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REVIEW

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# A review on current status of antiviral siRNA

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## Summary

Viral diseases like influenza, AIDS, hepatitis, and Ebola cause severe epidemics worldwide. Along with their resistant strains, new pathogenic viruses continue to be discovered so creating an ongoing need for new antiviral treatments. RNA interference is a cellular gene-silencing phenomenon in which sequence-specific degradation of target mRNA is achieved by means of complementary short interfering RNA (siRNA) molecules. Short interfering RNA technology affords a potential tractable strategy to combat viral pathogenesis because siRNAs are specific, easy to design, and can be directed against multiple strains of a virus by targeting their conserved gene regions. In this review, we briefly summarize the current status of siRNA therapy for representative examples from different virus families. In addition, other aspects like their design, delivery, medical significance, bioinformatics resources, and limitations are also discussed.

**KEYWORDS**

antiviral, clinical trials, delivery, design, limitations, resources, RNAi, siRNA, virus

## 1 | INTRODUCTION

Because of their high mutation rates, viruses have the potential to elude host defense systems as well as antiviral drugs and vaccines. Thus, development of new and alternate antiviral therapies has become important.[1,2] During the past decade, scientists have widely used the cellular RNA interference (RNAi) pathway approach to target a number of viral genes to restrain their expression.[3,4] In this pathway, long double-stranded RNA (dsRNA) precursors are split into short interfering RNAs (siRNAs) following which the RNA-induced silencing complex includes one of the siRNA strands and slices the complementary target mRNA using ATP.[5,6] Short interfering RNA technology has been exploited to target disease-causing genes as well as for functional studies.[7,8] Also, this strategy can target diverse types of viruses as even a tiny viral genome can provide several targetable regions. For example, siRNAs directed against different genes of deadly viruses like human immunodeficiency virus (HIV),[9,10] influenza virus (INFV),[11,12] hepatitis B virus (HBV),[13] SARS coronavirus (SARS-CoV),[14,15] human papillomavirus (HPV),[16] and West Nile virus (WNV)[17] in infected cells displayed encouraging results in inhibiting viral replication. Researchers have also used multiple siRNAs simultaneously to augment viral inhibition in a coordinated approach.[18,19] Short interfering RNAs for various human viruses like respiratory syncytial virus (RSV), hepatitis C virus (HCV), HBV, and HIV are also appearing in clinical trials, which further elucidate their importance in inhibiting viral infections.[20] Thus, siRNAs have emerged as practically modular and adaptable therapeutics for treating viral infections. In this review, we will discuss the use of siRNAs against different viral families, their therapeutic applications, and design and delivery considerations. Further limitations of siRNAs as antivirals and the remedial measures are also discussed.

**Abbreviations used:** BKV, polyomavirus BK; DENV, Dengue virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HIVsirDB, HIV siRNA database; HPV, human papillomavirus; INFV, influenza virus; MARV, Marburg virus; RISC, RNA-induced silencing complex; RNAi, RNA interference; RSV, respiratory syncytial virus; SARS-CoV, SARS coronavirus; siRNA, short interfering RNA; T-Ag, T antigen; UTR, untranslated region; VACV, vaccinia virus; VIRsiRNAdb, viral siRNA database; VIRsiRNApred, viral siRNA predictor; WNV, West Nile virus

## 2 | VIRUSES

Viruses are tiny obligate intracellular parasites, having either an RNA or a DNA genome enclosed by a virus-coded protein coat. Viruses depend on host cells for proliferation. They are classified on account of shape, genome structure, or mode of replication, and many classes of these pathogens cause a large number of diseases in different organisms.[21] Different species of viruses have varying types of infection processes; however, there are many general steps[22,23] that are briefly summarized

  

in Figure 1. Short interfering RNAs directed against specific viral/host genes can block the virus life cycle at any of the steps.

## 3 | RNA INTERFERENCE

RNAi is a cellular mechanism wherein small molecules of RNA hinder the expression of a particular gene(s) via counteracting the corresponding mRNA molecules that possess nucleotide sequences complimentary to the small RNA.[24] Historically, RNAi was identified by different terms such as quelling, cosuppression, and posttranscriptional gene silencing. In 1998, Mello and Fire illustrated a strong gene silencing caused by injecting dsRNA into *Caenorhabditis elegans*.[25] Further findings in the field of RNAi have been pictorially summarized in Figure 2.

