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Review

Aptamer-Mediated Antiviral Approaches for SARS-CoV-2

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Abstract

2020 and 2021 were disastrous years across the world, with the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus as a pandemic, which continues to be a top global health issue. There are still many countries and regions struggling to fight coronavirus disease 2019 (COVID-19), and, with the emergence of the various variants of the virus, we are still far from considering this global pandemic over. In addition to having good diagnostic tools and a variety of vaccines with high efficacy, it is of utmost importance to develop effective antiviral drugs or therapies to battle COVID-19. Aptamers known as the next-generation targeting elements can offer promising opportunities in developing antiviral drugs against SARS-CoV-2. This is owing to their high specificity and affinity, making them ideal for targeting ligands and neutralizers to impede both, viral entry and replication or even further enhance the anti-infection effects in the infected host cells. Also, aptamers are extremely attractive as they can be rapidly synthesized and scalable with a lower production cost. This work provides in-depth discussions on the potential of aptamers in therapeutic applications, their mode of action, and current progress on the use of aptamer-based therapies against SARS-CoV-2 and other viruses. The article also discusses the limitations associated with aptamer-based SARS-CoV-2-antiviral therapy with several proposed ideas to resolve them. Lastly, theranostic applications of aptamer nanoformulated dendrimers against viral infections are discussed.

Keywords: aptamers; antiviral drug; SARS-CoV-2; viral infection; dendrimer; nanoformulation

1. Introduction

Coronaviruses (CoVs) belong to a family of viruses that can cause mild to moderate respiratory tract illnesses. There are three well-known CoVs. These are severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2 [1]. Coronavirus disease 2019 (COVID-19) is caused by the virus, SARS-CoV-2. As of 23 July 2022, there have been more than 569 million confirmed SARS-CoV-2 cases globally, which included >6 million deaths reported [2]. Currently, despite there is an increasing number of vaccines being developed and approved since the outbreak, COVID-19 is still causing significant global health threats and economic burdens. In addition, molecular amplification of viral antigens and molecular antibody-based detection techniques have been developed for the clinical diagnosis of SARS-CoV-2 [3,4].

Despite these significant efforts toward diagnosis and vaccination, there are currently only two oral anti-viral COVID-19 drugs approved by the United States Food and Drug Administration (FDA) for emergency use authorization (EUA) [5,6]. These are Paxlovid and Lagevrio (Molnupiravir, MK-4482), both offering benefits over Veklury (Remdesivir) [7]. Paxlovid is a combination of the antiviral Nirmatrelvir (PF-07821332) and antiretroviral drug - ritonavir- used to treat human immunodeficiency virus (HIV). It works by disrupting viral replication by binding to the 3CL-like protease that is responsible for viral replication [8]. On the other hand, Lagevrio works as a nucleoside analog that inhibits the accurate replication of viral genetic materials, leading to the formation of non-infectious new viral particles [9]. Howbeit, the clinical trial data of Lagevrio has shown to be less effective than anticipated [10]. Also, the general challenges of inefficient viral medications, such as low specificity, unwanted side effects, and high mutation variability, are yet to be addressed. Hence, the search for antivirals remains a major research endeavour and a high priority.

Targeted drug delivery is a promising approach for disease diagnosis and treatments, owing to its capability to distinguish between malignant cells and healthy cells. Advancements in biotechnology appear to be promising in targeting and treating SARS-CoV-2 with the use of nucleic acid-based treatments such as aptamers [11]. The emergence of highly specific aptamers has revolutionized the sphere of targeted pharmaceutical applications as a new generation of targeting ligands [12]. A more specific antiviral treatment can be developed through the use of aptamers to promote the targeting of infected cells. It has been demonstrated that aptamer-navigated targeted drug delivery can enhance anti-cancer therapeutic outcomes [11–16]. Aptamer-mediated antiviral approaches can be devel-

oped to inhibit SARS-CoV-2 invasion against host cells or modulate the host immune system. In this review article, we first discuss the characteristics of aptamers and their mechanisms of action to reveal the promising potentials of aptamer-mediated antiviral treatments. We then discuss the use of aptamers to treat different infectious pathogens, with emphasis on the prospects of aptamers in targeting SARS-CoV-2. The application of aptamers as an antiviral system against SARS-CoV-2 is also discussed. We appraise the therapeutic applications of dendrimer nanoformulation of aptamers as antiviral drugs and theranostics for SARS-CoV-2.

