Received: 20 November 2021 | Revised: 25 January 2022 | Accepted: 14 February 2022

IET Nanobiotechnology

DOI: 10.1049/nbt2.12079

The Institution of Engineering and Technology WILEY

REVIEW

Antisense technology as a potential strategy for the treatment of coronaviruses infection: With focus on COVID-19

Seyedeh Hoda Alavizadeh[1,2] | Maham Doagooyan[3,4] | Fatemeh Zahedipour[3,5] | Shima Yahoo Torghabe[6] | Bahare Baharieh[3] | Firooze Soleymani[3] | Fatemeh Gheybi[1,3]

[1]Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

[5]Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[6]Department of Basic Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

Correspondence

Fatemeh Gheybi, Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran. Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, P.O. Box: 917794-8564, Mashhad, Iran.
Email: GheybiF@mums.ac.ir

Abstract

After the outbreak of coronavirus disease 2019 (COVID-19) in December 2019 and the increasing number of SARS-CoV-2 infections all over the world, researchers are struggling to investigate effective therapeutic strategies for the treatment of this infection. Targeting viral small molecules that are involved in the process of infection is a promising strategy. Since many host factors are also used by SARS-CoV-2 during various stages of infection, down-regulating or silencing these factors can serve as an effective therapeutic tool. Several nucleic acid-based technologies including short interfering RNAs, antisense oligonucleotides, aptamers, DNAzymes, and ribozymes have been suggested for the control of SARS-CoV-2 as well as other respiratory viruses. The antisense technology also plays an indispensable role in the treatment of many other diseases including cancer, influenza, and acquired immunodeficiency syndrome. In this review, we summarised the potential applications of antisense technology for the treatment of coronaviruses and specifically COVID-19 infection.

KEYWORDS

antisense, aptamers, coronavirus, COVID-19, DNAzymes, ribozymes, siRNA

1 | INTRODUCTION

The new strain of coronavirus, Severe Acute Respiratory Syndrome (SARS) coronavirus 2 (SARS-CoV-2) caused coronavirus disease 2019 (COVID-19) pandemic which has already led to a global health emergency [1]. The first COVID-19 cases were reported in December 2019 in eastern Asia, Wuhan, and China; the SARS-CoV-2 virus spread rapidly thereafter to other regions of the country within a month and subsequently affected the whole world. Since December 2019, the number of COVID-19 cases have risen to more than 55.6 million, which caused 1.34 million deaths throughout the world [2], and unfortunately thousands of people are dying due to COVID-19 complications [3]. Similar to the Middle East Respiratory Syndrome (MERS) and the SARS, SARS-CoV-2 could result in acute respiratory distress syndrome [4]. The SARS-CoV-2 is an enveloped β-coronavirus strain with a positive-sense single-stranded RNA genome of 26-32 Kb in length. The most prominent structural proteins of this virus include the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins.

The spike proteins enable the virus to enter the cell using the same ACE2[1] receptor as SARS-CoV, leading to the spread of the virus throughout the respiratory system of the host. For infectious viral entry, the spike protein is cleaved and then activated by the host protease enzyme [5]. The host cell protease belongs to a type II transmembrane serine protease, TMPRSS2[2], which revealed to cleave and activate SARS-CoV-2 spike protein in cell culture conditions. Thus, TMPRSS[2] could be regarded as a potential target for antiviral treatment and Camostat mesylate, a serine protease inhibitor, has shown to hamper the enzymatic activity of the TMPRSS2 enzyme [6, 7].

Right after entry, the virus starts proliferating through expressing and encoding its essential proteins that assist with the virus adaptation to the host human cell [8]. The high recombination rate of CoVs is due to the constant errors that occur in the transcription of the virus via RNA dependent RNA polymerase (RdRp) [9]; thus, CoVs are considered as zoonotic pathogens infecting a wide variety of animals and humans with a broad range of symptoms from asymptomatic course to multi-organ failure [10]. Several nonstructural proteins including RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (PLpro) are encoded by the viral genome. Crucial proteases that are involved in cleaving the translated viral polyproteins into effector proteins include 3CLpro and PLpro, while RdRp generates full-length negative-strand RNA as a template to form several viral genomic RNA [11, 12].

Effective therapy against SARS-CoV-2 includes the use of broad-spectrum antiviral agents or exploiting molecular therapeutics that intervene at any stages of the virus's life cycle to inhibit virus attachment and entrance into the host cells. Today, the world expects promising strategies to tackle novel coronavirus as quickly as possible. Many new prophylactic and combination therapy approaches have shown potential in protecting the lung from infections. For instance, molecular tools including Cas13a RNA-activated RNases have recently shown new paradigm for therapeutics against these pathogens [13]. Messenger RNA (mRNA)-encoded Cas13a used to mitigate SARS-CoV-2 infection, demonstrated a modest lung viral load reduction and a significant change in the pathophysiology of the infection [14, 15]. mRNA-based strategies for direct expression of neutralising antibodies in the lung tissue were also developed as a promising prophylaxis approach to prevent human respiratory syncytial virus (RSV) infections [14].

Antisense technology has been widely used as a potential therapeutic option to inhibit the specific gene expression. The antisense ODNs[3] play an indispensable role in the treatment of many diseases including cancer, influenza, and acquired immunodeficiency syndrome [16]. The antisense ODNs, usually 18–21 nucleotides in length, specifically hybridise to the target mRNA sequences through Watson-Crick base pairing, forming mRNA/DNA duplex that is subsequently cleaved and destroyed by RNAse H, an endonuclease that hydrolyses the RNA strand of the heteroduplex. The antisense ODNs are also capable of blocking mRNA translation physically. Short interfering RNAs (siRNAs) targeting the highly conserved regions of SARS-CoV-2 RNA can suppress the genetic disorders of the lungs. This approach could definitely assist with achieving a better treatment strategy that can reduce the pandemic threat of COVID-19 [7]. A combination therapeutic approach has recently suggested the use of SARS-CoV-2 specific RNA interference (RNAi) vectors in conjunction with systemic delivery of compstatin complement inhibitor to combat the cytokine storm associated with the progression and severity COVID-19 disease [17].

RNA-based vaccines including mRNA-1273 by Moderna and BNT162 by Pfizer/BioNTech have shown promising results for Covid-19 disease now [7, 18, 19]. In this review, the latest developments of antisense technology for the treatment of coronavirus and particularly COVID-19 are addressed.

2 | AN OVERVIEW OF THE MOLECULAR MECHANISMS INVOLVED IN SARS-CoV-2 PATHOGENICITY

2.1 | Viral attachment/penetration

SARS-CoV-2 virion possesses a nucleocapsid composed of genomic RNA (29.9kb) and phosphorylated nucleocapsid (N) protein. The nucleocapsid is covered by a phospholipid bilayer and contains two types of spike proteins: the haemagglutinin-esterase that is only found in some CoVs, and the spike glycoprotein trimmer (S) that exists in all CoVs. The viral envelope also contains the *M* and *E* proteins that are located across the *S* proteins. The SARS-CoV-2 can pass through the nasal and larynx mucosal membrane and enter the lungs through the respiratory tract. The virus uses the spike glycoprotein, a homotrimeric protein consists of S1 and S2 subunits in each spike monomers, to attach angiotensin-converting enzyme 2 (ACE2) highly expressed in the lungs, heart, renal system and gastrointestinal tract. This is actually a critical step for viral entry. Further, to provide a binding site for SARS-CoV-2, ACE2 cleaves the angiotensin (Ang) I to produce Angiotensin 1 to 9. Gene ontology studies revealed that the ACE2-expression is associated with several viral process-related genes including life cycle, replication machinery, assembly, and regulatory genes. This indicates that the ACE2-expressing cells facilitate the replication of SARS-CoV-2 in the lungs [20]. As mentioned earlier, cleaving and activating the spike protein by TMPRSS2 host cell protease, a type II transmembrane serine protease, is also considered a critical step for viral entry to the host cell [21, 22].

