

## REVIEW

### Pharmacological treatment of COVID-19: lights and shadows

Francesco Menzella MD<sup>1</sup>, Mirella Biava Msc<sup>2</sup>, Chiara Barbieri MD<sup>1</sup>, Francesco Livrieri MD<sup>1</sup>, Nicola Facciolongo MD<sup>1</sup>

<sup>1</sup>Department of Medical Specialties, Pneumology Unit, Arcispedale Santa Maria Nuova, Azienda USL di Reggio Emilia- IRCCS, Reggio Emilia, Italy;

<sup>2</sup>University of Rome "La Sapienza," Rome, Italy

#### Abstract

At the end of December 2019, a novel coronavirus, the severe acute respiratory syndrome coronavirus 2, caused an outbreak of pneumonia spreading from Wuhan, Hubei province, to the whole country of China and then the entire world, forcing the World Health Organization to make the assessment that the coronavirus disease (COVID-19) can be characterized as a pandemic, the first ever caused by a coronavirus. To date, clinical evidence and guidelines based on reliable data and randomized clinical trials for the treatment of COVID-19 are lacking. In the absence of definitive management protocols, many treatments for COVID-19 are currently being evaluated and tested worldwide. Some of these options were soon abandoned due to ineffectiveness, while others showed promising results. The basic treatments are mainly represented by antiviral drugs, even if the evidence is not satisfactory. Among the antivirals, the most promising appears to be

remdesivir. Corticosteroids and tocilizumab seem to guarantee positive results in selected patients so far, although the timing of starting therapy and the most appropriate therapeutic schemes remain to be clarified. Efficacy of the other drugs is still uncertain, and they are currently used as a cocktail of treatments in the absence of definitive guidelines. What will represent the real solution to the enormous problem taking place worldwide is the identification of a safe and effective vaccine, for which enormous efforts and investments are underway.

**Keywords:** antivirals, biologics, coronavirus, corticosteroids, pneumonia, severe acute respiratory syndrome.

#### Citation

Menzella F, Biava M, Barbieri C, Livrieri F, Facciolongo N. Pharmacological treatment of COVID-19: lights and shadows. *Drugs in Context* 2020; 9: 2020-4-6. DOI: [10.7573/dic.2020-4-6](https://doi.org/10.7573/dic.2020-4-6)

#### Introduction

At the end of December 2019, a novel coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an outbreak of pneumonia spreading from Wuhan, Hubei province, to the whole country of China and then the entire world, forcing the World Health Organization (WHO) to make the assessment that the coronavirus disease (COVID-19) can be characterized as a pandemic, the first ever caused by a coronavirus.<sup>1</sup>

The coronavirusidae family comprises pathogens that primarily target the human respiratory system. Of the other six coronaviruses known to infect humans, two, previously characterized as agents that are a great public health threat, present with major symptoms (severe acute respiratory syndrome [SARS]-CoV and the Middle East respiratory syndrome [MERS]-CoV), and four present with mild symptoms (HKU1, NL63, OC43, and 229E).<sup>2</sup> Coronaviruses (CoVs) (order Nidovirales, family Coronaviridae, subfamily Coronavirinae) are enveloped viruses with a positive sense, single-stranded

ribonucleic acid (RNA) genome. Based on genetic and antigenic criteria, CoVs have been organized into three groups:  $\alpha$ -CoVs,  $\beta$ -CoVs, and  $\gamma$ -CoVs.<sup>3</sup> Genome sequencing analysis attributed SARS-CoV-2 to the genus Betacoronavirus, within a subgenus (Sarbecovirus) that includes SARS-CoV (whereas MERS-CoV falls in a separate subgenus, Merbecovirus).<sup>4</sup> The origin of SARS-CoV-2 was explained with two possible scenarios: natural selection in an animal host before zoonotic transfer and natural selection in humans following zoonotic transfer.<sup>5</sup>

SARS-CoV-2 possesses the typical coronavirus structure with spike protein and other polyproteins, nucleoproteins, and membrane proteins, such as RNA polymerase, 3-chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and accessory proteins.<sup>6</sup> The spike protein of SARS-CoV-2 contains the 394 glutamine residue in the receptor-binding domain region of SARS-CoV-2, which is recognized by the critical lysine 31 residue on the human angiotensin 2 converting enzyme (ACE2) receptor.<sup>7</sup> Thus, SARS-CoV-2 uses the same ACE2 cell receptor and mechanism for entry into the host cell as SARS-CoV,<sup>8,9</sup> with a single N501T mutation in SARS-CoV-2's spike

protein that may have significantly enhanced its binding affinity for ACE2.<sup>7</sup>

Infection with SARS-CoV-2 results in mild and nonspecific symptoms, such as fever and cough (common symptoms), and nasal congestion, fatigue, loss of appetite and smell, body ache, and diarrhea (noted in a small number of patients). On the contrary, severe cases might rapidly progress to acute respiratory distress syndrome (ARDS), septic shock, and difficult-to-tackle metabolic acidosis and bleeding and coagulation dysfunction.<sup>10</sup> Suspicions have risen regarding the possibility that an excessive immune response – the so-called cytokine storm – associated with macrophage activated syndrome (MAS) may be driving COVID-19-related ARDS.<sup>11</sup> Specifically, a cytokine profile resembling MAS has been noted in COVID-19 patients, with increased interleukin (IL)-1 $\beta$ , IL-2, IL-6, IL-17, IL-8, tumor necrosis factor, and C-C motif chemokine ligand 2.<sup>12</sup> Current recommendations for the treatment of COVID-19 severe cases consist of using existing, approved therapies with proven safety profiles to address the immediate need to reduce the rising mortality.<sup>13</sup>

The aim of this review is to explore COVID-19 treatment options and describe the potential benefits or disadvantages of their use in severe cases.

