Anti-influenza therapy: the emerging challenge of resistance

Influenza is a common cause of respiratory infections, with an annual peak incidence in the late fall, winter and early spring. It is associated with significant morbidity and mortality, particularly in those at the extremes of life and with underlying medical conditions, as well as self-limited illness with reduced productivity in otherwise healthy individuals. Two classes of antiviral medications, M2 inhibitors and neuraminidase inhibitors, are widely available. Use of these agents is associated with reduction in severity and duration of illness, and may be associated with reduced risk of death in certain patient populations. They also are effective in preventing influenza, in addition to or instead of immunization. Unfortunately, widespread resistance to one of the two classes of drugs is now common among most circulating strains of influenza.

KEYWORDS: adamantine ■ amantadine ■ influenza ■ M2 inhibitor ■ neuraminidase inhibitor ■ oseltamivir ■ peramivir ■ resistance ■ rimantadine ■ zanamivir

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Influenza viruses cause annual epidemics of illness characterized by the rapid onset of constitutional and respiratory tract symptoms [1]. There are three subtypes of influenza: A, B and C [1]. Influenza A viruses are further classified into subtypes on the basis of their surface proteins (16 hemagglutinins and nine neuraminidases are currently recognized) [1]. Annually, influenza affects approximately 5–10% of adults, with higher rates in children [2]. In the USA, influenza causes an average of 36,000 deaths and 130,000–170,000 hospitalizations during each epidemic [3,4]. Hospitalization and death appear to be more common in the very young and very old patients, those with abnormal immune systems, and those with significant underlying medical conditions, particularly cardiopulmonary disease [2]. In addition, there is a significant financial impact of influenza in the USA annually, with US$8–12 billion in direct medical costs and economic losses due to lost productivity [5].

The cornerstone of prevention of influenza is vaccination [2]. These vaccines are reformulated each year to attempt to match circulating strains, and availability varies from year to year [2]. Vaccination has been shown to reduce the risk of hospitalization, morbidity and mortality among high-risk patients and be cost-effective, in part, secondary to retained productivity among working adults [2,5]. Recommendations for their use are updated annually, and the most recent advice should be consulted [2]. Unfortunately, response to vaccine is limited in the elderly and those with underlying immune compromise, such as transplant recipients [2,6]. As we have learned in the development of vaccines for H5N1 [7], developing novel vaccines directed against pandemic strains pose formidable challenges, and such vaccines will likely be either completely or partially unavailable during the first wave of infection. As a result of limitations of the current vaccines, antiviral and nonpharmacologic measures, such as social distancing, will play an important role in retarding the spread of the infection [2,8] during a pandemic.

Once infection is established, antiviral medications have been shown to decrease the severity of influenza, shorten the duration of illness, and reduce infectious and noninfectious complications [9,10].

Available anti-influenza agents

■ Approved agents

Currently, there are four drugs in two antiviral classes available for the prevention and treatment of influenza: M2 inhibitors (amantadine and rimantadine) and neuraminidase inhibitors (NAIs; zanamivir and oseltamivir) (Figure 1) [9,10]. Recently, the increase in resistance globally to both classes of agents has significantly limited the effectiveness of our current antiviral armamentarium [11–14].

M2 inhibitors

The M2 ion channel allows the influx of protons into the viral particle which, in turn, facilitates uncoating [9]. M2 inhibitors bind to the M2 ion

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channel and limit the influx of protons, resulting in its antiviral effect. Since the M2 protein is present only on influenza A viruses, M2 inhibitors have no activity against influenza B [9].

There are two US FDA-approved M2 inhibitors: amantadine (Symmetrel®, Endo, PA, USA) and rimantadine (Flumadine®, Forest Laboratories, NY, USA) [15]. Although the mechanism of action and spectrum of activity are similar, there are important pharmacokinetic differences between the two drugs [16]. Amantadine, which has a half-life of 12–18 h, is not extensively metabolized and is excreted unchanged in the urine [9]. Therefore, amantadine accumulates rapidly in patients with reduced renal function, including the elderly [9]. The most common side effects of amantadine are minor, reversible CNS effects, such as insomnia, dizziness and difficulty in concentrating [9]. Side effects appear to be more common among the elderly; confusion is noted in approximately 18% of elderly recipients [17]. In addition, amantadine use has been associated with seizures in individuals with a prior seizure disorder [18]. Dose adjustment of amantadine is therefore indicated in those with renal dysfunction and in the elderly [9,16]. In contrast, rimantadine is extensively metabolized, with less than 15% of the drug excreted unchanged in the urine [9,16]. As a result, rimantadine is associated with considerably fewer CNS side effects and is better tolerated by the elderly. Rimatadine needs to be adjusted in the elderly and those with severe renal dysfunction (<10 ml/min) [9,16].

Neuraminidase inhibitors

The influenza neuraminidase has enzymatic activity that cleaves terminal sialic acid residues and destroys the receptors recognized by viral hemagglutinin [10,19]. This activity is essential for release of virus from infected cells, for prevention of viral aggregates and for viral spread within the respiratory tract [19]. The NAIs are sialic acid analogs that potently and specifically inhibit influenza A and B neuraminidases by competitively and reversibly interacting with the active enzyme site [10].

