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

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Oligonucleotide antiviral therapeutics: Antisense and RNA interference for highly pathogenic RNA viruses

Kevin B. Spurgers, C. Matthew Sharkey, Kelly L. Warfield, Sina Bavari

United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA

Received 31 October 2007; accepted 6 December 2007

Abstract

RNA viruses are a significant source of morbidity and mortality in humans every year. Additionally, the potential use of these viruses in acts of bioterrorism poses a threat to national security. Given the paucity of vaccines or postexposure therapeutics for many highly pathogenic RNA viruses, novel treatments are badly needed. Sequence-based drug design, under development for almost 20 years, is proving effective in animal models and has moved into clinical trials. Important advances in the field include the characterization of RNA interference in mammalian cells and chemical modifications that can dramatically increase the in vivo stability of therapeutic oligonucleotides. Antisense strategies utilize single-stranded DNA oligonucleotides that inhibit protein production by mediating the catalytic degradation of target mRNA, or by binding to sites on mRNA essential for translation. Double-stranded RNA oligonucleotides, known as short-interfering RNAs (siRNAs), also mediate the catalytic degradation of complementary mRNAs. As RNA virus infection is predicated on the delivery, replication, and translation of viral RNA, these pathogens present an obvious target for the rapidly advancing field of sequence-specific therapeutics. Antisense oligonucleotides or siRNAs can be designed to target the viral RNA genome or viral transcripts. This article reviews current knowledge on therapeutic applications of antisense and RNA interference for highly pathogenic RNA viral infections.

© 2008 Elsevier B.V. All rights reserved.

Keywords: RNA virus; RNA interference; Antisense; siRNA; Phosphorodiamidate morpholino oligomer

1. Introduction

A number of RNA viruses are highly infectious, acutely pathogenic, and pose a significant threat to human health and public safety. For example, influenza A virus infects up to 20% of the U.S. population each year, causing approximately 36,000 deaths (http://www.cdc.gov/flu/keyfacts), and poses the continuing threat of a global pandemic that could kill millions (Johnson and Mueller, 2002). Dengue virus is endemic to tropical regions around the world and is reported to infect 80 million people each year, causing 500,000 cases of dengue hemorrhagic fever (Halstead, 2007). Newly emerging RNA viruses also endanger public health as evidenced by the 2002–2003 outbreak of severe acute respiratory syndrome (SARS), caused by a novel coronavirus, that claimed the lives of over 800 people after spreading to more than 30 countries (Drosten et al., 2003; Peiris et al., 2003; Satija and Lal, 2007).

Certain highly pathogenic RNA viruses may also impact public safety and national security through the intentional use of these agents as bioweapons. The Centers for Disease Control and Prevention (CDC) classify a number of RNA viruses, including Ebola, Marburg, Lassa and Machupo viruses, as Category A bioterrorism agents. High mortality rates, ease of production, potential dissemination by aerosol and lack of effective countermeasures characterize these pathogens. For example, although not a natural mode of human-to-human transmission, filoviruses are highly infectious via the aerosol route (Leffel and Reed, 2004). There are reports investigating the stability of lyophilized Marburg virus, and the ability to aerosolize these samples for lethal infection of non-human primates (Bazhutin et al., 1992; Leffel and Reed, 2004).

Effective vaccines or postexposure therapeutics for many RNA viruses are at an infant stage and are badly needed. Antisense oligonucleotides (ASOs) are single-stranded DNA
oligonucleotides that inhibit translation or mediate RNase H-dependent degradation of complementary target RNAs. The introduction of small, double-stranded RNA oligonucleotides (siRNAs) mediates the cleavage of target transcripts through activation of the RNA interference pathway. Small hairpin RNAs (shRNAs), expressed from DNA or viral vectors, also enter the RNA interference pathway after being processed into siRNAs. These reagents are being aggressively pursued as therapeutics for a variety of ailments, including viral infections. Viral genomic RNA, or viral mRNA molecules, can be directly targeted with the aim of disrupting viral replication and disease progression. In this regard, there is continuing preclinical success in using nucleic acid-based techniques to treat viral infections in laboratory animals (Bennink and Palmore, 2004; Lee and Rossi, 2004; Lim et al., 2006; Warfield et al., 2006). Here, we review various experimental oligonucleotide-based therapies and describe recent efforts to target RNA viruses (Table 1).

### Table 1
Selected recent reports of nucleic acid-based experimental therapeutics targeting RNA viruses

| Virus family | Virus | Treatment | Model | Summary | Reference |
|-------------|-------|-----------|-------|---------|-----------|
| Filoviridae | EBOV  | PMO       | Mouse, Guinea Pig, Rhesus Mouse | PMOs show efficacy in three animal models | Warfield et al. (2006) |
|             |       |           |       | VP35 PMO inhibits EBOV in vitro and protects mice, pre and postchallenge | Enterlein et al. (2006) |
|             |       | siRNA     | Guinea Pig | SNALP encapsulated siRNAs against L protect guinea pigs from challenge | Geisbert et al. (2006) |
|             | MARV  | siRNA     | Vero   | Reduction of target proteins and amount of released virus | Fowler et al. (2005) |
| Flaviviridae | JEV, WNV | PMO       | Vero, Mouse | Single PMO gives cross protection in cell culture. Partial in vivo efficacy against WNV | Deas et al. (2007) |
|             |       | PMO       | BHK    | Conjugated PMOs inhibit viral replication. Mechanism investigated in vitro | Holden et al. (2006) |
|             | JEV   | siRNA     | Mouse  | Conjugated, brain targeting siRNA given IV protects mice from JEV | Kumar et al. (2007) |
|             | JEV, WNV | shRNA, siRNA | Mouse | Injection of a single siRNA IC protects mice from JEV and WNV | Kumar et al. (2006) |
| Arenaviridae | VEEV  | siRNA     | BHK    | siRNA inhibits multiple strains or VEEV L and NP siRNA inhibit 5 Lassa isolates, LCMV and Mopeia virus | O’Brien (2007) |
|             | Lassa | siRNA     | Vero   | Reduction of target proteins and amount of released virus | Muller and Gunther (2007) |
| Coronaviridae | SARS Co-V | PMO       | Vero   | Conjugated PMOs against TRS inhibit cytopathology, viral titer and viral spread | Neuman et al. (2005) |
|             |       | siRNA     | Mouse, Rhesus | siRNA treatment reduces viral replication and spread, along with clinical signs of SARS, in an animal model | Li et al. (2005a) |
|             |       | 293T shRNA | Vero   | siRNAs developed against SARS Co-V sequence coding for membrane (M) | Qin et al. (2007) |
|             |       | shRNA     | Vero   | shRNA to orf 7a prevents viral gene expression and replication | Akerstrom et al. (2007) |
| Orthomyxoviridae | Influenza | PMO       | Vero, MDCK | Conjugated PMOs inhibit multiple strains of influenza A in cell culture | Ge et al. (2006) |
|             |       | ASO       | CEF, chicken | ASOs targeting NS1 protect chickens from lethal H5N1 infection | Wu et al. (2008) |
|             |       | siRNA     | Mouse   | siRNAs against PA and NP inhibit production of multiple virus subtypes and increase survival | Tompkins et al. (2004) |
|             |       | siRNA, shRNA | Mouse | siRNA or shRNA given IV reduce viral titer in lung tissue | Ge et al. (2004) |
|             |       | shRNA     | MDCK, Mouse | shRNAs targeting NP and M2, given IV, partially protect mice from H1N1 and H5N1 | Zhou et al. (2007) |
|             |       | MDCK, CEF, Chick Eggs | shRNAs inhibit cytopathic effects, virus titer and virus-induced apoptosis | Li et al. (2005b) |

