

Oseltamivir (Tamiflu[®]) and its potential for use in the event of an influenza pandemic

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Recent cross species transmission of avian influenza has highlighted the threat of pandemic influenza. Oseltamivir (Tamiflu[®]) has been shown to be effective in the treatment and prevention of epidemic influenza infection in adults, adolescents and children (≥ 1 year). Although oseltamivir has not been approved for prophylactic use in children, it has been shown to be effective. Oseltamivir is also active against avian influenza virus strains. Evidence suggests that lower doses or shorter durations of treatment/chemoprophylaxis other than those approved may not be effective and may contribute to emergence of viral resistance. Safety data from dose ranging studies show that 5 day courses of 150 mg twice daily for treatment and 6 week courses of 75 mg twice daily for prophylaxis were as well tolerated as the approved dose regimens. The use of oseltamivir in a pandemic is influenced by the goals of the pandemic plan developed by the responsible Government and Health Authority. To optimize use of antiviral medications, processes will be needed to collect, collate and report outcome data from treated patients and/or from use for chemoprophylaxis of pandemic influenza during the first-wave outbreaks. If oseltamivir is included in a national or regional pandemic plan, stockpiling of the material, either in the form of capsules or the bulk active pharmaceutical ingredient will be necessary. In the absence of a stockpile, there is no guarantee that an adequate supply of oseltamivir will be available.

Keywords: treatment, therapy, neuraminidase inhibitors

Background

This supplement is intended to review information concerning the potential use of oseltamivir phosphate (Tamiflu[®]) for the management of pandemic influenza. It provides information on the following topics:

- (i) The rationale supporting inhibition of neuraminidase as a management strategy for pandemic influenza, including the conditions under which this strategy would not be appropriate.
- (ii) Information on the biological properties of avian influenza in animal models and implications for dose selection and treatment period required in the event of a pandemic of avian influenza in humans.
- (iii) The *in vitro* activity of oseltamivir versus novel influenza neuraminidase enzymes.
- (iv) The *in vivo* activity of oseltamivir in animal models including potential pandemic strains.
- (v) Dose response and safety information derived from clinical studies with oseltamivir which are pertinent to the management of pandemic influenza.
- (vi) Published information on the use of oseltamivir for the prevention of inter-species transmission of avian influenza to abattoir workers and veterinary staff following experience in the Netherlands, Thailand and Vietnam.

(vii) Published information on the use of oseltamivir for the treatment of avian influenza in humans following experience in the Netherlands and Vietnam.

(viii) Information pertinent to storage, preparation and use of various dose forms of oseltamivir in the management of pandemic influenza.

(ix) Recommendations for additional animal and clinical research which should be conducted in the event of an outbreak caused by a novel influenza strain in order to provide specific advice based on evidence rather than extrapolation.

The biology of influenza A virus

Influenza virus is an enveloped RNA virus of the orthomyxovirus family with a genome that is composed of eight (influenza A and B) or seven (influenza C) segments. Influenza A and B are responsible for the annual outbreaks of epidemic influenza in humans. The virus capsid contains two major antigenic proteins, haemagglutinin (HA) and neuraminidase (NA). There are 16 different HA subtypes (H1–H16) and nine different NA subtypes (N1–N9), all of which have been found among influenza A viruses. Wild birds are the primary natural reservoir for all subtypes of influenza A viruses and are thought to be the source of influenza A viruses in all other animals. Although most influenza viruses cause asymptomatic or mild infection in birds,

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infection with certain strains of H5 and H7 viruses can cause widespread disease and death among wild and domestic birds such as chickens and turkeys.

Pigs are susceptible to avian, human and swine influenza viruses and thus have a potential to be infected with influenza viruses from different species (e.g. ducks and humans) at the same time. In this circumstance, active replication of both virus subtypes within the same host may result in reassortment of the genome RNA segments, creating a new virus containing a novel combination of HA and/or NA capsid proteins, a process known as antigenic shift. Antigenic shift may result in the emergence of a new influenza A subtype which can cross the species barrier and infect humans, who may have little or no immunity to the new virus. If the virus can be transmitted easily from person to person, an influenza pandemic could occur.

Influenza virus life cycle. The life cycle of influenza virus involves attachment to cell surface receptors, entry into the cell and uncoating of the viral RNA followed by replication of the viral genes inside the cell nucleus. After the synthesis of new copies of viral proteins and genes, these components assemble into progeny virus particles, which then exit the cell by budding from the cell surface.¹ The HA protein is responsible for virus binding to sialic acid containing receptors on host cells, mediating fusion between the viral envelope and the cell membrane.² The main role of NA, in contrast, is the release of newly manufactured virions from the cell. NA may have an additional role in the pathogenesis of influenza; by removing the sialic acids present in mucin, NA activity may facilitate virus penetration into the respiratory tract and ease of access to the surface respiratory mucosa. In some viruses, NA has also been shown to enhance virus pathogenicity and neurovirulence.³ The sialidase activity of NA also removes terminal sialic acid residues from both the HA and NA proteins, as well as host cell surface glycoproteins. Since the terminal sialic acid is critical for HA binding, the receptor-destroying activity of the NA counters the receptor-binding activity of the HA. In the absence of functional sialidase, progeny virions aggregate on the cell surface due to HA receptor-binding activity and fail to be released from the cell surface, limiting spread from cell to cell or, via infected respiratory secretions, from host to host.^{4,5} Influenza A viruses also contain a third protein, the M2 protein, which is an ion channel, controlling the pH of the endosome and permitting release of the ribonucleoproteins (RNPs) into the cell cytoplasm. The activity of all of these enzymes is required for efficient influenza A virus replication *in vivo*.¹

Antiviral treatment of influenza

The first antiviral treatments for influenza were the adamantane derivatives, amantadine and rimantadine, which are inhibitors of the M2 ion channel protein contained within the influenza virus. Whereas both agents have been shown to be effective, they are active only against influenza A virus (influenza B does not possess an M2 protein). Adamantane-resistant influenza A viruses emerge readily following use of both agents as the mutations required to produce resistance do not affect the ion channel function of the protein.⁶ These mutations may occur naturally among some avian influenza strains.⁷ These features contribute to the ease with which adamantane resistance develops in treated subjects.⁸ In contrast, the mutations required to give resistance to

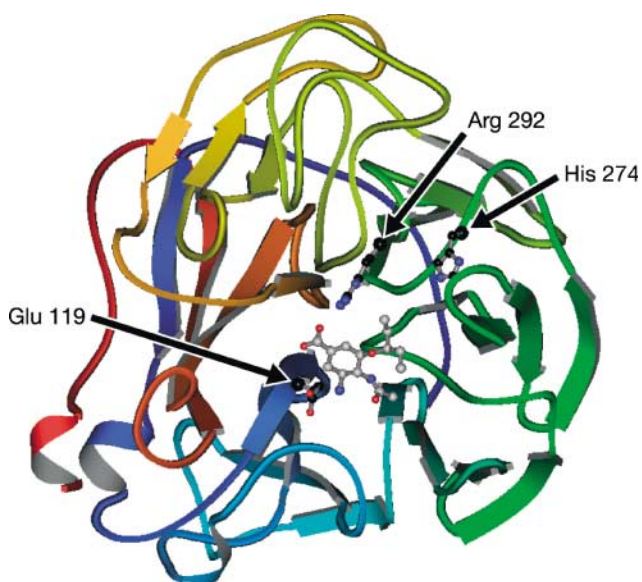


Figure 1. Diagram depicting the structure of neuraminidase (N1 and N2) in relation to bound oseltamivir. Arrows depict sites of known mutations in resistant subtypes of influenza virus.

the neuraminidase inhibitors are at the highly conserved active site of the neuraminidase enzyme (Figure 1). These mutations result in compromised neuraminidase activity and hence a lower potential for the emergence of resistance. The highly conserved nature of the active site of neuraminidase allows the neuraminidase inhibitors to be active against all influenza A and B subtypes. Two neuraminidase inhibitors, oseltamivir and zanamivir, are available in the EU for the treatment of influenza. Oseltamivir is orally active.^{9,10} Zanamivir however must be administered topically, by inhalation, or parenterally (iv), to be effective;^{11,12} only the inhaled product is commercially available.

20th Century influenza pandemics

Three influenza pandemics occurred during the 20th century. The first of these, from 1918 to 1920, was by far the most devastating with a death toll estimated to be between 20 and 50 million people. The source of this outbreak has never been satisfactorily identified. Although some evidence points to it starting in the USA in March 1918, other data indicate the virus may have first emerged in France as early as 1916.¹³ It is estimated that 50% of the global population became infected, of whom half suffered clinical illness, an attack rate of 25%. In some regions, e.g. Samoa and Alaska, the death rate was 25% of the population.¹⁴ Genetic characterization has shown the responsible virus to be of the A/H1N1 type.¹⁵

The clinical features of the infection were severe but mostly typical of influenza: rapid onset of fever, headache, myalgia, anorexia, nausea, vomiting and cough lasting 2–4 days. Epistaxis was a frequent but unusual symptom. Unusually, a proportion of patients died very quickly, having been rapidly overcome by a tracheobronchitis associated with dyspnoea and the appearance of mahogany spots around the mouth which coalesced into a heliotrope cyanosis. Post-mortem data from such patients indicated haemorrhagic lungs with an absence of pus. Of those patients who recovered, up to 18% subsequently developed pneumonia. The increase in mortality associated with this

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pandemic was notably greater in young adults than in other age groups, and in the elderly, mortality was actually decreased compared with normal influenza epidemics. Females <35 years accounted for 70% of all female influenza deaths.¹⁶

The pandemic of 1957–1958 originated in China and was transmitted mainly along sea lanes, spanning the globe within 6–7 months. Infection rates were similar to the 1918 pandemic, with 40–50% of people becoming infected, and 25% developing illness. The course of this infection was clinically typical of influenza, and most deaths arose due to pneumonia, although a higher than normal incidence of primary viral pneumonia was recorded. Mortality was confined mainly to the very young and the elderly. Nevertheless, the total death toll was estimated to be 1 million.¹⁴ Genetic analysis indicates that this pandemic was caused by an A/H2N2-type virus.

The 1968 influenza pandemic originated in Hong Kong, was the mildest of the 20th century pandemics, and was caused by an A/H3N2-type virus. In many regions, the virus seeded itself for up to 12 months before causing more serious disease in the second wave in 1969.¹³

Although excess mortality was mainly observed in the elderly, and attributable to secondary infections, in both the 1968 and 1957 pandemics, the relative increase in excess deaths was greater among young adults, as noted earlier for the 1918 pandemic. The partial protection of older persons is thought to be due to retained heterosubtypic immunity following prior exposure to similar antigens as children or young adults.¹⁶

Lessons learned from previous pandemic experience

Our knowledge of the clinical epidemiology of pandemic influenza is derived from information generated during the three influenza pandemics which occurred in the 20th century. Four elements of this experience are pertinent to the matters discussed in this report:

(i) The clinical features of pandemic influenza differ from those of epidemic influenza in that clinical progression within an individual may be significantly faster and associated with a significantly increased risk of fatal primary viral pneumonia. The relative increase in excess deaths is likely to be amongst young adults.

(ii) Antigenic cross-reactivity provides some protection against infection and ameliorates the pattern of illness within an individual as well as limiting spread within the community.

(iii) Among the population segment previously exposed to a strain sharing some cross antigenicity, mortality remains highest in those with a degree of existing co-morbidity (pre-existing cardiac disease, respiratory disease, renal disease, diabetes, immunosuppression or pregnancy).

