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Influenza viruses are a major threat to human health globally. In addition to further improving vaccine prophylaxis, disease management through antiviral therapeutics constitutes an important component of the current intervention strategy to prevent advance to complicated disease and reduce case-fatality rates. Standard-of-care is treatment with neuraminidase inhibitors that prevent viral dissemination. In 2018, the first mechanistically new influenza drug class for the treatment of uncomplicated seasonal influenza in 2 decades was approved for human use. Targeting the PA endonuclease subunit of the viral polymerase complex, this class suppresses viral replication. However, the genetic barrier against viral resistance to both drug classes is low, pre-existing resistance is observed in circulating strains, and resistant viruses are pathogenic and transmit efficiently. Addressing the resistance problem has emerged as an important objective for the development of next-generation influenza virus therapeutics. This review will discuss the status of influenza therapeutics including the endonuclease inhibitor baloxavir marboxil after its first year of clinical use and evaluate a subset of direct-acting antiviral candidates in different stages of preclinical and clinical development. (Translational Research 2020; 220:33–42)

Abbreviations: RBC = red blood cells; PK = pharmacokinetics; CDC = centers for disease control and prevention; IAV = influenza A virus; IBV = influenza B virus; RdRP = RNA-dependent RNA polymerase

INTRODUCTION

Influenza virus affects approximately 10% of the population during every season. In most healthy individuals, these infections predominantly result in relatively mild, self-limiting disease that remains restricted to the upper respiratory tract and does not require therapeutic intervention. Reflecting the overall high disease prevalence, however, the World Health Organization estimates that 3–5 million infections lead to severe disease that advances to the lower respiratory tract and viral pneumonia, resulting in up to 650,000 deaths annually. High-risk groups for severe influenza infection include older adults, the immunocompromized, pregnant women, people with underlying pulmonary conditions, and, to a lesser degree, the very young. Yearly vaccination is recommended for everyone older than 6 months of age, but vaccine efficacy varies substantially based on how well circulating viruses and vaccine strains are matched, patient age and patient influenza history. In the 2017/18 influenza season, for instance, vaccine efficacy against the predominant H3N2 strain was only 25%, leading to the highest mortality rate since the 2009 H1N1 pandemic. Although disease burden was particularly high in that season, vaccination efficacy was on average below 50% also in...
the preceding years also. Moreover, effectiveness of the influenza vaccine is particularly low in older adults, leaving one of the primary at-risk groups poorly protected (reviewed in 5). Due to these limitations to vaccine prophylaxis combined with continued high disease burden caused by seasonal influenza viruses, the threat of spill-over of highly pathogenic avian influenza viruses into the human population and a low barrier to viral escape of standard-of-care therapeutics (discussed in detail below), effective novel antiviral therapeutics are urgently needed for improved disease management especially in high risk patients and for heightened preparedness against the risk of future global pandemics.

**INFLUENZA VIRUS RESISTANCE TO ANTIVIRALS**

All currently approved influenza drugs interfere with viral protein function and therefore belong to the group of direct-acting antivirals (DAAs). In comparison with indirectly acting host-directed experimental antivirals, drugs of the DAA group have a lower tendency for undesirable side effects. However, rapid development of viral resistance has emerged as the predominant liability of DAAs, especially when directed against RNA viruses with error prone polymerases such as respiratory syncytial virus (RSV) or the influenza viruses.

Exemplifying the scope of the problem, the adamantanes, amantadine and, subsequently, rimantadine, were the first drugs approved for the treatment of influenza A virus (IAV) infections. These inhibitors target the viral M2 ion channel, preventing dissociation of the viral ribonucleoprotein (RNP) genome from the matrix protein by blocking M2-mediated diffusion of protons into virions located in maturing endosomes. For some IAV strains, they can also affect virion assembly by disturbing M2-mediated pH-equilibration of the late Golgi. However, the administration of the adamantanes has been discouraged by the CDC for more than a decade due to widespread pre-existing viral resistance. The 2019 influenza season, for instance, >99% of circulating H3N2 and H1N1 strains carried resistance mutations to amantadine. This high prevalence of resistance to the adamantanes is thought to directly reflect their extensive use in the poultry industry, which also increased the risk of prolonged exposure of human viruses to the drug, since the adamantanes were detectable in agricultural animals for up to 13 days after treatment. In addition to seasonal influenza virus strains, signature amantadine resistance mutations were detected in highly pathogenic avian H5N1 strains. To lower the risk of triggering the emergence of resistance against other influenza drug classes in circulating viruses, many countries including the United States and China have banned their use in agriculture.

