ACTA MICROBIOLOGICA BULGARICA Volume 39 / 1 (2023)

Review

Various therapies against SARS-CoV-2

Aikaterini Valsamatzi-Panagiotou, Martina Traykovska, Georgi Y. Miloshev, Robert Penchovsky*

Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

Abstract

An outbreak of coronavirus SARS-CoV-2 infection in December 2019 in Wuhan, a province of China, has caused a worldwide pandemic that led to devastating effects on healthcare systems and the economy worldwide. The contagiousness of the infection and the consequences of the disease in everyday life highlighted the great need for a suitable treatment against coronavirus as soon as possible. Therefore, lots of scientists all around the world focused on the discovery of a proper therapy against the virus. The present article explains the structure of the virus, the pathophysiology of the infection with SARS-CoV-2, and various therapies against SARS-CoV-2. The first data that concern the effectiveness of vaccines from the countries that have already started mass vaccinations are positive. However, it is very early to conclude about the efficacy of vaccines in the population. The appearance of novel virus mutations raises concerns and forces some countries to impose further restrictions. The latest and the most contagious variant, known as Omicron, seems to decrease the global pandemic significantly. New SARS-CoV-2 therapies are suggested based on antisense technology.

Keywords: SARS-CoV-2, therapies, vaccines, genome organization of SARS-CoV-2

Резюме

Избухване на инфекция с коронавирус SARS-CoV-2 през декември 2019 г. в Ухан, провинция на Китай, предизвика световна пандемия, която доведе до опустошителни ефекти върху здравните системи и икономиката в световен мащаб. Заразността на инфекцията и последствията от болестта в ежедневието подчертаха голямата необходимост от създаване на подходящо лечение срещу коронавирус възможно най-скоро. Затова много учени по целия свят се фокусираха върху откриването на подходяща терапия срещу вируса. Настоящата статия обяснява структурата на вируса, патофизиологията на инфекцията със SARS-CoV-2 и различните терапии срещу SARS-CoV-2. Положителни са първите данни, които касаят ефективността на ваксините от страните, които вече са започнали масово ваксиниране. Въпреки това е много рано да се правят заключения относно ефикасността на ваксините при населението. Появата на нови вирусни мутации буди безпокойство и принуждава някои страни да наложат допълнителни ограничения. Най-новият и най-заразният вариант, известен като Омикрон, изглежда намалява значително глобалната пандемия. Предложени са нови терапии за SARS-CoV-2, базирани на антисенс технология.

Introduction

The first coronavirus cases, which were reported in China, presented with acute atypical severe respiratory disease. It was then discovered that the novel virus has a very high homology, about 80%, with another virus that concerned humanity from 2002 to 2003 and caused acute respiratory distress syndrome (ARDS) while its mortality rate was high, named SARS-CoV. The similarity of these two viruses leads to the designation of the novel virus as SARS-CoV-2. The original scenario of the virus transmission concerns a seafood market in Wuhan province. It is believed that the infection was at first zoonotic, and after that, due to some mutations, the virus started to spread between humans, which had

* Corresponding author: robert.penchovsky@hotmail.com

a significant effect on the outbreak. Bats were considered the natural host, pangolins were the intermediate host, and humans were the terminal host of the infection.

The sources and routes of infection and the susceptible host are significant for transmitting infectious diseases. Coronavirus can be transmitted through droplets, respiratory aspirates, aerosols, contacts, and feces (Harrison *et al*., 2020; Machhi *et al.,* 2020; Rabaan *et al.*, 2020). The early outbreak data of the infection in China revealed that the mean incubation period of the virus was 6.4 days and can vary from 0 to 24 days. The fundamental reproductive values (Ro) are significant for infectious diseases. They were calculated at the beginning of the spread of the virus to indicate how many people can be infected from a person who suffers from coronavirus infection. The results revealed that the values were between 2.5 and 3 (Wang *et al*., 2020; Yuki *et al.,* 2020). In contrast to SARS, people infected with the new coronavirus SARS-CoV-2 may also be contagious during the incubation period of the infection before the onset of the symptoms or asymptomatic during the whole period of the disease. That is the worst scenario because, during this period, they can spread the virus in society without even knowing that they are carriers, leading to a rapid increment of new coronavirus cases (Surveillances, 2020).

Overview of the structure and genomic organization of the virus

Coronaviruses (CoVs), the most significant known viruses, are part of the *Nidovirales,* including the *Coronoviridae*, *Arteriviridae,* and *Roniviridae*. The *Coronoviridae* family is comprised of two subfamilies, *Coronavirinae* and *Toronovirinae*. The two subfamilies are often mistaken due to their common morphological features. They have similar structural proteins, genome organization, replication, and transcription. Furthermore, the *Coronovirinae* have been divided into several subgroups: alpha, beta, gamma, and delta coronaviruses. All the viruses from *Nidovirales* are RNA viruses. The genetic information is encoded in their RNA genome. All the families from the *Nidovirales* have large RNA genomes, with *Coronovirinae* identified to have the largest genome of approximately 30 kilobases (kb). Single-stranded RNA viruses envelop coronaviruses. There is one significant difference in *Nidovirales* families regarding the number, size, and type of structural proteins. That causes alterations in the structural morphology of the nucleocapsids and virions.

