Antiviral RNAi: Translating Science Towards Therapeutic Success

Priya S. Shah • David V. Schaffer

ABSTRACT Viruses continuously evolve to contend with an ever-changing environment that involves transmission between hosts and sometimes species, immune responses, and in some cases therapeutic interventions. Given the high mutation rate of viruses relative to the timescales of host evolution and drug development, novel drug classes that are readily screened and translated to the clinic are needed. RNA interference (RNAi)—a natural mechanism for specific degradation of target RNAs that is conserved from plants to invertebrates and vertebrates—can potentially be harnessed to yield therapies with extensive specificity, ease of design, and broad application. In this review, we discuss basic mechanisms of action and therapeutic applications of RNAi, including design considerations and areas for future development in the field.

KEY WORDS antiviral • gene therapy • RNA interference (RNAi) • viral escape

INTRODUCTION

Viruses are known for their genomic economy and prolific ability to mutate. Related to the former, instead of encoding large numbers of their own factors, viruses rely on a number of host factors to mediate their replication (1–6), a property that limits the number of viral molecules that can be therapeutically targeted, particularly by small molecule therapies. Furthermore, mutation rates as high as $10^{-3}$ errors per nucleotide per genome replication (7) rapidly endow viruses with impressive sequence diversity, which allows them to evade both host immune responses (8) and sample mutational paths that may yield strains resistant to antiviral therapeutics (9). As a result of the increasing incidence of resistance to the limited number of antiviral drugs (10,11), novel therapies must be explored—particularly ones that are readily designed and tested.

RNA interference (RNAi) is a recently discovered, evolutionarily conserved mechanism for regulating gene expression that has the potential to be harnessed as a therapeutic alternative to antiviral small molecule drugs. This process, in which cells can be primed to identify and degrade RNA in a sequence-specific manner, was first observed in petunias (12). Shortly thereafter, a similar phenomenon was found to have natural antiviral activity in plants (13,14), and the pathway responsible for this activity was identified in C. elegans as RNAi (15). The natural antiviral activity of RNAi demonstrated in C. elegans, D. melanogaster, and A. aegypti (16–18)—and importantly conservation of the pathway in vertebrates (19)—makes RNAi a particularly attractive antiviral therapy strategy. In this review, we focus on therapeutic applica-
tions of antiviral RNAi. We will discuss design considerations, including modes of expression and delivery strategies. We will also consider shortcomings in using RNAi as an antiviral therapy and how these challenges may be addressed in therapy design and translation to the clinic.

**MECHANISM**

The RNAi pathway is a highly conserved cellular mechanism for regulating gene expression. In invertebrate animals (Fig. 1a), long double-stranded RNA (dsRNA) is processed into ~22-nucleotide (nt) short interfering RNA (siRNA) duplexes by a cellular RNase-III enzyme called Dicer (20). One strand of this duplex is loaded into Argonaute as the guide strand to create an active RNA-induced silencing complex (RISC), and the choice of this strand is made based on the thermodynamic properties of the siRNA duplex (21,22). The guide strand is then used to direct RISC to complementary RNAs. Perfectly complementary RNAs are "sliced" by the RNase H-like activity of Argonaute (23), the major component of RISC (24). In vertebrates (Fig. 1b), the presence of long dsRNA precursors induces a non-specific immune response in addition to RNAi (25,26), and RNAi can be specifically triggered by directly introducing siRNA duplexes to cells (27) or through expression of smaller RNAs, such as short hairpin RNAs (shRNAs), that can be processed by Dicer into siRNA duplexes (28).

In a pathway with many similarities to RNAi, endogenously expressed microRNAs (miRNAs) also serve to regulate gene expression in plants and animals (29–31). miRNAs are generally expressed as longer hairpin RNAs called primary miRNAs (pri-miRNAs) and contain several mismatches in the ~33-nt stemloop (32). This pri-miRNA is processed by the cellular complex of Drosha and DGCR8 in animals (33) to generate a shRNA-like molecule called a precursor miRNA (pre-miRNA). The pre-miRNA is then processed by Dicer (34) and loaded into RISC in a manner similar to siRNAs (22). A defining property of miRNAs is that there are typically mismatches between the miRNA guide and the target mRNA, and as a result, miRNAs generally regulate gene expression through repression of translation instead of direct cleavage of the target (35,36), though RNA degradation can occur via alternate mechanisms such as decapping and deadenylation (37).

The sequence-specific nature of RNAi provides several advantages in developing antiviral treatments. First, since RNAi acts at the nucleotide level, the only information necessary to begin designing RNAi therapeutics is the target sequence itself, a consideration that can accelerate the rate at which potential therapies enter a drug development pipeline. Second, antiviral small molecules generally act by specific biochemical inhibition of a viral...
protein function, but accompanying non-specific interactions with host factors can result in detrimental side effects that negatively impact long-term patient health and compliance (38). RNAi can certainly have off-target effects through interactions with host mRNAs close in sequence to the viral target (39). However, unlike small molecule–protein interactions, nucleotide sequence and Watson–Crick base pairing provide a straightforward means to differentiate between the host (whose genome sequence is of course now known) and pathogen, and in principle a more specific inhibition of viruses could potentially be achieved and reduce the incidence of side effects.

**DESIGN OF THERAPEUTIC RNAi**

Viruses infecting a variety of hosts, ranging from mosquitoes to humans, have been therapeutically targeted by RNAi (Table 1). The ability of RNAi to target all types of viral genomes (ssDNA, dsDNA, RNA(+) and RNA(−) and dsRNA) means this versatile mechanism could be harnessed very broadly as an antiviral therapy. While RNAi targeting of many factors has shown inhibition of viral replication, when translating from proof of principle toward the clinic, it has been helpful to tailor an antiviral RNAi therapy based on the biology of the host-pathogen interaction. Below, we describe design considerations in developing such therapies.

**Target Choice**

It is ideal to choose an RNAi target that is essential for viral replication, which could be a viral or host factor. When targeting viral factors, it is important to consider at what point in the viral life cycle the target will be accessible for RNAi-mediated degradation. For example, in addition to using RNAi to target newly transcribed viral mRNAs, viruses with positive sense RNA genomes, such as hepatitis C virus (HCV) and human immunodeficiency virus (HIV), may be susceptible to degradation even earlier, during their initial infection and entry into a cell (40). In addition, alternative splicing of the viral genome may mean that some viral targets are present in all splice variants, while some are only present in early or late gene expression (41). Furthermore, recent computational modeling suggests that targeting regions that are transcribed early or included in many transcripts can help to maximize the impact of therapeutic RNA by degrading viral RNA early and often (42,43). Although viral factors represent clear targets that are likely to be essential to replication and are readily distinguished from host factors, the rapid evolution of viruses means that these targets can be mutated and selected for resistance to RNAi. While we will discuss viral resistance to RNAi in more detail below, two options to avoid resistance are to target a highly conserved viral factor or a less mutable host factor.

The primary advantage of a host target is that its sequence is constant relative to the rapidly evolving viral genome, though one clear complication is that it may be required for host function and health. Some host factors may not be essential, as is the case with inhibiting CCR5 expression in HIV infection (44,45). In other cases, host factors essential for maintenance of cell health can serve as effective targets if the level of knockdown required to inhibit viral replication does not affect the endogenous function of the factor, as was the case in vitro with knocking down the transcription elongation factors Cdk9 and CyclinT1 to suppress HIV infection (46). Nonetheless, when inhibiting host factors, careful screening for undesirable side effects should be conducted in cell culture and animal models prior to implementation in humans. Finally, as mentioned above, modeling efforts suggest that targeting early points of the viral life cycle is essential for therapeutic efficacy (42,43), and host factors used for viral entry or genome replication represent additional options that act early in the viral life cycle.

With the maturation of high throughput screening methods, a number of large-scale RNAi screens have recently been conducted to identify host factors involved in viral infections for HIV, Influenza A, HCV, and West Nile virus (WNV) (1–6), and hits emerging from these screens could feed into target validation efforts. In addition, expanding the types of viruses and the host cell lines used for such screens will increase the number of potential host targets for antiviral RNAi therapy, improve confidence in the screens when common hits emerge in multiple screens (47), and provide insights into whether particular factors are or are not essential to the healthy function of host cells.

Upon choosing a host or viral target for knockdown, a specific 19 to 22-nt sequence must be identified. Secondary structure of the target RNA, as well as the thermodynamic stability of both the siRNA duplex and guide-target duplex, can greatly affect the efficacy of RNAi. Generally, the RNAi target should be as unstructured as possible, particularly at the termini of the ~22-nt target region, in order to improve target accessibility to RISC (48,49). Additional thermodynamic properties associated with active siRNAs include low GC content, asymmetric instability of the siRNA duplex (meaning an effective duplex has lower internal stability at the 5’ end compared to the 3’ end of the antisense strand (50)), and asymmetric interactions between the guide strand and the target RNA within RISC (such that the 5’ end of the guide stand is responsible for surpassing a thermodynamic threshold for stability with the target RNA for effective degradation (48)). An optimal target will also have minimal off-target effects, just as an ideal small molecule pharmaceutical will have minimal interactions with targets beyond those therapeutically
Table 1: Plant and Animal Viruses Targeted with RNAi. Virus Name, Target Gene(s), Mode of RNAi Induction and Biological System are Listed

