

Newer influenza antivirals, biotherapeutics and combinations

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This summary provides an overview of investigational antiviral agents for influenza and of future directions for development of influenza therapeutics. While progress in developing clinically useful antiviral agents for influenza has been generally slow, especially with respect to seriously ill and high-risk patients, important clinical studies of intravenous neuraminidase inhibitors, antibodies and drug combinations are currently in progress. The current decade offers the promise of developing small molecular weight inhibitors with novel mechanisms of action, including host-directed therapies, new biotherapeutics and drug combinations, that should provide more effective antiviral

therapies and help mitigate the problem of antiviral resistance. Immunomodulatory interventions also offer promise but need to be based on better understanding of influenza pathogenesis, particularly in seriously ill patients. The development of combination interventions, immunomodulators and host-directed therapies presents unique clinical trial design and regulatory hurdles that remain to be addressed.

Keywords Antibodies, antivirals, combinations, influenza, immunomodulators, neuraminidase inhibitors.

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Introduction

The purpose of this summary is to provide an overview of where we stand with respect to investigational antiviral agents for influenza and of future directions for development of influenza therapeutics. This commentary is based on a presentation at the first isirv Antiviral Group meeting in November 2011 and focuses primarily on clinical studies. It updates previous reviews¹ and summaries based on presentations by the author at international meetings in 2006,^{2,3} 2008,⁴ and 2010.⁵ In addition, a number of review articles that provide more detailed consideration of pre-clinical and clinical aspects of influenza antiviral development have been published recently by others.^{6–12}

When considered historically, the development of the first class of influenza antivirals, the aminoadamantanes (amantadine, rimantadine), dates to nearly five decades ago in the 1960s. Studies on ribavirin administered by various routes and on intranasal interferons followed in the 1970s and 1980s, respectively, but did not lead to approval for influenza in most countries. Developmental work on the second class of currently available agents, the neuraminidase inhibitors (NAIs) (zanamivir, oseltamivir), took place during the 1990s, but overall progress on developing

clinically useful antivirals for influenza has been slow. In addition, global circulation of influenza A(H3N2) viruses resistant to the aminoadamantanes and of seasonal A(H1N1) viruses resistant to oseltamivir,^{13,14} as well as instances of oseltamivir resistance among the 2009 pandemic A(H1N1) viruses,¹⁵ are reminders of the very limited size of our current influenza antiviral armamentarium.

However, recent pre-clinical studies have identified interesting inhibitors of influenza virus replication, and several of these have just entered or are expected to enter into clinical development. Table 1 contains a representative list of those which have shown activity either in animal models of influenza or in some cases in infected humans. In addition to the viral targets of inhibitors with proven clinical utility (i.e. M2, NA), a variety of targets and approaches have been identified that could potentially be used for developing new inhibitors. At meetings organised by the National Institute of Allergy and Infectious Diseases in 2009¹⁶ and 2011,¹⁷ investigators provided detailed updates on many of these approaches. Substantial data have also emerged in regard to using dual or multiple inhibitors in combination, including one modality combining three available agents, to increase effectiveness and manage the problem of anti-viral resistance. With adequate funding

Table 1. Representative investigational anti-influenza agents and biotherapeutics with antiviral activity in animal models and/or humans

NA inhibitors (NAIs)
Peramivir (IV)*, zanamivir (IV)*, oseltamivir (IV)
A-315675 (oral)(120,121)
Long-acting NAIs (LANIs)
Laninamivir (topical)*
ZNV dimers (topical)*
Conjugated sialidase
DAS181 (topical)*
Protease inhibitors
Aprotinin (topical, IV) (122)
HA inhibitors and viral binding agents
Peptides- FluPep (topical) (123), Entry Blocker (topical) (124), HB80/36 (70), Flufirvitide (72,73)
Arbidol (oral) (125,126)
Cyanovirin-N (topical) (127)
Iota-carrageenan (topical) (128)
Pentraxin PTX3 (IP) (129)
Polymer bound 6' sialyl-N-acetylactosamine (topical)(130)
CYSTUS052 (topical) (131)
Recombinant human galectin-1 (topical)(132)
Polymerase inhibitors
Ribavirin (oral, IV, inhaled)(3)
Favipiravir/T-705 (oral)*
Viramidine (oral) (133)
Antisense oligonucleotides (IV, topical) (134,135)
M gene
Antisense oligonucleotide (AVI-7100) (topical, IV)*
NP inhibitors
Nucleozin (IP) (136,137)
Antisense oligonucleotides (IV) (93,138)
Interferons (139–143)
IFN inducers- poly-ICLC (topical) (144,145); (107), nitazoxanide (PO)*
RIG-I activator (5'PPP-RNA) (IV) (146)
Antibodies to viral proteins
Convalescent plasma, hyperimmune globulin*
Anti-HA, M2e, NA, NP*
Other topical agents
Cationic airway lining modulators (iCALM- topical)(16,147)
Surfactant nano-emulsions (topical) (148)
SOFA-HDV ribozymes targeting M, NS, NP (149)
Defective interfering particles (244 DI RNA in a cloned A/PR/8/34) (150)