The RNAi pathway processes dsRNA into 21 to 30 nucleotide-long RNA molecules that act as a module of a silencing machinery to distinctively suppress expression/function of an intended gene/genomic region (Figure 3). In particular, the silencing pathway involves chopping of dsRNA into siRNA that are typically 21 to 25 base pairs long dsRNA having dinucleotide overhangs on the 3′ termini. One of the siRNA strands (guide strand) is then incorporated into an RNA-induced silencing complex that degrades the target mRNA.[5,26] The siRNAs resulting from the original longer dsRNA are different from microRNAs as the latter in general have partial base pairing with a target mRNA and restrain the expression of several different genes having related sequences. Short interfering RNAs also differ from short hairpin RNAs as the latter have a hairpin turn and their expression in cells is usually achieved by means of bacterial/viral vectors.[27]

Short interfering RNAs characteristically base-pair completely and cause mRNA cleavage in a precise target region.[28] MicroRNAs are generated from introns or their own genes. Gene silencing can take place through mRNA cleavage or thwarting mRNA translation. The role of microRNAs is to regulate gene expression. In the plant kingdom, RNAi is broadcasted by the transportation of siRNAs amid cells via plasmodesmata.[29] RNAi was described as the "breakthrough of the year" in[30] 2002. RNAi is also believed to be a component of a primitive immune system based on nucleic acid recognition.[31] RNAi shields human cells from pathogenic viruses by silencing viral genes.[32,33] Moreover, small RNAs can cause genomic imprinting or facilitate in delineating tissue-specific transcription prototypes by adapting conformation of certain genome regions.[34]

## 4 | ANTIVIRAL POTENTIAL OF siRNAs

Short interfering RNAs can be used against all types of viral genomes, be it double- or single-stranded DNA/RNA. Also, several siRNAs can be used concurrently to maintain an extended antiviral effect. The following examples (Table 1) further illustrate the development of siRNA therapeutics as a novel antiviral strategy.

### 4.1 | DNA viruses

#### 4.1.1 | Papillomaviridae

This taxonomic group consists of nonenveloped DNA viruses. Infection by papillomaviruses can cause benign or cancerous tumors.[85] Viral

