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Current Opinion in Virology

Baloxavir marboxil: the new influenza drug on the market

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For the first time in nearly 20 years there is a new class of antiviral drug for influenza. The latest approved antiviral is baloxavir marboxil (trade name, Xofluza) which targets the endonuclease function of the viral PA polymerase subunit and prevents the transcription of viral mRNA. The most promising aspect of this new drug is its pharmacology which allows for effective treatment of influenza A or B virus infection with just a single dose. A clinical trial showed greater reductions in viral loads with baloxavir marboxil treatment compared with oseltamivir, although no difference in the time to alleviation of symptoms between these two drugs. With this new class of influenza drug comes exciting prospects for combination therapy with the neuraminidase inhibitors which may help to abate concerns about the development of resistance.

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Current Opinion in Virology 2019, **35**:14–18

This review comes from a themed issue on **Antiviral strategies**

Edited by **Margo A Brinton** and **Richard K Plemper**

For a complete overview see the Issue and the Editorial

Available online 8th March 2019

https://doi.org/10.1016/j.coviro.2019.01.006

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Introduction

Annually, influenza viruses have a major impact on global health, causing 3–5 million severe cases of disease and 1 million deaths [1]. The economic burden of seasonal epidemics is estimated to cost $90 billion for the U.S. alone, while pandemics may cost hundreds of billions of dollars [2]. Although vaccines for influenza are available, poor annual vaccine coverage, low immunogenicity in the elderly population, and occasional vaccine mismatches means that they do not deliver adequate levels of protection. For this reason, influenza antiviral drugs are essential and are used for both treatment and prophylaxis (Table 1).

Until this year, the only FDA approved antivirals for influenza viruses were the adamantanes or M2 ion channel inhibitors and the neuraminidase (NA) inhibitors (NAI). The M2 inhibitors amantadine and rimantadine were approved in 1966 and 1993 [3], respectively and they act by blocking the M2 ion channel activity (for influenza A viruses only), thus preventing uncoating of the viral genome. However, the increase in resistance to these drugs amongst circulating influenza A virus strains in the last two decades has been well documented. Before 2000, the worldwide frequency of resistant influenza A viruses was low (0.5–4%, depending on the strain) [4,5]. Currently over 95% of the isolated H3N2 and H1N1 influenza A viruses are resistant. The resistance is caused by one of several single residue mutations in the M2 pore (S31N, L26I, or V27A), which are sufficient to dramatically decrease the susceptibility to M2 inhibitors [6,7]. As a result of the rapid spread of these mutations that confer resistance, the Advisory Committee on Immunization Practices at the CDC recommended against the use of adamantanes for treatment of influenza A virus infections [8].

NAIs are still used clinically for uncomplicated influenza, and have been in use since oseltamivir and zanamivir were both FDA approved in 1999, followed by peramivir in 2014 [9,10]. These drugs act by preventing the release of newly formed virions from the infected cells, and since the residues and structural elements of NA in influenza A and B viruses are highly conserved, oseltamivir and zanamivir are broad inhibitors of these types of viruses [11,12]. Oseltamivir has several advantages over other NAIs in that it can be taken orally, is approved for use in infants, and it is well tolerated in diverse populations as well as patients with liver and renal complications [13]. Unfortunately, influenza A and B viruses can develop resistance to oseltamivir by acquiring mutations in the neuraminidase active site (H274Y, E119V) that are critical for drug binding [14–17], but not sialidase activity. Before 2007, oseltamivir resistant H1N1 viruses were reported at low frequencies (under 1% of clinical isolates), but increased to 95% worldwide during the 2008/2009 influenza season [18]. Fortunately, such high levels of resistance have not been observed amongst currently circulating H1N1 viruses (Summary of the 2017–2018 Influenza Season; URL https://www.cdc.gov/flu/about/season/flu-season-2017-2018.htm). In contrast, zanamivir resistance is infrequent with only a few reported cases of resistant influenza A and B viruses from clinical samples [19]. However, zanamivir is administered as an inhaled

Table 1

FDA-approved antiviral drugs for influenza

Drug class	Antiviral drug	Active against	Route	Treatment	Chemoprophylaxis	Resistance mutations
M2 ion channel inhibitors	Rimantadine	Influenza A viruses	Oral	Not Recommended	Not Recommended	S31N, L26I, V27A
	Amantadine	Influenza A viruses	Oral	Not Recommended	Not Recommended	S31N, L26I, V27A
Neuraminidase (NA) inhibitors	Oseltamivir	Influenza A and B viruses	Oral	Any age	3 months and older	H274Y, E119V
	Zanamivir	Influenza A and B viruses	Inhaled	7 years and older	5 years and older	I223R, E119V
	Peramivir	Influenza A and B viruses	Intravenous	2 years and older	Not Recommended	H274Y
Endonuclease (PA) inhibitors	Baloxavir marboxil	Influenza A and B viruses	Oral	12 years and older	Not Recommended	I38T/F/M

powder and is poorly tolerated in patients with underlying respiratory diseases. The advantage to peramivir is that it provides an intravenous option for treatment of patients two years and older. However, viruses bearing the H274Y mutation in NA that confers oseltamivir resistance are cross-resistant to peramivir as well [20,21].

