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Recent Patents on Development of Nucleic Acid-based Antiviral Drugs against Seasonal and Pandemic Influenza Virus Infections

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Abstract: Influenza viruses are etiological agents of deadly flu that continue to pose global health threats, and have caused global pandemics that killed millions of people worldwide. The global crisis involving the avian H5N1 influenza provides compelling reasons for accelerate fast track development of novel antiviral drugs against the potential pandemic virus. The availability of neuraminidase inhibitors such as oseltamivir (tamiflu) improves our ability to defend against influenza viruses, but the incidences of tamiflu-resistance are on the rapid rise. Nucleic acid-based antiviral drugs are promising classes of experimental antiviral drugs that have been shown in pre-clinical studies to be effective against seasonal and avian influenza viruses. The potency and versatility of these drugs make them potential candidates to be used in seasonal and pandemic influenza scenarios. The review will assess the recent patents, research and development of antisense oligonucleotides, small interfering RNA, immunomodulating RNA for the prevention and treatment of influenza infection.

Keywords: Patents, influenza infection, nucleic acid-based drugs, antisense, small interfering RNA.

INTRODUCTION

Influenza is a leading cause of human mortality and morbidity worldwide, and is responsible for resulting in loss of billions of dollars in health care costs. Approximately 36,000 people die of the influenza each year in the US alone. However, its most potent threat is its potential to cause deadly global pandemics. The 1918-19 Spanish influenza pandemic kill more than 50 millions [1], and highlighted the vulnerability of humans to pandemic influenza viruses. Currently global crisis posed by the emergence of the avian H5N1 influenza virus provides testament to the challenges of defending against a deadly virus that is unpredictable and ever changing. As the human death toll from the bird flu outbreaks continues to increase, the world is moving close to a potential influenza pandemic. With a reported case fatality rate over 50% [2], an influenza pandemic by a highly transmissible strain of avian H5N1 influenza virus could potentially kill millions of people worldwide.

Vaccination with trivalent influenza vaccine is effective in reducing the impact of the annual spread of seasonal influenza, although its prophylactic effectiveness can be significantly impacted by strain matching with circulating strains, strains used for vaccine production, and by virus mutations. Given the ability of influenza virus to undergo constant antigenic change, there is a compelling requirement to develop alternative prophylactic countermeasures to protect against seasonal and pandemic influenza. Antiviral therapy is used clinically to reduce the duration and severity of influenza, and stockpiling of antiviral drugs is an important component of many influenza pandemic preparedness plans developed by many western nations [3]. Currently there is a limited arsenal of antiviral compounds which includes M2 ion channel inhibitor) (amantadine, rimantidine), neuraminidase inhibitors (perimivir, oseltamivir and zanamivir). However analysis of recent avian H5N1 isolates from infected patients had revealed that these virus isolates are completely resistant to rimantidine and amantidine and are increasingly resistant to oseltamivir (tamiflu) [4]. In view of the steady increases in influenza viruses developing drug-resistance, and the global threat of a looming influenza pandemic, the requirement to develop novel antiviral drugs that are less likely to give rise to drug resistance becomes more urgent.

Rapid advances in viral genomics and gene-based drug design demonstrate that antisense oligonucleotides, siRNAs, ribozymes and DNAzymes are versatile in their mechanisms of action, can inhibit viral replication at the molecular level in the early phase of infection, and can be custom designed to match antigenic shifts, mutations or recombination in the virus. The pharmaceutical industry, academic research and defense departments have been quick to recognize the potential value of nucleic acid based antiviral agents. An outline of the current development activities (Table 1) reveals that indeed several newly established companies are employing a wide range of nucleic acid technological strategies to combat the threat of influenza. Advances in this area may be attributed to a keen appreciation of both public health and bio-defense considerations driving increased funding and R&D efforts in this area. This has resulted in a corresponding accumulation of intellectual property relating to the designs and development of nucleic acid-based anti-influenza drugs. This review will survey the recent and significant patents on designs and applications of nucleic acid-based drugs, and will provide an overview on their prophylactic and/or therapeutic applications against influenza virus infections.

