Antiviral Research 169 (2019) 104545

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Antivirals targeting the polymerase complex of influenza viruses

Edin J. Mifsud[a], Frederick G. Hayden[b], Aeron C. Hurt[a,c,*]

[a] WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia
[b] Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA
[c] Department of Microbiology and Immunology, University of Melbourne, Victoria, Australia

A B S T R A C T

Current influenza antivirals have limitations with regard to their effectiveness and the potential emergence of resistance. Encouragingly, several new compounds which inhibit the polymerase of influenza viruses have recently been shown to have enhanced pre-clinical and clinical effectiveness compared to the neuraminidase inhibitors, the mainstay of influenza antiviral therapy over the last two decades. In this review we focus on four compounds which inhibit polymerase function, baloxavir marboxil, favipiravir, pimodivir and AL-794 and discuss their clinical and virological effectiveness, their propensity to select for resistance and their potential for future combination therapy with the most commonly used neuraminidase inhibitor, oseltamivir.

1. Introduction

Antivirals play an important role in the treatment of influenza, but they also have limitations with regards to the time-dependent magnitude of their clinical effectiveness and the emergence of antiviral resistant viruses. In Japan, antivirals are used to treat large numbers of uncomplicated influenza cases (Sugaya, 2011), whereas in other parts of the world, they are used primarily for the treatment of high-risk outpatients or hospitalised influenza patients (Nguyen-Van-Tam et al., 2015). In addition, influenza antivirals are stockpiled by many countries for use in the event of an influenza pandemic or major outbreak of a novel strain for which vaccines are not available (Patel et al., 2017). Virus-specific vaccines are unlikely to be available until many months after the start of a pandemic, making antivirals the first line of defence in treating infected individuals and preventing infection amongst healthcare workers, first line responders, and others with a high risk of exposure.

Two classes of antivirals have been available in many countries for the treatment or prophylaxis of influenza over the last two decades, the adamantanes and the neuraminidase inhibitors (NAIs). However, new compounds targeting the influenza virus polymerase complex have recently progressed to or through late phase clinical trials. Two polymerase inhibitors (favipiravir, baloxavir marboxil) have been licensed in Japan and one (baloxavir) in the United States. In this review we examine the clinical and virological effectiveness data available for baloxavir, favipiravir, pimodivir and AL-794 and discuss their propensity to select for resistance, the consequences of resistance emergence, and the potential for combination therapy with NAIs.

2. Adamantanes and NA inhibitors

2.1. Adamantanes

Amantadine and rimantadine, which target the M2 ion channel of influenza A viruses, were the first anti-influenza compounds licensed. However these compounds have a high propensity to select for single amino acid substitutions in residues 26, 27, 30, 31 or 34 of the M2 protein resulting in reduced susceptibility or resistance (Deyde et al., 2007). In randomised controlled trials of non-hospitalised (outpatients) with influenza, treatment-emergent resistant variants were detected at high frequencies in both treated children (27%; 10/37) (Hall et al., 1987) and adults (39.4%; 26/66) (Hayden et al., 1989, Table 1). In hospitalised children under the age of 15, 80% (12/15) shed amantadine-resistant viruses following treatment (Goto et al., 2003). Importantly, the resistant variants showed no reductions in replicative fitness and transmissibility (Hay et al., 1991; Hayden et al., 1991). Younger children who shed amantadine-resistant viruses, experienced prolonged illness and secondary spikes of fever on either day 4 or 5 post-infection (Shobugawa et al., 2008). In household-based trials, the transmission of adamantane-resistant variants was associated with the failure of post-exposure rimantadine prophylaxis (Hayden et al., 1989). Adamantane-resistant A(H3N2) viruses possessing a S31N substitution in the M2 protein spread globally during the 2000's, and because of resistance in currently circulating A(H3N2) and A(H1N1)pdm09 viruses, these drugs are no longer recommended for clinical use.

* Corresponding author. WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.
E-mail address: aeron.hurt@influenzacentre.org (A.C. Hurt).

https://doi.org/10.1016/j.antiviral.2019.104545
Received 9 April 2019; Received in revised form 19 June 2019; Accepted 24 June 2019
Available online 25 June 2019

2.2. Neuraminidase inhibitors

The NAIs, the first rationally designed influenza antivirals to be developed, prevent the budding of influenza viruses from the host cell by inhibiting the enzymatic action of the neuraminidase (NA), which facilitates the release of viral progeny and spread from cell to cell (Moscona, 2005; Democratis et al., 2006; McKimm-Breschkin, 2013). There are four different NAIs that are licensed in various countries: oseltamivir, zanamivir, laninamivir, and peramivir. Oseltamivir, which was licensed in most countries in 2000, is the most commonly prescribed NAI globally and is considered the standard of care for the treatment of hospitalised influenza cases in many countries (Uyeki et al., 2018; World Health Organisation, 2018). If treatment is initiated within 36 h of symptom onset, randomised controlled trials demonstrated that oseltamivir is effective at reducing the time to alleviation of symptoms (TTAS) in uncomplicated cases of influenza by approximately 24 h. It is also effective in reducing lower respiratory tract complications leading to antibiotic use and all-cause hospitalisations (Treanor et al., 2000; Okoli et al., 2014; Dobson et al., 2015). However, data on the effectiveness of oseltamivir in severely ill or hospitalised influenza patients is limited to observational studies, and therefore potentially subject to bias and confounding. However, observational studies indicate that treatment with oseltamivir reduces the risk of pneumonia and mortality in severely ill or hospitalised influenza patients (Muthuri et al., 2014; Dobson et al., 2015; Venkatesan et al., 2017). In all settings, the greatest benefit is seen when oseltamivir is delivered as early as possible following symptom onset (Allen et al., 2006; Adisasmito et al., 2010; Muthuri et al., 2014).

The frequency of treatment emergent oseltamivir-resistant viruses is lower than that seen with the adamantanes (Table 1). In outpatients receiving oseltamivir, the frequency of resistant influenza A viruses in young children < 5 years of age (11.9%; 30/253) is higher than in older children and adults (1.4%; 13/909) (Kiso et al., 2004; Whitley et al., 2013; Lina et al., 2018). However, factors such as the virus type/subtype, immune status of the patient, and severity of illness can affect the frequency of resistance, with higher rates often observed in hospitalised children (Kiso et al., 2004), and particularly those who are immunocompromised and in whom virus replication is prolonged (Memoli et al., 2010; Zheng et al., 2010). This emphasizes the need to evaluate the potential benefits of combination therapy in reducing the rates of resistance. Currently, the frequency of oseltamivir resistance amongst circulating strains is currently quite low < 1% overall (Lackenby et al., 2018), although in some years the frequency of resistance amongst circulating A(H1N1)pdm09 viruses has exceeded 3% (Takashita et al., 2015). The most striking example of widespread oseltamivir resistance occurred in 2008 when seasonal A(H1N1) viruses with the H275Y NA amino acid substitution spread globally in the absence of selective drug pressure and replaced the oseltamivir-sensitive viruses of that subtype (Hurt et al., 2009). The fitness of H275Y variant viruses is attributed to two permissive mutations (R222Q and V234M) which occurred shortly before the wide-spread emergence viruses with the single H275Y substitution have reduced NA proteins on the cell surface. However, the addition of R222Q and V234M counteracted the detrimental fitness effect of the H275Y substitution (Bloom et al., 2010). *In vitro*, the H275Y variant viruses had a > 1000-fold decrease in oseltamivir sensitivity compared to the wildtype virus, and were clinically resistant to oseltamivir in observational studies from Japan (Kawamura et al., 2009; Saito et al., 2010). Many NA substitutions conferring reduced or highly reduced susceptibility to oseltamivir and sometimes other NAIs have been described (Hurt et al., 2016; Lackenby et al., 2018). But to date only influenza A(H1N1) viruses harbouring the H275Y substitution, which confers reduced susceptibility to both oseltamivir and peramivir, have circulated widely at the community level.

