Chapter 69
Antiviral Resistance in Influenza Viruses: Clinical and Epidemiological Aspects

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1 Introduction

Two classes of anti-viral agents, the M2 ion channel inhibitors (amantadine, rimantadine) and neuraminidase (NA) inhibitors (oseltamivir, zanamivir) are available for treatment and prevention of influenza in most countries of the world. The principle concerns about emergence of antiviral resistance in influenza viruses are loss of drug efficacy, transmission of resistant variants, and possible increased virulence or transmissibility of resistant variants (1). Because seasonal influenza is usually an acute, self-limited illness in which viral clearance occurs rapidly due to innate and adaptive host immune responses, the emergence of drug-resistant variants would be anticipated to have modest effects on clinical recovery, except perhaps in immunocompromised or immunologically naïve hosts, such as young infants or during the appearance of a novel strain. In contrast to the limited impact of resistance emergence in the treated immunocompetent individual, the epidemiologic impact of resistance emergence and transmission could be considerable, including loss of both prophylactic and therapeutic activity for a particular drug, at the household, community, or perhaps global level. Influenza epidemiology in temperate climates is expected to provide some protection against widespread circulation of resistant variants, as viruses do not persist between epidemics but rather are re-introduced each season and new variants appear often (2, 3).

However, the emergence and circulation of M2 ion channel inhibitor-resistant variants has been an important concern given their transmission fitness, detection in some animal influenza viruses and many human isolates of avian A(H5N1) virus, their frequent emergence during therapy in humans, and the increasing use of amantadine in regions of the globe like China that may be the sites for emergence of new drift variants or possibly pandemic strains. Indeed, the recent observations of global spread of M2 inhibitor-resistant A(H3N2) viruses, initially recognized in Asia (4, 5), illustrates the public health consequences of antiviral resistance in influenza viruses and has led to changes in policies for use of this antiviral class of drugs in many countries. The more recent and unexpectedly rapid dissemination of oseltamivir-resistant A(H1N1) viruses highlights the unpredictability of antiviral resistance emergence and spread (6, 6a). Antiviral resistance and its consequences are key factors that need to be considered by health authorities and governments when making decisions regarding the stockpiling of antivirals for response to pandemics or other influenza threats (7), although concerns about antiviral resistance, particularly to NA inhibitors, should not dissuade countries from developing adequate antiviral inventories for pandemic response (1, 8).

Factors that influence the clinical and epidemiologic importance of drug-resistant influenza viruses include the magnitude of phenotypic resistance, its frequency and rapidity of emergence, its stability and ability of resistant variants to compete with wild-type viruses in the absence of selective drug pressure, and the effects of resistance mutations on viral replication competence, pathogenicity, and transmissibility *in vivo*. In general, data to date indicate that mutations conferring either NA or M2 inhibitor resistance are not associated with worsened viral virulence, atypical influenza, or enhanced transmissibility in humans. In contrast to M2 inhibitor resistance, most but not all NA mutations conferring resistance in clinical isolates have been associated with reduced infectivity, replication, and pathogenicity in animal models of influenza. However, changes in other influenza genes segments, such as antigenic change in the hemagglutinin (HA), or perhaps compensatory mutations in the target genes may enhance viral fitness and be associated with widespread transmission

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and illness. The following sections/chapters review clinical and epidemiological data on antiviral resistance for the two classes of available anti-influenza agents. Information from experimental animal models of influenza is incorporated to supplement the limited data derived from clinical studies.

2 M2 Ion Channel Inhibitors (Amantadine, Rimantadine)

Amantadine was initially approved in the United States in 1966 for influenza A(H2N2) infections and then for all influenza A viruses in 1976; rimantadine was used in the former Soviet Union for decades and later approved for use in the United States in 1993. Rapid selection of drug-resistant variants was demonstrated over 30 years ago in early laboratory studies employing in vitro and in vivo passage in the presence of amantadine (9, 10), and the study of resistance was used to determine the mechanism of antiviral action of M2 inhibitors (reviewed in (11)). For human influenza viruses, resistance was shown to be due to point mutations in the M gene and corresponding single amino acid substitutions in the transmembrane region of the M2 protein (positions 26, 27, 30, 31, 34) that resulted in marked loss of phenotypic susceptibility in vitro. The clinical implications of resistance became apparent in studies during the 1980s of treated children (12), in whom a high frequency of resistance emergence was documented, and subsequently of households and nursing homes, where transmission of drug-resistant variants was implicated in failures of drug prophylaxis (13–15). Phenotypic resistance to M2 inhibitors is high-level and generally leads to loss of antiviral activity in vivo.

2.1 Detection of Resistance

Detection of M2 inhibitor resistance has usually relied on virus isolation from respiratory samples and susceptibility testing of virus in cell culture. Several assays have been described including plaque reduction, yield reduction, and ELISA (16). Following phenotypic analysis, genotypic M2 inhibitor resistance has been confirmed by nucleotide sequence analysis of the M2 gene and detection of the characteristic mutations. Genotypic detection can be accomplished quickly by the use of PCR-restriction length polymorphism (RFLP) analysis of the RNA extracted from respiratory samples using commercially available endonucleases for discrimination of point mutations in the M2 gene (17). Greater sensitivity in detecting resistant clones has been described with reverse transcription-polymerase chain reaction amplification of the RNA followed by sequencing of multiple clones (18). Recently the rapid pyro-

sequencing technique has been shown to be a reliable, high-throughput method for detecting genotypic resistance in large numbers of community isolates (4, 5). Following treatment, approximately 70–90% of amino acid substitutions in resistant viruses occur at position 31 and about 10% each are found at positions 27 and 30 (17). The distribution of resistance mutations depends on influenza A subtype, such that Ser31Asn predominates in A(H3N2) subtype whereas Val27Ala occurs with increased frequency in A(H1N1) subtype viruses (19). M2 proteins show considerable evolution in human and swine viruses, and the H3 and H1 subtype viruses have phylogenetically different M2 proteins (20). This may influence the mutations that are more advantageous for conferring M2 inhibitor resistance. Of note, the Ser31Asn mutation has been responsible for the resistant A(H3N2) and A(H1N1) variants that have recently circulated globally (4, 5).

2.2 Susceptibility of Field Isolates

Pandemic strains, including reassortants bearing the M gene of the 1918 virus (21), and earlier prototype epidemic viruses (13) have been susceptible to amantadine and rimantadine. Until recently, studies of community isolates of A(H3N2) viruses generally revealed low levels of primary resistance, approximately 1–3% (Table 1). A survey of 2,017 isolates from 43 countries during 1991–1995 detected resistance in only 0.8%; of the 16 persons with resistant isolates, two were receiving drug and four others were in potential contact with drug recipients (22). Of note, 4.5% of 198 isolates from Australia, collected between 1989 and 1995, showed resistance for unexplained reasons. Another survey of 1,813 field isolates collected 1968–1999 in the United Kingdom found resistance in 1.5% (23). Amantadine was approved of for use in Japan in 1998, and a Japanese study found resistant isolates in 3.4% of 179 children before starting antiviral treatment in the 1999–2000 season, although not in the preceding or succeeding seasons (24). A survey of 1,096 community isolates collected over four seasons in Canada from 1998 to 2002 found that 0.7% showed resistance mutations (Li, unpublished observations). During the same period, 20% of 138 viruses isolated during nursing home outbreaks, in which amantadine was often used for control, were resistant. Resistant isolates have also been reported without known drug exposure in nursing home residents (25).

However, the incidence of resistance in field isolates of A(H3N2) viruses increased dramatically in 2003 in isolates from China, perhaps related to increased use of over-the-counter amantadine after the emergence of severe acute respiratory syndrome (SARS) (4, 8). During the 2004–2005 influenza season, approximately 70% of the A(H3N2) isolates from China and Hong Kong and nearly 15% of those from

Table 1 Representative studies of M2 inhibitor susceptibility of influenza A field isolates from adults and children

	Site	Period	Method	Number tested by subtype	No. (%) resistant
Belshe et al. (13)	US	1978–1988	EIA, S	65 H1N1	0
				181 H3N2	5 (2.0%)[a]
Valette et al. (162)	France	1988–1990	EIA	28 H1N1	0
				77 H3N2	0
Ziegler et al. (22)	43 countries	1991–1995	EIA, S, PCR-RFLP	2,017	16 (0.8%)[b]
Dawson (23)	UK	1968–1999	EIA, Plaque	1,813	28 (1.5%)
Suzuki et al. (24)	Japan	1993–1998	Not stated	55	0
		1999–2000	Not stated	179	6 (3.4%)
Li 2003 (personal communication)	Canada	1998–2002	PCR-RFLP	1,096	8 (0.7%)
Shih et al. (163)	Taiwan	1996–1998	Plaque, S	84	1(1.2%)
Bright et al. (4)	Global	1994–2005	S	6,525 H3N2	392 (6.0%)
		1994–2002			0.3–1.8%
		2003–2005			12.3–13.3%[c]
		1998–2004		589 H1N1	2 (0.3%)
Bright et al. (5)	US	2005–2006	S	205 H3N2	193 (92.3%)
				8 H1N1	2 (25%)
Saito et al. (164)	Japan	2005–2006	S	354 H3N2	231 (65.3%)
Saito et al. (165)				61 H1N1	0
		2006–2007	S	632 H3N2	566 (89.6%)
				120 H1N1	77 (64.2%)
Barr et al. (27)	Australia, New Zealand, Asia, South Africa	2005	S	102 H3N2	43 (42%)
				37 H1N1	0
Deyde (28, 29)	Asia	2006–2007	S	235 H3N2	156 (66.4%)
				118 H1N1	87 (73.7%)
	Europe			71 H3N2	25 (35.2%)
				45 H1N1	27 (60.0%)
	North America			481 H3N2	327 (68.0%)
				519 H1N1	15 (2.9%)
	South America			77 H3N2	73 (94.8%)
				29 H1N1	0 (0)