2. Aptamers

2.1 Biophysical and Functional Properties of Aptamers

In recent years, aptamers have gained significant research attention for advanced cell targeting in pharmaceutical delivery and medicines, owing to their enhanced specificity, binding affinity, and sensitivity with low dissociation constants towards various molecular targets [13]. They are chemically synthesized, short, and single-stranded (ss) oligonucleotides of either single-stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (ssRNA) sequences that can selectively bind to a specific target by folding into unique three-dimensional (3D) structures [14]. Systematic Evolution of Ligands by Exponential Enrichment (SELEX) is an iterative selection process used to engineer aptamers through 10–15 repeated rounds of sequential target incubation, selections, amplifications, and enrichment until an enriched pool of specific target clones is formed to derive aptamers [15]. Specific aptamers to target molecules, bacteria, cells, tissues, endotoxin, and viruses can be engineered via SELEX with the modification of incubation conditions and selection strategies. Their ease in functionalization with diverse moieties such as biotin, redox label, fluorophore, and nanomaterials allows for a wide range of applications. In addition, aptamers have high selectivity and affinity with the chemical versatility of synthetic drugs and tuneable functional properties, highlighting their advantages as biological drugs [16,17].

Aptamers possess desirable bio-physiochemical properties such as small hydrodynamic size (5–15 K_d), high productivity, biocompatibility, biodegradability, and low or negligible immunogenicity as well as good thermal and chemical stability due to their strong phosphodiester bonds [14,18]. With their inherent advantages, aptamers have made them ideal candidates as therapeutic drugs, diagnostic tools, and delivery vehicles for targeted delivery. For instance, their smaller size (~2–3 nm in diameter) helps to prevent steric hindrance on the surface of targeted bacteria or viruses, allowing the binding of more recognition molecules on the cell surface compared to other targeting ligands such as antibodies. There are numerous studies on the effectiveness of aptamers in treating various chronic conditions. These include the Pegaptanib aptamer for treating vascular age-related macular degeneration [19]; NU172 aptamer for treating anticoagulation in cardiovascular disease [20]; nucleolin-specific AS1411 for targeting cancer cells such as renal carcinoma and acute myeloid leukemia [21]. There are also several other therapeutic aptamers under clinical trials [22–24].

2.2 Aptamer Binding Mechanism

In principle, aptamers interact with their targets by folding into unique 3D conformations, making them sensitive to changes in their sequences. For example, alterations or mutations that happen in the aptamer-target binding regions can affect the aptameric binding affinity. Aptamers can act through direct binding to relevant targets to interfere with the first stage of viral infection, blocking the viral attachment to the host cell [25]. In addition, random oligonucleotides or aptamers with no defined binding site have shown anti-viral activities against different viruses such as HIV and hepatitis C. This interaction capacity is attributed to the amphiphilic nature of the phosphorothioate and its poly-anionic nature [26–28].

Aptamers can potentially offer broad-spectrum inhibition to disrupt viral replication [29]. For instance, deoxyribonucleic acid (DNA) aptamers are reported to inhibit diverse primate lentiviral reverse transcriptase (RT) by attaching to the RT over a large surface area with an extended binding interface. Their binding is remarkably similar to the binding by natural nucleic acid substrates, with most of the contacts residing within their interface, making them compete with natural substrates for RT while remaining inert to the RT activity. An aptamer has been demonstrated to possess a thumb-and-fingers-open conformation upon binding with RT, indicating its unique specificity to the target without affecting the polymerization active site [29]. With their smaller size as compared to antibodies, aptamers can access and bind onto conserved or poorly accessible loops or epitopes to enhance their target binding interaction. For instance, an aptamer demonstrated a neutralization activity of IC_{50} <60 μg/mL in a study that targeted the dengue virus, which is stronger than cross-reactive antibodies in neutralizing all 4 serotypes. Guanine-rich aptamers can form G-quadruplex conformations with stacked G.G.G.G tetrads in the presence of potassium, leading to improved binding affinity and stability [30]. Aptamers can stimulate the host's innate and adaptive immune system against viral infections by activating certain pathways such as the RIG-1-mediated IFNβ production to block viral replication [31].

3. The Therapeutic Applications of Aptamers against Viruses

Scientific studies have determined some therapeutic effects of aptamers against viral infections. They can hamper viral attachment and replication by binding and inhibiting target molecules. Sullenger *et al.* [32] reported the first use of aptamers in treating HIV with trans-activation

response element-ribonucleic acid (TAR-RNA) aptamer to impede trans-activator of transcription (Tat)-mediated viral gene expression by targeting both Tat and cyclin T1 proteins in the human cluster of differentiation (CD)4+ T helper cells [32]. Several other studies have also used aptamers to target different HIV stages, including R1T and RT1t49(-5) DNA aptamers that inhibit the RT of primate lentiviral family [29]; and interleukin 6 receptor (IL-6R)-specific aptamer (16-mer DNA aptamer AID-1 [d(GGGT)$_4$]) that acts as the HIV-1 inhibitor with identical effects as the well-known Haemophilus influenzae type B (HIB) inhibitor T30923 [33]. The findings showed their inhibitory actions on the 3' processing activity and binding of HIV-1 integrase as well as inhibiting the HIV *de novo* infection. There are also studies on using aptamer-small interfering RNA (siRNA) complex to reduce the messenger RNA (mRNA) protease [34] and tat/regulator of virion protein expression (rev) protein expression of HIV [35] with reduced viral activity.