Although the overall levels of NAI resistance is currently low, it may be only a matter of time before the NAIs are rendered ineffective as happened with the M2 inhibitors. Studies indicate that some of these resistance mutations may have been present before antiviral drug exposure, suggesting that resistant viruses may have wild-type growth and transmissibility abilities allowing rapid spread in the population [4,22]. This concern has prompted the discovery and development of novel classes of influenza antivirals with a higher barrier to resistance.

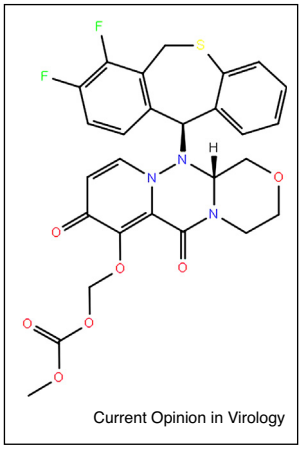
The most recent example is baloxavir marboxil, trade name Xofluza, which was approved by the FDA in October 2018, becoming the first influenza antiviral with a novel mechanism of action to be approved in almost two decades. It was developed by Shionogi Inc. and received its first approval in Japan in February 2018. Shionogi partnered with Roche for global development and commercialization of baloxavir marboxil, and is still seeking approval in other countries [23]. Baloxavir marboxil is expected to be available for use during the 2018–2019 influenza season in the Northern hemisphere and is approved for treatment of patients older than 12 years who present with uncomplicated influenza within 48 hours of the start of symptoms (Genentech Announces FDA Approval of XOFLUZA (Baloxavir Marboxil) for Influenza; URL: https://www.gene.com/media/press-releases/14761/2018-10-24/genentech-announces-fda-approval-of-xofl).

Discovery and antiviral mechanism of baloxavir marboxil

Baloxavir marboxil is unique in that it inhibits viral replication by targeting the endonuclease function encoded by the PA subunit of the viral polymerase complex. Influenza viruses have a trimeric polymerase complex composed of the PB1, PB2, and PA subunits, and the RNA-dependent RNA polymerase activity resides in the PB1 protein. The synthesis of viral mRNAs requires a host pre-mRNA to be used as a primer for viral transcription, and to achieve this the PB2 protein binds to the 5′-cap structure of host pre-mRNAs in the nucleus of the infected cell. For over two decades it was known that an endonuclease was required to cleave the captured pre-mRNA 10–13 nucleotides from the cap structure [24]. Studies showed that inhibiting this activity strongly decreased viral replication, supporting the notion that the endonuclease function would be a good antiviral target [25]. However, attempts by pharmaceutical companies to develop such an endonuclease inhibitor initially failed, likely due to lack of potent antiviral activity in the pre-clinical stages [26]. However, this was during a period when the location of the active site for the endonuclease was not clearly identified within the trimeric viral polymerase complex. It was not until 2009 that the structure of PA was determined by crystallography, and it was discovered that the viral endonuclease function resides in the N-terminus of the PA subunit [27]. Further study with residues 1–209 revealed highly conserved residues for metal ion binding (H41, E80, D108, and E119) and catalytic activity (K134) typical of type II endonucleases. Mutation of any of these residues results in loss of PA endonuclease activity [28].

These studies laid the groundwork for rational design of successful inhibitors targeting the active site of the PA endonuclease. Knowing the preferential binding of manganese ions that are essential for its activity, many groups used this to computationally design inhibitors with a metal ion binding structure that could specifically fit within the endonuclease site [29,30]. The most successful of these groups to date is Shionogi Inc., which designed baloxavir marboxil (Figure 1) based off of a metal ion binding integrase inhibitor for human immunodeficiency virus, dolutegravir. Baloxavir acid, the active form of baloxavir marboxil, was designed to be specific for the PA endonuclease. From co-crystal structural data of

Figure 1



Chemical structure of baloxavir marboxil.

baloxavir acid bound to PA, it can be seen that stable binding to the active site requires an interaction with two manganese ions and van der Waals interactions with residues-specific to the pocket [31••].