There are currently two NAIs approved by the FDA: zanamivir (Relenza™, GlaxoSmithKline, London, UK) and oseltamivir (Tamiflu®, Roche, Basel, Switzerland) [10]. Although each drug has unique pharmacologic properties, both have identical mechanisms of action and similar profiles of antiviral activity [10]. Zanamivir is not orally bioavailable and is therefore administered topically by dry-powder inhalation [10]. The proprietary inhaler device for delivering zanamivir is breath-actuated and requires a cooperative patient [20]. Zanamivir may cause bronchosconstriction with use; it should be used with caution in patients with underlying lung disease, and these patients should have ready access to a rapidly acting bronchodilator in the event of bronchospasm [10]. Few other side effects are noticed, since there is limited systemic exposure [10]. Oseltamivir carboxylate is an orally bioavailable ethyl ester prodrug of oseltamivir phosphate [10]. The prodrug is rapidly converted to active drug by intestinal and hepatic esterases. Oseltamivir is eliminated primarily by tubular secretion unchanged. As a result, oseltamivir requires dose adjustment in individuals with a creatinine clearance rate of less than 30 ml/min [10]. The most common side effect of oseltamivir is gastrointestinal upset, which is ameliorated with co-administration with food [10].

Investigational agents

The threat of an A/H5N1 pandemic and emerging resistance to both M2 inhibitors and NAIs has stimulated research into several investigational anti-influenza agents; these have been reviewed extensively elsewhere [21,22]. Many of the agents under current investigation are new NAIs [23,24]. Peramivir is also undergoing investigation as both an intravenous and an intramuscular formulation [23,24]. Two topical long-acting NAIs are also being developed; their major advantage is the less frequent dosing for prophylaxis. In addition, several compounds with novel mechanisms of action are being developed (Table 1) [21–24]. These newer agents typically remain active against viruses resistant to M2 inhibitors and NAIs, and have been recently reviewed elsewhere [21,22].

M2 inhibitor resistance

Resistance to the available M2 inhibitors has been recognized since early in their development. Mutations in the M2 inhibitor gene at one of five commonly recognized sites (position 26, 27, 30, 31 or 34 of the M2 protein) results in reduced binding of the M2 inhibitors or in enlargement of the pore diameter; by either mechanism, the function of the M2 pore is preserved in the presence of the inhibitor [25–27]. Resistance mutations do not affect transmissibility or replication fitness as compared with wild-type viruses; documented transmission from person to person has been well established [28]. Resistance affects both drugs in the class equally, and appears to be persistent over time [26,29].

Table 1. Investigational anti-influenza agents.

Class	Compound	Route
Neuraminidase inhibitors	Peramivir	i.v./i.m.
	Zanamivir*	i.v.
	Oseltamivir*	i.v.
	A-32278	Oral
	CS8959/R-118958	Topical
	Zanamivir dimers	Topical
Conjugated sialidase	DAS181	Topical
Hemagglutinin inhibitors	Cyanovirin-N	Topical
	Sialylglycopolymer	Topical
	Entry blocker	Topical
Polymerase inhibitors	Ribavirin	Topical, i.v., oral
	Viramidine	Oral
	T-705	Oral
siRNA		Topical, i.v.
Protease inhibitor	Aprotinin	Topical
Antibodies		i.v., i.m.
Interferons		i.m., s.c.
Interferon inducers	Poly (I) Poly (C)	Inhaled

**Zanamivir is approved for oral inhalation and oseltamivir is approved for oral therapy.*
i.m.: Intramuscular; i.v.: Intravenous; s.c.: Subcutaneous.
Data taken from [22–24].

During routine treatment with M2 inhibitors for documented influenza, resistant variants emerge frequently. Approximately 30% of adults treated with M2 inhibitors will have resistant variants detected during the course of their illness, with high frequency (up to 80%) of resistance emergence in immunocompromised patients, patients hospitalized for influenza and children [30–34]. Until recently, the frequency of M2 inhibitor resistance among seasonal isolates was low (1–3%) [14]. However, in recent years the prevalence of resistance to M2 inhibitors among circulating influenza A/H3N2 increased globally, and now the majority of influenza A/H3N2 globally is resistant to this class of drugs [14,35]. Resistance has resulted from the S31N substitution of the M2 inhibitor. M2 inhibitor resistance has also been documented in two important novel strains of influenza: A/H5N1 and swine-origin A/H1N1 [8,36]. Most clade 1 A/H5N1 viruses and all swine-origin A/H1N1 are resistant to the M2 inhibitors as a result of the S31N substitution, while most (~80%) of clade 2.1 A/H5N1 are resistant secondary to S31N or V27A substitution [8,36,37]. Of note, most of the clade 2.2 and 2.3 A/H5N1 viruses remain susceptible to M2 inhibitors [37].

There are currently no rapid tests that can screen for and identify the presence of M2 inhibitor resistance. M2 resistance may be diagnosed using plaque assay or gene sequencing. Although plaque assays are well described, they are not widely available [38]. Pyrosequencing methods for rapid analysis of mutations in the M2 gene associated with resistance have been described and are used in several reference laboratories [27,39]. Neither assay is typically available in most clinical laboratories. As a result, most clinicians rely on data generated from groups actively monitoring the resistance among circulating strains – in the USA this is actively carried out by the Centers for Disease Control and Prevention (CDC) [14].

