

The Novel Coronavirus (nCoV)

Július Rajčáni*

RT-Europe Non-profit Research Centre, Vár tér 2/E,
Mosonmagyaróvár, H-9200 Hungary.

***Correspondence:**

Július Rajčáni, Senior Scientist, 82103 Bratislava, Sabinovská
9, Slovakia.

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ABSTRACT

This review aims to assess the actual data from recent literature dealing with the novel Coronavirus (nCoV) isolated in the autumn of 2019. The nCoV has emerged in China namely in the city Wuhan (Hubei province), while the classical Coronavirus (cCoV) identified 18 years ago, came from a different region (province Guangdong). Extensive progression of nCoV represents a considerable danger for public health. The relatively great number of papers devoted to given topics in the last year (2020), also points at its importance. Despite of their non-selective acquisition, the collected data became useful for better understanding of virus replication, at description of virion structure as well as by interpretation of Coronavirus properties. Finally, the typical clinical signs of nCoV disease are briefly highlighted, not excluding the less closely related pathological states. At last but not least, relevant epidemiological data are presented when tracing the routes of nCoV spread in human population, with special regards to the region of Middle Europe.

Keywords

Coronavirus, Virion structure, Virus transmission, Clinical signs of disease.

Introduction

Within a relatively short period of 5-6 months (from March until September 2020) a novel Coronavirus (nCoV) has emerged in China (in the city of Wuhan, Hubei province), where from it spread all over the world. Some investigators called nCoV as Coronavirus 2019 (CoV-19), while the others termed it Coronavirus 2 (CoV-2). In the early stages of nCoV infection, cases of pneumonia have been reported, while other patients showed acute respiratory symptoms, namely a syndrome of rapidly developing acute respiratory distress (ARD) and/or an acute respiratory failure, both accompanied with serious complications leading to death [1]. In a proportion of infected subjects, the less severe cases revealed clinical complaints called Coronavirus Disease (COVID-19), an entity closely resembling to the previously described severe acute respiratory syndrome (SARS) [2]. Several details of the recent nCoV/CoV-2 outbreak have remained unclear, except for the fact that the novel virus has occurred in China and that (very probably) it has been transmitted by direct exposure [3,4]. In this respect, the recent nCoV outbreak has shown a possible link to the South

China Seafood market at City of Wuhan [5,6]. Despite of that the fish and wild animal market in Wuhan has remained in continuous performance selling live animals (such as poultry, bats, marmots and snakes), regardless that it became the source of dangerous infection [7]. Even though the majority of COVID-19 cases was reported in patients from continental China, also the island of Taiwan has been involved [8]. Unlike to previously described „classical” Coronavirus (cCoV), which had been isolated in a different Chinese province (Guangdong) 18 years ago (namely in the late autumn of year 2002), the nCoV spread quickly infecting nearly 110 million people worldwide, a proportion (about 3-4 %) of whom has developed the signs of severe lung disease¹.

Distribution

Due to the extensive spread of nCoV/CoV-2 as mentioned above, WHO declared the recent outbreak for public health emergency, i.e. a situation deserving wide international concern [11]. It has been recognized that valid healthcare measures may not be sufficient for preventing further nCoV/CoV-2 transfer. Especially

¹ Global case count (as of February 18, 08:24 ET, 2021): 110,013,841 confirmed cases and 2,432,695 deaths (2.2%). Histological details of lung disease (interstitial pneumonia) could be documented in piglets infected with porcine coronavirus, called the porcine respiratory and reproductive syndrome virus (PRRSV).

the spread of COVID-19 from asymptomatic carriers (i.e. via person-to-person contact) deserves much attention [9,12]. To stop a dangerous outcome, additional measures were recommended which practical effects should be under control to achieve the following goals: 1) Slowing down the spread of COVID-19 illness. 2) Providing enough time to implement the rules of local health care (in each cooperating state). 3) Achieving individual business protection. 4) Improving the actual education of local health authorities. 5) Instructing the general public how to avoid virus transmission of more disastrous extent (if possible). 6) And finally, to describing the signs of COVID-19 in the majority of local health guidelines [13] including an appropriate vaccine development along with optimal vaccination strategies [14]. In the course of 2020, as soon as the virus sequence became available the number of different vaccine has moved with unexpected speed. Different types of vaccine candidates, such as inactivated virus, live attenuated virus vaccines revealing a limited replication as well as various recombinant protein vaccines (especially the spike protein-based ones) and/or DNA vaccines have been developed. Of special interest are the novel specific mRNA based vaccines delivered via lipid nanoparticles; these represent an entirely new technology aiming to produce the immunogenic S protein in the body of vaccinated individual. It comes from above mentioned considerations that development and deployment of medical countermeasures, including precise diagnostics and therapeutic recommendations (not excluding the above-mentioned continuing efforts for vaccine development) has come into the focus of interest [15]. For achieving this, especially the asymptomatic COVID-19 cases should be assessed, based either on viral nucleic acid tests or on virus antigen detection [16].