## Methods of literature search

A search strategy based on validated keywords filters was devised to select articles regarding SARS-CoV-2 and related treatments. In detail, a selective search on PubMed was carried out up to April 2020, and research papers, international guidelines, case reports, and meta-analyses have been considered. The search strategy was based on the following keywords: coronavirus, SARS-CoV-2 pneumonia, COVID-19, severe acute respiratory syndrome, antivirals, corticosteroids, biologics, and anticoagulants. A total of 136 potential papers were identified in the first search through PubMed, and 72 of these were considered eligible for inclusion in this review by the authors. Because of the rapid developments in this area, we have also added and cited any newsworthy reports, as appropriate.

## Pharmacological treatments under evaluation

To date, clinical evidence and guidelines based on reliable data and randomized clinical trials (RCTs) for the treatment of COVID-19 are lacking. In the absence of definitive management protocols, many treatments for COVID-19 are currently being evaluated and tested worldwide (Table 1). Some of these options were soon abandoned due to ineffectiveness,<sup>14</sup> while others have shown promising results,<sup>15,16</sup> although it is still too early to have conclusive results, especially from rigorous RCTs. The use of many drug classes is producing numerous data, which are often contradictory. It is extremely difficult to evaluate the results of clinical studies during a pandemic. So

far, no therapy has been proven to be effective for treating the severe evolution of SARS-CoV-2 virus infection.<sup>17</sup>

## Antiviral drugs

Current antiviral treatments are mainly based on previous experiences (favipiravir) or on experimental drugs (remdesivir) used for the treatment of viral infections due to different viruses, such as influenza virus (InfV), Ebolavirus (EBOV), human immunodeficiency virus (HIV), MERS, and SARS.<sup>18</sup> These molecules act as nucleoside analogues, either in the form of adenine or guanosine, and target the RNA-dependent RNA polymerase (RDRP), causing the block of viral RNA synthesis.<sup>19</sup>

### Favipiravir

Favipiravir, a guanine analogue, was able to block the RDRP of SARS-CoV and MERS in vitro. Due to the high similarity of SARS-CoV-2 genome with SARS-CoV,<sup>20</sup> this treatment is considered a potential candidate for COVID-19, even if in vitro efficacy on SARS-CoV-2 has not been tested yet.<sup>21</sup> Favipiravir is now being assessed in RCTs recruiting patients with COVID-19, to evaluate its efficacy when combined with interferon- $\alpha$  or baloxavir marboxil (approved InfV inhibitor) (ChiCTR2000029548).

### Remdesivir

Remdesivir, a nucleotide analogue inhibitor of RDRP, is considered one of the most promising antivirals for the treatment of COVID-19. This drug has a broad antiviral spectrum against hepatitis B virus and HIV and MERS-CoV and SARS-CoV, both in vivo and in vitro.<sup>22,23</sup> The safety profile also appears reassuring. Two trials on the efficacy of remdesivir on mild-to-moderate (NCT04252664) and severe (NCT04257656) COVID-19 patients are currently ongoing in China; these studies may provide very important data on the efficacy of remdesivir. A recent work has shown that the antiviral activity of remdesivir begins immediately after the virus enters Vero E6 cells, carrying out its antiviral mechanism as a nucleotide analogue.<sup>15</sup> This drug acts as a competitor by incorporation with adenosine triphosphate to confuse viral RDRP, evading proofreading from viral exonuclease and causing a decrease in viral RNA production.<sup>24</sup>

A trial on remdesivir was conducted by the US National Institute of Allergy and Infectious Diseases on a cohort of 1063 patients. Preliminary data showed a positive effect in diminishing the time to recovery and reducing mortality rate. However, the latter result was not statistically significant.<sup>25</sup>

In a very recent randomized, double-blind, RCT, 237 patients with COVID-19 pneumonia and acute respiratory failure were randomly assigned in a 2:1 ratio to intravenous remdesivir or placebo infusions for 10 days. Unfortunately, also in this case, remdesivir was not associated with statistically significant clinical improvements.<sup>26</sup>

**Table 1. Ongoing RCTs for the treatment of COVID-19 with estimated completion date within 2020.**

| Drug name   | Type                           | Mechanism of action                                  | Approved conditions                        | Trials for COVID-19 treatment              | Intervention  | COVID-19 condition                           | Result date  | Clinical Trials Identifier |
|-------------|--------------------------------|--|--|--|---|--|--------------|----------------------------|
| Favipiravir | Antiviral, nucleoside analogue | Guanine analogue, blocks viral RdRP                  | InfV infection                             | Phase 3 Study - Active                     | Day 1: 1800 mg, BID; Day 2 and after: 600 mg, TID, for 14 days maximum  | COVID-19 moderate type                       | July 2020    | NCT04299724                |
|             |                                |  |  | Phase 3 – Not yet recruiting               | Lopinavir 800 mg per day + Ritonavir 200 mg per day + Favipiravir 2400 mg every 8 hours on Day 1, and a maintenance dose of 1200 mg BID   | COVID-19 for moderate to severe              | October 2020 | NCT04303299                |
|             |                                |  |  | Phase 3 – Not yet recruiting               | Darunavir 400 mg every 8 hours + Ritonavir 200 mg per day + Favipiravir 2400 mg, every 8 hours on Day 1, and a maintenance dose of 1200 mg BID plus Hydroxychloroquine 400 mg per day | COVID-19 moderate to critically ill patients | October 2020 | NCT04303299                |
|             |                                |  |  | Recruiting                                 | 1. Favipiravir (Day 1: 1600 mg BID, Days 2–7: 600 mg BID) + Tocilizumab (4 ~ 8 mg/kg)<br>2. Favipiravir (Day 1: 1600 mg BID, Days 2–7: 600 mg BID)<br>3. Tocilizumab (4 ~ 8 mg/kg)    | COVID-19                                     | May 2020     | NCT04310228                |
|             |                                |  |  | Not yet recruiting                         | Favipiravir (Day 1: 1600 mg, BID, Days 2–7: 600 mg BID)   | COVID-19                                     | June 2020    | NCT04333589                |
| Remdesivir  | Antiviral, nucleoside analogue | Adenosine analogue, terminates the nonobligate chain | Phase 3 on Ebola virus disease NCT03719586 | Phase 3 Study – Recruiting                 | Remdesivir 200 mg on Day 1 followed by Remdesivir 100 mg on Days 2–5 or 2–10  | Severe COVID-19                              | May 2020     | NCT04292899                |
|             |                                |  |  | Phase 3 Study – Recruiting                 | Remdesivir 200 mg on Day 1 followed by Remdesivir 100 mg on Days 2–5 or 2–10  | Moderate COVID-19                            | May 2020     | NCT04292730                |
|             |                                |  |  | Phase 3 Study – Recruiting                 | Remdesivir 200 mg on Day 1 followed by Remdesivir 100 mg for 9 days   | Mild Moderate COVID-19                       | April 2020   | NCT04252664                |
|             |                                |  |  | Phase 3 Study – Recruiting                 | Remdesivir 200 mg on Day 1 followed by Remdesivir 100 mg for 9 days   | Severe COVID-19                              | May 2020     | NCT04257656                |
|             |                                |  |  | Phase 2 Phase 3 Study – Not yet recruiting | 1. Remdesivir 200 mg on Day 1 followed by Remdesivir 100 mg up to 10 days<br>2. Hydroxychloroquine 800 mg x 2 loading dose followed by 400 mg x 2 every day up to 10 days             | COVID-19                                     | August 2020  | NCT04321616                |