As mentioned above, coronaviruses are enveloped in ssRNA viruses containing all the information that allows them to hijack the host cells and turns them into virus factories. The RNA genome contains a 5'-cap structure and a 3'-polyA tail. It is widely known that these structures are present in the mRNAs and protect them from hydrolytic degradation from the nuclease enzymes in the cell. These structures allow the RNA genome to act as mRNA translated into functional proteins, structural and non-structural. The 5'-UTR (untranslated region) contains stem-loops structures required for RNA replication and transcription. The 3'-UTR includes the facilities needed for the synthesis of the viral RNA. The whole genomic structure is 5'-UTR-leader sequence replicase-S (spike) gene-E (envelope) gene-M (membrane) gene-N (nucleocapsid) gene-3'-UTR poly-A tail (Fig. 1A). The non-structural proteins that have an essential role in viral RNA synthesis are replicase-transcriptase proteins. The translation of the genomic RNA mediates its expression. The replicase-transcriptase proteins are encoded in open-reading frame 1a (OFR1a) and ORF1b and are initially synthesized as two large proteins, pp1a and pp1ab. The synthesis of pp1ab involves programmed ribosomal frameshifting during translation ORF1a. During or after synthesis, these polyproteins are cleaved by virus proteinases into 16 proteins, nsp1 to nsp11 are encoded in ORF1a, and nsp12 to nsp16 are encoded in ORF1b.

The replicase-transcriptase and other viral proteins assemble into membrane-bound replication-transcription complexes (RTC). In the later period of infection, it appears that the NSP proteins encoded in ORF1b detach and diffuse to the cytosol. In addition to NSP proteins, RTC contains the viral N protein. Nsp1 to nsp16 have additional enzymatic activities besides being involved in the synthesis of viral RNA. During this synthesis, sub-genomic mRNAs are generated. Each contains a 5'-leader sequence corresponding to the 5'-end of the genome. This 5'-end leader is joined to an mRNA "body" representing sequences from the 3'-end poly(A) tail to a posit upstream of each genomic ORF encoding structural protein or NSP protein. Thus, in the genome are functional transcription-regulating sequence motifs (TRS) found in the 3'-end of the leader and in front of each ORF that will become the 5'-end in one subgenome-length mRNA. There are two main possibilities for generating subgenomic mRNAs: (1) discontinuous transcription occurs during the synthesis of minus-strand subgenome-length templates, and (2) the process of

discontinuous transcription resembles the mechanism of similarity-assisted or high-frequency copy-choice RNA recombination.

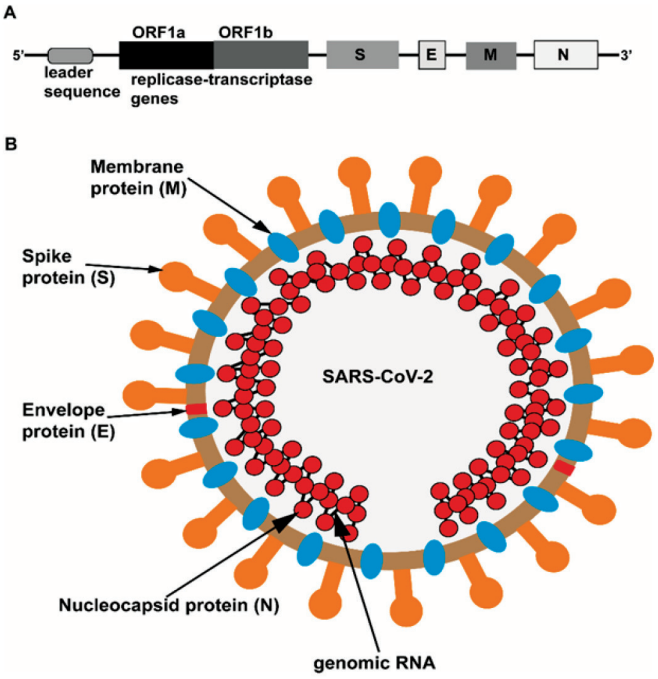

Fig. 1. Overview of the: (A) genomic structure of the viral RNA of SARS-CoV-2: near the 5'-end, there is a leader sequence, followed by the replicase-transcriptase genes that are replicated from two different ORFs (ORF1a and ORF1b), S gene, which encodes the S-spike protein, E gene, which encodes the E-envelope protein, M gene, which encodes the M-membrane protein, N gene, which encodes the N-nucleocapsid protein. (B) Structure of the SARS-CoV-2 virus. Inside the virus is the viral genomic RNA, which is covered by the nucleocapsid protein N. The viral envelope consists of the membrane protein M, envelope protein E, and spike protein S.

In terms of shape, SARS-CoV-2 is a spiky ball. The viral envelope consists of a lipid bilayer where the membrane (M), envelope (E), and spike (S) structural proteins are anchored. A nucleocapsid is formed from multiple copies of the nucleocapsid (N) protein inside the envelope, which binds to the positive-sense single-stranded RNA genome in continuous beads on a string-type conformation (Fig. 1).

The N protein constitutes the only protein in the nucleocapsid, comprising two separate domains, an N-terminal domain, and the C-terminal domain. Furthermore, there is evidence that this protein binds to specific RNA sequences, including the leader sequence, TRS sequence, and sequences located at the 3'-end of the viral genome. Thus, a scenario claims that the N protein acts as an RNA chaperone. That would be important for initiating minus-strand synthesis or template switching at the TRS element during discontinuous synthesis.