2. Antisense oligonucleotides (ASOs)

ASOs are single-stranded deoxyribonucleotide oligomers (~20 nucleotides) with a nucleotide sequence designed to be complementary to a target mRNA transcript (Chan et al., 2006). The ASO must be long enough to confer target specificity and to form a DNA/RNA hybrid of sufficient stability. However, as length increases, there is a greater probability the ASO will bind with partial complementarity to a non-target sequence. Typi-
specific base-pairing interactions between the ASO and target transcript result in the RNase H-mediated degradation of the target RNA and a subsequent reduction in the protein product of the targeted gene (Fig. 1). RNase H activity in mammalian cells is ubiquitous, constitutive, and is thought to normally play a role in DNA replication and transcription (Busen et al., 1977; Busen, 1980; Eder et al., 1993; Turchi et al., 1994). When targeted to ribosomal entry sites in the 5′ untranslated region of mRNA, ASOs with particular chemical modifications (discussed below) can inhibit translation in the absence of mRNA degradation.

Unmodified oligonucleotides are highly unstable in vivo (in circulation and within cells) due to rapid nuclease digestion. Therefore, ASOs are routinely customized with chemical modifications that increase stability and confer other desirable attributes. For example, Fomivirsen (Vitravene®, ISIS-2922) is an FDA-approved ASO antiviral drug with phosphodiester linkages that are modified by replacing a non-linking oxygen with a sulfur atom (phosphorothioate modification) (Fig. 2) (Orr, 2001; Geary et al., 2002). Phosphorothioate-modified ASOs are poor substrates for nucleases and thus show increased stability, increased serum half-life, and greater tissue retention. Although improved in terms of stability, these highly charged oligos have decreased affinity for their target mRNA, are not taken up efficiently by cells, and readily bind to serum and intracellular proteins, which may lead to diminished activity or off-target effects such as triggering of the complement cascade (Stein and Krieg, 1994; Krieg and Stein, 1995). Additional modifications to the 2′ position on the ribose ring have been developed to increase affinity to target mRNA and provide further resistance to degradation (Prakash and Bhat, 2007). Nucleotides with 2′ modifications, such as 2′-O-(2-methoxy)ethyl (2′-MOE), are not substrates for RNase H cleavage when bound to their target RNA (Fig. 2). Therefore, to retain this property, ASOs are

Fig. 1. Mechanism of action of antisense and RNA interference-based therapeutics. The formation of an ASO:RNA hybrid directs the RNase H-mediated cleavage of the target RNA molecule. PMO:RNA hybrids are not substrates of RNase H-mediated cleavage. Rather, when targeted near the AUG start codon, PMOs inhibit translation by preventing ribosome entry. Introduced synthetic siRNAs are incorporated into the multiprotein RISC complex where they direct the cleavage of complementary target RNA molecules.

Fig. 2. RNase H-dependent, modified ASO therapeutic design. A trinucleotide portion of an ASO is shown with phosphorothioate internucleotide linkages (A). The 3′-most nucleotide is 2′-O-(2-methoxy)ethyl (2′-MOE) modified (B). Both modifications can be found in “gapmer” ASOs which feature a phosphorothioate core (gap) flanked by 2′-modified nucleotides (2′ MOE wings).
Several ASO compounds are currently being evaluated in clinical trials for the treatment of a variety of ailments, including cancer, diabetes and viral infections (www.clinicaltrials.gov). Trials are now under way studying the intravenous delivery of ASO drug candidates targeting Bcl-2 (GenaSense®, Genta) and c-myc (Resten-NG®, AVI BioPharma), while completed trials have evaluated subcutaneously administered ASOs targeting protein tyrosine phosphatase 1B (ISIS 113715, Isis Pharmaceuticals), and hepatitis C virus (AVI-4065, AVI BioPharma). Despite these research efforts, effective compound delivery is still a major hurdle that must be overcome before ASO compounds find broad utility in the clinic. It is perhaps telling that the only ASO drug approved to date by the FDA, Fomivirsen, is administered locally into the confined space of the eye. Furthermore, despite some success of this ASO in the clinic, its use is now limited due to significant side effects (Mercerelli et al., 2007).

Conjugation of ASOs to cell-penetrating peptides is an increasingly common approach to improve cellular uptake (Pooga et al., 1998; Debart et al., 2007). A recent report demonstrates that conjugation of PMO (P-PMO) to the arginine-rich peptide (RXR)₄ increases elimination half-life and tissue retention (Amantana et al., 2007). However, the literature does not reveal a consistent correlation between peptide conjugation and increased efficacy (Enterlein et al., 2006; Tilley et al., 2007). P-PMOs are generally well tolerated by animal recipients, although toxicity can be observed as the dose increases (Amantana et al., 2007; Tilley et al., 2007). The nature of the treatment regimen may affect P-PMO toxicity. For example, Burrer et al. (2007) observed no treatment-associated toxicity when P-PMOs were administered to healthy mice. However, when P-PMO treatment followed viral challenge, significant toxicity was observed.