A small number of influenza A and B viruses with reduced peramivir, zanamivir and laninamivir sensitivity have been identified with different NA amino acid substitutions (e.g. S110F, D199G, N329K, D151G/D/N and S331R for influenza A viruses and I221V, T42A, P124T, P76S, H134Y/H, D197N, A200T, S246P, K125T, R150K and D197N for influenza B viruses), but these are rarely detected and some are cell culture derived substitutions (Lackenby et al., 2018).

3. Influenza polymerase inhibitors

The polymerase complex of influenza viruses is a heterotrimer composed of three protein subunits: polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase acidic protein (PA). Transcription and replication of the vRNA occurs in the nucleus of the infected cell, with the former initiated by a 'cap-snatching' process where the PB2 subunit binds the cap structure (7 mG) of a capped host RNA and the PA subunit subsequently cleaves the capped host RNA 10–15 nucleotides from the cap (Krug et al., 1979; Fodor et al., 1994). The resultant capped-oligonucleotides act as primers to copy the virus template (Krug et al., 1979). The RNA-dependent RNA polymerase, PB1, is responsible for RNA elongation (Lamb and Choppin, 1983; Su et al., 2010; Stevaert and Naesens, 2016; Krammer et al., 2018). Viral RNAs are imported to the nucleus where they are transcribed to mRNA. Replication occurs via a positive-sense complementary ribonucleoprotein cRNP intermediate. Transcription ceases when the polymerase complex reaches an oligo-U tract located upstream of the 5′ terminus of the un-transcribed vRNA resulting in a polyadenylation signal (Poon et al., 1999). The influenza polymerase complex is highly conserved and plays a pivotal role in the replication of the virus (Stevaert and

Table 1
Comparison of frequency of resistance following treatment of outpatients with different influenza antivirals.

Antiviral compound	Age group	Predominant influenza virus	Frequency of mutation	Amino acid substitutions	Reference
Rimantadine	1–15 years	Influenza A virus	27% (10/37)	M2/S31N	Hall et al. (1987)
	17-75-years	Influenza A virus	39.3% (26/66)	M2/S31N	Hayden et al. (1989)
Oseltamivir	1-5-years	A(H1N1)pdm09	16.1% (20/124)	NA/H275Y	Lina et al. (2018)
		A(H3N2)	7.7% (10/129)	NA/R292K	Lina et al. (2018)
		Influenza Bvirus	0% (0/40)		Whitley et al. (2013)
	> 5-years	A(H1N1)pdm09	1.7% (7/403)	NA/H275Y	Lina et al. (2018)
		A(H3N2)	1.2% (6/506)	NA/R292K	Lina et al. (2018)
		Influenza B virus	0% (0/178)		Whitley et al. (2013)
Baloxavir	< 12-years	A(H1N1)pdm09	0% (0/2)		Omoto et al. (2018)
		A(H3N2)	19.5% (15/77)	PA/I38T, PA/I38M	Omoto et al. (2018)
		Influenza B virus	0% (0/6)		Omoto et al. (2018)
	> 12-years	A(H1N1)pdm09	3.6% (4/112)	PA/I38T, PA/I38F	Uehara et al. (2019)
		A(H3N2)	10.9% (36/330)	PA/I38T	Uehara et al. (2019)
		Influenza B virus	0.8% (1/131)	PA/I38T	Uehara et al. (2019)
Pimodivir	18-65-years	Influenza A virus	6.4% (11/172)	PB2/S324 K/N/R, PB2/F325L, PB2/S337P PB2/K376 N/R, PB2/T378S, PB2/N510T	Finberg et al. (2018)

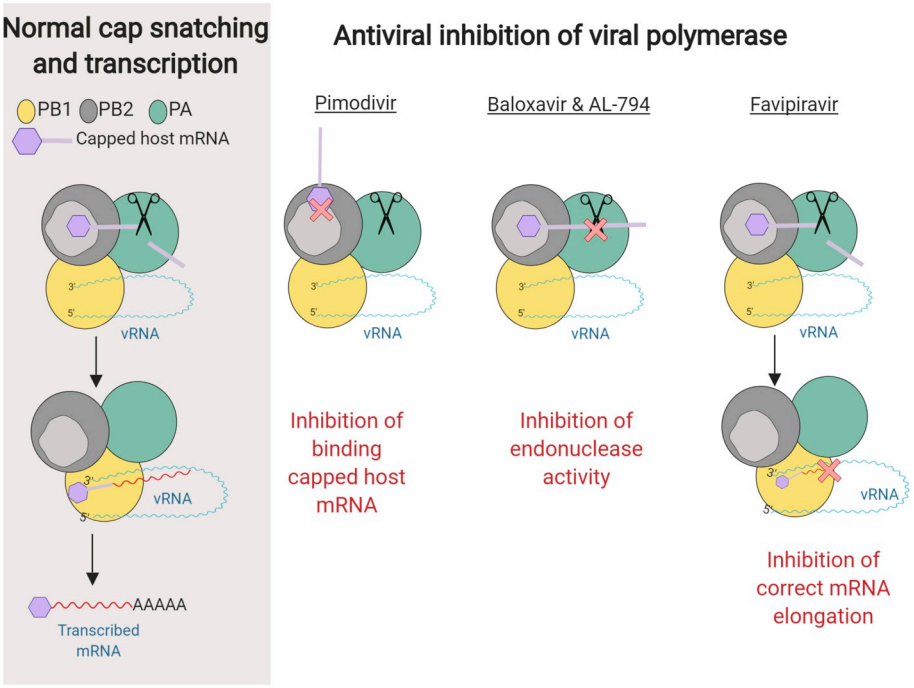

Fig. 1. Inhibition of the polymerase complex by influenza antivirals. Transcription of viral mRNA in cells infected with the influenza virus occurs in the nucleus. Capped 5′ host mRNA is bound to the PB2 protein, the first 10–20 nucleotides of host mRNA are subsequently cleaved by the PA protein and used as a viral template for vRNA elongation performed by PB1. Pimodivir inhibits the binding of host mRNA to PB2, whereas baloxavir and AL-794 prevent cap-snatching by the PA endonuclease, thereby impeding vRNA synthesis. Favipiravir acts as a purine nucleoside and is recognised as an alternate substrate leading to inaccurate vRNA synthesis. Image was created using BioRender.

Naesens, 2016) and therefore was identified as an attractive target for the development of new antiviral compounds. For example, *in silico* screens have identified a range of small molecules that can inhibit PA-PB1 interactions in HEK-293 T-cells, which were effective against both influenza A and B viruses (Muratore et al., 2012). The development and action of these pre-clinical compounds are beyond the scope of this review but are extensively described in Massari et al., (2015), Makau et al., (2017) and reviewed in Stevaert and Naesens (2016).

Below we discuss the clinical and virological effectiveness of baloxavir marboxil, favipiravir, pimodivir and AL-794 (Fig. 1) and elaborate on their propensity to select for resistance and potential for combination therapy with NAIs (Table 2). The *in vitro* susceptibility profiles, pharmacokinetics, tolerability, and clinical efficacy of favipiravir, pimodivir, and baloxavir marboxil and are described in (Hayden and Shindo, 2019).