The S protein forms the spikes that emanate from the surface of the virion and mediates the attachment with the host cell receptor. The protein utilizes an N-terminal signal sequence to access the endoplasmatic reticulum (ER). After binding the S, the protein is cleaved into two polypeptides, S1 and S2, by furin-like protease. Thus, S1 makes the receptor-binding domain (RBD), while S2 forms the stalk of the spike molecule.

The second structural protein, the M protein, gives the shape to the virion with three transmembrane domains. Despite its co-translationally insertion into the ER membrane, it does not contain a signal sequence. The E protein facilities the assembly and release of the virus, and it contains an N-terminal ectodomain and C-terminal end domain and has ion channel activity. However, this protein also has other functions. For instance, the ion channel activity in SARS-CoV is not required for viral replication but for pathogenesis. The S protein mediates the initial attachment of the virion with the host cell. Thus, the S protein can be considered a "multifunctional molecular machine" that facilities the entry of the coronavirus into the host cell. In the attachment process, the RBD is activated, essentially a 'hook" that grips onto host cells (Weiss and Navas-Martin, 2005; Chen *et al.,* 2020; Kirtipal *et al.,* 2020; Machhi *et al.*, 2020; Phan, 2020; Wang *et al.*, 2020).

Pathophysiology of SARS-CoV-2

SARS-CoV-2 targets a receptor known as angiotensin-converting enzyme 2 (ACE-2) (Li *et al.,* 2003). ACE-2 receptor is a type I transmembrane metallocarboxypeptidase responsible for the breakdown of angiotensin II to its metabolite's angiotensin-(1-9) and angiotensin-(1-7). It is a renin-angiotensin system (RAS) component and works explicitly as a negative regulator. RAS is an effective body fluid regulation system composed of two-axis, critical for regulating blood pressure. ACE is involved in the cleavage of angiotensin I to angiotensin II, which manages to bind and activate AT1R responsible for blood vessels' constriction. The ACE-2 is responsible for the inactivation of angiotensin II and the generation of its metabolite angiotensin-(1-7), which acts as a vasodilator through activating the Mas receptor. ACE-2 is expressed in the vascular endothelial cells of the heart, kidneys, brain tissue, testis, gastrointestinal tract, type

II alveolar cells of the lungs, type I alveolar cells, bronchial epithelial cells, fibroblasts, endothelial cells, and macrophages. Thus, it seems to play an essential role in regulating blood pressure and heart function. SARS-CoV-2 uses the ACE-2 receptor as a cellular entry receptor. More specifically, the Spike protein (S) plays an essential role in the entry of the virus. As mentioned above, S-proteins are composed of two functional units, S1 and S2. S1 contains the RBD, which binds directly to ACE-2, while the function of S2 is the fusion with the host cell membrane (Gurwitz, 2020; Leung *et al.*, 2020; Machhi *et al.,* 2020; Zhang *et al.*, 2020).

After replicating and synthesizing the subgenomic RNAs, the structural proteins S, E and M are translated, and together with the viral genome, they form a mature virion. For that purpose, the structural proteins are inserted at first into the endoplasmic reticulum (ER), and subsequently, they move along the secretory pathway into the endoplasmic-Golgi intermediate compartment (ERGIC). In this compartment, viral genomes are encapsulated by the N protein bud into the membranes of the ERGIC containing viral structural proteins, resulting in the formation of mature virions. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis. Thus, the newly released virions are ready to infect new cells, while apoptosis is triggered in the cells replicating the viral RNA.

The progression of the infection with the virus can be divided into three distinct phases an early infection phase, a pulmonary phase, and a hyper inflammation phase. The progression of each phase may differ among the patients. The most severe cases usually present secondary organ involvement. Once a person gets infected with the virus, the virus infiltrates the lung parenchyma and starts to increase. In this phase, the initial response of the organism is activated, consequently the innate immunity. During this phase, the patients develop mild symptoms of the infection. The next phase involves collateral tissue injury and the inflammatory process, which leads to hypoxemia, pulmonary damage, and cardiovascular stress. Systemic inflammation involves secondary organs amplifying the inflammatory response, which can occur even with reduced viral load. That is the third phase of the infection, also called ‘’cytokine storm’’. In this phase, the patients may develop multiple organ failures. The critical inflammatory biomarkers such as interleukin (IL)-6, IL-2, IL-7, tumor necrosis factor (TNF)-α, interferon-γ inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP) 1-α, granulocyte-colony stimulating factor (G-CSF), C-reactive protein (CRP), procalcitonin (PCT), and ferritin are elevated. In addition, serum amyloid A (SAA), D-dimer, and creatine kinase (CK) may also be elevated. The elevation of those biomarkers is closely related to higher mortality. The laboratory examinations of the infected patients infected with the virus in the acute phase usually show lymphocytopenia.

There is a belief that particular HLA haplotypes are associated with distinct genetic predispositions and that HLA variability can be correlated with the incidence of COVID-19. Although, further research is needed to determine the exact role of HLA alleles in patients infected with SARS-CoV-2 (Akhmerov and Marban, 2020; Martelli *et al.,* 2020; Pourbagheri-Sigaroodi *et al*., 2020; Soy *et al*., 2020; Tavasolian *et al.* 2020; Zhang *et al*., 2020).