(Continued)

Table 1. (Continued)

| Drug name           | Type                          | Mechanism of action   | Approved conditions                                | Trials for COVID-19 treatment      | Intervention   | COVID-19 condition | Result date    | Clinical Trials Identifier |
|---------------------|-------------------------------|---|--|------------------------------------|--|--------------------|----------------|----------------------------|
| Lopinavir/Ritonavir | Antiviral, protease inhibitor | Inhibits viral protease by forming an inhibitor-enzyme complex, preventing cleavage of polyproteins | HIV, SARS-CoV, MERS-CoV, HPV, HCoV-229E Infections | Phase 2 Study – Recruiting         | 1. Lopinavir/ritonavir 200 mg/100 mg, 2 tablets, BID for 7–10 days<br>2. Hydroxychloroquine 200 mg, 2 tablets, BID for 7–10 days   | Mild COVID-19      | May 2020       | NCT04307693                |
|                     |                               |   |  | Phase 4 Study – Recruiting         | 1. Abidol hydrochloride 0.2 g once, TID, 2 weeks<br>2. Oseltamivir 75 mg once, BID, 2 weeks<br>3. Lopinavir/ritonavir 500 mg once, BID, 2 weeks  | COVID-19           | February 2020  | NCT04255017                |
|                     |                               |   |  | Not yet recruiting                 | 1. ASC09/ritonavir (300 mg/100 mg) + standard treatment<br>2. Lopinavir/ritonavir tablet (200 mg/50 mg) + standard treatment   | COVID-19           | June 2020      | NCT04261907                |
| Tocilizumab         | Monoclonal antibody           | Targets the soluble or membrane IL6 receptors   | Rheumatoid arthritis or large vessels vasculitis   | Phase 2 Study – Recruiting         | Tocilizumab 8 mg/kg (up to maximum 800 mg per dose), 12 hours apart  | COVID-19           | December 2020  | NCT04317092                |
|                     |                               |   |  | Phase 2 Study – Recruiting         | 1. Tocilizumab (162 mg, 2 doses at 12 hours) + hydroxychloroquine (400 mg/12 hours Day 1 followed by 200 mg/12 hours for 6 days) + azithromycin (500 mg/day for 3 days)<br>2. Hydroxychloroquine (400 mg/12 hours Day 1 followed by 200 mg/12 hours for 6 days) + azithromycin (500 mg/day for 3 days) | COVID-19           | September 2020 | NCT04332094                |
|                     |                               |   |  | Phase 2 Study – Not yet recruiting | 8 mg/kg, maximum single dose 800 mg  | COVID-19           | October 2020   | NCT04335071                |
|                     |                               |   |  | Phase 2 Study – Active             | Single intravenous administration 8 mg/kg  | COVID-19           | April 2020     | NCT04315480                |

BID, twice a day; COVID-19, coronavirus disease; HCoV-229E, human coronavirus 229E; HIV, human immunodeficiency virus; HPV, human papilloma virus; IL, interleukin; InV, influenza virus; MERS-CoV; Middle East respiratory syndrome coronavirus; RDRP, RNA-dependent RNA polymerase; SARS-CoV, severe acute respiratory syndrome coronavirus; TID, three times a day.

## HIV protease inhibitors

Even if the activity on SARS-CoV-2 is uncertain, approved HIV protease inhibitors, such as lopinavir/ritonavir, were initially found to act on MERS-CoV and SARS-CoV and showed promising results in patients treated for SARS.<sup>27,28</sup> For these reasons, the combination was initially widely used for the treatment of SARS-CoV-2 infection, with uncertain results. A Chinese study on a small cohort of patients demonstrated clinical effectiveness of lopinavir/ritonavir in combination with arbidol, an antiviral drug usually used in China and Russia for InfV infection. However, this study did not show efficacy of lopinavir/ritonavir alone.<sup>29</sup> Currently, RCTs for the efficacy of a combination of lopinavir/ritonavir with interferon- $\alpha$  in patients with mild-to-moderate and severe-to-critical COVID-19 (ChiCTR2000029387 – ChiCTR2000029308) are ongoing. However, the first randomized, open-label, controlled trial involving 199 hospitalized adult patients with SARS-CoV-2 infection and respiratory failure unfortunately showed no benefit with lopinavir/ritonavir compared with standard of care.<sup>30</sup>

## Neuraminidase inhibitors

Neuraminidase inhibitors, such as oral oseltamivir and intravenous peramivir, have demonstrated significant clinical improvement in the treatment of patients with influenza in the past.<sup>31</sup> Oseltamivir has recently been used for SARS-CoV-2 suspected and confirmed patients in China.<sup>32</sup> Unfortunately, to date, no other data are available on their efficacy in COVID-19.

