The virus that shook the world: questions and answers about SARS-CoV-2 and COVID-19

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ABSTRACT
SARS-CoV-2 is an emerging human beta-coronavirus that caused the COVID-19 (Coronavirus Disease – 19) pandemic, the most significant health and social crisis in the last 100 years. SARS-CoV-2 is not only a respiratory virus; the symptoms of COVID-19 can include gastrointestinal, neurological, renal, cardiovascular and other complications. A large part of SARS-CoV-2 infected individuals are asymptomatic or have mild symptoms, around 20% of COVID-19 cases require hospitalization, and 5% can become critically ill. This review summarizes data on the biology of the virus and its pathological manifestations, antiviral immune response, information on the experimental models used in the related studies, treatment approaches and vaccine strategies.

Coronaviruses
General information
Coronaviruses (CoVs) belong to the subfamily Coronavirinae in the family Coronaviridae, order Nidovirales (according to the International Committee on Taxonomy of Viruses). CoVs are enveloped viruses, 65–125 nm in diameter, which possess a relatively large single-stranded, positive-sense RNA genome of around 27–32 kb - the largest known genomes for RNA viruses. Currently, the most famous virus from the coronaviridae family, SARS-CoV-2 has a genome length of 29.881 kb (GenBank No. MN908947) and encodes 9860 amino acids [1]. The name “Corona” represents the crown-like spikes on the outer surface of the virus (the word ‘corona’ means crown in Latin).

The morphology of coronavirus (left) and the organisation of the viral genomes of SARS-CoV, MERS-CoV and SARS-CoV-2 are illustrated in Figure 1.

The spike (S) glycoprotein is responsible for the binding of the coronavirus to the host cell (this function is performed by the S1 part that contains the receptor-binding domain of the virus - RBD) and mediates membrane fusion and virus entry (the S2 domain plays the leading role in this process) after processing by cellular transmembrane serine protease 2 (TMPRSS2) [2]. The S glycoprotein is also the main target for neutralizing antibodies [3] (see Figure 2A). The interaction of Spike’s RBD domain with the host receptor ACE2 is shown in Figure 2B.

Viral proteins
The understanding of the SARS-CoV viral proteins allows a rational approach to the development of more effective antiviral drugs [5–7]. The proteins of SARS-CoV (presented in Table 1) consist of two major polyproteins: ORF1a and ORF1ab (which cleave proteolytically to form 16 non-structural proteins); four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N); and eight accessory proteins: ORF3a, ORF3b (NP_828853.1, not present in SARS-CoV-2), ORF6, ORF7a, ORF7b, ORF8a, ORF8b and ORF9b (NP_828859.1, not present in SARS-CoV-2) [5]. None of the accessory SARS-CoV proteins is essential for viral replication, but some of them play a role in viral pathogenesis, for example, some of them modulate interferon signalling pathways
and the production of pro-inflammatory cytokines [8]. Like SARS-CoV, SARS-CoV-2 lacks the haemagglutinin esterase gene found in human coronavirus (hCoV) HKU1 [8]. The spike protein [2], envelope protein [9,10], membrane protein, nucleocapsid protein [11], 3CL protease [12], papain-like protease [13], RNA polymerase [14] and helicase [15] have been proposed as viable targets for antiviral drugs [16–18]. Examples of some viral proteins listed above are presented in Figure 3.

**Family members**

Based on their phylogenetic relationships and genomic structures, CoVs are divided into four genera: Alphacoronavirus (α), Betacoronavirus (β), Gammacoronavirus (γ) and Deltacoronavirus (δ) [19–22]. The alpha-CoVs and beta-CoVs infect only mammals inducing mainly respiratory, gastrointestinal and neurological diseases. The gamma-CoVs and delta-CoVs affect birds, but some of them can also be pathogenic for mammals.

Currently, there are seven coronaviruses known to be pathogenic for humans (SARS-CoV, MERS-CoV, 229E, NL63, OC43, HKU1, as well as the new SARS-CoV-2) and all of them have a zoonotic origin from bats, mice or domestic animals. Four of them, HCoV-229E and HCoV-NL63 (α-CoVs) as well as HCoV-OC43 and HCoV-HKU1 (β-CoVs), induce mild upper respiratory diseases
In this century, three highly pathogenic human beta-CoVs emerged: SARS-CoV, MERS-CoV and SARS-CoV-2. SARS-CoV caused the outbreak (2002-2003) of Severe Acute Respiratory Syndrome (SARS), which started in November 2002 in Guangdong province in China. The infected patients exhibited pneumonia symptoms with a diffused alveolar injury which led to acute respiratory distress syndrome (ARDS). There were more than 8000 infected individuals (mortality rate of around 9.6%) in more than 30 countries [24]. A decade later, in 2012, MERS-CoV was identified to induce Middle East Respiratory Syndrome (MERS). MERS was spread mainly in the Middle East (almost 80% of the cases were in Saudi Arabia); the virus is endemic in this part of the world, but there was an outbreak in South Korea in 2015. According to the World Health Organization [25], up to February 2020, there were >2500 laboratory-confirmed cases with a
fatality rate of 34.4%. The affected individuals suffered pneumonia, followed by ARDS and renal failure [26]. The SARS-CoV-2 outbreak began at the end of 2019 in Wuhan, Hubei province in China, and soon spread to many other countries. On January 30, 2020, the World Health Organization (WHO) declared “a public health emergency of international concern”; on March 11, the 2020 Covid-19 outbreak was announced a pandemic. SARS-CoV-2 has caused a global pandemic in only three months.

The first genomic sequence of SARS-CoV-2 was reported on January 10, 2020; the virus was isolated from a 41-year-old man who had worked at the animal market in Wuhan [1]. SARS-CoV and SARS-CoV-2 are very similar, with 82% nucleotide sequence homology.

**Cellular receptors, targeted tissues and organs**

The most-reported cell receptor for SARS-CoV and SARS-CoV-2 is the cellular exopeptidase angiotensin-converting enzyme 2 (ACE2). The physiological role of ACE2 lies in the regulation of heart and kidney functions and blood pressure control. The main substrate for ACE2 is angiotensin II, and ACE2 acts as a negative regulator of the renin–angiotensin–aldosterone system (RAAS) by catalysing the hydrolysis of the active angiotensin II to the inactive angiotensin 1-7 [27,28]. The cell receptor for MERS-CoV is dipeptidyl peptidase 4 (DPP4) [22,29,30]. The receptor-binding domains of both SARS-CoV and SARS-CoV-2 are reported to show high similarity; however, subtle differences between the two account for a slightly higher binding affinity between SARS-CoV-2 and the ACE2 receptor compared to SARS-CoV [31]. The SARS-CoV-2 spike binds the ACE2 receptor at least ten times more tightly than SARS-CoV does [32]. This difference alone can only partially explain the higher transmissibility of SARS-CoV-2. As in the case of SARS-CoV, the cellular transmembrane serine protease 2 (TMPRSS2) is also required for SARS-CoV-2 entry into the cell – for the priming of spike S protein. The SARS-CoV-2 spike protein has been demonstrated to possess a sequence of amino acids (not present in SARS-CoV) that allows it to be recognized and cleaved by an enzyme called furin [33,34]. It is currently unclear whether and how this sequence is related to the virulence of SARS-CoV-2. However, from experience with other viruses (influenza viruses), we know that such sequences in the receptor-binding protein contribute to their virulence [35].

Studies have detected the co-expression of transcripts encoding ACE2 and TMPRSS2 by single-cell RNA sequencing in various cell types, including nasal epithelial cells, type II alveolar cells, myocardial cells, proximal tubule cells of the kidney, ileum and oesophageal epithelial cells and bladder urothelial cells [36,37]. Autopsy studies have confirmed the presence of SARS-CoV-2 in multiple organs, such as the lungs, pharynx, heart, liver, brain, kidneys as well as in the blood, with the highest level found in the respiratory tract [38].

ACE2 also determines the potential host range for SARS-CoV/SARS-CoV-2. These viruses can infect humans, cats, dogs, pigs, Chinese hamsters, civets, horseshoe bats, but not mice and rats [39,40].

**Origin**

Research has recognized bats as the natural reservoirs of a large variety of viruses, including the known human coronaviruses. SARS-CoV and MERS-CoV were further transmitted to humans via civet cats and camels, respectively [41].

The novel coronavirus SARS-CoV-2 is also believed to have originated from bats, as its closest known relative (BatCoV RaTG13; GenBank: MN996532) was described in horseshoe bats (Rhinolophus affinis): the nucleotide homology between SARS-CoV-2 and BatCoV RaTG13 is 96.2% [42–44]. However, its intermediate host before transmission to humans is not fully clarified yet. Genomic and evolutionary evidence suggests that this role can be played by pangolins – ant-eating mammals often used in traditional Chinese medicine. SARS-CoV-2 and CoV isolated from pangolins share 99% genetic homology [40, 43, 45]. Additional investigations are required to confirm the intermediate zoonotic source/host(s) of SARS-CoV-2 that caused the transmission of the virus to humans, as this is crucial for our efforts to limit the spread of the virus.

