REVIEWS

REVIEW ARTICLE
DOI: https://doi.org/10.36233/0507-4088-61

Potential of application of the RNA interference phenomenon in the treatment of new coronavirus infection COVID-19

Evgeny A. Pashkov[1,2], Ekaterina R. Korchevaya[2], Evgeny B. Faizuloev[2], Oxana A. Svitich[1,2], Evgeny P. Pashkov[1], Dmitry N. Nechaev[1], Vitaliy V. Zverev[1,2]

[1]FSAEI HE I.M. Sechenov First Moscow State Medical University (Sechenov University) of the Ministry of the Health of Russia, 119991, Moscow, Russia;
[2]FSBRI «I.I. Mechnikov Research Institute of Vaccines and Sera», 105064, Moscow, Russia

COVID-19 has killed more than 4 million people to date and is the most significant global health problem. The first recorded case of COVID-19 had been noted in Wuhan, China in December 2019, and already on March 11, 2020, World Health Organization declared a pandemic due to the rapid spread of this infection. In addition to the damage to the respiratory system, SARS-CoV-2 is capable of causing severe complications that can affect almost all organ systems. Due to the insufficient effectiveness of the COVID-19 therapy, there is an urgent need to develop effective specific medicines. Among the known approaches to the creation of antiviral drugs, a very promising direction is the development of drugs whose action is mediated by the mechanism of RNA interference (RNAi). A small interfering RNA (siRNA) molecule suppresses the expression of a target gene in this regulatory pathway. The phenomenon of RNAi makes it possible to quickly create a whole series of highly effective antiviral drugs, if the matrix RNA (mRNA) sequence of the target viral protein is known. This review examines the possibility of clinical application of siRNAs aimed at suppressing reproduction of the SARS-CoV-2, taking into account the experience of similar studies using SARS-CoV and MERS-CoV infection models. It is important to remember that the effectiveness of siRNA molecules targeting viral genes may decrease due to the formation of viral resistance. In this regard, the design of siRNAs targeting the cellular factors necessary for the reproduction of SARS-CoV-2 deserves special attention.

Key words: *SARS-CoV-2 coronavirus; RNA interference; small interfering RNAs; gene knockdown*

For citation: Pashkov E.A., Korchevaya E.R., Faizuloev E.B., Svitich O.A., Pashkov E.P., Nechaev D.N., Zverev V.V. Potential of application of the RNA interference phenomenon in the treatment of new coronavirus infection COVID-19. *Problems of Virology* (*Voprosy Virusologii*). 2021; 66(4): 241-251.
DOI: https://doi.org/10.36233/0507-4088-61

For correspondence: Evgeny A. Pashkov, Junior Researcher of the Molecular Immunology Laboratory, FSBRI «I.I. Mechnikov Research Institute of Vaccines and Sera», 105064, Moscow, Russia; Postgraduate Student of the Department of Microbiology, Virology, and Immunology, FSAEI HE I.M. Sechenov First Moscow State Medical University (Sechenov University) of the Ministry of the Health of Russia, 119991, Moscow, Russia.
E-mail: pashckov.j@yandex.ru

Information about the authors:
Pashkov E.A., https://orcid.org/0000-0002-5682-4581
Korchevaya E.R., https://orcid.org/0000-0002-6417-3301
Faizuloev E.B., https://orcid.org/0000-0001-7385-5083
Svitich O.A., https://orcid.org/0000-0003-1757-8389
Pashkov E.P., https://orcid.org/0000-0002-2581-273X
Nechaev D.N., https://orcid.org/0000-0002-7592-3809
Zverev V.V., https://orcid.org/0000-0002-0017-1892

Contribution: Pashkov E.A. – writing of the text, conclusion; Korchevaya E.R. – writing of the text, conclusion; Faizuloev E.B. – collection and processing of the materials, writing of the text, conclusion; Svitich O.A. – resume, general edition, scientific editing; Pashkov E.P. – collection and processing of the materials, resume, general edition; Nechaev D.N. – collection and processing of the materials; Zverev V.V. – resume, general edition, scientific editing.

Funding. The research was funded by the State budget.
Conflict of interest. The authors declare no conflict of interest.

Received 19 May 2021
Accepted 14 July 2021
Published 31 August 2021

PROBLEMS OF VIROLOGY (VOPROSY VIRUSOLOGII). 2021; 66(4)
DOI: https://doi.org/10.36233/0507-4088-61
REVIEWS

НАУЧНЫЙ ОБЗОР

DOI: https://doi.org/10.36233/0507-4088-61

Потенциал применения явления РНК-интерференции в терапии новой коронавирусной инфекции COVID-19

Пашков Е.А.[1,2], Корчевая Е.Р.[2], Файзулоев Е.Б.[2], Свитич О.А.[1,2], Пашков Е.П.[1], Нечаев Д.Н.[1], Зверев В.В.[1,2]

[1]ФГАОУ ВО Первый Московский государственный медицинский университет им. И.М. Сеченова Минздрава России (Сеченовский Университет), 119991, Москва, Россия;
[2]ФГБНУ «Научно-исследовательский институт вакцин и сывороток им. И.И. Мечникова», 105064, Москва, Россия

Новая коронавирусная инфекция на сегодняшний день привела к гибели свыше 4 млн человек и представляет собой наиболее значимую проблему мирового здравоохранения. Первый зафиксированный случай COVID-19 отмечен в Китайской Народной Республике (КНР) (г. Ухань) в декабре 2019 г., а уже 11 марта 2020 г. Всемирная организация здравоохранения (ВОЗ) объявила пандемию в связи с быстрым распространением этой инфекции. Помимо поражения органов дыхания её возбудитель SARS-CoV-2 способен вызывать тяжёлые осложнения, которые могут затронуть практически все системы организма. В связи с недостаточной эффективностью терапии COVID-19 сохраняется острая необходимость в разработке эффективных специфических лекарственных средств. Среди известных подходов к созданию противовирусных препаратов весьма перспективным направлением является получение соединений, действие которых опосредовано механизмом РНК-интерференции. РНК-интерференция – регуляторный путь, при котором молекула малой интерферирующей РНК (миРНК; small interfering RNA, siRNA) подавляет экспрессию гена-мишени. Это явление позволяет быстро создать целую серию высокоэффективных противовирусных веществ при условии, что известна только последовательность матричной РНК (мРНК, mRNA) целевого вирусного белка. В настоящем обзоре рассматривается возможность клинического применения миРНК, направленных на подавление репродукции нового коронавируса SARS-CoV-2, с учётом опыта подобных исследований на моделях инфицирования SARS-CoV и MERS-CoV. Важно помнить, что эффективность использования молекул миРНК, нацеленных на вирусные гены, может снизиться из-за формирования к ним устойчивости патогена. В связи с этим особого внимания заслуживает дизайн миРНК, нацеленных на клеточные факторы, необходимые для репродукции SARS-CoV-2.

Ключевые слова: *коронавирус SARS-CoV-2; РНК-интерференция; малые интерферирующие РНК; нокдаун гена*

Для цитирования: Пашков Е.А., Корчевая Е.Р., Файзулоев Е.Б., Свитич О.А., Пашков Е.П., Нечаев Д.Н., Зверев В.В. Потенциал применения явления РНК-интерференции в терапии новой коронавирусной инфекции COVID-19. *Вопросы вирусологии*. 2021; 66(4): 241-251. DOI: https://doi.org/10.36233/0507-4088-61

Для корреспонденции: Пашков Евгений Алексеевич, младший научный сотрудник лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт вакцин и сывороток им. И.И. Мечникова», 105064, Москва, Россия; аспирант кафедры микробиологии, вирусологии и иммунологии ФГАОУ ВО Первый Московский государственный медицинский университет им. И.М. Сеченова Минздрава России (Сеченовский Университет), 119991, Москва, Россия. E-mail: pashckov.j@yandex.ru

Участие авторов: Пашков Е.А. – написание текста, заключение; Корчевая Е.Р. – написание текста, заключение; Файзулоев Е.Б. – сбор и обработка материалов, написание текста, заключение; Свитич О.А. – резюме, общая редакция, научное редактирование; Пашков Е.П. – сбор и обработка материалов, резюме, общая редакция; Нечаев Д.Н. – сбор и обработка материалов; Зверев В.В. – резюме, общая редакция, научное редактирование.