Other studies have reported on the use of aptamers to treat various infectious viruses such as hepatitis B virus and hepatitis C virus (HCV) by targeting the core protein and nonstructural protein 5B (NS5B) protein to hinder both the extracellular DNA synthesis and viral replication [36]. Lee and his team designed RNA aptamers containing 2′-hydroxyl- or 2′-fluoropyrimidines to target an enzyme called NS5B replicase of HCV via competitive sequestration of the target protein. As a result, the HCV replicon replication impeded human liver cells without causing escape mutant appearance, off-target effects, and cellular toxicity. This aptamer was also conjugated with galactose-polyethylene glycol or cholesterol to enhance liver-targeting delivery, *in vivo* availability, and cell transfection. Both genotype 1b and genotype 2a HCV JFH-1 RNA replication was successfully hindered, indicating a potential new therapeutic tool and feasible antiviral therapy for current SARS-CoV-2 therapeutics [36].

There are aptamers designed to treat herpes simplex-1 by targeting its glycoprotein D to block the viral entry [37]. A study has illustrated the use of a DNA aptamer with antiviral activity in neutralizing all 4 serotypes of Dengue viruses by specifically binding onto the dengue virus-2 envelop protein domain III; in the conserved loop between β_A and β_B strands. This DNA aptamer possesses a unique G-quadruplex structure and a sequence on the 5'-end that contributes to its high binding affinity by hindering the E protein attachment to the host receptor [38]. RNA aptamers have also been developed to target the viral protein 35 of the Ebola virus by competing with double-stranded RNA to antagonize the viral protein 35-nucleoprotein interaction. This led to the disruption of the Ebola polymerase cofactor function by prohibiting the interferon (IFN) inhibitory action of the viral protein 35 for effective treatment outcomes [39]. Also, a DNA aptamer has been shown to have high sensitivity against the different binding epitopes of the NS1 protein of the Zika virus. It can therefore be used as a detection agent in diagnosis with a detection limit of 100 ng/mL to identify Zika NS1 [40]. These findings suggest the potential application of aptamers as a targeting ligand capable of navigating antiviral drug molecules to the proper cellular site for improved therapeutic effects.

Furthermore, aptamers have been widely exploited as anti-influenza candidates in the drug development race to treat influenza viruses. Influenza viruses are characterized by their surface hemagglutinin (HA) glycoprotein that binds onto the sialic acid receptors of host cells and has been the target of various aptamers that aim to hinder viral attack and proliferation. A study has reported the efficacy of DNA aptamer BV02 against the swine flu virus (H1N1) by targeting the hemagglutinin viral protein responsible for the first stage of the viral infection–cell interaction [25]. The study also demonstrated that the binding of the aptamer against the influenza virus is more dependent on general two-dimensional (2D) structural motifs such as loops and C stretches, aptamer length, and repeating sequences of C nucleotides rather than sequence-specific, with 87% binding specificity detected. This mechanism of action is relevant to different influenza strains and can be extended to other viruses.

Another viral target for aptamers is the virus endonucleases that disrupt the virus transcription. DNA aptamers have been designed to target the N-terminal domain of the polymerase acidic (PA) protein of the H5NI virus with increased endonuclease inhibitory activity and antiviral efficacy [41]. Furthermore, PA$_N$-DNA aptamers have been shown to have cross-subtype protection against another influenza A virus subtypes such as H1N1, H7N7, and H7N9 with 50% of half-maximal inhibitory concentration (IC_{50}) of approximately 10 nM, potentially due to enriched guanine-cytosine (GC) with easier hairpin structural formation for a stronger binding affinity. The anti-viral effects work by aptameric substitutions in the enzyme active site to reduce viral fitness [41]. Aptamers are reported with the capability to distinguish between influenza type A and B or other closely related subtype strains. For instance, two RNA aptamers (P30-10-16 and A-20) were designed to bind against type A and B, respectively, with more than 15-fold higher affinity than anti-HA monoclonal antibodies in targeting influenza type A [42]. These reported studies as summarized in Table 1 (Ref. [25,29,32–36,38–43]) with the successful application of aptamers against different stages of viral infections and pathogenic diseases have provided a rationale for the use of nucleic acid-based treatments to target SARS-CoV-2 infection [29,32–36,38–43].