*See text for discussion of selected agents and additional references.
IN, intranasal; IP, intraperitoneal; IV, intravenous; SC, subcutaneous.

and agreement on feasible clinical study pathways to address regulatory concerns,^{18,19} the current decade offers the promise of progress in developing agents with novel mechanisms of action and of drug combinations that provide more effective therapies.

Antivirals in current clinical development

As shown in Table 2, there is a relatively short list of anti-influenza agents in advanced clinical development and a focus on NAIs, including three being developed for intravenous (IV) administration. Intravenous peramivir and the

inhaled long-acting NAI laninamivir are already approved in Japan, and peramivir also in South Korea. In addition, several novel agents that retain activity against influenza viruses resistant to the currently available classes of drug are also under clinical study.

Neuraminidase inhibitors

A medical need for parenteral antivirals in treating severe influenza has been recognised for many years. Intravenous administration of NAIs like zanamivir and peramivir can guarantee a rapid delivery of high-plasma drug levels in a reliable fashion. Indeed, the maximum plasma concentra-

Table 2. Selected influenza antiviral agents in advanced clinical development

Agent	Viral target	Sponsor	Route	Development phase
Zanamivir	NA	GSK	IV	Phase 3
Peramivir	NA	Biocryst, Shionogi	IV	Phase 3*†
Oseltamivir	NA	Roche	IV	Phase 3
Laninamivir (CS-8958)	NA	Biota, Daiichi-Sankyo	Inhaled	Phase 3*
Favipiravir (T-705)	Polymerase	Toyama	Oral	Phase 2-3
DAS181	HA receptor	Nexbio	Inhaled	Phase 1-2
Nitazoxanide	Possibly HA; IFN inducer	Romark	Oral	Phase 2

*Licensed in Japan.

†licensed in South Korea.

tions following IV zanamivir or peramivir are approximately 50-fold higher than those observed with double-dose (150 mg) oseltamivir, although the plasma AUC and minimum concentrations are closer.²⁰ Whether these pharmacologic differences will translate into greater antiviral activity, less frequent resistance emergence, and improved clinical outcomes remains to be determined.

While the available NAIs have inhibitory activity against influenza A and B viruses, their antiviral spectra and cross-resistance patterns vary by agent as they bind differently within the active site of the enzyme.^{11,13} In general, zanamivir and laninamivir have similar profiles of susceptibility. For example, the H275Y mutation confers high-level resistance to oseltamivir carboxylate and reduced susceptibility to peramivir in N1-containing viruses but does not substantially diminish susceptibility to zanamivir and laninamivir.²¹ Although peramivir has been reported to inhibit a laboratory strain of influenza A(H1N1) with H275Y,²² this particular mutation has emerged during *in vitro* passage with peramivir and also during its therapeutic use in an immunocompromised patient.²³ Furthermore, IV peramivir has not shown antiviral effects in treating infections due to oseltamivir-resistant A(H1N1)pdm09 infections.²⁴ Consequently, peramivir would not be reliable in treating such resistant variants, especially in immunocompromised hosts.