Abbreviations: S = M2 gene sequence analysis; PCR–RFLP = polymerase chain reaction–restriction length polymorphism; *EIA* enzyme immunoassay

[a] All resistant viruses from family members receiving rimantadine

[b] Over 80% of tested isolates were H3N2 subtype and all resistant ones were of this subtype. Separate analysis found that 9 (4.5%) of 198 strains from Australia, 1989–1995, were resistant

[c] In 2004–2005 the frequencies of resistance in H3N2 viruses were 73.8% in China, 69.6% in Hong Kong, 22.7% in Taiwan, 15.1% in South Korea, 4.3% in Japan, 30.0% in Canada, 19.2% in Mexico, 14.5% in USA, and 4.7% in Europe

the United States and Europe showed resistance due to a Ser31Asn mutation, and this frequency increased to over 90% in the United States during the 2005–2006 season (5). Resistant A(H3N2) viruses have spread widely in countries without substantial M2 inhibitor use (Table 1). This unprecedented global spread of A(H3N2) viruses with a specific mutation (Ser31Asn) has occurred despite the absence of a sustained selective drug pressure, possibly because the resistant M gene was incorporated into efficiently spreading HA antigenic variants, so-called "hitch-hiking" of a resistance marker (26). Initial phylogenetic analyses of the M2 and other viral genes, particularly HA, suggested a common lineage of these viruses (26, 27), but testing of greater numbers of resistant strains over several seasons has found no signature amino acid changes in the HA (28, 29). This experience clearly indicates that this

resistance mutation does not reduce transmissibility. Recently an increased frequency of resistant A(H1N1) viruses harboring this mutation, particularly in Asia, has also been recognized (Table 1). The public health consequence has been to make this class of antiviral drugs unreliable for prophylaxis or treatment currently. It remains to be seen to what extent the emergence of new antigenic variants will lead to the returned circulation of susceptible viruses, but almost all A(H3N2) viruses isolated in late 2008 continued to show adamantane resistance.

2.2.1 Swine and Avian Viruses

In addition, a characteristic feature of A(H1N1), A(H1N2), and A(H3N2) swine viruses circulating in Europe since 1987

has been the presence of a Ser31Asn mutation, as well as Val27Ala in some isolates, that confers resistance to M2 inhibitors (30, 31); such isolates have caused occasional human infections and are now present in Asia (32). Some swine isolates from the 1930s were found to harbor the same resistance mutation (Bean, unpublished observations). The introduction of human and avian genes and genetic reassortment among co-circulating subtypes has been found in swine influenza isolates in different parts of the world (31, 31a). The postulated role of the swine as intermediate hosts in the emergence of some novel human viruses highlight the potential risk of a new human epidemic or pandemic strains harboring primary M2 inhibitor resistance.

In addition, direct inter-species transmission of virus from birds or a reassortment event leading to acquisition of an M gene encoding resistance in a human strain provides another route for acquisition of the M2 inhibitor resistance in a human virus. One survey of avian isolates found that M2 inhibitor-resistant variants were not detected among 1979–1983 isolates, whereas 31% of H5 and 11% of H9 strains from Southeast Asia isolated in 2000–2004 carried M2 resistance mutations (33). In North America, resistant variants occurred among 16% of H7 viruses only, whereas H6 viruses were amantadine-sensitive. Resistance due to the Ser31Asn mutation was also confirmed in poultry A(H5N1) isolates collected between 2002 and 2005 in Northern China, which perhaps related to use of amantadine in chicken food or water (34). One highly pathogenic avian A(H7N7) isolate from a fatal human case in Holland in 2003 was resistant to amantadine in cell culture and in experimentally infected mice; resistance was linked to the HA protein and not to mutations in M2 (34a).

2.2.2 A(H5N1) Viruses

Although the initial human isolates of avian A(H5N1) viruses in Hong Kong in 1997 were M2 inhibitor susceptible (34b), more recent human isolates of clade 1 viruses have been resistant to M2 inhibitors (35, 36). One sequence analysis of 638 M2 genes of avian and human isolates of A(H5N1) viruses from 1996 to 2005 found resistance due to dual Ser31Asn and Leu26Ile mutations in almost all clade 1 isolates from Vietnam, Thailand, and Cambodia since 2003, observations that were consistent with a single introduction (37). M2 inhibitor-resistance due to the Ser31Asn mutation, or less often mutations at positions 27 or 30, was seen in a minority of isolates from China and Hong Kong SAR starting in 2003 and in only 2 of 32 Indonesian viruses. In 2005 the frequency of resistance detection was 83% in Vietnam but only 7% in China. Another survey of 55 avian A(H5N1) isolates from Southeast Asian countries found that all of the A(H5N1) viruses from Vietnam, Malaysia

and Cambodia contained dual-resistance mutations (L26I and S31N), while 4 of 6 strains from Indonesia were sensitive (38). However, subsequent studies indicate that an increasing proportion of Clade 2.1 isolates from Indonesia have been resistant (over 80% in 2006–2007), whereas over 90% clade 2.2 and 2.3 isolates from Eurasia, Africa, and China have been susceptible (A. Klimov, personal communication). These observations highlight the need for continued surveillance of drug susceptibility in new human and animal influenza viruses.

2.3 Resistance in Posttreatment Isolates

The rapid emergence of resistant variants in M2 inhibitor-treated patients has been found also in studies of experimentally infected animals. In a study using a chicken A(H5N2) virus, resistant viruses are detectable by 2–3 days after starting drug administration and persisted thereafter (39). A study in ferrets inoculated with a human influenza A(H3N2) virus detected M2 inhibitor resistance mutations in four of the nine amantadine-treated animals by day 6 after inoculation; in each instance two or more M2 gene mutations were identified (40). In contrast, intranasal zanamivir did not select for known neuraminidase resistance mutations under similar conditions.

2.3.1 Immunocompetent Patients

Resistant variants arise commonly and rapidly in M2 inhibitor-treated children and adults with acute influenza (Table 2). One study of adults found that resistant virus could be detected in 50% of six rimantadine recipients by day three of treatment, although the nasal lavage titers were lower than in placebo recipients shedding susceptible virus (41). Another study found that 33% of 24 adult and pediatric household members receiving rimantadine shed resistant virus on day 5 of treatment; none were positive when tested five days later (41). A larger pediatric trial found emergence of resistant virus in 27% of 37 rimantadine recipients, including 45% of those still virus positive on day 7, compared to 6% of 32 acetaminophen recipients (12). Resistant virus was detected as early as day 3 but was usually present on days 5–7. A study of Japanese children treated with amantadine found that 30% of 81 in the 1999–2000 season and 23% of 30 during the following season had resistant virus detected on day 3–5 after a 3-day course (19). Resistant variants were detected more frequently in A(H3N2)-infected children (33%) than in A(H1N1)-infected ones (20%). Another study employing sensitive molecular cloning detection methods found mutations conferring resistance in 80% of 15 hospitalized children

Table 2 Recovery of resistant influenza A during M2 inhibitor treatment

Study	Seasons	Patient group	Treatment	Number treated	No. (%) shedding resistant viruses
Hall et al. (12)	1983	Children	Rimantadine	37	10 (27%) H3N2
Hayden et al. (41)	1987–1989	Children	Rimantadine	21	6 (29%) H3N2
Hayden et al. (41)	1988–1989	Adults	Rimantadine	13	5 (38%) H3N2
Betts, personal communication		Elderly	Rimantadine	26	3 (11%)
Englund et al. (42)	1993–1994	Immunocompromised	Amantadine, Rimantadine	15	5 (33%) H3N2
Saito et al. (19)	1999–2001	Children	Amantadine	111	22 (33%) H3N2
					9 (20%) H1N1
Shirashi et al. (18)	1999–2001	Children (hospitalized)	Amantadine	15	8 (100%) H3N2
					4 (57%) H1N1

during or immediately after amantadine treatment (18). Nine (75%) of 12 children had 2–4 different resistance mutations detected in clones from a single sample, sometimes mixed with a wild-type virus. Viruses with the Ser31Asn mutation were more prevalent in A(H3N2)-infected children and those with Val27Ala in A(H1N1)-infected ones compared to other mutations, suggesting that they had greater replication competence in these subtypes (18).

2.3.2 Immunocompromised Hosts

Resistant influenza A viruses may be shed for prolonged periods in immunocompromised hosts, who can serve as a reservoir for nosocomial transmission. One study of adult bone marrow transplant and acute leukemia patients recovered resistant virus in 5 (33%) of 15 M2 inhibitor-treated patients and in five (83%) of six patients with illness who shed virus for ≥3 days (42). The median time between first and last virus isolation was 7 days with range up to 44 days. Death associated with influenza occurred in two of the five (40%) patients with resistant virus, compared to 5 of the 24 (21%) without, and prolonged illness was noted in several with protracted shedding. Other reports have documented prolonged shedding of resistant variants in immunocompromised hosts with or without continued drug exposure, including one transplanted SCID child who shed resistant virus for 5 weeks and one adult leukemia patient who shed resistant virus for ≥1 week off therapy (43). Another case report documented recovery of resistant virus >1 month after cessation of a course of amantadine, as well as shedding of mixtures of wild-type virus and variants with different resistance genotypes (44). Heterogeneous populations of resistant variants with sequential or dual mutations have been found in several immunocompromised hosts (42, 43). One stem cell transplant recipient shed the dually M2 inhibitor and oseltamivir-resistant virus for at least 5 months and probably over a period of 1 year (45). The prolonged shedding of resistant variants in immunocompromised hosts is consistent with the genetic stability of such variants observed in experimental animal models (39). However, early amantadine or rimantadine treatment in acute leukemia or hematopoietic stem cell transplant patients with drug-susceptible influenza A can be clinically beneficial and was associated with a significant reduction (35% versus 76%) in the risk of progression to pneumonia in one report (46).

