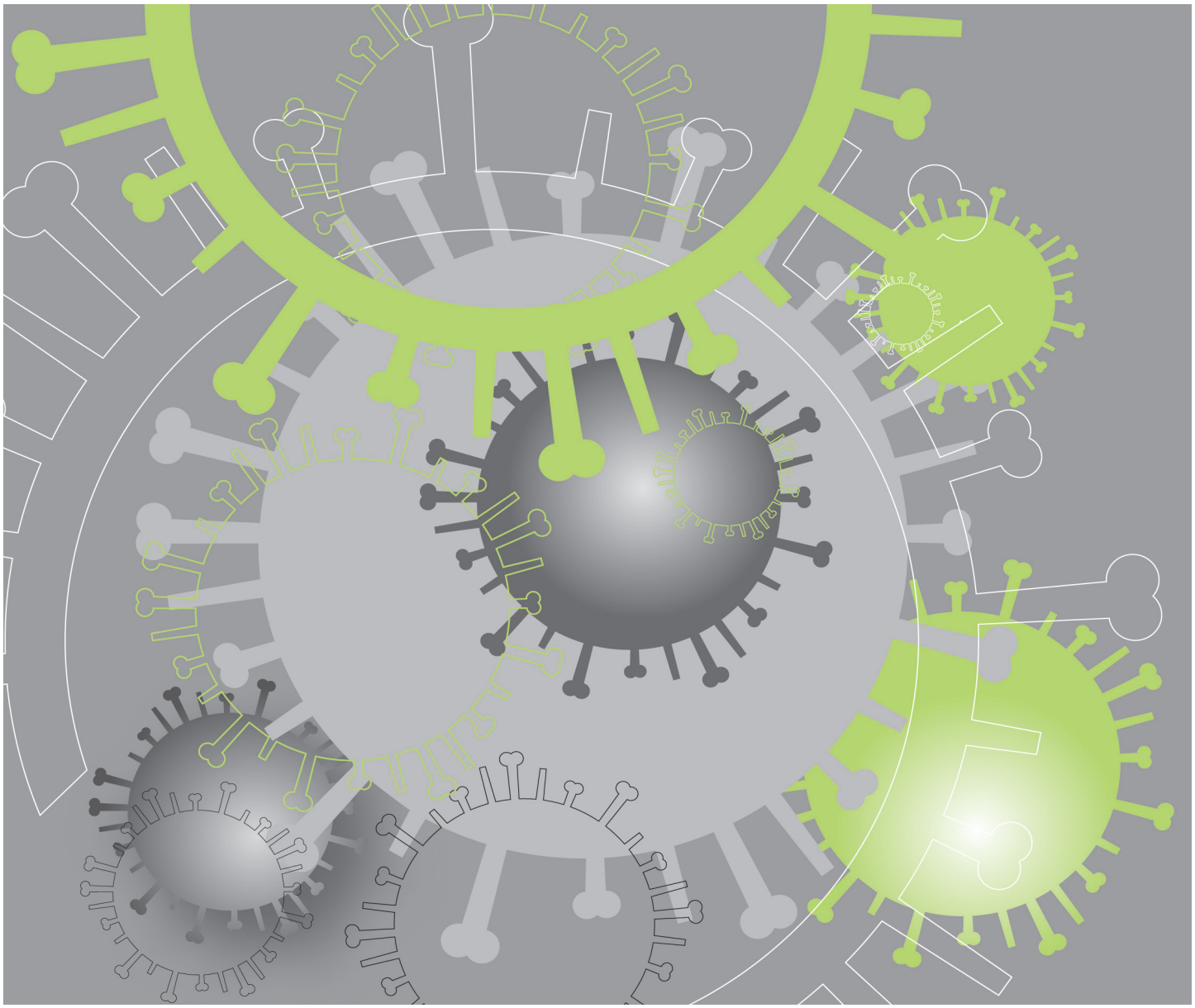
NISN

Neuraminidase Inhibitor Susceptibility Network

An Overview of Antiviral Drug Resistance Data presented at Options for the Control of Influenza VII

Hong Kong, September 2010



Contents

Introduction

At the time when the neuraminidase inhibitors (NAIs) zanamivir and oseltamivir became available for general clinical use the Neuraminidase Inhibitor Susceptibility Network (NISN) was one of a very few independent groups conducting antiviral resistance surveillance and testing as well as related studies in the influenza field [1,2,3]. However, prompted by more recent events, including: human infection associated with the epizootic of highly pathogenic avian H5N1 influenza, the unexpected circulation of a seasonal H1N1 virus carrying the H275Y neuraminidase (NA) mutation conferring resistance to oseltamivir and then the emergence of another H1N1 virus (from swine) which resulted in the 2009 influenza pandemic, many laboratories worldwide, particularly those in the World Health Organization (WHO) Global Influenza Surveillance Network (GISN), have now carried out surveillance of drug resistance of influenza viruses and studied the properties of those resistant viruses. As a consequence, much new data on drug resistance of influenza viruses and related topics were presented at the "Options VII" conference in September 2010; here NISN summarizes and comments on the new findings.

