



Drug Weaponry to Fight Against SARS-CoV-2

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The current outbreak of SARS-CoV-2 virus has caused a large increase in mortality and morbidity associated with respiratory diseases. Huge efforts are currently ongoing to develop a vaccine against this virus. However, alternative approaches could be considered in the fight against this disease. Among other strategies, structural-based drug design could be an effective approach to generate specific molecules against SARS-CoV-2, thus reducing viral burden in infected patients. Here, in addition to this structural approach, we also revise several therapeutic strategies to fight against this viral threat. Furthermore, we report ACE-2 genetic polymorphic variants affecting residues involved in close contacts with SARS-CoV-2 that might be associated to different infection risks. These analyses could provide valuable information to predict the course of the disease.

Keywords: coronavirus, SARS-CoV-2, Covid-19, spike, drugs, ACE-2, polymorphisms

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INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have been responsible for major outbreaks, causing the death of hundreds of patients (Ksiazek et al., 2003; Kuiken et al., 2003; Rota et al., 2003; Anderson et al., 2004; Assiri et al., 2013; De Wit et al., 2016; Song et al., 2019). The current pandemic spread of SARS-CoV-2 virus, responsible for the coronavirus disease 2019 (Covid-19), has driven health systems around the world to the edge of collapse. Covid-19 has a lower lethality rate than SARS and MERS, which emerged in 2002 and 2012, respectively. However, and precisely because of its lower fatality, SARS-CoV-2 virus has widely spread around the world. The rate of infection by this virus is superior to other common respiratory viruses, such as influenza, with a larger basic reproductive number (R_0), and a higher case fatality rate (CFR). Nonetheless, it is not possible to know exactly the infective and fatality rate numbers, as it is very likely that there are many undetected, infected people. What is certain is that the world is in a state of emergency in the fight against this disease. Several initiatives are currently on the way with special focus on development of vaccines to protect populations against this infection.

Vaccines are the main tool to prevent the spread of the infection in the long term. Unfortunately, they have little impact in patients already infected with the virus. Therefore, therapeutic drugs should be found to treat infected patients at hospitals. Several drugs are under consideration to deal with the disease, although most of them have not been specifically designed to target this virus. In the battery of available therapeutic drugs we can find antivirals, such as remdesivir, lopinavir, ritonavir, ribavirin, and fapiravir. Molecules affecting interactions of SARS-CoV-2 with different cell partners are also being used in clinical setups, albeit with no clear benefits. Some examples

are the anti-paludic chloroquine (Colson et al., 2020; Gao et al., 2020) and its derivative hydroxy-chloroquine (Liu et al., 2020) combined with azithromycin (Gautret et al., 2020), a broad-spectrum antibiotic. However, several studies question the effectiveness of chloroquine, and articles on this matter have been retracted (Mehra et al., 2020a,b). Recently, the WHO has recommended against the use of high doses of chloroquine for treatment of Covid-19.

A recent high-throughput mass spectrometry analysis found that SARS-CoV-2 interacts with multiple cellular systems (DNA replication, host translation machinery, RNA processing and replication, innate immune system pathways, vesicle trafficking, lipid modification, nuclear transport, mitochondria, etc.) (Gordon et al., 2020). Therefore, instead of trying to develop new drugs, some trials are underway to test compounds already approved for human use, in the hope of finding one that might affect those interactions. This strategy would try to stop the cell from making new viral particles out of viral RNA, thus preventing virus translation (Gordon et al., 2020). However, most of the efforts are focused on the viral spike protein that SARS-CoV-2 uses to infect cells, in a strategy that seeks to disrupt the virus entry. SARS-CoV-2 binds to the angiotensin conferring enzyme receptor (ACE-2) with high affinity, via the spike protein (Hoffmann et al., 2020; Wrapp et al., 2020). Therefore, the use of antibodies to neutralize the binding of the virus to ACE-2 has also been considered in the treatment of the disease (Kruse, 2020), as well as the use of peptides that mimic the interaction between the virus and ACE-2. Thus, molecules that could prevent the binding of the virus to ACE-2 would be of great interest to treat this disease. Here, we describe in detail the interactions between SARS-CoV-2 and ACE-2, and the main therapeutic strategies that are in consideration for the treatment of Covid-19 to inhibit virus entry or virus translation, but with a particular emphasis on the viral spike protein, since most of the efforts are focused on this target.

