

REVIEW ARTICLE

BIOLOGICAL FEATURES OF SARS-CoV-2 AND CURRENT APPROACHES TO ANTIVIRAL THERAPY AND VACCINATION: A REVIEW

Snjezana Zidovec Lepej¹, Petra Korac², Dijana Skoric³, Margarita Batovic¹, Paula Grskovic², Valerija Begic^{2,4}, Suzana Harabajsa^{2,5}, Leona Radmanic¹, Petra Simicic¹

Abstract: Since the first description of patients with pneumonia of unknown origin in Wuhan in December 2019, unprecedented efforts of the international scientific community led to the identification and molecular characterization of its etiological agent, e.g. SARS-CoV-2. The global pandemic of COVID-19 represents an outstanding challenge for the scientists and medical professionals worldwide. In this review, we discuss the most important aspects of SARS-CoV-2 biology and virology including antiviral and immunomodulatory treatment strategies as well as vaccine development.

¹ Department of Immunological and Molecular Diagnostics, University Hospital for Infectious Diseases "Dr. Fran Mihaljevic", Zagreb, Croatia

² Faculty of Science, Department of Biology, Division of Molecular Biology, University of Zagreb, Zagreb, Croatia

³ Faculty of Science, Department of Biology, Division of Microbiology, University of Zagreb, Zagreb, Croatia

⁴ Primary School "Sesvetski Kraljevec", Sesvetski Kraljevec, Croatia

⁵ Department of Pathology and Cytology, Division of Pulmonary Cytology Jordanovac, University Hospital Centre Zagreb, Zagreb, Croatia

Corresponding author:

Snjezana Zidovec Lepej
Department of Immunological and Molecular Diagnostics, University Hospital for Infectious Diseases "Dr. Fran Mihaljevic", Mirogojska 8, 10 000 Zagreb, Croatia
e-mail: szidovec@gmail.com

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INTRODUCTION

A cluster of patients with pneumonia of unknown origin in Wuhan, Hubei province, China alerted the local medical community in December 2019.¹ WHO China Country Office was informed about the cases on 31st December 2019.² The causative pathogen was first isolated via inoculation of bronchoalveolar lavage fluid into human airway epithelial cells as well as Vero E6 and Huh7 cell lines leading to the initial eight complete genome sequences of the 2019-new coronavirus.^{3,4} The first genomic sequence of the virus (WH-Human_1) was published on January 10th 2020.⁵ On February 11th, the International Committee for the Taxonomy of Viruses (ICTV) *Coronaviridae* Study Group (CSG) recognized this virus as forming a sister clade to the prototype human and bat severe acute respiratory syndrome coronaviruses (SARS-CoVs) of the species *Severe acute respiratory syndrome-related coronavirus* and designated it as SARS-CoV-2.⁶ The disease caused by SARS-CoV-2 has been termed COVID-19 (coronavirus disease 2019) by the WHO.⁷ The epidemic potential of SARS-CoV-2 was recognized very early on. Although initial four COVID-19 patients reported association with Huanan Seafood Wholesale Market that was promptly closed, subsequent epidemiological analysis showed that only 1% of patients in China had a direct contact with the live-animal market trade suggesting the importance of person-to-person transmission in the spread of SARS-CoV-2 infection.⁸ In addition, the first report on SARS-CoV-2 transmission from infected asymptomatic persons described in Germany and China indicated early on that the control of this infection without preventive measures (prophylactic antiviral drugs or vaccines) will represent a major challenge for the global scientific community.⁹ Indeed, by March 11 2020, COVID-19 had become a major global health concern and WHO declared the outbreak of SARS-CoV-2 a global pandemic.¹⁰ A total of 38,141,034 SARS-CoV-2 infections in 189 countries/regions as well as 1,086,315 deaths from COVID-19 have been reported as of October 14th 2020.¹¹

The aim of this review is to summarize the current knowledge on the most important features of SARS-CoV-2 biology including basic virology, evolutionary origin of the virus and transmission potential. In addition, current approaches to antiviral and immunomodulatory treatment of the COVID-19 disease as well as vaccine development strategies will be critically discussed.

BIOLOGICAL FEATURES OF SARS-CoV-2

Coronaviridae

Zoonoses are human diseases wherein a causal agent is acquired from a vertebrate animal acting as an agent's natural host.¹² About 60% of emerging infectious diseases are zoonotic and about 25% are of viral origin.¹³ Emerging viruses include viruses causing diseases in new hosts, appearing in new geographic areas, or both.¹⁴ In humans, they are mostly zoonotic and many of those representing a significant burden to public health and global economy are spill-overs from natural wildlife reservoirs (e.g. primate, rodent, bird, bat or other mammal hosts) to humans.¹³ Diverse socio-economic, demographic, environmental and ecological factors are drivers of emerging infectious diseases including viruses.^{13, 15} Enlarging arable areas of land, deforestation, loss of biodiversity, hunting, outdoor recreational activities, global transport and travel, political instabilities and migrations can increase the likelihood of human contacts with wildlife reservoirs of new viruses.¹⁶ It was evident that the frequency of these events increased in the last two decades of the 20th century. Unfortunately, this trend continues into this century. Only in the last two decades, it has been underpinned with extremely serious viral disease outbreaks like Ebola, SARS and MERS. With the most recent emergence of COVID-19, we are witnessing a further increase of emerging and re-emerging viruses with the potential of changing the history of mankind.¹⁵ The outbreaks of SARS in 2002, MERS in 2012 and COVID-19 in 2019 have etiological agents from the family *Coronaviridae* (<https://talk.ictvonline.org/>).¹⁷⁻¹⁹ Before these three 21st-century outbreaks, coronaviruses had been considered etiological agents of serious diseases in animals, especially pigs, and only mild respiratory diseases in immunocompetent humans. The members of this viral family are widespread in nature and have natural animal, mostly mammal or bird, hosts. ICTV-CSG currently recognizes 39 species classified in 27 subgenera, five genera and two subfamilies that belong to the family *Coronaviridae*, suborder *Cornidovirineae*, order *Nidovirales*, realm *Riboviria*.⁶ There are presently seven members of *Coronaviridae* family, subfamily *Coronavirinae*, infecting humans. Four human coronaviruses (HCoV-NL63, HCoV-229E belonging to genus *Alphacoronavirus* plus HCoV-OC43 and HKU1 belonging to *Betacoronavirus* genus) induce