3. RNA interference (RNAi)

RNA interference (RNAi) is an endogenous molecular pathway that plays a role in antiviral defense and gene regulation (Fritz et al., 2006). The human genome encodes specialized, non-coding transcripts termed microRNAs (miRNAs) that regulate the expression of cellular genes (Bartel, 2004; Ying et al., 2006). The first miRNAs discovered have a role during development in Caenorhabditis elegans (Lee et al., 1993; Reinhart et al., 2000). It is now estimated that 30% of all mammalian protein-coding genes, and diverse cellular processes, are regulated by miRNAs (I.e Sage and Agami, 2006). Long, polyadenylated primary miRNA transcripts, containing a hairpin structure with sequence homology to the target gene, are first processed in the nucleus to yield a shorter miRNA precursor (pre-miRNA). Pre-miRNAs are then shuttled to the cytoplasm where they are further processed by Dicer to yield mature, short, double stranded miRNAs (Bartel, 2004; Ying et al., 2006). The RNAi pathway is now widely exploited as a tool to silence the expression of specific target genes for experimental
or therapeutic benefit (Lee and Sinko, 2006). The RNAi pathway can be activated by introducing synthetic, double-stranded small interfering RNAs (siRNAs) into cells. Alternatively, small hairpin RNAs (shRNAs) can be expressed from transfected DNA plasmid vectors or from replication-deficient viral vectors (i.e., adenovirus, and lentivirus, among others). siRNAs are loaded directly into the RISC to mediate target hybridization and cleavage (Fig. 1). In contrast, shRNAs are cleaved by DICER before incorporation into the RISC. Sequence-based rules for designing siRNA molecules with optimal activity are becoming increasingly sophisticated and are available to the general research community (Elbashir et al., 2002; Henschel et al., 2004; Pei and Tuschl, 2006; Shah et al., 2007). Additionally, pre-designed siRNAs, and shRNA expression vectors, targeting nearly every gene in the human and mouse genome are available from commercial sources.

For in vivo use, siRNAs and ASOs face similar pitfalls, including stability, delivery and off-target effects. Phosphothioate linkage and 2′-O-methyl sugar modifications protect siRNAs from nuclease digestion and help prevent off-target stimulation of the type I interferon system (Sioud, 2007). In the pursuit of systemic and targeted delivery, siRNAs have been delivered within liposomes or conjugated to antibodies, cholesterol, RNA aptamers, or peptides (de Fougerolles et al., 2007). A recent report demonstrates the feasibility of organ-specific delivery of siRNA when given intravenously (Kumar et al., 2007). The authors utilized the ability of rabies virus glycoprotein G (RVG) to bind acetylcholine receptor on neuronal cells. A peptide from RVG shows neuronal cell specificity, and when conjugated to siRNA, enables delivery into this cell type. Importantly, RVG-siRNA, when administered to mice intravenously, is delivered to the brain, without accumulating in spleen or liver (Kumar et al., 2007). Although a highly successful siRNA delivery method in this animal model, it remains to be studied whether this general approach is safe in humans.

Often cited as an advantage of siRNAs and shRNAs, these molecules utilize an endogenous molecular pathway to achieve a desired effect. However, in some cases, this could account for observed treatment-induced toxicity. Grimm et al. (2006) using a total of 49 different shRNAs expressed from an adenovirus-associated virus vector, achieved high-level shRNA expression in mouse liver after intravenous injection. Forty seven percent of treated mice died within 2 months. Interestingly, morbidity correlated with an observed decrease in liver miRNA levels. The authors demonstrate that high-level shRNA expression can saturate, and interfere with, components of the endogenous miRNA pathway. Specifically, this saturation may involve the protein exportin-5 and occur at the level of miRNA nuclear export. In contrast, systemic administration of siRNAs can achieve gene silencing in the liver without toxicity and without a disruption in liver miRNA processing (John et al., 2007). One obvious explanation is that introduced siRNAs bypass early processing steps of miRNAs and shRNAs, and are loaded directly into the RISC complex. John et al. (2007) demonstrated siRNA-dependent silencing of liver-expressed transcripts, without a decrease in liver miRNA levels or a disruption in liver miRNA target-gene expression. Importantly, positive safety results have been achieved in phase I and II clinical trials for locally delivered siRNAs. These treatments include bevasiranib (Cand5), a siRNA for intravitreal inoculation, which targets vascular endothelial growth factor (Acuity Pharmaceuticals), and ALN-RSV01, an intranasally delivered product that targets the transcript for the nucleocapsid protein of respiratory syncytial virus (Alnylam Pharmaceuticals).

4. Antisense and siRNA as antiviral therapeutics

RNA virus infection requires delivery of the viral genome into cells, transcription of viral mRNA and subsequent translation of viral proteins that aid in genome replication, viral assembly and budding. Targeting viral RNA sequences with ASOs or siRNAs is a conceptually appealing strategy for treating RNA virus infections for several reasons. First, preventing the synthesis of even one critical viral protein could potentially disrupt the viral life cycle. Secondly, the sequence specificity of ASO and siRNA compounds potentially allows viral genes to be targeted without affecting host genes, thus decreasing or eliminating unwanted side effects. In addition, targeting viral sequences does not require an understanding of gene function. The feasibility of using ASOs and siRNAs to disrupt RNA virus gene expression was first demonstrated using Rous sarcoma virus and respiratory syncytial virus, respectively (Stephenson and Zamecnik, 1978; Bitko and Barik, 2001). Viral genome sequences are available, or can be easily acquired, allowing researchers rapidly to design and test novel nucleic acid-based, sequence-specific antiviral reagents. In support of this approach, there is an extensive and rapidly expanding record of using antisense and RNAi to target viruses for therapeutic benefit. Recent investigations of sequence-based reagents against highly pathogenic RNA viruses are discussed below.

4.1. Filoviruses

Ebola (EBOV) and Marburg (MARV) viruses have a single-stranded, negative-sense 19 kb RNA genome that codes for seven proteins. Viral replication and transcription depend on a complex of nucleoprotein (NP), VP30, VP35 and the RNA polymerase L. VP24 and VP40 are involved in budding of mature virions from the cell surface while glycoprotein (GP) is found embedded in the lipid bilayer surrounding the nucleocapsid. The filoviruses are listed as Category A bioterrorism agents by the CDC and must be handled under biosafety level 4 conditions. Both viruses cause severe hemorrhagic fever in humans with mortality rates of 30–90% (Thacker, 2003; Feldmann, 2006). Currently, there are no vaccines or therapeutics for treating filovirus infections in humans. For further information and perspective on filovirus drug development, see the article by Bausch et al. (2008).

