Review Article

The role of adjuvant immunomodulatory agents for treatment of severe influenza

David S. Hui[a,*], Nelson Lee[a,b], Paul K. Chan[c], John H. Beigel[d]

[a] Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[b] Division of Infectious Diseases, University of Alberta, Edmonton, Canada
[c] Department of Microbiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[d] Leidos Biomedical Research Inc, Support to National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

ABSTRACT

A severe inflammatory immune response with hypercytokinemia occurs in patients hospitalized with severe influenza, such as avian influenza A(H5N1), A(H7N9), and seasonal A(H1N1)pdm09 virus infections. The role of immunomodulatory therapy is unclear as there have been limited published data based on randomized controlled trials (RCTs). Passive immunotherapy such as convalescent plasma and hyperimmune globulin have some studies demonstrating benefit when administered as an adjunctive therapy for severe influenza. Triple combination of oseltamivir, clarithromycin, and naproxen for severe influenza has one study supporting its use, and confirmatory studies would be of great interest. Likewise, confirmatory studies of sirolimus without concomitant corticosteroid therapy should be explored as a research priority. Other agents with potential immunomodulating effects, including non-immune intravenous immunoglobulin, N-acetylcysteine, acute use of statins, macrolides, pamidronate, nitazoxanide, chloroquine, antiC5a antibody, interferons, human mesenchymal stromal cells, mycophenolic acid, peroxisome proliferator-activated receptors agonists, non-steroidal anti-inflammatory agents, mesalazine, herbal medicine, and the role of plasmapheresis and hemoperfusion as rescue therapy have supportive preclinical or observational clinical data, and deserve more investigation preferably by RCTs. Systemic corticosteroids administered in high dose may increase the risk of mortality and morbidity in patients with severe influenza and should not be used, while the clinical utility of low dose systemic corticosteroids requires further investigation.

1. Introduction

Seasonal influenza epidemics, as well as pandemics, are important causes of mortality and morbidity. The World Health Organization estimates that seasonal influenza results in 3–5 million cases of severe illness and between 250,000 and 500,000 deaths annually worldwide (WHO, 2016). Avian influenza A(H5N1) and A(H7N9) viruses continue to circulate in some poultry populations and have caused intermittent human cases since 1997 (Chan, 2002) and 2013 (Gao et al., 2013) respectively. In addition, sporadic human cases of avian influenza A(H6N1) (Wei et al., 2013), A(H5N6) (Jiang et al., 2017), and A(H10N8) (Chen et al., 2014) have emerged in recent years. The number of different avian influenza viruses infecting humans is a concern because it increases the probability that a strain will acquire human-to-human transmission capability, making the emergence of an influenza pandemic more likely (Trombetta et al., 2015).

Severe influenza is defined by the World Health Organization as clinical or radiographic evidence of lower respiratory tract disease (e.g. dyspnoea, tachypnoea, radiographic pneumonia, etc), central nervous system involvement (e.g. encephalopathy, encephalitis), severe dehydration, influenza associated with certain complications (e.g. renal failure, multiorgan failure, septic shock, rhabdomyolysis and myocarditis), or influenza that causes exacerbation of underlying chronic disease (e.g. asthma, chronic obstructive pulmonary disease, diabetes, or cardiovascular conditions such as congestive cardiac failure) or any other condition requiring hospital admission(WHO, 2010). However, most studies simply define severe influenza as influenza requiring hospitalization.

High levels of pro-inflammatory cytokines have been reported in patients with severe influenza A(H1N1)pdm09 virus infection (Lee et al., 2011c; To et al., 2010; Bradley-Stewart et al., 2013), A(H5N1) infection (To et al., 2001; Peiris et al., 2004; de Jong et al., 2006), and

A(H7N9) infection (Zhou et al., 2013; Chi et al., 2013). These cytokines may be caused by the viral infection (primary cytokines), or immune response (secondary cytokines) (Guo and Thomas, 2017). The high level of inflammatory cytokines are often called "cytokine storm" or "cytokine dysregulation", though it is difficult to discern a high level pro-inflammatory response due to severe disease from a dysregulated cytokine response.

While early antiviral therapy with a neuraminidase inhibitor (NAI) is associated with improved outcome in patients hospitalized with seasonal influenza (Lee et al., 2009, 2010), A(H1N1)pdm09 (Lee et al., 2011b; Louie et al., 2012; Muthuri et al., 2014) and A(H5N1) viruses (Adisasmito et al., 2010), significant number of deaths occur despite antivirals (e.g. 25% mortality despite antivirals in patients admitted to ICUs with influenza A(H1N1) (Louie et al., 2012]). Therefore, improving antivirals may not be a sufficient strategy by itself to minimize morbidity and mortality from severe influenza. Given the role of inflammation in the pathogenesis, the use of adjunctive immuno-modulating agents remains a topic of great interest (Hui et al., 2013). As many patients hospitalized with influenza are elderly subjects with co-morbid illness requiring drug treatment with some immuno-modulating properties (eg. statins, PPAR agonist, and COX-2 inhibitors), it is challenging to rely on retrospective observational studies in investigating the effects of these drugs on the clinical outcome in severe influenza. This review provides an update on the potential role of different immuno-modulatory agents and other adjunctive therapies in the management of patients hospitalized with severe influenza with a focus on the rationale and evidence for using these agents and their side effects. Agents with both an immunomodulatory effect and a direct antiviral effect are also discussed.

We searched publications in English on the MEDLINE, EMBASE and GOOGLE SCHOLAR up to December 31st, 2017 using the search terms "influenza" in combination with the terms "immunomodulating agents" or "adjunctive therapy". We selected publications relevant to clinical treatment and classified the immuno-modulating agents or therapeutic procedures as therapies with evidence of worsened patient outcome, therapies of uncertain benefits, and therapies with clinical evidence of improved patient outcome.

2. Therapies with evidence of worsened patient outcome

2.1. Systemic corticosteroids

Systemic corticosteroids are widely prescribed in patients with influenza complicated by critical illness (Delaney et al., 2016), based on the findings that marked elevation of pro-inflammatory cytokine levels was associated with a high mortality rate in severe influenza virus infections due to A(H5N1) (de Jong et al., 2006; Carter, 2007), A(H7N9) (Zhou et al., 2013), and A(H1N1)pdm09 virus (Lee et al., 2011a). Many clinicians administer corticosteroids in an attempt to halt disease progression and to improve clinical outcomes.

Except for a small number of studies (Kudo et al., 2012a; Diaz et al., 2012; Linko et al., 2011), the majority of observational studies have shown that corticosteroid therapy, often in the presence of some delay in initiation of NAI therapy, was associated with a higher risk of death, an increased rate of nosocomial pneumonia and developing critical disease compared with those who did not receive corticosteroid therapy (Martin-Loeches et al., 2011; Brun-Buisson et al., 2011; Kim et al., 2011; Han et al., 2011; Lee et al., 2015) (Table 1). While these studies did not adjust for baseline and time-varying confounders (such as worsening clinical status and the decision timing to start corticosteroid therapy), corticosteroid therapy should not be used alone for influenza treatment without antiviral cover (Han et al., 2011). Corticosteroids therapy may also increase risk of opportunistic infections such as invasive aspergillosis, as reported in two patients infected with A(H1N1) pdm09 virus who had received high dose corticosteroids for ARDS (Lat et al., 2010). Emergence of NA Arg292Lys mutation has been observed

in two patients with A(H7N9) virus infection who had been given corticosteroid treatment that resulted in treatment failure and a fatal outcome (Hui et al., 2013). In addition, a retrospective cohort study of patients who received corticosteroid therapy for upper respiratory tract infections, allergies or spinal diseases has shown that within one month of drug commencement, there was an increase in rates of sepsis (incidence rate ratio 5.30, 95%CI 3.80 to 7.41), fracture (1.87, 1.69 to 2.07) and venous thromboembolism (3.33, 2.78 to 3.99), which decreased over the subsequent 2 months. At prednisone equivalent doses of < 20 mg/day, the increased risk persisted (incidence rate ratio 4.02 for sepsis, 3.61 for venous thromboembolism, and 1.83 for fracture) (Waljee et al., 2017).

A meta-analysis of data from 13 studies predominantly related to the treatment of severe influenza A(H1N1)pdm09 virus infection has shown that high dose corticosteroid therapy was associated with an increase in overall mortality (OR 3.06, 95% CI 1.58 to 5.92) (Rodrigo et al., 2016). When restricted to a subgroup of 4 studies that reported a risk adjusted mortality, the OR was still 2.82 (95% CI 1.61 to 4.92). These data call for caution against the liberal use of high dose corticosteroids in managing patients with severe influenza.

Two studies in China have recently examined the relationship between the dosages of corticosteroids and mortality in A(H1N1)pdm09 and A(H7N9) virus infections. In comparisons to controls, high-dose corticosteroids (> 150 mg/d methylprednisolone or equivalent) was associated with increased risks in 30-day mortality (38.5% vs 7.7%, p = .021) and 60-day mortality (50% vs 15.4%, p = .022) and longer duration of viral shedding (15 vs 13 days, p = .039) in patients with influenza A(H7N9) viral pneumonia while there was no difference between low dose (25–150 mg/d methylprednisolone) and controls (Cao et al., 2016). Another study of patients hospitalized with A(H1N1) pdm09 virus infection in China has shown that corticosteroids overall did not influence either 30-day or 60-day mortality, but in a subgroup analysis among patients with PaO_2/FiO_2 < 300 mmHg, low-to-moderate-dose corticosteroids treatment (equivalent 25–150 mg/day of methylprednisolone) significantly reduced both 30-day mortality (aHR 0.49 [95% CI 0.32–0.77]) and 60-day mortality (aHR 0.51 [95% CI 0.33–0.78]), whereas high-dose (> 150 mg/day) corticosteroid therapy yielded no difference (Li et al., 2017).

Currently, low-dose corticosteroids (e.g. hydrocortisone 50 mg q6h) is recommended for treatment of refractory septic shock in patients with severe influenza (Bautista et al., 2010). The role of low dose adjuvant corticosteroids for patients with severe influenza is of great interest and should be addressed as a research priority. There is a well designed RCT on the role of low dose corticosteroid therapy to be conducted during the first wave of the next influenza pandemic in the UK (Lim et al., 2015).

3. Therapies of uncertain benefit

3.1. N-acetylcysteine (NAC)

N-acetylcysteine (NAC) is a modified form of the amino acid cysteine, with anti-oxidant properties. NAC was shown to inhibit the production of pro-inflammatory molecules in lung epithelial cells infected with the highly pathogenic influenza A(H5N1) viruses (Geiler et al., 2010) and inhibit mucin synthesis and pro-inflammatory mediators in alveolar type II epithelial cells infected with influenza viruses A and B (Mata et al., 2011).

In a murine model infected with an influenza virus strain A/ PR8(H1N1) adapted in mice, NAC demonstrated synergy with oseltamivir in protecting mice from lethal influenza infection, with a survival rate of 100% for the combination therapy vs 60% for oseltamivir alone (Garozzo et al., 2007). In BALB/c mice inoculated intra-nasally with A/ swine/HeBei/012/2008 (H9N2) viruses with or without NAC, NAC reduced pulmonary inflammation, pulmonary edema, myeloperoxidase activity, total cells, neutrophils, macrophages, TNF-α, IL-6, IL-1β and

Table 1
Observational studies: Systemic corticosteroids for patients hospitalized with severe influenza[a].

Authors	N	Timing of antiviral from illness onset	% IMV	% given SC	Outcome
Kudo et al., 2012a, 2012b	58	Median 2 days	0%	79.3%	No difference in time to fever alleviation and hospital length of stay.
Martin-Loeches et al., 2011	220	Mean 4–5 days	70.5%	57.3%	Early use of corticosteroids was associated with an increased rate of HAP (OR 2.2, 95% CI 1.0–4.8, p < .05).
Brun-Buisson et al., 2011	208 with ARDS	Median 5 days	100%	40%	Corticosteroid therapy associated with increased risk of death, more HAP, & a trend to a longer duration of ventilation.
Kim et al., 2011	245	Mean 4.5 days	66.1%	44%	Corticosteroid therapy was associated with increased 90-day mortality (adjusted OR, 2.20; 95%CI, 1.03–4.71). Steroid group more likely to have superinfection (secondary bacterial pneumonia or invasive fungal infection), & had more prolonged ICU stays than the no-steroid group.
Han et al., 2011	83	> 5 days	44.6%	20.5%	Of 17 patients who received early corticosteroids (≤72 h ILI onset), 71% subsequently developed critical disease[b] vs 39% of 66 patients who received late (> 72 h) or no corticosteroids (RRM-H = 1.8, 95% CI 1.2–2.8, after adjusting for underlying diseases & risk factors). Proportional hazards modeling: use of corticosteroids tripled the hazard of developing critical disease (HR = 2.9, 95% CI 1.3–6.2, after adjusting for the same summary variables).
Diaz et al., 2012	372 ICU cases	N/A	60.2%	36.6%	Mortality was not significantly higher in patients treated with corticosteroids vs those who were not (18.4% vs 17.4%, p = .806).
Linko et al., 2011	132 ICU cases	Mean 4.5 days	78% either IMV or NIV	55%	The crude hospital mortality was not different in patients with corticosteroid therapy compared to those without: 8 of 72 (11%, 95% CI 4–19%) vs. 2 of 60 (3%, 95% CI 0–8%) (P = .11).
Lee et al., 2015	2649 A(H3N2), A(H1N1)pdm09, B)	Median 2 days	11.5%	23.1%	Corticosteroid therapy ↑ risks of super-infections (9.7% vs 2.7%) & deaths (adj HR 1.7, 95% CI 1.1–2.6) when controlled for indications.
Cao et al., 2016.	288 with H7N9	Median 6.3 days	52.1%	70.8%	Compared with the patients who did not receive corticosteroids, those who received corticosteroids had a significantly higher 60-day mortality (adjusted HR, 1.98; 95% CI, 1.03–3.79; p = .04). Subgroup analysis showed that high-dose corticosteroid therapy (> 150 mg/d methylprednisolone or eqv) significantly increased both 30-day and 60-day mortality, whereas no significant impact was observed for low-to-moderate doses (25–150 mg/d methylprednisolone or eqv). The median viral shedding time was much longer in the group that received high-dose (15 d), compared with patients who did not receive corticosteroids (13 d; p = .039).
Li et al., 2017	2141	Median 6 days	19.4%	49.3%	Overall, corticosteroids did not influence either 30-day or 60-day mortality. In the subgroup analysis among patients with PaO$_2$/FiO$_2$ < 300 mmHg, low-to-moderate-dose corticosteroids treatment (equivalent 25–150 mg/day of methylprednisolone) significantly reduced both 30-day mortality (aHR 0.49 [95% CI 0.32–0.77]) and 60-day mortality (aHR 0.51 [95% CI 0.33–0.78]), while high-dose (> 150 mg/day) of corticosteroid therapy yielded no difference.