2.4 Transmissibility of Resistant Variants

The transmissibility of M2 inhibitor-resistant viruses has been demonstrated in animal models and in several clinical settings. Competition-transmission studies with an avian A/chicken/Pennsylvania/1370/83(H5N2) virus compared the transmissibility of wild-type virus with resistant variants possessing M2 substitutions at positions 27, 30, or 31 (39). Contact birds shedding resistant virus due to earlier incorporation of amantadine in the drinking water of donors (four days only) were caged with birds shedding susceptible virus, and the virus was allowed to transmit through three more sets of contact birds in the absence of selective drug pressure. Resistant virus was detected from the final set of contact birds in three of four experiments over four cumulative transmission cycles.

2.4.1 Households

Both amantadine and rimantadine are effective for postexposure prophylaxis of illness due to susceptible strains in household contacts, when ill index cases are not given concurrent treatment (Table 3). In contrast, two studies have found no significant reduction in secondary influenza illness in household contacts receiving either amantadine or rimantadine for postexposure prophylaxis, when the ill index cases received treatment with the same drug. One of these documented failures of prophylaxis due to infection by drug-resistant variants, most likely transmitted from the treated index cases (14). These findings indicate that the strategy of using M2

Table 3 Influenza prevention in households with postexposure prophylaxis (PEP)

| Study | Drug (age of contacts) | Season (predominant virus) | Index case treated | Influenza A illness in contacts | | PEP efficacy (%) |
| | | | | No./total evaluable (%) | | |
				Active	Control	
Galbraith et al. (166)	Amantadine (≥2 years)	1967–1968 (A/H2N2)	No	0/91 (0%)	12/90 (13%)	100
Bricaire (167)	Rimantadine (≥1 year)	1988–1989 (A/not stated)	No	8/151[a] (5%)	26/150[a] (17%)	70
Monto et al. (168)	Zanamivir (≥5 years)	2000–2001 (A/H3N2, B)	No	12/661 (2%)	55/630 (9%)	82
Welliver et al. (169)	Oseltamivir (≥13 years)	1998–1999 (A/H3N2, B)	No	4/493 (1%)	34/462 (7%)	89
Galbraith et al. (170)	Amantadine (≥2 years)	1968–1969 (A/H3N2)	Yes	5/43 (12%)	6/42 (14%)	6
Hayden et al. (14)	Rimantadine (≥1 year)	1987–1989 (A/H3N2, A/H1N1)	Yes	11/61 (18%)	10/54 (19%)	3
Hayden et al. (171)	Zanamivir (≥5 years)	1998–1999 (A/H3N2, B)	Yes	7/414 (2%)	40/423 (9%)	82
Hayden et al. (172)	Oseltamivir (≥1 year)	2000–2001 (A/H3N2, B)	Yes	11/400 (3%)	40//392 (10%)	73

[a]Clinical influenza

inhibitors for both index case treatment and postexposure prophylaxis in households should be avoided.

2.4.2 Chronic Care Facilities

Transmission of M2 inhibitor-resistant viruses is well documented in nursing home outbreaks of influenza A and may be manifested by a persistent or an increasing number of virus-positive patients despite amantadine prophylaxis. The recovery of the same genotype of resistant virus from multiple patients on prophylaxis or from patients or staff not receiving drug indicate ongoing transmission in this setting (15, 47). This is particularly true with multiple isolations of a less commonly observed resistant variant, as was found with nine isolates of a Leu26Phe variant in one nursing home outbreak (47). The frequency of instances in which amantadine or rimantadine have failed to control outbreaks because of resistance emergence is not well defined. In Canada amantadine was used for outbreak control in 29 influenza A outbreaks in chronic care facilities over 11 influenza seasons studied between 1989 and 2000 (48). In 22 (76%) instances, transmission was stopped within 2–3 days, whereas the outbreak was not controlled in seven (24%) instances. Susceptibility testing found emergence of M2 inhibitor resistance in three of six outbreaks with ongoing transmission; amantadine failure was associated with simultaneous prophylactic and therapeutic use of the drug and with treatment of a higher proportion of ill persons in the facility. Continuing outbreaks due to resistant virus were associated with a higher proportion of cases in shared rooms compared to those due to susceptible virus. During the 1999–2000 season another study found that only 59% of 200 influenza A outbreaks were controlled by amantadine (49); four of five amantadine outbreak failures were associated with circulation of susceptible virus, perhaps because of use of reduced amantadine doses in an effort to avoid side effects (50). Such findings emphasize the importance of proper isolation of treated persons and of

using NAI for treatment of ill/sick persons. However, failures of rimantadine prophylaxis due to resistant virus have been observed in nursing home residents despite use of oseltamivir treatment of ill/sick residents (51). Uncontrolled clinical experiences indicate that prophylaxis with inhaled zanamivir (47, 52) or oral oseltamivir (48, 50) are both effective in terminating such outbreaks. One controlled comparative study found that inhaled zanamivir prophylaxis was superior to oral rimantadine in protecting nursing home residents, largely because of the high frequency of M2 inhibition resistance in the nursing homes (53).

Resistant viruses have been recovered occasionally from patients receiving long-term amantadine for Parkinsonism (54). One study during the 1998–1999 season in Japanese nursing homes detected resistant viruses by PCR–RLFP analysis in elderly residents with influenza-like illness in 24% of 141 PCR positive nasopharyngeal samples, over 90% of which were due to Ser31Asn substitutions (17). Only 18% of the 34 patients with resistance detected were receiving amantadine at the time of sampling. The average frequency of resistance detection was nonsignificantly higher in the four homes using amantadine for therapy of ILI (28%) than in the four homes where it was used for Parkinsonism (16%). Such findings indicate that patients receiving amantadine for noninfluenza indications may serve as a source of drug-resistant virus under certain circumstances.

2.5 Pathogenicity

M2 inhibitor-resistant influenza A viruses appear to cause typical influenza illness without obviously enhanced or attenuated symptoms (14, 16, 41). Illness occurs in both the presence or absence of the drug, a finding that indicates the loss of antiviral effectiveness in vivo. In temporal relationship to increasing frequencies of M2 inhibitor resistance in Japan during the 2003–2006 seasons, amantadine treatment was

found to have decreasing clinical effectiveness, whereas that of oseltamivir did not change (55). In nursing home outbreaks residents developing influenza due to resistant strains have experienced serious illness, including fatal outcomes in some instances (56). Patients with infections caused by M2 inhibitor-resistant variants have no obvious reductions in the risks of pneumonia, hospitalization, or death compared to those with wild-type illness. One study of higher-risk patients during the 2004–2005 season found no differences in the number of symptoms, duration of illness, or frequency of hospitalization in comparing outcomes among 80 patients with A(H3N2) illness due to M2 inhibitor-susceptible virus to 72 patients infected with resistant viruses (57). While the M gene mutations do not appear to attenuate or potentiate the virulence of human influenza viruses, more subtle effects on biologic fitness cannot be excluded by studies to date. Occasionally wild-type virus replaces resistant variants after cessation of amantadine (18). As noted for some avian H7 viruses, this reversion in the absence of selective drug pressure suggests diminished replication competence of some resistant genotypes. However, the most common resistant variants with Ser31Asn have no apparent loss of replication competence or transmissibility.

Animal model studies have found that mutations in the M2 protein do not appear to attenuate or potentiate the virulence of influenza viruses compared to wild-type virus in the absence of drug administration. Resistant variants of an avian influenza A/chicken/Pennsylvania/1370/83(H5N2) with position 27, 30, or 31 substitutions were comparable to drug-susceptible virus in causing mortality in experimentally infected birds, although virulence in both susceptible and resistant viruses varied (39). As expected, amantadine administration protected against death in birds inoculated with wild-type virus but not a resistant variant. Studies with three pairs of epidemiologically linked human A(H3N2) subtype isolates, representing resistant variants with M2 substitutions at Val27Ala, Ala30Val, or Ser31Asn, found no differences in febrile responses, peak nasal viral titers, and nasal inflammatory cell counts in experimentally infected ferrets between the resistant variants and corresponding wild-type viruses (58). The variants retained their resistance phenotype during the short-term passage in ferrets. In an A/Udorn/307/72(H3N2) virus background, the Ser31Asn and Val27Thr mutations were associated with replication in cell culture and mice comparable to wild-type virus (59). Studies of a laboratory virus A/WSN(H1N1) that was genetically engineered to harbor different M2 resistance mutations found that none were associated with reduced replication in cell culture or diminished virulence in mice, and several mutations, including Ser31Asn and particularly the combination of Ser31Asn with Val27Ala were associated with increased weight loss and mortality in mice compared to susceptible virus (60). In general, it appears that M2 inhibitor-resistant human influenza A viruses that emerge in vivo do not differ substantially in replication ability or pathogenicity from drug-susceptible wild-type viruses.