Two preclinical studies have been published investigating PMOs as a treatment for EBOV infection, with both showing efficacy in animal models. PMOs were designed to inhibit translation of EBOV VP35, VP24, and L transcripts (Warfield et al., 2006). All three PMOs showed activity using an in vitro translation assay and reduced viral titer in cell culture. In a similar study, a different VP35-specific PMO also reduced virus yield in cell
RNAi-based drug candidates is the presence of virus-encoded siRNA-mediated therapy and warrant further investigation as gest that filovirus infections are susceptible to antisense and Vero cells 24 h after infection. Together, these studies sug-

As expected, this decreased the yield of virus released from a co-transfection assay or after MARV infection in Vero cells. siRNA efficiently reduced the level of its target transcript in (SNALP) (Geisbert et al., 2006). SNALP-encapsulated siRNAs somes can be used to form a stable nucleic acid-lipid particle increase the serum half-life of siRNAs in vivo, specialized lipo-

prove to be an effective therapy against filovirus infection. To PMO treatment survived lethal EBOV challenge. A third mon-

treated with the single VP35-targeting PMO survived EBOV challenge by a combination of parenteral routes. No monkeys given closer to challenge; probably due to differences in phar-

macokinetics and bioavailability between treatment regimens. Finally, anti-EBOV PMO treatment was investigated in rhesus macaques (Warfield et al., 2006). Monkeys were administered a single PMO (VP35) or a combination of three PMOs (VP35, VP24 and L) from 2 days before, through 9 days after EBOV challenge by a combination of parenteral routes. No monkeys treated with the single VP35-targeting PMO survived EBOV infection, but two of four monkeys receiving the combination PMO treatment survived lethal EBOV challenge. A third mon-

key in this treatment group remained aviremic, but succumbed to a secondary bacterial infection.

In addition to these promising PMO studies, siRNA may prove to be an effective therapy against filovirus infection. To increase the serum half-life of siRNAs in vivo, specialized liposo-

mies can be used to form a stable nucleic acid-lipid particle (SNALP) (Geisbert et al., 2006). SNALP-encapsulated siRNAs against EBOV L gene were efficacious in a guinea pig model of EBOV infection when given daily by i.p. injection, beginning 1 h after challenge. Viremia was not detected in any of the siRNA-
treated animals, although two of five siRNA-treated guinea pigs died (compared to five out of five dead control animals). Because these results could indicate therapy-dependent toxicity, a trial with a lower dose of SNALP L siRNAs was performed, and successfully protected 100% of challenged animals.

Recently, siRNAs were also generated to target MARV NP, VP30 and VP35 transcripts (Fowler et al., 2005). Each siRNA efficiently reduced the level of its target transcript in a co-transfection assay or after MARV infection in Vero cells. As expected, this decreased the yield of virus released from Vero cells 24 h after infection. Together, these studies sug-

gest that filovirus infections are susceptible to antisense and siRNA-mediated therapy and warrant further investigation as therapeutics for eventual use in humans.

A growing consideration for those developing antiviral RNAi-based drug candidates is the presence of virus-encoded RNA silencing suppressors (RSSs) (Voinnet, 2005). Although the presence and significance of an endogenous RNAi antiviral response in mammalian cells is still under scrutiny, it has been well established in plants, insects, and nematodes. It is now suggested that EBOV VP35 has RSS activity, as this protein can inhibit shRNA-mediated silencing of luciferase expression in cotransfection assays (Haasnoot et al., 2007). This RNAi inhibition by VP35 could not be attributed to interferon (IFN) antagonism, as this effect was also observed in Vero cells, which have a defective interferon pathway. Additionally, VP35 can functionally complement a mutant HIV-1 Tat pro-

tein. HIV-1 Tat exhibits RSS activity that is required for viral replication (Bennasser et al., 2005; Haasnoot et al., 2007). It is tempting to speculate that siRNA or ASO/PMO treatments targeting RSS protein encoding sequences, whether as part of a pool or as individual compounds, will be more efficacious than treatments that do not reduce RSS protein levels. This will likely be an important area of investigation in the near future.

4.2. Flaviviruses

Flaviviruses contain a ∼11 kb, plus-sense orientation, single-stranded RNA genome that encodes a single long polyprotein. Once generated, this polyprotein is cleaved to produce three structural, and seven non-structural proteins (Samuel and Diamond, 2006). The family Flaviviridae contains a number of important human pathogens. These include West Nile (WNV), yellow fever (YFV), Japanese encephalitis (JEV), and dengue (DENV) viruses. The significance of DENV infections has already been cited, and the increasing public health toll of JEV is described in another paper in this issue (Gould et al., 2008). In 2006 there were more than 4000 reported cases of WNV infection in the United States alone; most were neuroin-

vasive (http://www.cdc.gov). Importantly, there are currently no approved vaccines or antiviral therapies against DENV or WNV for use in humans.

PMOs have been used to inhibit flavivirus infections (WNV, JEV, SLEV and DENV) by targeting conserved sequences within the 5′ and 3′ untranslated regions of the viral genome (Deas et al., 2005, 2007; Kinney et al., 2005; Holden et al., 2006). These areas of the genome form critical secondary structures that are necessary for viral replication (Khromykh et al., 2003). Deas et al. (2005) identified a peptide-conjugated PMO (PPMO) target-

ging a highly conserved 3′ sequence element that exhibited potent anti-WNV activity in cell culture. In a subsequent report, this PMO was redesigned to achieve perfect complementarity to this sequence element in WNV, JEV, and St. Louis encephali-

tis virus (SLEV) (Deas et al., 2007). The redesigned oligo was effective against all three viruses in cell culture. PPMO and PMO targeting this 3′ sequence element were then tested in a mouse model of WNV infection. Although mice could tolerate higher doses of the PMO, compared to PPMO, this reagent did not protect virus-infected mice. In contrast, the PPMO, even when given at a more than 10-fold lower dose, increased survival and improved clinical correlates in mice infected with WNV. One can speculate that the conjugated peptide improved cellular uptake of the PMO and in this way improved efficacy.
Several groups have successfully inhibited WNV replication using siRNA-based approaches (Kachko et al., 2006; Ong et al., 2006). Importantly, by targeting highly conserved sequences, progress has been made in designing antisense or RNAi-based therapies that protect mice from challenge with multiple flaviviruses. By screening several siRNAs against the JEV genome, Kumar et al. (2006) identified a siRNA targeting the envelope protein (E) that provided significant protection from JEV infection in cell culture. A single dose of E-specific shRNA-expressing neurotropic lentivirus was able to provide complete protection (100% of mice survive) against lethal JEV challenge in mice when administered by intracranial (IC) injection. Although this approach successfully protected mice from JEV, lentivirus-based therapies may have a hard time finding their way into the clinic due to safety concerns stemming from viral integration.

siRNAs may offer a better choice because of the transient nature of their effect and the ease of dose regimen optimization. One hundred percent of mice injected IC with lipid-encapsulated siRNA targeting E, 30 min and 6 h after challenge, were protected from lethal JEV infection. Importantly, the authors were able to design a siRNA with near-perfect homology to a region in the E gene of both JEV and WNV. IC injection 30 min or 6 h after challenge provided almost complete protection against both JEV and WNV. Kumar et al. (2007) further demonstrated the in vivo efficacy of intravenously (IV) administered siRNA against JEV. A peptide derived from the glycoprotein of rabies virus (RVG) enables brain-specific delivery of conjugated siRNAs when given IV. Treatment with RVG-conjugated siRNA (against E protein) was initiated 4 h after challenge, and was repeated daily for 3 days. This treatment regimen did not induce a type I IFN response and protected 80% of mice from lethal encephalitis. Additional information on antiviral therapy for JEV is available in this issue (Gould et al., 2008).

