Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

Harnessing aptamers against COVID-19: A therapeutic strategy

Ali Mahmoudi [1,8], Seyedeh Hoda Alavizadeh [2,3,8], Seyedeh Atefeh Hosseini [1], Pouria Meidany [2], Maham Doagooyan [1], Yasaman Abolhasani [1], Zakieh Saadat [1], Fatemeh Amani [1], Prashant Kesharwani [4,5], Fatemeh Gheybi [1,2,*], Amirhossein Sahebkar [6,7,*]

[1] Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Iran
[2] Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India
[5] Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Chennai, India
[6] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[7] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

The novel coronavirus crisis caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) was a global pandemic. Although various therapeutic approaches were developed over the past 2 years, novel strategies with more efficient applicability are required to target new variants. Aptamers are single-stranded (ss)RNA or DNA oligonucleotides capable of folding into unique 3D structures with robust binding affinity to a wide variety of targets following structural recognition. Aptamer-based theranostics have proven excellent capability for diagnosing and treating various viral infections. Herein, we review the current status and future perspective of the potential of aptamers as COVID-19 therapies.

Keywords: Aptamers; COVID-19; Therapeutic applications

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the infectious pathogen SARS-CoV-2, resulted in one of the largest disease outbreaks of the 21st century. The ssRNA coronavirus causes lethal respiratory tract infections and is still in circulation through novel SARS-CoV-2 variants. SARS-CoV-2 is efficiently transmitted between humans through airway droplets or direct contact. Given its high transmissibility and the enormous death toll it caused worldwide, COVID-19 is considered a severe threat to global health. Numerous attempts have been made to discover its pathogenetic mechanisms, develop rapid indicative tools, efficacious preventive and therapeutic medications, and to determine cellular processes that could affect susceptibility to SARS-CoV-2 and drug response.[1]

Numerous antiviral drugs, antiparasitic agents, anti-inflammatory drugs,[2,3] convalescent plasma, as well as supportive measures, including O_2 therapy and antibiotics, were used to manage the disease. Urgent vaccine approval helped curb the spread of COVID-19 and prevent further transmission of SARS-CoV-2.[4] Furthermore, the development of novel preventive

* *Corresponding authors: Gheybi, F. (gheybif@mums.ac.ir), Sahebkar, A. (amir_saheb2000@yahoo.com).*
[8] Contributed equally to this work as first authors.

https://doi.org/10.1016/j.drudis.2023.103663

and/or therapeutic strategies for COVID-19 has been a research focus.

Aptamers are single-stranded oligonucleotides with high affinity and specificity for their target molecules. They have various applications, including bioimaging, and therapeutic potential, and are used as recognition elements in numerous detecting platforms.[5,6] Aptamers are randomly established from a library through the systematic evolution of ligands via exponential enrichment (SELEX).[7] They are 2–3 nm in diameter, which is smaller than antibodies, leading to les steric interference with the shallow coronavirus (~100 nm in diameter).[8] Their smaller size allows for their efficient binding to numerous molecules on various parts of the coronavirus. Given the chemical features of nucleic acids, aptamers could be chemically created and accurately altered with high thermal stability and minor batch-to-batch variations.[9] Furthermore, combining aptamers with other advanced technologies, including clustered regularly interspaced short palindromic repeats (CRISPR)/Cas and diagnostic applications of pathogens, has been reported.[10] These characteristics aim to achieve a more suitable transference, standardization, and storage.[11] Despite the extensive utility of antibody-based drugs and detection techniques in the clinic, their acceptance rate is limited because of their high price, antibody instability, and a variety of target restrictions.[12,13] Aptamers have shown the potential to target pathogens in a wide variety of infectious diseases,[14] including those of respiratory syncytial virus (RSV) and SARS.[15] In this review, we summarize several research approaches investigating the therapeutic potential of aptamers as promising tools for the therapy of SARS-CoV-2.

SELEX in aptamers

Aptamers are manufactured through an *in vitro* procedure known as SELEX, in which aptamers are isolated explicitly for the desired target. SELEX relies on replicative rounds of binding, amplification, and separating of nucleotides. The process is initiated by incubating the sequence pool with targets, such as nucleic acids, proteins, and other molecules of interest. The sequence pool comprises a nucleic acid library containing 10^{14}–10^{15} random 30–100-nucleotide variants flanked by preserved regions. The haphazard section includes the sequences with high affinity and high specificity to the unique target. In the next step, the binding sequences are preserved from the target, whereas the released nucleotides are excluded. The third phase is to create a recent sequence pool for the following round by purifying and amplifying the bound sequences. The SELEX method typically requires repeating rounds of selection and amplification (8–15 times) to obtain aptamer sequences with high affinity. Each round includes incubation of the sequence pool with the target molecules to allow binding. This is followed by the separation of bound (desired) and unbound (undesirable) sequences. The unwanted sequences, nontarget binders, and unbound aptamers are removed. Sometimes, the adverse selection step is carried out before incubation with the target, and sometimes after, to improve the specificity of the selected aptamers (Figure 1). Biotech companies provide comprehensive support for aptamer discovery and development. They facilitate aptamer selection by generating sequence pools and performing SELEX rounds. They synthesize selected aptamer sequences and chemically modify them to optimize properties. SELEX has been widely applied to develop aptamers against pathogenic microorganisms. However, the low variety of the DNA library sequence, contamination of target cells or PCR reagents, rigid nonspecific binding, low frequency of cellular target molecules, and alterations of cellular state, can adversely affect the development of aptamer sequences.[16]

Aptamer applications in viral infection

Aptamers have been described as robust research tools for the detection and therapy of viral infections.[17,18] A DNA aptamer was recently designed against the human herpes virus containing a similar sequence to an exon sequence of the gene encoding *N*-sulfoglucosamine sulfohydrolase (*SGSH*), which demonstrated a regulatory role in the blockade of the virus. Furthermore, a highly efficient aptamer was designed to recognise the Ebola virus (EBOV). The detection procedure involves sample mixing, separation, and signal acquisition, which are highly integrated and directed in a magnetism-controlled detection chip, displaying high biosafety and excessive potential for point-of-care detection.[19] A recent study designed and optimized 15 RNA aptamers that bind to the C-terminus helical region of the GP2 subunit of EBOV, consequently preventing the entry of EBOV into the target cell by constraining viral fusion with the cell membrane.[20] Recent studies demonstrated the potential for aptamers in therapies targeting SARS-CoV andSARS-CoV-2 (Table 1).