In treated patients the emergence of resistant virus may be associated with persistence of viral shedding and in some studies delays in resolution of illness in immunocompetent persons. Retrospective analysis of rimantadine-treated adult and pediatric family members infected with influenza A(H3N2) virus found that the third who had emergence of the resistant virus on therapy, experienced somewhat longer times to resolution of symptoms, fever, and possibly functional impairment compared to the two-thirds that did not shed resistant virus (41). However, both groups had more rapid illness recovery than placebo-treated persons. Another study of influenza A(H3N2) illness found that rimantadine-treated children had lower frequencies and titers of detectable virus on the second day of treatment but higher values on days 6 and 7 after treatment and a mean 1-day longer period of viral shedding, principally related to the emergence of resistant variants (12). Among rimantadine recipients, illness measures were initially improved compared to placebo, but 41% became worse on later days compared to 18% in the placebo. Rimantadine recipients with resistant virus tended to have increased illness scores on days 5 and 6 compared to those without. Although such studies do not prove that resistance emergence caused the delay in recovery, the persistence of symptoms combined with emergence of resistance could contribute to transmission of resistant virus from such persons, especially young children.

2.6 Treatment Alternatives

Amantadine and rimantadine share susceptibility and resistance, so that resistance to one M2 inhibitor confers high-level cross-resistance to the other one and to date the entire class of compounds targeting M2 protein. Because of their different mechanism of antiviral action, NA inhibitors (discussed below) retain full activity against M2 inhibitor-resistant viruses and are appropriate choices for both prophylaxis and treatment of suspected M2 inhibitor-resistant infections. Both oseltamivir and zanamivir have been used with apparent success in terminating ongoing institutional outbreaks in which amantadine-resistance was implicated (47, 48, 50, 52). One unanswered clinical question is whether combined treatment with an M2 and NA inhibitor reduces the likelihood of resistance emergence to either class of drugs. In vitro studies indicate that the combination reduces this risk (61). One small study comparing oral rimantadine monotherapy to rimantadine combined with aerosolized zanamivir in hospitalized adults found that

the only M2 inhibitor-resistant variants were detected in the rimantadine monotherapy group (62), and further studies of combination therapy are warranted in serious infections including those due to A(H5N1), when the infecting virus is known or likely to be M2 inhibitor susceptible (63, 63a).

The synthetic nucleoside ribavirin is also inhibitory for M2-inhibitor resistant influenza A and B viruses and is a therapeutic consideration. Aerosolized ribavirin has been studied in uncomplicated influenza and used in treating individuals with influenza pneumonia (reviewed in (64)). High (8.4 g in 2 days) but not low (1 g/day) dose oral ribavirin appears to reduce clinical illness in uncomplicated influenza (65, 66), and intravenous ribavirin has been used in severe infections with uncertain benefit (67). Other potential inhibitors have been reviewed (68, 69, 69a) and are briefly discussed below.

3 Neuraminidase Inhibitors (Zanamivir, Oseltamivir)

Antiviral resistance studies with the neuraminidase (NA) inhibitors were initiated shortly after their discovery. Initial efforts utilizing sequential passage in cell culture to select resistant variants found that changes in either viral HA or NA could confer resistance in vitro (reviewed in (70, 71)). The frequency and possible importance of resistance emergence during drug administration have been studied largely in the context of controlled clinical trials conducted in the late 1990s that served as the basis for approval of zanamivir and oseltamivir. Due to differences in drug binding interactions and structural differences in the enzyme active site, NA inhibitors show varying susceptibility patterns that depend on virus type and subtype (reviewed in (72, 73)). For example, several studies found that zanamivir is on average 3- to 10-fold more potent against influenza B NAs than oseltamivir but somewhat less active against influenza A N2s (74, 75). While the possible clinical importance of such differences is uncertain, the lower oseltamivir carboxylate susceptibility of influenza B relative to A NAs may have clinical consequences. Several Japanese studies have reported that oseltamivir therapy is somewhat less effective for treatment of influenza B compared to influenza A, particularly in younger children, as measured by time to defervescence and by reductions in viral titers in the upper respiratory tract (76, 77). In contrast, inhaled zanamivir appears comparably effective in influenza A and B infections (78). Zanamivir and oseltamivir have been available in many countries since 1999, but their actual extent of clinical use has been quite limited, except recently in Japan (79, 80). Consequently, the possible epidemiologic importance of resistance emergence and transmission has received limited direct study to date.

3.1 Detection of Resistance

Several phenotypic and genotypic assays are used to detect NA inhibitor resistance (6a). As noted above, changes in either NA or HA can result in antiviral resistance to NA inhibitors under laboratory conditions (reviewed in (70, 72)). This relates to the functional balance between the receptor binding activity of HA and the receptor-destroying activity of NA, such that HA mutations causing decreased dependence on NA action for viral elution from cells can lead to in vitro resistance to NA inhibitors. Furthermore, both the target enzyme and inhibitors exert extra-cellular effects. Unlike the situation for M2 inhibitors, cell-culture-based assays have not been validated for detecting phenotypic resistance in clinical isolates, in part because of the differences in cellular receptor specificity between human respiratory epithelium and most available cell culture types (reviewed in (71)). Recent, low passage clinical isolates often appear resistant to NA inhibitors in laboratory cell lines. Madin Darby canine kidney (MDCK) cell line that are stably transfected with human 2,6-sialyltransferase (SIAT1) to enhance expression of alpha 2,6-linked sialic acid and reduce that of alpha 2,3-linked ones overcome this limitation but has not been widely utilized to date (81, 82). Based on plaque size determinations, oseltamivir susceptibility in SIAT1-MDCK cells increases and appears to correlate well with the results in enzyme inhibition assays for clinical isolates (82). However, the levels of resistance observed in yield reduction assays with such cells may be much less than observed in enzyme inhibition assays for viruses with several clinically relevant oseltamivir resistance mutations, and some resistant variants (e.g., Arg292Lys) may not replicate sufficiently for assay (83).

HA binding efficiency and associated susceptibility to NA inhibitors are affected by amino acids in the receptor binding pocket, location and presence of oligosaccharide chains, and the structure of cellular receptors (84). Changes in HA that alter binding to the $\alpha 2,3$-linked sialic acid residues on typical MDCK cells may reduce susceptibility to NA inhibitors in vitro but not change binding to human cells expressing $\alpha 2,6$-linked residues. Consequently, HA mutations have been looked for in clinical isolates usually by comparing the sequence of pre- and posttherapy isolates and in some instances by examining changes in receptor affinity. HA variants that have reduced receptor affinity show cross-resistance in vitro to all NA inhibitors but in general retain susceptibility to NA inhibitors in animal models (71, 85). Of note, altering the HA receptor binding site of a clade 1 A(H5N1) virus by reverse genetics, including switch from $\alpha 2,3$ to $\alpha 2,6$ specificity, did not affect NA inhibitor susceptibility of the engineered viruses in differentiated human bronchial epithelial cells, although many receptor variants were less susceptible in MDCK and SIAT1-MDCK cells (85a).

In order to detect changes in NA susceptibility, most studies have utilized enzyme inhibition assays for phenotyping and sequence analysis of the NA gene to detect

relevant mutations (6a). Real-time RT-PCR using labeled probes and more recently pyro-sequencing methods have been used to rapidly detect specific NA mutations (86, 86a). Both fluorometric and chemiluminescent NA inhibition assays are available, but all assays have limitations and may not reliably detect resistant subpopulations (87). No clinically validated thresholds for resistance (absolute concentrations or fold-changes compared to wild-type) have been determined for the different NA inhibitors using NA inhibition assays. Depending on the drug, virus strain, and assay inhibitory concentrations for clinically and laboratory selected NA variants have shown at least 10-fold to over 1,000-fold reductions in susceptibility (87).

The NA mutation conferring resistance depends on the drug and virus type and subtype. For oseltamivir, His274Tyr confers resistance in N1 but not N2-containing viruses (88), whereas Arg292Lys and Glu119Val are the most common resistance mutations in N2-containing viruses. Because of the differences in interaction among drugs with the active enzyme site, varying patterns of cross-resistance are found for particular NA mutations. Importantly, zanamivir retains full inhibitory activity against variants with either the His274Tyr or Glu119Val mutation and partial activity against the Arg292Lys variant (87, 89).

3.2 Susceptibility of Field Isolates

Studies utilizing both phenotypic susceptibility testing and NA sequence analysis have rarely documented primary (de novo) resistance to the NA inhibitors in community isolates of human influenza viruses until the 2007–2008 season (below). Natural variation occurs in susceptibility patterns, and the range of inhibitory concentrations may vary by tenfold or more within an NA type or subtype. The possible clinical importance of such differences is unknown. Both zanamivir and oseltamivir have also been shown to be active in vitro and in vivo against virus containing the neuraminidase of the 1918 pandemic strain (21) and against A(H5N1) and other avian viruses (90–92). Both drugs are active against the nine NA subtypes recognized in nature (92).

3.2.1 Surveillance Studies to 2006

Assays of large numbers of pretreatment isolates and those from placebo recipients during controlled clinical trials in both adults and children (reviewed in (93)) did not detect naturally occurring resistant variants to either zanamivir or oseltamivir. One large survey of 1,054 influenza isolates collected between 1996 and 1999 through the World Health Organization's Global Influenza Surveillance Network (GISN) found no instances of neuraminidase

resistance to zanamivir or oseltamivir in enzyme inhibition assays (Table 4) (94). Sequence analysis of isolates with inhibition values above the 95% confidence limits and other isolates found variation in some previously conserved resides but no recognized resistance mutations. Similarly, no resistance was observed in over 3,000 pretreatment isolates collected in clinical trials of oseltamivir (95).

