

## Oseltamivir in human avian influenza infection

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Avian influenza A viruses continue to cause disease outbreaks in humans, and extrapulmonary infection is characteristic. *In vitro* studies demonstrate the activity of oseltamivir against avian viruses of the H5, H7 and H9 subtypes. In animal models of lethal infection, oseltamivir treatment and prophylaxis limit viral replication and improve survival. Outcomes are influenced by the virulence of the viral strain, dosage regimen and treatment delay; it is also critical for the compound to act systemically. Observational data on oseltamivir treatment in the early stages of disease suggest it is useful for improving survival in patients infected with H5 viruses, and drug-selected resistance has only rarely been reported. The WHO strongly recommends oseltamivir for the treatment of confirmed or suspected cases of human H5 infection and prophylaxis of those at high risk of infection. In addition to oral dosing, nasogastric administration appears to be a viable option for the management of severely ill patients, as is the use of higher doses and prolonged schedules. F. Hoffmann-La Roche Ltd, the manufacturer of oseltamivir, is developing a mathematical model to allow rapid prediction of appropriate dosage regimens for any future pandemic. Roche is also funding the Avian Influenza Registry, an online database that aims to collect information from clinicians worldwide on the course of avian influenza in humans.

**Keywords:** H5N1, treatment, prophylaxis, clinical, pre-clinical

### Introduction

Influenza A viruses cause recurrent epidemics with substantial human morbidity and mortality, and are also associated with pandemics.<sup>1</sup> As pandemic influenza A has its origins in avian influenza viruses, the highly pathogenic H5N1 viruses that have become panzootic in domestic and wild fowl have to be considered a serious threat.<sup>1–3</sup> H5N1 is principally a zoonosis, with transmission in the first instance typically from fowl, or fowl product, to humans, although the primary source of exposure cannot always be determined.<sup>4</sup> Recent evidence supports the occurrence of limited human to human transmission,<sup>4–9</sup> while evidence from Pakistan supports human to human to human (third-generation) transmission.<sup>8</sup>

The typical clinical manifestation of H5 infection is influenza-like illness, with the development of dyspnoea and progression to severe illness, usually typified by fulminant pneumonia complicated by acute respiratory distress syndrome (ARDS); multiorgan failure and death may follow.<sup>2,10,11</sup> Gastrointestinal disturbances may also be evident, and the illness may present as a gastroenteritis-like condition.<sup>12</sup> Neurological signs and symptoms have also been reported.<sup>13,14</sup> Cases of mild illness and asymptomatic infection are evident,<sup>4,15–18</sup> but the extent of these less dramatic infections remains largely unknown.

Common laboratory findings of abnormality include leucopenia, lymphopenia and thrombocytopenia.<sup>2,11</sup> Mortality is high; up

to 31 August 2009, of 440 laboratory-confirmed cases of H5N1 infection reported to the WHO since 2003, 262 (60%) were fatal.<sup>19</sup> Although most cases have been reported in South-East Asia,<sup>19</sup> Egypt accounts for the third greatest number of cases, and a significant outbreak was reported from Eastern Turkey.<sup>18</sup> Wild birds in a number of EU countries have tested positive for H5, and 14 outbreaks in domestic poultry had been reported in six member states by early December 2007.<sup>20</sup>

Avian viruses of the H7 subtype also cause human disease outbreaks, and appear to have a predilection for the conjunctiva, as well as causing respiratory illness. In an outbreak in the Netherlands, a H7N3 virus of avian origin was detected in 89 persons, of whom 85 presented with conjunctivitis and/or influenza-like illness.<sup>21</sup> Symptoms were generally mild, but a fatal case of pneumonia in combination with ARDS was reported. In the UK, three outbreaks of H7N2 disease with influenza-like illness and/or conjunctivitis have been reported;<sup>22–25</sup> in one of these outbreaks, three of the four cases documented were hospitalized.<sup>22</sup> Cases of H7 disease have also been reported in the USA (H7N2),<sup>26</sup> Canada (H7N3)<sup>27</sup> and Italy (H7N3).<sup>28</sup> In some of these outbreaks, seroconversion was not detectable in all patients with symptomatic disease. The reason for this is unclear, but it seems likely that some H7 infections go undetected.<sup>29</sup> Symptomatic infections with H9N2 viruses were reported in Hong Kong, but the illness appears to be mild.<sup>30</sup> Seropositivity for H9 has also been reported from China.<sup>31</sup>