In the past 2 decades, major research efforts have been directed towards the discovery of host-directed antiviral drugs, lured by the prospect of a high barrier of these therapeutics against the development of resistance. Mechanistically unique is the host-directed viral entry inhibitor DAS181, a recombinant sialidase fusion protein, which targets cellular sialic acid residues required for influenza virus infection. In contrast to this extracellular host target, the majority of experimental host-directed antivirals aim to modulate host immune responses or have been developed against intracellular processes that are critical for successful virus replication (reviewed in 17). The challenge, however, is the identification of a druggable intracellular target that has a major impact on virus replication but
is dispensable for host function. Reflecting the conundrum that antiviral efficacy and undesirable adverse effects of host-directed antivirals follow in most cases the same structure-activity relationship, no intracellularly acting host-directed candidate has been approved yet for clinical use against influenza viruses\textsuperscript{29,30} or other respiratory RNA viruses of the myxovirus and pneumovirus families.

**CLINICALLY USED DRUGS AND SELECTED DAA CANDIDATES IN ADVANCED STAGES OF DEVELOPMENT**

Table 1 shows influenza drugs currently approved and recommended for clinical use.

**Neuraminidase inhibitors.** Of the NAIs in clinical use, oseltamivir (Fig 1), peramivir, and zanamivir, oseltamivir carboxylate is the most frequently prescribed and considered standard-of-care for influenza management since essentially all circulating influenza viruses have acquired resistance to the adamantanes. Mechanistically, NAIs competitively inhibits the enzymatic activity of IAV and influenza B virus (IBV) neuraminidases, preventing cleavage of the terminal sialic acid residues from glycoproteins and carbohydrates displayed on the surface of mammalian cells and influenza virus particles. Binding of virions to uncleaved sialic acid then impairs virion release and dissemination.\textsuperscript{31}

Oseltamivir is orally bioavailable and well-tolerated. Reflecting this excellent safety profile, the drug has been approved for use also in pregnant women, pediatric patients, and neonates.\textsuperscript{29,32} As discussed above, clinical trials have shown that NAIs provide benefit when started early after infection.\textsuperscript{9,33} However, meta-analyses of clinical data revealed little effect on reducing influenza-associated complications or hospitalization,\textsuperscript{34-37} and some data suggested that late treatment with NAIs could have enhanced the risk of progression to severe disease in the 2009/2010 pandemic.\textsuperscript{38}

Resistance to oseltamivir can develop rapidly in both experimental settings and the clinic, and typically originates from substitutions at signature resistance sites in the viral NA protein such as H274Y and I223R (predominant in H1N1 and H5N1 viruses), and E119V, R292K, or N294S (predominant in H3N2 viruses).\textsuperscript{39,40} Major resistance mutations to zanamivir are NA substitutions Q136K and I223R (H1N1 viruses) and Q136K (H3N2 viruses), respectively.\textsuperscript{41} Whereas resistance to the NAIs is relatively rare in currently endemic influenza virus strains,\textsuperscript{42,43} the prevalence of escape mutations increased rapidly in the 2007/2008 influenza season\textsuperscript{44} and approximately 90% of strains circulating during the 2008/2009 season were resistant to NAIs,\textsuperscript{45-47} generating concern that this drug class could also be lost to resistance in the future.\textsuperscript{48}

**Neutralizing antibodies.** Although no biologics have been approved for influenza therapy so far, neutralizing antibodies (nAbs) in particular have been extensively tested. Substantiated by the precedent established, for instance, by nAbs used clinically against RSV\textsuperscript{49} and

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**Table 1. Influenza therapeutics currently approved for clinical use**

| Drug              | Target protein | Route of administration | Patient age group | Target virus |
|-------------------|----------------|-------------------------|-------------------|--------------|
| Baloxavir marboxil| PA             | oral (PO)               | ≥ 12 years        | IAV and IBV  |
| Oseltamivir       | NA             | oral (PO)               | ≥ 1 year          | IAV and IBV  |
| Peramivir         | NA             | Intravenous             | ≥ 2 years         | IAV and IBV  |
| Zanamivir         | NA             | Inhalation              | ≥ 5 years         | IAV and IBV  |
| Amantadine\*      | M2             | oral (PO)               | ≥ 1 year          | IAV          |
| Rimantadine\*     | M2             | oral (PO)               | ≥ 1 year          | IAV          |
| Favipiravir\*     | RdRP           | oral (PO)               | adults            | IAV and IBV  |

Abbreviations: IAV, influenza A virus; IBV, influenza B virus; RdRP, RNA-dependent RNA polymerase.