CORONAVIRUS STRUCTURE

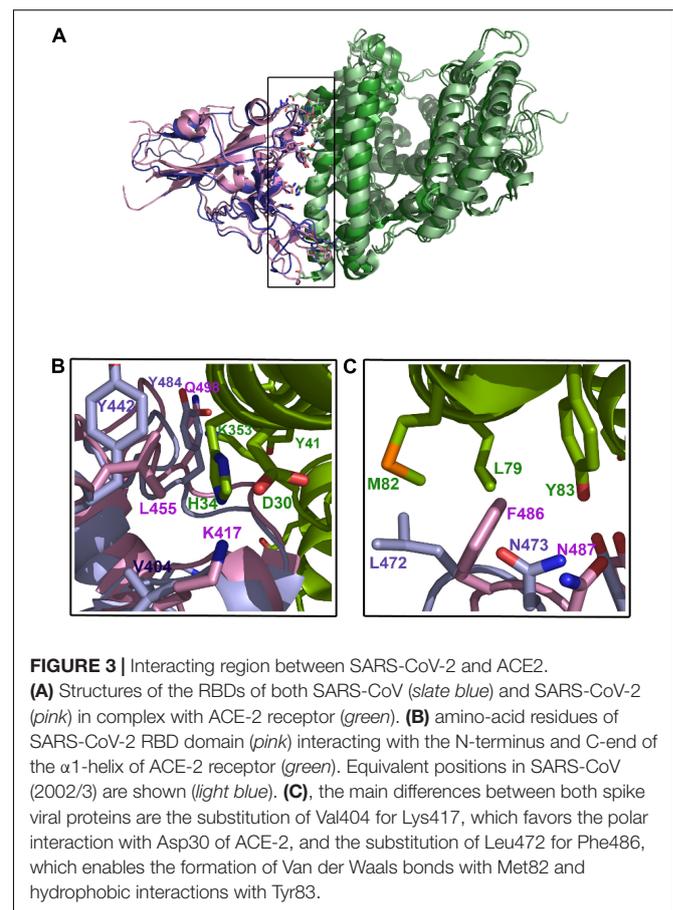
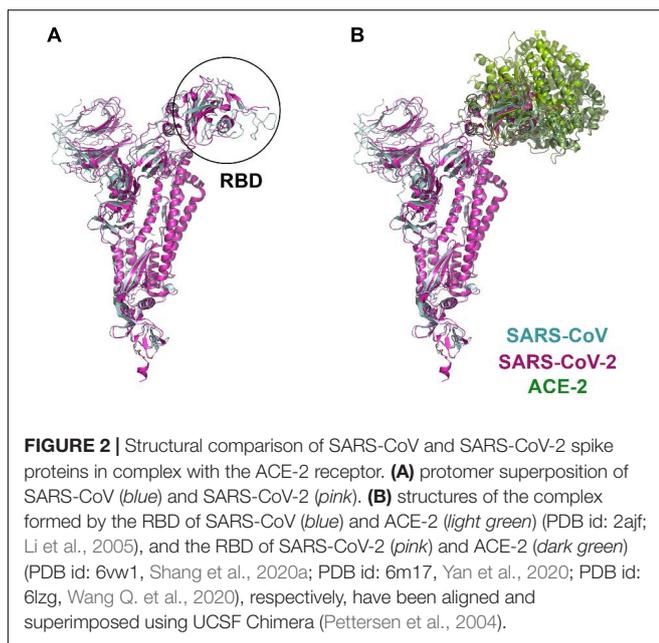
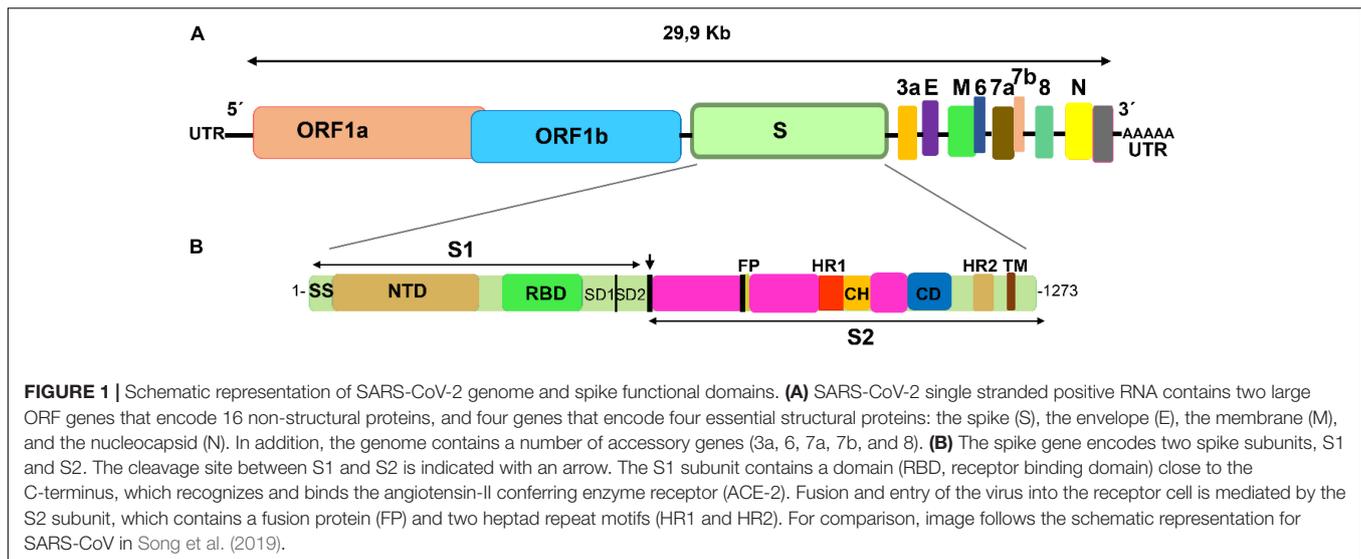
Coronaviridae is a family comprising several viral species (Lai et al., 2007; Adams and Carstens, 2012), seven of which are able to infect human cells (the *alphacoronavirus* HCoV-229E, HCoV-NL63, and the *betacoronavirus* HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) (Kin et al., 2015; Su et al., 2016). These pleomorphic membrane-enveloped viruses consist of a positive sense RNA molecule and four essential structural proteins: M (the most abundant glycoprotein in the membrane), E (an envelope small membrane protein), N (a nucleocapsid protein), and S (the spike protein, which is also a membrane glycoprotein) (Figure 1). The spike glycoproteins (S) form homotrimers that decorate the viruses (Du et al., 2009; Kirchdoerfer et al., 2016; Walls et al., 2016; Wrapp et al., 2020; Yan et al., 2020). The spike protein is essential for binding the receptor and for its entry into the infected cell (Gallagher and Buchmeier, 2001; Bosch et al., 2003; Li et al., 2006). During the course of infection the S protein is cleaved by host proteases in two fragments, the S1 and S2 subunits, which remain non-covalently bound in the prefusion conformation

