Special article

Neuraminidase Inhibitor Susceptibility Network position statement: antiviral resistance in influenza A/H5N1 viruses

Frederick Hayden[1], Alexander Klimov[2], Masato Tashiro[3], Alan Hay[4], Arnold Monto[5], Jennifer McKimm-Breschkin[6], Catherine Macken[7], Alan Hampson[8], Robert G Webster[9], Michèle Amyard[10] and Maria Zambon[11]*

[1]Division of Infectious Disease and International Health, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA
[2]Chief, Strain Surveillance Section, CDC, Atlanta, USA
[3]National Institute of Infectious Diseases, Tokyo, Japan
[4]MRC National Institute for Medical Research, London, UK
[5]University of Michigan School of Public Health, MI, USA
[6]CSIRO Division of Health Sciences and Nutrition, Melbourne, Australia
[7]Los Alamos National Laboratory, New Mexico, USA
[8]WHO Collaborating Centre for Influenza Reference & Research, Melbourne, Australia
[9]St Jude Children's Research Hospital, Memphis, TN, USA
[10]Université Claude Bernard, Lyon, France
[11]Health Protection Agency, London, UK

*Corresponding author: Tel: +1 434 924 5059; Fax: +1 434 924 9065; E-mail: FGH@Virginia.edu

The emerging epidemic of H5N1 avian influenza virus with spillover into the human population in Asia has provoked intense concern globally about the potential of these particularly pathogenic viruses to evolve with the capacity for human-to-human transmission with a consequent pandemic. The availability of antiviral drugs with activity against influenza A viruses and the recognition of drug-resistant variants to these drugs prompted the following report by a select group of the global experts – members of the Neuraminidase Inhibitor Susceptibility Network – on the best use of the available drugs, both for prophylaxis and treatment. The editors of *Antiviral Therapy* are pleased to be able to provide this document in an expeditious manner.

The NISN: an introduction

The global Neuraminidase Inhibitor Susceptibility Network (NISN) was established in 1999 to address public health and regulatory concerns regarding the potential emergence and consequences of drug resistance in influenza viruses following the introduction of the influenza neuraminidase inhibitor (NI) class of antiviral agents. The broad objectives of the Network are to (i) provide a coherent approach to global NI resistance monitoring from both public health and research perspectives; (ii) to examine data from the scientific literature and from specific monitoring programmes to make recommendations for appropriate general strategies and specific assays for monitoring resistance; (iii) to conduct longitudinal prospective surveillance for resistance emergence through a link with the existing WHO Global Influenza Surveillance Network; and (iv) to communicate this information to the scientific community [1].

Update on emerging antiviral resistance

Several recent reports on the detection of drug-resistant variants in human isolates of H5N1 and H3N2 influenza viruses have provoked considerable discussion regarding the possible implications for clinical management and pandemic antiviral stockpiling [2–4]. The principal concerns with respect to antiviral resistance in A/H5N1 and other influenza viruses are that (i) emergence during drug administration will result in failure to inhibit viral replication and be associated with clinical progression, similar to that which might

occur in the absence of drug administration, and (ii) resistant variants will transmit to contacts and spread in the community to cause failures of drug treatment and prophylaxis.

A decade-long surveillance study found marked increases over the past 3 years in the proportions of H3N2 community isolates that harbour a particular M2 inhibitor (amantadine/rimantadine) resistance mutation (Ser31Asn). During 2004–2005, over 70% of isolates from China and Hong Kong and nearly 15% of those from the USA and Europe displayed this resistance marker [2]. The same M2 inhibitor resistance marker was found in two A/H5N1 human isolates acquired in Fujian Province China in 2003 [5] and in one lineage of H5N1 viruses that has caused severe human infections in Thailand, Vietnam and Cambodia [6]. By contrast, most isolates from a second H5N1 lineage that is circulating in Indonesia and, more recently, in China, Mongolia, Russia, Turkey and Romania and that appears to have been spread via migratory waterfowl through central Asia to Europe, are amantadine sensitive. However, some avian and human isolates from this lineage in Indonesia possess M2 inhibitor resistance [A Klimov and A Hay, unpublished observations]. Of note, M2 inhibitor resistance was not found in avian influenza viruses collected in southeast Asia during 1979–1983 but was detected in 31% of 135 H5 and 11% of 47 H9 avian isolates collected during 2000–2004; 62% of the H5 and 100% of the H9 viruses had the Ser31Asn mutation as the basis for resistance [7].