only mild upper respiratory diseases in immunocompetent hosts. However, some of them can cause severe infections in infants, young children and elderly people.²⁰ The three highly pathogenic viruses for humans clustering within the *Betacoronavirus* (β -CoV) genus are SARS-CoV, MERS-CoV and the emerging virus first tentatively named 2019-nCoV then officially renamed SARS-CoV-2 by the ICTV-CSG.⁶ The nomenclature of SARS-CoV-2 is based on its genome characteristics as reflected in its taxonomical position within viral species Severe acute respiratory syndrome-related coronavirus, genus *Betacoronavirus*, subgenus *Sarbecovirus*.^{6, 21} All members of the family *Coronaviridae* form enveloped roughly spherical particles of 100-160 nm in diameter encasing a positive-sense, single-stranded RNA (+ssRNA) genome of 27-32 kb in size (Figure 1). The 5'-terminal two-thirds of the of 29,903-nucleotides-long SARS-CoV-2 genome encode a polyprotein 1a/1ab whose protein products are directly translated from the genomic RNA and cleaved by virus proteases.²¹ The cleavage products, especially the viral RNA dependent RNA polymerase (RdRp) and the proteins forming the replication-transcription complex, are essential for the genome transcription and replication. This part of the genome also encodes 16 non-structural proteins.²² In such a large +ssRNA viral genome, there is a large number of accessory genes specific for virus species but probably dispensable for some steps in the viral cycle.²⁰ The replication-transcription complex also enables the discontinuous synthesis of a number of subgenomic RNA molecules from the 3'-terminal third of the genome via -ssRNA intermediates. The production of subgenomic RNAs whose templates are nested within the genome came to define the superfamily *Nidovirales* (lat. nidus = nest) comprising a number of other virus families. The 3'-terminal part of the viral genome encodes structural virion components: envelope spike glycoproteins (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. Besides these main virion components, several accessory proteins are produced.^{21, 22} Although the function(s) of many of these proteins have yet to be determined, principal players and the steps of the viral cycle have been inferred from the similarities with other betacoronaviruses or revealed in the current research.²⁰⁻²³

ORIGINS OF SARS-CoV-2

The SARS epidemic, as one of the most important public health threats in the 21st century, prompted research revealing bats as the reservoir hosts of many SARS-like coronaviruses.²⁴ Whilst the human-infecting coronaviruses HCoV-OC43 and HCoV-HKU1 probably originated from rodents,^{20, 25} the rest of them (HCoV-NL63, HCoV-229E, SARS-CoV, MERS-CoV) have a presumed bat origin.^{20, 25-30} According to the available research data, the same applies to the latest SARS-CoV-2.^{21, 31, 32} As the current number of SARS-CoV-2 fully

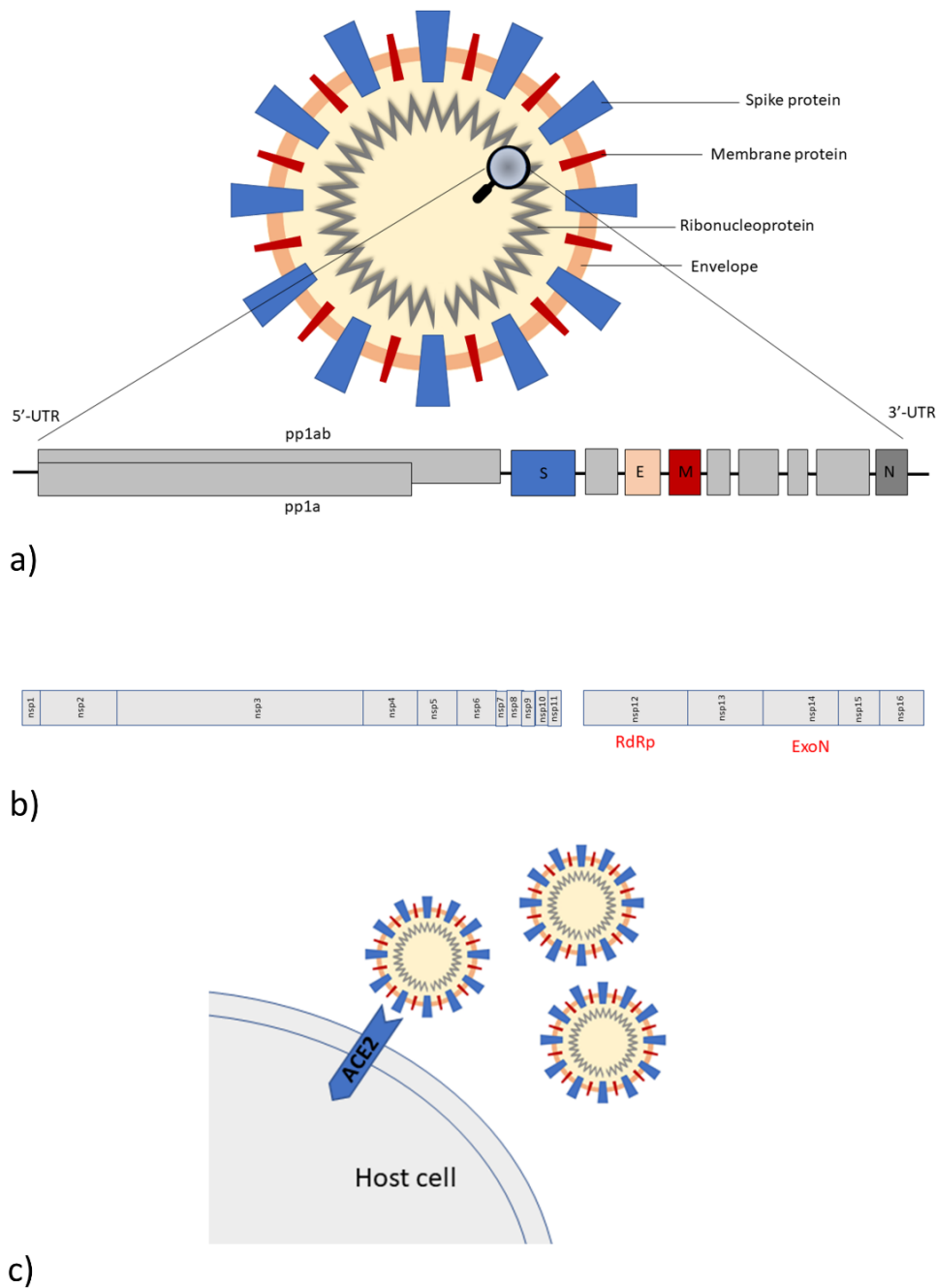


Figure 1. a) schematic representation of SARS-CoV-2 virion and its genome, b) non-structural proteins coded in orf1ab and orf1a, b) virus enters host cell via ACE2 receptor.

sequenced genomes is constantly rising, the comparative analyses of the viral genome support the natural origin of the virus, and there is no credible evidence to believe otherwise.³³ Insectivorous Chinese horseshoe bats (family Rhinolophidae) are pinpointed as particularly interesting group in the search for SARS-like coronavirus progenitors.^{31, 34} They can host different virus species and different viral populations within a species (quasispecies) facilitating recombination and the

emergence of new virus variants and species.^{29, 30} It is still early to know whether this originally bat virus preadapted to humans in another animal species like pangolin, or whether the zoonotic transfer was enabled by adaptive process in humans.^{31, 34} Data about the genome sequence and specific genetic similarities and differences between SARS-like coronaviruses should help in the understanding of zoonotic transfer and help prevent future zoonotic events.