4. Aptamer as a Potential Antiviral Candidate for SARS-CoV-2

The development of targeting ligands capable of binding to the spike protein of SARS-CoV-2 and blocking viral infection is of importance to COVID-19 therapy. Angiotensin-converting enzyme II (ACE2) is the receptor

Table 1. Therapeutic applications of aptamers against viruses.

Aptamers	Target molecules	Application	Reference
TAR-RNA aptamer	Tat and cyclin T1 proteins in human CD4+ T cells	HIV treatment	[32]
R1T and RT1t49(-5) DNA aptamers	RT of primate lentiviral family	Treatment of distinct stages of HIV	[29,43]
IL-6R-specific aptamer	HIV-1 inhibitor T30923	HIV treatment	[33]
Aptamer-siRNA complex	mRNA protease and tat/rev protein expression	HIV treatment	[34,35]
2′-hydroxyl- or 2′-fluoropyrimidines RNA aptamers	NS5B replicase	HCV inhibition	[36]
DNA aptamer	Dengue virus-2 enveloped protein domain III	Dengue virus treatment	[38]
RNA aptamers	Viral protein 35	Ebola virus treatment	[39]
DNA aptamer	Epitopes of the NS1 protein	Zika virus diagnosis	[40]
DNA aptamer BV02	Hemagglutinin viral protein	Swine flu virus (H1N1)	[25]
DNA aptamers	N-terminal domain of the polymerase acidic (PA) protein	H5NI virus inhibition	[41]
P30-10-16 and A-20 RNA aptamers	Type A and B of anti-HA monoclonal antibodies	Influenza type A	[42]

that SARS-CoV-2 uses to infect human host cells via the binding between the receptor-binding domain (RBD) of the spike glycoprotein and ACE2. This makes RBD an ideal target to develop drugs and vaccines against SARS-CoV-2. NEC Solution Innovators have developed an artificial DNA aptamer that targets SARS-CoV-2. The aptamer binds to the 3D structure of the RBD to prevent the viral spike protein from attaching to the ACE2 receptors on human host cells, thus blocking the viral entry. The results indicated a strong binding of the aptamer to three strains of SARS-CoV-2; the original strain (WK521 Wuhan strain) and two other mutant strains (TY7-501 Brazilian and QK002 UK strains) [44]. This work has the potential to facilitate the development of aptamers as antiviral drugs against COVID-19.

A serum-stable RNA aptamer has been engineered as a promising candidate to inhibit viral entry by binding to the RBD of COVID-19 spike protein with a picomolar range of binding efficiency, preventing interaction between host receptors and SARS-CoV-2 spike protein. This RNA aptamer was modified with 2-fluoropyrimidine to enhance its resistance against viral nuclease-mediated degradation and improve chemical stability. It was demonstrated with high specificity to distinguish SARS-CoV-2 from other viruses such as SARS-CoV and MERS, with no cross-reactivity and the capability to detect different COVID-19 variants [45]. This contrasts with recent studies that indicated antibodies with decreased affinity to new variants of protein spike [46]. Probably, this is due to the less sensitivity of aptamers towards single amino acid mutations as their targets are usually epitopes with larger and discontinuous structures [45]. A study has described the application of BC 007 aptamer (currently in clinical trial phase II for congestive heart failure) to target the DNA-susceptible peptide sequences in both the RBD region and RNA-dependent RNA polymerase of SARS-CoV-2. The findings showed that the BC 007 aptamer was able to fold into a quadruplex structure for high-affinity-specific binding. The preclinical and clinical assessments showed no toxicity. The tolerability test in addition to the anti-coagulatory effects of the BC 007 aptamer, highlighted its potential as an antiviral agent against COVID-19 infection [47]. Song and team have utilized both machine learning screening algorithm and ACE2 competition-based aptamer selection to identify two aptamers called CoV2-RBD-1C and CoV2-RBD-4C with hairpin structure and high binding affinities (K_d values of 5.8 nM and 19.9 nM, respectively) against several amino acid residues of RBD of SARS-CoV-2 [48]. These aptamers have the potential to hinder the binding and viral entry of SARS-CoV-2, suggesting their potential to be used for the prevention and treatment of COVID-19.