3.1. Baloxavir marboxil

Baloxavir marboxil (hereafter referred to as baloxavir) is a pro-drug that is metabolised into baloxavir acid, which directly inhibits the cap-dependent endonuclease activity of the PA protein of influenza A and B viruses, thereby inhibiting the cleavage of mRNA during the 'cap-snatching' process (Uehara et al., 2016). In 2018, oral baloxavir was the first polymerase inhibitor to be licensed for the treatment of uncomplicated influenza in Japan and the United States of America (Shionogi, 2018; US Food and Drug Administration, 2018). In Japan, baloxavir is licensed for use in both children and adults, with dosage dependent on age and weight. Individuals aged less than 12 years receive a single dose of up to 20 mg, whereas, individuals aged 12 years and over are administered a single dose of up to 80 mg depending on weight (Ministry of Health, 2018). In the US, where baloxavir is not licensed for use in children < 12 years of age, the current treatment regimen for adolescents and adults is a single, weight-based dose of either 40 or 80 mg (US Food and Drug Administration, 2018). Baloxavir acid has a long plasma retention time (half-life of 49–91 h) meaning that the drug can be delivered as a single dose in patients with uncomplicated influenza (Koshimichi et al., 2018), thereby avoiding compliance issues which can occur with antivirals like oseltamivir and inhaled zanamivir, which are administered twice daily for 5 days.

3.1.1. Safety and tolerability

In a phase I clinical trial, dose escalation from 6 mg to 80 mg of the

Table 2
Summary of polymerase inhibitor antivirals.

	Baloxavir	Favipiravir	Pimodivir	AL-794
Polymerase target	PA	PB1	PB2	PA
Influenza specificity	A, B, & C	A, B & C	A	A & B
Inhibitory for non-influenza viruses	No	Yes	No	No
Approved for influenza use	Japan, USA	Japan (but restricted)	No	No longer in development
Dosing route	Oral	Oral	Oral	Oral
Tested in combination with oseltamivir in clinical efficacy trials	In progress	In progress	Yes	No
Inhibitory for NAI resistant influenza A viruses	Yes	Yes	Yes	NA[a]
Antiviral-resistant variants identified *in vitro*	Yes	Yes	Yes	NA
Antiviral-resistant strains identified in clinical trials	Yes	No	Yes	NA
Most commonly identified amino acid substitutions in antiviral-resistant viruses	PA-I38 T/F/M, E23 K/G, A37T E119G	PB1-K229R	PB2-S324 K/N/R/I, F325L, S337P, K376 N/R, T378S, N510T M431I	N/A

[a] NA- Not applicable as the drug is no longer in development and experiments have not been performed.

Table 3
Ongoing clinical trials studying polymerase inhibitor effectiveness.

Antiviral	Current status	Future and ongoing clinical trials	Masking	Clinical trial reference number
Baloxavir	Licensed for use in uncomplicated outpatients in Japan and USA	Phase III: • Hospitalised patients	Double blind	NCT03684044
		• Pediatric patients between 1 and 12 years of age	Double blind	NCT03629184
		• Pediatric patients less than 1 year old	Double blind	NCT03653364
		• Post-exposure prophylaxis	Open labelled	JapicCTI-184180
Favipiravir	Limited licensure in Japan for use only in pandemics	Phase II: • Critically ill patients receiving current standard of care	Open labelled	NCT03394209
Pimodivir	Phase IIb	Phase III: • High risk patients	Double blind	NCT03381196
		• Hospitalised patients	Double blind	NCT03376321
AL-794	**Phase 1**	**Discontinued**		

drug was well tolerated in healthy adults, with no serious adverse events (SAEs) reported (Koshimichi et al., 2018). A total of 8 of 55 baloxavir-exposed individuals reported treatment-emergent adverse events (TEAE), which included headache, increased alanine aminotransferase, elevated eosinophil count and white blood cell count (Koshimichi et al., 2018). In phase II and III trials enrolling outpatients with uncomplicated influenza-like illness, baloxavir has been shown to be well tolerated in adults, adolescents, and in high risk patients with co-morbidities such as asthma and chronic lung disease (Hayden et al., 2018; Ison et al., 2018; Koshimichi et al., 2018). The proportions of baloxavir recipients experiencing TEAEs were similar to that of placebo recipients, although a recent update to baloxavir precautions in Japan, now states that bloody stool, epitaxis, hematuria, or other forms of bleeding may occur (Ministry of Health, 2019). An open-label phase II clinical study in influenza-infected children (aged between 6 months and 12 years of age) did not identify tolerance problems. Emesis was the most common TEAE noted (7.5%), although it typically occurred well after drug administration and at the age-related frequency reported in placebo-treated, influenza-infected children in earlier oseltamivir randomised controlled trials (Whitley et al., 2001; Silvennoinen et al., 2010; Fry et al., 2014).

3.1.2. Clinical and virological efficacy

Studies to date have examined the clinical and antiviral efficacy of baloxavir in comparison to placebo and oseltamivir treatment in outpatients with uncomplicated influenza. In a phase II clinical trial, treatment with 10, 20 or 40 mg of baloxavir reduced TTAS in predominantly A(H1N1)pdm09 infected patients by 23.5, 26.7 and 28.2 h, respectively when compared to placebo (Hayden et al., 2018). Similar results were observed in a phase III clinical study (CAPSTONE 1) involving predominantly A(H3N2) infected patients; baloxavir reduced TTAS by 26.5 h compared to placebo, but no significant difference in the TTAS was observed between oseltamivir and baloxavir (Hayden et al., 2018). A phase III clinical trial conducted in outpatients who were at increased risk of influenza complications due to co-morbidities (CAPSTONE 2), found that treatment with baloxavir significantly reduced time to improvement of influenza symptoms (TTIIS) by 29.1 h compared to placebo treatment (Ison et al., 2018). TTIIS with baloxavir treatment was 7.8 h faster than with oseltamivir treatment, although the difference was not statistically significant (Ison et al., 2018). An additional finding from the CAPSTONE 2 study was that for patients infected with influenza B viruses, baloxavir reduced TTIIS by 25.8 and 27.0 h faster than placebo and oseltamivir, respectively (Ison et al., 2018). The findings with oseltamivir treatment align with Japanese observational studies which reported lower efficacy of oseltamivir against influenza B compared to A virus infections (Kawai et al., 2006), and highlights a clear clinical benefit in the use of baloxavir over oseltamivir for treatment of type B influenza. The comparable clinical benefit of baloxavir for both influenza A and B viruses is observed even though the baloxavir half maximal effective concentration (EC_{50}) values of influenza B viruses are approximately 5-fold higher than that of influenza A viruses (Omoto et al., 2018). In CAPSTONE-2, the use of antibiotics and the incidence of influenza-related complications in high risk patients treated with baloxavir were significantly lower than in placebo-treated patients (4.1% and 7.6% respectively) (Ison et al., 2018).

In the clinical trials discussed above, viral shedding was quantitated either by detection of infectious virus in cell culture ($TCID_{50}$) or viral RNA using real-time RT-PCR. In the CAPSTONE-1 trial, baloxavir resulted in a 1000 fold-reduction in viral titers compared to placebo at 24 h post-treatment. In addition, a 100 fold-reduction at 24 h and a 48 h faster median time to infectious virus negativity were observed in baloxavir-treated patients compared to those treated with oseltamivir, even though a difference in the TTAS was not observed between these groups (Hayden et al., 2018). The median time for sustained cessation of viral shedding was 48 h post-treatment in the baloxavir group, compared to 72 h and 96 h in the oseltamivir and placebo groups (Hayden et al., 2018).

Four additional phase III clinical trials will be conducted in 2019 to evaluate the efficacy of baloxavir treatment in children less than 1 year of age, children between 1 and 12 years of age, hospitalised patients with influenza and a Japanese study to evaluate the prophylactic benefits of baloxavir in a household setting (Table 3).