The majority of new data, much of which was in the form of poster presentations, was on drug resistance of the 2009 pandemic H1N1 viruses, but further information on surveillance of seasonal viruses from 2008-9 was also presented. A broad range of information included clinical trial reports, animal model data, in vitro susceptibility assays and other in vitro studies of antiviral resistance*. Two comprehensive overview presentations were also given at the meeting and are included in the summary and comments on the significance and consequences of the new data.

For mutations in the N1 subtype NA, N1 amino acid residue numbering is used, and for the N2 subtype, N2 numbering.

*where data presented in a conference paper/ poster differ from those published in the conference proceedings, the former are included in this report to represent the most up-to-date data available.

Pandemic H1N1 Viruses

All presentations providing resistance data on pandemic influenza A H1N1 viruses isolated in 2009-2010 are summarized in Table 1 (with the exception of three individual case study reports, see below).

It is clear that, where evaluated, the incidence of resistance of the pandemic H1N1 viruses to the adamantanamines, amantadine and rimantadine, was almost universal. Thus these drugs should not have been used for therapy. In contrast, resistance to NAIs has been very low to date both in circulating viruses and in post-treatment samples from otherwise healthy individuals. This is despite the widespread use of oseltamivir and to a lesser extent zanamivir in treatment and prophylaxis.

Two overview lectures by Hayden [4] and Penn [5] included a summary of resistance data available prior to the Options VII meeting. They described a total of 304 oseltamivir-resistant pandemic H1N1 viruses, as reported to WHO to August 2010 (most of those identified at this meeting are included in this total), of which only 12% had no clear association with drug usage. The denominator (the total tested) is unknown but likely to be in excess of 20,000; which would give an incidence of less than 1.5%. (It should be noted, however, that the population is skewed towards those likely to carry resistant virus). This is consistent with many of the reports here with an incidence of 0.5-1.0%. There is no evidence of widespread or sustained community circulation. Many cases of the emergence of oseltamivir resistance and a few cases of resistance to zanamivir in pandemic H1N1 viruses have been in hospitalized patients most of whom were either immunocompromised (37% of the 304 total), often with a haematological malignancy, or less often severely ill in the context of underlying chronic illnesses. While resistance generally emerged only after four or more days of therapy, immunocompromised patients shed virus over many days or even weeks. The most common mutation was, as might be expected, H275Y in NA, conferring resistance to oseltamivir. However, a novel NA mutation I223R was reported in several instances [6- 9, 20, 57, 62], causing relatively modest resistance to both oseltamivir and zanamivir (approximately 40- and 10-fold increases in IC_{50} values, respectively). In two highly immunocompromised patients the emergence of the I223R occurred either after oseltamivir treatment or oseltamivir plus zanamivir treatment [6- 8]. In studies where the ratio of mutant and wild type viruses had been assessed [6, 8-11] the H275Y and I223R mutants were only a proportion, often less than 50%, of the total virus population. They were, nevertheless, classified as "resistant". As has previously been observed [12], provided effective immune responses develop (e.g. ref 13, 14) virus clearance may occur in immunocompromised individuals despite the presence of H275Y mutant virus

Where cross-resistance was examined for pandemic H1N1 mutants (e.g. ref 6, 8, 9), previous findings from seasonal H1N1 viruses were confirmed; the H275Y mutants were resistant to oseltamivir and peramivir but sensitive to zanamivir. The novel I223R mutation caused modest resistance to all three NAIs and in combination with H275Y significantly increased resistance to oseltamivir and peramivir, but only marginally to zanamivir. A novel combination of NA mutations Q313R and I427T caused resistance to both oseltamivir and zanamivir (10-40 fold and 3-20 fold increases in IC_{50} value, respectively) [9].