(Bosch et al., 2003; Belouzard et al., 2009; Millet and Whittaker, 2014; Walls et al., 2020). The S1 subunit contains a Receptor Binding Domain (RBD) which interacts with the cell receptor (Babcock et al., 2004; Li, 2015), whereas the S2 subunit acts in the fusion and entry into the cell (Walls et al., 2020). The S2 subunit is a multidomain protein consisting of a cytoplasmic domain, a transmembrane span, a fusion peptide, and two heptad repeats (HR1 and HR2) (Bosch et al., 2004; Liu et al., 2004; Xia et al., 2020b). These two heptad repeats oligomerize into a six-helix bundle fusion core, which is essential for viral integrity and infectivity. There has been much effort in developing peptides based on HR1 and HR2 structures to prevent infection by these viruses, mainly for MERS-CoV and HCoV-229E, with promising results (Gao et al., 2013; Lu et al., 2014; Sun et al., 2017; Xia et al., 2018). However, the entry pathway of MERS-CoV into the cell, mediated by the DPP4 receptor (Meyerholz et al., 2016), is different from that used by both SARS viruses, SARS-CoV and SARS-CoV-2, which bind to the ACE-2 receptor. Therefore, these peptides may be helpless in the current outbreak.

INTERACTIONS OF THE SPIKE PROTEIN WITH THE ACE-2 RECEPTOR

The spike S1 glycoprotein of SARS like viruses interacts very strongly with ACE-2, a protein receptor involved in the maturation of angiotensin, an essential peptide in vascular homeostasis (Donoghue et al., 2000; Crackower et al., 2002). ACE-2 receptor is a membrane protein, consisting of an N-terminal domain, named PD, and a C-terminal Collectrin-like domain (CLD) (Zhang et al., 2001). Structures of the ACE-2 PD domain in complex with SARS-CoV receptor domain (RBD) have been published (Li et al., 2005, PDB id: 2AJF). Recently, the atomic structure of the spike glycoprotein of SARS-CoV-2 has been determined (Wrapp et al., 2020, PDB id: 6VSB; Walls et al., 2020, PDB id: 6VXX and 6VYB; **Supplementary Figure S1**). Moreover, the structure of the complex formed between the RBD of the viral spike and ACE-2 receptor has also been solved (Shang et al., 2020b, PDB id: 6VW1; Yan et al., 2020, PDB id: 6M17 and 6M18; Wang Q. et al., 2020, PDB id: 6LZG; **Figure 2**). Despite the high degree of homology shared by the spike glycoproteins of SARS-CoV and SARS-CoV-2 viruses, monoclonal antibodies directed against the SARS-CoV of 2002/3 virus were not effective against the new SARS, revealing important differences between the two spike viral proteins (Wrapp et al., 2020).

