Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Antiviral combinations for severe influenza

Jake Dunning, J Kenneth Baillie, Bin Cao, Frederick G Hayden, on behalf of the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)

Observational data suggest that the treatment of influenza infection with neuraminidase inhibitors decreases progression to more severe illness, especially when treatment is started soon after symptom onset. However, even early treatment might fail to prevent complications in some patients, particularly those infected with novel viruses such as the 2009 pandemic influenza A H1N1, avian influenza A H5N1 virus subtype, or the avian influenza A H7N9 virus subtype. Furthermore, treatment with one antiviral drug might promote the development of antiviral resistance, especially in immunocompromised hosts and critically ill patients. An obvious strategy to optimise antiviral therapy is to combine drugs with different modes of action. Because host immune responses to infection might also contribute to illness pathogenesis, improved outcomes might be gained from the combination of antiviral therapy with drugs that modulate the immune response in an infected individual. We review available data from preclinical and clinical studies of combination antiviral therapy and of combined antiviral-immunomodulator therapy for influenza. Early-stage data draw attention to several promising antiviral combinations with therapeutic potential in severe infections, but there remains a need to substantiate clinical benefit. Combination therapies with favourable experimental data need to be tested in carefully designed aclinical trials to assess their efficacy.

Introduction

Influenza virus infection causes substantial morbidity and mortality, despite the availability of antiviral drugs and vaccines. WHO estimates that annual epidemics cause 3 million to 5 million cases of severe illness and 250,000–500,000 deaths worldwide.1 After the 2009 pandemic, outbreaks of that strain have continued to cause serious illness and increased mortality, particularly in young adults and children.2,3 The increase of zoonotic infections with avian influenza viruses is also of concern.

A second wave of avian influenza H7N9 occurred in China in late 2013 to early 2014, with high rates of severe illness and death in patients with confirmed infection, and the virus continues to circulate in poultry.4 For the first time,4 severe avian influenza A H10N8 infection in human beings has been described, and cases of avian influenza A H5N1 continue to be reported, including the first case of imported infection in North America.5

Various observational studies in seasonal, pandemic, or avian influenza H5N1 infections show that timely oseltamivir monotherapy can reduce the risk of severe influenza outcomes such as pneumonia and admission to hospital and lower mortality in hospital inpatients, including risk groups such as pregnant women and immunocompromised hosts.5,6 However, monotherapy has not prevented death in many patients with severe pandemic H1N1,6 H5N1,6 or H7N9 illness.7 Although various factors might account for these deaths, oseltamivir treatment is associated with incomplete antiviral responses in severely ill patients, in whom viral detection can persist in the upper and, more often, the lower respiratory tract for days to sometimes weeks during otrement.8,9

Furthermore, emergence of resistance during monotherapy has been a drawback in severe influenza, particularly in immunocompromised hosts9 and in avian H5N10 and H7N9 infections.9,10 Variants with highly reduced inhibition by oseltamivir in vitro have sometimes emerged within several days of initiation of therapy in severe influenza caused by pandemic H1N12,11 or avian viruses.12,13 Modelling studies based on human viral kinetics show that all possible single nucleotide mutations and a sizeable proportion of double ones are generated during an uncomplicated influenza infection.14 Whereas most of these mutations sustain a fitness cost, some variants show reduced inhibition and be selected during drug therapy. Therapeutic use of influenza antiviral combinations could increase antiviral potency and reduce resistance emergence; both of these effects could increase clinical effectiveness, especially in seriously ill or immunocompromised hosts. Additionally, combinations might allow dose-sparing in the event of drug shortages and possibly reduce risks of adverse drug effects.

The broad range of responses to infection, as seen by the high rates of clinically inapparent infections reported by seroepidemiological studies,7 shows that the severity of influenza is at least, in part, determined by host factors. Death in many cases is a result of irreversible lung injury related to both host inflammatory responses and direct cellular effects of viral infection (cytopathology and apoptosis). Hence, modulation of the host proinflammatory response might be a tractable complementary therapeutic strategy and offers an advantage compared with antivirals in avoiding emergence of drug-resistant variants. Furthermore, some host-cell pathways are essential for viral replication, so that some host-directed inhibitors have the potential to diminish both viral replication and harmful inflammatory responses.15,16 The well established therapeutic approach of antiviral combinations has received only limited clinical testing in influenza infections so far, and very little information is available from studies in hospitalised or severely ill patients with influenza. Consequently, no combinations of proven value for treatment of severe human influenza are now available. In this Review, we summarise published information regarding influenza antiviral combinations.
and comment on antiviral and immunomodulator combinations that have received preclinical and, in some instances, clinical investigation. The details of representative studies of antiviral combinations and of antiviral and immunomodulator combinations are listed in the appendix. Several of these regimens would be candidates for controlled studies in hospital inpatients with serious influenza infections, including patients infected with avian influenza A H7N9 virus, or in patients who are immunocompromised with an increase in the risk of severe disease and resistance emergence.

Historical perspectives
As previously reviewed,27,28 in-vitro studies showing enhanced antiviral activity against influenza A virus with dual drug regimens of amantadine and interferon date to 196838 and oseltamivir and ribavirin date to 1977.10 Combinations of amantadine and ribavirin were reported to increase survival in murine models of influenza A virus infection,19 although not of influenza B.20 Reduction in adamantane resistance emergence in vitro by combination of rimantadine and ribavirin was reported in 1980,32 a principle subsequently supported with amantadine and oseltamivir for many influenza A subtypes, including avian H5N1.33 The first triple influenza drug regimen (interferon alfa, rimantadine, and ribavirin) showing enhanced in-vitro activity against influenza A was described in 1984;34 the investigators also noted enhanced activity with combinations of ribavirin and interferon alfa for an influenza B virus. Once neuraminidase inhibitors became available, preclinical studies showed additive or synergistic in-vitro activity and increased survival in murine models with combinations of an adamantane with a neuraminidase inhibitor.45–46 The first randomised controlled trial of combination therapy in hospital inpatients, done by the Collaborative Antiviral Study Group in the late 1990s, showed that a regimen of oral rimantadine and nebulised zanamivir seemed to exert a slightly greater antiviral effect and prevent emergence of adamantane resistance compared with one of rimantadine and nebulised saline.46 A subsequent randomised controlled trial in outpatients reported evidence for possible antagonism with a combination of oral oseltamivir and inhaled zanamivir,47 an observation that emphasised the importance of detailed preclinical studies before embarking on clinical trials. Subsequent studies have led to several randomised controlled trials testing the safety and efficacy of various combinations of influenza antivirals (table I).

Antiviral combinations
Neuraminidase inhibitors
At present, circulating influenza viruses including avian influenza H7N9 and H5N1 are susceptible to neuraminidase inhibitors, and observational clinical data show reduced mortality with timely oseltamivir therapy in H5N1 disease.15 Most preclinical studies show at least additive and often synergistic interactions between neuraminidase inhibitors and antiviral drugs with different mechanisms of action. Consequently, oseltamivir or, if available, intravenous zanamivir or peramivir would be a logical choice for use as a foundation drug in testing of an antiviral combination.