Финансирование. Исследование выполнено за счет Государственного бюджета.
Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Поступила 19.05.2021
Принята к печати 14.07.2021
Опубликована 31.08.2021

Introduction

To date, the novel coronavirus infection (COVID-19) has claimed the lives of more than 4 million people, remaining the most pressing global health challenge [1]. SARS-CoV-2, the coronavirus that causes severe infectious disease COVID-19, belongs to the species *Severe acute respiratory syndrome-related coronavirus* of the genus *Betacoronavirus* of the family *Coronaviridae* [2]. The first case of COVID-19 was reported in Wuhan (People's Republic of China (PRC)) in December 2019, and on March 11, 2020, the World Health Organization (WHO) declared a global pandemic, as the infection rapidly

spread across the world [3]. The novel coronavirus primarily affects the respiratory tract, though it can cause complications involving not only the respiratory system, but also the cardiovascular, central nervous, and urinary systems. COVID-19 can progress to acute heart, renal, and respiratory failure, cardiomyopathy, cardiac arrhythmia, septic shock, cytokine storm; therefore, it poses particular risk for people with diabetes, chronic lung and kidney diseases, underlying cardiovascular diseases, and immunodeficiency disorders [4–11]. A number of studies show that in addition to standard respiratory manifestations of COVID-19, both adults and children are at high risk of developing multisystem inflammatory syndrome (MIS-A and MIS-C) resulting in gastrointestinal disorders, cardiac involvement, and shock syndrome [12–14].

The specific therapy for COVID-19 is difficult to find, as the recommended therapeutic agents are either insufficiently effective or their efficacy has not been definitively proven. It has been found that the lopinavir–ritonavir treatment had little or no effect on patients with COVID-19; the number of fatal cases among the patients receiving the above treatment was equal to the number of fatal cases among patients in the control group with standard care [15]. In their study, Joshi S. et al. point out that the condition of patients with mild to moderate COVID-19 improved when favipiravir treatment was administered; however, large randomized trials are required to demonstrate its clinical benefits such as shortening the disease course, early hospital discharge, and reducing the need for oxygen [16]. Another study addressing the effect of hydroxychloroquine on the progression of novel coronavirus infection demonstrated that administration of this therapeutic agent was associated with the increased risk of ventricular arrhythmia and a higher rate of fatal outcomes [17]. Interferons (IFNs) used in combating COVID-19 have quite a few limitations. Furthermore, Sa Ribero M. et al. found that late administration of IFN type I could be ineffective for disease treatment [18]. Currently, high expectations are pinned on vaccines aimed at developing active immunity against SARS-CoV-2. However, there are a number of problems impeding preventive mass vaccination: lack of data on duration of post-vaccination immune response; a large number of people who have contraindications to vaccination; people's low confidence in preventive vaccination; anti-vaccination (anti-vax) propaganda [19–23]. Increasing production of vaccines to immunize most of the world's population presents a huge problem.

Therefore, there is an urgent need for efficient therapeutics for treatment of COVID-19. One of the promising approaches in this field focuses on therapeutic agents having an RNA-interference-mediated effect [24].

At present, there are several known therapeutics positioned for treatment of other viral diseases and being evaluated in clinical trials. Promising results have been demonstrated by such antiviral therapeutics as miravirsen (Santaris Pharma) (administered for treatment of hepatitis C virus), pHIV7-shITAR-CCR5RZ (City of Hope Medical Center) (administered for treatment of human immunodeficiency virus (HIV) infection), ALN-RSV01 (Alnylam Pharmaceuticals) (administered for treatment of respiratory syncytial virus (RSV) infection) [25, 26].

This review is aimed to evaluate the therapeutic potential of small interfering RNAs (siRNAs) inhibiting SARS-CoV-2 replication, based on the previous studies of siRNAs for combating SARS-CoV and MERS-CoV infections.

Currently, there are no licensed siRNA-based antiviral therapeutics. However, the approval for clinical use has been granted to 2 therapeutics for treatment of rare genetic disorders: patisiran (Alnylam Pharmaceuticals) – familial amyloidotic polyneuropathy, and givosiran (Alnylam Pharmaceuticals) – acute hepatic porphyria [27, 28]. The available and approved RNAi-based therapeutics gives hope that similar antivirals will be created.

RNA interference: Biology and mechanism

RNA interference (RNAi) is a regulatory mechanism involving siRNA molecules inhibiting the expression of the target gene [29]. It was discovered by scientists A. Fire and C. Mello in 1998 in the nematode *Caenorhabditis elegans*. The investigators also identified the main characteristics typical of the above biological phenomenon:

– RNA interference involves cleavage of the messenger RNA (mRNA);

– the double-stranded RNA (dsRNA) specific for the complementary region of the target mRNA is more efficient than the single-stranded RNA (ssRNA);

– short dsRNA fragments are sufficient for gene knockdown [30].

The RNAi mechanism is a process during which endonuclease Dicer cleaves the foreign dsRNA into individual double-stranded fragments of up to 25 bp, which are known as siRNAs. The latter bind to the RISC complex (RNA-induced silencing complex) to target and destroy the mRNA [31].

Development of RNAi-based antiviral therapeutics

The SARS-CoV, MERS-CoV and SARS-CoV-2 genome sequences share many similarities [32, 33]. The mRNA sequences of most of their proteins are 79.6% similar to each other [34]. Early studies addressing the effect of siRNAs on SARS-CoV and MERS-CoV genomes demonstrated positive results for inhibition of the virus replication both *in vitro* and *in vivo*. Considering that SARS-CoV, MERS-

CoV and SARS-CoV-2 are phylogenetically related and belong to the same genus of the *Coronaviridae* family, the using of RNA interference in inhibition of SARS-CoV-2 replication holds great potential.

Classical approaches to development of antiviral drugs

The classical approach to constructing antiviral therapeutic agents focuses on the design of siRNAs, the inhibiting effect of which is targeted at the viral genome. In one of their early studies on identification of SARS-CoV targets for RNA interference, Meng B. et al. screened and identified siRNAs capable of blocking the expression of SARS-CoV genes. The authors synthesized envelope *E* and *RNA-dependent RNA-polymerase* (*RDRP*) genes to transfect in NIH 3T3 cells. The real-time (RT-) PCR test showed that both genes were expressed in the cells after the transfection. Then, 2 siRNAs for the *E* gene (*Ei1* and *Ei2*) and 4 siRNAs for the *RDRP* gene (*Ri1*, *-2*, *-3*, and *-4*) were synthesized and used for transfection in the same cells. In 48 hrs after the transfection, the inhibition of the gene expression was measured by using quantitative PCR. The test results showed that *Ei1* siRNA reduced the *E* gene expression by 89%, while *Ei2* siRNA downregulated the expression by 97%. The expression of the *RDRP* gene was almost completely blocked by *Ri1* and *Ri3* siRNAs targeting its mRNA sites at 118–140 and 394–415 bp positions, respectively. The efficiency of *Ri2* siRNA targeting the 224–245 bp site was 60%. At the same time, *Ri4* siRNA had no effect on the expression of the *RDRP* gene. The test was repeated three times, demonstrating similar results. Thus, a number of siRNAs that effectively blocked the expression of *E* and *RDRP* genes were identified. These molecules may be useful for further studies on the SARS-CoV replication cycle and can potentially be studied as therapeutic agents for treatment of SARS-CoV infection [35].

