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INFLUENZA ANTIVIRAL DRUG THERAPY – AN ACTUAL REWIEW

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ABSTRACT. Antiviral resistance means that a virus has changed in such a way that antiviral drugs are less effective or not effective at all in treating or preventing illnesses with that virus. In the last years Influenza viruses can become resistant to influenza antiviral drugs by some molecular mechanisms.In this our work we present a new data regarding: this antiviral drug-resistance of influenza viruses, some mehanisms of this resistance, the new techniques utilised for identification and monitoring of the mechanisms of this resistance and the most newest data obtained by the recent trials realised with a a single dose of *Baloxavir marboxil,* a first-in-class drug that inhibits a viral protein essential for influenza replication, wich significantly reduced the duration of flu symptoms, duration of fever, length of time of viral shedding, and levels of virus in the nose and throat, compared with placebo or oseltamivir among otherwise healthy people with the flu in three randomized controlled trials.
Keywords: influenza virus, virus resistance, antiviral therapy, the news drugs anti influenza viruses.
Abbreviation: hemagglutinin(HA), neuraminidase (NA), BXA (Baloxavir).

INFLUENZA VIRUSES – A GENERAL PRESENTATION

Influenza is a frequently underestimated, but vaccine preventable disease. The adamantane derivates rimantadine, amantadine and the neuraminidase inhibitors zanamivir and oseltamivir The prevalence of these viruses increased rapidly and nearby all viruses circulating during the following seasons were resistant to oseltamivir. The A(H1N1)pdm09 viruses replaced the former seasonal A(H1N1) subtype during the 2009–2017 influenza season.The resistance to neuraminidase inhibitors was detected in A(H1N1)pdm09, A(H3N2) and for influenza B viruses only sporadically and was treatment related mostly (http://www.cdc.gov/flu/keyfacts.htm. Accessed: March 11, 2013.)

The M2 ion channel protein of A(H1N1) pdm09 viruses is associated with the Eurasian avian-like swine lineage and thus show "natural" resistance to adamantane derivates. Therefore, only neuraminidase inhibitors are recommended for influenza treatment today, by except, experimentally therapy with the newest inhibitors.

The life cycle of the influenza virus begins with binding of the virus particles to the surface of the host cells. Binding is mediated by the interaction of viral hemagglutinin (HA) with sialyloligosaccharides on proteins and lipids of the cell membranes. Due to receptor-mediated endocytosis the virus is internalized into the host cell enclosed by an endosome. Triggered by low pH in late endosomes and mediated by M2 ion channel, a conformational change of HA induces the fusion of the viral and the endosomal membrane. This triggers the release of uncoated viral ribonucleoprotein (vRNP) complexes into the cytosol of the host cell cytoplasm. After transport of vRNP complexes into the nucleus, replication and transcription follows the amplification of vRNA and synthesis of mRNAs for viral protein synthesis. Newly assembled vRNPs are exported to the cytoplasm and assembled with viral proteins at budding sites within the host cell membrane, followed by the budding and, after cleavage by neuraminidase, release of influenza virions (Ault and colab., 2018). Minor changes in viral proteins (antigenic-drift) are caused by high genomic variability of influenza viruses.

Our rewiew summarizes the occurrence and spread of antiviral resistant influenza viruses and highlights the importance for developing and/or approving new antiviral compounds. Influenza viruses belong to the family Orthomyxoviridae that is characterized by the ability to attach on glycoproteins of host cell surfaces and a segmented genome composed of single stranded, negatively orientated ribonucleic acid (–ssRNA). Based on their molecular features and serological characteristics of their nucleoproteins and matrix proteins influenza viruses are divided into three genera: Influenzavirus A, Influenzavirus B and Influenzavirus C. Whereas infections with influenza C viruses are often symptomless in humans, influenza A and B viruses cause annual epidemics known as seasonal flu, and influenza A viruses also cause pandemics at random intervals. Influenza A viruses are zoonotic pathogens that can infect a broad range of species including birds, pigs and humans. According to the antigenic properties of their surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) influenza A viruses are further divided into 18 HA and 11 NA subtypes (H1–H16 and N1–N9 in wild waterfowl, H17, H18 and N10, N11 in bats). In comparison to influenza A viruses influenza B viruses are less variable (Brooks and colab., 2018).

The influenza viruses resistance is today a huge wordwide therapeutic problem.