3.1.3. Antiviral resistance

To evaluate the frequency of reduced baloxavir susceptibility amongst circulating viruses, and to determine the amino acid substitutions that may be responsible for such changes, clinical specimens from patients enrolled in the clinical trials were analysed before and after treatment. The most prevalent substitutions resulting in > 10-fold reductions in baloxavir sensitivity were at position 38 of the PA protein and involved changes from isoleucine (I) in the wildtype viruses to either threonine (T), phenylalanine (F) or methionine (M). In addition, a naturally occurring substitution from I to leucine (L) at position 38 was detected in an A(H1N1)pdm09 virus through routine surveillance and was shown to confer an ~8 fold reduction in baloxavir susceptibility (Gubareva et al., 2019). In adolescents and adults, the frequency of treatment-emergent influenza B PA/I38X variant viruses was relatively low (0.8%; 1/131 Table 1) (Omoto et al., 2018), but was higher in A(H1N1)pdm09 viruses (3.6%, 4/112), and substantially higher in A(H3N2) viruses (10.9%, 36/330) (Hayden et al., 2018; Uehara et al., 2019; Omoto et al., 2018) (Table 1). Most concerning was data from a pediatric study, in which A(H3N2) viruses were predominant, that reported 19.5% (15/77) of children aged < 12 years of age shed viruses with PA/I38X substitutions following baloxavir treatment (Omoto et al., 2018). Other PA amino acid substitutions, including E23 K/G, A37T and E119G, have been identified to reduce baloxavir susceptibility, although they have been detected only at low frequencies and reduce baloxavir sensitivity by < 3-fold.

Although transient increases in viral titers were observed in adult/

adolescent patients with PA/I38X variants, there was only a short (12 h) increase in the duration of symptoms compared with baloxavir-treated patients shedding sensitive viruses, and the TTAS remained shorter than observed in the placebo-treated patients (Uehara et al., 2019). In the pediatric cohort the emergence of I38X variants was associated with a rebound in viral replication, with titers increasing from day 3 to day 6 post-treatment, and resulting in the prolongation of viral shedding, which was also observed in adults and adolescents (Uehara et al., 2019). A clinical effect was observed in children shedding I38X variants, with the median TTAS in baloxavir-treated patients with I38X variants extended to 79.6 h compared to 42.8 h in baloxavir-treated patients without variants (Uehara et al., 2019). Furthermore, the median duration of fever was longer in children shedding viruses with an I38X substitution (29.5 h) compared with those shedding baloxavir-sensitive viruses (20.7 h) (Uehara et al., 2019). However, since there was no placebo or oseltamivir control group for comparison, it is not possible to determine whether children with variant emergence had prolonged illness relative to these interventions. Viruses with the I38X substitutions were more likely to emerge in children with low antibody titer (i.e. those not previously vaccinated or infected), suggesting that the lack of pre-existing immunity may lead to more prolonged viral replication and higher viral loads that increase the risk of variant emergence (Uehara et al., 2019).

In vitro analysis examining the viral fitness of different PA/I38X variant viruses has shown slightly reduced endonuclease activity and impaired replicative fitness *in vitro* (Omoto et al., 2018). A(H3N2) viruses containing the PA/I38T substitution are the most commonly detected variant. This substitution confers the largest decrease in baloxavir susceptibility, but these viruses also appear to be the fittest of the different I38X variants based on *in vitro* assays (Jones, 2018). During the first influenza season in Japan in which baloxavir has been licensed (2018–19), one A(H1N1)pdm09 virus with mixed PA/I38 T/F substitutions and five A(H3N2) viruses with either PA/I38T or PA/I38 T/M mixed substitutions were detected in children treated with baloxavir (Takashita et al., 2019a). As of March 18, 2019, 1.6% (2/125) A(H1N1)pdm09 viruses, 22.1% (25/113) of A(H3N2) viruses and 0% (0/6) influenza B viruses were found to have reduced susceptibility to baloxavir, although the treatment status and age of patients from which these viruses were collected is not described (National Institute of Infectious Diseases, 2019). In addition a small number of untreated close contacts acquired infections with A(H3N2) viruses containing the PA/I38T substitution (Takashita et al., 2019b). In other regions of the Asia Pacific, no viruses with reduced baloxavir susceptibility were detected between 2012 and 2018 (Koszalka et al., 2019).

3.1.4. Combination therapy of baloxavir marboxil with NAIs

Drug-drug interactions between baloxavir and oseltamivir were examined in healthy subjects, where 4 of 18 patients receiving both drugs reported TEAE with nausea being the most common symptom (Kawaguchi et al., 2018). No differences in the plasma concentrations of either active metabolite (oseltamivir carboxylate and baloxavir acid) were identified between subjects receiving either monotherapy or combination therapy suggesting that there was no significant drug-drug interactions in healthy subjects (Kawaguchi et al., 2018). Studies investigating the effectiveness of oseltamivir and baloxavir combination therapy have not yet been completed in humans. However, in mice, the combination of oseltamivir and baloxavir delivered four days post-infection was able to reduce the mortality rate associated with A/PR/8 (H1N1) infection compared to either oseltamivir or baloxavir monotherapy (Fukao et al., 2019). Combination therapy was also associated with lower pro-inflammatory cytokine (IL-6 and MCP-1) levels and lung pathology when compared to either monotherapy (Fukao et al., 2019). A placebo-controlled study investigating the effectiveness of baloxavir therapy together with standard of care (which typically involves the use of a NAI) compared with standard of care alone has recently been initiated in hospitalised patients (Table 3). Further research to determine if the frequency of baloxavir-induced I38X variant viruses can be reduced by combination therapy is of significant interest.

3.2. Favipiravir

Favipiravir, formerly known as T-705, is a pyrazinecarboxamide derivative, which acts as a purine nucleoside and therefore is recognized as an alternative substrate by the viral polymerase resulting in errors during viral RNA synthesis (Furuta et al., 2005). A recent study has demonstrated that favipiravir causes mutations by primarily acting as a guanine analogue and secondarily as an adenine analogue leading to transition mutations (Goldhill et al., 2018a). Currently, favipiravir has conditional marketing approval in Japan with strict regulations for its production and clinical use, that limit its use to the treatment of novel influenza viruses (i.e. non-seasonal influenza viruses) that are resistant to other available antivirals (Furuta et al., 2017). The restricted licensure was due to concerns that favipiravir has a risk for teratogenicity and embryotoxicity (Furuta et al., 2017). Early embryonic deaths in rats and teratogenicity in monkeys, mice, rats and rabbits has been observed in animals with exposure levels similar to clinical studies (Toyama Chemical, 2018).

Favipiravir has broad-spectrum activity against other RNA viruses including West Nile virus, poliovirus, Ebola virus and norovirus (Rocha-Pereira et al., 2012; Smither et al., 2014).

3.2.1. Safety and tolerability

In a phase I clinical study, doses ranging from 30 to 1600 mg/kg were well tolerated, and no serious adverse incidents were reported (Kobayashi et al., 2008). Despite being well tolerated in the aforementioned clinical study, favipiravir treatment can result in teratogenicity and/or embryotoxicity (Furuta et al., 2017). Less severe adverse events associated with favipiravir treatment include diarrhea (6.3%), nausea (0.8%), vomiting (0.5%) and elevated uric acid levels in the blood (5.6%) (Ministry of Health, 2018).

3.2.2. Clinical and virological efficacy

The efficacy of favipiravir to reduce the TTAS has been variable across trials in patients with uncomplicated influenza. Using a dose regimen of 1800 mg BID on day 1 and 800 mg BID on days 2–5 in a phase II and two phase III trials, the median TTAS in patients was only 15.0, 14.2 and 6.1 h shorter than in placebo-treated patients (Epstein, 2017; Hayden and Shindo, 2019). Virological burden, measured by $TCID_{50}$, was approximately 10-fold lower in favipiravir-treated patients on days 2 and 3 of the study than in placebo-treated patients (Epstein, 2017). Based on the modest effect on TTAS and the teratogenicity concerns, it appears unlikely that favipiravir will be further developed for use in uncomplicated influenza patients. However, a phase II clinical trial investigating the pharmacokinetics and efficacy of favipiravir in critically ill influenza patients who are concurrently receiving standard-of-care NAI therapy is currently being conducted in China (Table 3).