2.2 | Viral replication

The SARS-CoV-2 replication machinery includes several nonstructural proteins (Nsp 1–16), however, the functions of some

[1] Angiotensin-converting enzyme two
[2] Transmembrane protease, serine two
[3] Oligonucleotides

Nsps are not clear yet. The viral replication machinery includes some proteins and enzymes including RNA-dependent RNA polymerase (RdRp, Nsp12), zinc-binding helicase (HEL, Nsp13), RNA proofreading (Nsp14), mRNA capping (Nsp14, Nsp16), and uridylate-specific endoribonuclease activity (NendoU, Nsp15). Other non-structural proteins including Nsp7–Nsp10 exert regulatory roles in the process of replication and transcription. The Nsp1 protein as a major SARS-CoV-2 virulence factor is responsible for gene expression inhibition and intrinsic immune response suppression in the host cells. The *N* protein that is tightly bound to the RNA genome is involved in the replication machinery. Further, the *N* protein interacts with the *M* viral envelope protein that is involved in the packaging of the viral genome [23]. RdRp is a critical enzyme for the replication of RNA viruses. However, it possesses some other functions in enhancing the efficiency of the whole machinery. The active site of RdRp is conserved among various organisms and has been targeted in several viral infections [24] (Figure 1).

2.3 | SARS-CoV-2 main protease (Mpro) and papain-like protease papain-like protease

Papain-like protease and 3C-like protease (3CLpro) are the two cysteine proteases encoded by viral Nsp3 and Nsp5, respectively. The 3C-like protease (3CLpro) is also referred to as NSP5 or the main protease (Mpro). Papain-like protease and Mpro proteases are required for the replication of the virus and are potential targets for antiviral therapy. The Mpro is a homodimeric cysteine protease that represents almost 97% sequence identity with the corresponding Mpro protease of the SARS-CoV [25]. The PLpro cleaves Nsp1, Nsp2, Nsp3, and 3CLpro and processes the remaining 13 non-structural proteins. After their generation, Nsps assemble the viral replicase complex on the host membrane and begin replication and transcription of the viral genome. Papain-like protease cleaves ubiquitin and ubiquitin-like post-translational modifications from host proteins to escape the host immune system. The PLpro of SARS-CoV-2 shares about 83% sequence identity with the corresponding PLpro protease of the SARS-CoV. Several small-molecule inhibitors for PLpro catalytic activity have been proposed as potential therapeutic options [26, 27].

3 | ANTISENSE TECHNOLOGY AND CORONAVIRUS

Antisense therapeutics generally include DNA and RNA-based therapeutics. These are sequence-selective cleaving agents with the ability to downregulate the disease-causing genes through RNAi or catalytic cleavage of the transcripts. Several types of oligonucleotides in the field of antisense technology that have been used to combat CoVs infections are summarised in the following sections.

3.1 | Short interfering RNAs

Short interfering RNAs are 19–22 bp RNA duplexes with an average molecular weight of 12 KDa that inhibit the translation of mRNAs by combining their leading strand with RNA-induced silencing complex and binding to the complementary mRNA strands. RNA interference is naturally occurring in the cells and makes a great contribution in cell defensive mechanism against mobile genetic elements such as viruses [28]. Following the introduction of siRNAs into the cells, they can either downregulate the translation of the targeted mRNA or destroy the mRNA [29, 30]. The replication of a variety of human pathogenic viruses including HIV-1, poliovirus, hepatitis C virus, hepatitis B virus, and influenza virus have shown to be prohibited by RNAi in cell culture studies [31–33]. Short interfering RNAs targeting the replicase 1A region of SARS-CoV have shown to suppress cytopathiceffects in SARS-CoV-infected monkey kidney cells [34]. Similar studies were conducted investigating the efficacy of various siRNAs against SARS-CoV on Vero E6 or other cells in vitro [35, 36].

The most important limitation of siRNAs for in vitro or in vivo application is that naked short interfering RNAs (siRNA) is not capable of diffusing through the negatively charged cellular membrane due to its vigorous phosphate related charges. Thus, the success of siRNA therapy highly depends on appropriate viral and non-viral vectors for reaching the intracellular environment [37]. Several carriers have been used for safe and effective delivery of siRNA to the target area [38–40].

Short interfering RNAs targeting highly conserved regions in the RSV nucleocapsid protein mRNA were used in humans [41–43]. The siRNA was developed as 19 duplex along with two (2′-deoxy) thymidine overhangs on both 3′ ends, which prevent RNA degradation, by cellular nuclease enzymes. In a randomized, double-blind, placebo-controlled clinical trial, the naked siRNA administrated as nasal spray in elder patients (infected with RSV) showed to be safe and was well-tolerated [42, 43]. Further, in a second phase randomized, double blind, placebo controlled clinical trial, a significant reduction in a bronchiolitis obliterans syndrome risk was demonstrated in RSV patients with lung transplant using ALN-RSV01 [43, 44]. However, due to missing the primary endpoint in a second phase, the study was halted in 2014. The lack of efficiency was thought to be due to the emergence of resistant viruses, which is the most common complication when using a single siRNA molecule targeting a single-site of the viral genome. [43]. Therefore, using numerous siRNAs, which can target multiple genes in the target cell genome, can be promising for future investigations.

It has been demonstrated that RNAi particularly siRNAs, siHel1, siHel2, UC7, and siUTR3 could efficiently repress SARS-CoV-2 in vitro and in vivo which could be a potential therapeutic approach in treating COVID-19 [40]. Recently, AGO (Argonaute-crosslinking)-associated RNAs isolated via crosslinking immunoprecipitation (AGO CLIP: class II-associated invariant chain peptide) analysis were utilised to develop siRNAs with antifibrotic miRNA activity, which showed to suppress SARS-CoV-2 [45].