Yang *et al*. [49] discovered recently that the S1 protein of RBD can serve as a more stable binding site than RBD to enhance the kinetic and interaction time between aptamer-based drug molecules and the receptor, leading to a stronger interaction potency [50]. The same team also demonstrated a good dose-dependent inhibitory effect and neutralization performance of the designed aptamer (nCoV-S1-Apt) on S1/ACE2 binding and viral infection. This indicates the potential of the aptamer as a neutralizing antiviral molecule with the capability to block the binding of S1 protein to the host ACE2, thus, preventing viral transduction. Mutations that occur in the spike protein of SARS-CoV-2 serve as a challenge to aptamers targeting RBD-ACE2. The nucleocapsid protein is another target used to treat SARS-CoV-2. This is certainly since the nucleocapsid protein is highly conserved among different COVID strains, and thus, it can be used to overcome drug resistance. A DNA aptamer with K_d of 0.49 nm was developed to detect the presence of SARS-COV-2 via a sandwich-type interaction that forms a ternary complex with the nucleocapsid protein [51]. This aptamer can be used to develop aptamer-based antiviral therapy in treating COVID-19. This is further supported by another study that showed the effectiveness of an aptamer in targeting nucleocapsid protein as a potential antiviral candidate [52].

Aptamers are capable of blocking the infection and replication process of SARS-CoV-2 infection, as illustrated in Fig. 1, while siRNAs have the potential to cleave the viral RNA genome and hamper its proliferation. It is also possible that a little concentration of siRNA is sufficient to reduce viral RNA load significantly. Aptamer-siRNA chimeras have been reported to be an effective targeted antiviral therapy with dual functions to inhibit viral replication and neutralize the virus. siRNA is an exogenous agent used for gene manipulation and, thus, able to cleave the viral mRNA and alter gene function via gene silencing. There are numerous studies designated on the use of siRNAs against the mRNA of different target genes, which include envelope, membrane, nucleocapsid, and spike proteins to treat coronaviruses [53–56]. siRNAs with highly specific cleaving properties work by hindering their gene expression with complementary sequences to block their mRNA post-transcription and reduce protein levels [57]. On the other hand, aptamers can act as both targeting ligand and delivery system to direct siRNA to the targeted site of the viral infection cycle and minimize off-target effects. Many studies have demonstrated the efficacies of aptamer-siRNA chimeras as targeted delivery, anti-cancer, or anti-viral therapies. This includes studies against various cancers such as lung carcinoma, melanoma, breast, and lymphoma [58–61] as well as against HIV by targeting the cell surface markers such as CD4, C-C chemokine receptor type 5 (CCR5), and glycoprotein 120 (gp120) [34,62–64]. Therefore, presenting the potential of this combination as a useful dual-functioning antiviral treatment against SARS-CoV-2.

A recent clinical study [65] has demonstrated the efficacy of RNAi-encapsulated aptamer-functionalized lipid nanocarriers as an antiviral treatment against SARS-CoV-2 in a severely ill patient, with improvement observed in the ground-glass opacity in lungs after 6 days post-treatment. The findings indicated the functionality of the aptamer with good affinity against the spike protein (0.29 nM K_d value) and the antiviral action of RNAi, targeting nucleocapsid phosphoprotein with siRNA molecule. The spike protein-targeted aptamer worked as both a targeting element and delivery vector that binds onto the spike protein of SARS-CoV-2 to block viral entry as well as delivering RNAi to the targeted mRNA site, leading to reduced production of disease-causing proteins. This work presents the possibility of using aptamers and RNAi/siRNA as a powerful antiviral therapy with three main antiviral strategies that target the spike protein, inhibit the nucleocapsid protein (N protein), and work as a delivery vector that enhances high retention efficiency and permeability, and reduces off-target effects [65].

5. Potential Limitations of Aptamer-Based SARS-CoV-2 Antiviral Therapy

The fact that aptamers are susceptible to nuclease cleavage and rapid renal filtration can hamper their drug development and clinical applications. Structural modifications of aptamers can help to improve both their pharmacokinetics and pharmacodynamics. For instance, inverted nucleotides can be introduced as oligonucleotide terminal caps, and synthetic polymers such as polyethylene glycol (PEG) can be used for aptamer conjugation to elongate the *in vivo* stability of aptamers and improve their half-life [66]. It is also possible to modify aptamers with 2'fluoro, 2'-amino, or thiol-phosphate in the 2'-position or phosphate backbone to increase their resistance against nuclease degradation and binding affinity for better serum stability [45]. Unlike monoclonal antibodies, these modifications will not cause aptamers to lose their functional properties [67,68]. Also, the application of aptamers as molecular recognition agents may be constrained by nuclease degradation, and this can be resolved using their 'mirror' analogs which preserve their original features and resistance against nucleases [16]. The hydrophilic characteristics of aptamers have also limited the specificity of intracellular targets in addition to the safety aspects of aptameric intracellular delivery, which remains unknown and requires further research investigations. Some studies have demonstrated that aptameric complexes can stimulate the production of neutralizing antibodies as well as the interior accumulation of delivered cargoes and non-specific effects [69–72]. Therefore, more research investigations are also required to study the toxicity of aptamers to utilize their potential as antiviral agents fully.