(iv) Several waves of illness may occur during which the properties of the virus and the clinical pattern of illness within individuals and communities may alter.

During a pandemic, the number of people infected and the resultant mortality will be much higher than in a seasonal epidemic. Thus the ability of a government to deal with the chaos of a pandemic will be dependent upon their level of pandemic preparedness. It is for this reason that the WHO has provided broad guidance on key elements of a pandemic plan and has strongly urged all countries to develop their own detailed pandemic plans.¹⁷

Current events, particularly with avian influenza (see the following section) have brought into focus the potential for another influenza pandemic, with perhaps the most likely candidate strain being a modified avian H5N1 strain. Although first identified in 1997, there is, as yet, no candidate vaccine for this virus in production. It is also possible, of course, that a pandemic may arise from another source, precluding the possibility of advance vaccine preparation. Thus, pandemic planning for the control of influenza within communities, which is the responsibility of governments and responsible health authorities, needs to include options for control of influenza that involve the use of antiviral medications. In particular, the need for advance stockpiling of such agents, in order that sufficient quantities may be available at the time of a pandemic, should be addressed.

Avian influenza

Several instances of human infections and outbreaks following interspecies transmission of avian influenza have been reported since 1997.¹⁸ Most cases have resulted from contact with infected animals or contaminated surfaces. In 1997, the infection of 18 individuals with an unchanged avian influenza A virus (H5N1), six of whom died, was another sign that the threat of an influenza pandemic still exists, although serological investigations showed that human-to-human transmission was very rare.¹⁹ In 2003, H5N1 and H9N2 infections were confirmed in Hong Kong with several reported cases of mortality. Since January 2004, outbreaks of avian H5N1 influenza have been reported in several countries in Asia. These outbreaks were believed to have been contained earlier in the year, but beginning in June 2004, new deaths were reported in Vietnam caused by a highly pathogenic H5N1 avian influenza. This second H5 strain has some immunological differences to the 1997 strain suggesting that vaccines derived from the 1997 seed strains may be ineffective against it.²⁰

In 1999, H9N2 avian influenza was confirmed in two children in Hong Kong and several additional cases from mainland China. In 2003, another outbreak of H9N2 infection was confirmed in Hong Kong. The largest avian influenza outbreak occurred in the Netherlands in 2003 and involved an H7N7 virus. Recent evidence has emerged suggesting that approximately 1000 people, mainly farmers and poultry workers, were infected by the virus as a result of handling poultry.²¹ Of these, approximately 500 complained of symptoms consisting mainly of conjunctivitis and influenza-like illness.²² In another unexpected finding, those who developed symptoms after being infected passed the virus on to approximately 59% of their household contacts.²¹

The biological properties of these potential pandemic influenza strains have been explored in a series of animal models including the BALB/c mouse,²³ the cynomolgus monkey,²⁴ and the ferret.²⁵ Ferrets are naturally susceptible to infection with human influenza A and B viruses and their disease resembles that of human influenza. For these reasons, the ferret model system is widely used as a model for influenza virus pathogenesis and immunity studies.²⁵ Active viral replication began promptly and peaked early in ferrets. Clinical symptoms were more severe than those observed following infection of control groups with an epidemic H3N2 strain, although the H5N1 virus titres were 1000-fold lower than those of H3N2. Viral shedding from the respiratory tract continued for >7 days with peak virus titres

being reached on day 1 post-infection.²⁵ Among animals infected with H5N1 avian influenza, but not among those infected with epidemic H3N2 influenza, virus was also isolated from multiple systemic organs, although at substantially lower titres than from respiratory tract samples.²⁵ Histopathological features were consistent with broncho- and interstitial pneumonia with no evidence of secondary bacterial superinfection. The data from the BALB/c mouse are similar with all infected mice producing prompt clinical evidence of illness with rapid progression to a primary influenza virus pneumonia and death within 14 days. In mice, H5N1 infection failed to activate TGF- β production, which seemed to contribute to the virulence of the disease.²³

The primary lesion of experimental H5N1 virus infection in macaques was a severe necrotizing bronchointerstitial pneumonia similar in character and severity to that found in primary influenza virus pneumonia in humans.²⁴ The viral aetiology of this lesion was confirmed by demonstration of virus in tissues by PCR and immunocytochemistry, while bacterial superinfection was ruled out by the absence of visible bacteria in Gram-stained sections of pulmonary tissue and the histological character of the pulmonary lesions. The authors commented that, had the macaques survived, the regenerative changes in their lungs observed on day 7 post-infection would probably have developed into the organizing diffuse alveolar damage with interstitial fibrosis seen in two humans who died 1 month after H5N1 virus infection and contributed to the respiratory distress syndrome both developed, and from which they died.²⁴

Rationale supporting inhibition of neuraminidase as an option for control of pandemic influenza

As noted in the subsection 'Influenza virus life cycle' above, the influenza virus life cycle is dependent on the activity of the two capsid surface proteins, HA and NA. The viral HA is a fusion protein which binds to sialic (neuraminic) acid based cellular receptors on the epithelial cell membranes of the host species. Once bound to the cell membrane, viral nucleoproteins and viral RNA are incorporated into the epithelial cell nucleus where virus replication occurs. Newly formed virions emerge from the infected cell but remain bound to the sialic acid of receptors on the cell membrane unless cleaved from the cell surface by the viral NA. Recently, viral NA has also been shown to be involved in the initiation of influenza virus infection.²⁶ Thus NA activity is a key component of the virus life cycle.

Although nine different types of NA (N1–N9) have been recorded in the influenza viruses infecting different animal species, the active site of each of these subtypes shares a high degree of sequence identity. This makes it an attractive target for antiviral intervention as an effective inhibitor of one NA type is likely to have activity against the other subtypes.

As the viral NA is an antigen, NA-specific antibody can be generated. The effect of NA-specific antibody has been explored in animal species and been shown to protect against infection following exposure, and to ameliorate the illness associated with influenza virus infection in sero-susceptible animals; NA-specific vaccination has been shown to be protective in humans.^{27–32}

The protective effect of NA antibody in humans is thought to underlie the lower impact of the 1968 pandemic as the pandemic strain shared an NA (N2) which had been a feature

of the influenza viruses (H2N2 strains) circulating before the emergence of the pandemic strain, which possessed a novel HA (H3).

Oseltamivir

Oseltamivir is the orally-active prodrug of oseltamivir carboxylate which is a specific inhibitor of influenza virus NA. Oseltamivir has been shown to be clinically active for the treatment and chemoprophylaxis of influenza in adults and in children and is currently approved for use in 80 countries worldwide.

Activity of oseltamivir carboxylate in vitro

Oseltamivir carboxylate (OC), the active metabolite of the parent prodrug oseltamivir, is a potent and selective inhibitor of all influenza A NA subtypes.^{33,34} The OC concentrations required to inhibit NA activity described herein were measured by a modification of a previously described method [using 2'-(4-methylumbelliferyl)-D-N-acetyl neuraminic acid (MUNANA) as a substrate]. OC inhibits NA at IC₅₀s in the low nanomolar range.³⁵ The specificity of oseltamivir for influenza NA is high—even at a concentration of 1 mM, OC had little or no inhibitory activity against NA from sources other than influenza viruses.³⁴ Although only influenza viruses containing the NA subtypes N1 or N2 have circulated widely in man in the past 100 years, and could be the source of a future pandemic,^{36,37} it is also possible that a future pandemic may involve influenza A carrying one of the alternative NA subtypes, N3–N9.^{37,38} Each of the NA subtypes may combine with any of the HA subtypes (H1–H16). The activities of OC against representative N3–N9 NAs (IC₅₀ range 0.3–1.5 nM)^{33,34} are very similar to the geometric mean IC₅₀s using a similar assay (N1 = 1.54 nM, N2 = 0.43 nM) for approximately 900 clinical isolates collected worldwide over the period 1996–1999.³⁹ The IC₅₀ of OC for A/Chicken/Pennsylvania/1370/83 (H5N2) NA was 1.3 nM.⁴⁰

Antiviral tests *in vitro* against the recently circulating A/Vietnam/1194/04 (H5N1) showed that this strain was sensitive to OC, with an average 50% effective concentration for inhibition of plaque formation (EC₅₀) of < 10 nM.⁴¹

It is therefore likely that oseltamivir will be clinically effective against influenza A viruses containing any form of NA enzyme. However, as *in vitro* assays, particularly antiviral assays in cell culture,⁴² are not a reliable method to predict dose requirements, dose–response relationships must be explored in animal models.

Oral activity of oseltamivir in vivo in animals experimentally infected with human pandemic and avian influenza strains

Oseltamivir is orally active in a variety of species. Interspecies differences in esterase activity require that different doses are administered to ensure that plasma concentrations of the carboxylate achieve levels associated with antiviral activity. To achieve the same area under the curve (AUC) as an oral dose of 75 mg twice daily in humans, mice require an oseltamivir dose of 10 mg/kg, ferrets 5 mg/kg and chickens 120 mg/kg. Oseltamivir has been shown to be effective *in vitro* and *in vivo* in mice against a recombinant influenza A virus containing the H1 and N1 genes of the 1918 pandemic influenza virus.⁴³

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Transmission of avian influenza viruses to humans has been confirmed on several occasions since 1997. The efficacy of oseltamivir against these subtypes has been established *in vitro* and in various animal models.

The efficacy of oseltamivir against H5N1 and H9N2 viruses was demonstrated both *in vitro* and *in vivo* in a mouse model of influenza infection.^{44,45} When orally administered at doses of 1 and 10 mg/kg per day, oseltamivir prevented the death of mice infected with A/Hong Kong/156/97 (H5N1), mouse-adapted A/Quail/Hong Kong/G1/97 (H9N2), or human A/Hong Kong/1074/99 (H9N2) viruses. It also reduced virus titres in the lungs and prevented the spread of virus to the brain of mice infected with A/Hong Kong/156/97 (H5N1) and mouse-adapted A/Quail/Hong Kong/G1/97 (H9N2) viruses. When therapy was delayed until 36 h after exposure to the H5N1 virus, oseltamivir was still effective and significantly increased the number of survivors compared with control. Oral administration of oseltamivir (0.1 mg/kg per day) in combination with rimantadine (1 mg/kg per day) reduced the number of deaths of mice infected with 100 median lethal dose (MLD₅₀) of H9N2 virus and prevented the deaths of mice infected with 5 MLD₅₀ of virus. These findings were confirmed in a second experiment, in which oseltamivir compared favourably with zanamivir and with an experimental product RJW-270201, which is no longer in development.⁴⁶

In a chicken model of systemic influenza virus infection using the highly pathogenic influenza virus A/Chick/Victoria/1/85 (H7N7), oseltamivir treatment was associated with lower mortality than that of the control group.³⁴ Also in the chicken model, oseltamivir reduced transmission, morbidity and mortality of an H5N2 strain and prevented viral shedding.

There are, as yet, no data investigating the efficacy of oseltamivir used for the treatment or prevention of more recent (2003) H5N1 strains in animal model systems.

Human experience

Oseltamivir was used to control the transmission of H7N7 avian influenza virus to humans in the Netherlands.²² Oseltamivir treatment at 75 mg twice daily was provided to all new clinical cases and a prophylactic regimen (75 mg daily) was used to protect poultry workers and their families, which was to be continued until 2 days after their last exposure. In this context, permission was granted by the Dutch Health Authorities to use oseltamivir prophylactically for longer than the approved 6 week period (unofficially reported to have been up to 12 weeks of prophylactic use). However, oseltamivir prophylaxis was started late in the outbreak due to hesitancy about its optimal use and acceptance by the population. Nevertheless, towards the end of the outbreak, more patients took their antiviral medication. Protection against virus transmission was observed with avian influenza virus infection being detected in 5/52 (9.6%) untreated subjects compared with 1/38 (2.4%) of those who took oseltamivir (protective efficacy of 75%). As the numbers were small however, the difference was not statistically significant.