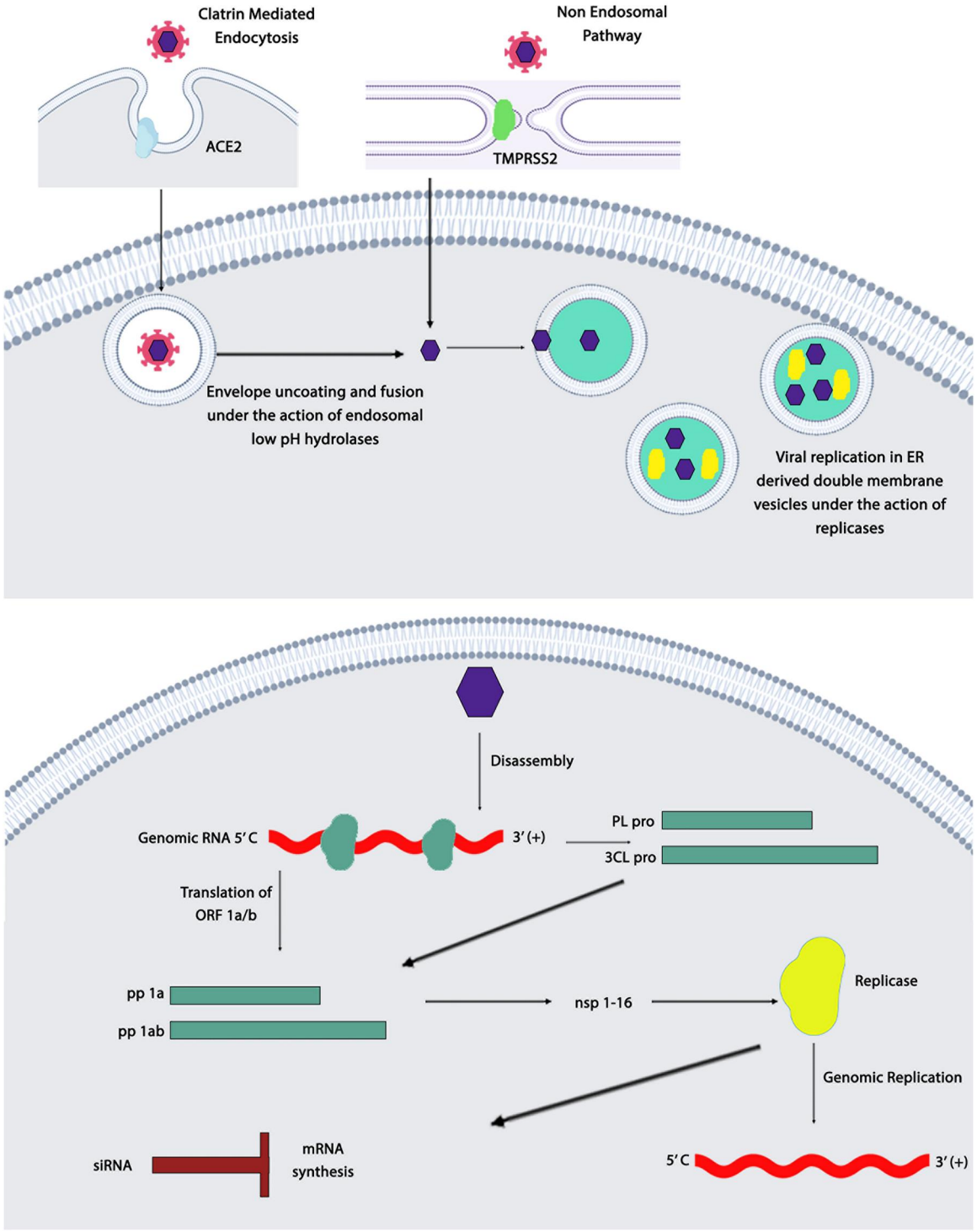

FIGURE 1 Potential effects of Short interfering RNAs (siRNAs) on silencing viral genes at the post-transcriptional level in COVID-19

Overall, siRNA therapy could be regarded as a potential therapeutic strategy to target the highly conserved regions of the SARS-CoV-2 RNA sequences. There are currently a number of research studies and patents investigating the ability of siRNAs to combat coronaviruses and some of the recent investigations are summarised in the following sections.

3.1.1 | Short interfering RNAs targeting coronavirus *S*, *M*, *N*, or *E* proteins

The genome of SARS-CoV expresses at least four crucial structural proteins including *N*, *S*, *M*, and *E* proteins. As mentioned earlier, the *N* protein is a RNA-associated virion

protein that makes a long and flexible helical nucleocapsid essential for viral RNA synthesis. While the S glycoprotein on the surface membrane of the virus is involved in virus binding and entrance into the host cells, the *M* and *E* proteins enhance virus budding and assembly, respectively [46, 47].

Due to the high rate of mutation of S protein [48], targeting its mRNA is regarded as a promising approach to inhibit SARS-CoV proliferation [49, 50]. Qin et al. used S-specific siRNAs to specifically inhibit SARS-CoV S glycoprotein expression in HEK 293T (human embryonic kidney 293T cells) cultured cells through blocking the accumulation of S mRNA [49]. Wu et al. explored the silencing potential of some synthetic siRNAs against SARS-CoV leader, transcription-regulating sequences, untranslated region (3′-UTR) and Spike coding sequence and indicated that siRNAs against Spike sequences and the 3′-UTR could prevent the replication of SARS-CoV in Vero-E6 cells [50, 51]. It has been indicated that DNA vector-driven siRNA can specially downregulate the S gene expression in SARS-CoV-infected 293T cells, and suggested that siRNA targeting the leader sequence result in a significant reduction in both mRNA and protein levels of the reporter genes in the cells [49, 52].

Intranasal delivery of a combination of siRNA (siSC2-5) targeting SARS-CoV ORF1 and *S* proteins have shown to decrease SARS-CoV pathogenesis [36, 53]. Double-stranded RNAs have shown to selectively target two segregate regions of the *M* protein mRNA including siRNA-M1 targeted the nucleotides 220–241, and siRNA-M2 targeted the nucleotides 460–480 within the mRNA of *M* protein. The efficiency of interference of these two siRNAs on SARS *M* protein gene expression was more than 70%. Several siRNA sequences have also been designed to specifically target the *M*, *N*, and *E* genes of SARS among which three siRNAs sequences have been shown to interact with the expression of GFP-M, GFP-N, and GFP-E. In addition, three siRNAs contained a mutation at the 3′ end of their sense strand. These mutations improved the function of siRNAs and indicated the increased inhibition of SARS virus gene expression compared to the original siRNAs [54]. There are reports on designing siRNAs targeting the sequences expressing the *N* protein as well as the *E* and RdRp genes [51]. In an in-silico study by Chowdhury et al., specific siRNAs for several conserved regions of N phosphoprotein and surface glycoprotein genes of SARS-CoV-2 were designed, which showed to suppress the expression of these proteins and inhibition of SARS-CoV-2 infection [55]. Chen et al. also designed 9 promising siRNA targeting *S*, *M*, *N*, open reading frame (ORF) 1ab, and ORF 3a in the SARS-CoV-2 genome, based on their computational experiment by analysing 143 high-quality strains in the 2019nCoVR database [12, 56].

3.1.2 | Short interfering RNAs targeting angiotensin-converting enzyme 2

Angiotensin-converting enzyme 2 is a cell membrane receptor involved in SARS-CoV attachment and entry. Angiotensin-converting enzyme 2 expression has shown to be successfully silenced in Vero E6 cells through siRNA containing the homologous sequence to a region of ACE2 under the U6 promoter [57]. Due to the vital role of ACE2 in virus entrance into the cell [58], it is considered as a therapeutic target for SARS-CoV-2 infection [59]. The efficacy of ACE2 silencing potential could be enhanced by using a multiple-hit strategy via combining several siRNAs that simultaneously target several mRNA sequences of SARS-CoV and its receptors. Moreover, recent studies have shown that viruses can escape siRNA targeting via fast mutagenesis thus, the synergistic effects of several siRNAs targeting various SARS-CoV genes have been investigated. He et al. revealed that siRNAs targeting several replicase, spike, envelope, and membrane genes could exert synergistic antiviral activities compared to a single siRNA alone at the saturated concentration [60].

Overall, by using siRNA technology researchers succeed to diminish the cellular ACE2 receptor expression and therefore the entry and replication of SARS-CoV into the cells. Though the hypothesis that silencing ACE2 can plunge or inhibit SARS-CoV infections was proved correct, there are still concerns regarding the efficacy of this approach at clinical context that requires further in-depth investigations [61].