Zoonotic transmission [46] of the virus to humans in the cases of SARS-CoV, MERS-CoV and SARS-CoV-2 is shown in Figure 4.

**Coronavirus disease 2019 (COVID-19)**

The initial site of infection of SARS-CoV-2 is the respiratory tract. The incubation period of SARS-CoV-2 is usually 1–14 days, mostly 3–7 days. However, it could also be longer depending on the age of the patients [47]. The clinical picture of the disease ranges from asymptomatic via mild and moderate respiratory symptoms to severe and even potentially life-threatening cardiovascular and pulmonary complications [48]. Fever is often the initial and major symptom of
COVID-19, which can be accompanied by dry cough, shortness of breath, muscle ache, dizziness, headache, sore throat, rhinorrhea, chest pain, diarrhoea, nausea and vomiting. In severe cases, patients quickly progress to develop acute respiratory syndrome, septic shock, metabolic acidosis and coagulopathy [22, 49,50]. Nearly 20% of patients develop severe condition [51], and the situation for 5% of them can become critical. Factors associated with increased mortality risk include male sex, advanced age, and presence of comorbidities, including hypertension, obesity, cardiovascular diseases, diabetes mellitus, cerebrovascular diseases [52,53]. Reportedly, about 2–11% of patients with COVID-19 have underlying chronic liver disease [53].

Although COVID-19 is mainly associated with respiratory pathology, it can also lead to several extrapulmonary manifestations such as gastrointestinal, neurological, renal, cardiovascular and other complications. Hyperglycaemia and ketoacidosis, ocular and dermatologic symptoms are found in these patients [54–56]. The diverse clinical picture is related to various factors, including the expression of the ACE2 receptor and protease Tmprss2 in multiple cells, organs and tissues; endothelial damage and thromboinflammation, dysregulation of immune responses, disturbed functioning of RAAS and dysbalance between Angiotensin II and Angiotensin 1-7 physiological arms [57].

**Cardiovascular manifestations**

There is abundant evidence that coronaviruses affect the cardiovascular system. [52]. Studies have suggested that the target cardiac cells of SARS-CoV-2 are pericytes characterized by high expression of ACE2. The pericyte injury due to virus infection may result in capillary endothelial cells dysfunction [58]. Cardiac complications of COVID-19 include acute myocarditis, arrhythmia, cardiogenic shock and even sudden death [48, 52]. Some authors have hypothesized that SARS-CoV-2 may induce myocardial injury via Nox2-related production of reactive oxygen species (ROS) [59]. Reports have demonstrated increased levels of troponin T and NT-proBNP, which are biomarkers of early myocardial damage, in SARS-CoV-2-infected patients [58, 60,61]. Individuals with COVID-19 often display underlying cardiovascular disease, and 19–33% of hospitalized patients experience cardiac injury [62]. Elevated troponin levels in patients with COVID-19 are associated with high mortality [63]. Moreover, studies have reported a sustained depression in left ventricular function in two-thirds of the patients recovered from a moderate to severe form of COVID-19 [64,65].

**Kidney damage**

Reports indicate that up to one in four patients in critical condition develop acute kidney injury (AKI). The pathophysiological developments of COVID-19 induced AKI can be both SARS-CoV-2 specific and non-specific. The COVID-specific mechanisms include cell damage caused by viral entry via the ACE2 receptor (which is characterized with a high renal expression), disruption of the RAAS, and elevated levels of pro-inflammatory cytokines. On the other hand, the non-specific pathways include hypovolemia, haemodynamic alterations, right heart failure, high levels of positive end-expiratory pressure (PEEP) in severe cases, where mechanical ventilation of the patient is required, elevated levels of pro-inflammatory cytokines as a result of the viral infection and thrombotic events, damage by nephrotoxic drugs and nosocomial
sepsis. At this point, no specific treatment for COVID-19 associated AKI is available [66].

**Neurological manifestations**

Neurological manifestations have been commonly reported in patients with COVID-19 [57, 67, 68]. Practically around half of patients experience a reduction or complete loss of taste or smell [69]. This fact is not surprising because respiratory viruses (including human coronaviruses) may disturb the central nervous system (CNS) due to their ability to induce direct damage in CNS as a result of replication and/or misdirected host immune responses that could be associated with autoimmunity in susceptible individuals. SARS-CoV-2 can affect the CNS via the olfactory tract in the early stages of infection by attacking the olfactory bulbs [70]. Olfactory and taste disorders are part of the complaints in some patients with COVID-19 [71]. Reports recognize neurological impairment and the demyelinating reaction as complications in case of severe COVID-19 [67].

**Liver complications**

ACE2 receptors are expressed in cholangiocytes of the liver. Observations have shown hepatic dysfunction possibly related to the direct cytopathic effect of the virus, an uncontrolled immune reaction, sepsis or drug-induced liver injury, in 14-53% of patients with COVID-19, particularly in those with severe disease [53, 72].

**Pancreas**

Pancreatic injury can occur in some COVID-19 patients [73]. In another study, the authors report mild pancreatic injury patterns in patients with COVID-19 pneumonia that can be due to direct viral involvement of the pancreas or from secondary enzyme abnormalities in the context of severe illness without substantial pancreatic injury [74]. On the other hand, there are reports of new-onset diabetes and severe metabolic complications of pre-existing diabetes. A global registry of patients with COVID-19-related diabetes has been established to clarify better the association between SARS-CoV-2 and diabetes (covidiab.e-dendrite.com).

**Coagulopathy**

Coagulopathy and thrombotic events can occur in association with SARS-CoV-2 infection. The origin of such events could be explained by various factors including direct viral effects, the disturbed balance between vasoconstrictor and vasodilator angiotensins, and the release of cytokines. There is an elevated production of pro-inflammatory cytokines interleukin 6 (IL-6), IL-17A, and tumour necrosis factor α (TNF-α) in the majority of patients with severe outcomes, and hypercoagulability is recognized as an essential hallmark of inflammation.

The initial coagulopathy in COVID-19 patients is associated with prominent elevation of D-dimer (fibrin degradation product, whose progressively increasing values indicate increased thrombin production and activation of fibrinolysis and can serve as a prognostic parameter indicating a worse outcome) and fibrin/fibrinogen-degradation products. Studies report a coagulopathy in up to 50% of patients with severe COVID-19 manifestations [75–77]. COVID-19 frequently runs a more complicated course in patients with comorbidities at a more advanced age, and this group of individuals also have an age-related increased risk of thrombosis.

**Reproductive system**

One of the most critical questions is whether SARS-CoV-2 can affect (and in what way) the reproductive health of men and women and human fertility. Furthermore, can vertical (mother-to-fetus) and sexual transmissions occur? These concerns are based on the following facts: i) The ACE2 receptor is found in the vagina, ovaries, uterus and placenta. Moreover, ACE2 is involved in the regulation of follicle maturation, and ovulation affects changes in the endometrium and embryo development. The degree of expression of this receptor changes during the menstrual cycle; ii) ACE2 is also expressed in the testes, and, according to some reports, orchitis could be a complication of SARS-CoV infection [76]. However, the viral load is thought to be too low to cross the blood-testis barrier, and the ACE2 concentration has been suggested to be insufficient to allow the virus to enter (for review of this and other routes of SARS-CoV-2 transmission see [78]). To date, there is no evidence of damage to the female reproductive system in COVID-19 patients. Additional studies are needed to clarify better whether or not, and to what extent SARS-CoV-2 can affect male reproductive health [79,80]. Sexual and vertical transmission of SARS-CoV-2 has not been confirmed.

**SARS-CoV-2 and children**

Liguoro et al. [81] have performed a systematic overview of the available data on clinical, laboratory and
Radiation findings in children with SARS-CoV-2 infection based on the analysis of sixty-two studies (conducted in the period Jan 1, 2020–May 1, 2020) and three reviews. The total number of children included is 7480, aged 0 to 18 years (mean age 7.6 years). This overview shows that infected children display mainly mild (42.5%) and moderate symptoms (39.6%), among which the most common are fever (51.6%) and cough (47.3%). Only 2% of children go to paediatric intensive care units. The estimated mortality is 0.08%. A higher proportion of more seriously ill children was found in newborns: 12%, with the most common sign being shortness of breath (in 40% of cases). Laboratory and radiological findings in children were mainly non-specific [81]. A multisystem inflammatory syndrome related to infection with SARS-CoV-2 has been reported in older children (known as MIS-C), manifested by severe abdominal pain, cardiac dysfunction and shock, with a phenotype resembling Kawasaki disease (KD). MIS-C can lead to severe and life-threatening illness in previously healthy children and adolescents [82,83]. The inflammatory response in MIS-C differs from the cytokine storm of severe acute COVID-19 and shares several features with KD [84]. Compared with children with KD, those with MIS-C show differences in T-cell subsets, the level of IL-17A expression (it is lower) and biomarkers associated with arterial damage. Also, various autoantibodies have been suggested to be involved in the pathogenesis of MIS-C [84].

