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Antiviral combinations for severe influenza

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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.

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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,[5] 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.[6]

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.[7–15] However, monotherapy has not prevented death in many patients with severe pandemic H1N1,[16] H5N1,[15] or H7N9 illness.[17] 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 otreatment.[17–20]

Furthermore, emergence of resistance during monotherapy has been a drawback in severe influenza, particularly in immunocompromised hosts[21] and in avian H5N1[22] and H7N9 infections.[17,23] Variants with highly reduced inhibition by oseltamivir in vitro have sometimes emerged within several days of initiation of therapy in severe influenza caused by by pandemic H1N1[21] or avian viruses.[17,23] 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.[24] 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,[25] 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.[26]

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.

See Online for appendix

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 1968[29] and ofrimantadine and ribavirin date to 1977.[30] Combinations of amantadine and ribavirin were reported to increase survival in murine models of influenza A virus infection,[30] although not of influenza B.[31] 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.[35–40] 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.[41] A subsequent randomised controlled trial in outpatients reported evidence for possible antagonism with a combination of oral oseltamivir and inhaled zanamivir,[42] 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 1).

Antiviral combinations

Neuraminidase inhibitors

At present, circulating influenza viruses including avian influenzas H7N9 and H5N1 are susceptible to neuraminidase inhibitors, and ovesrvational 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.[47] 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,[48] 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

	Drugs tested	Target population
Pharmacokinetic interactions	Oral oseltamivir + oral amantadine (NCT00416962)	Healthy volunteers
	Oral oseltamivir + oral favipiravir (unpublished)	Healthy volunteers
	Intravenous peramivir + oral rimantadine[43]	Healthy volunteers
	Intravenous peramivir + oral oseltamivir[43]	Healthy volunteers
	Intravenous zanamivir + oral oseltamivir[44]	Healthy volunteers
	Oral amantadine + oral ribavirin + oral oseltamivir (NCT00867139)	Healthy volunteers
Completed controlled trails of clinical efficacy	Oral rimantadine + nebulised zanamivir[4]	Hospitalised adults
	Oral oseltamivir + inhaled zanamivir[42]	Ambulatory adults
	Oral oseltamivir + pH1N1 convalescent plasma[45]	Critically ill patients
	Oral oseltamivir + pH1N1 hyperimmune globulin (NCT01617317)	Critically ill patients
	Oral oseltamivir + maxingshigan/yinqiaosan (NCT00935194)	Ambulatory adults
	Oral oseltamivir + sirolimus + corticosteroids[46]	Critically ill patients
	Oral amantadine + ribavirin + oseltamivir (TCAD; NCT01617317)	Critically ill patients
Continuing randomised controlled trials of clinical efficacy	Oral oseltamivir + convalescent plasma or hyperimmune globulin (NCT01052480)	Hospitalised adults
		High-risk outpatients
	Oral amantadine + ribavirin + oseltamivir (TCAD; NCT01227967)	Ambulatory adults
	Oseltamivir + nitazoxanide (NCT01610245)	

TCAD= triple combination antiviral drug.

***Table 1*: Representative antiviral combinations that have been studied or are presently in trials, by study type**

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).[52,53] 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.[52] 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,[54] and absence of inhibition in mice given neuraminidase inhibitors.[55] Of note, emergence of the Arg292Lys substitution has been reported as early as 2 days after initiation of oseltamivir in patients infected with iH7N9 and has been associated with poor clinical outcomes.[17,56] H7N9 variants with Arg292Lys replicate at least as well in Madin-Darby canine kidney-SIAT1 cells and primary human respiratory cells as susceptible virus[54] and show similar virulence in mice and transmissibility in guineapigs.[57] 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 oseltamivir[44] or of intravenous peramivir and oral oseltamivir[43] 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,[42] although the combination might have been more effective in reducing secondary transmission.[58] 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.[59] 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.[60] 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.[61] 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.[62] 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.[30,33,35,65] 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.[66]

Human studies of combinations of oral oseltamivir and amantadine[67] and of oral rimantadine and intravenous peramivir[43] 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.[41] 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.[68] Several oseltamivir and ribavirin dose combinations increased survival, reduced lung consolidation, and

reduced 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.[69] Ribavirin and oseltamivir have shown mainly additive interactions in preclinical assays with H5N1.[40,65,70,71] In mice infected with avian H5N1 virus, oseltamivir and ribavirin also showed exceptions of marginal synergy or slight antagonism at some dose combinations.[6]

In uncomplicated seasonal influenza, randomised controlled trials show that ribavirin monotherapy is ineffective at doses of 1 g per day[72] 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 teratogeniocity.[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.[48] 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.[66] 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.[80] 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.[81] A randomised controlled trial trial sponsored 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.[45] 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),[89] 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 globin 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),[90] group 2 haemagglutinins (including H3 and H7),[91] or both group 1 and 2 haemagglutinins.[92] 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 oseltamivir compared with either single agent.[93] These antibodies are just entering clinical trials at present but might offer broad-spectrum activity, especially if they possess sufficient avidity and can be delivered in sufficient quantity.