In the face of these developments, the antiviral medication oseltamivir (Tamiflu®; F. Hoffmann-La Roche Ltd) is recommended on the basis of observational data and systemic activity by the WHO as the primary treatment for H5N1 infection, with particular potential for reducing mortality if used in the early stages of disease.<sup>32,33</sup> Oseltamivir also appears active in the attempts to control disease outbreaks caused by H7 avian viruses.<sup>34,35</sup>

## Treatment experience

### Pre-clinical studies

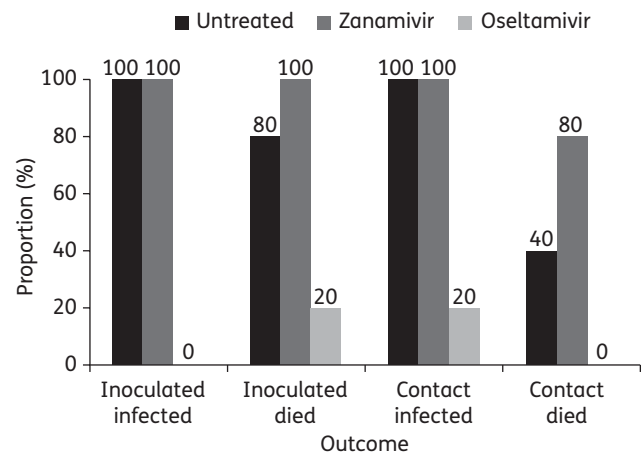
H5N1 viruses isolated from infected humans have been shown to be susceptible to oseltamivir in pre-clinical studies *in vitro* and in animal models.<sup>36–38</sup> Two studies focused on the influenza A/HK/156/97 (H5N1) strain that was transmitted to humans in Hong Kong in 1997 and killed 6 of 18 people infected. Leneva *et al.*<sup>38</sup> showed that oseltamivir carboxylate inhibited replication of the virus in Madin–Darby canine kidney (MDCK) cells with an EC<sub>50</sub> (50% effective concentration) of  $7.5 \pm 2.5 \mu\text{mol/L}$ . In this study, mice given 1 or 10 mg/kg oseltamivir after lethal challenge with A/HK/156/97 (H5N1) did not die and lost less weight than controls; mice treated with 0.1 mg/kg showed improved survival. In a similar study of the same viral strain, 90% of infected animals survived when given 10 mg/kg/day oseltamivir for 5 days starting 24 h after lethal infection.<sup>36</sup> When treatment was started 60 h after infection, 65% survived. Systemic efficacy was demonstrated by the prevention of spread of virus to the brain by daily dosages of 1 and 10 mg/kg. Oseltamivir was also active against the viral subtype A/quail/HK/G1/97 (H9N2) in both of these studies.<sup>36,38</sup>

The importance of early treatment has been shown by effects on more recent clinical isolates successfully treated with oseltamivir in animals. Ferrets were protected from lethal infection with influenza A/Vietnam/1203/04 (H5N1) by 5 mg/kg oseltamivir daily when started 4 h after infection, but 25 mg/kg daily was needed when treatment was started after 24 h.<sup>37</sup> A dosage of 10 mg/kg daily attenuated viral symptoms and blocked viral spread to internal organs when animals were challenged with the less pathogenic A/Turkey/15/06 strain.

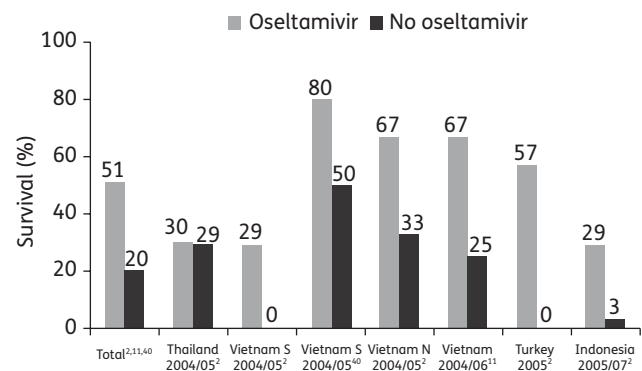
The significance of systemic action has been highlighted by results in chickens infected with influenza A/Chicken/Pennsylvania/1370/83 (H5N2). Chickens infected with virus were caged with contact chickens; treated birds received either intratracheal 1 mg/kg zanamivir or oral 120 mg/kg oseltamivir twice daily from 1 day before infection to 7 days afterwards. Monitoring for infection was carried out daily to day 8 and then on day 14. As indicated in Figure 1, zanamivir (which is distributed to the lungs only) had no effect on rates of infection and mortality while oseltamivir showed beneficial effects.<sup>39</sup>