DISCOVERY AND DESCRIPTION OF SARS-CoV-2

Current understanding of SARS-CoV-2 stems from data obtained in the initial studies that analyzed patients' samples in the first few weeks after the epidemic event was recognized (end of January/ beginning of February). Lu et al. conducted research on samples from nine patients connected to the Huanan Seafood Market.⁴ Common respiratory pathogens were excluded using commercially available kits, while previously published essays were used for the exclusion of SARS-CoV and MERS-CoV infection. Human airway epithelial cells were used for inoculation with bronchoalveolar lavage fluids or throat swabs from the patients in order to isolate a virus. Viral genomes were then sequenced using combinations of Sanger sequencing, high throughput sequencing on BGI, Illumina and Nanopore platforms, and the addition of rapid amplification of cDNA ends for terminal sequences. Phylogenetic analysis using the complete genome sequence showed that the new virus has the highest similarity with bat SARS-like coronaviruses: bat-SL-CoVZC45 and bat-SL-CoVZXC21 (87.99 % and 87.23 %, respectively). The lowest sequence identity based on specific coding regions between analyzed virus and bat SARS-like coronaviruses was detected for S gene (75 %). It was also shown that the new virus has relatively low genomic similarity to SARS-CoV (79%) and MERS-CoV (50%). Genomic organization was predicted to be similar to those of bat SARS-like coronaviruses and consisted of 12 open reading frames that code for proteins similar in length to those of bat viruses with the exception of spike protein. Spike protein, which has two basic domains: S1 and S2 in all *Coronaviridae* members, showed higher similarity when using receptor binding domain for comparison with SARS-CoV than with bat-SL-CoVZC45 and bat-SL-CoVZXC21. Such results suggested that novel virus can use angiotensin-converting enzyme 2 (ACE2) receptor on human cells as an entry point.⁴

In the same month, Zhu N et al. (2020) reported findings based on the analysis of lower respiratory tract samples from 3 patients.³⁵ Patients whose samples were analyzed were connected to the Huanan Seafood Market, and diagnosed with pneumonia of unknown cause. After exclusion of 18 common viruses and 4 bacteria using commercial diagnostic tests, high throughput sequencing using Illumina and Nanopore platforms was conducted in order to describe the viral genome and conduct phylogenetic analysis. Obtained genome sequences showed 86.9% similarity with the previously described bat SARS-like CoV genome and were relatively dissimilar to SARS-CoV and MERS-CoV genomes. Additionally, real-time PCR for the detection of the pan β -CoV RNA dependent RNA polymerase (RdRp) region confirmed that the new virus belongs to the family of RNA viruses, *Coronaviridae*. Virus particles were also visualized using transmission electron microscopy analysis of human airway epithelial

cells infected with the virus. Spherical particles with spikes 9 to 12 nm that resembled solar corona were observed.³⁵

Zhou P et al. (2020) analyzed samples from seven patients with severe pneumonia, mostly workers at seafood market, in order to find the cause of the symptoms.³⁶ Pan-CoV PCR primers were used as a first step and five positive samples were further investigated using high throughput sequencing, de novo assembly and targeted PCR. The genome of the new virus consisted of 6 open reading frames characteristically found in coronaviruses, as well as additional accessory genes. Although nucleotide sequences of most open reading frames showed less than 80 % similarity with corresponding open reading frames of SARS-CoV, replicase conserved domains within RdRp from ORF1ab had 94.4 % match with the one from SARS-CoV when amino acid sequences were compared. High sequence similarity between novel virus and bat SARS-like coronavirus RaTG13 was found based both on full-length genome and the RdRp and spike genes. Furthermore, the authors conducted infectivity study using HeLa cells that expressed human, Chinese horseshoe bat, civet, pig and mice ACE2 proteins. The novel virus was able to enter the human cells using all but mouse ACE2 receptors, and it did not use other known corona virus receptors.³⁶ Further studies confirmed interaction of the spike protein and ACE2 receptor.³⁷⁻³⁹

Data published in first few months after the outbreak were later confirmed and showed that SARS-CoV-2 is a +ss RNA virus that has 14 ORFs encoding 27 proteins, as was mentioned earlier (Figure 1a). Its 5'-terminus harbors open reading frame orf1ab and orf1a. These orfs contain 15 non-structural proteins (nsps) among which are nsp12 and nsp14 (Figure 1b). Nsp12 is a RdRp that has a pivotal role in the viral life-cycle, lacks host homologues and has a high level of sequence and structural conservation which makes it an optimal target for therapeutics.⁴⁰ However, there has been remarkably little biochemical characterization of nsp12 and a lack of fundamental data to guide the design of antiviral therapeutics and study their mechanism of action. A promising class of RdRp inhibitors are nucleoside analogues (NAs), small molecule drugs that are metabolized intracellularly into their active ribonucleoside 5'-triphosphate (RTP) forms and incorporated into the nascent viral RNA by error-prone viral RdRps. This can disrupt RNA synthesis directly via chain termination or lead to the accumulation of deleterious mutations within the viral genome. However, the post-replicative repair capacity provided by the nsp14 exonuclease (ExoN) that is essential for maintaining the integrity of the large ~30 kb genomes of coronaviruses reduces the antiviral effects of certain NAs. Despite this, several NAs are currently being used for the treatment of other viral infections and have been identified as potential anti-CoV candidates.⁴⁰ The 3'-terminus of SARS-CoV-2 genome harbors structural genes (spike (S), envelope (E), membrane (M), and

nucleocapsid (N) genes) and some accessory genes.⁴¹ As mentioned earlier, the spike gene is recognized as the viral genome sequence with the highest genetical similarity to SARS-CoV and has therapeutic value because of its function – binding to human cell receptor ACE2 (Figure 1c).