Therapeutics for SARS-CoV-2

There is no doubt that there are no specific antiviral drugs against SARS-CoV2. Meanwhile, many therapies have been used during the last months, and scientists are still trying to discover a proper treatment against the virus. The management of the infection is usually supportive and aims to the prevention of respiratory failure. The patients who present with hypoxia ($SatO_2$<94%) or symptoms of respiratory distress need oxygen therapy. If there is no improvement and acute lung injury, mechanical ventilation and intubation are necessary. Several drugs used against other medical diseases were tested to treat patients infected with coronavirus or improve their clinical picture during the pandemic. The field of polypharmacology gained attention due to the need to use several drugs simultaneously against SARS-CoV-2. The use of polypharmacology has several advantages, as the modulation of multiple biological targets simultaneously plays an essential role in efficacy, decreases the possibilities of resistance development, and improves the safety profile.

There is no evidence that antibacterial drugs as prophylaxis in patients positive for the virus have a protective effect on bacterial superinfections. Although an antibiotic from the category of macrolides, azithromycin was used in patients suffering from an infection with coronavirus and seemed to reduce the viral load and have anti-inflammatory and immunomodulatory effects. Another drug used against malaria and amoebiasis is named Chloroquine/hydroxychloroquine. At the beginning of the

pandemic, it was believed to have a good tolerability profile and be effective in patients with COVID-19. Indeed, combining hydroxychloroquine with azithromycin may cause side effects like prolonged QTc interval and elevation of liver enzymes. Therefore, the use of both drugs is not suggested anymore against coronavirus infection.

In September 2020, the WHO published a living guide for corticosteroids. The illness severity is divided into three categories critical, severe, and non-severe. Essential includes those patients who present with ARDS, septic shock, or other conditions that need further management, such as mechanical ventilation or vasopressor therapy. Severe consists of patients who present with hypoxia increased respiratory rate, or signs of severe respiratory distress. Non-severe are considered those patients who do not have any manifestations included in the categories mentioned above.

According to the guidance, the systemic use of corticosteroids is recommended to treat patients with severe and critical infections. In contrast, it is not recommended to treat patients with non-severe diseases. Evidence shows their use is related to an essential reduction in the mortality rate in severe and critical infections. The corticosteroid regimens used in the trials were methylprednisolone, dexamethasone, and hydrocortisone. Due to the high risk of ischaemic events and disseminated intravascular coagulation (DIC) at the early stage of the infection, and in case there is a 4-fold elevation of the value of D-dimer, the use of anticoagulation therapy is recommended.

The antiviral drugs used against COVID-19 and effective are Remdesivir and second-generation antiretroviral drugs Lopinavir/Ritonavir. The combination of antiretroviral medications seems to decrease the viral load. Most patients treated with antiretroviral drugs presented common side effects like nausea, diarrhea, and insomnia. Remdesivir may also cause elevated liver enzymes, prothrombin time, and blood sugar.

Another antiviral drug that seems to be more effective in comparison to Remdesivir is called Plitidepsin. It acts by inhibiting the host protein eEF1A of SARS-CoV-2. The drug has completed the Phase I/II clinical study against coronavirus and continues to Phase II/III. The *in vivo* efficacy and the cytotoxicity of Plitidepsin were tested in mice. The results are positive, which means that Plitidepsin may constitute a promising therapeutic agent against the virus.

A monoclonal antibody against IL-6, Tocilizumab, was also used successfully in Wuhan patients infected with the virus. In November 2020, the FDA authorized another monoclonal antibody from Eli Lilly named bamlanivimab for patients with a higher risk of manifesting more severe symptoms from infection with coronavirus. The first data are very positive, and its use seems to decrease the days of hospitalization and severe manifestation of the infection. However, safety and effectiveness need to be continuously evaluated. REGN-COV2 antibody combination (casirivimab/imdevimab) is also used in the fight against COVID-19. It is a cocktail which is consisted of two monoclonal antibodies. Using REGN-COV2 seems to decrease the viral load in symptomatic patients with high viral load and patients whose immune response was not initiated yet. These two antibodies act by targeting non-overlapping epitopes on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (Administration, 2020; Alina Baum, 2020; Bellera *et al.*, 2020; Cavalcanti *et al*., 2020; Chaudhari *et al*., 2020; Damle *et al.*, 2020; Hanff *et al.*, 2020; Organization, 2020; Pascarella *et al*., 2020; Singh *et al*., 2020; Sivapalan *et al*., 2020; Weinreich, 2021; White *et al.*, 2021).

Colchicine, administered in various cardiovascular conditions such as acute pericarditis and gout, was tested in the clinical course of patients with COVID-19 as a possible treatment (Deftereos *et al*., 2020; Della-Torre *et al.*, 2020). A study named COLCORONA was in progress to use Colchicine in non-hospitalized patients infected with the virus. In January 2021, it was announced by the Montreal Heart Institute found that there were positive results. It seems that the use of Colchicine in patients who fulfill some criteria reduces the mortality rate and the possibility of hospitalization, so Colchicine plays a significant role in the current therapeutic approaches against the virus (Jean-Claude Tardif, 2021).