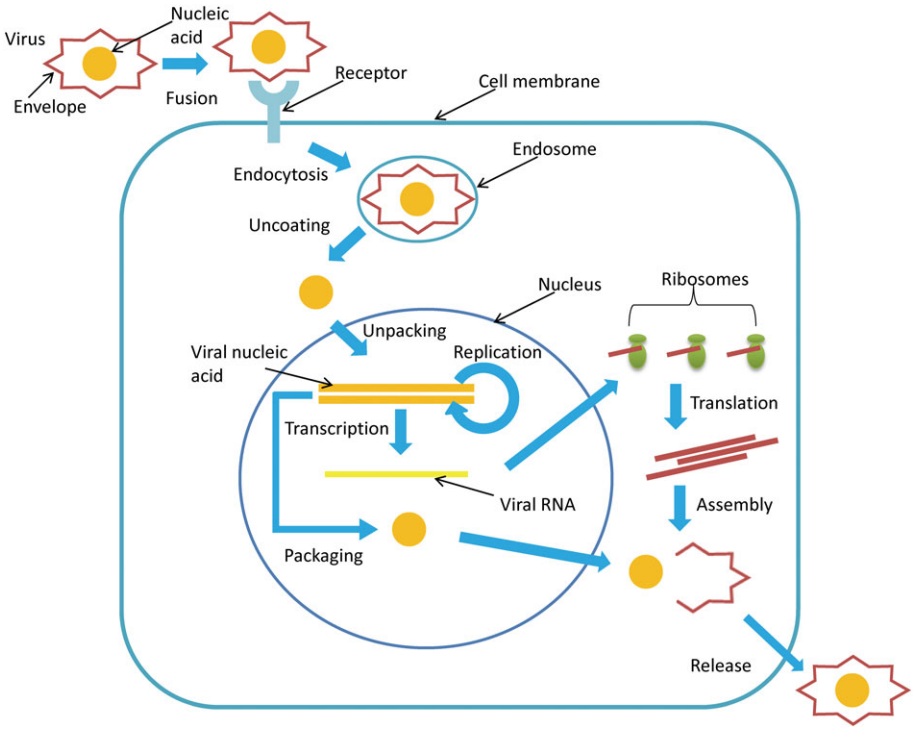


**FIGURE 1** The typical different stages of virus life cycle. (1) Attachment: In this step, the viral envelope glycoproteins attach to certain host cell membrane receptors. (2) Endocytosis: Here, the viral contents are taken up by the host cell. (3) Uncoating: Degradation of the viral capsid by host cell enzymes. (4) Growth: It involves translation and replication of the viral genes. (5) Assembly: The viral proteins assemble to enclose the viral genome. (6) Release: The mature virus particles escape from the host cell by budding/lysis. In RNA viruses, however, the viral genome is usually not integrated into the host genome, and hence, their RNA molecules are directly used as mRNAs for translation

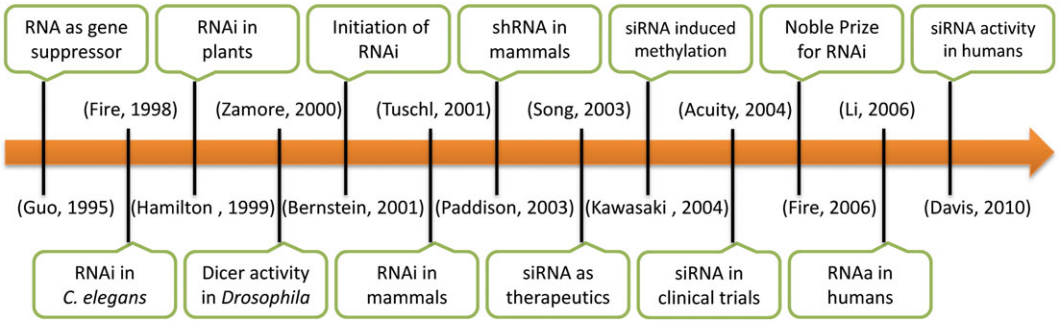


**FIGURE 2** Timeline depicting the sequential progression in the field of RNA interference (RNAi). shRNA, short hairpin RNA; siRNA, short interfering RNA

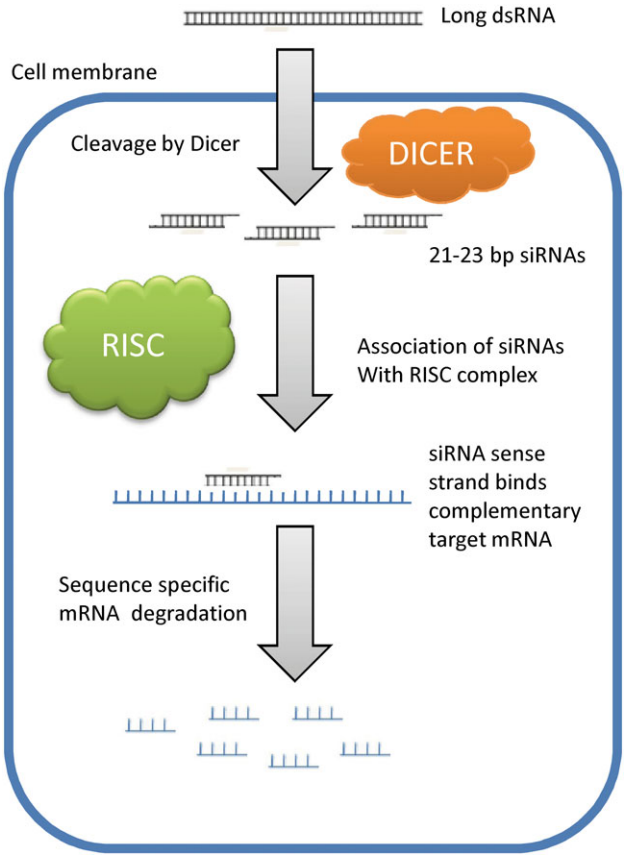


**FIGURE 3** In the RNA interference mechanism, double-stranded RNA (dsRNA) is chopped into short (21-25 nucleotides) interfering RNA (siRNA) molecules possessing dinucleotide overhangs on the 3′ termini. One siRNA strand is integrated into the RNA-induced silencing complex (RISC) that finally degrades the complementary target mRNA