Clinical signs

Infections occurring in the absence of any COVID-19 symptoms, either respiratory or gastrointestinal, may not show any significant abnormalities on chest radiograph [3,4]. Guan et al. [17] using a larger patient sample for estimation, suggested that the median incubation period might be 3 days only, but could be as long as 24 days. Noteworthy, the mean incubation time was estimated for 5.2 days, in a range from 2.1 to 11.1 days [18]. Some patients with SARS, which were defined as laboratory-confirmed COVID-19 cases, had respiratory symptoms even though their chest computed tomography (CT) but did not reveal signs of pneumonia [19]. Another patients with pneumonia manifested on their chest radiograph (defined for COVID-19 positive), then had both, the respiratory symptoms as well as pneumonia [20]. The latter category of positive individuals showed severe pneumonia along with a state of critical clinical conditions, such as shock and/or respiratory failure requiring mechanical ventilation. In some cases there even was a general organ failure needing special management [21]. In general, fever occurs with a probability ranging from 67% to 98%, cough by at least from 43% but up to 81%; the shortness of breath may be present in 31% to 55% of COVID-19 cases and finally, the frequency of myalgias remains as low as 3% to 11%, but never exceeding 44% [24]. Patients with pneumonia were older, with a higher prevalence of smoking history and more underlying

diseases. They were more likely to have fever, myalgia/fatigue, dyspnea, headache, and nausea/vomiting as compared to patients with a simple ARD by a statistical difference of $p < 0.05$. In addition, the pneumonia cases have presented higher white blood cell and neutrophil counts, while the simple ARD cases had rather a reduced leukocyte count [22]. The pneumonia patients, as a rule, received more antibiotics and/or antiviral therapy and later on, they were more likely to require oxygenation therapy, mechanical ventilator, extracorporeal membrane oxygenation and even renal replacement [23].

Transmission

Since the person-to-person transmission of nCoV has been clearly confirmed [20], asymptomatic individuals were recognized for potential sources of infection [24]. The identification of nCoV cases as well as their contacts has led to the assessment of travelers arriving from areas with more frequent and/or substantial virus transmission [25]. These conditions have got similar to and/or identical with those introduced in the course of influenza virus pandemic [26]. The differences in infectivity among the various coronavirus isolates were attributed to differences in the rigidity of their shells and/or another chosen protein, a parameter which could be evaluated using computational tools applied for predicting any intrinsic disorder predisposition [27]. The estimated reproductive number of 0.3 was obtained from considering a small number of infected persons who revealed not quite precise information as the onset of the outbreak concerns [19]. Thus, the reproductive number of nCoV/CoV-19 is likely to be similar (in the range from 2 to 3) either to that SARS-cCoV which had appeared during the pre-intervention period in year 2003 or to the pandemic of influenza virus A/H1N1 in year 2009 in US (estimated range from 1.3 to 1.7) [28,29]. Owing to these observations, a series of DNA vaccine candidates expressing different forms of the SARS-CoV-2 spike (S) protein have been developed and evaluated in rhesus macaques, namely (i) A full-length polypeptide (S). (ii) The given polypeptide at the cytoplasmic tail sequence deleted (S.dCT). (iii) The S polypeptide by both, the transmembrane domain and the cytoplasmic tail soluble ectodomain (S.dTM) deleted. (iv) The given polypeptide extended at the S1 domain by a foldon trimerization tag (S1). (v) The receptor-binding domain of given polypeptide only but along with the foldon trimerization tag (RBD). (vi) A perfusion-stabilized soluble ectodomain of the given polypeptide deleted at a furin cleavage site (showing two proline mutations), and finally, the given polypeptide just with the foldon trimerization tag added: all these were assessed for their immunogenicity and protective efficacy [14,30].