SARS-CoV-2 TRANSMISSION

As SARS-CoV-2 is a virus that mainly targets upper respiratory pathways (and lungs), similarly to SARS-CoV and MERS-CoV, it is supposed that they share the same main pathway of transmission through droplets released by coughing or sneezing that remain in the air or on the surrounding surfaces. A healthy individual can be infected by inhaling the aerosol containing the viral particles or through direct contact between mucous membranes in the nose and mouth and infected surfaces.^{42, 43}

It remains unclear whether mucous membranes in eyes could also be exploited as an entrance point by respiratory viruses, as there are conflicting reports regarding the presence of SARS-CoV in samples of tears collected from infected individuals⁴⁴⁻⁴⁶, as well as SARS-CoV-2.^{47,48} Still, the lack of eye protection was associated with an increased risk of SARS-CoV transmission from infected patients to health care workers during the 2003 Toronto SARS outbreak⁴⁹ and is assumed to be the reason why a Chinese respiratory specialist was infected with SARS-CoV-2 in January 2020 despite wearing a protective suit and N95 respirator.⁵⁰

A smaller percentage of patients (2-10%) diagnosed with SARS-CoV-2 displayed gastrointestinal symptoms, such as diarrhea or abdominal pain, alongside respiratory symptoms. Moreover, in some cases, it was observed that the gastrointestinal symptoms had developed before fever and respiratory symptoms.⁵¹ Also, it is known that ACE2, a membrane receptor through which SARS-CoV-2 enters lung cells⁵², is also expressed on the cells of intestinal tract, mostly in the glandular cells of gastric, duodenal, and rectal epithelia.⁵³ It has not yet been established that gastrointestinal symptoms in mentioned cases are caused by SARS-CoV-2, nor has it been confirmed yet that fecal–oral transmission is another means by which SARS-CoV-2 is spread. However, the virus has been detected in the faeces⁵³, as have both SARS-CoV and MERS-CoV^{54, 55}, so it is possible that SARS-CoV-2 could also be transmitted this way.

Viral RNA has also been detected in plasma or serum of 15% of the first Wuhan SARS-CoV-2 patients, but at very low concentrations, regardless of the severity of the symptoms.⁵⁶ Therefore, the possibility of transmission of the virus through blood donations cannot be overlooked, with donations by asymptomatic carriers posing the greatest danger. However, blood collection establishments are not currently under obligation to undertake any specific SARS-CoV-2-related actions,

since there are no data suggesting a risk of transfusion transmission of SARS-CoV-2.^{57, 58} A case report from South Korea described a 21-year-old man diagnosed with very severe aplastic anemia in November 2019 who received a platelet transfusion from a SARS-CoV-2 infected individual who displayed no symptoms at the time of the donation. The recipient was tested multiple times for SARS-CoV-2 post transfusion; all the results were negative.⁵⁹

There has been no evidence of vertical transmission of SARS-CoV-2 from mother to child during childbirth or through breastmilk so far. Zhu H et al. (2020) examined samples taken from amniotic fluid, umbilical cord blood and throat swabs of babies born to SARS-CoV-2 positive mothers immediately after delivery, as well as milk samples⁶⁰, while Chen H et al. (2020) examined only throat swabs of newborns.⁶¹ No sample in either study tested positive for SARS-CoV-2. A case study by Chen S et al. examined placentas of three women with diagnosed SARS-COV-2 infection during pregnancy. The viral RNA was not detected in newborns, and the placentas displayed no histopathological changes connected to SARS-CoV-2 infection.⁶²

SARS-CoV-2 STABILITY

SARS-CoV-2 was shown to remain viable in aerosols for three hours, with a reduction in infectious titer similar to SARS-CoV in the same conditions. Both viruses displayed longest viability on stainless steel and plastic, and viable SARS-CoV-2 was detected up to 72 hours after application to these surfaces, though its estimated half-life is considerably shorter (around six and seven hours respectively).⁶³ Chin et al. (2020) found that no infectious SARS-CoV-2 could be detected on stainless steel and plastic only on day 7. Infectious virus could not be found on glass and banknotes after four days, which is a considerably longer period of incubation compared to printing and tissue papers (three hours)⁶⁴, copper (four hours), cardboard (24 hours)⁶³, or even wood and cloth (two days)⁶³ at room temperature and 40-60% relative humidity. The estimated half-life of the virus in aerosol and on copper was about an hour, around four on cardboard⁶³ and five hours on glass⁶⁴ while it was somewhat longer on banknotes (around eight hours).⁶⁴ These results suggest that SARS-CoV-2 remains viable on smooth surfaces considerably longer. A detectable level of infectious virus could still be present on the outer layer of a surgical mask even after a week.⁶⁴

SARS-CoV-2 is highly stable at 4°C but sensitive to heat, and the time for its inactivation is gradually reduced upon the temperature increase. It also remains viable in a wide range of pH values at room temperature. On the other hand, no infectious virus could be detected after 5-minute incubation at room temperature in various disinfectants, which suggests that the virus is susceptible to standard disinfection methods.⁶⁴

CURRENT APPROACHES TO ANTIVIRAL THERAPY AND VACCINATION

SARS-CoV-2 infection in humans and development of coronavirus disease COVID-19

Clinical presentations of SARS-CoV-2 infection exhibit a broad spectrum of severity and progression patterns, ranging from asymptomatic infection to mild, severe or critical COVID-19 disease.⁶⁵ The most commonly reported signs and symptoms of COVID-19 include fever, dry cough, fatigue, myalgia, chest tightness and pain, sore throat, shortness of breath, dyspnea, rhinorrhea etc. Although the main target for SARS-CoV-2 infection are the lungs, distribution patterns of SARS-CoV-2 receptor ACE2 in various tissues and organs are associated with damage to the cardiovascular, gastrointestinal and central nervous systems observed in COVID-19.⁶⁶ Initial clinical symptoms sub-acutely progress to respiratory distress and acute respiratory distress syndrome (ARDS) in about 8-19% of patients (depending on age, sex, genetics and comorbidities) with about 14% of patients requiring supplemental oxygen and approximately 5% needing mechanical ventilation.⁶⁶