### Clinical studies

Evidence pointing to the activity of oseltamivir against avian influenza in pre-clinical studies is supported by data in humans who have contracted H5N1. A summary of results from clinical case series in patients who contracted clade 1 or 2 influenza A (H5N1) viruses and who recovered after treatment with oseltamivir is shown in Figure 2.<sup>2,11,40</sup> Overall, results from uncontrolled



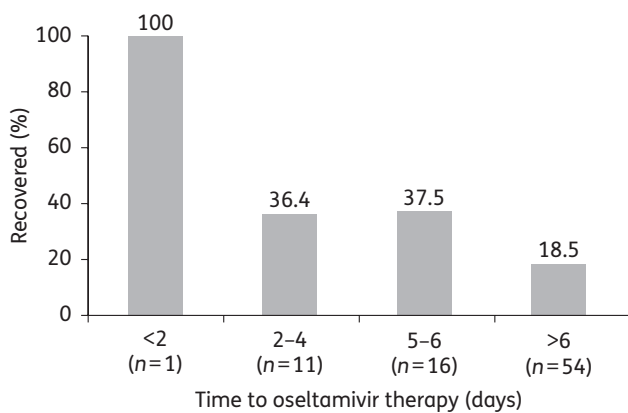
**Figure 1.** Infection and mortality in chickens inoculated with and in contact with influenza A/Chicken/Pennsylvania/1370/83 (H5N2);  $n=5$  chickens per group. 'Infection' refers to systemic infection indicated by virus isolation from the cloaca (with or without tracheal infection). Figure derived from data in Meijer *et al.*<sup>39</sup>



**Figure 2.** Survival among H5N1-infected patients treated with oseltamivir. Figure derived from data in WHO Writing Committee,<sup>2</sup> Liem *et al.*<sup>11</sup> and Hien *et al.*<sup>40</sup>

studies suggest that survival is improved ~2-fold by the use of oseltamivir, with early therapy being recommended.<sup>2,11,40</sup> However, the WHO recommends that late presentation should not preclude the initiation of oseltamivir therapy.<sup>32</sup>

Mortality remains high despite use of oseltamivir, but delayed initiation of treatment appears to be a key factor, as exemplified by the most recent analysis of data from Indonesia,<sup>41</sup> experience in Egyptian patients<sup>42</sup> and pooling of results for the years 2004–06 from Vietnam, Thailand, Indonesia and Turkey.<sup>43</sup> Indonesia has put in place surveillance and referral systems for human H5N1 infections since February 2004, and data are available for 127 patients with confirmed onset from June 2005 to February 2008 (this includes 54 included in a previous data set with a case fatality rate of 76%).<sup>44</sup> Of the 127 patients, 103 (81%) died; 88 patients were treated with oseltamivir (median time to treatment of 7 days). Patients with early oseltamivir treatment showed significantly increased survival ( $P=0.03$  for trend; Figure 3).<sup>41</sup> Pooled data from Vietnam, Thailand, Indonesia and Turkey show similar survival in patients treated with oseltamivir (13/34; 38%) and those not treated (4/16; 25%).<sup>43</sup>



**Figure 3.** Rates of recovery according to time to oseltamivir treatment in patients infected with influenza A/H5N1 in Indonesia from June 2005 to February 2008. Initiation of treatment within 2 days was associated with significantly lower mortality than initiation after 5–6 days or later ( $P < 0.0001$ ). Figure derived from data in Kandun *et al.*<sup>41</sup>

However, when time to treatment was accounted for, patients receiving oseltamivir within 5 days of onset showed 53% survival (8/15) compared with 26% of those who received oseltamivir  $\geq 6$  days after onset of disease (5/19).