Despite the fact that the low incidence of the H275Y NA mutation in pandemic H1N1 viruses suggests that these mutants are less fit than the wild-type virus, there is evidence that the mutant virus can be transmitted directly from person to person. Phuong et al [15] (abstract only) presented strong circumstantial evidence for transmission of H275Y mutant virus between otherwise healthy adults in prolonged close contact. Strong evidence for person to person transmission between hospitalized, lymphopenic haematology patients, presented by Moore et al [16], was supported by phylogenetic analysis showing 4 patients (in an outbreak of 11 infected patients) clearly being infected with resistant virus by direct transmission. Their conclusion that zanamivir should be considered as first choice for therapy and outbreak control in these circumstances is uncontroversial.

Seasonal Influenza Viruses

Surveillance for resistance to amantadine and NAIs among influenza viruses isolated in India from 2004-2009, using one step diagnostic RT-PCR, was reported by Potdar et al [17]. H3N2 isolates (204/248), possessing a S31N mutation in the M2 protein, were resistant to amantadine, with the incidence gradually increasing from none in 2004 to resistance being predominant in 2009. In contrast only 3/145 seasonal H1N1 isolates were resistant to amantadine, one isolate in each year from 2007-2009. The earlier two isolates carried a S31N and the final isolate carried a L26F mutation in M2. No resistance to NAIs was observed in any of 194 H3N2 isolates. Also no resistance to NAIs was observed in 93 H1N1 viruses isolated prior to December 2008. However in 2009 oseltamivir-resistant seasonal H1N1 viruses carrying the H275Y NA mutation were predominant in most parts of India.

Similar studies were reported for 2001-2009 isolates from Vietnam by Phuong et al [15] (abstract only). Resistance was detected by gene sequencing and virus NA sensitivity to NAI using MUNANA as a substrate. Resistance to amantadine, due to S31N in M2, in H3N2 viruses rose steadily from 3.2% (1/31) in 2003 to 60% (18/30) in 2007 and 100% (3/3) in 2008. For seasonal H1N1 viruses the incidence of resistance to amantadine (S31N) was lower and detected at 11% (2/18) in 2006 and 19.5% (9/46) in 2008. This mutation was also seen in 3/11 highly pathogenic H5N1 influenza viruses of clade 1 in 2004-2005 but not in more recent viruses of clade 2.3.4. The H3N2 viruses were all sensitive to both oseltamivir and zanamivir. However, among H1N1 isolates resistance to oseltamivir reached 76.2% (32/42) in 2008 (presumably associated with a H275Y NA mutation, but not stated). The authors also reported a novel NA resistance mutation, I117V, conferring resistance to oseltamivir in two human highly pathogenic H5N1 isolates from 2007 and 2008 (characterized by both sequencing and NAI sensitivity assay for the 2008 isolate). This mutation was also detected in 2/11 poultry H5N1 isolates in 2007.

Similar observations of increasing amantadine (rimantadine) resistance were made on isolates from Russia [18]. Using a cell-based ELISA assay, resistance of H3N2 viruses to rimantadine increased from 14% (12/84) in 2003-2004 to 89% (114/128) in 2008-2009. For H1N1 viruses the rimantadine resistance frequencies were 25% (2/8) for 2005-2006 and 48% (21/44) for 2006-2007. They also recorded a rise in oseltamivir resistance in H1N1 viruses from 49% (22/45) in 2007-2008 to 92% (24/26) during the 2008-2009 epidemic season. H3N2 viruses remained sensitive to oseltamivir and zanamivir. All viruses, including those resistant to other antiviral agents, remained sensitive to arbidol (an agent approved in the Russian Federation).

Sheu et al [19] pointed out that two clades of seasonal H1N1 viruses with differing drug susceptibility patterns circulated in 2008-2010: clade 2B largely resistant to oseltamivir but sensitive to amantadine, and clade 2C susceptible to oseltamivir but largely resistant to amantadine. Differences in their geographical distribution helps to explain the differences in resistance incidence cited above. Each clade thus provided a treatment option. However, the extensive drug resistance screening program performed at CDC, Atlanta, with samples from USA and globally, detected 28 isolates with dual oseltamivir and adamantanamine resistance due to H275Y in N1 and S31N in M2, respectively. These arose through different pathways including reassortment between clades; however, it was not clear how many of the dual resistant mutants may have been associated with antiviral treatment (possibly 3), nor was the size of the denominator given. Subjects infected with such viruses have more limited treatment options with zanamivir being the only widely approved antiviral with inhibitory activity.

In addition to surveillance of H1N1 pandemic viruses (Table 1) Takashita et al [20] also reported resistance data for 2009-2010 seasonal influenza isolates. Four seasonal H1N1 viruses from Laos were all resistant to oseltamivir, but no resistance was seen among 82 H3N2 isolates or 98 influenza B isolates.