Metformin is a drug mainly used for the treatment of diabetes mellitus. The pleiotropic effects of metformin have led to its widespread utility in medicine. Although it was discovered as a drug against influenza nowadays, it is widely used in patients who suffer from diabetes mellitus. The drug's mechanism of action involves decreasing the glucose levels in the organism, and at the same time, its use seems to have favorable effects on lipid metabolism. The use of metformin in patients suffering from COVID-19 may offer cardiopulmonary protection. The actual mechanism of metformin‘s action that may also seem effective

against COVID-19 involves activating AMPK, which phosphorylates ACE-2 on Ser-680. That post-translational modification of ACE-2 decreases its ubiquitination and leads to the extension of the half-life of ACE-2, which seems protective for the lungs. Metformin may be considered a promising agent against SARS-CoV2. It appears to prevent the virus's entry and the detrimental sequelae. There is a hypothesis that patients with metformin have higher circulating ACE-2 levels and lower mortality and morbidity than those who do not take metformin. Although there is already evidence that some drugs are effective against Covid-19, more research should be done in this field, and the benefit-risk should be calculated before the massive use of a therapy (Malhotra *et al*., 2020; Sharma *et al*., 2020).

Convalescent plasma (CP) therapy is also used in combination with other medications in those patients who suffer from a severe form of the infection. CP has been used since the 20 century, and it was tried successfully in other coronaviruses and the H1N1 pandemic with satisfactory efficacy and safety. It is a passive immunization strategy in which the plasma of patients recovered from the virus is used. ABO type of the convalescent plasma transfused should be compatible with the patient's ABO type. Some studies show that a dose of CP reduces the viral load and improves clinical outcomes. Although, it is not clear if the CP has benefits in treating patients. We should remember that plasma transfusions can result in adverse events. More research should be done to evaluate the potential antiviral and immunomodulatory effects of CP in COVID-19 and the benefits and safety of this type of therapy (Duan *et al.*, 2020; Li *et al*., 2020; Piechotta *et al*., 2020; Rojas *et al*., 2020).

A novel therapy was tested in animals and, more specifically, in mice and seems very promising. It concerns a high-affinity human antibody domain. More specifically, VH ab8 is a heavy chain variable domain that interferes directly with the ACE-2 binding to RBD and is involved in the potent neutralization of SARS-CoV-2. It seems to exhibit prophylactic as well as therapeutic efficacy in mice, which is very positive because the possibility of the effectiveness of the therapy in humans is higher when the experiments in the phase of research are done *in vivo* in comparison to the results of the experiments which are done *in vitro* (Li *et al*., 2020).

Alternative approaches

What if we could control the gene expression of the S and the other structural proteins and the replicase gene responsible for replicating the viral RNA? With the outbreak of SARS-CoV in 2003, Japanese scientists turned their attention to using RNA as a target to fight against the virus. The invention was based on the use of RNA interference (RNAi) molecules or double-stranded RNA (dsRNA) as therapeutic agents against SARS in humans (Tao *et al*., 2005). Preferably, the RNAi molecules target the replicase region of the SARS virus. RNA interference decreases or inhibits the expression of nucleic acids against which the RNAi is directed (Spurgers *et al*., 2008). RNAi is also called post-translational gene silencing. Since the only RNA molecules usually found in the cytoplasm of a cell are molecules of mRNA, the cell has enzymes that recognize and cut double-stranded RNA (dsRNA) into fragments leading to the lack of protein synthesis. Their approach has been successful as they manage to inhibit the replication of the SARS virus isolated from a patient sample (Tan *et al.*, 2004; Ma *et al*., 2007).

Novel technologies for drug development are urgently needed. However, the main obstacles to discovering and developing novel drugs are the research and clinical trials cost, the risk of developing something new, and the time needed to create new medicines (Aghila Rani *et al*., 2020). Some novel agents which can be used against SARS-CoV-2 are called antisense oligonucleotides (ASOs). ASOs are short single-stranded nucleic acids with an optimal length of 20-25 nucleotides. They should attach a carrier that may be a cell-penetrating peptide (CPPs) or a lipoprotein transferred to a specific preselected target (Traykovska *et al*., 2021; Traykovska and Penchovsky, 2022). They hybridize complementary to the target, and as a result, they manage to regulate the expression of genes. There are ASOs already approved on the market against neurodegenerative diseases and atherosclerosis. Indeed they can be used effectively against bacterial and viral infections. Their mechanism of action is very well understood, and in case of antibiotic resistance or new mutations, they can be redesigned and reused. As we already described, the genome of the COVID-19 virus is ssRNA, in which all the essential genes are located in the 5'-end of the RNA, which is transcribed into mRNAs. By finding the nucleotide sequence of these genes, which is done by specialized software programs, we can design ASOs to bind with the complementary sequence of the targeted mRNA specifically. Suppose we target the 5'-end of the mRNA responsible for synthesizing the replicase-transcriptase genes. In that

case, we could inhibit the translation and synthesis of the essential proteins involved in replicating the genomic RNA. These advantages are significant and render them an up-and-coming therapeutic agent for the future against various bacterial and viral infections (Spurgers *et al*., 2008; Penchovsky and Traykovska, 2015; Penchovsky, 2019; Valsamatzi-Panagiotou, 2020; Penchovsky and Popova, 2021) (Fig. 2).

