



# The Possible Role of Novel Coronavirus 2019 Proteins in the Development of Drugs and Vaccines

Hadi Esmaili Gouvarchin Ghaleh<sup>1</sup>, Mohammad Reza Karimi<sup>2</sup>, Parisa Rezaat<sup>2</sup>, Masomeh Bolandian<sup>1</sup>, Majid Mirzaei Nodoushan<sup>1</sup>, Mahdieh Farzanehpour<sup>1,2\*</sup>

<sup>1</sup>Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>2</sup>Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran

**Corresponding Author:** Mahdieh Farzanehpour, Assistant Professor, Ph.D. in Virology, Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel: +98-9122122110, Email: farzanehpourmahdieh1399@gmail.com

Received April 19, 2020; Accepted June 1, 2020; Online Published June 13, 2020

## Abstract

While the world has faced an epidemic disease resulted from severe acute respiratory syndrome coronavirus (SARS-CoV), a disease which has derived from a new coronavirus that has a high contagious power causing severe illness in some individuals that may even lead to death, no vaccine or special effective treatment has been offered yet. By noticing the genetic similarity between Middle East respiratory syndrome coronavirus (MERS) and SARS-CoV viruses with SARS-CoV-2 and especially between SARS-CoV and SARS-CoV-2, the aim of this research was to express the proteins of the SARS-CoV-2 virus including the spike (S), nucleocapsid (N) and other proteins in the virus and their function in entering host cells, virus replication and production on one hand, and developing possible drugs that are effective in treating this coronavirus infection by targeting the above-mentioned proteins. On the other hand, the researchers are looking to offer a possible vaccine to prevent infection with this virus by using the proteins of this virus.

**Keywords:** COVID-19 Disease, SARS-CoV-2, SARS-CoV-2 Viral Proteins, Possible Antiviral Treatments, Possible Vaccine

**Citation:** Esmaili Gouvarchin Ghaleh H, Karimi MR, Rezaat P, Bolandian M, Mirzaei Nodoushan M, Farzanehpour M. The possible role of novel coronavirus 2019 proteins in the development of drugs and vaccines. J Appl Biotechnol Rep. 2020;7(2):63-73. doi:10.30491/JABR.2020.230039.1220.

## Introduction

Coronaviruses are a great group of viruses with positive single-stranded RNA that belong to the Coronaviridae family. These viruses usually infect animals such as birds and mammals. Coronaviruses are known as viruses that cause mild infections in the human population and are generally considered as harmless pathogens.<sup>1,2</sup> However, the emerging of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) showed that coronaviruses can also cause severe and even deadly respiratory infections in human beings. Agents causing SARS-CoV and MERS-CoV are identified as beta coronaviruses with the zoonotic origin. The first case of SARS was identified in Foshan, China in 2002 and then in 2003, it caused an endemic situation and 8098 cases of SARS were reported worldwide, with 774 cases of death, thus the mortality rate was estimated 9%-10%.<sup>2</sup> The first case of MERS was seen in Saudi Arabia in June 2012 and since then 2495 cases of infection have been reported which have caused 858 death cases in infected patients. Therefore, a mortality rate of 34.4% has been estimated.<sup>3</sup> In December 2019, some cases of Atypical Pneumonia were observed in Wuhan China (Hubei province) that was clinically similar to viral pneumonia and its reason was a new coronavirus. It was identified very fast

and it was named the novel coronavirus 2019 (COVID-19). At the present time, the epidemic resulted from this virus has caused concern all over the world due to its great contagion. This virus, since its first inception in Wuhan, which was about five months ago, has infected thousands of people and as a result the World Health Organization (WHO) has announced the status of Epidemic and Health Emergency.<sup>4,5</sup> In addition, the WHO has estimated the mortality rate of this disease as 4% in its first emergency session.<sup>6</sup> These incidents show that the threat of coronaviruses should not be underestimated and it is necessary to increase our information related to this viral family about their structure, replication mechanism and interactions with their host in order to find an appropriate treatment and to produce vaccines. These types of Coronavirus outbreaks and long-term threats for interspecies transfer will result in epidemic and recrudescence similar to viral infections which should be taken seriously.<sup>7</sup>

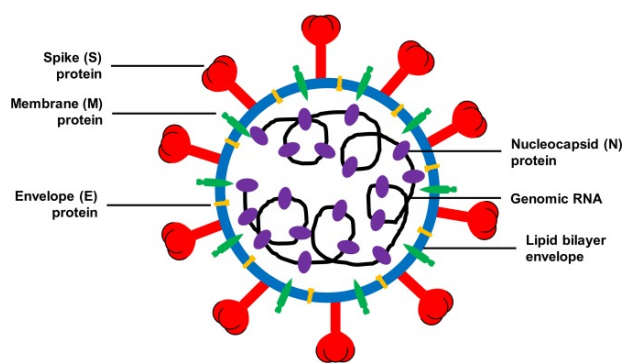
The SARS-CoV-2 is the seventh member of the Coronavirus family that causes severe respiratory diseases such as SARS in human beings. The new SARS-CoV-2 similar to SARS-CoV and MERS-CoV, belongs to the beta coronavirus genus and its genome size is 30 kilobases. Similar to other coronaviruses, this virus has many structural and non-structural proteins including spike protein (S), envelope protein (E), membrane

protein (M), and Nucleocapsid protein (N), which are its structural proteins and the other proteins of this virus include non-structural and auxiliary proteins.<sup>8</sup> Considering the fact that the SARS-CoV-2 has been recently identified, little immunologic data about this virus is available, for example information about its immunogenic epitopes that stimulate antibody secretion or reaction of T-Cell. The primary study performed based on phylogenetic analysis of the whole genome,<sup>9,10</sup> and the possible mechanism of entering the cell, and the utilization mechanism of human cell receptor have shown that SARS-CoV-2 is very similar to SARS-CoV.<sup>10,11</sup> Analysis of the new coronavirus genome sequence, taken from patients during the epidemic, showed that it is almost similar to SARS-CoV and their sequences are similar to each other for about 79.5%.<sup>12</sup> The SARS-CoV-2, similar to the SARS virus, enters the target cell through endosomal pathways and also uses the similar receptor that is angiotensin-converting enzyme 2 (ACE2) to enter the cell.<sup>12,13</sup> Due to great similarity between these 2 viruses it may be possible to use the previous studies done on the response of body immunity against SARS-CoV proteins to make medicine or vaccine for SARS-CoV-2.