Several issues emerge when the possibility of dual neuraminidase inhibitor therapy is considered. The use of dual neuraminidase inhibitors combinations might offer the possibility of reduced resistance emergence because of differing antiviral resistance profiles among these drugs.48 However, data have not substantiated this potential advantage. Combinations of oseltamivir and zanamivir or peramivir show concentration-dependent additive to antagonistic antiviral effects for H1N1 viruses in vitro, whereas another study reported that combinations of oseltamivir and peramivir show mainly additive activities in vitro and in mice.49 These findings are consistent with

![Table 1: Representative antiviral combinations that have been studied or are presently in trials, by study type](https://www.thelancet.com/infection/Vol_14/December_2014)

| Drugs tested                                                                 | Target population                        |
|------------------------------------------------------------------------------|------------------------------------------|
| Oral oseltamivir + oral amantadine (NCT00416962)                             | Healthy volunteers                       |
| Oral oseltamivir + oral favipiravir (unpublished)                            | Healthy volunteers                       |
| Intravenous parmeravir + oral oseltamivir                                   | Healthy volunteers                       |
| Intravenous zanamivir + oral oseltamivir                                     | Healthy volunteers                       |
| Oral amantadine + oral ribavirin + oral oseltamivir (NCT00867139)            | Healthy volunteers                       |
| Oral rimantadine + nebulised zanamivir                                      | Hospitalised adults                      |
| Oral oseltamivir + inhaled zanamivir                                         | Ambulatory adults                        |
| Oral oseltamivir + pH1N1 convalescent plasma                                 | Critically ill patients                  |
| Oral oseltamivir + pH1N1 hyperimmune globulin (NCT01617317)                 | Critically ill patients                  |
| Oral oseltamivir + maxingshigan/yinqiaosan (NCT00935194)                     | Ambulatory adults                        |
| Oral oseltamivir + sirolimus + corticosteroids                               | Critically ill patients                  |
| Oral amantadine + ribavirin + oseltamivir (TCAD; NCT01617317)                | Critically ill patients                  |
| Oral oseltamivir + convalescent plasma or hyperimmune globulin (NCT01524880) | Hospitalised adults                      |
| Oral amantadine + ribavirin + oseltamivir (TCAD; NCT01227967)                | High-risk outpatients                    |
| Oseltamivir + nitazoxanide (NCT01610245)                                     | Ambulatory adults                        |

TCAD= triple combination antiviral drug.
the similarities in chemical structure of oseltamivir and peramivir. In either instance, no greater antiviral effects than the more potent drug of the two would be expected.

Influenza A neuraminidases form two groups on phylogenetic analysis: group 1 (N1, N4, N5, and N8) and group 2 (N2, N3, N6, N7, and N9). Each group has distinctive structural features, including a protein loop, the 150-loop, that has an open active-site conformation in group 1 neuraminidases and a closed active-site conformation in group 2 neuraminidases.50 Although both oseltamivir and zanamivir inhibit both groups to a similar degree, some oseltamivir-resistant viruses show neuraminidase group specificity that is mediated by specific aminoacid changes.47,51

N9 aminoacid substitutions selected in vitro by oseltamivir include several also seen in H3N2 viruses in patients given oseltamivir (eg, Arg292Lys and Glu119Val in N2 numbering).32,53 Although the catalytic site Arg292Lys substitution causes a marked fitness loss in H3N2, it also confers highly reduced inhibition by oseltamivir (>1000-fold reduction) in enzyme inhibition assays and reduced inhibition by zanamivir and to greater extent by peramivir.12 This substitution (Arg294Lys in N9 numbering) also causes highly reduced inhibition by oseltamivir and peramivir and reduced inhibition by zanamivir in avian influenza H7N9 in vitro,34 and absence of inhibition in mice given neuraminidase inhibitors.35 Of note, emergence of the Arg292Lys substitution has been reported as early as 2 days after initiation of oseltamivir in patients with H7N9 and has been associated with poor clinical outcomes.7,56 H7N9 variants with Arg292Lys replicate at least as well in Madin-Darby canine kidney-SIAT1 cells and primary human respiratory cells as susceptible virus46 and show similar virulence in mice and transmissibility in guineapigs.27 Such findings show that the fitness effects of Arg292Lys in the N9 background are much less than those reported for H3N2 viruses and that this substitution might render all available neuraminidase inhibitors clinically ineffective.

In healthy volunteers, the combined administration of intravenous zanamivir and oral oseltamivir16 or of intravenous peramivir and oral oseltamivir16 shows no important pharmacokinetic interactions. One randomised double-blind, placebo-controlled trial identified slower virological and clinical responses in patients given combined therapy with oseltamivir and inhaled zanamivir compared with oseltamivir alone in treatment of uncomplicated, mostly H3N2 influenza in adults,48 although the combination might have been more effective in reducing secondary transmission.48 A small randomised controlled trial in outpatients infected with 2009 pandemic H1N1 virus did not show obvious differences in antiviral effects with the combination compared with oseltamivir alone.49 However, an observational study in oseltamivir-pretreated, severely ill patients with pandemic H1N1 virus showed that late switch to intravenous zanamivir was associated with sustained viral-load reductions in three of three patients given zanamivir monotherapy but in only three of ten given combined therapy with oseltamivir.50 Another open-label trial in critically ill patients with pandemic H1N1 virus reported persistent viral detection, with 75% still RNA positive for influenza at 7 days after the start of treatment, despite administration of a combination of higher dose oral oseltamivir and inhaled zanamivir.51 Consequently, these results raise concerns about adverse interactions for combinations of zanamivir and oseltamivir; further preclinical assessment, including enzyme inhibition studies, is warranted before use in clinical practice.

Adamantanes
Most circulating or threatening influenza viruses at present are adamantane-resistant, including avian A H7N9, pandemic H1N1, avian H10N8, and seasonal H3N2 viruses. However, adamantane resistance is variable in avian H5N1 viruses, and many isolates have been susceptible.42 In preclinical studies with adamantane-susceptible influenza A virus, the combined use of an adamantane with a neuraminidase inhibitor or ribavirin generally shows additive or synergistic interactions in vitro,30,37,63,64 and increased survival in murine models of influenza, including avian H5N1 virus.43,51,55 However, if an influenza virus is adamantane-resistant, no additional survival benefit or antiviral effect has been shown when adamantane has also been given compared with oseltamivir or ribavirin monotherapy, although one study reported increased survival with dual combinations of amantadine and oseltamivir or ribavirin for an adamantane-resistant 2009 pandemic H1N1 virus in mice.58

Human studies of combinations of oral oseltamivir and amantadine29 and of oral rimantadine and intravenous peramivir41 have shown no important pharmacokinetic interactions. One placebo-controlled but underpowered trial of nebulised zanamivir in patients with influenza A who had been admitted to hospital, all of whom were given rimantadine, showed trends towards faster cough resolution and lesser risk of adamantane resistance emergence.46 Consequently, a dual regimen of an adamantane and neuraminidase inhibitor would be a reasonable initial treatment regimen for serious influenza when the infecting strain is probably susceptible to both drug classes.