Wang Y. et al. studied the efficiency of siRNAs targeting the gene of the membrane (M) protein of SARS-CoV. The two most highly conserved regions (221–242 and 466–486 bp) of the nucleotide sequence of M protein mRNA were selected as targets; 2 *si-M1* and *si-M2* siRNAs were designed to target these regions. The *M* gene was fused with the *EGFP* gene to construct a *pEGFP-M* plasmid, which was further transfected into HEK 293 cell culture. The effect of siRNAs on the *M* gene expression was meas-ured both by RT-PCR and *EGFP* expression. The using of *si-M1* and *si-M2* demonstrated an 8-fold reduction in the *M* gene expression due to its inhibition. Higher doses of *si-M1* resulted in a 2–3-fold reduction as compared to the initial value [36].

Zhao P. et al. showed the efficiency of the newly designed plasmid vectors carrying genes of small hairpin RNAs (shRNAs), which targeted the *N* gene of SARS-CoV, in their tests on mice (*Mus musculus*). The obtained plasmid vectors *pN-EGFP*, *pU6-shN388* and *pUC18* carrying sequences of *N* gene and siRNAs were injected intramuscularly to 6-week-old female BALB/c mice. The animals were randomly divided into two groups: the experimental and the control groups (4 mice in each one). Every 4 days, one mouse from each group was sacrificed so that samples of muscular tissue could be obtained for the further analysis of the *N* and *EGFP* expression. The PCR test showed that siRNAs (plasmid *pU6-shN388*) retained its inhibitory effect during 16 days after the injection. The expression level of mRNA of SARS-CoV *N* gene in mouse muscles went down to 19, 17, 21, and 23% compared to the control plasmid on days 4, 8, 12, and 16 after the infection [37].

The high inhibitory effect of siRNAs on HKU-39849 strain of SARS-CoV in Vero cell culture was described by Wang Z. et al. A total of 6 plasmids encoding siRNAs targeting different sites of the viral genome were constructed. Vero cells were transfected with plasmids and infected with SARS-CoV. The highest effect was demonstrated by siRNA plasmids *pSR02* and *pSR03* targeting the viral RNA polymerase. The virus titer showed a 48-fold decrease with *pSR02* and a 96-fold decrease with *pSR03* as compared to the infected and non-transfected cells. In addition, the plasmids effectively reduced the synthesis of N protein and 3CL proteinase. The authors suppose that these siRNAs can be used for development of specific therapeutic agents [38].

The successful application of siRNAs targeting the viral genome was demonstrated by the study conducted by Shi Y. et al. The researchers selected 26 siRNA sequences specific for *E*, *M*, and *N* genes of SARS-CoV. *Nos. 5, 6, and 16* siRNAs targeting the mRNA of E, M, and N proteins, respectively, suppressed the gene expression in Vero cells by 70% when used at 30 nM. The scientists believe that the clinical application of the selected siRNAs may provide an effective therapeutic strategy for combating SARS (severe acute respiratory syndrome) infection [39].

The spike (S) protein is one of the potential targets for siRNAs. It plays a key role in SARS-CoV's entry to the host cell by binding to the receptor of angiotensin-converting enzyme 2 (ACE2) and mediating fusion of the viral envelope and cell membrane [40]. In their study, Wu C. et al. provide the data on the inhibition of replication of the Hong Kong strain of SARS-CoV by using a number of siRNAs, which also target mRNA of the viral *S* protein. The authors designed 7 siRNAs targeting S protein (*siSARS-S1, -S2, -S3*), the *3'-UTR*

region essential for replication and transcription of the viral RNA genome (*siSARS-3'-UTR*); 1 siRNA targeted the transcription-regulating sequence (TRS) region (*siSARS-TRS*). They also designed *siSARS-L* siRNA to target the leader sequence region of each 5' subgenomic mRNA (sgRNA). The obtained siRNAs were used to transfect Vero cells (4×10^4 cells/well) at 100 pM per well; then the cells were infected with SARS-CoV at a multiplicity of infection (MOI) of 0.01. When ones were microscopically examined for cytopathic effect (CPE), it was found that the cells transfected with *siSARS-S2* or *siSARS-S3* showed no marked changes, while the cells transfected with the other *siSARS* had rounded up and shrunk. RT-PCR showed that *siSARS-S2* and *siSARS-S3* inhibited the synthesis of viral nucleic acid by 85–90%; all the other siRNAs demonstrated much lower effect. The study found that the above constructs were able to effectively inhibit the replication of SARS-CoV *in vitro*. However, further studies are required to identify optimum doses of siRNAs and other parameters to use this approach in clinical practice [41].

Qin Z. et al. transfected HEK 293T cells with plasmids *pEGFP-S* containing fragments of SARS-CoV *S* gene to measure antiviral activity of candidate siRNAs. The latter (*S-siRNA1* and *S-siRNA2*) were checked for absence of homology with cell genes to prevent any risk of non-specific knockdown and further used to transfect the cell culture. Very mild fluorescence was detected in the *pEGFP-S* transfected cells; the similar decrease was observed in additional tests repeated at least three times, thus proving the inhibition of the *S* gene expression and degrading of the target mRNA. The quantitative real-time PCR (qRT-PCR) analysis showed that the transcript level of the gene decreased 9–10-fold in the siRNA transfected cells [42].

The efficiency of siRNAs targeting the specific gene is confirmed by the tests conducted by Li B. et al. on the laboratory model of rhesus macaques (*Macaca mulatta*) using the PUMC01 strain of SARS-CoV, which was grown in Vero cell culture. The tests were performed by using 2 siRNAs, *siSC2* and *siSC5* targeting the SARS-CoV genome in the regions encoding the S protein and NSP12 (*ORF1b*). The authors provided the following arguments supporting their choice: 1) the selected siRNAs showed a 100% homology to TOR-2 and PUMC01 strains; 2) the efficiency of these siRNAs was demonstrated in the previous studies and tends to increase with their duplex transfection; 3) their targeted sequences share no homology with the human genome, thus preventing any unforeseen risk of non-specific gene knockdown [43–46]. Two siRNA duplexes, *siCONa* and *siCONb*, with no homology to either the human genome or the viral genome, were chosen as the negative control. To assess the efficiency of the selected siRNAs, the researchers used a luciferase-based reporter gene system (*pCI-scLuc*) containing both *siSC2* and *siSC5* sequences. The co-transfection of *pCI-scLuc* and *siSC2/siSC5* into Vero cells showed that the combination of these siRNAs targeting the S protein can specifically inhibit the luciferase expression. To identify a viable carrier for siRNA delivery, two carriers were used: D5W solution [47] and Infasurf solution [48]. The co-delivery of *pCI-scLuc* plasmid with *siSC2/siSC5* in the D5W solution resulted in a higher level of reporter gene expression and a stronger RNAi effect than those observed for the delivery in the Infasurf solution. Out of the total number ($n = 21$) of the laboratory animals, 20 macaques (divided into 5 groups) were infected with SARS-CoV at a dose of 1×10^5 $TCID_{50}$ (the dose infecting 50% of tissue culture) in 1 ml phosphate buffered saline (PBS) through intranasal instillation (internal control was 1 macaque without infection). The animals were also administered *siSC2/siSC5* or the combination of *siCONa* and *siCONb* at 30 mg per dose in 3 ml D5W solution. All the infected macaques developed SARS-like symptoms, but those who received specific siRNAs had significantly lower body temperatures (~38.7 °C, close to the normal body temperature of this species). The RT-PCR was used to analyze oropharyngeal swab samples. No viral RNA was detected in 75% of the samples from the macaques treated with the specific siRNA. Thus, the test results for the *siSC2-5* molecule demonstrate that it can be seen as a potential therapeutic agent [49].

Åkerström S. et al. described the inhibition of SARS-CoV replication by using siRNAs targeting the viral sgRNA encoding 7a/7b, 3a/3b and S proteins. The Vero E6 cells were transfected with siRNA plasmid constructs by using electroporation. The *GFP* marker under the *CMV* promoter control was used as a reporter molecule. The cells were transfected with siRNAs and infected with SARS-CoV. One day after the infection, the culture fluid was collected and titrated on Vero E6 cells for CPE values. All the 3 lines of transfected cells demonstrated a ~70% inhibition of the viral replication as compared to the control group. Interestingly, one of the selected siRNAs (*siRNA 7*) appeared to inhibit both the target *sgRNA* 7 and the *sgRNA* 8 more efficiently, providing a knockdown of 4 accessory proteins [50].