Virion structure

The structural proteins (and/or glycoproteins) at any Beta-coronavirus (B-CoV) strain are encoded by four regular structural genes, namely the spike glycoprotein (S, former E2), the envelope glycoprotein (E, former sM), the membrane glycoprotein (M, former E1) and the nucleocapsid protein (N) [15]. The 29.7 kb long sequence of the single stranded negative sense viral RNA (vRNA) has an untranslated region at its 5'-end (5'-UTR) along with a

short leader sequence (LS) which continues into the two relatively long open reading frames (ORF 1a/b) encoding the corresponding polyproteins (Plp1a and Plp1b). These both become cleaved by an endogenous peptidase to form at least 13-15 non-structural polypeptides (NSP) involved mainly in vRNA replication [31,32]. Another four genes encoding structural proteins are interrupted by regions specifying the so-called accessory proteins (in the case of CoV-2 these are the following: ORF 3a, ORF 3b, ORF6, ORF7a, ORF7b, ORF 8a, ORF 8b and ORF9). Some of these are located in between S and E sequences (ORF 3a/3b), but the majority between the M and N genes (with exception of ORF 9 which is positioned within the N sequence). The vRNA ends with a short untranslated region of the 3'-UTR sequence [18,33]².

Replication

The life cycle of CoV-2 in the susceptible host cells begins by binding of S protein to corresponding cellular receptor, namely the angiotensin converting enzyme 2 (ACE2). Understanding how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) hijacks epithelial cells and infiltrates the lung, as well as other organs and tissues, is essential for developing treatment strategies and vaccines against this highly contagious virus. Another major goal is to fully elucidate the mechanisms by which SARS-CoV-2 bypasses the innate immune system and induces what is called **cytokine storm**, which increases mortality. Currently, SARS-CoV-2 is thought to evade innate antiviral immunity, undergo endocytosis, and fuse with the host cell membrane by exploiting ACE2 receptors and the protease TMMPRSS2, with cathepsin B/L as alternative protease, for entry into the epithelial cells of tissues vulnerable to developing coronavirus disease 2019 (CoV-19) symptoms. However, the incorporation of new and unique binding sites, i.e., O-linked glycans, and the preservation and augmentation of effective binding sites (N-linked glycans) on the outer membrane of SARS-CoV-2 may represent other strategies of infecting the human host [39]. The surface glycoprotein S of coronaviruses mediating the attachment and entry into target cells is composed of 2 subunits, S1 and S2. The S1 subunit contains the N-terminal domain (NTD) and a receptor-binding domain (RBD), the latter encompassing a receptor-binding motif (RBM). The S2 contains a fusion peptide (FP), heptad repeat 1 (HR1) and 2 (HR2) domains, and a transmembrane (TM) and a cytoplasmic (CP) domain. After S1 binding to a membrane receptor, the FP is inserted into the cell membrane to promote fusion with the viral membrane, a process that depends on proteolytic cleavages at the S1/S2 site to separate S1 and S2 and at the S2 site to generate a mature FP [50]. The entry point for the virus is ACE2, which is a component of the counteracting hypotensive axis of renin angiotensin system

² Taken together, the nCoV genome has the following genes:

5'-LS--p65/Plp1a/ORF1a/NSP1--Plp1b/ORF1b/NSP2 (poliovirus type protease)--NSP3 (putative PL pro-domain)--NSP4 (associates with NSP3)--NSP5 (3CL-pro-domain)--NSP6 (complex with NSP3)--NSP7 (primase component)--NSP8 (primase component)--NSP9 (nucleic acid binding)--NSP10 (associates with NSP14)--NSP11(short peptide)--NSP12 (RNA polymerase)--NSP13 (helicase/NTPase)--NSP14 (exonuclease) --NSP15 (endoribonuclease)-NSP16 (methyl-transferase)-S-E-M-N-3'.