The hallmark of severe COVID-19 pathogenesis is the hyperactivation of the immune response to SARS-CoV-2 that includes massive infiltration of activated monocytes, macrophages and lymphocytes into the pulmonary interstitium that is, in addition to vasculitis and hypercoagulability, associated with development of ARDS.⁶⁷ Increased synthesis of high concentrations of proinflammatory cytokines and biological response modifiers including IL-1, IL-2, IL-6, IL-7, IL-10, TNF- α , granulocyte colony stimulating factor, interferon (IFN)- γ inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1- α (MIP1- α) in response to SARS-CoV-2 infection, which is often referred to as a “cytokine storm”, plays a central role in COVID-19 pathogenesis and is associated with multiple organ failure and death observed in the severe form of the disease.⁶⁷

Antiviral and immunomodulatory treatment strategies in COVID-19

Current therapeutic strategies in COVID-19 include supportive care and broad-spectrum antibiotics, supplemented initially by antiviral drugs intended to inhibit SARS-CoV-2 replication and followed by immunomodulatory drugs aimed at inhibiting the immune system.⁶⁸ Recent studies on SARS-CoV-2 molecular virology provided an excellent scientific background for the repurposing of existing antiviral drugs for the treatment of COVID-19. Currently used antiviral drugs that inhibit key steps in the SARS-CoV-2 replication cycle include entry inhibitors (receptor-binding, fusion and endosomal-acidification inhibitors),

RdRp inhibitors (nucleoside and nucleotide analogues) and 3CL protease inhibitors.^{69, 70} So far, several direct acting antiviral drugs (umifenovir, remdesivir, favipravir, lopinavir/ritonavir, chloroquine phosphate and hydroxychloroquine) have been considered by international or national regulatory agencies (recommended for authorization, approved for emergency use, approved or retracted from clinical use) (reviewed in Table 1).

Entry inhibitors

Umifenovir is a viral entry inhibitor that interferes with the fusion of the viral envelope and cell membrane by interfering with clathrin-mediated endocytosis.⁷¹⁻⁷³ *In vitro*, umifenovir inhibits SARS-CoV-2 replication in Vero cells by blocking or impeding the trimerization of the viral S glycoprotein that is crucial for the viral adherence step.^{74, 75} This small indole derivative is approved for clinical use as prophylaxis and treatment of influenza in Russia and China and has been approved for the treatment of COVID-19 in China based on mainly retrospective national studies.⁷⁶⁻⁷⁸

Chloroquine phosphate and its derivative hydroxychloroquine are aminoquinolines that have been traditionally used for the prophylaxis and treatment of malaria and for the treatment of various autoimmune diseases. Both molecules exhibit antiviral activity; chloroquine phosphate inhibits phosphorylation of the SARS-CoV-2 receptor by interfering with the viral binding to the receptor, while hydroxychloroquine increases pH within the endosomes, inhibits endosome-lysosome fusion and prevents the release of viral molecules into target cells. In addition, hydroxychloroquine is an immunomodulator that interferes with macrophage activation and cytokine synthesis. Since hydroxychloroquine is metabolized into chloroquine, both mechanisms of antiviral activity are expected to be observed *in vivo*. Both molecules exhibit antiviral activity against several RNA viruses including SARS-CoV, MERS-CoV and SARS-CoV-2 *in vitro*.⁷⁹ Despite the encouraging early results from clinical trials conducted in China and the subsequent approval of the drug by the Chinese medical authorities, results of a retrospective multicenter cohort study of 1438 hospitalised COVID-19 patients showed that treatment with hydroxychloroquine and/or azithromycin failed to influence mortality compared to placebo.⁸⁰⁻⁸¹

Chloroquine phosphate and hydroxychloroquine are by far the most controversial drugs used in COVID-19 pandemic, not only because of the public non-expert endorsement of their prophylactic uses but also because of the retraction of the largest clinical study published in *Lancet* by Mehra et al. (2020) due to the inability of the authors to validate the primary data source (multinational registry of 96,032 patients collected by a private company).^{82, 83}

Table 1. Selected antiviral drugs used for the treatment of COVID-19 disease

Name	Antiviral drug class and mechanism of action	Antiviral activity <i>in vitro</i> and <i>in vivo</i>	Previous clinical research/use, current approval status and selected clinical data for COVID-19 treatment
Umifenovir	- entry inhibitor - blocks trimerisation of viral S glycoprotein	<i>In vitro</i> : SARS-CoV-2; influenza virus A, influenza virus B, Ebola virus, Lassa virus, HBV, HCV, HHV-8, VZV, polioviruses	- approved for treatment of influenza (China, Russia) - approved for COVID-19 treatment (China, 2020) - shorter time to viral clearance compared with lopinavir/ritonavir arm (n=50 patients) ⁷⁸ - higher reduction in mortality in the umifenovir arm compared with lopinavir/ritonavir or oseltamivir arms ⁷⁷
Remdesivir	- RdRp inhibitor (adenosine analogue)	<i>In vitro</i> : SARS-CoV-2, SARS-CoV, MERS, CoV, HcoV-OC43, HcoV-229, Ebola virus, Marburg virus, Parainfluenza type 3 virus, Nipah virus, Hendra virus, measles virus, mumps virus, RSV <i>In vivo</i> : MERS-CoV	- discouraging results of the Ebola clinical trial ⁸⁶ - recommended by EMA for authorisation in the European Union for COVID-19 treatment (June 25th, 2020) ⁸⁷ - emergency use authorisation by the FDA in COVID-19 (May 1st, 2020) - reduction in time to recovery from severe COVID-19 compared with placebo and numerically lower mortality rate in the remdesivir group (open-label, phase 3, randomised clinical trial, n=1063 patients) ⁸⁸
Favipravir	RdRp inhibitor (guanosine inhibitor)	<i>In vitro</i> : SARS-CoV-2, influenza viruses, Ebola virus, Lassa virus, Yellow Fever virus, Chikungunya virus, noroviruses and enteroviruses	- approval for new or re-emerging influenza in Japan (2014) - a trend towards improved survival in proof-of-concept Ebola trial ⁹² - approved for COVID-19 treatment in Russia, China and India - shorter times to SARS-CoV-2 negative RNA assay, mean time of antipyretic use and cough remission time were reported in the favipravir versus umifenovir group but failure to demonstrate between-group differences in the primary end point defined as clinical improvement by day 7 did not differ (randomized clinical trial) ⁹⁴ - better treatment effect in terms of disease progression (radiological assessment) and time to viral clearance of favipravir compared with a combination of lopinavir and ritonavir (IFN-alpha was administered to both groups via aerosol inhalation) in patients with moderate or mild COVID-19 ⁹³
Lopinavir and ritonavir	Protease inhibitor and cytochrome CPY3A4 inhibitor	<i>In vitro</i> : HIV-1, SARS-CoV-2, SARS-CoV <i>In vivo</i> : HIV-1, SARS-CoV	- approved for the treatment of HIV-1 infection - therapeutic option for COVID-19 in China - 19 clinical trials in COVID-19 patients failed to confirm clinical efficacy - no benefit of lopinavir/ritonavir beyond standard-of-care treatment (randomised, open-label clinical trial, n=199 patients) ⁹⁸
Chloroquine phosphate, hydroxychloroquine	Aminoquinolines - inhibition of ACE2 phosphorylation, increase in the endosomal pH	<i>In vitro</i> : SARS-CoV-2, SARS-CoV, MERS-CoV, influenza viruses, Chikungunya virus	- approved for prophylaxis, the treatment of malaria and the treatment of autoimmune diseases - approved for COVID-19 treatment in China - chloroquine use associated with reduction of symptom duration and prevention of pneumonia exacerbation in COVID-19 patients compared to controls (n=100 patients) ⁸⁰ - treatment with hydroxychloroquine and/or azithromycin failed to influence mortality compared to placebo (retrospective multicenter cohort study, n=1438 hospitalised COVID-19 patients) ⁸¹