In their recent study, Gallicano G. et al. found that siRNAs and microRNAs (miRNAs) with the predicted specificity to SARS-CoV-2 S protein suppressed its expression in HEK 293T and hpTC cells. Cell cultures were transfected with the plasmid expressing S protein.

The cells were further treated with synthetic siRNA (*siRNA1-Sense*, *siRNA2-Sense*) and miRNA (*miRNA1-hsa-miR624-5p* and *miRNA2-hsa-miR510-3p*) reagents targeting mRNA of the protein. By using the RT-PCR test, the authors also found that all the siRNAs and miRNAs, alone or in combination, could suppress S protein production 2.5–5-fold at the 200 nM. It is assumed that the above approach can be used as an innovative strategy for inhibiting replication of respiratory coronaviruses [51]. The study deserves special attention for its using miRNAs, natural cellular factors of RNA interference.

Table 1 summarizes the data on the viral target genes which suppression led to an effective reduction of SARS-CoV reproduction according to the data of independent studies.

Most of the studies addressing the antiviral activity of siRNAs towards MERS-CoV rarely move beyond theory. Nur S. et al. used the siDirect 2.0 program to design several siRNAs for the *ORF1ab* gene, including screening of any off-target effects [33]. Sohrab S. et al. describe siRNAs targeting the same gene to inhibit MERS-CoV replication in Vero culture [52].

On the other hand, J. Millet and G. Whittaker, when studying the entry of MERS-CoV to the cell, found that the HEK 293T cells transfected with siRNAs suppressing furin expression became less susceptible to the infection caused by the above virus. The cells showed a knockdown of furin mRNA levels by 62.5% and decreased susceptibility to MERS-CoV pseudotyped viral particles (MERSpp). Furthermore, the overexpression of the proteolytic enzyme in the cells restored susceptibility to infection, thus suggesting that furin can have an important role in MERS-CoV replication [53].

Alternative techniques for constructing antiviral agents

Another approach to design of antiviral constructs offers specific siRNAs targeting mRNAs of cellular genes. ACE is known as a cellular receptor for SARS-CoV [54]. Lu C. et al. constructed a number of plasmids encoding small hairpin RNAs (shRNAs) targeting *ACE2* mRNA. The authors transfected Vero E6 cells with 2 shRNAs targeting A4 and C4 sites of *ACE2* mRNA. Then, the cells were infected with SARS-CoV at MOI = 1; 0,1; 0,01; 0,001. The replication of the infectious agent was blocked in ACE2-silenced A4 cells at low inoculation doses ($1 \times 10^{-1} – 1 \times 10^{-3}$), while at the inoculation dose of MOI = 1, no difference in viral loads was noted between Vero E6 (control) and A4 cells at different time points after the infection. The researchers believe that the suppression of the *ACE2* activity can inhibit SARS-CoV replication. However, they warn that the selective elimination of *ACE2* in the organs vulnerable to SARS-CoV can lead to unexpected consequences such as respiratory disorders and cardiac dysfunction [55–57].

Cellular factors participating in replication of different coronaviruses at different stages of their life cycle were studied by de Wilde A. et al. To identify the host factors involved in SARS-CoV replication, the authors screened siRNA libraries targeting the human kinome. As protein kinases are key regulators in many cellular functions, studies on suppression of their gene expression help identify factors and signaling pathways promoting

Table 1. Viral genes which knockdown led to a significant decrease in the reproduction of SARS-CoV-2
Таблица 1. Вирусные гены, нокдаун которых приводил к значительному снижению репродукции SARS-CoV-2

Gene name Название генов	Function Функция	References Ссылки
E	Transport of ions across the virus envelope Перенос ионов через мембрану вируса	[35, 39]
RDRP gene	Synthesis of new RNA molecules Синтез новых молекул РНК	[35, 38]
M	Ensuring the stability of the virus envelope Стабилизация вирусной мембраны	[39]
N	Formation of the nucleocapsid Образование нуклеокапсида	
Spike gene	Ensuring binding to the cellular receptor ACE2 and penetration of the virus into the cell Обеспечение связывания с клеточным рецептором ACE2 и проникновения вируса в клетку	[41, 49, 51]
3'-UTR	3' untranslated region 3'-нетранслируемая область	[41]
TRS	Transcriptional regulatory sequence Регуляторная последовательность транскрипции	
ORF1B	Open reading frame Открытая рамка считывания	[49]

or preventing coronavirus replication. For SARS-CoV, the screening resulted in the identification of 40 proviral and 90 antiviral proteins. The analysis of signaling pathways made it possible to identify factors participating in cellular processes, including cellular immune response and metabolism of complex lipids. These factors may play role in development of the infection caused by the above pathogen. Several factors were selected for thorough examination during the subsequent tests. The cells treated with siRNAs targeting COPB2 demonstrated a pronounced antiviral effect involving reduced expression of SARS-CoV proteins and a nearly 100-fold reduction in the virus reproduction. The knockdown of COPB2-related proteins, COPB1, GBF1 and a number of others, also showed their importance for SARS-CoV replication. The results of the first screening showed that the reduced expression of protein kinase R (PKR) increased the virus replication, while the validation tests demonstrated the increased expression of SARS-CoV protein and virus replication after PKR depletion. Besides, cyclin-dependent kinase 6 (CDK6) is another host factor important for virus replication [58, 59].

Table 2 shows a number of cellular genes that play an important role in the life cycle of coronaviruses. The knockdown of these genes led to a decrease in SARS-CoV reproduction.

Conclusion

Therefore, the development of specific RNAi-based therapeutics offers a promising avenue for studies focused on COVID-19 treatment. The available and approved RNAi-based therapeutics for genetic disorders (patisiran and givosiran) suggest that similar antivirals can be created for COVID-19 treatment. It is believed that downregulation of viral replication at early stages of SARS-CoV-2 infection can significantly reduce the risk of development of severe disease forms. Note that siRNAs can target viral mRNAs as well as cellular factors and signaling pathways participating in virus replication at different stages.

On the other hand, it should be remembered that the application of siRNAs targeting viral genes may result in development of virus resistance due to point substitutions in the viral genome; besides, they may non-specifically inhibit the expression of cellular genes and have an adverse impact on the cells. To prevent virus resistance to siRNA agents due to mutability and genetic diversity of viruses, the principle of combined effect can be used when

Table 2. Cellular genes which knockdown led to a significant decrease in the reproduction of SARS-CoV-2
Таблица 2. Клеточные гены, нокдаун которых приводил к значительному снижению репродукции SARS-CoV-2

Gene name Название гена	Function Функция	References Ссылки
ACE2 (*ACEH*)	Receptor for the entry of SARS-CoV-2 into the cell Специфический рецептор для входа SARS-CoV-2 в клетку	[55, 59]
EIF2AK2	Phosphorylation of the translation initiation factor EIF2S1 Фосфорилирование фактора инициации трансляции EIF2S1	[59]
CDK5R2	Encoding of the specific activator of CDK5 kinase Кодирование специфического активатора киназы CDK5	[58]
GBF1	Participation in vesicular transfer by activating factor 1 of adenosine diphosphate ribosylation Участие в везикулярном переносе путём активации фактора 1 рибозилирования аденозиндифосфата	
COPB1	Encoding of one of the protein subunits associated with transport in the Golgi complex Кодирование одной из белковых субъединиц, связанных с транспортом к комплексу Гольджи	
COPB2	Encoding of one of the protein subunits associated with transport in the Golgi complex Кодирование одной из белковых субъединиц, связанных с транспортом к комплексу Гольджи	
CDK6	Catalytic subunit of the protein kinase complex, which is important for the passage of the G1 phase of the cell cycle and the G1/S transition Каталитическая субъединица протеинкиназного комплекса, важная для прохождения фазы G1 клеточного цикла и перехода к G1/S	
CLK1	The encoded protein in the nucleus which phosphorylates the serine/arginine-rich proteins involved in pre-mRNA processing with releasing them into the nucleoplasm Кодируемый белок в ядре, фосфорилирует богатые серином/аргинином белки, участвующие в процессинге пре-мРНК, высвобождая их в нуклеоплазму	
ABL1	Protein tyrosine kinase which involved in a variety of cellular processes including cell division, adhesion, differentiation, and stress response Белковая тирозинкиназа, участвует во множестве клеточных процессов, включая деление, адгезию, дифференцировку и стрессовую реакцию	
Furin	Proteolysis of the S2 subunit Протеолиз S2 субъединицы	[53]

dealing with independent targets in the transcriptomes of both the virus and the host cell supporting its replication. The efficient targeted *in vivo* delivery of siRNAs remains an unsolved problem. All these and other problems need further studies, both theoretical and experimental.