Neuraminidase inhibitor resistance

Resistance to NAIs has been recognized since early in the development of these approved agents. Since the neuraminidase and hemagglutinin have a close functional relationship, viral resistance to NAIs can arise through mutations in either glycopeptides. Neuraminidase mutations affect the binding site and reduce affinity for the inhibitors, while mutations in the hemagglutinin reduce the affinity for its receptor [29,40,41]. Although new mutations conferring resistance are constantly being recognized, several key mutations have been well described (TABLE 2) [42,43]. Resistance to one NAI does not predict resistance to other NAIs. This is mostly related to differences in the chemical structures of the drugs. Oseltamivir, for example, has a bulky side chain, requiring the neuraminidase to undergo a significant conformational change in order to bind the active site. Any mutation

Table 2. Commonly recognized neuraminidase mutations associated with neuraminidase inhibitor resistance.

Location of residues	Mutation	Viral subtypes identified with mutation	Neuraminidase inhibitor susceptibility		
			Oseltamivir	*Zanamivir*	*Peramivir*
Neuraminidase – functional	R292K	H3N2, H5N1, B	R	RS	R
	D151E	H3N2	RS	R	–
	R152K	B	R*	R/RS*	R/RS
	R224K	H3N2	R	R	–
	E276D	H3N2	R	R	–
	R371K	H3N2	R	R	–
Neuraminidase – framework	H274Y	H1N1, H3N2, H5N1	R*	S	S/R
	N294S	H1N1, H5N1	R	RS	–
	E119V	H3N2, B	R	S	S
	E119D	H3N2	S	R	S
		B	R	R	R
	E119G‡	H3N2, H5N1, B	S/RS	R	S/R
	E119A	B	R	R	R

**Susceptibility maintained when mutation present in H3N2 virus.*
‡Not genetically stable in H3N2 and H5N1 viruses.
R: Resistant; RS: Reduced susceptibility; S: Susceptible.
Data taken from [42,64].

that inhibits this conformational change would be expected to reduce the binding affinity of oseltamivir, while not affecting other inhibitors that do not require such conformational changes (i.e., zanamivir) [42,44]. In addition, differences in the neuraminidase protein of type 1 (N1, N4, N5 and N8) and type 2 (N2, N3, N6, N7 and N9) influenza A viruses would also predict differential effects of mutations and may explain differences in NAI susceptibility that are seen between subtypes of influenza [43].

Resistance to NAIs appears to occur less frequently in immunocompetent patients [40]. In clinical studies, resistance has been detected in 0.4% of adults [45]. Higher levels of resistance have been described in children (2.9–27.3%), particularly in Japan [33,46–49]. During the 2007–2008 influenza season, influenza A/H1N1 that had a H274Y mutation conferring oseltamivir resistance was recognized with variable frequency throughout Europe among circulating strains [12]. Over the subsequent months, it became apparent that this resistant virus was detected globally, and during the 2008–2009 influenza season in the USA, nearly all circulating A/H1N1 viruses were resistant to oseltamivir secondary to the H274Y mutation [11,50]. Interestingly, previously recognized oseltamivir-resistant mutants containing the H274Y mutation were associated with reduced replication *in vitro* and reduced virulence in mice and ferrets [40,41,51,52]. Recent studies suggest that the presence of other mutations in the circulating oseltamivir-resistant A/H1N1 viruses may enhance their fitness through enhancing neuraminidase function, which in turn counteracts the attenuation of substrate affinity brought on by the H274Y mutation [53]. The net effect of all of the mutations present in this virus may confer a competitive advantage, explaining the persistence and global spread of this mutant. There is no clear association with prior exposure to or regional use of oseltamivir and the emergence of this resistant strain [11,12]. There are no significant differences between cases of oseltamivir-resistant and oseltamivir-susceptible influenza in terms of demographic characteristics, underlying medical illness or clinical symptoms [11].

Resistance to oseltamivir can occur during treatment of influenza and has best been described in immunocompromised patients and those with severe infections caused by A/H5N1 [29,54–56]. Although it is clinically challenging to detect in real-time, there appears to be evidence of progressive influenza disease with an associated high mortality rate, particularly for A/H5N1 infections, when this occurs [29,54].