To further investigate the clinical features, disease course and impact of antiviral treatment in human H5N1 infections, Roche is funding the AVEX Avian Influenza Registry, an online database that aims to collect information from clinicians worldwide.<sup>45</sup> This will be one of the largest sources of such information available. Data from 177 patients in eight countries were available for initial analysis as of August 2009.<sup>45</sup> The median age of patients was 20 years (range: 1.25–67 years) and 49% were male. Treatment with oseltamivir significantly improved crude survival rates for patients who received at least one dose [45/78 (58%) versus 10/87 (11%) for patients who received no antiviral;  $P < 0.0001$ ]. A significant survival benefit over no antiviral treatment remained even when oseltamivir was not initiated until days 6–8 of illness [12/23 (52%) patients given oseltamivir surviving versus 20/101 (20%) for no antiviral;  $P = 0.003$ ], but early treatment provided the greatest impact on mortality [survival on days 1–2; 6/8 (75%) among patients treated with oseltamivir versus 45/153 (29%) untreated patients;  $P = 0.014$ ].<sup>45</sup>

## Prophylaxis experience

### Pre-clinical studies

Oseltamivir was effective as prevention in the murine studies of the 1997 Hong Kong virus discussed earlier when 5 day courses were started 4 h before potentially lethal infection with A/HK/156/97 (H5N1); 100% survival was reported with daily dosages  $\geq 0.1$  mg/kg (Table 1).<sup>36,38</sup>

Dose-dependent effects have also been reported in prophylactic studies focusing on the more recent A/Vietnam/1203/04 (H5N1) virus, originally isolated from a throat swab from a fatally infected person.<sup>46,47</sup> A 5 day regimen of 10 mg/kg/day protected 50% of mice and increased duration of survival (Table 1); an increase in duration of therapy to 8 days improved efficacy and conferred survival rates of up to 80%. This viral variant is more

virulent in mice than the earlier influenza subtype A/HK/156/97 (H5N1), which points to the potential need for more prolonged therapy at higher dosages.<sup>47</sup> In a more recent study, 100% survival was reported in ferrets protected with 10 day oseltamivir started before infection with influenza A/Vietnam/1203/04 (H5N1) (Table 1).<sup>46</sup> When oseltamivir was given twice daily to these animals, clinical signs, systemic virus distribution and organ pathology were also prevented.

### Clinical studies

There is currently only limited information available regarding the efficacy of oseltamivir for the prevention of human infection with avian influenza virus. No clinical trial has evaluated oseltamivir for the prevention of H5N1.<sup>33</sup> However, modelling of an emerging outbreak in rural Asia has predicted the benefit of mass targeted antiviral prevention in terms of delaying or stopping spread of disease, and the WHO already has a stockpile of oseltamivir for this purpose.<sup>2</sup>

Kang *et al.*<sup>48</sup> detailed an analysis of 2512 people involved in the management of influenza A/Chicken/Korea/ES/03 (H5N1) that caused 19 outbreaks in seven provinces in Korea in 2003–04. All those directly involved in the culling operations were equipped with personal protective equipment, were vaccinated with commercial seasonal influenza vaccine and received oseltamivir as prophylaxis. None of the persons studied developed any influenza-like symptoms or fever, although nine individuals seroconverted (0.36%), showing that they had been directly exposed to the virus but that infection was not associated with clinical signs (see later for discussion of seroconversion data from animals and patients given oseltamivir).

An updated review by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) published in December 2007 of antiviral medicinal products for potential use during a pandemic also noted that ‘during the 2003 avian influenza H7N7 outbreak in the Netherlands, oseltamivir with the recommended prophylactic dose of 75 mg once daily seemed to be effective to protect poultry workers and their close contacts’.<sup>34</sup> The use of oseltamivir as prophylaxis was also linked to a significant reduction in the risk of conjunctivitis (a common extrapulmonary symptom of H7N7 infection).<sup>35</sup> These data also support a rationale for the potential utility of oseltamivir in avian influenza A infections of subtypes other than H5N1. Lim *et al.*<sup>49</sup> presented a case study of an outbreak of highly pathogenic avian influenza A (H5N1) on a farm in the UK in 2007. Information was available for 482 people considered at risk of exposure to H5N1 during the outbreak. Oseltamivir was provided as post-exposure prevention to 11 farm workers exposed at the time of the outbreak, none of whom was subsequently hospitalized or reported to have developed clinical symptoms of H5N1 infection.