3.1.3 | Short interfering RNAs targeting RNA replicase and RNA dependent RNA polymerase (RdRp)

According to studies, viral RNA polymerase is an appropriate target for inhibition of SARS-CoV-2 replication and drug development [62]. The SARS-CoV-2 bears 14 ORFs encoding 27 proteins, thus RdRp that is located in ORF1b could be considered as a potential therapeutic target [63]. In a recent experiment, it was demonstrated that the transfection of SARS-CoV-2-infected Vero E6 cells with siRNAs targeting RdRp could significantly prohibit SARS-CoV-2 replication and infection. The siRNA silencing of the RdRp has shown to result in a remarkable decrease in the viral titre, and suppression of viral infection in the Golden Syrian hamster and rhesus macaque [64]. As previously noted, the US20050004063 patent application showed six different siRNAs (SARSi-1–SARSi-6) capable of targeting SARS-CoV replicase 1A region and suppressing virus infection and replication. This invention also revealed other SARSis with the potential of targeting *S*, *E*, *N*, and *M* genes and the subsequent suppression of coronavirus infection and replication in FRhk-4 cells. Other studies identified siRNAs that could target RdRp, helicase, nucleoprotein N, and proteolytic enzymes genes of SARS-CoV. These siRNAs were capable of hampering the BJ01 strain SARS virus replication while the most vigorous effect was observed with proteolytic enzyme-targeting siRNA [54]. Scientists used commercially available vector pSUPER.retro for designing siRNAs that target replicase of SARS-CoV (1a region of the genome). They have demonstrated that siRNAs delivered by pSUPER vector were able to suppress the replication of SARS-CoV in Vero E6 cells, which ultimately suppress the cytopathic effects of SARS-CoV on Vero cells [65–68]. In another study, siRNAs designed to target the ultra-conserved regions in the

RNA-dependent RNA polymerase (RdRp), Helicase (Hel), and 5′ untranslated region (5′ UTR) have shown to repress virus proliferation and inhibit the disease progress. It is of note that chemical modifications used to stabilise siRNAs leads to a longer-term expression and in vivo persistence [69] which proved to be a promising treatment against this disease [40].

Khaitov et al., have recently developed a variety of in silico-designed siRNAs for targeting SARS-CoV-2 RNA-dependent RNA polymerase (RdRp). They have shown that siR-7 modification with Locked nucleic acids could increase siRNA stability and that formulation with the peptide dendrimer KK-46 could enhance cellular uptake. Further study in Syrian Hamster model for SARS-CoV-2 infection has shown to boost the antiviral capacity and induce a remarkable reduction of the virus titre and lung inflammation in animals exposed to inhalation of siR-7-EM/KK-46 [56].

3.2 | Ribozymes

Ribozymes are RNA molecules with catalytic activity that are capable of cleaving the phosphodiester bonds of nucleic acids. Ribozymes are divided into two classes including the hammerhead and hairpin ribozymes known for their small size [70, 71]. It has been shown that a chimaeric RNA/DNA ribozyme could recognise the GUC with loop structures and common conserved regions in the genome of coronaviruses [54]. Fukuda et al. developed a novel therapeutic ribozyme for the treatment of SARS-CoV and other coronaviruses associated infections. To exert therapeutic action, ribozyme specifically recognises and targets the GUC base sequence located in the loop region on the mRNA of SARS-CoV or other coronaviruses. The complementary base sequence on the ribozyme is derived by removing, inserting, or changing bases without altering their binding affinity. The ribozyme was designed as an RNA/DNA chimaeric structure with an RNA structure in the conserved region and a DNA structure in other regions containing phosphorothioate modification at the 3' end to avoid in vivo decomposition. A mismatch-ribozyme was also designed to compare the effectiveness of the ribozyme in cleaving mRNA of SARS-CoV, which demonstrates the significance of sequence specificity for therapeutic effectiveness [72, 73]. Previously, it was shown that a chimaeric DNA/RNA hammerhead ribozyme could suppress the expression of SARS-CoV RNA in 3T3 cells transfected with a recombinant plasmid, suggesting this ribozyme as an effective treatment for SARS-CoV infection [74, 75]. Ribozymes are RNA molecules capable of targeting and cleaving the transcript or RNA genomes of the virus and repeating the process with another RNA target [76]. This is considered as a distinctive feature of ribozyme to cleave ample of specific molecular targets [77]. Ribozymes play a crucial role in the suppression of human immunodeficiency virus gene expression and proliferation [78, 79]. Synthetic ribozyme has shown to be capable of cleaving SARS-CoV RNA thus, inhibiting RNA expression of SARS-CoV [75, 80]. However, the cleavage site and binding arms should be considered while designing ribozyme to target the special RNA sequence [81].

3.3 | Antisense oligonucleotides

Antisense oligonucleotides (ASOs) have been developed to prevent or treat SARS-CoV infection and to detect the virus in human samples. Ionis Pharmaceuticals designed a hybrid DNA/RNA ASO to break down the pseudoknot in the frameshift site of the SARS-CoV RNA [54]. Among 26 antisense phosphorothioate oligonucleotides (PS-ODNs) targeting different sites within the ORFs of *E*, *M*, and *N* proteins, 12 antisense oligos were shown to decrease the target gene expression by over 50% efficiency in Vero E6 cells. Taken together, results indicated a sequence-specific down-regulation of a 20 mer antisense PS-ODN in Vero E6 cells, where the antisense oligo suppressed the expression of the E, M, and N genes of SARS-CoV [82]. Though ASOs could be designed to detect and treat SARS-CoV-2 infection, the delivery of ASOs to the lung tissue remains a big challenge [83, 84].

3.4 | DNAzymes

DNAzymes are DNA-based catalysts capable of performing a special chemical reaction and can act as potential diagnostic and therapeutic agents [85, 86]. They are usually used to cleave and make linear scaffolds with minimal fixed sequence overhangs [87]. It has been shown that the DNAzyme could efficiently cleave the 5′UTR of SARS-CoV in vitro, indicating the 5′UTR as a promising potential molecular target. DNAzymes bear intrinsic physicochemical benefits compared to other nucleic acid-based agents including greater target flexibility, reduced chemical modification, relative resistance to degradation by nuclease, and low cost of synthesis. Hence, they may be considered as a clinically attractive new class of anti-SARS therapeutic agent [88]. To inhibit the viral replication, DNAzyme was used to target 5′-UTR of a highly conserved fragment in the SARS-CoV genome. The in vitro study indicated the efficacy of a mono-DNA enzyme (Dz-104) with a 10–23 catalytic motif. In mammalian cell culture, the Dz-104 cleaved the SARS-CoV RNA molecule and decreased the expression of the SARS-CoV 5′UTR-eGFP fusion RNA [74, 89].

3.5 | Aptamers

Oligonucleotide aptamers have favourable advantages over antibodies such as selectivity, sensitivity, safety, reproducibility, as well as cost-effectiveness. Aptamers can be administered in the upper respiratory system as aerosol particles where they can enter the epithelial cells [90]. Systematic evolution of ligands by exponential enrichment (SELEX)[4] has been widely used for the synthesis of nucleic acid and protein aptamers for the diagnosis of genetic disorders, detection of microbes and biosensing like for screening the influenza virus through

[4]Systemic evolution of ligands through exponential enrichment

targeting the antigenic proteins [91, 92]. It has been shown that aptamers can quickly and effectively be inhaled in the upper respiratory tract in the form of aerosolised particles and inhibit the SARS-CoV-2 entrance into the host cells with low toxicity [90]. RNA aptamers were shown to be capable of binding and suppressing the double-stranded DNA unwinding of the SARS-CoV helicase. RNA aptamers have also been shown to possess a particular affinity for SARS-CoV nucleocapsid which confirms their potential as therapeutic agents [54]. Another approach that has been used to combat SARS-CoV is to design aptamers against the ACE-2 receptor; this could be achieved through protein-based SELEX that recognise sequence candidates with high target sensitivity and specificity in a relatively short amount of time. Short interfering RNAs or microRNAs conjugated aptamers that could selectively target viral genes have been considered as potential therapeutic approach for the treatment of COVID19. However, there are still challenges regarding the delivery of aptamers including their rapid plasma clearance following IV injection [93, 94].