oncogenes of HPV types 16 and 18 can cause cervical cancer. To counteract this, siRNAs have been engaged against the antiapoptotic HPV E6 oncogene, resulting in selective and substantial cell death of HeLa cancer cells.[86] Short interfering RNAs have also been used in several combinations against HPV-16 and HPV-18 targeting their E6 or E7 gene resulting in considerable reduction in viral replication in HeLa cell line. It is noteworthy to mention that the siRNAs did not show any harmful effect on control cells, which indicates meager off-target effects.[87] This illustrates that siRNA treatment has the potential to suppress the progression of cervical cancer.

**TABLE 1** Development of antiviral short interfering RNAs by various researchers against a range of pathogenic viruses

| S. No. | Virus Family | Genome Type | Reference |
|---|---|---|---|
| 1 | Papillomaviridae | DNA viruses | 16,35,36 |
| 2 | Polyomaviridae | | 37,38 |
| 3 | Poxviridae | | 39-41 |
| 4 | Hepadnaviridae | | 42-44 |
| 5 | Herpesviridae | | 45-47 |
| 6 | Reoviridae | Double-stranded RNA viruses | 48,49 |
| 7 | Arenaviridae | Negative-strand RNA viruses | 50-52 |
| 8 | Paramyxoviridae | | 53-55 |
| 9 | Rhabdoviridae | | 56-58 |
| 10 | Bunyavirales | | 59-61 |
| 11 | Filoviridae | | 62-64 |
| 12 | Orthomyxoviridae | | 65-67 |
| 13 | Picornaviridae | Positive-strand RNA viruses | 68-70 |
| 14 | Togaviridae | | 71,72 |
| 15 | Coronaviridae | | 15,73,74 |
| 16 | Flaviviridae | | 75-77 |
| 17 | Hepeviridae | | 78-80 |
| 18 | Retroviridae | | 81-84 |

### 4.1.2 | Polyomaviridae

Polyomaviruses have a double-stranded circular DNA genome.[88] They are clinically relevant because they cause Merkel cell carcinoma, a very aggressive type of squamous cancer. Merkel cell polyomavirus T antigen (T-Ag) protein is involved in replication and plays a major role in viral infection. Merkel cell polyomavirus activity can be restrained through rationally designed siRNA molecules for the treatment of Merkel cell carcinoma at the genome level.[37] Similarly, the polyomavirus BK (BKV) has been found to induce malignant transformation. Repression of the T-Ag oncogene has been shown to hinder the transformation of the cells. Short interfering RNAs designed to target the BKV T-Ag were reported to restrain its expression in pRPc cell lines. Blocking of T-Ag results in diminished growth rate of BKV-transformed cells and thus suppresses tumorigenicity.[38]

### 4.1.3 | Poxviridae

Poxviruses have a single, linear, double-stranded DNA and infect both vertebrates and invertebrates. Vaccinia virus (VACV) is the quintessential member of the Poxviridae.[89] The VACV produces a dsRNA

binding protein, E3L, which hampers host defense mechanisms like interferon. E3L-specific siRNAs inhibited virus replication in HeLa cells by 98% as compared with control infection.[39] In addition, both early and late gene expressions of VACV could be blocked by siRNA treatment.[90]

#### 4.1.4 | Hepadnaviridae

Hepadnaviridae include enveloped viruses with a partially double-stranded genome. The viruses of this family can cause liver infections in animals including humans.[91] Hepatitis B virus is the most well-known member of this group, as it is one of the leading causes of liver cirrhosis and hepatocellular carcinoma. Short interfering RNA developed against the surface antigen region significantly reduced the level of viral transcripts as well as the secretion of viral antigens in mice.[92]

#### 4.1.5 | Herpesviridae

This family includes important pathogenic viruses like Epstein-Barr virus and herpes simplex virus. Epstein-Barr virus is responsible for the maintenance of the tumor phenotype in many cancer types. It was found that EBNA1 is universally expressed in all Epstein-Barr virus–associated tumors. Short interfering RNAs generated against the EBNA1 mRNA are able to inhibit its translation and thus block tumor survival in HeLa cells.[93] Similarly, the herpes simplex virus glycoprotein E is responsible for cell-to-cell spread and immune evasion. Targeting glycoprotein E with siRNAs suppressed its expression and function in HaCaT cells.[94]

### 4.2 | dsRNA viruses

#### 4.2.1 | Reoviridae

Reoviruses have a genome of about 10 segments of dsRNA. Plasmid-based vectors expressing siRNAs targeting the μNS, μ2, and σNS genes of the T3D strain of reovirus considerably blocked multiple steps in the viral replication machinery in 293T cells.[48] These studies further illustrate the usefulness of siRNAs both as therapeutic agents and as important means for the investigation of relationship between structure and function of viral proteins.