(RAS). Bradykinin is a potent part of the vasopressor system that induces hypotension and vasodilation and is degraded by ACE and enhanced by the angiotensin1-9 produced by ACE2. Analysis on gene expression data from cells in broncho-alveolar lavage fluid (BALF) from COVID-19 patients that were used to sequence the virus. Comparison with BALF from controls identifies a critical imbalance in the (RAS) represented by decreased expression of ACE in combination with increases in ACE2, renin, angiotensin, key RAS receptors, kinogen and many kallikrein enzymes that activate it, and both bradykinin receptors [12,34,35]. Recent reports indicate hypotension is highly associated with COVID-19 patients once in the hospital. The RAS is an important pathway linked to these conditions because it maintains a balance of fluid volume and pressure using several cleavage products of the peptide angiotensin (AGT) and their receptors [36]. The most well studied peptide is angiotensin II (Ang II), which typically generates vasoconstriction and sodium retention via the AGT-R1 receptor as well as causes vasodilation and natriuresis when bound to the AGT-R2 receptor. The RAS also includes several other lesser-known peptides that may be highly important; for example, Ang₁₋₇ binds to the MAS1 receptor, generating anti-inflammatory and vasodilatory effects, while Ang₁₋₉ binds to the AGT-R2 receptor. Ang II is produced by the enzyme ACE, whereas Ang₁₋₇ is generated by the combination of ACE and ACE2 activity while Ang₁₋₉ by ACE2 alone.

The presence of SARS-CoV-2 causes to decrease the levels of ACE in lung cells, while the levels of ACE2 increase. This, in turn, increases the level of bradykinin molecules (an event referred to as a '**Bradykinin Storm**'). Several studies have shown that bradykinin induces pain and causes blood vessels to expand and getting leaky, which leads to swelling and/or inflammation in surrounding tissue. A vicious positive feedback loop such as des-Arg (9)-bradykinin- and bradykinin-mediated inflammation → injury → inflammation, was found likely to precipitate by life threatening respiratory complications related to COVID-19 [43]. The leading cause of mortality in COVID-19 patients is the cytokine storm syndrome associated with inflammation. The COVID-19 patients might have higher levels of several pro-inflammatory cytokines and chemokines. Namely, the blood laboratory profile of COVID-19 patients exhibits lymphopenia, leukopenia, thrombocytopenia, and RNA-emia, along with the increased levels of aspartate aminotransferase. Several data confirm that dysregulated BK signaling is involved in COVID-19 respiratory complications. Summing up, SARS-CoV infection depletes ACE2, which increases levels of des-Arg (9)-bradykinin (DABK), a bioactive metabolite that is associated with lung injury and inflammation.

After receptor binding, a conformation change within the S protein facilitates the fusion of virion membrane with the cell membrane, an event that activates the cellular transportation pathway to endoplasmic reticulum (ER). The virus coded RNA polymerase transcribes a series of sub-genomic mRNAs from the vRNA by a process called discontinuous transcription. These mRNA molecules have a common end but different initiation sequences,

situated closely to the corresponding initiation codon. On the ribosomes, which are either free or attached to the membranes of cytoplasmic ER, the newly formed transcripts are translated into relevant viral proteins. When passing through the Golgi apparatus the structural viral proteins are glycosylated. Since the ORF1a and ORF1b sequences encode relatively large genes, they are translated into long polypeptides (PLP1a and PLP1b), which are cleaved into shorter polypeptides by means of a virus coded papain-like proteinase. The structural virus proteins along with the newly formed RNA genome are subsequently assembled into virions, which are transported back to the cell membrane and then released from infected cell.

Inhibitors

The use of the B2R blocker, Icatibant, seems promising for patients with unremitting respiratory distress caused by COVID-19 (Icatibant is the trade name of FIRAZYR as provided by Takeda, Tokyo, Japan). Another promising drug called Favipiravir (T-705), a synthetic prodrug, has been found to possess antiviral activity not only against the influenza virus, but also against Ebola, arenaviruses, bunya viruses, filoviruses, the West Nile virus, the yellow fever virus, the foot-and-mouth-disease virus and Lassa virus. Favipiravir acts by inhibiting the viral RdRp enzyme, allowing its insertion into viral RNA while sparing the cellular DNA. Wang et al. [37] found that the high concentrations of Favipiravir (EC50: 61.88 μ M) were needed to inhibit SARS-CoV-2 infection in Vero cells. Thus, it is difficult to ascertain the basis on which the current dose of this drug has been established in SARS-CoV-2. Despite this uncertainty, the dose in clinical use in most countries, including India, is 1800 mg bid on day 1, followed by 800 mg bid on days 2 - 14. Favipiravir may emerge as a valuable drug in the treatment of mild to moderate symptomatic SARS CoV- 2 infected cases [38]. Favipiravir, a drug that has a similar mechanism of action to Remdesivir but is orally administered, is nevertheless an emerging antiviral agent that is worth considering in mild to moderate cases. The preliminary results have been encouraging with small but significant improvement in time to clinical recovery and a two-day shorter viral shedding time. Thus, Favipiravir showed some therapeutic responses on COVID-19 in terms of disease progression and viral clearance.