Ribavirin
Combinations of ribavirin with neuraminidase inhibitors have shown variable interactions. Peramivir and ribavirin showed synergistic activity for an H1N1 virus in vitro and enhanced survival effects when given orally in combination compared with suboptimum doses of either agent.60 Several oseltamivir and ribavirin dose combinations increased survival, reduced lung consolidation, and
reviewed lung viral titres in influenza B compared with suboptimum doses of single agents, whereas one dosing regimen of oseltamivir and ribavirin showed no greater effects than ribavirin alone for mice injected with A/New Caledonia/20/99 H1N1.49 Ribavirin and oseltamivir have shown mainly additive interactions in preclinical assays with H5N1.60,61,62 In mice infected with avian H5N1 virus, oseltamivir and ribavirin also showed exceptions of marginal synergy or slight antagonism at some dose combinations.63

In uncomplicated seasonal influenza, randomised controlled trials show that ribavirin monotherapy is ineffective at doses of 1 g per day72 and only marginally benefits clinical manifestations at higher doses of 8-4 g given over 2 days.73 Aerosolised and intravenous ribavirin preparations have been used with possible benefit in severely ill patients with influenza,74,75 and a combination of oral ribavirin and amantadine was used in the treatment of influenza pneumonia in a pregnant patient who survived.76 However, an assessment by US Food and Drug Administration experts concluded that the data from compassionate use reports of intravenous ribavirin in influenza were inconclusive in terms of clinical benefits and also pointed out the potential safety issues associated with ribavirin, such as haemolytic anaemia and teratogenicity.77

**Triple-combination antiviral drug (TCAD) treatment**

A TCAD regimen (Adamas, Emeryville, CA, USA) of three available agents (amantadine, ribavirin, and oseltamivir) shows synergistic activity in vitro against not only influenza A viruses that are susceptible,78 but also those resistant to the adamantanes or oseltamivir at baseline, including adamantane-resistant 2009 pandemic H1N1 virus.49 TCAD was more inhibitory than any of the dual combinations and was also more effective at preventing resistance emergence during in-vitro passage.79 Murine model studies reported greater survival than with dual combinations for an adamantane-susceptible H5N1 virus and also for an adamantane-resistant pandemic H1N1 virus.80 However, virological data were not provided to establish whether the improved survival was associated with greater antiviral effects or possibly reduced resistance emergence in vivo.

In a small cohort of highly immunocompromised patients infected with influenza, TCAD recipients did not have emergence of resistance-associated substitutions; the regimen was reasonably well tolerated over 10 days and provided the target blood concentrations of the individual drugs.81 A retrospective observational study of critically ill adults infected with pandemic H1N1 virus suggest non-significant trends towards reduced 14 day (17% vs 35%; p=0.08) and 90 day (46% vs 59%; p=0.23) mortality in TCAD recipients compared with those receiving oseltamivir monotherapy.82 A randomised controlled trial performed by the National Institute of Allergy and Infectious Disease comparing TCAD with oseltamivir monotherapy for ambulatory high-risk patients is in progress (NCT01227967), and controlled studies in hospital inpatients seem to be warranted.

**Favipiravir**

Favipiravir or T-705 (Toyama Chemical Co, Tokyo, Japan) is a novel influenza polymerase inhibitor that is active against influenza A, B, and C viruses including adamantane-resistant or oseltamivir-resistant variants.82 Favipiravir and oseltamivir show concentration-related additive or synergistic effects for influenza A viruses in vitro and, depending on dose and timing, on survival in mice infected with various influenza viruses.83 Combination of suboptimum doses of favipiravir and oseltamivir afforded 60–80% protection and improved bodyweights during infection in a lethal H5N1 model (one designed to give an infectious dose that is predictably associated with 100% mortality, and combinations of favipiravir and peramivir also showed synergy in survival and enhanced antiviral effects compared with sub-optimum doses of each compound alone for the treatment of 2009 pandemic H1N1 virus in mice.84 Limited testing has recorded no pharmacokinetic interactions of oral favipiravir and oseltamivir in healthy participants. However, dose adjustments in the setting of renal insufficiency are still to be identified, and the recommended dose regimen varies with the target population (Asian and white). In Japan, where favipiravir has been approved for treatment of novel or re-emerging influenza virus infections (limited to cases in which other anti-influenza virus drugs are ineffective or not sufficiently effective),85 a phase 3 study in uncomplicated influenza showed similar antiviral effects as oseltamivir.86 A phase 2 treatment study completed in adults reported evidence for symptom alleviation,87 and a large phase 3 randomised controlled trial in uncomplicated disease was initiated during the 2013–14 season (NCT02008344).

In view of the available preclinical data, oral favipiravir would be an especially interesting candidate for study of combination therapy with a neuraminidase inhibitor in serious influenza viral infection.

**Neutralising antibodies**

Convalescent plasma containing virus-specific neutralising antibodies has been used with apparent benefit in neuraminidase inhibitor-treated patients with severe H5N1 infection.88 In a cohort study in neuraminidase inhibitor-treated, critically ill patients infected with 2009 pandemic H1N1 virus, crude mortality was reduced from 55% in non-treated patients to 20% in 20 patients receiving convalescent plasma (p=0.011), and substantial reductions in nasopharyngeal viral load (quantity of viral RNA copy number in volume of sample) on treatment days 3–7 and in plasma cytokines and chemokines were recorded compared with controls.89 In a similar critically ill group of patients receiving neuraminidase inhibitor therapy, a small double-blind
randomised controlled trial of hyperimmune globulin containing high neutralising antibody titres to influenza pandemic H1N1 virus was associated with no mortality when treatment was given within 5 days of illness onset compared with 40% in those receiving pre-2009 intravenous immunoglobulin (p=0.04), although overall mortality did not differ between the groups when those with delayed administration were included (five of 17 treated and four of 17 untreated). These findings suggest that the combination of neuraminidase inhibitor therapy with neutralising antibodies in the form of convalescent plasma or hyperimmune globulin would be an appropriate choice for study in patients with severe H7N9 illness once available.

Broad-spectrum neutralising monoclonal antibodies that target conserved epitopes on the stem of viral haemagglutinin and inhibit fusion have therapeutic activity in animal models of influenza for group 1 haemagglutinins (including H1, H2, H5, and H9),

9 group 2 haemagglutinins (including H3 and H7),

or both group 1 and 2 haemagglutinins.

One pan-influenza antihaemagglutinin stem monoclonal antibody (designated 39.29) increased survival in mice infected with A/PR/8/34 H1N1 virus when combined with antihaemagglutinin and neuraminidase.102 Inhaled DAS181 showed great antiviral effects in a phase 2 randomised controlled trial in uncomplicated influenza, although no demonstrable effects on illness resolution.

The inhalation route might prove difficult in severe illness, but several case reports have shown apparent clinical benefit and no serious adverse events when used for treatment of serious parainfluenza virus in immuno-compromised hosts.

Further studies in uncomplicated influenza are in progress (NCT01740063).

Arbidol is an oral antiviral available used for influenza treatment in Russia (where it has been available over-the-counter since 1990) and some other countries. It has broad spectrum inhibitory effects for many enveloped RNA viruses, but also specifically targets influenza antihaemagglutinin-mediated membrane fusion. Arbidol-resistant variants selected in vitro have substitutions in the antihaemagglutinin 2 subunit. Arbidol shows dose-related antiviral effects and survival in murine model studies, and experiments in cell culture have shown synergistic effects when combined with adamantanes, ribavirin, or neuraminidase inhibitors.

Few randomised controlled trial data on its clinical and antiviral efficacy are available, but it seems to be generally well tolerated when used for influenza prophylaxis or treatment in Russian studies, and further studies are in progress. Arbidol would be an interesting candidate for combination studies, particularly in countries where it is already being used as monotherapy.