Since introduction of the drugs into clinical practice, continued surveillance detected phenotypic resistance to oseltamivir in 8 of 2,287 community isolates (0.35%) collected during the influenza seasons from 1999 to 2002 (Table 4), four of which showed reduced susceptibility to zanamivir (80). Three isolates had a recognized resistance mutation (His274Tyr in H1N1; Asp198Glu and Ile222Thr in B) but other several new NA mutations were detected that might have contributed to reduced susceptibility. As these viruses were not obtained from persons taking a NA inhibitor, the results would indicate that either transmission of resistant variants was occurring from treated persons or that low level of de novo resistance occurs. Similarly, an Australian study of 532 strains collected between 1998 to 2002 (Table 4) found only one instance of apparent resistance (75), an influenza B/Perth/211/2001 isolate that contained a mixed population with resistant variants harboring a Asp198Glu mutation (reported above) (96, 97). A survey of 1,550 isolates collected worldwide in 2000–2002 reported very few outlier results and no confirmed isolates with resistance (98). A study in France did not find changes in A(H3N2) susceptibility to either agent nor recognized resistant variants over 3 years from 2002 to 2005, although four variants with deficient NA and resistance to both drugs in cell culture were described (99). Surveys of community isolates in Japan, which has had the highest per capita use of oseltamivir in the world, have found low frequencies of oseltamivir-resistance in influenza A (79) and B (100, 101) viruses. During the 2003–2004 season 0.3% of 1,180 H3N2 isolates harbored oseltamivir resistance mutations (79), whereas no resistance was found in influenza B viruses that season or in A(H3N2) and A(H1N1) isolates during the subsequent season (Table 4). However, 3.0% of 132 H1N1 isolates during the 2005–2006 season had the His274Tyr mutation that confers oseltamivir resistance (102). During the 2004–2005 season 1.7% of 422 influenza B isolates from untreated persons showed reduced susceptibility to neuraminidase inhibitors; four of these persons were likely infected in the community and three through household contact (100). These findings likely indicate low-level transmission of resistant variants in the community during periods of substantial oseltamivir use.

In addition to examining the frequencies of resistant variants in community isolates, most studies have not found evidence for secular trends indicating reduced NAI suscepti-

Table 4 Representative studies of oseltamivir and zanamivir susceptibility of field isolates of influenza A and B viruses to 2006-7

Study	Location	Seasons	Assay	No. tested	No. (%) resistant	Mutations detected
McKimm-Breschkin et al. (94)	Worldwide	1999–2002	NAI-FA, NAI-CL, S	139 A/N1	0	
				767 A/N2	0	
				148 B	0	
Hurt et al. (75)	Australia, South East Asia, Oceania	1998–2002	NAI-FA	235 A/N1	0	
				169 A/N2	0	
				128 B	1[a]	Asp197Glu
Hurt et al. (173)		2001–2006	NAI-FA	288 A/N1	1	His274Tyr
				540 A/N2	0	
				270 B	1[a]	Asp197Glu
Bovin and Goyette (74)	Canada	1999–2000	NAI-CL	38 H3N2	0	
				40 H2N1	0	
				23 B	0	
Mungall et al. (98)	Worldwide	2000–2002	NAI-CL	567 A/N2	0	
				271 A/N1	0	
				712 B	0	
Monto et al. (80)	Worldwide	1999–2002	NAI-CL, S	922 A/N2	3 (0.3%)	Gln41Gly, Gln226His
				622 A/N1	3 (0.5%)	His274Tyr, Tyr155His, Gly248Arg
				743 B	2 (0.3%)	Asp198Glu, Ile222Thr
Ferraris et al. (99)	France	2002–2005	NAI-FA, S	788 H3N2	0[b]	
Escuret et al. (99a)	France	2005–2006		151 H1N1	3 (2.0%)	1 His274Tyr, 2[c]
				225 B	1	1Asp198Tyr
NISN (79)	Japan	2003–2004	NAI-CL, S	1,180 H3N2	3 (0.3%)	2 Glu119Val, 1 Arg292Lys
				171 B	0	
Hatakeyama et al. (100)	Japan	2004–2005	NAI-FA, S	422 B	7 (1.7%)	3 Asp198Asn, 3 Ile222Thr, 1 Ser250Gly[d]
NISN (102)	Japan	2004–2005	NAI-CL, S	558 H3N2	0	
				60 H1N1	0	
		2005–2006	S	250 H3N2	0	
				132 H1N1	4 (3.0%)	4 His274Tyr
				61B	0	
		2006–2007	S	54 H1N1	0	
				134 H3N2	0	
				119 B	0	

NAI neuraminidase inhibition; *CL* chemiluminescence; *FA* fluorescence; *S* sequence analysis of neuraminidase gene; *NISN* Neuraminidase Inhibitor Susceptibility Network. Amino acid numbering based on N2 neuraminidase.

[a] One B/Perth/211/2001 isolate had 7- to 9-fold reduced susceptibility to zanamivir and 14- to18-fold to oseltamivir compared to the mean inhibitory concentrations of influenza B strains and contained a mixed population including resistant variants with a Asp197Glu mutation (96)

[b] Four isolates (0.5%) with NA deficiency were found to be resistant to NA inhibitors in cell culture-based assays

[c] Two A(H1N1) isolates had 9- and 30-fold reduced susceptibility to zanamivir but no loss of oseltamivir susceptibility nor apparent NA mutation (99a).

[d] Asp198Asn confers resistance to both oseltamivir and zanamivir, Ile222Thr to oseltamivir, and Ser250Gly to zanamivir (100).

bility of influenza NAs since the drugs have been introduced into practice. However, one survey in the United Kingdom found that the susceptibility of influenza B, but not influenza A, NAs to both oseltamivir and zanamivir had decreased by over 50% since 1997 (101). Such changes may be related to natural genetic evolution in viral NA unrelated to drug use but emphasize the importance of continued surveillance.

3.2.2 A(H1N1) Viruses

In January 2008, WHO was notified about a high prevalence (75%) of oseltamivir resistance due to the His274Tyr muta-tion in seasonal influenza A(H1N1) viruses in Norway (6, 6a). Depending on the assay methods, this mutation is associated with 350-fold to >1,500-fold reductions in N1 susceptibility in enzyme inhibition assays (87, 89, 103, 104) and lack of response in vivo (104a, 119, 120). Subsequent surveillance through WHO's GISN and the European Influenza Surveillance Scheme (EISS) found an overall 16% frequency of resistance in community A(H1N1) isolates during the 2007–8 northern hemisphere season (105, 105a). However, many countries were unaffected and wide variations in resistance prevalence existed within Europe and in different regions of the world. In comparison, no viruses with the His274Tyr mutation were detected among 139 A(H1N1)

isolates collected globally through GISN from 1996 to 1999 (94) and this mutation was rarely detected in community isolates subsequently, except for the 2005-6 season in Japan (Table 4) (80, 102). The unexpected high prevalence of oseltamivir resistance in A(H1N1) viruses in many parts of the world within a single season indicated efficient person-person transmission. Available evidence indicated that selective drug pressure was not driving this phenomenon, and Japan had a notably low rate of resistant A(H1N1) viruses. Although several oseltamivir-resistant A(H1N1) variants belonging to clade 2B were detected globally, a predominate antigenic drift variant A/Brisbane/59/2007(H1N1) lineage, that is resistant to oseltamivir but susceptible to zanamivir and M2 inhibitors (105, 105a) emerged and continued to circulate in the southern hemisphere and subsequently northern hemispheres in 2008–2009. These viruses replicated efficiently and caused typical influenza illness including severe and sometimes fatal infections (104a). The circulation of H1N1 viruses naturally resistant to oseltamivir emphasizes that genetic variations may result in variations in sensitivity to oseltamivir in the absence of apparent selective drug pressure.

Sporadic zanamivir resistance occurs at low frequency in community A(H1N1) isolates (80, 99a). Variants that show about 100-fold less susceptibility to zanamivir but sensitivity to oseltamivir, related to a Q136K mutation in NA, have been reported from the Philippines and Australia (106).

3.2.3 A(H5N1) Viruses

Almost all avian A(H5N1) viruses isolated from birds and humans have been susceptible to NA inhibitors. However, one survey of avian A(H5N1) isolates collected between 2004 and 2006 in Southeast Asia found that two of 55 viruses showed approximately 4- to 16-fold reduced susceptibility to oseltamivir and one of these had 63-fold decreased susceptibility to zanamivir (107). Novel NA mutations of potential significance (Ile117Val, Val116Ala) were detected in these isolates. In addition, rare avian isolates harboring the His274Tyr mutation have been detected (108, 109), and one study reported that recent avian A(H5N1) positive samples have preexisting resistant subpopulations possessing this mutation (109). One clade 1 A/Vietnam/JP36-2/05 virus was transmissible in ferrets and showed apparent emergence of the H274Y mutation in an infected recipient animal (109a). Avian A(H5N1) viruses possessing an Asn294Ser NA mutation that confers 12- to 15-fold or greater reductions in oseltamivir susceptibility have been detected in two fatal cases before initiation of oseltamivir therapy and also in some isolates from birds (110).