Neuraminidase inhibitor resistance can be diagnosed by plaque assays, neuraminidase inhibition assays, and neuraminidase and hemagglutinin gene sequencing [40,57–59]. Unfortunately, standard cell lines, such as MDCK, do not provide reliable or consistent assessments of antiviral potency of NAIs [59–62]. This effect is the result of a mismatch between the receptor types in human airway epithelial cells and cell culture systems, and results in reduced requirement for neuraminidase activity [42,60,61]. Cell lines that

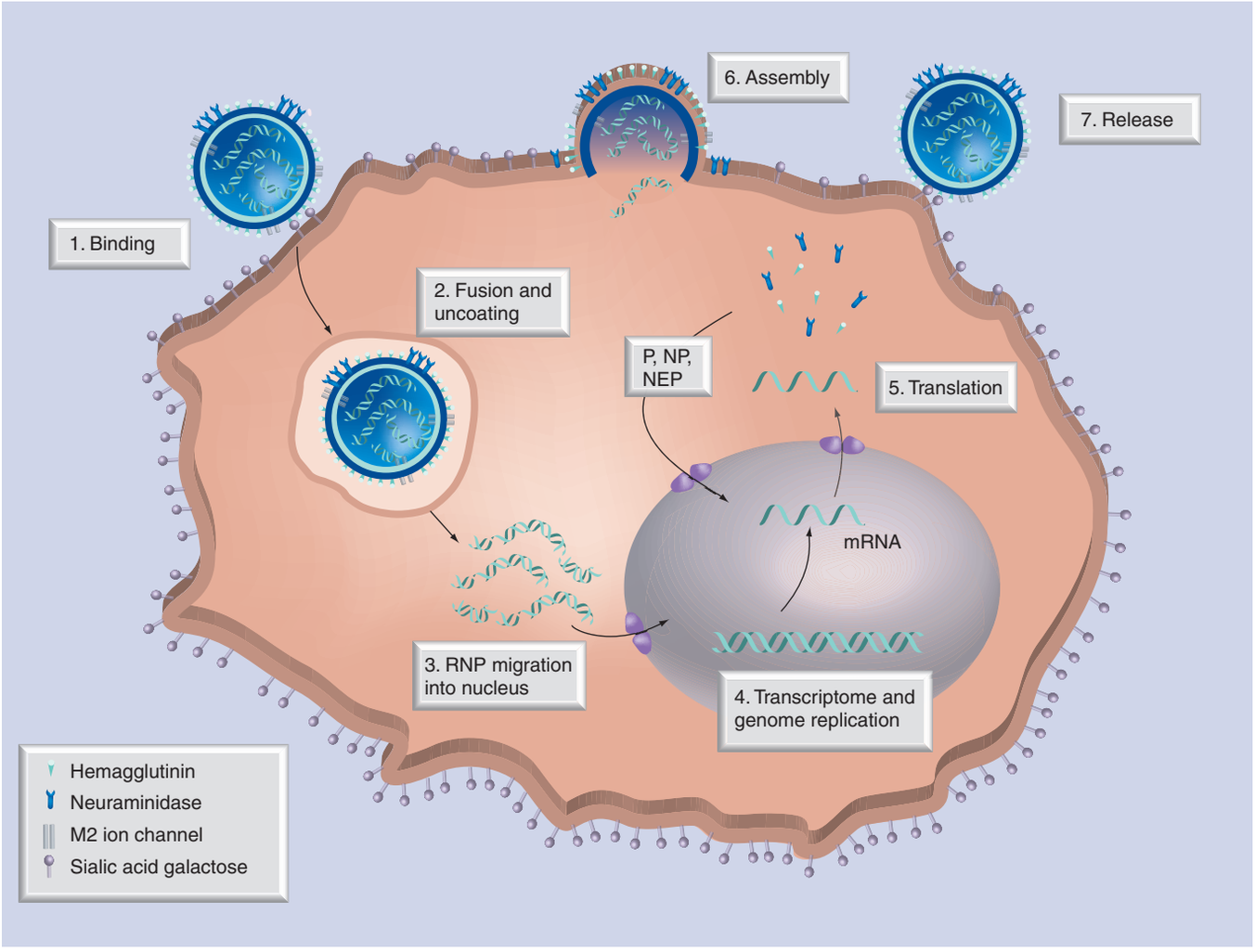

Figure 1. The influenza A virus replication cycle. The available antivirals inhibit either the M2 ion change (replication step 2) or the neuraminidase (replication step 7).
NEP: Nuclear export protein; NP: Nucleoprotein; P: Polymerase; RNP: Ribonucleoprotein.
Reproduced with permission from [21].

have been stably transfected with the human 2,6-sialyltransferase gene have been developed and provide more reliable results when using cell cultures [60,61]. These lines are not widely available and are not frequently used to screen clinical isolates. The neuraminidase inhibition assay has been one of the most widely used assays for detecting resistance [63]. Despite being easy to perform, interpreting results may sometimes be challenging, and it may not detect variants with hemagglutinin mutations only [40,42,57]. The assay has been modified recently for clinical studies, since viral neuraminidase levels in samples from the nose and throat may be too low to assay using this method. This modification involves growing the virus in cell culture initially, and therefore sensitivity may be compromised by both reduced replication potential of resistant viruses as compared with wild-type virus and reduced enzymatic activity in resistant variants [42]. Genotypic analysis through sequencing is now utilized by most groups as it can be applied to primary clinical samples and detects the presence of both neuraminidase and hemagglutinin mutations [38,40,42]. Many laboratories have begun screening specifically for the H274Y mutation to allow more rapid identification of oseltamivir resistance in strains predicted to contain this strain [64,65]. Unfortunately, few of these techniques are widely available in clinical laboratories. There are systems for quickly screening for resistance in development, but none are currently commercially available.

Clinical management of influenza in the era of antiviral resistance

The management of influenza is currently complicated by circulating strains of influenza with

Executive summary

Influenza

- Influenza causes 36,000 deaths and 130,000–170,000 hospitalizations annually in the USA.