In one case report, a respiratory sample collected on the fourth day of oseltamivir treatment was shown to contain an A/H5N1 virus mixture (A/Vietnam/30408/2004) that was partially resistant to oseltamivir by neuraminidase (NA) inhibition assay and contained both drug-susceptible and drug-resistant virus clones [3]. The majority of the resistant clones had the His274Tyr NA mutation, which is known to confer high-level oseltamivir resistance in N1 NAs; some had an Asn294Ser mutation, which was associated with moderate oseltamivir resistance. This sample was collected from a teenager who had received 3 days of oseltamivir prophylaxis with once-daily dosing but who, in retrospect, was symptomatic and most probably already infected before starting the drug. Increased fever and respiratory complaints on prophylaxis prompted an increase to a standard twice-daily therapeutic dose of oseltamivir, after which no further samples were positive for virus and recovery ensued.

A recent second report describes two patients with emergence of oseltamivir-resistant A/H5N1 during or after therapy in association with fatal outcomes [4]. One 13-year-old girl who presented with fever and focal pneumonia received conventional therapeutic doses of oseltamivir starting 1 day after illness onset and continuing for total of 5 days. She developed increasing pneumonia and respiratory distress on the fourth treatment day in association with detection of A/H5N1 virus harbouring the His274Tyr mutation in her pharynx and died 3 days later (2 days after cessation of therapy) with continued pharyngeal detection of resistant virus. An 18-year-old patient had resistant virus with the His274Tyr mutation isolated 3 days after completion of a 5-day oseltamivir treatment that had been started on the sixth day of illness; she died on the 20th day of respiratory failure. No autopsies were performed and no other viral data are available. Including these two patients, there was only one survivor among nine who presented 2–7 days (median, 6 days) after illness onset and had detectable virus at the end of therapy (n=3) or who did not have serial samples collected (n=6). In comparison, all 4 patients who started treatment 4–8 days after illness onset and who had undetectable pharyngeal RNA levels at the end of 5 days of therapy survived.

Based on these recent observations and other data, members of the NISN provide the following commentary and guidance. This statement supplements two earlier ones that have examined other aspects of antiviral resistance in influenza viruses [8,9] and will be updated as new information becomes available.

M2 inhibitors

Mutations in influenza viruses that confer resistance *in vitro* to M2 inhibitors or oseltamivir also lead to loss of drug effectiveness *in vivo*. For example, in a ferret model of the A/H5N1 infection, oseltamivir reduced virus titres and febrile responses due to a wild-type, susceptible virus clone, but not due to one of the oseltamivir-resistant (His274Tyr) clones [3]. Given the high frequencies of M2 inhibitor resistance in human isolates of A/H5N1 [6] and A/H3N2 [2] subtype viruses in certain parts of the world, this class of drugs cannot be relied upon for clinical management. However, if the circulating strain were known to be susceptible to M2 inhibitors, these drugs would offer a less expensive alternative for prophylaxis and, in the absence of NA inhibitors, treatment. Controlled prophylaxis studies established that M2 inhibitors partially protected against illness due to pandemic strains in 1968 and 1977 [10]. Consequently, although NA inhibitors are the preferred choice as the principal component of antiviral stockpiles, M2 inhibitors could provide a useful addition for preventing illness due to susceptible strains.

Monotherapy with M2 inhibitors of drug-susceptible A/H5N1 infection, as for human influenza, would be expected to lead to emergence of resistant variants

and their transmission to close contacts. Treated patients should be appropriately isolated, in part to prevent transmission of resistant variants. Combinations of an M2 inhibitor and an NA inhibitor show enhanced antiviral activity *in vitro* and in animal models of influenza due to M2-inhibitor-susceptible viruses [11,12] and might be expected to reduce the likelihood of resistance emergence, although the latter has not been proven. Such combinations have received very limited clinical study in human influenza [13], and controlled studies of their use in treating A/H5N1 and severe human influenza virus illness due to M2-inhibitor-susceptible strains appear warranted.