### 4.4. Alphaviruses

Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne, enveloped RNA virus of the genus *Alphavirus* that is found in Central and South America, and has been extensively studied in the laboratory (Weaver et al., 2004). Its positive-sense, single-stranded RNA genome codes for seven genes, four non-structural proteins (nsp1-nsp4) and three structural proteins (E1, E2, and capsid). Although lethal human infections are rare, acute encephalitis can develop after exposure. VEEV can also be easily transmitted by aerosol, and is considered a possible agent of bioterrorism. There is currently no licensed vaccine or antiviral therapy for human use.

siRNAs against nsp1, nsp4, and E1 have been investigated for the ability to inhibit VEEV replication in vitro (O’Brien, 2007). Four individual siRNAs were designed to target sequences in these genes conserved in six VEEV strains. When a pool of all four siRNAs was transfected into BHK 21 cells (defective for IFN production), antiviral activity was observed against all VEEV strains tested. Although the inhibition of viral replication was only transient, this study raises the possibility that pools of siRNAs could be used to target multiple genetic strains of a particular virus.

### 4.5. SARS-associated coronavirus (SARS Co-V)

SARS emerged as an atypical pneumonia in Guangdong Province, China, in late 2002, then quickly spread to southeast Asia and North America, eventually appearing in more than 30 countries and causing more than 800 deaths (Satija and Lal, 2007). Death occurred in approximately 3–10% of infected individuals following flu-like symptoms, which included fever, malaise and dry cough, leading to acute respiratory distress with diffuse alveolar damage. An intensive research effort identified a novel coronavirus (SARS Co-V) as the causative agent of SARS (Drosten et al., 2003; Peiris et al., 2003; Rota et al., 2003).

Like other coronaviruses, the RNA genome of SARS Co-V is large (~30 kb), single-stranded, in the positive sense orientation, capped, and polyadenylated. Neuman et al. (2005) describe the design and testing of peptide-conjugated PMOs complementary to several regions of the SARS Co-V genome. These targeted regions include the AUG start codon of the replicase gene (orf 1a/1b), the orf 1b ribosomal frameshift point, the 5′ UTR transcription regulatory sequence (TRS), and the 3′ UTR. PMOs TRS1 and TRS2 were designed to target the TRS consensus sequence and disrupt secondary structure formed at this point in the genome. Vero cells were treated with PMOs and then...
infected with SARS Co-V, TRS1 and TRS2, more so than other tested PMOs, reduced the cytopathic effects of viral infection, reduced viral yield and viral spread, and inhibited viral genomic RNA synthesis.

A number of preclinical studies have been completed investigating the ability of siRNAs to prevent SARS Co-V replication and cytopathic effects, and were recently reviewed (Wu and Chan, 2006). These experiments, which targeted several regions of the SARS Co-V genome, demonstrated that siRNAs, or shRNAs, can reduce target gene expression, viral RNA levels, and viral yield. Zheng et al. (2004) conducted an initial in vitro screen of 48 siRNAs covering the entire SARS Co-V genome that identified two promising siRNAs targeting the spike protein-coding region and the NSP12 region. These siRNAs were further evaluated in vivo. siRNAs were administered in combination, intranasally, to rhesus macaques before, during, or after intranasal challenge with SARS Co-V (Li et al., 2005a). Each siRNA treatment regimen successfully repressed viral replication and spread in the lungs, and prevented the development of clinical signs of SARS. Although siRNA treatment did not completely eliminate the presence of SARS Co-V, efficacy was demonstrated by reduced viral loads in oropharyngeal swabs and lung tissue sections, less severe diffuse alveolar damage and diminished fever with no treatment-associated toxicities or safety concerns (Li et al., 2005a).

4.6. Influenza A

Influenza A viruses contain eight segments of single-stranded, negative sense genomic RNA which code for 11 known proteins (Cheung and Poon, 2007). Multiple subtypes of influenza A virus exist based on antigenic variation of envelope-associated hemagglutinin (HA) and neuraminidase (NA) proteins (i.e., H1N1, H3N2 and H5N1). While vaccines can protect individuals from specific viral subtypes, the emergence of novel strains poses the risk of deadly pandemic (Johnson and Mueller, 2002). H5N1 is a highly virulent avian influenza virus that can also infect humans. Currently, the virus does not spread easily from person to person. With the acquisition of this ability, the consequences of an influenza H5N1 pandemic could be truly devastating.

Since the mid-1990s, ASOs have been investigated as potential therapies for influenza A virus infection. Antisense phosphodiester and phosphorothioate oligos can successfully decrease expression of viral proteins PB2 and PA in cell culture (Hatta et al., 1996, 1997). Phosphorothioate oligos targeting PB2 have also shown efficacy in vivo. Lipid encapsulated ASOs targeting the AUG start codon of PB2, given IV, protected mice from lethal challenge with influenza A/PR/8/34 (Mizuta et al., 1999). This treatment effectively reduced viral RNA levels in mouse lung, decreased viral titers in the lung, and limited damage to lung tissue. More recently, it was reported that 2′-O-methyl-modified ASOs targeting NS1, delivered intranasally, protected chickens from lethal H5N1 infection (Wu et al., 2008). PMOs have also been designed and found to inhibit multiple strains of influenza A virus (Ge et al., 2006). Peptide-conjugated PMOs (P-PMOs) were designed to bind to the AUG start codon region of four genes (PA, PB1, PB2, and NP). Additional P-PMOs were designed to target four terminal regions of the NP gene, which should interrupt viral RNA/RNA interactions required for RNA synthesis. Six of eight P-PMOs successfully inhibited H1N1 production in Vero cells. Based on these results, two P-PMOs were tested for activity against a panel of influenza A subtypes, including the highly pathogenic H5N1. Notably, pretreatment with P-PMOs targeting the start codon of PB1, or the terminal region of NP, effectively inhibited H5N1 growth in MDCK cells. Postinfection protocols also showed some success (Ge et al., 2006).