### Seroconversion data

Information about the production of antibodies to avian influenza viruses during oseltamivir treatment is limited, but findings to date suggest that oseltamivir does not prevent the development of humoral immune responses to influenza A (H5N1) that are adequate to prevent re-infection.

**Table 1.** Effects on survival of oseltamivir as prevention started 4 h before infection with avian influenza A viruses in animal models

Reference (animal type)	Virus	Treatment	Dosage (mg/kg)	Survivors/total	Mean survival (days)	
Govorkova <i>et al.</i> <sup>36</sup> (mice)	A/HK/156/97 (H5N1)	oseltamivir ×5 days	0.01	2/6	11.3	
			0.1	6/6	16.0	
			1.0	5/5	16.0	
		control		0/11	6.3	
		A/quail/HK/G1/97 (H9N2)	oseltamivir ×5 days	0.01	5/11	12.2
	0.1			4/11	11.9	
	1.0			11/11	16.0	
	10.0		12/12	16.0		
	control			0/11	8.0	
	Leneva <i>et al.</i> <sup>38</sup> (mice)	A/HK/156/97 (H5N1)	oseltamivir ×5 days	0.1	4/5	14.0
1.0				5/5	>16.0	
10.0				5/5	>16.0	
control				0/4	7.5	
A/quail/HK/G1/97 (H9N2)			oseltamivir ×5 days	0.1	5/10	9.8
		1.0		9/9	>16.0	
		10.0		10/10	>16.0	
		100.0	10/10	>16.0		
		control		0/10	4.0	
A/HK/1074/99 (H9N2)		oseltamivir ×5 days	0.1	3/5	11.2	
			1.0	5/5	>16.0	
			10.0	5/5	>16.0	
		control		2/5	8.8	
		Boltz <i>et al.</i> <sup>46</sup> (ferrets)	A/Vietnam/1203/04 (H5N1)	oseltamivir ×10 days	5	3/3
10					3/3	
control				0/3		
Yen <i>et al.</i> <sup>47</sup> (mice)	A/Vietnam/1203/04 (H5N1)	oseltamivir ×5 days	0.1	0/10	10.7	
			1.0	0/10	12.2	
			10.0	5/10	20.1	
		control		0/10	9.6	
		oseltamivir ×8 days	0.1	1/10	11.6	
			1.0	6/10	12.1	
			10.0	8/10	13.0	
		control		0/10	11.0	

Antibody production has been reported in ferrets inoculated with H5N1 virus and subsequently treated with oseltamivir, which supports the contention that the drug does not interfere with serum antibody production.<sup>37</sup> Treated animals surviving lethal challenge with influenza A/Vietnam/1203/04 (H5N1) showed levels of HI antibody to influenza A/Vietnam/1203/04 (H5N1) that were low but nevertheless sufficient to provide complete protection against lethal rechallenge. Similar results were reported in animals initially challenged with influenza A/Turkey/15/06 (H5N1), although homologous HI titres were considerably higher (Table 2). Boltz *et al.*<sup>46</sup> subsequently also reported low but sufficient homologous HI titres in treated ferrets surviving lethal challenge with A/Vietnam/1203/04 (H5N1), with higher titres of the heterologous virus A/HK/213/03 (H5N1). No clinical signs of infection were seen in any ferrets undergoing lethal rechallenge with influenza A/Vietnam/1203/04 (H5N1). Overall, these findings are compatible with the premise that, rather than preventing

infection, prophylaxis limits the course of influenza infection within its early stages and prior to the onset of symptomatic disease.

Seroconversion has also been noted in humans treated with oseltamivir for exposure to avian influenza, notably the nine individuals (see earlier) who seroconverted after exposure to influenza A/Chicken/Korea/ES/03 (H5N1).<sup>48</sup> In a retrospective study of avian influenza in 705 persons involved in the 2003 outbreak of H7N7 virus in the Netherlands, a modified HI assay showed the presence of anti-H7 antibodies in 49% of 508 persons exposed to poultry and 64% of 63 persons exposed to infected humans.<sup>35</sup> These findings are consistent with those observed in seasonal influenza.