Oral VX-787 (Vertex Pharmaceuticals, Boston, MA, USA) has a novel mechanism of action selective for influenza A viruses and is active against neuraminidase-inhibitor variants and adamantane-resistant variants and would probably show enhanced activity in combination with neuraminidase inhibitors. Oral VX-787 is reported to have positive antiviral and clinical effects in a phase 2 experimental infection study in human beings at highest dose tested of 1200 mg once, followed by 600 mg daily for 4 days.

AVI-7100 (Serepta, Cambridge, MA) is a small-interfering RNA (siRNA) construct designed to inhibit the translation of both the matrix protein and the M2 ion channel by targeting their shared translation initiation start site. This modified phosphorodiamidate
morpholino oligomer has enhanced resistance to enzymatic degradation, improves pharmacological properties, and limits the potential for non-specific immunomodulatory effects. Ferret studies with an oseltamivir-resistant H1N1 virus have shown disease moderation and reduced viral titres after topical or intraperitoneal administration, including reduced transmission in ferrets after intranasal administration.111,112 Although oseltamivir was not inhibitory in this model, the combination of intranasal or intraperitoneal AVI-7100 with oral oseltamivir tended to reduce nasal viral titres to greater extent than AVI-7100 alone.111 The National Institute of Allergy and Infectious Disease is conducting a phase 1, randomised placebo-controlled trial to assess the safety, tolerability, and pharmacokinetics of single and multiple doses of intravenous AVI-7100 in healthy participants (NCT01747148).

**Antiviral and immunomodulator combinations**

Several potential immunomodulatory agents have been proposed for adjunctive influenza treatment, mainly those directed against excessive proinflammatory host responses to infection.27,28,113,114 Many of these have shown activity in animal models and new candidates, such as the agonist of human complement component 5a (C5a) termed EP67,115 the retinoic acid-inducible gene 1 (RIG-1) agonist 5′triphosphate RNA,116 and the Toll-like receptor 4 antagonist Eritoran (EisaiCo, Tokyo, Japan)117 continue to be reported. For example, studies suggest that the endogenous lipid mediator protectin D1 is downregulated during severe influenza and that exogenous administration exerts antiviral effects and improves outcome from severe influenza in a mouse model.118 Agents with dual mechanisms of action have also been described: the cyclo-oxygenase 2 (COX-2) inhibitor naproxen was shown to inhibit influenza nucleoprotein and exert antiviral effects in a murine model.119

Depending on the particular model, drugs with either proinflammatory or anti-inflammatory effects have shown benefits in animal models. However, few immunomodulators have been studied in combination with influenza antivirals in preclinical studies, and none have been studied in adequately powered randomised controlled trials in serious human influenza (appendix). Furthermore, the unclear relation of disease pathogenesis in animal models, especially murine ones, with human influenza, and the heterogeneity of factors contributing to severe human influenza,119 means that the predictive value of immunomodulator activity in animal models of influenza studies is unclear. One obvious concern is that downregulation of important innate immune responses could contribute to inadequate control of viral replication and be associated with worsened clinical outcomes.

However, some immunomodulatory agents such as the mammalian target of rapamycin (mTOR) inhibitors120 or inhibitors of the Raf–MEK–ERK pathway120 seem to target host cell pathways essential for viral replication. The mTOR inhibitor everolimus shows antiviral effects and disease mitigation in a lethal murine model of influenza.120 A small, open-label randomised controlled trial in critically ill adults infected with 2009 pandemic H1N1 virus pip who were all given oral oseltamivir and systemic corticosteroids showed both more rapid improvements in respiratory function (as shown in parameters of gas exchange and need for mechanical ventilation) and reduced viral detection on day 7 in recipients of the mTOR inhibitor sirolimus compared with no treatment, although there was no overall difference in mortality.121 The potential value of mTOR inhibitors in severe influenza warrants further study. Immunomodulatory agents that have increased survival in combination with influenza antivirals in murine models include N-acetylcysteine,123,124 a topical sphingosine analogue designated AAL-R that inhibits various proinflammatory cytokine and chemokine responses,125 the COX-2 inhibitor celecoxib with mesalazine,126 thymosin,127 topical surfactant,128 and probenecid.129 In addition to the enhanced antiviral activity reported with combined probenecid and oseltamivir treatment in a mouse model of influenza,129 this combination is particularly interesting since probenecid inhibits oseltamivir carboxylate excretion and potentially enables either boosting of blood concentrations or dosing-sparing.130

**Macrolides and statins**

The use of widely available, low cost drugs with immunomodulatory activity has been promoted as a possible treatment strategy,130 but so far no prospective randomised controlled trials of such agents have been completed in patients with severe influenza illness. In mice infected with avian H5N1 virus, simvastatin given with oseltamivir did not improve the efficacy of oseltamivir monotherapy,131 whereas a preliminary study reported some disease-modifying effects with a triple regimen of oseltamivir, simvastatin, and fenofibrate compared with oseltamivir alone.132 Observational studies from the 2009 pandemic did not find improved outcomes in severely ill patients given neuraminidase inhibitors and various immunomodulatory therapies including macrolides and statins.133 One retrospective analysis in uncomplicated influenza did not find that the addition of clarithromycin to oseltamivir improved clinical outcomes.134 In a prospective observational study of critically ill patients with influenza without evidence of bacterial co-infection, treatment with macrolides was not associated with improved survival.135 However, several retrospective studies have reported substantial mortality benefits in patients taking statins who were subsequently admitted to hospital for seasonal influenza136 or pneumonia.137,138 Other studies have not shown such results, and the possible benefit of starting statins at the time of influenza onset or treatment in...
hospital has not been reported. One randomised controlled trial in an intensive care unit suggested reduced risks of ventilator-associated pneumonia and mortality with addition of pravastatin therapy in patients needing mechanical ventilation; further studies are warranted.

Interferons

Interferon alfa shows enhanced anti-influenza activity in vitro with other antivirals, in addition to its immunomodulatory properties. Some evidence shows that severely ill patients with influenza have deficient endogenous interferon responses. Systemic interferon is active in a murine model of H5N1 virus infection and in a macaque model of H1N1 virus infection. Systemic interferon-alfacon-1 in combination with systemic glucocorticoids showed possible benefit and adequate tolerance in treatment of patients with severe acute respiratory syndrome coronavirus, (SARS) infection but systemic interferon has not been studied in serious influenza until now.

Glucocorticoids

Systemic glucocorticoids have been frequently used for treatment of influenza-associated pneumonia and acute respiratory distress syndrome (ARDS), including up to 60% of hospital inpatients with avian influenza A H7N9 illness. Almost all of these patients have been given concurrent antiviral therapy. However, extension of viral replication has been identified in patients with seasonal influenza given systemic glucocorticoids for management of chronic obstructive pulmonary disease or asthma, and large observational studies from the 2009 pandemic reported that systemic glucocorticoid administration for pneumonia or ARDS was associated with increases in secondary bacterial and fungal infections, prolongation of intensive care unit stay, and sometimes higher mortality in intensive care unit patients. Reports of patients infected with H7N9 virus suggest that glucocorticoid use might also be a risk factor for antiviral resistance emergence. Consequently, their use for influenza-associated pneumonia or ARDS should best be limited to controlled clinical studies.