In adults with uncomplicated influenza, single IV doses of peramivir (300 or 600 mg) were superior to placebo²⁵ and comparable to a 5-day course of oseltamivir,²⁶ but IV peramivir was no better than oseltamivir in treating adults infected with oseltamivir-resistant seasonal A(H1N1) virus harbouring the H275Y mutation. Peramivir in daily IV doses (200 or 400 mg once daily for 5 days) was comparable to oseltamivir in hospitalised adults and did not select for resistance,²⁷ but a once-daily dose of 300 mg appeared less effective than one of 600 mg.²⁸ Peramivir was used on both compassionate use and Emergency Use Authorization bases in the United States for treating severe pandemic

2009 A(H1N1) illness,²⁹ and controlled studies in hospitalised patients are in progress.

Intravenous zanamivir was used extensively on a compassionate use basis during the 2009 pandemic, particularly for treating suspected or proven oseltamivir resistance,^{30–32} and a phase III trial is currently in progress to compare IV zanamivir to oral oseltamivir in hospitalised patients. In a small, phase II study,³³ hospitalised patients with high frequencies of severe illness (40% requiring mechanical ventilation), co-morbidities and prior oseltamivir therapy were initiated on IV zanamivir at a median of 5 days after symptom onset when they still had, despite oseltamivir treatment, high levels of viral RNA in nasopharyngeal samples. Zanamivir in this setting was temporally associated with median viral RNA load reductions of nearly two log₁₀ over the subsequent 4–5 days of administration. It remains to be determined whether even more rapid and profound antiviral inhibition might be possible with combinations of antivirals.

Inhalation of the NAI laninamivir prodrug (termed CS-8958) provides prolonged duration of antiviral activity in animal models³⁴ and prolonged presence of laninamivir in humans.³⁵ Laninamivir has an antiviral spectrum similar to zanamivir²¹ and was found to be superior to oseltamivir in treating children infected with oseltamivir-resistant seasonal A(H1N1) virus.³⁶ Single inhaled doses of laninamivir (20 mg or 40 mg) were comparable to 5 days of oseltamivir in adults,³⁷ although for unclear reasons it was not superior in treating adults infected with oseltamivir-resistant seasonal A(H1N1) virus. Inhaled dimers of zanamivir are also in early clinical development.^{38,39}

Conjugated sialidase

DAS181 is a novel fusion construct that includes the catalytic domain from *Actinomyces viscosus* sialidase linked with an epithelium-anchoring domain of human amphiregulin.⁴⁰ This sialidase removes both the human-like α 2,6- and avian-like α 2,3-linked sialic acids from cellular receptors,

and hence, this agent has a broad range of activity for influenza viruses, including those resistant to the amino-adamantanes and NAIs. Resistance has been difficult to select during *in vitro* passage and appears low-level (3- to 18-fold reductions in susceptibility).⁴¹ When administered topically, DAS181 shows inhibitory activity in animal models, including infections due to avian A(H5N1) and A(H1N1)pdm09 viruses.^{42,43} DAS181 is also inhibitory for parainfluenza viruses *in vitro* and in the cotton rat model⁴⁴; inhaled DAS181 has been given on compassionate use basis to hematopoietic stem cell and lung transplant patients with severe PIV infection with apparent benefit.^{45,46}

In a phase II randomised, controlled trial (RCT) of this agent for treating uncomplicated influenza,⁴⁷ 264 previously healthy adults with acute influenza were randomised to receive treatment with a single 10-mg inhalation of DAS181, once-daily inhalations for 3 days or placebo in a double-blinded fashion. Throat gargle virus titres, the primary virologic end point, showed significantly greater declines between the day of enrolment and the following day in the active groups compared with placebo. This accelerated clearance of pharyngeal virus continued to day 5 in the group that received DAS181 treatment over 3 days but was not seen with a single administration. This trial showed an encouraging antiviral effect, although this was not associated with greater improvement in symptom resolution. The reasons for this apparent discrepancy remain to be clarified but may relate to the relatively mild influenza illness in these patients. More work needs to be done to assess the tolerability and efficacy of different topical formulations of this novel host-directed inhibitor for potential influenza management.

Favipiravir

Favipiravir, previously designated T-705, also has a unique mechanism of antiviral action, so that it has inhibitory activity against both NAI- and aminoadamantane-resistant viruses.^{48,49} After undergoing intracellular metabolism (ribosylation and phosphorylation), so that it has a nucleoside-like configuration, the triphosphate inhibits influenza RNA polymerase.⁵⁰ *In vitro* favipiravir is active against all influenza types (A, B, C) at relatively low concentrations (0.01–0.5 µg/ml), and higher concentrations also show activity against some other RNA viruses.⁵⁰ Oral favipiravir is active in murine models of influenza, including lethal A(H5N1),⁴⁹ and shows synergistic interactions with oseltamivir.⁵¹ Favipiravir-resistant variants have not been reported to date.