Influenza A-specific siRNAs have proven efficacious in mice. In a protocol that preferentially delivers nucleic acids to the lung, siRNAs specific for NP and PA (Ge et al., 2004), or shRNAs targeting NP and M2 (Zhou et al., 2007) were administered intravenously in complex with a cationic polymer (PEI). In both studies, siRNA or shRNA treatment reduced viral titers in lung tissue. shRNAs against NP and M2 also increased survival in mice infected with influenza A strains H1N1 or H5N1. Another group also has demonstrated that siRNAs targeting PA and NP can reduce influenza A virus production in the lungs of infected mice and increase survival (Tompkins et al., 2004). Combination treatment with PA and NP siRNAs provided 100% protection from lethal challenge. Importantly, this same antiviral activity was seen against multiple strains of influenza A, including H5N1. IFN-α was not detected in serum or lung tissue homogenates from mice treated with siRNA; evidence that the observed antiviral effect is not due to IFN production. For additional information on treating seasonal and avian influenza, see the review by Beigel and Bray (2008).

5. Conclusion

Based on preclinical studies, antisense and siRNA-based antiviral compounds appear to hold a great deal of promise and will increasingly find their way through the FDA approval process and into the clinic. However, delivery remains a significant challenge. As mentioned, the only FDA-approved ASO drug is administered locally. A recent report of peptide-mediated, organ-specific delivery of siRNA after systemic administration is an important advance. Such delivery methods have the potential to increase the efficacy of many ASO and siRNA compounds. The development of systemic and postexposure treatment regimens will be critical for the successful and widespread use of these compounds. Further, we expect the efficacy of these drug candidates to improve as ASO and siRNA design rules become even better understood and entire viral genomes are systematically screened for appropriate targets.

Acknowledgements

A portion of the research described herein was sponsored by the Defense Threat Reduction Agency JSTO-CBD and the Medical Research and Material Command. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.
References

Akerstrom, S., Mirazimi, A., Tan, Y.J., 2007. Inhibition of SARS-CoV replication cycle by small interfering RNAs silencing specific SARS proteins. 7a/7b, 3a/3b and S. Antiviral Res. 73 (3), 219–227.

Amantana, A., Moulton, H.M., Cate, M.L., Reddy, M.T., Whitehead, T., Hassinger, J.N., Youngblood, D.S., Iversen, P.L., 2007. Pharmacokinetics, biodistribution, stability and toxicity of a cell-penetrating peptide-morpholino oligomer conjugate. Bioconjug. Chem. 18 (4), 1325–1331.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2), 281–297.

Bausch, D., Sprecher, A.G., Jeffs, B., Bounouadouki, P., 2008. Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs under outbreak conditions. Antiviral Res. 78, 150–161.

Bazhutin, N.B., Belanov, E.F., Spiridonov, V.A., Voitenko, A.V., Krivenchuk, N.A., Krotov, S.A., Omel’chenko, N.I., Tereshchenko, A., Khomichev, V.V., 1992. The effect of the methods for producing an experimental Marburg virus infection on the characteristics of the course of the disease in green monkeys. Vopr. Virusol. 37 (3), 153–156.

Beigel, J., Bray, M., 2008. Current and future therapy of severe influenza A virus infections. Antiviral Res. 78, 91–102.

Bennasser, Y., Le, S.Y., Benkirane, M., Jeang, K.T., 2005. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. Immunity 22 (5), 607–619.

Bennink, J.R., Palmore, T.N., 2004. The promise of siRNAs for the treatment of influenza. Trends Mol. Med. 10 (12), 571–574.

Bitko, V., Barik, S., 2001. Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses. BMC Microbiol. 1, 34.

Burrer, R., Neuman, B.W., Ting, J.P., Stein, D.A., Moulton, H.M., Iversen, P.L., Kuhn, P., Buchmeier, M.J., 2007. Antiviral effects of antisense morpholino oligomers in murine coronavirus infection models. J. Virol. 81 (11), 5637–5648.

Busen, 1980. Purification, subunit structure, and serologicai analysis of calf thymus ribonuclease H I. J. Biol. Chem. 255 (19), 9434–9443.

Busen, W., Peters, J.H., Hausen, P., 1977. Ribonuclease H levels during the cokinetics, biodistribution, stability and toxicity of a cell-penetrating peptide-morpholino oligomer conjugate. Bioconjug. Chem. 18 (4), 1325–1331.

Eder, P.S., Waldner, R.Y., Waldner, J.A., 1993. Substrate specificity of human RNase H1 and its role in excision repair of ribose residues misincorporated in DNA. Biochimie 75 (1/2), 123–126.

Elbashir, S.M., Harborth, J., Weber, K., Tuschl, T., 2002. Analysis of gene function in somatic mammalian cells using small interfering RNAs. Methods 26 (2), 199–213.

Enterlein, S., Warfield, K.L., Swenson, D.L., Stein, D.A., Smith, J.L., Gamble, C.S., Kroeker, A.D., Iversen, P.L., Bavari, S., Muhlderger, E., 2006. VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. Antimicrob. Agents Chemother. 50 (3), 984–993.

Feldmann, H., 2006. Marburg hemorrhagic fever—the forgotten cousin strikes. N. Engl. J. Med. 355 (9), 866–869.

Fowler, T., Bamberg, S., Moller, P., Klinkd, H.D., Meyer, T.F., Becker, S., Rudel, T., 2005. Inhibition of Marburg virus protein expression and viral release by RNA interference. J. Gen. Virol. 86 (Pt 4), 1181–1188.

Fritz, J.H., Girardin, S.E., Philpott, D.J., 2006. Innate immune defense through RNA interference. Sci. STKE 2006 (339), pe27.

Ge, Q., Filip, L., Bai, A., Nguyen, T., Eisen, H.N., Chen, J., 2004. Inhibition of influenza virus production in virus-infected mice by RNA interference. Proc. Natl. Acad. Sci. U.S.A. 101 (23), 8676–8681.

Ge, Q., Fastey, M., Kobasa, D., Puthavathana, P., Lupfer, C., Bestwick, R.K., Iversen, P.L., Chen, J., Stein, D.A., 2006. Inhibition of multiple subtypes of influenza A virus in cell cultures with morpholino oligomers. Antimicrob. Agents Chemother. 50 (11), 3724–3733.