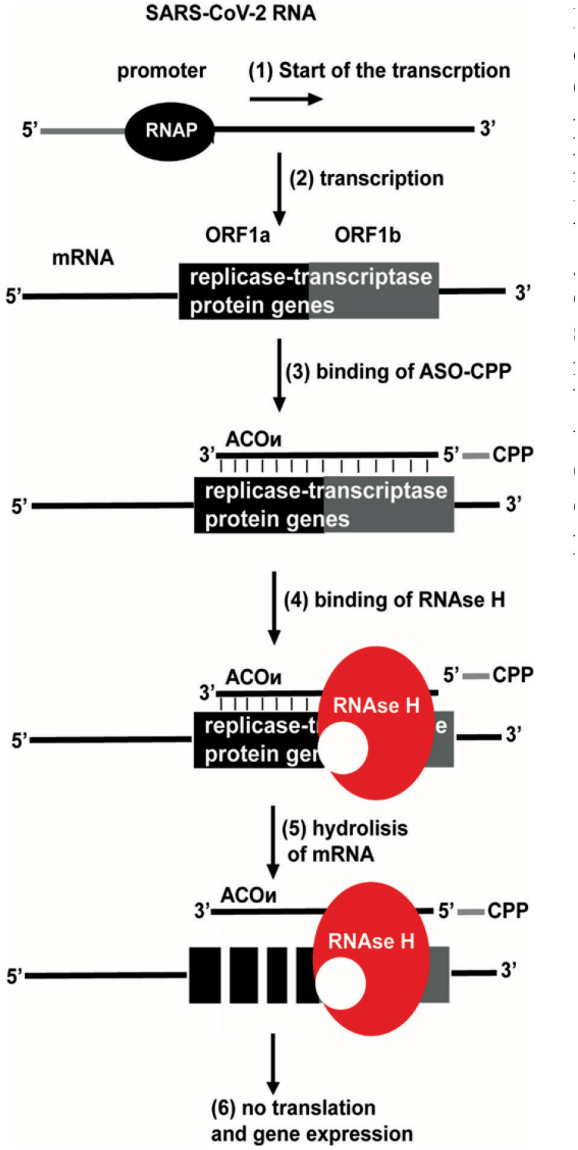

Fig. 2. Applying the antisense technology to control the gene expression of the SARS-CoV-2 genomic RNA. (1) The RNA polymerase enzyme (RNAP) binds with the promoter of the SARS-CoV-2 genomic RNA. (2) After binding, RNAP triggers the transcription of the replicase-transcriptase genes from ORF1a and ORG1b. (3) ASO enters the cell, binds with the target sequence, and forms a double-stranded molecule. (4) RNAse H recognizes and binds with the double-stranded molecule, which triggers the target mRNA's hydrolysis (5). That leads to no translation and gene expression of the essential transcriptase proteins.

Research about the correlation between sunlight and the mortality rate of people infected with SARS-CoV-2 was conducted in Edinburgh. The results indicated that people living in areas with high UVA rays had a lower mortality risk than those with lower levels of UVA. The researchers believe this is attributed to the release of nitric oxide after sunlight exposure, which seems to reduce the ability of the virus to replicate. These are very positive results, although further research is needed to be done to be used as a method to decrease the mortality rate (Cherrie *et al*., 2021).

Discussion

An effective vaccine or medication is the only possibility to return to pre-COVID-19 societal behavior. Global cooperation and teamwork are very significant in the fight against the virus. Mass vaccinations have already started, although the appearance of new variants raises concerns about the efficacy of the vaccines against them. The immunization of the population results in the production of antibodies against the spike protein of the virus. Although some of the mutations which came up concern the spike protein as the mutations in RBD. That is the main reason why the effectiveness of vaccines may be reduced against these new variants. More specifically, the South African variant seems to be the one that threatens the vaccine's efficacy. It is generally believed that vaccination is more efficient than adaptive immunity due to the high titers of antibodies. The vaccines work based on protective antibody responses, neutralizing antibodies as the most common mechanism of action, although there is an additional elicitation of CD4+ or CD8+ T cells. Nevertheless, the antibodies produced because of vaccination act against only one protein, the spike protein, the leading region where the novel mutations appeared.

On the other hand, when a person has been infected with the virus, the adaptive immunity (humoral and cellular) is activated, which consists of B cells (antibody-producing cells), CD4+ T cells (helper T cells), and CD8+ T cells (cytotoxic, or killer, T cells) which are very important for the protection against viral infections. Therefore, there is a great need to develop poly mRNA vaccines with different antigens to produce more antibodies. In addition, it is believed that using poly mRNA vaccines will reduce the appearance of mutations.

Conclusion

There is no specific drug that is effective against coronavirus. The treatment of patients who

suffer a severe infection includes mechanical ventilation and a combination of medications. Although mass vaccinations have already started, the most successful ways to protect ourselves from the disease are social distancing, isolation, lockdowns, and PPE. That is because many of the population should be vaccinated to achieve herd immunity. Many drugs were tested and used as possible treatments against coronavirus, and some seem to play an essential role in reducing mortality. The use of vaccines massively is promising for the future. However, the appearance of new variants of the virus, which seem more contagious, raises conflicts about the efficacy of some vaccines against them. The new-sequencing technologies are powerful diagnostic tools for detecting new variants. The first data available from countries that have already managed to vaccinate a substantial amount of the population is very positive, which raises hope that the quality of life will improve. Globally, as of June 10th, 2022, there have been 532,201,219 confirmed cases of COVID-19, including 6,305,358 deaths, reported to WHO. As of June 6th, 2022, 11,854,673,610 vaccine doses have been administered (https://covid19.who.int/). However, COVID-19 spread by less than 10% compared to its peak worldwide due to vaccination and the Omicron variant, which seems to decrease the global pandemic significantly. However, we must stay vigilant and develop novel drugs against SARS-CoV-2 that are easily adaptive to its mutations, such as ASOs.