3.2.3. Antiviral resistance

Viruses isolated from patients before and after treatment enrolled in clinical trials were examined for favipiravir susceptibility and amino acid substitutions which may confer reduced susceptibility. Despite the occurrence of a number of substitutions in proteins of the polymerase complex of post-treatment viruses, none had significant reductions in favipiravir susceptibility, although one PA substitution (L666F) was found to reduce polymerase activity (Takashita et al., 2016). Serial passage of influenza viruses in increasing concentrations of favipiravir has been performed in a small number of studies. One study found that favipiravir did not select for specific resistance mutations, but rather induced transversion mutations (G-A and C-T) in the NP gene resulting in non-viable viruses (Baranovich et al., 2013). Another study found that serial passage of an A(H1N1)pdm09 virus in the presence of increasing favipiravir concentrations selected for a K229R substitution in

the PB1 that conferred a 30-fold reduction in favipiravir susceptibility. Although the K229R substitution significantly reduced viral fitness, the addition of a P653L substitution in the PA gene was found to increase polymerase activity and reversed the detrimental fitness effect (Goldhill et al., 2018b). The K229R substitution was also shown to alter favipiravir susceptibility when introduced into A(H3N2) and A(H7N9) viruses demonstrating its effect across multiple subtypes (Goldhill et al., 2018b).

3.2.4. Combination therapy of favipiravir with oseltamivir

Several studies conducted in mice, have demonstrated the synergistic relationship between oseltamivir and favipiravir, highlighting the potential application of these compounds in combination. For example, the co-administration of oseltamivir (20 mg/kg/day) and favipiravir (50 mg/kg/day) to mice, 96 h after infection with a lethal dose of A(H5N1) virus, protected all animals from death, whereas either favipiravir or oseltamivir monotherapy protected only 40% (4/10) of animals (Marathe et al., 2016). Using an immunocompromised mouse model with A(H1N1)pdm09 virus infection, oseltamivir and favipiravir combination therapy significantly delayed mortality, reduced lung viral burden and significantly reduced levels of IL-10, IL-12(p70), MIP-1α and TNF-α compared to untreated mice or mice treated with oseltamivir monotherapy (Baz et al., 2018). Due to prolonged shedding of the virus in immunocompromised mice, viruses developed oseltamivir-resistance via a H275Y NA mutation, but the viruses remained sensitive to favipiravir (Baz et al., 2018). A similar study by Kiso et al., also found that in immunocompromised mice, favipiravir together with either oseltamivir or laninamivir, increased survival time compared with monotherapy, but combination therapy did not suppress the emergence of oseltamivir-resistant variants (Kiso et al., 2017).

3.3. Pimodivir

Pimodivir (JNJ-63623872; formerly VX-787) is a compound that targets the PB2 subunit and prevents the polymerase from binding the 7-methyl GTP cap structures on the host capped RNA (Clark et al., 2014) thus inhibiting viral gene transcription. Due to structural differences in the PB2 cap-binding pocket, pimodivir is only effective against influenza A viruses but not influenza B viruses (Clark et al., 2014).

3.3.1. Safety and tolerability

In a phase IIa clinical study, volunteers were experimentally infected with an A(H3N2) influenza virus and a total of 4 groups were treated with either 100 mg or 400 mg of pimodivir or they received a loading dose of 1200 mg or 900 mg followed by once daily doses of 600 mg for a total of 5 days (Trevejo et al., 2017). In the Phase IIb TOPAZ trial, which investigated antiviral efficacy in otherwise healthy patients, dose-related diarrhea (16.9%, 25/148) was the most commonly reported TEAE, with nausea (4.0%, 6/148) and vomiting (2.7%, 4/148) reported less frequently (Finberg et al., 2018).

3.3.2. Clinical and virological efficacy

In individuals experimentally infected with an A(H3N2) influenza virus, clinical symptoms were reduced by 43.0 h (1.8 days) in the 1200/600 mg group of pimodivir-treated individuals compared to placebo-treated individuals (Trevejo et al., 2017). In the TOPAZ trial there were trends for accelerated TTAS with pimodivir treatment, but there was no statistically significant differences between placebo and either 300 mg or 600 mg doses of pimodivir (Finberg et al., 2018). In both TOPAZ and another phase IIb trial OPAL, which investigated antiviral efficacy in hospitalised patients, the combination of oseltamivir and pimodivir was also examined (see below).

In the human challenge study, significant reductions in viral shedding area under the curve (AUC) and peak viral load were observed in pimodivir-treated individuals (1200/600 mg) compared with placebo, following both $TCID_{50}$ or real-time RT-PCR analysis (Trevejo et al., 2017). In the TOPAZ study, treatment with 600 mg or 300 mg pimodivir BID led to dose-dependant viral AUC reductions of −4.5 day × log10 copies/mL and −3.6 day × log10 copies/mL respectively, compared to placebo, and reduced time to virus negativity by18% and 13% for viral RNA and by 28% and 29% for infectious virus, respectively (Finberg et al., 2018).

Two phase III clinical studies investigating the effects of pimodivir in high risk (DIAMOND) or hospitalised patients (SAPPHIRE) are currently underway (Table 3). In both trials, 600 mg of pimodivir will be administered twice daily for a total of 5 days together with standard of care (which typically involves an NAI), compared to placebo with standard of care (Table 3). The primary outcomes of these trials will determine if pimodivir alters the time to resolution of influenza related symptoms or the participant's clinical status as assessed by a hospital recovery scale.

Delayed pimodivir treatment (3 or 10 mg/kg) in mice 24–96 h following infection with A/Vietnam/1203/2004 (H5N1) virus was shown to be effective at protecting from death when compared to OST, which failed to protect the animals (Byrn et al., 2015). The results from Bryn et al. suggest that delayed pimodivir treatment can potentially be utilised to combat novel or highly pathogenic influenza virus infections (Byrn et al., 2015).

3.3.3. Antiviral resistance

Following serial *in vitro* passage of A(H3N2) and A(H1N1)pdm09 viruses in the presence of increasing pimodivir concentrations, PB2 substitutions S324I/N/R and N510T were detected and found to reduce susceptibility (Clark et al., 2014; Byrn et al., 2015). In the experimental infection study, 9.7% (7/72) of volunteers shed viruses with reduced pimodivir susceptibility. Viruses with a PB2/M431I substitution were detected in four patients conferring a 57-fold decrease in drug sensitivity (Trevejo et al., 2017). Further *in vitro* examination of the variant virus revealed a reduction in replication compared to a wildtype virus (Trevejo et al., 2017). In the phase IIb TOPAZ clinical trial, the frequency of patients shedding viruses with PB2 amino acid substitutions (including S324 K/N/R, F325L, S337P, K376 N/R, T378S and N510T) which reduced pimodivir susceptibility was 6.9% (4/58) and 10.5% (6/57) in the 300 mg and 600 mg pimodivir dose groups respectively (Finberg et al., 2018).

3.3.4. Combination therapy of pimodivir with oseltamivir

Pimodivir was the first compound which inhibits the polymerase complex to be tested in combination with oseltamivir in a randomised control trial. The combination of pimodivir (600 mg) and oseltamivir (75 mg) in healthy volunteers demonstrated no clinical drug-drug interactions and the drug combinations resulted in no serious adverse incidents reported in otherwise healthy and hospitalised patients (Deleu et al., 2018; Finberg et al., 2018; Leopold, 2018). The most common TEAE in both studies was diarrhea (Deleu et al., 2018; Finberg et al., 2018; Leopold, 2018).