*not recommended for antiviral treatment against currently circulating influenza virus strains due to high level of preexisting resistance

\*conditionally approved in Japan against emerging influenza viruses resistant to other antivirals

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**Fig 1.** Chemical structures of oseltamivir carboxylate, baloxavir marboxil, favipiravir, EIDD-2801, and pimodivir.
Ebola virus infection, antibody-based therapeutics are typically well-tolerated and show favorable pharmacokinetic profiles. The influenza virus HA head domain containing the receptor binding sites (RBCs) is highly immunogenic and thus a primary target for nAbs. However, these head-directed anti-influenza nAbs typically show a very narrow indication spectrum, hampering their clinical development. Although some nAbs directed against the more conserved RBC have been identified, alternative targeting of the less variable stalk domain of the HA trimer has attracted major attention in recent years, due to cross-reactivity with multiple HA subtypes.

Three influenza virus HA stalk-targeting broadly neutralizing Abs (bnAbs), MHAA4548A, MEDI8852, and VIS410, have advanced to phase 2 clinical trials and demonstrated some antiviral efficacy when dosed therapeutically, accelerating symptom resolution and reducing virus replication. A half-life of approximately 3 weeks in humans makes therapeutic antibodies compatible with attractive single-dose administration. Whereas no resistant viruses emerged in these clinical trials, 2 viral escape pathways from HA stalk-directed antibodies have been identified experimentally: after viral adaptation in cell culture, resistant variants either carried a point mutation in the stalk epitope preventing antibody binding, or harbored substitutions that enhanced HA fusion activity and therefore allowed viral entry in the presence of antibody. These experimental resistance mutations affected viral fitness, however, reducing viral replication in cell culture.

Major concerns for the use of broadly reactive Abs against uncomplicated seasonal influenza are the target restriction to IAVs, the lack of oral bioavailability, a necessity for high-dose injections as a consequence of poor distribution from blood to the upper airways, and the high manufacturing costs of biologics. Since both IAVs and IBVs are human pathogens and clinically relevant, efficacy against pathogens of both genera should constitute a developmental objective of next-generation drugs. This target breadth cannot be achieved with traditional bnAbs. However, engineering of multidomain antibodies has been explored experimentally and proof-of-concept efficacy against both IAVs and IBVs has been demonstrated in mice, potentially illuminating a path to overcome this limitation. Furthermore, better compliance from the large patient group of nonhospitalized adults suffering from uncomplicated disease is expected when the drug is orally bioavailable and amenable to cost-effective manufacture. bnAbs appear therefore best suited for use in high risk groups or hospitalized patients with complicated disease.

Inhibitors of the viral polymerase complex. In the last decade, the viral RNA-dependent RNA polymerase (RdRP) complex has emerged as an important target for novel small-molecule therapeutics that have progressed to advanced development and/or clinical testing. Influenza virus RdRP is comprised of 3 subunits, the PB1, PB2, and PA proteins, that are responsible for both replication and transcription of the 8 genome segments. The segments do not exist as naked RNA but are encapsidated by viral NP proteins, forming RNP complexes. Whereas replication requires the generation of complementary positive polarity RNA intermediates (cRNA) that are then copied into progeny negative polarity segments (vRNPs), viral message is directly synthesized from vRNPs. Since the influenza virus RdRP lacks enzymatic activity to form 5’ mRNA cap structures, endonuclease activity of the PA subunit is instrumental for the generation of bioactive viral mRNAs through transfer of 5’-capped RNA primers derived from host mRNAs in a cap-snatching mechanism. PB2 is involved in binding of the capped primers, whereas the PB1 subunit harbors enzymatic activity for phosphodiester bond formation. The overall replication strategy and RdRP organization is highly conserved across all IAV subtypes and among IAVs and IBVs, increasing the likelihood that therapeutic targeting of the polymerase complex will result in the development of broad-spectrum influenza virus inhibitors.

**Baloxavir marboxil** – founding member of the PA endonuclease inhibitor class. Licensed in Japan and the United States since 2018, baloxavir marboxil (Fig 1) is the first new influenza drug class approved in 2 decades for the treatment of uncomplicated influenza infection in patients older than 12 years of age. Taken orally, baloxavir marboxil is a prodrug that prevents cap-snatching by blocking PA endonuclease activity when hydrolyzed to free baloxavir acid. A single dose is typically sufficient for clinical benefit, and efficacy is similar to, or better than, current standard-of-care. In clinical trials, participants showed significantly lower viral burden 1 day after initiation of treatment compared to placebo groups, and the time to alleviation of clinical signs was decreased on average by approximately 26 hours, which resembled the improvements that can be achieved with NAIs. Quite remarkably, twice daily experimental treatment of mice infected with a lethal dose of a highly pathogenic avian IAV resulted in complete survival of the animals and reduction of viral load in the respiratory tract. Similarly profound efficacy was observed in immunocompromized mice. In addition to activity against IAVs, IBVs and influenza viruses of the C and D genera are susceptible to baloxavir inhibition, making the drug a truly broad-spectrum influenza virus inhibitor.
resolved without further intervention. Side effects included headaches and increased eosinophil and white blood cell counts. PK properties in pediatric recipients were similar to those seen in adults, and a trial involving 107 pediatric patients receiving 1 weight-adjusted dose of baloxavir marboxil established equivalent efficacy in adults and children against uncomplicated influenza. 