## Chloroquine and hydroxychloroquine

Chloroquine sulfate and phosphate salts were both marketed as antimalarial drugs. Hydroxychloroquine has been widely used as an antimalarial and in autoimmune diseases, such as lupus and rheumatoid arthritis (RA). These are drugs with a good safety profile with mild and transient side effects, if correctly dosed. In case of overdose or prolonged treatments, they can cause cardiomyopathies and QT prolongation.<sup>33</sup>

Chloroquine has also been used in various chronic viral diseases. In HIV infection, no positive results emerged, so the drug was not included in the recommended panel for the treatment of HIV.<sup>34</sup> The only viral infection in which hydroxychloroquine showed any efficacy was found in chronic hepatitis C, especially if associated with interferon pegylated plus ribavirin.<sup>35</sup>

Interestingly, Wang and colleagues evaluated various antivirals and chloroquine in vitro, concluding that this is highly effective in controlling SARS-CoV-2 replication.<sup>15</sup> Some data indicate that chloroquine interferes with the ability of SARS-CoV-2 to acidify lysosomes; moreover, it inhibits cathepsins, allowing the cleavage of the spike protein of SARS-CoV-2.<sup>36</sup>

In a recent study, chloroquine phosphate was found to be superior to control in the treatment of COVID-19 pneumonia, improving clinical and imaging aspects and shortening the course of the disease.<sup>37</sup> In a nonrandomized clinical trial on a small group of patients, a significant improvement in viral clearance was demonstrated. Furthermore, the combination with azithromycin was significantly more effective for the reduction or disappearance of the viral load compared with hydroxychloroquine monotherapy.<sup>38</sup>

On the contrary, in a study with a small cohort of patients hospitalized for severe SARS-CoV-2 infection, no strong antiviral activity or clinical efficacy of the combination of hydroxychloroquine and azithromycin was found.<sup>39</sup> The strong discrepancy of what emerged from the results of these studies highlights the fundamental need of RCTs to assess the efficacy of these drugs in the treatment of COVID-19. Conclusions from the published data are that there is currently no evidence of the efficacy of chloroquine for the treatment of COVID-19. The use should be limited to clinical studies to further clarify its role in the management of COVID-19.<sup>40</sup>

## Corticosteroids

The evidence on the efficacy of corticosteroids is conflicting, and the setting of application of these drugs needs further clarification. In the absence of reliable data, the WHO interim guidance on COVID-19 management does not recommend their routine use.<sup>41</sup> Furthermore, according to version 7 of the National Health Commission of China guidelines, corticosteroids should be used carefully in the case of SARS-CoV-2 infection.<sup>42</sup>

In a recent study in patients with ARDS due to COVID-19, a significant increase in survival was found in those who received methylprednisolone treatment ( $p=0.003$ ).<sup>43</sup>

In a retrospective study on a cohort of critically ill patients with SARS-CoV-1, appropriate use of corticosteroids was found to significantly reduce hospitalization mortality and duration.<sup>44</sup> All this without increasing superinfections and other important complications.

Another study in intensive care unit patients treated with corticosteroids had no conclusions due to the small sample size.<sup>10</sup> Corticosteroids associated with invasive and noninvasive mechanical ventilation should be considered to prevent progression to ARDS, especially in severe patients.<sup>45</sup> The Chinese Thoracic Society has defined an expert consensus statement on the use of corticosteroids for the treatment of COVID-19 pneumonia.<sup>46</sup> According to this document, corticosteroids should be used with caution in critically ill patients, and the dosage should be low to moderate ( $\leq 0.5$ – $1$  mg/kg per day of methylprednisolone or equivalent) with a short course ( $\leq 7$  days). Other authors argue that RCTs are needed to obtain more precise indications on the correct use of these important drugs.<sup>47</sup>

## Biologic drugs

### Tocilizumab

Tocilizumab (atlizumab) is a humanized IgG1k monoclonal antibody that targets the soluble or membrane IL-6 receptors (SIL-6R and MII-6R). This drug has so far been used in the treatment of autoimmune diseases such as RA or large vessels vasculitis.<sup>48</sup> As for SARS-CoV-2 infection, it is now known that the serum levels of inflammatory mediators are proportional to the severity of the clinical picture.<sup>10</sup> In these patients, exaggerated immunological responses can trigger cytokine storms and cause damage to multiple organs. The increase in IL-6 levels can also be a sensitive biomarker of clinical worsening and serious organ damage. Tocilizumab can block two fundamental inflammatory factors, IL-6 and granulocyte-macrophage colony-stimulating factor, thereby reducing the level of inflammation. A multicenter RCT to evaluate the efficacy and safety of tocilizumab in the treatment of moderate patients at high risk of evolution toward serious and critical illness (registration number: ChiCTR2000029765) was recently concluded. Results of this trial are not yet available; however, they are assumed to be positive in patients with elevated IL-6 levels.

### Sarilumab

Sarilumab is a fully human monoclonal IgG1 antibody targeting soluble and membrane IL-6 receptors, inhibiting IL-6-mediated signal transduction mediated by these receptors.<sup>49</sup> This biologic is approved as subcutaneous treatment in patients over 18 years of age with moderate-to-severe active RA refractory or intolerant to one or more disease-modifying antirheumatic drugs.<sup>50</sup> Based on the experience of tocilizumab, sarilumab is recently being used in the treatment of patients with severe forms of COVID-19. Five RCTs are ongoing (ClinicalTrials.gov Identifier: NCT04315298, NCT04327388, NCT04324073, NCT04322773, and NCT04321993), the results of which will be of great value.