| Virus | Target | Mode | Model | Reference |
|-------|--------|------|-------|-----------|
| ALCV  | L Polymerase, Z mRNA | si-, shRNA | HEK 293T, Vero, A549 | (150) |
| ALV   | tvb, env(B) | shRNA | DF-1 cells | (151) |
| BDV   | VP1 | shRNA | Vero cells | (152) |
| Coxsackie | CRE (2C) | shRNA | BALB/c | (153) |
| Coxsackie | 3C pro | siRNA | Rhabdomyosarcoma cells | (154) |
| Dengue | pre-membrane CDS | IR RNA | Aedes aegypti | (64) |
| Dengue | Env | siRNA | Human DCs, NOD/SCID humanized mice | (155) |
| EAV   | ORF1, ORF2b, ORF7 | si-, shRNA | AP9-R, BHK-21 cells | (156) |
| EBV   | Zta | shRNA | NA NPCs | (157) |
| ENT-70 | 3Dpol | siRNA | Rhabdomyosarcoma cells | (158) |
| ENT-71 | 3′UTR, 2C, 3C, 3D | siRNA | Rhabdomyosarcoma cells | (159) |
| ENT-71 | 3Dpol | si-, sh-, lhRNA | Suckling mice | (160) |
| ENT-71 | VP1, VP2 | siRNA | Rhabdomyosarcoma cells | (161) |
| FMDV  | 1D, Pol3D | shRNA | Guinea pig, Pigs | (81) |
| FMDV  | VP1 | shRNA | BHK-21 cells, suckling mice | (162) |
| FMDV  | 3P, 3D | shRNA | BHK-21 cells | (163) |
| GB Virus B | 5′ UTR | shRNA | Marmoset primate model | (72) |
| HBV   | HBsAg/POL | shRNA | HepG2.2.15 cells | (164) |
| HBV   | pre S2/6 | shRNA | Huh-7 cells | (165) |
| HBV   | Core, Pol, X | shRNA | CS7BL/6J, NOD SCID mouse model | (166) |
| HBV   | X, Core | shRNA | Huh-7 cells | (167) |
| HBV   | Pol, X | shRNA | Huh-7 | (168) |
| HBV   | HBsAg, pre-genomic RNA | shRNA | HepG2.2.15 cells, BALB/c | (169) |
| HCMV  | UL54, IE2 | siRNA | primary fibroblasts, U373 cells | (170) |
| HCV   | 5′ NTR, IRES | siRNA | En5-3, 2-3c cells | (171) |
| HCV   | IRES, NS5b, CD81 | shRNA | Huh-7 cells | (111) |
| HCV   | NS3, NS5b | siRNA | Huh-7 cells | (172) |
| HCV   | NS5B | siRNA | Huh-7 HCV Replicon cells | (173) |
| HCV   | C | shRNA | HepG2 | (83) |
| HCV   | La, PTB, hVAP-33 | shRNA | Huh-7 cells | (174) |
| HCV   | NS5b | siRNA | Huh-7 | (96) |
| HCV   | IRES, NS5b, CD81 | shRNA | Huh-6, Huh-7, NOD/SCID mice | (107) |
| HCV   | E2, NS3 | tsRNA | Huh-7 | (175) |
| HCV   | IRES, NS5b | siRNA | Huh-7, HepG2, HeLa | (176) |
| HEV   | helicase, replicase, 3′CAE | shRNA | HepG2 | (177) |
| HIV-I | Tat | shRNA | H9 cells | (94) |
| HIV-I | Gag | shRNA | PBMCs | (178) |
| HIV-I | TRBP | shRNA | HeLa cells | (99) |
| HIV-I | Nef | shRNA | SupT1 cells | (179) |
| HIV-I | Tat | shRNA | human | (66) |
| HIV-I | ALIX, ATG16, TRBP | shRNA | HEK293T, SupT1 cells | (100) |
| HIV-I | RT | shRNA | HeLa | (126) |
| HIV-I | Vif, TAR, Nef | shRNA | Magi cells, PBMCs | (40) |
| HIV-I | CCR5 | shRNA | BLT mice | (84) |
| HIV-I | TAR | shRNA | SupT1s | (89) |
| HIV-I | Tat | shRNA | CD34+ HSCs | (180) |
| HIV-I | LTR, Gag, Pol, Vif, Tat, Env, Vpu | shRNA | HEK 293A | (112) |
| HIV-I | LTR, Gag, Pol, Vif, Env, Nef | shRNA | HeLa | (109) |
| HIV-I | Pol, U3 | sh-, lhRNA | MT-4, PBMCs | (181) |
Table 1 (continued)

| Virus | Target | Mode | Model | Reference |
|-------|--------|------|-------|-----------|
| HIV-1 | Gag, Pol, Vif, Rev, Env, Gag | shRNA | HeLa, HEK293FT | (58) |
| HIV-1 | Tat, Nef, LTR, Pol | shRNA | SupT1, PBMCs | (110) |
| HIV-1 | 5′ UTR, Gag, Pol, Tat/Rev | shRNA | SupT1 | (182) |
| HIV-1 | 5′ UTR, Gag, Pol, Vif, Tat/Rev, Nef/LTR | shRNA | SupT1 | (182) |
| HIV-1 | CycT1, CDK-9 | siRNA | HeLa | (46) |
| HIV-1 | CXCR4, Fasl | siRNA | SX22-1, HEK293-005 | (101) |
| HIV-1 | PARP-1 | siRNA | HeLa, J111 | (102) |
| HIV-1 | Arp2/3 | shRNA | HEK293, H9 cells | (104) |
| HIV-1 | Sam68 | shRNA | HEK293T, HeLa SSKH | (105) |
| HIV-1 | hRIP | siRNA | HeLa, HL2/3, Jurkat, primary macrophages | (183) |
| HIV-1 | CCR5, Vif, Tat | siRNA | ND/SCID/IL2γ−/− Hu-PBL mice | (67) |
| HIV-1 | CD4, CCR5, CXCR4 | shRNA | Magi, PBMCs | (44) |
| HIV-1 | Int, mut-Int | shRNA | SupT1 | (123) |
| HIV-1 | Pol, Tat, Rev, Nef | shRNA | SupT1 | (184) |
| HIV-1 | 5′LTR, Gag, Pol, Tat/Rev | shRNA | SupT1 | (185) |
| HIV-1 | Gag, Pro, Int, Tat/Rev | shRNA | SupT1 | (186) |
| HIV-1 | Env/Rev | shRNA | SupT1 | (187) |
| HIV-1 | Nef, mut-Nef | shRNA | SupT1 | (124) |
| HPV | E6, E7 | siRNA | Human cervical carcinoma cells | (188) |
| HPV | E6 | si-, shRNA | HeLa | (189) |
| HRV-16 | 5′UTR, VP1-4, 2A, 2C, 3A, 3C, 3D, 4B, 5B | siRNA | HeLa | (190) |
| HSV-1 | glycoprotein E | siRNA | human keratinocytes, in vitro | (191) |
| HTLV-I | Gag, Env | siRNA | HEK293 | (192) |
| HTLV-I | TORC3 | siRNA | HEK293T | (103) |
| HV-6B | U38 DNA Polymerase | siRNA | SupT1 | (193) |
| Influenza A | NP, PA, PB-1 | siRNA | CS7BL6 | (194) |
| Influenza A | M2, NP | shRNA | MDCK | (195) |
| Influenza A | NP, PA | siRNA | BALB/cAnNR | (196) |
| Influenza A | PB1, PB2, PA, NP, MP | siRNA | A549 cells | (197) |
| Influenza A | NP, M2 | siRNA | MDCK cells, BALB/c | (198) |
| Influenza A | Caveolin-1 | shRNA | MDCK cells | (106) |
| JEV | Env | si-, shRNA | BHK-21, Neuro2A, Vero cells, BALB/c | (67) |
| JEV | Env | siRNA-peptide | Neuro2A cells | (73) |
| Marburg | NP, VP35, VP30 | siRNA | HeLa CCL-2, Vero cells | (199) |
| MDV | gB, UL29 | shRNA | chicken embryo fibroblasts, chickens | (119) |
| Monkeypox | A6R, EBL | siRNA | LLC-MK2 cells, in vitro | (200) |
| NDV | Matrix | shRNA | Chicken embryo fibroblasts | (201) |
| ONNV | P3 | dsRNA | Anopheles gambiae | (147) |
| Parainfluenza | P | siRNA | A549 cells, BALB/c | (62) |
| Parainfluenza | F, HN | siRNA | A549 cells | (202) |
| PCV-1 | Rep | shRNA | PK15 cells | (203) |
| PCV-2 | ORF1, ORF2 | shRNA | PK15 cells, BALB/c | (204) |
| PCV-2 | Rep | shRNA | PK15 cells | (203) |
| PEMV | 2C, 2B, 3C and 3D | siRNA | BHK-21 cells | (205) |
| Polio | Capsid, P3 | siRNA | HeLa S3, P19 mouse carcinoma cells | (206) |
| Polio | Capsid, P3 | siRNA | HeLa S3, MEFs | (95) |
| PPV | P1, HC-Pro | IR RNA | Nicotiana benthamiana | (63) |
| PRRSV | ORF 7 | shRNA | MARC-I45 | (207) |
| Rabies | Nucleocapsid | shRNA | Neuro2A cells | (208) |
Arenavirus Lymphocytic Choriomeningitis Virus, ALV Avian Leukosis Virus, BDV Bursal Disease Virus, ENTV-70 Enterovirus 70, ENTV-71 Enterovirus 71, EBV Epstein Barr Virus, EAV Equine Arteritis Virus, FMDV Foot and Mouth Disease Virus, HBV Hepatitis B Virus, HCMV Human Cytomegalovirus, HCV Hepatitis C Virus, HEV Hepatitis E Virus, HIV-6B Herpesvirus 6B, HSV-1 Herpes Simplex Virus 1, HIV-1 Human Immunodeficiency Virus 1, HPV Human Papilloma Virus, HTLV-1 Human T Lymphotropic Virus 1, HRV-16 Human Rhinovirus 16, JEV Japanese Encephalitis Virus, MDV Marek’s Disease Virus, NDV Newcastle Disease Virus, ONNV O’nyong nyong virus, PCV-1 Porcine Circovirus 1, PCV-2 Porcine Circovirus 2, PEMV Porcine Encephalomyocarditis Virus, PPV Plum Pox Virus, PRRSV Porcine Reproductive and Respiratory Syndrome Virus, RSV Respiratory Syncytial Virus, SARS-CoV Severe Acute Respiratory Syndrome Corona Virus, VSV Vesicular Stomatitis Virus, WNV West Nile Virus, YHV Yellow Head Virus. This Table is Not Comprehensive, But is Meant to Provide Both Breadth and Depth of Viruses Targeted by RNAi in the Last 10 Years.