Geary, R.S., Henry, S.P., Grilone, L.R., 2002. Fomiviren: clinical pharmacology and potential drug interactions. Clin. Pharmacokinet. 41 (4), 255–260.

Goldman, T.W., Hensley, L.E., Kagan, E. Yu, E.Z., Geisbert, J.B., Daddario-DiCaprio, K., Fritz, E.A., Jahrling, P.B., McClintock, K., Phelps, J.R., Lee, A.C., Judge, A., Jeffs, L.B., MacLachlan, L. 2006. Postexposure protection of guinea pigs against a lethal ebola virus challenge is conferred by RNA interference. J. Infect. Dis. 193 (12), 1650–1657.

Gould, E., Solomon, T., Mackenzie, J., 2008. Does antiviral therapy have a role in the control of Japanese encephalitis? Antiviral Res. 78, 140–149.

Grimm, D., Streetz, K.L., Jopling, C.L., Storm, T.A., Pandey, K., Davis, C.R., Marion, P., Salazar, F., Kay, M.A., 2006. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. Nature 441 (7092), 537–541.

Gunther, S., Lenz, O., 2004. Lassa virus. Crit. Rev. Clin. Lab. Sci. 41 (3–4), 339–390.

Hassanin, J., de Vries, W., Geutjes, E.J., Prins, M., de Haan, P., Berkhout, B., 2007. The ebola virus VP35 protein is a suppressor of RNA silencing. PLoS Pathog. 3 (6), e86.

Halstead, S.B., 2007. Dengue. Lancet 370, 1644–1652.

Hatta, T., Nakagawa, Y., Takai, K., Nakada, S., Yokota, T., Takaku, H., 1996. Inhibition of influenza virus RNA polymerase and nucleoprotein genes expression by unmodified, phosphorothioated, and liposomally encapsulated oligonucleotides. Biochem. Biophys. Res. Commun. 223 (2), 341–346.

Hatta, T., Takai, K., Nakada, S., Yokota, T., Takaku, H., 1997. Specific inhibition of influenza virus RNA polymerase and nucleoprotein genes expression by liposomally endocapsulated antisense phosphorothioate oligonucleotides: penetration and localization of oligonucleotides in clone 76 cells. Biochem. Biophys. Res. Commun. 232 (2), 545–549.

Henschel, A., Buchholz, F., Habermann, B., 2004. DEQOR: a web-based tool for the design and quality control of siRNAs. Nucleic Acids Res. 32, W113–W120 (Web Server issue).

Holden, K.L., Stein, D.A., Pierson, T.C., Ahmed, A.A., Clyde, K., Iversen, P.L., Harris, E., 2006. Inhibition of dengue virus translation and RNA synthesis by a morpholine oligomer targeted to the top of the terminal 3′ stem-loop structure. Virology 344 (2), 439–452.

Jason, T.L., Koropatnick, J., Berg, R.W., 2004. Toxicology of antisense therapeutics. Toxicol. Appl. Pharmacol. 201 (1), 66–83.

Johnson, N., Constien, R., Akinc, A., Goldberg, M., Moon, Y.A., Spranger, M., Hadwiger, P., Soutschek, J., Vornlocher, H.P., Manoharan, M., Stoffel, M., Langer, R., Anderson, D.G., Horton, J.D., Kotelliansky, V., Bumcroft, D., 2007. Effective RNAi-mediated gene silencing without interruption of the endogenous microRNA pathway. Nature 449 (7163), 745–747.

Johnson, N.P., Mueller, J., 2002. Updating the accounts: global mortality of the 1918–1920 ‘Spanish’ influenza pandemic. Bull. Hist. Med. 76 (1), 105–115.

Kachko, A.V., Ivanova, A.V., Protopopova, E.V., Netesov, V., Lokev, V.B., 2006. Inhibition of West Nile virus replication by short interfering RNAs. Dokl. Biochem. Biophys. 410, 260–262.
C. elegans

Lee, N.S., Rossi, J.J., 2004. Control of HIV-1 replication by RNA interference.

le Sage, C., Agami, R., 2006. Immense promises for tiny molecules: uncovering miRNA functions. Cell Cycle 5 (13), 1415–1421.

Khromykh, A.A., Kondratieva, N., Sgro, J.Y., Palmenberg, A., Westaway, E.G., Khan, S.H., Goba, A., Chu, M., Roth, C., Healing, T., Marx, A., Fair, J., Guttieri, O.R.M., 2001. Technology evaluation: fomivirsen, Isis Pharmaceuticals Inc.

Orr, R.M., 2001. Technology evaluation: fomivirsen. Isis Pharmaceuticals Inc/CIBA vision. Curr. Opin. Mol. Ther. 3 (3), 288–294.

Pei, Y., Tuschl, T., 2006. On the art of identifying effective and specific siRNAs. Nat. Methods 3 (9), 670–676.

Khan, S.H., Goba, A., Chu, M., Roth, C., Healing, T., Marx, A., Fair, J., Guttiere, M., Ferro, P., Imes, T., Monagin, C., Garry, R., Bausch, D., 2008. New opportunities for field research on the pathogenesis and treatment of Lassa fever. Antiviral Res. 78, 103–115.

Khromykh, A.A., Kondratieva, N., Sgro, J.Y., Palmberg, A., Westaway, E.G., 2003. Significance in replication of the terminal nucleotides of the flavivirus genome. J. Virol. 77 (19), 10623–10629.

Kinney, R.M., Huang, C.Y., Rose, B.C., Kroeker, A.D., Dreher, T.W., Iversen, P.L., Stein, D.A., 2005. Inhibition of dengue virus serotypes 1-4 in vero cell cultures with morpholino oligomers. J. Virol. 79 (8), 5116–5128.

Krieg, A.M., Stein, C.A., 1995. Phosphorothioate oligodeoxynucleotides: antisense or anti-protein? Antisense Res. Dev. 5 (4), 241.

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

Kumar, P., Wu, H., McBride, J.L., Jung, K.E., Kim, M.H., Davidson, B.L., Lee, S.K., Shankar, P., Manjunath, N., 2007. Transvascular delivery of small interfering RNA to the central nervous system. Nature 448 (7149), 39–43.

Lee, S.H., Sinko, P.J., 2006. siRNA—getting the message out. Eur. J. Pharm. Sci. 27 (5), 401–410.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., Kim, V.N., 2005b. The nuclear RNase III Drosha initiates microRNA processing. Nature 425 (6956), 415–419.

Leffel, E.K., Reed, D.S., 2004. Marburg and Ebola viruses as aerosol threats. Biodef. Insights 2 (3), 186–191.