Sequence comparison between the SARS-CoV and SARS-CoV-2 spike proteins revealed 76% identity (**Supplementary Figure S2**). The main sequence variations are precisely in the RBD domain involved in ACE-2 interaction. Although the interface between ACE-2 and SARS-CoV-2 (Yan et al., 2020) is similar to that of ACE-2 with SARS-CoV (Li et al., 2005), a close inspection of this area reveals some important differences (Wang Y. et al., 2020). Among these, substitutions of Asn⁴⁷⁹ and Thr⁴⁸⁷ in SARS-CoV for Gln⁴⁹³ and Asn⁵⁰¹ in SARS-CoV-2, respectively, seem to be particularly relevant, as they are key residues in the interaction with the ACE-2 receptor. Up to six other modifications in SARS-CoV-2 are present in



this region (Arg⁴²⁶ → Asn⁴³⁹, Tyr⁴⁴² → Leu⁴⁵⁵, Leu⁴⁴³ → Phe⁴⁵⁶, Phe⁴⁶⁰ → Tyr⁴⁷³, Leu⁴⁷² → Phe⁴⁸⁶, and Tyr⁴⁸⁴ → Gln⁴⁹⁸; SARS-CoV → SARS-CoV-2 mutations) (Supplementary Figure S3). In addition to these substitutions, it is worth noting the change of Val⁴⁰⁴ in SARS-CoV for Lys⁴¹⁷ in SARS-CoV-2 (Figure 3). The introduction of a positive charge in this position contributes to the formation of a salt bridge with residue Asp³⁰ in ACE-2 (Yan et al., 2020). Likewise, the change of Leu⁴⁷² for Phe⁴⁸⁶ seems to favor the formation of strong Van der Waals interactions with Met⁸² in the receptor (Yan et al., 2020). Other residues in this region of interaction between SARS-CoV and SARS-CoV-2 with ACE-2 are well conserved. Overall, these modifications seem to increase the stability of the complex formed between the RBD of SARS-CoV-2 and ACE-2 receptor, which is in agreement with the

high affinity ($K_d = 15$ nM) reported for the binding between both proteins (Wrapp et al., 2020). This extraordinary affinity could explain the high rates of infection observed in Covid-19.

A recent analysis of 5,349 SARS-CoV-2 genomes (Phelan et al., 2020) has found numerous SNPs (Single Nucleotide

Polymorphisms), although at low frequencies. Only three mutations are more prevalent, two mutations in the nucleocapsid protein and one in the spike protein (D614G). This D614G mutation has been suggested to increase the morbidity and mortality of the disease (Eaaswarkhanth et al., 2020; Korber et al., 2020). In any case, despite this mutation, the spike protein is relatively stable, which is good news for the development of vaccines. Nonetheless, a close inspection of further mutations is paramount in order to prevent failures in the design of a vaccine.

Finally, another point to consider is the glycosylation of the spike protein, since it could facilitate the hiding of specific epitopes, which in turn would neutralize antibody recognition by the immune system. Glycosylation has been described for a number of coronaviruses (Walls et al., 2019; Yang et al., 2020). A recent site-specific mass spectrometry analysis (Watanabe et al., 2020) has enabled mapping of the glycosylation sites on the viral SARS-CoV-2 spike. These viral glycans were found to be different from typical host glycans. They are mainly oligomannosides and complex fucoside-derived sugars localized in 22 different sites on the spike protein (Watanabe et al., 2020). It is not clear yet how this glycosylation pattern affects the interaction with the ACE-2 receptor but this type of study could provide a framework to develop future glycoprotein-based vaccines.

ACE-2 GENETIC VARIANTS THAT MIGHT BE ASSOCIATED WITH A DIFFERENT RISK FOR COVID-19 OUTCOME

Recently, it has been shown that ACE-2 expression levels may be critical for the susceptibility and outcome of Covid-19 (Cao Y. et al., 2020). In a systematic analysis of ACE-2 coding-region variants and expression quantitative trait loci (eQTL) variants, the authors found that East Asian populations have increased allele frequencies in the eQTL variants associated with high ACE-2 expression, suggesting an enlarged susceptibility to SARS-CoV and SARS-CoV-2 infection. However, no clear evidence of resistant mutations for coronavirus spike-protein binding was detected (ChinaMAP and 1KGP databases). In this context, a genetic analysis for coding variants affecting ACE-2 expression is of particular interest, since ACE2 gene polymorphisms and ACE2 mRNA expression might influence the disease outcome (Devaux et al., 2020).

Genetic variants in the ACE-2 receptor affecting the interaction with the spike protein might be associated with a different risk of SARS-CoV-2 infection. The ACE-2 gene is associated with 6634 variant alleles (Ensembl GRCh38.p13), and three of them are missense variants that affect three essential residues involved in close contacts between the RBD of the spike protein and the ACE-2 receptor (**Table 1**). The missense variants E37K and E329G would affect H-bonds and essential polar contacts with residues of the spike protein, whereas M82I variant could have a bearing on the van der Waals interactions established with a leucine or a phenylalanine in SARS-CoV and SARS-CoV-2 viruses, respectively. In particular, molecular modelling of E329G variant showed noticeable variations in the

interactions with the viral spike protein (Hussain et al., 2020). Although these are very low-frequency missense variants, found in the gnomAD database (MAF < 0.01), it might be interesting to investigate whether they are associated with a lower risk of infection, corresponding to that percentage of the population that shows very weak symptoms or are practically asymptomatic.

