Targeting pandemic influenza: a primer on influenza antivirals and drug resistance

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The emergence of the 2009 H1N1 pandemic influenza A virus, as well as constant antigenic drift of seasonal influenza, underscores the remarkable versatility of this virus in adapting to the human population. While vaccines are the principal public health defence against influenza, rapid vaccine development can be a daunting task. Antiviral drugs offer the promise of inhibiting influenza regardless of its genetic variations. However, the rapid rise of resistance to several antivirals has highlighted the need for developing novel therapeutics with reduced drug resistance potential. In this review, we will summarize the effects of the currently licensed anti-influenza drugs as well as the candidates in development against the seasonal and the 2009 H1N1 pandemic influenza A virus with an emphasis on drug resistance.

Keywords: flu, DAS181, H274Y, oseltamivir, zanamivir, peramivir

Background

The 1918–19 influenza pandemic had an estimated mortality of 40–100 million deaths worldwide and is considered the worst public health catastrophe of the 20th century.1 Ninety-one years later in April 2009, the CDC examined two cases of febrile respiratory illness in Southern California that were caused by a novel swine influenza strain (H1N1).2 The number of confirmed cases of this new influenza strain increased over time to >30000 in over 74 countries by June 2009 and as a result the WHO changed its pandemic alert from phase 5 to full pandemic phase 6. As of December 2009, a total of 289 paediatric deaths from influenza have been reported to the CDC.4 To date, children and young adults appear to be most affected. The average age of those who have been hospitalized or died from pandemic H1N1 thus far ranges from 20 to 37 years and patients with asthma have been found to be the most common among hospitalized patients in the USA.5 In addition, by February 2010, 248 oseltamivir-resistant H1N1 viruses have been detected worldwide, characterized and reported to the WHO.5

The 2009 pandemic H1N1 virus appears to have arisen from genetic re-assortments between influenza viruses from humans, birds and pigs.7 The human H1N1 influenza virus that circulated from 1918 to 1957 probably came from an avian origin and then disappeared, with its reappearance in 1977.8 Sporadic cross-species infections with swine influenza to humans have been noted since 1958. Most notably, an outbreak of swine influenza occurred among military recruits in 1976 in Fort Dix, New Jersey.9 Cross-species infection of human influenza H1N1 in pigs was confirmed in 1979.10 In 1981 the first influenza of avian origin was described in European swine. The first triple re-assortment swine influenza virus identified in recent years in North America not only had components of the swine influenza virus, but also had components of both avian and human influenza.11 Similarly, the 2009 pandemic H1N1 virus has components of Eurasian and North American swine, North American avian and North American human influenza viruses.12,13

IFVs belong to the Orthomyxoviridae family of RNA viruses. Both type A and type B viruses have eight segmented negative-strand RNA genomes enclosed in a lipid envelope derived from the host cell. The viral envelope is covered with spikes that are composed of three proteins: haemagglutinin (HA) that attaches virus to host cell receptors and mediates fusion of viral and cellular membranes; neuraminidase (NA) that facilitates release of new viruses from the host cell; and a small number of M2 proteins that serve as ion channels. The antiviral drugs approved today target two of the viral envelope proteins: M2 and NA. These drugs include the M2 inhibitors, the adamantanes (amantadine and rimantadine), and the NA inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza). Several drug candidates against novel molecular targets involved in different steps in the influenza virus life cycle are currently in clinical trials. Various antiviral approaches against influenza virus are delineated in Figure 1. In this review, we will summarize the effects of the currently licensed anti-influenza drugs as well as the candidates in development against the seasonal and the 2009 H1N1 pandemic influenza virus with an emphasis on drug resistance. Our search strategy included the National Library of Medicine (PubMed) and manufacturers’ trial databases available on the internet.
Figure 1. (a) Current pathogen-targeted antiviral approaches to influenza. NAIs block release of the virion, which halts viral replication, adamantanes block the release of viral RNA into the cytoplasm through the M2 channel and RNA polymerase inhibitors directly block transcription in the nucleus. (b) Host-targeted antiviral approaches to influenza. Sialidases cleave/remove the sialic acids on the respiratory epithelium, the receptors for both influenza and parainfluenza viruses. Hyperimmune serum contains pre-existing antibodies to HA that may block binding of virus to host cells.
Licensed antivirals for influenza: pathogen-targeted approaches