Influenza virus causes an infectious disease annually associated with 290,000 to 650,000 deaths and 3 to 5

million cases of severe illness worldwide. In addition, pandemics, caused by newly emerging reassortment viruses, can have a devastating impact globally. Therefore, continued efforts are necessary to improve vaccines and anti-viral drugs as countermeasures.

Two classes of antivirals are currently available for clinical use, neuraminidase inhibitors:

- ***NAIs: oseltamivir, zanamivir, peramivir*** *and*
- ***M2 ion-channel inhibitors (amantadine, rimantadine).***

However, circulating influenza viruses are now largely resistant to the M2 inhibitors. Moreover, the antiviral potency of the NAIs is relatively moderate and another concern for this class of drugs is the emergence of resistance, as occurred during the 2008 to 2009 season when oseltamivir-resistant H1N1 was prevalent. Therefore, more effective antiviral agents, with a novel mechanism of action, are required for the treatment and prevention of influenza virus infections.

What are the influenza viruses species?

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Whereas infections with influenza C viruses are often symptomless in humans, influenza A and B viruses cause annual epidemics known as seasonal flu, and influenza A viruses also cause pandemics at random intervals.

Influenza A viruses are zoonotic pathogens that can infect a broad range of species including birds, pigs and humans. According to the antigenic properties of their surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) influenza A viruses are further divided into 18 HA and 11 NA subtypes (H1–H16 and N1–N9 in wild waterfowl, H17, H18 and N10, N11 in bats).

Influenza B viruses are less variable. They are antigenically related to either B/Victoria/2/87 or B/Yamagata/16/88 and are distinguished into two lineages which are referred to as the Yamagata and the Victoria lineage (Guharoy and colab., 2004; Jefferson and colab., 2009)

The life cycle of the influenza virus begins with binding of the virus particles to the surface of the host cells. Binding is mediated by the interaction of viral hemagglutinin (HA) with sialyloligosaccharides on proteins and lipids of the cell membranes. Due to receptor-mediated endocytosis the virus is internalized into the host cell enclosed by an endosome. Triggered by low pH in late endosomes and mediated by M2 ion channel, a conformational change of HA induces the fusion of the viral and the endosomal membrane. This triggers the release of uncoated viral ribonucleoprotein (vRNP) complexes into the cytosol of the host cell cytoplasm. After transport of vRNP complexes into the nucleus, replication and transcription follows the amplification of vRNA and synthesis of mRNAs for viral protein synthesis. Newly assembled vRNPs are exported to the cytoplasm and assembled with viral proteins at budding sites within the host cell membrane, followed by the budding and, after cleavage by neuraminidase, release of influenza virions.

Minor changes in viral proteins (antigenic-drift) are caused by high genomic variability of influenza viruses. Due to GMS Infectious Diseases 2017, the lack of a proof-reading activity of the viral polymerase point mutations in the vRNA occur that might result in amino acid exchanges of the antigenic epitopes of the HA and NA proteins. This worsens the recognition and neutralization (abolishment of infectivity) of influenza viruses by the host's immune system antibodies and thus may lead to immune evasive virus variants.

Antigenic-drift is the cause for influenza epidemics by weakening the original bond between antigen and antibodies produced by vaccination, or infection induced immunity.

Antigenic shift results from a reassortment between at least two influenza subtypes resulting in a new influenza subtype. This may generate a novel virus able to infect the majority of the population due to their lack of immunity towards the unknown pathogen leading to high infection rates and pandemic spreads. Both mechanisms, point mutations as well as reassortment, can affect the efficacy of anti-influenza drugs. Influenza occurs globally with an estimated annual attack rate of 5–10% in adults and 20–30% in children.

Currently most influenza vaccines contain three different influenza strains (trivalent): two influenza A strains (A(H1N1) and A(H3N2) subtypes) and one influenza B strain (Victoria or Yamagata lineage). Starting from the influenza season 2014–2015 new quadrivalent combination vaccines containing four different influenza strains are increasingly becoming available in the European Union/European Economic Area (EU/EEA). These vaccines contain two influenza A strains (A(H1N1) and A(H3N2) subtypes) and two influenza B strains (Victoria and Yamagata lineages). Annual vaccinations with the current antigen combination are recommended by WHO even when the antigen composition of the vaccine is unchanged compared with the previous season. In Germany, the Standing Committee on Vaccination (STIKO) recommends annual vaccination in autumn as a standard vaccination for all persons aged 60 years and older, and where indicated in specific groups of persons e.g. children, adolescents and adults with an increased health risk resulting from an underlying disease, all pregnant women, persons at increased risk, e. g., medical personnel, persons in establishments dealing extensively with the public, as well as persons who may be possible sources of infection by caring for individuals at particular risk (Brooks and colab., 2012; http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm294057.htm. ; http://www.fda.gov/NewsEvents/Newsroom/PressAnn

