


Antiviral Approaches against Influenza Virus

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SUMMARY Preventing and controlling influenza virus infection remains a global public health challenge, as it causes seasonal epidemics to unexpected pandemics. These infections are responsible for high morbidity, mortality, and substantial economic impact. Vaccines are the prophylaxis mainstay in the fight against influenza. However, vaccination fails to confer complete protection due to inadequate vaccination coverages, vaccine shortages, and mismatches with circulating strains. Antivirals represent an important prophylactic and therapeutic measure to reduce influenza-associated morbidity and mortality, particularly in high-risk populations. Here, we review current FDA-approved influenza antivirals with their mechanisms of action, and different viral- and host-directed influenza antiviral approaches, including immunomodulatory interventions in clinical development. Furthermore, we also illustrate the potential utility of machine learning in developing next-generation antivirals against influenza.

KEYWORDS antiviral agents, drug resistance mechanisms, influenza, machine learning, monoclonal antibodies

INTRODUCTION

The public health measures intended to curtail SARS-CoV-2 have suppressed the circulation of influenza viruses for the 2020–2021 season (1). However, as coronavirus disease 2019 (COVID-19) restrictions relax worldwide, influenza is reemerging in the United States (US) (2) and globally (1). An estimate of past seasons places the number of deaths between ~291,000 and 646,000 globally in typical years (3), and ~12,000 to 51,000 in the US (4). The individuals with a greater risk of severe disease from influenza include people >65 years of age, children <2 years of age, individuals with comorbidities (i.e., asthma, heart, liver, kidney disease, obesity, etc.), and immunocompromised people (i.e., HIV, leukemia, and others on immunosuppressants) (5).

Influenza is primarily a respiratory disease, and organ systems outside the lungs represent an underappreciated aspect of influenza pathogenesis. Some extrapulmonary complications reported in influenza infection include renal (6), neurological (7), and cardiac (8). In addition, myocarditis, a rare but substantial side effect resulting from mRNA SARS-CoV-2 vaccination (9, 10) and SARS-CoV-2 infection (10), occurs during influenza infections (11).

The health impacts of influenza also have far-reaching effects on the economy. One way to estimate the economic impact of influenza is to assess both direct and indirect costs. These influenza-associated costs include medical care expenses and lost earnings. The estimated economic burden of influenza in the US alone is between 6.3 and 25.3 billion US dollars annually, with the most significant percentage impacting ages 18 to 49 (12).

Effective treatments and preventive measures, including vaccines and antivirals, can reduce health and economic burdens. However, the substantial diversity of influenza viruses impacts these measures. Influenza viruses belong to *Orthomyxoviridae* and are classified into A, B, C, and D types. Influenza A, B, and C viruses can infect humans. Types A and B cocirculate as the primary seasonal strains causing mild to severe

respiratory infections and other complications in humans. Yearly vaccine formulations thus incorporate both types. Influenza viruses are further subdivided into subtypes and lineages based on antigenic characteristics and genetic sequences of the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) (13). Currently, 18 HA and 11 NA (14) subtypes are found in nature for influenza A virus (IAV). Based on its HA, IAV can be categorized into group 1 and group 2.

In contrast, influenza B viruses (IBVs) do not belong to groups or subtypes but are classified into two major lineages, B/Yamagata and B/Victoria. The naming conventions for influenza viruses hint at their diversity. For instance, an IAV designated A/Tasmania/503/2020 is an H3N2 component in the Flucelvax quadrivalent vaccine product for 2021 to 2022 in the US. It is so named because it was the 503rd human isolate from the island state of Tasmania, Australia, possessing an H3 HA and N2 NA subtype isolated in 2020.

Influenza diversification occurs by two main mechanisms, antigenic shift and antigenic drift. When two different influenza viruses within an influenza type coinfect the same cells within an individual, the mixing and matching of viral genome segments occur. A change in HA and NA antigenic characteristics can occur due to this reassortment, and this process is called antigenic shift. For example, the 2009 pandemic virus, initially known as “swine” flu, is a triple-reassortant virus because it contains gene segments of avian-, human-, and swine-origin IAVs (15). Pandemic influenza strains, including the 1918 Spanish flu A (H1N1), 1957 Asian influenza A (H2N2), 1968 Hong Kong influenza A (H3N2), and the 2009 pandemic influenza A (H1N1)pdm09, arose due to antigenic shifts. Antigenic drift, a much slower process, refers to the accumulated genetic mutations within the viral genome over time. Antigenic drift and shift have implications for the genesis of an epidemic, pandemic, and drug-resistant influenza viruses. Such a dynamic viral diversity is also the reason why it is necessary to update vaccines annually.

Vaccination is currently the best method to protect against morbidity and mortality from influenza infection. However, vaccine effectiveness varies by year, population under study, and strain. Overall, the vaccine effectiveness ranged from 10% to 60% in the US from 2004 to 2021 (16). The factors involved include vaccine mismatch (17–19), preexisting influenza immunity, age, weight, biological sex, and immune status (20–25). Efforts to improve vaccine efficacy are a complex problem of urgent concern, as both host and viral factors play crucial roles. Meanwhile, alternatives are needed to fill the gaps caused by vaccine-related issues for prophylactic and therapeutic interventions.

Antiviral drugs have been critical tools in the struggle against influenza viruses. The Centers for Disease Control and Prevention (CDC) recommends four antiviral drugs to treat influenza, Oseltamivir phosphate, Zanamivir, Peramivir, and Baloxavir marboxil (BXM). Oseltamivir, Zanamivir, and Peramivir are NA inhibitors that block NA activity and viral egress from cells, while Baloxavir inhibits viral replication by inhibiting the polymerase acidic protein (PA) (26). These drugs are not a cure but can reduce the time to clinical resolution (27, 28). NA inhibitors and Baloxavir are also recommended for treating humans infected with avian influenza viruses, A(H5N1), A(H7N9), and A(H5N6) (29).

The emergence of drug-resistant influenza strains can render antivirals ineffective (30). The matrix protein 2 (M2) inhibitors, like Amantadine used since the 1960s (31) against seasonal influenza, are no longer recommended. The widespread prevalence of M2 mutations conferring resistance—occurring initially in A(H3N2) viruses between 2003 and 2006 and A(H1N1) viruses in 2009—resulted in the discontinuation of M2 inhibitors in treating influenza (32–36). In addition, NA inhibitor-resistant (36) and Baloxavir-resistant (27) strains have emerged, though their prevalence can change rapidly depending on the specific drug in question and the background strain where the mutation occurs (30, 37). Improving therapeutics against influenza can fill in gaps in vaccine efficacy that are likely to persist in the near and long term, considering the unpredictable nature of seasonal and pandemic influenza outbreaks. This review

focuses on antiviral and immunomodulatory drugs that are US Food and Drug Administration (FDA)-approved or in clinical development against the influenza virus.

INFLUENZA VIRION AND REPLICATION IN HOST CELLS

Influenza is a negative-sense single-stranded RNA (ssRNA) virus with segmented genome encapsidated by the viral nucleoprotein (NP) (38). The virion is usually spherical or elliptical, ranging in size from 80 to 120 nm in diameter, and sometimes filamentous, reaching more than 20 μ m in length (39). The host plasma membrane forms the lipid envelope protecting the viral genome. Two viral glycoproteins, HA and NA, along with small amounts of matrix protein 2 (M2), are present on the surface of the virions (40). M2 forms ion channels through the lipid layers. The matrix protein 1 (M1) forms a lining underneath the lipid bilayer to maintain the morphology of the virion (41). The envelope, along with the viral proteins, encloses and protects the virion core, which contains the viral ribonucleoprotein (vRNP) complexes and the nuclear export protein (NEP) (42). Each viral RNA (vRNA) contains conserved sequences at their 3' and 5' ends and ranges in size from 890 bp to 2,341 bp (43). NP wraps around each of the genomic RNA segments that also contains a copy of the RNA-dependent RNA polymerase (RdRp) consisting of polymerase acidic protein (PA), polymerase basic protein 1 (PB1), and polymerase basic protein 2 (PB2) subunits (39). The IAV and IBV viruses carry eight negative-strand RNA segments, whereas the C and D viruses contain seven segments each (44, 45). The genome of the IAV encodes 12 proteins essential for efficient replication in host cells and the release of mature virions. These proteins consist of the three proteins that make up the vRNA polymerase subunits (PA, PB1, and PB2), the viral glycoproteins HA and NA, M1, M2, NP, PB1-F2, and nonstructural proteins 1 (NS1) and 2 (NS2) or NEP (46, 47).