Non-structural Protein 10 (Nsp10) enzyme is one of the discovered proteins of life-threatening SARS-CoV which bears NTPase/helicase activity. Similar to Flaviviridae NS3 protein function, Nsp10 unwinds viral dsDNA. Jang et al. modified Nsp10 using a specific aptamer RNA, ES15, which bears secondary structure including stem-loop structure with repeats of AG. This aptamer has shown a significant suppression of the viral enzyme activity in a dose-dependent manner in vitro [95, 96]. Nucleocapsid (*N*) protein, one of the most abundant proteins of SARS-CoV, is regarded as another potential target for designing aptamers. The use of a nanoarray aptamer chip with an RNA aptamer was reported by Ahn et al, which showed the capacity of detecting SARS *N* protein at a concentration of 2 pg/ml. In another study, it was demonstrated that DNA aptamers against the non-structural Nsp13 protein suppressed the helicase activity of Nsp13 protein required for SARS-CoV replication [74, 97]. There are many patents associated with antisense therapy of coronaviruses that have recently been reviewed by Sajid et al [98] and can be exploited for RNAi-based therapy of anti-SARS-CoV-2 as well. Some of the siRNA-based patents are summarised in Table 1. Isis Pharmaceuticals, Inc. has developed a modified 2MOE ASO with a phosphodiester backbone targeting various regions in SARS-CoV-2 genes that showed no significant toxicity on Vero C1008 cells [54, 99].

4 | ANTISENSE DELIVERY SYSTEMS

Various lipid-based nanoparticles have been used as efficient vectors to deliver mRNA vaccines for the treatment of COVID-19 [52]. Using an appropriate delivery system is a critical aspect for effective clinical applications of ASOs in both therapeutic and prophylactic context. The delivery of naked or unprotected antisense sequences would potentially expose them to the enzymatic degradation, resulting in several side effects including toxicity, instability, and reticuloendothelial system and kidneys clearance. Therefore, it is essential to optimise specific nanocarriers for the delivery of these fragile molecules to their target site. Li et al. used a glucose D5W (D-glucose in water) to deliver the chemically synthesised siRNAs into the airway of Rhesus macaques. Though results were promising regarding SARS-CoV replication suppression, the siRNA contents delivery into the animal's airway epithelial cells and the maintenance time of transfected siRNA duplexes in vivo remained unclear [100, 101].

Techniques for gene delivery to the airway systems have been significantly improved over the last decade mainly

TABLE 1 List of some patents associated with antisense therapy of coronaviruses

Patent no.	Type of antisense	Patent title	Year
WO2005023083	siRNA	Target mRNA reduction; no significant Toxic in COVID-19	2020
CN1548054	siRNA	Medicine for preventing and treating SARS coronavirus	2003
CN1569233	siRNA	Method for manufacturing gene medicine for preventing and treating SARS and its medicinal prescription	2003
WO2005019410	siRNA	RNAi agents for anti-SARS coronavirus therapy	2004
WO2005023083A3 US7339051B2	Antisense oligonucleotide	Compositions and methods for the treatment of severe acute respiratory syndrome (SARS)	2004
US7687475B2	siRNA	RNA interference in respiratory epithelial cells	2005
CN1648249A	siRNA	Small molecular interference ribonucleic acid for inhibiting SARS virus gene expression	2004
CN20061114168	siRNA	Small interfering RNA for restraining SARS coronavirus *M* protein gene expression, encoding gene and application thereof	2006
US20050004063A1	siRNA	Inhibition of SARS-associated coronavirus (SCoV) infection and replication by RNA interference	2004
US8653252B2	siRNA	Short interfering RNA (siRNA) analogues	2004
CN20061027475	siRNA	SARS coronavirus disturbance RNA and its uses	2006

through viral vector approaches. Adenoviral vectors using helper vectors have long been applied for gene delivery to the airway epithelial cells in mice and rabbits. The gene delivery using viral vectors has been shown to be highly efficacious, and oligos that have been delivered by viral vectors lasted for a longer time in the transfected cells, however, adverse effects including inflammation occurred. Concern regarding host immune responses towards the viral vectors can be overcome by utilising anti-inflammatory drugs. On the other hand, adeno-associated viral vectors were identified to express siRNA in the mouse liver with high efficiency and without evoking host immune responses.

Short interfering RNA has shown sufficient potential to be used as a safe and specific option against COVID-19 disease by interfering with mRNA of the virus and blocking the virus replication [102]. Recently a database of SARS-CoV-2 targets for small interference RNA (siRNA) based therapeutic has been introduced with the aim of facilitating the development of novel antivirals against Covid-19 [103]. Short interfering RNAs delivery systems have been widely used for other diseases as well for instance, ICS-283 system identified by Schiffelers and Storm was used for the systemic delivery of therapeutic siRNA to the pathological angiogenesis sites. Further, many other approaches for intravenous delivery of siRNA have been recently reported by Dykxhoorn and Leiberman [51, 104, 105]. Delivery of antisense oligos could be applicable for the treatment of COVID19 in the clinic. Alnylam Pharmaceuticals (USA) designed and synthesised more than 350 siRNAs that target highly conserved regions of the SARS-CoV-2 genome. However, effective vectors are needed for the successful delivery of siRNAs to the direct site of infection [7, 104]. In SARS-CoV-2 infection, the primary receptors are located at ciliated cells of the lungs and the viral transmission occurs via direct personal contact or droplets of saliva from the infected person. Accordingly, designing methods for effective delivery of antiviral drugs into the lung epithelial cells is crucially important. In this regard, Conti et al. reported on the use of an in vitro aerosol system based on poly (amidoamine) dendrimer nanocarriers for siRNA delivery to the lung epithelial cells. However, the efficiency of siRNA delivery using this system was shown to be insufficient [7, 66, 106]. Another important issue is the number of cells that are required to be transfected in COVID-19 clinical course. For instance, in traditional gene therapy, 25% transfection efficiency would be a great success, however, it might hardly make any difference in the course of the viral infection [107]. An experiment on monkey models showed that intratracheal administration of siRNA-D5W solution complex targeting spike and Nsp12 coding genes effectively suppressed SARS-CoV replication and SARS-like symptoms [74, 108].

The delivery of siRNAs could be enhanced by using novel nanocarriers such as lipid, inorganic, or polymeric nanoparticles [109, 110]. Natural or synthetic polymeric nanoparticles such as polyethyleneimine and chitosan have also been investigated as gene carriers. Chitosan-based nanoparticles have been reported to induce protective immunity against several infectious diseases and have been used in the formulation of some vaccines such as the hepatitis B virus vaccine. Therefore, these types of gene carriers should be evaluated for specific delivery of antisense sequences in COVID-19 cases [109, 111]. It was demonstrated that siRNA delivery through the IV injection of cationic liposomes could result in highly effective gen silencing and protein knockdown in either endothelial or epithelial cells of the lung tissue which could have implication in treating COVID-19 disease [38, 40]. Another study investigated the efficacy of small interfering RNA (siRNA) therapeutic targeting highly conserved regions of the SARS-CoV-2 virus using a novel lipid nanoparticle delivery system. The encapsulation of siRNAs in lipidic nanoparticles illustrated repression of the virus in the lungs and improved the survival of treated mice suggesting a highly promising treatment approach against COVID-19 [40].