**Adamantanes (M2 ion channel blockers)**

The first class of antivirals developed for the treatment of influenza was the adamantanes, which include both amantadine and rimantadine.\(^\text{14}\) Amantadine was first approved for treatment and prophylaxis in 1967, while rimantadine was approved in 1993 by the US FDA. These drugs are M2 ion channel blockers and are thought to interfere with viral uncoating inside the cell. Adamantanes are effective against influenza A virus, but not influenza B virus. A meta-analysis review of published clinical studies indicates that amantadine prophylaxis resulted in a relative reduction of 61% of influenza A; when used as a treatment it shortened the duration of fever by 1 day.\(^\text{15}\) Rimantadine appears to be equally effective for prophylaxis, preventing ~71% of influenza A cases, and decreasing the duration of fever by ~1 day when used as a treatment.\(^\text{15}\) Amantadine, and to a lesser extent rimantadine, may be associated with neuropsychiatric side effects such as insomnia, confusion, hallucinations, ataxia, difficulty in concentration, depression, dizziness and tremor; indeed, they are also used in the treatment of Parkinson’s Disease. These drugs may also cause gastrointestinal side effects including anorexia, nausea and vomiting.\(^\text{15}\)

Adamantanes are limited in their clinical use for influenza virus because of widespread drug resistance. Amantidine-resistant influenza A virus was first detected in 1981. In the USA, the incidence of amantadine resistance rose from <20% in 2003 to 92% in 2005.\(^\text{16}\) Although this dramatic increase in resistance may have been due to increased usage of these drugs, some increases have been noted in countries where amantadine use was low, such as Australia.\(^\text{16}\) The genetic basis of adamantane resistance is M2 gene mutations.\(^\text{16}\) Up to 80% of resistant virus have a Ser31→Asn mutation, ~10% have mutations at position 27 or 30 and up to 2% have mutation at position 26. Resistance to adamantanes develops very rapidly in the influenza A virus background. In cell culture, susceptible viruses became resistant after only three or five passages in the presence of 2 μg/mL amantadine. Naturally occurring influenza A virus can be viewed as mixtures of susceptible and resistant strains and the latter can be selected within 2–3 days of starting amantadine therapy.\(^\text{16}\) About one-third of patients may shed resistant virus upon treatment with an adamantane.\(^\text{16}\) Resistant viruses appear to have similar virulence to the wild-type virus.\(^\text{16}\)

With regard to 2009 pandemic H1N1, both of the approved adamantanes have lost their inhibitory activity towards the M2 channel due to the mutation of Ser31, as discussed above.\(^\text{11,13,17}\) The M gene within this new pandemic influenza virus is reportedly similar to the M gene in the Eurasian swine virus, which confers resistance to both amantadine and rimantadine.

**NA inhibitors (NAIs)**

At present, the predominant type of antivirals used for the treatment and prophylaxis of influenza are NAIs. NAIs interfere with the release of new influenza virus particles from infected cells, preventing the infection of new cells. In general, this class of antivirals is associated with less toxicity than the adamantanes. Unlike the adamantanes, the NAIs are effective against both influenza A and B.

The inhalational form of zanamivir (Relenza®; GlaxoSmithKline) was the first in the class of NAIs approved by the US FDA for the prophylaxis and treatment of influenza in July 1999.\(^\text{18,19}\) The second drug in this same class, oral oseltamivir (Tamiflu®; Roche) was approved in October 1999.

According to a meta-analysis of eight randomized controlled trials of zanamivir and nine trials of oseltamivir, both drugs are effective in reducing the median time to alleviation of influenza symptoms.\(^\text{20}\) For zanamivir, these studies, in an intention to treat population, suggested a median time to alleviation of symptoms that ranged from 0.78 days in healthy adults to 1 day in children. For oseltamivir, the meta-analysis of the intention to treat population suggested a median reduction in time to alleviation of symptoms of 0.86 for healthy adults to 0.87 days for children. In addition, in a Cochrane review in 2005, a decreased risk of complications from influenza was noted in trials of NAIs.\(^\text{21}\) This effect on the alleviation of symptoms in otherwise healthy adults was confirmed in a recent update to an earlier Cochrane review of the efficacy of both licensed NAIs. However, in this recent review, the authors judged existing data as being either inconclusive or insufficiently compelling to support prior claims that oseltamivir has a demonstrably beneficial effect upon preventing the secondary complications of influenza infection in previously healthy adults.\(^\text{22}\)

In summary, the NAIs appear to have modest effects against the symptoms of influenza in otherwise healthy adults. However, data are still lacking on the effects in high-risk populations defined as those <65 years of age, those with chronic medical conditions (including heart and respiratory conditions) or those who are immunocompromised. In addition, the benefit from NAIs has been demonstrated primarily in studies where treatment was initiated within 48 h in otherwise previously healthy adults. Hence, evidence that benefit may still be realized in immunocompromised individuals when treatment is initiated longer than 48 h after onset of symptoms will require additional studies.\(^\text{23}\)