A survey of the most recent and significant patents on nucleic acid-based drugs, which is reflective of rapid advances made in this subject area, is shown in Table 1 [5-33]. This overview of recent patent activity reveals a diverse range of nucleic acid-based drug designs and strategies. These novel or improved drug designs can be broadly classified into 4 major classes: antisense oligonucleotides, immunomodulating nucleic acids (CpG oligonucleotides and ds RNA), catalytic nucleic acids (ribozymes and DNAzymes) and small interfering RNA (siRNA). Upon reviewing these patents and patent applications, it is particularly significant that most of these drug candidates have demonstrated anti-influenza activity primarily in established tissue culture cell lines and/or animal infection model systems. Thus review of these gene based strategies is limited by the relative paucity of strong clinical data. The diversity inherent in exploiting the influenza gene sequence yields both enormous flexibility to this area of antiviral treatment and in the same measure adds complexity to their pharmaceutical development particularly in terms of modes of delivery, toxicology and potential non-specific activity. Nevertheless the current patents reveal the

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Table 1. Current Anti-Influenza Pharmaceutical Development Activities

Company	Anti influenza Drug	Type	Phase	Representative Patent/Application
AVI Biopharma	NeuGene™ (AVI 60010)	Antisense (Morpholino)	Phase I	20070004661
Replicor Inc.	Rep9	PS - oligonucleotide	Preclinical	20050196382
Coley Pharmaceutical Group	CpG7909 (Promune™) CpG10101 (Actilon - withdrawn)	Oligonucleotide TLR-9 Agonists	PhaseI PhaseII (withdrawn)	20050256073
Hemispherex Biopharma	Ampligen™ Mismatched dsRNA Poly A:Poly U	poly(I):poly(C12U)	Preclincial Preclinical	20060035859
IRX Therapeutics	MIMP (5 methyl inosine monophosphate)	APC and T-cell stimulator	Preclinical	5614504 20050148538
Multicell Technologies	MCT-465	dsRNA	Preclinical	EP1485403 20050222060
Nastech	G00101	siRNA	Preclinical	20040242518
Sima Therapeutics		siRNA	Preclinical	20060293271 20060293272 20060217337
Alnylam	ALN-Flu01	siRNA	Preclinical	20060035254
Protiva	ProFlu™	siRNA	Preclinical	20050064595
BioDelivery Sci	Bioral™ siRNA	siRNA	Preclinical	20050013855

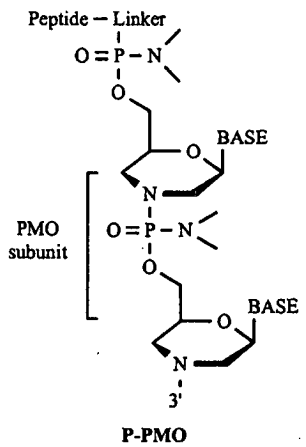
state-of-the-art in nucleic acid based approaches for anti-influenza drug development. Their modes of action, current status of development and potential applications of each of these approaches directed against influenza are outlined summarized here.

A) ANTISENSE OLIGONUCLEOTIDES

Antisense oligonucleotides have been developed as a means of gene blockade by specifically hybridizing to target mRNA sequences [34]. Antisense oligonucleotides can be designed to bind to coding region of virus mRNA thereby interfering viral protein synthesis, or bind to promoter region or initiation codon thus stopping the initiation of viral protein translation. In addition, the binding of antisense oligos to the mRNA form duplexes recognized by the cellular enzyme RNAse H, which in turn cleaves the viral mRNA. These antisense effects result in the silencing of viral protein expression and inhibition of viral replication. The most successful antiviral application of this technology is the marketed phosphorothioate (PS) oligonucleotide Fomiversin which is an antisense directed against cytomegalovirus (CMV) infections in the human eye [35]. Several antisense oligonucleotides have been designed for antiviral applications against influenza virus infection [36]. These antisense oligonucleotides were directed against the translation initiation codons in the PB2 and PA genes that encode for the influenza virus RNA polymerase, and were found to be effective in the inhibition of PB2 and PA gene expressions in cultured cells. Treatment of influenza A virus-infected mice with PB2 antisense encapsulated in cationic liposomes significantly prolonged overall survival rates, reduced lung virus loads and pulmonary consolidations [36].

However, the PS oligonucleotides are attributed to non specific effects and are susceptible to nuclease degradation in the body [34].