The ACE-2 receptor forms a dimer, which can fluctuate between two conformations, “open” and “closed” (Towler et al., 2004; Yan et al., 2020). However, when ACE-2 is bound to the RBD of the spike protein, only the closed state is present, which suggests that residues stabilizing the dimer interface might be also essential for virus infection. **Table 2** shows genetic variants in the ACE-2 receptor affecting essential residues involved in dimerization. Missense variants affecting this interface might also be associated to a lower risk of SARS-CoV-2 infection.

In addition, recent studies on the biology of viral infection also indicate that there might be a sex predisposition to develop Covid-19, with men more prone to being infected (Cai, 2020; Ciaglia et al., 2020; Guan et al., 2020). It is worth noting that the ACE-2 gene is located on the X chromosome and, therefore, allele variants associated to an increased risk of infection would affect women and men differently. As ACE-2 genetic variants are at different frequencies in the general population, it might be worthwhile investigating whether they confer a genetic predisposition for a different risk of infection. This knowledge would be of great interest to improve disease management, but additional investigation will be required to confirm such a putative association.

THERAPEUTIC STRATEGIES

Immunotherapy

Antibodies that recognize the epitope domain of previous SARS-CoV have been reported (Sui et al., 2004; Ter Meulen et al., 2004; Qiu et al., 2005; van den Brink et al., 2005), as well as monoclonal antibodies (m396 and S230.15) that neutralize SARS-CoV/ACE2 interaction (Zhu et al., 2007). Some of these antibodies (m396) bind to the RBD of the viral spike with high affinity (Prabakaran et al., 2006), thus preventing the binding of the virus to ACE-2. Unfortunately, none of these antibodies were able to recognize SARS-CoV-2 (Wrapp et al., 2020). Therefore, production of specific monoclonal antibodies against SARS-CoV-2 should be a priority. A cryptic epitope highly conserved in the RBD of SARS-CoV and SARS-CoV-2 has been identified (Yuan et al., 2020) and antibodies (CR3022) directed against it have been shown to bind with high affinity *in vitro* (Tian et al., 2020). Generation of antibodies by phage display could also be an alternative strategy. In that sense, antibodies already developed against HR1 and HR2 domains of the S2 spike of SARS-CoV (Elshabrawy et al., 2012) and other viruses (Shin et al., 2019) could provide leads to generate specific antibodies against SARS-CoV-2. However, more research is needed before it can be translated to clinical practice. Other alternative approaches, such as purifying polyclonal antibodies from animals, do not look promising. Passive immunotherapy with antibodies from plasma of already infected people might be an alternative therapy (Walker and Burton, 2018). Unfortunately, the variability

TABLE 1 | ACE-2 missense variants affecting residues involved in SARS-CoV and SARS-CoV-2 spike-protein binding.

ACE-2 variant	Polymorphism	Allele frequency*	Codons	SARS-CoV interaction	SARS-CoV-2 interaction
E37K	rs146676783	9.1×10^{-5}	GAA/AAA	Y491	Y505
M82I	rs766996587	1.4×10^{-4}	ATG/ATT/ATA	L472	F484
E329G	rs143936283	9.1×10^{-5}	GAA/GGA	R426	N439

*Source: Genome aggregation database (gnomAD database).

TABLE 2 | ACE-2 missense variants affecting residues involved in ACE-2 dimerization.

ACE-2 variant	polymorphism	Allele frequency*	Codons	ACE-2 dimer interaction
N638S	rs183135788	1.8×10^{-4}	AAT/AGT	R652, Q653
S709R	rs1052746182	2.8×10^{-5}	AGC/CGC	R716
R710H	rs370187012	4.6×10^{-5}	CGT/CAT	N636
R710C	rs901495523	9.2×10^{-5}	CGT/TGT	N636
R716H	rs200540199	1.4×10^{-4}	CGT/CAT	S709,D713
R716C	rs144869363	2.8×10^{-5}	CGT/TGT	S709,D713

*Source: Genome aggregation database (gnomAD database).

in plasma samples and viral cargo of previously infected people makes this approach less precise and reproducible. Another clinical trial for the treatment of Covid-19 is based on Tocilizumab, a humanized monoclonal antibody commercialized by Roche to treat rheumatoid arthritis, which is being used in Covid-19 infected patients with promising results (Fu et al., 2020; Guo et al., 2020).

Novel strategies that rely on targeting the viral receptor protein in the cell surface, preventing the binding and entry of the virus inside the cell, could be more promising. For instance, fusions of immunoglobulin Fc with a soluble fraction of ACE-2 has been proposed to prevent the binding of the virus to the receptor (Kruse, 2020). As additional benefit, this therapy would supplement ACE-2 levels during infection, and it would boost the immune system to generate lasting immunity (Kruse, 2020).