Therapeutic aptamers

SARS-CoV-2 structural features comprise a single positive strand of RNA, constituting its genome, which is covered by nucleocapsid (N) protein. The 30-kb viral genome comprises an open reading frame (ORF) 1a and 1bb at the 5′-end, which encode nonstructural proteins (pp1a and pp1ab). The 3′-end of the genome encodes essential structural proteins, such as spike glycoprotein (S), envelope protein (E), N protein, membrane protein (M), as well as accessory proteins (ORF 3, 6, 7a, 7b, 8, and 9b). The virus uses its S protein to attach to angiotensin-converting enzyme (ACE2) receptors, which are highly concentrated in the lungs and intestine[21] (Figure 2). Here, we provided an overview of the therapeutic applications of aptamers based on various proteins of SARS-CoV-2.

Aptamers interfering with the S protein–ACE2 interaction

The S protein condenses the specific spikes on the surface of SARS-CoV-2. It is responsible for virus entry to the host cell by fusing it with the plasma membrane. The protein has two significant subdomains: S1 and S2. S1 contains a receptor-binding domain (RBD) and is responsible for providing stability during the perfusion state. S2 contains all components that are used for fusion (Figure 2). S protein, primarily its S1 domain, is highly immunogenic and essential for host cell infection and, thus, has been the target of frequent antibody and vaccine research. Previous research showed that COVID-19 binds to host cells through interactions with human ACE2.[22]

Oligonucleotide-based strategies can be best performed by either hindering viral S protein interactions or interfering with

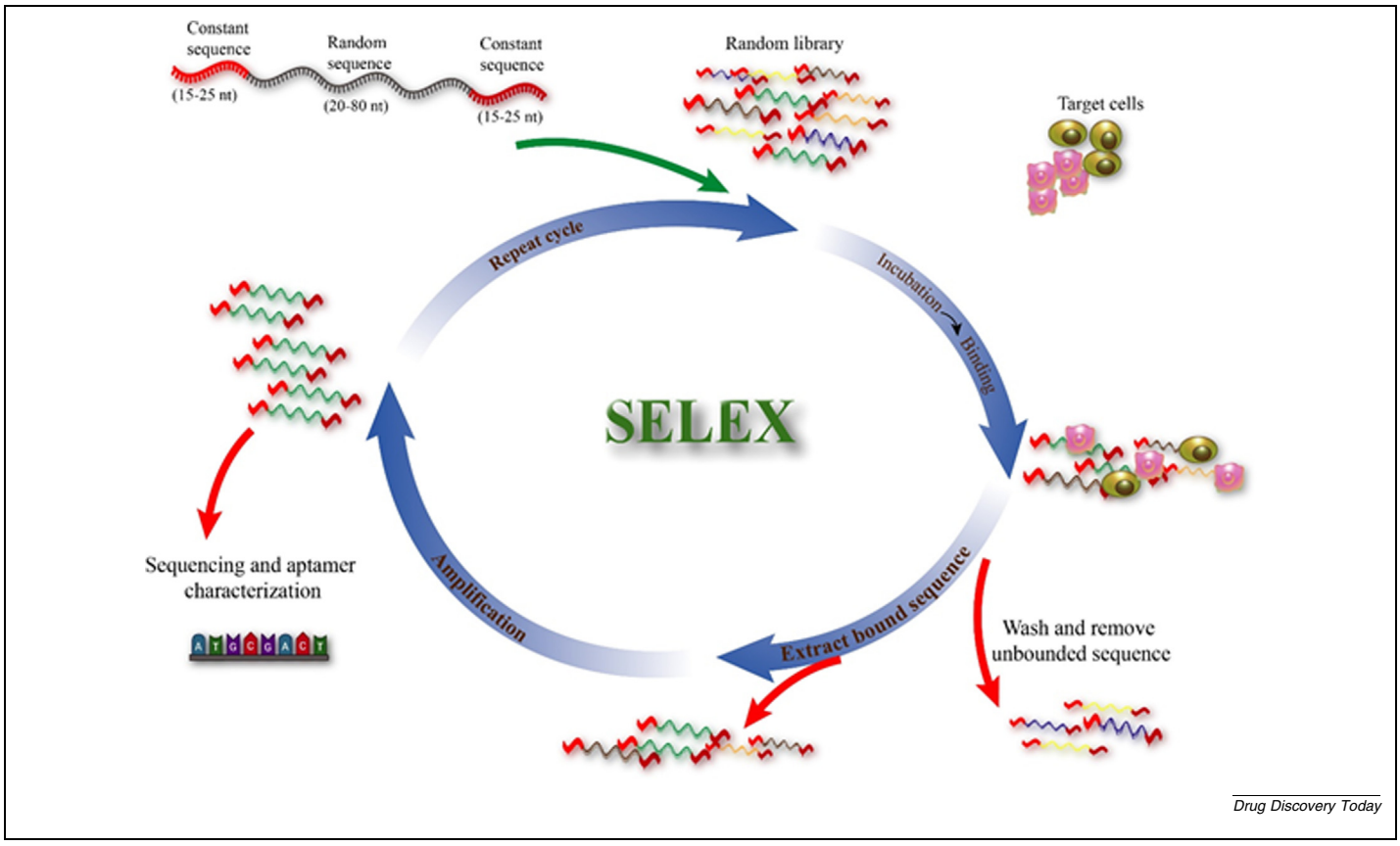

FIGURE 1

Systematic evolution of ligands via exponential enrichment (SELEX) begins with the production of various libraries of DNA or RNA molecules; then, the library is incubated alongside the targets to assemble aptamers and target structures. After that, the libraries are multiplied with PCR for subsequent enrichment rounds, with oligonucleotides separated out from the target attached aptamers. Multiple rounds are run until the library converges on to a pool of sequences with affinity to the target. In the final step, the enriched aptamer sequences are identified by sequencing.

its replication. The former approach is achieved by the aptamer via the protein–nucleic acid interface or by direct targeting of the genome through antisense oligonucleotide (ASO)-mediated gene silencing or small interfering RNA (siRNA). Gene silencing methods have shown to decrease the inflammatory aftermath in the lungs in patients critically ill with COVID-19. Rossi *et al.* theorized that highly preserved regions inside the replicate polyprotein-encoding regions of the SARS-COV-2 genome could be desirable gene silencing targets. Another preference is to use aptamers for inhibiting ACE2 (Figure 3). Aptamers have a faster development time compared with protein-based SELEX, and can quickly identify candidate sequences with high target specificity and sensitivity in a short period. This supports the vision of using aptamers for various applications.[23]

In recent years, the simple selection processes and structural stability of several DNA aptamers, such as CoV2-6C3, cb-CoV2-6C3, aptamer-1, aptamer-2, and nCoV-S1-Apt1, have gained attention. However, RNA aptamers have more complex 3D structures.[14] Although both SARS-CoV and SARS-CoV-2 S proteins have general structural homology, none of the three SARS-CoV RBD-directed monoclonal antibodies could bind to SARS-CoV-2 RBD. Consequently, there is a crucial need to recognize efficient identification molecules for SARS-CoV-2 RBD. Although neutralizing antibodies have been extensively applied in biology and clinical medicine, aptamers have various advantages, including less immunogenicity as well as facile engineering and chemical modification. SARS-CoV-2 binds to host cells via the interface of the S protein and ACE2 on host cells. Following receptor detection, the virus genome along with its nucleocapsid is released into the cytoplasm of the host cell. PP1a and PP1ab are two factors that facilitate the control of host ribosomes in the translation of viral proteins.