A number of oligonucleotide chemical modifications have been introduced, most specifically here the phosphorodiamidate morpholino oligonucleotides (PMO) [37]. The uncharged backbone is attributed with better uptake into target host cells and is reported to lead to greatly reduced non-antisense effects (Fig. 1). Recently Ge *et al.* have demonstrated that an influenza specific morpholino oligonucleotide - conjugated to an alanine rich peptide to aid in cellular uptake - designed to base pair with FLUAV RNA sequences that are highly conserved across viral subtypes and



P-PMO

Fig. (1). Structural representation of the peptide conjugated phosphorodiamidate morpholino antisense structure. Adapted from ref. [38]

considered critical to the FLUAV biological-cycle [38]. Several PMO were highly efficacious, and two PMO targeted to the AUG translation start site region of PB1 to the 3'-terminal region of nucleoprotein viral genome proved to be potent against several other FLUAV strains [38], including A/WSN/33 (H1N1), A/Memphis/8/88 (H3N2), A/Eq/Miami/63 (H3N8), A/Eq/Prague/56 (H7N7), and the highly pathogenic A/Thailand/1(KAN-1)/04 (H5N1). The novel mopholino oligonucleotides have also recently been found to be similarly effective against several strains of the Dengue fever virus [39] and have recently been tested in clinical trials for anti-tumor applications [40]. The anti-influenza drug AVI60010 NeuGene antisense oligonucleotide is currently being developed by AVI Biopharma. AVI60010 antisense has been demonstrated *in vitro* to inhibit multiple types of influenza including the highly pathogenic H5N5 avian influenza virus [38]. Preclinical studies are ongoing and an investigational new drug (IND) submission is planned for this product.

Single stranded RNA has also been investigated for use as an anti-influenza agent. A recent patent described Wong *et al.* (US6544958) Table **2** described synthetic ribonucleotide oligonucleotides (RNO) which were designed to suppress the gene expression of heamagglutinin protein, a surface spike protein of influenza A responsible for virus attachment to target host cells. The single stranded RNO 15-mers were evaluated in murine influenza models and were demonstrated to be effective in both prophylaxis and treatment of the disease and could be effective delivered as naked RNO or encapsulated in liposomes [41]. The sequences that were active in protecting and treating mice against influenza virus were found to be either sense or antisense strands while control random sequence did lacked anti-influenza activity. Recent work suggested that ssRNA from viral sources may result in the production of high levels of type-1 interferon (IFN-α/β). ssRNA stimulated interferon production is mediated either in the cytoplasm or the endosome. In the cytoplasm, this effect is mediated through RIG-1 (RNA helicase enzymes retinoic acid–inducible gene 1), an element of the innate immune system which recognizes 5'-phosphorylated ssRNA [42]. Such an effect has also been seen with transfection of cells with siRNA [43]. In the endosome the TLR-7 receptor mediates the production of interferon upon interaction with ssRNA [44]. Further work will be required to determine whether antisense or interferon mediated effects are responsible for the anti-influenza effects observed with these RNOs.

In addition to antisense oligonucleotides, anti-influenza oligonucleotide drug products that do not rely on sequence complementarity are also being developed [45, 46]. Replicor is developing randomer PS development of Rep9, an anti-influenza randomer PS-oligonucleotide which has been reported to be effective against H5N1 influenza (A/Vietnam/120I3/04) and which prevents the spread of influenza when administered as aerosols to the lungs of mice. It has been demonstrated for some time that polyanionic compounds such as polysulfones, sulfated polysaccharides and phosphorothioate modified nucleotides inhibit the fusion of viruses to the host cell surface [46]. This process was further characterized by researchers at Replicor who demonstrated that long chain phosphorothioate oligonucleotide randomers act as anti-viral agents [47]. Using HIV-1 as a model virus they demonstrated that PS oligomers of optimal lengths (~40mer) blocked viral fusion by a mechanism involving blocking gp41 six helix bundle formation. As gp41 represents the type I fusion protein the data suggests that the other viruses which employ type I proteins including, influenza, ebola and coronavirus could be susceptible to this form of PS-randomer antiviral activity.