ouncements/2007/ucm108892.htm.; Blanton and colab., 2017; https://www.cdc.gov/flu/protect/vaccine/egg-allergies.htm; Lee and colab., 2010; Kilbourne and coalb., 2006; http://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm; Gubareva and colab., 2000).

The mutations of influenza strain viruses and their resistance to antiviral molecules

Minor changes in viral proteins (antigenic-drift) are caused by high genomic variability of influenza viruses. The lack of a proof-reading activity of the viral polymerase point mutations in the vRNA occur that might result in amino acid exchanges of the antigenic epitopes of the HA and NA proteins. This worsens the recognition and neutralization (abolishment of infectivity) of influenza viruses by the host's immune system antibodies and thus may lead to immune evasive virus variants.

Antigenic-drift is the cause for influenza epidemics by weakening the original bond between antigen and antibodies produced by vaccination, or infection induced immunity (Drake, 1993)

Antigenic shift results from a reassortment between at least two influenza subtypes resulting in a new influenza subtype. This may generate a novel virus able to infect the majority of the population due to their lack of immunity towards the unknown pathogen leading to high infection rates and pandemic spreads. Both mechanisms, point mutations as well as reassortment, can affect the efficacy of anti-influenza drugs. Influenza occurs globally with an estimated annual attack rate of 5–10% in adults and 20–30% in children. Both influenza A and influenza B viruses cause seasonal epidemics and out-of-season sporadic cases and outbreaks. A typical influenza is marked by sudden onset of fever (≥38.5°C), dry cough, sore throat, body aches and pains, and headaches. Other symptoms may include general fatigue, sweating, rhinorrhea, but also nausea, vomiting, and diarrhea. In more severe cases, infection can lead to pneumonia, bacterial superinfection and even death. Nevertheless, not all patients fall ill with typical symptomatology. The duration of the disease is usually 5–7 days, which can be considerably longer depending on complications and risk factors. Influenza is a vaccine preventable disease and influenza vaccines have been available for use in Europe since the 1960s. Currently most influenza vaccines contain three different influenza strains (trivalent): two influenza A strains (A(H1N1) and A(H3N2) subtypes) and one influenza B strain (Victoria or Yamagata lineage). Starting from the influenza season 2014–2015 new quadrivalent combination vaccines containing four different influenza strains are increasingly becoming available in the European Union/European Economic Area (EU/EEA). These vaccines contain two influenza A strains (A(H1N1) and A(H3N2) subtypes) and two influenza B strains (Victoria and Yamagata lineages). Annual vaccinations with the current antigen combination are recommended by WHO even when the antigen composition of the vaccine is unchanged compared with the previous season (Frederick and colab., 2018). In Germany, the Standing Committee on Vaccination (STIKO) recommends annual vaccination in autumn as a standard vaccination for all persons aged 60 years and older, and where indicated in specific groups of persons e.g. children, adolescents and adults with an increased health risk resulting from an underlying disease, all pregnant women, persons at increased risk, e. g., medical personnel, persons in establishments dealing extensively with the public, as well as persons who may be possible sources of infection by caring for individuals at particular risk.

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Both mechanisms, point mutations as well as reassortment, can affect the efficacy of anti-influenza drugs. Influenza occurs globally with an estimated annual attack rate of 5–10% in adults and 20–30% in children. The duration of the disease is usually 5–7 days, which can be considerably longer depending on complications and risk factors (Robinson and colab., 2017; Grohskopf and colab., 2017; Troy and colab., 2018)

The currently available influenza medications

In Germany prescription medicines from two classes of active substances are approved for prevention and therapy of influenza infection.