[a] Severe influenza due to A(H1N1)pdm09 virus infection unless otherwise stated; SC= Systemic corticosteroids; HAP = hospital-acquired pneumonia; IMV = invasive mechanical ventilation; NIV = noninvasive ventilation.
[b] A critical case = any confirmed, patients hospitalized with ≥1 of the following: death, respiratory failure, septic shock, failure of ≥2 extra pulmonary organs, mechanical ventilation, or ICU admission.

CXCL-10 in broncho-alveolar lavage (BAL) fluid. In addition, NAC significantly inhibited the levels of TLR4 protein and TLR4 mRNA in the lungs while pharmacological inhibitors of TLR4 (E5564) led to similar effects as those determined for NAC in A(H9N2) swine influenza virus-infected mice (Zhang et al., 2014).

Despite the scientific basis, there is only one case report that high dose NAC, administered at 100 mg/kg daily as a continuous IV infusion, appeared to be effective in improving the clinical status by reducing C-reactive protein and oxygen requirement in a 48-year-old previously healthy female, who had presented with severe pneumonia and septic shock due to A(H1N1)pdm09 influenza (Lai et al., 2010). However, it was difficult to interpret the efficacy of NAC in this case report as there was concomitant treatment with higher than licensed dose of oseltamivir (150 mg twice-daily). As NAC is readily available, and its side effects (cough, dyspnea) are uncommon (Tarrant et al., 2017), more clinical data based on RCT design are needed to evaluate the role of NAC in the management of severe influenza.

3.2. Statins

Statins are competitive inhibitors of the enzyme HMG-CoA reductase and commonly prescribed for treatment of hypercholesterolemia. Statins have anti-inflammatory and immunomodulatory effects (e. g., repressing the induction of MHC-II by interferon (IFN)-γ and subsequent T-lymphocyte activation) (Weitz-Schmidt et al. 2001; Jain and Ridker, 2005). As such, it has been argued that statins may have a potential role for treatment of severe influenza (Fedson, 2009).

A number of studies have examined the effects of statins on influenza in mice with generally negative results in terms of reduction of survival rates or viral loads (Liu et al., 2009; Radigan et al., 2012; Belser et al., 2013; Gluck et al., 2013) (Table 2a). For example, simvastatin did not reduce morbidity, mortality, or viral load of mice infected with either A(H1N1) or A(H5N1) viruses while a combination of simvastatin and oseltamivir did not improve the effectiveness of oseltamivir alone following infection with highly pathogenic avian A(H5N1) influenza virus in mice despite some reductions in lung cytokine production (Belser et al., 2013). In another study of mice infected with influenza A/PR/8/34 (H1N1) virus, simvastatin given orally or intra-peritoneally resulted in lower survival rates and in more distinct weight loss than virus-infected control mice. Furthermore, neither the viral load in lungs and tracheas nor histopathological lesions was reduced by simvastatin treatment (Gluck et al., 2013). Epidemiologic studies of patients on chronic statins when they developed influenza have yielded generally favorable results in terms of some protection against death and hospitalization (Kwong et al., 2009; Brett et al., 2011; Vandermeer et al., 2012; Laidler et al. 2015; Brassard et al., 2017) (Table 2b). In a study of the UK Clinical Practice Research Datalink to identify all patients aged ≥ 30 years diagnosed with influenza-like illness during 1997–2010, including 5181 statin users matched to 5181 non-users, the 30-day incidence of hospitalization or death was 3.5% among statin users vs 5.2% in non-users, resulting in a 27% lower incidence with statin use (cumulative incidence ratio: 0.73, 95%CI: 0.59–0.89). However, the protective effect of statins was less certain among new users and those with concomitant chronic illness predisposing to influenza complications such as respiratory and cardiac disease. The data suggest that the beneficial effects of statins on influenza-related adverse outcomes may be due to a healthy user bias (Brassard et al., 2017).

In a multicenter trial in which patients with sepsis-associated ARDS randomly assigned to receive either enteral rosuvastatin or placebo, rosuvastatin did not improve clinical outcomes in patients with sepsis-associated ARDS and might have contributed to hepatic and renal organ dysfunction (National Heart, Lung, and Blood Institute ARDS Clinical Trials Network, 2014). A one year follow up of the same cohort has shown no significant difference in cumulative survival in the rosuvastatin group vs placebo (58% vs 61%; p = .377) (Dinglas et al., 2016). Another RCT in adults with suspected ventilator associated pneumonia

has shown that adjunctive simvastatin therapy did not reduce day-28 mortality in comparisons with placebo (Papazian et al., 2013).

There are currently no published human data based on RCTs on the acute use of statins in the management of severe influenza. As the drugs are relatively cheap and readily available, further research is warranted to examine their role as an adjunctive therapy in animal models (eg mice and ferrets) or in human clinical studies. The major side effects of statins include myopathy, rhabdomyolysis, liver damage, and type 2 diabetes (Allen and Mamotte, 2017). An ongoing study is evaluating atorvastatin vs placebo, in addition to standard care, for decreasing inflammatory markers in acute influenza (ClinicalTrials.gov Identifier: NCT02056340)

3.3. Macrolides

Macrolide antibiotics (e.g. erythromycin, clarithromycin, azithromycin) have anti-inflammatory effects in addition to their antibacterial properties; in vitro, they are shown to downregulate pro-inflammatory cytokines/chemokines, inhibit signal transduction and adhesion molecules expression, and regulate inflammatory cell functions (Kanoh and Rubin, 2010; Zarogoulidis et al., 2012; Parnham et al., 2014).

In cultured human tracheal epithelial cells infected with influenza A(H3N2) viruses, clarithromycin reduced viral titers and cytokines in supernatant fluids and viral RNA in the cells. Clarithromycin also reduced expression of susceptibility to influenza virus infection apart from reducing expression of sialic acid α2,6Gal on the tracheal mucosal surface, and the number and fluorescence intensity of acidic endosomes in the cells from which viral RNPs enter the cytoplasm (Yamaya et al., 2010). In an in vitro model using Mardin-Darby canine kidney (MDCK) cells and human lung epithelial cells, clarithromycin has been show to decrease intracellular viral replication via inhibition of viral protein production. (Miyamoto et al., 2008). Limited data based on the murine models have suggested that macrolides could reduce cytokine production and severity of pneumonia (Kanoh and Rubin, 2010; Min and Jang, 2012).

The effects of macrolides on humans infected with influenza viruses are shown in Table 3. In patients with mild seasonal influenza A without pneumonia, addition of clarithromycin led to a shorter duration of cough in patients who were without cough at the onset of pyrexia (Ishii et al., 2012). Addition of azithromycin to oseltamivir in patients with A(H1N1)pdm09 influenza without pneumonia led to a lower maximum temperature than that in the mono-group on days 3 through 5 (p = .048), without significant differences in the expression levels of inflammatory cytokines and chemokines between the 2 groups. (Kakeya et al., 2014).

The utility of macrolides was evaluated in an observational study of critically ill patients in Spain infected with influenza A(H1N1)pdm09 and a primary viral pneumonia without secondary infection. Patients with macrolide-based treatment had lower ICU mortality in the univariate analysis (19.2 vs. 28.1%, p = .02); however, a propensity score analysis showed no effect of macrolide-based treatment on ICU mortality (OR = 0.87; 95% CI 0.55–1.37, p = .5) (Martin-Loeches et al., 2013).

In a separate multicenter trial in adults hospitalized for laboratory-confirmed influenza, participants were randomized to receive oseltamivir-azithromycin (n = 25) or oseltamivir alone (n = 25) groups (Lee et al., 2017). There was faster reduction in plasma concentrations of pro-inflammatory cytokines IL-6, CXCL8/IL-8, IL-17, CXCL9/MIG, sTNFR-1, IL-18, and CRP in the oseltamivir-azithromycin group. The % reduction in plasma concentrations from baseline was: IL-6 (-83% vs -60%), CXCL8/IL-8(-81% vs -58%), IL-17 (-74% vs -34%), and CXCL9/MIG (-71% vs -56%) respectively when comparing the oseltamivir-azithromycin group vs oseltamivir monotherapy. There was a trend toward faster symptom resolution (β-0.463, 95%CI-1.297, 0.371) but no difference in viral RNA decline (p = .777) and culture-

Table 2
Studies of the effects of statins on influenza.

	Study design	Key findings
a) Animal studies		
Liu et al., 2009	BALB/c mice infected with A(H5N1), A(H3N2), or A(H1N1) influenza virus	Combination of 50 μg statin + 200 μg caffeine ameliorated lung damage and inhibited viral replication, and appeared to be at least as effective as oseltamivir and ribavirin. However, the statin/caffeine combination seemed to be more effective when administered preventatively, rather than as treatment.
Radigan et al., 2012	A murine model of influenza A virus infection,	Administration of rosuvastatin had no effect on viral clearance after infection or on mortality.
Belser et al., 2013	Mice infected with A(H1N1) or A(H5N1) viruses.	Simvastatin did not reduce morbidity, mortality, or viral load. A combination of simvastatin and oseltamivir did not improve the effectiveness of oseltamivir alone following highly pathogenic avian influenza A(H5N1) virus infection in mice despite modest reductions in lung cytokine production.
Gluck et al., 2013	This study evaluated the efficacy of simvastatin against influenza A/PR/8/34 (H1N1) virus infection in BALB/c-mice. In the first study, simvastatin was administered orally. To achieve high plasma levels, intraperitoneal application was used in a second study.	Treatment with simvastatin resulted in lower survival rates and in more distinct body mass loss in comparison to virus-infected control mice. Furthermore, the viral load in lungs and tracheas as well as histopathological lesions were not reduced by simvastatin.
b) Human epidemiology studies		
Kwong et al., 2009	A population-based cohort study over 10 influenza seasons (1996–2006) in Ontario, Canada with propensity-based matching.	Chronic use of statins showed small protective effects against pneumonia hospitalization ([OR] 0.92; 95% CI 0.89–0.95), 30-day pneumonia mortality (0.84; 95% CI 0.77–0.91), and all-cause mortality (0.87; 95% CI 0.84–0.89). However, these positive effects were reduced substantially following multivariate adjustment for confounding factors.
Brett et al., 2011	A retrospective case-control study of the UK Influenza Clinical Information Network database of 1520 patients hospitalized with A(H1N1)pdm09 influenza from April 2009 to January 2010.	No statistically significant association between pre-admission statin use and the severity of outcome in patients aged ≥ 35 years [adjusted OR: 0.81 (95% CI: 0.46–1.38); n = 571]. Following adjustment for age, sex, obesity and indication for statins, there was no statistically significant association between pre-admission statin use and the severity of outcome.
Vandermeer et al., 2012	A study of hospitalized adults in 10 states in the USA during the 2007-08 influenza season, which was analyzed to evaluate the association between receiving statins and influenza-related death.	Statins treatment before or during hospitalization was associated with a protective adjusted odds-of-death of 0.59 (95%CI 0.38–0.92), following adjustment for age, race, comorbid diseases, influenza vaccination and antiviral administration.
Laidler, et al. 2015	A study using population-based, influenza hospitalization surveillance data, propensity score-matched analysis, and Cox regression to determine if there was an association between mortality (within 30 days of a positive influenza test) and statin treatment among hospitalized cohorts from 2 influenza seasons (October 1, 2007 to April 30, 2008 and September 1, 2009 to April 31, 2010).	Hazard ratios for death within the 30-day follow-up period were 0.41 (95%CI, 0.25–0.68) for a matched sample from the 2007–2008 season and 0.77 (95% CI, 0.43–1.36) for a matched sample from the 2009 pandemic. The data suggest a protective effect of statins against death from influenza among patients hospitalized in 2007–2008 but not during the pandemic.
Lee et al., 2015	A retrospective study of factors influencing outcomes of adults hospitalized for seasonal and A(H1N1)pdm09 influenza in 2008–2011 in 3 cities (Hong Kong, Singapore and Beijing; N = 2649).	Chronic statin use decreased death risks (adjusted HR 0.44, 95% CI 0.23–0.84)
Brassard et al., 2017	A study of the UK Clinical Practice Research Datalink to identify all patients aged ≥ 30 years diagnosed with influenza-like illness during 1997–2010. The study cohort included 5181 statin users matched to 5181 non-users.	The 30-day incidence of hospitalization or death was 3.5% in statin users vs 5.2% in non-users, resulting in a 27% lower incidence with statin use (cumulative incidence ratio: 0.73, 95%CI: 0.59–0.89). However, the protective effect of statins was less pronounced among new users and those with concomitant chronic illness predisposing to influenza complications such as respiratory and cardiac disease.

negativity rates. Additional *ex vivo* studies confirmed reduced induction of IL-6 (p = .017) and CXCL8/IL-8 (p = .005) with azithromycin (Lee et al., 2017). These findings demonstrate that azithromycin can reduce cytokine levels and do not impair viral clearance.

The major side effects of macrolides include diarrhea, leucopenia, deafness and increased risk of cardiovascular deaths in the elderly subjects with high baseline risks for cardiovascular disease (Ray et al., 2012). The role of a triple combination therapy (oseltamivir, clarithromycin and naproxen) is discussed under section 4.

3.4. Peroxisome proliferator-activated receptors agonists

The PPAR agonists are a group of medications that act on the peroxisome proliferator-activated receptor. Apart from its clinically useful lipid-lowering activity, there is evidence that gemfibrozil (a PPAR-α agonist) can inhibit production of pro-inflammatory cytokines (Calkin et al., 2006). There are several studies (Budd et al., 2007; Bauer et al., 2010; Aldridge et al., 2009) based on the mice model that showed some anti-inflammatory effects of PPAR-γ agonist against influenza virus infection with survival advantage (Table 4). TNF inducible nitric oxide synthase producing dendritic cells (tipDC) accumulate in the lungs in significant numbers during lethal influenza infections, where they acquire, process, and present influenza antigen in the context of MHC class I antigen to CD8 T cells (Aldridge et al., 2009). During lethal influenza infections, the chemokines MCP-1 and MCP-3 accumulate to significantly higher levels in the airways, resulting in increased recruitment of tipDCs. Piglitazone diminishes tipDC recruitment, while allowing for protective CD8 T-cell expansion. Smoke exposure prior to influenza infection has been shown to increase lung inflammation with influenza infection (Robbins et al., 2006), and pioglitazone significantly attenuated the exaggerated inflammation observed in smoke-exposed influenza infected mice (Bauer et al., 2010).

The US Food and Drug Administration (FDA) communicated the potential cardiovascular risk of thiazolidinediones (rosiglitazone and pioglitazone) in 2007 and required a Risk Evaluation and Mitigation Strategy (REMS) for rosiglitazone in 2010. It also communicated in 2010 the potential risk of bladder cancer with pioglitazone use (Hsu et al., 2015). The role of the alternative anti-inflammatory drugs such as PPAR-γ agonists should be further explored for treatment of severe influenza in the animal model, but clinical utility may be limited by these toxicities.