**Modes of transmission**

SARS-CoV-2 can be transmitted through direct, indirect or close contact with infected people through secretions such as saliva and respiratory secretions or respiratory droplets (expelled during a cough, sneeze, speech, song or even a simple exhalation). The size of these respiratory droplets is critical because it determines how far they will travel and how long they will stay in the air. The so-called respiratory droplets are >5-10 μm in diameter, whereas those that are <5 μm in diameter are referred to as droplet nuclei or aerosols [85]. In the case of respiratory droplets, infection occurs at a distance within 1 m from the infected person, when they enter the mouth, nose or eyes of a susceptible individual. These larger droplets quickly fall to the ground, but being smaller and lighter aerosols can stay suspended in the air and travel a more extended way (it is not completely clear how much exactly) [85–87]. Aerosol generation can occur during some medical procedures and is associated with increased risk of respiratory virus transmission (including SARS-CoV-2) to healthcare workers [87,88]. COVID-19 transmission through aerosol has been reported [89–91].

Respiratory secretions (droplets and aerosols) can contaminate surfaces and objects, creating fomites [85]. Viable SARS-CoV-2 virus and/or RNA detected by reverse transcription polymerase chain reaction (RT-PCR) can be found on those surfaces for various periods ranging from hours to days, depending on the ambient environment (including temperature and humidity), type of surface (the type of material and its relief - smooth, rough), exposure to sunlight. Literature reports surface persistence for up to 9 days, but disinfection can be achieved successfully using 70% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within 1-minute exposure. Other agents such as 0.05-0.2% benzalkonium chloride and 0.02% chlorhexidine digluconate are reported as less effective [92]. There are no specific reports to demonstrate fomite transmission directly. This is not surprising because people who have come in contact with contaminated surfaces have usually also been in physical contact with the infectious person [87].

Other possible modes of SARS-CoV-2 transmission have also been discussed. The virus detection in urine and stool has been documented [50, 93–96]. SARS-CoV-2 RNA can be detected in feces for an extended period, even after respiratory samples have tested negative and patients are asymptomatic. According to the available literature, the transmission of SARS-CoV-2 in humans through feces or urine has not been confirmed [87]. Currently, there is no evidence of SARS-CoV-2 transmission through breast milk [97]. WHO recommends that mothers with suspected or confirmed COVID-19 should be encouraged to initiate or continue to breastfeed [98].

Although SARS-CoV-2 has been detected in the amniotic fluid, in the cord blood and the placentas of infected women, the ability of this virus to undergo intrauterine or transplacental transmission from infected pregnant women to their foetuses has not been confirmed [99,100]. Experts warn that full clarification of this issue requires further research.

Viral transmission occurs during close and prolonged contact, such as a shared household or stays in enclosed places for 15 min or more with an infected person and without precautions [101].

SARS-CoV-2 RNA can be detected in people 1–3 days before the onset of clinical symptoms. The highest viral loads (measured by RT-PCR) appear on the day of symptom onset and then gradually decrease over time [86,87,102,103]. One of the
challenges for preventive control of SARS-CoV spreading is that people infect others before they became ill [104–106].

**Factors associated with SARS-CoV-2 susceptibility and pathogenesis**

One of the most intriguing questions is why people react differently to the virus: some people do not develop symptoms at all, or the clinical picture is mild, while others develop a severe clinical picture that can lead to a life-threatening condition. The answer to this question is undoubtedly ambiguous and includes factors from the virus, the host and the environment.

**Host factors**

The risk of a more severe course of COVID-19 and a fatal outcome is increased in individuals with underlying comorbidities (including obesity, diabetes, cardiovascular and lung conditions and cancer) and those with impaired immune system, regardless of age, and in older people who are more likely to have these conditions. Data show that men are more vulnerable than women.

Various hypotheses have been proposed to explain higher morbidity and mortality in the elderly. Potential roles of the hallmarks of ageing including immunosenescence, inflammation and inflammasomes, genomic instability, mitochondrial dysfunction, epigenetic alterations, biological clocks such as telomere attrition, impaired autophagy and several other pathobiochemical changes have been discussed in relation with COVID-19. Intensive studies have focussed on the differences between cellular/molecular mechanisms of biological responses between young, middle aged and older people as well as between males and females to improve our knowledge about host–coronavirus interactions and prophylactic and therapeutic strategies [107,108].

Severe COVID-19 clinical picture is observed (although less frequently) in younger people without known concomitant diseases. What is the role of our genetics and epigenetics? Analysis of the genome of about 4,000 people from Italy and Spain shows a correlation between specific genetic variants and the severity of COVID-19 [109]. The study includes 835 patients and 1255 control participants from Italy and 775 patients and 950 control participants from Spain; all the patients had severe COVID-19 (pulmonary failure). In total, the authors analyzed 8,582,968 single-nucleotide polymorphisms and conducted a meta-analysis of the two case-control panels. As a result of their efforts, a 3p21.31 cluster of six genes (SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6 and XCR1) was identified as the genetic susceptibility locus in patients with COVID-19 with respiratory insufficiency.

Further studies are needed to clarify better the significance of these genes for virus infection and the severity of the disease. It is interesting to note that the product of one gene (SLC6A20, which encodes the sodium-imino acid (proline) transporter 1 - SIT1) interacts with the ACE2 viral receptor. Other genes encode chemokine receptors (such as CC motif chemokine receptor 9 - CCR9, and the C-X-C motif chemokine receptor 6 - CXCR6) and are involved in the immune response against pathogens. The study also confirmed the potential importance of ABO blood group antigens for virus infection and disease: higher risk for blood group A and a lower one for blood group O as compared with other blood groups [109]. There are data that specific HLA polymorphisms are related to the susceptibility and protection from SARS-CoV-1 infection [110,111]. Authors have suggested that epigenetic dysregulation of ACE2 and interferon-regulated genes is associated with increased COVID-19 susceptibility and severity in lupus patients [112].

**Viral factors**

Although researchers have described thousands of mutations and different variants and subvariants of the virus, there is currently no evidence that any mutation or variant is responsible for the development of a more severe clinical picture and higher mortality or to be associated with a milder disease [113,114]. Environmental factors are poorly understood. However, there is some evidence that suggests a possible correlation between air pollution and a more severe clinical course of disease [115].

**Model systems**

Suitable model systems are required to study virus biology and behaviour, various aspects of its pathogenicity, as well as to identify effective strategies for prevention and treatment. Cell cultures (primary cell cultures and permanent cell lines) have been used for many years in experimental and clinical virology as well as in the design and development of antiviral agents and vaccine production.

Cell lines established from African green, cynomolgus, rhesus monkey as well as buffalo green monkey kidney (such as BGM, CV-1, FRhK, LLC-Mk2, MA-104,
pCMK, Vero E6) are susceptible to productive SARS-CoV infection. Reaching a high viral titer is not always accompanied by a specific cytopathic effect [116].

The novel coronavirus SARS-CoV-2 was isolated in pathogen-free human airway epithelial (HAE) cells [117] as well as from Vero E6 (kidney epithelial cells of African green monkey, clone E6) and Huh7 (human hepatoma) cell lines. An engineered TMPRSS2-expressing VeroE6 cell line that is highly susceptible to SARS-CoV-2 infection and useful for virus isolating and propagating has been obtained [39].

Cell cultures are valuable model systems, but they also have their disadvantages and limitations. Thus, as a result of prolonged cultivation in laboratory conditions, many genetic and epigenetic changes can occur in the permanent cell lines, moving them away from the characteristics of the initial tissue/organ from which they are derived. Primary cell cultures represent better biological features of the starting material. However, their application is associated with other difficulties, including a limited capacity for cell division and short life, heterogeneity (it is not possible to obtain the same primary cell culture twice even following the same protocol), different expression levels of genes crucial to infectivity, including host receptors. Sometimes it is not easy to find source material which is the case, for instance, with donors for HAE cells. Also, the conventional monolayer (2D) cell cultures do not reproduce adequately virus-host cell interactions. 3D cell cultures, as well as organ-on-a-chip models, are improved next generation in vitro models that can represent better the properties of biological systems in vivo.