The WHO recommended oseltamivir for treatment of clinically confirmed cases and for post-exposure prophylaxis to control recent H5N1 avian influenza outbreaks. Hien *et al.*⁴⁶ reported the clinical and epidemiological findings of 10 confirmed human cases of avian influenza (H5N1) who presented to hospitals in Vietnam between December 2003 and January 2004.

Oseltamivir treatment began well after symptoms were established (> day 10) in only five of the cases, four of whom died.

Experience from studies conducted in epidemic seasons pertinent to use in a pandemic

Treatment of influenza. Oseltamivir is currently indicated for the treatment of influenza in patients aged ≥ 1 year. The adult dosage is 75 mg twice daily, with weight-based unit dosing using the suspension for children (Table 1). Dose adjustment is only necessary in patients with severe renal impairment, as evidenced by a creatinine clearance (CL_{CR}) < 30 mL/min.

The effectiveness of oseltamivir in the treatment of influenza infection has been demonstrated in a range of clinical studies. The clinical benefit of oseltamivir treatment of influenza infection in adults, using a pooled dataset from these 10 studies, has been summarized.⁴⁷ The key benefits of oseltamivir treatment were earlier resolution of illness combined with earlier return to normal health and ability to carry out normal activities. In patients with co-morbid conditions, mainly cardiac and chronic respiratory diseases, overlap of symptoms between influenza and the underlying conditions precluded demonstration of earlier resolution of *all* symptoms (the primary end point). However, the duration of febrile illness (fever, myalgia and chills/sweats) was significantly reduced.

The benefits of oseltamivir treatment are not restricted to the resolution of influenza symptoms. There is clear evidence that oseltamivir treatment leads to a reduction in secondary lower respiratory tract complications [infections (LRTI), bronchitis, pneumonia] and hospitalizations.⁴⁸ Relative to placebo-treated patients, overall, those receiving oseltamivir were 55% less likely to develop an influenza-related LRTI requiring antibiotic treatment. In patients considered at increased risk of such complications, there was a 34% decrease in LRTIs requiring antibiotics and a 59% reduction in hospitalizations.

The benefits of oseltamivir treatment have also been demonstrated in children aged ≥ 1 year.⁴⁹ Oseltamivir treatment resolved illness 36 h earlier than in the placebo arm. In young children, the main secondary complication arising from influenza infection is otitis media. This study demonstrated the ability of oseltamivir treatment to reduce the incidence of new diagnoses of otitis by 44%, a clear demonstration of the benefits of a

Table 1. Current recommended dose and duration of oseltamivir for treatment and prophylaxis of influenza

	Dose (oral)	Duration
Treatment		
Adults and adolescents (≥ 13 years)	75 mg twice daily	5 days
Children (> 1 year)		
≤ 15 kg	30 mg twice daily	5 days
> 15–23 kg	45 mg twice daily	
> 23–40 kg	60 mg twice daily	
> 40 kg	75 mg twice daily	
Prophylaxis		
Adults and adolescents (≥ 13 years)		
close contact	75 mg once daily	≥ 7 days
community outbreak	75 mg once daily	up to 6 weeks

systemic therapy. In children with mild to moderate asthma, there was a small but significant improvement in the forced expiratory volume in 1 s (FEV₁).

Data have recently also been published detailing the use of oseltamivir in preventing complications arising from influenza in immunocompromised patients following bone marrow transplantation.⁵⁰ Although not a controlled study, by comparison with data from the same centre in previous seasons, the authors concluded that oseltamivir use was well tolerated and that it had played an important role in preventing influenza complications in this population.

An obvious concern in the pandemic context is the requirement that treatment begins within 48 h of the onset of symptoms. The practical implications of this are significant even in a normal influenza epidemic, but are substantially greater in a pandemic situation. It is well recognized that by the time symptoms become apparent, viral replication and transmission may have already been ongoing for perhaps 24 h. Not surprisingly therefore, it has been demonstrated that increased benefit can be obtained by providing oseltamivir as soon as possible after the onset of symptoms.⁵¹

While authorities are concerned to understand whether treatment may be effective if started later, data from the animal models of avian influenza (see the section ‘Lessons learned from previous pandemic experience’ above) confirm that viral replication peaks early and late intervention may not be successful.⁴⁴ Intervention should not be postponed beyond the 48 h approved for epidemic influenza and given the rapidity of clinical deterioration noted in infected cases, treatment plans should emphasize treatment intervention at the earliest possible time in suspected cases.

Prophylaxis. Oseltamivir is currently indicated for the prophylaxis of influenza in adults aged ≥ 13 years (Table 1). Data from a recent study demonstrate preventive efficacy in children aged ≥ 1 year.⁵² Registration studies examined ‘seasonal’ prophylaxis in adults⁵³ and in the frail elderly,⁵⁴ and ‘post-exposure’ prophylaxis (PEP) in a family setting.⁵⁵ The outcomes of these studies are summarized in Table 2.

The overall conclusions from these studies were that oseltamivir, administered at a dose of 75 mg once daily for a period of 7–42 days:

- (i) Reduced the incidence of laboratory-confirmed clinical influenza by up to 92% ($P \leq 0.001$).
- (ii) Reduced the incidence of influenza A and B virus infection.
- (iii) Reduced the proportion of subjects shedding influenza virus.
- (iv) Reduced the incidence of clinically diagnosed complications of influenza (e.g. bronchitis, sinusitis and pneumonia).
- (v) Did not prevent the formation of a specific antibody response to influenza infection.
- (vi) Did not result in the development of resistance.

Protective efficacy was maintained whether subjects were at risk of influenza only from an encounter with an individual in the community or were in close and prolonged contact with an infected individual. Efficacy was demonstrated regardless of pre-existing immune status. There was no evidence that the protective efficacy of oseltamivir in adults and children ≥ 13 years was altered by age, gender, geographic region, or the presence of pre-existing co-morbidity [particularly chronic obstructive pulmonary disease (COPD)].

Data from a further PEP study⁵³ have shown that treatment of influenza is not an effective stratagem for preventing transmission of influenza in close contact scenarios. This study provides clear evidence that control of an influenza outbreak (epidemic or pandemic) is not possible solely by treating ill cases as they develop. Rather, a strategy involving prophylaxis is essential if virus transmission between individuals is to be interrupted and the outbreak curtailed and controlled.

In 2004, Hayden *et al.* reported the outcomes of a study⁵² which compared the effectiveness of expectant treatment with that of treatment plus PEP in preventing secondary spread of influenza within households. All index cases were treated with oseltamivir and contacts were randomized by household to

Table 2. Number of subjects with laboratory-confirmed clinical influenza during the dosing period in studies of influenza prophylaxis

Study	Oseltamivir			<i>P</i> value	Treatment effect ^a [95% confidence intervals]
	Placebo	75 mg once daily	75 mg twice daily		
Welliver <i>et al.</i> ⁵⁵ ITTIINAB	24/200	2/205		<0.001	92% [72–98%]
Welliver <i>et al.</i> ⁵⁵ ITTH	34/462	4/493		<0.001	89% [72–96%]
Hayden <i>et al.</i> ⁵³ ITT	25/519	6/520	7/520	<0.001	76% [42–90%]
Peters <i>et al.</i> ⁵⁴ ITT	12/272	1/276		<0.01	72% [36–88%]
Peters <i>et al.</i> ⁵⁴ ITT				<0.01	92% [37–99%]
Pooled data from Hayden <i>et al.</i> , ⁵³ and Peters <i>et al.</i> ⁵⁴	37/791	7/796		<0.001	81% [58–92%]

ITT, intent to treat; ITTH, intent to treat (index case influenza infected); ITTIINAB, intent to treat [index case influenza infected; contact not influenza infected (culture positive) at baseline]. Number of laboratory-confirmed clinical influenza cases was determined between days 1 and 7 inclusive for Welliver *et al.* (7 day dosing) and between days 4 and 42 inclusive for all other studies (42 day dosing).

^aTreatment effect = proportionate reduction in cases of laboratory-confirmed influenza illness.

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receive either expectant treatment (75 mg twice daily for 5 days), if illness developed, or post-exposure prophylaxis (75 mg once daily for 10 days). PEP provided an additional protective efficacy against proven influenza of 58.5% for households and 68% for individual contacts compared with treatment alone. Excluding contacts who were already virus infected at baseline, the protective efficacy of treatment plus PEP over symptomatic treatment alone increased to 78.8% and 84.5% for households and contacts, respectively. In contrast to the earlier PEP study reported by Welliver *et al.*,⁵⁵ the trial included children aged ≥ 1 year as both index cases and contacts. The fact that children are a significant vector in influenza virus transmission is well recognized and, in fact, more than 50% of reported secondary influenza cases in this study were children aged 1–12 years. Amongst paediatric contacts managed expectantly, the incidence of influenza illness was almost three-fold higher in the 1–12 year age group than among contacts aged ≥ 13 years (24% versus 8%). PEP reduced the incidence of febrile influenza illness by 55% among paediatric contacts and by 80% among those who were not already infected with influenza at study start. Another important point to emerge from this study was the fact that, despite simultaneous treatment and prophylaxis within the same household, no virus resistant to oseltamivir was detected in any participant in the study.

Outbreak control in institutionalized populations presents unique challenges, particularly if the populations are frail from disease or age. Oseltamivir has been used successfully to control several outbreaks in nursing home populations. Simultaneous treatment and prophylaxis with oseltamivir have also been used successfully to control influenza A outbreaks in Canadian nursing homes, in some cases after amantadine failure.⁵⁶ Similar results have been reported where oseltamivir use controlled an influenza B outbreak in an elderly nursing home population.⁵⁷ Outbreak control was achieved following an average of 15 days of prophylaxis, although the period ranged from 9 to 23 days in the individual institutions.

The maximum duration of influenza prophylaxis with oseltamivir employed during the registration studies was 6 weeks, a period which was chosen to reflect the normal maximum duration over which influenza may persist in a particular local area. The maximum dose studied was 75 mg twice daily. As circumstances may differ somewhat in a pandemic situation, any data indicating the safety of higher doses or more prolonged exposure are pertinent. A recent publication⁵⁷ describes the use of oseltamivir for prolonged (8 weeks) prophylaxis in a paediatric cancer centre. The population ($n=32$) were immunocompromised by chemotherapy or bone marrow transplantation and were aged 6–23 years. No influenza infection was noted during the study period and the side effects of oseltamivir were few and acceptable (gastrointestinal upset in five of the subjects). Similarly, the registration studies showed that the safety profile of twice- versus once-daily oseltamivir given over 6 weeks for seasonal prophylaxis was identical.

Data produced since registration of oseltamivir for prophylaxis against influenza have led to the extension of the age range to include children aged ≥ 1 year, and raised the duration of exposure for which safety data are available from 6 to 8 weeks.