NA-inhibitor resistance

Currently, there is no indication that circulating avian A/H5N1 viruses, which serve as the source for human infections, have developed resistance to NA inhibitors. Among 97 A/H5N1 human and poultry isolates tested by the World Health Organization (WHO) Collaborating Center at the Centers for Disease Control and Prevention (CDC), Atlanta, only a single sample from the patient described above (A/Vietnam/30408/2004) has shown partial resistance to oseltamivir [14,15; and A Klimov, personal communication]. The likelihood that oseltamivir-resistant variants will transmit and circulate at the community level appears to be much lower than the documented frequent circulation of M2-inhibitor-resistant H3N2 variants in many countries [2]. NISN surveillance detected only one variant with the His274Tyr mutation among 622 H1N1 community isolates collected worldwide from persons without known oseltamivir exposure during the first 3 years (1999–2002) after introduction of oseltamivir into clinical practice [AS Monto, submitted for publication]. However, oseltamivir use was low during this period. During the 2003–20044 season when a large increase in oseltamivir use occurred in Japan, only three A/H3N2 isolates with known oseltamivir-resistance mutations, two Glu119Val and one Arg292Lys, were identified among nearly 1,200 community isolates collected across Japan from patients without known oseltamivir exposure [9]. These observations suggest that a very low level of transmission may have occurred. In animal models of influenza, the His274Tyr mutation in A/H1N1 or A/H5N1 viruses confers 100-fold or greater reduced infectiousness [16,17] and is associated with over 10-fold reductions in virus replication compared with the susceptible, wild-type virus [3,16]. However, transmission studies in ferrets indicate that oseltamivir-resistant A/H1N1 (His274Tyr) and A/H3N2 (Glu119Val) viruses can transmit from one animal to another [17]. Further animal studies with H5N1 virus genetically engineered to possess the His274Tyr mutation are in progress [E Govorkova and R Webster, personal communication]. Although the predictive value of such animal model observations for human influenza is uncertain, it is essential that both human and animal isolates of A/H5N1 viruses, representing all lineages, be provided to WHO- or FAO/OIE-qualified laboratories for drug susceptibility testing and characterization.

Frequency of oseltamivir resistance

The detection of resistant A/H5N1 variants during oseltamivir therapy is not unexpected, given the experience with oseltamivir treatment of human influenza in children, particularly if suboptimal dosing regimens are used. One earlier study of 43 A/H1N1-infected Japanese children found that 16% shed resistant variants with the His274Tyr resistance mutation during or after oseltamivir treatment [18]. A similar frequency of detecting phenotypically resistant variants (18%) was observed in hospitalized children infected with A/H3N2 virus during or after oseltamivir treatment [19]. In these reports, some of the patients may have received suboptimal dosing of the drug, which may not suppress viral replication adequately and so provide the appropriate conditions for the emergence of resistant mutants. The frequency of resistance emergence during oseltamivir treatment of A/H5N1 paediatric patients is uncertain, because little sequential viral sampling has been performed, but it is likely to be no less than that observed in A/H1N1-infected children. The recent study in Vietnam detected emergence of the His274Tyr mutation in 2 of 8 (25%) oseltamivir-treated patients from whom sequential pharyngeal samples were collected for analysis [4]. Whenever possible, sequential respiratory samples should be collected from A/H5N1-infected patients receiving antivirals for subsequent analysis in qualified laboratories. NISN is prepared to help in this process.

Consequences of oseltamivir resistance

The clinical consequences of oseltamivir resistance emergence during therapy of H5N1 infections are at present uncertain. Many oseltamivir-treated patients with A/H5N1 disease have died, but usually treatment has been initiated late in the illness, when pneumonia was already present [20,21]. However, as described above, patients may have clinical progression despite receiving oseltamivir at conventional doses relatively early after H5N1 illness onset [4]. Establishing a causal relationship between viral persistence in the upper respiratory tract and clinical failure is confounded by lack of information regarding lower respiratory tract

virology, pulmonary damage due to host pro-inflammatory mediators, and secondary bacterial infections. In uncomplicated human influenza, emergence of resistance to oseltamivir has been detected generally on day 4 or 6 of therapy and has not been associated with apparent clinical deterioration in children [22]. However, one study found rebounds in viral replication after emergence of the H274Y variant in two individuals with experimentally induced A/H1N1 infection [23]. Such observations suggest that if there were early resistance emergence during antiviral therapy of A/H5N1 illness, it may be associated with clinical failure, but further studies are needed. Currently, the available data do not indicate that potential oseltamivir resistance should be a deterrent to its stockpiling for pandemic response.

Oseltamivir treatment regimens

At present, it is uncertain whether higher doses or longer oseltamivir treatment regimens might reduce the likelihood of emergence of resistant variants and/or provide greater clinical benefit in severe infections due to A/H5N1 or human influenza viruses. In one murine model study using a human isolate of A/H5N1 from 2004, higher oseltamivir doses (10 mg/kg/d compared with 1 mg/kg/d) and more prolonged administration (8 days instead of 5 days) were required to inhibit viral replication compared to a human A/H5N1 isolate from 1997 [24]. This was related to the higher levels of replication of the former virus; drug-resistant variants were not detected [24]. Further studies in a ferret model of A/H5N1 disease are in progress. If dose regimens requiring more oseltamivir or perhaps antiviral combinations help in reducing resistance emergence, it would have important implications for clinical management and stockpiling decisions. On the other hand, rigorous data are needed to determine whether short course (for example, 3 days) or other reduced dose regimens are as clinically effective as standard ones, including the possible consequences with respect to resistance emergence. Until such data become available, NISN advises use of the approved therapeutic 5-day regimen of oseltamivir, with consideration of longer treatment if clinical deterioration or inadequate response is observed.