Animal models

Certain animal species have long been established as important model systems for investigating human pathology. Approximately 90% of the laboratory animals used in biomedical studies are mice, rats and other rodents [118]. ACE2 of mice cannot interact with SARS-CoV-2, so these laboratory animals cannot be used as experimental models without genetic modifications (for example, transfection of human ACE2) [40]. For a detailed review that recently focussed on animal models in SARS-CoV-2 research, see [119]. Here we outline the most notable examples in brief.

Small animals

The high genetic similarity (>80%) between SARS-CoV and SARS-CoV-2 and the fact that both viruses use the same cellular receptor (ACE2) makes it possible to use the models developed for the investigations of SASR to study the new coronavirus and COVID-19. Among them are F344 rats where SARS-CoV was adapted to replicate and induce severe disease as a result of a mutation (single amino acid substitution) at spike S glycoprotein recognizing cellular ACE2 [120]. Mouse-adapted models of SARS-CoV (SARS-CoV MA15) have also been developed [121].

Genetically modified small animals can be applied to investigate the pathogenicity of novel coronaviruses as well as in the search for effective treatment options. Among the benefits of this type of models are their relatively low cost and high reproducibility of experiments as well as the availability of detailed information about their biological characteristics [118, 122,123]. A ferret and Syrian hamster model of SARS-CoV-2 infection and transmission that recapitulates aspects of human disease have been reported. These models are expected to be useful for understanding SARS-CoV-2 pathogenesis, as well as the search for new prophylactic and therapeutic approaches [124,125].

Non-human primates

Non-human primates (NHPs) provide highly valuable animal models for research related to human health and pathology because of at least two reasons: i) they have close phylogenetic relationship with humans; ii) among all other laboratory animals, they most closely resemble the development and maturation of the human immune system. What is more, the similarities in lung development and structure between human and NHPs that are not observed in rodents make them very suitable for studies in the field of lung pathologies and treatment [118, 122,123].

Designing a suitable model of COVID-19 is an essential next step towards a better understanding of the mechanisms associated with the pathogenesis of SARS-CoV-2, as well as easier identification of both preventive and treatment solutions.

Immunity

SARS-CoV-2 is a new virus, so the entire human population generally lacks specific immunity against it, and people are susceptible to infection.

The antiviral immune response involves two steps:

1. an early, non-specific phase (usually occurs in the first 5-7 days after infection) where a key role plays the synthesis and secretion of type I interferons (IFN-I) such as IFN-α and IFN-β. This first line
of defense is essential because it is critical to the fate of the virus, i.e. whether it will be successfully controlled or spread to the host.

ii. a later, antigen-specific phase involving adaptive immunity performed by T and B lymphocytes. Cytotoxic CD8+ T cells directly kill virus-infected cells. Through cytokine signalling, CD4+ T cells stimulate B cells directly to produce virus-specific antibodies and to support other defending cell populations indirectly [126,127].

Like other viruses, the new coronavirus implements different mechanisms to escape the host immune response. It can be assumed that the immune evasion mechanism of SARS-CoV-2 potentially resembles those of the other two highly pathogenic human beta coronaviruses SARS-CoV and MERS-CoV. Some of the proteins synthesized by SARS-CoV-2 (such as N protein, a novel short protein encoded by orf3b) may counteract the antiviral effect of IFN-β [29, 128,129].

Acute SARS-CoV-2 infection has been found to result in a reduction of T lymphocytes, natural killer cells, monocytes and dendritic cells [130]. A decreased number of CD4+ and CD8+ T cells, as well as lower IFN-γ production that correlates with disease severity, has been reported in COVID-19 patients [51].

The main target of neutralizing antibodies to coronaviruses, including SARS-CoV-2 is the S glycoprotein [2]. Antibodies against the nucleoprotein are also generated [131,132].

IgM and IgG antibodies have been reported to show similar dynamics in COVID-19 disease. There are data that specific IgA response tends to appear earlier and is stronger and more persistent than the IgM response [133–135]. IgA levels in severe cases were significantly higher than those in mild or moderate cases [136].

Adequately regulated, both the innate and adaptive immune responses are needed to control the infection and clear the virus successfully. On the other hand, the excessive inflammation, local generation of a large number of free radicals and an exaggerated immune response can lead to the so-called “cytokine storm” and subsequent progression to acute lung injury/acute respiratory distress syndrome and in the worst scenario multi-organ failure and death.

**Cytokine storm**

The “cytokine storm” is associated with hyperactivation of the transcription factor NF-kappa B (NF-κB) and sudden acute release of different pro-inflammatory cytokines and chemokines such as IL-6, IL-1, tumour necrosis factor-alpha, and interferons. SARS-CoV-2 directly activated NF-κB via pattern recognition receptors [137].

Downregulation of ACE2 due to the binding of SARS-CoV-2 to its cellular receptor leads to an increase in angiotensin II (Ang II). In addition to its vasoconstrictor function, angiotensin II also expresses pro-inflammatory effects via interaction with its AT1 receptor (AT1R). AngII-AT1R axis stimulated NF-κB and disintegrin and metalloprotease 17 (ADAM17), which leads to the following consequences: generation of epidermal growth factor receptor (EGFR) ligands and TNFα that are NF-κB stimulators; trans-signalling of IL-6-sIL-6Ra complex, in which the gp 130-mediated activation of STAT3 occurs in the lung epithelial cells [61, 137–140].

The cytokine storm, which results in exacerbation of the severe acute respiratory distress syndrome reported in COVID-19 patients, is induced by NF-κB hyperactivation. Even though SARS-CoV-2 itself can activate NF-κB, hyperactivation of NF-κB is achieved by the IL-6 amplifier, through the simultaneous activation of both NF-κB and STAT3.

Increased levels of cytokines IL-1β, IL-7, IL-8, IL-9, IL-10, FGF (fibroblast growth factor), G-CSF (Granulocyte colony-stimulating factor), GM-CSF (Granulocyte-macrophage colony-stimulating factor), IFN-γ, IP-10 (Interferon gamma-induced protein 10), MCP-1 (monocyte chemoattractant protein-1), MIP-1A and MIP1-B (macrophage inflammatory proteins), PDGF (Platelet-derived growth factor), TNF-α and VEGF (vascular endothelial growth factor) have been reported in patients with severe COVID-16.

Such hypercytokinemia is a frequently occurring event also in other severe infections with SARS-CoV, MERS-CoV as well as H5N1 and H7N9 influenza viruses. It is often associated with disease progression and outcome [61, 137–140]. The correlation between levels of inflammatory cytokines (G-CSF, IP10, MCP1, MIP1A, and TNF-α) and disease severity has been observed [61]. There are data that the IL-6/IFN-γ ratio is higher in severe than in moderate COVID-19 patients and could be connected with an enhanced cytokine storm [141].

Different strategies for reducing the cytokine storm in the treatment of COVID-19 are discussed. Among them is the application of artificial-liver blood purification [142] and angiotensin receptor blockers [143].

**Antibody-dependent enhancement**

Antibody-dependent enhancement (ADE) is a paradoxical phenomenon in host–pathogen biology, in which...
the presence of specific antibodies can be beneficial to the virus. It is crucial not only for the understanding of viral pathogenesis but also for the development of adequate antiviral strategies, especially vaccines.

The ADE is a process in which suboptimal virus-specific antibodies enhance the entry of the virus, and in some cases, the replication of the virus, into monocytes/macrophages and granulocytic cells through interaction with Fc and/or complement receptors. ADE has been reported in vitro and in vivo for various viruses of public health and veterinary importance, including coronaviruses [144,145].

ADE has been observed in some preclinical animal models vaccinated with experimental SARS-CoV vaccines [146,147]. Avoiding this reaction is one of the significant challenges in developing the vaccine against COVID-19.

Some authors have suggested that ADE of SARS-CoV-2 infection due to prior exposure to other locally circulating coronaviruses with similar antigenic epitopes may explain the severity of cases observed in the Hubei province of China [148]. It is not yet clear whether ADE is linked to SARS-CoV-2 infection, but finding an answer to this question is one of the hot topics in biomedicine at the moment [149].

**Pre-existing immunity**

Undoubtedly, one of the most intriguing questions is whether the presence of a cross-immune response against the human coronaviruses inducing common colds (hCoV-ICC) - 229E, NL63, OC43 and HKU1, can contribute to the milder course of SARS-CoV-2 infection. This hypothesis is based on the following facts: i) hCoV-ICC, on the one hand, and SARS-CoV-2, on the other, share many common characteristics, including transmissibility and the ability to infect and multiply in the upper respiratory tract; ii) 229E, NL63, OC43 and HKU viruses are widely distributed and each of us has probably encountered them many times in our lives – it has been estimated that ≥90% of adults have IgG antibodies specific to these four viruses [150].