The M2 ion channel inhibitor - ***amantadine*** belongs to the group of adamantanes and blocks the release of viral RNA into the cytoplasm of the host cell. This effect is achieved with therapeutic dosage of the active substance only with influenza A not with influenza B viruses because of different structure of the ion channel in both influenza species. Due to the current resistance patterns of circulating viruses, the clinical use of adamantanes is not recommended presently.Thus, amantadine is currently not used for the treatment of influenza infections.(Drake and colab., 1993; Bell and colab., 2006; Auewarakul and colab., 2007; http://www.who.int/influenza/human_animal_interface/EN_GIP_20120810CumulativeNumberH5N1cases.pdf .; Matsuzaki and colab., 2007; Medline, 2012).

The other inhibitories therapeutic molecules (Susanne Duwe. GMS Infectious Diseases 2017, Vol. 5, ISSN 2195-8831

- ***Favipiravir (Avigan),*** an RNA synthesis inhibitor, was approved in Japan in 2014 although the indication is limited for treatment of novel influenza viruses unresponsive to other agents.
- ***Pimodivir*** **(**JNJ-63623872, VX-787), a PB2 cap-binding inhibitor, has demonstrated virological efficacy alone and in combination with oseltamivir in uncomplicated influenza, but is only active against influenza A viruses.Therefore, continued efforts to discover and develop influenza drugs with improved properties are still required.
- **The compounds oseltamivir (Tamiflu™) and zanamivir (Relenza™**) belong to the group of neuraminidase inhibitors and were approved and authorized by the European Medicines Agency (EMA) for prevention and treatment of influenza in the European Union/European Economic Area (EU/EEA) in 2002 and 1999. They inhibit selectively the neuraminidase of influenza A and B viruses. Thus, the release of new viruses from infected cells is prevented. Oseltamivir is admitted by EMA for adults and children including full-term newborns. It represents a prodrug and is available as oseltamivir phosphate in the form of capsules and as a powder for oral suspension. The compound is biotransformed by esterases in the intestinal tract and in the liver into the active metabolite oseltamivir carboxylate. The most common possible side effects include nausea, vomiting and headache.

Antiviral therapy in neonates and young infants was therefore mixed with lactose and inhaled as a dry powder. Undesirable side effects are headache, diarrhea, nausea, and vomiting.

Zanamivir is approved by EMA for treatment and prevention of influenza infections in patients from five years old. EMA issued in 2011 a summary on compassionate use for intravenous (i.v.) zanamivir in a specific targeted population and should be considered only to treat i.v. critically ill adults and children having a life-threatening condition. In 2014 the neuraminidase inhibitors oseltamivir and zanamivir have been subject for critically discussion concerning their effectiveness and safety, as well as the appropriateness of stockpiling these drugs for use in future influenza.

An expert opinion from the European Center for Disease Prevention and Control (ECDC) and national authorities as well as national professional virological, medical and therapeutical associations (GfV e.V., DVV e.V, PEG e.V) confirmed earlier assessments that there is no significant.

A new antiviral drug shows promise for treating acute, uncomplicated influenza — including avian and oseltamivir-resistant strains - in adults and children older than age 12 years, new research shows.

In this general review we present this recent study focused on Baloxavir therapy.

The name of this new inhibitor is: BALOXAVIR MARBOXIL. A single dose of Baloxavir marboxil, a first-in-class drug that inhibits a viral protein essential for influenza replication, significantly reduced the duration of flu symptoms, duration of fever, length of time of viral shedding, and levels of virus in the nose and throat compared with placebo or oseltamivir among otherwise healthy people with the flu in two randomized controlled trials. (Frederick and colab., 2018).

The heterotrimeric RNA-dependent RNA polymerase (RdRp) of influenza virus is composed of subunits PA, PB1 **and PB2.** It is responsible for replication and transcription of the segmented, single-stranded viral RNA genome (vRNA) in the nucleus of infected cells.Transcription of viral mRNAs occurs through a unique "cap-snatching" mechanism. This involves high-jacking host RNA polymerase II by binding of nascent capped transcripts to the PB2 subunit followed by cleavage at nucleotides 10–13 by *the cap-dependent endonuclease (CEN) in the PA subunit.* The short, capped oligomers so generated serve as primers for transcription of viral mRNA by the RdRp function of the PB1 subunit. The viral transcripts are poly-adenylated by a stuttering mechanism at a conserved U-rich region of the template vRNA for translation to functional proteins after nuclear export.

Since cap-snatching is essential for virus replication, the cap-binding, endonuclease and RdRp activities are all attractive targets for small molecule inhibitors, and indeed several novel compounds targeting the polymerase are under active clinical development.