Available anti-influenza agents

- Both M2 inhibitors (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) are commercially available for the prevention and treatment of influenza.
- A wide range of investigational anti-influenza compounds are under development; many are neuraminidase inhibitors with novel administration, pharmacokinetics or activity against resistant variants. A sialidase (DAS-181) and a protease inhibitor (T-705) both have novel mechanisms of action and are entering advanced stages of clinical development.

M2 inhibitor resistance

- Mutations in the gene result in resistance to all drugs in the class; the S31N mutation is responsible for resistance in naturally circulating A/H3N2 strains currently.
- M2 inhibitor resistance is diagnosed by plaque assay or gene sequencing; neither is widely available clinically.

Neuraminidase inhibitor resistance

- Mutations in either the neuraminidase or the hemagglutinin gene result in resistance to the neuraminidase inhibitors; frequently resistance is subtype- and drug-specific, with most oseltamivir-resistant variants remaining susceptible to zanamivir.
- The H274Y mutation is responsible for resistance in most circulating A/H1N1 viruses.
- Neuraminidase inhibitor resistance is diagnosed by plaque assay, neuraminidase inhibition assay or sequencing; none of the assays are widely available clinically.

Clinical management of influenza in the era of antiviral resistance

- Currently, clinical management of influenza depends on attempting to diagnose the subtype of virus (human A/H1, novel swine-origin A/H1, A/H3) and the recommendations of health authorities, such as the Centers for Disease Control and Prevention, who monitor the changing resistance pattern in circulating viruses.

Conclusion

- Advances in methods of diagnosing resistance in clinical isolates are required; these assays need to be easy to use so that they can be implemented at most hospitals or at the point of care.
- Studies of antivirals with novel mechanisms of action and combinations of antivirals should be carried out to assess the optimal management of influenza in the era of widespread antiviral resistance.

resistance to one of the two classes of available anti-influenza agents. Until reliable resistance testing is widely available in the clinical laboratory, management requires attempting to identify the virus, optimally to the subtype, and reliance on guidance from national reference laboratories, such as the CDC, who monitor for resistance and make treatment recommendations based on up-to-date data [2,101]. There are commercially available antigen detection kits that can differentiate between influenza A and B, in addition to commercially available and home-brew molecular methods that can provide further subtyping of the hemagglutinin of the virus present in clinical samples [2,66,67]. Identifying the virus to at least the subtype level may allow more tailored use of antivirals. Since the resistance in circulating strains is continually changing, consultation with current recommendations of health authorities is recommended [2,101,102]. This is particularly important with regard to the emerging pandemic associated with the swine-origin influenza A/H1N1. This reassorted virus has spread globally and is currently universally resistant to the M2 inhibitors [103]. A few isolates have been identified that have developed resistance to oseltamivir during the course of therapy [68,69]. As a result, management will be guided by emerging data on resistance and optimal treatment, which will be available through the CDC.

Future perspective

Influenza naturally changes over time, resulting in a high risk of emergence of antiviral resistance. Experience has proven that resistance will emerge for all anti-influenza agents and that this resistance may spread globally [11,12,39]. Likewise, resistance may emerge over a treatment course, particularly in patients with suppressed immune systems and those critically ill with novel viruses; when this does occur, morbidity and mortality may be high [29,54]. As a result, it is critical that advances are made to make antiviral resistance testing widely available in most clinical laboratories. Although dual-resistant variants have rarely been isolated, they have not yet circulated globally. If this does occur, there may be limited options for treatment unless new drugs with novel mechanisms of action are developed and approved for clinical use. Likewise, new paradigms in the management of influenza need to be considered

– particularly the use of antiviral combinations or selective rotation of antivirals [70]. The use of these combinations could improve the likelihood that therapy would be active against all circulating strains. In addition, the combination could potentially reduce the emergence of resistant mutants, as has already been demonstrated in one clinical study of combination therapy [71]. Further studies of combination therapy are clearly warranted.

Financial & competing interests disclosure

Dr Ison has been the site principal investigator of studies involving oseltamivir and peramivir. He has been a paid speaker of Abbott Molecular and ViraCor Laboratories. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Hayden FG, Palese P: Influenza Virus. In: *Clinical Virology – 2nd edition.* Richman DD, Whitley RJ, Hayden FG (Eds). ASM Press, Washington, DC, USA, 891–920 (2002).

2 Fiore AE, Shay DK, Broder K *et al.*: Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR Recomm. Rep.* 57(RR-7), 1–60 (2008).

3 Thompson WW, Shay DK, Weintraub E *et al.*: Influenza-associated hospitalizations in the United States. *JAMA* 292(11), 1333–1340 (2004).

▪ **Provides data on the rates of hospitalization related to influenza in the USA.**

4 Thompson WW, Shay DK, Weintraub E *et al.*: Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 289(2), 179–186 (2003).

5 Nichol KL, Lind A, Margolis KL *et al.*: The effectiveness of vaccination against influenza in healthy, working adults. *N. Engl. J. Med.* 333(14), 889–893 (1995).