For prevention, three trials of zanamivir and four trials of oseltamivir were examined in one meta-analysis.\(^\text{20}\) For seasonal prophylaxis, a 69% relative reduction in the incidence of influenza was observed in the zanamivir-treated group compared with controls. In post-exposure prophylaxis studies, an 81% relative reduction in infection was observed for zanamivir-treated patients. For oseltamivir, a 74% relative reduction in the incidence of influenza infection was observed in seasonal influenza studies and a 90% relative reduction was observed in post-exposure prophylaxis studies. An investigational intravenous form of zanamivir has also been used in a preventative virus challenge model and was shown to decrease shedding and infection.\(^\text{24}\)

In general, the NAIs are well tolerated. However, post-licensing reports have indicated that zanamivir may cause cough, bronchospasm or even death in patients with pre-existing pulmonary disease and therefore this antiviral is contraindicated in patients with serious underlying respiratory diseases.\(^\text{25}\) The most frequent side effects for oseltamivir have been transient nausea, vomiting and abdominal pain occurring in up to 10% of patients.\(^\text{26}\) There have also been some post-marketing reports of cases of delirium
and abnormal behaviour leading to injury and in some cases deaths after NAI use.26

The NAIs were designed to resemble sialic acid and thus it was initially believed that any mutation within NA that reduced affinity to the NAIs would also result in decreased fitness of the virus. Thus resistance to these drugs was initially thought to have little clinical consequence. Initial studies of drug resistance to oseltamivir indicated that viruses prior to 2006–7 that contained the H274Y mutation in the NA gene (histidine to tyrosine at codon 274 in N2 nomenclature or H275Y in N1 nomenclature) were less virulent.27 However, in 2008, the CDC reported that ∼99% of seasonal H1N1 isolates were oseltamivir resistant.28 In addition, more recent clinical studies suggest that virulence and replication capacity of these resistant viruses is maintained. Most interestingly, around the world, some of the highest rates of oseltamivir resistance have been found in countries where the drug has been used infrequently. Thus transmission of oseltamivir resistance is now considered to have occurred in the absence of direct selective drug pressure.29 It is unclear, however, why zanamivir resistance appears to remain less common if higher exposure to oseltamivir does not necessarily account for all cases of drug resistance to the latter agent.

In 2009, the CDC reported that almost 100% of seasonal H1N1 influenza was resistant to oseltamivir.30 In general, zanamivir retains full inhibitory activity against several NA subtypes in the presence of mutations responsible for oseltamivir resistance. However, while resistance to zanamivir may be uncommon, it has been noted after treatment of immunocompromised individuals.31,32 More recently, the WHO has documented a unique zanamivir resistance mutation in the NA gene occurring in ~2.3% of H1N1 isolates with equivalent fitness.33

The initial reports of the first 37 isolates of the 2009 H1N1 pandemic suggest that all strains are susceptible to both oseltamivir and zanamivir.13 In early 2009 the US FDA issued an Emergency Use Authorization (EUA) to allow for the use of oseltamivir in paediatric patients <1 year old. In addition, an EUA was also issued for both oseltamivir and zanamivir to allow use in patients symptomatic for >2 days or who have complicated illness requiring hospitalization.34

Although the majority of 2009 H1N1 pandemic isolates that have been tested remain susceptible to the NAIs, concern exists that with seasonal and pandemic influenza co-circulating, there is the potential for incorporation of the H274Y mutation into pandemic H1N1, resulting in oseltamivir-resistant pandemic virus (Figure 2). Indeed the CDC has reported two cases of oseltamivir resistance from isolates of two transplant patients and two campers so far.35,36 Additional clusters of H274Y-dependent resistance in pandemic H1N1 infection have also been documented in other immunocompromised patients in North Carolina and Wales UK by the WHO.37 In addition, a number of 2009 H1N1 pandemic cases have been reported worldwide that have been documented to be resistant to oseltamivir. For example, resistant 2009 H1N1 pandemic viruses have been reported in Denmark and Japan in patients previously treated with oseltamivir.38 Perhaps more concerning is a case reported from Hong Kong of a 16-year-old girl infected with H1N1 virus with the H274Y mutation without prior exposure to oseltamivir, suggesting secondary transmission of the resistant virus.39 As of November 2009, the CDC has observed 0.5% of 2009 H1N1 pandemic
viruses to be resistant to oseltamivir while individual clusters of cases continue to be reported. In contrast, the intravenous formulations of peramivir have shown significant decreases in the median time to alleviation of symptoms in 405 subjects with acute influenza also failed to show similar effects. The use of intravenous zanamivir to be useful in treating an immunosuppressed girl with H274Y resistant 2009 H1N1. If the use of intravenous zanamivir is successful, these studies may permit clinicians to switch to using parenteral forms of one or both agents in critically ill patients whose absorption of these drugs when administered by their conventional oral or inhalational routes may be problematic.