Other investigational antiviral drugs

Several antivirals that possess novel mechanisms of action and show anti-influenza activity in initial clinical studies would also be options for potential combination regimens with neuraminidase inhibitors or other approved drugs.[28,94]

Nitazoxanide is an approved oral antiparasitic agent with a well defined safety profile and human pharmacology. In vitro, nitazoxanide and its active metabolite tizoxanide inhibit influenza virus replication at submicromolar concentrations.[95,96] Nitazoxanide is reportedly both an interferon inducer and an inhibitor of influenza antihaemagglutinin maturation through a novel mechanism of action.[97] Scarce in-vitro testing shows evidence for synergy with combinations of tizoxanide and neuraminidase inhibitors in preclinical testing.[96] A placebo-controlled phase 2 randomised controlled trial in uncomplicated influenza showed great antiviral effects and clinical benefit (roughly a 21 h reduction in illness duration) at a dose of 600 mg twice a day compared with placebo.[98] Under the sponsorship of the Biomedical Advanced Research and Development Authority (BARDA), a large phase 3 randomised placebo-controlled trial has been initiated to compare nitazoxanide monotherapy, oseltamivir monotherapy, and the combination of oseltamivir and nitazoxanide for the treatment of uncomplicated influenza (NCT01610245).

Inhaled DAS181 (Ansun Biopharma, San Diego, CA, USA) is a conjugated sialidase that destroys the sialic acid-bearing receptors on host cells that are used by influenza A and B and parainfluenza viruses, to initiate infection. Topically applied DAS181 is active in murine and ferret models of influenza including H5N1, H7N9, and other influenza viruses.[55,99,100] Because of its novel host-directed mechanism of action, DAS181 is active against viruses resistant to existing agents[99,101] and has a low risk of resistance emergence. Longlasting in-vitro passage of influenza viruses in the presence of DAS181 leads to emergence of unstable variants with three to 18-fold reductions in susceptibility that contain substitutions in antihaemagglutinin and neuroaminidase.[102] Inhaled DAS181 showed great antiviral effects in a phase 2 randomised controlled trial in uncomplicated influenza, although no demonstrable effects on illness resolution.[103] 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 immunocompromised hosts.[104,105] 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.[106] 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.[107] Arbidol shows dose-related antiviral effects and survival in murine model studies,[108] and experiments in cell culture have shown synergistic effects when combined with adamantanes, ribavirin, or neuraminidase inhibitors.[106,109] 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 neuroaminidase 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.[110]

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.[111,112] 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-I) 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,[120] 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) inhibitors[121] or inhibitors of the Raf–MEK–ERK pathway[122] 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.[121] A small, open-label randomised controlled trial in critically ill adults infected with 2009 pandemic H1N1 virus pjp 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.[46] 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,[114] 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 osetltamivir, 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 influenza[136] 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;[139] further studies are warranted.

Interferons

Interferon alfa shows enhanced anti-influenza activity in vitro with other antivirals,[29,34] in addition to its immunomodulatory properties. Some evidence shows that severely ill patients with influenza have deficient endogenous interferon responses.[140,141] Systemic interferon is active in a murine model of H5N1 virus infection[142] and in a macaque model of H1N1 virus infection.[143] 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[144] 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.[23] 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,[145] 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.[146–149] Reports of patients infected with H7N9 virus suggest that glucocorticoid use might also be a risk factor for antiviral resistance emergence.[17,56] 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.[150] The mechanisms of CTMs in the treatment of influenza are complex and incompletely understood. Maxingshigan, one formulation of CTM, directly inactivates influenza A virus, idisrupting 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-yinqiaosan, and the combination of both in treatment of outpatients with 2009 pandemic influenza A.[151] 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