On other alternative approach to influenza therapeutics, recently developed is ***Baloxavir acid (S-033447; BXA) and its prodrug - baloxavir marboxil (S-033188; BXM), in which a phenolic hydroxyl group was added to enhance oral absorption of BXA***.

Following successful preclinical trials and a safety trial in which healthy volunteers received a single dose (up to 80 mg) of baloxavir without evident safety concerns, researchers conducted phase 2 and phase 3 randomized controlled trials to assess the safety and efficacy of single-dose baloxavir treatment in otherwise healthy persons with acute influenza.

The phase 2 trial was a double-blind, placebo-controlled, dose-ranging, randomized trial of single doses of baloxavir (10, 20, or 40 mg) or placebo. The trial enrolled 400 Japanese adults 20 to 64 years of age with acute influenza [predominantly A(H1N1)pdm09 virus] between December 2015 and March 2016.

The double-blind, placebo- and oseltamivir-controlled, randomized phase 3 (CAPSTONE-1) trial enrolled 1436 outpatients, 12 to 64 years of age, with influenza-like illness in the United States and Japan between December 2016 and March 2017. Patients aged 20 to 64 years were randomly assigned in a ratio of 2:1:2 to receive a single one-time oral dose of 40 or 80 mg of baloxavir according to body weight, a placebo, or 75 mg of oseltamivir twice daily for 5 days.

Patients aged 12 to 19 years were randomly assigned in a ratio of 2:1 to receive a single dose of baloxavir or a placebo. Those who weighed less than 80 kg received 40 mg of baloxavir, and those who weighed more than 80 kg received 80 mg.

BXA was developed by rational molecular design based on the two-metal pharmacophore concept for dolutegravir (DTG), a strand transfer inhibitor of human immunodeficiency virus (HIV) integrase. Both CEN and HIV integrase use two divalent metal ions as cofactors for their endonuclease activities. Since DTG binds to these ions in the HIV integrase active site, the metal-chelating chemical scaffold of DTG could also be used for the development of CEN inhibitors. The authors of this initially studies are screened the metal-chelating compounds by CEN enzymatic assays, followed by a cellular phenotypic screen and then optimized the chemical structure to improve the pharmacokinetics and safety. This culminated in the generation of the compounds BXA and BXM that target the CEN in the PA proteins of influenza A and B viruses. Because amino acids in the active site of CEN are well conserved across seasonal, pandemic, and highly pathogenic avian influenza viruses, BXA/BXM has the potential to be a new broad-spectrum, therapeutic class of anti-influenza therapy. Whereas single doses of BXM achieved a profound decline in viral titers of swabs, resulting in a amelioration of influenza symptoms in uncomplicated influenza in clinical studies, information on the mechanism of BXA binding to PA and potential determinants for reduced susceptibility are limited. Some aminoacid substitutions are detected in the clinical studies of BXM and the impact they have on drug susceptibility and viral replicative capacity. For understanding better the molecular mechanisms involved, are determined the co-crystal structures of wild-type and I38T endonuclease domains from influenza A and B viruses with bound BXA. The virological and structural which have demonstrated some results regarding a highlight importance of substitutions at PA residue *Ile38* in the mechanism of reduced sensitivity to BXM and BXA.

A multi-center, randomized, double-blind, controlled phase 2 study was conducted during the 2015-6 influenza seasons with BXM in Japanese adults aged 20–64 years with uncomplicated influenza (Japic CTI-153090). In the subsequent 2016-7 influenza seasons, an open-label study was conducted with BXM in otherwise healthy pediatric patients aged 6 months to <12 years with uncomplicated influenza (Japic CTI-163417). *In the clinical trials*, baseline variant monitoring was conducted to evaluate BXA susceptibility of the viruses in the baseline samples from nasopharyngeal/pharyngeal swabs. In addition, monitoring was performed in paired pre- and last positive swab samples from BXM treated patients to identify treatment-emergent AA substitutions that were associated with reduced susceptibility to BXA. In order to investigate the impact of the substitutions, reverse genetics was employed to generate *the recombinant variant viruses* in the corresponding influenza type/subtype, *followed by drug sensitivity testing* by means of plaque reduction with BXA and favipiravir or NA inhibition assays with oseltamivir acid.