### SARS-CoV-2 Viral Proteins

Based on genetic characteristics, the Coronaviridae family is categorized into 4 types including alpha coronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus. Coronaviruses RNA genome with 26 to 32 thousand bases is one of the greatest genomes among RNA viruses and the diameter of the virus particle in the coronaviruses is about 125 nanometers.<sup>14</sup> Coronaviruses genome expression is complicated. One role of proteins in the Coronaviridae family is to replicate and produce the gene. In addition, some of coronavirus proteins that are expressed in cells contaminated with this virus, play a role in interactions between the host and the coronavirus. These interactions between the coronavirus and the host result in a suitable environment condition for the replication of the coronavirus, host gene expression changes, and neutralizing the host's antiviral defense system and therefore are main factors of virus pathogenesis.<sup>15</sup> The SARS-CoV-2 codes at least 27 proteins and non-structural protein genes make two-thirds of the coronavirus genome, and this virus will have 15 non-structural proteins, 4 structural proteins, and 8 auxiliary proteins.<sup>8</sup> The structural proteins of this virus include the spike protein (S), enveloping protein (E), membrane protein (M), nucleocapsid protein (N). The S, E, and M proteins are in virus membrane, M and E proteins involve in virus production while the N protein is necessary for the RNA genome assembly (Figure 1).<sup>1</sup>

Therefore, it can be speculated that glutamine amino acid, due to its long chain, polarity and capability of hydrogen bindings, can give higher stability to the protein in the novel coronavirus 2019. This mutation within NSP2 protein happens on an area similar to endosome-associated protein that is similar to the avian infectious bronchitis virus that plays a great role in virus pathogenesis. For amino acid in position 1010 (corresponding with position 192 in NSP3 protein), the similar area in Bath SARS-like coronavirus and SARS virus have a polar amino acid and a non-polar amino



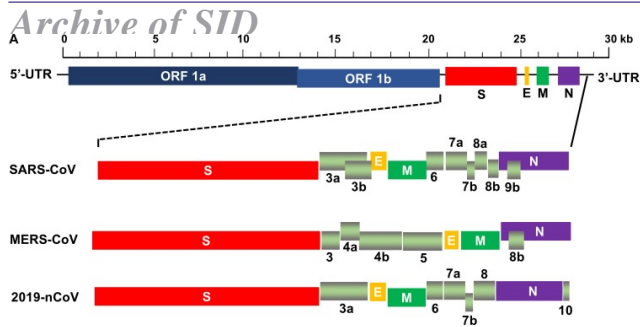
**Figure 1.** SARS-CoV-2 Virus Structure. The figure shows four structural proteins of the virus that are Spike proteins (S), Envelope proteins (E), Membrane proteins (M), Nucleocapsid proteins (N), together with virus RNA.<sup>1</sup>

acid respectively while SARS-CoV-2 has proline amino acid. In this case, it can be supposed that due to the special stiffness and steric bulge of proline, the molecular structure of SARS-CoV-2 may have a local perturbation in the conformation in comparison to the other 2 viruses and this mutation in NSP3 happens in the proximity of proteins similar to phosphatase that also exists in SARS corona virus and has a great role in the replication process of this virus in cells.<sup>16</sup> In addition, recently, high-resolution crystalline structure of SARS-CoV-2 NendoU endoribonuclease is determined and this structure shows similarity to the structure of NSP15 of SARS and MERS for 88% and 51% respectively.<sup>17</sup> The NSP15 is an endoribonuclease that plays a role in the virus replication and therefore, the replication speed of virus will reduce through slowing down or emitting it.<sup>18</sup> Thus it can be stated that inhibitors made for SARS-CoV NSP15, will have a great chance for inhibiting SARS-CoV-2 homologue too, but MERS NendoU inhibitors are unlikely to inhibit this enzyme.<sup>17</sup>

Spike protein (S) which is a trimeric protein and is on the surface, is a structural protein that is on the virion outer envelope (viral particle) and is responsible for being bound to ACE2. Spike glycoprotein of SARS-CoV, MERS-CoV and SARS-CoV-2 has 1104 to 1273 amino acids and includes a subunit amino(N)-terminal S1 and a subunit carboxyl (C)-terminal S2 (Figure 2).<sup>19</sup>

In subunit S1, the receptor binding domain (RBD) produces 200 pieces that contains two subdomains: core and external subdomains.<sup>20,21</sup> The RBD core subdomain is responsible for making S trimmer particles<sup>22</sup> and the external subdomain includes 2 exposed rings on the surface that will be bound to ACE2.<sup>11</sup> Therefore, this protein has a great role in the entrance of the virus in the host cell, virus infectiousness and its pathogenesis and is considered as an important target for establishing treatment and making vaccines against SARS-CoV and MERS-CoV.

Finally, the genetic similarity of the structural proteins of SARS-CoV-2 to SARS-CoV was studied and it was observed that according to the genome phylogenetic characteristics, SARS-CoV-2 is more similar to SARS-CoV than to MERS-CoV.<sup>9,10</sup> The accuracy of this fact is studied for structural proteins S, E, M and N and a comparison based on reference sequence showed the fact that proteins M, N and E of SARS-



**Figure 2.** Structural Diagram of Spike Glycoprotein of SARS-CoV, MERS-CoV and SARS-CoV-2.<sup>19</sup>

CoV-2 and SARS-CoV have about 90% genetic similarity. This is while, although protein S has a similarity, but this similarity is less compared to the other proteins. This similarity between SARS-CoV-2 and MERS-CoV was far less for all proteins (Table 1).

This table demonstrates the percentage of genetic similarity of structural proteins of SARS-CoV-2 with the structural proteins of SARS-CoV and MERS-CoV, using the reference sequence of each corona virus for computation of genetic similarity percentage. Although MERS-CoV is a virus that has infected people more recently and also had more recurrence and caused epidemic in 2012, 2015, and 2018,<sup>3</sup> the similarity of SARS-CoV-2 to SARS-CoV is higher. Therefore, considering the genetic similarity between the structural proteins of SARS-CoV-2 and SARS-CoV, efforts are made to use performed studies for SARS-CoV in order to make vaccine or medicine, for SARS-Cov-2.<sup>23-28</sup>

### Anti-Virus Treatment During COVID-19 Outbreak

At the beginning of diseases outbreaks, people usually do not have a correct concept of the virus vulnerability against anti-viral drugs. Taking into consideration the drugs used for SARS, it can be said that Ribavirin as an anti-virus drug, has been effective on many RNA viruses but it was not so useful for fighting SARS. The effect of Ribavirin on SARS-CoV replication in lab is extremely variable based on the cell type used for the test. Some of the patients suffering from SARS were cured with ribavirin and corticosteroids that was accompanied with some side effects.<sup>29</sup> In some countries Alfa Interferon was used together with immunoglobulin or thymosin and had therapeutic effects.<sup>30</sup> It is believed that interferon beta is better than interferon alfa and that interferon alfa modified by polyethylene glycol has prevented SARS-CoV infection, reduces virus replication and has reduced histopathology during the treatment.<sup>31</sup>

In order to make possible drugs for the COVID-19 disease, the viral protease is modeled. In these studies, some drugs are chosen from the drugs in the markets, expensive self-made drugs and drugs in drug resource databases. These drugs include natural and biologically active products and traditional Chinese medicines which were likely to have therapeutic effects against this disease. According to the previous studies on SARS and computer simulations, some old drugs such as cinnamon, vitamin B and cyclosporine A

**Table 1.** Comparison of structural proteins of SARS-CoV-2 with proteins of SARS-CoV and MERS-CoV

	M Protein	N Protein	E Protein	S Protein
MERS- CoV	6.3%	7.6%	30.5%	4.6%
SARS- CoV	90.1%	90.6%	94.7%	76%

can be effective against SARS-CoV-2. Immune suppressants such as cyclosporine A which prevent the binding of virus nucleocapsid protein to cyclophilin A of the host cell that has peptidyl-prolyl cis-trans isomerase activity. It was previously shown that cyclosporine A and interferon compound prevent virus reproduction and reduce tissue damage caused by coronavirus infection in the lungs and human bronchitis. By using protein-protein interaction techniques, Pfefflerle et al found some interactions between CypA and non-structural protein No. 1 (NSP1) of the SARS-CoV virus. They also tested CsA in some coronaviruses and noticed a kind of coronavirus inhibition.<sup>32</sup>