Several reports contrasted the high incidence of resistance to oseltamivir in seasonal H1N1 viruses in 2008-2009 with the low incidence in pandemic H1N1 viruses in 2009-2010. Okomo-Adhiambo et al [21], using the NA-Star™ NA inhibition assay showed that 93.3% of 1533 seasonal H1N1 viruses, collected globally, were resistant to oseltamivir compared with only 0.7%

of 2259 pandemic H1N1 viruses. All resistant viruses harbored the H275Y NA mutation which caused cross-resistance to peramivir (subset of 1058 tested). No resistance to NAIs was observed among H3N2 viruses (n=834) and only one case of resistance (NA D198E) was seen in influenza B isolates (n=914). One note of caution was that several variants with mutations in residue D151 of NA of both N1 and N2 subtypes were found with reduced NAI susceptibility. However, mutations causing substitution of D151 by G,N,E or A are known to be artifacts due to cell culture selection [21-24].

As part of a clinical study of the effectiveness of NAIs in Japan, comparing data from 2007-2008 and 2008-2009, some resistance testing was performed on pre-treatment virus samples by gene sequencing [25]. None of the 44 H1N1 viruses analyzed from the 2007-2008 influenza season carried the H275Y NA mutation compared to 100% of the 88 viruses analyzed in 2008/09. None of the 34 pandemic H1N1 viruses analyzed to date carried the H275Y mutation.

Naranzul et al [26] reported resistance data for influenza viruses isolated in Mongolia, using a chemiluminescence-based NA inhibition assay. All viruses were sensitive to oseltamivir with the exception of 1/37 seasonal H1N1 viruses from 2008-2009 and 1/262 pandemic H1N1 viruses. No resistance to oseltamivir was seen in 18 influenza B isolates. High level resistance to amantadine was observed in 13/14 isolates (93%).

Clinical Trails

The results of a clinical trial of the use of inhaled zanamivir in Japanese children aged 2-14 years was presented by Yates et al [27]. This post-approval study was conducted over three influenza seasons from 2006 to 2009, i.e. against seasonal influenza. Pharyngeal swabs were taken at baseline, during and post treatment and virus expanded in cell culture. Resistance was analyzed by population and clonal sequencing on both expanded virus and swabs, and virus sensitivity to zanamivir assessed using the NA-Star™ assay. Virus isolates from 273 patients were examined. Swabs (719) were taken allowing 481 samples, including post-treatment samples from 229 patients, to be analyzed, 248 from influenza A H1N1 infections, 126 from influenza A H3N2 and 107 from influenza B infections. No clear evidence of drug selected resistance to zanamivir was found. One pre-treatment sample (H1N1) contained a N70S mutation in NA which resulted in decreased (46-fold) sensitivity to zanamivir, and two cell culture samples (H1N1) from one patient had a 300 fold decrease in sensitivity to zanamivir and contained virus carrying the Q136K NA mutation, previously shown to confer resistance to zanamivir [28]. This mutation was absent in the corresponding swab and therefore deemed likely to be a cell culture selection artifact. However, direct detection of this mutation in a sample from a zanamivir-exposed ferret infected with H5N1 virus has been recently reported by Hurt et al [29]; further studies of original samples from zanamivir-treated humans infected with N1 viruses are therefore warranted.

It is very reassuring that treatment of influenza infection in children with inhaled zanamivir did not give rise to resistance. However, direct comparison of data from this trial with data on the selection of virus resistant to oseltamivir in Japanese children, as reported by Kiso [30], is not entirely valid as all the children in which virus resistant to oseltamivir was selected were age 3 years or less (i.e. under the age of general licensed usage of inhaled zanamivir).

Schutten et al [31] reported a clinical trial (IRIS study) of oseltamivir against pandemic H1N1 virus in which the emergence of resistance was not detected in 167 treated patients (aged one or more years).

Four posters reported clinical trials [32, 33] or animal studies [34, 35] of treatment with peramivir. None contained any resistance data, which is unfortunate as emergence of resistance to peramivir in vivo is the least well characterized of the advanced NAIs. Similarly, Sugaya [36] presented data from a clinical trial of laninamivir treatment of oseltamivir-resistant seasonal H1N1 infection in Japanese subjects, but gave no data on the selection of resistance by laninamivir.