## Antibiotic and antifungal agents

The latest version of the guidelines issued by the Chinese National Health Commission for the diagnosis and treatment of COVID-19 infection and the WHO interim guidelines advise against the unnecessary use of antibiotics, even on a broad spectrum.<sup>42</sup> These should be used in the event of an increase in procalcitonin (PCT), in the case of hospital-acquired pneumonia or ventilator-associated pneumonia, in case of microbiological tests indicative of bacterial superinfection. If fungal infection is suspected, voriconazole is indicated for *Aspergillus* infections, while fluconazole is indicated for *Candida* spp. infections. For pneumocystis pneumonia in immunosuppressed patients, the drugs to be considered are sulfamethoxazole and caspofungin.<sup>51</sup>

### Teicoplanin

Teicoplanin is a first-generation glycopeptide with antimicrobial activity against aerobic and anaerobic Gram-positive bacteria including multi-resistant *Staphylococci*. This antibiotic has shown efficacy in the past against numerous viruses, such as EBOV, InfV, flavivirus, hepatitis C, HIV, MERS-CoV, and SARS-CoV.<sup>52,53</sup> The antiviral activity has recently been confirmed against SARS-CoV-2.<sup>54</sup> It will be necessary to confirm these results and the possible use of teicoplanin in COVID-19 through RCTs.

## Anticoagulants

It is now known that about 20% of patients with COVID-19 have clotting alterations; thrombosis of lungs, liver, and other organs; and marked increase in D-dimer.<sup>10,32</sup> Anticoagulant therapy should be administered carefully in clinical practice or in case of surgery. In these cases, platelet transfusion, administration of fresh frozen plasma, or more commonly low molecular weight heparin (LMWH) is recommended. In critically ill patients, anticoagulant therapy is recommended if no contraindications are present. Recently, new evidence has appeared on coagulopathies and the appearance of antiphospholipid antibodies with consequent multiple heart attacks in patients with SARS-CoV-2 infections.<sup>55</sup> Large cohorts of severe COVID-19 patients showed a high risk of disseminated intravascular coagulation and venous thromboembolism. Low molecular weight heparin therapy is related to a higher survival rate in patients with severe COVID-19.<sup>56</sup>

In light of these data, it is even more important to reiterate the importance of anticoagulant therapy in severe Covid-19 patients.

## Other potential treatments

The concern about the possibility that drugs blocking the renin-angiotensin system (RAS) might increase the risk of developing a life-threatening SARS-CoV-2 infection could be due to the fact that the ACE2 receptor allows the entry of coronavirus into cells.<sup>57</sup> However, there are no data to support the possibility that ACE inhibitors or angiotensin II receptor blockers (ARBs) favor the entry of coronaviruses by increasing the expression of ACE2 in humans.

RAS dysfunction is present in patients with COVID-19, but clinical outcomes of RAS inhibitor therapy, for example, with angiotensin converting enzyme inhibitors (ACE inhibitors) or ARBs are currently unknown, and there is no evidence for their suspension. In a retrospective study of 417 patients with COVID-19, patients treated with an ACEI or ARB had a better prognosis and lower levels of IL-6 in peripheral blood.<sup>58</sup> In addition, therapy with these drugs had increased CD3 and CD8 T-cell counts in peripheral blood and reduced viral load. These data could indicate that the treatment with an ACEI or ARB

may have positive effects on a more favorable evolution of the COVID-19 infection.

To assess more clearly the potential benefits of ARBs, such as valsartan or losartan, on the evolution of COVID-19, RCTs are ongoing (NCT04335786, NCT04335123, and NCT04312009). Only when the data of these studies are published, it will be possible to define the potential benefits or the risks related to these treatments.

## Future directions: the search for the vaccine

Exploring and understanding the immunogenicity of COVID-19 are essential for developing the most effective vaccine. However, evidence on the immunogenicity of SARS-CoV-2 is limited. The genome of the SARS-CoV-2 is over 80% identical to the SARS-like bat CoV, and studies on B-cells and T-cells epitopes have revealed high homology between SARS-CoV and SARS-CoV-2 proteins.<sup>59</sup> Previously, studies on SARS-CoV-1 vaccines revealed that the S protein on the surface of the virus is an ideal target for a vaccine, as antibody responses directed against it showed promising results in protecting from infection in mouse models.<sup>60,61</sup> Moreover, while B-cell response

toward SARS-CoV gave limited protection over time,<sup>62</sup> T-cell response provided long-term protection, even up to 11 years post-infection, and are thus considered as a potential target for vaccines against coronaviruses.<sup>63</sup> Research efforts are aimed at identifying the ideal SARS-CoV-2 epitopes, against which directing vaccines is increasing. Many vaccines are currently under development (Table 2), following different strategies.<sup>58,64</sup> Whole virus vaccines are currently being investigated by companies such as Johnson & Johnson.<sup>65</sup> They have the advantage of eliciting a strong immune response, through the stimulation of toll-like receptors; however, they require longer testing to guarantee safety.<sup>66</sup> Furthermore, subunit vaccines, which rely on stimulating an immune response against the viral S-protein preventing its binding with the cell-receptor, are already under investigation by companies such as Novavax, Clover Biopharmaceuticals, and different consortia in the USA and Australia.<sup>67–69</sup> Finally, nucleic acids vaccines, both based on deoxyribonucleic acid and RNA platforms, are being considered by biotech companies, such as Inovio Pharmaceuticals and Moderna Therapeutics, both at phase I stage (NCT04336410 and NCT04283461). These latter formulations have not been successful in eliciting protective immunity in humans so far; however, expectations are high that this approach might eventually be successful.<sup>70</sup>