In the baseline monitoring of the phase 2 study, the viruses collected from nasopharyngeal/pharyngeal swabs were propagated in MDCK-SIAT1 cells to obtain sufficient quantity for phenotypic analysis.

The treatment-emergent monitoring in the phase 2 study identified I38T or I38F substitutions in A/H1N1 viruses as conferring more than 10-fold reductions in BXA susceptibility,whereas E23K viral mutation had a significant but lesser impact. Given that I38 is highly conserved and that I38T and I38F were not detected in A/H1N1, A/H3N2 or type B viruses according to the NCBI database suggested *that the A/H1N1 I38T or I38F viruses emerged as a result of exposure to BXA therapy.*

BXA sensitivity of viruses with amino acid substitutions detected in a pediatric study

Similar monitoring was performed with the pediatric study in the subsequent influenza season. In the baseline monitoring, the viruses in nasopharyngeal/pharyngeal swabs were propagated in MDCK cells to obtain sufficient amount of virus stocks, and the EC_{50} values of BXA were determined by the *ViroSpot assay*.

In the treatment-emergent variant monitoring of the pediatric study, viral RNAs collected from 77 patients who had the paired swabs available at baseline and the last positive, were extracted without virus amplification to avoid introduction of unwanted mutations. The PA regions were genotyped by Sanger sequencing at the lower limit of sequencing defined of 4 $\log_{10}10$ (viral particle/mL). . Extensive sequencing of the viral RNA from 25 patients who exhibited rebound in viral titre resulted in identification of the I38T change in A/H3N2 virus for three patients. Drug testing showed that the FCs of A/H3N2 with the single mutation of I38T or I38M were 56.59 and 13.77-fold, respectively.

Effect of resistance mutations to other endonuclease inhibitors on susceptibility to BXA

It has been reported that PA E119D confers resistance to the compound L-742,001 that targets the CEN with a metal chelating mechanism similar to BXA3. The authors of theses studyes are evaluated the antiviral effect of BXA to type A and B viruses with the PA mutations E119D and E120D, respectively.They found that E119D conferred slightly reduced susceptibility to BXA by 6.46 and 4.51-fold in A/H1N1 and A/H3N2, respectively, while E120D in type B virus did not have a significant impact.

An very important result of this trials are

Cross-resistance of BXA, favipiravir and oseltamivir acid

To test for cross-resistance, we evaluated the sensitivity to favipiravir with the panel of PA variant viruses. This revealed no cross-resistance.one of the NA inhibitor-resistant mutations, NA/H274Y, was introduced in A/H1N1 and the susceptibility to BXA was determined. Whilst NA/H274Y results in a more than 200-fold reduction in susceptibility to oseltamivir acid, BXA showed potent activity against these viruses. No significant cross resistance to the NA inhibitor was observed for any of PA variant viruses generated in this study). Together, no cross-resistance of the viruses with less susceptibility to BXA was observed for favipiravir and oseltamivir acid.

Structural analysis of influenza A and B WT and I38T endonuclease with bound BXA

To give structural insight into the mechanism of reduced susceptibility of the I38T variant, the study team are co-crystallised BXA with the PA endonuclease domain from influenza A and B virus polymerase with either I38 or T38. The four co-crystal structures were determined at 1.8 to 2.3 Å resolution. In addition, a structure of the influenza B domain with the I38T mutation was determined without bound compound at 1.8 Å resolution.

SOME CONCLUSIONS

This very complexes studies shows that a minority of treated patients select for influenza variants that escape the high potency of BXA and we give a structure-based mechanistic explanation of how the most prominent of these, I38T, reduces susceptibility to the drug. However, these variant viruses have reduced fitness and may not propagate in the absence of the selective pressure imposed by the compound. It remains to be seen how this might impact future use of the drug. Importantly, no correlation of the emergence of I38T or other substitutions with clinical influenza symptoms or relapse/persistence of fever has emerged so far. However, these variant viruses have reduced fitness and may not propagate in the absence of the selective pressure imposed by the compound. It remains to be seen how this might impact future use of the drug. Importantly, no correlation of the emergence of I38T or other substitutions with clinical influenza symptoms or relapse/persistence of fever has emerged so far.

Probably, a most important conclusion of this trials,are that: *"A further large scale clinical investigations will be needed to shed more light on the relationship between the treatment-emergent variants and clinical symptoms. Finally, our results provide markers of reduced susceptibility to BXM which will be of value in future surveillance of treated populations."*

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