B) CATALYTIC DNA AND RNA (DNAZYMES AND RIBOZYMES)

Catalytic DNA and RNA, (DNAzymes and ribozymes respectively), like morpholino oligonucleotides, have RNAase H independent mechanisms of action [48]. The thermodynamic energy of hybridization of these oligonucleotides drives a catalytic core to cleave the RNA of the target site resulting in gene blockade. Ribozymes against DNAzymes are entirely synthetic and require modified nucleotides (3' inversion, PS, etc) for stability against nucleases in the binding sequences flanking the phosphodiester "catalytic core" nucleotides (Fig. **2**). Ribozymes may be transcribed *in situ* from a plasmid or retrovirus, such as those targeted against HIV or are also designed and made synthetically [49]. Recently, DNAzymes have been described that are effective against influenza A viruses. DNAzyme drug candidates capable of cleaving the sequence at the AUC initiation codon of the PB2 gene in influenza A were designed and characterized [50, 51]. Cell culture studies revealed that these DNAzymes were effective in reducing levels of influenza levels by 99% in MDCK cells [50,51]. Using a different strategy, Lazareve *et al.* [52] constructed cell lines that endogenously expressed ribozymes targeted against a region of the influenza PB1 gene conserved across several strains. These cell lines were demonstrated to have substantial resistance to influenza infection reducing the virus levels up to 94% versus. While both ribozymes and DNAzymes have shown promise in as therapeutic agents in experimental models, a great deal of preclinical work will be required to show promise to support further clinical study DNAzyme [53].

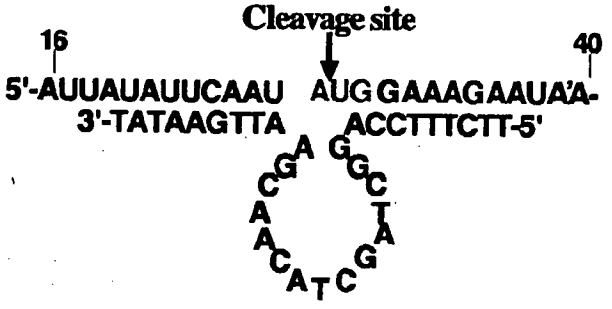


Fig. (2). Schematic representation of the structure of a DNAzyme binding to target RNA. Adapted from ref. [51]

C) IMMUNOMODULATORY NUCLEIC ACIDS

CpG Oligonucleotides

Amongst the non-hybridization dependant effect of PS oligonucleotides is the CpG activation of B cells via the Toll-like receptor 9 (TLR-9). The extent of activation is dependant up on the sequence context around the CpG motifs of non-methylated DNA [54]. Much effort has been expended designing CpG containing oligonucleotides as immunomodulators and adjuvants. Immunomodulating oligonucleotides form a second class of antiviral compounds. Coley Pharmaceutical group has developed a range of oligonucleotide TLR-9 receptor agonists such as CpG 7909 (Promune™) and CpG10101 (Actilon). CpG 7909 was demonstrated in early (Phase Ib) trials to enhance the efficacy of fluvarix influenza vaccine and has shown promise in as a late phase (II and III) anticancer adjuvant [55]. However, early pre-clinical and clinical success does not guarantee a clinical success. Such is the case for Actilon (CpG10101) where shortly after fastracking by the FDA, clinical trials were cancelled due to insufficient evidence of efficacy due to poor Phase II results against HCV.

CpG oligonucleotides have also been demonstrated to induce immunological responses to protect experimental animals against multiple lethal dose challenge with influenza A virus [56]. Wong *et al* demonstrated that liposome encapsulation could be used for the intranasal delivery of CpG oligos in a mouse influenza model.