Table 3
The effects of macrolide on humans with influenza infection.

Authors	Study design	Key findings
Ishii et al., 2012	A RCT of young patients in Japan with mild seasonal influenza A without pneumonia who had received early NAI therapy	Addition of clarithromycin did not result in a better outcome, apart from a shorter duration of cough in patients who were without cough at the onset of pyrexia.
Martin-Loeches et al., 2013.	A prospective, observational, multicenter study of critically ill A(H1N1)pdm09 influenza patients with primary viral pneumonia without any secondary bacterial infection across 148 ICUs in Spain (n = 733) with A (H1N1)pdm09 virus infection with primary viral pneumonia and severe respiratory failure.	Macrolide-based treatment was administered to 190 (25.9%) [Clarithromycin in 99 (52.1%), azithromycin in 90 (47.4%), and erythromycin in 1 (0.5%)]. Macrolide-based treatment was not associated with improved survival.
Kakeya et al., 2014	A prospective, randomized, clinical trial of oseltamivir (n = 56) vs oseltamivir and azithromycin combination therapy (n = 51) for influenza without pneumonia.	There were no significant differences in the expression levels of inflammatory cytokines and chemokines between the 2 groups. The maximum temperature in the combo-group was lower than that in the mono-group on D3 through D5 (p = .048), particularly on D4 (p = .037).
Lee et al., 2017.	In a RCT of patients randomized to the oseltamivir-azithromycin (n = 25) or oseltamivir groups (n = 25), with similar baseline characteristics [age, 57 ± 18 years; A(H3N2), 70%].	There was faster reduction in pro-inflammatory cytokines IL-6, CXCL8/IL-8, IL-17, CXCL9/MIG, sTNFR-1, IL-18, and CRP in the oseltamivir-azithromycin group. There was a trend toward faster symptom resolution (β-0.463, 95%CI-1.297, 0.371) but no difference in viral RNA decline (P = .777) and culture-negativity rates. Additional ex vivo studies confirmed reduced induction of IL-6 (P = .017) and CXCL8/IL-8 (P = .005) with azithromycin.

Table 4
Studies of the effects of PPAR agonists on influenza infection in mice.

Authors	Study design	Key findings
Budd et al., 2007	Mice given gemfibrozil intraperitoneally at 60 mg/kg once daily (n = 46) from days 4–10 following intranasal infection and compared to a controlled group.	Survival in BALB/c mice infected with influenza A/Japan/305/57 (H2N2) virus increased from 26% in vehicle-treated mice (n = 50) to 52% in mice given gemfibrozil.
Bauer et al., 2010	In a study examining the effects of systemic corticosteroids (dexamethasone) vs the PPAR-γ agonist pioglitazone on the outcome of influenza, C57BL/6 mice were exposed to room air or cigarette smoke for 4 days, then inoculated with a mouse adapted A(H1N1) virus, to mimic influenza and COPD.	Smoke-exposed mice were noted to have an exacerbated inflammatory response following infection. Dexamethasone treatment reduced mononuclear cells in the broncho-alveolar lavage (BAL) of smoke-exposed, virus-infected mice, while pioglitazone reduced mononuclear cells and neutrophils in the BAL and increased peripheral CD4$^+$ and CD8$^+$ T cells.
Aldridge et al., 2009	Mice were challenged with virulent influenza A viruses, including circulating avian A(H5N1) strains.	An increased selective accumulation of a particular dendritic cell (DC) subset, the TNF/iNOS-producing (tip) DCs, was noted in airways of mice with pneumonia. These tipDCs appear to be required for the further proliferation of influenza-specific CD8$^+$ T cells in the infected lung, because blocking their recruitment in CCR2-knockout mice decreased the numbers of CD8$^+$ effectors and reduced virus clearance However, treatment with the PPAR-γ agonist pioglitazone reduced tipDC trafficking, and this might moderate the potentially lethal consequences of excessive tipDC recruitment, without abrogating CD8$^+$ T cell expansion or compromising virus control.

3.5. Nitazoxanide

Nitazoxanide (NTZ) is an oral antiparasitic drug with both antiviral and immuno-modulatory effects. Antiviral activity of NTZ and its circulating metabolite, tizoxanide, has been demonstrated in several cell lines after infection with influenza A (H1N1, H3N2 and H5N9) and influenza B virus with EC50s between 0.3 and 1 μg/mL (Rossignol, 2014). Tizoxanide inhibits the replication of influenza viruses at the post-translational level by selectively blocking the maturation of the viral hemagglutinin at a stage preceding resistance to endoglycosidase H digestion, thus impairing hemagglutinin intracellular trafficking and insertion into the host plasma membrane (Rossignol et al., 2009). Anti-inflammatory effects include upregulation of IFN and various IFN-inducible genes (Rossignol, 2014). There are *in vitro* data showing synergistic effect of NTZ and oseltamivir against influenza A viruses (Belardo et al., 2015).

In a Phase 2b/3 study of low risk acute uncomplicated influenza A or B (age 12–64 years and without underlying medical conditions that put them at high risk for complications), 650 subjects were randomized to receive NTZ 300 mg bd, NTZ 600 mg bd, or placebo. Treatment with NTZ 600 mg bd for 5 days was associated with reduction in duration of symptoms 95.5 h (84.0–108.0) compared to placebo (116.7 h (95% CI 108.1–122.1) (p = .0084), whereas the 300 mg bd cohort was not significantly different than the control [109.1 h (96.1–129.5, p = .52)] (Haffizulla et al., 2014). Influenza-infected subjects receiving NTZ 600 mg also showed significant reductions in TCID50 viral titers on study Days 1–3 vs the placebo group (p = .0006). No resistance was observed for influenza viruses collected from NTZ-treated subjects, and no adverse effect on the humoral immune response was observed. Adverse events were similar among the three treatment groups (Haffizulla et al., 2014).

There are recently completed studies of NTZ vs oseltamivir for treatment of acute uncomplicated influenza (ClinicalTrials.gov Identifier: NCT02612922), ± NTZ ± oseltamivir (2 × 2 design) in an acute uncomplicated influenza (ClinicalTrials.gov Identifier: NCT01610245), and NTZ vs placebo + standard care in severe acute respiratory illness (SARI) (ClinicalTrials.gov Identifier: NCT03168282), but no data is available for these studies at this time. Until the data from these studies is public and appropriately peer reviewed, the use or further study of NTZ for the treatment of influenza should be cautioned.

3.6. Chloroquine

Chloroquine is an antimalarial drug which acts to raise lysosomal pH, leading to inhibition of both the fusion of autophagosomes with lysosomes and lysosomal protein degradation. Chloroquine has *in vitro* activity against both A(H1N1) and A(H3N2) influenza strains at concentrations achievable *in vivo* at the doses used for malaria prophylaxis and treatment of connective-tissue diseases (Ooi et al., 2006; Di Trani et al., 2007). Chloroquine did not prevent influenza infection nor diminish weight loss in mice infected with a mouse-adapted influenza A(H1N1) strain, and had only minimal activity in limiting viral replication in ferrets infected with an adapted A(H3N2) strain (Vigerust and McCullers, 2007). Chloroquine inhibited autophagy in mouse lung induced by A(H5N1) but viral loads and proinflammatory cytokines were not significantly affected (Yan et al., 2013). The major side effects of chloroquine in humans include headache, diarrhea, nausea and blurred vision. Furthermore, it did not prevent influenza infection in a healthy community population (Paton et al., 2011). The limited published data put chloroquine and its derivatives at a lower priority to be evaluated as a candidate drug for clinical treatment of severe influenza.

3.7. Pamidronate

Pamidronate is a bisphosphonate that has been used clinically for treatment of hypercalcemia and Paget's disease (Siris et al., 2006). Pamidronate exhibited protective effects against influenza infection caused by seasonal influenza A(H1N1) and avian A(H5N1) viruses in both *in vitro* and *in vivo* models, and the antiviral effects of pamidronate were dependent on Vδ2-T cells and mediated by their cytokine secretion and cytotoxicity against virus-infected host cells (Tu et al., 2011; Qin et al., 2012). Early treatment with intraperitoneal pamidronate reduced morbidity and mortality of A(H7N9)-infected mice through controlling both viral replication and inflammation in affected lungs. Delayed treatment with pamidronate starting Day 3 after infection could still reduce disease severity in infected mice and improve chance of survival, whereas oral oseltamivir starting at the same time showed no therapeutic effects. Antiviral effects of pamidronate were partly mediated by IFN-γ secreted from human Vδ2-T cells while human Vδ2-T cells could directly kill virus-infected host cells in a perforin, granzyme B- and CD137- dependent manner (Zheng et al., 2015).

The major side effects of pamidronate include acute systemic inflammatory reaction, ocular inflammation, renal failure, nephrotic syndrome, electrolyte imbalance, and osteonecrosis of the maxilla and mandible (Tanvetyanon and Stiff, 2006) and atypical femoral fractures (Powell et al., 2012). Given the potential side effects, pamidronate-based therapy would not be a high priority candidate for clinical trial on treatment of severe influenza infection.

3.8. Anti-C5a antibody

C5a is a complement cleavage peptide that may trigger the

formation of neutrophil extracellular traps (NETs) and release of histone proteins to the extracellular compartment during acute lung injury (ALI)/ARDS. NETs may activate platelets to release TGFβ, which is involved in tissue remodeling during the later phases of ALI/ARDS (Bosmann and Ward, 2014). Following inoculation of African green monkeys with A(H7N9) viruses and then treatment intravenously with IFX-1 at 5 mg/kg, a novel neutralizing specific antihuman C5a antibody 30 min after virus inoculation, anti-C5a treatment in A(H7N9)-infected monkeys led to a reduction in ALI, with reduction in lung histopathological injury and less lung infiltration of macrophages and neutrophils when the monkeys were sacrificed at Day 3. There was also a decrease in systemic inflammatory response and reduction in virus titers in the infected lungs (Sun et al., 2015).

3.9. Human mesenchymal stromal cells

ALI, including impaired alveolar fluid clearance, is a serious complication of severe respiratory virus infection. In vitro, influenza A(H5N1) infection impaired alveolar fluid clearance much more than seasonal virus. This impairment is mediated by the release of soluble factors from infected cells, leading to down- regulation of alveolar Na^+ and Cl^- transporters. Mesenchymal stromal cells (MSC) prevented or reduced this effect in vitro and in vivo in A(H5N1)-infected aged mice through secretion of soluble paracrine growth factors (Ang1 and KGF) (Chan et al., 2016). However, in C57Bl/6 mice that were infected with influenza A/Puerto Rico/8/34 (mouse-adapted H1N1 virus) or influenza A/Mexico/4108/2009 (swine-origin pandemic H1N1 virus) and administered human or mouse MSCs via the tail vein, either pre- or post-infection, the regimen failed to improve survival, decrease pulmonary inflammation/inflammatory cell counts or prevent ALI. MSCs administered in combination with oseltamivir also failed to improve outcomes (Darwish et al., 2013). In another study of 6- to 8-week-old C57BL/6 mice that were infected intra-nasally with A(H9N2) virus to induce ALI, MSC treatment significantly reduced A(H9N2)-induced ALI and was associated with reduced pulmonary inflammation (Li et al., 2016). MSCs promote an anti-inflammatory and highly phagocytic macrophage phenotype through extracellular vesicle (EV)-mediated mitochondrial transfer in ARDS. MSC-induced changes in macrophage phenotype critically depend on enhancement of macrophage oxidative phosphorylation. Murine alveolar macrophages treated with MSC-derived EVs ameliorate lung injury in vivo (Morrison et al., 2017). More work is needed in the animal model (eg mice and ferrets) to examine the role of MSCs in combination with antiviral treatment for ALI in severe influenza.

3.10. Mycophenolic acid

Mycophenolic acid, or its prodrug mycophenolate mofetil (MMF), is widely used as an immunomodulatory agent in transplant recipients and other patients with autoimmune diseases (Villarroel et al., 2009; Volkmann et al., 2017). Mycophenolic acid has broad-spectrum in vitro antiviral activity against influenza A(H1N1), A(H3N2), A(H7N9), and B viruses in the cell-protection assay while the antiviral effect of mycophenolic acid was completely reverted by guanosine supplementation. Plaque reduction assays showed that mycophenolic acid had antiviral activity against multiple clinical isolates of influenza A(H1N1), A(H3N2), A(H7N9) and B viruses (IC50 < 1 μM) (To et al., 2016). In a study that examined the anti-viral activity of MMF against A/ Vietnam/1194/2004 (H5N1) virus infection using MDCK cells and mice, the IC_{50} of MMF (0.94 μM) was similar to that of zanamivir (0.87 μM) in A(H5N1) virus-infected MDCK cells based on ELISA. Interestingly, MMF inhibited A(H5N1) viral mRNA replication and protein expression completely for about 8 h in time-course assays following commencement of treatment. In addition, all mice survived while viral titers in lungs were markedly reduced by MMF treatment (Cho et al., 2017). The protective mechanism of MMF against A(H5N1)

virus infection was due to inhibition of cellular inosine monophosphate dehydrogenase (IMPDH) by exogenous guanosine, which inhibits viral mRNA and protein expression. Moreover, IFN-β, IL-1β, IL-6, and IP-10 mRNA expression levels were substantially downregulated in MDCK cells with MMF treatment (Cho et al., 2017). It is important to note that while MMF has good inhibitory effect against Middle East Respiratory Syndrome coronavirus in vitro (Chan et al., 2013), all marmosets treated with MMF developed severe and/or fatal disease with higher mean viral loads than the untreated animals (Chan et al., 2015). More work is needed in mice and ferret models to understand the potential role of mycophenolic acid for treatment in severe influenza.

3.11. Herbal medicine

In a RCT of relatively mildly affected patients aged 15–59 yrs hospitalized with influenza A(H1N1)pdm09, but without pneumonia, there was significant reduction in the estimated median time to fever resolution seen with oseltamivir, maxingshigan-yinqiaosan (MY), and a combination of oseltamivir and MY vs the control group. Ephedra is the main ingredient of MY and the biological effects of MY are due to its ephedrine and pseudo-ephedrine content. Time to fever resolution was reduced by 19% (CI, 0.3%–34%; $P = .05$) when comparing oseltamivir + MY vs oseltamivir alone. However, there was no significant difference in symptom scores and data on serial viral kinetics were not available (Wang et al., 2011). Another RCT of patients aged 16–65 yrs with uncomplicated influenza A(H1N1)pdm09 found no significant differences between patients treated with herbal medicine Lianhuaqingwen capsule (LHC) or Oseltamivir in terms of the median duration of illness (LHC 69 h vs. Oseltamivir 85 h, p > .05) and the median duration of viral shedding (LHC 103 h vs. Oseltamivir 96 h, p > .05) (Duan et al., 2011). A Cochrane review of the role of Chinese Medicinal herbs for influenza based on 18 studies and 2521 participants has concluded that the methodological quality of the majority of studies was poor and more high quality RCTs with larger sample sizes are needed to evaluate the therapeutic role of herbal medicine (Jiang et al., 2013).