**Peramivir**

Peramivir (BioCryst) is an NAI under development for the treatment and prevention of influenza. Early studies against human challenge virus using oral peramivir showed some beneficial treatment effects on viral shedding for both influenza A and B viruses, but failed to demonstrate effects as a prophylaxis, due to low peramivir concentrations with the oral form. Further development of this antiviral drug focused on either an intramuscular formulation or an intravenous formulation. A 344 patient treatment study of the intramuscular formulation of peramivir in the treatment of acute influenza failed to show significant decreases in time to alleviation of symptoms compared with placebo. A second study of the same intramuscular formulation in 405 subjects with acute influenza also failed to show significant decreases in the median time to alleviation of symptoms. In contrast, the intravenous formulations of peramivir have shown activity for treatment of influenza in certain populations. For example, in a study of 137 hospitalized patients, intravenous peramivir showed activity defined as time to clinical stability, with activity comparable to that of oral oseltamivir. In a Phase III study of intravenous peramivir in 1099 subjects with acute influenza, non-inferiority to oseltamivir was demonstrated with regard to time to alleviation of symptoms.

In general, viruses that contain the H274Y mutation for oseltamivir are less susceptible to peramivir as well. For example, in a study of clinical isolates from South-east Asia and Africa, wherein 64% of H1N1 isolates had the H274Y mutation, this mutation resulted in an average reduction in susceptibility to oseltamivir and peramivir of 1466-fold and 527-fold, respectively.

In the initial 13 clinical isolates of 2009 H1N1 pandemic reported by the CDC, all cases were susceptible to NAIs including peramivir, while they were consistently resistant to the adamantanes. In summary, intravenous peramivir may have utility for hospitalized patients during current and future pandemics. However, its large-scale use may be limited as the intramuscular form has shown less activity.

On 23 October 2009 the FDA granted peramivir an EUA for intravenous administration for the treatment of certain adult and paediatric patients with 2009 H1N1 pandemic influenza. Recently peramivir has received marketing approval in Japan.

**T-705**

T-705 (favipiravir; Toyama Chemical) is an investigational antiviral drug that undergoes conversion into a nucleotide analogue that inhibits influenza virus RNA polymerase, similar to ribavirin. As this drug appears to be more selective than other RNA polymerase inhibitors, it may also have less toxicity. However, clinical safety data collection is not yet complete. T-705, either alone or in combination with licensed NAIs, is being investigated in Phase II clinical studies for the treatment of influenza.

There are few published data on resistance to this drug. In addition there are limited published data on the activity of this drug against 2009 H1N1 pandemic virus. Pre-clinical studies by Itoh et al. suggest that T-705 is active against pandemic H1N1 strains both in vitro and in vivo.

**CS-8958**

CS-8958 (laninamivir; Biota) is a long-acting NAI. It is structurally related to zanamivir, but has a longer half-life. A recent Phase III clinical trial of CS-8958 in 1000 patients demonstrated that a single dose of CS-8958 was comparable to a standard course of oseltamivir with respect to time to alleviation of symptoms. A study in children has reported similar results. Although there are limited published data on resistance to this drug, one would assume that it may have a similar profile to zanamivir. However, it is possible that the longer half-life may also allow for additional resistance as well, if mutations occur with prolongation of exposure. One pre-clinical study has suggested that CS-8958 has activity in vivo and in vitro against the 2009 H1N1 pandemic virus.