Although coronaviruses have a lot of similarities, they have undergone a series of fundamental genetic changes and in order to identify the goal for anti-virus treatments for this disease, structural similarities between SARS-CoV and SARS-CoV-2 should be determined and should be searched for those proteins that are common in some coronaviruses. Some efforts were made to make sure that all scientific data related to this virus such as data related to treatment and new research is available to scientific forums. For example, <http://ghddi.aliab.io/targeting2019-ncov> has been established by the Global Health Drug Discovery Institute (GHDDI) so that empirical data related to the coronavirus, the homology models of SARS-CoV-2 targets as well as SARS-CoV and MERS-CoV protein targets are placed on it. Among the discussed targets, attacking SARS-CoV and other coronaviruses, enzymes related to reproduction such as proteases are important.<sup>12</sup> Drugs that inhibit proteases can prevent replication of polypeptides necessary for virus and they also reduce the risk of mutations that cause virus drug resistance. This happened for SARS-CoV, because inhibitors would inhibit the main proteases that were involved in its replication and it was one of the most important tools in eliminating epidemic.<sup>33</sup> When the target is determined, computer methods are used to study the repeated use of previous drugs in order to identify appropriate drugs. By using this method, lopinavir and ritonavir, 2 HIV-1 protease inhibiting drug, were identified that could inhibit the main protease.<sup>34</sup> Similarity between two main proteases of SARS-CoV and the main protease of SARS-CoV-2 is close to 96.1% and therefore it can be used as a target for screening drugs that can inhibit replication of this virus. For instance, endopeptidase C30 that is called 3C-like proteinase or 3C-like protease (3CLP) of Coronavirus or main protease of coronavirus ( $M^{pro}$ ), is a homodimeric cysteine protease and is a member of the enzymes family that are found in the polypeptide of coronaviruses.<sup>35</sup>

This protease fractures polypeptides into polypeptides which are necessary for replication and transcription.<sup>36,37</sup> After translation of messenger RNA for making polypeptides, 3CLP first separates from polypeptide to become an adult

*Archive of SID*

enzyme.<sup>38</sup> The 3CLP will then separate all other 11 structural proteins. The 3CLP has an important role in virus replication and is an interesting target against the Human SARS virus.<sup>39</sup>

Using the drug through computation method is an effective method to find new indications for drugs that exist.<sup>40,41</sup> Computing method reusing drugs, is an integrated method that includes virtual medicinal library screening for finding appropriate drugs and in this method, methods such as molecular similarity and homology modeling are used for making model of the target. In addition, binding computation and molecular docking are used to predict drug-target interactions and the degree of the adhesion bind.<sup>42</sup>

Resistance establishment against present viral drugs as well as reoccurrence of viral infections is one of the most important problems in discovering anti-virus drugs. Using present drugs, through computational method has been a method to identify drugs for infectious diseases such as Ebola, Zika, Dengue fever and influenza.<sup>43</sup> This method is also used for identifying and finding drugs for SARS-CoV and MERS-CoV.<sup>44,45</sup> After the outbreak of the epidemic of COVID-19, this computation method was also used for it and results of some research in this field have been published. For instance, for searching drugs that have high capacity for binding with the main protease of SARS-CoV, four micro molecule drugs such as tegobuvir, nelfinavir, bictegravir and prulifloxacin were identified that can be used to cure COVID-19.<sup>46</sup> These four molecules were selected by high-throughput computational screening of a library of 8000 experimental and approved drugs, small molecules were obtained from DrugBank and using the structures and sequences of SARS-CoV main protease were downloaded from the PDB database. Molecular similarity search was performed by using a strategy based on the similar sequences of the structure-revealed molecules. The crystal structure of the main protease monomer was used as a target protein for molecular docking and a protein-ligand interaction analysis was performed on the resulting 690 candidates. Toxins, neurologic drugs, and antitumor drugs with strong side effects were discarded from the initial set of 690 candidates leaving 50 molecules with the capability to bind the SARS-CoV main protease. After filtering for the approved drugs and performing further kinetic and biochemical analysis, the four remaining drugs were prulifloxacin, bictegravir, nelfinavir, and tegobuvir. Interestingly, Nelfinavir, an HIV-1 protease inhibitor to treat HIV, was also predicted to be a potential inhibitor of COVID-19 main protease by another computational-based study combining homology modelling, molecular docking and binding free energy calculation.<sup>47</sup>

In this study, the main COVID-19 protease structures were modeled using the SARS homologue (PDB ID: 2GTB) as a template. Molecular docking was performed and 1903 approved drugs were tested against the model. Based on the docking score and after further three-dimensional similarity analysis, 15 drugs were selected. Ten additional new models of the main COVID-19 protease were used for additional docking analysis of these 15 drugs. Six drugs (nelfinavir, praziquantel, pitavastatin, perampanel, eszopiclone, and

zopiclone) had good binding modes and were selected for further analysis. Binding free energy calculation was performed for four of the 6 drugs and nelfinavir was selected as the most promising candidate.<sup>48</sup> In another study, the main COVID-19 protease was also used as a target to find repurposing candidates through computational screening among clinically approved medicines. The study identified a list of 10 commercial medicines that may form hydrogen bonds to key residues within the binding pocket of COVID-19 main protease and which may also have a higher tolerance to resistance mutations.<sup>49</sup>

In addition to the above mentioned research, drugs such as interferon  $\alpha$ , Kaletra (lopinavir/ritonavir), and chloroquine phosphate have been reported in the latest version of the guidelines for the prevention and treatment of pneumonia caused by coronavirus.<sup>50</sup> Interferon  $\alpha$  is an antiviral which is generally used for hepatitis treatment, but its ability to inhibit SARS-CoV proliferation in *in vitro* has been reported.<sup>51</sup> Lopinavir/ritonavir is a medicine for the human immune deficiency virus (HIV) that is used in combination with other drugs to treat adults and children over 14 days of age. Chu et al found out lopinavir/ritonavir has anti-SARS-CoV activities in *in vitro* and *in vivo* studies.<sup>52</sup> Chloroquine is an anti-Malaria drug that appears to block the entry stage of the virus into cells and provides sufficient opportunity for the virus to be cleared by the immune system. Studies in 2006 show that hydroxychloroquine is less toxic than chloroquine and is a broad spectrum antiviral.<sup>53</sup>

The lopinavir/ritonavir compound has antiviral activity similar to lopinavir. Therefore, it is expected that the therapeutic effect is largely driven by lopinavir.<sup>47,54</sup> Antiviral system IFN (IFN- $\alpha$ /beta) is an important part of the intrinsic immune defense.<sup>55</sup> The IFN- $\alpha$ /beta are cytokines secreted by cells that are exposed to viruses or parts of them.<sup>56</sup> All nucleated cells are able to respond to viruses by expressing an IFN receptor (consisting of two subunits: IFNAR-1 and IFNAR-2).<sup>57</sup> Interferon gamma also has antiviral activities, but its most important feature is that it is considered as an intermediary for antiviral receptors.<sup>56</sup> As a result, treatment with high-dose of IFN Type I and III has shown obvious effects on SARS-CoV and MERS-CoV in cell culture, this is while MERS-CoV is more sensitive than SARS-CoV in cell culture.<sup>58</sup> In addition, membrane proteins 1,2,3 produced by interferon limits the entry of many enveloped viruses, including SARS-CoV and other new viruses.<sup>59,60</sup> In addition, other medications can be effective in treating new coronavirus infection, such as Arbidol. Arbidol is an antiviral drug used to treat the flu virus. One study found that Arbidol could effectively inhibit SARS-CoV-2 infection under laboratory conditions.<sup>61</sup> Darunavir is the second generation of HIV-1 proteinase inhibitors. On February 4, 2020, Chinese researchers announced that Darunavir inhibited new coronavirus infection in *in vitro*. In addition, Transmembrane protease, serine 2 (TMPRSS2) inhibitors and Bcr-Abl tyrosine-kinase inhibitors (TKI) such as imatinib have also been able to control the virus. Hoffmann et al have shown that SARS-CoV-2 use the SARS-CoV, ACE2 receptors and cellular protein TMPRSS2 to enter target cells.<sup>12</sup>