Chinese traditional medicine (CTM)

The use of CTMs in the treatment of seasonal influenza has a history of several thousands of years. The mechanisms of CTMs in the treatment of influenza are complex and incompletely understood. Maxingshigan, one formulation of CTM, directly inactivates influenza A virus, disrupting adsorption, and protecting cells from becoming infected. The administration of Chinese herbs might also have beneficial immunomodulatory effects, but few clinical trials have been reported about the effects of combination therapy in influenza. One multicentre, prospective non-blinded randomised controlled trial compared the efficacy and safety of oseltamivir, maxingshigan-ynqiaoisan, and the combination of both in treatment of outpatients with 2009 pandemic influenza A. Both individual agents accelerated fever resolution compared with placebo, and the combination showed a borderline statistically significant reduction in time to fever resolution compared with oseltamivir alone (median hours to alleviation of fever, 20-0 [95% CI 17-0 to 24-0] vs 15-0 [12-0 to 18-0]). However, no difference in the reduction of symptom scores and duration of viral shedding between combination therapy and oseltamivir monotherapy were found. Other clinical studies
planning and implementation. It also requires careful consideration of the ethical implications, including the potential for harm and the need for informed consent.

Declaration of interests

JDB, JKB and BC declare no competing interests. FGH has served as an unpaid consultant to many companies engaged in development and marketing of influenza antivirals since 2008 to present. He was a member of the Neuraminidase Inhibitor Susceptibility Network (NISN) from 2008–11 with honoraria paid to the University of Virginia; NISN received funding from Roche and GSK. Both FGH and the University of Virginia have received fees for his testimony in legal cases involving neuraminidase inhibitors. FGH served on an independent data safety and monitoring board for an influenza vaccine trial sponsored by Sanofi Pasteur, as consultant to GSK on respiratory virus vaccines, and as a consultant to Hologic on respiratory virus diagnostics with honoraria paid to the University of Virginia.

Acknowledgments

This Review was written by members of the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) to assist colleagues in WHO and in China with clinical research responses to the outbreak of avian influenza A H7N9 virus infections in 2013–14. We thank Mike Wilson (Research and Data Services, Claude Moore Health Sciences Library, University of Virginia Health System) and Min Wang (Institute of Medical Information [IMI] & Library, Chinese Academy of Medical Sciences and Peking Union Medical College [CAMS & PUMC]) for assistance with the scientific literature search, and Lisa Cook (University of Virginia School of Medicine) for her help with manuscript preparation.

References

1. WHO. Influenza (seasonal). Fact sheet; March, 2014. http://www.who.int/mediacentre/factsheets/fs231/en/index.html (accessed June 23, 2014).
2. Health Protection Agency. Surveillance of influenza and other respiratory viruses in the UK 2010–2011. May, 2011 report. http://www.hpa.org.uk/Publications/InfectionsDiseases/Influenza/10/InfluenzaVirus/epi/FinalReport23May11.pdf (accessed June 23, 2014).
3. Centers for Disease Control and Prevention. Influenza (flu). 2013–2014 influenza season week 18 ending May 3, 2014. http://www.cdc.gov/flu/weekly/ (accessed June 23, 2014).
4. European Centre For Disease Prevention and Control. Human infection with a novel avian influenza A(H7N9) virus, China. Third update; Jan 27, 2014. http://www.ecdc.europa.eu/en/publications/Publications/influenza-AH7N9-China-2013-01-27-January2014-27-January2014.pdf (accessed June 23, 2014).
5. Chen H, Yuan H, Gao R, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. Lancet 2014; 383: 714–21.
6. WHO. Human infection with avian influenza A (H5N1) virus-update. Global Alert and Response (GAR); Jan 9, 2014. http://www.who.int/csr/don/2014_01_09_h5n1/en/index.html (accessed June 23, 2014).
7. Kumar D, Michaels MG, Morris MI, et al. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect Dis 2010; 10: 511–26.
8. Yu H, Liao Q, Yuan Y, et al. Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1 infection. J Infect Dis 2010; 201: 1411–17.
9. Louie JK, Yang S, Acosta M, et al. Treatment with neuraminidase inhibitors for critically ill patients with influenza A(H1N1)pdm09. Clin Infect Dis 2012; 55: 1198–204.
10. Muthuri SG, Myles PR, Venkatesan S, Leonardi-Bee J, Nguyen-Van-Tam JS. Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–2010 influenza A(H1N1) pandemic: a systematic review and meta-analysis in hospitalized patients. J Infect Dis 2013; 207: 533–63.
11. Hsu J, Santesso N, Mustafa R, et al. Antivirals for treatment of influenza: a systematic review and meta-analysis of observational studies. Ann Intern Med 2012; 156: 512–24.
12. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA 2010; 303: 1517–25.
13. Dubar G, Azria E, Tesniere A, et al. French experience of 2009 A/H1N1pdm influenza in pregnant women. PLoS One 2010; 5: e13112 .
Muthuri SG, Venkatesan S, Myles PR, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. Lancet Respir Med 2014; 2: 395–404.

Chan PK, Lee N, Zaman M, et al. Determinants of antiviral effectiveness in influenza A subtype H5N1. J Infect Dis 2012; 206: 1559–66.

Nukiwa N, Kamigaki T, Oshiti H. Fatal cases of pandemic (H1N1) 2009 influenza despite their early antiviral treatment in Japan. Clin Infect Dis 2010; 51: 993–94.

Hu Y, Lu S, Song Z, et al. Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance. Lancet 2013; 381: 2273–79.

To KK, Hung IF, Li FW, et al. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin Infect Dis 2010; 50: 850–59.

Lee N, Ison MG. Diagnosis, management and outcomes of adults hospitalized with influenza. Antivir Ther 2012; 17: 143–57.

Malato L, Llavador V, Marmier E, et al. Pandemic influenza A(H1N1) 2009: molecular characterisation and duration of viral shedding in intensive care patients in Bordeaux, south-west France, May 2009 to January 2010. Euro Surveill 2011; 16: 19776.

Hurt AC, Chotpitayasunondh T, Cox NJ, et al. Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. Lancet Infect Dis 2011; 12: 240–48.

de Jong MD, Tran TT, Truong HK, et al. Osel tamivir resistance during treatment of influenza A (H5N1) infection. N Eng J Med 2005; 353: 2667–72.

Gao HN, Lu HZ, Cao B, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. N Engl J Med 2013; 368: 2277–85.

Perelson AS, Rong L, Hayden FG. Combination antiviral therapy for influenza: predictions from modeling of human infections. J Infect Dis 2012; 205: 1642–45.

Hsieh YH, Tsai CA, Lin CY, et al. Asymptomatic ratio for seasonal H1N1 influenza infection among schoolchildren in Taiwan. BMC Infect Dis 2014; 14: 80.

Ludwig S. Disruption of virus-host cell interactions and cell signaling pathways as an anti-viral approach against influenza virus infections. Biol Chem 2011; 392: 837–47.

Hayden FG. Combinations of antiviral agents for treatment of influenza virus infections. J Antimicrob Chemother 1986; 18 (suppl B): 177–83.

Hayden FG. Newer influenza antivirals, biotherapeutics and combinations. Influenza Other Respir Viruses 2013; 7 (suppl 1): 63–75.

Lavrov SV, Vrenkina EI, Orlov TA, Galegov GA, Soloviev VD, Zhdanov VM. Combined inhibition of influenza virus reproduction in cell culture using interferon and amantadine. Nature 1968; 217: 556–57.