**Table 2. COVID-19 vaccines development programs.**

| Sponsor  | Vaccine type   | Status  | ClinicalTrials.gov Identifier |
|--|--|---|-------------------------------|
| Shenzhen Geno-Immune Medical Institute   | Lentiviral-vector vaccine/artificial antigen presenting cells (COVID-19/aAPC)  | Phase 1 Study – Recruiting                    | NCT04299724                   |
| Shenzhen Geno-Immune Medical Institute   | Lentivirus vectors expressing Covid-19 minigene SMENP (LV-SMENP-DC) and CTLs   | Phase 1, Phase 2 Studies – Recruiting         | NCT04276896                   |
| Symvivo Corporation  | Bac_TRL-Spike (live <i>Bifidobacterium longum</i> , engineered to deliver plasmids containing synthetic DNA encoding SARS-CoV-2 S-protein) | Phase 1 Study – Not yet recruiting            | NCT04334980                   |
| University of Oxford   | ChAdOx1 nCoV-19 (ChAdOx1 viral vector)   | Phase 1, Phase 2 Studies – Not yet recruiting | NCT04324606                   |
| Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China | Recombinant novel coronavirus vaccine (Adenovirus type 5 vector, Ad5-nCoV, encoding for Spike protein)                                     | Phase 2 Study – Not yet recruiting            | NCT04341389                   |
| CanSino Biologics Inc.   | Recombinant novel coronavirus vaccine (Adenovirus type 5 vector, Ad5-nCoV, encoding for Spike protein)                                     | Phase 1 Study – Active                        | NCT04313127                   |
| National Institute of Allergy and Infectious Diseases                          | mRNA-1273 (RNA – nucleic acid vaccine)   | Phase 1 Study – Recruiting                    | NCT04283461                   |
| Inovio Pharmaceuticals   | INO-4800 (DNA – nucleic acid vaccine)  | Phase 1 Study – Recruiting                    | NCT04336410                   |

aAPC, antigen presenting cells; COVID-19, coronavirus disease; CTL, cytotoxic T-lymphocytes; DNA, deoxyribonucleic acid; LV-SMENP, Lentiviral minigene vaccine; mRNA, messenger RNA; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

## Conclusions

At the moment, treatment of COVID-19 unfortunately has more shadows than lights. The basic treatments are mainly represented by antiviral drugs such as remdesivir, chloroquine, or lopinavir/ritonavir, even if the evidence for their use is not satisfactory. Among the antiviral agents, the most promising appears to be remdesivir, though this needs to be confirmed by ongoing RCTs. However, many questions remain open about pathogenesis, the molecular mechanism of input, viral replication, and immunological pathways, in order to identify

targeted therapies. Corticosteroids and tocilizumab seem to guarantee positive results in selected patients so far, although the timing of starting therapy and the most appropriate therapeutic schemes remain to be clarified. Efficacy of the other drugs is still uncertain, and they are currently used in combination and in variable ways in the absence of definitive guidelines. What will represent the real solution to the enormous problem taking place worldwide is the identification of a safe and effective vaccine, for which enormous efforts and investments are underway.

**Contributions:** FM and MB conceived the manuscript outline, contributed to all the sections, and revised the whole manuscript. CB and FL drafted the paragraphs concerning antibiotics and anticoagulants. NF reviewed and approved the manuscript. All the authors read and approved the final version. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

**Disclosure and potential conflicts of interest:** Francesco Menzella participated in contracted research and clinical trials for Novartis and Sanofi. He has received lecture fees and advisory board fees from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Mundipharma, and Novartis. The other authors report no conflicts of interest in this work. The International Committee of Medical Journal Editors (ICMJE) Potential Conflicts of Interests form for the authors is available for download at: <https://www.drugsincontext.com/wp-content/uploads/2020/05/dic.2020-4-6-COI.pdf>

**Acknowledgements:** None.

**Funding declaration:** There was no funding associated with the preparation of this article.

**Copyright:** Copyright © 2020 Menzella F, Biava M, Barbieri C, Livrieri F, Facciolongo N. Published by Drugs in Context under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

**Correct attribution:** Copyright © 2020 Menzella F, Biava M, Barbieri C, Livrieri F, Facciolongo N. <https://doi.org/10.7573/dic.2020-4-6>. Published by Drugs in Context under Creative Commons License Deed CC BY NC ND 4.0.

**Article URL:** <https://drugsincontext.com/pharmacological-treatment-of-covid-19:-lights-and-shadows>

**Correspondence:** Francesco Menzella, Department of Medical Specialties, Pneumology Unit, Azienda USL di Reggio Emilia- IRCCS, Viale Amendola 2, 42122 Reggio Emilia, Italy. [francesco.menzella@ausl.re.it](mailto:francesco.menzella@ausl.re.it)

**Provenance:** submitted; externally peer reviewed.

**Submitted:** 13 April 2020; **Peer review comments to author:** 30 April 2020; **Revised manuscript received:** 30 April 2020; **Accepted:** 30 April 2020; **Publication date:** 19 May 2020.

**Drugs in Context** is published by BioExcel Publishing Ltd. Registered office: Plaza Building, Lee High Road, London, England, SE13 5PT.

BioExcel Publishing Limited is registered in England Number 10038393. VAT GB 252 7720 07.