Using an ACE2 competition-based aptamer selection approach, Song *et al.* showed an improved K_d value of CoV2-RBD-1C and CoV2-RBD-4C aptamers with limit of detection (LOD) of 5.8 nM and 19.9 nM, respectively. Simulated interaction modeling proposed that the two aptamers could have the same binding sites on ACE2 or SARS-CoV-2 RBD. Moreover, these aptamers provided a prospect for generating new probes for the identification of SARS-CoV-2. They could aid in diagnosing and treating SARS-CoV-2 by creating a novel means for in-depth research of the coronavirus infection procedure.[11]

ssDNA-aptamer (BC 007) was shown to be capable of neutralizing pathogenic autoantibodies[24,25] because they occur in the heart as well as circulatory system diseases.[26] BC 007 showed a perfect safety profile in Phase I trials (NCT02955420), which led to its successful entry into Phase II clinical testing.[27] BC 007 can also bind to vital proteins of SARS-CoV-2, including

TABLE 1

Summary of antiviral aptamers designed for SARS-COV therapeutic applications

Selected aptamer	Aptamer type	Target	Organism	Refs
NG1	ssDNA	Helicase	SARS-COV	41
NG3	ssDNA	Helicase	SARS-COV	41
3′-Inverted thymidine aptamer NG8	ssDNA	Helicase	SARS-COV	41
30-biotin aptamer NG8	ssDNA	Helicase	SARS-COV	41
ES15-(1,2,3,4,5,6)	RNA	NTPase/helicase	SARS-COV	42
	Peptide	S protein	SARS-COV-2	30
BC-007	ssDNA	S protein, RNA polymerase	SARS-COV-2	29

DNA-susceptible peptide structures, such as the RNA-dependent RNA polymerase of SARS-CoV-2 and the RBD of the S protein (repurposing). Overall, BC 007 displayed specific binding for both sequences in a therapy-relevant concentration range, as shown by nuclear magnetic resonance (NMR), circular dichroism (CD) spectroscopy, and isothermal titration calorimetry (ITC) analyses.[28]

In a recent study, an 18-residue-long peptide aptamer was designed using bacterial Thioredoxin A as the scaffold protein. The peptide–aptamer tertiary structure was prepared and docked to the RBD of the SARS-CoV-2 S protein. Its binding was found to be similar to the pattern of ACE2 binding. Moreover, it showed remarkable stability in a 100 ns (number of nanoseconds) molecular dynamics simulation. Overall, it was suggested that this peptide aptamer could be considered for the treatment of SARS-CoV-2.[29] Recently, highly resistant 2′-fluoro-arabino nucleic acid (FANA), and xeno-nucleic acid (XNA) aptamers formed by SELEX through targeting the SARS-CoV-2 S-protein RBD were shown to strongly block the RBD and the subsequent ACE2 binding.[30]

Neutralizing antibodies against SARS-CoV-2 have several drawbacks, including their potential for antibody-dependent enhancement (ADE) and their large size, which makes them unsuitable for intranasal delivery. To avoid these limitations, CoV2-6 aptamer was applied to inhibit ACE2 binding to S-protein RBD by using molecular docking. It has been indicated that, by engineering aptamer binding to the region of S-protein RBD that is involved in ACE2–receptor engagement, SARS-CoV-2 infection could be successfully blocked. In further studies, CoV2-6 was engineered as a circular bivalent aptamer (cb-CoV2-6C3) to improve aptamer stability, binding potential, and efficacy. cb-CoV2-6C3 revealed efficient binding to S-protein RBD (Kd: 0.13 nM), and considerable SARS-CoV-2 virus inhibition (IC_{50}: 0.42 nM). Furthermore, the cb-CoV2-6C3 aptamer was stable in serum for 12 h and was preserved at room temperature for 14 days. Overall, the results indicated that CoV2-6 could prevent ACE2 binding to S-protein RBD, inhibiting SARS-CoV-2 infection.[31] Other bead-based SELEX experiments indicated the potential of DNA aptamers in targeting purified RBD proteins with high affinity and further proved the blockade of RBD–ACE2 interaction and host cell infection.[32] Researchers used the capillary electrophoresis (CE)-based SELEX method to develop a range of DNA aptamers, including nCoV-S1-Apt1, which can target the RBD S1 subunit and disrupt the interaction between S1 and ACE2.[33]

Among various RNA aptamers, RBD-PB6 indicated efficient viral blockade by inhibiting RBD binding to the ACE2 receptor. This aptamer has a strong affinity (in the nanomolar range) for the S protein of the beta variant of SARS-CoV-2 and can effectively inhibit the alpha variants with K_d values of 38 and 12 nM, respectively. Additionally, a 2′-fluoropyrimidine-modi fied aptamer was found to be effective against both SARS-CoV-2 and wild-type virus-like particles (VLPs), including the D614G spike variants. It did not show any cross-reactivity to Middle East respiratory syndrome (MERS) or SARS-CoV-1 S proteins.[34]

To dominate the mutations frequently occurring in the RBD of SARS-CoV-2 variants, Sun *et al*. used a cocktail of three neutralizing aptamers (CoV2-RBD-1C, CoV2-RBD-4C, and CoV2-6C3) by attaching them to a gold nanoparticle (gold NP) core. The conjugated multivalent aptamer system showed improved stability because of the nuclease deactivation feature of gold NPs and efficiently blocked various epitopes on the S proteins. The aptamer–gold NP conjugate demonstrated an affinity within the pM range and showed efficient toxicity against various variants, including pseudotyped SARS-CoV-2 N501Y, D614G, K417N: E484K:N501Y [35], and Omicron.[36] A recent study identified DNA-based aptamers known as slow off-rate modified aptamers (SOMAmers) that can bind to fragments of the SARS-CoV-2 S protein (S1, S2, and RBD). Several of these aptamers were capable of preventing the S protein from binding to ACE2 and neutralized the virus *in vitro*. The most potent aptamer in this research was SL1111, which inhibits spike–ACE2 binding and neutralizes all tested variants. SL1111 appears to bind to a spike surface conserved across variants. These aptamers showed promise as COVID-19 diagnostics or therapeutics tools, and SL1111 is being developed as a candidate treatment requiring *in vivo* testing.[37]