Most clade 1 A(H5N1) viruses from 2004 to 2005 appear to have increased susceptibility to oseltamivir carboxylate compared to 1997 isolates from Hong Kong or human A(H1N1) viruses in enzyme inhibition and cell culture assays (111, 112, 114, 114a, 114b). The increased oseltamivir susceptibility has been postulated to be related to amino acid changes at residues 248 and 252 surrounding the active site (111). The susceptibility of A(H5N1) viruses to oseltamivir, but not to zanamivir, varies up to 30-fold in enzyme inhibition assays with clade 2 viruses being less susceptible than early clade 1 viruses (113, 113a). While the clinical importance of such susceptibility variations is uncertain, these differences in in vitro susceptibility correspond to some extent with the dose levels of oseltamivir needed to reduce replication in murine and ferret treatment models (114). Despite increased susceptibility of some clade 1 viruses, their virulence and rapid replication kinetics require higher oseltamivir doses to inhibit replication in animal models (112, 114, 114a, 114b). Resistance emergence has been documented very uncommonly in murine and ferret treatment models to date (63a, 112, 114a, 114b).

3.2.4 HA Mutations

Clinical isolates possessing HA mutations that induce cross-resistance to NA inhibitors in cell culture, but no NA changes, have been described. One such HA variant (Arg229Ile) showed reduced binding to MDCK cell receptors and over 100-fold reduced susceptibility in MDCK cells but full susceptibility in ferrets (85). Similarly, apparent reductions in the zanamivir susceptibility of circulating A(H3N2) viruses in MDCK cell culture are linked to specific changes in HA (Leu226Ile/Val) that alter receptor binding without associated NA mutations (115), but zanamivir has been shown to be effective against H3N2 viruses in controlled clinical trials.

3.3 Resistance in Posttreatment Isolates

Oseltamivir-resistant viruses with NA mutations have been detected in clinical trials when the drug has been used for influenza treatment (reviewed in (95)) (Table 5). No zanamivir-resistant viruses have been recovered in zanamivir-treated immunocompetent hosts, although the number of paired isolates studied has been low and limited by the need for pharyngeal or lower respiratory isolates (71, 116). For both zanamivir and oseltamivir, no resistant variants have been detected in immunocompetent persons receiving drug for chemoprophylaxis of seasonal influenza to date (117).

3.3.1 Immunocompetent Hosts

In natural infections, oseltamivir-resistant variants have been detected much more commonly in treated children than adults

Table 5 Frequency of resistance emergence to oseltamivir or zanamivir during treatment of influenza A and B virus infections

Drug/Study	Population	Assay	Virus type	Number of isolates tested	No. (%) resistant	Mutations detected
Oseltamivir						
Gubareva et al. (119)	Adults	NAI, S	A/H1N1	54	2 (4%)	2 His274 Tyr
Roberts et al. (118)	Adults	NAI, S	A/H3N2	418	5 (1%)	4 Arg292Lys, 1 Glu119Val
Whitley et al. (123)[a]	Children – outpatient	NAI, S	A & B	150 A 66 B	10 (6.7 %) 0	8 Arg292Lys, 1 Glu119Val, 1 His274Tyr
Kiso et al. (124)[a]	Children – outpatient + hospitalized	Cloning + S	A/H3N2	50	9 (18%)	6 Arg292Lys, 2 Glu119Val, 1 Asn294Ser
Ward et al. (122)[a]	Children – outpatient + hospitalized	NAI, S	A/H1N1	43	7 (16%)	7 His274Tyr
Hatakeyama et al. (100)	Children – outpatient	NAI, S	B	74	1 (1.4%)	Gly402Ser
Democratis et al. (125)	Children – outpatient	S	A/H1N1	11	3 (27%)	3 His274Tyr
			A/H3N2	34	1 (3%)	Arg292Lys
			B	19	0	
Zanamivir						
Barnett et al. (116)	Adults	NAI, S	A + B	41	0	

[a] These pediatric studies used a 2 mg/kg dose of oseltamivir that has been shown to give reduced drug exposure because of more rapid clearance in children under the age of 5 years. Insufficient drug exposure may have contributed to resistance emergence in these studies

[b] This influenza B isolate showed 7-fold and 4-fold reduced susceptibility to zanamivir and oseltamivir, respectively and high IC50 values to oseltamivir (100).

(Table 5). Analysis of samples from over 2,500 influenza patients treated with oseltamivir as outpatients indicates that the frequency of resistance detection has been about 10-fold lower in adults than children (118) (Table 4). Resistance may emerge more readily in influenza A infections but has been reported in influenza B (100). In contrast to in vitro observations, no HA resistance mutations have been detected in those with NA mutations conferring oseltamivir resistance (118).

Among 54 volunteers experimentally infected with an A(H1N1) virus, oseltamivir-resistant variants with His274Tyr mutation were detected in two subjects in association with apparent rebounds in viral replication (119). In addition, this study found that oseltamivir-treated subjects were less likely than placebo to have late viral isolates showing reversion of the egg-adapted inoculum virus to a human receptor HA genotype. The His274Tyr finding suggests that HA mutations with reduced affinity for human receptors might have a replication advantage over viruses with human receptor preference during oseltamivir use in humans. This mutation has also been detected in infected children (Table 5), immunocompromised hosts (below) and in several patients infected with A(H5N1) virus (120, 121). One study of A(H1N1)-infected children from Japan reported a frequency of 16% resistance emergence with this mutation (122). In two A(H5N1) patients, including the one who was treated within 2 days of the onset of illness onset and had resistant virus detected on day four of the treatment, and the emergence of the His274Tyr resistance mutation in the upper respiratory tract was associated temporally with persistent viral replication and fatal outcome (121). Another A(H5N1)-infected patient had emergence of resistant clones with this mutation during oseltamivir administration at prophylactic doses but survived and had a cleared virus after the dose was increased (120).

One treatment study of outpatient children, most of whom had influenza A(H3N2) illness, detected resistant variants in 5.5% of 182 patients who were culture positive and for whom adequate data were obtained (Table 5) (123). The resistant variants were all influenza A, typically detected on day 6, and were not recovered on day 10. The clinical course of oseltamivir-treated patients who shed resistant variants has not differed appreciably from those who did not shed such variants (117, 123). Another Japanese study of mostly hospitalized children that utilized molecular techniques for detection of resistant clones found that 18% of 50 influenza A(H3N2)-infected children harbored viruses with NA mutations conferring resistance (124). The viruses with NA mutations fully replaced the wild-type in three cases and co-existed with wild-type in six others; they emerged as early as on day 4 of the treatment, persisted to day 7, and appeared to be associated with more prolonged shedding. The use of weight-based dosing for children in Japan, as contrasted with unit dosing in most countries, is associated with lower drug exposure in young children and has been postulated to be a major factor in the higher frequency of resistance detected in these studies. However, a recent study found the His274Tyr mutation emerge in 3(27%) of 11 A(H1N1)-infected children receiving weight-adjusted doses (125), although the frequencies of influenza A/H3N2 and B virus resistance detection were low in this trial (Table 4). The higher frequency of resistance emergence in young children, likely experiencing

their first or second influenza infection, may be relevant to the expected frequency of resistance during NA inhibitor use in a pandemic or outbreak due to a novel virus (124).

3.3.2 Immunocompromised Hosts

Several case reports have documented the emergence of NA resistance in highly immunocompromised hosts with influenza but the risk has not been well defined. During several weeks of therapy with inhaled zanamivir, one 18-month-old bone marrow transplant recipient had prolonged shedding of an influenza B virus, first with an HA mutation (Thr198Ile) that reduced affinity for human cell receptors and altered antigenicity and later with a dual variant that also possessed an NA catalytic site mutation (Arg152Lys) conferring 1,000-fold reduction in neuraminidase susceptibility by enzyme inhibition assay (126). Of note, the later isolate showed a tenfold increase in susceptibility to zanamivir in several cell cultures, a finding that demonstrates the unreliability of cell culture-based phenotypic assays. A 23-year-old male who underwent bone marrow transplantation for acute lymphocytic leukemia documented persistent influenza A infection of the upper respiratory tract for 18 months despite sequential courses of amantadine, oseltamivir, rimantadine, and ultimately zanamivir (45). An influenza A(H1N1) isolate with dual resistance to M2 inhibitors and oseltamivir, but not zanamivir, and possessing an His274Tyr substitution in NA was documented for the last six months of his life. Several immunocompromised patients have experienced emergence of influenza A(H3N2) viruses harboring both Glu119Val in NA and M2 inhibitor resistance mutations, perhaps fostered by sequential antiviral therapy (127, 128). One of these patients died with continued replication of dually resistant virus, whereas two others survived, including one who had persistent excretion of resistant virus for eight months after cessation of oseltamivir (128). In addition, one instance of apparent failure of oseltamivir prophylaxis for influenza B with subsequent emergence of a resistant variant with an Asp198Asn has been reported (127). Quasi-species of resistant and susceptible subpopulations may be present. However, the frequency of resistance emergence is undefined in such patients, and oseltamivir appears to be a useful therapy in most immunocompromised patients (129, 129a). One prospective study of 38 bone marrow transplant patients with acute influenza treated with oseltamivir, including 12 before engraftment, reported only two episodes of pneumonia and no influenza-related deaths (129). Antigen positivity was detected for 7 days or more in 8% of those treated but no resistance studies were performed. Oseltamivir is also active in an immunocompromised SCID mouse model, although resistant variants arise in some treated animals (129b). In a study of seven bone marrow transplant patients given inhaled zanamivir, treatment was continued until excretion of virus ceased (median 15 days, range 5–44 days) (130); despite four presenting with evidence for lower respiratory involvement, symptoms resolved promptly and no influenza mortality occurred. Careful virologic monitoring of immunocompromised hosts treated with anti-influenza agents is warranted to document clearance of infection.