Legend: hepatitis C virus (HCV), hepatitis B virus (HBV), human herpesvirus-8 (HHV-8), vesicular stomatitis virus (VZV), respiratory syncytial virus (RSV), RNA-dependent RNA polymerase (RdRp), coronavirus disease 2019 (COVID-19)

RdRp inhibitors

Remdesivir is a phosphoramidate adenosine (nucleotide) analogue that acts as delayed chain terminator and inhibits RdRp of various RNA viruses. It

is a direct-acting antiviral molecule that incorporates into nascent viral RNA, resulting in premature termination of the viral RNA synthesis.⁸⁴ Remdesivir is a pro-drug that is metabolized within cells into an alanine metabolite by a sequence of hydrolytic steps that

start with esterase-mediated hydrolysis of a phosphoramidate carrier into a monophosphate derivative and, subsequently, into an active triphosphorylated analogue of adenosine by intracellular nucleoside-phosphate kinases. Remdesivir exhibits antiviral activity against human and animal coronaviruses including SARS-CoV, MERS-CoV, SARS-CoV-2, HCoV-OC43 and HCoV-229 *in vitro*.^{79, 84, 85} Despite discouraging results of the clinical trial in Ebola patients conducted in Congo, remdesivir was proven to be safe for use in humans and subsequently immediately entered clinical trials for COVID-19.⁸⁶ On June 25th 2020, remdesivir become the first COVID-19 therapeutic option recommended by European Medicinal Agency (EMA) for authorization in the EU for the treatment of patients with pneumonia who require supplemental oxygen therapy.⁸⁷ The recommendation was mainly based on the results of an open-label, phase 3, randomized controlled US National Institute of Allergy and Infectious Diseases (NIAID)-ACTT-1 clinical trial of 1063 patients that reported reduced time to recovery from severe COVID-19 associated with remdesivir treatment compared to placebo (11 vs. 15 days).⁸⁸

Favipravir is a pyrazinecarboxamide derivative and guanosine analogue that inhibits viral replication via competitive inhibition of viral RdRp as well as by induction of lethal RNA transversion mutations resulting in a non-viable viral phenotype.⁸⁹ Shannon et al. (2020) showed a 12-fold increase in G-to-A and C-to-U transition mutations in SARS-CoV-2-infected Vero cells cultivated in the presence of favipravir leading to a further reduction in the already low cytosine content (17.6%) of the viral genome.⁹⁰ Increased mutation frequency was associated with a high diversity of viral variants in favipravir-treated infected cells as well as with a reduction of virus-induced cytopathic effect, reduction of viral load and lower virion yield.⁹⁰ Favipravir is a pro-drug that is metabolised into its active form favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP) by human hypoxanthine guanine phosphoribosyl-transferase. *In vitro*, favipravir can inhibit the replication of a wide range of RNA viruses including influenza viruses and SARS-CoV-2.^{75, 91} Prior to COVID-19 pandemics, clinical use of favipravir has been limited to the treatment of non-seasonal influenza in Japan (since 2014). In addition, favipravir showed a trend towards improved survival in Ebola patients treated in a clinical trial carried out in Guinea.⁹² Based on the results from COVID-19 clinical experience and trials conducted in China and Russia, favipravir become the first approved antiviral drugs for COVID-19 treatment in China (March 2020) and, more recently, in Russia and India as a generic drug.⁹³⁻⁹⁷

Protease inhibitors

The majority of data on COVID-19 treatment using protease inhibitors comes from clinical trials employing

a combination of lopinavir (inhibitor of HIV-1 aspartyl protease) and ritonavir (cytochrome CYP3A4 enzyme inhibitor), which is used to increase the bioavailability of lopinavir. Although coronaviruses encode a different class of proteases (cysteine protease), *in vitro* data suggest that the lopinavir/ritonavir combination might inhibit coronavirus 3CL1pro protease. Despite initial encouraging results from China on the use of lopinavir/ritonavir in combination with ribavirin and confirmed antiviral activity against SARS-CoV-2 *in vitro*, more than 19 clinical trials in COVID-19 failed to confirm clinical efficiency of this therapeutic regimen.⁹⁸⁻¹⁰⁰ Nevertheless, lopinavir/ritonavir combination remains an approved therapeutic option for the treatment of COVID-19 in China.