In a phase II randomised, double-blind controlled trial in Japan, oral favipiravir (600 mg BID twice daily for 1 day followed by 600 mg daily for 4 days) gave a similar mean time to illness alleviation when compared to oseltamivir (approximately 50 hours in both groups), whereas a lower

favipiravir dose was less effective.⁵² Pharmacokinetic studies have shown that there is a need for both initial loading doses and dose adjustments based on weight and perhaps ethnicity. A large phase III treatment study of ambulatory patients with uncomplicated influenza has been conducted in Japan and other Asian countries. The time courses of resolution of virus detection in the upper respiratory tract, based on titres of infectious virus, were comparable in the favipiravir (1200 mg once followed by 400 mg on day 1 and then 400 mg BID for 4 days) and oseltamivir groups.⁵² A phase II placebo-controlled RCT treatment study in adults aged 55–80 years (favipiravir doses of 1000 mg BID on 1 day and then 400 mg BID for 4 days versus 1200 mg BID on 1 day and then 800 mg BID for 4 days) is in progress in the USA and other countries. While the clinical efficacy and safety data remain to be published from these studies, the available data show that favipiravir exerts antiviral effects in humans. These proof-of-concept findings confirm that influenza viral polymerase is an important target for antiviral development.

Nitazoxanide

Nitazoxanide, an oral antiparasitic agent, has interesting immunomodulatory effects, including up-regulation of various interferon and interferon-inducible genes. In addition, it has been reported to exert a specific influenza inhibitory effect related to blockade of HA maturation.⁵³ A recent phase II RCT compared two different doses of nitazoxanide (300 or 600 mg twice daily for 5 days) to placebo in ambulatory patients with suspected influenza.⁵⁴ Among 257 influenza-infected persons, the time to alleviation of symptoms, similar to the end point that was used in the pivotal NAI trials, was shorter by about 20 hours in the high-dose group compared with placebo. This study also found evidence for an antiviral effect with an approximate 1 log₁₀ reduction in treatment day 1 virus titres in the high-dose group compared with placebo. The 300-mg dose groups showed intermediate effects. These interesting results need confirmation, but given the extensive safety record of this drug and its unique mechanisms of action, it might be particularly interesting for use in combination with other antiviral agents.

Antibodies

Interest in antibody therapies of influenza has been stimulated in part by observations from the use of convalescent blood products as therapy in pneumonia patients during the 1918 pandemic.⁵⁵ Although these studies were not RCTs and used various forms of blood products, a retrospective analysis found a very dramatic reduction in overall mortality (crude case-fatality, 16% in treated versus 37% in controls), particularly if the products were administered within 4 days of a pneumonia diagnosis (19% compared to

59% with delayed treatment).⁵⁵ More recent anecdotal reports of administering convalescent plasma for treatment of severe avian A(H5N1)⁵⁶ and 2009 pandemic A(H1N1)⁵⁷ illness have also indicated benefit. A case-control study of convalescent 2009 pandemic A(H1N1) plasma, selected to have relatively high neutralising antibody titres, compared outcomes in 20 patients given plasma and 70 controls, all of whom were critically ill in intensive care (94% receiving mechanical ventilation) and already receiving oseltamivir therapy.⁵⁷ The crude case-fatality was much lower with convalescent plasma compared with no treatment (20% versus 55%), and there was also a suggestion of some acceleration of virus clearance. Because of these promising observations, a RCT is now being mounted through the NIAID to determine whether addition of convalescent plasma adds to oseltamivir therapy in seriously ill hospitalised patients.

This area has also received increased interest because of the identification of conserved epitopes on the stem region of the influenza haemagglutinin (HA). The 16 HA subtypes can be divided into two phylogenetic groups, designated group 1 (containing H1, H2, H5, H9, and others) and group 2 (containing H3, H7, and others). Hetero-subtypic, neutralising monoclonal human antibodies that are therapeutically active after passive transfer in mice and ferrets have been identified for both group 1^{58–62} and more recently group 2 HAs.⁶³ In one case, an antibody that recognises HA subtypes in both groups to variable extent has been reported.⁶⁴ These antibodies target conserved sites on the stem region and prevent the conformational changes in HA needed for membrane fusion during replication.^{59,60} Several of these antibodies have gone into initial clinical studies or are about to so. These broad spectrum neutralising anti-HA monoclonal antibodies and possibly ones directed to other relatively conserved epitopes on M2e,^{65,66} which appear to mediate cellular cytotoxicity and require intact Fc receptors,⁶⁷ and possibly NA⁶⁸ or NP,⁶⁹ offer the interesting prospect of combination therapies with small molecular weight inhibitors and activity against influenza viruses resistant to NAIs and/or aminoadamantanes.