6 Ison MG, Hayden FG: Viral infections in immunocompromised patients: what's new with respiratory viruses? *Curr. Opin. Infect. Dis.* 15(4), 355–367 (2002).

7 Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M: Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N. Engl. J. Med.* 354(13), 1343–1351 (2006).

8 The Writing Committee of the World Health Organization: Consultation on avian influenza A (H5N1) infection in humans. *N. Engl. J. Med.* 353(13), 1374–1385 (2005).

▪▪ **A key review of salient features related to H5N1 disease in humans, including epidemiology, risk factors, prevention and management.**

9 Hayden FG, Aoki FY: Amantadine, rimantadine, and related agents. In: *Antimicrobial Therapy and Vaccines.* Yu VL, Merigan TCJ, Barriere SL (Eds). Williams & Wilkins, MD, USA, 1344–1365 (1999).

10 Moscona A: Neuraminidase inhibitors for influenza. *N. Engl. J. Med.* 353(13), 1363–1373 (2005).

11 Dharan NJ, Gubareva LV, Meyer JJ *et al.*: Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* 301(10), 1034–1041 (2009).

▪▪ **A detailed report of the emergence and spread of oseltamivir-resistant H1N1 viruses, with details about clinical course and how it relates to susceptible strains.**

12 Lackenby A, Hungnes O, Dudman SG *et al.*: Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. *Euro. Surveill.* 13(5) (2008).

▪▪ **An outline of the epidemiology and virology of the emergence of oseltamivir-resistant H1N1 in Europe.**

13 Bright RA, Shay D, Bresee J *et al.*: High levels of adamantane resistance among influenza A (H3N2) viruses and interim guidelines for use of antiviral agents – United States, 2005–2006 influenza season. *MMWR Morb. Mortal. Wkly Rep.* 55(2), 44–46 (2006).

14 Bright RA, Shay DK, Shu B, Cox NJ, Klimov AI: Adamantane resistance among influenza A viruses isolated early during the 2005–2006 influenza season in the United States. *JAMA* 295(8), 891–894 (2006).

▪▪ **The hallmark paper that highlighted the spread of M2-resistant H3N2 viruses in the USA and its impact on clinical care of patients.**

15 Nicholson KG, Wood JM, Zambon M: Influenza. *Lancet* 362(9397), 1733–1745 (2003).

16 Hayden FG, Minocha A, Spyker DA, Hoffman HE: Comparative single-dose pharmacokinetics of amantadine hydrochloride and rimantadine hydrochloride in young and elderly adults. *Antimicrob. Agents Chemother.* 28(2), 216–221 (1985).

17 Keyser LA, Karl M, Nafziger AN, Bertino JS Jr: Comparison of central nervous system adverse effects of amantadine and rimantadine used as sequential prophylaxis of influenza A in elderly nursing home patients. *Arch. Intern. Med.* 160(10), 1485–1488 (2000).

18 Atkinson WL, Arden NH, Patriarca PA, Leslie N, Lui KJ, Gohd R: Amantadine prophylaxis during an institutional outbreak of type A (H1N1) influenza. *Arch. Intern. Med.* 146(9), 1751–1756 (1986).

19 Colman PM, Hoyne PA, Lawrence MC: Sequence and structure alignment of paramyxovirus hemagglutinin-neuraminidase with influenza virus neuraminidase. *J. Virol.* 67(6), 2972–2980 (1993).

20 Diggory P, Fernandez C, Humphrey A, Jones V, Murphy M: Comparison of elderly people's technique in using two dry powder inhalers to deliver zanamivir: randomised controlled trial. *BMJ* 322(7286), 577–579 (2001).

21 Beigel J, Bray M: Current and future antiviral therapy of severe seasonal and avian influenza. *Antiviral Res.* 78(1), 91–102 (2008).

▪ **A detailed review of anti-influenza agents currently under study for the treatment of influenza of man.**

22 Hayden F: Developing new antiviral agents for influenza treatment: what does the future hold? *Clin. Infect. Dis.* 48(Suppl. 1), S3–S13 (2009).

▪ **A review of anti-influenza agents currently under study for the treatment of influenza in man.**

23 De Clercq E: Antiviral agents active against influenza A viruses. *Nat. Rev.* 5(12), 1015–1025 (2006).

24 Ong AK, Hayden FG: John F. Enders lecture 2006: antivirals for influenza. *J. Infect. Dis.* 196(2), 181–190 (2007).

25 Astrahan P, Kass I, Cooper MA, Arkin IT: A novel method of resistance for influenza against a channel-blocking antiviral drug. *Proteins* 55(2), 251–257 (2004).

26 Hay AJ, Zambon MC, Wolstenholme AJ, Skehel JJ, Smith MH: Molecular basis of resistance of influenza A viruses to amantadine. *J. Antimicrob. Chemother.* 18(Suppl. B), 19–29 (1986).

27 Hurt AC, Ho HT, Barr I: Resistance to anti-influenza drugs: adamantanes and neuraminidase inhibitors. *Expert Rev. Anti Infect. Ther.* 4(5), 795–805 (2006).

28 Sweet C, Hayden FG, Jakeman KJ, Grambas S, Hay AJ: Virulence of rimantadine-resistant human influenza A (H3N2) viruses in ferrets. *J. Infect. Dis.* 164(5), 969–972 (1991).