The analyses showed that an excess of the substance called hyaluronic acid could degrade harmful enzymes, Hyaluronic acid can absorb water in amounts corresponding to more than 1,000 fold of its own weight forming a hydrogel. The **Bradykinin-Storm**-induced leakage of fluid into the lungs combined with the excess hyaluronic acid would likely result in a Jello-like substance that is preventing oxygen uptake and carbon dioxide release in the lungs of severely affected COVID-19 patients. This explains why Bradykinin Storm may be responsible for the more severe symptoms of COVID-19.

There is no clinically approved antiviral drug available to be used against COVID-19. There is no specific antiviral medication available for COVID-19 treatment, for patients with severe

infection the health care providers generally recommend to treat the symptoms by using oxygen therapy. However, a few broad-spectrum antiviral drugs have been evaluated as well. For example, for potential antiviral treatment of human nCoV drugs such as Lopinavir/Ritonavir (400 mg/100 mg per dose) have been recommended. In addition, nucleoside analogues, neuraminidase inhibitors, Remdesivir, the peptide EK1, arbidol, RNA synthesis inhibitors (such as TDF, 3TC) and/or certain anti-inflammatory drugs including IFN-alpha (5 million Units/dose) were tested. IFN-alpha is a broad-spectrum antiviral substance, which has been used, to treat hepatitis B [39]. Lopinavir is a protease inhibitor showing anti-CoV activity *in vitro*. It has been used to treat infection by human immune deficiency virus (HIV), together with Ritonavir as a booster. For SARS treatment, there was found that in contrast to Ribavirin alone, patients treated with Lopinavir/Ritonavir as well as Ribavirin had a lower risk of the ARD syndrome and/or death [15,37]. Namely, Ribavirin can effectively reduce the virus titer in experimental infection of mice improving the lung tissue damage. The effect of the latter drug combination may be better than the treatment using Lopinavir/Ritonavir along with interferon [40]. As shown in mice infected with a previously isolated CoV causing the so-called Middle East Respiratory Syndrome (MERS), Remdesivir may have the best CoV treatment potential. The MERS-CoV was first identified in 2012; since then, over 400 cases were registered [21]. The cell receptor for MERS-CoV is the dipeptidyl peptidase 4 (DPP4/8CD26), while dromedary camels are believed to be the reservoir for virus transmission.

China has relied on the use of the anti-viral drug Favilavir to treat the symptoms of COVID-19. This medication was initially developed by Toyama Chemical to treat nose and throat infections. Although the results of the study have not yet been published, it has been assumed that the drug has proven effective (at least in part) in treating symptoms of COVID-19 in a clinical trial of more than 70 patients with minimal side effects. Another new antiviral drug Favilavir was approved in Japan in 2014 to treat influenza, but currently it has been also used for treating COVID-19 [41], but not by the U.S. Food and Drug Administration (FDA). Remdesivir (GS-5734) is a broad-acting antiviral drug originally designed to target Ebola as it had been developed by Gilead Sciences. Remdesivir inhibits viral replication through premature termination of vRNA transcription, i.e. by disrupting virus reproduction. China announced that the clinical trials for Remdesivir have been officially started in Wuhan by testing its efficacy against COVID-19; an additional single clinical trial has been approved by FDA in United States. However, the efficacy and claiming safety of Remdesivir still needs further clinical trials. Chloroquine and Hydroxychloroquine, drugs used to treat malaria and arthritis, respectively, were recommended by the National Health Commission of the People's Republic of China COVID-19 for treatment. As mentioned above, Chloroquine and Hydroxychloroquine are drugs used to treat malaria, as well as chemoprophylaxis; and certain inflammatory conditions to include rheumatoid arthritis, lupus and a rare blood disorder called *porphyria cutanea tarda*. They have been approved by the U. S. FDA to be tested against COVID-19. Researchers