3.12. COX-2 inhibitors with/without other immune modulators

Selective COX-2 inhibitors are non-steroidal anti-inflammatory drugs (NSAID) that target COX-2, an inducible enzyme responsible for inflammatory processes and immune responses. COX-2 inhibitors have been shown to suppress A(H5N1) virus replication in human macrophages (Lee et al., 2011c).

The effects of COX-1 and COX-2 inhibitors in influenza A viral infection were examined in mice which were given a COX-1 inhibitor (SC-560), a COX-2 inhibitor (celecoxib) or no inhibitor beginning 2 weeks prior to influenza A viral infection (200 PFU) and throughout the course of the experiment. Treatment with SC-560 significantly increased mortality and was associated with profound hypothermia and greater weight loss than celecoxib or control groups. Levels of TNF-a and G-CSF were significantly attenuated in the SC-560 and celecoxib groups vs control while IL-6 levels were much lower in BAL fluid of celecoxib-treated mice vs control and vs the SC-560 group (Carey et al., 2010).

The effect of celecoxib in combination with mesalazine and zanamivir was assessed in BALB/c mice challenged with influenza A/ Vietnam/1194/2004 (H5N1) virus, with treatment started 48 h after viral inoculation to simulate a real-life scenario (Zheng et al., 2008). Survival rates and survival time were improved in the group that received zanamivir, celecoxib, and mesalazine, compared to zanamivir alone. Significantly higher levels of $CD4^+$ and $CD8^+$ T cells and less pulmonary inflammation were also noted in the group receiving the triple therapy. Zanamivir alone reduced viral load, but without any significant effect on lung inflammation or mortality. Mesalazine can inhibit both lipoxygenase and COX pathways, leading to reductions in pro-inflammatory cytokines and eicosanoids, and therefore

deactivation of inflammatory cells such as macrophages and neutrophils. (Zheng et al., 2008).

In a study of BALB/c mice inoculated intra-nasally with an A(H7N9) virus isolated from a chicken in a wet market epidemiologically linked to a fatal human case, (A/chicken/Zhejiang/DTID-ZJU01/2013 [CK1]), there was heavy alveolar inflammation and pulmonary hemorrhage, associated with high pulmonary levels of proinflammatory cytokines. In the mouse lung cell line LA-4, CK1 also induced high levels of IL-6 and cyclooxygenase-2 (COX-2) mRNA. Administration of the antiviral zanamivir did not significantly improve survival in mice infected with CK1, but co-administration of celecoxib in combination with zanamivir improved survival and lung pathology (Li et al., 2014). It is important to note that use of NSAID during episodes of acute respiratory infections, especially parenteral NSAIDs, was associated with an increased risk of acute myocardial infarction (Wen et al., 2017). More clinical studies are needed on the therapeutic role of a COX-2 inhibitor in combination with an NAI ± other immunomodulating agent for treatment of severe influenza (section 4).

3.13. Interferons(IFNs)

Type I IFNs (e.g., IFN-α/β) and type III interferons (IFN-λ) are important mediators of the innate immune response against influenza virus infection (Hsu et al., 2012). Recognition of influenza A viral components by Toll-like receptors (TLR) (eg TLR3 and/or TLR7) retinoic acid-inducible gene I, and melanoma differentiation associated factor-5 (Crotta et al., 2013) upregulates the expression of type I and type III IFNs, which induce numerous IFN-stimulated genes (ISGs), resulting in viral restriction and activation of the adaptive immune response (Durbin et al., 2013).

Patients hospitalized with severe influenza had evidence of deficient innate immune responses with lower plasma levels of IFN-α and monocyte chemoattractant protein–1 (MCP-1) (Agrati et al., 2010) while the fatal cases were unable to mount sufficient serology response early (Guihot et al., 2014). The innate immune response to influenza viruses involves production of IFN-α/β, which plays an important role in virus clearance during the initial stage of infection. In IFN α receptor knock-out mice, type I IFNs are responsible for direct resolution of viral load and limitation of acute lung injury through suppression of immunopathology caused by influenza A virus via IL-10 production (Arimori et al., 2013). Low-dose IFN-α protected mice against lethal A(H5N1) viral infection and was also effective against A(H1N1)pdm09 virus *in vitro* and in mice (Haasbach et al., 2011). An *intro* study has shown that antiviral agents with different mechanisms of action against seasonal influenza viruses (e.g., oseltamivir combined with IFN-λ1) exerted a significantly greater synergistic effect than co-treatment with drugs that target the same signaling pathway (i.e., IFN-β plus IFN-λ1) *in vitro* (Ilyushina and Donnelly, 2014). Thus a combination of exogenous IFN plus oseltamivir may be a potential therapy for treating influenza infections.

3.14. Antipyretics

A systematic review and meta-analysis of animal studies involving mice and chicks has shown that treatment with antipyretics including aspirin, paracetamol and Diclofenac for influenza infection increased the risk of mortality with a pooled odds ratio of 1.34 (1.04–1,73) (Eyers et al., 2010). However, a RCT has shown that early administration of intravenous acetaminophen 1 g q6h vs placebo for treatment of fever due to probable infection did not affect the number of ICU-free days or mortality rate at Day 28 or at Day 90 (Young et al., 2015). Another RCT of patients aged 18–65 yrs has shown that regular paracetamol 1 g qid vs oral placebo for 5 days had no effect on viral shedding, temperature or clinical symptoms in patients with influenza confirmed by PCR (Jefferies et al., 2016).

3.15. Therapeutic procedures

The polymyxin (PMX) B-immobilized fiber column, first introduced in Japan in 1994, is an extracorporeal device using PMX-B fixed to polystyrene-derived fibers, which can remove circulating endotoxin and reduce various cytokines through direct hemoperfusion (Ronco and Klein, 2014)). Three case reports suggest that PMX column hemoperfusion could reduce hypercytokinemia (elevated IL6, IL8, interferon-gamma, and high-mobility group box-1) and improve clinical outcome in patients with A(H1N1)pdm09 (Takeda et al., 2010; Binh et al., 2015) or A(H5N1) virus infection (Kudo et al., 2012b) complicated by ARDS despite treatment with NAI. It has been suggested that treatment with PMX hemoperfusion and oseltamivir is effective in improving ARDS due to influenza only if initiated early (Binh et al., 2015).

Therapeutic plasma exchange (TPE) or plasmapheresis is an extracorporeal blood purification technique designed to remove large-molecular-weight substances from the plasma. A pediatric case series described three children (aged 8, 11 and 17 yrs) with severe A(H1N1)pdm09 influenza complicated by ARDS and hemodynamic instability who required invasive mechanical ventilation and inhaled nitric oxide, and extracorporeal membrane oxygenation in one patient (Patel et al., 2011). TPE was provided as a rescue strategy to these patients with dramatic subsequent reductions in blood lactate levels, oxygen requirement, inotropic support and pediatric logistic organ dysfunction scores. All patients made good functional recovery. It is difficult to judge the therapeutic effect of PMX B-immobilized fiber column hemoperfusion or TPE based on findings of uncontrolled case reports, especially with concomitant use of NAI.

4. Therapies with clinical evidence of improved patient outcome

4.1. Passive immunotherapy- immune plasma, standard immunoglobulin (IVIG) and hyperimmune IVIG

Since 1890, when Behring and Shibasaburō demonstrated the first use of passive immunotherapy to treat diphtheria and tetanus (Behring and Kitasato, 1890), plasma has been used to treat severe infectious diseases including Spanish flu of 1917–1918 (Luke et al., 2006), and severe acute respiratory syndrome (SARS) in 2003 (Soo et al., 2004), Middle East respiratory syndrome (MERS) (Mo and Fisher, 2016), and most recently, the Ebola epidemic in West Africa (van Griensven et al., 2016). It is therefore not surprising that this modality has been used to treat severe influenza.

Immune globulin for intravenous use (IVIG) contains concentrated globulin preparations made from pooled human plasma (Gelfand. 2012). IVIG has several benefits over plasma treatment. IVIG can be given as a smaller volume (thus less concern with fluid overload), there is no ABO blood matching needed, and IVIG provides a more uniform product (as compared to individual plasma units with different antibody titers).

The presumed functional component of both immune plasma and IVIG is the IgG fraction. IgG may have effects however by either non-specific immune modulatory effects through the IgG constant fragment (Fc fragment) or the dimeric antigen binding fragment (F(ab')₂ fragment)(Schwab and Nimmerjahn, 2013). The Fc fragment is important for the pro-inflammatory activities of IgG, both the humoral arm and the cellular arm of the innate immune system. The IgG Fc fragment may also play a role in the anti-inflammatory effects. However, in influenza models, protection was also afforded by purified F(ab')2 but not Fc fragments derived from IVIG, supporting a specific antibody-mediated mechanism of protection rather than immune modulation (Rockman et al., 2017). Prior studies comparing the F(ab')2 fragments to whole non-immune IgG have shown the F(ab')2 fragment sufficient for protection where was the non-immune antibody was not protective (Lu et al., 2006).

4.1.1. Immune plasma

Plasma therapy uses plasma from patients who have fully recovered from or were vaccinated to an infection to treat those with the same infection. One meta-analysis that reviewed reports from the 1918 A(H1N1) influenza pandemic suggested that early administration of convalescent blood products reduced the risk of death from pneumonia (overall mortality reduced from 37 to 16%, 95% CI 15–27%) (Luke et al., 2006). Convalescent plasma was administered as an adjunctive treatment with a favorable outcome in a patient in China with severe A(H5N1) influenza pneumonia and multi-organ failure despite high-dose oseltamivir (Zhou et al., 2007) and in another patient with severe A(H7N9) infection with progression to ARDS despite treatment with a standard dose of oseltamivir (Wu et al., 2015).

A prospective multicenter case-control study evaluated the use of convalescent plasma for severe influenza A(H1N1)pdm09 (Hung et al., 2011). Ninety-three participants that had clinical deterioration despite optimal antiviral treatment and that required intensive care were enrolled. All subjects were offered immune plasma, with a neutralizing antibody titer of > 1:160. The 20 participants that accepted the plasma were compared to the 73 subjects that did not accept the plasma. Mortality was 20% in the group that accepted the plasma vs 54% in those that declined. The control arm mortality was higher than anticipated for a similar severity of illness (Duggal et al., 2016). However, the authors concluded that plasma was effective in reducing mortality, respiratory tract viral load, and serum cytokine levels in the treatment group.

A phase 2 study of patients in the USA with severe influenza A or B (hypoxia or tachypnea), who were randomly assigned to either 2 units (or pediatric equivalent) of convalescent plasma (HAI titer at least 1:80) plus standard care or standard care alone (99% received antivirals, of which 98% was oseltamivir monotherapy). This study showed no differences in the primary endpoint of normalization of respiratory status by Day 28 (67% vs 53%, p = .069) (Beigel et al., 2017). Multiple secondary endpoints were also not uniformly conclusive, including fewer days in the hospital after randomization (median 6 vs. 11, p = .13), fewer participants with hospital readmissions (2 vs. 7, p = .096), fewer participants with ICU admissions (57% vs. 69%, p = .097), and fewer days on mechanical ventilation (median 0 vs. 3, p = .14), disposition after hospital discharge (p = .029) and clinical status at Day 7 (death, in ICU, hospitalized on oxygen, hospitalized not on oxygen, not hospitalized but not returned to normal activities, or not hospitalized and returned to normal activities) (p = .020). Fewer plasma plus standard care participants had serious adverse events compared with standard care alone recipients (nine [20%] of 46 vs 20 [38%] of 52, p = 0·041), the most frequent of which were ARDS (one [2%] vs two [4%] patients) and stroke (one [2%] vs two [4%] patients). Transfusion-related acute lung injury (TRALI), and transfusion associated circulatory overload (TACO) are potentially life threatening While the study was underpowered, the results supported development of a larger ongoing plasma treatment study using clinical status on Day 7 by ordinal scale as the primary endpoint (ClinicalTrials.gov Identifier: NCT02572817).

4.1.2. Standard IVIG

In vitro studies have shown that standard human IVIG lots manufactured often have detectable HAI and neutralizing antibody titers against circulating and non-circulating strains (Hong et al., 2011; Kubota-Koketsu et al., 2012; Tian et al., 2016; Onodera et al., 2017). (Table 5). Undiluted IVIG is typically 6%, 10%, or 20% protein (e.g 20 g/100 ml), which is roughly 6, 10, or 20-fold more IgG than a typical serum concentration. Undiluted IVIG has moderate HAI and neutralizing titers (e.g. HAI 1:31–1:128 for a non-circulating H2N2) (Kubota-Koketsu et al., 2012), though when diluted to physiologic concentrations, titers to non-circulating strains are closer to 1:10. (Hong et al., 2011). A study using ferrets has shown that IVIG administered at the time of influenza virus exposure led to a significant

reduction in lung viral load following challenge of ferrets with A(H1N1) pdm09 virus. In the lethal influenza A(H5N1) model, the majority of ferrets given IVIG survived challenge in a dose dependent manner. (Rockman et al., 2017).

High dose standard IVIG (2 g/kg given for Kawasaki disease) has been shown to increase micro-neutralization (MN) titers in recipients from 1:6.9 (5.4–9.0) to 1: 35.9 (30.3 42.4), and HAI titers from 5.0 (5.0–5.0) to 12.5 (9.9–15.6). (Hong et al., 2011). However, this dose is not practical and may carry risk of thrombosis or renal dysfunction (Gammunex Package insert), so interest has turned to immune IVIG products.