Ferret Models

Kawaoka [37] addressed the potential of oseltamivir-resistant (H275Y) pandemic H1N1 virus to transmit using a ferret model [38], in which infected (donor) animals and naïve (recipient) animals were housed in adjacent cages separated by a double mesh barrier. Thus transmission was by respiratory droplet (RD) rather than by direct nose to nose or nose to fur contact between animals. (This RD model may be the preferable protocol for future studies; see also [39]). In this model the resistant virus did transmit from animal to animal but transmission was delayed compared to wild-type for one pair. Duan et al [39] compared the transmissibility of a H275Y mutant and corresponding wild-type H1N1 pandemic virus in ferrets using both the direct contact and respiratory droplet models. In the former both mutant and wild-type transmitted with similar efficiency while by droplet transmission only the wild-type transmitted, showing a differentiation more in keeping with clinical and surveillance observations. When ferrets were infected with a 50/50 mixture of mutant and wild-type virus the wild-type outgrew the mutant in the donor animals and only wild-type was transmitted by droplet transmission. The reduced NA function and delayed virus growth in vitro may in part explain less efficient transmission of some oseltamivir-resistant pandemic H1N1 variants [40, 41].

In studies in which donor and recipient ferrets were co-housed, Hurt et al [43] were unable to demonstrate any benefit of either donor treatment or recipient prophylaxis with oseltamivir on the transmission of drug sensitive (wild-type) H1N1 pandemic virus between animals; this result is not consistent with general clinical experience. Oseltamivir-resistant (H275Y) virus emerged in one of the two treated donor animals. Both Hurt et al [43] and Govorkova et al [44] used infection of donor ferrets with mixtures of two viruses in varying ratios (e.g. 100/0; 80/20; 50/50; 20/80; 0/100) to assess competitive fitness (virus-virus interactions within the host) and relative transmissibility of the virus pairs to recipient ferrets. Using this novel technique, Hurt et al [45] demonstrated that the R292K mutation in H3N2 virus severely disabled the virus, so that it was outgrown by wild-type in donor ferrets and did not transmit. However, the H275Y mutant of a seasonal H1N1 (2007) virus was only marginally less fit and still able to transmit to recipient ferrets. Both observations are consistent with previous findings in ferrets [46, 47]. Govorkova [48] showed that the H275Y NA mutation had different effects depending on the genetic background of the virus. Introduction of the H275Y mutation into a recombinant highly pathogenic H5N1 virus A/Vietnam/1203/04 had much less effect on viral fitness and transmissibility than introduction of the same mutation into the NA of A/Turkey/15/06 (H5N1) virus. This confirms that the fitness change due to a particular mutation may also be dependent on the genetic background as well as the NA sequence.

In Vitro Methods For Resistance Testing

Evaluation of the sensitivity of viral NA to the NAIs has been performed for several years by enzyme inhibition assays using either MUNANA as substrate with local protocols and locally prepared reagents or the Applied Biosystems chemiluminescent assay kit with NA-Star™ as substrate. Applied Biosystems presented two new alternatives. Mihali et al [49] described a standardized, packaged kit version of the MUNANA assay (NA-Fluor™) with a protocol closely based on, and giving IC_{50} values similar to, the protocols provided on the NISN website [50]. Use of this standardized assay should provide more comparable IC_{50} data across laboratories and less reagent preparation time, though obviously at a cost. More innovatively, Miller et al [51] described a new substrate for chemiluminescent assay of NA inhibition, NA-XTD™, which has an extended-glow light signal ($T_{1/2}$ decay time for light emission is about 2 hours compared with about 10 minutes for NA-Star™). This eliminates the need for reagent injection and allows signal measurement for up to several hours after assay completion. This new substrate is also provided in assay kit form.

Lerdsamran et al [52] pointed out correctly that should resistance to NAIs arise through mutations in HA, which confer weaker receptor binding, such mutants would not be detected by NA inhibition assays. They therefore compared NA inhibitions assays with assays measuring inhibition of virus replication in MDCK cell culture and found apparent resistance in the cell-based assays not seen by NA enzyme inhibition. They were clearly unaware of the history of this problem and of the guidelines set out by NISN warning of potential false positives for resistance using unmodified MDCK cell assays [1] and of the development of MDCK-SIAT1 cells [53] and other similar cell lines [54] specifically produced to try to solve this problem of a reliable cell culture-based phenotypic assay.