3.4 Transmissibility of Resistant Variants

Human to-human transmission of NA inhibitor resistant variants appeared to be rare until the 2007–2008 season, although the number of studies examining this question was small and the surveys of community isolates discussed above show the potential. One study during an influenza B epidemic in Japan provided strong epidemiologic and virologic evidence for transmission of oseltamivir-resistant variants among siblings in three households (100). However, in contrast to M2 inhibitors, oral oseltamivir and inhaled zanamivir are both effective for postexposure prophylaxis of influenza in household settings, whether the ill index cases are treated or not with the same drug (Table 3). However, the oseltamivir-resistant A(H1N1) viruses with His274Tyr mutation spread efficiently during the 2007–2009 seasons and caused household transmission and several nosocomial outbreaks (6).

The reduced fitness and replication competence of certain NA mutations appears to correlate with reduced transmissibility in animal models. One study inoculated ferrets intranasally with comparable infectious doses of a clinical isolate of wild-type influenza A/Sydney/5/97(H3N2) virus or its oseltamivir-resistant variant containing the Arg292Lys substitution and found that the mutant virus was associated with lower infectivity (50% versus 100%), 10- to 100-fold lower nasal viral titers, and lack of transmission to susceptible contact animals, in contrast to 100% transmission with wild-type virus (131). One donor ferret inoculated with the Arg292Lys-containing resistant virus transmitted wild-type virus to several contacts, but it is unclear whether this resulted from reversion of the resistant variant, emergence of a wild-type subpopulation, or cross-contamination during the experiment. In contrast, similar studies with an influenza A(H3N2) virus possessing the Glu119Val resistance substitution found that the variant was as transmissible as the parental, wild-type virus and resulted in comparable nasal viral titers in both donor and recipient animals (132). In guinea pigs recombinant human influenza A/H3N2 viruses with the Glu119Val or dual Glu119Val and Iso222Val mutations had similar infectivity as wild-type and were transmitted efficiently by direct contact; however, in contrast to wild-type virus, the oseltamivir-resistant viruses transmitted poorly or not at all by aerosol (132a). An influenza A(H1N1)

Table 6 Effects of NA mutations that confer oseltamivir resistance on viral fitness measures in representative influenza A and B viruses

Virus (subtype) (reference)	Mutation	Enzyme activity or stability (% of parental virus)	Infectivity in mice/ferret	Replication in ferret	Transmissibility in ferret
A/Wuhan/359/95(H3N2) Yen et al. (83) A/Wuhan-like/98(H3N2) Herlocher et al. (132)	Glu119Val	↓	↓/–[a]	–	–
A/Wuhan/359/95(H3N2) Yen et al. (83) A/Sydney/5/97(H3N2) Herlocher et al. (131); Carr et al. (133)	Arg292Lys	↓↓ (2%)	↓ (>100-fold) / ↓ (>100-fold)	↓↓ Reversion to wild-type observed	0 or ↓↓
A/Texas/36/91(H1N1) Ives et al. (135) A/New Caledonia-like/01(H1N1) Herlocher et al. (132)	His 274 Tyr	–	↓ (>1,000-fold) / ↓ (>100-fold)	– or ↓[b]	– (1–2 day delay)
A/WSN/33(H1N1) Abed et al. (104)	His 274 Tyr Asn294Ser	NR NR	–/NR ↓/NR	NR NR	NR NR
A/Puerto Rico/8/34(H1N1) Yen et al. (136)	His274Tyr Asn294Ser	↓[c] ↓[c]	–/NR –/NR	NR NR	NR NR
A/Hanoi/30408/05(H5N1) Le et al. (120) A/Vietnam/1203/04(H5N1) Yen et al. (136)	His274Tyr Asn294Ser	↓ ↓	–/– –/–	↓ NR	NR NR
B/Rochester/02/2001 Mishin et al. (89)	Asp198Asn	NR	NR	–	NR
B/Memphis/20/96 Gubareva et al. (126) B/Beijing/1/87 Jackson et al. (139)	Arg152Lys	↓↓ (3–5%)	NR / ↓	↓	NR

– no change compared to wild-type; ↓ decreased; *O* absent; *NR* not reported

[a] One ferret study reported that infectivity was decreased at least 100- to 1,000-fold, and the resistant variant reverted to wild-type (175) but another study indicate full retention of replication and transmissibility in ferrets (132)

[b] Differing results from two studies with different A(H1N1) viruses

[c] Despite reductions, the A(H5N1) neuraminidase retained high levels of enzymatic activity that greatly exceeded that of A/PR8(H1N1) virus (136)

harboring the His274Tyr mutation required a 100-fold higher inoculum to infect donor ferrets, but once infected, they transmitted infection to contact animals with a delay of 1–3 days compared to wild-type virus. These studies indicate that the degree of compromise in transmissibility varies with the particular NA mutation and ranges from the severely to the minimally compromised. The oseltamivir-resistant A(H1N1) viruses that have extensively transmitted during the 2007–2008 season remain to be studied in such models.

3.5 Pathogenicity

The highly conserved nature of the NA enzyme active site and the lack of circulating influenza strains in which NA is absent are postulated reasons that NA mutations would likely reduce the biologic fitness of the virus (95, 117). Detailed studies of the infectivity and virulence of clinical isolates possessing different NA mutations have been con-

ducted in experimentally infected animals. Most, but not all, of these NA variants show markedly reduced enzyme activity or stability and reduced fitness in animals compared to their drug-susceptible parents (Table 6). An influenza B virus with an Arg152Lys mutation showed only 3–5% of the enzymatic activity of its parent and was less infectious and associated with lower nasal viral titers in infected animals (126). Even when inoculated into ferrets at an infectious dose ratio of 60:1 (mutant:parent), the susceptible parent outgrew the mutant in the absence, but not in the presence, of zanamivir treatment. An A/Sydney/5/97(H3N2) virus containing a Arg292Lys substitution, replicated as well as a wild-type in MDCK cell culture but showed approximately 100-fold reduced infectivity and 10- to 1,000-fold lower lung viral titers in experimentally infected mice (133). In ferrets the resistant variant was approximately 100-fold less infectious than the wild-type and was associated with significantly lower nasal viral titers, nasal inflammatory cell counts, and for lower viral inocula, febrile responses. Similarly, an A/Victoria/3/75(H3N2) variant possessing

both the Arg292Lys mutation and hemagglutinin substitutions was about 10,000-fold less infectious in mice (134). In comparison, an influenza A(H3N2) with the Glu119Val mutation appears to replicate to comparable levels and cause similar febrile responses compared to its respective parental viruses (132) (Table 6).

Oseltamivir-resistant A(H1N1) viruses that circulated in the 2007–2008 season replicated in vitro as well as susceptible strains (134a) and caused typical influenza illness including complications, hospitalizations, fatalities in previously healthy and high-risk hosts (6, 104a). Findings with clinical isolates of influenza A(H1N1) virus possessing the His274Tyr mutation have varied in animal model studies (Table 6). One found that an A/Texas/36/91(H1N1) variant replicated less well in MDCK cell culture and that its infectivity and/or replication were severely compromised in mice and ferrets (135) (Table 6). In addition, inocula that resulted in comparable levels of replication in the upper respiratory tract were associated with reduced nasal inflammatory cell and febrile responses in ferrets compared to the wild-type virus. In contrast, a study of an A/New Caledonia(H1N1) variant found that, although less infectious for ferrets, infected animals had similar nasal viral titers and febrile responses as those infected with the wild-type virus (132). A laboratory virus A/WSN(H1N1) genetically engineered to posses the His274Tyr mutation was as virulent and replication competent in mice as the parental virus (104). However, a clone from a clinical H5N1 isolate with this mutation grew less well in ferrets (>10-fold reduction in lung titers) and was less pathogenic compared to an oseltamivir susceptible clone (120). Of note, while oseltamivir inhibited replication of the susceptible virus in the ferret model, it had no effect on replication of resistant virus. As predicted by in vitro susceptibility testing, zanamivir was inhibitory for both viruses. However, studies of another clade 1 A(H5N1) virus harboring the His274Tyr mutation found no differences in viral replication levels or lethality compared to susceptible, wild-type virus in mice (136).

The Asn294Ser mutation has been recognized in both N2 and N1-containing viruses; it is associated with much greater loss of in vitro susceptibility in N2 than N1 viruses but retains susceptibility to zanamivir (104, 120). For one laboratory adapted A(H1N1) virus, this mutation has been associated with reduced replication in cell culture and in mice, as well as reduced lethality in mice (104). Lower infectivity, replication, and pathogenicity for ferrets has been reported following infection by an A(H3N2) virus with this mutation (presented by J Oxford, 9th ISRVI, Hong Kong, March 2007). Studies of an A(H5N1) virus with this mutation indicated no reduction in pulmonary viral replication or lethality in mice (136).

3.6 Treatment Alternatives

The patterns of NA inhibitor cross-resistance vary by virus type and subtype, such that zanamivir retains inhibitory generally activity for the most common resistant variants that emerge during therapeutic use of oseltamivir. Zanamivir is fully inhibitory for oseltamivir-resistant variants possessing the Glu119Val substitution in N2 or His274Tyr or Asn294Ser in N1 (87, 104). Depending on the virus and assay, zanamivir is partially inhibitory for resistant variants with Arg292Lys substitution in N2, in that the loss of susceptibility is about 5- to 25-fold compared to the wild-type (83, 87, 89, 104, 138). Inhaled zanamivir has been used in treating immunocompromised hosts (130), including a few who had virologic failure on oseltamivir (104a, 138), but it has not been systematically studied in oseltamivir-resistant infections. Patients with pneumonia may not respond (138a). Oseltamivir is not inhibitory for the Arg152Lys mutation in influenza B NA that confers reduced susceptibility to zanamivir (139).