4.1.3. Hyperimmmune IVIG

The plasma used to manufacture IVIG is chosen because of certain attributes, such as high titer anti-influenza antibodies, and is called hyperimmune IVIG. The protective efficacy of the IVIG manufactured from human plasma collected before the influenza pandemic due to A(H1N1)pdm09 virus and post-pandemic immune IVIG preparations was evaluated in a SCID mouse challenge model. Post-pandemic immune IVIG (HI titer 1:1280) provided complete protection from lethality of SCID mice against A(H1N1)pdm09 virus challenge: 100% of mice survived for 29 days post-challenge vs 50% of mice survival in the pre-pandemic IVIG (HI titer 1:70) group vs 40% survival in the buffer control group. Mice were administered dilutions of the IVIG (from 1:16 to undiluted). Protection was dose dependent, and there was a highly significant correlation between circulating *in vivo* HI and MN antibody titers and mice survival (nonparametric Spearman correlation r = 0.9, P < .0001) (Hohenadl et al., 2014)

A RCT of patients hospitalized with severe A(H1N1)pdm09 virus infection in 5 hospitals in Hong Kong compared treatment with immune IVIG prepared from plasma of persons who had recovered from the disease against treatment with normal IVIG manufactured before 2009 (Hung et al., 2013). Thirty-five patients with severe influenza A(H1N1) pdm09 infection on standard antiviral treatment requiring intensive care and ventilator support were randomized to receive immune IVIG (17 patients) or non-immune IVIG (18 patients). Immune IVIG treatment was associated with significantly lower Day 5 and 7 post-treatment viral load when compared to the control (p = .04 and p = .02 respectively). Subgroup multivariate analysis of the 22 patients who received treatment within 5 days of symptom onset demonstrated that immune IVIG treatment was the only factor that independently reduced mortality [OR: 0.14, 95% CI, 0.02–0.92; p = .04]. (Hung et al., 2013). An ongoing study is evaluating immune IVIG vs placebo, in addition to standard care, for patients hospitalized with severe influenza, and using the clinical status on Day 7 (by ordinal scale) as the primary endpoint (ClinicalTrials.gov Identifier: NCT02287467).

4.2. Triple combination therapy with oseltamivir, clarithromycin and naproxen

A phase 2b/3 RCT of adults hospitalized for A(H3N2) infection in 2015 has shown that a triple combination treatment of 2 days of clarithromycin 500 mg, naproxen 200 mg and oseltamivir 75 mg twice daily, followed by 3 days of oseltamivir reduced both 30-day (0.9% vs 8.2%) and 90-day mortality (1.9% vs 10%) and hospital length of stay vs oseltamivir 75 mg twice daily without placebos for 5 days as control (Hung et al., 2017). Multivariate analysis showed that the triple combination therapy was the only independent factor associated with lower 30-day mortality (OR:0.06; 95%CI, 0.004–0.94; P = .04). The findings were associated with significantly faster reductions in viral load and pneumonia severity index and fewer patients with ≥5% NAI-resistant A(H3N2) virus quasispecies detected (Hung et al., 2017). However, the study design did not allow assessment of the possible individual contributions of naproxen or clarithromycin. A larger confirmatory study that includes patients presenting later in their illness is a high priority for this readily available combination. Naproxen inhibits virus

Table 5
Studies of the effects of IVIG on influenza.

Authors	Study design	Main findings
Hong et al., 2011	A study that analyzed prepandemic IVIG and sera from Kawasaki disease patients (who had received IVIG as the standard treatment for autoimmune vasculitis) for A(H1N1)pdm09 virus-specific antibodies by micro-neutralization and hemagglutination inhibition,	All 6 IVIG preparations tested had significant levels of cross-reactive specific antibody, at a concentration of 2.0 g/dL of immunoglobulin. Sera from 18 of 19 Kawasaki disease patients had significant increases in cross-reactive-specific antibody after receiving 2.0 g/kg of prepandemic IVIG.
Kubota-Koketsu et al., 2012	*In vitro* study of human IVIG manufactured from 1993 to 2010 in Japan.	Significant neutralizing activities against influenza A(H2N2) viruses have been observed in lots of human IVIG
Tian et al., 2016	A study that analyzed sera from 96 healthy adult donors in southern China and commercially available IVIG manufactured in 2014 by microneutralization test for the titers of neutralizing antibodies (Nab) to several most common respiratory viruses including respiratory syncytial virus (RSV), seasonal influenza A, enterovirus 71 (EV71), coxsackievirus A16 (CA16),	A high proportion of samples from healthy adults were positive for NAbs (> 16) to all the viruses except Ad55. A different proportion of these samples had high NAb titers (> 512) for influenza A (25%), Ad3 (17.71%), RSV (9.38%), EV71 adenovirus type 3 (Ad3) and adenovirus type 55 (Ad55).(1.04%), CA16 (3.13%), and Ad55 (4.17%). Commercially available IVIG was found to have high NAb titers to influenza A (> 1000).
Rockman et al., 2017	This study examined the potential of human IVIG to ameliorate influenza infection in ferrets exposed to either the A(H1N1)pdm09 or highly pathogenic avian influenza A(H5N1) virus.	IVIG administered at the time of influenza virus exposure led to a significant reduction in lung viral load following challenge of ferrets with A(H1N1)pdm09 virus. In the lethal A(H5N1) model, the majority of ferrets given IVIG survived challenge in a dose dependent manner. Protection was also afforded by purified F(ab')2 but not Fc fragments derived from IVIG, supporting a specific antibody-mediated mechanism of protection.
Onodera et al., 2017	In this study, variations in hemagglutination inhibition (HI) and neutralizing antibody titers against seasonal influenza viruses [A(H1N1), A(H3N2), and B influenza virus strains] in IVIG lots manufactured from 1999 to 2014 were examined. In addition, the importance of monitoring the reactivity of IVIG against seasonal influenza viruses with varying antigenicity was evaluated.	IVIG manufactured from 1999 to 2014 exhibited significant HI & MN titers vs all investigated strains of seasonal influenza.

replication by interfering with the binding of virus RNA to nucleoprotein (Lejal et al., 2013).

4.3. Sirolimus

Sirolimus, also known as rapamycin, is a macrolide compound which has an inhibitory effect on the mechanistic target of rapamycin (mTOR) pathway. mTOR plays a central role in regulating many fundamental cell processes, from protein synthesis to autophagy, while deregulated mTOR signaling is implicated in the progression of cancer and diabetes, as well as the aging process (Saxton and Sabatini, 2017). Several genes in the phosphatidylinositol 3′-kinase (PI3K)-AKT-mTOR pathway have been shown to support broad-spectrum viral replication including influenza *in vitro* by RNA interference. Everolimus is a mTOR inhibitor which has *in vitro* antiviral activity against influenza A virus. In a lethal mouse infection model of influenza A (H1N1 and H5N1) virus infection, everolimus treatment (1 mg/kg/day) significantly delayed death although it could not prevent mortality. Histo-pathological findings showed decreased pulmonary hemorrhage and lung weights in everolimus treated mice in response to infection (Murray et al., 2012).

In a mouse model, sirolimus was given at the time of influenza A/PR/8/34 (H1N1) virus inoculation, and continued for 28 days. Mice treated with sirolimus had more body weight loss during the illness, and a higher viral load on Day 10 than controls that did not receive sirolimus. However, there was a trend, suggesting lower lung inflammation score as seen in histology specimens (mean score 9.0 ± 4.5 vs 11.5 ± 4.5, p = .335), and improved compliance on mechanical ventilation in the mice that received sirolimus, consistent with drug-promoting apoptosis that lowered experimental lung injury associated with influenza infection (Alsuwaidi et al., 2017).

In an open-label prospective RCT of critically ill patients infected with influenza A(H1N1)pdm09 requiring invasive mechanical ventilation (IMV), addition of sirolimus 2 mg/d for 14 days to oseltamivir 75 mg bd for 10 days and prednisolone 20 mg/d for 14 days (n = 19) was associated with a higher frequency of liberation from IMV (84.2 vs 47.4%, p = .04), a shorter duration of IMV (13.8 vs 33 days, p = .03) and a higher chance of achieving LRT viral RNA negativity by Day 7 (75% vs 33%, p < .05) compared to those treated with oseltamivir and prednisolone without addition of sirolimus (n = 19) (Wang et al., 2014). However, the study did not collect serial samples for quantitative virology nor did it assess the immunologic impact (i.e., serum or bronchoalveolar lavage cytokine levels) of the intervention.

The adverse effects of sirolimus and everolimus include leukopenia, thrombocytopenia, diarrhea, stomatitis, hypercholesterolemia, and rarely interstitial pneumonitis (Moes et al., 2015).

5. Conclusions

Currently there are no immunomodulatory agents that have been conclusively proven to be of benefit in severe influenza. Passive immunotherapy in the form of convalescent plasma or immune IVIG may be useful as an adjunct therapy for severe influenza, and definitive studies are ongoing. The triple combination of oseltamivir, clarithromycin, and naproxen for severe influenza was demonstrated to be effective in one RCT, but these findings need to be confirmed. Likewise, as sirolimus has been used with apparent benefit in critically ill influenza patients in the context of a RCT, confirmatory studies of this approach without concomitant corticosteroid therapy should be explored as a research priority. The efficacy of other agents with potential immunomodulating effects, including NAC, acute use of statins, macrolides, pamidronate, NTZ, IFNs, chloroquine, antiC5a antibody, human mesenchymal stromal cells, PPAR agonists, COX 2 inhibitors, mesalazine, herbal medicine, and the role of plasmapheresis and PMX hemoperfusion as rescue therapy, deserve more investigation preferably by RCTs. Systemic corticosteroids when administered in high dose may increase the risk of mortality and morbidity in patients with severe influenza and should not be used. The role of low dose systemic corticosteroid requires further evaluation as a research priority.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2018.01.002.

References

Adisasmito, W., Chan, P.K., Lee, N., Oner, A.F., Gasimov, V., Zaman, M., Bamgboye, E., Dogan, N., Starzyk, K., Dreyer, N.A., Toovey, S., 2010. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a Global Patient Registry. J. Infect. Dis. 202, 1154–1160.

Agrati, C., Gioia, C., Lalle, E., Cimini, E., Castilletti, C., Armignacco, O., Lauria, F.N., Ferraro, F., Antonini, M., Ippolito, G., Capobianchi, M.R., Martini, F., 2010. Association of profoundly impaired immune competence in H1N1v-infected patients with a severe or fatal clinical course. J. Infect. Dis. 202, 681–689.

Aldridge Jr., J.R., Moseley, C.E., Boltz, D.A., Negovetich, N.J., Reynolds, C., Franks, J., Brown, S.A., Doherty, P.C., Webster, R.G., Thomas, P.G., 2009. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. Proc Natl Acad Sci U S A. 106, 5306–5311.

Allen, S.C., Mamotte, C.D., 2017. Pleiotropic and adverse effects of statins-do epigenetics play a role? J. Pharmacol. Exp. Ther. 362, 319–326.

Alsuwaidi, A.R., George, J.A., Almarzooqi, S., Hartwig, S.M., Varga, S.M., Souid, A.K., 2017. Sirolimus alters lung pathology and viral load following influenza A virus infection. Respir. Res. 18, 136.

Arimori, Y., Nakamura, R., Yamada, H., Shibata, K., Maeda, N., Kase, T., Yoshikai, Y., 2013. Type I interferon limits influenza virus-induced acute lung injury by regulation of excessive inflammation in mice. Antivir. Res. 99, 230–237.

Bauer, C.M., Zavitz, C.C., Botelho, F.M., Lambert, K.N., Brown, E.G., Mossman, K.L., Taylor, J.D., Stämpfli, M.R., 2010. Treating viral exacerbations of chronic obstructive pulmonary disease: insights from a mouse model of cigarette smoke and H1N1 influenza infection. PLoS One 5, e13251.

Bautista, E., Chotpitayasunondh, T., Gao, Z.C., Harper, S.A., Hayden, F.G., Hui, D.S., Kettner, J.D., Kumar, A., Lim, M., Nicholson, K.G., Penn, C., Shaw, M., Shindo, N., Uyeki, T.M., 2010. Clinical aspects of pandemic influenza a (H1N1) 2009. N. Engl. J. Med. 362, 1708–1719.

Behring, E., Kitasato, S., 1890. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. Dlsch. Med. Wochenschr 16, 1113.

Beigel, J.H., Tebas, P., Elie-Turenne, M.C., Bajwa, E., Bell, T.E., Cairns, C.B., Shoham, S., Deville, J.G., Feucht, E., Feinberg, J., Luke, T., Raviprakash, K., Danko, J., O'Neil, D., Metcalf, J.A., King, K., Burgess, T.H., Aga, E., Lane, H.C., Hughes, M.D., Davey, R.T., IRC002 study team, 2017. Immune plasma for the treatment of severe influenza: an open-label, multicentre, phase 2 randomised study. Lancet Respir Med. 5, 500–511.

Belardo, G., Cenciarelli, O., La Frazia, S., Rossignol, J.F., Santoro, M.G., 2015. Synergistic effect of nitazoxanide with neuraminidase inhibitors against influenza A viruses in vitro. Antimicrob. Agents Chemother. 59, 1061–1069.

Belser, J.A., Szretter, K.J., Katz, J.M., Tumpey, T.M., 2013. Simvastatin and oseltamivir combination therapy does not improve the effectiveness of oseltamivir alone following highly pathogenic avian H5N1 influenza virus infection in mice. Virology 439, 42–46.

Binh, N.G., Manabe, T., Co, D.X., Tuan, N.D., Thach, P.T., Kudo, K., 2015. Polymyxin-B-immobilized-fiber column hemoperfusion with oseltamivir treatment for ARDS due to influenza H1N1/09. Respirol Case Rep 3, 57–60.

Bosmann, M., Ward, P.A., 2014. Protein-based therapies for acute lung injury: targeting neutrophil extracellular traps. Expert Opin. Ther. Targets 18, 703–714.

Bradley-Stewart, A., Jolly, L., Adamson, W., Gunson, R., Frew-Gillespie, C., Templeton, K., Aitken, C., Carman, W., Cameron, S., McSharry, C., 2013. Cytokine responses in patients with mild or severe influenza A(H1N1)pdm09. J. Clin. Virol. 58, 100–107.

Brassard, P., Wu, J.W., Ernst, P., Dell'Aniello, S., Smiechowski, B., Suissa, S., 2017. The effect of statins on influenza-like illness morbidity and mortality. Pharmacoepidemiol. Drug Saf. 26, 63–70.

Brett, S.J., Myles, P., Lim, W.S., Enstone, J.E., Bannister, B., Semple, M.G., Read, R.C., Taylor, B.L., McMenamin, J., Nicholson, K.G., Nguyen-Van-Tam, J.S., Openshaw, P.J., Influenza clinical information Network (FLU-CIN), 2011. Pre-admission statin use and in-hospital severity of 2009 pandemic influenza A(H1N1) disease. PLoS One 6, e18120.

Brun-Buisson, C., Richard, J.C., Mercat, A., Thiébaut, A.C., Brochard, L., REVA-SRLF A/H1N1v 2009 registry group, 2011. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 183, 1200–1206.

Budd, A., Alleva, L., Alsharifi, M., Koskinen, A., Smythe, V., Müllbacher, A., Wood, J., Clark, I., 2007. Increased survival after gemfibrozil treatment of severe mouse influenza. Antimicrob. Agents Chemother. 51, 2965–2968.

Calkin, A.C., Cooper, M.E., Jandeleit-Dahm, K.A., Allen, T.J., 2006. Gemfibrozil decreases atherosclerosis in experimental diabetes in association with a reduction in oxidative stress and inflammation. Diabetologia 49, 766–774.