Although efficacy of baloxavir marboxil and NAI s were comparable, baloxavir marboxil provides full benefit after a single oral dose, giving the drug an advantage over current standard-of-care. Consistent with distinct protein targets of baloxavir marboxil and the NAI s, viruses harboring signature NAI resistance mutations were shown to remain susceptible to inhibition by baloxavir marboxil. 

Despite the exciting safety and efficacy performance of baloxavir marboxil, resistant viruses emerged in up to 9.7% of adult clinical trial participants and 23.4% of children. In several cases, the emergence of resistance coincided with a rebounds in shed virus titers and prolonged manifestation of clinical signs. Resembling resistance profiles generated for different PA endonuclease inhibitor chemotypes in cell culture, the signature hotspot for escape from baloxavir marboxil is PA residue 38, for which several substitutions (PA I38T/M/F) have been described. The initial characterization of PA I38 mutant viruses in cell culture suggested that resistance may carry a severe fitness penalty. However, subsequent follow-up analyses of the fitness of H1N1 and H3N2 strains containing PA I38 mutants in the ferret model revealed that pathogenesis and transmission success of resistant and the corresponding sensitive viruses were equivalent. In mice and ferrets, both loss of animal body weight and lung viral loads were identical after infection with resistant or sensitive viruses, and substitutions at PA I38 remained stable over 4 passages through mice. A small percentage of influenza viruses circulating in the United States in the 2016/2017 and 2017/2018 seasons, and thus prior to approval of baloxavir marboxil, contained mutations at PA residue 38. Moreover, a resistant H3N2 virus was detected in a pediatric patient without prior exposure to baloxavir marboxil, supporting that this mutant strain spread between humans. Even though baloxavir marboxil has not yet been used extensively in the clinic and long-term field data are scarce, the available evidence indicates that the genetic barrier against resistance to the drug is low and that escape comes with little penalty to viral fitness, pathogenesis, or transmission success. Accordingly, there is considerable risk that pre-existing resistance to baloxavir marboxil could become widespread with increasing duration of clinical use.

Favipiravir (T-705) - broad-spectrum ribonucleoside analog inhibitor of RNA viruses. T-705 is a broad-spectrum pyrazinecarboxamide (Fig 1) that is converted intracellularly to a ribonucleoside analog through phosphoribosylation, thus competitively targeting the viral RdRP complex. Anabolism efficiency can vary between cells of different tissue origin, but has been shown to be low in disease-relevant primary human airway epithelium cells. The drug has broad-spectrum antiviral activity in cell culture, inhibiting RNA viruses of the arenavirus, bunyavirus, flavivirus, alphavirus, norovirus, picornavirus, paramyxovirus, and rhabdovirus families, in addition to influenza viruses.

Mechanistically, T-705 is incorporated into newly synthesized RNA by the viral polymerase in place of purines but not pyrimidines, resulting in increased frequencies of C-to-U and G-to-A transition mutations as demonstrated for influenza viruses and hepatitis C virus both in vitro and in vivo. Collapse of virus replication is considered to be a consequence of error catastrophe or lethal mutagenesis, resulting from the accumulation of random low-frequency mutations in the viral genome (Fig 2). The genetic barrier of T-705 against resistance is favorably high, although a recent study has identified a pair of point mutations in the PB1 (K229R) and PA (P659L) polymerase subunits that reduce viral sensitivity to the compound by approximately 30-fold in cell culture.

Safety testing revealed that T-705 is teratogenic and embryotoxic at concentrations close to the approved human dose. Although conditionally licensed in Japan for the treatment of pathogenic influenza viruses resistant to other therapeutics, these safety concerns prevent drug administration to some high-risk groups such as children or pregnant women. Activity assessment against other potential viral targets in animal models furthermore revealed that in vivo efficacy is quite poor and dependent on extremely high dose concentrations. These results lower the clinical value of T-705 and undermine its use against viruses predominantly associated with pediatric diseases, such as pathogens of the pneumovirus and paramyxovirus families.