▪ **An important study that defined that M2-resistant influenza appears to be equally virulent as the wild-type virus.**

29 Ison MG, Gubareva LV, Atmar RL, Treanor J, Hayden FG: Recovery of drug-resistant influenza virus from immunocompromised patients: a case series. *J. Infect. Dis.* 193(6), 760–764 (2006).

30 Hall CB, Dolin R, Gala CL *et al.*: Children with influenza A infection: treatment with rimantadine. *Pediatrics* 80(2), 275–282 (1987).

31 Hayden FG, Belshe RB, Clover RD, Hay AJ, Oakes MG, Soo W: Emergence and apparent transmission of rimantadine-resistant influenza A virus in families. *N. Engl. J. Med.* 321(25), 1696–1702 (1989).

▪▪ **A key early study that documented the emergence and transmission of M2 inhibitor resistance among family members exposed to individuals treated with M2 inhibtors.**

32 Hayden FG, Sperber SJ, Belshe RB, Clover RD, Hay AJ, Pyke S: Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob. Agents Chemother.* 35(9), 1741–1747 (1991).

33 Kiso M, Mitamura K, Sakai-Tagawa Y *et al.*: Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 364(9436), 759–765 (2004).

34 Englund JA, Champlin RE, Wyde PR *et al.*: Common emergence of amantadine- and rimantadine-resistant influenza A viruses in symptomatic immunocompromised adults. *Clin. Infect. Dis.* 26(6), 1418–1424 (1998).

35 Deyde VM, Xu X, Bright RA *et al.*: Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. *J. Infect. Dis.* 196(2), 249–257 (2007).

36 Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S *et al.*: Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N. Engl. J. Med.* 360(25), 2605–2615 (2009).

▪▪ **Key review of the emergence of swine-origin influenza virus H1N1 in the USA and Mexico.**

37 Cox NJ: FDA H5N1 Update: classification of H5N1 viruses and development of vaccine reference strains. US Food and Drug Administration Vaccines and Related Biological Products Advisory Committee (2007).

38 Ison MG, Mishin VP, Braciale TJ, Hayden FG, Gubareva LV: Comparative activities of oseltamivir and A-322278 in immunocompetent and immunocompromised murine models of influenza virus infection. *J. Infect. Dis.* 193(6), 765–772 (2006).

39 Bright RA, Medina MJ, Xu X *et al.*: Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 366(9492), 1175–1181 (2005).

40 Gubareva LV: Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. *Virus Res.* 103(1–2), 199–203 (2004).

▪ **A detailed review of neuraminidase inhibitor (NAI) resistance mechanisms.**

41 McKimm-Breschkin J, Trivedi T, Hampson A *et al.*: Neuraminidase sequence analysis and susceptibilities of influenza virus clinical isolates to zanamivir and oseltamivir. *Antimicrob. Agents Chemother.* 47(7), 2264–2272 (2003).

42 Aoki FY, Boivin G, Roberts N: Influenza virus susceptibility and resistance to oseltamivir. *Antivir. Ther.* 12(4 Pt B), 603–616 (2007).

▪▪ **A detailed review of NAI resistance mechanisms and testing strategies.**

43 Lackenby A, Thompson CI, Democratis J: The potential impact of neuraminidase inhibitor resistant influenza. *Curr. Opin. Infect. Dis.* 21(6), 626–638 (2008).

44 Collins PJ, Haire LF, Lin YP *et al.*: Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. *Nature* 453(7199), 1258–1261 (2008).

45 Roberts NA: Treatment of influenza with neuraminidase inhibitors: virological implications. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 356(1416), 1895–1897 (2001).

46 Shiraishi K, Mitamura K, Sakai-Tagawa Y, Goto H, Sugaya N, Kawaoka Y: High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. *J. Infect. Dis.* 188(1), 57–61 (2003).

47 Ward P, Small I, Smith J, Suter P, Dutkowski R: Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J. Antimicrob. Chemother.* 55(Suppl. 1), I5–I21 (2005).

48 Whitley RJ, Monto AS: Prevention and treatment of influenza in high-risk groups: children, pregnant women, immunocompromised hosts, and nursing home residents. *J. Infect. Dis.* 194(Suppl. 2), S133–S138 (2006).

49 Stephenson I, Democratis J, Lackenby A *et al.*: Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. *Clin. Infect. Dis.* 48(4), 389–396 (2009).

50 Gooskens J, Jonges M, Claas EC, Meijer A, van den Broek PJ, Kroes AM: Morbidity and mortality associated with nosocomial transmission of oseltamivir-resistant influenza A(H1N1) virus. *JAMA* 301(10), 1042–1046 (2009).

▪▪ **A case series highlighting the importance of oseltamivir resistance among hospitalized adults, including its potential for nosocomial spread.**

51 Weinstock DM, Zuccotti G: The evolution of influenza resistance and treatment. *JAMA* 301(10), 1066–1069 (2009).

52 Hayden FG: Antiviral resistance in influenza viruses--implications for management and pandemic response. *N. Engl. J. Med.* 354(8), 785–788 (2006).