In addition, the identification of highly conserved regions in the HA stalk has led to the identification of peptides that are able to bind potently and inhibit the fusogenic activity of HA.^{70,71} One of these peptides, a 16-mer called flufirviride, has progressed into initial clinical development.^{72,73}

Antiviral combinations

The fact that combinations of influenza antivirals offer the possibilities of enhanced potency and reduced resistance emergence, as well as potential dose-sparing, is a well-established concept. Work in this field started over 40 years ago with an amantadine and interferon combination.⁷⁴ About 25 years ago, the first triple drug combination

including interferon, rimantadine and ribavirin was described.⁷⁵ Subsequent pre-clinical studies have indicated that if an influenza A virus is aminoadamantane-susceptible, synergistic interactions *in vitro* and increased survival in murine models of influenza, including A(H5N1), are observed when the aminoadamantane is combined with a NAI or ribavirin.^{76–78} If a virus is aminoadamantane resistant, no consistent benefit has been found in using the aminoadamantane in combination with oseltamivir or ribavirin. Ribavirin and oseltamivir show primarily additive interactions *in vitro* and in murine models of A(H5N1),^{78–80} whereas favipiravir and NAIs show dose-related additive to synergistic effects for influenza A viruses *in vitro* and on survival in mice.⁵¹ Combinations of oseltamivir and zanamivir showed concentration-related additive to antagonistic antiviral effects for A(H1N1)pdm09 viruses *in vitro*,⁸¹ whereas combinations of oseltamivir and peramivir showed primarily additive activities *in vitro* and in mice.⁸² These reports did not describe possible effects in preventing resistance emergence, although such a benefit was seen with aminoadamantane and oseltamivir combinations for a range of aminoadamantane-susceptible influenza A viruses.⁷⁶

An increasing number of human studies have been done to assess influenza antiviral combinations, most often examining possible pharmacokinetic interactions and tolerability with currently available agents (e.g. oral oseltamivir + amantadine, oral oseltamivir + favipiravir, IV peramivir + oral rimantadine, IV peramivir + oral oseltamivir, IV zanamivir + oral oseltamivir).^{83–85} In general, these combinations appear to be adequately tolerated without important pharmacokinetic interactions. However, the number of combinations that have been tested for efficacy in humans in controlled trials is much more limited. One placebo-controlled trial of nebulised zanamivir in hospitalised influenza A-infected patients, all of whom were given rimantadine, was under-enrolled but found interesting trends towards faster cough resolution and lesser risk of rimantadine resistance emergence.⁸⁶ In contrast, a recent double-blind, placebo-controlled RCT highlighted the potential for antagonism with dual NAI use, when it found slower virologic and clinical responses in those given combined therapy with oseltamivir and inhaled zanamivir compared with oseltamivir alone in uncomplicated influenza.⁸⁷ Consequently, combinations of zanamivir and oseltamivir need further evaluation before being used in clinical practice. As indicated previously, a controlled study of convalescent 2009 pandemic A(H1N1) plasma combined with oseltamivir therapy is ongoing in hospitalised patients under NIAID sponsorship.

One triple drug regimen with three available agents (amantadine, ribavirin, oseltamivir) showed synergistic activity *in vitro* against not only influenza A viruses that