REFERENCES

1. WHO. Coronavirus (COVID-19) Dashboard. Coronavirus Disease (COVID-19) Dashboard. Available at: https://covid19.who.int/ (accessed July 29, 2021).
2. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* 2020; 5(4): 536–44. https://doi.org/10.1038/s41564-020-0695-z
3. WHO Director-General's opening remarks at the media briefing on COVID-19 – 11 March 2020. Available at: https://www.who.int/ru/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (accessed July 29, 2021).
4. Hanff T.C., Harhay M.O., Brown T.S., Cohen J.B., Mohareb A.M. Is there an association between COVID-19 mortality and the renin-angiotensin system? A call for epidemiologic investigations. *Clin. Infect. Dis.* 2020; 71(15): 870–4. https://doi.org/10.1093/cid/ciaa329
5. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020; 395(10223): 497–506. https://doi.org/10.1016/s0140-6736(20)30183-5
6. Wu J., Li J., Zhu G., Zhang Y., Bi Z., Yu Y., et al. Clinical features of maintenance Hemodialysis patients with 2019 novel Coronavirus-infected pneumonia in Wuhan, China. *Clin. J. Am. Soc. Nephrol.* 2020; 15(8): 1139–45. https://doi.org/10.2215/cjn.04160320
7. Mao L., Jin H., Wang M., Hu Y., Chen S., He Q., et al. Neurologic manifestations of hospitalized patients with Coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.* 2020; 77(6): 683–90. https://doi.org/10.1001/jamaneurol.2020.1127
8. Perico L., Benigni A., Remuzzi G. Should COVID-19 concern nephrologists? Why and to what extent? The emerging impasse of angiotensin blockade. *Nephron.* 2020; 144(5): 213–21. https://doi.org/10.1159/000507305
9. Xu Z., Shi L., Wang Y., Zhang J., Huang L., Zhang C., et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 2020; 8(4): 420–2. https://doi.org/10.1016/s2213-2600(20)30076-x
10. Jordan R.E., Adab P., Cheng K.K. Covid-19: risk factors for severe disease and death. *BMJ.* 2020; 368: m1198. https://doi.org/10.1136/bmj.m1198
11. Coronavirus disease 2019 (COVID-19). Complications. Available at: https://bestpractice.bmj.com/topics/en-gb/3000201/complications (accessed July 29, 2021).
12. Yasuhara J., Kuno T., Takagi H., Sumitomo N. Clinical characteristics of COVID-19 in children: A systematic review. *Pediatr. Pulmonol.* 2020; 55(10): 2565–75. https://doi.org/10.1002/ppul.24991
13. Panigrahy N., Policarpio J., Ramanathan R. Multisystem inflammatory syndrome in children and SARS-CoV-2: A scoping review. *J. Pediatr. Rehabil. Med.* 2020; 13(3): 301–16. https://doi.org/10.3233/prm-200794
14. García-Salido A., de Carlos Vicente J.C., Belda Hofheinz S., Balcells Ramírez J., Slöcker Barrio M., Leóz Gordillo I., et al. Spanish Pediatric Intensive Care Society working group on SARS-CoV-2 infection. Severe manifestations of SARS-CoV-2 in children and adolescents: from COVID-19 pneumonia to multisystem inflammatory syndrome: a multicentre study in pediatric intensive care units in Spain. *Crit. Care*. 2020; 24(1): 666. https://doi.org/10.1186/s13054-020-03332-4
15. Cao B., Wang Y., Wen D., Liu W., Wang J., Fan G., et al. A trial of Lopinavir–Ritonavir in adults hospitalized with severe Covid-19. *N. Engl. J. Med.* 2020; 382(19): 1787–99. https://doi.org/10.1056/nejmoa2001282
16. Joshi S., Parkar J., Ansari A., Vora A., Talwar D., Tiwaskar M., et al. Role of favipiravir in the treatment of COVID-19. *Int. J. Infect. Dis.* 2021; 102: 501–8. https://doi.org/10.1016/j.ijid.2020.10.069
17. Cavalcanti A.B., Zampieri F.G., Rosa R.G., Azevedo L.C.P., Veiga V.C., Avezum A., et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate Covid-19. *N. Engl. J. Med.* 2020; 383(21): 2041–52. https://doi.org/10.1056/NEJMoa2019014
18. Sa Ribero M., Jouvenet N., Dreux M., Nisole S. Interplay between SARS-CoV-2 and the type I interferon response. *PLoS Pathog*. 2020; 16(7): e1008737. https://doi.org/10.1371/journal.ppat.1008737
19. Onishchenko G.G., Sizikova T.E., Lebedev V.N., Borisevich S.V. Analysis of promising approaches to COVID-19 vaccine development *[Analiz perspektivnykh napravleniy sozdaniya vaktsin protiv COVID-19]*. *BIOpreparaty. Profilaktika, diagnostika, lechenie.* 2020; 20(4): 216–27. https://doi.org/10.30895/2221-996X-2020-20-4-216-227 (in Russian)
20. Glover R.E., Urquhart R., Lukawska J., Blumenthal K.G. Vaccinating against covid-19 in people who report allergies. *BMJ.* 2021; 372: n120. https://doi.org/10.1136/bmj.n120
21. Smith M. Vaccine safety: medical contraindications, myths, and risk communication. *Pediatr. Rev.* 2015; 36(6): 227–38. https://doi.org/10.1542/pir.36-6-227
22. Gallup. One in Three Americans Would Not Get COVID-19 Vaccine. Available at: https://news.gallup.com/poll/317018/one-three-americans-not-covid-vaccine.aspx (accessed July 29, 2021).
23. da Costa C.B.P., Martins F.J., da Cunha L.E.R., Ratcliffe N.A., Cisne de Paula R., Castro H.C. COVID-19 and Hyperimmune sera: A feasible plan B to fight against coronavirus. *Int. Immunopharmacol.* 2021; 90: 107220. https://doi.org/10.1016/j.intimp.2020.107220
24. Weng Y., Xiao H., Zhang J., Liang X.J., Huang Y. RNAi therapeutic and its innovative biotechnological evolution. *Biotechnol. Adv.* 2019; 37(5): 801–25. https://doi.org/10.1016/j.biotechadv.2019.04.012
25. Janssen H.L., Reesink H.W., Lawitz E.J., Zeuzem S., Rodriguez-Torres M., Patel K., et al. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* 2013; 368(18): 1685–94. https://doi.org/10.1056/nejmoa1209026
26. Qureshi A., Tantray V.G., Kirmani A.R., Ahangar A.G. A review on current status of antiviral siRNA. *Rev. Med. Virol.* 2018; 28(4): e1976. https://doi.org/10.1002/rmv.1976
27. Hoy S.M. Patisiran: first global approval. *Drugs*. 2018; 78(15): 1625–31. https://doi.org/10.1007/s40265-018-0983-6
28. Center for drug evaluation and research. Multi-discipline review. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf (accessed July 29, 2021).
29. Agrawal N., Dasaradhi P.V., Mohmmed A., Malhotra P., Bhatnagar R.K., Mukherjee S.K. RNA interference: biology, mechanism, and applications. *Microbiol. Mol. Biol. Rev*. 2003; 67(4): 657–85. https://doi.org/10.1128/mmbr.67.4.657-685.2003
30. Fire A., Xu S.Q., Montgomery M.K., Kostas S.A., Driver S.E., Mello C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391(6669): 806–11. https://doi.org/10.1038/35888
31. Pashkov E.A., Faizuloev E.B., Svitich O.A., Sergeev O.V., Zverev V.V. The potential of synthetic small interfering RNA-based antiviral drugs for influenza treatment *[Perspektiva sozdaniya spetsificheskikh protivogrippoznykh preparatov na osnove sinteticheskikh malykh interferiruyushchikh RNK]*. *Voprosy virusologii*. 2020; 65(4): 182–90. https://doi.org/10.36233/0507-4088-2020-65-4-182-190 (in Russian)
32. Kannan S., Shaik Syed Ali P., Sheeza A., Hemalatha K. COVID-19 (Novel Coronavirus 2019) – recent trends. *Eur. Rev. Med. Pharmacol. Sci*. 2020; 24(4): 2006–11. https://doi.org/10.26355/eurrev_202002_20378