53 Rameix-Welti MA, Enouf V, Cuvelier F, Jeannin P, van der Werf S: Enzymatic properties of the neuraminidase of seasonal H1N1 influenza viruses provide insights for the emergence of natural resistance to oseltamivir. *PLoS Pathog.* 4(7), E1000103 (2008).

▪▪ **A critical study that defined the molecular virology of oseltamivir resistance among circulating H1N1 viruses in Europe.**

54 de Jong MD, Tran TT, Truong HK *et al.*: Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N. Engl. J. Med.* 353(25), 2667–2672 (2005).

55 Gubareva LV, Matrosovich MN, Brenner MK, Bethell RC, Webster RG: Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J. Infect. Dis.* 178(5), 1257–1262 (1998).

56 Weinstock DM, Gubareva LV, Zuccotti G: Prolonged shedding of multidrug-resistant influenza A virus in an immunocompromised patient. *N. Engl. J. Med.* 348(9), 867–868 (2003).

57 Gubareva LV, Webster RG, Hayden FG: Detection of influenza virus resistance to neuraminidase inhibitors by an enzyme inhibition assay. *Antiviral Res.* 53(1), 47–61 (2002).

58 McKimm-Breschkin JL: Management of influenza virus infections with neuraminidase inhibitors: detection, incidence, and implications of drug resistance. *Treat Respir. Med.* 4(2), 107–116 (2005).

59 Zambon M, Hayden FG: Position statement: global neuraminidase inhibitor susceptibility network. *Antiviral Res.* 49(3), 147–156 (2001).

60 Hatakeyama S, Sakai-Tagawa Y, Kiso M *et al.*: Enhanced expression of an α2,6-linked sialic acid on MDCK cells improves isolation of human influenza viruses and evaluation of their sensitivity to a neuraminidase inhibitor. *J. Clin. Microbiol.* 43(8), 4139–4146 (2005).

61 Matrosovich M, Matrosovich T, Carr J, Roberts NA, Klenk HD: Overexpression of the α-2,6-sialyltransferase in MDCK cells increases influenza virus sensitivity to neuraminidase inhibitors. *J. Virol.* 77(15), 8418–8425 (2003).

▪ **A study documenting the limitations associated with standard cell lines in detecting antiviral resistance emergence in influenza isolates.**

62 Tisdale M: Monitoring of viral susceptibility: new challenges with the development of influenza NA inhibitors. *Rev. Med. Virol.* 10(1), 45–55 (2000).

63 Potier M, Mameli L, Belisle M, Dallaire L, Melancon SB: Fluorometric assay of neuraminidase with a sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) substrate. *Anal. Biochem.* 94(2), 287–296 (1979).

64 Lackenby A, Democratis J, Siqueira MM, Zambon MC: Rapid quantitation of neuraminidase inhibitor drug resistance in influenza virus quasispecies. *Antivir. Ther.* 13(6), 809–820 (2008).

65 Guo L, Garten RJ, Foust AS *et al.*: Rapid identification of oseltamivir-resistant influenza A(H1N1) viruses with H274Y mutation by RT-PCR/restriction fragment length polymorphism assay. *Antiviral Res.* 82(1), 29–33 (2009).

66 Pabbaraju K, Tokaryk KL, Wong S, Fox JD: Comparison of the Luminex xTAG respiratory viral panel with in-house nucleic acid amplification tests for diagnosis of respiratory virus infections. *J. Clin. Microbiol.* 46(9), 3056–3062 (2008).

67 Merante F, Yaghoubian S, Janeczko R: Principles of the xTAG respiratory viral panel assay (RVP Assay). *J. Clin. Virol.* 40(Suppl. 1), S31–S35 (2007).

68 Centers for Disease Control and Prevention (CDC): Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients – Seattle, Washington, 2009. *MMWR Morb. Mortal. Wkly Rep.* 58(32), 893–896 (2009).

69 Centers for Disease Control and Prevention (CDC): Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis – North Carolina, 2009. *MMWR Morb. Mortal. Wkly Rep.* 58(35), 969–972 (2009).

70 McCaw JM, Wood JG, McCaw CT, McVernon J: Impact of emerging antiviral drug resistance on influenza containment and spread: influenza of subclinical infection and strategic use of a stockpile containing one or two drugs. *PLoS ONE* 3, E2362 (2008).

71 Ison MG, Gnann JW Jr, Nagy-Agren S *et al.*: Safety and efficacy of nebulized zanamivir in hospitalized patients with serious influenza. *Antivir. Ther.* 8(3), 183–190 (2003).

▪ **The only study of combination therapy for influenza in man that documented a reduced rate of resistance emergence with combination therapy.**

Websites

101 CDC issues interim recommendations for the use of influenza antiviral medications in the setting of oseltamivir resistance among circulating influenza A (H1N1) viruses, 2008–2009 influenza season. *CDC Health Advisory* (2008) http://www2a.cdc.gov/HAN/ArchiveSys/ViewMsgV.asp?AlertNum=00279

102 Main website for all seasonal influenza information from the CDC www.cdc.gov/flu

103 CDC website with antiviral recommendations with regard to the pandemic influenza A/H1N1 2009 www.cdc.gov/h1n1flu/recommendations.htm