are susceptible⁸⁸ but also those resistant to the amantadine or oseltamivir at baseline, including A(H1N1)pdm09 virus.⁸¹ This triple regimen, termed TCAD, was more inhibitory than any of the dual combinations and was also more effective at preventing resistance emergence during *in vitro* passage.⁸⁹ Murine model studies indicated that amantadine contributes to the activity of TCAD and also enhances the activity of oseltamivir in a dual combination, in increasing survival following infection by amantadine-resistant A(H1N1)pdm09 virus,⁹⁰ although the mechanisms have not been clarified. TCAD has been studied in a small cohort of highly immunocompromised patients with influenza at the Fred Hutchinson Cancer Centre in Seattle [Janet Englund, presented at ICAR, April 2010]. Those who received the triple regimen did not show the emergence of new resistance mutations, and the regimen was reasonably well-tolerated over 10 days and provided the target blood levels of the individual drugs. A retrospective Korean study of critically ill adults with influenza A(H1N1)pdm09 infection suggested trends towards lower 14-day (17% versus 35%; $P = 0.08$) and 90-day (46% versus 59%; $P = 0.23$) mortality in TCAD recipients compared with those receiving oseltamivir monotherapy.⁹¹ A RCT trial sponsored by NIAID comparing TCAD to oseltamivir monotherapy for ambulatory high-risk patients is in progress.

There are a number of possibilities with regard to future combinations of antivirals and of antivirals combined with biotherapeutics including nitazoxanide and therapeutic antibodies, as well as immunomodulators. Combining antivirals with different mechanisms of action, for example, a polymerase inhibitor-like favipiravir with a NAI, would be especially interesting for treating more severe forms of influenza or infections in immunocompromised hosts. A large number of potential immunomodulatory agents have been proposed for adjunctive influenza treatment, many of which have shown activity in animal models (Table 3). For example, one recent report looked at a strategy of targeting sphingosine-1-phosphate (S1P) receptors with a sphingosine analog, designated AAL-R, to inhibit various pro-inflammatory cytokine and chemokine responses.⁹² In a murine model of A(H1N1)pdm09 infection, intratracheal application of AAL-R alone had a beneficial effect (survival increased to 82% compared to 21% in vehicle control and to 50% with oseltamivir), and when combined with oseltamivir, 96% survival was observed.

Future directions

The following section provides a highly selected commentary on novel approaches for developing more effective influenza therapeutics. The reader is referred to the many recent reviews regarding compounds in pre-clinical development for established and alternative (e.g. polymerase, nucleoprotein) targets.^{6–10,12,16,17}

RNA inhibition

There have been a number of interesting preclinical reports regarding antisense strategies and the use of siRNAs for treating influenza⁹³ and other respiratory viruses.⁹⁴ One that has moved forward clinically is AVI-7100, a phosphorodiamidate morpholino oligomer containing three modified linkages (PMO*plus*) that is designed to interfere with the translation of both the M1 and M2 mRNAs of influenza A virus (AVI Biopharma Inc, Bothell, WA, USA). These two proteins are products of splice variants from the same genome segment and share the same translation initiation start site which is targeted by this oligomer. The unique backbone structure allows for better delivery of the antisense oligomer to infected cells, and the molecule has been shown to have good activity against influenza A viruses in both cell culture and in animal model studies. In a ferret model of oseltamivir-resistant A(H1N1)pdm09 virus infection, this antisense molecule given either intraperitoneally or intranasally was associated with significant antiviral effects in terms of reduced nasal and BAL virus loads and lesser illness.⁹⁵ Intravenous administration of this molecule is now being examined in a dose-ranging phase I study. Depending on subsequent findings, this agent might eventually be an option for use in combination with other parenteral agents in more seriously ill patients.

Cellular targets

Another area of active investigation is the interaction between influenza virus and various host cell factors, at both the RNA and protein levels, to identify host cellular pathways essential for virus replication that might be amenable to inhibition, as a basis for treatment of acute infection.¹⁷ This approach of host-directed therapies has also been promoted because of the very low likelihood of resistance emergence and its potential applicability to multiple respiratory viruses.

Influenza infection results in the activation of various intracellular signalling responses, some of which the virus uses to ensure efficient replication. Two particular pathways have been established as suitable targets for inhibition of virus replication in murine models: the Raf/MEK/ERK mitogenic kinase cascade (involved in nuclear export of viral RNPs) and the IKK/NF- κ B module, the activation of which affects both several steps in replication and host innate immune responses.¹² Topical application of acetylsalicylic acid (aspirin), an inhibitor of IKK2, showed antiviral effects in mice,⁹⁶ although systemic aspirin was associated with increased mortality in several influenza animal models.⁹⁷

Recently, multiple groups using different RNAi genome-wide screening systems have published on the complexity of these interactions and identified possible targets in influenza, as well as several candidate inhibitors.^{98–101} Integrated analysis of five screens determined that 85 cellular factors

Table 3. Examples of potential adjunctive influenza treatments tested in animal models or used in humans