Preclinical data for baloxavir marboxil

In vitro assays demonstrate that baloxavir acid inhibits PA endonuclease activity (IC_{50} of 2.5 nM), without affecting the RNA-dependent RNA polymerase activity of PB1 or cap binding activity of PB2. Importantly, it is active against both influenza A and B viruses, although there is a distinct difference in potency with EC_{90} values reported in the sub nanomolar range for influenza A viruses versus single digit values for influenza B viruses. Inhibition of a wide range of influenza viruses has been demonstrated, including viruses carrying the oseltamivir resistance mutation (H274Y), laboratory, clinical, and zoonotic strains. Baloxavir acid is extremely potent in cell culture with reported EC_{90} values 2–3 logs lower than those of the approved NAIs, which provided optimism for a good clinical outcome [31••].

Given that baloxavir acid has a distinct mechanism of action from that of the NAIs, their potential to work as a combination therapy has been investigated. *In vitro*, a drug combination study determined that baloxavir acid and all the approved NAIs had synergistic antiviral activity. Furthermore, combinations of baloxavir marboxil and oseltamivir protected mice from a lethal influenza infection better than either drug alone. Remarkably, a dose of baloxavir marboxil 30 times lower than a protective dose of 15 mg/kg was sufficient to confer protection when used in combination with oseltamivir. In contrast to oseltamivir alone, protective doses of baloxavir marboxil (alone or in combination) significantly decreased viral lung titers 24 hours after treatment and were associated with significantly lower levels of inflammatory cytokines (IL-6 and IFN-γ) compared to oseltamivir alone [32••]. Collectively, these animal studies indicated that baloxavir marboxil can protect from lethal influenza infections, has the potential to reduce viral shedding, and can reduce damage to the lung due to virus induced inflammation.

Clinical data for baloxavir marboxil

In a phase III clinical trial (CAPSTONE-1), a single dose of baloxavir marboxil was superior to placebo in alleviating influenza symptoms in a double-blind, randomized trial of 1436 patients (aged 12–64 years) diagnosed with uncomplicated influenza A or B from Japan and the United States. Within 48 hours of symptoms, patients were given a single oral dose of baloxavir marboxil (40 mg or 80 mg, depending on weight), or 75 mg of oseltamivir twice daily for 5 days, or placebo. The median time of alleviation of symptoms in groups treated with baloxavir marboxil was shorter versus placebo (53.7 hours versus 80.2 hours, $P < 0.001$); however, it was not different from that of oseltamivir (53.8 hours). Despite no difference in clinical outcome baloxavir marboxil was superior to oseltamivir in reducing viral loads, with a median duration of infectious virus detection of 24 hours versus 72 hours for oseltamivir ($P < 0.001$) and 96 hours for placebo ($P < 0.001$). It was also notable that the median time for the resolution of fever was shorter with baloxavir marboxil than placebo (24.5 hours versus 42.0 hours, $P < 0.001$). The median time to return to usual health was shorter with baloxavir marboxil than placebo, although it was not a significantly difference ($P = 0.06$) [33••].

Baloxavir marboxil was well tolerated with only 4.4% of patients reporting minor adverse events related to the trial regimen, such as diarrhea and nausea. A similar number of patients reported the same adverse events for the placebo (3.9%) and oseltamivir (8.4%) groups, mitigating any concern that these events are unique to baloxavir marboxil. Similarly, only a few non-serious adverse events were reported during phase I and phase II trials with baloxavir marboxil, including a phase I trial looking for adverse events in 18 healthy adults treated with combinations of baloxavir marboxil and oseltamivir [23,33••,34•,35].

Resistance data on baloxavir marboxil

Since baloxavir marboxil targets a viral protein, there is a valid concern that drug resistance could develop relatively easily. To date, there are reports of influenza viruses with a reduced susceptibility to baloxavir marboxil isolated from cell cultures and also from patients in the clinical trials. In two phase II trials with adult and pediatric patients with non-complicated influenza, resistant viruses were detected before (baseline) and after treatment with baloxavir marboxil (treatment-emergent). Baseline strains with polymorphisms that conferred resistance (like A36V) were mostly rare and did

not result in more than sevenfold reduction in susceptibility. However, the most significant treatment emergent strains came from five adult patients infected with influenza A/H1N1 and 15 pediatric patients with influenza A/H3N2 viruses, which contained I38T, I38M, or I38F mutations that had at least a 10-fold reduction in susceptibility. *In vitro*, influenza A/WSN/33 virus serially passaged in the presence of baloxavir acid also generated I38T mutants that showed 20–40-fold reduced susceptibility [36••]. The observation that I38 in PA is conserved in >99.9% of influenza viruses supports the notion that the mutations arise due to direct pressure from baloxavir marboxil. Furthermore, in the CAPSTONE-1 trial, influenza H3N2 strains with I38T/M mutations were isolated from 9.7% of baloxavir marboxil recipients starting as early as 24 hours post initiation of treatment. This specific group also experienced a longer median time to alleviation of symptoms versus baloxavir marboxil recipients without these substitutions (63.1 hours versus 49.6 hours). The percentage of patients shedding infectious virus on day five was also elevated for the group with I38T/M variants (91%) versus baloxavir marboxil recipients without these variants (7%) and placebo recipients (31%) [33••].