In Vitro Studies Of Resistance

The emergence of a fit, transmissible oseltamivir-resistant (H275Y) seasonal H1N1 virus in 2007 was unexpected. Wu et al [55] reported that in haemagglutination inhibition and virus neutralization assays the H275Y mutant was antigenically distinguishable from corresponding 275H wild-type virus, and suggested that NA-dependent antigenic variation may have played a role in the emergence of the oseltamivir-resistant epidemic strain.

The potential for reassortment between a seasonal H1N1 virus carrying the H275Y NA mutation and a pandemic H1N1 virus to produce an oseltamivir-resistant pandemic virus has been a concern. Ferraris et al [56] addressed the likelihood of this possibility. They produced 50 reassortants in MDCK cells between a seasonal H1N1 (275Y) virus (2007) and a pandemic H1N1 (275H) virus (2009) and selected three, representing different internal and external gene combinations, but all carrying the 275Y NA from the 2007 virus. In vitro these reassortants were essentially as fit as their parental strains. They could all infect mice but with slightly different phenotypes. The relevance of the mouse model is perhaps questionable, but the fitness of the reassortants leaves open the potential for such a reassortment event in nature.

An alternative approach was taken by Abou-Jaoudé et al [57]. They introduced into both H1N1 pandemic virus and H5N1 virus mutations in NA at positions 222 and 344, which are known to impact the activity of NA in seasonal H1N1 viruses. Potentially compensatory alterations in enzyme kinetics and viral phenotypes were observed, especially with variants at residue 344, emphasizing the possibility for such changes to occur in nature and produce fitter oseltamivir-resistant viruses.

CONCLUSIONS

Continued surveillance of seasonal viruses has revealed no unexpected events, but has served to highlight the consequence of seasonal H1N1 virus acquiring a H275Y NA mutation, and the potential for dual resistance to NA and M2 inhibitors. Prior to 2000, influenza A viruses carrying NA or M2 resistance mutations had not become dominant epidemic species. As a consequence, prophylaxis and therapy of type A influenza has become complicated, with A(H3N2) infection requiring oseltamivir or zanamivir but not amantadine, and seasonal H1N1 infection requiring amantadine or zanamivir but not oseltamivir. It is possible that such a situation could occur again, since there is no complete explanation of the emergence of oseltamivir resistance of the seasonal H1N1 viruses. It is therefore crucial that there is continued antiviral susceptibility surveillance using both genetic and phenotypic methodologies.

Although oseltamivir-resistant H1N1 has now been largely superseded by oseltamivir-sensitive pandemic H1N1 virus, detection of the H275Y mutation at low levels in post-treatment samples from pandemic H1N1 patients and in sporadic clusters associated with transmissible oseltamivir resistance indicates the need for continued vigilance, and an understanding of the structural and functional pathways to the generation of a virus capable of overcoming the fitness deficit usually associated with this NA mutation.

Studies on pandemic H1N1 viruses indicate that the emergence of a readily transmissible NAI-resistant pandemic virus is still a possibility; in ferret models, transmission studies by respiratory droplets appear to better reflect clinical observations and infection with mixed virus populations are an excellent tool for evaluation of in vivo viral fitness and transmissibility.

Amantadine resistance in circulating H3N2 and pandemic H1N1 viruses continues without decline, indicating that such viruses do not have any apparent fitness disadvantages in the absence of drug selection pressure. The novel NAI resistance mutation I117V and the Q313R plus I427T combination identified in H3N2 viruses need further characterization. As culture-based selection of antiviral resistance can occur e.g. Q136R, it is also important to recognize artifacts arising from in vitro studies which require confirmation from analyses of individual clinical material.

With the increase in the pipeline of antiviral agents for influenza, new assay methods to detect antiviral resistance are being developed. These new methodologies to improve early detection of antiviral resistance and improve the correlation between phenotypic, genotypic and clinical resistance are important tools to evaluate for use in surveillance programmes as well as mechanistic studies.