6. Dendrimer Nanoformulations against Viral Infections

Some limitations of aptamers in the development of antiviral drugs can be addressed through formulation with novel materials. Dendrimers are hyper-branched polymers with several functional groups and efficient molecular structures, as well as an inner shell, symmetric core, and outer shell [73]. Dendrimers in nanoforms are identified to be beneficial in biomedical fields for drug delivery, bioimaging, and biosensor applications due to their high surface-to-volume ratio and exclusive properties compared to traditional dendrimers [74]. Dendrimers have been studied as potential nanoformulations for targeted and controlled delivery of antiviral agents as listed in Table 2 (Ref. [43,75–82]) [83]. Vacas-Córdoba *et al*. [75] showed that polyanionic carbosilane dendrimers namely G2-NF16 and G3-S16, functionalized with two naphthylsulfonate and sulfate possess significant antiviral activity against human immunodeficiency virus (HIV). The study indicated that the dendrimer possessed an enhanced ability to inhibit the virus at the initial fusion stage with the host cell. Also, the functional groups halted the transmission of viral particles by

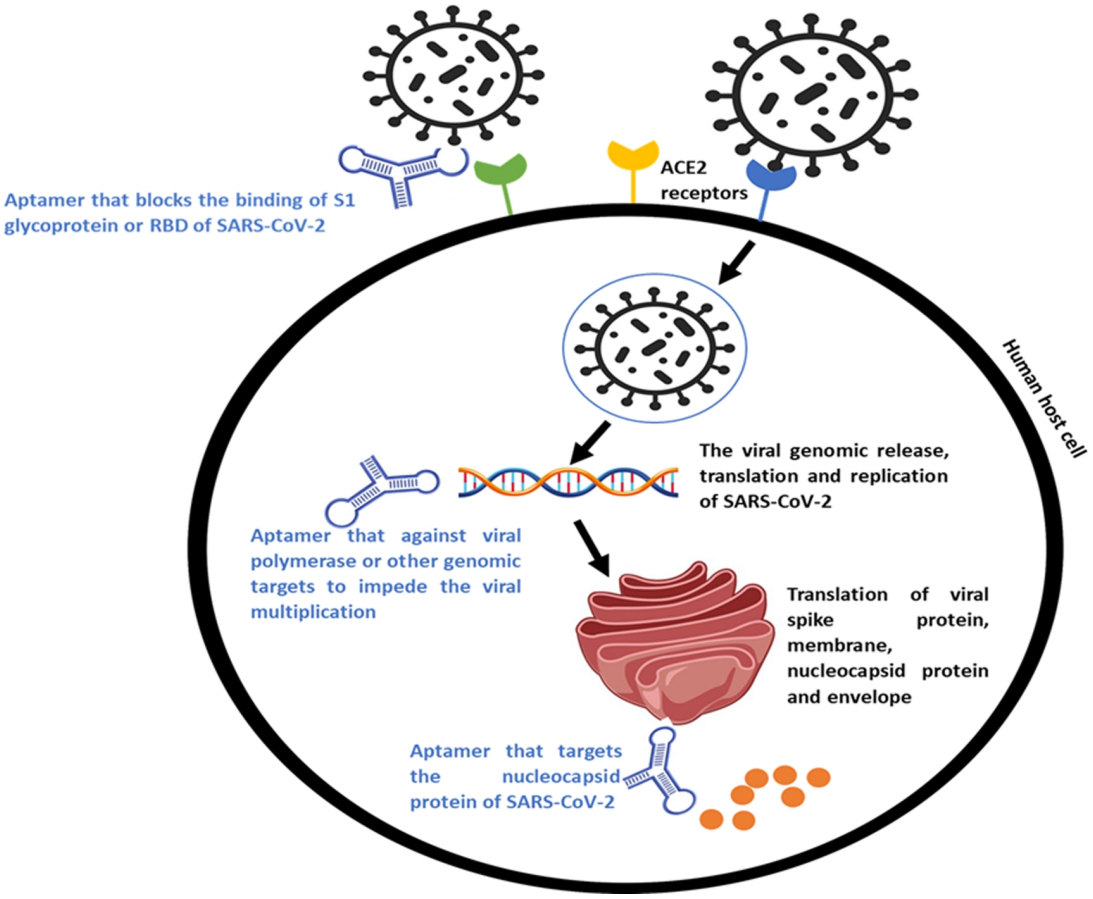

Fig. 1. Aptamers serve as a potential antiviral molecule with the capability to target different stages of SARS-CoV-2 viral entry and replication.