Figure 1 shows the life cycle of the virus in host cells. The influenza virus enters the host by infecting the epithelial cells in the respiratory tract (in humans) or the intestinal tract (in birds) (48). The binding of the viral HA protein to *N*-acetylneuraminic (sialic) acid expressed on the host cell surface facilitates the virus attachment to the host cells. Sialic acids are nine-carbon acidic monosaccharides found at the termini of many glycoproteins. The binding of the sialic acid to either carbon 3 or carbon 6 of the galactose results in forming either α -2,3 or α -2,6 configurations. The HA protein has specificity for either α -2,3 or α -2,6 linkages (49). After binding to the host receptors, the virus gets internalized by the receptor-mediated endocytosis in the endosome. M2 ion channel proteins facilitate proton transport, resulting in the acidification of the virion. Acidification triggers a conformational change in the HA protein and induces fusion of the viral envelope with the endosome and disrupts the internal protein-protein interactions in the virions, thus releasing the viral RNPs into the host cytoplasm (50). The viral genome is imported into the nucleus for replication. The NP and all three viral RNA polymerase subunits carry the nuclear localization signals (NLSs). In addition, the importin α/β -dependent nuclear import pathway also takes part in the nuclear import of vRNPs (51–55). After importing into the nucleus, the viral RNA's transcription for viral protein synthesis and replication occurs. The RNA polymerase complex of vRNP initiates transcription and replication (45, 56). The primer-independent replication of the virus occurs *de novo* and goes through a cRNA intermediate to create more vRNA copies (45, 56).

The influenza virus NP monomers encapsulate the genome to form a double-helix structure capped by the subunits of the RNA polymerase components PA, PB1, and PB2 at one end. The newly formed viral RNA and polymerase complexes (RNPs) traffic to the plasma membrane in a rat sarcoma virus (Ras)-related Rab GTPase protein 11-dependent manner for assembling new virions (57). While the replication of the viral genome is a primer-independent process, transcription of the genome is a primer-dependent process. Since the viral RNA polymerase (RNAP) lacks capping ability, the RNAP uses a process termed "cap snatching" for generating 5' capped RNA primers used for mRNA synthesis (58). The viral RNAP binding to a serine 5' phosphorylated host RNA polymerase II C-terminal domain (CTD) initiates the cap-snatching process

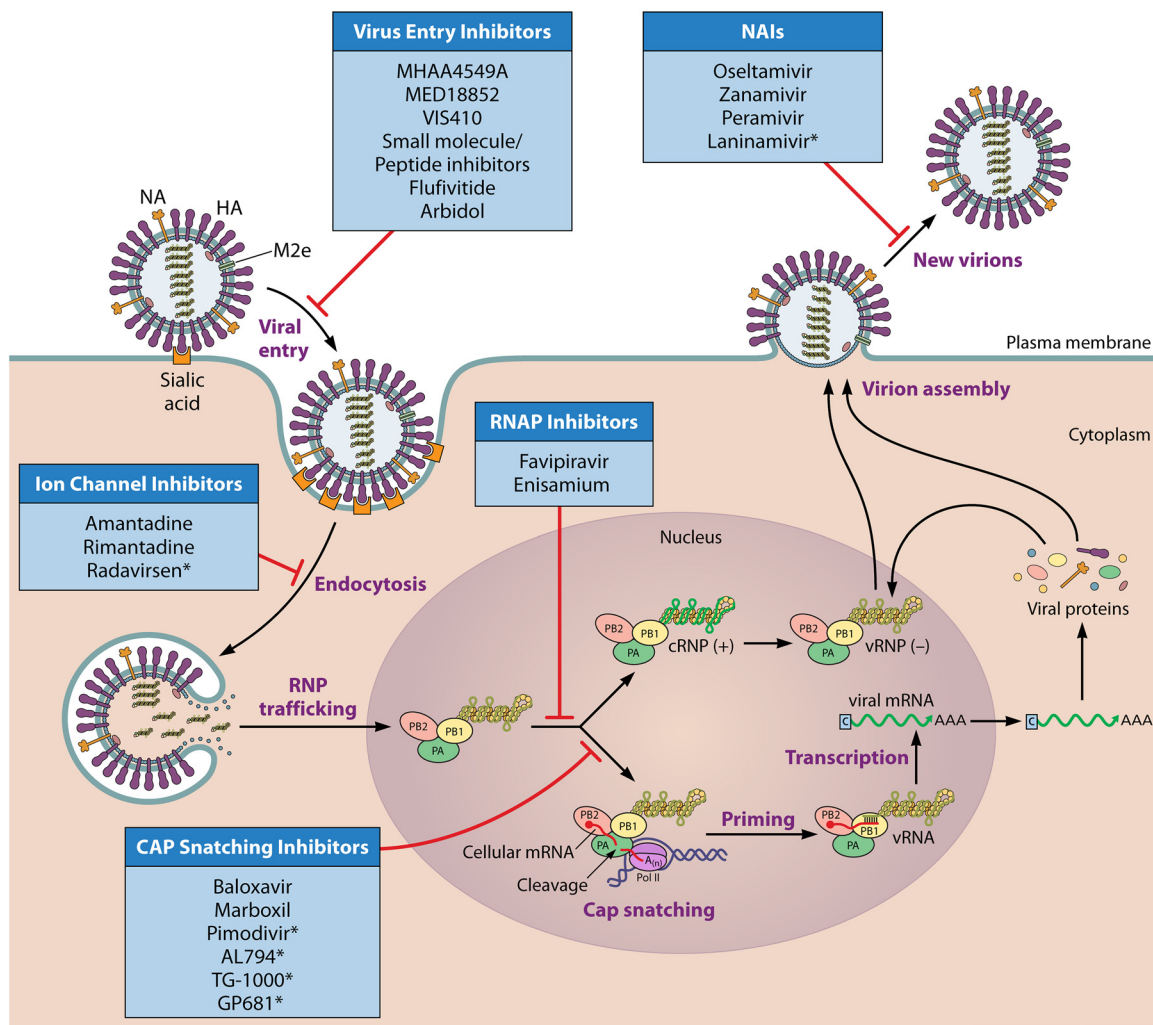


FIG 1 Schematics showing the life cycle of the influenza virus and sites of action of anti-influenza drugs. Influenza virus enters the cell by attaching to the epithelial cell surface by binding the viral hemagglutinin (HA) to sialic acid receptors expressed on the surface of host cells. Once the virus is internalized through endocytosis and fusion, the viral M2 protein forms a channel facilitating the release of the viral genome in the cytoplasm. The viral RNPs are imported into the nucleus, where they undergo the process of cap snatching, as viral RNAP lacks capping abilities. Further, it is replicated and transcribed into mRNA to facilitate the viral protein expression and participate in synthesizing the genomic RNA for incorporation into new progeny viruses. The release of new virus particles to the extracellular milieu is promoted by the viral neuraminidase (NA). Steps at which different antiviral drugs target the virus life cycle are shown in blue boxes. RNA polymerase (RNAP) inhibitors either inhibit the RNA replication or cap-snatching properties of RNAP. The amantadine class of drugs blocks the internalization and uncoating of the virus. However, neuraminidase inhibitors prevent viruses from budding and dispersing. Similarly, as indicated in the figure, mAb and small-molecule inhibitors also potentially inhibit the entry of the virus. Drugs in clinical trials are denoted by asterisk.