Helicase enzyme

Viral helicase has a crucial role in viral genome replication, which could be considered a probable target for therapeutic applications against viral pathogens. The SARS-CoV helicase involves various biological processes, including recombination, conformational catalytic procedures, replication, chromatin remodeling, Holliday movement junctions, and DNA repair. Therefore, SARS-CoV NTPase/Helicase (nsP10) could be a potential target for developing anti-SARS-CoV agents.[38,39] Different drugs can target helicase and prevent its function by obstructing its binding to the nucleic acids.[40] The study presented an attractive RNA aptamer library for nsP10 of SARS. This RNA aptamer efficiently inhibited the nucleic acid activity of the helicase and ATPase[41] as an anti-SARS-CoV agent applicant. Given the high resemblance of the nsp10 sequence arrangement in coron-

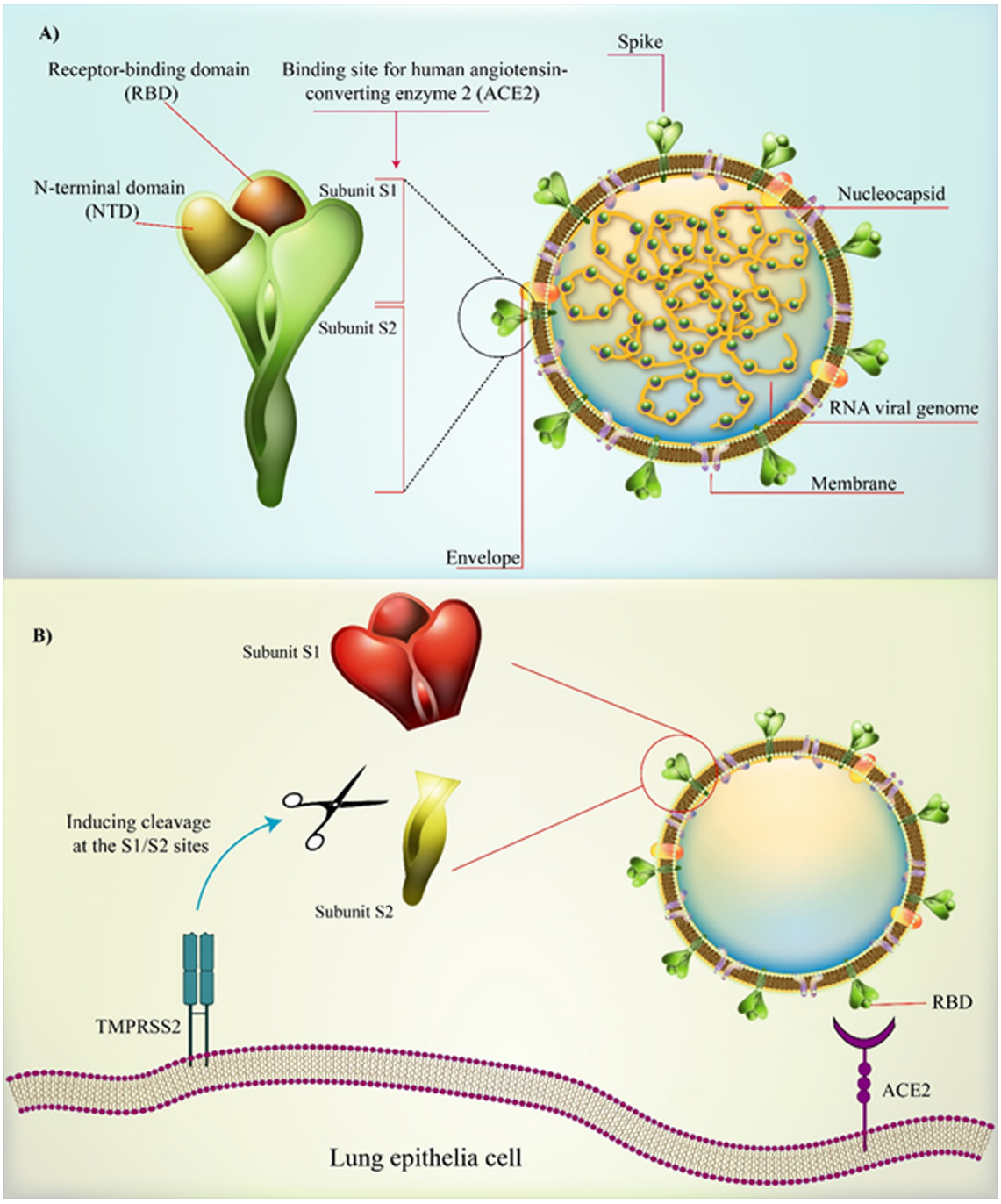

FIGURE 2

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) structural features and possible targets for detection and treatment. SARS-CoV-2 comprises four crucial structural proteins: envelope (E), membrane (M), nucleocapsid (N), and spike glycoprotein (S). The S protein is a suitable target for aptamer selection because it has as an essential role in the pathogenesis of the virus. The S protein comprises three identical monomers and two distinct subunits: the S1 subunit, located at the external layer of the virus, contains a receptor-binding domain (RBD) and an N-terminal domain (NTD), wherasS2 is a membrane-bound subunit **(a).** The RBD is the binding site for human angiotensin-converting enzyme 2 (ACE2), which mediates host cell recognition. Transmembrane protease serine 2 (TMPRSS2) activates the S protein by cleavage at the S1/S2 sites, enhancing viral entry within the host cell **(b)**.

aviruses, it is proposed that it could also be investigated against SARS-CoV-2.[42] Another therapeutic DNA aptamer has also been recently introduced against the SARS-CoV nsp13 protein, which could capably hinder the role of ATPase in the SARS-CoV helicase.[43]

Other targets

Previous investigations utilized DNA and nuclease-resistant RNA aptamers to target the N protein of SARS-CoV.[44,45] However, given the current situation, the conserved nature of the N protein, and its affinity for nucleic acids, the modular and customiz-