In otherwise healthy patients in the TOPAZ trial, treatment with both pimodivir (600 mg) and oseltamivir (75 mg) nonsignificantly reduced the TTAS by 17% when compared to placebo (Finberg et al., 2018; McKimm-Breschkin, Jiang et al., 2018). Hospitalised patients enrolled in the OPAL trial, treated with both pimodivir (600 mg) and oseltamivir (75 mg) did not show any significant differences in time to recovery when compared to those receiving oseltamivir monotherapy (Leopold, 2018). However, in a subset of patients enrolled in the same study, treatment with oseltamivir and pimodivir, within 72 h of symptom onset, led to faster viral clearance, resolution of symptoms, return to usual activities and return to usual health when compared to oseltamivir monotherapy (Leopold, 2018). Compared to patients who received placebo or pimodivir 600 mg BID monotherapy, combination therapy resulted in 8.6 day × log_{10}copies/ml and 4.1 day × log_{10}copies/ml reductions in AUC viral RNA loads, and 31% and

16% faster reductions in time to virus negativity, respectively (Finberg et al., 2018). In hospitalised patients, combination therapy did not reduce viral burden or the duration of hospital stay when compared to oseltamivir monotherapy alone (Leopold, 2018). However, in the subset of patients treated within 3 days of symptom onset, combination therapy showed trends towards improved clinical and virologic outcomes compared to oseltamivir monotherapy. Only one of the 57 outpatients (1.8%) receiving both pimodivir and oseltamivir developed an amino acid substitution altering pimodivir susceptibility (Finberg et al., 2018). No such variants were observed in hospitalised patients receiving the combination, suggesting that combination therapy may reduce the frequency of viruses with reduced pimodivir sensitivity compared to pimodivir monotherapy.

3.4. AL-794

AL-794, also known as ALS-033794/JNJ-6415580, is an ester prodrug of ALS-033719, which selectively binds to the endonuclease domain of the PA, inhibiting endonuclease activity of both influenza A and B viruses (Jekle et al., 2016). In a human challenge study, healthy individuals were infected with an A(H3N2) virus and 12 h later were treated orally with either 50 mg or 150 mg of AL-794 twice daily for 5 days or placebo (Yogaratnam et al., 2019).

AL-974 was tolerated by individuals with no serious adverse events noted. The most frequent TEAE reported were headaches and light headedness.

When compared to placebo, those taking AL-794 displayed a dose-dependent effect with 150 mg regimen reducing peak viral burden by approximately 10 fold, resulting in 58% decrease in viral RNA titer AUC, 35 h reduction in time to influenza RNA negativity and mean symptom scores (Yogaratnam et al., 2019). Pharmacokinetic analysis found that exposure levels of AL-794 varied significantly based on prior food consumption and gender, and because a single dose which was both well-tolerated and effective against influenza virus infection could not be established, the development of the drug has been discontinued (Witek, 2018).

4. Conclusion

The polymerase complex plays an essential role in the influenza virus replication cycle and as such is a major target for the development of small molecule inhibitors. It is hoped that the current generation of polymerase inhibitors, particularly when used in combination with NAIs, may enhance our repertoire of antiviral options including more effective treatment for severely ill or hospitalised patients. Compounds that remain effective when administered later in the illness course (i.e. > 48 h post-symptom onset) and have a low propensity for resistance would be ideal. When used as monotherapy, both pimodivir and baloxavir are associated with the frequent emergence of variants with reduced susceptibility, although preliminary data for pimodivir and oseltamivir suggest that combination therapy with a NAI reduces the frequency of variant emergence. The use of a combination of antiviral compounds that could rapidly reduce viral replication and limit the emergence of antiviral resistant strains remains an attractive approach. Future studies with a combination of baloxavir and oseltamivir will not only provide insights into variant emergence but will hopefully provide a potent virological effect and improved clinical outcomes for the treatment of hospitalised or severely ill patients.

Acknowledgment

The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.antiviral.2019.104545.

References

Adisasmito, W., Chan, P.K.S., Lee, N., et al., 2010. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a global patient registry. J. Infect. Dis. 202 (8), 1154–1160.
Allen, U.D., Aoki, F.Y., Stiver, H.G., 2006. The use of antiviral drugs for influenza: recommended guidelines for practitioners. Can. J. Infect Dis. Med. Microbiol. 17 (5), 273–284.
Baranovich, T., Wong, S.-S., Armstrong, J., et al., 2013. T-705 (favipiravir) induces lethal mutagenesis in influenza a H1N1 viruses in vitro. J. Virol. 87 (7), 3741–3751.
Baz, M., Carbonneau, J., Rhéaume, C., et al., 2018. Combination therapy with oseltamivir and favipiravir delays mortality but does not prevent oseltamivir resistance in immunodeficient mice infected with pandemic A(H1N1) influenza virus. Viruses 10 (11), 610.
Bloom, J.D., Gong, L.I., Baltimore, D., 2010. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. Science (New York, N.Y.) 328 (5983), 1272–1275.
Byrn, R.A., Jones, S.M., Bennett, H.B., et al., 2015. Preclinical activity of VX-787, a first-in-class, orally bioavailable inhibitor of the influenza virus polymerase PB2 subunit. Antimicrob. Agents Chemother. 59 (3), 1569–1582.
Clark, M.P., Ledeboer, M.W., Davies, I., et al., 2014. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. J. Med. Chem. 57 (15), 6668–6678.
Deleu, S., Kakuda, T.N., Spittaels, K., et al., 2018. Single- and multiple-dose pharmacokinetics and safety of pimodivir, a novel, non-nucleoside polymerase basic protein 2 subunit inhibitor of the influenza A virus polymerase complex, and interaction with oseltamivir: a Phase 1 open-label study in healthy volunteers. Br. J. Clin. Pharmacol. 84 (11), 2663–2672.
Democratis, J., Pareek, M., Stephenson, I., 2006. Use of neuraminidase inhibitors to combat pandemic influenza. J. Antimicrob. Chemother. 58 (5), 911–915.
Deyde, V.M., Xu, X., Bright, R.A., et al., 2007. Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. J. Infect. Dis. 196 (2), 249–257.
Dobson, J., Whitley, R.J., Pocock, S., et al., 2015. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. The Lancet 385 (9979), 1729–1737.
Epstein, C., 2017. Experience with favipiravir in phase 3 and implications for future studies. In: 5th Isirv-AVG Conference; Prevention and Treatment of RVIs: Antivirals, Traditional Therapies and Host-Directed Interventions, (Shanghai, China).
Finberg, R.W., Lanno, R., Anderson, D., et al., 2018. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. J. Infect. Dis 219 (7), 1026–1034.
Fodor, E., Pritlove, D.C., Brownlee, G.G., 1994. The influenza virus panhandle is involved in the initiation of transcription. J. Virol. 68 (6), 4092–4096.
Fry, A.M., Goswami, D., Nahar, K., et al., 2014. Efficacy of oseltamivir treatment started within 5 days of symptom onset to reduce influenza illness duration and virus shedding in an urban setting in Bangladesh: a randomised placebo-controlled trial. Lancet Infect. Dis. 14 (2), 109–118.
Fukao, K., Noshi, T., Yamamoto, A., et al., 2019. Combination treatment with the cap-dependent endonuclease inhibitor baloxavir marboxil and a neuraminidase inhibitor in a mouse model of influenza A virus infection. J. Antimicrob. Chemother. 74 (3), 654–662.
Furuta, Y., Komeno, T., Nakamura, T., 2017. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc. Jpn. Acad. B 93 (7), 449–463.
Furuta, Y., Takahashi, K., Kuno-Maekawa, M., et al., 2005. Mechanism of action of T-705 against influenza virus. Antimicrob. Agents Chemother. 49 (3), 981–986.
Goldhill, D.H., Langat, P., Xie, H., et al., 2018a. Determining the mutation bias of favipiravir in influenza using next-generation sequencing. J. Virol. 93 (2), e01217–01218.
Goldhill, D.H., te Velthuis, A.J.W., Fletcher, R.A., et al., 2018b. The mechanism of resistance to favipiravir in influenza. Proc. Natl. Acad. Sci. Unit. States Am. 115 (45), 11613–11618.
Goto, H., Sakai-Tagawa, Y., Shiraishi, K., et al., 2003. High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. J. Infect. Dis. 188 (1), 57–61.
Gubareva, L.V., Mishin, V.P., Patel, M.C., et al., 2019. Assessing baloxavir susceptibility of influenza viruses circulating in the United States during the 2016/17 and 2017/18 seasons. Euro Surveill. 24 (3), 1800666.
Hall, C.B., Dolin, R., Gala, C.L., et al., 1987. Children with influenza a infection: treatment with rimantadine. Pediatrics 80 (2), 275–282.
Hay, A.J., Sweet, C., Hayden, F.G., et al., 1991. Virulence of rimantadine-resistant human influenza a (H3N2) viruses in ferrets. J. Infect. Dis. 164 (5), 969–972.
Hayden, F.G., Belshe, R.B., Clover, R.D., et al., 1989. Emergence and apparent transmission of rimantadine-resistant influenza a virus in families. N. Engl. J. Med. 321 (25), 1696–1702.
Hayden, F.G., Shindo, N., 2019. Influenza virus polymerase inhibitors in clinical development. Curr. Opin. Infect. Dis. 32 (2), 176–186.
Hayden, F.G., Sperber, S.J., Belshe, R.B., et al., 1991. Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. Antimicrob. Agents Chemother. 35