### 4.3 | Negative-strand RNA viruses

#### 4.3.1 | Arenaviridae

Arenaviruses contain a segmented RNA genome with 2 single-stranded ambisense RNAs.[95] Arenaviruses are often responsible for fatal hemorrhagic fever in people, and there is a lack of effective medications to tackle their infection. The lymphocytic choriomeningitis virus is a key model used in the investigation of arenavirus linked pathogenesis. Short interfering RNAs directed against L polymerase and Z viral mRNAs stall viral growth in HEK 293T host cells.[50]

#### 4.3.2 | Paramyxoviridae

Paramyxoviridae are negative-sense single-stranded RNA viruses.[96] The RSV is an important member of this group. It causes lung infection in young and the aged people worldwide. Short interfering RNAs targeting the RSV P gene in a BALB/c mice model showed less lung pathogenesis or pulmonary inflammation and generated a strong antiviral reaction when later given RSV.[97]

#### 4.3.3 | Rhabdoviridae

Rabies virus (RV) is a member of family Rhabdoviridae and causes fatal zoonotic disease in both humans and animals. It is responsible for 50 000 to 55 000 human deaths annually in Asia and Africa.[56] Brandao et al used siRNAs against rabies virus nucleoprotein mRNA, which decreased virus titers in BHK-21 cells.[57] Other workers developed siRNAs targeting rabies virus glycoprotein and nucleoprotein showing remarkable knockdown effects and significant inhibition of RV multiplication and release.[56]

#### 4.3.4 | Bunyavirales

This order of viruses, previously known as the Bunyaviridae family, includes enveloped, segmented negative-stranded RNA viruses.[98] Because of lack of effective vaccines and therapies for bunyaviruses in humans, siRNAs afford an attractive alternative. For example, the model Hazara virus of genus *Nairovirus* was inhibited by siRNAs targeting the NP gene in A549 cells.[59] Similarly, siRNAs developed against the agnoprotein of John Cunningham virus, the etiological agent of the demyelinating progressive multifocal leukoencephalopathy disease, proved to be an effective inhibitor of John Cunningham virus infection in nude mice.[99]

#### 4.3.5 | Filoviridae

The Filoviridae family comprises filamentous single-stranded negative-sense RNA viruses such as Ebola and Marburg virus (MARV), which can cause severe disease in humans.[100] Short interfering RNA targeting the Zaire ebolavirus nucleoprotein decreased viral titers after infection in 293T cells.[62] Short interfering RNAs were used to degrade MARV nucleocapsid transcripts (NP, VP35, and VP30) in HeLa CCL-2 cells. Down-regulation of VP30 also caused a strong decline in the expression of other MARV proteins and virus release, suggesting the role of VP30 in viral transcription and replication.[63]

#### 4.3.6 | Orthomyxoviridae

Influenza virus causes one of the most common infections in humans. Although some antiviral drugs are available, their use is limited by possible emergence of resistant virus. Ge et al found that siRNAs pertaining to conserved regions of the INFV genome can inhibit its replication in C57BL/6 mice.[101,102]

### 4.4 | Positive-strand RNA viruses

#### 4.4.1 | Picornaviridae

Picornaviruses are nonenveloped RNA viruses and have an icosahedral capsid. Coxsackievirus and poliovirus are two of the well-known viruses in this group.[103] Treatment with siRNAs significantly decreased cell death in parallel with a reduction in coxsackievirus B3 replication in HeLa cells. Also, the efficient coxsackievirus B3–specific siRNA displayed antiviral activity in other related enteroviruses such as CVB1, CVB5, CVB6, coxsackievirus A9, and Echo6 in HeLa cells.[104]