Acknowledgments

This work was financially supported by Bulgarian National Science Fund under Grant No. KP-06-H63/1/13.12.2022 and partially by the Operational Program "Science and Education for Smart Growth" 2014-2020, co-funded by the European Union through the European structural and investment funds: Project BG-05M2OP001-1.002-0019 "Clean technologies for a sustainable environment–water, waste, energy for a circular economy" (Clean&Circle CoC) by funding of the expert's labor.

References

Administration, F. a. D. (2020). Coronavirus (COVID-19) Update: FDA Authorizes monoclonal antibody for treatment of COVID-19.

Akhmerov, A., E. Marban (2020). COVID-19 and the heart. *Circ. Res.* **126**: 1443-1455.

Baum, A., D. Ajithdoss, R. Copin, A. Zhou, K. Lanza, N. Negron *et al*. (2020). REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**: 1110-1115.

Bellera, C. L., M. Llanos, M. E. Gantner, S. Rodriguez, L. Gavernet, M. Comini, A. Talevi (2020). Can drug repurposing strategies be the solution to the COVID-19 crisis? *Expert Opin. Drug Discov.* **16**: 605-612.

Cavalcanti, A. B., F. G. Zampieri, R. G. Rosa, L. C. P. Azevedo, V. C. Veiga *et al*. (2020). Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N. Engl. J. Med.* **383**: 2041-2052.

Chaudhari, R., L. W. Fong, Z. Tan, B. Huang, S. Zhang (2020). An up-to-date overview of computational polypharmacology in modern drug discovery. *Expert Opin. Drug Discov.* **15**: 1025-1044.

Cherrie, M., T. Clemens, C. Colandrea, Z. Feng, D. J. Webb, R.B. Weller, C. Dibben (2021). Ultraviolet a radiation and COVID-19 deaths in the USA with replication studies in England and Italy. *BJD* **185**: 363-370.

Damle, B., M. Vourvahis, E. Wang, J. Leaney, B. Corrigan (2020). Clinical pharmacology perspectives on the antiviral activity of azithromycin and use in COVID-19. *Clin. Pharmacol. Ther.* **108**: 201-211.

Deftereos, S. G., G. Siasos, G. Giannopoulos, D. A. Vrachatis, C. Angelidis *et al*. (2020). The Greek study in the effects of colchicine in COvid-19 complications prevention (GRECCO-19 study): Rationale and study design. *Hellenic J. Cardiol.* **61**: 42-45.

Della-Torre, E., F. Della-Torre, M. Kusanovic, R. Scotti, G. A. Ramirez, L. Dagna, M. Tresoldi (2020). Treating COVID-19 with colchicine in community healthcare setting. *Clin. Immunol.* **217**: 108490.

Duan, K., B. Liu, C. Li, H. Zhang, T. Yu, J. Qu *et al*. (2020). Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc. Natl. Acad. Sci. USA* **117**: 9490-9496.

Gurwitz, D. (2020). Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev. Res.* **81**: 537-540.

Hanff, T. C., A. M. Mohareb, J. Giri, J. B. Cohen, J. A. Chirinos (2020). Thrombosis in COVID-19. *Am. J. Hematol.* **95**: 1578-1589.

Harrison, A. G., T. Lin, P. Wang (2020). Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends Immunol.* **41**: 1100-1115.

Jean-Claude Tardif, N. B., Ph. L. L'Allier, D. Gaudet, B. Shah, M. H. Pillinger *et al*. (2021). Efficacy of colchicine in non-hospitalized patients with COVID-19. *medRxiv 22*.

Li, L., W. Zhang, Y. Hu, X. Tong, S. Zheng, J. Yang *et al*. (2020). Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: A randomized clinical trial. *JAMA* **324**: 460-470.

Li, W., M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne *et al.* (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**: 450-454.

Li, W., A. Schäfer, S. S. Kulkarni, X. Liu, D. R. Martinez, C. Chen *et al*. (2020). High potency of a bivalent human VH domain in SARS-CoV-2 animal models. *Cell* **183**: 429-441.

Ma, Y., C.-Y. Chan, M.-L. He (2007). RNA interference and antiviral therapy. *World J. Gastroenterol: WJG* **13**: 5169.

Machhi, J., J. Herskovitz, A. M. Senan, D. Dutta, B. Nath, M. D. Oleynikov *et al*. (2020). The natural history, pathobiology, and clinical manifestations of SARS-CoV-2 infections. *J. Neuroimmune. Pharmacol.* **15**: 359-386.

Malhotra, A., M. Hepokoski, K. C. McCowen, Y. J. S. J

(2020). ACE2, Metformin, and COVID-19. *iScience* **23**: 101425.

Martelli, A., V. Citi, V. Calderone (2020). Recent efforts in drug discovery on vascular inflammation and consequent atherosclerosis. *Expert Opin. Drug Discov.* **16**: 411–427.

Organization, W. H. (2020). Corticosteroids for COVID-19. 25.

Pascarella, G., A. Strumia, C. Piliego, F. Bruno, R. Del Buono, F. Costa, S. Scarlata, F. E. Agro (2020). COVID-19 diagnosis and management: a comprehensive review. *J. Intern. Med.* **288**: 192-206.

Penchovsky, R., K. B. Popova, A. V. Panagiotou (2021). New drug discovery strategies for targeting drug resistant bacteria. *Environ. Chem. Lett.* 19, pp. 1995–2004.