EIDD-2801 – next-generation influenza virus inhibitor candidate in early development. A search for inhibitors with broad antirespiratory RNA virus activity yielded the ribonucleoside analog N4-hydroxycytidine (NHC), which showed oral efficacy against RSV and both highly-pathogenic avian and seasonal influenza viruses in mouse models. Mechanistic characterization revealed that the compound is efficiently anabolized to the bioactive tri-phosphate form in ex vivo primary human airway epithelium cultures and in animal tissues, followed by incorporation as cytidine into viral RNA by influenza virus polymerases. Tautomeric interconversions then cause base-pairing of the incorporated analog either as cytosine or uracil, resulting in high-frequency random C-to-U and G-to-A transition
mutations in viral RNA replication or transcription products and thus inhibition through error catastrophe. Additional studies have expanded the antiviral target spectrum of NHC to viruses of the flavivirus, alphavirus, coronavirus, and togavirus families, demonstrating broad anti-RNA virus activity.

Whereas rodent PK properties of NHC were good, oral bioavailability of the drug in non-human primates such as cynomolgus macaques was limited. This liability was addressed through the development of the prodrug EIDD-2801, a 5'-isopropylester (Fig 1) of NHC, which demonstrated good oral bioavailability in all species tested including nonhuman primates. Upon intestinal absorption, EIDD-2801 was efficiently hydrolyzed to free NHC. Oral daily doses of 14 mg/kg EIDD-2801 were reportedly efficacious against H1N1 and H3N2 IAV strains in the ferret model, causing significant reduction of viral load in both the upper and lower respiratory tract and alleviation of clinical signs. The compound showed potent antiviral activity in disease-relevant well-differentiated human airway epithelium model cultures, inhibiting both IAV and IBV strains with active concentrations around 0.08 μM and selectivity indices >1700.

EIDD-2801/NHC appears to establish an unusually high barrier against viral resistance, since several studies have unsuccessfully tried to induce viral escape through dose-escalation and sublethal fixed-dose adaptation strategies. Next-generation sequencing of the resulting treatment-experienced virus populations revealed an elevated number of low-frequency mutations, but none of these became allele-dominant or mediated robust resistance to the drug. Although these results are encouraging, adaptation through serial passaging in vivo will likely be necessary to better predict whether resistance to EIDD-2801/NHC could ultimately emerge.

Toxicity concerns are a major potential liability of ribonucleoside analogs as underscored, for instance, by the compromised safety profile of T-705. A primary source for adverse effects is incorporation of the ribonucleoside analogs into host RNAs by nuclear and mitochondrial RNA polymerases. The integrity of mitochondrial transcripts is particularly at risk due to the proofreading inability of mitochondrial RNA polymerases in contrast to nuclear RNA Pol II. However, NHC was incorporated by human mitochondrial polymerases in in vitro studies and an elevated frequency of NHC-characteristic transition events was noted after prolonged exposure of primary human airway epithelium cells to the drug. However, the transition mutation frequency in ferret lung tissue was unchanged from that in controls after extended treatment of animals with 200 mg/kg daily dose and thus over 14-times the efficacious dose for influenza therapy. Potential adverse effects of EIDD-2801 on embryo development are untested at present, and formal 2-species multi-dose toxicity studies will be required to establish initial human doses examined in phase 1 clinical trials.

**Pimodivir (VX-787)** – PB2 inhibitor in clinical development. Pimodivir is a cyclohexyl carboxylic acid analogue (Fig 1) that inhibits influenza virus replication