Another strategy has been to generate fusion proteins containing the extracellular domain of human ACE2 and the Fc region of the human immunoglobulin IgG1 to neutralize SARS-CoV or SARS-CoV-2 spike proteins *in vitro* (Lei et al., 2020). As the authors state, these fusion proteins have potential applications in the diagnosis, prophylaxis, and treatment of SARS-CoV-2.

Peptides That Prevent Interactions Between ACE-2 and SARS-CoV-2

In addition to the generation of antibodies, treatment with antiviral peptides could provide an alternative route of therapy to avoid virus entry. In contrast to small inhibiting molecules, peptides have the advantage of disrupting relatively large protein-protein interfaces. Antiviral peptides were designed against MERS-CoV (Gao et al., 2013; Lu et al., 2014; Sun et al., 2017) and also against SARS-CoV virus in order to disrupt the interaction between the spike protein and the ACE-2 receptor (Hu et al., 2005) or affect the interface of the spike trimer (Zheng et al., 2005). Peptides selected against the S2 spike subunit (Yuan et al., 2004; Xia et al., 2018) and peptides including short sequences of the RBD of SARS-CoV were also proposed (Struck et al., 2012). Recently, and based on the pan-coronavirus virus fusion inhibitor

EK1, developed for SARS-CoV and MERS-CoV (Xia et al., 2019), other lipopeptides, such as EK1C4, have been produced (Xia et al., 2020a), which could be effective in the treatment of Covid-19.

Close inspection of the amino acid substitutions in the interacting region between SARS-CoV-2 spike protein and ACE-2 receptor provides a framework to generate a library of antigenic peptides. These peptides should block the interaction of the virus with target cells. To this respect, some reports have been recently published in which peptides specifically designed to bind SARS-CoV-2 spike protein have been generated (Zhang et al., 2020). These peptides should be able to disrupt SARS-CoV-2 RBD-ACE2 interface. One of these peptides, named SBP1, is a 23-mer molecule including amino acid residues 21–43 from ACE-2 α 1-helix (IEEQAKTFLDKFNHEAEDLFYQS) (**Supplementary Figure S4**). This peptide is able to bind the RBD of SARS-CoV-2 at nanomolar levels (Zhang et al., 2020), but it is unknown if this peptide is tolerated by the immune system.

Interestingly, another short region in ACE-2 involved in closed contacts with the spike can be appreciated (residues 325–354) (**Supplementary Figure S3**), which could be a lead to develop other effective peptides. The affinity or stability of such a putative peptide has not been assayed, but it might be worth testing it, alone or in combination with other peptides.

The use of a soluble fraction of ACE-2, instead of small peptides, to snatch viral particles has been suggested (Batlle et al., 2020). Such soluble ACE-2 recombinant protein has already been used in the treatment of angiotensin II-dependent hypertension (Wysocki et al., 2010), but its potential use in the treatment of Covid-19 is unknown.

Specific Proteases

Another therapeutic alternative is the use of specific proteases. The spike protein of previous SARS-CoV is cleaved by a host transmembrane Type II serine protease TMPRSS2 (Glowacka et al., 2011; Reinke et al., 2017). This cleavage results in the activation of the spike protein, which, in turn, facilitates the entry of the virus into the cell. Thus, development of therapeutic drugs

targeting this protein could also open an alternative route in the treatment of patients (Simmons et al., 2011). In this regard, some serine protease inhibitors targeting TMPRSS2 were proposed (Kawase et al., 2012; Zhou et al., 2015). Recently, it has been shown that SARS-CoV-2 entry into the cells is also mediated not only by ACE-2 but also by TMPRSS2 (Hoffmann et al., 2020). Moreover, this entry is blocked by camostatate mesylate, a TMPRSS2 serine protease inhibitor, which makes this molecule a potential drug to be used in clinical trials (Hoffmann et al., 2020). Endosomal cysteine proteases, like cathepsin B and L (CatB/L), might also be inhibited with compounds such as E64 (Hoffmann et al., 2020). Interestingly, these authors also found that sera from patients infected with SARS-CoV neutralized SARS-CoV-2 entry into the cell.

In silico analyses of putative protease SARS-CoV-2 inhibitors are being used to identify potential therapeutic compounds (Pant et al., 2020). Among the potential inhibitors found in this analysis, there are some already described antivirals, such as lopinavir and ritonavir, and other FDA approved drugs, like cobicistat and darunavir.

Furin-Like Enzymes

Binding and release of viral particles is activated by specific cellular proprotein convertases, such as furin, trypsin, and cathepsin-L (Seidah and Prat, 2012; Millet and Whittaker, 2015; Izaguirre, 2019). Therefore, development of drugs that target the furin-like process mechanisms constitutes another avenue of research. For instance, teicoplanin, an antibiotic used to treat *Staphylococcus* infection, has been found to inhibit cathepsin-L in several coronavirus, including SARS-CoV-2 (Baron et al., 2020). Based on the crystal structure of furin, several inhibitors, like 2,5-dideoxystreptamine-mediated inhibitor, were previously described and tested in clinical trials against SARS-CoV (Dahms et al., 2017). This drug is now a promising candidate for Covid-19 treatment. Some cell furin-like proteases are able to target the spike protein of several coronavirus species, including SARS-CoV-2 (Coutard et al., 2020). Interestingly, the furin cleavage site in SARS-CoV-2 site differs from that present in other coronavirus (Coutard et al., 2020). However, as furin-like molecules are involved in multiple metabolic pathways, any drug targeting these molecules might have serious adverse effects.