**Antivirals in development for influenza: host-targeted approaches**

**Hyperimmune plasma**

Beginning even as early as the severe 1918 Spanish flu pandemic, clinicians have been experimenting with the infusion of so-called hyperimmune plasma derived from convalescent patient volunteers recently recovered from influenza to patients with severe acute influenza infection, both human and avian in origin. Although successful outcomes have been reported in scattered cases, all such reports to date have been either anecdotal in nature or have occurred outside of the bounds of a rigorous clinical trial infrastructure. Furthermore, there have been recent reports of IgG2 subclass deficiency having been identified in some patients with pandemic H1N1 that may help explain the
severity of the disease in certain high-risk individuals including pregnant women. Such a deficiency, if indeed causal and/or associated with more severe disease outcomes, could possibly be corrected by infusion with hyperimmune plasma harvested from recovered cases of pandemic H1N1 infection or from vaccinated donors. Thus, there is a need to study scientifically the safety and possible efficacy of hyperimmune plasma (either convalescent or post-vaccination in origin) as a potential therapeutic adjunct to conventional antiviral treatment in the treatment of severe cases of pandemic H1N1 infection as well as conventional seasonal influenza. Such clinical trials are either planned or already underway in the case of severe hospitalized 2009 H1N1 pandemic cases.

**DAS181**

DAS181 (Fludase™, NexBio, Inc.) is a sialidase catalytic domain/amphiregulin (AR) glycosaminoglycan binding sequence fusion protein. It is a recombinant fusion protein composed of a sialidase and a cationic sequence tag on the C-terminus. The entire DAS181 gene encodes 415 amino acids, which is composed of the initiation methionine followed by 393 amino acids derived from the sequence of Actinomyces viscosus sialidase catalytic domain (AvCD) and 21 amino acids derived from human AR glycosaminoglycan (GAG)-binding sequence.

The mechanism of action for this drug is unique in that the AvCD sialidase domain in DAS181 selectively cleaves sialic acids from the host cells, thereby rendering them inaccessible to influenza viral particles, which require sialic acids as receptors. By binding to the negatively charged GAGs on the surface of airway epithelial cells, the cationic C-terminal AR tag anchors DAS181 on the respiratory epithelium, thereby improving treatment potency and retention of the investigational drug on the airway surface. Because it is host-directed towards the sialic acid receptors on airway epithelium, it can also prevent the binding of other respiratory viruses that also utilize these receptors (e.g. parainfluenza).

DAS181 has in vitro and in vivo pre-clinical activity against numerous seasonal influenza strains as well as highly pathogenic avian influenza strains (H5N1). It has recently been demonstrated to be effective against viral clinical isolates with the H274Y mutation. DAS181 has also shown activity in vitro, in vivo and ex vivo against the 2009 H1N1 pandemic strains. A critical question is the resistance potential for this host-targeting approach. To assess the drug resistance potential for this approach, several influenza virus strains were subjected to extensive growth in the presence of low levels of DAS181. DAS181-resistant mutants were identified in this study, but the level of resistance was relatively low. Therefore, DAS181-resistant mutants could still be inhibited with only slightly higher DAS181 concentrations in vitro and in mice. In the absence of drug, the DAS181-resistant mutants exhibited attenuated phenotypes in vitro and in vivo. Furthermore, DAS181 resistance was lost shortly after removing the drug, indicating instability of the DAS181 resistance phenotype. Genotyping and molecular analysis indicated that DAS181 resistance may be due to broadened HA-binding specificity and reduced NA function, leading to imbalanced HA and NA activities and consequent loss of fitness in the absence of the drug. (Dr. Jeffrey Larson, NexBio, Inc., personal communication.)

Another theoretical clinical concern is the potential that desialylation of the airway surface might increase the risk of pneumococcal infection by unmasking certain cryptic receptors for the bacteria. However, studies have demonstrated that DAS181 treatment in healthy animals does not increase bacterial colonization. Furthermore, recent studies have demonstrated that DAS181 treatment in animals infected with influenza and challenged with Streptococcus pneumoniae were protected from bacterial superinfection and lethal secondary pneumonia compared with untreated animals.

Phase I clinical studies of DAS181 have been completed and the drug was generally well tolerated with no severe adverse events noted. Phase II studies have begun, which will measure changes in viral shedding in subjects with influenza infection.

**Conclusions**

2009 H1N1 pandemic virus has resulted because of influenza’s ability to evolve over time, making effective vaccine and drug development difficult. Vaccines remain the primary public health response to a pandemic. Antiviral drugs also play a significant role against influenza, particularly for those who are considered at high risk or not effectively immunized. A recent US Presidential Council of Advisors on Science and Technology report recommended that there be an expedited licensure of intravenous formulations of antivirals, as well as the development of new influenza drugs that target the host, in order to reduce the potential for antiviral resistance. Antiviral medications are prioritized in the current 2009 H1N1 pandemic for persons who are hospitalized and those <5 years of age, those ≥65 years, pregnant women and persons with chronic medical conditions. Evolving drug resistance is an ongoing obstacle that should be recognized particularly for pathogen-targeted approaches.

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