## Dose optimization

Whereas the majority of cases of H5N1 reported to date have been managed with the approved oseltamivir regimen of



**Table 2.** Results of rechallenge of ferrets with a lethal dose of homologous H5N1 after earlier oseltamivir treatment; all three rechallenged ferrets survived with no clinical signs of disease; reproduced from Govorkova *et al.*<sup>37</sup> with permission

Virus	Oseltamivir regimen used to treat initial infection	Rechallenge virus dose (EID <sub>50</sub> /ferret)	Range of pre-challenge HI titres (3 weeks after initial inoculation, expressed as reciprocal values)		
			A/Vietnam/1203/04	A/HK/213/03	A/Turkey/15/06
A/Vietnam/1203/04	25 mg/kg/day (24 h delay)	10 <sup>2</sup>	20–40	80–160	not tested
A/Turkey/15/06	10 mg/kg/day (24 h delay)	10 <sup>7</sup>	20–40	320–640	160–320

EID<sub>50</sub>, 50% egg infectious dose; HI, haemagglutinin inhibition.

75 mg, current dosages approved for seasonal influenza may need to be modified to combat effectively a potential pandemic virus based on the highly pathogenic influenza A/H5N1 strain. A higher dosage (e.g. 150 mg twice daily in adults) and increased duration of therapy may be reasonable in light of the high levels of replication of influenza A/H5N1, observations of progressive disease despite early treatment with standard dosages in some patients, and the apparent safety of higher dosages of oseltamivir in patients with seasonal influenza.<sup>2</sup>

The safety of a dosage of 150 mg twice daily given for 5 days has been demonstrated in two randomized and controlled studies in which 454 adult patients received this regimen for the treatment of acute seasonal influenza infection.<sup>50,51</sup> In these studies, the 150 mg regimen was shown to be as effective as the 75 mg regimen, with no apparent effect of the increased dosage on the incidence or severity of adverse events, most of which were transient and gastrointestinal in nature. Favourable tolerability at the 150 mg dose level was also reported in a study of experimental influenza B infection,<sup>52</sup> a dose-ranging study<sup>53</sup> and three pharmacokinetic studies.<sup>54–56</sup>

Higher doses have also been well tolerated. One study exposed 391 healthy volunteers to dosages of oseltamivir up to 450 mg twice daily for 5 days.<sup>57</sup> In this trial, in which dosages of 75 and 225 mg twice daily were also given, nausea, vomiting and headache were most commonly reported, and there was no apparent effect of oseltamivir at any dosage on QTc intervals or other electrocardiogram (ECG) parameters. No serious adverse events were reported. There was a dosage-related increase in gastrointestinal adverse events, with the majority of instances of nausea and vomiting being seen in the two highest dosage groups. Most were of mild to moderate severity and were transient. In other studies, oseltamivir was well tolerated at single doses of up to 675 mg<sup>58</sup> and 1000 mg,<sup>59</sup> and twice daily doses of up to 500 mg for 7 days.<sup>59</sup>

Given that individuals infected with influenza A/H5N1 are usually seriously ill (often with pneumonia) and need mechanical ventilation, the efficacy of nasogastric administration of oseltamivir is of considerable interest. Taylor *et al.*<sup>60</sup> investigated the pharmacokinetics of nasogastrically administered oseltamivir at a dose of 150 mg twice daily for 10 days in three severely ill patients, two with H5N1 (including one pregnant female) and one with influenza A/H3N2. Treatment commenced on day 6 of illness or later in all patients. Virus was cleared from two patients (one male infected with H5N1 and one elderly female with H3N2) after 5 days of treatment

although the female subsequently died of respiratory failure. The pregnant female also died of respiratory failure. Oseltamivir was well absorbed and converted extensively into oseltamivir carboxylate, with trough concentrations exceeding the IC<sub>50</sub> (50% inhibitory concentration) for the H5N1 virus isolated from the pregnant female by 545- to 3956-fold. Good absorption and therapeutic concentrations were also detected after nasogastric administration of oseltamivir to a child with influenza B-associated encephalitis.<sup>61</sup>

Animal data show that benefit might be obtained through the use of longer courses of therapy,<sup>47</sup> and this approach has been investigated in several human studies in seasonal influenza. Influenza prophylaxis with oseltamivir in the community has been given for up to 6 weeks in a study in children aged 1–12 years,<sup>62</sup> in two large studies in 1559 adults<sup>63</sup> and in >500 vaccinated frail elderly persons in the residential care setting.<sup>64</sup> Prophylactic dosing has been used successfully and safely for 8 weeks in a study in 32 immunocompromised children and young people<sup>65</sup> and, more recently, for 12 weeks in a placebo-controlled study involving 477 solid organ and stem cell transplant patients aged ≥1 year.<sup>66</sup> Oseltamivir prophylaxis has also been given for up to 13 weeks in at-risk healthcare workers.<sup>67</sup> In all reported studies, oseltamivir has been well tolerated and has reduced the incidence of infection substantially. An extended duration of prophylactic dosing is of particular relevance to healthcare workers in a pandemic situation, as these personnel play a key role in managing and containing an outbreak while being exposed to the virus over extended periods.