## Archive of SID

Therefore, TMPRSS2 inhibitors are treatment choices which actually block cell entry.<sup>62</sup> Nafamostat mesylate, an existing drug used for disseminated intravascular coagulation (DIC), effectively blocked MERS-CoV S proteins initiated cell fusion by targeting TMPRSS2, and inhibited MERS-CoV infection of human lung epithelium-derived Calu-3 cells. In this study a quantitative fusion assay dependent on SARS-CoV-2 S protein, ACE2 and TMPRSS2 established, and it was found that nafamostat mesylate potently inhibited the fusion while camostat mesylate was about 10-fold less active. Furthermore, nafamostat mesylate blocked SARS-CoV-2 infection of Calu-3 cells with an EC 50 around 10 nM, which is below its average blood concentration after intravenous administration through continuous infusion. These findings, together with accumulated clinical data regarding its safety, make nafamostat a likely candidate drug to treat COVID-19 (Figure 3).<sup>63</sup>

Thus, one of the most important goals in the treatment of patients suffering from COVID-19 is viral proteins that play the main role in virus replication. Targeting them, will reduce the mortality of this virus, which has become a pandemic and has taken the lives of more than 150 000 people (<https://www.worldmeters.info/coronavirus>). Table 2 briefly refers to the drugs which may have affected SARS-CoV-2 proteins and prevented viral infection.

### Making a Preventive Vaccine for SARS-CoV-2

Considering the rapid increase in the novel coronavirus 2019 infection, efforts for making an effective vaccine have begun in many countries. By obtaining knowledge from the development route of SARS-CoV and MERS-CoV vaccines, many research groups have started to make SARS-CoV-2 vaccine just in few weeks after the COVID-19 outbreak. Choosing the target antigen and the base of vaccine is done according to SARS-CoV and MERS-CoV vaccine studies which are summarized in Table 3.<sup>1,64,65</sup>

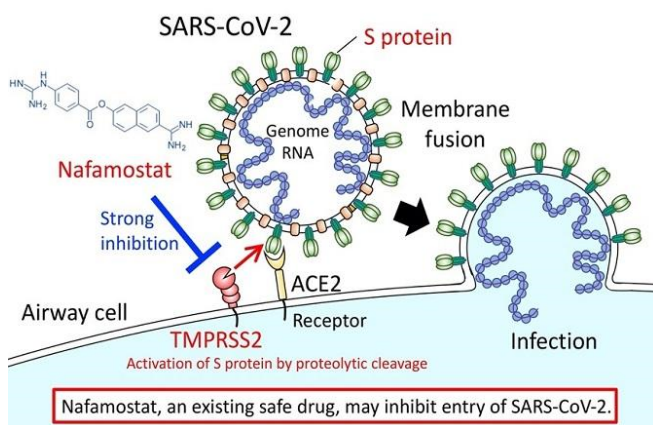
In Table 3, selected antigens and the bases that are tested for SARS-CoV and MERS-CoV clinical and pre-clinical

studies are described. As summarized in Table 3, acid nucleic based vaccine, same as DNA vaccine, has shown the most advanced platform in replying to the newfound pathogens. For instance, during the outbreak of the Zika virus, the DNA vaccine was the first vaccine candidate that entered clinical trial (NCT02809443)<sup>66</sup> less than 1-year after the outbreak. In addition, considering the present advances in technology, mRNA vaccines, are another type of nucleic acid-based vaccines that are discussed as disruptive vaccine technology. The mRNA vaccines that have been recently designed have improved the stability and output of protein translation and therefore can induce strong immune replies.<sup>67</sup> According to studies, only 23% and 16% of T cell and B cell epitopes of SARS-CoV are similar to SARS-CoV-2 respectively and no mutation was observed in the sequences of SARS-CoV-2 until February 21, 2020,<sup>68</sup> which is an important sign for the possibility of the establishment of T cell or an antibody reaction against SARS-CoV-2 and T cell epitopes that are similar to SARS-CoV-2.<sup>69-71</sup> The results of many surveys of patients suffering from SARS-CoV-2 especially in the convalescence phase has been shown that the immune response against the structural proteins (S and N) of the virus is more than non-structural proteins.<sup>72</sup>

In regards to the B cell, results of empirical studies<sup>20,73</sup> demonstrate that antibodies originated from SARS-CoV that target the binding receptor motif in the subunit S1 of the spike protein of SARS-CoV-2, may not be so effective and in this case, it is due to genetic mismatch in identified structural epitopes that target this domain. Linear epitopes of the B cell of SARS-CoV in subunit S2 may be more promising candidates for stimulating immune response. Some of these epitopes in SARS-CoV are similar to SARS-CoV-2 and primary results demonstrate their capability in making neutralizing antibodies and cross-reactions.<sup>63</sup> Therefore, the vaccine that stimulates antibodies which target S2 linear epitopes, can be effective and should be inspected in future research. In addition, some studies have shown that since the full-length spike (S) or S1 that contains the RBD can induce neutralizing antibodies that prevent connection and infection in the host cell, it is considered as a good antigen for producing vaccines (Figure 4).<sup>74-76</sup>

These epitopes are the ideal candidate to formulate a multi-epitopic peptide vaccine, not only because of being selected from the linear B-cell epitopic region but also due to the fact that their antigenic property was confirmed. Moreover, the molecular docking of vaccine components with the TLR-5 proves the significance and effectiveness of these epitopes as an ideal vaccine candidate against SARS-COV-2. However, these immunoinformatic analyses require several in vitro and in vivo validations before formulating the vaccine to resist (Figure 5).<sup>20</sup>

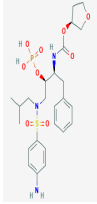
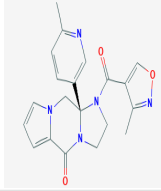
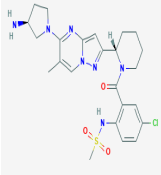
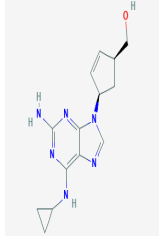
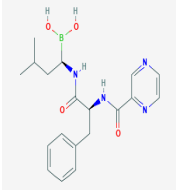
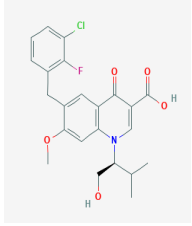
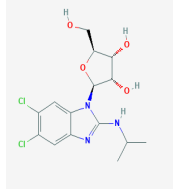
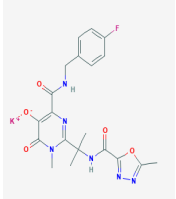
In addition to the above mentioned research, in late February 2020, Glaxo Smith Kline (GSK) announced a collaboration with Chinese firm Clover Biopharmaceuticals to assess a coronavirus (COVID-19) vaccine candidate.<sup>80</sup> It is said that this collaboration will involve the use of Clover's protein-based coronavirus vaccine candidate (COVID-19 S-Trimer) with the GSK's adjuvant system. By applying their