Galegov GA, Pushkarskaya NL, Ohrsova-Senova NP, Zhdanov VM. Combined action of ribavirin and rimantadine in experimental murine virus infections. EXPERIMENTA 1977; 33: 905–06.

Wilson SZ, Knight V, Wyde PR, Drake S, Couch RB. Amantadine and ribavirin aerosol treatment of influenza A and B infection in mice. Antimicrob Agents Chemother 1987; 17: 642–8.

Pushkarskaya NL, L’vov ND, Galegov GA. Prevention of the formation of a mutant influenza A virus resistant to rimantadine (alpha-methyl-1-adamananthemethylyl hydrochloride) by using ribavirin (1-beta-D-riburano5-1,2,4-triazole-3-carboxamide). Vopr Virusol 1980; 3: 303–06.

Ilyushina NA, Bovin NV, Webster RG, Govorkova EA. Combination chemotherapy, a potential strategy for reducing the emergence of drug-resistant influenza A variants. Antiviral Res 2006; 70: 121–31.

Hayden FG, Schlepuskin AN, Pushkarskaya NL. Combined interferon-alpha 2, rimantadine hydrochloride, and ribavirin inhibition of influenza virus replication in vitro. Antimicrob Agents Chemother 1994; 35: 53–57.

Ilyushina NA, Hoffmann E, Salomon R, Webster RG, Govorkova EA. Amantadine-oseltamivir combination therapy for H5N1 influenza virus infection in mice. Antivir Ther 2007; 12: 363–70.

Leneva IA, Roberts N, Govorkova EA, Goloubeva OG, Webster RG. The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H1N2) influenza viruses. Antiviral Res 2000; 48: 101–15.

Govorkova EA, Fang HB, Tan M, Webster RG. Neuraminidase inhibitor-rimantadine combinations exert additive and synergistic anti-influenza virus effects in MDCK cells. Antimicrob Agents Chemother 2004; 48: 4855–63.

Galabov AS, Simeonova L, Gegova G. Rimantadine and oseltamivir demonstrate synergistic combination effect in an experimental infection with type A (H3N2) influenza virus in mice. Antivir Chem Chemother 2006; 17: 251–58.

Bantia S, Kellogg D, Parker CD, Babu YS. Combination of peramivir and rimantadine demonstrate synergistic antiviral effects in sub-lethal influenza A (H3N2) virus mouse model. Antiviral Res 2010; 88: 276–80.

Madren LK, Shipman Jr C, Hayden FG. In vitro inhibitory effects of combinations of anti-influenza agents. Antivir Chem & Chemother 1995; 2: 109–13.

ison MG, Gazzan IJ V, Nagy-Agren S, et al. Safety and efficacy of nebulized zanamivir in hospitalized patients with serious influenza. Antivir Ther 2003; 8: 183–90.

Duval X, van der WS, Blanchon T, et al. Efficacy of oseltamivir-zanamivir combination compared to each monotherapy for seasonal influenza: a randomized placebo-controlled trial. PLoS Med 2010; 7: e1000362.

Atiee G, Lasseter K, Baughman S, et al. Absence of pharmacokinetic interaction between intravenous peramivir and oral oseltamivir or rimantadine in humans. J Clin Pharmacol 2012; 52: 1410–19.

Pukrittayakamee S, Jittamala P, Stempniewska K, et al. An open-label crossover study to evaluate potential pharmacokinetic interactions between oral oseltamivir and intravenous zanamivir in healthy Thai adults. Antimicrob Agents Chemother 2011; 55: 4050–57.

Hung IF, To KK, Lee CK, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin Infect Dis 2011; 52: 447–56.

122 Wang CH, Chung FT, Lin SM, et al. Adjunctive treatment with a mammalian target of rapamycin inhibitor, sirolimus, and steroids improves outcomes in patients with severe H1N1 pneumonia and acute respiratory failure. Crit Care Med 2014; 42: 313–21.

Bovin G. Detection and management of antiviral resistance for influenza viruses. Influenza Other Respir Viruses 2013; 7 (suppl 1): 18–23.

Nguyen JT, Hoopes JD, Le MH, et al. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. PLoS One 2010; 5: e1332.

Smeed DF, Hurst BL, Wong MH, et al. Combinations of oseltamivir and peramivir for the treatment of influenza A (H1N1) virus infections in cell culture and in mice. Antiviral Res 2010; 88: 34–48.

Russell RJ, Haire LF, Stevens DJ, et al. The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Nature 2006; 443: 45–49.

Collins PJ, Haire LF, Lin YP, et al. Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature 2008; 453: 1258–61.

Smith BJ, McKimm-Bresnick JL, McDonald M, Fernley RT, Varghese JN, Colman PM. Structural studies of the resistance of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Nat Commun 2013; 4: 2854.
Review