We do not yet have a definitive answer to this question. However, pre-existing SARS-CoV-2-cross-reactive T cell responses (directed against S glycoprotein and other proteins) have been proven in healthy individuals not exposed to SARS-CoV-2, indicating some potential for pre-existing immunity in the human population [150,151]. One thing is for sure, studying the immune response to hCoV-ICC will help us to be better oriented in the situation with SARS-CoV-2 and the COVID-19 pandemic.

**Effectivity of the immune response**

We do not yet understand the durability of the immune responses to SARS-CoV-2. The available experience with human coronaviruses that cause common colds indicates that they do not always induce long-lasting antibody responses, which, in turn, makes the reinfection of a person with the same virus possible [152,153]. Specific antibodies were found in individuals that survived SARS-CoV or MERS-CoV infections 2–3 years later [154,155] and even after a more extended period in the case of MERS survivors [153] but it is not clear whether they are enough to prevent reinfection.

Our knowledge about possible re-infection by SARS-CoV-2 is limited, but it is well known that reinfections by coronaviruses occur. For hCoV-ICC, the reinfection times ranged between 6 and 105 months and frequently occurred at 12 months after the initial infection [156]. Such information on the duration of protective immunity is essential to predict a possible second wave of an epidemic.

Both CD4+ (T helper cells) and CD8+ (cytotoxic, or killer, T cells) lymphocytes have been reported to play an important role in protective immunity against SARS-CoV-2 infection and are associated with milder disease. Low amount of naive T cells (which may be due to ageing) are recognized as immunological risk factors predisposing for severe COVID-19 [157]. Individuals who have recovered from COVID-19 have been reported to possess high levels of both neutralizing antibodies and T cells. In contrast to severe cases, milder cases of COVID-19 show more significant numbers of memory CD8+ T cells in the respiratory tract [150, 158,159].

Antiviral antibodies against SARS-CoV-2 were reported not to decline within four months after diagnosis [160]. It is still unclear what titer of neutralizing antibodies is sufficient to confer protection against infection.

Some sex differences in immune responses that underlie COVID-19 disease outcomes have been described, including higher plasma levels of IL-8 and IL-18 in male patients, whereas females demonstrate more pronounced T cell activation [161]. Such differences can at least partially explain the more severe symptoms and higher mortality among men than among women.

Estimates show that a large part (40–75%) of SARS-CoV-2 infected people are asymptomatic or have mild symptoms. These individuals seem to develop low levels of antibody-mediated immunity which poses a significant challenge for establishing herd immunity [162–164].
Genetic stability and mutations

Coronaviruses, including SARS-CoV-2, possess an RNA proofreading machine (ExoN) that can protect them from the increased mutation rate typical of other RNA viruses [165,166]. The following reasons can at least partially contribute to explain the genetic stability of SARS-CoV-2: i) It is new to humankind and therefore does not meet with an already existing immune response against it; ii) We do not have widespread specific anti-coronavirus agents to treat the infection; hence, there is little selective pressure. SARS-CoV-2 mutation rate is thought to be twice as low as that of the influenza virus.

However, the virus is changing, and SARS-CoV-2 today is certainly not what it was at the beginning of the pandemic. The genome of the virus was first sequenced at the beginning of 2020, and on January 11, 2020, it was published in a specialized database for genetic information, then immediately found a place in "Nature" journal [1]. What has happened to the virus in the past nine months?

The first news in this regard was announced by Tang et al. [167]. According to the data available at that time, at least 149 sites of mutations were identified across the genome of 103 sequenced strains of SARS-CoV-2 allowing the identification of two viral subtypes, termed L and S subtype [167]. Later, the phylogenetic network analysis of 160 complete genomes of human SARS-Cov-2 revealed the presence of three variants designated as A, B and C [168]. These subtypes/variants seem to have different geographical distribution and transmission ability.

A more recent study conducted by Mercatelli and Giorgi [113] from the University of Bologna, Italy, also attempts to answer this question. The authors systematized the genomic analyses of 48 635 virus samples isolated and sequenced in laboratories around the world and available because of the collective endeavour of the GISAID consortium.

Although a total of 353 341 mutation events were identified compared to the reference viral genome NC_045512.2 Wuhan, the results show that the number of mutations that each sample carries is relatively low (average 7.23 per sample) and more than 15 mutation events are detected in very few isolates. Interestingly, the samples originating mainly from Asia (256 samples in total) did not differ from the reference one, while the remaining 48,379 samples carry at least one mutation. There was a difference in the average number of mutations per sample between countries, but not between continents.

The authors identify six phylogenetic groups G (named after the Spike D614G mutation), GH, GR, S, V and L with different characteristic mutations, frequency of occurrence and geographical distribution:

The original lineage “L,” corresponds to the reference genome NC_045512.2. Clade S appeared in early 2020; clades V and G, after mid-January; and GR and GH, in late February 2020.

Clade G and its derivatives GH and GR are the most common: they represent about 74% of all analyzed samples. The G and GR clades are most common in Europe and Italy. This study [113] did not detect GH in Italy, but it was often found in France and Germany. GH is the most common variant in North America, while GR predominates in South America. In Asia, the prevalence of G, GH and GR is also increasing. Clade S is identified in some areas in the USA and Spain, and L and V are gradually disappearing.

Another study presents data based on the genome analysis of more than 90,000 SARS-CoV-2 isolates. The results suggest that every two viruses isolated from any two individuals in any two parts of the world differ on average by only ten nucleotides [114].

Despite the slow rate of mutation, scientists have identified and categorized more than 12,000 mutations. One of them is the most common and is detected in around 97% of the cases; this is the mutation D614G (aspartate to glycine change in protein position 614 of the S protein, presented in Figure 5). This mutation is not located within the receptor-binding region of the S protein. A recent study of different SARS-CoV-2 S protein variants using pseudoviruses (see Table 2) linked the D614G mutation alone to higher infectivity but did not observe it to result in a change of sensitivity to neutralizing antibodies [169].

It is not excluded that different viral variants may cause COVID-19 with various rates of severity. Up to now, however, there is no mutation associated with a higher risk to public health.

Treatment strategies

SARS-CoV-2 is a new virus, so at the moment there is no specific and universally accepted treatment and vaccine for it. Supportive care is also crucial to the treatment provided to COVID-19 patients and includes approaches to managing clinical symptoms (such as fever), fluid management, oxygen therapy, optimal management of complications (including antibiotic and/or antifungal treatment) and underlying comorbidities.
The wide spread of the disease all over the world raises the urgency of the search for a targeted anti-SARS-CoV-2 agent. Currently, the efforts of specialists are focussed in two directions:

1. Drug repurposing. The choice is based on the following factors: registered drugs with known pharmacokinetics, pharmacodynamics and toxicological profiles; some of them showed an effect in the treatment of SARS and/or MERS; promising activity against COVID-19 has been observed in small clinical trials (in China and other countries); they can be produced quickly and in large quantities. Examples of such medications are chloroquine and hydroxychloroquine, lopinavir/ritonavir, remdesivir, favipiravir (see Figure 6). In general, the repurposed agents can be divided into two main groups: i) drugs that are expected to attack the replication and life cycle of the SARS-CoV-2 directly, and ii) those that affect the immune response by stimulating innate immunity or suppressing unregulated abnormal immune/inflammatory reactions and prevent or relieve the induced damage. These drugs have been included in large clinical trials initiated by the WHO and by France, the United Kingdom, Canada, etc. Brief information about some of them is presented in Table 3. The interaction of these treatments with other drugs used in the patients should be monitored carefully [22, 48, 177, 193, 200].

Currently, the results of only several trials investigating the potential benefits of remdesivir in the fight
against COVID-19 are available [171, 178–181]. On May 1, 2020 the U.S. Food and Drug Administration (FDA) [202] granted Veklury (remdesivir) authorization for emergency use for the treatment of suspected or laboratory-confirmed COVID-19 in hospitalized adult and paediatric patients with severe disease. This decision was based on the preliminary results of the randomized, double-blind clinical placebo-controlled trial of 1062 patients by Beigel et al. [181] (ACTT-1 trial), which concluded that remdesivir shortened recovery time from 15 to 11 days (later revised to 10 days in the final report). The emergency use authorization was later expanded on August 28, 2020 to include all hospitalized COVID-19 patients regardless of severity of disease [203]. On October 22, 2020 Veklury was approved by the FDA [204] for treatment of hospitalized COVID-19 patients aged 12 and older and weighing 40 kg or more. The analysis of the evidence of remdesivir’s effectiveness, leading to its approval, included the findings from the trials by Beigel et al. [181], Goldmann et al. [179] and Spinner el al. [180]. However, the interim results from the larger SOLIDARITY trial, which is coordinated by the WHO and aims to assess the effectiveness of remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon-β1a, has concluded that none of the investigated drugs had a significant clinical benefit (with regards to hospital stay duration, overall mortality and initiation of ventilation) compared to standard-of-care without a drug [171]. The apparent disagreement between the findings of the reported trials may be at least partially explained by differences in trial design, patient heterogeneity and stage of disease progression of patients at enrolment. Large multicenter trials also need to account for the differences which arise from studying patients/volunteers from different countries/geographic locations, such as variations in the provided medical care and potential pharmacogenetic and other variability of local populations. As such, at the time of writing of this review, it is still early to know for certain if remdesivir will gain widespread approval outside of the USA, and more research is needed to carefully evaluate its clinical effects.