Zanamivir

Zanamivir retains full *in vitro* activity against the oseltamivir-resistant N1 NAs harbouring the His274Tyr mutation [25,26] and was shown to inhibit the replication of both the oseltamivir-susceptible and oseltamivir-resistant A/H5N1 virus clones of A/Vietnam/30408/2004 in ferrets [3]. All avian and human A/H5N1 isolates tested so far at CDC, including this partially resistant isolate, are susceptible to zanamivir (14; A Klimov, personal communication). The use of inhaled zanamivir in human A/H5N1 infection has not been reported, and data are needed from severe human influenza to indicate whether it is safe and therapeutically effective in pneumonic disease. The presence of advanced pneumonia and findings of extrapulmonary dissemination in some patients with A/H5N1 illness are important considerations regarding delivery of inhaled zanamivir to sites of viral replication beyond the pharynx and tracheobronchial tree. Uncertainties also exist regarding the bioavailability of oral oseltamivir in critically ill patients, and an injectable NA inhibitor would be advantageous for treating severe disease due to A/H5N1 or human influenza viruses. However, inhaled zanamivir would be a therapeutic consideration if oseltamivir resistance were likely to be present. Inhaled zanamivir is highly effective for prophylaxis of human influenza, although not approved currently for this indication in many countries. Studies of its protective efficacy against A/H5N1 infection in humans would be valuable. At present inhaled zanamivir, as well as oseltamivir, would be an appropriate choice for pandemic response stockpiles.

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References

1. Zambon M, Hayden FG; Global Neuraminidase Inhibitor Susceptibility Network. Position statement: global neuraminidase inhibitor susceptibility network. *Antiviral Research* 2001; **49:**147–156
2. Bright RA, Medina MJ, Xu X, Perez-Oronoz G, Wallis TR, Davis XM, Povinelli L, Cox NJ & Klimov AI. Incidence of amantadine resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *The Lancet* 2005; **366:**1175–1181.
3. Le QM, Kiso M, Someya K, Sakai YT, Nguyen TH, Nguyen KH, Pham ND, Ngyen HH, Yamada S, Muramoto Y, Horimoto T, Takada A, Goto H, Suzuki T, Suzuki Y & Kawaoka Y. Avian flu: Isolation of drug-resistant H5N1 virus. *Nature* 2005; **437:**1108.
4. de Jong M, Thanh TT, Khanh TH, Hien V, Smith G, Chau NV, Cam BV, Qui, PT, Ha DQ, Guan Y, Peiris JSM, Hien, TT & Farrar J. Oseltamivir resistance during treatment of influenza H5N1. *New England Journal of Medicine* 2005; **353:**25–30.
5. Peiris JS, Yu WC, Leung CW, Cheung CY, Ng WF, Nicholls JM, Ng TK, Chan KH, Lai ST, Lim WL, Yuen KY & Guan Y. Re-emergence of fatal human influenza A subtype H5N1 disease. *The Lancet* 2004; **363:**617–619.
6. Hien TT, Liem NT, Dung NT, San LT, Mai PP, Chau NV, Suu PT, Dong VC, Mai LTQ, Thi NT, Khoa DB, Phat LP, Truong NT, Long HT, Tung CV, Giang LT, Tho ND, Nga LH, Tien NTK, San LH, Tuan LV, Dolecek C, Thanh TT, de Jong M, Schultsz C, Cheng P, Lim W, Horby P, the World Health Organization International Avian Influenza

Investigative Team & Farrar J. Avian influenza A (H5N1) in 10 patients in Vietnam. *New England Journal of Medicine* 2004; **350:**1179–1188.