### 4.4.2 | Togaviridae

This group of viruses contains linear, single-stranded, positive-sense RNA.[105] One of its members, the Chikungunya virus, is the causative agent of chikungunya fever, which has emerged as an important arboviral infection of public health concern. Short interfering RNAs targeting the conserved segments of nsP3 and E1 mRNAs of the pathogen were able to drastically reduce the virus titer in Vero cells.[71]

### 4.4.3 | Coronaviridae

Severe acute respiratory syndrome, also known as SARS-CoV, is a member of the Coronaviridae family whose members have single-stranded positive-sense RNA genomes. Short interfering RNAs targeting the 3′ untranslated regions (UTRs) of the pathogen inhibit the replication of SARS-CoV in Vero-E6 cells.[106] Li et al demonstrated that siRNAs used against SARS-CoV provided relief from viral fever, decreased viral levels, and lower acute diffuse alveoli damage in macaques.[107]

### 4.4.4 | Flaviviridae

This family encompasses spherical enveloped viruses with linear, single-stranded positive RNA genome. It includes many important human viruses like HCV, Dengue virus (DENV), and WNV.[108] Hepatitis C virus infection can cause permanent damage to the liver, hepatocellular carcinoma, and death. Seo et al targeted multiple segments of the 5′ UTR of the virus genome using siRNA and achieved up to 85% reduction in activity in Huh-7 cells.[109] In a similar approach, siRNAs were designed against WNV 3′ UTR and then expressed from a plasmid-based system in Vero cells resulting in suppression of WNV replication in a sequence-specific manner and also indicating the function of 3′ UTR in WNV pathogenesis.[110] Dengue virus causes severe disease that threatens public health globally in tropical and subtropical places. Exogenously introduced siRNA directed against the conserved 5′ cyclization sequence segment of the DENV genome effectively decreased the viral titers of multiple DENV strains in mice illustrating the potential of siRNAs to tackle genetically varied dengue strains.[111] Zika virus had recently posed as a global health threat prompting intense research to control the pathogen.[112] Scientists have found that the conserved 3′ UTR of the virus genome plays a crucial function in its replication. Therefore, rationally designed siRNAs against the 3′ UTRs have been predicted to inhibit the pathogen.[113,114] Studies have also shown that siRNA-directed silencing of host endoplasmic reticulum membrane complex protein components halted the replication of multiple Zika virus strains in HeLa cells.[115]

### 4.4.5 | Hepeviridae

Hepeviridae mainly includes hepatitis E virus, a key source of waterborne hepatitis in adults that has particularly high mortality in pregnant women. Short interfering RNAs developed against the helicase and replicase genes of hepatitis E virus were found to be effective in inhibiting virus replication in A549 as well as HepG2 cells.[78,79]

### 4.4.6 | Retroviridae

Retroviridae is a family of enveloped single-stranded RNA viruses that replicate in a host cell through the process of reverse transcription.[116] It has been noted that siRNAs can inhibit HIV-1 growth by aiming the genes for the host CD4 cell receptor, or the viral Gag and Nef proteins in Magi-CCR5, HeLa-CD4, and H9 T cells. Studies report that siRNA molecules efficiently hinder preintegration as well as postintegration infection phases in the HIV life cycle.[117]

## 5 | CLINICAL TRIALS

Because of the large therapeutic potential offered by siRNAs against the pathogenic viruses, many of them are being considered for medical use in the near future. A few siRNA-based antivirals have already entered clinical trials, eg, ALN-RSV01 for RSV targeting its nucleocapsid gene (phase II),[118] NucB1000 directed against 4 different targets (Pre-C, Pre-S1, Pre-S2, and X) of HBV (phase I),[119] SPC3649 directed against miR-122 for HCV (phase II),[120] pHIV7-shI-TAR-CCR5RZ targeting multiple genes (Tat, Tar, and CCR5) for HIV (phase I),[121] and TKM-Ebola directed against multiple transcripts (L, VP24, and VP35) of EBOV (phase I).[122]

RNAi therapy has also been proved to be useful against animal viruses such as Marek's disease virus in chicken,[123] foot and mouth disease virus in pigs,[124] and O'nyong-nyong virus in mosquitoes.[125] The significance of RNAi in the treatment of viral infections was further reviewed.[20,126] Figure 4 briefly summarizes the role of gene silencing in combating pathogenic viruses.