(9), 1741–1747.
Hayden, F.G., Sugaya, N., Hirotsu, N., et al., 2018. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. N. Engl. J. Med. 379 (10), 913–923.
Hurt, A.C., Besselaar, T.G., Daniels, R.S., et al., 2016. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014-2015. Antivir. Res. 132, 178–185.
Hurt, A.C., Ernest, J., Deng, Y.-M., et al., 2009. Emergence and spread of oseltamivir-resistant A(H1N1) influenza viruses in oceania, south east Asia and South Africa. Antivir. Res. 83 (1), 90–93.
Ison, M.G., Portsmouth, S., Yoshida, Y., et al., 2018. Phase 3 Trial of Baloxavir Marboxil in High Risk Influenza Patients (CAPSTONE-2 Study). ID Week Advancing Science Week San Francisco.
Jekle, A., Stoycheva, A., Hendricks, R.T., et al., 2016. Preclinical antiviral activity and ADME characteristics of the novel influenza endonuclease inhibitor AL-794. Options IX meeting. In: Annual Scientific Conference for International Society for Influenza and Respiratory and Viral Disease, (Chicago).
Jones, J.C., 2018. Susceptibility of Influenza Viruses to the Novel Cap-dependent Endonuclease Inhibitor Baloxavir Marboxil 6th Advances in Respiratory Virus Therapeutics. (Washington).
Kawaguchi, N., Koshimichi, H., Ishibashi, T., et al., 2018. Evaluation of drug–drug interaction potential between baloxavir marboxil and oseltamivir in healthy subjects. Clin. Drug Investig. 38 (11), 1053–1060.
Kawai, N., Ikematsu, H., Iwaki, N., et al., 2006. A comparison of the effectiveness of oseltamivir for the treatment of influenza a and influenza B: a Japanese multicenter study of the 2003–2004 and 2004–2005 influenza seasons. Clin. Infect. Dis. 43 (4), 439–444.
Kawamura, K., Hirotsu, N., Iwaki, N., et al., 2009. Clinical effectiveness of oseltamivir and zanamivir for treatment of influenza a virus subtype H1N1 with the H274Y mutation: a Japanese, multicenter study of the 2007–2008 and 2008–2009 influenza seasons. Clin. Infect. Dis. 49 (12), 1828–1835.
Kiso, M., Mitamura, K., Sakai-Tagawa, Y., et al., 2004. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. The Lancet 364 (9436), 759–765.
Kiso, M., Yamashita, M., Ito, M., et al., 2017. Combination therapy with neuraminidase and polymerase inhibitors in nude mice infected with influenza virus. J. Infect. Dis. 217 (6), 887–896.
Kobayashi, O., Noto, M., Sakurai, T., et al., 2008. T-705, a novel anti-influenza virus compound - the safety, tolerability and pharmacokinetics in human. Int. J. Infect. Dis. 12, e299.
Koshimichi, H., Ishibashi, T., Kawaguchi, N., et al., 2018. Safety, tolerability, and pharmacokinetics of the novel anti-influenza agent baloxavir marboxil in healthy adults: phase I study findings. Clin. Drug Investig. 38 (12), 1189–1196.
Koszalka, P., Tilmanis, D., Roe, M., et al., 2019. Baloxavir marboxil susceptibility of influenza viruses from the Asia-Pacific, 2012–2018. Antivir. Res. 164, 91–96.
Krammer, F., Smith, G.J.D., Fouchier, R.A.M., et al., 2018. Influenza. Nat. Rev. Dis. Prim. 4 (1), 3.
Krug, R.M., Broni, B.A., Bouloy, M., 1979. Are the 5′ ends of influenza viral mrnas synthesized in vivo donated by host mRNAs? Cell 18 (2), 329–334.
Lackenby, A., Besselaar, T.G., Daniels, R.S., et al., 2018. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors and status of novel antivirals, 2016–2017. Antivir. Res. 157, 38–46.
Lamb, R.A., Choppin, P.W., 1983. The gene structure and replication of influenza virus. Annu. Rev. Biochem. 52 (1), 467–506.
Leopold, L., 2018. Hospital Recovery Scale- the Pimodivir Experience with an Ordinal Scale 6th ISIRV AVS Conference. (Washington, USA).
Lina, B., Boucher, C., Osterhaus, A., et al., 2018. Five years of monitoring for the emergence of oseltamivir resistance in patients with influenza A infections in the Influenza Resistance Information Study. Influenza and Other Respir. Viruses 12 (2), 267–278.
Makau, J.N., Watanabe, K., Ishikawa, T., et al., 2017. Identification of small molecule inhibitors for influenza a virus using in silico and in vitro approaches. PLoS One 12 (3), e0173582.
Marathe, B.M., Wong, S.-S., Vogel, P., et al., 2016. Combinations of oseltamivir and T-705 extend the treatment window for highly pathogenic influenza A(H5N1) virus infection in mice. Sci. Rep. 6, 26742.
Massari, S., Nannetti, G., Desantis, J., et al., 2015. A broad anti-influenza hybrid small molecule that potently disrupts the interaction of polymerase acidic protein–basic protein 1 (PA-PB1) subunits. J. Med. Chem. 58 (9), 3830–3842.
McKimm-Breschkin, J.L., 2013. "Influenza Neuraminidase Inhibitors: Antiviral Action and Mechanisms of resistance." Influenza and Other Respiratory Viruses 7 Suppl 1(Suppl Suppl 1). pp. 25–36.
McKimm-Breschkin, J.L., Jiang, S., Hui, D.S., et al., 2018. Prevention and treatment of respiratory viral infections: presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV Antiviral Group conference. Antivir. Res. 149, 118–142.
Memoli, M.J., Hrabal, R.J., Hassantoufighi, A., et al., 2010. Rapid selection of oseltamivir- and peramivir-resistant pandemic H1N1 virus during therapy in 2 immunocompromised hosts. Clin. Infect. Dis. : Off. Publ. Infect. Dis. Soc. Am. 50 (9), 1252–1255.
Ministry of Health, L. a. W., 2018. Report on the Deliberation Results. Retrieved 21/1/2019, 2019.
Ministry of Health, L. a. W., 2019. Revisions of Precautions Baloxavir Marboxil. PMDA.
Moscona, A., 2005. Neuraminidase inhibitors for influenza. N. Engl. J. Med. 353 (13), 1363–1373.
Muratore, G., Goracci, L., Mercorelli, B., et al., 2012. Small molecule inhibitors of influenza A and B viruses that act by disrupting subunit interactions of the viral polymerase. Proc. Natl. Acad. Sci. Unit. States Am. 109 (16), 6247–6252.
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. The Lancet Respir. Med. 2 (5), 395–404.
National Institute of Infectious Diseases, 2019. Antiviral Resistance Surveillance in Japan (As of March 4 2019). Retrieved 12/03/2019, 2019.
Nguyen-Van-Tam, J.S., Venkatesan, S., Muthuri, S.G., et al., 2015. Neuraminidase inhibitors: who, when, where? Clin. Microbiol. Infect. 21 (3), 222–225.
Okoli, G.N., Otete, H.E., Beck, C.R., et al., 2014. Use of neuraminidase inhibitors for rapid containment of influenza: a systematic review and meta-analysis of individual and household transmission studies. PLoS One 9 (12), e113633.
Omoto, S., Speranzini, V., Hashimoto, T., et al., 2018. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci. Rep. 8 (1), 9633.
Patel, T.S., Cinti, S., Sun, D., et al., 2017. Oseltamivir for pandemic influenza preparation: maximizing the use of an existing stockpile. Am. J. Infect. Contr. 45 (3), 303–305.
Poon, L.L.M., Pritlove, D.C., Fodor, E., et al., 1999. Direct evidence that the poly(A) tail of influenza a virus mRNA is synthesized by reiterative copying of a U track in the virion RNA template. J. Virol. 73 (4), 3473–3476.
Rocha-Pereira, J., Jochmans, D., Dallmeier, K., et al., 2012. Favipiravir (T-705) inhibits in vitro norovirus replication. Biochem. Biophys. Res. Commun. 424 (4), 777–780.
Saito, R., Sato, I., Suzuki, Y., et al., 2010. Reduced effectiveness of oseltamivir in children infected with oseltamivir-resistant influenza a (H1N1) viruses with His275Tyr mutation. Pediatr. Infect. Dis. J. 29 (10), 898–904.
Shionogi, 2018. XOFLUZATM (Baloxavir Marboxil) Tablets 10mg/20mg for the Treatment of Influenza Types A and B Launched in Japan. Retrieved March 14, 2018, from. http://www.shionogi.co.jp/en/company/news/2018/pmrltj0000003oid-att/e180314.pdf.
Shobugawa, Y., Saito, R., Sato, I., et al., 2008. Recurrence and persistence of fever in children who developed amantadine-resistant influenza viruses after treatment. Tohoku J. Exp. Med. 214 (2), 129–138.
Silvennoinen, H., Lehtinen, P., Heinonen, S., et al., 2010. Early oseltamivir treatment of influenza in children 1–3 Years of age: a randomized controlled trial. Clin. Infect. Dis. 51 (8), 887–894.
Smither, S.J., Eastaugh, L.S., Steward, J.A., et al., 2014. Post-exposure efficacy of Oral T-705 (Favipiravir) against inhalational Ebola virus infection in a mouse model. Antivir. Res. 104, 153–155.
Stevaert, A., Naesens, L., 2016. The influenza virus polymerase complex: an update on its structure, functions, and significance for antiviral drug design. Med. Res. Rev. 36 (6), 1127–1173.
Su, C.-Y., Cheng, T.-J.R., Lin, M.-I., et al., 2010. High-throughput identification of compounds targeting influenza RNA-dependent RNA polymerase activity. Proc. Natl. Acad. Sci. Unit. States Am. 107 (45), 19151–19156.
Sugaya, N., 2011. Widespread use of neuraminidase inhibitors in Japan. J. Infect. Chemother. 17 (5), 595–601.
Takashita, E., Ejima, M., Ogawa, R., et al., 2016. Antiviral susceptibility of influenza viruses isolated from patients pre- and post-administration of favipiravir. Antivir. Res. 132, 170–177.
Takashita, E., Kawakami, C., Morita, H., et al., 2019a. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. Euro Surveill. 24 (3), 1800698.
Takashita, E., Kawakami, C., Ogawa, R., et al., 2019b. Influenza A(H3N2) virus exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019. Euro Surveill. 24 (12), 1900170.
Takashita, E., Meijer, A., Lackenby, A., et al., 2015. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. Antivir. Res. 117, 27–38.
Toyama Chemical, 2018. AVIGAN Tablets.
Treanor, J.J., Hayden, F.G., Vrooman, P.S., et al., 2000. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. J. Am. Med. Assoc. 283 (8), 1016–1024.
Trevejo, J.M., Asmal, M., Vingerhoets, J., et al., 2017. Pimodivir treatment in adult volunteers experimentally inoculated with live influenza virus: a Phase IIa, randomized, double-blind, placebo-controlled study. Antivir. Ther. 23.
Uehara, T., Hayden, F.G., Kawaguchi, K., et al., 2019. Treatment emergent influenza virus variants with reduced baloxavir susceptibility: impact on clinical and virologic outcomes in uncomplicated influenza. JID (J. Infect. Dis.) (submitted for publication).
Uehara, T., Shishido, T., Ishibashi, T., et al., 2016. Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus, Leads to Rapid and Profound Viral Load Reduction. Options for the Control of Influenza. (Chicago, USA).
US Food and Drug Administration, 2018. FDA approves new drug to treat influenza. Retrieved October 24, 2018, from. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm624226.htm.
Uyeki, T.M., Bernstein, H.H., Bradley, J.S., et al., 2018. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenza.
Venkatesan, S., Myles, P.R., Leonardi-Bee, J., et al., 2017. Impact of outpatient neuraminidase inhibitor treatment in patients infected with influenza A(H1N1)pdm09 at high risk of hospitalization: an individual participant data metaanalysis. Clin. Infect.