7. Ilyushina NA, Govorkova EA & Webster RG. Detection of amantadine-resistant variants among avian influenza viruses isolated in North America and Asia. *Virology* 2005; **341:**102–106.
8. NISN. NISN statement on antiviral resistance in influenza viruses. *Weekly Epidemiological Record* 2004; **79:**306–308.
9. NISN. Use of influenza antivirals during 2003-2004 and monitoring of neuraminidase inhibitor resistance. *Weekly Epidemiological Record* 2005; **80:**156.
10. Hayden FG. Perspectives on antiviral use during pandemic influenza. *Philosophical Transactions of the Royal Society of London* 2001; **356:**1877–1884.
11. Leneva IA, Roberts N, Govorkova EA, Goloubeva OG & Webster RG. The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H9N2) influenza viruses. *Antiviral Research* 2000; **48:**101–115.
12. Govorkova EA, Fang HB, Tan M & Webster RG. Neuraminidase inhibitor-rimantadine combinations exert additive and synergistic anti-influenza virus effects in MDCK cells. *Antimicrobial Agents and Chemotherapy* 2004; **48:**4855–4863.
13. Ison MG, Gnann JW Jr, Nagy-Agren S, Treannor J, Paya C, Steigbigel R, Elliott M, Weiss HL, Hayden FG; NIAID Collaborative Antiviral Study Group. Safety and efficacy of nebulized zanamivir in hospitalized patients with serious influenza. *Antivir Therapy* 2003; **8:**183–190.
14. WHO. WHO intercountry-consultation. Influenza A/H5N1 in humans in Asia. Manila, Philippines, 6–7 May 2005. World Health Organization; 2005 [cited 6 December 2005]. Available from: http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_GIP_2005_7/en/
15. Medina M, Bright RA, Xu X, Cox N & Klimov AI. Susceptibility of influenza A(H5N1) and H3N2 viruses isolated over the last three yers to neuraminidase inhibitors. *Second European Influenza Conference*. 11–14 September 2005, St Julian's, Malta. Abstract 62.
16. Ives JA, Carr JA, Mendel DB, Tai CY, Lambkin R, Kelly L, Oxford JS, Hayden FG & Roberts NA. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and *in vivo*. *Antiviral Research* 2002; **55:**307–317.
17. Yen HL Herlocher LM, Hoffmann E, Matrosovich MN, Monto AS, Webster RG, Govorkova EA. Neuraminidase inhibitor-resistant influenza viruses may differ substantially in fitness and transmissibility. *Antimicrobial Agents & Chemotherapy* 2005; **49:**4075–4084
18. Ward P, Small I, Smith J, Suter P & Dutkowski R. Oseltamivir (Tamiflu(R)) and its potential for use in the event of an influenza pandemic. *The Journal of Antimicrobial Chemotherapy* 2005; **55(Suppl 1):**i5–21.
19. Kiso M, Mitamura K, Sakai-Tagawa Y, Shiraishi K, Kawakami C, Kimura K, Hayden FG, Sugaya N & Kawaoka Y. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *The Lancet* 2004; **364:**759–765.
20. Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, Chunsuthiwat S, Sawanpanyalert P, Kijphati R, Lochindarat S, Srisan P, Suwan P, Osotthanakorn Y, Anantasetagoon T, Kanjanawasri S, Tanupattarachai S, Weerakul J, Chaiwirattana R, Maneerattanaporn M, Poolsavathitikool R, Chokephaibulkit K, Apisarnthanarak A & Dowell SF. Human disease from influenza A (H5N1), Thailand, 2004. *Emerging Infectious Diseases* 2005; **11:**201–209.
21. The Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza. Avian influenza A (H5N1) infection in humans. *The New England Journal of Medicine* 2005; **353:**1374–1385.
22. Jackson HC, Roberts N, Wang Z & Belshe R. Management of influenza: Use of new antivirals and resistance in perspective. *Clinical Drug Investigations* 2000; **20:**447–454.
23. Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y & Hayden FG. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *Journal of Infectious Diseases* 2001; **183:**523–531.
24. Yen HL, Monto AS, Webster RG & Govorkova EA. Virulence may determine the necessary duration and dosage of oseltamivir treatment for highly pathogenic A/Vietnam/1203/04 (H5N1) influenza virus in mice. *Journal of Infectious Diseases* 2005; **192:** 665–672.
25. McKimm-Breschkin J, Trivedi T, Hampson A, Hay A, Klimov A, Tashiro M, Hayden F & Zambon M. Neuraminidase sequence analysis and susceptibilities of influenza virus clinical isolates to zanamivir and oseltamivir. *Antimicrobial Agents & Chemotherapy* 2003; **47:**2264–2272.7
26. Mishin VP, Hayden FG & Gubareva LV. Susceptibilities of Antiviral-resistant influenza viruses to novel neuraminidase inhibitors. *Antimicrobial Agents and Chemotherapy* 2005; **49:**4515–4520.

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