Antivirals

Various essential stages in the viral life cycle, such as RNA synthesis, are susceptible to be targeted by drugs. In that sense, several antivirals are being used in the treatment of patients affected by Covid-19 (Table 3). Among them we can find adenosine nucleotide analogs, such as remdesivir, a broad spectrum antiviral agent with activity against a number of different virus, including Ebola virus (Siegel et al., 2017; Tchesnokov et al., 2019) and pathogenic coronavirus SARS-CoV and MERS-CoV (De Wit et al., 2016). Remdesivir inhibits viral RNA polymerase, thus reducing viral load. Remdesivir has been approved by the FDA to be used in the treatment of patients infected with SARS-CoV-2, since compassionate treatment with remdesivir has been reported to improve the outcome in several patients (Grein et al., 2020). Combined treatment of remdesivir

with chloroquine has also been suggested to inhibit SARS-CoV-2 (Wang Y. et al., 2020). Other tested antivirals such as lopinavir, an HIV-1 protease inhibitor which, combined with ritonavir, was used against SARS and MERS (Chu et al., 2004; Chan et al., 2015) has also been tried with Covid-19 patients (Cao B. et al., 2020). Ribavirin, a nucleoside analog developed against influenza and hepatitis C virus (Feld and Hoofnagle, 2005; Te et al., 2007), has also being used in animal models infected with MERS in combination with interferons (IFNs) (Al-Tawfiq et al., 2014), but its use against respiratory diseases could not be advisable, as it reduces hemoglobin concentration (Sheahan et al., 2020). A combination therapy with lopinavir, ritonavir, ribavirin, and interferon α has been proposed as treatment of MERS-CoV infections (Kim et al., 2015). Favipiravir, an RNA dependent RNA polymerase (RdRp) inhibitor (Furuta et al., 2013), has been used against Ebola and Marburg virus (Bixler et al., 2018), influenza (Goldhill et al., 2018), and many other RNA viruses.

However, none of these antivirals were specifically designed against SARS-CoV-2. Therefore, development of specific antivirals against SARS-CoV-2 is a foremost objective.

Other Potential Therapeutic Treatments

An alternative strategy could be the use of small interfering RNA (siRNA) to target directly the viral RNA (Qureshi et al., 2018). However, administration of these molecules to the patients presents practical problems. A similar approach was tried against Ebola and, despite of initial success in preclinical animal tests (Thi et al., 2015), it failed in clinical trials.

Another approach could be to target viral assembly. The formation of the S spike trimer might not be a fast and direct process, so there is a chance to block the quaternary structure of the spike (Bongini et al., 2020). Analysis of protein-protein interfaces of the spike has led to the prediction of ligands (Ergoloid, Darifenacin, 5-methyl-tetrahydrofolic acid, Buclizine, Saquinavir, Solifenacin, Sorafenib, tetrahydrofolic acid) that potentially could affect this process (Bongini et al., 2020).

In addition to the generation of specific peptides directed to neutralize SARS-CoV-2 spike or the action of the serine protease TMPRSS2 already mentioned in the previous section, other targets, such as the viral non-structural proteins (*nsp*) or the accessory proteins (envelope, nucleocapsid, and membrane) could be considered. Coronavirus *nsp*s are relatively well conserved (Totura and Bavari, 2019; Kim et al., 2020). Among these, 3C-like protease (3CLpro), papain-like protease (PLpro), and RNA-dependent RNA polymerase (RdRp) have been suggested to be targets for antiviral drug discovery (Totura and Bavari, 2019). In contrast to the structural and *nsp* proteins, amino acid conservation in accessory proteins is low, which makes them unsuitable drug targets.

A recent mass spectrometry analysis identified 332 putative human targets for therapeutic drugs (Gordon et al., 2020). Screening of over 60 potential leads resulted in two classes of molecules that reduced effectively viral infectivity. These molecules were protein biogenesis inhibitors (zotatifin, ternatin-4, and PS3061) and ligands of Sigma1 and Sigma2 receptors (haloperidol, PB28, PD-144418, and hydroxychloroquine) (Gordon et al., 2020). In addition, clemastine, cloperastine,

TABLE 3 | Drugs proposed for the treatment of SARS-CoV-2 infected patients.