Cao, B., Gao, H., Zhou, B., Deng, X., Hu, C., Deng, C., Lu, H., Li, Y., Gan, J., Liu, J., Li, H., Zhang, Y., Yang, Y., Fang, Q., Shen, Y., Gu, Q., Zhou, X., Zhao, W., Pu, Z., Chen, L., Sun, B., Liu, X., Hamilton, C.D., Li, L., 2016. Adjuvant corticosteroid treatment in adults with influenza a (H7N9) viral pneumonia. Crit. Care Med. 44, e318–328.

Carey, M.A., Bradbury, J.A., Rebolloso, Y.D., Graves, J.P., Zeldin, D.C., Germolec, D.R., 2010. Pharmacologic inhibition of COX-1 and COX-2 in influenza A viral infection in mice. PLoS One 5, e11610.

Carter, M.J., 2007. A rationale for using steroids in the treatment of severe cases of A/H5N1 avian influenza. J. Med. Microbiol. 56, 875–883.

Chan, J.F., Chan, K.H., Kao, R.Y., To, K.K., Zheng, B.J., Li, C.P., Li, P.T., Dai, J., Mok, F.K., Chen, H., Hayden, F.G., Yuen, K.Y., 2013. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J. Infect. 67, 606–616.

Chan, J.F., Yao, Y., Yeung, M.L., Deng, W., Bao, L., Jia, L., Li, F., Xiao, C., Gao, H., Yu, P., Cai, J.P., Chu, H., Zhou, J., Chen, H., Qin, C., Yuen, K.Y., 2015. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J. Infect. Dis. 212, 1904–1913.

Chan, M.C., Kuok, D.I., Leung, C.Y., Hui, K.P., Valkenburg, S.A., Lau, E.H., Nicholls, J.M., Fang, X., Guan, Y., Lee, J.W., Chan, R.W., Webster, R.G., Matthay, M.A., Peiris, J.S., 2016. Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. Proc Natl Acad Sci U S A 113, 3621–3626.

Chan, P.K., 2002. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. Clin. Infect. Dis. 34 (Suppl. 2), S58–S64.

Chen, Y., Yuan, H., Gao, R., Zhang, J., Wang, D., Xiong, Y., Fan, G., Yang, F., Li, X., Zhou, J., Zou, S., Yang, L., Chen, T., Dong, L., Bo, H., Zhao, X., Zhang, Y., Lan, Y., Bai, T., Dong, J., Li, Q., Wang, S., Zhang, Y., Li, H., Gong, T., Shi, Y., Ni, X., Li, J., Zhou, J., Fan, J., Wu, J., Zhou, X., Hu, M., Wan, J., Yang, W., Li, D., Wu, G., Feng, Z., Gao, G.F., Wang, Y., Jin, Q., Liu, M., Shu, Y., 2014. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. Lancet 383, 714–721.

Chi, Y., Zhu, Y., Wen, T., Cui, L., Ge, Y., Jiao, Y., Wu, T., Ge, A., Ji, H., Xu, K., Bao, C., Zhu, Z., Qi, X., Wu, B., Shi, Z., Tang, F., Xing, Z., Zhou, M., 2013. Cytokine and chemokine levels in patients infected with the novel avian influenza A (H7N9) virus in China. J. Infect. Dis. 208, 1962–1967.

Cho, J., Yi, H., Jang, E.Y., Lee, M.S., Lee, J.Y., Kang, C., Lee, C.H., Kim, K., 2017. Mycophenolic mofetil, an alternative antiviral and immunomodulator for the highly pathogenic avian influenza H5N1 virus infection. Biochem. Biophys. Res. Commun. 494, 298–304.

Crotta, S., Davidson, S., Mahlakoiv, T., Desmet, C.J., Buckwalter, M.R., Albert, M.L., Staeheli, P., Wack, A., 2013. Type I and type III interferons drive redundant amplification loops to induce a transcriptional signature in influenza-infected airway epithelia. PLoS Pathog. 9, e1003773.

Darwish, I., Banner, D., Mubareka, S., Kim, H., Besla, R., Kelvin, D.J., Kain, K.C., Liles, W.C., 2013. Mesenchymal stromal (stem) cell therapy fails to improve outcomes in experimental severe influenza. PLoS One 8, e71761.

de Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., Smith, G.J., Chau, T.N., Hoang, D.M., Chau, N.V., Khanh, T.H., Dong, V.C., Qui, P.T., Cam, B.V., Ha do, Q., Guan, Y., Peiris, J.S., Chinh, N.T., Hien, T.T., Farrar, J., 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat. Med. 12, 1203–1207.

Delaney, J.W., Pinto, R., Long, J., Lamontagne, F., Adhikari, N.K., Kumar, A., Marshall, J.C., Cook, D.J., Jouvet, P., Ferguson, N.D., Griesdale, D., Burry, L.D., Burns, K.E., Hutchison, J., Mehta, S., Menon, K., Fowler, R.A., Canadian critical care trials group H1N1 collaborative, 2016. The influence of corticosteroid treatment on the outcome of influenza A(H1N1pdm09)-related critical illness. Crit. Care 20, 75.

Di Trani, L., Savarino, A., Campitelli, L., Norelli, S., Puzelli, S., D'Ostilio, D., Vignolo, E., Donatelli, I., Cassone, A., 2007. Different pH requirements are associated with divergent inhibitory effects of chloroquine on human and avian influenza A viruses. Virol. J. 4, 39.

Diaz, E., Martin-Loeches, I., Canadell, L., Vidaur, L., Suarez, D., Socias, L., Estella, A., Gil Rueda, B., Guerrero, J.E., Valverdú-Vidal, M., Vergara, J.C., López-Pueyo, M.J., Magret, M., Recio, T., López, D., Rello, J., Rodriguez, A., H1N1 Semicyuc-Ciberes-REIPI working group (GETGAG), 2012. Corticosteroid therapy in patients with primary viral pneumonia due to pandemic (H1N1) 2009 influenza. J. Infect. 64, 311–318.

Dinglas, V.D., Hopkins, R.O., Wozniak, A.W., Hough, C.L., Morris, P.E., Jackson, J.C., Mendez-Tellez, P.A., Bienvenu, O.J., Ely, E.W., Colantuoni, E., Needham, D.M., 2016. One-year outcomes of rosuvastatin versus placebo in sepsis-associated acute respiratory distress syndrome: prospective follow-up of SAILS randomised trial. Thorax 71, 401–410.

Duan, Z.P., Jia, Z.H., Zhang, J., Liu, S., Chen, Y., Liang, L.C., Zhang, C.Q., Zhang, Z., Sun, Y., Zhang, S.Q., Wang, Y.Y., Wu, Y.L., 2011. Natural herbal medicine Lianhuaqingwen capsule anti-influenza A (H1N1) trial: a randomized, double blind, positive controlled clinical trial. Chin Med J (Engl) 124, 2925–2933.

Duggal, A., Pinto, R., Rubenfeld, G., Fowler, R.A., 2016. Global Variability in Reported Mortality for Critical Illness during the 2009-10 Influenza A(H1N1) Pandemic: a Systematic Review and Meta-Regression to Guide Reporting of Outcomes during Disease Outbreaks. PLoS One 11, e0155044.

Durbin, R.K., Kotenko, S.V., Durbin, J.E., 2013. Interferon induction and function at the mucosal surface. Immunol. Rev. 255, 25–39.

Eyers, S., Weatherall, M., Shirtcliffe, P., Perrin, K., Beasley, R., 2010. The effect on mortality of antipyretics in the treatment of influenza infection: systematic review and meta-analysis. J. Roy. Soc. Med. 103, 403–411.

Fedson, D.S., 2009. Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: why they are needed and how they might work. Influenza. Respi Viruses 3, 129–142.

Gao, R., Cao, B., Hu, Y., Feng, Z., Wang, D., Hu, W., Chen, J., Jie, Z., Qiu, H., Xu, K., Xu, X., Lu, H., Zhu, W., Gao, Z., Xiang, N., Shen, Y., He, Z., Gu, Y., Zhang, Z., Yang, Y., Zhao, X., Zhou, L., Li, X., Zou, S., Zhang, Y., Li, X., Yang, L., Guo, J., Dong, J., Li, Q., Dong, L., Zhu, Y., Bai, T., Wang, S., Hao, P., Yang, W., Zhang, Y., Han, J., Yu, H., Li, D., Gao, G.F., Wu, G., Wang, Y., Yuan, Z., Shu, Y., 2013. Human infection with a novel avian-origin influenza A (H7N9) virus. N. Engl. J. Med. 368, 1888–1897.

Garozzo, A., Tempera, G., Ungheri, D., Timpanaro, R., Castro, A., 2007. N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection. Int. J. Immunopathol. Pharmacol. 20, 349–354.

Geiler, J., Michaelis, M., Naczk, P., Leutz, A., Langer, K., Doerr, H.W., Cinatl Jr., J., 2010. N-acetyl-l-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. Biochem. Pharmacol. 79, 413–420.

Gelfand, E.W., 2012. Intravenous immune globulin in autoimmune and inflammatory diseases. N. Engl. J. Med. 367, 2015–2025.

Glück, B., Schmidtke, M., Walther, M., Meerbach, A., Wutzler, P., 2013. Simvastatin

treatment showed no prophylactic effect in influenza virus-infected mice. J. Med. Virol. 85, 1978–1982.

Guihot, A., Luyt, C.E., Parrot, A., Rousset, D., Cavaillon, J.M., Boutolleau, D., Fitting, C., Pajanirassa, P., Mallet, A., Fartoukh, M., Agut, H., Musset, L., Zoorob, R., Kirilovsky, A., Combadière, B., van der Werf, S., Autran, B., Carcelain, G., FluBAL Study Group, 2014. Low titers of serum antibodies inhibiting hemagglutination predict fatal fulminant influenza A(H1N1) 2009 infection. Am. J. Respir. Crit. Care Med. 189 1240–1209.

Guo, X.J., Thomas, P.G., 2017. New fronts emerge in the influenza cytokine storm. Semin. Immunopathol. 39, 541–550.

Haasbach, E., Droebner, K., Vogel, A.B., Planz, O., 2011. Low-dose interferon Type I treatment is effective against H5N1 and swine-origin H1N1 influenza A viruses in vitro and in vivo. J. Interferon Cytokine Res. 31, 515–525.

Haffizulla, J., Hartman, A., Hoppers, M., Resnick, H., Samudrala, S., Ginocchio, C., Bardin, M., Rossignol, J.F., US nitazoxanide influenza clinical study group, 2014. Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. Lancet Infect. Dis. 14, 609–618.

Han, K., Ma, H., An, X., Su, Y., Chen, J., Lian, Z., Zhao, J., Zhu, B.P., Fontaine, R.E., Feng, Z., Zeng, G., 2011. Early use of glucocorticoids was a risk factor for critical disease and death from pH1N1 infection. Clin. Infect. Dis. 53, 326–333.

Hohenadl, C., Wodal, W., Kerschbaum, A., Fritz, R., Howard, M.K., Farcet, M.R., Portsmouth, D., McVey, J.K., Baker, D.A., Ehrlich, H.J., Barrett, P.N., Kreil, T.R., 2014. Hyperimmune intravenous immunoglobulin containing high titers of pandemic H1N1 hemagglutinin and neuraminidase antibodies provides dose-dependent protection against lethal virus challenge in SCID mice. Virol. J. 11, 70.

Hong, D.K., Tremoulet, A.H., Burns, J.C., Lewis, D.B., 2011. Cross-reactive neutralizing antibody against pandemic 2009 H1N1 influenza A virus in intravenous immunoglobulin preparations. Pediatr. Infect. Dis. J. 30, 67–69.

Hsu, A.C., Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, P.M., Wark, P.A., 2012. Critical role of constitutive type I interferon response in bronchial epithelial cell to influenza infection. PLoS One 7, e32947.

Hsu, J.C., Ross-Degnan, D., Wagner, A.K., Zhang, F., Lu, C.Y., 2015. How did multiple FDA actions affect the utilization and reimbursed costs of thiazolidinediones in US medicaid? Clin. Ther. 37, 1420–1432.

Hui, D.S., Lee, N., Chan, P.K., 2013. Adjunctive therapies and immunomodulatory agents in the management of severe influenza. Antivir. Res. 98, 410–416.

Hung, I.F., To, K.K., Lee, C.K., Lee, K.L., Chan, K., Yan, W.W., Liu, R., Watt, C.L., Chan, W.M., Lai, K.Y., Koo, C.K., Buckley, T., Chow, F.L., Wong, K.K., Chan, H.S., Ching, C.K., Tang, B.S., Lau, C.C., Li, I.W., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2011. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin. Infect. Dis. 52, 510–519.

Hung, I.F., To, K.K., Lee, C.K., Lee, K.L., Yan, W.W., Chan, K., Chan, W.M., Ngai, C.W., Law, K.I., Chow, F.L., Liu, R., Lai, K.Y., Lau, C.C., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2013. Hyperimmune intravenous immunoglobulin treatment: a multicentre double-blind randomized controlled trial for patients with severe A(H1N1) pdm09 infection. Chest 144, 464–473.

Hung, I.F., To, K.K., Chan, J.F., Cheng, V.C., Liu, K.S., Tam, A., Chan, T.C., Zhang, A.J., Li, P., Wong, T.L., Zhang, R., Cheung, M.K., Leung, W., Lau, J.Y., Fok, M., Chen, H., Chan, K.H., Yuen, K.Y., 2017. Efficacy of clarithromycin-naproxen-oseltamivir combination in the treatment of patients hospitalized for influenza A(H3N2) infection: an open-label randomized, controlled, phase IIb/III trial. Chest 151, 1069–1080.

Ilyushina, N.A., Donnelly, R.P., 2014. In vitro anti-influenza A activity of interferon (IFN)-λ1 combined with IFN-β or oseltamivir carboxylate. Antivir. Res. 111, 112–120.

Ishii, H., Komiya, K., Yamagata, E., Yatera, K., Chojin, Y., Yamamoto, H., Mukae, H., Kadota, J., 2012. Clarithromycin has limited effects in non-elderly, non-severe patients with seasonal influenza virus A infection. J. Infect. 64, 343–345.

Jefferies, S., Braithwaite, I., Walker, S., Weatherall, M., Jennings, L., Luck, M., Barrett, K., Siebers, R., Blackmore, T., Beasley, R., Perrin, K., Pi study group, 2016. Randomized controlled trial of the effect of regular paracetamol on influenza infection. Respirology 21, 370–377.

Jain, M.K., Ridker, P.M., 2005. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat. Rev. Drug Discov. 4, 977–987.

Jiang, H., Wu, P., Uyeki, T.M., He, J., Deng, Z., Xu, W., Lv, Q., Zhang, J., Wu, Y., Tsang, T.K., Kang, M., Zheng, J., Wang, L., Yang, B., Qin, Y., Feng, L., Fang, V.J., Gao, G.F., Leung, G.M., Yu, H., Cowling, B.J., 2017. Preliminary epidemiologic assessment of human infections with highly pathogenic avian influenza A(H5N6) virus, China. Clin. Infect. Dis. 65, 383–388.