33. Nur S.M., Hasan M.A., Amin M.A., Hossain M., Sharmin T. Design of potential RNAi (miRNA and siRNA) molecules for Middle East respiratory syndrome coronavirus (MERS-CoV) gene silencing by computational method. *Interdiscip. Sci*. 2015; 7(3): 257–65. https://doi.org/10.1007/s12539-015-0266-9
34. Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579(7798): 270–3. https://doi.org/10.1038/s41586-020-2012-7
35. Meng B., Lui Y.W., Meng S., Cao C., Hu Y. Identification of effective siRNA blocking the expression of SARS viral envelope E and RDRP genes. *Mol. Biotechnol*. 2006; 33(2): 141–8. https://doi.org/10.1385/mb:33:2:141
36. Wang Y., Cao Y.L., Yang F., Zhang Y., Wang S.H., Liu L. Small interfering RNA effectively inhibits the expression of SARS coronavirus membrane gene at two novel targeting sites. *Molecules*. 2010; 15(10): 7197–207. https://doi.org/10.3390/molecules15107197
37. Zhao P., Qin Z.L., Ke J.S., Lu Y., Liu M., Pan W., et al. Small interfering RNA inhibits SARS-CoV nucleocapsid gene expression in cultured cells and mouse muscles. *FEBS Lett*. 2005; 579(11): 2404–10. https://doi.org/10.1016/j.febslet.2005.02.080
38. Wang Z., Ren L., Zhao X., Hung T., Meng A., Wang J., et al. Inhibition of severe acute respiratory syndrome virus replication by small interfering RNAs in mammalian cells. *J. Virol*. 2004; 78(14): 7523–7. https://doi.org/10.1128/jvi.78.14.7523-7527.2004
39. Shi Y., Yang D.H., Xiong J., Jia J., Huang B., Jin Y.X. Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. *Cell Res*. 2005; 15(3): 193–200. https://doi.org/10.1038/sj.cr.7290286
40. Xiao X., Dimitrov D.S. The SARS-CoV S glycoprotein. *Cell Mol. Life Sci*. 2004; 61(19-20): 2428–30. https://doi.org/10.1007/s00018-004-4257-y
41. Wu C.J., Huang H.W., Liu C.Y., Hong C.F., Chan Y.L. Inhibition of SARS-CoV replication by siRNA. *Antiviral. Res*. 2005; 65(1): 45–8. https://doi.org/10.1016/j.antiviral.2004.09.005
42. Qin Z.L., Zhao P., Zhang X.L., Yu J.G., Cao M.M., Zhao L.J., et al. Silencing of SARS-CoV spike gene by small interfering RNA in HEK 293T cells. *Biochem. Biophys. Res. Commun*. 2004; 324(4): 1186–93. https://doi.org/10.1016/j.bbrc.2004.09.180
43. Chen Z., Zhang L., Qin C., Ba L., Yi C.E., Zhang F., et al. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. *J. Virol*. 2005; 79(5): 2678–88. https://doi.org/10.1128/jvi.79.5.2678-2688.2005
44. Qin C., Wang J., Wei Q., She M., Marasco W.A., Jiang H., et al. An animal model of SARS produced by infection of *Macaca mulatta* with SARS coronavirus. *J. Pathol*. 2005; 206(3): 251-9. https://doi.org/10.1002/path.1769
45. Haasnoot P.C., Cupac D., Berkhout B. Inhibition of virus replication by RNA interference. *J. Biomed. Sci*. 2003; 10(6 Pt. 1): 607–16. https://doi.org/10.1159/000073526
46. Zheng B.J., Guan Y., Tang Q., Du C., Xie F.Y., He M.L., et al. Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. *Antivir. Ther*. 2004; 9(3): 365–74.
47. Ghanayem N.S., Yee L., Nelson T., Wong S., Gordon J.B., Marcdante K., et al. Stability of dopamine and epinephrine solutions up to 84 hours. *Pediatr. Crit. Care. Med*. 2001; 2(4): 315–7. https://doi.org/10.1097/00130478-200110000-00005
48. Thomas N.J., Hollenbeak C.S., Lucking S.E., Willson D.F. Cost-effectiveness of exogenous surfactant therapy in pediatric patients with acute hypoxemic respiratory failure. *Pediatr. Crit. Care Med*. 2005; 6(2): 160–5. https://doi.org/10.1097/01.pcc.0000154965.08432.16
49. Li B.J., Tang Q., Cheng D., Qin C., Xie F.Y., Wei Q., et al. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat. Med*. 2005; 11(9): 944–51. https://doi.org/10.1038/nm1280
50. Åkerström S., Mirazimi A., Tan Y.J. Inhibition of SARS-CoV replication cycle by small interference RNAs silencing specific SARS proteins, 7a/7b, 3a/3b and S. *Antiviral Res*. 2007; 73(3): 219–27. https://doi.org/10.1016/j.antiviral.2006.10.008
51. Gallicano G.I., Casey J.L., Fu J., Mahapatra S. Molecular targeting of vulnerable RNA sequences in SARS CoV-2: identifying clinical feasibility. *Gene Ther*. 2020; 1–8. https://doi.org/10.1038/s41434-020-00210-0
52. Sohrab S.S. et al. Antiviral Activity Evaluation of siRNAs Against MERS-CoV in Vero Cell Culture. *Applied Microbiology*. London; 2020.
53. Millet J.K., Whittaker G.R. Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *Proc. Natl. Acad. Sci. USA*. 2014; 111(42): 15214–9. https://doi.org/10.1073/pnas.1407087111
54. Li W., Moore M.J., Vasilieva N., Sui J., Wong S.K., Berne M.A., et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003; 426(6965): 450–4. https://doi.org/10.1038/nature02145
55. Lu C.Y., Huang H.Y., Yang T.H., Chang L.Y., Lee C.Y., Huang L.M. siRNA silencing of angiotensin-converting enzyme 2 reduced severe acute respiratory syndrome-associated coronavirus replications in Vero E6 cells. *Eur. J. Clin. Microbiol. Infect. Dis*. 2008; 27(8): 709–15. https://doi.org/10.1007/s10096-008-0495-5
56. Hanff T.C., Harhay M.O., Brown T.S., Cohen J.B., Mohareb A.M. Is There an Association Between COVID-19 Mortality and the Renin-Angiotensin System? A Call for Epidemiologic Investigations. *Clin. Infect. Dis*. 2020; 71(15): 870–4. https://doi.org/10.1093/cid/ciaa329
57. Cheng H., Wang Y., Wang G.Q. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J. Med. Virol*. 2020; 92(7): 726–30. https://doi.org/10.1002/jmv.25785
58. de Wilde A.H., Wannee K.F., Scholte F.E., Goeman J.J., Ten Dijke P., Snijder E.J., et al. A kinome-wide small interfering RNA screen identifies proviral and antiviral host factors in severe acute respiratory syndrome coronavirus replication, including double-stranded RNA-activated protein kinase and early secretory pathway proteins. *J. Virol*. 2015; 89(16): 8318–33. https://doi.org/10.1128/jvi.01029-15
59. de Wilde A.H., Snijder E.J., Kikkert M., van Hemert M.J. Host factors in coronavirus replication. *Curr. Top. Microbiol. Immunol*. 2018; 419: 1–42. https://doi.org/10.1007/82_2017_25