## Resistance to oseltamivir in H5N1 viruses

Most neuraminidases of H5N1 viruses appear to be susceptible to oseltamivir. Comparison of virus isolates from Asia from 1997 to 2005 found that viral neuraminidases from strains isolated in 2004 and 2005 were ~10 times more susceptible to oseltamivir than H5N1 isolates from 1997 and 2003 and a H1N1 isolate from 2004.<sup>68</sup> Fifty-three of 55 strains of H5N1 virus from Australia and South-East Asian countries analysed between 2004 and 2006 were shown to be susceptible to neuraminidase inhibitors, their IC<sub>50</sub> values being similar to those for H1N1 strains circulating in this region between 2001 and 2006.<sup>69</sup> The remaining two strains exhibited higher IC<sub>50</sub> values of oseltamivir than wild-type viruses. However, these values represented only small decreases in susceptibility, and would not be expected to be associated with reduced clinical efficacy at recommended doses. A second

example of such a natural variant was reported in Egypt, where the N294S mutation was found in H5N1 virus isolates taken before and after oseltamivir treatment from two infected patients who later died.<sup>70</sup> This mutation reduced susceptibility to the drug by 12–15 times. Recent data confirm that the N294S mutation, and the H274Y mutation that confers high level resistance to oseltamivir, are rare in circulating H5N1 viruses; of 676 isolates, only 1 contained N294S and 4 contained H274Y.<sup>71</sup>

The available information suggests that the incidence of selection of clinically significant resistance during treatment of H5N1 infection with oseltamivir is low. In a study of Indonesian patients treated with oseltamivir for H5N1 infections, no H275Y mutants were found in samples taken from 75 patients on admission or taken from 25 patients during the first 3 days of treatment follow-up.<sup>72</sup> Indeed, only three cases of infection with oseltamivir-resistant A/H5N1 viruses (all H275Y mutants) have been reported, all in Vietnamese patients. A patient who received oseltamivir treatment at the prevention dose (75 mg once daily) for 4 days despite being symptomatic recovered after a dose increase led to clearance of the virus.<sup>9</sup> In two other patients, both of whom died, oseltamivir treatment was given at the correct dose, but was started late in one case (sixth day of illness).<sup>73</sup> In all three cases, resistant virus was isolated after treatment had started.

## Combination chemotherapy

For other viral infections, combination chemotherapy has been successfully used to improve efficacy and/or reduce the emergence of drug resistance. By combining oseltamivir with an antiviral that has an alternative mechanism of action, similar advantages might be obtained. Amantadine and rimantadine are established antiviral agents for influenza that have a mechanism of action different from that of oseltamivir, and work by inhibiting the M2 ion channel. Recent publications suggest that combination therapy with either agent and oseltamivir could be advantageous over monotherapy.<sup>74–79</sup> Addition of ribavirin to this combination has also been investigated.<sup>80</sup>

## Clinical pharmacokinetics

In a recent study, Morrison *et al.*<sup>74</sup> investigated the pharmacokinetic profiles of amantadine and oseltamivir in healthy human volunteers. Seventeen subjects took part in the study, and each received amantadine and oseltamivir as monotherapy and as combination chemotherapy, with an appropriate wash-out between each regimen. Compared with monotherapy, neither drug had any clinically significant effect on the pharmacokinetics of the other when co-administered. There was also no increase in adverse events for the combination versus oseltamivir or amantadine monotherapy. The authors concluded that combination chemotherapy with oseltamivir and amantadine appeared safe and was without pharmacokinetic consequences.<sup>74</sup>