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REFERENCES

1. Reeves JD, Gallo SA, Ahmad N, et al. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc Natl Acad Sci U S A 2002;99:16249–54.
2. Umino Y, Kohama T, Sato TA, Sugira A, Klenk HD, Rott R. Monoclonal antibodies to three structural proteins of Newcastle disease virus: biological characterization with particular reference to the conformational change of envelope glycoproteins associated with proteolytic cleavage. J Gen Virol 1990;71(Pt 5):1189–97.
3. Garten R, Blanton L, Elal AIA, Alabi N, Barnes J, Biggerstaff M, et al. Update: influenza activity in the United States during the 2017–18 season and composition of the 2018–19 influenza vaccine. MMWR Morb Mortal Wkly Rep 2018;67:634–42.
4. Doyle JD, Chang JR, Kim SS, et al. Interim estimates of 2018-19 seasonal influenza vaccine effectiveness—United States, February 2019. MMWR Morb Mortal Wkly Rep. 2019;68:135–9.
5. Dugan HL, Henry C, Wilson PC. Aging and influenza vaccine-induced immunity. Cell Immunol 2019:103998.
6. Baccam P, Beauchemin C, Macken CA, Hayden FG, Perelson AS. Kinetics of influenza A virus infection in humans. J Virol 2006;80:7590–9.
7. Dobson J, Whitley RJ, Pocock S, Monto AS. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. Lancet 2015;385:1729–37.
8. Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. Cochrane Database Syst Rev 2014;348:CD008965.
9. Aoki FY, Macleod MD, Paggiaro P, et al. Early administration of oral oseltamivir increases the benefits of influenza treatment. J Antimicrob Chemother 2003:51:123–9.
10. Trenor JJ, Hayden FG, Vroomon PS, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. JAMA. 2000;283:1016–24.
11. Nicholson KG, Aoki FY, Osterhaus AD, et al. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet. 2000;355:1845–50.
12. Vennula SV, Zhao J, Liu J, Wang X, Biswas S, Hewlett I. Current approaches for diagnosis of influenza virus infections in humans. Viruses 2016;8:96.
13. Jackson RJ, Cooper KL, Tappenden P, et al. Oseltamivir, zanamivir and amantadine in the prevention of influenza: a systematic review. J Infect 2011;62:14–25.
14. Centers for Disease Control and Prevention. Influenza antiviral medications: summary for clinicians; 2019. Available at: https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm#Treatment. (Accessed February 27, 2020).
15. DeVincenzo JP, Whitley RJ, Mackman RL, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N Engl J Med 2014;371:711–22.
16. Chemaly RF, Dadwal SS, Bergeron A, et al. A phase 2, randomized, double-blind, placebo-controlled trial of presatovir for the treatment of respiratory syncytial virus upper respiratory tract infection in hematopoietic-cell transplant recipients. Clin Infect Dis 2019;[epub ahead of print].
17. Hussain M, Galvin HD, Haw TY, Nutsford AN, Hussain M. Drug resistance in influenza A virus: the epidemiology and management. Infect Drug Resist 2017;10:121–34.
proton-selective ion channel. Proc Natl Acad Sci U S A 2008;105:10967–72.

20. Stouffer AL, Acharya R, Salom D, et al. Structural basis for the function and inhibition of an influenza virus proton channel. Nature 2008;451:596–9.

21. Wang J, Qiu JX, Soto C, DeGrado WF. Structural and dynamic mechanisms for the function and inhibition of the M2 proton channel from influenza A virus. Curr Opin Struct Biol 2011;21:68–80.

22. Shiraishi K, Mitamura K, Sakai-Tagawa Y, Goto H, Sugaya N, Kawaoka Y. High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. J Infect Dis 2003;188:57–61.

23. Li KS, Guan Y, Wang J, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 2004;430:209–13.

24. Parry J. Use of antiviral drug in poultry is blamed for drug resistant strains of avian flu. BMJ 2005;331:10.

25. You X, Yang S, Zhao J, et al. Study on the abuse of amantadine in tissues of broiler chickens by HPLC-MS/MS. J Vet Pharmacol Ther 2017;40:539–44.

26. Principi N, Camilloni B, Alunno A, Polinori I, Argentiero A, Esposito S. Drugs for influenza treatment: is there significant prevalence of amantadine resistance among circulating European porcine influenza A viruses. J Gen Virol 2009;90:900–8.

27. Guan Y, Poon LL, Cheung CY, et al. H5N1 influenza: a protean disease. Nature 2004;430:209–13.

28. Wang J, Qiu JX, DeGrado WF. Structural and dynamic mechanisms for the function and inhibition of an influenza virus proton channel. Nature 2008;451:596–9.

29. Bassetti M, Castaldo N, Carnevali A. Neuraminidase inhibitors as a strategy for influenza treatment: pros, cons and future perspectives. Expert Opin Pharmacov 2019;20:1711–8.

30. Ebel MH. Oseltamivir and zanamivir have limited effect on symptoms and do not reduce hospitalisation or serious complications of influenza. Evid Based Med 2014;19:211.

31. Jefferson T, Jones M, Dosil P, Spencer EA, Onakpoya I, Henehan CJ. Oseltamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. BMJ 2014;348:g2545.

32. Michelis B, Van Puyenbroeck K, Verhoeven V, Vermeire E, Coenen S. The value of neuraminidase inhibitors for the prevention and treatment of seasonal influenza: a systematic review of systematic reviews. PLoS One 2013;8:e60348.

33. Tai CY, Escarpe PA, Sidwell RW, et al. Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor GS 4071. Antimicrob Agents Chemother 1998;42:3234–41.

34. Gubareva LV. Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. Virus Res 2004;103:199–203.

35. Han J, Perez J, Schafer A, et al. Influenza virus: small molecule therapeutics and mechanisms of antiviral resistance. Curr Med Chem 2018;25:5115–27.