### Table 1 (continued)

| Virus | Target | Mode | Model | Reference |
|-------|--------|------|-------|-----------|
| Rotavirus | VP4, VP7 | siRNA | MA104 | (209) |
| Rotavirus | VP4 | siRNA | MA104 | (210) |
| RSV | P | siRNA | A549 cells, BALB/c | (62) |
| RSV | Nucleocapsid | siRNA | human | (143) |
| RSV | PF | siRNA | A549 cells | (211) |
| SARS-CoV | Replicase IA | siRNA | FRHK-4 cells | (212) |
| SARS-CoV | S, E, M and N | shRNA | FRHK-4 cells | (213) |
| SARS-CoV | RdRp | shRNA | 293, HeLa, Vero-E6 cells | (214) |
| SARS-CoV | Leader, TRS, 3’UTR, Spike | siRNA | Vero E6 | (98) |
| SARS-CoV | ORF1b, ORF2 | siRNA | FRHK-4 cells | (215) |
| VSV | M, RdRp | siRNA | HEp-2 | (202) |
| WNV | 3’ UTR | siRNA | Vero cells, in vitro | (216) |
| WNV | Nucleocapsid | si-, shRNA | BHK-21, Neuro2A, Vero cells, BALB/c | (217) |
| YHV | Protease, Polymerase, Helicase | dsRNA | Shrimp Primary Cells | (218) |

*ALCV Arenavirus Lymphocytic Choriomeningitis Virus, ALV Avian Leukosis Virus, BDV Bursal Disease Virus, ENTV-70 Enterovirus 70, ENTV-71 Enterovirus 71, EBV Epstein Barr Virus, EAV Equine Arteritis Virus, FMDV Foot and Mouth Disease Virus, HBV Hepatitis B Virus, HCMV Human Cytomegalovirus, HCV Hepatitis C Virus, HEV Hepatitis E Virus, HIV-6B Herpesvirus 6B, HSV-1 Herpes Simplex Virus 1, HIV-1 Human Immunodeficiency Virus 1, HPV Human Papilloma Virus, HTLV-1 Human T Lymphotropic Virus 1, HRV-16 Human Rhinovirus 16, JEV Japanese Encephalitis Virus, MDV Marek’s Disease Virus, NDV Newcastle Disease Virus, ONNV O’nyong nyong virus, PCV-1 Porcine Circovirus 1, PCV-2 Porcine Circovirus 2, PEMV Porcine Encephalomyocarditis Virus, PPV Plum Pox Virus, PRRSV Porcine Reproductive and Respiratory Syndrome Virus, RSV Respiratory Syncytial Virus, SARS-CoV Severe Acute Respiratory Syndrome Corona Virus, VSV Vesicular Stomatitis Virus, WNV West Nile Virus, YHV Yellow Head Virus.

Computational efforts can also aid in improving target prediction. There are a number of general-use websites for siRNA design developed by industry and academia that consider thermodynamic requirements of the siRNA and potential off-target effects, though these resources are not designed to address the need to target highly conserved regions in viruses to mitigate viral escape. Two design services have been developed to fill the niche for antiviral RNAi therapy design. siVirus is a web-based application that streamlines antiviral RNAi design by implementing several algorithms for creating functional siRNAs, targeting highly conserved regions of the virus, and considering potential off-target effects (52). Currently, the software has sequence data to identify highly conserved regions of HIV, SARS, HCV, and influenza, and as second and even third generation sequencing technologies lower the cost of large-scale sequencing (33), the known genetic diversity of many viruses and the capabilities of siVirus will presumably grow.

Another freely available piece of software specifically for viral siRNA design is CAPSID (54), which searches for active siRNAs with minimized potential off-target effects in highly conserved regions of the viral genome, with guidance from user-provided sample virus sequences. This flexibility means CAPSID is more widely applicable to different viruses; however, limited user-provided sequence data could bias the results. It should be noted that the impact of computational modeling on antiviral siRNA design is not limited to target selection, and additional insights that other classes of computational work have provided in addition to siRNA sequence selection will be discussed below.

Library approaches can also be used to identify effective targets that do not necessarily conform to the general thermodynamic and structural rules outlined above by probing every possible 19 to 22-nt target individually. The ever-decreasing cost of oligonucleotide synthesis and multiplexing technology brings the potential for complete siRNA coverage of shorter viral genomes into reach (55,56). Alternatively, methods for generating such libraries using enzymatic approaches have been developed such that near complete siRNA coverage can be achieved by processing genomic DNA or cDNA into shRNAs using a combination of specialized restriction endonucleases and loop adaptors (57–61).
Method of Induction

The method of RNAi induction, or the mechanism by which the RNAi pathway is triggered for therapeutic purposes, can also have considerable impacts on efficacy, depending on the type of infection being treated. As mentioned earlier, RNAi can be triggered using a variety of effector agents, including synthetic siRNAs and gene-encoded shRNAs, and the choice among such options can have important implications for therapeutic applications. For example, acute, existing infections can be treated by administration of synthetic siRNAs, though delays in the initiation of treatment following the initial infection can limit the therapeutic benefits (62). On the other hand, treatment of chronic infections and prophylactic use of RNAi to prevent the inception of a viral infection may require sustained induction through repeated delivery of synthetic siRNAs or possibly gene-encoded effector agents such as shRNAs (though the risk/benefit ratio of the latter must be considered). Such sustained induction could be accomplished in invertebrates by genetically engineering inverted repeat RNA (IR RNA) or siRNA expression cassettes into the host cell genome (63–65). For vertebrates, sustained induction will likely be accomplished by gene-encoded siRNA expression in genetically engineered cells (66) or periodic administration of synthetic siRNAs (67,68). Depending on the nature of the host (invertebrate versus vertebrate), the nature of the infection (acute or chronic), the treatment objective (preventative or pre-existing), and the necessity and difficulty of maintaining sustained expression (single or repeated administration), one method of RNAi induction may be more desirable than another.

Method of Delivery

Once the mode of induction is chosen, a delivery strategy must also be determined. Synthetic siRNAs can simply be administered as naked RNA, though poor RNA stability can limit the efficacy of this approach. siRNA stability can be improved by chemical modification (69–71), and delivery can be enhanced by encapsulation of the siRNA in synthetic vehicles such as cationic liposomes (72), though directing the siRNAs to specific cells of interest remains a challenge. To address this problem, siRNAs have been conjugated to targeting antibodies, peptides, or aptamers that target particular cell types or infected cells (67,68,73).

For gene-encoded RNAi effectors, naked plasmid DNA administration is an option, though delivery efficiency is typically very limited. For higher efficiency, expression cassettes can be delivered via viral vectors (66). The use of viral vectors can further improve one’s ability to target cells of interest. For example, lentiviral vectors can be used for systemic delivery to both dividing and non-dividing cells, while retroviral vectors limit infection to dividing cell populations such as stem cells (74). Systemic delivery generally results in targeting the liver and spleen (75), and recently discovered variants of vectors such as adeno-associated virus (AAV) can also transduce muscle and even the central nervous system upon systemic injection (76). Alternatively, direct injection into the desired tissue could aid in targeting particular cells of therapeutic interest (77). In the future, systemic or direct administration could also aid in transitioning from the ex vivo delivery approach recently used to generate hematopoietic stem cells protected from HIV replication (66) into a more readily administered in vivo delivery method. Despite their delivery advantages, lenti- and retroviral vectors can pose a risk of genotoxicity due to vector integration (78). This risk can be decreased through the use of other viral vectors, such as AAV, that provide stable gene expression from episomal viral genomes without an explicit need for integration (79), engineered retroviral vectors that exhibit zinc-finger-mediated site-specific integration (80), or adenoviral vectors for transient gene expression (81). Cellular expression of siRNAs also affords further degrees of control. For example, tissue-specific and viral infection-specific promoters have been shown to drive siRNA production only in therapeutically relevant cells (82,83), thus reducing the risk of off-target effects. Delivery can be further improved by functionalization of the delivery vehicle via chemical or protein conjugation (84,85), vector pseudotyping (73,86), or engineering the viral capsid (87,88). Targeted delivery can reduce the total amount of siRNA or expression cassette required for treatment, thus reducing the potential risk of off-target effects, as well as potentially the cost of therapy.

Additional Computational Insight

Computational models of antiviral RNAi have also provided significant insights into therapy design. Two studies have considered long-term antiviral RNAi therapy and identified critical constraints on therapy parameters that could significantly affect therapy outcome. In the first investigation, a stochastic model was developed to understand how specific therapy parameters impact efficacy of and viral escape from an anti-HIV siRNA (42). This model was the first of its kind to simulate the molecular level detail of virus replication and response to therapeutic RNAi. Depending on RNAi inhibition efficiency, the model suggests that two to four targets are required to maintain long-term inhibition without escape. It also predicts that a threshold exists for the size of the reservoir of unprotected cells (cells not harboring siRNAs) tolerable for therapy success, and this prediction was subsequently validated in vitro (89). A more recent study simulates HIV infection in lymphoid tissue (90). This larger in vivo-like model evaluates efficacy in a biologically relevant engraftment efficiency range of 1% to
20% and finds that larger reservoirs of unprotected cells may not be detrimental to therapy efficacy, provided that the RNAi therapy is able to degrade incoming viral genomes prior to integration. This latter capability is debated (40,91), however, and RNAi therapy may need to be combined with another therapy capable of targeting the incoming viral genome for full efficacy. Further testing of predictions and expansion of these models to simulate other viruses and alternative modes of RNAi induction will continuously improve their predictive power and applicability.