Proposed agent	Comment/Influenza model system
Glucocorticoids	IN reduced inflammation in cotton rats (151), but systemic delivery ineffective for A(H5N1) in mice (152); strong observational evidence for harmful effects in severe human influenza (see text)
Statins	Oral rosuvastatin ineffective in murine model (153), but combined statin/caffeine IN or oral inhibited viral replication (154). Reduced mortality reported in hospitalised influenza patients on prior therapy (see text)
Gemfibrozil	IP therapy increased survival in mice (155)
Pioglitazone	PPAR- γ agonist beneficial for A(H5N1) in mice by decreasing tipDC trafficking to lung (156). Pre-treatment with pioglitazone or rosiglitazone reduced influenza mortality in one murine model (157). AICAR (aminoimidazole carboxamide ribonucleotide), an activator of AMP-activated protein kinase (AMPK) that stimulates PPARs, is also active in mice (157).
PF-04178903	Prophylactic SC delivery of CCR2 blocker increased survival and decreased inflammatory markers in mice (158)
AAL-R	Topical sphingosine-1-phosphate receptor agonist active in mice alone and in combination with oseltamivir (92)
Cyclo-oxygenase inhibitors	Cox-2 inhibitor (celecoxib) beneficial with NAI for treating A(H5N1) in mice but ineffective alone (159). Pre-treatment with Cox-1 inhibitor (SC-560) associated with hypothermia, weight loss, and increased mortality in mice (160)
N-acetyl-cysteine ⁺	Dose-related protection alone and with antivirals in mice (161); case report of possible benefit (162)
Chloroquine	Ineffective in mice and ferrets (163), and in oral prophylaxis RCT in humans (164)
Bacterial lysate	Prophylactic topical delivery increased survival and decreased virus titres in mice (106)
Erythromycin	IP delivery increased survival and modulated immune measures in mice (165)
Ketotifen	Oral mast cell degranulation inhibitor reduced inflammatory mediators in H5N1-infected mice and was highly protective in combination with oseltamivir (166)
Pamidronate	Increased survival and antiviral effects in humanised mice (167)
Allopurinol	Oral allopurinol, an inhibitor of xanthine oxidase, and IV superoxide dismutase, an oxygen radical scavenger, reduced mortality in mice (168)
Cocaine	Modest antiviral effect after IP dosing in mice (169)
CpG oligonucleotides	Topical TLR-9 agonist protective in mice (144)
3M-011	IN TLR7/8 agonist active in mice (170)
<i>Lactobacillus pentosus</i> and <i>plantarum</i>	IN delivery of strain S-PT84 protective against influenza A challenge in mice (171). Oral prophylaxis with killed strains showed dose-related immunostimulatory effects, reduced lung virus titres, and increased survival in mice (172,173)
TJS-064	Oral traditional Chinese herbal therapy active in mice (174)
Maxingshigan-Yinqiaosan	Oral traditional Chinese therapy comparable to oseltamivir in fever resolution in RCT in uncomplicated influenza A(H1N1)pdm09 (175)
Nitric oxide inhalation	No antiviral or beneficial clinical effects in mice (176)
<i>Echinacea</i> extract	Oral use reduced weight loss and inflammation measures in mice (177)
PUL-042	Inhaled oligodeoxynucleotide and lipoprotein immunotherapeutic that protects against various pathogens in mice (17)
Clara cell protein CC10	Topical dosing of recombinant human CC10 showed antiviral effects in cotton rats (17)
Gabexate mesilate	Protease inhibitor that reduces cytokine responses in mice after IP dosing (178)
Green tea (catechins)	Oral catechins and theanine prophylaxis RCT showed possible reduced influenza infections (179)
Chitin microparticles	IN prophylaxis increased A(H5N1) survival in mice (180)
Isoquercetin	Plant-derived polyphenolic with antiviral effects in mice after IP delivery (181)
Cannabis	Oral Δ^9 -tetrahydrocannabinol increased virus loads and decreased inflammatory responses in mice (182)
Resveratrol	Antiviral effects and increased survival of mice after IP delivery (183)
Slit2N	IV treatment reduces endothelial hyper-permeability and mortality after H5N1 infection in mice (184).