Of crucial importance is an awareness of the mechanisms by which oseltamivir is effective in prophylaxis. Data reported by Hayden *et al.* (1999)⁵³ and Peters *et al.* (2001)⁵⁴ clearly demonstrate that oseltamivir does *not* prevent the indi-

vidual from being infected by the virus, and hence raising an immune response to it. Rather, the drug works by preventing productive viral replication and release of virus from infected cells, such that the infection remains, in almost all cases, sub-clinical or associated with only minor symptoms. Hayden *et al.* (1999)⁵³ reported that 55/519 (10.6%) placebo recipients became infected with influenza, of whom 19 (36%) developed a febrile illness compared with 55/1043 (5.3%) oseltamivir recipients who were infected, of whom 7/55 (13%) developed a febrile illness. In an elderly population,⁵⁴ not only did a greater number of subjects with evidence of infection (a four-fold or higher rise in type-specific HAI antibody) develop influenza illness—12/19 (63%) on placebo compared with 1/13 (~8%) of oseltamivir recipients, but also the group geometric mean increase in antibody titre was greater among oseltamivir recipients than among placebo recipients. The ability of the individual to be protected from the consequences of influenza infection, while still mounting an immune response sufficient to protect them against subsequent exposure, will be even more crucial in a pandemic situation than in the normal epidemic situation.

We would contend, therefore, that two key messages derive from the oseltamivir dataset and should be included in any pandemic guidance:

- (i) Control of the spread of influenza infection is not possible solely by the treatment of symptomatic cases as they emerge.
- (ii) Prophylaxis with oseltamivir in the immunocompetent individual will not prevent the development of an antibody response, which may in turn offer a measure of protection against antigenically cross reacting viruses of the same strain.

‘At-risk’ populations

With normal epidemic influenza infections, ‘at-risk’ populations are defined as those deemed most likely to suffer secondary consequences following viral infection, most usually pneumonia and bronchitis. Populations considered to be ‘at risk’ include the elderly, those with chronic respiratory or cardiac conditions, diabetes, renal disease, pregnant women and children under 1 year of age.

Efficacy has been demonstrated in the elderly and in patients with co-morbid daily cardiac and respiratory disease as detailed above in the sections ‘Treatment of influenza’ and ‘Prophylaxis’ and in published references.^{19,48} Protective efficacy of 92% was demonstrated in an elderly population where 80% had been vaccinated for the current influenza season.⁵⁴ Clinical benefit has also been reported when oseltamivir has been used for treatment or prophylaxis in paediatric cancer patients,⁵⁸ the elderly,⁵⁹ and bone marrow transplant recipients.⁵⁰

Pregnant women

Historical data from the influenza pandemics of 1918 and 1957 illustrate the potential risks of influenza in pregnant women and their fetuses. In the 1918–1919 pandemic, mortality associated with infection during pregnancy was reported to be over 50%, with higher rates of mortality reported in the later stages of pregnancy.⁶⁰ During the 1957 pandemic, the obstetrical literature reported that 50% of the women of child-

bearing age who died of influenza were pregnant.^{61,62} Furthermore, 10% of all influenza deaths reported during the 1957 influenza season were in pregnant women, with the majority occurring during the latter half of pregnancy. Influenza in pregnant women during the pandemics of 1918 and 1957 was associated with pneumonia. In half the cases of influenza pneumonia in pregnant women, pregnancy was interrupted due to spontaneous abortion or delivery. Since that time, literature reports have shown that epidemic influenza illness is common in women in the second and third trimesters of pregnancy during the influenza season. Pregnant women may be at higher risk of developing serious complications of influenza infection than the normal healthy adult population.^{63–68}

There are no data from studies investigating oseltamivir treatment of pregnant women. Oseltamivir showed no evidence of fetal toxicity or teratogenicity in animal testing (Roche: data on file). Data from pregnancies reported during clinical trials and in subsequent post-marketing experience reveal no evidence that receipt of oseltamivir results in a significant risk of fetal abnormality (see ‘Background’ above). Nevertheless, in the absence of controlled data from pregnant women, oseltamivir should be used in pregnant or lactating women only if the benefit is considered to outweigh the potential risk to the unborn child.

Children < 1 year of age

The effect of influenza in the neonate or very young child is also associated with substantial morbidity. In a prospective study of children infected with influenza before the age of 1 year, about one-third of the infections occurred in the first 6 months of life.⁶⁹ Excess rates of hospitalization due to acute respiratory disease have been consistently reported in young children.^{70,71} Rates of hospitalization during the influenza season are known to be highest in the very young as well as the very old.⁷² In 2002, a toxicology study in juvenile rats was completed which demonstrated high mortality associated with CNS penetration and accumulation of the prodrug and carboxylate at levels >800-fold higher than the levels expected in children of ~1 year of age (Roche: data on file). Brain levels of oseltamivir in rats decreased with increasing age, reflecting the fact that the blood–brain barrier in the 7-day-old rat appears to be incompletely matured. There is general agreement that the human blood–brain barrier is fully developed by the age of 1 year. There are no data from clinical studies in children under 1 year of age and oseltamivir is not approved for use in this group. Oseltamivir should be used in children under 1 year of age only if the benefit is considered to outweigh the potential risk.

Virological considerations

In a pandemic situation, direct antiviral activity against a novel strain would be helpful to reduce the overall period of risk of transmission to direct contacts. Indeed, during the first wave of a pandemic, before the availability of vaccines, appropriate antiviral use will be the only option for control of the virus.

Oseltamivir treatment of epidemic influenza was associated with reductions in the proportion of patients shedding virus, reduced titres of virus in respiratory secretions and a reduced overall viral load (AUC viral titres) in young otherwise healthy adults,^{9,10} the elderly⁴⁷ and in children.⁴⁹ In young,

otherwise healthy, influenza-infected adults (who provided nose and throat swabs for influenza virus culture at baseline and days 2, 4 and 6) the proportion of subjects still shedding virus on day 4 was lower on active treatment than on placebo (28% for the 75 mg and 23% for the 150 mg oseltamivir treatment groups, compared with 36% in the placebo group, $P=0.013$). Mean and median virus titres were also ~1 log lower in the subjects treated with oseltamivir than in the placebo group at each post-baseline assessment up to day 6 (when virus shedding had ceased in most cases). Compared with the median value in the placebo group (128.3 log₁₀ TCID₅₀/h/mL), the AUC of virus titre was reduced by 17% to 91.9 log₁₀TCID₅₀/h/mL ($P=0.016$) in the 75 mg twice daily dose group and by 22% to 90.2 log₁₀TCID₅₀/h/mL ($P=0.0002$) in the 150 mg twice daily dose group, respectively. Similar results were obtained from studies in the elderly and in children. The data indicate that subjects treated with oseltamivir stopped shedding virus earlier than did subjects receiving placebo.

Virological resistance

Virological resistance to NA inhibitors can be acquired by alteration of the drug target (NA) or via alterations in the affinity of HA for cell surface receptors.

There is no evidence for the existence of naturally occurring oseltamivir-resistant variants. This is supported by the observation that all pre-treatment samples from clinical studies revealed no instances of virus with reduced sensitivity to oseltamivir. This was further supported by data from a separate study of over 1000 clinical isolates taken from influenza surveillance before the launch of the NA inhibitors.³⁹

Mutations in neuraminidase. *In vitro* studies have shown that, as expected, given the high sequence conservation of NA enzymes, influenza virus with decreased sensitivity to OC owing to mutations in the viral NA can only be generated with difficulty. Mutations observed are NA specific. Three mutations have been generated *in vitro* in influenza A N1 or A N2. Experiments have not been conducted with viruses carrying other NA subtypes. The *in vitro* data generated using N1 and N2 strains were generally predictive of the mutations found following treatment of infected adults and children. Subsequent experiments (see the section ‘Properties of resistant viruses’ below) showed that the presence of these mutations reduced the biological fitness of affected viruses. Thus the risk of transmission of oseltamivir-resistant virus in clinical practice is low.

Mutations in haemagglutinin. After eight passages in the presence of oseltamivir, two mutations of viral HA have been observed with a minor decrease in susceptibility to oseltamivir (by 8.6-fold). However, owing to the slight decrease in sensitivity and other considerations, it seems unlikely that mutations in HA will be of major significance.⁷³ Indeed, contrary to the observation that HA mutations generally precede NA mutations during *in vitro* resistance selection studies,⁷⁴ there is no evidence for the selection of HA mutations in conjunction with NA mutations in influenza in the clinical setting.^{75–77}

Resistance in clinical samples

Influenza virus samples studied during clinical trials. The primary assay to determine the emergence of resistance was a

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change in NA phenotype between pre- and post-treatment samples, supported by extensive genotypic studies. Consistent with *in vitro* data, resistance mutations arose at a low frequency during oseltamivir treatment. They were subtype specific, H274Y being identified in N1 NA and R292K in N2. There was additionally a very low frequency of E119V, found only in N2 NA. When detected, resistant viruses arose late in treatment, always on or after day 4, and were often present as a mixture of both wild-type virus and resistant virus, in which the wild-type dominated. The clinical course of patients carrying resistant epidemic influenza is generally indistinguishable from those still carrying wild-type virus at the end of treatment.⁷⁷

Among the otherwise healthy adults enrolled in the dose ranging trials, three of the patients harbouring resistant virus received oseltamivir 75 mg twice daily and one patient received 150 mg twice daily. As of July 2004, the incidence of oseltamivir resistance seen in clinical trial samples was 0.33% (4/1228) in adults/adolescents (≥ 13 years), 4.0% (17/421) in children (1– ≤ 12 years) and 1.26% overall.

Other reports of resistance to oseltamivir. A recent publication reported oseltamivir-resistant viruses in 9/50 (18%) Japanese children treated with oseltamivir for influenza A (H3N2) infection.⁷⁷ The children were young (median age 3.7 years) with several <1 year of age (contrary to the approved label). Consistent with our findings, resistance was not selected until at least day 4 of treatment. The predominant mutation was R292K. Two instances of E119V were also found. One novel mutation, N294S, was identified. Again consistent with our findings, there were no accompanying mutations in the viral HA.

A Roche-sponsored clinical trial JV16284, was conducted in Japanese children in 2000/2001 (Roche: data on file). The predominant circulating virus was an N1 strain. Virus with resistant phenotype was found in 7/43 (16.3%) children—all were due to the H274Y mutation in influenza A (H1N1). In this study, as with the recently published data,⁷⁷ children received a dose of 2 mg/kg body weight twice daily. Three 1-year-old children were among those found to harbour resistant virus in Study JV16284.

Oseltamivir carboxylate is excreted via the kidney and renal excretion rates are inversely proportional to age in children <12 years of age. Outside of Japan, the approved dose of oseltamivir is a weight-based unit dosing regimen with administration using a syringe device. These dosing strategies act to increase plasma concentrations of OC relative to the 2 mg/kg dose, particularly among pre-school age children, in whom there was some evidence of under-exposure relative to adults and children of school age.

The apparently higher incidence of resistance seen in Study JV16284⁷⁸ and Kiso *et al.*⁷⁷ may be a result of under-exposure to oseltamivir in young children. In Study JV16284, 30/70 (42.9%) of the children were aged 1–3 years, and in Kiso *et al.*, 25 of the 33 (75%) children who had positive post-treatment viral samples were in this low age range. These children were likely to be encountering their primary infection with influenza, and under-exposure to an antiviral drug in this immunologically naive population would permit continued viral replication and thereby increase the risk of the emergence of resistant virus.

This hypothesis is supported by data from the study reported by Hayden *et al.* (2004).⁵² Children enrolled in this study received the weight-based unit doses approved outside of Japan.

No resistance was identified in any of the 147 children treated, including the 26 children aged ≤ 5 years (mean 3.76, median 4, range 1–5 years). If these interpretations are correct, the implication is that the treatment of pandemic influenza in a totally naive population may require either higher exposures or longer treatment periods, or both, to reduce the potential for emergent resistant virus.