Parenterally administered NA inhibitors are under clinical investigation at present. Of note, zanamivir is highly active after intravenous administration (140), which provides peak plasma concentrations that are approximately 100-fold higher than those achieved after oral oseltamivir administration. Peramivir retains at least partial inhibitory activity against many variants with oseltamivir resistance mutations (103) and has been shown to be active after intravenous or intramuscular injection in animal models of influenza (137, 141), including infections due to A(H5N1) viruses (142). Phase 2 human trials of parenteral peramivir are in progress (69a). Other NA inhibitors inhibitory for most oseltamivir-resistant variants and zanamivir dimers that have prolonged duration of antiviral effect after topical application are currently under development (143). These may provide NA inhibitor prevention and perhaps treatment alternatives in the future.

Because of their differing mechanism of antiviral action, M2 inhibitors generally retain activity against influenza A viruses resistant to NA inhibitors and would be appropriate agents for prophylaxis or treatment of suspected NA inhibitor-resistant infections, if the circulating strain was known to be susceptible. Ribavirin would also be expected to be inhibitory for influenza A and B viruses resistant to the NA inhibitors, but there are no reports of its use in human influenza infections due to such variants. Ribavirin combined with a neuraminidase inhibitor exerts additive to synergistic antiviral activity in vitro (144, 145). In mice experimentally infected with influenza A, the combination of orally administered ribavirin and peramivir was associated with improved survival relative to ribavirin alone but not to peramivir alone (144). A combination of ribavirin and oseltamivir was no more effective than ribavirin alone against a lethal influenza A(H1N1) infection but superior to single agents against

influenza B (146), whereas this combination showed additive effects against two highly pathogenic H5N1 influenza viruses in mice (114a). Further studies of such ribavirin-NA inhibitor combinations are warranted to determine whether this strategy offers the possibility of treating severe influenza, particularly that due to M2 inhibitor-resistant viruses.

4 Modeling Studies

Various mathematical models have been developed to estimate the emergence and transmission of drug-resistant influenza viruses and the associated implications for antiviral effectiveness (147–150). At the population level, the transmission fitness of resistant variants is the key determinant of their impact, in addition to the frequency of resistance emergence (148, 149). In addition to out-competing the wild-type virus in the presence of selective drug pressure, substantial transmission of resistant variants would require that their absolute transmissibility be sufficient to enable sustained spread. During seasonal influenza, even high rates of oseltamivir use are predicted to not cause substantial resistance transmission in the community as long as viral fitness is compromised (151). When this model assumed a 10% relative transmissibility of oseltamivir-resistant variants compared to the wild-type virus, even extensive use of oseltamivir for treatment (40% coverage of ill persons) resulted in low levels of resistant variants circulating in the community after several seasons of use (151). A closed population outbreak model predicted that the combination of antiviral treatment and prophylaxis would reduce the total number of infected persons but increase the fraction of resistant infections compared to treatment only of ill persons (149). Some of these models have attempted to determine the importance of drug resistance emergence by making linkages between viral replication dynamics at the individual level and transmission of resistant virus in particular populations (152, 153). Resistance emergence at the individual level depends strongly on specific within-host dynamics of influenza infections, including viral mutation rate, the fitness costs of resistance mutations, and the effects of host immune responses and antiviral administration on replication.

In a pandemic scenario, the ability of antiviral interventions to lower attack rates depends heavily on the magnitude of antiviral effectiveness, the timing of their application, the fitness costs, if any, of resistance and development of compensatory mutations (153a). Very early antiviral treatment, particularly prophylaxis, would be predicted to reduce the risk of resistance emergence in some models (152, 153). However, even very low rates of resistance emergence in persons receiving antiviral treatment or prophylaxis would strongly promote the spread of resistant

variants in a pandemic (150). Prophylaxis, by eliminating competition from drug-susceptible virus, might increase substantially the number of resistant cases, if resistant variants have sufficient transmission fitness (149, 150). The extent of antiviral use is predicted to affect the extent of resistance transmission, in part because inhibiting the spread of drug-susceptible strains with antiviral interventions in the absence of an effective vaccine would keep a pool of susceptibles to resistant strains. Modeling predicts that when two drugs are available, allocating different drugs to cases and contacts is likely to be most effective at constraining resistance emergence (147). Resistance concerns indicate the importance of stockpile diversification.

However, many assumptions used in these models have been based primarily on observations from experimental influenza infections of immunocompetent young adults and sometimes animals, so that they require validation by studies in seasonal influenza, whenever possible. Furthermore, their predictive values in pandemic influenza are uncertain. For example, in a pandemic the emergence of resistant variants that are transmissible is more likely with a higher intrinsic reproduction number (R_0) of the wild-type virus (152). In addition, it is possible that an initially less fit resistant variant will acquire further compensatory mutations that would allow it to replicate as well as be a susceptible, wild-type virus in the absence of selective drug pressure. The recent experiences with widespread circulation of M2 inhibitor-resistant A(H3N2) and A(H1N1) viruses and of oseltamivir-resistant A(H1N1) viruses underline this potential.

5 Implications and Future Research Directions

The available evidence indicates that future pandemic and epidemic influenza A viruses may show de novo resistance to M2 inhibitors, whereas it is much less likely that such a strain would show primary resistance to NA inhibitors (1). Of concern, M2 inhibitor resistance has been documented in high frequencies of recent A(H3N2) and A(H1N1) clinical isolates, selected swine isolates resulting in human infection, and in many recent human isolates of avian influenza A(H5N1) viruses. Furthermore, the frequency of resistance emergence during therapy is substantially higher with M2 inhibitors than NA inhibitors and, for both classes, is higher in children than adults. Higher frequencies of antiviral resistance emergence would be expected in treatment of pandemic compared to interpandemic influenza. However, the recent circulation of oseltamivir-resistant A(H1N1) viruses in the absence of selective drug pressure highlights the uncertainty with regard to future neuraminidase inhibitor susceptibility patterns. Thus, the clinical and epidemiologic implications

of antiviral resistance in a future pandemic influenza virus cannot be predicted with confidence, and mechanisms to rapidly monitor the susceptibility patterns of circulating strains are needed to guide recommendations for antiviral use in both seasonal and pandemic influenza.

5.1 Current Clinical Use of Antivirals

Because of high antiviral resistance frequencies in epidemic influenza A strains, M2 inhibitors are not reliable for prophylaxis or treatment at present. They remain valuable agents for prophylaxis of influenza A illness, providing that the circulating strain is likely to be susceptible, and are an option for treatment of oseltamivir-resistant A(H1N1) illnesses when zanamivir cannot be used, as with recently circulating oseltamivir-resistant A(H1N1) viruses. They are also an option for combined treatment with NA inhibitors in serious infections when the locally circulating strains are susceptible. This strategy also applies for A(H5N1) virus infections (63, 63a, 154).

M2 and NA inhibitors retain activity against variants resistant to the other class, although dual resistance has emerged in highly immunocompromised hosts. Consequently, NA inhibitors can be used for treatment and prevention of M2 inhibitor-resistant virus infections and, when circulating strains are M2 inhibitor susceptible, vice versa. While M2 inhibitor resistance confers resistance to all drugs in this class, NA inhibitor resistance patterns vary with drug and virus type/subtype, such that zanamivir retains activity for many, including His274Tyr and Asn294Ser in N1, Glu119Val in N2, and Iso222Thr in B, but not all oseltamivir-resistant variants. Several influenza B (e.g., Asp198Asn, Asp198Asn, Arg152Lys) and A/N1 (Tyr155His) mutations result in cross-resistance, and the Arg292Lys mutation in N2 causes substantially reduced zanamivir susceptibility. Zanamivir is an important alternative for prophylaxis and treatment in uncomplicated illness, especially when resistance to other available agents is suspected. However, the effectiveness of inhaled zanamivir in serious lower respiratory illness including A(H5N1) disease is uncertain, and resistance to zanamivir has also been reported rarely in community isolates of A(H1N1). These uncertainties reinforce the importance of continued monitoring of community isolates to assess whether increasing frequencies of resistance might affect drug effectiveness.

5.2 Future Research Directions

A substantial number of unanswered questions remain regarding antiviral drug resistance in influenza viruses. New NA mutations in N1, N2, and B neuraminidases continue to be recognized and their associated phenotypic susceptibility and fitness consequences require study (6a, 174). The role of compensatory mutations in the target proteins that enhance viral fitness are not well understood. Further data on the duration and levels of drug-resistant variants in the upper and lower respiratory tract of treated persons, in comparison to those observed in nontreated persons or in those treated with the other class of antivirals would be helpful in predicting the likelihood of transmission and whether there are differences between drug-susceptible and resistant viruses for either M2 or NA inhibitors. From a therapeutic perspective it remains to be established whether alternative dosing regimens or, especially in seriously ill persons, combinations of antivirals might be able to prevent or mitigate the frequency of resistance emergence and improve clinical outcomes. For management of individual patients, particularly seriously ill or immunocompromised hosts, the development of improved assays to rapidly detect resistant variants would enable selection of appropriate initial antivirals for treatment and therapeutic monitoring. Continued surveillance of antiviral susceptibility patterns in human and animal influenza viruses, especially community isolates in countries with higher antiviral use, and for resistance transmission in high-risk epidemiologic settings is needed.

The development of antiviral agents with activity against viruses resistant to currently available agents remains a priority. In addition to the parenteral neuraminidase inhibitors discussed above, other potential inhibitors with activity in animal models, including activity against A(H5N1) virus, include the polymerase inhibitor T-705 (155, 156), neutralizing antibodies (157–159), and the receptor-destroying sialidase DAS181 (160, 161). These approaches are entering into clinical study at present (69a).

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