Evidence has demonstrated that administration of dexamethasone is effective for suppression of excessive immune response in critically ill patients [205]. On July 16, 2020, FDA added dexamethasone to COVID-19 temporary compounding lists.

Anticoagulant therapy with heparin was also associated with better prognosis in severe COVID-19 patients [206]. In the search for practical approaches to prevent COVID-19-associated thrombosis and complications and to improve treatment results, scientists have proposed several antithrombotic drugs, including heparin, FXII inhibitors, fibrinolytic drugs, nafamostat and dipyridamole, many of which with pleiotropic anti-inflammatory or antiviral effect [207].

Nevertheless, in the urgency and early stages of anti-SARS-CoV-2 drug discovery, one should be careful in promoting new indications for known drugs. Namely, the American Heart Association has
### Table 3. Repurposed therapeutic agents for the treatment of COVID-19 currently in clinical trials.

| Product | [References] | Initially developed for the treatment of | Mechanism of antiviral activity | Side effects | Remarks |
|---------|--------------|------------------------------------------|---------------------------------|--------------|---------|
| Chloroquine and Hydroxychloroquine | [170–176] | Malaria, also applied in autoimmune diseases. | Interfere with the glycosylation of the cellular receptor ACE2, block fusion by increasing endosomal pH | The arm of the RECOVERY clinical trial with hydroxychloroquine was ended in June, 2020 after it was concluded that there was no benefit in hospitalized patients. Similar findings were reported from the SOLIDARITY trial. | Hypersensitivity reactions, mild to moderate elevation of liver enzymes levels, etc. |
| Remdesivir (GS-5734, Veklury) | [171, 177–186] | Ebola virus disease | Nucleotide (adenosine) analogue, inserts into viral RNA and further inhibits the RNA-dependent RNA polymerase resulting in premature termination during virus transcription | Remdesivir received approval by the FDA (Oct 22, 2020) for hospitalized COVID-19 patients as well as a one-year conditional marketing authorization from the European Commission due to the urgency of the COVID-19 pandemic. | |
| Lopinavir/Ritonavir (Kaletra) | [62, 177, 187–189] | HIV/AIDS Ritonavir (a potent cytochrome P450 3A4 inhibitor) inhibits the metabolism of lopinavir, resulting in more favourable lopinavir bioavailability | HIV-1 protease inhibitors | Remdesivir received approval by the FDA (Oct 22, 2020) for hospitalized COVID-19 patients as well as a one-year conditional marketing authorization from the European Commission due to the urgency of the COVID-19 pandemic. | It is associated with many adverse reactions and drug interactions due to the potent inhibition of CYP3A4. The most frequent side effects observed are mild to moderate diarrhea, nausea and vomiting; can prolong the QT interval. The RECOVERY clinical trial (and later SOLIDARITY) concluded that lopinavir/ritonavir is not an effective therapeutic option for COVID-19 and ended the treatment arm in late June, 2020. |
| Favipiravir (Avigan) | [62, 190–192] | Influenza virus (Developed for influenza virus strains resistant to Tamiflu) | Inhibitor of the RNA-dependent RNA polymerase by structurally resembling guanine | Contraindicated in pregnant women due to its teratogenicity and embryotoxicity in animals; can prolong the QT interval. | Well tolerated molecule; its efficacy in the treatment of COVID-19 patients has been demonstrated in some studies. |
| Umifenovir (Arbidol™) | [177, 193–196] | A small indole-derivate molecule; licensed in Russia and China for oral prophylaxis and treatment of infections with influenza A and B viruses and other respiratory viruses | Prevents viral host cell entry and virus infection by inhibition of fusion via suppression of clathrin-mediated endocytosis | | Well tolerated molecule; its efficacy in the treatment of COVID-19 patients has been demonstrated in some studies. |
| Dexamethasone | [197–199] | A synthetic corticosteroid approved by the FDA in 1958 as a broad-spectrum immunosuppressor; it is about 30 times as active and with longer duration of action (2-3 days) than cortisone. Included in the WHO model list of essential medicines since 1977, used in a range of conditions, including inflammatory disorders and certain cancers | Overall effects on innate and adaptive immunity, limits the production of and damaging effects of cytokines. | The large randomized trial RECOVERY shows that low dose dexamethasone reduces 28-day mortality from around 41% to 27% for ventilated patients and from 25% to 20% among those needing oxygen. The benefit was only seen in patients with severe COVID-19 and was not observed in individuals with milder disease. | FDA added dexamethasone to COVID-19 temporary compounding lists on July 16, 2020. |
recognized chloroquine and hydroxychloroquine as agents which can cause direct myocardial toxicity. Furthermore, studies compromising chloroquine and hydroxychloroquine activity against SARS-CoV-2 have been published [208]. Similarly, other drugs such as favipiravir and kaletra (lopinavir/ritonavir) as well as several antibiotics commonly used for the treatment of patients with COVID-19, for instance, azithromycin, can prolong the QT interval [62, 209] and cause other difficulties [210].

1. Design and development of effective new anti-SARS-CoV-2 agents. Drug design can be directed at several major targets (spike protein, envelop protein, membrane protein, protease, nucleocapsid protein, haemagglutinin esterase and helicase) [16, 211]. Despite requiring a more extended development and approval process, the search for novel therapeutic agents may yield more specific and compelling treatment options and possibly a common drug solution for coronaviruses [212]. For a broad-spectrum coronavirus antiviral to be developed, it must be able to target either viral or host components, which are vital to the replication cycle of the virus or the disease development in the host. Of course, the targeted factors must be present and highly conserved within a broader range of CoVs to ensure efficacy in future emergent CoVs [122].

Brief information about other strategies proposed for the treatment of COVID-19 patients is presented below.

**Plasma therapy**

Though new to the current pandemic, serum therapy has been used (in various forms) since the 1890s when it was introduced in medicine by Emil von Behring for the treatment of diphtheria and tetanus – the first Nobel Prize in physiology and medicine in the twentieth century was awarded in 1901 for this achievement. A direct antiviral effect of virus-neutralizing antibodies in convalescent plasma is believed to be the main mechanism of action, but indirect effects may also contribute to improved viral clearance, inflammation and organ function [123, 213]. Many clinical trials are currently underway to determine the benefit of plasma therapy compared to standard treatment with COVID-19 or other alternative therapy. They are also expected to answer several important questions related to the application of plasma therapy, including the accuracy and predictive value of antibody testing of donors and patients, optimal donor and patient selection, optimal timing and schedule of application [123, 213]. Plasma therapy is thought to be more appropriate for patients with moderate symptoms before the virus has severely damaged tissues and organs.

**Monoclonal antibodies**

Studies have reported human monoclonal antibodies, as well as single-domain antibody fragments isolated from llama and alpaca that can recognize and neutralize SARS-CoV-2. Such biotechnologically derived antibodies/antibody fragments are expected to assist in the diagnosis, prevention and treatment of this infection [214–218].

Of interest are the results obtained with two neutralizing monoclonal antibodies (COV2-2196 and COV2-2130), which recognize and bind simultaneously to regions of the S protein of SARS-CoV-2 virus in a synergistic manner. In two models of SARS-CoV-2 infection in mice, passive transfer of COV2-2196, COV2-2130, or a combination of both antibodies, protected the animals from weight loss and reduced viral load and levels of inflammation in the lungs. Moreover, application of ACE2-blocking monoclonal antibodies (COV2-2196 or COV2-2381) as monotherapy protects rhesus macaques from SARS-CoV-2 infection [219].

Tocilizumab and sarilumab are monoclonal antibodies that inhibit interleukin-6 (IL-6) and are approved for the treatment of rheumatoid arthritis. Currently, they are also being investigated as therapeutic agents for COVID-19 [220,221].

**Interferon**

Due to its antiviral and immunomodulatory properties, interferon is also an attractive strategy for the treatment of SARS-CoV-2 infections, alone or in combination with other therapeutic agents [222–225].