Table 2. Recent Anti-Influenza Patents and Patent Applications

Patent	Author	Title	Assignee	Date
Antisense Oligonucleotides				
5,194,428 [5]	Agrawal *et al.*	Inhibition of influenza virus replication by oligonucleotide phosphorothioates	Mt Sinai School of Medicine	Mar 16, 1993
5,580,767 [6]	Cowert *et al.*	Inhibition of influenza viruses by antisense oligonucleotides	Isis Pharmaceuticals	Dec 3, 1996
5,637,573 [7]	Agrawal *et al.*	Influenza virus replication inhibiting analogues and their pharmaceutical compositions	Authors	Jun 10, 1997
6,326,487 [8]	Peyman *et al.*	3 Modified oligonucleotide derivatives	Aventis Pharma Deutchland	Dec 4, 2001
6,495,675 [9]	Takaku *et al.*	Pharmaceutical composition for treating for preventing influenza, and novel capped oligonucleotide	Chiba Institute of Technology, China	Dec 17, 2002
6,683,167 [10]	Metelev *et al.*	Hybrid oligonucleotide phosphorothioates	University of Massachusetts	Jan 27, 2004
7,045,609 [11]	Metelev *et al.*	Hybrid oligonucleotide phosphorothioates	University of Massachusetts	May 16, 2006
20070004661 [12]	Stein *et al.*	Antisense antiviral compound and method for treating influenza viral infection	AVI Biopharma	Jan 4, 2007
6,544,958 [13]	Wong *et al.*	Therapy of respiratory influenza virus infection using free and liposome-encapsulated ribonucleotides	Defence R&D Canada – Suffield	April 8, 2003
Ribozymes				
6,258,585 [14]	Draper	Method and reagent for inhibiting influenza virus replication	Ribozyme Pharmaceuticals (now Sirna)	Jul 10, 2001
Immunomodulatory/ Non Complementary Nucleic Acids				
5614504 [15]	Hadden *et al*	Method of making inosine monophosphate derivatives and immunopotenitating uses thereof	The University of South Florida	Apr 21, 1995
EP1650218 [16]	Yu	The artificial CPG single strand deoxidation oligonucleotide and its antiviral uses	Changchun Huapu Biotechnology Co.	Jul 26, 2007
20050148538 [17]	Hadden *et al.*	Adjuvant formulations for bacterial and virus vaccines and method for making same	IMP Therapeutics	Jul 7, 2005
20050196382 [18]	Vaillant *et al.*	Antiviral oligonucleotides targeting viral families	Replicor Inc.	Sept 8, 2005
20050222060 [19]	Bot *et al.*	Composition and methods to initiate or enhance antibody and major-histocompatability class I or class II-restricted T-cell responses by using immunomodulatory, non-coding RNA motifs	Astral Inc. (Multicell Technologies)	Oct 6, 2005
20050256073 [20]	Lipford *et al.*	Immunostimulatory viral RNA oligonucleotides	Coley Pharmaceuticals	Nov 17, 2005
dsRNA				
6,468,558 [21]	Wong *et al*	Liposome encapsulated poly ICLC	Defence R&D Canada	Oct 22, 2002
6,506,559 [22]	Fire *et al.*	Genetic inhibition by double-stranded RNA	Carnegie Institute of Washington	Jan 14, 2003
20060035859 [23]	Carter *et al.*	Treating severe and acute viral infections	Hemespherex Biopharma	Feb 16, 2006

(Table 2) Contd....

Patent	Author	Title	Assignee	Date
siRNA				
20040242518 [24]	Chen *et al.*	Influenza Therapeutic	M.I.T.	Dec 2, 2004
EP1647595 [25]	Berkhout *et al*	Nucleic acids against viruses in particular HIV	Universiteit van Amsterdam	Oct 15, 2004
20050013855 [26]	Gould-Fogerite *et al*	Cochleate compositions directed against expression of proteins	BioDelivery Sciences	Jan 20, 2005
20050058982 [27]	Han *et al.*	Modified small interfering RNA molecules and methods of use	Chiron Corporation	Mar 17, 2005
20050064595 [28]	MacLachlan *et al.*	Lipid encapsulated interfering RNA	Protiva Biotherapeutics Inc.	Mar 24, 2005
20060084620 [29]	McCray *et al.*	RNA interference in respiratory epithelial cells	University of Iowa	Apr 20, 2006
20060217337 [30]	McSwiggen *et al.*	RNA interference mediated inhibition of gene expression using chemically modified short interfering nucleic acid (SINA)	Sirna Pharmaceuticals	Sept 28, 2006
20060275265 [31]	Pal *et al.*	Potent inhibition of influenza virus by specifically designed short interfering RNA	Authors	Dec 7, 2006
20060293271 [32]	McSwiggen *et al.*	RNA interference mediated inhibition of gene expression using chemically modified short interfering nucleic acid (SINA)	Sirna Pharmaceuticals	Dec 28, 2006
20060293272 [33]	McSwiggen *et al.*	RNA interference mediated inhibition of gene expression using chemically modified short interfering nucleic acid (SINA)	Sirna Pharmaceuticals	Dec 28, 2006

These pre-clinical studies demonstrated that 5 μg of naked oligonucleotide given 5 days prior to infection offered protection to 50% of mice infected with 10 LD50 influenza A. Liposome encapsulation of the CpG oligos increased the survival rate to 80% [56].