58 Carrat F, Duval X, Toubah F, et al. Effect of oseltamivir, zanamivir or oseltamivir-zanamivir combination treatments on transmission of influenza in households. Antivir Ther 2012; 17: 1085–90.
59 Escuret V, Cornu C, Boutique F, et al. Oseltamivir-zanamivir bitherapy compared to oseltamivir monotherapy in the treatment of pandemic 2009 influenza A(H1N1) virus infections. Antiviral Res 2012; 96: 110–17.
60 Fiaraj PL, van der Vlies E, Beersma MF, et al. Evaluation of the antiviral response to zanamivir administered intravenously for treatment of critically ill patients with pandemic influenza A (H1N1) infection. J Infect Dis 2011; 204: 777–82.
61 Petersen E, Keld DB, Ellermann-Eriksen S, et al. Failure of combination oral oseltamivir and inhaled zanamivir antiviral treatment in ventilator- and ECMO-treated critically ill patients with pandemic influenza A (H1N1) infection. J Infect Dis 2011; 204: 783–87.
62 Govorkova EA, Baranovich T, Seiler P, et al. Antiviral resistance among highly pathogenic influenza A (H5N1) viruses isolated worldwide in 2002–2012 shows need for continued monitoring. Antiviral Res 2013; 101: 349–53.
63 Hayden FG, Douglas RG Jr, Simons R. Enhancement of activity against influenza viruses by combinations of antiviral agents. Antimicrob Agents Chemother 1980; 18: 536–41.
64 Burlington DB, Meiklejohn G, Mostow SR. Anti-influenza A activity of combinations of amantadine and ribavirin in ferret tracheal ciliated epithelium. J Antimicrob Chemother 1983; 11: 7–14.
65 Smee DF, Hurst BL, Wong MH, Bailey KW, Morrey JD. Effects of double combinations of amantadine, oseltamivir, and ribavirin on influenza A (H5N1) virus infections in cell culture and in mice. Antimicrob Agents Chemother 2009; 53: 2120–28.
66 Nguyen JT, Smee DF, Barnard DL, et al. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. PLoS One 2012; 7: e31060.
67 Morrison D, Roy S, Rayner C, et al. A randomized, crossover study to evaluate the pharmacokinetics of amantadine and oseltamivir administered alone and in combination. PLoS One 2007; 2: e1305.
68 Smee DF, Bailey KW, Morrison AC, Sidwell RW. Combination treatment of influenza A virus infections in cell culture and in mice with the cyclopentane neuraminidase inhibitor RW-270201 and ribavirin. Chemotherapy 2002; 48: 88–93.
69 Smee DF, Wong MH, Bailey KW, Sidwell RW. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses. Antivir Chem Chemother 2006; 17: 185–92.
70 Ilyushina NA, Hay A, Yilmaz N, Boon AC, Webster RG, Govorkova EA. Oseltamivir-ribavirin combination therapy for highly pathogenic H5N1 influenza virus infection in mice. Antimicrob Agents Chemother 2009; 53: 2120–28.
71 Smee DF, Bailey KW, Morrison AC, Sidwell RW. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses. Antivir Chem Chemother 2006; 17: 185–92.
72 Smith CB, Charette RP, Fox JP, Cooney MK, Hall CE. Lack of effect of oral ribavirin in naturally occurring influenza A virus (H1N1) infection. J Infect Dis 1980; 140: 548–54.
73 Stein DS, Creticos CM, Jackson GG, et al. Oral ribavirin treatment of influenza A and B. Antimicrob Agents Chemother 1987 Aug; 31: 1285–87.
74 Ray CG, Icenogle TB, Minnich LL, Copeland JG, Grogan TM. The use of intravenous ribavirin to treat influenza virus-associated acute myocarditis. J Infect Dis 1989; 159: 829–36.
75 Hayden FG, Sable CA, Connor JD, Lane J. Intravenous ribavirin by constant infusion for serious influenza and parainfluenza virus infection. Antivir Ther 1996; 1: 51–56.
76 Kirshon B, Faro S, Zarawin RK, Sarno TC, Carpenter RJ. Favorable outcome after treatment with amantadine and ribavirin in a pregnancy complicated by influenza pneumonia. A case report. J Reprod Med 1988; 33: 399–401.
77 Chan-Tack KM, Murray JS, Binkrant DJ. Use of ribavirin to treat influenza. N Eng J Med 2009; 361: 171–174.
78 Nguyen JT, Hoopes JD, Smee DF, et al. Triple combination of oseltamivir, amantadine, and ribavirin displays synergistic activity against multiple influenza virus strains in vitro. Antimicrob Agents Chemother 2009; 53: 4115–26.
79 Hoopes JD, Driebe EM, Kelley E, et al. Triple combination antiviral drug (TCAD) composed of amantadine, oseltamivir, and ribavirin impairs propagation of drug-resistant influenza A virus. PLoS One 2011; 6: e22978.
80 Seo S, Englund JA, Nguyen JT, et al. Combination therapy with amantadine, oseltamivir and ribavirin for influenza A infection: safety and pharmacokinetics. Antivir Ther 2012; 18: 377–86.
81 Kim WY, Young SG, Huh JW, et al. Triple-combination antiviral drug for pandemic H1N1 influenza virus infection in critically ill patients on mechanical ventilation. Antimicrob Agents Chemother 2011; 55: 5703–9.
82 Furuta Y, Gobow BN, Takahashi K, Shiraiki K, Smee DF, Barnard DL. Favipiravir (T705), a novel viral RNA polymerase inhibitor. Antiviral Res 2013; 100: 446–54.
83 Smee DF, Hurst BL, Wong MH, et al. Effects of the combination of favipiravir (T705) and oseltamivir on influenza A virus infections in mice. Antimicrob Agents Chemother 2010; 54: 126–33.
84 Tarbet EB, Maekawa M, Furuta Y, Baba YS, Morrey JD, Smee DF. Combinations of favipiravir and oseltamivir for the treatment of pandemic influenza A/California/04/2009 (H1N1) virus infections in mice. Antiviral Res 2012; 94: 103–10.
85 Toyama Chemical Co., The new drug application approval of “AVIGAN® tablet 200mg” in Japan for the anti-influenza virus drug. March 24, 2014, http://www.toyama-chemical.co.jp/jp/english/news/news140324e.html (accessed June 23, 2014).
86 Kobayashi O, Kashwagi S, Iwamoto A. Clinical effectiveness and safety of favipiravir, a novel anti-influenza drug with a selective inhibition activity against viral RNA polymerase. S1st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) Chicago, IL, USA; Sept 9–11, 2011. V-405.
87 Epstein C. A phase 2, randomized, double blind, placebo-controlled, multicenter study evaluating the safety and pharmacokinetics of different dosing regimens of favipiravir (T705) in adult subjects with uncomplicated influenza. Abstract O-905. Options for Control of Influenza VII; Cape Town, South Africa; Sept 3–10, 2013.
88 Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H1N1) infection. N Engl J Med 2007; 357: 1450–51.
89 Hung IF, To KK, Lee CK, et al. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest 2013; 144: 464–73.
90 Ekiert DC, Wilson IA. Broadly neutralizing antibodies against influenza virus and prospects for universal therapies. Curr Opin Virol 2012; 2: 134–41.
91 Ekiert DC, Friesen RH, Bhanja G, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. Science 2011; 333: 843–50.
92 Corti D, Voss J, Gamblin SJ, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science 2011; 333: 850–56.
93 Nakamura G, Chai N, Park S, et al. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza antibodies. Cell Host Microbe 2013; 14: 93–103.
94 Wathen MW, Barro M, Bright RA. Antivirals in seasonal and pandemic influenza—future perspectives. Influenza Other Respir Viruses 2013; 7: 1–16.
95 Ashton IV, Callan RL, Rao S, Landolt GA. In vitro susceptibility of canine influenza A (H1N8) virus to zanamivir and oseltamivir. Vet Med Int 2010; 2010: 1–5.
96 Belardo G, La Frazia S, Cenciarelli O, Carta S, Rossignol JF, Santoro MG. A novel potential anti-influenza drug, acting in synergism with neuraminidase inhibitors [Internet]. Paper presented at: 49th Infectious Disease Society of America Annual Meeting: New Approaches to Anti-Viral Therapy; Boston, MA; Oct 29–31, 2011. https://idea.confex.com/idaa/2011/webprogram/ Paper11075.html (accessed April 29, 2014).
97 Rossignol JF, La Frazia S, Chiappa L, Ciacci A, Santoro MG. Thiazolidines, a new class of anti-influenza molecules targeting viral hemagglutinin at the post-translational level. J Biol Chem 2009; 284: 29798–808.
98 Rossignol JF, Samudrals S, Hoppers M, Haffizulla J. A randomized, double-blind, placebo (PCB) controlled clinical trial of nitazoxanide (NTZ) in adults and adolescents with acute uncomplicated influenza [Internet]. Paper presented at the 49th Infectious Disease Society of America Annual Meeting; Boston, MA; Oct 20–23, 2011 (cited Apr 29, 2013); DSA Annual Meeting, Boston. LB-15, Oct 22, 2011. https://idaa.confex.com/idaa/2011/webprogram/Paper33028.html (accessed June 23, 2014).

99 Malakho VP, Aschenbrenner LM, Smeef DF, et al. Sialidase fusion protein as a novel broad-spectrum inhibitor of influenza virus infection. Antimicrob Agents Chemother 2006; 50: 4700–79.

100 Beulser JA, Lu X, Szettrer KJ, et al. DAS181, a novel sialidase fusion protein, protects mice from lethal avian influenza H5N1 virus infection. J Infect Dis 2007; 196: 1493–99.

101 Tiriana-Balzer GB, Gohareva LV, Nicholls J, et al. Novel pandemic influenza A(H1N1) viruses are potently inhibited by DAS181, a sialidase fusion protein. PLoS One 2009; 4: e7788.