A major advantage of theranostic nanoparticles is to enhance the specific delivery of therapeutics into the target cells. Numerous theranostic nanoparticles have shown promising results to combat against various viral infections including SARS or MERS coronaviruses, which could be similarly applied to SARS- CoV-2 as coronaviruses have the same key spike proteins. [112]. Overall, nanoparticle assisted delivery ensures that therapeutics reach the targeted sites while minimise adverse reactions to the normal tissues. Various types of nanoparticles including organic (i.e. lipid, polymer, and dendrimer), inorganic (i.e. gold), and virus-like or self-assembling protein nanoparticles were investigated against viral infections including SARS or MERS coronaviruses. In each case, the physicochemical properties of nanoparticles such as shape, size and the surface charge could considerably affect the success of the treatment. One important issue is to design CoV-specific siRNAs with no cross-reactivity to the human genome. Despite exciting results, there are still many challenges to avoid the nonspecific toxicity of therapeutics. It has been reported that the main routes of toxicity that greatly contribute to the challenges of RNAi drug development include immunogenicity of siRNA, excipients toxicity, and nonspecific and off-target RNAi activity. Some of these challenges could be overcome by applying chemical components verified for low toxicity, and by opting specific delivery systems like lipid-based nanoparticles or non-viral vectors. Further, the sequence features of siRNAs might provide crucial essential information regarding the toxicity profile of the final therapeutic [66].

5 | CONCLUSION AND FUTURE PERSPECTIVES

Since the outbreak of COVID-19, concerted efforts have been made to develop effective drugs and vaccines against the new 2019 coronavirus. Existing information about other types of coronaviruses such as SARS and MERS were helpful in this regard, as they share many similar characteristics. Drug development for SARS-CoV-2 infection aims at identifying inhibitors that target the viral replication and alleviates symptomatic results of their infections leading to severe disease

and/or death. To reduce the inflammation in the lungs and other organs as the major contributing factor in the mortality of COVID-19, gene activation or silencing approaches have been extensively used. Since the introduction of RNAi in the late 1990s, this technology has become a fundamental tool for suppressing or silencing target genes related to virulence and pathogenesis. Antisense includes many types of sequences such as siRNA, ASOs, ribozyme, DNAzyme, and aptamers with different features and mechanisms of action. Many structural and non-structural viral genes were identified that can be targeted by RNAi thus, makes this area a potential therapeutic tool to combat the infection. The design of oligo therapeutics is cost-effective for manufacturing, and several chemical modifications make it easier to optimise them during an emergency when rapid drug development is vital. A huge body of evidences have identified the potential targets in SARS-CoV-2; however, the most appropriate sequence targets of SARS-CoV-2 remain unknown. Further, though the approval of recent siRNA-based therapies was promising for anti-viral agents, direct delivery of oligonucleotides to the lungs is still a big challenge, and finding safe and efficacious delivery vehicles needs further investigations.

CONFLICT OF INTEREST

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Maham Doagooyan https://orcid.org/0000-0003-2962-571X
Fatemeh Gheybi https://orcid.org/0000-0001-9209-251X

REFERENCES

1. Wang, L.-s., et al.: A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. p. 105948
2. Abbasi-Oshaghi, E., et al.: Diagnosis and treatment of coronavirus disease 2019 (COVID-19): laboratory, PCR, and chest CT imaging findings
3. Jin, Y., et al.: Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses. 12(4), 372 (2020)
4. Pan, F., et al.: Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia, p. 200370. Radiology (2020)
5. Glowacka, I., et al.: Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J. Virol. 85(9), 4122–4134 (2011)
6. Depfenhart, M., et al.: Potential new treatment strategies for COVID-19: is there a role for bromhexine as add-on therapy? Internal and Emergency Medicine, 1 (2020)
7. Ghosh, S., Firdous, S.M., Nath, A.: siRNA could be a potential therapy for COVID-19. EXCLI journal. 19, 528 (2020)
8. Le Mercier, P., et al.: ViralZone: recent updates to the virus knowledge resource, vol. 8. F1000Research (2019)
9. Ernst, T., et al.: Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat. Genet. 42(8), 722–726 (2010)
10. Yin, Y., Wunderink, R.G.: MERS, SARS and other coronaviruses as causes of pneumonia. Respirology. 23(2), 130–137 (2018)
11. Báez-Santos, Y.M., St John, S.E., Mesecar, A.D.: The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antiviral Res. 115, 21–38 (2015)
12. Chen, W., et al.: Computational identification of small interfering RNA targets in SARS-CoV-2. Virol. Sin. 1–3 (2020)
13. Kaczmarek, J.C., Kowalski, P.S., Anderson, D.G.: Advances in the delivery of RNA therapeutics: from concept to clinical reality. Genome Med. 9(1), 1–16 (2017)
14. Tiwari, P.M., et al.: Engineered mRNA-expressed antibodies prevent respiratory syncytial virus infection. Nat. Commun. 9(1), 1–15 (2018)
15. Svitkin, Y.V., et al.: N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. Nucleic Acids Res. 45(10), 6023–6036 (2017)
16. Shanmugaraj, B., et al.: Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pac. J. Allergy. Immunol. 38(1), 10–18 (2020)
17. Naqvi, R.A., Shukla, D., Naqvi, A.R.: SARS-CoV-2 targeting by RNAi and host complement inhibition: a two-pronged subterfuge for COVID-19 treatment. Immunity, inflammation and disease (2021)
18. Corbett, K.S., et al.: SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 586(7830), 567–571 (2020)
19. Laczkó, D., et al.: A single immunization with nucleoside-modified mRNA vaccines elicits strong cellular and humoral immune responses against SARS-CoV-2 in mice. Immunity. 53(4), 724–732 (2020). e7
20. Di Gennaro, F., et al.: Coronavirus diseases (COVID-19) current status and future perspectives: a narrative review. Int. J. Environ. Res. Publ. Health. 17(8), 2690 (2020)
21. Martinez, M.A.: Compounds with therapeutic potential against novel respiratory 2019 coronavirus. Antimicrob. Agents. chemothe. 64(5) (2020)
22. Kawase, M., et al.: Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. J. Virol. 86(12), 6537–6545 (2012)
23. Romano, M., et al.: A structural view of SARS-CoV-2 RNA replication machinery: RNA synthesis, proofreading and final capping. Cells. 9(5), 1267 (2020)
24. Joshi, R.S., et al.: Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J. Biomol. Struct. Dyn., 1–16 (2020)
25. Bafna, K., Krug, R.M., Montelione, G.T.: Structural similarity of SARS-CoV2 Mpro and HCV NS3/4A proteases suggests new approaches for identifying existing drugs useful as COVID-19 therapeutics. ChemRxiv (2020)
26. Shin, D., et al.: Inhibition of papain-like protease PLpro blocks SARS-CoV-2 spread and promotes anti-viral immunity (2020)
27. Best, R.B., Hummer, G.: Optimized molecular dynamics force fields applied to the helix− coil transition of polypeptides. J. Phys. Chem. B. 113(26), 9004–9015 (2009)
28. Chi, X., Gatti, P., Papoian, T.: Safety of antisense oligonucleotide and siRNA-based therapeutics. Drug. Discov. Today. 22(5), 823–833 (2017)
29. de Fougerolles, A., et al.: RNA interference in vivo: toward synthetic small inhibitory RNA-based therapeutics. In: Methods in enzymology, pp. 278–296. Elsevier (2005)
30. Elbashir, S.M., et al.: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 411(6836), 494–498 (2001)
31. Gitlin, L., Karelsky, S., Andino, R.: Short interfering RNA confers intracellular antiviral immunity in human cells. Nature. 418(6896), 430–434 (2002)
32. McCaffrey, A.P., et al.: Inhibition of hepatitis B virus in mice by RNA interference. Nat. Biotechnol. 21(6), 639–644 (2003)
33. Guidotti, L.G., et al.: Viral clearance without destruction of infected cells during acute HBV infection. Science. 284(5415), 825–829 (1999)
34. Wong, S.S., Yuen, K.: The severe acute respiratory syndrome (SARS). J. Neurovirol. 11(5), 455–468 (2005)