Double Stranded RNA (dsRNA)

Double stranded (ds) RNA has long been known to be a strong mediator of a non-specific immune response acting as a TLR-3 agonist resulting in stimulation of interferon-α, -β and -γ production [57,58]. Several examples of dsRNA immunomodulation employed for the treatment and prophylaxis against influenza, including have been reported [59-61]. Poly ICLC is a synthetic double-stranded polyriboinosinic-polyribocytidylic acid (poly IC) stabilized with poly-L -lysine and carboxymethyl cellulose (LC). When poly ICLC was encapsulated and/or complexed to liposomes, the duration in window of protection against influenza infection, and the safety profile were enhanced in mice [60,61]. Preclinical studies in mice have demonstrated that protection against seasonal influenza virus could last up to 21 days [61, 62]. Recent studies have shown that liposomal poly ICLC was found to be effective in the protection of mice against lethal challenge of avian influenza A/H5N1/chicken/Henan strain [61].

A related nucleic acid-based immunostimulator, ampligen®, which is a mismatched ds RNA poly (I):poly ($C_{12}U$) developed by Hemispherx Biopharma Inc. Ampligen has been used in antiviral applications. As with poly IC, this dsRNA stimulates the 2′-5′ oligoadenylate synthetase/RNase L pathway for viral RNA destruction. Published work on its effectiveness has only been reported for coxsackie B3 virus [62], Nipah virus [63] and in clinical trials for HIV [64]. Methyl inosine monophosphate (MIMP) is another immunomodulating nucleic acid drug that has been demonstrated to have anti-influenza activity during development by IRX Therapeutics [65]. Recent studies, (IRX Therapeutics) have questioned the effectiveness of this compound [66]. In the earlier studies, MIMP was protective against aerosol delivered mouse adapted influenza A in a strain of outbred mice. However, when the MIMP was tested in a mouse adapted influenza A inoculated in an inbred mouse strain (BALB/c) by intranasal administration, the drug was shown to be ineffective. The authors correctly concluded that care must be taken to consider the age, strain and routes of administration when extrapolating data from preclinical *in vivo* models of viral disease.

D) siRNA

Small interfering RNA (siRNA) is one of the most active areas in nucleic acid research. The 2006 Nobel Prize winning research on genetic interference observed first in nematode *Caenorhabditis elegans* by Fire and Mello [67] led soon after to its characterization in mammalian cells [68]. Here double stranded RNA (dsRNA) directs sequence specific degradation of messenger RNA (mRNA). The process involves the cutting of small (~25 mer) dsRNA from large dsRNA (Fig. 3). This is part of the naturally occurring process used by these eukaryotic cells as defence mechanisms against viruses and transposons. The discovery that these siRNA could mediate sequence-specific gene silencing effect had generated much excitement in the biotechnology sector in that these siRNA molecules can either be synthetically produced in large scale, or can be expressed by ribozyme or lentiviral vector expression systems [69].