Dis. 64 (10), 1328–1334.
Whitley, R., Hayden, F.G., Reisinger, K., et al., 2001. Oral oseltamivir treatment of influenza in children. Pediatr. Infect. Dis. J. 20 (2), 127–133.
Whitley, R.J., Boucher, C.A., Lina, B., et al., 2013. Global assessment of resistance to neuraminidase inhibitors, 2008–2011: the influenza resistance information study (IRIS). Clin. Infect. Dis. 56 (9), 1197–1205.
Witek, J., 2018. Overview of RSV and Influenza Programmes. International Society for Influenza and Other Respiratory Virus Diseases -Advances in Respiratory Viral Therapeutics. (Washington).
World Health Organisation, 2018. Standard Guidelines for the Clinical Management of Severe Influenza Virus Infections Initial Guideline Development Group (GDG) Meeting. Retrieved 11/2/2019, 2019.
Yogaratnam, J., Rito, J., Kakuda, T.N., et al., 2019. Antiviral activity, safety, and pharmacokinetics of AL-794, a novel oral influenza endonuclease inhibitor: results of an influenza human challenge study. J. Infect. Dis. 219 (2), 177–185.
Zheng, B., Lau, C.C.Y., Tse, H., et al., 2010. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin. Infect. Dis. 50 (6), 850–859.