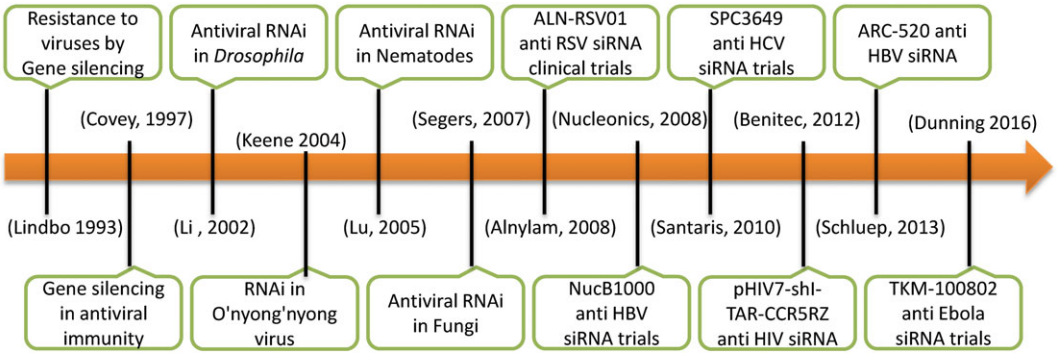


**FIGURE 4** Timeline summarizing different stages in the course of development of RNA interference (RNAi)–based therapeutics against viruses. HBV, hepatitis B virus; HCV, hepatitis C virus; siRNA, short interfering RNA

## 6 | DESIGN

Designing siRNAs with high antiviral activity is a challenging task. There are many features that influence siRNA efficacy including GC (guanine-cytosine) content, nucleotides at siRNA termini, thermodynamic properties, siRNA structure, and accessibility of the target site.[127,128] Also, the siRNAs should be developed against conserved target sites to prevent viral escape due to mutations.[129] It has been suggested that a single target site might not be enough for durable viral silencing so using multiple siRNAs against several target sites can also prevent viral escape.[19] In addition, chemically modified nucleotides can also be used to enhance the stability or reduce off-target effects of siRNAs,[130-132] eg, modified siRNAs used against HBV infection were found to have significantly increased half-life and activity in human serum as compared with the unmodified siRNAs.[133]

## 7 | DELIVERY

Unaided siRNAs are not capable to penetrate the cellular membrane due to their negative charge. Besides, siRNAs have been shown to elicit immune response and are vulnerable to nuclease enzymes. Thus, suitable delivery agents are required to efficiently transport the siRNAs to the target cells. Delivery of siRNAs to the desired cells can lessen the amount of siRNA necessary for silencing and also circumvent off-target effects.[20]

Short interfering RNAs are delivered in a number of ways, eg, by encapsulation in synthetic vehicles such as cationic liposomes/nanoparticles or siRNAs conjugated to cell penetrating peptides or specific antibodies against the infected cells.[134] Liposomes are frequently used as delivery mediums for a wide range of drugs including siRNAs. This method often involves cationic lipids to overcome the negative charge associated with siRNAs.[135] Lately, stable nucleic acid lipid particles have been used to efficiently stabilize and transport siRNAs.[136] Morrissey et al used stable nucleic acid lipid particles to deliver siRNAs against HBV, which efficiently inhibited the virus in mice.[137] Next, the polymer-based siRNA delivery vehicles (polyplexes) afford high structural and physicochemical flexibility while shielding the siRNAs against nucleases.[138] Likewise, inorganic nanoparticles generated from calcium phosphate, gold, carbon, and iron oxides are sometimes preferred to transport siRNAs due to their small size and increased permeability in comparison with liposomes and polyplexes. Occasionally, surface ligands are incorporated with the nanoparticles to enhance selective targeting of the diseased cells.[139] Plasmid and viral vectors are also used as expression cassettes for sustained silencing effect. However, use of plasmids limits the delivery efficiency as compared with the viral vectors.[140,141] Alternatively, direct injection into the infected tissue may also help in targeting the specific cells.[134]