(59). The PB2 subunit binds to the host RNAs containing a cap 1 structure, and the PA endonuclease cleaves approximately 9 to 14 nucleotides downstream of the 5' cap, resulting in generation of primers with the 3'-hydroxyl group (56, 60, 61). The PB1 subunit plays a crucial role in the RNA polymerase's assembly and catalytic function. The RNAP reaches a uridine-rich sequence at about 16 to 17 nucleotides from the 5' end of the vRNA template, triggering termination and polyadenylation. This results in the generation of viral mRNA transcripts identical to the cellular transcripts, thus enabling the viral mRNAs to evade the host's innate immune recognition.

Further, it also enables the nuclear export of mRNA into the cytoplasm of infected cells, leading to their translation for the production of viral proteins (62). The cytosolic ribosomes and the endoplasmic reticulum-associated ribosomes translate the viral mRNAs (NP, PB1, PB2, PA, NS1, NS2, and M1) and viral glycoproteins-associated mRNA (HA, NA, M2), respectively (63). The importin α/β proteins shuttle the newly synthesized

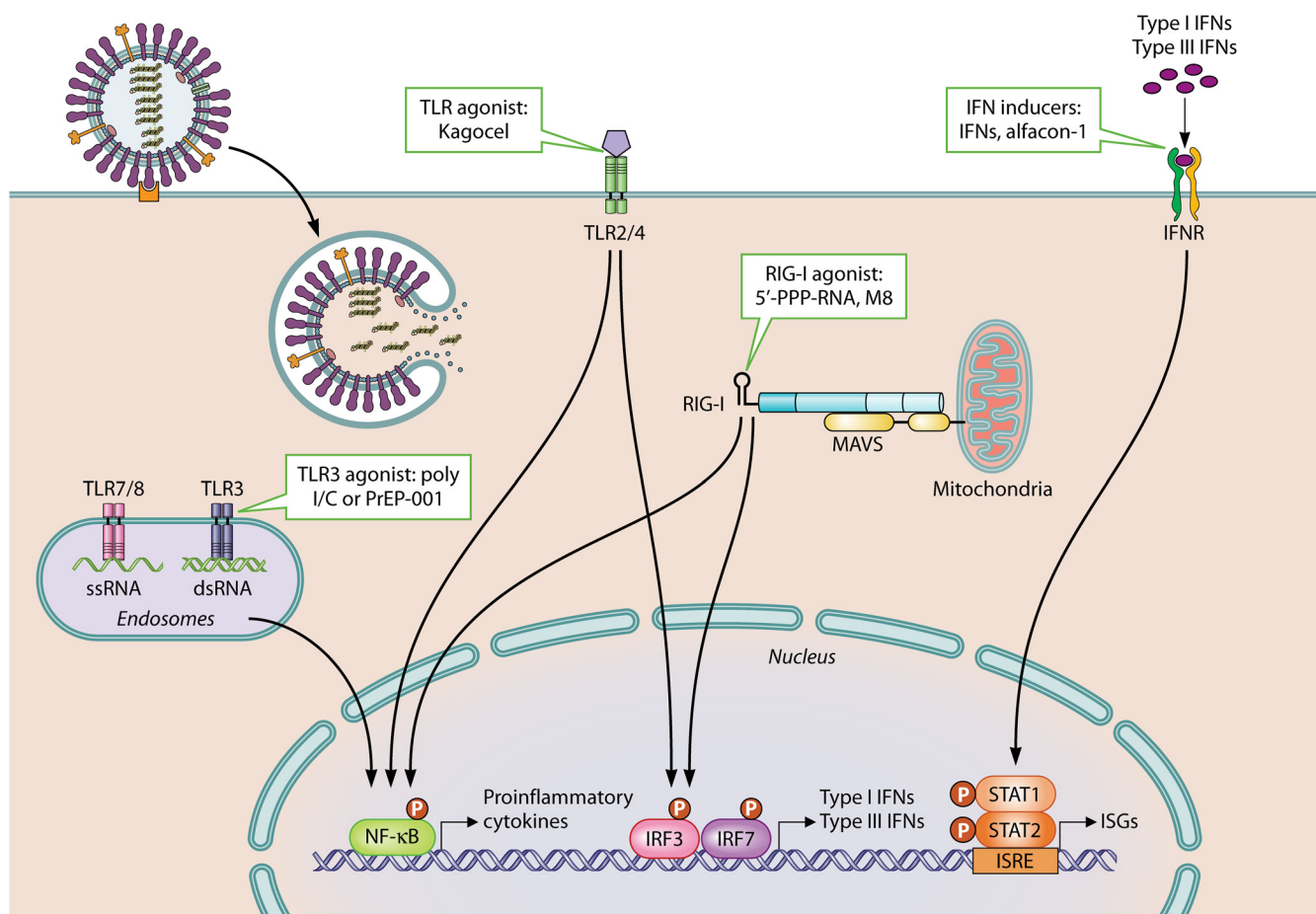


FIG 2 Host innate immune sensing of the influenza virus by RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs). The cytosolic and endosomal RNA sensors, RLRs and TLRs, can detect genomic RNA, dsRNA, and small RNA molecules produced during viral replication. An activated RLR, RIG-I, undergoes a conformational change that allows the RLRs to recruit other proteins and trigger the IFN-signaling pathway. On the other hand, TLR3 and TLR7/8 initiate the antiviral IFN program by recruiting the signal adaptor molecules that subsequently activate downstream kinases and transcription factors to elicit the production of the IFNs and proinflammatory cytokines. Agonists and inducers as potent stimulators of the innate antiviral response in pipeline as antiviral drugs are indicated.

NP and polymerase subunits from the cytosol to the nucleus. These imported viral polymerases increase the rate of viral RNA synthesis. Subsequently, the viral proteins HA, NA, and M2 traffic to the host cell membrane to assemble the mature virion, and the viral M1 and NEP proteins facilitate this process (44). Further, the sialidase activity of the NA removes the sialic acid residues from the N-linked glycans present on the HA and NA of the viral envelope, leading to the release of the newly assembled virions (44, 64).

INNATE IMMUNE RECOGNITION AND VIRAL EVASION

To invade the host tissues, the virus must overcome the mucus layer lining the respiratory and oral mucosa (65). Once the virus enters the host cells, the host activates cellular defenses to clear the infection. Initial recognition of the influenza virus involves the Pattern recognition receptors (PRRs) recognizing the components of the pathogen called pathogen-associated molecular patterns (PAMPs) or components of the cells referred to as damage-associated molecular patterns (DAMPs). The three main classes of PRRs involved in the recognition of the influenza virus are Toll-like receptors (TLRs), the retinoic acid-inducible gene-like (RIG-I) receptors (RLRs), and the nucleotide oligomerization domain (NOD)-like receptor family (NLRs) pyrin-containing 3 (NLRP3) (Fig. 2) (66–68). The RLR family sensors, consisting of RIG-I, melanoma differentiation factor 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), recognize single- or double-stranded RNA (dsRNA) molecules (69). The most studied NLR is NLRP3,

which leads to the activation of the inflammasome pathway, which plays a vital role during influenza virus infections (70, 71). Monocytes, macrophages, dendritic cells (DCs), neutrophils, and epithelial cells express NLRP3. NLRP3 is activated by several PAMPs and DAMPs, including dsRNA, the viral M2 protein, and the reactive oxygen species (71). In addition to the cytoplasmic receptors, TLRs on the cell membrane are also crucial for antiviral response (69, 72). For influenza virus recognition, TLR3, TLR7/8, and RIG-I, which recognize dsRNA and ssRNA, play vital roles (73, 74).

The recognition of PAMPs by PRRs activates several transcription factors, including interferon regulatory factors 3 and 7 (IRF3 and IRF7) and nuclear kappa light chain enhancer of activated B cell (NF- κ B), resulting in type I and type III interferon (IFN) secretion (75). These IFNs bind to their receptors and induce the expression of interferon-stimulated genes (ISGs) that mediate various antiviral functions. IFNs induction also leads to the release of several proinflammatory cytokines and chemokines, the activation and modulation of antigen-specific T-cell and B-cell responses, and the recruitment of cells such as neutrophils, monocytes, and natural killer cells to the sites of infection. The dendritic cells (DCs) in the lungs are classified as migratory conventional DCs (cDCs), plasmacytoid DCs (pDCs), and monocyte-derived DCs, which play an important role after exposure to influenza virus infection (76, 77). The lung-migratory cDCs, following uptake of viral antigens, migrate to secondary lymphoid organs, where they present viral antigens to the naive B and T cells to induce adaptive immune responses. Both innate and adaptive responses contribute to the dampening of influenza virus infection, leading to protection and viral clearance (78, 79).