Liu, B.J., Tang, Q., Cheng, D., Qin, C., Xie, F.Y., Wei, Q., Xu, J., Liu, Y., Zheng, B.J., Woodle, M.C., Zhong, N., Lu, P.Y., 2005a. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat. Med. 11 (9), 944–951.

Li, Y.C., Kong, L.H., Cheng, B.Z., Li, K.S., 2005a. Inhibition, escape, and attenuated growth of severe acute respiratory syndrome coronavirus treated with antisense morpholino oligomers. J. Virol. Nov 16 (epub ahead of print).

Lim, T.W., Yuan, J., Liu, Z., Qiu, D., Sall, A., Yang, D., 2006. Nucleic-acid-based antiviral agents against positive single-stranded RNA viruses. Curr. Opin. Antiviral Res. 2 (1), 20–29.

L.J., Bellini, W.J., 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300 (5624), 1394–1399.

Pooga, M., Hallbrink, M., Zorko, M., Langel, U., 1998. Cell penetration by antisense peptide-phosphorodiamidate morpholino oligomer conjugate: a balance between virulence, innate and adaptive immunity, and viral evasion. J. Virol. 80 (19), 9349–9360.

Prakash, T.P., Bhat, B., 2007. 2′-Modified oligonucleotides for antisense therapeutics. Curr. Top. Med. Chem. 7 (7), 641–649.

Qin, Z.L., Zhao, P., Cao, M.M., Qi, Z.T., 2007. siRNAs targeting terminal sequences of the SARS-associated coronavirus membrane gene inhibit M protein expression through degradation of M mRNA. J. Virol. Methods 145 (2), 146–154.

Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettiger, J.C., Rougvie, A.E., Horvitz, H.R., Ruvkun, G., 2000. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 403 (6722), 901–906.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Incenog, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Tong, S., Tamin, A., Lowe, L., Face, M., DeRisi, J.L., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Kiszak, T.G., Rollin, P.E., Sanchez, A., Liflick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Anderson, L.J., Bellini, W.J., 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300 (5624), 1394–1399.

Samuel, M.A., Diamond, M.S., 2006. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J. Virol. 80 (19), 9349–9360.

Satija, N., Lal, S.K., 2007. The molecular biology of SARS coronavirus. Ann. N.Y. Acad. Sci. 1102, 26–38.

Shah, J.K., Garner, H.R., White, M.A., Shames, D.S., Minna, J.D., 2007. siRNA interference Information Resource, a web-based tool for siRNA sequence design and analysis and an open access siRNA database. BMC Bioinformatics. 8, 178.

Sioud, M., 2007. RNA interference and innate immunity. Adv. Drug Deliv. Rev. 59 (2), 153–163.

Sontheimer, E.J., 2005. Assembly and function of RNA silencing complexes. Nat. Rev. Mol. Cell Biol. 6 (2), 127–138.

Stein, C.A., Krieg, A.M., 1994. Problems in interpretation of data derived from in vitro and in vivo use of antisense oligodeoxynucleotides. Antisense Res. Dev. 4 (2), 67–69.

Stephenson, M.L., Zamecnik, P.C., 1978. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc. Natl. Acad. Sci. U.S.A. 75 (1), 285–288.

Summerton, J., 1999. Morpholin antisense oligomers: the case for an RNAse H-independent structural type. Biochim. Biophys. Acta. 1489 (1), 141–158.

Summerton, J.E., 2007. Morpholin, siRNA, and S-DNA compared: impact of structure and mechanism of action on off-target effects and sequence specificity. Curr. Top. Med. Chem. 7 (7), 651–660.

Thacker, P.D., 2003. An Ebola epidemic simmers in Africa: in remote region, outbreak shows staying power. JAMA 290 (3), 317–319.

Tilley, L.D., Mellbye, B.L., Puckett, S.E., Iversen, P.L., Geller, B.L., 2007. Antisense peptide-phosphorodiamidate morpholino oligomer conjugate: dose–response in mice infected with Escherichia coli. J. Antimicrob. Chemother. 59 (9), 66–73.

Tompkins, S.M., Lo, C.Y., Tumpey, T.M., Epstein, S.L., 2004. Protection against lethal influenza virus challenge by RNA interference in vivo. Proc. Natl. Acad. Sci. U.S.A. 101 (23), 8682–8686.

Turchi, J.J., Huang, L., Murante, R.S., Kim, Y., Bambara, R.A., 1994. Enzymatic completion of mammalian lagging-strand DNA replication. Proc. Natl. Acad. Sci. U.S.A. 91 (21), 9803–9807.

Voinnet, O., 2005. Induction and suppression of RNA silencing: insights from viral infections. Nat. Rev. Genet. 6 (3), 206–220.

Warfield, K.L., Swenson, D.L., Olinger, G.G., Nichols, D.K., Pratt, W.D., Blouch, R., Stein, D.A., Aman, M.J., Iversen, P.L., Bavari, S., 2006. Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. PLoS Pathog. 2 (1), e1.

Weaver, S.C., Ferro, C., Barrera, R., Boshell, J., Navarro, J.C., 2004. Venezuelan equine encephalitis. Annu. Rev. Environ. 40, 141–174.
Wu, C.J., Chan, Y.L., 2006. Antiviral applications of RNAi for coronavirus. Expert Opin. Investig. Drugs 15 (2), 89–97.

Wu, Y., Zhang, G., Li, Y., Jin, Y., Dale, R., Sun, L.Q., Wang, M., 2008. Inhibition of highly pathogenic avian H5N1 influenza virus replication by RNA oligonucleotides targeting NS1 gene. Biochem. Biophys. Res. Commun. 365 (2), 369–374.

Ying, S.Y., Chang, D.C., Miller, J.D., Lin, S.L., 2006. The microRNA: overview of the RNA gene that modulates gene functions. Methods Mol. Biol. 342, 1–18.

Zheng, B.J., Guan, Y., Tang, Q., Du, C., Xie, F.Y., He, M.L., Chan, K.W., Wong, K.L., Lader, E., Woodle, M.C., Lu, P.Y., Li, B., Zhong, N., 2004. Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. Antivir. Ther. 9 (3), 365–374.

Zhou, H., Jin, M., Yu, Z., Xu, X., Peng, Y., Wu, H., Liu, J., Liu, H., Cao, S., Chen, H., 2007. Effective small interfering RNAs targeting matrix and nucleocapsid protein gene inhibit influenza A virus replication in cells and mice. Antiviral Res. 76 (2), 186–193.