## 8 | BIOINFORMATICS RESOURCES

Although a large number of databases, design, and prediction algorithms are available for mammalian siRNAs, very few bioinformatics resources have been developed so far regarding viral siRNAs despite their huge potential and data availability. The viral siRNA database, available at http://crdd.osdd.net/servers/virsirnadb, covers the details of siRNAs targeting 42 important human viruses. The database provides not only detailed information about siRNA sequence, target virus and gene, cell line, assay, and inhibition but also useful siRNA analysis tools including siTarAlign that aligns the siRNA sequence with genome sequences of representative viruses.[142] On similar lines, the HIV siRNA database, available at http://crdd.osdd.net/raghava/hivsir, furnishes details of experimentally tested siRNAs/short hairpin RNAs aiming diverse HIV genome segments. Further, the "HivsirMut" subdatabase, accessible at http://crdd.osdd.net/raghava/hivsir/hiv-esc-seq.php, provides information of escape mutations together with nucleotide mismatch amid the target genes and the siRNA molecules and their effect on siRNA efficacy.[143]

As all siRNAs developed to inhibit a certain gene are not equally successful, a variety of wide-ranging siRNA design rules and prediction methods have been published. The most basic procedures for siRNA design were grounded on frequency of certain nucleotides at multiple locations in siRNA sequence as anticipated by Elbashir et al,[144] Reynolds et al,[145] Ui-Tei et al,[146] Amarzguioui et al,[147] and Jagla et al.[148] siVirus web server, accessible at http://sivirus.rnai.jp, makes use of these guidelines to select effective siRNAs against viruses. siVirus provides siRNA sequences directed against conserved regions of viruses like HIV, HCV, INFV, and SARS-CoV with minimum off-target effects.[149] Although many machine learning techniques like boosted genetic programming,[150] artificial neural network,[151] and support vector machine[152] have been used to design mammalian siRNAs, however, their performances were not satisfactory for viral siRNAs. This may be due to the fact that these methods were not trained on viral siRNAs. Viral siRNA predictor, available at http://crdd.osdd.net/servers/virsirnapred, the original algorithm for calculating inhibition potential of viral siRNAs, is a support vector machine–based method for predicting the activity of viral siRNA. This algorithm was developed using published viral siRNAs using many features such as nucleotide frequency, thermodynamic factors, and nucleotide location to predict the competence of siRNAs targeting pathogenic viruses.[153] These online resources (Table 2) will facilitate the

**TABLE 2** Bioinformatics resources dedicated to the analysis, prediction, and archival of antiviral short interfering RNAs (siRNAs)

| S. No. | Resource | Description | URL | Reference |
|---|---|---|---|---|
| 1 | siVirus | Antiviral siRNA design | http://sivirus.rnai.jp/ | 149 |
| 2 | HIVsirDB | HIV siRNA database | http://crdd.osdd.net/raghava/hivsir | 143 |
| 3 | VIRsiRNAdb | Viral siRNA database | http://crdd.osdd.net/servers/virsirnadb/ | 142 |
| 4 | VIRsiRNApred | Viral siRNA prediction | http://crdd.osdd.net/servers/virsirnapred/ | 153 |

researchers in selecting or designing efficient siRNAs for antiviral therapeutic development.

## 9 | LIMITATIONS AND FUTURE IMPLICATIONS

Short interfering RNA–mediated gene silencing has surfaced as a potent strategy to study cellular networks as well as to precisely knockdown the disease causing factors. However, siRNAs are confronted by a few shortcomings like virus escape, inefficient cellular uptake, poor stability, off-target effects, and immunostimulation.[154-156] Virus escape can be overcome by targeting conserved viral genes[149] or those factors involved in negative-feedback regulation.[3] Cellular uptake can be improved by using synthetic nanoparticles composed of polymers, lipids, and conjugates and also by incorporating cell-specific targeting ligands in the carriers.[140] As far as siRNA stability is concerned, chemical modifications like the 2′-fluoro and thioate linkages may be used to prolong the half-life of siRNAs.[157] Also, detailed identification of the cellular pathways of immunorecognition of RNA can allow the development of methods to avoid immunostimulatory oligonucleotide motifs during siRNA design.[158] Also, multiple siRNA expression vectors may be used to maximize the long-term inhibition.[159] It would also be helpful to make use of bioinformatics approaches to identify potential target sites as well as to design siRNAs with optimum features for preliminary experiments.[160]

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### CONFLICT OF INTEREST

The authors have no competing interest.

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