COMPLICATIONS

Viral Escape

The ease of RNAi design can increase the number of therapies in a development pipeline by providing a large number of potential siRNA targets within a single viral gene that can readily be tested in cell culture and preclinical animal models; however, these therapies face the same challenges that conventional antiviral therapies encounter in the clinic: viral escape. A single nucleotide substitution in an RNAi target site within the viral genome can result in complete loss of interference, depending on the location and the nature of the resulting mismatch (92). In addition to nucleotide substitution in the target (Fig. 2), viral resistance has emerged by deletion of the target, indirect structural rearrangement of the targeted region (93), and even mutation of promoter/enhancer elements far from the target site to increase viral gene transcription and thereby overwhelm the RNAi machinery with elevated numbers of viral transcripts (89). Such viral escape from RNAi suppression has been documented in HIV, HCV, and poliovirus (94–96). In addition, both RNA and DNA viruses pose the additional problem of pre-existing quasispecies diversity (97,98), such that RNAi-resistant clones may already be present within the host prior to therapeutic RNA administration (9).

A number of strategies have been suggested to circumvent or prevent the emergence of resistance to RNAi. As mentioned above, viruses often co-opt many host factors to facilitate viral replication, and targeting host factors involved in viral replication instead of viral factors is likely to reduce the risk of viral escape, as a virus cannot mutate a host factor. Such a strategy has been demonstrated to inhibit replication of HIV, human T lymphotropic virus (HTLV), Influenza A, and HCV (44,84,99–107).

An effective host target is not always available or sufficient to circumvent viral escape, and another approach is to target highly conserved regions of the viral genome. Regions constrained by overlapping reading frames, important functional codons (start codons, primer binding sites), and structured nucleic acid features (IRES, packaging signals, splicing sites, export signals) may be less able to

Fig. 2 Mechanisms of viral escape from RNAi. The viral RNA is shown in grey, and the region targeted by RNAi is highlighted in blue. (a) A point mutation (red star) within the target can partially interrupt base-pairing between the guide and target RNAs. (b) Deletion of a non-essential target can eliminate base-pairing between the guide and target. (c) A point mutation outside the target can result in a structural rearrangement of the target making it inaccessible to the guide strand for base-pairing. (d) Mutation of the viral promoter can increase the total number of viral transcripts produced by the virus and overwhelm the RNAi pathway.
tolerate mutations that compromise their function (108). A secondary advantage of targeting highly conserved regions is that the same RNAi target may be effective against multiple subtypes, serotypes, or genotypes, as demonstrated with HIV and HBV (98,109). However, as mentioned earlier, direct mutation of the viral target is not always necessary for viral escape, and targeting a highly conserved element does not guarantee long-term inhibition without escape (89).

Another strategy to reduce escape is to target multiple factors with RNAi. Such a combinatorial RNAi approach can result in synergistic suppression of viral replication, and it has, for example, also been demonstrated to delay the onset of escape in HIV (110). A multi-target approach has also been combined with the previously mentioned two strategies of targeting highly conserved viral factors and less mutable host factors to inhibit HIV and HCV replication (67,111). It has been computationally estimated that at least four RNAi targets would be required to successfully circumvent viral escape (42,112), yet maintaining expression and activity of multiple siRNAs is not trivial and can be problematic. For example, it was shown in vivo that high levels of exogenous siRNAs can competitively interfere with the endogenous activity of miRNAs and the natural function of the RNAi pathway (113). Additionally, competition among the various exogenous siRNAs themselves for RNAi machinery may lower the overall benefit attained from using multiple siRNAs and bias the interference towards a single siRNA that is selectively incorporated into RISC (114), thus eliminating any advantage gained from a combinatorial approach.

In the case of chronic infections or prophylactic prevention of infection, in which sustained and long-term expression is desired, production of multiple siRNAs from a single vector or plasmid can be difficult to engineer and maintain. Several strategies to achieve this have been pursued, each with its own advantages and shortcomings. The most straightforward is to include repeated expression cassettes in a single backbone, as was done for up to seven shRNA cassettes, each targeting a different HIV gene and driven by a RNA Polymerase III (Pol III) H1 promoter (112). While this strategy is elegant in the ease of its design, it suffers from difficulties in implementing the therapy, as repeated sequences can be eliminated by recombination during transduction of host cells, especially when mediated by retroviral vectors (115,116). In an effort to reduce repeated sequences, alternative promoters for siRNA production have been used in place of the traditionally used H1 and U6 Pol III promoters, including the 7SK Pol III and U1, TRE, and CMV Pol II promoters (116,117). While these options reduce the levels of repeated sequence present in any single construct, each promoter may require fine-tuning for optimized expression in various hosts and tissues (118,119) and thus complicate therapy design.

Finally, multiple siRNAs can be expressed from a single promoter using long hairpin RNA (lhiRNA) or polycistronic miRNA expression strategies, in which a number of siRNAs can be excised and processed from a single mRNA precursor (120,121). These strategies reduce the likelihood of cassette deletion, but the activity of each siRNA relies on efficient processing, which can actually vary with its specific placement within the extended RNA product. In addition, for a therapy with advantages in ease and speed of design, such elegant combinations may involve considerable engineering that could slow development.

An alternative delivery strategy for combinatorial therapy was recently investigated in silico (122), in which the individual components of the combination were either equally distributed among all cells in the population or compartmentalized into different cell subpopulations. Depending on the efficacy of the therapy and the relative fitness of resistant mutants, the compartmentalized strategy could provide therapeutic benefits similar to those of an equally distributed combination therapy, yet it avoids the extra engineering required for sustained combinatorial RNAi induction and potentially circumvents the risk of overwhelming the RNAi pathway with numerous therapeutic siRNAs in a single cell.

As an alternate strategy to combat resistance, it has been shown in some studies that evolution of resistance to specific siRNAs is due to specific mutations at particular base positions in the virus, and combinatorial delivery of multiple siRNAs whose sequences correspond to the parent virus and the most likely escape mutants could preemptively reduce the risk of viral escape (123). However, not all variant siRNAs are effective in inhibiting replication of the corresponding mutant virus, most likely due to changes in the thermodynamic characteristics of the siRNA (124). Furthermore, it is unclear whether this is the best strategy if the mutants do not exist at the start of treatment, and the corresponding siRNAs could thus compete with more effective siRNAs targeting wild-type strains.

Combinatorial strategies can also be extended to encompass other antiviral therapies. A combination of novel RNA-based gene therapies that includes RNAi, a ribozyme, and a RNA decoy is currently being tested in an important clinical trial for HIV (66). By analogy, a coxsackievirus therapy combining RNAi with an antibody that inhibits viral uptake has been tested in vitro (125). Combinations of RNAi and conventional therapies have also been tested in vitro and in vivo. RNAi targeting HCV was successfully combined with the traditional interferon therapy to inhibit HCV replication synergistically in vivo (107). Such combinations can also be advantageous in contending with existing resistance. In another example, RNAi was combined with a nucleoside reverse transcription inhibitor (NRTI) to inhibit HIV mixtures that contain wild
type and NRTI-resistant strains (126), and this may be a particularly fruitful line of application, as the resistance mechanisms to first line antiretrovirals are often well documented (9). RNAl combined with a NRTI was also shown to effectively suppress viral replication of both wild type and RNAl-resistant virus using low NRTI concentrations that alone were insufficient to inhibit wild type replication (89). Thus, RNAl can serve to lower the dosage needed for conventional therapies, decreasing the likelihood of side effects and improving the likelihood of continued patient compliance that is required to avoid the development of resistance to existing therapies.

**RNAl Suppressors and Hijackers**

While the natural role of RNAl as an antiviral mechanism in many organisms is considered advantageous for translating RNAl into a general antiviral therapy, this advantage can also lead to complications, as many viruses that infect these organisms have evolved mechanisms to suppress RNAl or hijack the pathway for their own uses. Viral suppressors of RNAl were first discovered in plant viruses (127,128) and have since been identified in viruses infecting invertebrate and vertebrate animals (129–133), though the extent to which RNAl plays a natural role in antiviral defense in mammals and the extent to which viral proteins truly suppress this innate antiviral RNAl have been questioned (134). Nonetheless, viruses of certain hosts have evolved a variety of mechanisms to subvert innate antiviral RNAl (135–137), and it is possible that sustained RNAl treatment in these hosts may force even more evolution of the suppressors to subvert both innate and therapeutic RNAl. As final examples, it may be challenging to use RNAl as a therapy against Ebola, HIV, and La Crosse virus, in particular, because these viruses have documented RNAl suppressors that function in their mammalian hosts or mosquito vectors (129,130,133), and RNAl in conjunction with another therapy that inhibits the suppressor function may be a promising strategy.

Finally, HIV and HSV, notorious for their capacity to form latent infections, have been shown to use endogenous miRNAs or virus-derived miRNAs to modulate viral gene expression during latency (138,139). Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), and SV40 have also been shown to generate viral miRNAs that are thought to play a role in the development and maintenance of latency for EBV and HCMV (140,141) and immune evasion in SV40 (142). While elucidation of such mechanisms for innate RNAl suppression, immune evasion, and latency can help to guide the development of new therapy strategies, the ability of viruses to inhibit the RNAl pathway and hijack RNAl resources for regulation of viral gene expression could limit the number of genes that could reasonably be targeted by a RNAl therapy before overwhelming this pathway.