While the host mounts a defense response to clear the virus infection, the virus has also developed strategies to overcome the host immune system. Understanding these pathways provides insights into building better therapeutic interventions or targeting the host's signaling pathways to overcome the viral evasive mechanisms. The viral NS1 protein is the leading player in overcoming the host response. In infected cells, NS1 is rapidly expressed to high levels and interferes with the host's pathways at multiple levels to antagonize the host innate immune response (80). Specifically, NS1 inhibits the induction of type I IFNs by inhibiting the RIG-I-mediated antiviral signaling pathway (81–84). In addition, NS1 can bind to host protein kinase R (PKR) (85) and eukaryotic translation initiation factor 4G (eIF4G) and promotes the translation of mRNA (82, 86). Segment 3 of the influenza genome encodes protein PA-X, which also blocks the antiviral responses. In infected cells, PA-X is known to selectively degrade the host's mRNAs and noncoding RNAs, which is beneficial for viral replication due to the attenuation of the antiviral responses (87). Thus, downregulating the activity of viral NS-1 or PA-X or enhancing the expression of the host RIG-I signaling pathway offers opportunities to develop intervention strategies to combat influenza infections.

FDA-APPROVED ANTIVIRAL DRUGS FOR INFLUENZA

Anti-influenza compounds can be effective in controlling viral infections during epidemics or pandemics. The critical events in the viral life cycle (viral binding to cellular receptors, viral entry, fusion and release of RNP, transcription, translation of viral proteins, assembly, and viral budding) can be hindered by antiviral compounds, as shown in Fig. 1. M2 ion channel inhibitors or adamantanes (not recommended anymore), NA inhibitors (sialic acid analogs), and a polymerase inhibitor (metal-chelating compound), which target viral entry, replication, release, and spread, are three classes of antiviral drugs approved by the FDA to control influenza virus infections. In Table 1, currently used FDA-approved drugs and their mode of action are summarized, and Fig. 3 shows their structures.

M2 Ion Channel Inhibitors

Both Amantadine and Rimantadine belong to the adamantane class of drugs (88). The FDA approved Amantadine (Symmetrel) in 1976 and Rimantadine (Flumadine) in 1994 (89) to prevent and treat IAV (90) in the US. Adamantanes served as the first-choice antivirals against IAV outbreaks for many years (91, 92). Both Amantadine

TABLE 1 FDA-approved antivirals currently being used for the treatment of influenza

Characteristic	Oseltamivir	Zanamivir	Peramivir	Baloxavir
Activity	IAV and IBV	IAV and IBV	IAV and IBV	IAV and IBV, including NAI-resistant strains
Antiviral target	Neuraminidase	Neuraminidase	Neuraminidase	Endonuclease
Mechanism of action	Impairs virus release	Impairs virus release	Impairs virus release	Blocks viral transcription
Administration/duration	Oral	Inhaled	Intravenous	Oral
Treatment	Any age	7 yrs and older	6 mo and older	12 yrs and older
Prophylaxis	3 mo and older	5 yrs and older	Not recommended	Postexposure prophylaxis 12 yrs and older
Plasma half-life (h)	6–10	2.5–5.1	12–24	79.1 h
Peak time (h)	3–4	1–2	2–4	3.5–4 h
C _{max} ^a (ng/mL)	259	39	34	68.9 for 40 mg, 82.5 for 80 mg
Protein binding (%)	3	<10	<30	93–94
Clearance	99% kidneys	Urine and feces, unabsorbed	90% renal	Feces and urine
Pro(s)	Impairs release of both IAV and IVB	Impairs release of both IAV and IVB, can be inhaled	Impairs release of both IAV and IVB	Blocks transcription from IAV and IVB
Cons	Should be given within the first 48 h of symptom onset	Should be given within the first 48 h of symptom onset	Should be given within the first 48 h of symptom onset	Cannot be given with dairy products, calcium-fortified beverages, or laxatives and antacids
	Dose adjustment is required for renal dysfunction	Not to be used in patients with allergies to lactose, asthma, or COPD	Diarrhea, bronchitis, nausea, and sinusitis	Diarrhea, bronchitis, nausea, and sinusitis
	Nausea, vomiting, and dizziness	Bronchospasm, bronchitis, cough, sinusitis		
Resistance marker(s)	H275Y (H1N1, H1N1 pandemic), R292K (H7N9)	R294K (H7N9)	H275Y (H1N1)	I38T (H1N1 pandemic, H3N2)
References	152, 418–420	419, 421	152, 419	192, 422, 423

^aC_{max}, maximum concentration of drug in serum.

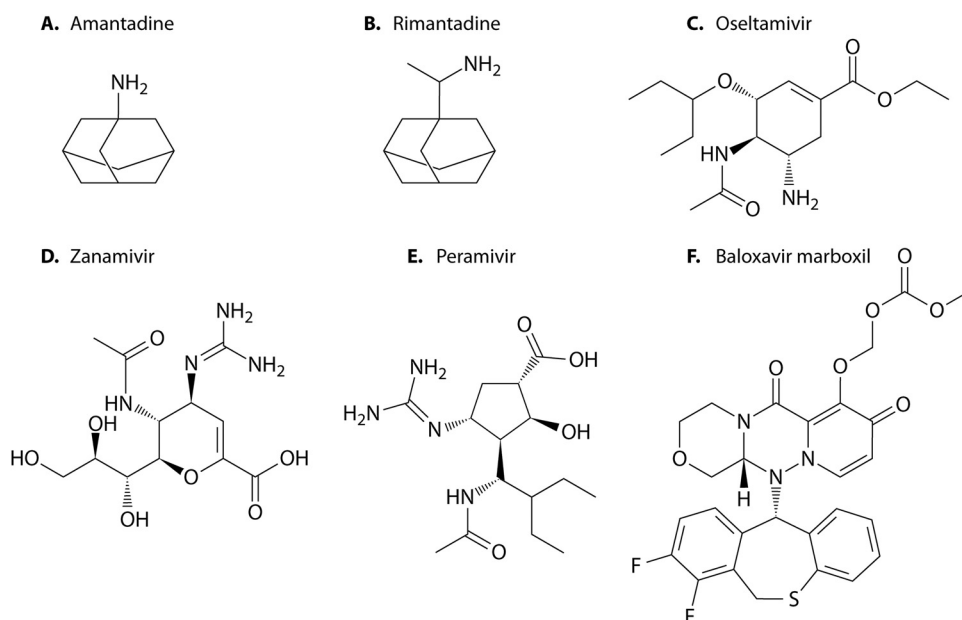


FIG 3 Structures of FDA-approved antivirals for influenza. (A) Amantadine (IUPAC name, adamantan-1-amine). (B) Rimantadine (IUPAC name, 1-(1-adamantyl)ethanamine). (C) Oseltamivir (IUPAC name, ethyl (3R,4R,5S)-4-acetamido-5-amino-3-pentan-3-yloxy-cyclohexene-1-carboxylate). (D) Zanamivir (IUPAC name, (2R,3R,4S)-3-acetamido-4-(diaminomethylideneamino)-2-[(1R,2R)-1,2,3-trihydroxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid). (E) Peramivir (IUPAC name, (1S,2S,3S,4R)-3-[(1S)-1-acetamido-2-ethylbutyl]-4-(diaminomethylideneamino)-2-hydroxycyclopentane-1-carboxylic acid). (F) Baloxavir marboxil (IUPAC name, [(3R)-2-[(11S)-7,8-difluoro-6,11-dihydrobenzo[c][1]benzothiepin-11-yl]-9,12-dioxo-5-oxa-1,2,8-triazatricyclo[8.4.0.0.3,8]tetradeca-10,13-dien-11-yl]oxymethyl methyl carbonate).

and its derivative, Rimantadine, target and inhibit the function of IAV M2. However, the IBV M2 ion channel is not sensitive to the adamantanes class of drugs (93). The most common side effects of these M2 inhibitors are anxiety, insomnia, dizziness, headache, and gastrointestinal upset (94, 95).