Jiang, L., Deng, L., Wu, T., 2013. Chinese medicinal herbs for influenza. Cochrane Database Syst Rev. 3 CD004559.

Kakeya, H., Seki, M., Izumikawa, K., Kosai, K., Morinaga, Y., Kurihara, S., Nakamura, S., Imamura, Y., Miyazaki, T., Tsukamoto, M., Yanagihara, K., Tashiro, T., Kohno, S., 2014. Efficacy of combination therapy with oseltamivir phosphate and azithromycin for influenza: a multicenter, open-label, randomized study. PLoS One 9, e91293.

Kanoh, S., Rubin, B.K., 2010. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin. Microbiol. Rev. 23, 590–615.

Kim, S.H., Hong, S.B., Yun, S.C., Choi, W.I., Ahn, J.J., Lee, Y.J., Lee, H.B., Lim, C.M., Koh, Y., Korean Society of Critical Care Medicine H1N1 Collaborative, 2011. Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. Am. J. Respir. Crit. Care Med. 183, 1207–1214.

Kubota-Koketsu, R., Yunoki, M., Okuno, Y., Ikuta, K., 2012. Significant neutralizing activities against H2N2 influenza A viruses in human intravenous immunoglobulin lots manufactured from 1993 to 2010. Biologics 6, 245–247.

Kudo, K., Takasaki, J., Manabe, T., Uryu, H., Yamada, R., Kuroda, E., Kobayashi, N., Matsushita, T., 2012a. Systemic corticosteroids and early administration of antiviral

agents for pneumonia with acute wheezing due to influenza A(H1N1)pdm09 in Japan. PLoS One 7, e32280.

Kudo, K., Binh, N.G., Manabe, T., Co, D.X., Tuan, N.D., Izumi, S., Takasaki, J., Minh, D.H., Thuy, P.T., Van, V.T., Hanh, T.T., Chau, N.Q., 2012b. Clinical preparedness for severe pneumonia with highly pathogenic avian influenza A (H5N1): experiences with cases in Vietnam. Respir Investig 50, 140–150.

Kwong, J.C., Li, P., Redelmeier, D.A., 2009. Influenza morbidity and mortality in elderly patients receiving statins: a cohort study. PLoS One 4, e8087.

Lai, K.Y., Ng, W.Y., Osburga Chan, P.K., Wong, K.F., Cheng, F., 2010. High-dose N-acetylcysteine therapy for novel H1N1 influenza pneumonia. Ann. Intern. Med. 152, 687–688.

Laidler, M.R., Thomas, A., Baumbach, J., Kirley, P.D., Meek, J., Aragon, D., Morin, C., Ryan, P.A., Schaffner, W., Zansky, S.M., Chaves, S.S., 2015. Statin treatment and mortality: propensity score-matched analyses of 2007-2008 and 2009-2010 laboratory-confirmed influenza hospitalizations. Open Forum Infect. Dis. 2 (1) ofv028.

Lat, A., Bhadelia, N., Miko, B., Furuya, E.Y., Thompson 3rd, G.R., 2010. Invasive aspergillosis after pandemic (H1N1) 2009. Emerg. Infect. Dis. 16, 971–973.

Lee, N., Chan, P.K., Hui, D.S., Rainer, T.H., Wong, E., Choi, K.W., Lui, G.C., Wong, B.C., Wong, R.Y., Lam, W.Y., Chu, I.M., Lai, R.W., Cockram, C.S., Sung, J.J., 2009. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J. Infect. Dis. 200, 492–500.

Lee, N., Choi, K.W., Chan, P.K., Hui, D.S., Lui, G.C., Wong, B.C., Wong, R.Y., Sin, W.Y., Hui, W.M., Ngai, K.L., Cockram, C.S., Lai, R.W., Sung, J.J., 2010. Outcomes of adults hospitalized with severe influenza. Thorax 65, 510–515.

Lee, N., Chan, P.K., Wong, C.K., Wong, K.T., Choi, K.W., Joynt, G.M., Lam, P., Chan, M.C., Wong, B.C., Lui, G.C., Sin, W.W., Wong, R.Y., Lam, W.Y., Yeung, A.C., Leung, T.F., So, H.Y., Yu, A.W., Sung, J.J., Hui, D.S., 2011a. Viral clearance and inflammatory response patterns in adults hospitalized for pandemic 2009 influenza A(H1N1)Virus pneumonia. Antivir. Ther. 16, 237–247.

Lee, N., Chan, P.K., Lui, G.C., Wong, B.C., Sin, W.W., Choi, K.W., Wong, R.Y., Lee, E.L., Yeung, A.C., Ngai, K.L., Chan, M.C., Lai, R.W., Yu, A.W., Hui, D.S., 2011b. Complications and outcomes of pandemic 2009 influenza a (H1N1) in hospitalized adults – how are they different from seasonal influenza? J. Infect. Dis. 203, 1739–1747.

Lee, N., Leo, Y.S., Cao, B., Chan, P.K., Kyaw, W.M., Uyeki, T.M., Tam, W.W., Cheung, C.S., Yung, I.M., Li, H., Gu, L., Liu, Y., Liu, Z., Qu, J., Hui, D.S., 2015. Neuraminidase inhibitors, superinfection and corticosteroids affect survival of influenza patients. Eur. Respir. J. 45, 1642–1652.

Lee, N., Wong, C.K., Chan, M.C., Yeung, E.S., Tam, W.W., Tsang, O.T., Choi, K.W., Chan, P.K., Kwok, A., Lui, G.C., Leung, W.S., Yung, I.M., Wong, R.Y., Cheung, C.S., Hui, D.S., 2017. Anti-inflammatory effects of adjunctive macrolide treatment in adults hospitalized with influenza: a randomized controlled trial. Antivir. Res. 144, 48–56.

Lee, S.M., Gai, W.W., Cheung, T.K., Peiris, J.S., 2011c. Antiviral effect of a selective COX-2 inhibitor on H5N1 infection in vitro. Antivir. Res. 91, 330–334.

Lejal, N., Tarus, B., Bouguyon, E., Chenavas, S., Bertho, N., Delmas, B., Ruigrok, R.W., Di Primo, C., Slama-Schwok, A., 2013. Structure-based discovery of the novel antiviral properties of naproxen against the nucleoprotein of influenza A virus. Antimicrob. Agents Chemother. 57, 2231–2242.

Li, C., Zhang, A.J., To, K.K., Lee, A.C., Zhu, H., Wu, H.W., Chan, J.F., Chen, H., Hung, I.F., Li, L., Yuen, K.Y., 2014. Avian influenza A H7N9 virus induces severe pneumonia in mice without prior adaptation and responds to a combination of zanamivir and COX-2 inhibitor. PLoS One 9, e107966.

Li, H., Yang, S.G., Gu, L., Zhang, Y., Yan, X.X., Liang, Z.A., Zhang, W., Jia, H.Y., Chen, W., Liu, M., Yu, K.J., Xue, C.X., Hu, K., Zou, Q., Li, L.J., Cao, B., Wang, C., National influenza A(H1N1)pdm09 clinical investigation group of China, 2017. Effect of low-to-moderate-dose corticosteroids on mortality of hospitalized adolescents and adults with influenza A(H1N1)pdm09 viral pneumonia. Influenza. Respir Viruses 11, 345–354.

Li, Y., Xu, J., Shi, W., Chen, C., Shao, Y., Zhu, L., Lu, W., Han, X., 2016. Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. Stem Cell Res. Ther. 7, 159.

Lim, W.S., Brittain, C., Duley, L., Edwards, S., Gordon, S., Montgomery, A., Nguyen-Van-Tam, J., Read, R., Whitham, D., Whynes, D., Woodhead, M., Wootton, D., 2015. Blinded randomised controlled trial of low-dose Adjuvant Steroids in Adults admitted to hospital with Pandemic influenza (ASAP): a trial 'in hibernation', ready for rapid activation. Health Technol. Assess. 19, 1–78 vii-viii.

Linko, R., Pettilä, V., Ruokonen, E., Varpula, T., Karlsson, S., Tenhunen, J., Reinikainen, M., Saarinen, K., Perttilä, J., Parviainen, I., Ala-Kokko, T., FINNH1N1-Study group, 2011. Corticosteroid therapy in intensive care unit patients with PCR-confirmed influenza A(H1N1) infection in Finland. Acta Anaesthesiol. Scand. 55, 971–979.

Liu, Z., Guo, Z., Wang, G., Zhang, D., He, H., Li, G., Liu, Y., Higgins, D., Walsh, A., Shanahan-Prendergast, L., Lu, J., 2009. Evaluation of the efficacy and safety of a statin/caffeine combination against H5N1, H3N2 and H1N1 virus infection in BALB/c mice. Eur. J. Pharmaceut. Sci. 38, 215–223.

Louie, J.K., Yang, S., Acosta, M., Yen, C., Samuel, M.C., Schechter, R., Guevara, H., Uyeki, T.M., 2012. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. Clin. Infect. Dis. 55, 1198–1204.

Lu, J., Guo, Z., Pan, X., Wang, G., Zhang, D., Li, Y., Tan, B., Ouyang, L., Yu, X., 2006. Passive immunotherapy for influenza A H5N1 virus infection with equine hyperimmune globulin F(ab')2 in mice. Respir. Res. 7, 43.

Luke, T., Kilbane, E., Jackson, J., Hoffman, S., 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Ann. Intern. Med. 145, 599–609.

Martin-Loeches, I., Lisboa, T., Rhodes, A., Moreno, R.P., Silva, E., Sprung, C., Chiche, J.D., Barahona, D., Villabon, M., Balasini, C., Pearse, R.M., Matos, R., Rello, J., ESICM H1N1 Registry Contributors, 2011. Use of early corticosteroid therapy on ICU

admission in patients affected by severe pandemic (H1N1)v influenza A infection. Intensive Care Med. 37, 272–283.

Martín-Loeches, I., Bermejo-Martin, J.F., Vallés, J., Granada, R., Vidaur, L., Vergara-Serrano, J.C., Martín, M., Figueira, J.C., Sirvent, J.M., Blanquer, J., Suarez, D., Artigas, A., Torres, A., Diaz, E., Rodriguez, A., Semicyuc/REIPI/CIBERES H1N1 working group, 2013. Macrolide-based regimens in absence of bacterial co-infection in critically ill H1N1 patients with primary viral pneumonia. Intensive Care Med. 39, 693–702.

Mata, M., Morcillo, E., Gimeno, C., Cortijo, J., 2011. N-acetyl-L-cysteine (NAC) inhibit mucin synthesis and pro-inflammatory mediators in alveolar type II epithelial cells infected with influenza virus A and B and with respiratory syncytial virus (RSV). Biochem. Pharmacol. 82, 548–555.

Miyamoto, D., Hasegawa, S., Sriwilaijaroen, N., Yingsakmongkon, S., Hiramatsu, H., Takahashi, T., Hidari, K., Guo, C.T., Sakano, Y., Suzuki, T., Suzuki, Y., 2008. Clarithromycin inhibits progeny virus production from human influenza virus-infected host cells. Biol. Pharmaceut. Bull. 31, 217–222.

Min, J.Y., Jang, Y.J., 2012. Macrolide therapy in respiratory viral infections. Mediat. Inflamm. 2012, 649570.

Mo, Y., Fisher, D., 2016. A review of treatment modalities for Middle East Respiratory Syndrome. J. Antimicrob. Chemother. 71, 3340–3350.

Moes, D.J., Guchelaar, H.J., de Fijter, J.W., 2015. Sirolimus and everolimus in kidney transplantation. Drug Discov. Today 20, 1243–1249.

Morrison, T.J., Jackson, M.V., Cunningham, E.K., Kissenpfennig, A., McAuley, D.F., O'Kane, C.M., Krasnodembskaya, A.D., 2017. Mesenchymal stromal cells modulate macrophages in clinically relevant lung injury models by extracellular vesicle mitochondrial transfer. Am. J. Respir. Crit. Care Med. 196, 1275–1286.

Murray, J.L., McDonald, N.J., Sheng, J., Shaw, M.W., Hodge, T.W., Rubin, D.H., O'Brien, W.A., Smee, D.F., 2012. Inhibition of influenza A virus replication by antagonism of a PI3K-AKT-mTOR pathway member identified by gene-trap insertional mutagenesis. Antivir. Chem. Chemother. 22, 205–215.

Muthuri, S.G., Venkatesan, S., Myles, P.R., et al., 2014. 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 2, 395–404.

National Heart, Lung, and Blood Institute ARDS Clinical Trials Network,Truwit, J.D., Bernard, G.R., Steingrub, J., Matthay, M.A., Liu, K.D., Albertson, T.E., Brower, R.G., Shanholtz, C., Rock, P., Douglas, I.S., deBoisblanc, B.P., Hough, C.L., Hite, R.D., Thompson, B.T., 2014. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. N. Engl. J. Med. 370, 2191–2200.

Onodera, H., Urayama, T., Hirota, K., Maeda, K., Kubota-Koketsu, R., Takahashi, K., Hagiwara, K., Okuno, Y., Ikuta, K., Yunoki, M., 2017. Neutralizing activities against seasonal influenza viruses in human intravenous immunoglobulin. Biologics 11, 23–30.

Ooi, E.E., Chew, J.S., Loh, J.P., Chua, R.C., 2006. _In vitro_ inhibition of human influenza A virus replication by chloroquine. Virol. J. 3, 39.

Papazian, L., Roch, A., Charles, P.E., Penot-Ragon, C., Perrin, G., Roulier, P., Goutorbe, P., Lefrant, J.Y., Wiramus, S., Jung, B., Perbet, S., Hernu, R., Nau, A., Baldesi, O., Allardet-Servent, J., Baumstarck, K., Jouve, E., Moussa, M., Hraiech, S., Guervilly, C., Forel, J.M., STATIN-VAP study group, 2013. Effect of statin therapy on mortality in patients with ventilator-associated pneumonia: a randomized clinical trial. J. Am. Med. Assoc. 310, 1692–1700.

Parnham, M.J., Erakovic Haber, V., Giamarellos-Bourboulis, E.J., Perletti, G., Verleden, G.M., Vos, R., 2014. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacol Ther. 143, 225–245.

Patel, P., Nadwani, V., Vanchiere, J., Conrad, S.A., Scott, L.K., 2011. Use of therapeutic plasma exchange as a rescue therapy in 2009 pH1N1 influenza-An associated respiratory failure and hemodynamic shock. Pediatr. Crit. Care Med. 12, e87–e89.

Paton, N.I., Lee, L., Xu, Y., Ooi, E.E., Cheung, Y.B., Archuleta, S., Wong, G., Wilder-Smith, A., 2011. Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial. Lancet Infect. Dis. 11, 677–683.