Drug	Target	Virus	References
80R scFv mAb	spike	SARS-CoV	Sui et al., 2004
CR3014 mAb	spike	SARS-CoV	Ter Meulen et al., 2004; van den Brink et al., 2005
CR3006 mAb	spike	SARS-CoV	van den Brink et al., 2005
CR3013 mAb			
CR3015 mAb			
CR3009 mAb	N	SARS-CoV	van den Brink et al., 2005
CR3018 mAb			
CR3022 mAb	spike	SARS-CoV-2	Tian et al., 2020; Yuan et al., 2020
m396 mAb	spike	SARS-CoV	Zhu et al., 2007
S230.15		SARS-CoV-2	
ACE2-Fc/IgG	spike	SARS-CoV-2	Kruse, 2020; Lei et al., 2020
HR1-1, HR2-18	spike	SARS-CoV	Yuan et al., 2004
⁴³⁸ YKYRYL ⁴⁴³	spike	SARS-CoV	Struck et al., 2012
229E-HR1P 229E-HR2P	spike	SARS-CoV	Xia et al., 2018
Cobicistat, Darunavir	spike	SARS-CoV-2	Pant et al., 2020
EK1	spike	SARS-MERS	Xia et al., 2019
EK1C4	spike	SARS-CoV-2	Xia et al., 2020a
Ergoloid, Darifenacin, 5MTH-folic acid, Buclizine, Saquinavir, Solifenacin, Sorafenib	spike	SARS-CoV-2	Bongini et al., 2020
Camostate mesylate	TMPRSS2	SARS-CoV SARS-CoV-2	Kawase et al., 2012; Hoffmann et al., 2020
E64d	CatB/P13pc	SARS-CoV SARS-CoV-2	Kawase et al., 2012; Hoffmann et al., 2020
Glycyrrhizin, Aescin Reserpine	3CLpro	SARS-CoV	Wu et al., 2004
Teicoplanin	CatB/P13pc	SARS-CoV-2	Baron et al., 2020
DDOS inhibitor	Furin	SARS-CoV	Dahms et al., 2017
Remdesivir	viral RNA polymerase	Ebola SARS-CoV-2	Siegel et al., 2017; Tchesnokov et al., 2019 Grein et al., 2020; Wang Y. et al., 2020
Lopinavir/ Ritonavir	3CLpro (nsp)	SARS-CoV MERS SARS-CoV-2	Chu et al., 2004; Wu et al., 2004; Chan et al., 2015; Kim et al., 2015; Cao B. et al., 2020
Ribavirin + Kaletra	RdRp	Influenza HCV MERS SARS-CoV	Feld and Hoofnagle, 2005; Te et al., 2007; Al-Tawfiq et al., 2014; Sheahan et al., 2020; Wang Y. et al., 2020
Favipiravir	RdRp	Ebola/Marburg Influenza SARS-CoV-2	Furuta et al., 2013; Bixler et al., 2018; Goldhill et al., 2018; Wang M. et al., 2020
zotatifin, ternatin-4, PS3061, haloperidol, PB28, clemastine, cloperastine, siramesine	several cell functions	SARS-CoV-2	Gordon et al., 2020
Tocilizumab	IL6 receptors	SARS-CoV-2	Fu et al., 2020; Guo et al., 2020
chloroquine Hydroxy-chloroquine		SARS-CoV-2	Goleson et al., 2020; Gao et al., 2020; Gaudrel et al., 2020; Liu et al., 2020; Meres et al., 2020b

Compounds affecting the entry of the virus into human cells include antibodies (light yellow), small peptides and drugs disrupting binding to the ACE-2 receptor (dark yellow), and specific proteases (salmon). Strategies affecting virus translation involve furin-like enzymes (pink), antivirals (light blue), and other inhibitors affecting essential proteins and cell functions (light green). Chloroquine and derivatives (red), although initially proposed as potential drugs to treat SARS-CoV-2, have been lately dismissed.

siramesine, zotatifin, and progesterone were also identified as potential drugs in this study.

OUTLOOK

Much effort is currently ongoing to find a specific vaccine to protect populations against the threat of SARS-CoV-2. However,

the development of a new vaccine is a long process that will come too late for hundreds of thousands of already infected people. Antiviral therapeutics such as remdesivir, lopinavir, ritonavir, favipiravir, or hydroxy-chloroquine, combined with immunotherapy, could work, and some of them are being used in the front line against the disease. However, new drugs that target specifically the new virus are needed to increase our weaponry in the fight of this pandemic emergency. To this end,

small peptides containing the sequence of the ACE receptor directly involved in SARS-CoV-2 binding might be a promising alternative in the fight against this pandemic emergency. The detailed analysis of ACE-2 spike viral protein interactions carried out in this work suggests putative peptides that might fulfill this requirement, as well as certain ACE2 genetic variants that might be associated with a lower risk of infection. These molecules, combined with antiviral strategies and drugs targeting other mechanisms of viral infection, could provide effective therapies for the management of the disease.

AUTHOR CONTRIBUTIONS

EC and IA wrote the article. IA designed and prepared all the figures. EC analyzed the polymorphisms and compiled data in

Tables 1, 2. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmolb.2020.00204/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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