-S. Zheng, K. Zhao, Q.-J. Chen, F. Deng, L.-L. Liu, B. Yan, F.-X. Zhan, Y.-Y. Wang, G.-F. Xiao, and Z.-L. Shi. 2020b. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*:10.1038/s41586-41020-42012-41587.

Zhou, Y., P. Vedantham, K. Lu, J. Agudelo, R. Carrion, Jr., J. W. Nunneley, D. Barnard, S. Pöhlmann, J. H. McKerrow, A. R. Renslo, and G. Simmons. 2015. Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res* 116:76-84.

Zou, Z., Y. Yan, Y. Shu, R. Gao, Y. Sun, X. Li, X. Ju, Z. Liang, Q. Liu, Y. Zhao, F. Guo, T. Bai, Z. Han, J. Zhu, H. Zhou, F. Huang, C. Li, H. Lu, N. Li, D. Li, N. Jin, J. M. Penninger, and C. Jiang. 2014. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nature Communications* 5 (1):3594.