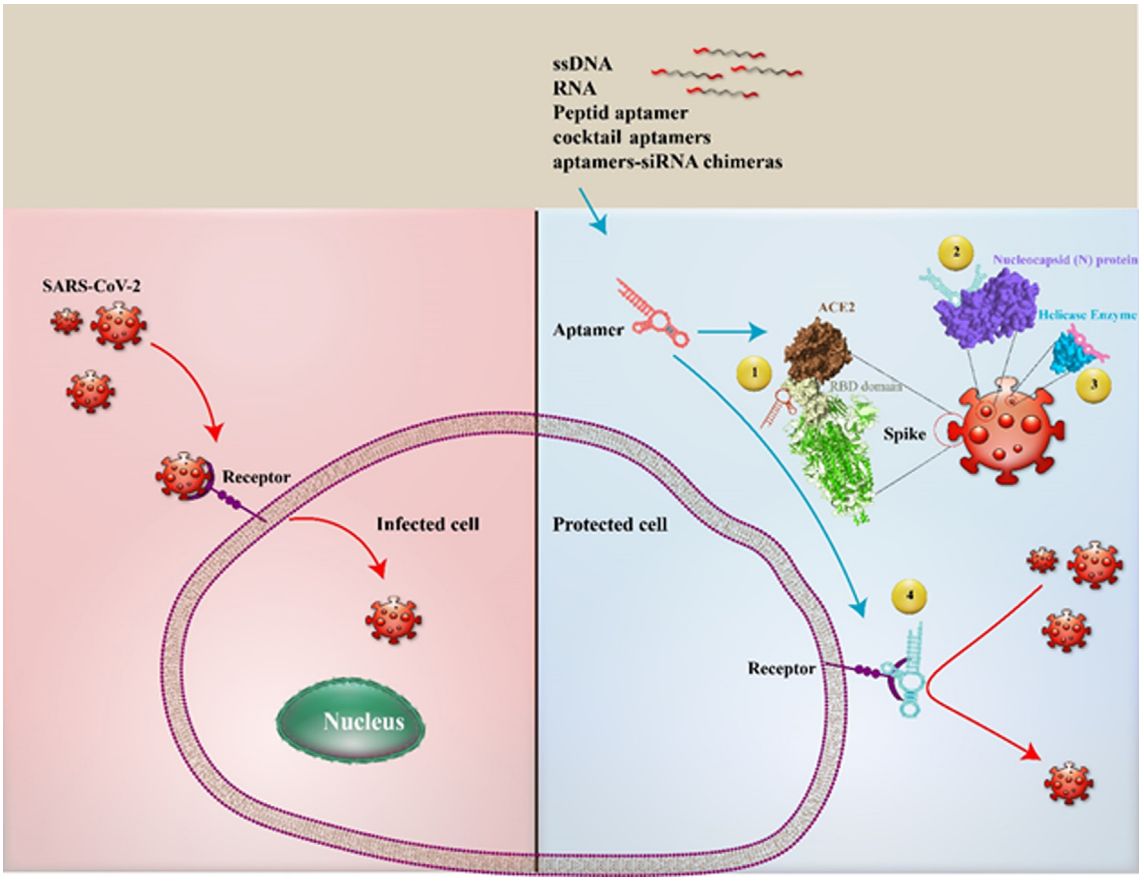

FIGURE 3

Therapeutic mechanisms of aptamers for treating Coronavirus 2019 (COVID-19). (1) Blocking the receptor-binding domain (RBD) on severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2); (2) blocking nucleocapsid (N) proteins on SARS-CoV-2; and (3) blocking the helicase of SARS-CoV-2; and (4) blocking human angiotensin-converting enzyme 2 (ACE2) on lung epithelial cells.

able properties of aptamers make them appealing for repurposing and developing into a potential therapeutic molecule against SARS-CoV-2.[46] Additionally, there are aptamers designed to target the S protein, which has been shown to block S-TLR4 signaling involved in SARS-CoV-2 pathogenesis and inflammation. The ST-6 aptamer, along with its two truncated versions (ST-6–1 and ST-6–2), exhibited promising results as a potential therapeutic candidate for SARS-CoV-2 therapy.[47]

Aptamer conjugation with advanced technology: new insight into COVID-19 therapeutics

Given the success of genome-targeting strategies and accessibility to the RNA genome sequence of SARS-CoV-2 (GenBank: MN908947), one strategy could be to target the viral RNA genome for degradation. Therefore, using siRNAs, RNA aptamers or ASOs, and nucleoside analogs to the SARS-CoV RNA genome could provide essential insights and promising therapeutic targets for SARS-CoV-2 treatment.[48] Numerous aptamer-mediated siRNA delivery systems have been advanced to improve RNA interference (RNAi) effectiveness for targeted delivery. Nevertheless, effective and harmless delivery of siRNA into the target cells remains a major concern for the clinical progress of siRNA-based therapeutics.[49]

Aptamer conjugation to siRNAs, ASOs, and CRISPR has emerged as a promising strategy for targeted delivery of these therapeutic agents. This approach involves the attachment of an aptamer with binding affinity to a specific cell surface marker to the therapeutic agent, allowing for targeted delivery to the desired cells. Therefore, one promising strategy in COVID-19 therapy is the conjugation of aptamers to siRNA, which could be attained by either covalent linkage or complementation (e.g., annealing), resulting in aptamer–siRNA chimeras. Aptamers with binding affinity to SARS-CoV-2 proteins, including the S protein, for instance, could serve as targeted delivery agents for siRNAs, ASOs, and other therapeutics that inhibit vital viral functions. By providing specific guidance to the virus-infected cells, aptamers could enhance their potency, reduce off-target effects, and prolong the duration of action against COVID-19. A recent study developed a circular aptamer-ASO chimera (circSAptNASO) containing an aptamer that binds the S protein and an ASO that inhibits the N gene. The chimera inhibited both S-induced inflammation and viral N gene-dependent replication, displaying efficacy against the original strain and omicron vari-

ant, which highlights the potential of aptamers as synergistic treatments.[50] Further studies revealed that aptamer-functionalized lipid nanoparticles encapsulating siRNAs could target the nucleocapsid phosphoprotein. Molecular docking and antiviral tests indicated a significant inhibition of infection in human lung cells. Furthermore, inhalation of this formulation was correlated with an improved lung inflammation/opacity state in a patient with severe COVID-19, demonstrating a promising therapeutic approach.[51] By leveraging RNAi and targeted delivery, this strategy has the potential for developing safe and effective anti-COVID-19 treatments. In this codelivery approach, while nanoparticles protect siRNAs from degradation and facilitate cellular uptake/endosomal escape, the high affinity/specificity of aptamers for the S protein allows for selective targeting of virus-infected cells. Noticeable benefits of aptamer–siRNA chimeras over other methodologies (e.g., protein-based systems) are simplicity (single-component conjugates), ease of manufacturing, and low immunogenicity. Given that the human immune system does not typically recognize nucleic acids as external molecules, aptamer–siRNA chimeras create less non-specific immune system activation compared with protein-mediated delivery systems.[52]