## Pre-clinical efficacy

The activity of the oseltamivir/amantadine combination has been studied *in vitro* and in mice infected with seasonal and

avian influenza viruses.<sup>75,77</sup> In MDCK cells infected with influenza A/Hong Kong/1/68 (H3N2) or influenza A/PR/8/34 (H1N1), a combination of oseltamivir and amantadine reduced viral replication to a greater extent than that seen with either antiviral alone.<sup>75</sup> In mice infected with lethal doses of the same mouse-adapted H1N1 or H3N2 strains, oseltamivir or amantadine monotherapy conferred 50%–60% survival, while complete protection was achieved with an oseltamivir/amantadine combination in which oseltamivir was administered at a lower dose than that used for monotherapy.<sup>75</sup> The combination was also effective against amantadine-resistant H1N1. In a further study, mice were given preventative doses of oseltamivir and amantadine, either separately or in combination.<sup>77</sup> A lethal dose of amantadine-susceptible or amantadine-resistant influenza A/Vietnam/1203/04 (H5N1) was then administered. Combination chemotherapy provided 60%–90% greater protection than monotherapy, depending on the amantadine dose. The efficacy of the combination against amantadine-resistant H5N1 was comparable to that with oseltamivir alone. Oseltamivir monotherapy produced dose-dependent protection against both amantadine-susceptible and amantadine-resistant viruses.

Combination therapy with oseltamivir and rimantadine has also been studied *in vitro* and *in vivo*. In MDCK cells infected with influenza A/New Caledonia/20/99 (H1N1) or A/Panama/2007/99 (H3N2), amantadine plus rimantadine effectively reduced virus yields and was more effective than either agent alone.<sup>78</sup> Combination chemotherapy also provided greater protection against lethal infection and prolonged survival versus monotherapy in mice infected with influenza A/Aichi/2/68 (H3N2).<sup>79</sup>

## Resistance

In addition to improved efficacy, combination chemotherapy with oseltamivir and amantadine or rimantadine could also decrease the emergence of resistant variants. Ilyushina *et al.*<sup>76</sup> investigated this property in MDCK cells infected with human seasonal influenza A/Nanchang/1/99 (H1N1) and A/Panama/2007/99 (H3N2) or avian influenza A/Hong Kong/156/97 (H5N1). Yields of all three viruses were significantly reduced when the cells were treated with a combination of oseltamivir and amantadine. While mutations associated with reduced antiviral susceptibility were noted with oseltamivir and amantadine monotherapy after repeated passages in MDCK cells, no mutations of the haemagglutinin, neuraminidase or M2 proteins were detected when the medications were used in combination.

## Ongoing studies

To address the issues surrounding dosing requirements in a future pandemic, Roche is developing a mathematical model based on viral kinetics in the presence and absence of oseltamivir therapy to facilitate rapid prediction of appropriate regimens.<sup>81</sup>

Roche is also supporting an open-label clinical study that will compare the efficacy and safety of oseltamivir monotherapy with oseltamivir/amantadine combination chemotherapy for 5 days in adults aged between 18 and 65 years with confirmed influenza A infections (clinicaltrials.gov reference NCT00830323). As published evidence on other combination chemotherapies is currently lacking, the study will also investigate the activity of

oseltamivir when used in combination with zanamivir, a second neuraminidase inhibitor. The study, which is being conducted in France, aims to recruit 60 subjects. A further randomized, double-blind, placebo-controlled study in France (clinicaltrials.gov reference NCT00799760) will compare the efficacy and safety of combination therapy with oseltamivir and zanamivir versus oseltamivir and zanamivir monotherapy (plus placebo to maintain the blind) in patients aged  $\geq 12$  years. Each drug will be taken at the standard dose for 5 days, whether alone or in combination, and patients will be stratified by time to treatment start from symptom onset ( $< 36$  h and  $\geq 36$  h). The target recruitment is 300 patients per group. The results of these studies should further inform influenza pandemic planning strategies.

## Conclusions

Avian influenza A viruses continue to cause disease outbreaks in humans. Animal models of avian infection demonstrate that oseltamivir is active against H5, H7 and H9 viruses when given as treatment or prophylaxis; outcomes are influenced by the virulence of the viral strain, dosage regimen and treatment delay. Observational data on oseltamivir treatment in human disease suggest its usefulness for reducing the frequency of H5-associated mortality, while resistance has only rarely emerged during treatment. On this basis and the systemic action of the drug, the WHO recommend oseltamivir as the primary intervention for the treatment and prevention of human H5N1 disease. Modified regimens and antiviral combinations may further improve the control of viral replication in these patients.

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