Properties of resistant viruses. Viruses carrying each of the three most common NA resistance mutations to OC seen in the clinic (E119V and R292K in N2 and H274Y in N1) have been studied in more detail in animal models to further characterize their transmissibility and virulence. Contrary to the statement of Kiso *et al.*,⁷⁷ a study was carried out with low passage clinical isolates, none of which had any changes in the HA.⁷⁹ Each resistant isolate was compared with the corresponding pre-treatment clinical isolate from the same patient to confirm this.⁷⁹

The overall fitness (infectivity, replicative ability and pathogenicity) of the virus carrying the R292K mutation in the N2 NA gene was reduced in the ferret model of influenza infection.⁷⁹ This confirmed previous studies in mice using *in vitro* selected mutant virus.⁷⁴ The R292K-carrying clinical isolate was not transmitted between ferrets under conditions where the corresponding wild-type virus was transmitted to 100% of contacts.⁸⁰ Similarly, evaluation of viruses carrying the H274Y mutation (N1) in the mouse and ferret models of infection also revealed that this mutant is less virulent/infective and is approximately 100-fold less likely to be transmitted than the corresponding wild-type.^{81,82}

The situation concerning the E119V mutation (N2) is less clear. In mice, it was established that the infectivity of E119V virus was less than that of the wild-type. In a repeated ferret study (in the first study, external factors complicated the interpretation of the data), the infectivity/replicative ability of E119V was reduced by 100-fold compared with wild-type. However, in a further study of transmission in the ferret, in which higher infectious titres were used, resistant virus was of similar infectivity to wild-type and transmitted from infected animals to non-infected animals.⁸³

A mathematical model was developed to look specifically at the dynamics of influenza transmission to predict the potential emergence and spread of drug-resistant virus.⁸⁴ The model demonstrates that the biological properties of the resistant viruses militate against the spread of virus, even when used in a high proportion of the population and for both treatment and prophylaxis.

The new N294S mutant virus identified by Kiso *et al.*⁷⁷ requires characterization, as will any future new mutations which may be identified in N1 or N2 NA. Similarly, should a future pandemic involve a virus carrying one of the alternative NAs N3–N9, additional research would be needed to identify and characterize any further subtype-specific mutants emerging during treatment.

In summary, the common phenotypic and genotypic changes observed in clinical influenza samples to date indicate that oseltamivir-resistant influenza viruses arise infrequently and are, in the main, biologically impaired with regard to infectivity and potential for transmission.

Continuing surveillance. The global Neuraminidase Inhibitor Susceptibility Network (NISN) was established in 1999 to address public health and regulatory concerns regarding the

potential emergence of resistance, and consequences of drug resistance, following the introduction of the NA inhibitor class of drugs to the market.⁴² In a post-marketing period, 3/2691 (~0.1%) clinical influenza samples showed resistance mutations.⁸⁵ The NISN continues to monitor for resistance to the NA inhibitors on a global level with particular emphasis on those regions with the highest clinical usage of NA inhibitors, currently Japan.

Prompted by the report of resistance in Japanese children treated with oseltamivir,⁷⁷ the NISN has issued a statement⁸⁵ and emphasized that concerns about resistance to NA inhibitors should not dissuade countries from continuing to develop adequate drug stockpiles. A similar position was taken by Moscona⁸⁶ while emphasizing the need to further study the properties and incidence of resistance to the NA inhibitors. The NISN continues to monitor the situation in Japan and globally, and a study employing over 1000 recent clinical isolates is in progress, specifically designed to seek evidence for generalized resistant virus transmission in Japan.

Safety considerations

Assessment of data from the clinical trial programme, retrospective studies from a US health insurance database and of post-marketing surveillance data provide a comprehensive review of the safety of oseltamivir in clinical use in subjects older than 1 year of age.^{87,88} No important safety concerns have evolved which might limit the suitability of oseltamivir for the treatment and prevention of influenza in all approved patient populations.

Experience from clinical trials

Adult treatment studies. In a total of 2554 patients (including patients on placebo, 75 mg twice daily and 150 mg twice daily oseltamivir) participating in the major studies in the treatment of influenza, the most frequently reported adverse events were nausea and vomiting.⁸⁷ These events were transient and generally occurred with first dosing. These events did not lead to

patient discontinuation of study drug in the vast majority of instances.

Some adverse events occurred more frequently in patients taking oseltamivir compared with those taking placebo. The adverse events that occurred most frequently are shown in Table 3. This summary includes healthy young adults and at-risk patients (patients at higher risk of developing complications associated with influenza, e.g. elderly patients and patients with chronic cardiac or respiratory disease). The incidence of the two main drug-related events, nausea and vomiting, are slightly increased by doubling the dose to 150 mg twice daily but both doses appear to be well tolerated in this population.

In general, the adverse event profile in the 'at-risk' patients in treatment studies was qualitatively similar to healthy young adults.

Paediatric treatment studies. A total of 1032 children aged 1–12 years (including 698 otherwise healthy children aged 1–12 and 334 asthmatic children aged 6–12) participated in studies of oseltamivir given for the treatment of influenza. A total of 515 children received treatment with oseltamivir suspension.

The most frequently reported adverse event was vomiting. Other events reported more frequently by oseltamivir-treated children included abdominal pain, epistaxis, ear disorder and conjunctivitis. These events generally occurred once, resolved despite continued dosing and did not cause discontinuation of treatment in the vast majority of cases.

Oseltamivir had no adverse effect on pulmonary function in children with asthma, where small improvements in peak flow and FEV₁ were observed and asthma exacerbations were decreased in the active treatment group.

Prophylaxis studies. A total of 3434 subjects (adolescents, healthy adults and elderly) participated in field studies of chemoprophylaxis of epidemic influenza; 1480 received the dose of 75 mg once daily for up to 6 weeks. Adverse events were qualitatively very similar to those seen in the treatment studies, despite a longer duration of dosing. There were no clinically relevant differences in the safety profile of the 942 elderly

Table 3. Summary of adverse events [*n* (%)] in the pooled adult population enrolled in studies of oseltamivir treatment of influenza^{9,10}

Adverse event	Placebo (<i>n</i> = 1050)	Oseltamivir 75 mg twice daily (<i>n</i> = 1057)	Oseltamivir 150 mg twice daily (<i>n</i> = 447)
Vomiting	32 (3.0)	85 (8.0)	53 (11.9)
Nausea	71 (6.8)	113 (10.7)	68 (15.2)
Vertigo	6 (0.6)	9 (0.9)	5 (1.1)
Abdominal pain	21 (2.0)	23 (2.2)	9 (2.0)
Fatigue	7 (0.7)	8 (0.8)	7 (1.6)
Pneumonia	8 (0.8)	9 (0.9)	2 (0.4)
Insomnia	10 (1.0)	11 (1.0)	8 (1.8)
Headache	16 (1.5)	17 (1.6)	13 (2.9)
Dyspepsia	6 (0.6)	6 (0.6)	6 (1.3)
Sore throat	8 (0.8)	6 (0.6)	5 (1.1)
Cough	12 (1.1)	10 (0.9)	9 (2.0)
Herpes simplex	12 (1.1)	9 (0.9)	5 (1.1)
Nasal congestion	10 (1.0)	6 (0.6)	6 (1.3)
Dizziness	31 (3.0)	20 (1.9)	10 (2.2)
Bronchitis	52 (5.0)	39 (3.7)	0 (0.0)
Diarrhoea	84 (8.0)	58 (5.5)	26 (5.8)

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subjects, who received oseltamivir or placebo, compared with the younger population.⁸⁷

Gastrointestinal problems were reported with similar overall frequency in subjects receiving oseltamivir once daily for post-exposure prophylaxis and in those receiving twice daily treatment of influenza-like illness in an influenza family transmission study.⁵² The incidence of vomiting was more frequent following twice daily administration for treatment than once daily administration for prophylaxis in both children and adults.

Post-marketing experience

Spontaneous reports. Oseltamivir was first launched in 1999 and approximately 20 million patients have been exposed to the drug since the introduction into the market. Approximately 1 out of 10 000 patients was reported to have experienced ≥ 1 adverse events. During the review period, about 4000 events were reported, of which 25% were considered serious. These concerned oseltamivir phosphate taken either as capsules or in the form of powder for suspension. More than 80% of all reports originate from Japan, where oseltamivir capsules were launched in February 2001 and the powder for suspension in April 2003. The adverse events reported may or may not be related to oseltamivir therapy.

The most frequently reported events were consistent with the events observed during clinical trials: nausea, vomiting, diarrhoea and dizziness. In addition, hypersensitivity reactions mainly allergic skin reactions such as rash, dermatitis, urticaria, eczema, face oedema and erythema were reported rarely. In very rare cases, more severe reactions such as Stevens-Johnson-Syndrome and erythema multiforme were reported. Very rare cases of hepatitis and elevated liver enzymes have been reported in patients with influenza-like illness receiving oseltamivir.

Overdose. As yet, there is no experience with overdose, although the anticipated manifestations of acute overdose would be nausea, with or without accompanying emesis. Single doses of up to 1000 mg of oseltamivir have been well tolerated apart from nausea and/or vomiting.

Pregnancy and lactation. At present, insufficient data are available in pregnant women taking the drug to enable an evaluation of the potential risk for oseltamivir to cause fetal malformations or fetal toxicity. Oseltamivir should therefore be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

To date, there have been 61 reports of oseltamivir therapy during pregnancy. Among these, there were 10 reports of abortion (of which six were therapeutic terminations). Single cases of trisomy 21 and anencephaly have been reported; in both cases, the causality was assessed as not related to treatment with oseltamivir. The majority of pregnancies reported resulted in the birth of a normal baby.

In lactating rats, oseltamivir and the active metabolite are excreted in milk. It is not known whether oseltamivir and the active metabolite are excreted in human milk, but extrapolation of the animal data provides estimates of 0.01 and 0.3 mg/day for the respective compounds. Oseltamivir should therefore be used only if the potential benefit for the lactating mother justifies the potential risk for the nursing infant.

Oseltamivir—formulations and indications

A key concern during any influenza pandemic will be ensuring an adequate supply of appropriate anti-influenza medications. It will take many months to prepare a vaccine against any new pandemic strain and antiviral drugs will be needed to help control the outbreak before the generation of a vaccine.

No manufacturer would be able to immediately produce enough drug to meet the potential demand during a pandemic, as the demand is likely to be orders of magnitude higher than the demand during seasonal epidemics. The manufacturing process for oseltamivir is complex, and requires approximately 12 months from raw materials to finished product.

Oseltamivir can, however, be stockpiled in advance for later use during a pandemic. To this end, two options are offered for governments to consider when building a stockpile of oseltamivir for pandemic use. These options are:

- (i) To stockpile the marketed formulations: (a) 75 mg capsules; (b) a powder for reconstitution as an oral suspension.
- (ii) To stockpile the Active Pharmaceutical Ingredient (API; oseltamivir phosphate), as a powder for reconstitution as a solution in a pandemic situation (see the section 'Magistral formulation' below).

Marketed formulation

The 75 mg capsules can be used for both treatment and prophylaxis in adolescent and adult patients. The powder formulation is primarily intended to enable children to receive the drug, but may also be used by adults who are unable to swallow capsules. The powder for suspension and the 75 mg capsules have been shown to be bioequivalent dosage forms. Both capsules and powder formulations can be stockpiled for use in a pandemic situation. Distribution and dispensing of the marketed form in a pandemic could be effected simply and rapidly.

Magistral formulation

The API is a water-soluble dry powder, which is chemically stable when stored in drums. When dissolved in water to a concentration of 15 mg/mL, the solution is stable for 3 weeks at 25°C or for 6 weeks at 5°C. The powder is readily soluble in water (containing sodium benzoate as preservative) and provides a clear odourless solution.