**ROOM FOR IMPROVEMENT**

RNAl therapies present considerable therapeutic opportunities, and several RNAl therapies have reached the clinic in the relatively short time this class of therapeutics has been under development. To date, four antiviral RNAl therapies have entered into clinical trials (Table II), for respiratory syncytial virus (RSV), HBV, HCV, and HIV (66,143–145). These trials represent major advances in the antiviral RNAl therapy field, and they can also provide insights that may accelerate future clinical RNAl efforts.

First, initial success in translating therapies to the clinic may come from synthetic siRNAs as the therapeutic effector, at least for acute infections, as they may pose fewer risks compared to their gene-encoded counterparts. Such an approach was successfully used to protect patients from RSV infection during a clinical trial (143). On the other hand, it is desirable to treat a chronic infection such as HIV with sustained RNAl from gene-modified cells, as was recently utilized by DiGiusto and colleagues (66). Practically speaking, the more advanced drug development process for synthetic siRNAs compared to gene-encoded shRNAs may result in faster clinical evaluation of siRNAs in the short term; however, sustained expression will be necessary for a long-term solution to chronic infections such as HIV and HSV, notorious for their capacity to form latent infections.

| Virus | Target | Delivery and mode of induction | Stage | Sponsors | Year started | Reference |
|-------|--------|--------------------------------|-------|----------|--------------|-----------|
| RSV   | Nucleocapsid | siRNA nasal spray          | Phase II | Aplylam   | 2008         | (143)     |
| HBV   | Four different HBV targets | Intravenous injection of liposome-encapsulated shRNA expression plasmid | Phase I | Nucleonics | 2007         | (144)     |
| HCV   | miR-122 | Subcutaneous injections of LNA | Phase II | Santaris   | 2010         | (219)     |
| HIV   | tat/rev | Ex vivo transduction by retroviral vector | Phase I | City of Hope, Benetc   | 2007         | (66)      |
as HIV. Taken together, RNAi stands to make a considerable impact in the treatment of both acute and chronic viral infections.

Additionally, as several large pharmaceutical companies exit the human RNAi therapy field (146), treating non-human hosts such as livestock may represent a test bed that provides insights to aid longer-term human therapeutic development. RNAi in non-human hosts will likely also face fewer regulatory limitations, such that therapies may be commercialized faster and alleviate concerns about viability. In a recent example, chickens were prophylactically treated to induce RNAi targeting Marek’s disease virus (MDV) and shown to have increased resistance to infection (119). Such a strategy could potentially be used to combat H5N1, a particularly lethal strain of influenza that is usually transmitted to humans via close contact with poultry. Similarly, RNAi was prophylactically induced in pigs to target foot and mouth disease virus (FMVD), which also infects humans (81). Given some anatomical and physiological similarities between pigs and humans, a great deal could be learned about efficacy, delivery and long-term viability by conducting such smaller studies on non-human hosts and translating these results to humans.

There are also a number of therapeutic RNAi applications for invertebrates that could have significant impacts on public health. Mosquitoes serve as vectors for a number of tropical viruses, including Dengue virus, O’nyong nyong virus (ONNV), WNV, and yellow fever virus (YFV). Priming mosquitoes with ONNV dsRNA was shown to decrease the spread of the virus in the insect host (147), and this strategy could serve as a general mechanism to control the transmission of additional vector-borne viruses. More recently, dengue virus-resistant transgenic mosquitoes were created using inverted repeat RNA expression cassettes that specifically activate in the mosquito midgut after a blood meal (64). These mosquitoes demonstrated significantly reduced transmission of dengue virus, though the stability of RNAi expression over multiple generations decreased (148). Further research into population replacement strategies could make this elegant strategy viable (149).

CONCLUSION

Since the mechanism of RNAi was first elucidated in C. elegans, its use to treat viral infections has itself spread like an epidemic. The ability to design a therapy based on simple sequence information and thermodynamic guidelines has the potential to accelerate therapeutic development. Furthermore, as the number of virus-specific RNAi computational design tools grows, target prediction based on viral constraints, such as sequence conservation, will improve. Delivery remains a challenge for all RNAi therapy applications, but progress in targeting and transduction will progressively alleviate these shortcomings. Furthermore, computational efforts have elucidated important therapy parameters for clinical success, such as the number of RNAi targets and the efficiency of transduction, and can help define targets for further improvements. Finally, while RNAi targeting human viruses importantly have entered the clinic, the treatment of livestock and vectors using antiviral RNAi should not be overlooked. Not only may these applications be easier to commercialize due to lower regulatory barriers, the results could also be generalized and translated into clinical and public health benefits for people.

REFERENCES

1. Brass AL, Dykhoom DM, Benita Y, Yan N, Engelman A, Xavier RJ, et al. Identification of host proteins required for HIV infection through a functional genomic screen. Science. 2008;319(5865):921–6.
2. Karlas A, Machuy N, Shin Y, Pleisner KP, Artarini A, Heuer D, et al. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. Nature. 2010;463(7282):818–22.
3. Kung R, Zhou Y, Ellell D, Diamond TL, Bonamy GM, Irelan JT, et al. Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. Cell. 2008;135(1):49–60.
4. Krishnan MN, Ng A, Sukumaran B, Gilfoyl FD, Uchil PD, Sultana H, et al. RNA interference screen for human genes associated with West Nile virus infection. Nature. 2008;455(7210):242–5.
5. Randall G, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, et al. Cellular cofactors affecting hepatitis C virus infection and replication. Proc Natl Acad Sci USA. 2007;104(31):12884–9.
6. Zhou H, Xu M, Huang Q, Gates AT, Zhang XD, Castle JC, et al. Genome-scale RNAi screen for host factors required for HIV replication. Cell Host Microbe. 2008;4(5):495–504.
7. Duffy S, Shackleton LA, Holmes EC. Rates of evolutionary change in viruses: patterns and determinants. Nat Rev Genet. 2008;9(4):267–76.
8. Kamp CW, CO, Adami, C, Bornholdt, S. Viral evolution under the pressure of an adaptive immune system-optimal mutation rates for viral escape. Complexity. 2002.
9. Glavel F, Hance AJ. HIV drug resistance. N Engl J Med. 2004;350(10):1023–35.
10. Hayden FG, de Jong MD. Emerging influenza antiviral resistance threats. J Infect Dis. 2011;203(1):6–10.
11. Smith RJ, Okano JT, Kahn JS, Bodine E.N, Blower S. Evolutionary dynamics of complex networks of HIV drug-resistant strains: the case of San Francisco. Science. 2010;327(5966):697–701.
12. Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell. 1990;2(4):279–89.
13. Al-Kaff NS, Covey SN, Kreike MM, Page AM, Pinder R, Dale PJ. Transcriptional and posttranscriptional plant gene silencing in response to a pathogen. Science. 1998;279(5359);2113–5.
Antiviral RNAi: Translating Science Towards Therapeutic Success