**Modulation of renin-angiotensin-aldosterone system**

Agonists of the ACE2-angiotensin1-7-Mas receptors signaling pathway are thought to have promising therapeutic potential in COVID-19 patients. This fact is not surprising, since the binding of SARS-CoV-2 to its cellular ACE2 receptor expresses at least two adverse effects on human health. First, it marks the beginning of a viral infection. Secondly, it prevents a cascade of...
reactions, including the formation of angiotensin 1-7 (angiotensin 1-7 is derived from angiotensin II with the involvement of ACE2, and ACE2 is already "occupied" by the virus, resulting in a lack of angiotensin 1-7 and an excess of angiotensin II).

By binding to specific receptors (so-called Mas receptors - MasR), angiotensin 1-7 counteracts the damaging effects resulting from the interaction of angiotensin II with its AT1 receptor (AT1R), including the stimulation of inflammation and fibrosis. This mechanism has been recognized as the driving force not only of acute respiratory distress syndrome and lung fibrosis (including fibrosis that is not aetiologically related to SARS-CoV-2) but also of several other socially significant pathological conditions, including diabetes, hypertension, cardiovascular and kidney diseases. Therefore, the use of agonists in the ACE2 - Angiotensin 1-7 - MasR pathway has been recognized as a promising option for the prevention of severe COVID-19 and other pathological conditions [226,227].

**Mesenchymal stem cells**

The idea of administering mesenchymal stem cells (MSCs) in COVID-19 patients is based on their immunomodulatory properties, which may contribute to the prevention of cytokine storm, as well as their regenerative capacity, which will be beneficial in lung recovery. MSC therapy has several benefits that make it advantageous compared to other stem cell treatment strategies [228], namely: i) An intensive proliferative capacity and possibility to obtain MSCs in sufficient numbers; ii) MSCs are available in adult individuals and can be obtained from different tissues (adipose tissues, dental pulp, bone marrow, menstrual blood, etc.); iii) Avoidance of ethical, legal and social problems; iv) Being multipotent cells, MSCs are able to self-renew and differentiate into several cellular lineages. For a more in-depth review, see [229]. In general, various clinical trials have documented MSC therapy to be a safe and effective treatment approach [230].

**Vitamin D**

Vitamin D has been suggested to play a beneficial role in reducing inflammation in COVID-19 patients by increasing the anti-inflammatory cytokines and decreasing the levels of the pro-inflammatory cytokines [231]. Treatment of vitamin D deficiency leads to reduced expression of DPP4/CD26 – a molecule that has been recognized as a possible co-receptor of SARS-CoV-2 and is essential for its virulence [231,232]. This explains the increased interest of scientists in inhibitors of DPP4/CD26.

**Ribavirin**

Ribavirin, a guanosine analogue that interferes with the replication of RNA and DNA viruses, has been expected to be potentially beneficial for the treatment of COVID-19, since it was approved for treating respiratory syncytial virus (RSV) infection and was used extensively during the SARS and MERS outbreaks (usually in combination with interferon, i.e. IFN-α or IFN-β). However, this drug shows the undesirable adverse effect of reducing haemoglobin, which is harmful to patients in respiratory distress. The therapeutic efficacy of ribavirin in SARS-CoV-2 infection has not been confirmed yet [200,201,233,234].

**Protease inhibitors**

Clinically approved anti-Hepatitis C virus drug boceprevir as well as a pre-clinical inhibitor against feline infectious peritonitis coronavirus (FIPV), GC376, have been reported to inhibit SARS-CoV-2 in cell cultures. The mechanism of action of both compounds is based on their ability to affect the coronavirus main protease (Mpro, also called 3CLpro). This protease is recognized as a promising drug target, as it is essential for processing and maturation of the viral polyprotein. What is more, a sterilizing additive effect has been observed as a result of the combined application of GC376 with remdesivir [235].

**Ivermectin**

Ivermectin is an FDA approved antiparasitic agent discovered in 1978 with more than 2.5 billion doses distributed in the last 30 years. A Nobel Prize in Physiology or Medicine in 2015 was awarded to William C. Campbell and Satoshi Omura for the discovery of ivermectin [236]. Antimicrobial and antiviral effects, as well as immunomodulatory and anticancer activities of ivermectin, have also been reported [236–238]. Ivermectin inhibits the in vitro replication of some RNA viruses (including dengue virus, Zika virus, yellow fever virus, Avian influenza A virus, etc.) and DNA viruses (such as Equine herpes type 1, BK polyomavirus, pseudorabies, porcine circovirus 2 and bovine herpesvirus 1 [237,238]. This drug has been found to inhibit SARS-CoV-2 in cell culture and is proposed in COVID-19 as a candidate for new drug use,
immediately attracting the interest of the scientific community [239].

Specialists are faced with two questions: 1) Whether ivermectin will show its antiviral activity in vivo; 2) And if so, whether this will happen in doses that are safe for humans [239–241]. Studies have explored whether ivermectin should not be administered in children aged less than 5 years and/or weighing less than 15 kg. It is also not advised for use in patients with liver or kidney dysfunction [242].

**Phytochemicals and natural products**

Various phytochemicals have been reported to possess antiviral and immunomodulating properties. Among them are phytochemicals such as flavonoids. Some of them (i.e. luteolin, myricetin, quercetin, apigenin, etc.) modulate the activity of NLRP3 inflammasome influencing in this way the inflammatory response to SARS viruses [193]. Chinese herbal drugs from traditional medicine have also been considered as an alternative approach for prevention of COVID-19 in high-risk populations. The increased interest in Chinese traditional medicine (CTM) is not accidental, because the SARS outbreak in 2002-2003 provided a lot of experience with its application. One of the reasons for the dramatic decline in SARS mortality since the end of May 2003 in Beijing is believed to be the use of CTM as a supplement to conventional therapy. The efficacy of CTM in COVID-19, however, has not yet been clinically proven [201, 233].

**Bacteriophages**

The potential use of bacteriophages in the fight against COVID-19, in particular in the treatment of antibiotic-resistant secondary bacterial infections, is discussed [243].

**Colchicine**

Colchicine is a known drug used nowadays for treatment of gout, rheumatologic diseases and chronic pericarditis. It has a prominent anti-inflammatory activity by interacting with neutrophil inflammasome complex, thus inhibiting interleukin, IL-6 [244]. As such, it has a potential to decrease the effects of the cytokine storm induced by SARS-CoV-2. Two recent studies have reported the effects of colchicine on hospitalized COVID-19 patients. The first one used a 7-point scale to assess the effectiveness of colchicine to reduce disease worsening, and observed that worsening occurred significantly less in the colchicine group (p = 0.02) [245]. The second study observed a significant reduction of mortality in colchicine-treated COVID-19 patients compared to those under treatment regimens without colchicine (16.3% vs. 37.1%, p = 0.001) [246]. Nevertheless, these promising results should be further scrutinized and confirmed in larger randomized trails.

**Vaccines**

The development and introduction of a successful vaccine is an important long-term strategy to prevent COVID-19 outbreaks in the future if the virus becomes endemic and causes periodic seasonal epidemics. It is targeted at front-line healthcare professionals, as well as people at increased risk of SARS-CoV-2 infection and more severe disease: elderly and individuals with comorbidities (such as hypertension, cardiovascular and lung diseases, obesity, diabetes, etc.).

Immunogenic peptides that can serve as potential targets for a SARS-CoV-2 vaccine have been identified by in silico design [247]. Different options regarding candidate antigens (such as whole virion, spike S protein, nucleocapsid N protein, membrane M protein, envelope E protein), adjuvants, model systems and technologies have been discussed in the choice of vaccine strategy. Various types of vaccines have been under consideration, including inactivated vaccines, live-attenuated vaccines, subunit, mRNA and DNA vaccines, vectored vaccines, synthetic peptide or epitope vaccines [248]. Each vaccine platform and strategy has its immunological and technological advantages and disadvantages. The presence of various vaccine candidates based on different approach is valuable for at least two reasons: i) this is a new human virus, the immune response against it has not yet been completely studied; ii) the vaccine is expected to work in people of different ages, including the elderly and with various comorbidities. Different groups of people may benefit from different types of vaccines.

Three months after the SARS-CoV-2 genome sequencing (announced on January 11, 2020), at least 78 candidates for the vaccine against COVID-19 have been developed, and five of them were already in the first phase of the clinical trial for human safety, tolerability and immunogenicity [153]. As of October 19, 2020, there were 44 candidate vaccines for COVID-19 in clinical evaluation (10 of them in Phase III of clinical trials) and 149 vaccines in preclinical development [249].