The API is exactly the same material which would normally be mixed with excipients and used to prepare the approved capsule and dry powder formulations. However, it avoids the additional costs of product refinement and packaging, thereby also providing the least expensive route for stockpiling.

Oseltamivir dissolves readily following oral administration in capsule or suspension form and as such it is expected that the systemic bioavailability of oseltamivir carboxylate following an API solution would be equivalent to the capsule and suspension formulations. Oseltamivir has a bitter taste; however, taste considerations should pale into insignificance when patients are faced with treating a potentially fatal illness or to reducing the potential for contracting an infectious disease with a high risk of mortality. Patients could also be informed that they may take the taste away afterwards, e.g. by having a strongly flavoured fruit drink or chewing flavoured gum.

Considerations for dosing and duration of treatment/prophylaxis of pandemic influenza

Viral replication and clinical pattern of illness

Data from experimental infection in animal models suggest that viral replication of potential pandemic influenza strains follows a pattern similar to epidemic strains in the same species. In general, viral titres of avian strains are low, but reach a peak rapidly following initial infection. These data indicate that the currently recommended time window between start of symptoms and start of treatment remains the maximum period over which clinical efficacy may be assumed. In contrast, the virulence (i.e. clinical disease) associated with pandemic or avian infection appears to be significantly worse. In some species, virus is detectable outside of the respiratory tract. If this were to be the case in humans in the course of a pandemic, oseltamivir (which is distributed within total body water) would be preferable to inhaled topical influenza therapy (zanamivir).

Animal model data show some evidence of protracted viral shedding. In these circumstances, a longer period of dosing may be necessary to constrain rebound viral replication. Higher drug doses might be required to exert comparable antiviral effects and clinical benefits in a pandemic compared with epidemic infections.⁸⁹ In the early phases of a pandemic, it therefore will be important to monitor the effectiveness of the currently approved dose and duration of treatment, and to take appropriate steps to increase these, should control appear inadequate.

One further consequence of insufficient dosing is the possible emergence of oseltamivir-resistant influenza strains. Recently published data from Japan⁷⁷ acknowledge the potential role of inadequate dosing and/or shortened treatment duration in the emergence of a higher level of resistant virus than previously seen. It is therefore imperative that currently approved dosing regimens are not decreased in an effort to make the drug supply 'go further'.

Prophylaxis

Prophylactic therapy with oseltamivir phosphate is considered to provide protection from influenza while the agent is being taken. As detailed above in the section 'Prophylaxis', safety data have now been published to indicate the absence of significant adverse reactions for up to 8 weeks of once-daily dosing at 75 mg per day and there is anecdotal information regarding dosing for up to 12 weeks in the recent avian influenza outbreak in the Netherlands (see the section 'Human experience' above). In view of concerns about the potential duration of viral shedding by individuals infected with a pandemic strain, the normal duration of post-exposure prophylaxis of 7–10 days should also be considered as the minimum period for which protection would be required in such circumstances. For particularly high-risk individuals (any person who will be repeatedly exposed to infected patients), chemoprophylaxis may be required for the entire duration of the outbreak within the community.

These conclusions are supported by information from epidemic modelling. Stilianakis *et al.*⁹⁰ used data from an influenza A outbreak in a boarding school in 1978 to model the impact of several strategies on the development and transmission of resistant virus in both an epidemic and pandemic situation.

The model predicted that in a pandemic, chemoprophylaxis represents the most beneficial strategy followed by the combination of treatment and prophylaxis. Chemoprophylaxis or a combination of prophylaxis and treatment during a pandemic was shown in this circumstance to reduce symptomatic cases by 41%. Drug resistance following use of an M2 inhibitor (all that was available at the time) with either strategy would be 13–22% of total symptomatic infections.

A second model⁸⁴ was developed to look specifically at the dynamics of influenza transmission and treatment to predict the potential emergence of drug resistance over many years in a large population. The number of infections prevented per individual treated was used as the outcome measure. When treatment demand is low (~6%) symptomatic treatment outperforms prophylaxis. However, prophylaxis outperformed symptomatic treatment once demand exceeded 30%. The model also showed that at high levels of prophylaxis of 80%, such as might be envisaged in a pandemic, influenza transmission could be dramatically lowered and the outbreak effectively controlled.

A recent publication by Longini *et al.*⁹¹ adds weight to these findings. The authors used stochastic epidemic simulations to investigate the effectiveness of targeted antiviral prophylaxis to contain influenza. In this strategy, close contacts of suspected index influenza cases take antiviral agents prophylactically. The authors compared targeted antiviral prophylaxis with vaccination strategies and modelled an influenza pandemic similar to the influenza A virus (H2N2) that caused the pandemic of 1957–1958. In the absence of intervention, the model predicted an influenza illness attack rate of 33% of the population [95% confidence interval (CI): 30, 37] and an influenza death rate of 0.58 deaths/1000 persons (95% CI: 0.4, 0.8). With the use of targeted antiviral prophylaxis, if 80% of the exposed persons maintained prophylaxis for up to 8 weeks, the epidemic would be contained, and the model predicted a reduction to an illness attack rate of 2% (95% CI: 0.2, 16) and a death rate of 0.04 deaths/1000 persons (95% CI: 0.0003, 0.25).

Van Genugten *et al.*⁹² assessed the potential impact of no intervention, influenza vaccination of either the total population or risk groups, pneumococcal vaccination or therapeutic use of NA inhibitors on hospitalization rates during a pandemic outbreak. The therapeutic use of antiviral treatment was estimated to reduce hospitalizations and deaths by 50%. However, sensitivity analysis demonstrated that this prediction was sensitive to the assumptions made. Chemoprophylaxis was not assessed. The biological properties of the recent strains (see section 'Avian influenza' above) and the observed outcomes from post-exposure prophylaxis studies^{52,55} suggest that the base assumptions, and thus the resulting analysis, underlying this model were incorrect.

It is relevant here to note that epidemic modelling was used, taking epidemiology data directly from the field, during the Sudan Acute Respiratory Syndrome (SARS) outbreak. It appeared that the modelling exercise was helpful in refining the management strategies in affected cities and in promoting the need for, and persuading the public to obey, the SARS containment processes defined from the pattern of transfection and spread.

Outbreak control (treatment versus prophylaxis)

One of the key priorities during an influenza pandemic is likely to be to limit the extent of local outbreaks. Appropriate strategies

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therefore need to be clearly identified and communicated. The major problem with any outbreak is that infected individuals can begin shedding virus, and hence infecting contacts, for 1–2 days before the onset of their acute symptoms, i.e. before they are aware that they are infected. There is likely to be debate as to the relative merits of treatment and prophylaxis under these circumstances, particularly as limited drug availability may lead some to consider an approach involving treatment of infected cases only, rather than more widespread prophylaxis.

As summarized above in the section ‘Prophylaxis’, the study reported by Hayden *et al.*⁵² on the management of influenza in households provides clear evidence that such an approach will be suboptimal, and will lead to continued rounds of infection amongst contacts. Comparison of the outcomes of our two PEP studies (Welliver *et al.*⁵⁵ and Hayden *et al.*⁵²) suggests that there is no additional protective efficacy obtained by treatment of the index case combined with prophylaxis of the contacts over that provided by prophylaxis alone (Table 4).

Experience of the use of oseltamivir in the control of nursing home outbreaks of influenza has shown the effectiveness of a strategy of combined treatment and prophylaxis with oseltamivir.^{56,57,93} Indeed, in several of the affected institutions, oseltamivir was used successfully after the failure of amantadine to control the outbreak. To date, in every outbreak where it has been used, oseltamivir has successfully brought it under control. Oseltamivir prophylaxis was well tolerated, and treatment of infection appeared to be associated with clinically important reductions in the rates of serious complications and antibiotic use. The majority of these data are derived from epidemic influenza in populations with some degree of immunity, either through vaccination or from prior influenza infection. It is not possible to say for certain if these outcomes would apply during pandemic exposure. However, as noted above, several groups have engaged in modelling and prediction exercises to assess scenarios that might best contain an outbreak and limit the impact of important secondary outcomes, namely hospitalizations and death and in general they reached similar conclusions.

These data therefore suggest that, in a pandemic, a combined strategy of treatment and prevention is significantly more likely to result in effective outbreak control than is a strategy based solely on treatment of cases as they arise.

Table 4. Effect of index case treatment on prophylactic effectiveness

	Index case treated	Index case not treated
Households		
Hayden <i>et al.</i> ; case definition ⁵²	78.8%	88.9%
Welliver <i>et al.</i> ; case definition ⁵⁵	63.8%	88.9%
Contacts		
Hayden <i>et al.</i> ; case definition ⁵²	84.5%	90.7%
Welliver <i>et al.</i> ; case definition ⁵⁵	70.9%	91.9%

Hayden *et al.* case definition = fever $\geq 37.8^{\circ}\text{C}$ [100°F] plus cough and/or coryza.

Welliver *et al.* case definition = fever $>99^{\circ}\text{F}$ plus ≥ 1 respiratory symptom and ≥ 1 constitutional symptom.

Accuracy of clinical diagnosis

Over recent years, the extent and accuracy of influenza surveillance have increased dramatically. The European Influenza Surveillance System (EISS) was launched in 1996, supported by the European Commission, and continues to grow. There are currently 22 surveillance networks within the EISS system, with a further six affiliated. Sentinel physicians take nose and throat swabs from suspected cases and forward these to reference laboratories for typing. The resultant information on the presence, proportion of cases and infecting strains is then made available. Full information is provided on their website: <http://www.eiss.org>.⁹⁴

The limitation of the EISS system is the time taken for full laboratory characterization of the submitted swab samples and the delay in reporting the results. Alternate, real-time, surveillance systems based on the use of near-patient diagnostic testing (NPT) or PCR are in use in several countries. The advantage of this approach is that up-to-date information on the presence and spread of influenza can be communicated on a much faster basis than the surveillance laboratory-based systems. The approach is complementary to conventional surveillance laboratory-based systems by providing physicians with good local and up-to-date information regarding the presence of confirmed influenza within their region. It is also a very valuable tool in those areas where national surveillance networks have not been established. Some NPT tests differentiate influenza A and B, but do not sub-type the strain—that information still requires appropriate culture within the reference laboratories. Experience with an NPT surveillance system has recently been described.⁹⁵

Within a pandemic situation, the extent of influenza surveillance would be anticipated to increase dramatically, coordinated by WHO. Similarly, as the pandemic is recognized, the ‘defining’ clinical symptoms will be widely communicated, even among the public via public announcements in the electronic media or press articles. Further, concerns over inappropriate prescription of anti-influenza medication will be far outweighed by the desire to stem the spread of the outbreak. Given the relatively benign safety profile of oseltamivir (see above), and its acceptability for long-term prophylaxis, there should be few, if any, adverse consequences of oseltamivir being given inappropriately to a person misdiagnosed with influenza.

Overall conclusions and recommendations

Oseltamivir has been shown to be efficacious and well tolerated in all populations studied and has been approved for use in most regions of the world. The data derived from published studies (see sections on ‘Treatment of influenza’ and ‘Prophylaxis’ above) were obtained during epidemic outbreaks of influenza. These were generally characterized as causing disease of mild to moderate intensity within populations with some immunity. The secondary complications resulting from these infections were, for the most part, mild and most often had the highest impact in the very young and the elderly. These data all indicate that 75 mg twice daily for 5 days is the well tolerated and efficacious dose for treatment in adults and that 75 mg once daily provides a high level of protection against clinical influenza in children and adults, including the elderly.