14. Ratcliffe F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. Science. 1997;276(5318):1558–60.
15. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391(6660):806–11.
16. Li H, Li WX, Ding SW. Induction and suppression of RNA silencing by an animal virus. Science. 2002;296(5571):1319–21.
17. Schott DH, Cureton DK, Whelan SP, Hunter CP. An antiviral role for the RNA interference machinery in Caenorhabditis elegans. Proc Natl Acad Sci USA. 2005;102(51):18420–4.
18. Myles KM, Wiley MR, Morazzani EM, Adelman ZN. Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes. Proc Natl Acad Sci USA. 2008;105(50):19938–43.
19. Cerutti H, Casas-Mollano JA. On the origin and functions of RNA-mediated silencing: from protists to man. Curr Genet. 2006;50(2):81–99.
20. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. 2001;409(6818):363–6.
21. Gu S, Jin L, Zhang F, Huang Y, Grimm D, Rossi JJ, et al. Thermodynamic stability of small hairpin RNAs highly influences the loading process of different mammalian Argonautes. Proc Natl Acad Sci USA. 2011;108(22):9208–13.
22. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell. 2003;115(2):209–16.
23. Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. Science. 2004;305(5689):1434–7.
24. Rivas FV, Tolía NH, Song JJ, Aragon JP, Liu J, Hannon GJ, et al. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat Struct Mol Biol. 2005;12(4):340–5.
25. Samuel-Abraham S, Leonard JN. Staying on message: design principles for controlling nonspecific responses to siRNA. FEBS Lett. 2010;584(23):60–6.
26. Ratcliff F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. Science. 1997;276(5318):1558–60.
27. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391(6660):806–11.
28. Schott DH, Cureton DK, Whelan SP, Hunter CP. An antiviral role for the RNA interference machinery in Caenorhabditis elegans. Proc Natl Acad Sci USA. 2005;102(51):18420–4.
29. Myles KM, Wiley MR, Morazzani EM, Adelman ZN. Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes. Proc Natl Acad Sci USA. 2008;105(50):19938–43.
30. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of small microRNA-like RNAs in mammalian cells. Cell. 2001;107(2):227–37.
31. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell. 2003;115(2):209–16.
32. Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. Science. 2004;305(5689):1434–7.
33. Rivas FV, Tolía NH, Song JJ, Aragon JP, Liu J, Hannon GJ, et al. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat Struct Mol Biol. 2005;12(4):340–5.
34. Samuel-Abraham S, Leonard JN. Staying on message: design principles for controlling nonspecific responses to siRNA. FEBS Lett. 2010;584(23):60–6.
35. Ratcliff F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. Science. 1997;276(5318):1558–60.
variable virus genomes. Biochem Biophys Res Commun. 2009;384(4):431–5.
55. Kosuri S, Eroshenko N, Leproust EM, Super M, Way J, Li JB, et al. Scalable gene synthesis by selective amplification of DNA pools from high-fidelity microchips. Nat Biotechnol. 2010;28(12):1295–9.
56. Bassik MC, Lebbink RJ, Churchman LS, Ingolia NT, Patena W, LeProust EM, et al. Rapid creation and quantitative monitoring of high coverage siRNA libraries. Nat Methods. 2009;6(6):443–5.
57. Luo B, Heard AD, Lodish HF. Small interfering RNA production by enzymatic engineering of DNA (SPEED). Proc Natl Acad Sci USA. 2004;101(15):5494–9.
58. Pongratz C, Yazdanpanah B, Kashkar H, Lehmann MJ, Krausslich HG, Krooke M. Selection of potent non-toxic inhibitory sequences from a randomized HIV-1 specific lentiviral short hairpin RNA library. PLoS One. 2010;5(10):e13172.
59. Sen G, Wehrman TS, Myers JW, Blau HM. Restriction enzyme-generated siRNA (REGS) vectors and libraries. Nat Genet. 2004;36(2):183–9.
60. Shirane D, Sugao K, Namiki S, Tanabe M, Iino M, Hirose K. Enzymatic production of RNAi libraries from cDNAs. Nat Genet. 2004;36(2):190–6.
61. Silva JM, Li MZ, Chang K, Ge W, Golding MC, Riddle RJ, et al. Second-generation shRNA libraries covering the mouse and human genomes. Nat Genet. 2005;37(11):1281–8.
62. Bitko V, Musiyenko A, Shuyalova E, Barik S. Inhibition of respiratory viruses by nasally administered siRNA. Nat Med. 2005;11(1):50–5.
63. Di Nicola-Negri E, Brunetti A, Tavazza M, Iardi V. Hairpin RNA-mediated silencing of Plasm pox virus P1 and HC-Pro genes for efficient and predictable resistance to the virus. Transgenic Res. 2005;14(6):598–94.
64. Franz AW, Sanchez-Vargas I, Adelman ZN, Blair CD, Beatty BJ, James AA, et al. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified Aedes aegypti. Proc Natl Acad Sci USA. 2006;103(11):4198–203.
65. Wakiyama M, Matsumoto T, Yokoyama S. Drosophila U6 promoter-driven short hairpin RNAs effectively induce RNA interference in Schneider 2 cells. Biochem Biophys Res Commun. 2005;331(4):1163–70.
66. DiGiusto DL, Krishnan A, Li L, Li H, Li S, Rao A, et al. RNAi-based gene therapy for HIV with lentiviral vector-modified CD4+ cells in patients undergoing transplantation for AIDS-related lymphoma. Sci Transl Med. 2010;2(36):36ra43.
67. Kumar P, Ban HS, Kim SS, Wu H, Pearson T, Greiner DL, et al. T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. Cell. 2008;134(4):577–86.
68. Neff CP, Zhou J, Remling L, Kuruvilla J, Zhang J, Li H, et al. An Aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4+ T cell decline in humanized mice. Sci Transl Med. 2011;3(66):66ra6.
69. Choug S, Kim YJ, Kim S, Park HO, Choi YC. Chemical modification of siRNAs to improve serum stability without loss of efficacy. Biochem Biophys Res Commun. 2006;342(3):919–27.
70. Bramsen JB, Laursen MB, Nielsen AF, Hansen TB, Bus C, Langkjaer N, et al. A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity. Nucleic Acids Res. 2009;37(9):2067–81.
71. Braasch DA, Jensen S, Liu Y, Kaur K, Arar K, White MA, et al. siRNA interference in mammalian cells by chemically-modified RNA. Biochemistry. 2003;42(26):7967–75.
72. Yokota T, Iijima S, Kubodera T, Ishii K, Katakai Y, Ageyama N, et al. Efficient regulation of viral replication by siRNA in a non-human primate surrogate model for hepatitis C. Biochem Biophys Res Commun. 2007;361(2):294–300.
73. Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, et al. Transvascular delivery of small interfering RNA to the central nervous system. Nature. 2007;448(7149):39–43.
74. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature. 2002;415(6875):1030–4.
75. Pan D, Gunther R, Duan W, Wendell S, Kaemmerer W, Kafri T, et al. Biodistribution and toxicity studies of VSVG-pseudotyped lentiviral vector after intravenous administration in mice with the observation of in vivo transduction of bone marrow. Mol Ther. 2002;5(1):19–29.
76. Fousse KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol. 2009;27(1):59–65.
77. Stein CS, Martins I, Davidson BL. The lymphocytic choriomeningitis virus envelope glycoprotein targets lentiviral gene transfer vector to neural progenitors in the murine brain. Mol Ther. 2005;11(3):382–9.
78. Hacen-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wullfraat N, Leboulch P, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science. 2003;302(5644):413–9.
79. Flosse TR, Alfone SA, Conrad C, McGrath SA, Solow R, Oba H, et al. Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. Proc Natl Acad Sci USA. 1993;90(22):10613–7.
80. Liu KM, Klimczak R, Yu JH, Schaffer DV. Specific insertions of zinc finger domains into Gal-Pos yield engineered retroviral vectors with selective integration properties. Proc Natl Acad Sci USA. 2010;107(28):12475–80.
81. Chen W, Liu M, Yao J, Yan W, Wei X, Chen J, et al. Adenovirus-mediated RNA interference against foot-and-mouth disease virus infection both in vitro and in vivo. J Virol. 2006;80(7):3559–66.
82. Snyder LL, Esser JM, Pachuk CJ, Steel LF. Vector design for liver-specific expression of multiple interfering RNAs that target hepatitis B virus transcripts. Antiviral Res. 2008;80(1):36–44.
83. Strayer DS, Feitelson M, Sun B, Matskevich AA. Paradigms for conditional expression of RNA interference molecules for use against viral targets. Methods Enzymol. 2005;392:227–41.
84. Kim SS, Peer D, Kumar P, Subramanya S, Wu H, Ashana D, et al. RNAi-mediated CCR5 silencing by LFA-1-targeted nanoparticles prevents HIV infection in BLT mice. Mol Ther. 2010;18(2):370–6.
85. Ponnazhagan S, Mahendra G, Kumar S, Thompson JA, Castillas Jr M. Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked ligands. J Virol. 2002;76(24):12900–7.
86. Kobinger GP, Weiner DJ, Yu QC, Wilson JM. Filovirus-pseudotyped lentiviral vector can efficiently and stably transduce airway epithelia in vivo. Nat Biotechnol. 2001;19(3):225–30.
87. Excoffon KJ, Koerber JT, Dickey DD, Muns HD, Keshavjee S, Kaspar BK, et al. Directed evolution of adeno-associated virus to an infectious respiratory virus, Proc Natl Acad Sci USA. 2005;102(10):3685–6.
88. Jang JH, Koerber JT, Kim JS, Asuri P, Vazin T, Bartel M, et al. An evolved adeno-associated viral variant enhances gene delivery and gene targeting in neural stem cells. Mol Ther. 2011.
89. Leonard JN, Shah PS, Burnett JC, Schaffer DV. HIV evades RNA interference directed at TAR by an indirect compensatory mechanism. Cell Host Microbe. 2008;4(5):484–94.
90. Applegate TL, Birkett DJ, McIntyre GJ, Jaramillo AB, Symonds G, Murray JM. In silico modeling indicates the development of HIV-1 resistance to multiple siRNA gene therapy differs to standard antiretroviral therapy. Retrovirology. 2010;7:83.
91. Westerhout EM, ter Brake O, Berkhout B. The virion-associated incoming HIV-1 RNA genome is not targeted by RNA interference. Retrovirology. 2006;3:57.
92. Du Q, Thonberg H, Wang J, Wahlestedt C, Liang Z. A systematic analysis of the silencing effects of an active siRNA at all single-nucleotide mismatched target sites. Nucleic Acids Res. 2005;33(5):1671–7.
93. Westerhout EM, Ooms M, Vink M, Das AT, Berkhout B. HIV-1 can escape from RNA interference by evolving an alternative structure in its RNA genome. Nucleic Acids Res. 2005;33(2):796–804.
94. Boden D, Posch O, Lee F, Tucker L, Ramratnam B. Human immunodeficiency virus type 1 escape from RNA interference. J Virol. 2005;77(21):11331–5.
95. Gütlin L, Karelsky S, Andino R. Short interfering RNA confers intracellular antiviral immunity in human cells. Nature. 2002;418(6896):430–4.
96. Wilson JA, Richardson CD. Hepatitis C virus replicons escape RNA interference induced by a short interfering RNA directed against the NS5b coding region. J Virol. 2005;79(11):7030–8.
97. Lauring AS, Andino R. Quasispecies theory and the behavior of RNA viruses. PLoS Pathog. 2010;6(7):e1001003.
98. Wu HL, Huang LR, Huang CC, Lai HL, Liu CJ, Huang YT, et al. RNA interference-mediated control of hepatitis B virus and emergence of resistant mutant. Gastroenterology. 2005;128(3):709–16.
99. Christiansen HS, Daher A, Soye KJ, Frankel LB, Alexander MR, Laine S, et al. Small interfering RNAs against the TAR RNA binding protein, TRBP, a Dicer cofactor, inhibit human immunodeficiency virus type 1 long terminal repeat expression and viral production. J Virol. 2007;81(10):5121–31.
100. Eekels JJ, Geerts D, Jeeninga RE, Berkhout B. Long-term inhibition of HIV-1 replication with RNA interference against cellular co-factors. Antiviral Res. 2010.
101. Ji J, Wernli M, Klimkait T, Erb P. Enhanced gene silencing by the application of multiple specific small interfering RNAs. FEBS Lett. 2003;552(2–3):247–52.
102. Kameoka M, Nukuzuma S, Iwaya A, Tanaka Y, Ota K, Ibata K, et al. RNA interference directed against Poly(ADP-Ribose) polymerase 1 efficiently suppresses human immunodeficiency virus type 1 replication in human cells. J Virol. 2004;78(3):12931–4.
103. Koga H, Oshihama T, Shimotohno K. Enhanced activation of tax-dependent transcription of human T-cell leukemia virus type I (HTLV-I) long terminal repeat by TORC3. J Biol Chem. 2004;279(51):52978–83.
104. Komano J, Miyachi K, Matsuda Z, Yamamoto N. Inhibiting the Arp2/3 complex limits infection of both intracellular mature vaccinia virus and primate lentiviruses. Mol Biol Cell. 2004;15(12):5197–207.
105. Modem S, Badri KR, Holland TC, Reddy TR. Sam68 is absolutely required for Rev function and HIV-1 production. Nucleic Acids Res. 2005;33(3):873–9.
106. Sun L, Hemgard GV, Susanto SA, Wirth M. Cavoolin-1 influences human influenza A virus (H1N1) multiplication in cell culture. Virol J. 2010;7:108.
107. Pan Q, Henry SD, Metselaar HJ, Scholte B, Kwekkeboom J, Tilanus HW, et al. Combined antiviral activity of interferon-alpha and RNA interference directed against hepatitis C without affecting vector delivery and gene silencing. J Mol Med. 2009;87(7):713–22.
108. von Eije KJ, ter Brake O, Berkhout B. Human immunodeficiency virus type 1 escape is restricted when conserved genome sequences are targeted by RNA interference. J Virol. 2008;82(6):2895–903.
109. Naito Y, Nohtomi K, Onogi T, Uenishi R, Ui-Tei K, Saigo K, et al. Optimal design and validation of antiviral siRNA for targeting HIV-1. Retrovirology. 2007;4:30.
110. ter Brake O, Konstantinova P, Ceylan M, Berkhout B. Silencing of HIV-1 with RNA interference: a multiple shRNA approach. Mol Ther. 2006;14(6):883–92.
111. Henry SD, van der Wegen P, Metselaar HJ, Tilanus HW, Scholte BJ, van der Laan IJ. Simultaneous targeting of HIV-1 replication and viral binding with a single lentiviral vector containing multiple RNA interference expression cassettes. Mol Ther. 2006;14(4):485–93.
112. McIntyre GJ, Groneman JL, Yu YH, Tran A, Applegate TL. Multiple shRNA combinations for near-complete coverage of all HIV-1 strains. AIDS Res Ther. 2011;8(1):1.
113. Grimm D, Streetz KL, Joling CL, Storm TA, Pandey K, Davis CR, et al. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. Nature. 2006;441(7092):537–41.
114. Castanotto D, Sakurai K, Lingeman R, Li H, Shively L, Aagaard L, et al. Combinatorial delivery of small interfering RNAs reduces RNAi efficacy by selective incorporation into RISC. Nucleic Acids Res. 2007;35(15):5154–64.
115. McIntyre GJ, Yu YH, Tran A, Jaramillo AB, Arndt AJ, Millington ML, et al. Cassette deletion in multiple shRNA lentiviral vectors for HIV-1 and its impact on treatment success. Virol J. 2009;6:184.
116. ter Brake O, t Hoof K, Liu YP, Gentilivre M, von Eije KJ, Berkhout B. Lentiviral vector design for multiple shRNA expression and durable HIV-1 inhibition. Mol Ther. 2008;16(3):557–64.
117. Stegemeier F, Hu G, Rickles RJ, Hannon GJ, Elledge SJ. A lentiviral microRNA-based system for single-copy polymerase II-regulated RNA interference in mammalian cells. Proc Natl Acad Sci USA. 2005;102(37):13212–7.
118. Bannister SC, Wise TG, Cahill DM, Doran TJ. Comparison of chicken 7SK and U6 RNA polymerase III promoters for short hairpin RNA expression. BMC Biotechnol. 2007;7:79.
119. Lambeth LS, Zhao Y, Smith LP, Kgosana L, Nair V. Targeting Marek’s disease virus by RNA interference delivered from a herpesvirus vaccine. Vaccine. 2009;27(2):298–306.
120. Liu YP, Haasnoot J, ter Brake O, Berkhout B, Konstantinova P. Inhibition of HIV-1 by multiple siRNAs expressed from a single microRNA polycistronic. Nucleic Acids Res. 2008;36(9):2811–24.
121. Saamyun S, Arbutneth P, Weinberg MS. Deriving four functional anti-HIV siRNAs from a single Pol III-generated transcript comprising two adjacent long hairpin RNA precursors. Nucleic Acids Res. 2010.
122. Aviran S, Shah PS, Schaffer DV, Arkin AP. Computational models of HIV-1 resistance to gene therapy elucidate therapy design principles. PLoS Comput Biol. 2010;6(8).
123. Schopman NC, ter Brake O, Berkhout B. Anticipating and blocking HIV-1 escape by second generation antiviral shRNAs. Retrovirology. 2010;7:32.
124. ter Brake O, Berkhout B. A novel approach for inhibition of HIV-1 by RNA interference: counteracting viral escape with a second generation of siRNAs. J RNA Gene Silencing. 2005;1(2):56–65.
125. Werk D, Pinkert S, Heim A, Zeichhardt H, Grunert HP, Poller W, et al. Combination of soluble cossackievirus-adenovirus receptor and anti-cossackievirus siRNAs exerts synergistic antiviral activity against cossackievirus B3. Antiviral Res. 2009;83(3):298–306.
126. Huelsmann PM, Rauch P, Allers K, John MJ, Metzner KJ. Inhibition of drug-resistant HIV-1 by RNA interference. Antiviral Res. 2006;69(1):1–8.
Identification and characterization of RNA silencing in vitro. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. Nucleic Acids Res. 2008;36(2):666–75.