IN, intranasal; IP, intraperitoneal; SC, subcutaneous; IV, intravenous.

were identified in two or more of the influenza virus screens, of which 50 were considered to have druggable properties and 34 were also needed for influenza replication *in vitro*.^{102,103} In particular, these analyses found that the vATPase and COPI complexes, the ribosomal mRNA splicing and nuclear trafficking machinery, and kinase-reg-

ulated signalling are all required for efficient replication of influenza A virus. Such cross-comparisons at the pathway level rather than the gene level reveal more common features that might provide potential targets for antiviral drug development, either influenza-specific or broader in spectrum.¹⁰⁴ Of course, even in the context of short-term

inhibitor administration, the targeting of cellular functions raises important tolerability concerns that will require careful safety studies in key patient populations.

Adjunctive therapies

Another area of considerable interest has been adjunctive treatments for influenza, primarily those directed against excessive pro-inflammatory host responses to infection.¹⁰⁵ Animal model studies have identified a wide range of agents with apparent beneficial effects (Table 3), but there are few for which clinical data have been developed. Depending on the particular model, agents with either pro-¹⁰⁶ or anti-inflammatory effects (Table 3) have been reported as showing benefit. This in part relates to the complexity of host responses leading to acute lung injury and differences among model systems.¹⁰⁷

The use of currently available drugs with immunomodulatory activity, well-characterised safety profiles, and low production costs has been promoted as a possible treatment strategy.¹⁰⁸ Some epidemiologic studies have reported substantial mortality benefits in patients taking statins who were subsequently hospitalised for influenza¹⁰⁹ or pneumonia,¹¹⁰ but the results are not consistent across studies.¹¹¹ The possible benefit of starting such drugs at the time of influenza onset or hospitalisation have not been reported to date, although one ICU-based, open-label RCT suggested reduced risks of ventilator-associated pneumonia and mortality associated with the addition of pravastatin therapy in patients requiring mechanical ventilation.¹¹² In contrast, while systemic glucocorticoids have been frequently used for treating influenza pneumonia and associated ARDS, studies from the 2009 A(H1N1) pandemic have found that glucocorticoids in such patients were associated with prolongation of virus replication, increases in secondary bacterial and fungal infections and higher rates of mortality in ICU patients.^{113–117} Consequently, one needs to be very cautious in terms of the particular immunomodulatory intervention, its therapeutic potency and its timing of use in relation to the type and course of respiratory illness. For example, studies of severe or fatal influenza viral pneumonia during seasonal¹¹⁸ and pandemic 2009¹¹⁹ outbreaks have found evidence for deficiency in interferon responses that appear key to controlling virus replication. Consequently, the possibility of using immunomodulatory interventions will need to consider the particular target population and goal of either suppressing adverse host responses or supplementing deficient ones, such that clinical trials will be challenging.

Summary

In conclusion, it is clear that medical needs exist for more effective therapies for severe influenza, particularly in those

who are hospitalised and in immunocompromised hosts. Considerable progress has been made in the clinical development of intravenous NAIs and to an increasing extent other novel antivirals and biotherapeutics for influenza management. In addition to optimisation of dosing regimens of existing drugs, combination therapies offer great promise going forward. Selective immunomodulatory interventions, in conjunction with antivirals to control replication, are another promising area for investigation, but the particular type(s) and timing of intervention need to be based on a better understanding of disease pathogenesis. Detailed pathogenesis studies to improve understanding of the relationships between virologic measures, biomarkers and clinical outcomes are needed, as are strategies for linking these findings to inform improved therapeutic monitoring approaches, particularly in seriously ill patients. In addition, the study of combination interventions, immunomodulators and host-directed therapies presents unique regulatory hurdles,^{18,19} and the pathways to efficient study and eventual marketing of such interventions require clarification.

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Conflicts of interest

From 2008 to present, Dr. Hayden has been an unpaid consultant to multiple companies engaged in the development or marketing of influenza antivirals, including AVI Biopharma, Biocryst, Crucell, GSK, Nexbio, Roche, Romark, Toyama and Visterra whose agents are included in this review. Since 2008 to present, the University of Virginia has received funding from the Wellcome Trust for his part-time work as influenza research coordinator at the Trust and from SAIC on behalf of the NIAID for his work as consultant the Southeast Asia Infectious Diseases Clinical Research Network. From 2008 to 2011, the University also received honoraria for his participation in the Neuraminidase Inhibitor Susceptibility Network which received funding from Roche and GSK.

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