For all manuscript and submissions enquiries, contact the Editor-in-Chief [gordon.mallarkey@bioexcelpublishing.com](mailto:gordon.mallarkey@bioexcelpublishing.com)

For all permissions, rights and reprints, contact David Hughes [david.hughes@bioexcelpublishing.com](mailto:david.hughes@bioexcelpublishing.com)

## References

1. WHO. Rolling updates on coronavirus. Disease (COVID-19). <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen>. Accessed April 9, 2020.
2. Corman VM, Muth D, Niemeyer D, Drosten C. Hosts and sources of endemic human coronaviruses. *Adv Virus Res.* 2018;100:163–188. <https://doi.org/10.1016/bs.aivir.2018.01.001>
3. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Virology.* 2019;16(1):69. <https://doi.org/10.1186/s12985-019-1182-0>
4. Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell.* 2020;181:223–227. <https://doi.org/10.1016/j.cell.2020.03.035>

5. Andersen KG, Rambaut A, Lipkin WI, et al. The proximal origin of SARS-CoV-2. *Nat Med*. Published online March 17, 2020. <https://doi.org/10.1038/s41591-020-0820-9>
6. Shereen MA, Khan S, Kazmi A, et al. COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. *J Adv Res*. 2020;24:91–98. <https://doi.org/10.1016/j.jare.2020.03.005>
7. Wan Y, Shang J, Graham R, et al. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol*. 2020;94(7). <https://doi.org/10.1128/JVI.00127-20>
8. Gralinski LE, Menachery VD. Return of the coronavirus: 2019-nCoV. *Viruses*. 2020;12(2):135. <https://doi.org/10.3390/v12020135>
9. Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*. 2020;63(3):457–460. <https://doi.org/10.1007/s11427-020-1637-5>
10. 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. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)
11. McGonagle D, Sharif K, O'Regan A, et al. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev*. 2020;102537. <https://doi.org/10.1016/j.autrev.2020.102537>
12. Wan S, Yi Q, Fan S, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). *MedRxiv*. Published online February 12, 2020. <https://doi.org/10.1101/2020.02.10.20021832>
13. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020;395(10229):1033–1034. [https://doi.org/10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0)
14. Stower H. Lopinavir-ritonavir in severe COVID-19. *Nat Med*. Published online April 9, 2020. <https://doi.org/10.1038/s41591-020-0849-9>
15. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020;30(3):269–271. <https://doi.org/10.1038/s41422-020-0282-0>
16. Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. *N Engl J Med*. 2020;382:929–936. <https://doi.org/10.1056/NEJMoa2001191>
17. Du YX, Chen XP. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. *Clin Pharmacol Ther*. Published online April 4, 2020. <https://doi.org/10.1002/cpt.1844>
18. Lu CC, Chen MY, Chang YL. Potential therapeutic agents against COVID-19: what we know so far. *J Chin Med Assoc*. Published online April 1, 2020. <https://doi.org/10.1097/JCMA.0000000000000318>
19. De Clercq E. New nucleoside analogues for the treatment of hemorrhagic fever virus infections. *Chem Asian J*. 2019;14(22):3962–3968. <https://doi.org/10.1002/asia.201900841>
20. 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. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8)
21. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov*. 2020;19(3):149–150. <https://doi.org/10.1038/d41573-020-00016-0>
22. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med*. 2017; 9(396):eaal3653. <https://doi.org/10.1126/scitranslmed.aal3653>
23. de Wit E, Feldmann F, Cronin J, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *Proc Natl Acad Sci U S A*. 2020;117(12):6771–6776. <https://doi.org/10.1073/pnas.1922083117>
24. Agostini ML, Andres EL, Sims AC, et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *mBio*. 2018;9(2):e00221-18. <https://doi.org/10.1128/mBio.00221-18>
25. BBC News. Remdesivir: Drug has 'clear-cut' power to fight coronavirus. <https://www.bbc.com/news/health-52478783>. Accessed April 30, 2020.
26. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. Published online April 29, 2020. [https://doi.org/10.1016/S0140-6736\(20\)31022-9](https://doi.org/10.1016/S0140-6736(20)31022-9)
27. 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. <https://doi.org/10.1136/thorax.2003.012658>
28. Zumla A, Chan JF, Azhar EI, et al. Coronaviruses - drug discovery and therapeutic options. *Nat Rev Drug Discov*. 2016;15(5):327–347. <https://doi.org/10.1038/nrd.2015.37>
29. Deng L, Li C, Zeng Q, et al. Arbidol combined with LPV/r versus LPV/r alone against corona virus disease 2019: a retrospective cohort study. *J Infect*. 2020. <https://doi.org/10.1016/j.jinf.2020.03.002>
30. Cao B, Wang Y, Wen D, et al. A Trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med*. Published online March 18, 2020. <https://doi.org/10.1056/NEJMoa2001282>
31. Chow EJ, Doyle JD, Uyeki TM. Influenza virus-related critical illness: prevention, diagnosis, treatment. *Crit Care*. 2019;23(1):214. <https://doi.org/10.1186/s13054-019-2491-9>

32. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. Published online February 7, 2020. <https://doi.org/10.1001/jama.2020.1585>
33. Frisk-Holmberg M, Bergqvist Y, Englund U. Chloroquine intoxication [letter]. *Br J Clin Pharmacol*. 1983;15:502–503. <https://doi.org/10.1111/j.1365-2125.1983.tb01540.x>
34. Chauhan A, Tikoo A. The enigma of the clandestine association between chloroquine and HIV-1 infection. *HIV Med*. 2015;16:585–590. <https://doi.org/10.1111/hiv.12295>
35. Helal GK, Gad MA, Abd-Ellah MF, et al. Hydroxychloroquine augments early virological response to pegylated interferon plus ribavirin in genotype-4 chronic hepatitis C patients. *J Med Virol*. 2016;88:2170–2178. <https://doi.org/10.1002/jmv.24575>
36. Simmons G, Bertram S, Glowacka I, et al. Different host cell proteases activate the SARS-coronavirus spike-protein for cell–cell and virus–cell fusion. *Virology*. 2011;413:265–274. <https://doi.org/10.1016/j.virol.2011.02.020>
37. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci. Trends*. Published online March 16, 2020. <https://doi.org/10.5582/bst.2020.01047>
38. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. Published online March 20, 2020. <https://doi.org/10.1016/j.ijantimicag.2020.105949>
39. Molina JM, Delaugerre C, Goff JL, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Med Mal Infect*. 2020. <https://doi.org/10.1016/j.medmal.2020.03.006>
40. Cortegiani A, Ingoglia G, Ippolito M, et al. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *J Crit Care*. Published online March 30, 2020. <https://doi.org/10.1016/j.jcrc.2020.03.005>
41. WHO. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. [https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). Accessed April 9, 2020.
42. National Health Commission of the People’s Republic of China. Guideline for the diagnosis and treatment of COVID-19 infections (version 1–7). 2020. [http://www.nhc.gov.cn/yzygj/zcwj2/new\\_zcwj.shtml](http://www.nhc.gov.cn/yzygj/zcwj2/new_zcwj.shtml). Accessed April 9, 2020.
43. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA*. Published online March 13, 2020. <https://doi.org/10.1001/jamainternmed.2020.0994>
44. Chen RC, Tang XP, Tan SY, et al. Treatment of severe acute respiratory syndrome with glucocorticoids: the Guangzhou experience. *Chest*. 2006;129:1441–52. <https://doi.org/10.1378/chest.129.6.1441>
45. Xu Z, Shi L, Wang Y, Zhang J, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8(4):420–422. [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X)
46. Zhao JP, Hu Y, Du RH, et al. Expert consensus on the use of corticosteroid in patients with 2019-nCoV pneumonia (in Chinese). *Zhonghua Jie He He Hu Xi Za Zhi*. 2020;43:183–184. <https://doi.org/10.3760/cma.j.issn.1001-0939.2020.03.008>
47. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet*. 2020;395(10223):473–475. [https://doi.org/10.1016/S0140-6736\(20\)30317-2](https://doi.org/10.1016/S0140-6736(20)30317-2)
48. Kaneko Y, Kato M, Tanaka Y, et al. Tocilizumab discontinuation after attaining remission in patients with rheumatoid arthritis who were treated with tocilizumab alone or in combination with methotrexate: results from a prospective randomised controlled study (the second year of the SURPRISE study). *Ann Rheum Dis*. 2018;77(9):1268–1275. <https://doi.org/10.1136/annrheumdis-2018-213416>
49. Scott LJ. Sarilumab: first global approval. *Drugs*. 2017;77(6):705–712. <https://doi.org/10.1007/s40265-017-0724-2>
50. Lamb YN, Deeks ED. Sarilumab: a review in moderate to severe rheumatoid arthritis. *Drugs*. 2018;78(9):929–940. <https://doi.org/10.1007/s40265-018-0929-z>
51. Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis*. 2016;63(5):e61–e111. <https://doi.org/10.1093/cid/ciw353>
52. Zhou N, Pan T, Zhang J, et al. Glycopeptide antibiotics potently inhibit cathepsin L in the late endosome/lysosome and block the entry of ebola virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). *J Biol Chem*. 2016;291:9218–9232. <https://doi.org/10.1074/jbc.M116.716100>
53. Colson P, Raoult D. Fighting viruses with antibiotics: an overlooked path. *Int J Antimicrob Agents*. 2016;48:349–352. <https://doi.org/10.1016/j.ijantimicag.2016.07.004>
54. Zhang J, Ma X, Yu F, et al. Teicoplanin potently blocks the cell entry of 2019-nCoV. *BioRxiv*. Published online February 13, 2020. <https://doi.org/10.1101/2020.02.05.935387>
55. Zhang Y, Xiao M, Zhang S, et al. Coagulopathy and antiphospholipid antibodies in patients with Covid-19. *N Engl J Med*. Published online April 8, 2020. <https://doi.org/10.1056/NEJMc2007575>

56. Tang N, Bai H, Chen X, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost*. Published online March 27, 2020. <https://doi.org/10.1111/jth.14817>
57. Danser AHJ, Epstein M, Batlle D. Renin-angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin-angiotensin system blockers. *Hypertension*. Published online March 25, 2020. <https://doi.org/10.1161/HYPERTENSIONAHA.120.15082>
58. Meng J, Xiao G, Zhang J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microbes Infect*. 2020;9(1):757–760. <https://doi.org/10.1080/22221751.2020.1746200>
59. 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):254. <https://doi.org/10.3390/v12030254>
60. Yang ZY, Kong WP, Huang Y, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature*. 2004;428:561–564. <https://doi.org/10.1038/nature02463>
61. Graham RL, Becker MM, Eckerle LD, et al. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nat. Med*. 2012;18:1820–1826. <https://doi.org/10.1038/nm.2972>
62. 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;186:7264–7268. <https://doi.org/10.4049/jimmunol.0903490>
63. 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. <https://doi.org/10.1016/j.vaccine.2016.02.063>
64. Grifoni A, Sidney J, Zhang Y, et al. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell Host Microbe*. 2020;27(4):671–680. <https://doi.org/10.1016/j.chom.2020.03.002>
65. Johnson & Johnson. Johnson & Johnson Announces a Lead Vaccine Candidate for COVID-19; Landmark New Partnership with U.S. Department of Health & Human Services; and Commitment to Supply One Billion Vaccines Worldwide for Emergency Pandemic Use. <https://www.jnj.com/johnson-johnson-announces-a-lead-vaccine-candidate-for-covid-19-landmark-new-partnership-with-u-s-department-of-health-human-services-and-commitment-to-supply-one-billion-vaccines-worldwide-for-emergency-pandemic-use>. Accessed April 10, 2020.
66. Jiang S, Bottazzi ME, 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. <https://doi.org/10.1586/erv.12.126>
67. Coleman CM, Liu YV, Mu H, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. *Vaccine*. 2014;32(26):3169–3174. <https://doi.org/10.1016/j.vaccine.2014.04.016>
68. 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. <https://doi.org/10.1016/j.xphs.2017.04.037>
69. 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 Vaccines Immunother*. 2014;10(3):648–658. <https://doi.org/10.4161/hv.27464>
70. Chen WH, Strych U, Hotez PJ, et al. The SARS-CoV-2 vaccine pipeline: an overview. *Curr Trop Med Rep*. Published online March 3, 2020. <https://doi.org/10.1007/s40475-020-00201-6>