Mechanism of action. The tetrameric M2 protein belongs to type III transmembrane (TM) proteins known as viroporins (96, 97). Embedded in the viral membrane, M2 forms an ion channel (98). There are ~14 to 68 molecules of M2 per virion (99). The M2 protein allows acidification and uncoating, which is necessary to import vRNPs into the nucleus. It also plays an important role in viral assembly (100) and budding (101). The Amantadine-sensitive ion channel from IAV is well characterized, with several X-ray and nuclear magnetic resonance (NMR) structures (102–105). IAV M2 consists of three regions, the extracellular domain, the transmembrane domain (TMD), and the cytosolic tail. The N-terminal domain from amino acid residues 1 to 22 is required for M2 incorporation into virion (103, 106). The M2 TMD region is conserved in all the human, swine, equine, and avian strains of IAV (107–109). The minimal functional IAV M2 TMD comprised of amino acid residues from 22 to 46 is essential for tetramerization and retaining conductance features like a full-length protein (96, 110). Moreover, the M2 ion channel is affected by both the pH and the surrounding membrane environment (102). The wild-type (WT) M2 ion channel of IAV has proton/cation exchange activity and is selective for protons over Na^+ and K^+ ions (111, 112).

After viral entry, the ion channel allows protons from host cell endosomes to enter the virion core. The influx of protons makes the core acidic, allowing M1 and vRNPs to dissociate (113). Blocking the M2 protein ion channel activity with Amantadine and Rimantadine can prevent the uncoating step (114). M2 also plays an essential role during the transport of newly synthesized HA protein across the *trans*-Golgi network (TGN) to the cell surface. M2 equilibrates the pH of the TGN with that of the host cell cytoplasm and prevents premature conformational rearrangement of HA (115, 116). Blocking this activity of the IAV M2 protein ion channel with Amantadine and

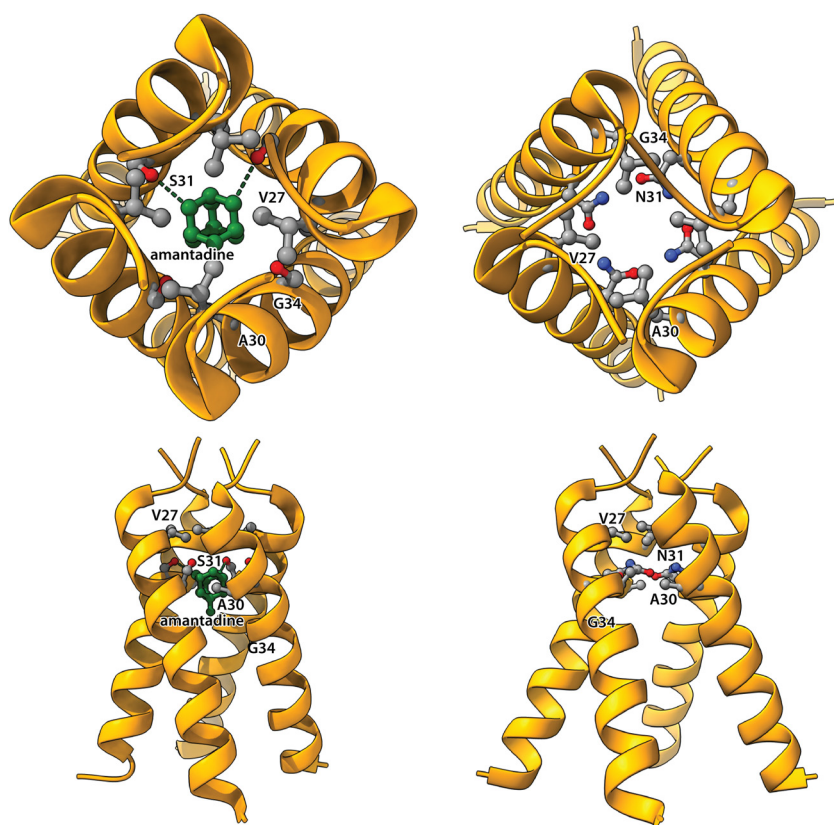


FIG 4 Solved structures of IAV M2 wild type (PDB accession no. 6BKK) with Amantadine bound (left) and the Ser31Asn mutant (PDB accession no. 6MJH), which blocks Amantadine binding (right). The top left structure depicts the IAV M2 wild type (orange) from a top-down perspective, i.e., looking through the transmembrane channel. The Ser31 residues from all four chains in the quaternary structure interact with the bound Amantadine (green). The bottom left vignette depicts a 90° rotation of the structure to show the overall structural geometry of the wild-type IAV M2. The top right structure represents the Ser31Asn mutant IAV M2, which blocks Amantadine binding. The asparagines from each chain interact with one another to cause a structural change to the protein, which prevents the Amantadine to bind yet maintains protein function. The Ser31Asn mutation in the IAV M2 protein renders the antiviral Amantadine ineffective.

Rimantadine drugs can prevent the fusion of the virus and host-cell membranes (117). The adamantane class of drugs targets the TMD of IAV M2 (108). Amantadine (100 μ M) treatment can block 90% of the A(H3N2) M2 channel activity (107, 118). Structural studies revealed that the TM pore contains the drug-binding site (119–122). Several studies confirmed that the pore-binding site is Amantadine's drug-binding site in M2 (123–125). Crystal structures of M2 further showed that the His37 and Trp41 residues were most conserved in the the IAV M2 TMD (109) and essential for ion channel activity. His37 is required for high proton selectivity, and Trp41 is needed for opening and closing the pore, acting as the channel gate (126, 127). Mutating His37 abolishes proton selectivity and low pH activation properties of M2 (128). Several other amino acid residues critical for ion channel activity were also identified by site-directed mutagenesis (129, 130). The crystal structure of M2 in complex with the inhibitor further showed that the drug molecule interacted with Val27, Ala30, Ser31 (Fig. 4, left), and Gly34 residues (108).

Solid-state NMR spectroscopy demonstrated a low- and high-affinity drug-binding location in the IAV M2 TMD channel. The M2 channel gets occluded when the drugs bind to these sites (119). Another study suggested inhibitor binding outside the M2 channel (117). A recent study investigated the oligomerization of the full-length IAV M2 TMD with various concentrations of Amantadine. The authors observed that IAV M2 could assemble into a range of oligomeric states and Amantadine perturbed

oligomerization. These findings suggest that Amantadine could directly affect ion channel activity or induce conformational changes in the M2 ion channel (102).

Resistance to M2 ion channel inhibitors. Drug resistance-conferring substitutions occur in the TM region of the M2 molecule. Influenza viruses with Ser31Asn (Fig. 4, right), a mutation of M2 started circulating before the wide use of Amantadine. In clinical isolates, even a single substitution in the amino acid at position 26, 27, 30, 31, or 34 of the M2 protein conferred drug resistance (34, 131–133). Recombinant A(H1N1) viruses engineered with Leu26Phe, Val27Ala, Ala30Thr, Ser31Asn, Gly34Glu, and Val27Ala/Ser31Asn mutations in the M2 gene also showed that these mutations could cause Amantadine resistance (32). M2 mutants retained virulence and remained transmissible between humans. Amantadine inhibited the wild-type IAV M2 channel activity but not the Ser31Asn IAV M2 channel (134), which are two mechanisms by which IAV avoids blockage of its M2 ion channel function. Stouffer et al. proposed a model where drug binding physically blocks the channel's pore, halting proton flow (108). The drug-resistant mutations in the IAV M2 TMD do not permit the binding of drugs to the channel. Another study showed that proton flow continued through the channel despite the drug binding to M2 TMD. Regardless of the drug binding in the channel pore, an increase in the channel diameter retained function (135). The resistance to adamantanes can also emerge during treatment (133, 136). Surveillance studies worldwide reported increased M2 resistance to adamantanes in A(H3N2) and A(H1N1) viruses (33, 34), and almost 95% of resistant viruses had the Ser31Asn change, whereas only about 1% had the Val27Ala substitution. Leu26Phe, Ala30Thr, Gly34Glu, and Lys38Phe were extremely rare (<0.2%) (133). These resistant variants displayed no reduction in replicative fitness or transmissibility (137).