**Figure 3.** The Possible Mechanisms of Some Different Drugs Which May Affect SARS-CoV-2 Proteins by a Schematic Diagram (Nafamostat effectively blocks SARS-CoV-2 S proteins initiated cell fusion by targeting TMPRSS2, and prevents from cell infection).<sup>63</sup>

Table 2. Drugs Affecting SARS-CoV-2 Proteins and Their Site of Effect and Structures

Drugs	Site of effect	Structure
Cyclosporine A	It prevents binding of virus Nucleocapsid protein to cyclophilin A of the host cell that has peptidyl prolyl cis-trans isomerase activity <sup>32</sup>	
Kaletra, Lopinavir and Ritonavir	They inhibit main protease (3CLP) <sup>34,35</sup>	
Tegobuvir	It has high capacity for binding with main protease <sup>46</sup>	
Nelfinavir	It has high capacity for binding with main protease <sup>46,48</sup>	
Bictegravir	It has high capacity for binding with main protease <sup>46</sup>	
Prulifloxacin	It has high capacity for binding with main protease <sup>46</sup>	
Chloroquine and Hydroxychloroquine (less toxic)	They appear to block the entry stage of the virus into cells <sup>53</sup>	
Darunavir	A protease inhibitor <sup>62</sup>	
Nafamostat	It is a transmembrane protease, serine 2 (TMPRSS2) inhibitors which block cell entry <sup>62</sup>	
Imatinib	It is a Bcr-Abl tyrosine-kinase inhibitors (TKI) which block cell entry <sup>62</sup>	
Tipranavir	An antiretroviral protease inhibitor <sup>62</sup>	

Drugs	Site of effect	Structure
Fosamprenavir	Protease inhibitor <sup>62</sup>	
Enzaplavir	Viral fusion protein inhibitor <sup>62</sup>	
Presatovir	Viral fusion protein inhibitor <sup>62</sup>	
Abacavir	Protease inhibitor <sup>62</sup>	
Bortezomib	Protease inhibitor <sup>62</sup>	
Elvitegravir	Protease inhibitor <sup>62</sup>	
Maribavir	A selective ATP competitor of viral UL97 kinase, which is involved in viral nuclear maturation events <sup>62</sup>	
Raltegravir	Protease inhibitor <sup>62</sup>	

Source: National Center for Biotechnology Information. PubChem Database, CID=58406357, <https://pubchem.ncbi.nlm.nih.gov/compound>. Accessed on May 16, 2020.

Table 3. Selected antigens and vaccine bases that are studied considering SARS-CoV and MERS- CoV proteins)

Vaccine Platform	Immunogen	Phase	Advantage	Disadvantage
DNA	Full-length Spike, or S1 • IM follow by electroporation	Phase I, II (NCT03721718)	<ul style="list-style-type: none"> <li>• Rapid production</li> <li>• Easy design and manipulation</li> <li>• Induce both B and T cells responses</li> </ul>	<ul style="list-style-type: none"> <li>• Efficient delivery system required</li> <li>• Induce lower immune responses when compare with live vaccine</li> </ul>
Viral vector	Full-length Spike or S1 • Vector used: ChAd or MVA	Phase I (NCT03399578, NCT03615911)	<ul style="list-style-type: none"> <li>• Excellence in immune induction</li> </ul>	<ul style="list-style-type: none"> <li>• Varies inoculation routes may produce different immune responses</li> <li>• Possible TH2 bias</li> </ul>
Subunit	Full-length Spike, S1, RDB, nucleocapsid • Formulated with various adjuvants and/or fused with Fc	Preclinical	<ul style="list-style-type: none"> <li>• High safety profile</li> <li>• Consistent production</li> <li>• Can induce cellular and humoral immune responses</li> </ul>	<ul style="list-style-type: none"> <li>• Need appropriate adjuvant,</li> <li>• Cost-effectiveness may vary</li> </ul>
Virus-like particles	RDB, S or Co-expressing of S1, M, and E • Produced in baculovirus	Preclinical	<ul style="list-style-type: none"> <li>• Multimeric antigen display</li> <li>• Preserve virus particle structure</li> </ul>	<ul style="list-style-type: none"> <li>• Require optimum assembly condition</li> </ul>

ChAd: Chimpanzee Adenovirus Vector; MVA: Modified Vaccinia Ankara.

Trimer-Tag technology, Clover has manufactured an S-Trimer subunit vaccine using a rapid mammalian cell culture-based expression system. The Trimer-Tag is an advanced drug development platform, which enables the production of novel, covalently trimerized fusion proteins that can better target previous drug gable pathways. Accordingly, a consortium led by Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine (including University of Texas Medical Branch and New York Blood Center) has developed and tested a subunit vaccine comprised of only the RBD of the SARS-CoV S-protein.<sup>81-83</sup> When formulated on alum, the SARS-CoV RBD vaccine elicits high levels of protective immunity on the homologous virus challenge. An advantage of the RBD-based vaccine is its ability to minimize host immune potentiation.<sup>78</sup> Initial findings that the SARS-CoV and SARS-CoV-2 RBDs exhibit more than 80% amino acid similarity and bind to the same ACE2 receptor offer an opportunity to develop either protein as a subunit vaccine. Recently, Generex announced that it is developing a COVID-19 vaccine following a contractual agreement with a Chinese consortium comprised of China Technology Exchange, Beijing Zhonghua Investment Fund Management, Biology Institute of Shandong Academy of Sciences, and Sinotek-Advocates International Industry Development. It is said that the company will utilize its Ii-Key immune system activation technology to produce a

COVID-19 viral peptide for human clinical trials.<sup>6,83,84</sup>

As a result, similarity between proteins of SARS-CoV-2 and SARS-CoV virus as well as the study of protection immune solidarity and long-term immune memory in individuals during convalescence can be helpful in designing preventive measures such as producing vaccine for COVID-19.

### Discussion and Conclusion

The epidemic of COVID-19 has made a lot of concern all over the world due to its high contagion. From its first occurrence in Wuhan, China which was about 3 months ago, this virus has infected thousands of people and its infection speed is increasing day to day. Due to its person to person transfer in China and its spread speed in other countries, the WHO announced Epidemic and Health Emergency. Knowing

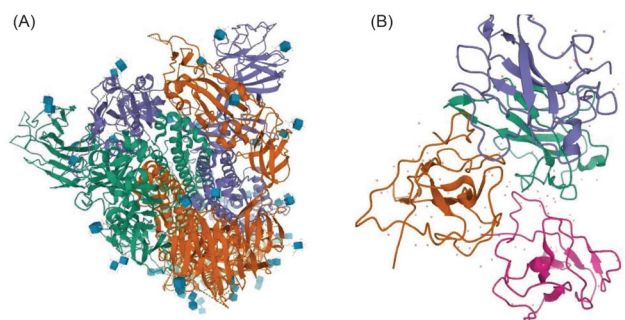


Figure 4. Structure of Some Protein Components of the SARS-CoV-2. (A) 2019-nCoV spike glycoprotein with a single receptor-binding domain.<sup>77</sup> (B) SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain.<sup>78</sup> Source: Protein Data Bank (PDB), <https://www.rcsb.org>