Sullivan GS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature. 2005;435 (7042):682–6.

Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, et al. Identification and characterization of human cytomegalovirus-encoded microRNAs. J Virol. 2005;79(18):12905–9.

DeVincenzo J, Lambkin-Williams R, Wilkinson T, Chehelsky J, Nochur S, Walsh E, et al. A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. Proc Natl Acad Sci USA. 2010;107 (19):8800–5.

Haussecker D. The business of RNAi therapeutics. Hum Gene Ther. 2008;19(5):451–62.

127. Anandalakshmi R, Pruss GJ, Ge X, Marathe R, Mallory AC, Smith TH, et al. A viral suppressor of gene silencing in plants. Proc Natl Acad Sci USA. 1998;95(22):13079–84.

128. Voinnet O, Pinto YM, Baulcombe DC. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc Natl Acad Sci USA. 1999;96(24):14147–52.

129. Bennasser Y, Le SY, Benkirane M, Jeang KT. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. Immunity. 2005;22(3):607–19.

130. Haasnoot J, de Vries W, Geutjes EJ, Prins M, de Haan P, Berkhouit B. The Ebola virus VP25 protein is a suppressor of RNA silencing. PLoS Pathog. 2007;3(6):e86.

131. Li WX, Li H, Lu R, Li F, Dus M, Atkinson P, et al. Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. Proc Natl Acad Sci USA. 2004;101(5):1350–5.

132. Lu R, Maduro M, Li F, Li HW, Broitman-Maduro G, Li WX, et al. Animal virus replication and RNAi-mediated antiviral silencing in Caenorhabditis elegans. Nature. 2005;436 (7053):1040–3.

133. Soldan SS, Plassmeyer ML, Matukonis MK, Gonzalez-Scarano F, La Crosse virus nonstructural protein NSs counteracts the effects of short interfering RNA. J Virol. 2005;79(1):234–44.

134. Umbach JL, Cullen BR. The role of RNAi and microRNAs in animal virus replication and antiviral immunity. Genes Dev. 2009;23(10):1511–64.

135. Chao JA, Lee JH, Chapados BR, Debler EW, Schneemann A, Williamson JR. Dual modes of RNA-silencing suppression by Flock house virus B2 suppressors of RNA silencing. Proc Natl Acad Sci USA. 2005;102(11):952–7.

136. Nayak A, Berry B, Tassetto M, Kunitomi M, Acevedo A, Deng C, et al. Cricket paralysis virus antagonizes Argonaute 2 to modulate antiviral defense in Drosophila. Nat Struct Mol Biol. 2010;17(5):547–54.

137. Singh G, Popli S, Hari Y, Malhotra P, Mukherjee S, Bhatnagar RK. Suppression of RNA silencing by Flock house virus B2 protein is mediated through its interaction with the PAZ domain of Dicer. FEBS Lett. 2009;585(16):1045–57.

138. Huang J, Wang F, Argyris E, Chen K, Liang Z, Tian H, et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nat Med. 2007;13(10):1241–7.

139. Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature. 2008;454 (7205):780–3.

140. Barth S, Pfuhl T, Maniani A, Elses C, Roemer K, Kremmer E, et al. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. Nucleic Acids Res. 2008;36(2):666–75.

141. Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature. 2005;435 (7042):682–6.

142. Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, et al. Identification and characterization of human cytomegalovirus-encoded microRNAs. J Virol. 2005;79(18):12905–9.

143. DeVincenzo J, Lambkin-Williams R, Wilkinson T, Chehelsky J, Nochur S, Walsh E, et al. A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. Proc Natl Acad Sci USA. 2010;107 (19):8800–5.