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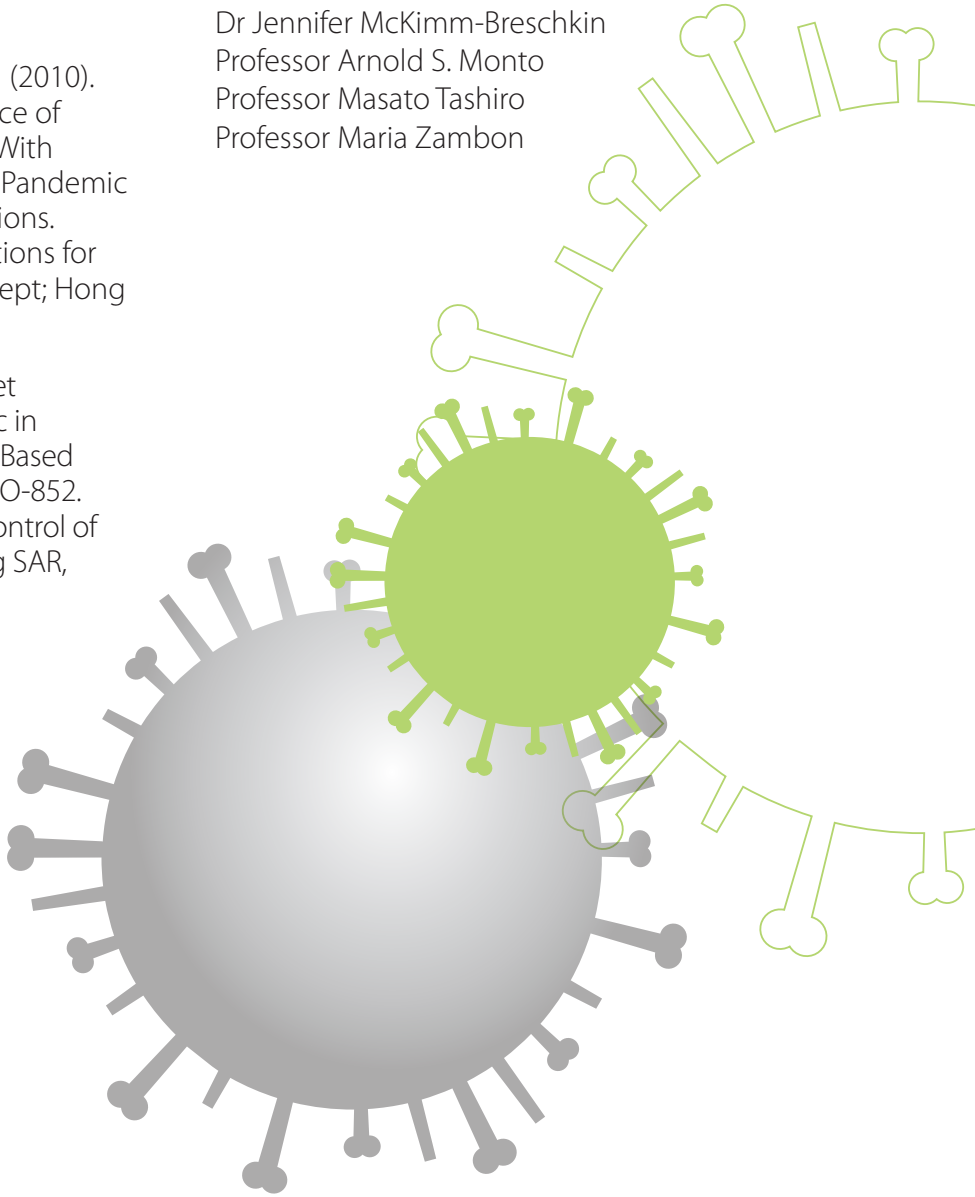

Table 1. Reports of Resistance to Neuraminidase Inhibitors in Pandemic H1N1 Virus

Author and Reference	Abstract no.	Country	Nature of Samples	Assay Method(s)	Resistance Incidence	Mutations Detected	Comments
Gubareva [9]	O-821	USA plus 72 countries	General surveillance. Many post-oseltamivir treatment	MDCK cell culture. Multiple genotyping and phenotyping assays of NA and M2	Amantadine 99.9% (n=4287) Oseltamivir 0.9% (71/8083)	S31N in M2 Most H275Y in NA; 1 case I223R; 1 case I223R + H275Y; 1 case Q313R + I427T	Many mixed H275Y + WT Cross-resistant Refer to accompanying text
Tang [10]	P-179	Singapore	2 high-risk hospitalized children oseltamivir treated	NA sequencing	2/2	H275Y	Mixed wt/mutant recovered over time
Lackenby [11]	P-186	UK	Self-sampling oseltamivir treated community	NA pyrosequencing NAI phenotyping MUNANA	0/323 pre-treatment 3/760 on treatment 0/51 post treatment	H275Y/H	All 3 mixed wt/mutant. Mutant <50%
Lackenby [13]	P-185	UK	Infected children, adults and household contacts	MDCK SIAT-1 culture NA pyrosequencing NAI phenotyping MUNANA	3/71	H275Y	2 immunocompromised adults. 1 child with H275Y virus pre-treatment
Pechirra [14]	P-187	Portugal	Random patient samples	MDCK cell culture(n= 153) NA and M2 sequencing (n = 53) NAI phenotyping	100% M2 1/53 NA	S31N H275Y	1 high-risk child initially under-dosed with oseltamivir
Phuong [15]	P-163	Vietnam	Not specified	NA sequence +limited phenotyping	7/275	H275Y	Evidence of transmission of H275Y virus

Key: NAI - neuraminidase inhibitor; PEP - post exposure prophylaxis; wt - wild type; ICU - intensive care unit

N.B. Where data presented on a conference poster differ from that published in the conference proceedings, the former are included in this table to represent the most up-to-date data available.