Virus strains from human, avian, and swine reservoirs have M2 ion channel mutations. A majority of H5N1 double mutants, reported between 1996 and 2005 from Vietnam, Cambodia, Malaysia, and Thailand, carried Ser31Asn and Leu26Ile M2 substitutions (138). M2 mutations with Ser31Asn and Val27Ala were also dominant among circulating H5N1 viruses (139). In summary, resistance to this class of drugs severely impacted their effectiveness against many influenza strains, including the 2009 A(H1N1)pdm09 pandemic strain (34, 90). In 2006, the CDC recommended discontinuing the use of both Amantadine and Rimantadine for the treatment of influenza (140).

The IBV M2 structure became available recently (141). The closed and open IBV M2 channels differ significantly from the closed and open IAV M2 channels. Also, IBV M2 has polar pore-facing residues and conducts protons inward and outward. In contrast, IAV M2 has a hydrophobic pore and conducts protons inward. Further research and development of next-generation antiviral agents that target both IAV and IBV M2 channels with the least potential to generate resistant strains are needed.

NA Inhibitors

The NA enzyme comprises four identical subunits (each ~470-amino-acid [aa] residues), with discrete structural domains comprising the cytoplasmic tail, the TM region, the stalk, and the catalytic head (142, 143). The NA enzyme cuts α -ketosidically linked Neu5Ac residues on the ends of various glycoproteins or glycolipids (144). NA inhibitors (NAIs), the first structure-based rationally designed inhibitors for the influenza virus, specifically target the active site of NA (145, 146). Stalling the catalytic activity of the NA enzyme by NAIs results in the formation of virus aggregates and prevents virus release, thus effectively limiting reinfection (147).

Several *in vitro* studies conducted in the 1970s demonstrated the ability of sialic acid analogs to inhibit NA activity (147–149). Further advances in the field came when the crystal structure of the NA molecule became available in the early 1980s. These studies paved the way for the discovery of several effective NA inhibitors, including three (Zanamivir, Oseltamivir, and Peramivir) approved in the US for clinical use (Table 1). The FDA approved Zanamivir (Relenza Diskhaler) and Oseltamivir (Tamiflu) in 1999 for the treatment of IAV and IBV in the US (150, 151). In addition, the FDA approved

Peramivir (Rapivab) for treatment of influenza infections in adults in 2014 (26). NAIs shorten the length of treatment, decrease hospitalization time in intensive care unit patients, and reduce the risk of death (152).

The prodrug Oseltamivir phosphate is a sialic acid analog and is hydrolyzed to its active form, Oseltamivir carboxylate. It is an oral antiviral treatment for acute influenza in adults and children over 2 weeks old (up to 48 h after the onset of symptoms) and for chemoprophylaxis in adults and children over 1 year old. The CDC recommends the use of oral Oseltamivir for the treatment of influenza in infants (<14 days old). Although not part of the FDA-approved indications, the CDC and the American Academy of Pediatrics recommend Oseltamivir for chemoprophylaxis in 3- to 12-month-olds and Oseltamivir in liquid form is approved to treat infants over 2 weeks of age or older (153, 154). Adverse effects reported for Oseltamivir include vomiting, nausea, abdominal pain, delirium, and anemia (26, 28). Zanamivir (inhaled) is used for the treatment of uncomplicated acute influenza infection (both A and B) within 2 days after the onset of symptoms (adults and children aged over 7 years old). Each Relenza Rotadisk (GSK) contains four blisters. Each blister holds a powder mixture of 5 mg of Zanamivir and 20 mg of lactose (155). Early treatment of uncomplicated influenza with Zanamivir leads to a reduction in severity of symptoms. It is also recommended for chemoprophylaxis of influenza in adults and children over 5 years old. Its use is contraindicated for individuals with severe milk protein allergy since each blister contains 20 mg of lactose or milk protein.

Adverse effects of Zanamivir include fever, myalgia, cough, headache, bronchospasm, nausea, and vomiting (26). Peramivir is approved for treating uncomplicated acute influenza within 48 h of symptoms onset (patients aged over 18 years old). However, it is not approved for use in children or for prophylaxis. Adverse drug-related outcomes include diarrhea, constipation, insomnia, and hypertension (26). Recently, a new preparation of Zanamivir, as a solution for intravenous administration, called Dectova, was authorized for use in the European Union. Intravenous Zanamivir, or Dectova, is used in seriously ill and hospitalized patients infected with IAV or IBV (156). It allows the treatment of patients whose medical condition does not allow the use of medications suitable for oral administration or inhalation (157).

Mechanism of action. The current understanding of NA's active site comes from several structural and biochemical studies (150, 158, 159). There are several catalytic residues (Arg118, Asp151, Arg152, Arg224, Glu276, Arg292, Arg371, and Tyr406; N2 numbering) which interact with sialic acids and participate in catalysis. These framework residues (Glu119, Arg156, Trp178, Ser179, Asp198, Ile222, Glu227, His274, Glu277, Asn294, and Glu425; N2 numbering) stabilize and maintain the functional structure of the active site by an extensive network of hydrogen bonds (160–162). These framework residues that do not interact with sialic acid support the catalytic residues. The active site of the NA enzyme consists of five regions based on the binding environment, S1 (Arg118, Arg292, and Arg371 positively charged electrostatic region), S2 (Glu119 and Glu227 negatively charged region), S3 (Trp178 and Ile222 hydrophobic region), S4 (a hydrophobic region derived from the side chains of Ile222, Ala246, and the hydrophobic face of Arg224), and S5 (mixed polarity, Glu276 and Ala246) (159). The left structure in Fig. 5 shows Oseltamivir (green) firmly bound to the NA wild-type protein (orange) through interactions with numerous residues, including Arg224, Glu276, Asn294, Tyr406, Arg371, and Arg292. Based on crystal structures, group 1 and group 2 NAs exist in at least two conformations due to orientation of "loop 150." Structurally, the group 1 NAs show a cavity next to the catalytic active site formed due to the movement of loop 150 because of the binding of the substrate within the active site. The NAs from group 2 lack this cavity (163, 164).

NA inhibitors (Oseltamivir, Zanamivir, and Peramivir) mimic the enzyme's natural substrates. These sialic acid analogs competitively inhibit NA activity by binding to conserved residues in its active site (144, 159, 165). The crystal structure of Neu5Ac2en or DANA (2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid), a transition-state analog of sialic acid, in complex with NA, allowed further characterization of the catalytic site