The vaccines that are currently in Phase III clinical trials [249] are prepared by:
• Oxford University, UK, and Astra Zeneca: a chimp-panzee adenovirus vectored vaccine (ChAdOx1 nCoV-19, also known as AZD-1222) expressing the SARS-CoV-2 spike protein [250]. The development of ChAdOx1 nCoV-19 is based on promising human studies with adenovirally vectored vaccines against MERS (ChAdOx1-MERS vaccine) [250] and tuberculosis (ChAdOx1-TB vaccine) [251];

• American biotechnological company Moderna, which has experience with mRNA-based MERS vaccines, developed jointly with the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, the first COVID-19 vaccine that entered clinical trial in humans, on March 16, 2020: mRNA encoding a prefusion-stabilized SARS-CoV-2 S protein encapsulated in lipid nanoparticles (mRNA-1273);

• Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation: heterologous vaccine consisting of a recombinant adenoviruses types 26 and 5, both of them carrying the gene coding for spike glycoprotein of SARS-CoV-2 (rAd26-S + rAd5-S) [252];

• Janssen Pharmaceutical Companies: Ad26COV51 vaccine based on adenovirus 26 vector expressing S protein (Ad5-nCoV);

• BioNTech/Fosun Pharma/Pfizer: mRNA–lipid nanoparticle vaccine encoding the S protein RBD (known as BNT162b1);

• CanSino Biological Inc/Beijing Institute of Biotechnology: a vaccine based on adenovirus type 5 vector expressing S protein (Ad5-nCoV);

• SARS-CoV-2 inactivated vaccines produced by Sinovac Research & Development Co LTD; Wuhan Institute of Biological Products/Sinopharm; Beijing Institute of Biological Products/Sinopharm.

• Novavax Inc. – A full-length recombinant SARS-CoV-2 glycoprotein nanoparticle vaccine (NVX-CoV2373) that contains Novavax’s patented saponin-based adjuvant [253].

To be able to enter clinical practice, the vaccine against SARS-CoV-2 must meet a number of norms and standards such as quality, safety and efficacy: i) to be safe; ii) to provide adequate immunity preventing viral infection and disease; iii) to be useful also against possible variants of the virus that can appear.

i. Providing adequate immunity to prevent viral infection and disease. Several pieces in the puzzle of the SARS-CoV-2 immunity are still missing. Among the most intriguing questions to be answered is the involvement of the humoral and cellular immune response, as well as the duration of immunological memory and its protective value. It is not yet clear what immunity is sufficient to ensure the efficacy of the vaccine, and whether it will prevent infection or just alleviate clinical manifestation. Vaccines have been developed for some animal coronaviruses that cause significant economic losses to the swine industry, such as transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) [258]. Although these vaccines protect against the pathogen, they do not answer the question of how long the created immunity would last, since the targeted farm animals are raised for food production and usually do not live long enough.

What is more, the vaccine for SARS-CoV-2 has to be able to induce a stable, protective immune response in people of all ages, including the elderly (≥ 60 years of age). Some data indicate that because of the so-called “immunosenescence”, elderly people have increased sensitivity to infections and reduced response to immunizations [259].

i. Being useful also against possible variants of the virus that can appear. The data available at the moment show that the genome of the virus is stable and does not pose a difficulty for diagnosis as well as for the development of drugs and vaccines [113,114]. As mentioned before, due to their RNA proofreading machine (ExoN), coronaviruses, including SARS-CoV-2, are less prone to mutations compared to other RNA viruses [165,166]. Besides, as SARS-CoV-2 is widespread throughout the world, indicating that its strategy is successful, we can assume that it is not subjected to extreme mutations. Moreover, people now do not have an immune response to this new virus and specific antiviral agents are not available, i.e. there are no factors to provoke the evolution towards a higher
variability of the virus. Monitoring the genomic stability of the virus is one of the major tasks of biomedical science to provide adequate diagnosis, prevention and treatment. Over 114,000 viral genomic sequences of SARS-CoV-2 have been shared until September 25, 2020, via GISAID (see www.gisaid.org).

A fundamental question to consider is how to create an effective vaccine in the face of possible virus mutation. There are generally two approaches to this: 1) Targeting the conservative regions of the immunogenic part of the viral genome; 2) Creating a universal vaccine platform, which would allow substitution of the target viral nucleotide sequence, thus allowing the necessary adjustments to be made to ensure guaranteed effectiveness with time.

The existence of an association between immunization with a tuberculosis Bacille Calmette-Guérin (BCG) vaccine and a lower COVID-19 incidence/mortality rate is also discussed [260–262]. At present, this hypothesis has not been confirmed. Experts warn that conclusions in this regard can be made only based on concrete evidence obtained from targeted research, and not only from the analysis of retrospective data. This is because there are considerable differences between countries such as socio-economic status, demographic structure, time of onset of the epidemic, national control strategies to limit the spread of COVID-19, etc. [263]. Clinical studies are underway to establish a causal relationship between BCG vaccination and protection against severe COVID-19, especially for frontline healthcare workers [260].

However, there are data indicating the non-targeted protective effects of BCG vaccine (reduced morbidity and mortality) against various RNA and DNA viruses in humans (such as Respiratory syncytial virus; RSV) and animals (for example herpes and influenza viruses in mice). BCG vaccine may be involved in non-specific immunotherapy of cutaneous and genital warts induced by human papillomaviruses (HPV) and has been associated with improved response to vaccines against influenza virus and hepatitis B virus. These activities can be at least partially explained by stimulation of cross-reactive T-cell response as well as antibody response against secondary viral infections. In addition, interleukin IL-1β, a pro-inflammatory cytokine with important function for antiviral immunity, can be secreted by epigenetically modified monocytes/macrophages [264]. BCG remains the standard immunotherapy treatment for patients with high-risk non-muscle-invasive bladder cancer globally for more than 40 years [260]. Some authors have proposed a possibility that vaccines for other diseases such as measles, mumps, rubella (MMR vaccine) can create cross-resistance for SARS-CoV-2 to explain at least partially the higher resistance of children to COVID-19 as compared to the elder population [123, 265]. This proposition can be rationalized through a 30 amino acid sequence homology between the Spike (S), Fusion (F1) and Envelope (E1) glycoprotein of SARS-CoV-2, measles and rubella viruses, respectively. In silico investigation suggests the sequence mentioned above as antigenic epitopes in measles and rubella, which in turn could encourage the idea that immunity acquired through the MMR vaccine may as well give some protective advantage against SARS-CoV-2 [266].

Almost all the vaccines developed are for parenteral administration. The respiratory mucosal vaccine strategy has also been suggested to be promising for the early control or clearance of SARS-CoV-2, especially in the high-risk elderly population [149].

When discussing the vaccine, along with the scientific and technological challenges, we must not forget one more important thing - once created, the vaccine must reach billions of people around the world, and as soon as possible. The cooperation and interaction between science and business, between countries and institutions, are needed to achieve this goal. An excellent example in this direction is the mechanism set up by Global Alliance for Vaccines and Immunization (GAVI), Coalition for Epidemic Preparedness Innovations (CEPI) and WHO (COVID-19 Vaccines Global Access - COVAX Facility) to raise funds to provide vaccines to residents in countries with different incomes, ensuring coordinated, rapid, transparent and equitable access for all of them. COVAX will maintain a buffer of doses of vaccines for emergency humanitarian use, including for dealing with severe outbreaks before they go out of control.

**Conclusions**

The COVID-19 pandemic has presented enormous medical, scientific, social, economic and moral challenges to the world. The new disease was announced at the very end of 2019, without anyone at that moment suspecting the trials that lie ahead for all of us. At the time of writing this paper (October 30th, 2020), there were 44 592 789 confirmed cases and 1 175 553 deaths from COVID-19 globally.

Over the past ten months, through the unprecedented efforts of medical doctors and scientists from all countries and various specialties, a huge (but still
far from sufficient) step has been made towards a better understanding of the biology and behaviour of the virus and the immune response against it, alongside with creating and improving diagnostic methods and treatment protocols. Clinical trials are underway with repurposed therapeutic agents (two of them, dexamethasone and remdesivir, have already been approved for the treatment of severe cases) and vaccine candidates (at least ten of which are already in the third phase of clinical trials). Experts have focussed their efforts on the development of specific new antiviral drugs; the genome of the specific virus is carefully monitored; the scientific community is looking for the factors that determine the susceptibility to a viral infection and its severity. Yes, the COVID-19 pandemic is a challenge in many ways, and the price paid by humanity is measured in pain, suffering and lost lives. But COVID-19 is also an opportunity for the triumph of the human spirit, professionalism and cooperation, of empathy and mercy; of everything that makes us people.

COVID-19 is neither the first nor the last pandemic. It is worth mentioning that the time intervals between the SARS, MERS and COVID-19 outbreaks are 10 and 7 years, respectively.

Moreover, in a situation like the present one, it is important for each of us and all of us as a society, as well as for governments and international organizations, to be able to learn from this experience. Importantly, to invest in medicine, science, education and new technologies; to rethink our attitude towards nature; to learn to discover the possibilities and solutions that lie in even the most significant problems.

Sharing knowledge and ideas is one of the essential steps towards overcoming the COVID-19 crisis.

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