35. Yi, S., et al.: Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. Cell Res. 15(3), 193–200 (2005)
36. Li, B.-j., et al.: Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat. Med. 11(9), 944–951 (2005)
37. Reischl, D., Zimmer, A.: Drug delivery of siRNA therapeutics: potentials and limits of nanosystems. Nanomed. Nanotechnol. Biol. Med. 5(1), 8–20 (2009)
38. McCaskill, J., et al.: Efficient biodistribution and gene silencing in the lung epithelium via intravenous liposomal delivery of siRNA. Mol. Ther. Nucleic Acids. 2, e96 (2013)
39. Wu, S.Y., et al.: Development of a novel method for formulating stable siRNA-loaded lipid particles for in vivo use. Pharmaceut. Res. 26(3), 512–522 (2009)
40. Idris, A., et al.: A SARS-CoV-2 targeted siRNA-nanoparticle therapy for COVID-19. Molecular Therapy (2021)
41. Pinnaduwage, P., Schmitt, L., Huang, L.: Use of a quaternary ammonium detergent in liposome mediated DNA transfection of mouse L-cells. Biochim. Biophys. Acta Biomembr. 985(1), 33–37 (1989)
42. Kong, W.H., et al.: Cationic solid lipid nanoparticles derived from apolipoprotein-free LDLs for target specific systemic treatment of liver fibrosis. Biomaterials. 34(2), 542–551 (2013)
43. Yonezawa, S., Koide, H., Asai, T.: Recent advances in siRNA delivery mediated by lipid-based nanoparticles. Adv. Drug Deliv. Rev. (2020)
44. Lee, S., et al.: Solid polymeric microparticles enhance the delivery of siRNA to macrophages in vivo. Nucleic Acids Res. 37(22) p. e145, (2009)
45. Ahn, S.H., et al.: AGO CLIP-based imputation of potent siRNA sequences targeting SARS-CoV-2 with antifibrotic miRNA-like activity. Sci. Rep. 11(1), 1–15 (2021)
46. Chang, Z., Babiuk, L.A., Hu, J.: Therapeutic and prophylactic potential of small interfering RNAs against severe acute respiratory syndrome. BioDrugs. 21(1), 9–15 (2007)
47. Holmes, K.V.: SARS-associated coronavirus. N. Engl. J. Med. 348(20), 1948–1951 (2003)
48. Ruan, Y., et al.: Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet. 361(9371), 1779–1785 (2003)
49. Qin, Z.-l., et al.: Silencing of SARS-CoV spike gene by small interfering RNA in HEK 293T cells. Biochem. biophys. res. commun. 324(4), 1186–1193 (2004)
50. Wu, C.-J., et al.: Inhibition of SARS-CoV replication by siRNA. Antivir. Res. 65(1), 45–48 (2005)
51. Chang, Z., Babiuk, L.A., Hu, J.: Therapeutic and prophylactic potential of small interfering RNAs against severe acute respiratory syndrome: progress to date. BioDrugs: clinical immunotherapeutics, biopharmaceuticals and gene therapy. 21(1), 9–15 (2007)
52. Ullah, A., et al.: Nanoparticles-assisted delivery of antiviral-siRNA as inhalable treatment for human respiratory viruses: a candidate approach against SARS-COV-2. Nano Select. 1(6), 612–621 (2020)
53. Dyall, J., et al.: Middle East respiratory syndrome and severe acute respiratory syndrome: current therapeutic options and potential targets for novel therapies. Drugs. 77(18), 1935–1966 (2017)
54. Liu, C., et al.: Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Publications (2020)
55. Chowdhury, U.F., et al.: A computational approach to design potential siRNA molecules as a prospective tool for silencing nucleocapsid phosphoprotein and surface glycoprotein gene of SARS-CoV-2. bioRxiv (2020)
56. Khaitov, M., et al.: Silencing of SARS-CoV-2 with modified siRNA-peptide dendrimer formulation. Allergy (2021)
57. Lu, C.-Y., et al.: 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. 27(8), 709–715 (2008)
58. Hoffmann, M., et al.: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell (2020)
59. Lei, C., et al.: Potent neutralization of 2019 novel coronavirus by recombinant ACE2-Ig. BioRxiv (2020)
60. He, M.-L., et al.: Kinetics and synergistic effects of siRNAs targeting structural and replicase genes of SARS-associated coronavirus. FEBS Lett. 580(10), 2414–2420 (2006)
61. Lu, C.Y., et al.: 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: official publication of the European Society of Clinical Microbiology. 27(8), 709–715 (2008)
62. Chang, Y.-C., et al.: Potential therapeutic agents for COVID-19 based on the analysis of protease and RNA polymerase docking (2020)
63. Wu, F., et al.: A new coronavirus associated with human respiratory disease in China. Nature. 579(7798), 265–269 (2020)
64. Gu, S.H., et al.: A Small interfering RNA lead targeting RNA-dependent RNA-polymerase effectively inhibit the SARS-CoV-2 infection in Golden Syrian hamster and Rhesus macaque. bioRxiv (2020)
65. Wu, C.-J., Chan, Y.-L.: Antiviral applications of RNAi for coronavirus. Expet. Opin. Invest. Drugs. 15(2), 89–97 (2006)
66. Uludağ, H., et al.: Prospects for RNAi therapy of COVID-19. Front. Bioeng. Biotechnol. 8, 916 (2020)
67. Zhang, R., et al.: Inhibiting severe acute respiratory syndrome-associated coronavirus by small interfering RNA. Chin. Med. J. 116(08), 1262–1264 (2003)
68. Li, T., et al.: siRNA targeting the leader sequence of SARS-CoV inhibits virus replication. Gene. Ther. 12(9), 751–761 (2005)
69. Selvam, C., et al.: Therapeutic potential of chemically modified si RNA: recent trends. Chem. Biol. Drug Des. 90(5), 665–678 (2017)
70. Kumar, B., et al.: Nucleic acid-mediated cleavage of M1 gene of influenza A virus is significantly augmented by antisense molecules targeted to hybridize close to the cleavage site. Mol. Biotechnol. 51(1), 27–36 (2012)
71. Asha, K., et al.: Advancements in nucleic acid based therapeutics against respiratory viral infections. J. Clin. Med. 8(1), 6 (2019)
72. Kumar, V., Jung, Y.S., Liang, P.H.: Anti-SARS coronavirus agents: a patent review (2008 - present). Expert. Opin. Ther. Pat. 23(10), 1337–1348 (2013)
73. Fukuda, N., et al.: Ribozyme to cleave coronavirus gene. US Patent 20100273997 (2010)
74. Asha, K., et al.: Advancements in nucleic acid based therapeutics against respiratory viral infections. J. Clin. Med. 8(1), 6 (2018)
75. Fukushima, A., et al.: Development of a chimeric DNA-RNA hammerhead ribozyme targeting SARS virus. Intervirology. 52(2), 92–99 (2009)
76. Ohkawa, J., et al.: Ribozymes: from mechanistic studies to applications in vivo. J. Biochem. 118(2), 251–258 (1995)
77. Vaish, N.K., Kore, A.R., Eckstein, F.: Recent developments in the hammerhead ribozyme field. Nucleic. Acids. Res. 26(23), 5237–5242 (1998)
78. Scherer, L., Rossi, J., Weinberg, M.: Progress and prospects: RNA-based therapies for treatment of HIV infection. Gene. Ther. 14(14), 1057–1064 (2007)
79. Puerta-Fernandez, E., et al.: Inhibition of HIV-1 replication by RNA targeted against the LTR region. Aids. 19(9), 863–870 (2005)
80. Buzayan, J.M., Gerlach, W.L., Bruening, G.: Non-enzymatic cleavage and ligation of RNAs complementary to a plant virus satellite RNA. Nature. 323(6086), 349–353 (1986)
81. Heidenreich, O., et al.: Ribozyme-mediated RNA degradation in nuclei suspension. Nucleic. Acids. Res. 23(12), 2223–2228 (1995)
82. Shi, Y., et al.: Antisense downregulation of SARS-CoV gene expression in Vero E6 cells. J. Gene. Med. 7(1), 97–107 (2005)
83. Youngren-Ortiz, S.R., et al.: Aerosol delivery of siRNA to the lungs. Part 1: rationale for gene delivery systems. KONA. Powder. Part. J, 2016014 (2016)