Penchovsky, R., M. Traykovska (2015). Designing drugs that overcome antibacterial resistance: where do we stand and what should we do? *Expert Opin. Drug Discov.* **10**: 631-650.

Phan, T. (2020). Genetic diversity and evolution of SARS-CoV-2. *Infect. Genet. Evol.* **81**: 104260.

Pourbagheri-Sigaroodi, A., D. Bashash, F. Fateh, H. Abolghasemi (2020). Laboratory findings in COVID-19 diagnosis and prognosis. *Clin. Chim. Acta.* **510**: 475-482.

Rabaan, A. A., S. H. Al-Ahmed, S. Haque, R. Sah, R. Tiwari, Y. S. Malik *et al*. (2020). SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview. *Infez. Med.* **28**: 174-184.

Rani, K. G. A, M. A. Hamad, D. M. Zaher, S. M. Sieburth, N. Madani, T. H. Al-Tel (2020). Drug development post COVID-19 pandemic: toward a better system to meet current and future global health challenges. *Expert Opin. Drug Discov.***16**: 365-371.

Rojas, M., Y. Rodriguez, D. M. Monsalve, Y. Acosta-Ampudia, B. Camacho, J. E. Gallo *et al*. (2020). Convalescent plasma in Covid-19: Possible mechanisms of action. *Autoimmun. Rev.* **19**: 102554.

Sharma, S., A. Ray, B. Sadasivam (2020). Metformin in COVID-19: A possible role beyond diabetes. *Diabetes Res. Clin. Pract*. **164**: 108183.

Singh, A. K., A. Singh, R. Singh, A. Misra (2020). Remdesivir in COVID-19: A critical review of pharmacology, pre-clinical and clinical studies. *Diabetes Metab. Syndr.* **14**: 641-648.

Sivapalan, P., C. S. Ulrik, R. D. Bojesen, T. S. Lapperre, J. V. Eklof, K. E. J. Hakansson *et al*. (2020). Proactive prophylaxis with azithromycin and hydroxychloroquine in hospitalised patients with COVID-19 (ProPAC-COVID): A structured summary of a study protocol for a randomised controlled trial. *Trials* **21**: 513.

Soy, M., G. Keser, P. Atagunduz, F. Tabak, I. Atagunduz, S. Kayhan (2020). Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin. Rheumatol.* **39**: 2085-2094.

Spurgers, K. B., C. M. Sharkey, K. L. Warfield, S. Bavari (2008). Oligonucleotide antiviral therapeutics: antisense and RNA interference for highly pathogenic RNA viruses. *Antiviral Res.* **78**: 26-36.

Surveillances, V. (2020). The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) - China, 2020. *China CDC Weekly* **2**: 113-122.

Tan, E. L., E. E. Ooi, C.-Y. Lin, H. C. Tan, A. E. Ling, B. Lim, L. W. Stanton (2004). Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. *Emerg. Infect. Dis.* **10**: 581.

Tao, P., J. Zhang, N. Tang, B.-q. Zhang, T.-C. He, A.-l. Huang (2005). Potent and specific inhibition of SARS-CoV antigen expression by RNA interference. *Chin. Med. J.* (Engl) **118**: 714-719.

Tavasolian, F., M. Rashidi, G. R. Hatam, M. Jeddi, A. Z. Hosseini, S. H. Mosawi, E. Abdollahi, R. D. Inman (2020). HLA, immune response, and susceptibility to COVID-19. *Front. Immunol.* **11**: 601886.

Traykovska, M., R. Penchovsky (2022). Engineering Antisense Oligonucleotides as Antibacterial Agents That Target FMN Riboswitches and Inhibit the Growth of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. *ACS Synth. Biol.* **11**: 1845–1855

Traykovska, M., K. B. Popova, R. Penchovsky (2021). Targeting glmS Ribozyme with Chimeric Antisense Oligonucleotides for Antibacterial Drug Development. *ACS Synth. Biol.* **10**: 3167-3176.

Valsamatzi-Panagiotou, A., K. Popova, R. Penchovsky (2020). Drug Discovery for Targeting Drug-Resistant Bacteria. *Sustain. Agric. Rev. 46.* C. Springer. **46**: 205-228.

Wang, F., R. M. Kream, G. B. Stefano (2020). An Evidence Based Perspective on mRNA-SARS-CoV-2 Vaccine Development. *Med. Sci. Monit.* **26**: e924700.

Weinreich, D. M., S. Sivapalasingam, T. Norton, S. Ali, D. Pharm, H. Gao *et al*. (2021). REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. NEJM **384**: 238-251.

Weiss, S. R., S. Navas-Martin (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol. Mol. Biol. Rev.* **69**: 635-664.

White, K. M., R. Rosales, S. Yildiz, T. Kehrer, L. Miorin, E. Moreno *et al*. (2021). Plitidepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A. *Science* **371**: 926-931.

Yuki, K., M. Fujiogi, S. Koutsogiannaki (2020). COVID-19 pathophysiology: A review. *Clin. Immunol.* **215**: 108427.

Zhang, J. J., X. Dong, Y. Y. Cao, Y. D. Yuan, Y. B. Yang, Y. Q. Yan, C. A. Akdis, Y. D. Gao (2020). Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* **75**: 1730-1741.

Zhang, X., S. Li, S. Niu (2020). ACE2 and COVID-19 and the resulting ARDS. *Postgrad. Med. J.* **96**: 403-407.