	Specific considerations
Availability of drug	Regulatory approval status, generic availability, cost, importation restrictions
Route of administration	
Inhaled/topical	Delivery device features; nebulisation potential in infants Effective delivery in severe and pneumonic disease; bronchospasm risk
Oral	Ease of administration, widest applicability, appropriate formulation for children
Injected	Reliable delivery in seriously ill patients, need for injection equipment and higher resourced setting
Complexity of dosing regimen	Compatibility of co-formulation (eg, potential single-dose form) Applicability across age spectrum (infants, elderly people) Applicability in low resource setting Compliance
Adverse event profile	Risk-benefit profile in outpatients and specific target populations Safety in infants and pregnancy
Antiviral potency and spectrum	Results from preclinical models Ability to rapidly control replication
Drug interactions	Pharmacokinetic and pharmacodynamic interactions with other influenza antivirals Pharmacokinetic interactions with other therapies (eg, immunosuppressive drugs)
Antiviral resistance emergence	Frequency, rapidity, cross-resistance patterns, viral fitness consequences
Immunomodulatory effects	Timing of initiation and cessation of intervention Target population based on disease pathogenesis
Cost	Effect on breadth of use Cost-benefit in outpatients Availability in low-resource settings

***Table 2*: Considerations in selecting antiviral combinations for study**

Search strategies and selection criteria

The primary searches for relevant studies for inclusion were done through PubMed between Jan 1, 1994 and May 1, 2014, in English with the following disease terms: "influenza" or "influenza, human". The results of these searches were crossed with the drug terms "interferons" or "oseltamivir" or "zanamivir" or "neuraminidase inhibitor" or "amantadine" or "peramivir" or "rimantadine" or "ribavirin" or "vaccines, combined" or "drug therapy, combination" or "combined drug therapy" or "combined antiviral therapy" or "antiviral therapy", and the identified abstracts were screened by one or more researchers. Earlier studies were also identified from the files of the senior author. We focused on studies of approved drugs and those in active clinical development that might be considered for near-term clinical study or use in combinations. Articles that studied investigational drugs that had not progressed in development (eg, in-vitro studies of plant extracts) or were inaccessible because of language barriers were not included. In addition, we searched three Chinese databases: Sinomed (former Chinese Biomedical Literature Database); China Knowledge Resource Integrated Database; and the WANFANG Database. Observational clinical studies with no control groups or those without laboratory-confirmed influenza virus detection were not included.

involving CTMs (appendix) have had limitations with respect to clarity on study methods (ie, randomisation procedures, blinding, and placebo controls) and adequacy of adverse-event reporting.

Future directions

Because of the many potential combinations that might be considered for study, various criteria need to be thought about to select the most appropriate interventions to take forward into clinical testing (table 2). For example, based on the availability in China at present, antiviral combinations of oseltamivir or intravenous peramivir with ribavirin, convalescent plasma, or some other form of neutralising antibody, or perhaps with nitazoxanide would be candidates for testing in serious influenza illness including that caused by avian H7N9.[56] Likewise, the strategies for clinical testing will need careful consideration. Since doing randomised controlled trials in seriously ill hospital inpatients is particularly difficult and resource intensive,[120] initial proof-of-concept studies might be done in alternative populations such experimentally infected volunteers[152] or outpatients with influenza. For antiviral combinations, the initial goal is to show tolerability and greater antiviral effects than with monotherapy. Consequently, smaller clinical studies that examine virological endpoints (quantitative virology and resistance emergence) might suffice to find out which combinations to take forward into large studies in risk groups or hospital inpatients.

For combinations involving immunomodulatory interventions, the challenges are great because the goal is disease amelioration through modulation of host responses. Unfortunately, our understanding of the heterogeneity and dynamics of immune responses in serious human influenza and their relation to disease pathogenesis is limited. The possibility of using immunomodulatory interventions will need to consider the particular target population and disease stage, the goal of either suppressing adverse host responses or supplementing deficient ones, and the timing of initiating and ceasing the intervention in relation to the course of illness. Adaptive clinical trial designs offer greater flexibility and efficiency through planned modifications, such as changes to sample size and treatment arms based on statistical analysis of data generated in the early stages of a trial.[153] By incorporating objective endpoints, such designs would enable the study of several different combinations simultaneously.

Contributors

FGH conceived the Review and wrote the first draft. JD reviewed the scientific literature on combination antiviral therapy, developed the associated appendix, and provided key revisions to the text. JKB reviewed the scientific literature on combination antiviral and immunomodulatory therapy, developed the associated appendix, and provided key revisions to the text. BC reviewed the scientific literature on combinations involving traditional Chinese medicine and provided associated updates to the appendices and text. All authors contributed to editing the final draft of the Review and all authors approved the final version.

Declaration of interests

JD, 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.

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