Peiris, J.S., Yu, W.C., Leung, C.W., Cheung, C.Y., Ng, W.F., Nicholls, J.M., Ng, T.K., Chan, K.H., Lai, S.T., Lim, W.L., Yuen, K.Y., Guan, Y., 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet 363, 617–619.

Powell, D., Bowler, C., Roberts, T., Garton, M., Matthews, C., McCall, I., Davie, M., 2012. Incidence of serious side effects with intravenous bisphosphonate: a clinical audit. QJM 105, 965–971.

Qin, G., Liu, Y., Zheng, J., Xiang, Z., Ng, I.H., Malik Peiris, J.S., Lau, Y.L., Tu, W., 2012. Phenotypic and functional characterization of human γδ T-cell subsets in response to influenza A viruses. J. Infect. Dis. 205, 1646–1653.

Radigan, K.A., Urich, D., Misharin, A.V., Chiarella, S.E., Soberanes, S., Gonzalez, A., Perlman, H., Wunderink, R.G., Budinger, G.R., Mutlu, G.M., 2012. The effect of rosuvastatin in a murine model of influenza A infection. PLoS One 7, e35788.

Ray, W.A., Murray, K.T., Hall, K., Arbogast, P.G., Stein, C.M., 2012. Azithromycin and the risk of cardiovascular death. N. Engl. J. Med. 366, 1881–1890.

Robbins, C.S., Bauer, C.M., Vujicic, N., Gaschler, G.J., Lichty, B.D., Brown, E.G., Stämpfli, M.R., 2006. Cigarette smoke impacts immune inflammatory responses to influenza in mice. Am. J. Respir. Crit. Care Med. 174, 1342–1351.

Rockman, S., Lowther, S., Camuglia, S., Vandenberg, K., Taylor, S., Fabri, L., Miescher, S., Pearse, M., Middleton, D., Kent, S.J., Maher, D., 2017. Intravenous immunoglobulin protects against severe pandemic influenza infection. EBio Med. 19, 119–127.

Rodrigo, C., Leonardi-Bee, J., Nguyen-Van-Tam, J., Lim, W.S., 2016. Corticosteroids as adjunctive therapy in the treatment of influenza. Cochrane Database Syst. Rev. 3, CD010406.

Ronco, C., Klein, D.J., 2014. Polymyxin B hemoperfusion: a mechanistic perspective. Crit. Care 18, 309.

Rossignol, J.F., La Frazia, S., Chiappa, L., Ciucci, A., Santoro, M.G., 2009. Thiazolides, a new class of anti-influenza molecules targeting viral hemagglutinin at the post-translational level. J. Biol. Chem. 284, 29798–29808.

Rossignol, J.F., 2014. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. Antivir. Res. 110, 94–103.

Saxton, R.A., Sabatini, D.M., 2017. mTOR signaling in growth, metabolism, and disease. Cell 169, 361–371.

Schwab, I., Nimmerjahn, F., 2013. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat. Rev. Immunol. 13, 176–189.

Siris, E.S., Lyles, K.W., Singer, F.R., Meunier, P.J., 2006. Medical management of Paget's disease of bone: indications for treatment and review of current therapies. J. Bone Miner. Res. 21 (2), 94–98.

Soo, Y.O., Cheng, Y., Wong, R., Hui, D.S., Lee, C.K., Tsang, K.K., Ng, M.H., Chan, P., Cheng, G., Sung, J.J., 2004. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. Clin. Microbiol. Infect. 10, 676–678.

Sun, S., Zhao, G., Liu, C., Fan, W., Zhou, X., Zeng, L., Guo, Y., Kou, Z., Yu, H., Li, J., Wang, R., Li, Y., Schneider, C., Habel, M., Riedemann, N.C., Du, L., Jiang, S., Guo, R., Zhou, Y., 2015. Treatment with anti-C5a antibody improves the outcome of H7N9 virus infection in African green monkeys. Clin. Infect. Dis. 60, 586–595.

Takeda, S., Munakata, R., Abe, S., Mii, S., Suzuki, M., Kashiwada, T., Azuma, A., Yamamoto, T., Gemma, A., Tanaka, K., 2010. Hypercytokinemia with 2009 pandemic H1N1 (pH1N1) influenza successfully treated with polymyxin B-immobilized fiber column hemoperfusion. Intensive Care Med. 36, 906–907.

Tanvetyanon, T., Stiff, P.J., 2006. Management of the adverse effects associated with intravenous bisphosphonates. Ann. Oncol. 17, 897–907.

Tarrant, B.J., Le Maitre, C., Romero, L., Steward, R., Button, B.M., Thompson, B.R., Holland, A.E., 2017. Mucoactive agents for chronic, non-cystic fibrosis lung disease: a systematic review and meta-analysis. Respirology 22, 1084–1092.

Tian, X., Jiang, Z., Ma, Q., Liu, Q., Lu, X., Liu, W., Liao, X., Zhou, R., Su, X., Luo, Q., 2016. Prevalence of neutralizing antibodies to common respiratory viruses in intravenous immunoglobulin and in healthy donors in southern China. J. Thorac. Dis. 8, 803–812.

To, K.F., Chan, P.K., Chan, K.F., Lee, W.K., Lam, W.Y., Wong, K.F., Tang, N.L., Tsang, D.N., Sung, R.Y., Buckley, T.A., Tam, J.S., Cheng, A.F., 2001. Pathology of fatal human infection associated with avian influenza A H5N1 virus. J. Med. Virol. 63, 242–246.

To, K.K., Hung, I.F., Li, I.W., Lee, K.L., Koo, C.K., Yan, W.W., Liu, R., Ho, K.Y., Chu, K.H., Watt, C.L., Luk, W.K., Lai, K.Y., Chow, F.L., Mok, T., Buckley, T., Chan, J.F., Wong, S.S., Zheng, B., Chen, H., Lau, C.C., Tse, H., Cheng, V.C., Chan, K.H., Yuen, K.Y., 2010. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin. Infect. Dis. 50, 850–859.

To, K.K., Mok, K.Y., Chan, A.S., Cheung, N.N., Wang, P., Lui, Y.M., Chan, J.F., Chen, H., Chan, K.H., Kao, R.Y., Yuen, K.Y., 2016. Mycophenolic acid, an immunomodulator, has potent and broad-spectrum _in vitro_ antiviral activity against pandemic, seasonal and avian influenza viruses affecting humans. J. Gen. Virol. 97, 1807–1817.

Trombetta, C., Piccirella, S., Perini, D., Kistner, O., Montomoli, E., 2015. Emerging influenza strains in the last two decades: a threat of a new pandemic? Vaccines 3, 172–185.

Tu, W., Zheng, J., Liu, Y., Sia, S.F., Liu, M., Qin, G., Ng, I.H., Xiang, Z., Lam, K.T., Peiris, J.S., Lau, Y.L., 2011. The aminobisphosphonate pamidronate controls influenza pathogenesis by expanding a gammadelta T cell population in humanized mice. J. Exp. Med. 208, 1511–1522.

van Griensven, J., Edwards, T., de Lamballerie, X., Semple, M.G., Gallian, P., Baize, S., Horby, P.W., Raoul, H., Magassouba, N., Antierens, A., Lomas, C., Faye, O., Sall, A.A., Fransen, K., Buyze, J., Ravinetto, R., Tiberghien, P., Claeys, Y., De Crop, M., Lynen, L., Bah, E.I., Smith, P.G., Delamou, A., De Weggheleire, A., Haba, N., Ebola-Tx Consortium, 2016. Evaluation of convalescent plasma for Ebola virus disease in Guinea. N. Engl. J. Med. 374, 33–42.

Vandermeer, M.L., Thomas, A.R., Kamimoto, L., Reingold, A., Gershman, K., Meek, J., Farley, M.M., Ryan, P., Lynfield, R., Baumbach, J., Schaffner, W., Bennett, N., Zansky, S., 2012. Association between use of statins and mortality among patients hospitalized with laboratory-confirmed influenza virus infections: a multistate study. J. Infect. Dis. 205, 13–19.

Vigerust, D.J., McCullers, J.A., 2007. Chloroquine is effective against influenza A virus _in vitro_ but not _in vivo_. Influenza. Respir Viruses 1, 189–192.

Villarroel, M.C., Hidalgo, M., Jimeno, A., 2009. Mycophenolate mofetil: an update. Drugs Today 45, 521–532.

Volkmann, E.R., Tashkin, D.P., Li, N., Roth, M.D., Khanna, D., Hoffmann-Vold, A.M., Kim, G., Goldin, J., Clements, P.J., Furst, D.E., Elashoff, R.M., 2017. Mycophenolate mofetil versus placebo for systemic sclerosis-related interstitial lung disease: an analysis of scleroderma lung studies I and II. Arthritis Rheumatol. 69, 1451–1460.

Waljee, A.K., Rogers, M.A., Lin, P., Singal, A.G., Stein, J.D., Marks, R.M., Ayanian, J.Z., Nallamothu, B.K., 2017. Short term use of oral corticosteroids and related harms among adults in the United States: population based cohort study. BMJ 357, j1415.

Wang, C., Cao, B., Liu, Q.Q., Zou, Z.Q., Liang, Z.A., Gu, L., Dong, J.P., Liang, L.R., Li, X.W., Hu, K., He, X.S., Sun, Y.H., An, Y., Yang, T., Cao, Z.X., Guo, Y.M., Wen, X.M., Wang, Y.G., Liu, Y.L., Jiang, L.D., 2011. Oseltamivir compared with the Chinese traditional therapy maxingshigan-yinqiaosan in the treatment of H1N1 influenza: a randomized trial. Ann. Intern. Med. 155, 217–225.

Wang, C.H., Chung, F.T., Lin, S.M., Huang, S.Y., Chou, C.L., Lee, K.Y., Lin, T.Y., Kuo, H.P., 2014. Adjuvant 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. 42, 313–321.

Wei, S.H., Yang, J.R., Wu, H.S., Chang, M.C., Lin, J.S., Lin, C.Y., Liu, Y.L., Lo, Y.C., Yang, C.H., Chuang, J.H., Lin, M.C., Chung, W.C., Liao, C.H., Lee, M.S., Huang, W.T., Chen, P.J., Liu, M.T., Chang, F.Y., 2013. Human infection with avian influenza A H6N1 virus: an epidemiological analysis. Lancet Respir Med. 1, 771–778.

Weitz-Schmidt, G., Welzenbach, K., Brinkmann, V., Kamata, T., Kallen, J., Bruns, C., Cottens, S., Takada, Y., Hommel, U., 2001. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat. Med. 7, 687–692.

Wen, Y.C., Hsiao, F.Y., Chan, K.A., Lin, Z.F., Shen, L.J., Fang, C.C., 2017. Acute respiratory infection and use of nonsteroidal anti-inflammatory drugs on risk of acute myocardial infarction: a nationwide case-crossover study. J. Infect. Dis. 215, 503–509.

World Health Organization, 2010. WHO Guidelines for Pharmacological Management of Pandemic (H1N1) 2009 Influenza and Other Influenza Viruses. Available at: http://www.who.int/csr/resources/publications/swineflu/h1n1_use_antivirals_20090820/en/, Accessed date: 13 December 2017.

World Health Organization, 2016. Influenza (Seasonal) Fact Sheet. Available at: http://www.who.int/mediacentre/factsheets/fs211/en/, Accessed date: 23 October 2017.

Wu, X.X., Gao, H.N., Wu, H.B., Peng, X.M., Ou, H.L., Li, L.J., 2015. Successful treatment of avian-origin influenza A (H7N9) infection using convalescent plasma. Int. J. Infect. Dis. 41, 3–5.

Yamaya, M., Shinya, K., Hatachi, Y., Kubo, H., Asada, M., Yasuda, H., Nishimura, H., Nagatomi, R., 2010. Clarithromycin inhibits type a seasonal influenza virus infection in human airway epithelial cells. J. Pharmacol. Exp. Ther. 333, 81–90.

Yan, Y., Zou, Z., Sun, Y., Li, X., Xu, K.F., Wei, Y., Jin, N., Jiang, C., 2013. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell Res. 23, 300–302.

Young, P., Saxena, M., Bellomo, R., Freebairn, R., Hammond, N., van Haren, F., Holliday, M., Henderson, S., Mackle, D., McArthur, C., McGuinness, S., Myburgh, J., Weatherall, M., Webb, S., Beasley, R., HEAT investigators; Australian and New Zealand intensive care society clinical trials group, 2015. Acetaminophen for fever in critically ill patients with suspected infection. N. Engl. J. Med. 373, 2215–2224.

Zarogoulidis, P., Papanas, N., Kioumis, I., Chatzaki, E., Maltezos, E., Zarogoulidis, K., 2012. Macrolides: from *in vitro* anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. Eur. J. Clin. Pharmacol. 68, 479–503.

Zhang, R.H., Li, C.H., Wang, C.L., Xu, M.J., Xu, T., Wei, D., Liu, B.J., Wang, G.H., Tian, S.F., 2014. N-acetyl-l-cystine (NAC) protects against H9N2 swine influenza virus-induced acute lung injury. Int Immunopharmacol 22, 1–8.

Zheng, B.J., Chan, K.W., Lin, Y.P., Zhao, G.Y., Chan, C., Zhang, H.J., Chen, H.L., Wong, S.S., Lau, S.K., Woo, P.C., Chan, K.H., Jin, D.Y., Yuen, K.Y., 2008. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. Proc Natl Acad Sci U S A 105, 8091–8096.

Zheng, J., Wu, W.L., Liu, Y., Xiang, Z., Liu, M., Chan, K.H., Lau, S.Y., Lam, K.T., To, K.K., Chan, J.F., Li, L., Chen, H., Lau, Y.L., Yuen, K.Y., Tu, W., 2015. The therapeutic effect of pamidronate on lethal avian influenza a H7N9 virus infected humanized mice. PLoS One 10, e0135999.

Zhou, B., Zhong, N., Guan, Y., 2007. Treatment with convalescent plasma for influenza A (H5N1) infection. N. Engl. J. Med. 357, 1450–1451.

Zhou, J., Wang, D., Gao, R., Zhao, B., Song, J., Qi, X., Zhang, Y., Shi, Y., Yang, L., Zhu, W., Bai, T., Qin, K., Lan, Y., Zou, S., Guo, J., Dong, J., Dong, L., Zhang, Y., Wei, H., Li, X., Lu, J., Liu, L., Zhao, X., Li, X., Huang, W., Wen, L., Bo, H., Xin, L., Chen, Y., Xu, C., Pei, Y., Yang, Y., Zhang, X., Wang, S., Feng, Z., Han, J., Yang, W., Gao, G.F., Wu, G., Li, D., Wang, Y., Shu, Y., 2013. Biological features of novel avian influenza A (H7N9) virus. Nature 499, 500–503.