Author and Reference	Abstract no.	Country	Nature of Samples	Assay Method(s)	Resistance Incidence	Mutations	Comments
Okomo-Adhiambo [21]	P-157	USA	Global surveillance samples	Phenotyping: NA-Star™ pyrosequencing and full NA sequencing	0.7% (n=2259)	H275Y	
Ikematsu [25]	P-162	Japan	Clinical trial (n= 361) oseltamivir treated	NA sequencing	0/20 isolates tested		53% patients teenage
Takashita [20]	P-175	Japan	Random selection to Aug 2010: NAI treated, PEP and untreated	NA sequencing Phenotyping: NA-Star™	65/6789	H275Y 1 case I223R	Most of 65 oseltamivir treated or PEP
Naranzul [26]	P-164	Mongolia	Surveillance (2008-2010) (n= 317, of which 262 pandemic)	NA: chemiluminscence assay M2: pyrosequencing + M gene sequencing	NA: 1/262 M2 : 100%	NA not stated M2 mostly S31N	
Schutten [31]	P-167	Asia, Europe, USA	Prospective surveillance of illness & treatment response (n=339)	H275Y discrimination: RT-PCR; Genotyping: HA/NA/MA; Phenotyping: NA-Star™	1/339	H275Y	2yr old received oseltamivir prior to study
Deng [58]	P-180	Australia	Hospitalized patients: several ICU and extended oseltamivir treatment	NA pyrosequencing NAI phenotyping	5/129	H275Y	Mixed wt/mutant recovered over time
Lee [59]	O-828	Hong Kong	Hospitalized adults	Not specified (genotyping NA?)	0/25 long term shedders		
Lee [60]	P-177	South Korea	Many with chronic/ refractory infections	NA and M2 sequencing	NA: 11/1085 M2: 100% (n=1113)	10 H275Y 1 H275Y + I117M	Some of 11 repeat samples from same patients

Key: NAI - neuraminidase inhibitor; PEP - post exposure prophylaxis; wt - wild type; ICU - intensive care unit

N.B. Where data presented on a conference poster differ from that published in the conference proceedings, the former are included in this table to represent the most up-to-date data available.

Author and Reference	Abstract no.	Country	Nature of Samples	Assay Method(s)	Resistance Incidence	Mutations	Comments
Meijer [61]	O-827	The Netherlands	Cases + contacts from start of outbreak (n=126). Pre- and post- oseltamivir treatment	RT-PCR sequencing NA Phenotyping NAI	0/126 pre- or post- oseltamivir Phenotypic: none identified to date		
Meijer [62]	P-193	The Netherlands	Mix of sentinel surveillance, hospitalized, deceased, oseltamivir treatment failures (n=>900)	NA: sequencing or H275Y RT-PCR Some phenotyping of isolates	19/1250	18: H275Y 1 case: H275Y + I223R on zanamivir	18 immunocompromised or haematological malignancy; 1 patient untreated
Santos [63]	O-851	Portugal	Community + hospital. 163/577 more severe cases	Genotying NA + NAI phenotyping MUNANA	2/96 tested	Both I223V	3-4 fold increase in IC50 to oseltamivir
Wang [64]	P-150	Australia	In- and out- patients 26/80 oseltamivir treated	NA: RT-PCR Phenotyping: NA-Star TM	3/26 treated 0/17 pre-treatment 0/54 outpatients	H275Y	Resistant = 1 child; 1 adult; 1 immunocompromised. H275Y after 9 days oseltamivir
Rousset [57]	O-852	France	Pandemic phase positive samples: many post-treatment	Pyrosequencing NA H275Y TR-PCR Phenotyping NA	11/(denominator unclear)	10 H275Y 1 H275Y + I223R	10/11 resistant from immunocompromised treated patients

Key: NAI - neuraminidase inhibitor; PEP - post exposure prophylaxis; wt - wild type; ICU - intensive care unit

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