There are no clinical data available for the use of oseltamivir in a pandemic. Although preclinical data indicate that the 75 mg dose may provide adequate antiviral activity against a novel

strain, the possibility remains that, given a situation where little or no immunity to a pandemic strain exists in a population, higher doses and/or longer durations of therapy may be warranted. Data available to date indicate that, during normal seasonal (epidemic) influenza, the approved doses of oseltamivir for treatment and prophylaxis are efficacious and well tolerated. In the absence of other information, the approved dose and duration of treatment/chemoprophylaxis represent the minimum required for the management of pandemic influenza. Lower doses or shorter durations of treatment/chemoprophylaxis are not supported by data and may contribute to the development of resistant virus. In these circumstances, it is reassuring that the safety data from dose ranging studies show that 5 day courses of 150 mg twice daily for treatment, and 6 week courses of 75 mg twice daily for prophylaxis, were as well tolerated as the approved dose regimens.

The recommendations for use of oseltamivir in a pandemic are influenced by the goals of a particular government and their strategy for managing infection within their populations. If the goal is reduction in complications, hospitalizations and deaths and the consequent utilization of resources, then treatment seems a viable option. If the goal is outbreak control to prevent further spread, then prophylaxis with or without treatment of the ill would be the option of choice. Scenario analysis or mathematical modelling would be useful to determine which factors might influence the choice of options. The lag between the appearance of a pandemic strain and the availability of an appropriate vaccine should also be factored into the consideration of scenarios.

Given these issues, a number of recommendations should be proposed to governments and health authorities struggling to develop pandemic plans:

- (i) Define objectives/goals in a pandemic. Define what mix of treatment and prophylaxis will be required to meet these objectives. This will provide the foundation determining the magnitude of the stockpile required by an individual government.
- (ii) In the absence of other information, the approved dose and duration of treatment/chemoprophylaxis represent the minimum required for the management of pandemic influenza. Lower doses or shorter durations of treatment/chemoprophylaxis are not supported by data and may contribute to the development of resistant virus.
- (iii) In a pandemic, unlike an epidemic, most of the population will be immunologically naive and therefore doses of oseltamivir higher than that approved may be needed to blunt virus spread.
- (iv) Longer periods of prophylaxis may also be required.
- (v) Governments need to establish a mechanism to monitor the effectiveness of oseltamivir or any other antiviral that they employ during the pandemic. Plans and processes should be made in advance to collect, collate and report outcome data from patients using oseltamivir for the treatment and prophylaxis of pandemic influenza during the first-wave outbreaks; the data should be used to optimize the use of the product [as per recommendations (iii) and (iv)].
- (vi) Collection of nose and throat swabs and virological testing would be needed to provide information on the rate of emergence of resistant strains and the biological properties of these would need to be rapidly assessed in animal models.

(vii) Mathematical modelling and scenario testing may help to determine the best strategy within a particular community. Field data would be essential to refine the model predictions and provide information on optimized management strategies.

(viii) The use of oseltamivir in the control of nursing home outbreaks of influenza has shown the effectiveness of the strategy of combined treatment and prophylaxis. Data from a household transmission study have shown that treating the ill family member alone does not stem the outbreak.

(ix) If oseltamivir is included in a plan to handle a new pandemic, stockpiling of the material, either in the form of capsules or the bulk active material will be necessary because the surge capacity will not be able to meet the demand once a pandemic is declared. In the absence of stockpiles, there is no guarantee that an adequate supply of oseltamivir will be available.

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Transparency declaration

All authors are currently full time employees of F. Hoffmann-La Roche Ltd.

References

1. Lamb, R. A. & Krug, R. M. (2001). Orthomyxoviridae: the viruses and their replication. In *Fields Virology*, 4th edn (Knipe, D. M. & Howley, P. M. Eds), pp. 1487–531. Lippincott, Williams, and Wilkins, Philadelphia, PA, USA.
2. Wiley, D. C. & Skehel, J. J. (1987). The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annual Review of Biochemistry* **56**, 365–94.
3. Goto, H., Wells, K., Takada, A. *et al.* (2001). Plasminogen-binding activity of neuraminidase determines the pathogenicity of influenza A virus. *Journal of Virology* **75**, 9297–301.
4. Palese, P., Tobita, K., Ueda, M. *et al.* (1974). Characterization of temperature-sensitive influenza virus mutants defective in neuraminidase. *Virology* **61**, 397–410.
5. Schulman, J. L. & Palese, P. (1975). Susceptibility of different strains of influenza A virus to the inhibitory effects of 2-deoxy-2,3-dehydro-n-trifluoroacetylneuraminic acid (FANA). *Virology* **63**, 98–104.
6. Scholtissek, C., Quack, G., Klenk, H. D. *et al.* (1998). How to overcome resistance of influenza A viruses against adamantane derivatives. *Antiviral Research* **37**, 83–95.
7. Wainright, P. O., Perdue, M. L., Brugh, M. *et al.* (1991). Amantadine resistance among hemagglutinin subtype 5 strains of avian influenza virus. *Avian Diseases* **35**, 31–9.
8. Hayden, F. G., Belshe, R. B., Clover, R. D. *et al.* (1989). Emergence and apparent transmission of rimantadine-resistant influenza A viruses in families. *New England Journal of Medicine* **321**, 1696–702.
9. Treanor, J. J., Hayden, F. G., Vrooman, P. S. *et al.* (2000). Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. *Journal of the American Medical Association* **283**, 1016–24.

Oseltamivir (Tamiflu®) and its potential for use in an influenza pandemic

10. Nicholson, K. G., Aoki, F. Y., Osterhaus, A. D. M. E. *et al.* (2000). Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. *Lancet* **355**, 1845–50.
11. Calfee, D. P., Peng, A. W., Cass, L. M. *et al.* (1999). Protective efficacy of intravenous zanamivir in experimental human influenza A virus infection. *Antimicrobial Agents and Chemotherapy* **43**, 1616–20.
12. Hayden, F. G., Osterhaus, A. D., Treanor, J. U. J. *et al.* (1997). Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. GC167 Influenza Study Group. *New England Journal of Medicine* **337**, 874–80.
13. Oxford, J. (2000). Influenza A pandemics of the 20th century with special reference to 1918: virology, pathology and epidemiology. *Reviews of Medical Virology* **10**, 119–33.
14. Potter, C. W. (2001). A history of influenza. *Journal of Applied Microbiology* **91**, 572–9.
15. Taubenberger, J. K., Reid, A. H., Krafft, A. E. *et al.* (1997). Initial genetic characterization of the 1918 'Spanish' influenza virus. *Science* **275**, 1793–6.
16. Nguyen-Van-Tam, J. & Hampton, A. W. (2003). The epidemiology and clinical impact of pandemic influenza. *Vaccine* **21**, 1762–8.
17. http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_RMD_2004_8/en/ (19 November 2004, date last accessed).
18. <http://www.cdc.gov/flu/avian/index.htm> (19 November 2004, date last accessed).
19. De Jong, J. C., Rimmelzwaan, G. F., Fouchier, R. A. *et al.* (2000). Influenza virus: a master of metamorphosis. *Journal of Infection* **40**, 218–28.
20. Horimoto, T., Fukuda, N., Iwatsuki-Horimoto, K. *et al.* (2004). Antigenic differences between H5N1 viruses isolated from humans in 1997 and 2003. *Journal of Veterinary Medical Science (Tokyo)* **66**, 303–5.
21. Enserink, M. (2004). Bird flu infected 1000, Dutch researchers say. *Science* **306**, 590b.
22. Koopmans, M., Wilbrink, B., Conyn, M. *et al.* (2004). Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* **363**, 587–93.
23. Dybing, J. K., Schultz-Cherry, S., Swayne, D. E. *et al.* (2000). Distinct pathogenesis of Hong Kong-origin H5N1 viruses in mice compared to that of other highly pathogenic H5 avian influenza viruses. *Journal of Virology* **74**, 1443–50.
24. Kuiken, T., Rimmelzwaan, G. F., Van Amerongen, G. *et al.* (2003). Pathology of human influenza A (H5N1) virus infection in Cynomolgus Macaques (*Macaca fascicularis*). *Veterinary Pathology* **40**, 304–10.
25. Zitzow, L. A., Rowe, T., Morken, T. *et al.* (2002). Pathogenesis of avian influenza A (H5N1) viruses in ferrets. *Journal of Virology* **76**, 4420–9.
26. Matrosovich, M. N., Matrosovich, T. Y., Gray, T. *et al.* (2004). Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *Journal of Virology* **78**, 12665–7.
27. Kilbourne, E. D., Laver, W. G., Schulman, J. L. *et al.* (1968). Antiviral activity of antiserum specific for an influenza virus neuraminidase. *Journal of Virology* **1**, 281–8.
28. Schulman, J. L., Khakpour, M. & Kilbourne, E. D. (1968). Protective effects of specific immunity to viral neuraminidase on influenza virus infection in mice. *Journal of Virology* **2**, 778–86.
29. Murphy, B. R., Kasel, J. A. & Chanock, R. M. (1972). Association of serum anti-neuraminidase antibody with resistance to influenza in man. *New England Journal of Medicine* **286**, 1329–32.
30. Couch, R. B., Kasel, J. A., Gerin, J. L. *et al.* (1974). Induction of partial immunity to influenza by a neuraminidase-specific vaccine. *Journal of Infectious Diseases* **129**, 411–9.
31. Beutner, K. R., Chow, T., Rubi, E. *et al.* (1979). Evaluation of a neuraminidase-specific influenza A virus vaccine in children: antibody responses and effects on two successive outbreaks of natural infection. *Journal of Infectious Diseases* **140**, 844–50.
32. Clements, M. L., Betts, R. F., Tierney, E. L. *et al.* (1986). Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. *Journal of Clinical Microbiology* **24**, 157–60.
33. Roberts, N. A., Wiltshire, H. R., Mendel, D. B. *et al.* Oseltamivir carboxylate is effective against all subtypes of influenza neuraminidase. Poster # 135, ASM Biodefense Research Meeting, Baltimore, March 2003. <http://www.asmbiodefense.org/tuepos.asp> (15 January 2005, date last accessed).
34. Mendel, D. B., Webster, R. G., Roberts, N. A. (1999). Inhibition of avian influenza neuraminidases by GS4071 (Ro 64-0802) *in vitro*. Roche Research Report W-143039, 2 February 1999.
35. Potier, M., Mameli, L., Belisle, M. *et al.* (1979). Fluorometric assay of neuraminidase with a sodium (4-Methylumbelliferyl- α -D-N-Acetylneuraminate) substrate. *Analytical Biochemistry* **94**, 287–96.
36. Li, K. S., Xu, K. M., Peiris, J. S. *et al.* (2003). Characterization of H9 subtype influenza viruses from the ducks of southern China: a candidate for the next influenza pandemic in humans? *Journal of Virology* **77**, 6988–94.
37. Trampuz, A., Prabhu, R. M., Smith, T. F. *et al.* (2004). Avian influenza: a new pandemic threat? *Mayo Clinic Proceedings* **79**, 523–30.
38. Webster, R. G. (1997). Predictions for future human influenza pandemics. *Journal of Infectious Diseases* **176**, Suppl. 1, S14–S19.
39. McKimm-Breschkin, J., Trivedi, T., Hampson, A. *et al.* (2003). Neuraminidase sequence analysis and susceptibilities of influenza virus clinical isolates to zanamivir and oseltamivir. *Antimicrobial Agents and Chemotherapy* **47**, 2264–72.
40. Meijer, A., van der Goot, J. A., Koch, G., *et al.* (2004). Oseltamivir reduces transmission, morbidity and mortality of highly pathogenic avian influenza in chickens. In *Options for the Control of Influenza V*, (Kawaoka, Y., Ed), pp. 455–8. International Congress Series 1263, Elsevier BV, Netherlands.
41. Balasingham, S., Manvell, R., Shell, W. *et al.* (2004). Antiviral activity of oseltamivir carboxylate (Tamiflu) against a potential human pandemic influenza A virus (chicken H5N1). In *Program and Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004*. Abstract 3839. American Society for Microbiology, Washington, DC, USA.
42. Zambon, M., Hayden, F. G. and the Global Neuraminidase Inhibitor Susceptibility Network. (2001). Position statement: global neuraminidase inhibitor susceptibility network. *Antiviral Research* **49**, 147–56.
43. Tumpey, T. M., Garcia-Sastre, A., Mikulasova, A. *et al.* (2002). Existing antivirals are effective against influenza viruses with genes from the 1918 pandemic virus. *Proceedings of the National Academy of Sciences, USA* **99**, 13849–54.
44. Leneva, I. A., Roberts, N., Govorkova, E. A. *et al.* (2000). 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* **48**, 101–15.
45. Govorkova, E. A., Leneva, I. A., Goloubeva, O. G. *et al.* (2001). Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. *Antimicrobial Agents and Chemotherapy* **45**, 2723–32.
46. Hien, T. T., Liem, N. T., Dung, N. T. *et al.* (2004). Avian influenza (H5N1) in 10 patients in Vietnam. *New England Journal of Medicine* **350**, 1179–88.
47. Singh, S., Barghoorn, J., Bagdonas, A. *et al.* (2003). Clinical benefits with oseltamivir in treating influenza in adult populations. *Clinical Drug Investigation* **23**, 561–9.
48. Kaiser, L., Wat, C., Mills, T. *et al.* (2003). Impact of oseltamivir treatment on influenza-related lower respiratory tract