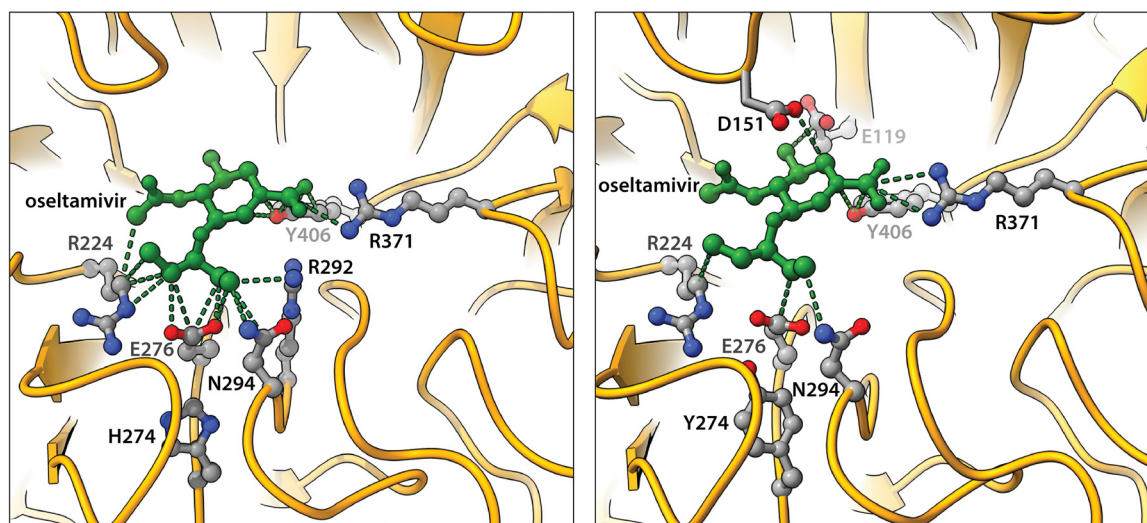


FIG 5 N1 neuraminidase (NA) wild type (PDB accession no. [2HU0](#)) with bound Oseltamivir (left) and NA H274Y mutant (PDB accession no. [3CLO](#)) with bound Oseltamivir (right). The left structure shows Oseltamivir (green) firmly bound to the NA wild-type protein (orange) through interactions with numerous residues, including Arg224, Glu276, Asn294, Tyr406, Arg371, and Arg292. The right structure shows the H274Y mutant, which results in a modified binding pocket. While the residue at 274 does not directly interact with the bound antiviral molecule, the mutation from histidine to tyrosine negatively affects the binding affinity of the drug through changes in the overall binding pocket, as seen by the reduced number of interactions on the right. The H274Y mutation in the NA protein renders the antiviral Oseltamivir less effective.

(166). The NA inhibitor complex showed several amino acid residues that directly bind DANA (167). In summary, NAIs closely mimic their natural substrate or enzyme transition-state complex and fit in the active site pockets due to an energetically favorable interaction (168).

Resistance to NAIs. NA inhibitors, especially Oseltamivir, are widely used in the US and have demonstrated clinical effectiveness against influenza viruses (seasonal and emerging) (169, 170). Since NAIs resemble the natural substrate (sialic acid), it was reasoned that developing drug-resistant viable mutant viruses would be difficult. However, drug-resistant mutants were reported *in vitro* (171) and in the clinic (172, 173). A(H1N1) viruses resistant to Oseltamivir were detected worldwide during the 2007 to 2008 influenza season (174). By 2009, most circulating seasonal A(H1N1) viruses were resistant to Oseltamivir. These were eventually displaced by the emerging A(H1N1)pdm09 viruses. A(H1N1) virus with a single His275Tyr amino acid change resulted in resistance to Oseltamivir (162). The His275Tyr mutation near the drug binding site of NA (163) perturbed the conformation of NA, allowing a decrease in the binding free energy of the His275Tyr NA-Oseltamivir complex (162, 175–179). The structure in Fig. 5, right, shows the His274Tyr mutant with a modified binding pocket. While the residue at 274 does not directly interact with the bound antiviral molecule, the mutation from histidine to tyrosine negatively affects the binding affinity of the drug through changes in the overall binding pocket. Several NA substitutions or deletions have been associated with reduced inhibition by one or more NAIs (summarized on the WHO global influenza surveillance and response system website) (180). Resistant H5N1 viruses with substitutions His275Tyr (His274Tyr) and/or Asn294Ser (Asn295Ser) were isolated from humans and bird species (181). There is a difference in the ability of NAIs, especially Oseltamivir, to inhibit the enzyme activity of H5N1 viruses from two clades, 1 and 2, as there are amino acid differences (e.g., Tyr252) which have been shown to affect the baseline susceptibility in neuraminidase inhibitor (NI) assay (182, 183).

Global surveillance of influenza viruses' susceptibility to NAI was conducted by the Neuraminidase Inhibitor Susceptibility Network (NISN) since Oseltamivir and Zanamivir entered the global markets in 1999. Since 2012 to 2013, the Antiviral Working Group

(AVWG) of the Global Influenza Surveillance and Response System (GISRS) of WHO has been performing this activity (184). WHO Collaborating Centers collected a total of 10,641 influenza viruses worldwide between 2013 and 2014. Around 2% of the viruses showed reduced inhibition to at least one NA inhibitor (185). Surveillance conducted in 2017 to 2018 assessing 15,409 viruses reported the occurrence of viruses with reduced susceptibility to NAIs being 0.8% (186).

The Oseltamivir-resistant strains (His274Tyr, Ile117Val, Glu119Ala, and Arg292Lys) have emerged and have an impact on the use and effectiveness of NAIs (187). These Oseltamivir-resistant strains remain sensitive to Zanamivir (156). Therefore, NA inhibitors remain an important option for treating influenza virus-infected subjects.

Inhibitors of Viral Polymerase

Influenza virus RdRp is a heterotrimeric complex with three subunits, PA, PB1, and PB2, (60), which perform both RNA transcription and replication processes. Therefore, viral polymerase remains a very attractive target for the design of new anti-influenza therapeutics (188). Baloxavir marboxil (BXM) (Table 1) is a novel inhibitor of the viral RNA polymerase. BXM got its first approval in Japan (February 2018), followed by the US (October 2018) and Europe (January 2021), for treating IAV and IBV infections (27, 189). Since influenza viruses lack their capping enzymes, they snatch the cap from nascent host RNAs. This strategy also makes viral mRNAs structurally indistinguishable from the host's mRNAs and exploits the host's cellular pathways for nuclear export and further processing of its genetic components (178). BXM is a prodrug of Baloxavir acid (BXA), which can specifically target the cap-dependent endonuclease activity of the PA subunit of the influenza virus. It also inhibits viral strains resistant to NAIs (29, 177). BXA is the first anti-influenza drug that inhibits the viral polymerase activity from several subtypes of influenza A viruses [A(H1N2), A(H5N1), A(H5N2), A(H5N6), A(H7N9), and A(H9N2)] (190). An *in vitro* study by Mishin et al. showed that BXA inhibitor has broad activity and inhibits replication of type A, B, C, and D viruses (191).

A recent multicenter, randomized, placebo-controlled trial further evaluated the clinical outcomes of this inhibitor during the 2018–2019 flu season. The study assessed the post-exposure efficacy of a single-dose BXM in preventing influenza infection in household contacts. The study found a significant decrease in clinical influenza cases among BXM treated versus the placebo group (13.6% placebo group versus 1.9% BXM group) (192). In addition, the risk of viral infection, irrespective of indications, was lower with BXM than with placebo. Based on these outcomes, in 2020, the FDA further expanded the use of BXM as post-exposure intervention, which allows treatment of those 12 years and older who come in contact with the infected individuals (193). The data from clinical trials showed that a single dose of BXM can reduce viral titers and lower disease symptoms (194). The most common adverse effects reported were headache, hematuria, pharyngitis, and increased alanine aminotransferase level (28, 192).

Mechanism of action. In brief, the influenza PB2 subunit first captures a nascent host RNA using its m7G cap-binding motif. Next, the PA endonuclease activity hydrolyzes the phosphodiester bond 8 to 14 nucleotides downstream of the 5' cap (195). This process generates capped RNA primers to initiate transcription by the PB1 subunit (196). The crystal structure of PA endonuclease displays a (Pro)Asp...X_n...(Asp/Glu)X Lys where X is any amino acid motif, required for metal ion coordination, like other known endonucleases (60). The active site of PA harbors a cluster of three conserved acidic residues (Gln80, Asp108, and Gln119) and His41 residue involved in binding to divalent metal ions. Baloxavir exhibits its inhibitory activity by binding to these catalytic metal ions in the enzyme's active site (60, 197, 198). The left structure of Fig. 6 depicts the binding mode of Baloxavir (green) in the binding pocket of the wild-type PA protein (orange). Baloxavir efficiently targets influenza virus replication by inhibiting the initiation of mRNA synthesis.