84. Lim, T.W., et al.: Nucleic-acid-based antiviral agents against positive single-stranded RNA viruses. Curr. Opin. Mol. Therapeut. 8(2), 104–107 (2006)
85. Saran, R., Liu, J.: A silver DNAzyme. Anal. Chem. 88(7), 4014–4020 (2016)
86. Feng, Q.-M., et al.: Self-assembled DNA tetrahedral scaffolds for the construction of electrochemiluminescence biosensor with programmable DNA cyclic amplification. ACS Appl. Mater. Interfaces. 9(20), 17637–17644 (2017)
87. Dobrovolskaia, M.A., Bathe, M.: Opportunities and challenges for the clinical translation of structured DNA assemblies as gene therapeutic delivery and vaccine vectors, pp. e1657. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology (2020)
88. Wu, S., et al.: An efficient RNA-cleaving DNA enzyme can specifically target the 5'-untranslated region of severe acute respiratory syndrome associated coronavirus (SARS-CoV). J. Gene Med. 9(12), 1080–1086 (2007)
89. Wu, S., et al.: An efficient RNA-cleaving DNA enzyme can specifically target the 5′-untranslated region of severe acute respiratory syndrome associated coronavirus (SARS-CoV). J. Gene Med: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications. 9(12), 1080–1086 (2007)
90. Liebich, S.: Potent SELEX aptamer-based therapeutic method for novel SARS-CoV2 virus disease (COVID-19)
91. Chen, A., Yang, S.: Replacing antibodies with aptamers in lateral flow immunoassay. Biosens. Bioelectron. 71, 230–242 (2015)
92. Eissa, S., et al.: Electrochemical SELEX technique for the selection of DNA aptamers against the small molecule 11-deoxycortisol. ACS Applied Bio Materials. 2(6), 2624–2632 (2019)
93. Rossi, J.J., Rossi, D.: Oligonucleotides and the COVID-19 pandemic: a perspective. Nucleic Acid Therapeut. (2020)
94. Metelev, V., Oretskaya, T.: Modified oligonucleotides: new structures, new properties, and new spheres of application. Russ. J. Bioorg. Chem. 47(2), 339–343 (2021)
95. Wandtke, T., Woźniak, J., Kopiński, P.: Aptamers in diagnostics and treatment of viral infections. Viruses. 7(2), 751–780 (2015)
96. Jang, K.J., et al.: Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. Biochem. biophys. res. commun. 366(3), 738–744 (2008)
97. Shum, K.T., Tanner, J.A.: Differential inhibitory activities and stabilisation of DNA aptamers against the SARS coronavirus helicase. Chembiochem. 9(18), 3037–3045 (2008)
98. Sajid, M.I., et al.: Overcoming barriers for siRNA therapeutics: from bench to bedside. Pharmaceuticals. 13(10), 294 (2020)
99. Sajid, M.I., et al.: siRNA therapeutics for the therapy of COVID-19 and other coronaviruses. Mol. Pharm. 18(6), 2105–2121 (2021)
100. Chang, Z., Babiuk, L.A., Hu, J.: Therapeutic and prophylactic potential of small interfering RNAs against severe acute respiratory syndrome: progress to date. BioDrugs. 21(1), 9–15 (2007)
101. Chang, Z., Hu, J.: RNAi therapeutics: can siRNAs conquer SARS? Gene. Ther. 13(11), 871–872 (2006)
102. Niktab, I., et al.: Design of advanced siRNA therapeutics for the treatment of COVID-19. Meta Gene. 29, 100910 (2021)
103. Medeiros, I.G., et al.: A small interfering RNA (siRNA) database for SARS-CoV-2. Sci. Rep. 11(1), 1–10 (2021)
104. Hodgson, J.: The pandemic pipeline. Nat. Biotechnol. 38(5), 523–532 (2020)
105. Dykxhoorn, D.M., Lieberman, J.: Knocking down disease with siRNAs. Cell. 126(2), 231–235 (2006)
106. Conti, D.S., et al.: Poly (amidoamine) dendrimer nanocarriers and their aerosol formulations for siRNA delivery to the lung epithelium. Mol. Pharm. 11(6), 1808–1822 (2014)
107. Kruse, R.L.: Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China. F1000Research, 9, p. 72-72 (2020)
108. Aljaberi, A., et al.: siRNA as a potential therapy for COVID-19. Current Drug Delivery (2021)
109. Itani, R., Tobaiqy, M., Al Faraj, A.: Optimizing use of theranostic nanoparticles as a life-saving strategy for treating COVID-19 patients. Theranostics. 10(13), 5932–5942 (2020)
110. Habtemariam, S., et al.: Lessons from SARS and MERS remind us of the possible therapeutic effects of implementing a siRNA strategy to target COVID-19: shoot the messenger!. J. Cell Mol. Med. 24(17), 10267–10269 (2020)
111. Itani, R., Tobaiqy M., Al Faraj A.: Optimizing use of theranostic nanoparticles as a life-saving strategy for treating COVID-19 patients
112. Li, Y., et al.: RNA interference functions as an antiviral immunity mechanism in mammals. Science. 342(6155), 231–234 (2013)

How to cite this article: Alavizadeh, S.H., et al.: Antisense technology as a potential strategy for the treatment of coronaviruses infection: With focus on COVID-19. IET Nanobiotechnol. 16(3), 67–77 (2022). https://doi.org/10.1049/nbt2.12079