Innovative approaches to antivirals

The majority of innovative approaches in antiviral therapy of COVID-19 are focused on entry inhibitors targeting the ACE2 receptor. Since unselective inhibition of ACE2 could induce alterations in the ACE2/angiotensin-1 to -7/Mas axis and its anti-inflammatory effect, recombinant human ACE2 molecule that could bind to virions prior to their attachment to target cells is explored as a possible entry inhibitor. Monteil et al. (2020) showed that recombinant human ACE2 inhibits SARS-CoV-2 replication in cellular and embryonic stem-cell derived organoids by 1,000-5,000-fold, providing a scientific foundation for the further development of this inhibitor.¹⁰¹ In addition, a new generation of entry inhibitors focus on host proteins important for the initial stage of SARS-CoV-2 replication, such as TMPRSS2 and CatB/L.¹⁰² Inhibition of viral protein nuclear transport represents another potential therapeutic strategy for RNA viruses.¹⁰³ Recently, Caly et al. (2020) showed that the anti-parasitic drug ivermectin reduces SARS-CoV-2 replication *in vitro*, opening a possibility for the repurposing of the drug for COVID-19 as well.¹⁰⁴

Immunomodulators in COVID-19

Numerous specific and non-specific immunomodulatory drugs/treatments have been employed for COVID-19 treatment including: convalescent plasma, corticosteroids (dexamethasone), cytokine receptor antagonists targeting IL-6 and IL-1 (anakinra, tocilizumab, sarilumab, siltuximab), growth factor inhibitors targeting granulocyte-macrophage colony-stimulating factors (gimsilumab, lenzilumab, namilumab) and vascular endothelial growth factor (bevacizumab), JAK inhibitors (baricitinib, ruxolitinib), macrolides (azithromycin) and interferons (recombinant IFN- α 2a, IFN- α 2b, IFN- β 1a and IFN- β 1b) with variable rates of success (selected data presented in Table 2).¹⁰⁵⁻¹¹⁰ Innovative approaches to immunomodulation

Table 2. Selected immunomodulatory therapeutic strategies in COVID-19

Name	Drug class	Mechanism of action	Previous clinical research/use, current approval status and selected clinical data for COVID-19 treatment
Dexamethasone	corticosteroid	- inhibition of proinflammatory signals via binding to the glucocorticoid receptor	- a variety of inflammatory and autoimmune diseases - 10-day dexamethasone treatment (randomised clinical trial, n=6425) ¹⁰⁸ - 28-day mortality reduced with dexamethasone in the overall cohort (21.6% vs 24.6% usual care; p<0.001) - reduced mortality by 35% in those on mechanical ventilation (29.0% vs. 40.7%; p = 0.003), and by 20% in those treated with oxygen (21.5% vs. 25.0%; p=0.0021)
Convalescent plasma	biological response modifier	- antiviral activity (neutralising antibodies) - immunomodulatory activity	- historical clinical use with variable results (SARS-CoV, MERS-CoV, Ebola virus, influenza viruses) - August 23rd 2020, Emergency Authorisation use by the FDA - encouraging results from case reports, case series and preliminary results of clinical trials and expanded access programmes (few completed) - significant reduction in mortality within 28 days in COVID-19 patients receiving convalescent plasma ¹⁰⁷ - preliminary non-peer reviewed results ¹⁰⁵ showed significantly lower 7-day mortality rate in patients transfused early (within 3 days of diagnosis) compared with patients transfused 4 or more days after diagnosis (8.7% vs. 11.9%, p<0.001), similar observations for 30-day mortality and for receiving high versus low titre IgG plasma
Tocilizumab	recombinant humanized monoclonal antibody specific for IL-6 receptor	- inhibition of IL-6 mediated signalling and biological activity	- approved for the treatment of rheumatoid arthritis, systemic juvenile idiopathic arthritis, cytokine release syndrome - observational, retrospective cohort study recently showed a trend association towards reduced mortality among COVID-19 patients treated with tocilizumab at the intensive care unit. ¹⁰⁶ - a prospective series of 100 hospitalised COVID-19 patients with pneumonia and ARDS requiring ventilatory support showed significant clinical improvement upon tocilizumab treatment. ¹⁰⁹

Legend: interleukin-6 (IL-6), acute respiratory distress syndrome (ARDS), coronavirus disease 2019 (COVID-19)

in COVID-19 include the use of stem cell-derived NK-cells (currently used in oncology) that might boost innate antiviral immune responses, as well as mesenchymal stem cell therapy that restores endothelial permeability and exhibits anti-inflammatory activity.¹¹¹

Traditional Chinese Medicine (TCM)

Since the beginning of the pandemics in Wuhan, self-made or commercially available TCM (reviewed by Yang et al. 2020) has been used for COVID-19, alone or in combination with conventional antiviral drugs.¹¹² By February 2020, TCM has been used in 60,107 confirmed COVID-19 patients (estimated 85.2% of total confirmed cases in China within that time period).¹¹²

Antiviral (inhibition of 3Clpro, RdRp and inhibition of S protein interaction with ACE2) and immunomodulatory (reduction of cytokine synthesis) properties of TCM documented *in vitro* and in animal models as well as favorable clinical effects documented in SARS-CoV epidemic, make TCM an important COVID-19 treatment option in China. However, the clinical effect of TCM is currently difficult to interpret due to the lack of randomised or placebo-controlled clinical trials.

SARS-CoV2 vaccine development

The availability of COVID-19 genome sequences and previous experience in the development of candidate vaccines for SARS-CoV and MERS-CoV contributed to the rapid development of 145 candidate SARS-CoV-2 vaccines, with 34 of them undergoing clinical evaluation (updates available by the WHO).¹¹³

The “ideal” vaccine is expected to induce a strong humoral immune response based on long-lasting neutralizing antibodies as well as specific T-cell immunity, but other parameters such as safety and manufacturing and logistical challenges have to be carefully evaluated.¹¹⁴ The most promising SARS-CoV-2 vaccines are focused on protein subunit (54 candidates) and nucleic acid (37 candidates) technologies, but other vaccine development strategies are likely to play an important contribution as well.¹¹³ Currently there are seven main vaccine development strategies that include:

DNA vaccines

DNA vaccines are based on plasmid DNA encoding one or several viral antigens (S, M or N) that are expressed

in host cells.^{114, 115-117} The synthesized protein induces the MHC class I pathway leading to cell mediated immune response, or it is released outside of the host cell where it acts as an exogenous antigen presented by MHC class II pathway to the CD4⁺ T-cells, leading subsequently to the induction of humoral immunity.¹¹⁸ Some of the candidates have already been demonstrated to activate both humoral and cell mediated immune response against SARS-CoV-2 in nonhuman primates, guinea pigs and transgenic mice.^{116, 119} DNA vaccines that are currently in clinical trials are focused on S glycoprotein but differ in the approach to the plasmid delivery (electroporation for the INO-4800 vaccine or a hybrid transporter protein within live *Bifidobacterium longum* by the bacTRL-Spike vaccine).^{116, 117} Nevertheless, no DNA vaccine has been approved for use in humans so far. Possible challenges for this type of vaccines include efficacy of plasmid delivery, potential risk for integration into the genome, toxicity and lower immunogenicity compared to live or attenuated vaccines.^{118, 120}