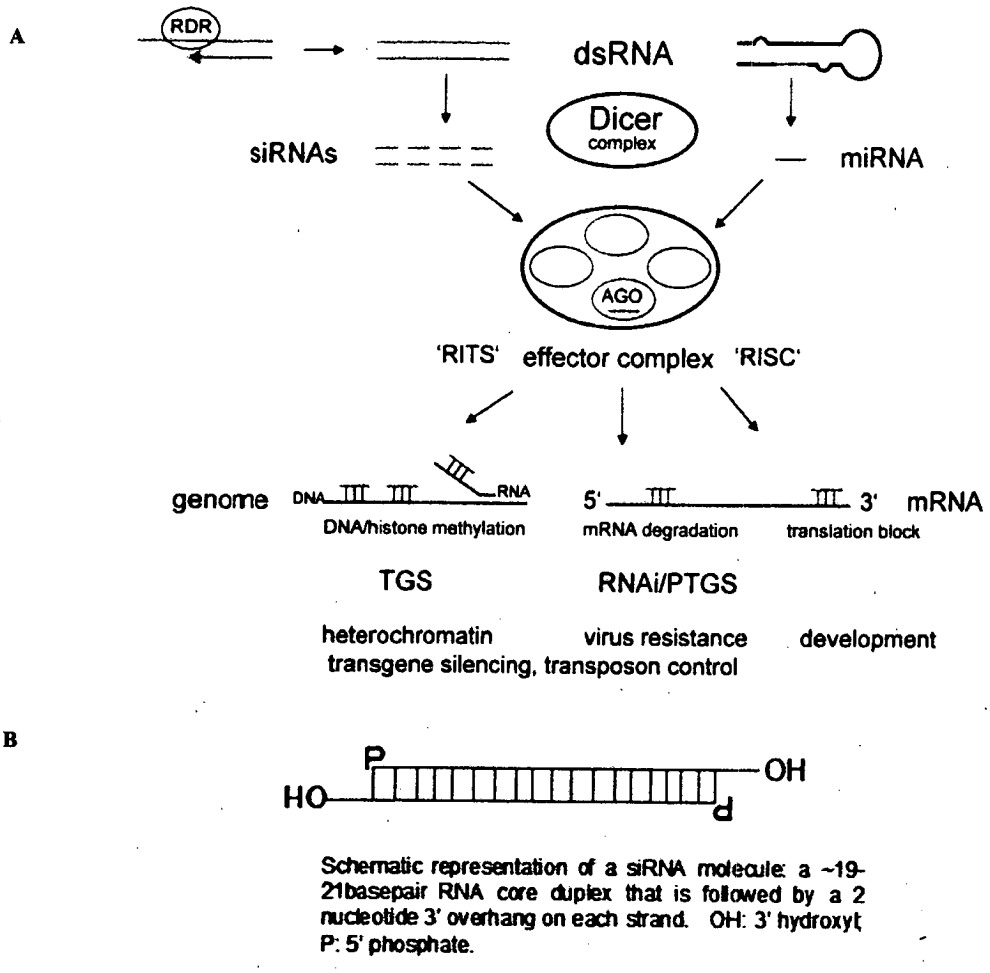


Fig. (3). Overview of intracellular siRNA production. **A.** Representation of the cellular gene silencing process. Naturally occurring RNAi is initiated by the dsRNA-specific endonuclease, called Dicer, which processively cleaves long dsRNA into double-stranded fragments between 21 and 25 nucleotides long, termed short interfering RNA (siRNA) [68]. siRNAs are then incorporated into a protein complex (RNA Induced Silencing Complex or RISC) that recognizes and cleaves target mRNAs resulting in Translational Gene Silencing (TGS) on the genome or Post Translational Gene Silencing (PTGS) at the mRNA level. **B.** Schematic structure of siRNA. Adapted from ref. [40]

siRNA are well suited to be used as antiviral agents. Since its discovery, siRNA technology platform has been successfully used to treat and/or prevent viral diseases including hepatitis C [70], HIV [71] and influenza [72]. siRNA have also been designed to target the nucleocapsid protein (NP) as well as polymerase (PA) RNAs of influenza A virus [73,74]. These siRNA were found to suppress viral mRNA, virion and complementary RNA levels in cell culture and chicken embryos [75]. In a mouse study, treatment of influenza-infected mice with siRNAs specific for NP and PA protected mice against lethal virus challenge and caused significant reduction of virus titers in the lungs. The protection was specific and was not mediated by an interferon response. Furthermore, this specific siRNA treatment was later found to be effective against the highly pathogenic avian influenza viruses of both H5 and H7 subtypes [76]. In another study in mice, siRNAs specific against conserved regions of NP and PA, found to be very effective to prevent or treat influenza virus infection. In this study, the antiviral activity of siRNAs were similar whether given intravenously when complexed with polycation carrier, or transcribed from a DNA expression vector [77].

Recently, Nastech has obtained G00101 antiviral RNAi from Galenea Corp and is taking the anti-influenza into preclinical studies. No less than 3 companies Protiva, Sirna Therapeutics (Formerly Ribozyme Therapeutics) and BioDelivery Sci have initiated preclinical studies with siRNA directed against influenza. Each of these companies is introducing not only siRNA as a drug but development is also driven by the introduction of enabling delivery systems and nucleotide chemistries to potentiate these novel therapeutics.