144. Hausecker D. The business of RNAi therapeutics. Hum Gene Ther. 2008;19(5):451–62.
Antiviral RNAi: Translating Science Towards Therapeutic Success

163. Kahana R, Kuznetzova I, Rogel A, Shemesh M, Hai D, Yadin H, et al. Inhibition of foot-and-mouth disease virus replication by small interfering RNA. J Gen Virol. 2004;85(Pt 11):3213–7.

164. Jia F, Zhang YZ, Liu CM. Stable inhibition of hepatitis B virus expression and replication in HepG2.2.15 cells by RNA interference based on retrovirus delivery. J Biotechnol. 2007;128(1):32–40.

165. Keck K, Volper EM, Spengler RM, Long DD, Chan CY, Ding Y, et al. Rational design leads to more potent RNA interference against hepatitis B virus: factors affecting silencing efficiency. Mol Ther. 2009;17(3):536–47.

166. McCaffrey AP, Nakai H, Pandey K, Huang Z, Salazar FH, Xu H, et al. Inhibition of hepatitis B virus in mice by RNA interference. Nat Biotechnol. 2003;21(6):639–44.

167. Shalomai A, Shaul Y. Inhibition of hepatitis B virus expression and replication by RNA interference. Hepatology. 2003;37(4):764–70.

168. Wu CJ, Huang HW, Liu CY, Hong CF, Chan YL. Inhibition of SARS-CoV replication by siRNA. Antiviral Res. 2005;65(1):45–8.

169. Ying RS, Zhu C, Fan XG, Li N, Tian XF, Liu HB, et al. Hepatitis B virus is inhibited by RNA interference in cell culture and in mice. Antiviral Res. 2007;73(1):24–30.

170. Wuchus L, Truss M, Hagemeier C. Inhibition of human cytomegalovirus replication by small interfering RNAs. J Gen Virol. 2004;85(Pr 1):179–84.

171. Chevalier C, Saudnier A, Benureau Y, Flechet D, Delgrange D, Colbere-Garapin F, et al. Inhibition of hepatitis C virus infection in cell culture by small interfering RNAs. Mol Ther. 2007;15(8):1452–62.

172. Kapadia SB, Brideau-Andersen A, Chisari FV. Interference of hepatitis C virus RNA replication by short interfering RNAs. Proc Natl Acad Sci USA. 2003;100(4):2014–8.

173. Trejo-Avila L, Elizondo-Gonzalez R, Trujillo-Murillo Kdel C, Zapata-Benavides P, Rodriguez-Padilla C, Rivas-Estilla AM, et al. Antiviral therapy: inhibition of Hepatitis C Virus expression by RNA interference directed against the NS5B region of the viral genome. Ann Hepatol. 2007;6(3):174–80.

174. Zhang J, Yamada O, Sakamoto T, Yoshida H, Iwai T, Matsushita Y, et al. Down-regulation of viral replication by adenosinoviral-mediated expression of siRNA against cellular cofactors for hepatitis B virus. Virology. 2004;329(1):135–43.

175. Shin D, Lee H, Kim SI, Yoon Y, Kim M. Optimization of linear double-stranded RNA for the production of multiple siRNAs targeting hepatitis C virus. RNA. 2009;15(5):898–901.

176. Wang Y, Kato N, Jazag A, Dharel N, Otsuka M, Taniguchi H, et al. Double-stranded RNA small interfering RNA targeting matrix and nucleocapsid proteins of influenza (H1N1) virus. Antiviral Res. 2010;85(3):559–66.

177. Zhiqiang W, Yaowu Y, Fan Y, Jian Y, Yongfeng H, Lina Z, et al. Antisense RNase technology inhibits influenza virus production in virus-infected mice by RNA interference. Proc Natl Acad Sci USA. 2007;104(11):4027–32.

178. Liu YP, von Eije KJ, Schopman NC, Westerink JT, ter Brake O, Haasnoot J, et al. Combinatorial RNAi against HIV-1 using extended short hairpin RNAs. Mol Ther. 2009;17(10):1712–23.

179. Das AT, Brummelkamp TR, Westerhout EM, Vink M, Van der Eerden J, Schermer J, et al. Short interfering RNA-mediated inhibition of HIV-1 by targeting partially complementary viral sequences. Nucleic Acids Res. 2009;37(18):6194–204.

180. Liu YP, von Eije KJ, Berkhourt B. Probing the sequence space available for HIV-1 evolution. AIDS (London, England). 2008;22(14):1875–7.

181. Sengers R, Pauls E, Armand-Ugon M, Clotet-Codina I, Moncunill G, Clotet B, et al. HIV-1 resistance to the anti-HIV activity of a shRNA targeting a dual-coding region. Virology. 2008;372(2):421–9.

182. Jiang M, Milener J. Selective silencing of viral gene E6 and E7 expression in HPV-positive human cervical carcinoma cells using small interfering RNAs. Methods Mol Biol. 2005;292:401–20.

183. Butz K, Ristriani T, Hengsternann A, Denk C, Schefller M, Hoppe-Seyler F. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. Oncogene. 2003;22(38):5938–45.

184. Pipps KM, Martinez A, Lu J, Heinz BA, Zhao G. Small interfering RNA molecules as potential anti-human rhinovirus agents: in vitro potency, specificity, and mechanism. Antiviral Res. 2004;61(1):49–55.

185. Bhuyan PK, Kariko K, Capodici J, Lubinski J, Hook LM, Friedman HM, et al. Short interfering RNA-mediated inhibition of herpes simplex virus type 1 gene expression and function during infection of human keratinocytes. J Virol. 2004;78(19):10276–81.

186. Haddad R, Kashima S, Rodrigues ES, Azevedo R, Palma PV, Magalhaes DA, et al. Silencing of HTLV-I Rev cofactor hIRP is required for viral replication. Proc Natl Acad Sci USA. 2005;102(11):4027–32.

187. Bowler T, Bamberg S, Muller P, Klenk HD, Meyer TF, Becker S, et al. Inhibition of Marburg virus replication and viral release by RNA interference. J Gen Virol. 2005;86(Pt 4):1181–8.
200. Alkhalil A, Strand S, Mucker E, Huggins JW, Jahrling PB, Ibrahim SM. Inhibition of monkeypox virus replication by RNA interference. Virol J. 2009;6:188.

201. Yin R, Ding Z, Liu X, Mu L, Cong Y, Stoeger T. Inhibition of Newcastle disease virus replication by RNA interference targeting the matrix protein gene in chicken embryon fibroblasts. J Virol Methods. 2010;167(1):107–11.

202. Barik S. Control of nonsegmented negative-strand RNA virus replication by siRNA. Virus Res. 2004;102(1):27–35.

203. Sun M, Liu X, Cao S, He Q, Zhou R, Ye J, et al. Inhibition of porcine circovirus type 1 and type 2 production in PK-13 cells by small interfering RNAs targeting the Rep gene. Vet Microbiol. 2007;123(1–3):205–9.

204. Liu J, Chen L, Chua H, Du Q, Kwang J. Inhibition of porcine circovirus type 2 replication in mice by RNA interference. Virology. 2006;347(2):422–33.

205. Jia H, Ge X, Guo X, Yang H, Yu K, Chen Z, et al. Specific small interfering RNAs-mediated inhibition of replication of porcine encephalomyocarditis virus in BHK-21 cells. Antiviral Res. 2008;79(2):95–104.

206. Gitlin L, Stone JK, Andino R. Poliovirus escape from RNA interference: short interfering RNA-target recognition and implications for therapeutic approaches. J Virol. 2005;79(2):1027–35.

207. He YX, Hua RH, Zhou YJ, Qiu HJ, Tong GZ. Interference of porcine reproductive and respiratory syndrome virus replication on MARC-145 cells using DNA-based short interfering RNAs. Antiviral Res. 2007;74(2):83–91.

208. Israsena N, Supavanwong P, Ratanaasetth N, Khasaplod P, Hemachuda T. Inhibition of rabies virus replication by multiple artificial microRNAs. Antiviral Res. 2009;84(1):76–83.

209. Arias CF, Dector MA, Segovia L, Lopez T, Camacho M, Isa P, et al. RNA silencing of rotavirus gene expression. Virus Res. 2004;102(1):43–51.

210. Dector MA, Romero P, Lopez S, Arias CF. Rotavirus gene silencing by small interfering RNAs. EMBO J. 2002;3(12):1175–80.

211. Bitko V, Barik S. An endoplasmic reticulum-specific stress-activated caspase (caspase-12) is implicated in the apoptosis of A549 epithelial cells by respiratory syncytial virus. J Cell Biochem. 2001;80(3):441–54.

212. He ML, Zheng B, Peng Y, Peiris JS, Poon LL, Yuen KY, et al. Inhibition of SARS-associated coronavirus infection and replication by RNA interference. JAMA. 2003;290(20):2665–6.

213. He ML, Zheng BJ, Chen Y, Wong KL, Huang JD, Lin MG, et al. Development of interfering RNA agents to inhibit SARS-associated coronavirus infection and replication. Hong Kong Med J. 2009;15(3 Suppl 4):28–31.

214. Lu S, Cullen BR. Adenovirus VAI noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. J Virol. 2004;78(23):12868–76.

215. Zheng BJ, Guan Y, Tang Q, Du C, Xie FY, He ML, et al. Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. Antivir Ther. 2004;9(3):365–74.

216. Anthony KG, Bai F, Krishnan MN, Fikrig E, Koski RA. Effective siRNA targeting of the 3′ untranslated region of the West Nile virus genome. Antiviral Res. 2009;82(3):166–8.

217. Kumar P, Lee SK, Shankar P, Manjunath N. A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med. 2006;3(4):e96.

218. Tirasophon W, Roshorm Y, Panyim S. Silencing of yellow head virus replication in penaeid shrimp cells by dsRNA. Biochem Biophys Res Commun. 2005;334(1):102–7.

219. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Person R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010;327(5962):198–201.