blocking the interaction of gp120-CD4, which eventually impeded the bond formation between the virus and the cell surface of the target. Fröhlich *et al*. [84] derived dimers, trimers, and dendrimers from Artemisinin, which is a pure sesquiterpene with 1, 2, and 4-trioxane ring that can be extracted from the plant, *Artemisia annua*. The study revealed that the dendrimer possessed enhanced antiviral activity against the human cytomegalovirus (HCMV) strain by inhibiting their green fluorescent protein (AD69-GFP) [84]. Furthermore, Romanowski *et al*. [76] demonstrated the synthesis of SPL7013, an astodrimer sodium with the core of divalent benzhydryl amine (BHA) and four lysine branches generations with hydrophobic naphthalene disulfonic acid groups capped on their outermost branches along with high anionic charged dendrimer surface. In this study, the surface-modified dendrimer was determined to possess anti-viral efficacy against a type 5 isolate of clinical adenovirus (HAdV5) in an adenovirus 5 (Ad5)/New Zealand white (NZW) rabbit ocular replication model.

It is noteworthy that astodrimer sodium (SPL 7013) was initially introduced as a vaginal antimicrobial agent for the prevention of HIV and Herpes Simplex Virus (HSV), and is currently utilized as an antiviral lubricant in condom products [85–87]. Moreover, Sepúlveda-Crespo *et al*. [77] demonstrated that the polyanionic carbosilane dendrimer G2-S16 with a silica core and 16 sulfonate end groups can inhibit HIV-1 viral ribonucleic acid (RNA) in the vagina of humanized bone marrow-liver-thymus (BLT) mice model. The study showed that the dendrimers possess the ability to block the interaction of gp120/CD4 and halt the cell-to-cell transmission of the virus in the host via non-specific and multifactorial antiviral efficacy. Maciel *et al*. [78] more recently developed a new family of chemically very stable anionic poly(alkylideneamine) dendrimers (G1-G3) functionalized with carboxylate or sulfonate terminal groups, demonstrating that generation 1 has potent activity against R5-HIV-1NLAD8 and X4-HIV-1NL4.3 isolates. This activity comes from the fact that these dendrimers act directly over the circulating viral particles by blocking their entry into the host cells. The *in vivo* studies in BALB/c mice also confirmed the G1-C8 or S8 dendrimers' capacity to be used against HIV-1 infection. The achieved results are not only similar to the carbosilane dendrimers, G2-S16, but were obtained using a lower dendrimer's generation and a smaller number of anionic terminal groups. Importantly, these anionic poly(alkylideneamine) dendrimers of generation 1 prevent HIV-1 infection without the need to be combined with other antiviral drugs (combined therapy) [78].

Table 2. Summary of some dendrimer formulations against viral infections.

Dendrimer	Formulation/functionalization	Antiviral efficacy	Reference
Polyanionic carbosilane dendrimers G2-NF16 and G3-S16	Functionalized with naphthylsulfonate and sulfate	Human immunodeficiency virus	[75]
Sulfonated SPL7013 dendrimer	Viva Gel®	Viruses causing vaginal infection	[43]
SPL7013 topical astodrimer	High anionic charge	Clinical adenovirus (HAdV5)	[76]
Polyanionic carbosilane dendrimer G2-S16	16 sulfonate end groups	HIV-1	[77]
PEG 600 dendrimer	Silver nanoparticles	HIV-1	[79]
Janus-like dendrimer	Peptides and glycoproteins	Herpes Simplex virus type 1	[80]
4th and 5th generation PAMAM glycodendrimers	Copper nanoparticles, functionalized with shikimic acid	Dengue and Zika viruses	[82]
PAMAM dendrimer	Telbivudine, Adefovir, Tenofovir, Entecavir, and Lamivudine	Hepatitis virus	[81]
1st, 2nd, and 3rd generation poly (alkylideneamine) dendrimers	Sulfonate and carboxylate terminal groups with nitrile termini	Human immunodeficiency virus type-1 (HIV-1)	[78]