36. Lee N, Hurt AC. Neuraminidase inhibitor resistance in influenza: a clinical perspective. Curr Opin Infect Dis 2018;31:520–6.

37. Nitsch-Osuch A, Brydak LB. Influenza viruses resistant to neuraminidase inhibitors. Acta Biochim Pol 2014;61:505–8.

38. Hauge SH, Budman S, Borgen K, Lackenby A, Hungnes O. Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. Emerg Infect Dis 2009;15:155–62.

39. Esposito S, Molteni CG, Colombo C, et al. Oseltamivir-induced resistant pandemic A/H1N1 influenza virus in a child with cystic fibrosis and Pseudomonas aeruginosa infection. J Clin Virol 2010;48:62–5.

40. van der Vlies E, Schutten M, Fraaij P, Boucher C, Osterhaus A. Influenza virus resistance to antiviral therapy. Adv Pharmacol 2013;67:217–46.

41. McLaurin KK, Farr AM, Wade SW, Diakum DR, Stewart DL. Respiratory syncytial virus hospitalization outcomes and costs of full-term and preterm infants. J Perinatol 2016;36:990–6.

42. Mulangu S, Dodd LE, Davey RT Jr., Tshiani Mbaya O, Proschan M, Mukadi D, et al. A randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med 2019;381:293–303.

43. Laursen NS, Wilson IA. Broadly neutralizing antibodies against influenza viruses. Antiviral Res 2013;98:476–83.

44. Ekiert DC, Kashyap AK, Steel J, et al. Cross-neutralization of influenza A viruses mediated by a single antibody loop. Nature 2012;489:526–32.

45. Tsibane T, Ekiert DC, Krause JC, et al. Influenza human monoclonal antibody 1F1 interacts with three major antigenic sites and residues mediating human receptor specificity in H1N1 viruses. PLoS Pathog 2012;8:e1000307.

46. Baranovich T, Jones JC, Russier M, et al. The hemagglutinin stem-binding monoclonal antibody VS410 controls influenza virus-induced acute respiratory distress syndrome. Antimicrob Agents Chemother 2016;60:2118–31.

47. Corbi D, Voss J, Farni J, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science 2011;333:850–6.

48. Dreyfus C, Laursen NS, Kwaks T, et al. Highly conserved protective epitopes on influenza B viruses. Science 2012;337:1343–8.

49. Ekiert DC, Friesen RH, Bhabha G, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. Science 2011;333:843–50.

50. Kallewaard NL, Corbi D, Collins PJ, et al. Structure and function analysis of an antibody recognizing all influenza A subtypes. Cell 2016;166:596–608.
59. Tharakaraman K, Subramanian V, Cain D, Sasisekharan V, Sasisekharan R. Broadly neutralizing influenza hemagglutinin stem-specific antibody CR8020 targets residues that are prone to escape due to host selection pressure. Cell Host Microbe 2014;15:644–51.

60. Hershberger E, Sloan S, Narayan K, et al. Safety and efficacy of monoclonal antibody VIS410 in adults with uncomplicated influenza A infection: Results from a randomized, double-blind, phase-2, placebo-controlled study. EBioMedicine 2019;40:574–82.

61. McBride JM, Lim JJ, Burgess T, et al. Phase 2 randomized trial of the safety and efficacy of MHAAl459A, a broadly neutralizing monoclonal antibody, in a human influenza A virus challenge model. Antimicrob Agents Chemother 2017;61.

62. Ali SO, Takas T, Nyborg AC, et al. A phase 2a study to evaluate the safety of MED18852 in outpatient adults with acute, uncomplicated influenza A. Open Forum Infect Dis 2017;4:519.

63. Chai N, Swem LR, Reichtel M, et al. Two escape mechanisms of influenza A virus to a broadly neutralizing stalk-binding antibody. PLoS Pathog 2016;12:e1005702.

64. Kotey E, Lukosiytė D, Quaye O, Ampofo W, Awandare G, Iqbal M. Current and novel approaches in influenza management. Vaccines (Basel) 2019;7:53.

65. Laursen NS, Friesen RHE, Zhu X, et al. Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin. Science 2018;362:598–602.

66. Ortin J, Martin-Benito J. The RNA synthesis machinery of negative-stranded RNA viruses. Virology 2015;479:532–44.

67. Pflug A, Lakarska M, Resa-Infante P, Reich S, Cusack S. Structural insights into RNA synthesis by the influenza virus transcription-replication machine. Virus Res 2017;234:103–17.

68. De Vlugt C, Sikora D, Pelchat M. Insight into Influenza: an update on its structure, functions, and significance for antiviral drug design. Med Res Rev 2016;36:1127–73.