These resistance mutations, and the ease at which they arise, constitute a clear concern as they impact the clinical efficacy of baloxavir marboxil. However, it is uncertain whether viruses bearing these mutations are equally fit, and most importantly, whether they can transmit efficiently in humans. An *in vitro* study suggested that recombinant influenza A viruses with the I38T mutation are attenuated for growth in cell culture. The structural data also indicate that I38 in PA is required for a critical interaction with the RNA substrate as well as binding to baloxavir acid. The I38T/M/F mutation removes a critical methyl group required for binding to baloxavir acid, and simultaneously makes this binding surface less hydrophobic, which is required for RNA binding. However, the same study showed that I38T and I38M substitutions in the influenza B virus PA do not attenuate virus growth [36••]. This is likely due to another residue (M34) that contributes more to the binding of RNA than I38 in the case of influenza B viruses. However, the clinical impact of an influenza B virus with an I38 substitution is unknown as there are no reports of their isolation.

Conclusions

The approval of baloxavir marboxil as an influenza antiviral drug is a major step forward as it represents a new drug class — endonuclease inhibitors, for influenza therapy. The key advantage of baloxavir marboxil is that only a single dose is required which should increase compliance. It does not appear to have a significant clinical advantage over oseltamivir as measured by time to alleviation of symptoms, but its ability to reduce viral loads within 24 hours of treatment makes it superior in preventing virus spread. It is possible that the quick reduction in viral load could mitigate the subsequent inflammation and damage to the lung tissue, resulting in a better clinical outcome, but this remains to be tested and may only be seen in cases of complicated influenza. The approval of baloxavir marboxil now opens the door to the use of combination therapy for influenza and the initial pre-clinical data on baloxavir marboxil/oseltamivir combinations are encouraging. In particular, this approach may be required to raise the barrier to resistance as the selection of baloxavir marboxil-resistant viruses with only a single dose of drug is concerning.

Future developmental milestones for baloxavir marboxil include approval for use in children under age 12, and in patients with risk factors for influenza complications. A phase III clinical trial to address the latter is underway (CAPSTONE-2). Also, the efficacy of baloxavir marboxil and NAI combination therapies and the ability to improve clinical outcome when starting treatment after 48 hours has yet to be evaluated in a clinical trial. Independent of clinical trials, investigators will need to remain vigilant in evaluating the incidence and transmissibility of resistant strains.

Acknowledgment

The authors were supported in part by National Institutes of Health grants R21AI131608 (to MLS) and R56AI139015 (to MLS).

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The authors characterize baloxavir acid as a selective inhibitor of PA endonuclease, and demonstrate its potent broad spectrum activity against influenza viruses. This is the first published study demonstrating baloxavir marboxil's mechanism of action, pan-influenza activity, and characterizing the resistance mutations *in vitro*.

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The authors demonstrate the ability to protect mice from a lethal infection of influenza virus with baloxavir marboxil alone, and in combination with oseltamivir. With an *in vitro* combination study, they also demonstrate that baloxavir acid works synergistically with all approved neuraminidase inhibitors, including laninamivir. These studies support that combination therapy could work in humans, and would be beneficial versus monotherapy.

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A landmark article summarizing successful the phase two and phase three (CAPSTONE-1) clinical trials with baloxavir marboxil that led to its approval in Japan and the U.S. Overall, treatment with baloxavir marboxil was superior in reducing viral loads in patients versus both oseltamivir and placebo treatment. It was also superior in significantly reducing the median time to alleviation of symptoms versus placebo. Similar levels of adverse events were reported with each treatment arm, suggesting that no additional side effects occur with baloxavir marboxil treatment.

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In this phase I study with healthy adults, the authors determined that there were no additional adverse events to patients or affects to the mutual pharmacokinetics with baloxavir marboxil and oseltamivir combination treatments versus either drug alone. This study demonstrates that the combination treatment is safe in humans, and warrants further investigation in phase two clinical trials.

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The authors isolated viruses with reduced susceptibillity to baloxavir marboxil from two phase 2 clinical trials, and identified a key resistance mutation in PA (I38T/M/F substitution does disrupt binding to baloxavir acid, it impairs replicative fitness *in vitro* for influenza A viruses, and to a lesser extent, influenza B viruses. This study does ease the concern of viable resistance mutations that could arise from baloxavir marboxil treatment, and provides markers to monitor in future treated populations.