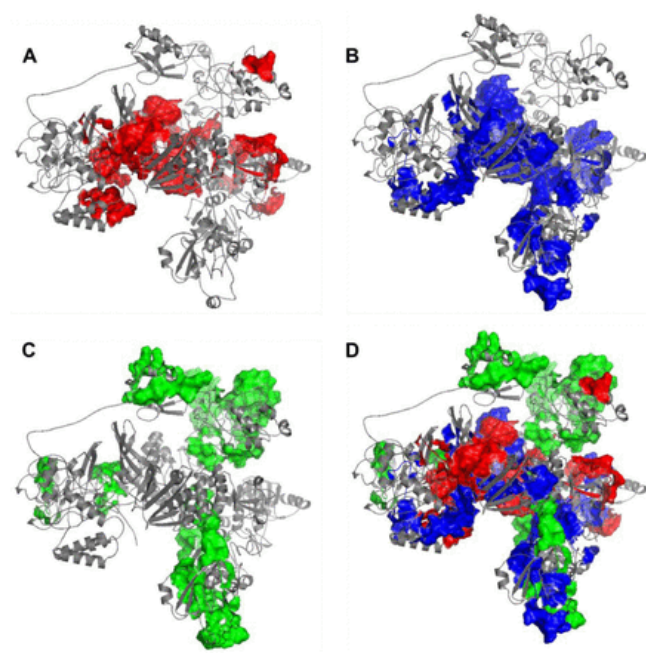


Figure 5. Predicted 3D Structure of nsp3 Protein Highlighted with (A) MHC-I T Cell Epitopes (red), (B) MHC-II (blue) T Cell Epitopes, (C) Linear B Cell Epitopes (Green), and the Merged Epitopes. The MHC-I epitopes are more internalized, MHC-II epitopes are more mixed, and B cells are more shown on the surface.<sup>79</sup>



**Archive of SID**

binding mechanisms helps finding correct resources for the virus and finding preventive and therapeutic measures. The spike protein of crystal SARS-CoV-2 is analyzed by cryo electronic microscope to find the transacting places between RBD of spike protein of SARS-CoV-2 with ACE2 of human beings or other host receptors. In addition, scientists have also studied other proteins of this virus and their roles in structure, replication and production of this new virus. Generally, noticing that these set of identified epitopes for SARS-CoV are similar to epitopes of SARS-CoV-2, they can be a good guide for making vaccines for SARS-CoV-2. Also, more empirical studies (B cell and T cell tests) must be carried out in order to determine the capability of epitopes for stimulating reaction against SARS-CoV-2. This will help the correction of epitopes set based on immunogenicity that is an important task in immunogenic design.

In conclusion, structural and non-structural proteins of SARS-CoV-2 are the main targets for developing vaccines and it is found that immunity response against structural proteins S and N are more long termed and among S protein subdomains, the vaccine that stimulates antibodies which target S2 linear epitopes, can be effective and should be inspected in future research. In addition, Zhang et al have shown that full-length spike (S) or S1 that contains RBD, since it can induce neutralizing antibodies that prevent connection and infection in the host cell, is considered as a good antigen for making vaccines.<sup>85</sup>

In the field of drug production, establishing computing methods for designing compounds which can cure coronavirus infections is a main challenge. Results indicate that this new method is cost-effective and timed to make new drugs to cure coronavirus infections. In spite of economic and social effects of coronavirus infections and the possibility of more dangerous coronaviruses outbreaks, there is no effective anti-viral drug for treating coronavirus. Noticing severe contagion and distribution of coronaviruses, the new virus can periodically act as a consequence of interspecies infections and awkward events. In general, this research clarified the importance of previous empirical and clinical studies performed in the field of SARS-CoV and by using them beside newfound data for SARS-CoV-2 and their importance for finding appropriate chemical drugs such as tipranavir, fosamprenavir, enzaplatovir, presatovir, abacavir, bortezomib, elvitegravir, maribavir, raltegravir, montelukast, deoxyrhapontin, polydatin, chalcone, disulfiram, carmofur, shikonin, ebselen, tideglusib, PX-12, TDZD-8, cyclosporin A, and cinanserin and herbal medicines such as *Rhizoma Polygoni Cuspidati* and *Radix Sophorae Tonkinensis* may contain substances that are effective against SARS-COV-2 and vaccine to confront COVID-19 epidemic. Results of this research can fill some data gaps about COVID-19, while this epidemic exists and scientists are gathering data about this new virus.

**Authors' Contributions**

All Authors contributed equally to this study.

**Conflict of Interest Disclosures**

The authors declare they have no conflicts of interest.

**Acknowledgments**

Authors wish to thank all the staff of Applied Virology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran and the Research Center for Clinical Virology, Tehran University of Medical Sciences, and Tehran, Iran for their cooperation in the study procedure.

**References**

1. Song Z, Xu Y, Bao L, et al. From SARS to MERS, thrusting coronaviruses into the spotlight. *Viruses*. 2019;11(1). doi:[10.3390/v11010059](https://doi.org/10.3390/v11010059).
2. World Health Organization (WHO). Update 49-SARS Case Fatality Ratio, Incubation Period. Geneva, Switzerland: WHO; 2003.
3. World Health Organization (WHO). Middle East Respiratory Syndrome Coronavirus (MERS-CoV). WHO; 2015.
4. Meo SA, Alhowikan AM, Al-Khlaiwi T, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Rev Med Pharmacol Sci*. 2020;24(4):2012-2019. doi:[10.26355/eurrev\\_202002\\_20379](https://doi.org/10.26355/eurrev_202002_20379).
5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi:[10.1016/s0140-6736\(20\)30183-5](https://doi.org/10.1016/s0140-6736(20)30183-5).
6. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:[10.1056/NEJMoa2001017](https://doi.org/10.1056/NEJMoa2001017).
7. World Health Organization (WHO). Statement on the Second Meeting of the International Health Regulations (2005) Emergency Committee Regarding the Outbreak of Novel Coronavirus (2019-nCoV). Geneva, Switzerland: WHO; 2020.
8. Menachery VD, Yount BL Jr, Debink K, et al. Corrigendum: a SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med*. 2016;22(4):446. doi:[10.1038/nm0416-446d](https://doi.org/10.1038/nm0416-446d).
9. Wu A, Peng Y, Huang B, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe*. 2020;27(3):325-328. doi:[10.1016/j.chom.2020.02.001](https://doi.org/10.1016/j.chom.2020.02.001).
10. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273. doi:[10.1038/s41586-020-2012-7](https://doi.org/10.1038/s41586-020-2012-7).
11. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395(10224):565-574. doi:[10.1016/s0140-6736\(20\)30251-8](https://doi.org/10.1016/s0140-6736(20)30251-8).
12. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *BioRxiv*. 2020. doi:[10.1101/2020.01.31.929042](https://doi.org/10.1101/2020.01.31.929042).
13. Zhou P, Yang XL, Wang XG, et al. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *BioRxiv*. 2020. doi:[10.1101/2020.01.22.914952](https://doi.org/10.1101/2020.01.22.914952).
14. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. 2020;5(4):562-569. doi:[10.1038/s41564-020-0688-y](https://doi.org/10.1038/s41564-020-0688-y).
15. Ji W, Wang W, Zhao X, Zai J, Li X. Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol*. 2020;92(4):433-440. doi:[10.1002/jmv.25682](https://doi.org/10.1002/jmv.25682).
16. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. Host factors in coronavirus replication. In: Tripp RA, Tompkins SM, eds. *Roles of Host Gene and Non-coding RNA Expression in Virus Infection*. Cham: Springer; 2017. p. 1-42.
17. Saikatendu KS, Joseph JS, Subramanian V, et al. Structural basis of severe acute respiratory syndrome coronavirus ADP-ribose-1'-phosphate dephosphorylation by a conserved domain of nsP3. *Structure*. 2005;13(11):1665-1675. doi:[10.1016/j.str.2005.07.022](https://doi.org/10.1016/j.str.2005.07.022).