The CRISPR and CRISPR/Cas systems have also been the center of attention for various essential applications, including genome editing and biosensor development, because of adjustable mechanical features, favorable biocompatibility, and cleavage activity.[53] Although several studies have explored the use of CRISPR technology to enhance the performance of aptamers in detecting COVID-19, there have been no studies to combine these technologies for therapeutic purposes.[54,55] It appears that combining their complementary strengths through targeted approaches could enhance CRISPR targeting by guiding nucleases to the specific viral proteins, thus maximizing virus disruption and replication inhibition. Developing aptamer–CRISPR complexes that bind to SARS-CoV-2 could improve the selective delivery of editing agents to the viral genome, enabling precision cutting with reduced off-target effects and improved safety and selectivity. In general, combining aptamers and CRISPR allows for developing several therapeutics concurrently. Using multiplex therapies that target different components (replication enzymes, entry proteins, and RNA) provides a multipronged attack needed against fast-mutating viruses, such as SARS-CoV-2.

Aptamer administration and kinetics

Aptamers can be delivered through various routes, including intravenous, intranasal, oral, and topical administration. Intravenous administration is the most commonly used route for aptamer delivery, where aptamers are administered directly into the bloodstream. However, this route has limitations, including potential immunogenicity and rapid clearance from the circulation.[56] In general, a particular problem with unprotected phosphodiester DNA aptamers is their short circulation lifetime in the blood because of the presence of serum nuclease or clearance by different organ extraction, including lung and kidneys.[57] Studies with chemically stabilized antiangiogenic Ribozyme (ANGIOZYME™) in healthy volunteers indicated that, following intravenous or subcutaneous administration, they could readily be distributed throughout the body and be taken up by target cells.[58] Intranasal administration of aptamers is a non-invasive delivery route that provides direct access to the respiratory tract, which is the primary site of SARS-CoV-2 infection. The small size of aptamers enables aptamer formulation into gels and aerosols, which could be applied for intranasal administration or nebulization as a straightforward delivery strategy into the respiratory system.[31] Although oral administration is regarded as a non-invasive route that directs the systemic delivery of aptamers, it could be challenging to achieve sufficient bioavailability because of the gastrointestinal barrier.[59]

Aptamers conjugated with different moieties, including lipids or polymers, could enhance their pharmacokinetic and biodistribution properties. This strategy could resolve the problem of instability in the biological media as well as crossing the cellular membranes to reach the site of action. Furthermore, by improving the stability of aptamers through bioconjugates, their lifetime could be extended in the blood, which would facilitate their uptake by target cells.[5] By complexing the aptamer with a protein, its circulation time could be greatly improved.[57] There is also evidence that liposome vesicles could be applied for directing aptamers into intracellular targets.[60,61] Although in most cases, the preferred administration route of aptamer–conjugated nanocarriers is via intravenous injection, some studies have used topical administration for the delivery of aptamer-functionalized carriers loaded with antitumoral drugs to treat skin cancer.[62]

Disadvantages of aptamers

Although aptamers have received much attention over the past decade and are potential candidates for various detection and therapeutic uses, they have some unresolved limitations. The first is that the selection process of most aptamers is performed *in vitro*, whereas the marked molecular structures vary and are more complicated *in vivo*. Thus, the aptamers might not be as efficient and functional as anticipated for *in vivo* studies. Furthermore, they are subject to cross-binding with other molecules that are not their target, which could restrict the utilization of aptamers for diagnostic applications. Therefore, considering all aspects and the precise outline of the selection procedure, it appears essential to improve aptamer specificity. Many types of virus and complex structures need more precise SELEX procedures to develop specific and/or universal antimicrobial aptamers. Furthermore, unchanged aptamers (particularly RNA aptamers) are commonly sensitive to digestion by cellular nucleases in body fluids or within cells. Consequently, varying chemical modifications are needed to improve the *in vivo* stability of aptamers. The most common modification is to replace the sulfur atom with non-bridging O_2 in the DNA or RNA phosphate backbone. Moreover, alterations of RNA aptamers are performed at the 2′-position on the ribose ring. Modified RNA aptamers indicate a half-life of 100 h in human serum, which is significantly improved compared with seconds associated with unmodified RNA.[63]

Additionally, small-sized aptamers are simply filtered out through glomerular capillaries.[64] The typical approach is to conjugate to polyethylene glycol (PEG),[65] liposome,[66] cholesterol,[67] an antibody,[68] or other nanomaterials to increase the circulation time of aptamers.[69] PEG has been broadly used to expand the cir-

culation half-life of these biological agents. Another crucial challenge is the delivery of aptamers into the lungs. Finally, scale-up and mass production of these potential biological agents will be necessary.[48]

Concluding remarks and future perspectives

Given the development of aptamers for numerous viruses, such as HIV, hepatitis C virus (HCV), and SARS-CoV, there is significant potential for also developing an extensive range of aptamers for genomic RNA and multiple proteins of SARS-CoV-2. Furthermore, aptamers are mostly constrained as sensing modules, although they can also be widely used as therapeutic inhibitory agents. This aspect of aptamers remains to be fully explored. We anticipate extensive developments in aptamer technology, where the next steps would be to develop multifunctional aptamers with the capability of recognizing and inhibiting various targets. This would be of significance for therapeutic applications, particularly in pandemic infections, such as COVID-19.

Only two aptamer-based technologies have reached clinical trials thus far: a saliva-based DNA aptamer (NCT04974203) and the SARS-CoV-2 therapeutic aptamer ApTOLL, developed to a Phase Ib randomized multicentre placebo-controlled clinical trial including patients hospitalized with COVID-19 at risk of cytokine storm syndrome (NCT05293236). Structural analysis of the previously established aptamers could be beneficial in aptamer repurposing and screening to develop favorable aptamers for diagnostics and therapeutic applications against SARS-CoV-2.

The S protein is a potential target for novel drugs to protect against SARS-CoV-2. However, no US Food and Drug Administration (FDA)-approved aptamers have been reported against the S protein of this virus. ACE2, as a receptor for SARS coronaviruses with a significant role in controlling respiratory distress syndrome (ARDS), has been considered a possible therapeutic and diagnostic target.[70] Researchers recently developed stable and biologically active aptamers that inhibit SARS-CoV-2 infection with high affinity and potency by blocking the S protein RBD–ACE2 receptor interaction. These aptamer-blocking strategies provide a novel approach for COVID-19 treatment beyond antibodies. Helicase also has a crucial role in viral proliferation and replication and could be regarded as a useful target for developing coronavirus aptamers for potential therapeutic applications. Likewise, the N protein has also been recognized as a key target to hinder COVID-19 because of its role in synthesizing viral RNA.