RNA vaccines

Development of the mRNA vaccines is currently based on two different strategies: (1) Non-replicating mRNA-based vaccines encode antigen of interest and contain 5' and 3' untranslated regions (UTRs) or (2) virally derived, self-amplifying RNAs that encode not only the antigen but also the viral replication machinery that enables intracellular RNA amplification and abundant protein expression.¹²¹ All RNA vaccines require appropriate delivery systems due to unmodified naked mRNA being rapidly digested by ribonucleases and innate immune response.¹²² Therefore, candidate SARS-CoV-2 mRNA vaccines are based on the use of liquid nanoparticles as a stabilizer. Several candidate vaccines of this type are in clinical trials, most importantly mRNA-1273 candidate vaccine that codes for the S protein. If approved, mRNA-based vaccine would be the first-of-its kind vaccine approved for use in humans.^{113, 118}

Subunit vaccines

Subunit or protein-based vaccines are composed of highly purified antigens, either synthetic peptides or recombinant proteins used to generate a protective immune response.^{123, 124} Candidate subunit SARS-CoV-2 that are currently evaluated are based on the full-length spike (S) protein, receptor-binding domain (RBD), non-RBD S protein fragments and non-S structural proteins.¹²⁵ Since highly purified proteins in subunit vaccines are usually not inherently immunogenic, carefully formulated adjuvants need to be used to ensure their immunogenicity. Recombinant S-trimeric SARS-CoV-2 protein candidate vaccine developed by the University of Queensland uses “molecular clamp”

transformative technology that is based on the synthesis of viral surface proteins and their subsequent “clamping” in a pre-fusion form. Alternatively, NVX-CoV2373 vaccine candidate is based on Matrix-M adjuvant along a full-length SARS-CoV-2 spike glycoprotein that is incorporated into a nanoparticle formulation.^{114, 124}

Viral vector vaccines

Vaccines based on non-replicating or replicating viral vectors exhibit excellent immunogenicity and are able to induce both humoral and cellular specific immune responses. The development of SARS-CoV-2 candidate vaccines of this type is mainly based on non-replicating adenovirus-based vectors¹¹³ due to high immunogenicity, possibility of administration via oral or nasal mucosa and safety (absence of integration into host cell genome).¹²² However, high prevalence of neutralizing antibodies specific for adenoviruses in the population can have a negative impact on the long-term immunogenicity of this type of vaccines.^{126, 127}

Attenuated vaccines

Live-attenuated vaccines have a long history of success and are the most frequently used vaccines in humans.¹²⁸ They are usually produced by a series of cultivation of the microorganism under suboptimal conditions or via successive passages in cell cultures that lead to the attenuation of virulence while preserving the immunogenic potential. This existing infrastructure is a big advantage for further development of SARS-CoV-2 vaccine.¹²⁹ Attenuated vaccines are also more efficient than other platforms in providing a long-lasting protective immunity since they persist for longer period of time, present the complete array of viral antigens to the immune system and deliver antigens to the appropriate cell compartments to produce proteins for efficient MHC I class presentation and generating a T cell response.¹¹⁸ The main problem in the application of attenuated vaccines is the possibility of reverting to the wild-type virulence.¹³⁰ Therefore, the risk of revertants makes attenuated vaccines inappropriate for infants, immunocompromised or elderly individuals.¹¹⁸ Besides, creating infectious clones takes more time because of their large genome size, requirement for dedicated biosafety level facilities and the need for extensive safety testing.^{114, 129}

Inactivated vaccines

Inactivated virus vaccines or whole killed virus (WKV) vaccines consist of pathogens that are no longer able to infect and replicate, but they have retained their ability to act as immunogens.¹¹⁸ Pathogens are usually inactivated physically or chemically (e.g. formaldehyde

or radiation)¹¹⁵, like in a case of the SARS-CoV-2 clinical candidate CoronaVac from Sinovac Biotech Ltd. (formerly PiCoVacc) that used β -propiolactone to inhibit viral membrane fusion in a dose-dependent manner.¹³¹ Inactivated vaccines represent an attractive solution for SARS-CoV-2 vaccine development, due to their previous successful use, excellent safety profiles, simple formulation (no need for adjuvants) and large-scale manufacturing infrastructure.¹¹⁴ Moreover, by exposing the same epitopes which a virus would have otherwise presented, these vaccines are very good at eliciting strong immune responses.¹¹⁸ Vaccine candidates of this type also displayed a good cross-neutralization to different SARS-CoV-2 strains.¹³¹ However, this technology has several limitations. Beside the main issue of raising biosafety concerns due to the risk of vaccine preparations containing the infectious virus, *in vivo* experiments with a candidate SARS-CoV inactivated vaccine in mice caused a Th2-type immunopathology, indicating development of hypersensitivity.¹²⁸

Virus-like particle (VLP) vaccines

Virus-like particles (VLPs) are macromolecular complexes that resemble the structure of the virus but lack its genetic material, enabling them to mimic the native structure of viruses without being infective.¹³² These features make VLP-vaccines excellent potential candidates for the development of safe and effective SARS-CoV-2 vaccines which can efficiently stimulate innate and adaptive immune responses.¹²³ VLP-based vaccines have mainly been designed to target B-cells and induce strong antibody responses following the activation of CD4⁺ T-cells and antigen presentation by MHC class II molecules.¹³³ In addition, VLP-based vaccines also enable MHC class I-mediated antigen presentation and activation of antigen-specific CD8⁺ T-cells.¹³³

Bacterial, insect, yeast and mammalian cells expression systems have been widely used in the production of VLPs.¹²³ However, candidate vaccine from Medicago Inc., as the only VLP vaccine in SARS-CoV-2 clinical evaluation, is based on a plant-derived system.¹¹³ An attractive feature of this type of transient expression system, which is based on deconstructed viral vectors and *Nicotiana benthamiana* as a host, is that it can allow for immediate exploitation of plants as efficient biofactories with rapid and large production of injectable vaccines.¹³³ Still, VLPs as a preventive vaccine have some considerable problems, such as viral mutations that might allow the virus to evade antibody-mediated neutralization.¹²³ Plant-based vaccines with transient nuclear genome transformation also have other drawbacks; they require purification of the antigen to eliminate toxic compounds from the host (e.g., alkaloids), as well as bacterial residues such as endotoxins coming from *Agrobacterium*-mediated delivery of viral vectors.¹³³

CONCLUSION

SARS-CoV-2 infection and COVID-19 disease are likely to remain an unprecedented challenge on the global level for an unknown period of time. Further research on the molecular features of the virus, its origin and underlying pathogenesis of the disease will contribute to the development of novel therapeutic strategies and vaccines.

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