ЛИТЕРАТУРА

1. WHO. Coronavirus (COVID-19) Dashboard. Coronavirus Disease (COVID-19) Dashboard. Available at: https://covid19.who.int/ (accessed July 29, 2021).
2. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* 2020; 5(4): 536–44. https://doi.org/10.1038/s41564-020-0695-z
3. WHO Director-General's opening remarks at the media briefing on COVID-19 – 11 March 2020. Available at: https://www.who.int/ru/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (accessed July 29, 2021).
4. Hanff T.C., Harhay M.O., Brown T.S., Cohen J.B., Mohareb A.M. Is there an association between COVID-19 mortality and the renin-angiotensin system? A call for epidemiologic investigations. *Clin. Infect. Dis.* 2020; 71(15): 870–4. https://doi.org/10.1093/cid/ciaa329
5. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020; 395(10223): 497–506. https://doi.org/10.1016/s0140-6736(20)30183-5
6. Wu J., Li J., Zhu G., Zhang Y., Bi Z., Yu Y., et al. Clinical features of maintenance Hemodialysis patients with 2019 novel Coronavi-

rus-infected pneumonia in Wuhan, China. *Clin. J. Am. Soc. Nephrol.* 2020; 15(8): 1139–45. https://doi.org/10.2215/cjn.04160320
7. Mao L., Jin H., Wang M., Hu Y., Chen S., He Q., et al. Neurologic manifestations of hospitalized patients with Coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.* 2020; 77(6): 683–90. https://doi.org/10.1001/jamaneurol.2020.1127
8. Perico L., Benigni A., Remuzzi G. Should COVID-19 concern nephrologists? Why and to what extent? The emerging impasse of angiotensin blockade. *Nephron.* 2020; 144(5): 213–21. https://doi.org/10.1159/000507305
9. Xu Z., Shi L., Wang Y., Zhang J., Huang L., Zhang C., et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 2020; 8(4): 420–2. https://doi.org/10.1016/s2213-2600(20)30076-x
10. Jordan R.E., Adab P., Cheng K.K. Covid-19: risk factors for severe disease and death. *BMJ.* 2020; 368: m1198. https://doi.org/10.1136/bmj.m1198
11. Coronavirus disease 2019 (COVID-19). Complications. Available at: https://bestpractice.bmj.com/topics/en-gb/3000201/complications (accessed July 29, 2021).
12. Yasuhara J., Kuno T., Takagi H., Sumitomo N. Clinical characteristics of COVID-19 in children: A systematic review. *Pediatr. Pulmonol.* 2020; 55(10): 2565–75. https://doi.org/10.1002/ppul.24991
13. Panigrahy N., Policarpio J., Ramanathan R. Multisystem inflammatory syndrome in children and SARS-CoV-2: A scoping review. *J. Pediatr. Rehabil. Med.* 2020; 13(3): 301–16. https://doi.org/10.3233/prm-200794
14. García-Salido A., de Carlos Vicente J.C., Belda Hofheinz S., Balcells Ramírez J., Slöcker Barrio M., Leóz Gordillo I., et al. Spanish Pediatric Intensive Care Society working group on SARS-CoV-2 infection. Severe manifestations of SARS-CoV-2 in children and adolescents: from COVID-19 pneumonia to multisystem inflammatory syndrome: a multicentre study in pediatric intensive care units in Spain. *Crit. Care*. 2020; 24(1): 666. https://doi.org/10.1186/s13054-020-03332-4
15. Cao B., Wang Y., Wen D., Liu W., Wang J., Fan G., et al. A trial of Lopinavir–Ritonavir in adults hospitalized with severe Covid-19. *N. Engl. J. Med.* 2020; 382(19): 1787–99. https://doi.org/10.1056/nejmoa2001282
16. Joshi S., Parkar J., Ansari A., Vora A., Talwar D., Tiwaskar M., et al. Role of favipiravir in the treatment of COVID-19. *Int. J. Infect. Dis.* 2021; 102: 501–8. https://doi.org/10.1016/j.ijid.2020.10.069
17. Cavalcanti A.B., Zampieri F.G., Rosa R.G., Azevedo L.C.P., Veiga V.C., Avezum A., et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate Covid-19. *N. Engl. J. Med.* 2020; 383(21): 2041–52. https://doi.org/10.1056/NEJMoa2019014
18. Sa Ribero M., Jouvenet N., Dreux M., Nisole S. Interplay between SARS-CoV-2 and the type I interferon response. *PLoS Pathog*. 2020; 16(7): e1008737. https://doi.org/10.1371/journal.ppat.1008737
19. Онищенко Г.Г., Сизикова Т.Е., Лебедев В.Н., Борисевич С.В. Анализ перспективных направлений создания вакцин против COVID-19. *БИОпрепараты. Профилактика, диагностика, лечение.* 2020; 20(4): 216–27. https://doi.org/10.30895/2221-996X-2020-20-4-216-227
20. Glover R.E., Urquhart R., Lukawska J., Blumenthal K.G. Vaccinating against covid-19 in people who report allergies. *BMJ.* 2021; 372: n120. https://doi.org/10.1136/bmj.n120
21. Smith M. Vaccine safety: medical contraindications, myths, and risk communication. *Pediatr. Rev.* 2015; 36(6): 227–38. https://doi.org/10.1542/pir.36-6-227
22. Gallup. One in Three Americans Would Not Get COVID-19 Vaccine. Available at: https://news.gallup.com/poll/317018/one-three-americans-not-covid-vaccine.aspx (accessed July 29, 2021).
23. da Costa C.B.P., Martins F.J., da Cunha L.E.R., Ratcliffe N.A., Cisne de Paula R., Castro H.C. COVID-19 and Hyperimmune sera: A feasible plan B to fight against coronavirus. *Int. Immunopharmacol.* 2021; 90: 107220. https://doi.org/10.1016/j.intimp.2020.107220
24. Weng Y., Xiao H., Zhang J., Liang X.J., Huang Y. RNAi therapeutic and its innovative biotechnological evolution. *Biotechnol. Adv.* 2019; 37(5): 801–25. https://doi.org/10.1016/j.biotechadv.2019.04.012
25. Janssen H.L., Reesink H.W., Lawitz E.J., Zeuzem S., Rodriguez-Torres M., Patel K., et al. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* 2013; 368(18): 1685–94. https://doi.org/10.1056/nejmoa1209026
26. Qureshi A., Tantray V.G., Kirmani A.R., Ahangar A.G. A review on current status of antiviral siRNA. *Rev. Med. Virol.* 2018; 28(4): e1976. https://doi.org/10.1002/rmv.1976
27. Hoy S.M. Patisiran: first global approval. *Drugs*. 2018; 78(15): 1625–31. https://doi.org/10.1007/s40265-018-0983-6
28. Center for drug evaluation and research. Multi-discipline review. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf (accessed July 29, 2021).
29. Agrawal N., Dasaradhi P.V., Mohmmed A., Malhotra P., Bhatnagar R.K., Mukherjee S.K. RNA interference: biology, mechanism, and applications. *Microbiol. Mol. Biol. Rev*. 2003; 67(4): 657–85. https://doi.org/10.1128/mmbr.67.4.657-685.2003
30. Fire A., Xu S.Q., Montgomery M.K., Kostas S.A., Driver S.E., Mello C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391(6669): 806–11. https://doi.org/10.1038/35888
31. Пашков Е.А., Файзулоев Е.Б., Свитич О.А., Сергеев О.В., Зверев В.В. Перспектива создания специфических противогриппозных препаратов на основе синтетических малых интерферирующих РНК. *Вопросы вирусологии.* 2020; 65(4): 182–90. https://doi.org/10.36233/0507-4088-2020-65-4-182-190
32. Kannan S., Shaik Syed Ali P., Sheeza A., Hemalatha K. COVID-19 (Novel Coronavirus 2019) – recent trends. *Eur. Rev. Med. Pharmacol. Sci*. 2020; 24(4): 2006–11. https://doi.org/10.26355/eurrev_202002_20378
33. Nur S.M., Hasan M.A., Amin M.A., Hossain M., Sharmin T. Design of potential RNAi (miRNA and siRNA) molecules for Middle East respiratory syndrome coronavirus (MERS-CoV) gene silencing by computational method. *Interdiscip. Sci*. 2015; 7(3): 257–65. https://doi.org/10.1007/s12539-015-0266-9
34. Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579(7798): 270–3. https://doi.org/10.1038/s41586-020-2012-7
35. Meng B., Lui Y.W., Meng S., Cao C., Hu Y. Identification of effective siRNA blocking the expression of SARS viral envelope E and RDRP genes. *Mol. Biotechnol*. 2006; 33(2): 141–8. https://doi.org/10.1385/mb:33:2:141
36. Wang Y., Cao Y.L., Yang F., Zhang Y., Wang S.H., Liu L. Small interfering RNA effectively inhibits the expression of SARS coronavirus membrane gene at two novel targeting sites. *Molecules*. 2010; 15(10): 7197–207. https://doi.org/10.3390/molecules15107197
37. Zhao P., Qin Z.L., Ke J.S., Lu Y., Liu M., Pan W., et al. Small interfering RNA inhibits SARS-CoV nucleocapsid gene expression in cultured cells and mouse muscles. *FEBS Lett*. 2005; 579(11): 2404–10. https://doi.org/10.1016/j.febslet.2005.02.080
38. Wang Z., Ren L., Zhao X., Hung T., Meng A., Wang J., et al. Inhibition of severe acute respiratory syndrome virus replication by small interfering RNAs in mammalian cells. *J. Virol*. 2004; 78(14): 7523–7. https://doi.org/10.1128/jvi.78.14.7523-7527.2004
39. Shi Y., Yang D.H., Xiong J., Jia J., Huang B., Jin Y.X. Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. *Cell Res*. 2005; 15(3): 193–200. https://doi.org/10.1038/sj.cr.7290286
40. Xiao X., Dimitrov D.S. The SARS-CoV S glycoprotein. *Cell Mol. Life Sci*. 2004; 61(19-20): 2428–30. https://doi.org/10.1007/s00018-004-4257-y