Ardestani *et al.* [79] utilized dicyclohexylcarbodiimide with dimethyl sulfoxide (DMSO) and polyethylene glycol 600 (PEG 600) as the dendrimer core for the fabrication of first- and second-generation dendrimers. Later, the dendrimers were conjugated with silver nanoparticles to form anionic linear globular dendrimers with exclusive antiretroviral activity. It is evident from the study that the resultant dendrimers possessed an ability to deliver silver nanoparticles for controlled inhibition of human immunodeficiency virus-1 (HIV-1) single-cell replicable (SCR) virions pseudotyped by vesicular stomatitis viral G-protein (VSVG) [79]. Falanga *et al.* [80] synthesized a novel Janus-like dendrimer with peptides derived from glycoproteins of Herpes Simplex virus type 1 (HSVT1) for the inhibition of the same viral strains. In this work, the Janus dendrimer was fabricated by a combination of copper-catalyzed bio-orthogonal cycloaddition of 1, 3-dipolar alkyne, or azide to obtain photoinitiated monofunctional, thiol-ene coupling and bifunctional conjugates of peptidodendrimer. The resultant dendrimers were determined to possess HSVT1 virus inhibition activity during the early and late stages of the infection process [80]. Bayat *et al.* [81] proposed the use of antiviral drugs, such as Telbivudine, Adefovir, Tenofovir, Entecavir, and Lamivudine, entrapped in PAMAM dendrimer to suppress hepatitis virus growth. The study revealed that the drugs, such as Adefovir and Entecavir, entrapped in dendrimer possess an enhanced ability to inhibit the virus compared to the standalone drug counterparts [81]. This suggests that standalone dendrimers or drug/nanoparticles entrapped dendrimers may possess some antiviral efficacy.

7. Dendrimer Nanoformulations Drugs and Theranostics

Several dendrimer-formulated drugs or biomolecules have been recently proposed to be beneficial for the inhibition of SARS-CoV-2. Khaitov *et al.* [88] extracted 15 siRNAs via an *in-silico* approach and screened them based on SARS-CoV-2 genes fused with the reporter gene of luciferase from a firefly. The screened siRNA, named siR-7, was regulated via locked nucleic acid to obtain stable siRNA and formulated with KK-46 peptide dendrimer. The resultant peptide dendrimer formulation was identified to possess the ability to inhibit SARS-CoV-2 and was proposed to be beneficial for the treatment of COVID-19 via inhalation [88]. Mignani *et al.* [89] proposed that functionalized dendrimers can be utilized to target SARS-CoV-2 and inhibit their growth and spread. They indicated that biocompatible dendrimers can serve as a novel nanocarrier of antiviral drugs to eliminate SARS-CoV-2 [89].

Farzin *et al.* [90] prepared an exclusive nanoscale genosensor for the early detection of COVID-19 via SARS-CoV-2 viral RNA polymerase sequence. In this study, a redox probe with silver ions on the surface of hexathia-18-crown-6 was incorporated with a carbon paste electrode. The carbon electrode was modified with silicon quantum dot-coated polyamidoamine (PAMAM) and chitosan dendrimer, essential for the probe sequence immobilization of aminated oligonucleotides. The study showed that the dendrimer-based voltammetric genosensor was beneficial in detecting RNA-dependent RNA polymerase sequence of SARS-CoV-2 in human sputum samples with a 0.3 pM limit of detection [90].

In recent times, the antiviral advantages of aptamers and/or siRNA and dendrimers have led to the emergence of aptamer formulations of dendrimers to exhibit potential antiviral efficacy. Zhou *et al.* [91] reported a dicer substrate small interfering-RNA conjugated with cationic PAMAM dendrimer for the inhibition of HIV-1 infection in a RAG-hu humanized mouse model. The study showed that the formulation protected the host from the depletion of CD4+ T-cells induced by the virus without any measurable toxic-

ity. However, the study also reported the accumulation of the formulation in the liver and peripheral blood mononuclear cells [91]. Vivian [92] proposed the development of a novel biosensor via dendrimer-gold platforms and conjugated with an either an aptamer for the detection of HIV in biological samples [50]. These reports show the potential of aptamer-dendrimer combinations in the development of a wide range of theranostics for viruses and viral diseases, including SARS-CoV-2.

8. Conclusions

Aptamers possess remarkable potential as they can be synthesized with high specificity for a wide range of targets, making them relevant as affinity ligands capable of rendering antiviral functionalities. Ongoing mutations in SARS-CoV-2 constitute a major challenge that affects the effectiveness of vaccines and drugs for COVID-19. Hence, the highly specific properties of aptamers are extremely attractive to accommodate viral mutations for the development of new and improved antiviral therapy against SARS-CoV-2. This is further supported by numerous reported studies that have demonstrated the useful application of aptamers and aptamer-conjugated dendrimers in diagnosing and treating different viral infections. Their preclinical safety and efficacy profiles provide ample rationale to promote the development of aptamer-based antiviral therapies against SARS-CoV-2.

Author Contributions

KXT and JJ prepared the initial draft of the review. JR and MKD reviewed and helped to improve the quality of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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