18. Kim Y, Jedrzejcak R, Maltseva NI, et al. Crystal structure of Nsp15 endoribonuclease NendoU from SARS-CoV-2. *Protein Sci.* 2020. doi:10.1002/pro.3873.
19. Ortiz-Alcantara J, Bhardwaj K, Palaninathan S, Frieman M, Baric R, Kao C. Small molecule inhibitors of the SARS-CoV Nsp15 endoribonuclease. *Virus Adapt Treat.* 2010;2(1):125-133. doi:10.2147/maat.s12733.
20. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020;367(6483):1260-1263. doi:10.1126/science.abb2507.
21. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science.* 2005;309(5742):1864-1868. doi:10.1126/science.1116480.
22. Lu G, Hu Y, Wang Q, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature.* 2013;500(7461):227-231. doi:10.1038/nature12328.
23. Yuan Y, Cao D, Zhang Y, et al. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nat Commun.* 2017;8:15092. doi:10.1038/ncomms15092.
24. Yang ZY, Kong WP, Huang Y, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature.* 2004;428(6982):561-564. doi:10.1038/nature02463.
25. Graham RL, Becker MM, Eckerle LD, Bolles M, Denison MR, Baric RS. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nat Med.* 2012;18(12):1820-1826. doi:10.1038/nm.2972.
26. Wang J, Wen J, Li J, et al. Assessment of immunoreactive synthetic peptides from the structural proteins of severe acute respiratory syndrome coronavirus. *Clin Chem.* 2003;49(12):1989-1996. doi:10.1373/clinchem.2003.023184.
27. Liu X, Shi Y, Li P, et al. Profile of antibodies to the nucleocapsid protein of the severe acute respiratory syndrome (SARS)-associated coronavirus in probable SARS patients. *Clin Diagn Lab Immunol.* 2004;11(1):227-228. doi:10.1128/cdli.11.1.227-228.2004.
28. Liu WJ, Zhao M, Liu K, et al. T-cell immunity of SARS-CoV: Implications for vaccine development against MERS-CoV. *Antiviral Res.* 2017;137:82-92. doi:10.1016/j.antiviral.2016.11.006.
29. Channappanavar R, Fett C, Zhao J, Meyerholz DK, Perlman S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *J Virol.* 2014;88(19):11034-11044. doi:10.1128/jvi.01505-14.
30. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet.* 2003;361(9374):2045-2046. doi:10.1016/s0140-6736(03)13615-x.
31. Zhao Z, Zhang F, Xu M, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. *J Med Microbiol.* 2003;52(Pt 8):715-720. doi:10.1099/jmm.0.05320-0.
32. Pfeifferle S, Schöpf J, Kögl M, et al. The SARS-coronavirus-host interactome: identification of cyclophilins as target for pan-coronavirus inhibitors. *PLoS Pathog.* 2011;7(10):e1002331. doi:10.1371/journal.ppat.1002331.
33. Haagmans BL, Kuiken T, Martina BE, et al. Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat Med.* 2004;10(3):290-293. doi:10.1038/nm1001.
34. Xia B, Kang X. Activation and maturation of SARS-CoV main protease. *Protein Cell.* 2011;2(4):282-290. doi:10.1007/s13238-011-1034-1.
35. Nukoolkarn V, Lee VS, Malaisree M, Aruksakulwong O, Hannongbua S. Molecular dynamic simulations analysis of ritonavir and lopinavir as SARS-CoV 3CL(pro) inhibitors. *J Theor Biol.* 2008;254(4):861-867. doi:10.1016/j.jtbi.2008.07.030.
36. Fan K, Wei P, Feng Q, et al. Biosynthesis, purification, and substrate specificity of severe acute respiratory syndrome coronavirus 3C-like proteinase. *J Biol Chem.* 2004;279(3):1637-1642. doi:10.1074/jbc.M310875200.
37. Thiel V, Ivanov KA, Putics Á, et al. Mechanisms and enzymes involved in SARS coronavirus genome expression. *J Gen Virol.* 2003;84(Pt 9):2305-2315. doi:10.1099/vir.0.19424-0.
38. Goetz DH, Choe Y, Hansell E, et al. Substrate specificity profiling and identification of a new class of inhibitor for the major protease of the SARS coronavirus. *Biochemistry.* 2007;46(30):8744-8752. doi:10.1021/bi0621415.
39. Adedeji AO, Sarafianos SG. Antiviral drugs specific for coronaviruses in preclinical development. *Curr Opin Virol.* 2014;8:45-53. doi:10.1016/j.coviro.2014.06.002.
40. Yang H, Xie W, Xue X, et al. Design of wide-spectrum inhibitors targeting coronavirus main proteases. *PLoS Biol.* 2005;3(10):e324. doi:10.1371/journal.pbio.0030324.
41. Vanhaelen Q, Mamoshina P, Aliper AM, et al. Design of efficient computational workflows for in silico drug repurposing. *Drug Discov Today.* 2017;22(2):210-222. doi:10.1016/j.drudis.2016.09.019.
42. Karaman B, Sippl W. Computational drug repurposing: current trends. *Curr Med Chem.* 2019;26(28):5389-5409. doi:10.2174/0929867325666180530100332.
43. Ozdemir ES, Halakou F, Nussinov R, Gursoy A, Keskin O. Methods for Discovering and Targeting Druggable Protein-Protein Interfaces and Their Application to Repurposing. *Methods Mol Biol.* 2019;1903:1-21. doi:10.1007/978-1-4939-8955-3\_1.
44. Mani D, Wadhvani A, Krishnamurthy PT. Drug repurposing in antiviral research: a current scenario. *J Young Pharm.* 2019;11(2):117-121. doi:10.5530/jyp.2019.11.26.
45. Dyllal J, Coleman CM, Hart BJ, et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. *Antimicrob Agents Chemother.* 2014;58(8):4885-4893. doi:10.1128/aac.03036-14.
46. Dyllal J, Gross R, Kindrachuk J, et al. Middle East respiratory syndrome and severe acute respiratory syndrome: current therapeutic options and potential targets for novel therapies. *Drugs.* 2017;77(18):1935-1966. doi:10.1007/s40265-017-0830-1.
47. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun.* 2020;11(1):222. doi:10.1038/s41467-019-13940-6.
48. Xu Z, Peng C, Shi Y, et al. Nelfinavir was predicted to be a potential inhibitor of 2019-nCoV main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. *BioRxiv.* 2020. doi:10.1101/2020.01.27.921627.
49. Zhavoronkov A, Zagribelnyy B, Zhebrak A, et al. Potential non-covalent SARS-CoV-2 3C-like protease inhibitors designed using generative deep learning approaches and reviewed by human medicinal chemist in virtual reality. *ChemRxiv.* 2020. doi:10.26434/chemrxiv.12301457.v1.
50. Liu X, Wang XJ. Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines. *J Genet Genomics.* 2020;47(2):119-121. doi:10.1016/j.jgg.2020.02.001.
51. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther.* 2020;14(1):58-60. doi:10.5582/ddt.2020.01012.
52. Chu CM, Cheng VC, Hung IF, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax.* 2004;59(3):252-256. doi:10.1136/thorax.2003.012658.
53. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. *PLoS Med.* 2006;3(9):e343. doi:10.1371/journal.pmed.0030343.
54. Su B, Wang Y, Zhou R, et al. Efficacy and tolerability of lopinavir/ritonavir- and efavirenz-based initial antiretroviral therapy in HIV-1-infected patients in a tertiary care hospital in Beijing, China. *Front Pharmacol.* 2019;10:1472. doi:10.3389/fphar.2019.01472.
55. Boffito M, Arnaudo I, Raiteri R, et al. Clinical use of lopinavir/