complications and hospitalizations. *Archives of Internal Medicine* **163**, 1667–72.

49. Whitley, R. J., Hayden, F. G., Reisinger, K. S. *et al.* (2001). Oral oseltamivir treatment of influenza in children. *Pediatric Infectious Disease Journal* **20**, 127–33.
50. Machado, C. M., Boas, L. S., Mendes, A. V. *et al.* (2004). Use of oseltamivir to control influenza complications after bone marrow transplantation. *Bone Marrow Transplantation* **34**, 111–4.
51. Aoki, F. Y., Macleod, M. D., Paggiaro, P. *et al.* (2003). Early administration of oral oseltamivir increases the benefits of influenza treatment. *Journal of Antimicrobial Chemotherapy* **51**, 123–9.
52. Hayden, F. G., Belshe, R., Villanueva, C. *et al.* (2004). Management of influenza in households; a prospective, randomized comparison of oseltamivir treatment with or without postexposure prophylaxis. *Journal of Infectious Diseases* **189**, 440–9.
53. Hayden, F. G., Atmar, R. L., Schilling, M. *et al.* (1999). Use of selective oral neuraminidase inhibitor oseltamivir to prevent influenza. *New England Journal of Medicine* **341**, 1336–43.
54. Peters, P. H., Jr, Gravenstein, S., Norwood, P. *et al.* (2001). Long-term use of oseltamivir in the prophylaxis of influenza in a vaccinated frail older population. *Journal of the American Geriatric Society* **49**, 1025–31.
55. Welliver, R., Monto, A. S., Carewicz, O. *et al.* (2001). Effectiveness of oseltamivir in preventing influenza in household contacts. A randomized controlled trial. *Journal of the American Medical Association* **285**, 748–54.
56. Bowles, S. K., Lee, W., Simor, A. E. *et al.* (2002). Use of oseltamivir during influenza outbreaks in Ontario nursing homes, 1999–2000. *Journal of the American Geriatric Society* **50**, 608–16.
57. Parker, R., Loewen, N. & Skowronski, D. (2001). Experience with oseltamivir in the control of a nursing home influenza B outbreak. *Canadian Communicable Disease Report* **27**, 37–40.
58. Chik, K. W., Li, C. K., Chan, P. K. *et al.* (2004). Oseltamivir prophylaxis during the influenza season in a paediatric cancer centre: prospective observational study. *Hong Kong Medical Journal* **10**, 103–6.
59. Shijubo, N., Yamada, G., Takahashi, M. *et al.* (2002). Experience with oseltamivir in the control of nursing home influenza A outbreak. *Internal Medicine* **41**, 366–70.
60. Harris, J. W. (1919). Influenza occurring in pregnant women. *Journal of the American Medical Association* **72**, 978–83.
61. Freeman, D. W. & Barno, A. (1959). Deaths from Asian influenza associated with pregnancy. *American Journal of Obstetrics and Gynecology* **78**, 1172–5.
62. Greenberg, M., Jacobziner, H., Pakter, J. *et al.* (1958). Maternal mortality in the epidemic of Asian influenza. New York City, 1957. *American Journal of Obstetrics and Gynecology* **76**, 897–902.
63. Hakoda, S. & Nakatani, T. (2000). A pregnant woman with influenza A encephalopathy in whom influenza A/Hong Kong virus (H3) was isolated from cerebrospinal fluid. *Archives of Internal Medicine* **160**, 1041–5.
64. Yawn, D. H., Pyeatt, J. C., Joseph, J. M. *et al.* (1971). Transplacental transfer of influenza virus. *Journal of the American Medical Association* **216**, 1022–3.
65. Kort, B. A., Cefalo, R. C. & Baker, V. V. (1986). Fatal influenza A pneumonia in pregnancy. *American Journal of Perinatology* **3**, 179–82.
66. Mullooly, J. P., Barker, W. H. & Nolan, T. F. (1986). Risk of acute respiratory disease among pregnant women during influenza A epidemics. *Public Health Reports* **101**, 205–11.
67. Neuzil, K. M., Reed, G. W., Mitchel, E. F., Jr *et al.* (1999). Influenza-associated morbidity and mortality in young and middle-aged women. *Journal of the American Medical Association* **281**, 901–7.
68. Irving, W. L., James, D. K., Stephenson, T. *et al.* (2000). Influenza virus infection in the second and third trimesters of pregnancy: a clinical and seroepidemiological study. *British Journal of Obstetrics and Gynaecology* **107**, 1282–9.
69. Glezen, W. P., Decker, M. & Perrotta, D. M. (1997). Influenza virus infections in infants. *Pediatric Infectious Disease Journal* **16**, 1065–8.
70. Glezen, W. P., Decker, M. & Perrotta, D. M. (1987). Survey of underlying conditions of persons hospitalized with acute respiratory disease during influenza epidemics in Houston, 1978–1981. *American Review of Respiratory Disease* **136**, 550–5.
71. Glezen, W. P., Greenberg, S. B., Atmar, R. L. *et al.* (2000). Impact of respiratory virus infections on persons with chronic underlying conditions. *Journal of the American Medical Association* **283**, 499–505.
72. Neuzil, K. M., Zhu, Y., Griffin, M. R. *et al.* (2002). Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. *Journal of Infectious Diseases* **185**, 147–52.
73. Tai, C. Y., Escarpe, P. A., Sidwell, R. W. *et al.* (1998). Characterization of human influenza virus variants selected *in vitro* in the presence of the neuraminidase inhibitor GS4071. *Antimicrobial Agents and Chemotherapy* **42**, 3234–41.
74. McKimm-Breschkin, J. L. (2000). Resistance of influenza viruses to neuraminidase inhibitors—a review. *Antiviral Research* **47**, 1–17.
75. Roche. (2004). Summary of Viral Resistance Data in the Treatment and Prophylaxis of Adults and Children for Tamiflu—Roche Research Report 1015254, Update, May 2004.
76. Roberts, N. A. (2001). Treatment of influenza with neuraminidase inhibitors; virological implications. *Philosophical Transactions of the Royal Society of London B* **356**, 1895–7.
77. Kiso, M., Mitamura, K., Sakai-Tagawa, Y. *et al.* (2004). Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* **364**, 759–65.
78. JV16284: Phase II clinical trial of oseltamivir phosphate for an influenza treatment in pediatric patients; Roche, data on file.
79. Carr, J., Ives, J., Kelly, L. *et al.* (2002). Influenza virus carrying neuraminidase with reduced sensitivity to oseltamivir carboxylate has altered properties *in vitro* and is compromised for infectivity and replicative ability *in vitro*. *Antiviral Research* **54**, 79–88.
80. Herlocher, M. L., Carr, J., Ives, J. *et al.* (2002). Influenza virus carrying an R292K mutation in the neuraminidase gene is not transmitted in ferrets. *Antiviral Research* **54**, 99–111.
81. Ives, J. A., Carr, J. A., Mendel, D. B. *et al.* (2002). The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leaves virus severely compromised both *in vitro* and *in vivo*. *Antiviral Research* **55**, 307–17.
82. Carr, J., Roberts, N. & Herlocher, L. (2002). Further study of the transmission in ferrets of influenza A/H1N1 virus carrying a H274Y neuraminidase mutation for Tamiflu®, oseltamivir phosphate. *Roche Research Report* 1008171.
83. Herlocher, M. L., Truscon, R., Elias, S. *et al.* (2004). Transmission studies in ferrets of influenza viruses resistant to the antiviral oseltamivir. *Journal of Infectious Diseases* **190**, 1627–30.
84. Ferguson, N. M., Mallett, S., Jackson, H. *et al.* (2003). A population-dynamic model for evaluating the potential spread of drug-resistant influenza virus infections during community-based use of antivirals. *Journal of Antimicrobial Chemotherapy* **51**, 977–90.
85. NISN. (2004). Statement on antiviral resistance in influenza viruses. *Weekly Epidemiological Record* **79**, 306–8.
86. Moscona, A. (2004). Oseltamivir resistant influenza? *Lancet* **364**, 733–4.

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87. Dutkowski, R., Thakrar, B., Froehlich, E. *et al.* (2003). Safety and pharmacology of oseltamivir in clinical use. *Drug Safety* **26**, 787–801.
88. Nordstrom, B. L., Oh, K., Sacks, S. T. *et al.* (2004). Skin reactions in patients with influenza treated with oseltamivir: a retrospective cohort study. *Antiviral Therapy* **9**, 187–95.
89. Hayden, F. G. (2001). Perspectives on antiviral use during pandemic influenza. *Philosophical Transactions of the Royal Society of London B* **356**, 1877–84.
90. Stilianakis, N. I., Perelson, A. S. & Hayden, F. G. (1998). Emergence of drug resistance during an influenza epidemic: insights from a mathematical model. *Journal of Infectious Diseases* **177**, 863–73.
91. Longini, I. M., Jr, Halloran, M. E., Nizam, A. *et al.* (2004). Containing pandemic influenza with antiviral agents. *American Journal of Epidemiology* **159**, 623–33.
92. Van Genugten, M. L., Heijnen, M. L. & Jager, J. C. (2003). A surveillance system for the real-time reporting of influenza activity. *Disease Management and Health Outcomes* **12**, 197–206.
93. Monto, A. S., Rotthoff, J., Teich, E. *et al.* (2004). Detection and control of influenza outbreaks in well-vaccinated nursing home populations. *Clinical Infectious Diseases* **39**, 459–64.
94. <http://www.eiss.org> (19 November 2004, date last accessed).
95. Uphoff, H., Groniewicz, I., Soriano, M. *et al.* (2004). A surveillance system for the real-time reporting of influenza activity. *Disease Management and Health Outcomes* **12**, 197–206.