Overall, given all the potential targets and their coupling with advanced molecular methods, aptamers could serve as sensitive diagnostics and therapeutics in the near future. However, further evidence is still needed, especially from clinical trials, to support these possibilities.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Cameroni E et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature*. 2022;602:664–670.
2. Di Toro F et al. Impact of corticosteroid therapy on outcomes of persons with SARS-CoV-2, SARS-CoV, or MERS-CoV infection: a systematic review and meta-analysis. *Leukemia*. 2020;34:1503–1511.
3. Kemp SA et al. SARS-CoV-2 evolution during treatment of chronic infection. *Nature*. 2021;592:277–282.
4. Bozgeyik I. Therapeutic potential of miRNAs targeting SARS-CoV-2 host cell receptor ACE2. *Meta Gene*. 2021;27 100831.
5. Ni S et al. Recent progress in aptamer discoveries and modifications for therapeutic applications. *ACS Appl Mater Interfaces*. 2021;13:9500–9519.
6. Wu L et al. Aptamer-based detection of circulating targets for precision medicine. *Chem Rev*. 2021;121:12035–12105.
7. Lyu C, Khan IM, Wang Z. Capture–SELEX for aptamer selection: a short review. *Talanta*. 2021;229 122274.
8. Li L et al. Nucleic acid aptamers for molecular diagnostics and therapeutics: advances and perspectives. *Angew Chem Int Ed*. 2021;60:2221–2231.
9. Elskens JP, Elskens JM, Madder A. Chemical modification of aptamers for increased binding affinity in diagnostic applications: current status and future prospects. *Int J Mol Sci*. 2020;21:4522.
10. Roueinfar M, Templeton HN, Sheng JA, Hong KL. An update of nucleic acids aptamers theranostic integration with CRISPR/Cas technology. *Molecules*. 2022;27:1114.].
11. Song Y et al. Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein. *Anal Chem*. 2020;92:9895–9900.
12. Resch B. Product review on the monoclonal antibody palivizumab for prevention of respiratory syncytial virus infection. *Hum Vaccin Immunother*. 2017;13:2138–2149.
13. Seo HB, Gu MB. Aptamer-based sandwich-type biosensors. *J Biol Eng*. 2017;11:11.
14. Zhang Y, Juhas M, Kwok CK. Aptamers targeting SARS-COV-2: a promising tool to fight against COVID-19. *Trends Biotechnol*. 2023;41:528–544.
15. Ahmadi M et al. Safety and efficacy of nanocurcumin as add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a pilot randomized clinical trial. *Neurotherapeutics*. 2018;15:430–438.
16. Xinran Z, Wu J, Gu J, Shen L, Mao L. Application of aptamers in virus detection and antiviral therapy. *Front Microbiol*. 2019;10:1462.
17. Ren S et al. Designer DNA nanostructures for viral inhibition. *Nat Protoc*. 2022;17:282–326.
18. Chakraborty B et al. Aptamers for viral detection and inhibition. *ACS Infect Dis*. 2022;8(4):667–692.
19. Hong S-L, Xiang M-Q, Tang M, Pang D-W, Zhang Z-L. Ebola virus aptamers: from highly efficient selection to application on magnetism-controlled chips. *Anal Chem*. 2019;91:3367–3373.
20. Teng Y et al. Computer-designed orthogonal RNA aptamers programmed to recognize Ebola virus glycoproteins. *Biosafety Health*. 2019;1:105–111.
21. Wang Q et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell*. 2020;181:894–904.
22. Wrapp D et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020;367:1260–1263.
23. Rossi JJ, Rossi D. Oligonucleotides and the COVID-19 pandemic: a perspective. *Nucleic Acid Ther*. 2020;30:129–132.
24. Haberland A et al. Aptamer BC 007 - a broad spectrum neutralizer of pathogenic autoantibodies against G-protein-coupled receptors. *Eur J Pharmacol*. 2016;789:37–45.
25. Müller J et al. The DNA-based drug BC 007 neutralizes agonistically acting autoantibodies directed against G protein-coupled receptors–successful mode of action demonstrated in clinical phase 1 trial. *Chim Oggi*. 2019;2:65–67.
26. Wallukat G, Schimke I. Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. *Semin Immunopathol*. 2014;36:351–363.
27. Becker NP et al. A Three-part, randomised study to investigate the safety, tolerability, pharmacokinetics and mode of action of BC 007, neutraliser of