41. Wu C.J., Huang H.W., Liu C.Y., Hong C.F., Chan Y.L. Inhibition of SARS-CoV replication by siRNA. *Antiviral. Res*. 2005; 65(1): 45–8. https://doi.org/10.1016/j.antiviral.2004.09.005
42. Qin Z.L., Zhao P., Zhang X.L., Yu J.G., Cao M.M., Zhao L.J., et al. Silencing of SARS-CoV spike gene by small interfering RNA in HEK 293T cells. *Biochem. Biophys. Res. Commun*. 2004; 324(4): 1186–93. https://doi.org/10.1016/j.bbrc.2004.09.180
43. Chen Z., Zhang L., Qin C., Ba L., Yi C.E., Zhang F., et al. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. *J. Virol*. 2005; 79(5): 2678–88. https://doi.org/10.1128/jvi.79.5.2678-2688.2005
44. Qin C., Wang J., Wei Q., She M., Marasco W.A., Jiang H., et al. An animal model of SARS produced by infection of *Macaca mulatta* with SARS coronavirus. *J. Pathol*. 2005; 206(3): 251-9. https://doi.org/10.1002/path.1769
45. Haasnoot P.C., Cupac D., Berkhout B. Inhibition of virus replication by RNA interference. *J. Biomed. Sci*. 2003; 10(6 Pt. 1): 607–16. https://doi.org/10.1159/000073526
46. Zheng B.J., Guan Y., Tang Q., Du C., Xie F.Y., He M.L., et al. Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. *Antivir. Ther*. 2004; 9(3): 365–74.
47. Ghanayem N.S., Yee L., Nelson T., Wong S., Gordon J.B., Marcdante K., et al. Stability of dopamine and epinephrine solutions up to 84 hours. *Pediatr. Crit. Care. Med*. 2001; 2(4): 315–7. https://doi.org/10.1097/00130478-200110000-00005
48. Thomas N.J., Hollenbeak C.S., Lucking S.E., Willson D.F. Cost-effectiveness of exogenous surfactant therapy in pediatric patients with acute hypoxemic respiratory failure. *Pediatr. Crit. Care Med*. 2005; 6(2): 160–5. https://doi.org/10.1097/01.pcc.0000154965.08432.16
49. Li B.J., Tang Q., Cheng D., Qin C., Xie F.Y., Wei Q., et al. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat. Med*. 2005; 11(9): 944–51. https://doi.org/10.1038/nm1280
50. Åkerström S., Mirazimi A., Tan Y.J. Inhibition of SARS-CoV replication cycle by small interference RNAs silencing specific SARS proteins, 7a/7b, 3a/3b and S. *Antiviral Res*. 2007; 73(3): 219–27. https://doi.org/10.1016/j.antiviral.2006.10.008
51. Gallicano G.I., Casey J.L., Fu J., Mahapatra S. Molecular targeting of vulnerable RNA sequences in SARS CoV-2: identifying clinical feasibility. *Gene Ther*. 2020; 1–8. https://doi.org/10.1038/s41434-020-00210-0
52. Sohrab S.S. et al. Antiviral Activity Evaluation of siRNAs Against MERS-CoV in Vero Cell Culture. *Applied Microbiology*. London; 2020.
53. Millet J.K., Whittaker G.R. Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *Proc. Natl. Acad. Sci. USA*. 2014; 111(42): 15214–9. https://doi.org/10.1073/pnas.1407087111
54. Li W., Moore M.J., Vasilieva N., Sui J., Wong S.K., Berne M.A., et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003; 426(6965): 450–4. https://doi.org/10.1038/nature02145
55. Lu C.Y., Huang H.Y., Yang T.H., Chang L.Y., Lee C.Y., Huang L.M. siRNA silencing of angiotensin-converting enzyme 2 reduced severe acute respiratory syndrome-associated coronavirus replications in Vero E6 cells. *Eur. J. Clin. Microbiol. Infect. Dis*. 2008; 27(8): 709–15. https://doi.org/10.1007/s10096-008-0495-5
56. Hanff T.C., Harhay M.O., Brown T.S., Cohen J.B., Mohareb A.M. Is There an Association Between COVID-19 Mortality and the Renin-Angiotensin System? A Call for Epidemiologic Investigations. *Clin. Infect. Dis*. 2020; 71(15): 870–4. https://doi.org/10.1093/cid/ciaa329
57. Cheng H., Wang Y., Wang G.Q. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J. Med. Virol*. 2020; 92(7): 726–30. https://doi.org/10.1002/jmv.25785
58. de Wilde A.H., Wannee K.F., Scholte F.E., Goeman J.J., Ten Dijke P., Snijder E.J., et al. A kinome-wide small interfering RNA screen identifies proviral and antiviral host factors in severe acute respiratory syndrome coronavirus replication, including double-stranded RNA-activated protein kinase and early secretory pathway proteins. *J. Virol*. 2015; 89(16): 8318–33. https://doi.org/10.1128/jvi.01029-15
59. de Wilde A.H., Snijder E.J., Kikkert M., van Hemert M.J. Host factors in coronavirus replication. *Curr. Top. Microbiol. Immunol*. 2018; 419: 1–42. https://doi.org/10.1007/82_2017_25