69. Aschenbrenner DS. FDA approves new antiviral for influenza. JAMA 2019;322:2855–6.
97. de Avila AI, Gallego I, Soria ME, et al. Lethal mutagenesis of hepatitis C virus induced by favipiravir. PLoS One 2016;11: e0164691.
98. Arias A, Thorne L, Goodfellow I. Favipiravir elicits antiviral mutagenesis during virus replication in vivo. Elife 2014;3: e03679.
99. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci 2017;93:449–63.
100. Goldhill DH, Langat P, Xie HY, et al. Determining the mutation bias of favipiravir in influenza virus using next-generation sequencing. J Virol 2019;93:e01217-18.
101. Goldhill DH, Te Velthuis AJW, Fletcher RA, et al. The mechanism of resistance to favipiravir in influenza. Proc Natl Acad Sci U S A 2018;115:11613–8.
102. Nagata T, Lefor AK, Hasegawa M, Ishii M. Favipiravir: a new medication for the Ebola virus disease pandemic. Disaster Med Public Health Prep 2015;9:79–81.
103. Delang L, Abdelnabi R, Neyts J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res 2018;153:85–94.
104. Yamada K, Noguchi K, Kimitsuki K, et al. Reevaluation of the efficacy of favipiravir against rabies virus using in vivo imaging analysis. Antiviral Res 2019;172:104641.
105. Jochmans D, van Nieuwkoop S, Smits SL, Neyts J, Fouchier RA, van den Hoogen BG. Antiviral activity of favipiravir (T-705) against a broad range of paramyxoviruses in vitro and against human metapneumovirus in hamsters. Antimicrob Agents Chemother 2016;60:4620–9.
106. Toots M, Yoon JJ, Cox RM, et al. Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia. Sci Transl Med 2019;11: eaax586.
107. Stuyver LJ, Whitaker T, McBrayer TR, et al. Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture. Antimicrob Agents Chemother 2003;47:244–54.
108. Urakova N, Kuznetsova V, Crossman DK, et al. Beta-D-N-4-hydroxycytidine is a potent anti-alphavirus compound that induces a high level of mutations in the viral genome. J Virol 2018;92:e01965-17.
109. Agostini ML, Pruijssers AJ, Chappell JD, et al. Small molecule antiviral beta-D-N (4)-hydroxycytidine inhibits a proofreading-intact coronavirus with a high genetic barrier to resistance. J Virol 2019 [epub ahead of print].
110. Reynard O, Nguyen XN, Alazard-Dany N, Barateau V, Cimarelli A, Volchkov VE. Identification of a new ribonucleoside inhibitor of Ebola virus replication. Viruses 2015;7:6233–40.
111. Painter GR, Bowen RA, Bluemling GR, et al. The prophylactic and therapeutic activity of a broadly active ribonucleoside analogue in a murine model of intranasal venezuelan equine encephalitis virus infection. Antiviral Res 2019;171:104597.
112. Lu G, Bluemling GR, Mao S, et al. Simple in vitro assay to evaluate the incorporation efficiency of ribonucleotide analog 5'-triphosphates into RNA by human mitochondrial DNA-dependent RNA polymerase. Antimicrob Agents Ch 2018:62: e01830-17.
113. Feng JY, Xu YL, Barauskas O, et al. Role of mitochondrial RNA polymerase in the toxicity of nucleotide inhibitors of hepatitis C virus. Antimicrob Agents Ch 2016:60:806–17.
114. Sultana S, Solotchi M, Ramachandran A, Patel SS. Transcriptional fidelities of human mitochondrial POLRMT, yeast mitochondrial Rpo41, and phage T7 single-subunit RNA polymerases. J Biol Chem 2017;292:18145–60.
115. Sticher ZM, Lu G, Mitchell DG, et al. Analysis of the potential for N(4)-hydroxycytidine to inhibit mitochondrial replication and function. Antimicrob Agents Chemother 2020:64.
116. Smee DF, Barnard DL, Jones SM. Activities of JNJ63623872 and oseltamivir against influenza A H1N1pdm and H3N2 virus infections in mice. Antivir Res 2016;136:45–50.
117. Byrn RA, Jones SM, Bennett HB, et al. Preclinical activity of VX-787, a first-in-class, orally bioavailable inhibitor of the influenza virus polymerase PB2 subunit. Antimicrob Agents Ch 2015;59:1574–87.
118. Finberg RW, Lanno R, Anderson D, et al. 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 2019;219:1026–34.
119. Clark MP, Ledeboer MW, Davies I, et al. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. J Med Chem. 2014;57:6668–78.