Recent Advances in Delivery of Antiviral Nucleic Acids

Nucleic acid-based antiviral agents are extremely versatile in their antiviral mechanisms of action. Whether these drugs exert their anti-influenza activity through induction of broad-spectrum antiviral immune responses (ds RNA, CpG oligos), or inhibition of gene expression and viral replication at the molecular level (antisense, siRNA), the delivery of these antiviral agents to the sites of virus infection is one of the greatest challenges. Drug delivery systems are of paramount importance in their therapeutic applications of nucleic acid-based drugs as these delivery vehicles enhance the transport of these highly charged macromolecules across cell membranes, as well as protecting them against nuclease degradation in the body for both local (regional) and systemic applications. Such systems can include cationic polymers or lipids, particles, liposomes, viral vectors, peptides and chemical modifications. Drug delivery systems such as liposomes and

nanoparticles are effective in targeting nucleic acid-based drugs to the site of viral replication, thereby avoiding potential toxicity to non-infected organs. The use of liposomes to deliver dsRNA poly ICLC has been shown to enhance antiviral efficacies against influenza virus infection, as well as reducing adverse drug effects in the body [60, 61]. Viral vectors such as adenovirus and baculovirus are also very effective and commonly used in the delivery of nucleic acid-based drugs, and they permit expression of these nucleic acid-based drugs at the transfection site. For a comprehensive reviews of the recent developments in the area of delivery of nucleic acid-based antiviral agents, particularly for anti-influenza applications, readers are encouraged to refer to these reference review articles [78,79].

CURRENT & FUTURE DEVELOPMENTS

In light of growing drug-resistance of seasonal and avian influenza viruses to antiviral drugs, and the increasing global threat of a potential avian H5N1 influenza pandemic, the need to fast track development of new antiviral drugs to combat influenza has never been more urgent. Nucleic acid-based antiviral agents may have a significant role to play as novel weapons to the existing arsenal of existing antiviral drugs against seasonal or avian influenza viruses. This survey of the recent patents on nucleic acid-based antiviral agents reveals that these drugs are versatile in their mode of action in that they can be designed to elicit protective and broad-spectrum antiviral immune responses, interfere with viral replication, suppress gene expression of key viral proteins, or cleave viral mRNAs. The potency and versatility of these drugs make them potential candidates for used in seasonal or pandemic influenza situations. The fundamental issue with this class of drug is whether the promising efficacy seen in preclinical studies in animals can be fully translated in human patients.

Comparing the potential of these nucleic acid approaches would depend upon the strategy for use. Drugs based on immunomodulation such as poly ICLC or CpG could be ideal where the strategy requires non-specificity, for example, in cases where the virus mutates or swap genetic materials with virus from another host species. Such a strategy could be important in forming a rapid first line of defense. Alternatively the use of modified antisense or siRNA may be preferred if specificity and potentially relatively fewer side-effects is desired. Overall, predicting success is difficult to predict because so few nucleic acid based drugs have made the transition from the bench to the clinic and ultimately the market. Indeed as with developing any novel product, issues such as manufacturability, toxicity, scalability and stability are all important for bringing a new therapeutic entity to clinical and commercial use. Furthermore it will be quite likely that both the nucleic acid drug and the accompanying (and previously untried) delivery system will have to be clinically evaluated. For example despite its potential potency and specificity against influenza gene targets, siRNA may be particularly unstable and would require a delivery system to be effective *in vivo*. Such a delivery system may raise additional stability, toxicity and cost issues of its own. Thus would such a drug be cost-effective enough to be made widely available for prevention and treatment of pandemic influenza? Such an analysis may prove telling for each class of nucleic acid based drug.

Ultimately however, clinical efficacy and safety will be the most important factors in judging the success of any nucleic acid based drug as an anti-influenza agent. Thus it is important to guard against unrealistic expectations from nucleic acid-based drugs. As with conventional pharmaceutical therapeutics many of these drugs have failed at various stages of clinical trials. As these drugs are entering the various phases of clinical studies against various cancer and infectious diseases, preliminary results from a small number of studies appear to indicate that these drugs are relatively safe and well tolerated in patients [70]. Nevertheless, the therapeutic usefulness of the nucleic acid-based antiviral agents against influenza infection will need to be determined in phase II and III studies in humans. It is to be hoped that amongst the many approaches and targets available, clinical success will further guide ongoing development and innovation.

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