102 Tiriana-Balzer GB, Sanders RL, Hedlund M, et al. Phenotypic and genotypic characterization of influenza virus mutants selected with the sialidase fusion protein DAS181. J Antimicrob Chemother 2011; 66: 15–28.

103 Moss RB, Hansen C, Sanders RL, Hawley S, Li T, Steigbigel RT. A phase II study of DAS181, a novel host directed antiviral for the treatment of influenza infection. J Infect Dis 2012; 206: 1844–51.

104 Chen YB, Driscoll JP, McAfee SL, et al. Treatment of parainfluenza 3 infection with DAS181 in a patient after allogeneic stem cell transplantation. Clin Infect Dis 2011; 53: 677–80.

105 Drozdz DR, Limaye AP, Moss RB, et al. DAS181 treatment of severe parainfluenza type 1 pneumonia in a lung transplant recipient. Transpl Infect Dis 2013; 15: E28–12.

106 Borisink YS, Leneva IA, Pecheur EI, Polyak SJ. Arbidol: a broad-spectrum antiviral compound that blocks viral fusion. Curr Med Chem 2008; 15: 997–1005.

107 Leneva IA, Russell BJ, Borisink YS, Hay AJ. Characteristics of arbidol-resistant mutants of influenza virus: implications for the mechanism of anti-influenza action of arbidol. Antiviral Res 2009; 81: 132–40.

108 Shi L, Yang H, He J, et al. Antiviral activity of arbidol against influenza A virus, respiratory syncytial virus, rhinovirus, coxsackie virus and adenovirus in vitro and in vivo. Arch Virology 2007; 152: 1467–55.

109 Leneva IA, Pediakina IT, Gus'skova TA, Glushakov RG. Sensitivity of various influenza virus strains to arbidol. Influence of arbidol combination with different antiviral drugs on reproduction of influenza virus A. Ter Arkh 2005; 77: 84–88 (in Russian).

110 Verteck Press Release. VX-787 showed significant antiviral activity and reduced the severity and duration of influenza symptoms in phase 2 challenge study. http://investors.vrx.com/releasedetail.cfm?ReleaseIds=748857 (accessed June 23, 2014).

111 Iversen PL, Mourich DV, Voss T. AVI-7100 is effective in oseltamivir resistant H1N1 infected ferrets. Abstract F1-13725a. Presented at the 51st Interscience conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, IL, USA; Sept 17–20, 2011.

112 Iversen PL, Mourich DV, Voss T, Bothwell WA. AVI-7100 prevents transmission from oseltamivir resistant H1N1 viral infected to naïve ferrets. Presented at the 51st Interscience conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, IL, USA; Sept 17–20, 2011.

113 Hui DS, Lee N, Chan PK. Adjunctive therapies and immunomodulatory agents in the management of severe influenza. Antiviral Res 2013; 98: 410–16.

114 Haasch B, Hartmayer C, Platz O. Combination of MEK inhibitors and oseltamivir leads to synergistic antiviral effects after influenza A virus infection in vitro. Antivir Res 2013; 98: 319–24.

115 Leal J, Tarus B, Bouguen V, et al. Structure-based discovery of the novel antiviral properties of naproxen against the nucleoprotein of influenza A virus. Antimicrob Agents Chemother 2013; 57: 2231–42.

116 Ion MG, de Jong MD, Giglino KJ, et al. End points for testing influenza antiviral treatments for patients at high risk of severe and life-threatening disease. J Infect Dis 2010; 201: 1654–62.

117 Murray JL, McDonald NJ, Sheng J, et al. Inhibition of influenza A virus replication by antagonism of a PI3K-akt-mTOR pathway member identified by gene-trap insertional mutagenesis. Antivir Chem Chemother 2012; 22: 205–15.

118 Mortina M, Kuhm K, Ichikawa A, et al. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 2013; 153: 112–25.

119 Lejal N, Tarus B, Bouguen V, et al. Structure-based discovery of the novel antiviral properties of naproxen against the nucleoprotein of influenza A virus. Antimicrob Agents Chemother 2013; 57: 2231–42.

120 Iversen PL, Mourich DV, Voss T, Bothwell WA. AVI-7100 is effective in oseltamivir resistant H1N1 infected ferrets. Abstract F1-13725a. Presented at the 51st Interscience conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, IL, USA; Sept 17–20, 2011.

121 Murray JL, McDonald NJ, Sheng J, et al. Inhibition of influenza A virus replication by antagonism of a PI3K-akt-mTOR pathway member identified by gene-trap insertional mutagenesis. Antivir Chem Chemother 2012; 22: 205–15.
139 Makris D, Manoulakas E, Komnou A, et al. Effect of pravastatin on the frequency of ventilator-associated pneumonia and on intensive care unit mortality: open-label, randomized study. Crit Care Med 2011; 39: 2440–46.

140 Baron S, Isaacs A. Absence of interferon in lungs from fatal cases of influenza. BMJ 1962; 1: 18–20.

141 Agrati C, Gioia C, Lalle E, et al. Association of profoundly impaired immune competence in H1N1v-infected patients with a severe or fatal clinical course. J Infect Dis 2011; 202: 681–89.

142 Szretter KJ, Gangappa S, Belser JA, et al. Early control of H5N1 influenza virus replication by the type I interferon response in mice. J Virol 2009; 83: 5825–34.

143 Matzinger SR, Carroll TD, Fritts L, McChesney MB, Miller CJ. Exogenous IFN-alpha administration reduces influenza A virus replication in the lower respiratory tract of rhesus macaques. PLoS One 2011; 6: e29255.

144 Loutfy MR, Blatt LM, Siminovitch KA, et al. Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA 2003; 290: 3222–28.

145 Lee SM, Cheung CY, Nicholls JM, et al. Hyperinduction of cyclooxygenase-2-mediated proinflammatory cascade: a mechanism for the pathogenesis of avian influenza H5N1 infection. J Infect Dis 2008; 198: 525–35.

146 Brun-Buisson C, Richard JC, Mercat A, Thiebaut AC, Brochard L. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. Am J Respir Crit Care Med 2011; 183: 1200–06.

147 Martin-Loeches I, Lisboa T, Rhodes A, et al. Use of early corticosteroid therapy on ICU admission in patients affected by severe pandemic (H1N1)v influenza A infection. Intensive Care Med 2011; 37: 272–83.

148 Xi X, Xu Y, Jiang L, Li A, Duan J, Du B. Hospitalized adult patients with 2009 influenza A(H1N1) in Beijing, China: risk factors for hospital mortality. BMC Infect Dis 2010; 10: 256.

149 Kim SH, Hong SB, Yun SC, et al. Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. Am J Respir Crit Care Med 2011; 183: 1207–14.

150 Chan K. Chinese medicinal materials and their interface with western medical concepts. J Ethnopharmacol 2005; 96: 1–18.

151 Wang C, Cao B, Liu QQ, et al. Oseltamivir compared with the Chinese traditional therapy maxingshigan-yinqiaosan in the treatment of H1N1 influenza: a randomized trial. Ann Intern Med 2011; 155: 27–25.

152 Hayden FG. Experimental human influenza: observations from studies of influenza antivirals. Antivir Ther 2012; 17: 133–41.

153 Kairalla JA, Coffey CS, Thomann MA, Muller KE. Adaptive trial designs: a review of barriers and opportunities. Trials 2012; 13: 145.