- ritonavir in a salvage therapy setting: pharmacokinetics and pharmacodynamics. *AIDS*. 2002;16(15):2081-2083. doi:10.1097/00002030-200210180-00015.
56. tenOever BR. The evolution of antiviral defense systems. *Cell Host Microbe*. 2016;19(2):142-149. doi:10.1016/j.chom.2016.01.006.
  57. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 2014;32:513-545. doi:10.1146/annurev-immunol-032713-120231.
  58. Bekisz J, Schmeisser H, Hernandez J, Goldman ND, Zoon KC. Human interferons alpha, beta and omega. *Growth Factors*. 2004;22(4):243-251. doi:10.1080/08977190400000833.
  59. Ziebeck F, Weber M, Eickmann M, et al. Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. *J Virol*. 2013;87(9):5300-5304. doi:10.1128/jvi.03496-12.
  60. Bailey CC, Zhong G, Huang IC, Farzan M. IFITM-family proteins: the cell's first line of antiviral defense. *Annu Rev Virol*. 2014;1:261-283. doi:10.1146/annurev-virology-031413-085537.
  61. Huang C, Lokugamage KG, Rozovics JM, Narayanan K, Semler BL, Makino S. SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. *PLoS Pathog*. 2011;7(12):e1002433. doi:10.1371/journal.ppat.1002433.
  62. Delang L, Abdelnabi R, Neyts J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. *Antiviral Res*. 2018;153:85-94. doi:10.1016/j.antiviral.2018.03.003.
  63. Hoffmann M, Schroeder S, Kleine-Weber H, Müller MA, Drosten C, Pöhlmann S. Nafamostat mesylate blocks activation of SARS-CoV-2: new treatment option for COVID-19. *Antimicrob Agents Chemother*. 2020;64(6). doi:10.1128/aac.00754-20.
  64. Yamamoto M, Kiso M, Sakai-Tagawa Y, et al. The anticoagulant nafamostat potently inhibits SARS-CoV-2 infection in vitro: an existing drug with multiple possible therapeutic effects. *BioRxiv*. 2020. doi:10.1101/2020.04.22.054981.
  65. Yong CY, Ong HK, Yeap SK, Ho KL, Tan WS. Recent advances in the vaccine development against middle east respiratory syndrome-coronavirus. *Front Microbiol*. 2019;10:1781. doi:10.3389/fmicb.2019.01781.
  66. Schindewolf C, Menachery VD. Middle East respiratory syndrome vaccine candidates: cautious optimism. *Viruses*. 2019;11(1). doi:10.3390/v11010074.
  67. Tebas P, Roberts CC, Muthumani K, et al. Safety and immunogenicity of an anti-Zika Virus DNA vaccine - preliminary report. *N Engl J Med*. 2017. doi:10.1056/NEJMoa1708120.
  68. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. *Ther Deliv*. 2016;7(5):319-334. doi:10.4155/tde-2016-0006.
  69. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. *Viruses*. 2020;12(3). doi:10.3390/v12030254.
  70. Tang F, Quan Y, Xin ZT, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. *J Immunol*. 2011;186(12):7264-7268. doi:10.4049/jimmunol.0903490.
  71. Peng H, Yang LT, Wang LY, et al. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virology*. 2006;351(2):466-475. doi:10.1016/j.virol.2006.03.036.
  72. Fan YY, Huang ZT, Li L, et al. Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. *Arch Virol*. 2009;154(7):1093-1099. doi:10.1007/s00705-009-0409-6.
  73. Ng OW, Chia A, Tan AT, et al. Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. *Vaccine*. 2016;34(17):2008-2014. doi:10.1016/j.vaccine.2016.02.063.
  74. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*. 2020;181(2):281-292.e286. doi:10.1016/j.cell.2020.02.058.
  75. Al-Amri SS, Abbas AT, Siddiq LA, et al. Immunogenicity of candidate MERS-CoV DNA vaccines based on the spike protein. *Sci Rep*. 2017;7:44875. doi:10.1038/srep44875.
  76. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. *Nat Rev Microbiol*. 2009;7(3):226-236. doi:10.1038/nrmicro2090.
  77. Du L, Zhao G, He Y, et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. *Vaccine*. 2007;25(15):2832-2838. doi:10.1016/j.vaccine.2006.10.031.
  78. Bhattacharya M, Sharma AR, Patra P, et al. Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): Immunoinformatics approach. *J Med Virol*. 2020;92(6):618-31. doi:10.1002/jmv.25736.
  79. Kang S, Yang M, Hong Z, et al. Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. *Acta Pharm Sin B*. 2020. doi:10.1016/j.apsb.2020.04.009.
  80. Clover Biopharmaceuticals. Clover Initiates Development of Recombinant Subunit-Trimer Vaccine for Wuhan Coronavirus (2019-nCoV). 2020.
  81. Jiang S, Bottazzi ME, Du L, et al. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. *Expert Rev Vaccines*. 2012;11(12):1405-1413. doi:10.1586/erv.12.126.
  82. Chen WH, Chag SM, Poongavanam MV, et al. Optimization of the production process and characterization of the yeast-expressed SARS-CoV recombinant receptor-binding domain (RBD219-N1), a SARS vaccine candidate. *J Pharm Sci*. 2017;106(8):1961-1970. doi:10.1016/j.xphs.2
  83. Ong E, Wong MU, Huffman A, He Y. COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning. *BioRxiv*. 2020. doi:10.1101/2020.03.20.000141.
  84. Chen WH, Du L, Chag SM, et al. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. *Hum Vaccin Immunother*. 2014;10(3):648-658. doi:10.4161/hv.27464.
  85. Zhang J, Zeng H, Gu J, Li H, Zheng L, Zou Q. Progress and prospects on vaccine development against SARS-CoV-2. *Vaccines (Basel)*. 2020;8(2):E153. doi:10.3390/vaccines8020153.

Surf and download all data from SID.ir: [www.SID.ir](http://www.SID.ir)

Translate via STRS.ir: [www.STRS.ir](http://www.STRS.ir)

Follow our scientific posts via our Blog: [www.sid.ir/blog](http://www.sid.ir/blog)

Use our educational service (Courses, Workshops, Videos and etc.) via Workshop: [www.sid.ir/workshop](http://www.sid.ir/workshop)