pathogenic autoantibodies against G-protein coupled receptors in healthy, young and elderly subjects. *Clin Drug Investig*. 2020;40:433–447.
28. Weisshoff H et al. Aptamer BC 007-Efficient binder of spreading-crucial SARS-CoV-2 proteins. *Heliyon*. 2020;6:e05421.
29. Devi A, Chaitanya NSN. Designing of peptide aptamer targeting the receptor-binding domain of spike protein of SARS-CoV-2: an in silico study. *Mol Divers*. 2022;26:157–169.
30. Alves Ferreira-Bravo I, DeStefano JJ. Xeno-nucleic acid (XNA) 2′-fluoro-arabino nucleic acid (FANA) aptamers to the receptor-binding domain of SARS-CoV-2 S protein block ACE2 binding. *Viruses*. 2021;13:1983.
31. Sun M et al. Aptamer blocking strategy inhibits SARS-CoV-2 virus infection. *Angew Chem Int Ed*. 2021;60:10266–10272.
32. Liu X et al. Neutralizing aptamers block S/RBD-ACE2 interactions and prevent host cell infection. *Angew Chem, Int Ed Engl*. 2021;60:10273–10278.
33. Yang G et al. Identification of SARS-CoV-2–against aptamer with high neutralization activity by blocking the RBD domain of spike protein 1. *Signal Transduct Target Ther*. 2021;6:227.
34. Valero J et al. A serum-stable RNA aptamer specific for SARS-CoV-2 neutralizes viral entry. *Proc Natl Acad Sci USA*. 2021;118 e2112942118.
35. Sun M et al. Spherical neutralizing aptamer inhibits SARS-CoV-2 infection and suppresses mutational escape. *J Am Chem Soc*. 2021;143:21541–21548.
36. Sun M et al. Spherical neutralizing aptamer suppresses SARS-CoV-2 Omicron escape. *Nano Today*. 2022;44 101499.
37. Gelinas AD et al. Broadly neutralizing aptamers to SARS-CoV-2: a diverse panel of modified DNA antiviral agents. *Mol Ther Nucleic Acids*. 2023;31:370–382.
38. Kwong AD, Rao BG, Jeang KT. Viral and cellular RNA helicases as antiviral targets. *Nat Rev Drug Discov*. 2005;4:845–853.
39. Adedeji AO et al. Mechanism of nucleic acid unwinding by SARS-CoV helicase. *PLoS ONE*. 2012;7:e36521.
40. Frick DN. Helicases as antiviral drug targets. *Drug News Perspect*. 2003;16:355–362.
41. Jang KJ, Lee N-R, Yeo W-S, Jeong Y-J, Kim D-E. Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. *Biochem Biophys Res Commun*. 2008;366:738–744.
42. Ciotti M et al. COVID-19 outbreak: an overview. *Chemotherapy*. 2019;64:215–223.
43. Shum KT, Tanner JA. Differential inhibitory activities and stabilisation of DNA aptamers against the SARS coronavirus helicase. *Chembiochem*. 2008;9:3037.
44. Cho SJ, Woo HM, Kim KS, Oh JW, Jeong YJ. Novel system for detecting SARS coronavirus nucleocapsid protein using an ssDNA aptamer. *J Biosci Bioeng*. 2011;112:535–540.
45. Ahn DG et al. RNA aptamer-based sensitive detection of SARS coronavirus nucleocapsid protein. *Analyst*. 2009;134:1896–1901.
46. Parashar NC, Poddar J, Chakrabarti S, Parashar G. Repurposing of SARS-CoV nucleocapsid protein specific nuclease resistant RNA aptamer for therapeutics against SARS-CoV-2. *Infect Genet Evol*. 2020;85:104497.
47. Yang G et al. Aptamer blocking S-TLR4 interaction selectively inhibits SARS-CoV-2 induced inflammation. *Signal Transduct Target Ther*. 2022;7:1–3.
48. Zhou H, Fang Y, Xu T, Ni WJ, Shen AZ, Meng XM. Potential therapeutic targets and promising drugs for combating SARS-CoV-2. *Br J Pharmacol*. 2020;177:3147–3161.
49. Sivakumar P, Kim S, Kang HC, Shim MS. Targeted siRNA delivery using aptamer-siRNA chimeras and aptamer-conjugated nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2019;11:e1543.
50. Yang G et al. Efficient targeted delivery of bifunctional circular aptamer-ASO chimera to suppress the SARS-CoV-2 proliferation and inflammation. *Small*. 2023;19:2207066.
51. Saify Nabiabad H, Amini M, Demirdas S. Specific delivering of RNAi using Spike's aptamer-functionalized lipid nanoparticles for targeting SARS-CoV-2: a strong anti-Covid drug in a clinical case study. *Chem Biol Drug Des*. 2022;99:233–246.
52. Kruspe S, Giangrande PH. Aptamer–siRNA chimeras: discovery, progress, and future prospects. *Biomedicines*. 2017;5:45.
53. Zhu C et al. CRISPR/Cas systems accelerating the development of aptasensors. *Trends Anal Chem*. 2023;158 116775.
54. Han C et al. CRISPR/Cas12a-derived electrochemical aptasensor for ultrasensitive detection of COVID-19 nucleocapsid protein. *Biosens Bioelectron*. 2022;200 113922.
55. Peinetti AS et al. Direct detection of human adenovirus or SARS-CoV-2 with ability to inform infectivity using DNA aptamer-nanopore sensors. *Sci Adv*. 2021;7:eabh2848.
56. Nimjee SM, White RR, Becker RC, Sullenger BA. Aptamers as therapeutics. *Annu Rev Pharmacol Toxicol*. 2017;57:61–79.
57. Dougan H, Lyster DM, Vo CV, Stafford A, Weitz JI, Hobbs JB. Extending the lifetime of anticoagulant oligodeoxynucleotide aptamers in blood. *Nucl Med Biol*. 2000;27:289–297.
58. Sandberg JA et al. Pharmacokinetics and tolerability of an antiangiogenic ribozyme (ANGIOZYME™) in healthy volunteers. *J Clin Pharmacol*. 2000;40:1462–1469.
59. Park JP et al. Screening and development of DNA aptamers specific to several oral pathogens. *J Microbiol Biotechnol*. 2015;25:393–398.
60. Li X, Wu X, Yang H, Li L, Ye Z, Rao Y. A nuclear targeted Dox–aptamer loaded liposome delivery platform for the circumvention of drug resistance in breast cancer. *Biomed Pharmacother*. 2019;117 109072.
61. Plourde K, Derbali RM, Desrosiers A, Dubath C, Vallée-Bélisle A, Leblond J. Aptamer-based liposomes improve specific drug loading and release. *J Control Release*. 2017;251:82–91.
62. Rata DM et al. Topical formulations containing aptamer-functionalized nanocapsules loaded with 5-fluorouracil–an innovative concept for the skin cancer therapy. *Mater Sci Eng C*. 2021;119 111591.
63. Shigdar S et al. Aptamers as theranostic agents: modifications, serum stability and functionalisation. *Sensors (Basel)*. 2013;13:13624–13637.
64. Huang L, Sullenger B, Juliano R. The role of carrier size in the pharmacodynamics of antisense and siRNA oligonucleotides. *J Drug Target*. 2010;18:567–574.
65. Lee CH et al. Inhibition of hepatitis C virus (HCV) replication by specific RNA aptamers against HCV NS5B RNA replicase. *J Virol*. 2013;87:7064–7074.
66. Ara MN et al. An aptamer ligand based liposomal nanocarrier system that targets tumor endothelial cells. *Biomaterials*. 2014;35:7110–7120.
67. Lee CH, Lee SH, Kim JH, Noh YH, Noh GJ, Lee SW. Pharmacokinetics of a cholesterol-conjugated aptamer against the hepatitis C virus (HCV) NS5B protein. *Mol Ther Nucleic Acids*. 2015;4:e254.
68. Heo K et al. An aptamer-antibody complex (oligobody) as a novel delivery platform for targeted cancer therapies. *J Control Release*. 2016;229:1–9.
69. Liao J, Liu B, Liu J, Zhang J, Chen K, Liu H. Cell-specific aptamers and their conjugation with nanomaterials for targeted drug delivery. *Expert Opin Drug Deliv*. 2015;12:493–506.
70. Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM. Angiotensin-converting enzyme 2 (ACE2) in disease pathogenesis. *Circ J*. 2010;74:405–410.