have found that both drugs have *in vitro* activity against cCoV as well as nCoV/CoV-2, with hydroxychloroquine having a relatively higher potency. Based on these results, chloroquine and hydroxychloroquine are currently recommended for treatment of hospitalized COVID-19 patients in several countries, including in the U. S. One Chinese study showed that when chloroquine was tested on more than 100 patients, it had superior results compared to a control drug inhibiting the exacerbation of pneumonia, improving lung-imaging findings, promoting virus negative conversion and shortening the disease course [17]. However, both Chloroquine and Hydroxychloroquine cause frequent side effects, such as worsening vision, nausea, digestive disorders and in more severe cases can lead to heart failure. As recently reported, a man in Arizona died and his wife was in critical condition when taking Chloroquine just prophylactically in order to prevent CoV-2 infection. Another anti-viral drug originally designed for influenza, called Favipiravir, was also used in the management of COVID-19 (until now, out of 32 studies registered on *clinicaltrials.gov* only 3 have been completed) [42].

Lopinavir/Ritonavir are sold by AbbVie under the name Kaletra; originally, they have been designed to treat the acute immune deficiency syndrome (AIDS). To evaluate the efficacy of Lopinavir/Ritonavir for CoV-2 infection, 99 patients with positive tests were treated with given drug combination. Surprisingly, no benefit as compared to the standard care was observed in latter study [31]. However, in South Korea, a 54-year-old man who was given a combination of these two medications had a significant and substantial decrease in the levels of the Beta-coronavirus. According to the WHO, there may be benefits when using Lopinavir/Ritonavir in combination with additional drugs such as interferon- β , Oseltamivir and/or Ribavirin [41]. As described above, the treatment of SARS and MERS has been mainly focused on using drugs with more general antiviral activity, rather than on data obtained at experimental therapy of animal infection models such as primates or rodents [43].

Reviewing the published trials has consistently shown that a high proportion of theirs might had a relatively low power at detecting clinically meaningful effects. Such situation can pose ethical concerns either when recruiting patients to investigation treatment without answering the research question properly, or if subjecting more patients than necessary to a potentially inferior treatment even when sufficient information is already available to answer the research question. Sample size re-estimation (SSR) methods investigate the validity of such assumptions and increase the sample size if necessary. The so called “promising zone” [44] concept appeals to the researchers suggesting that the trial conditions should be planned following sample size calculations made before it starts. Incorrect assumptions can result in a trial which is either underpowered or overpowered. The so-called promising zone should be implemented in the range of identifying trials, but using the promising zone can be difficult due to the lack of SSR methodology reports. Incorrect assumptions can result by under- or overpowered trials, initiating ethical concerns.

Immune response

The immune response is expected being similar to that described for other coronaviruses. Given its activity on the interferon pathway, and the manner in which it dysregulates innate immunity, the use of additional treatments directed at modulating or containing this could be of interest. Furthermore, circulating SARS-CoV-2-specific CD8+ and CD4+ T cells were identified in 70% and 100% of COVID-19 convalescent patients, respectively. CD4+ T cell responses to spike, the main target of most vaccine efforts, were robust and correlated with the magnitude of the antiSARS-CoV-2 IgG and IgA titers. The M, spike, and N proteins each accounted for 11%–27% of the total CD4+ response, with additional responses commonly targeting certain peptides, such as nsp3, nsp4 and the proteins encoded by ORF3a, and ORF8, respectively. There should be noted that SARS-CoV-2 infection of pregnant women does not lead to fetal damage and/or mortality, unlike to other coronaviruses, i.e. the classical SARS-cCoV and/or the MERS-CoV. Thus, to date there is no evidence for intrauterine transmission of SARS-CoV-2 to the neonate [45].

Conclusions

The novel Coronavirus discussed in this paper comes from a different region of China than the classical virus identified nearly 18 years ago. Mutations of the viral S protein are probably responsible for the greater ability of nCoV to spread in human population as well as in the body of infected individuals. The treatment of sick patients (who comprise a relatively low portion of infected persons) with drugs such as Remdesivir, Lopinavir/Ritonavir and/or Chloroquine (and its derivatives) has been neither convincing nor clearly successful. More hope comes from active immunization which has been recently introduced using a novel category of vaccines, containing either the specific mRNA or a recombinant DNA molecule encoding the S protein, regarded for the most efficient tool of the virulence of infectious virus particles.

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