Resistance to Baloxavir. Growing viruses in the presence of drugs can result in the generation of drug-resistant variants. *In vitro* passages of influenza A(H1N1) virus in

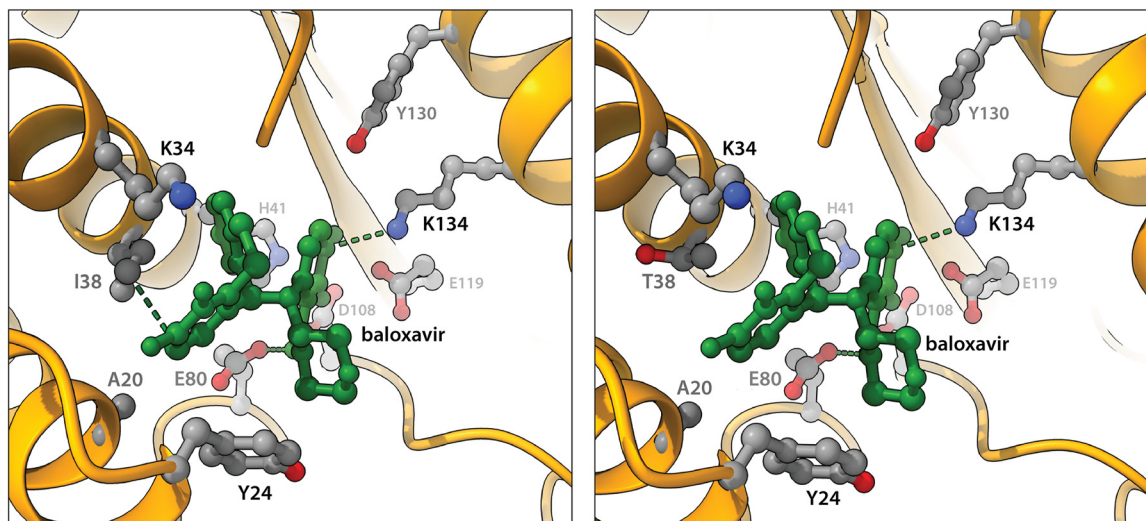


FIG 6 Polymerase acidic (PA) protein wild type (PDB accession no. 6F56) bound with Baloxavir (left) and PA Ile38Thr mutant (PDB accession no. 6F57) bound with Baloxavir (right). The left structure depicts the binding mode of Baloxavir (green) in the binding pocket of the wild-type PA protein (orange). The right structure shows Baloxavir bound to the mutant PA protein, where the Threonine does not interact with the Baloxavir, reducing the binding affinity of the antiviral to the mutated binding pocket. In addition, the I38T mutation in the PA protein renders the antiviral Baloxavir less effective.

Madin-Darby bovine kidney (MDBK) cells treated with BXA resulted in Ile38Thr PA mutant virus (190). The Ile38Thr substitution did not significantly alter the replication kinetics of rescued (H1N1) Ile38Thr and (H3N2) Ile38Thr PA variants in Madin-Darby canine kidney (MDCK) cells (199). Mutant viruses of Ile38 residue in the PA active site were isolated clinically (27, 192, 194). The Ile38Thr substitution also reduced antiviral effects of Baloxavir in clinical trials (194, 200, 201). Crystal structures of the PA-Baloxavir complex revealed several active site residues involved in interacting with the drug (Fig. 6, right) (194). The most prevalent substitutions resulting in reductions in Baloxavir activity involved changes from Ile at residue 38 in the wild-type viruses to Thr, Phe, or Met. In addition, the Ile38Thr mutation perturbed van der Waals interactions with the inhibitor in the binding pocket (191, 194, 202). Ile38Thr does not interact with Baloxavir; it reduces the binding affinity of the antiviral to the mutated binding pocket and renders the antiviral Baloxavir less effective.

In patients treated with Baloxavir, during the 2018–2019 season, mutations (Ile38Thr, Ile38Phe, and Ile38Met) in the PA active site from A(H1N1)pdm09 and A (H3N2) influenza viruses were detected (203, 204). A single dose of BXM was compared to placebo or Oseltamivir in a phase 3 randomized, double-blind study. This study found that 9.7% of viruses isolated (36/370) developed mutations of Ile38 residue 3 to 9 days after treatment (201). Eleven cases infected with influenza A(H3N2) viruses, including one patient untreated with Baloxavir, had the PA Ile38Thr substitution (205). Another A(H1N1)pdm09 virus with the PA Glu23Lys mutation detected in a pediatric patient without Baloxavir treatment showed reduced susceptibility to Baloxavir (206). Such human-to-human transmission events of resistant variants are concerning and require monitoring and surveillance (203, 207). In Japan, Baloxavir is approved for treatment of children less than 12 years old. However, there is a high rate of resistance emergence in children treated with Baloxavir (208). Ile38Thr and Ile38Met in A(H3N2) variants detected during post-treatment monitoring showed almost 10-fold reductions in BXA susceptibility in pediatric patients (194). Treatment-emergent substitutions [Ile38Asn from A(H1N1)pdm09 and Ile38Arg in A(H3N2)] were also detected from the CAPSTONE-2 study (209). PA mutations at Ala37Thr and Glu199Gly had relatively minor effects compared to mutations at residue 38 (194). In addition, 13,523 PA sequences deposited to the Global Initiative on Sharing All Influenza Data (GISAID) or NCBI Influenza Virus Resource (NCBI-IVR) databases (2017 to 2018) were screened for

mutations associated with reduced Baloxavir susceptibility (186). This analysis showed that the frequency of viruses with reduced susceptibility to PA inhibitors was low (0.08%). Another study determined BXA 50% effective concentration (EC_{50}) values by plaque reduction assays in MDCK cells and reported EC_{50} values for A(H3N2) virus with PA Glu23Arg (EC_{50} , 5.7 nM) and PA-Ile38Thr (EC_{50} , 25 nM) mutations. They found a steep increase in EC_{50} of the drug from 5.7 nM to 975 nM for the double mutant Glu23Arg/Ile38Thr (210), thus potentially impacting the treatment efficacy.

WHO's AVWG summarizes and posts updates on PA mutations associated with decreased susceptibility *in vitro* (211). Regular surveillance and monitoring for the emergence of seasonal influenza (both A and B variants) and tracking changes in the susceptibility of new viral mutants to inhibitors are crucial to ensure the accuracy of treatment guidelines.

STRATEGIES TO OVERCOME DRUG RESISTANCE WITH NEXT-GENERATION ANTIVIRALS

Significant progress has been made in addressing M2 resistance with the newer generation of inhibitors (212, 213). DeGrado and coworkers used the power of molecular dynamics (MD) simulations to solve the resistance problem and developed a novel inhibitor called spiro-adamantyl amine (214). The inhibitor blocks proton conductance in the WT and Val27Ala mutant M2 channels (215). This is a very promising discovery given the fact that the M2 Val27Ala mutation became transmissible and provided proof of concept that MD simulations can be used for the discovery of inhibitors of targets (104, 105, 216, 217). The drug resistance mechanism of M2-Ser31Asn channel blockers has been extensively studied using multiple viruses in different cell lines (218). The Leu46Pro (134) and Arg45His (219) mutations have been identified from serial viral passage experiments *in vitro*. Compounds such as isoxazole-conjugated Adamantane bind AM2 with the Ser31Asn mutation and inhibit channel activity (134). Wang and coworkers discovered three compounds, (3s,5s,7s)-N-((5-(3-methoxythiophen-2-yl)isoxazole-3-yl)methyl)Adamantane-1-amine, (1s,3r,5R,7S)-3-(((5-(3-methoxythiophen-2-yl)isoxazole-3-yl)methyl)amino)Adamantane-1-ol, and (1s,3r,5R,7S)-3-(((5-(2-(methylthio)phenyl)isoxazole-3-yl)methyl)amino)Adamantane-1-ol), which significantly inhibited single (M2-Ser31Asn) and double (M2-Ser31Asn Leu26Ile and M2-Ser31Asn Val27Ala M2) ion channel mutants (139). The significant outcome of inhibition of the drug-resistant M2 double mutants is remarkable. These studies further advance the field and provide insights for developing novel antivirals.

Oseltamivir treatment can also result in resistant viruses (NA-His275Tyr, Arg292Lys, and Asn294Ser), although these Oseltamivir-resistant strains remain sensitive to Zanamivir. Several groups are working to develop a newer generation of inhibitors. Naturally occurring compounds from several microbes and medicinal plants have been used to refine and develop synthetic influenza antivirals (152). For example, a flavonoid compound, Kaempferol-3-O-[(4''5''-O-isopropylidene)- α -L-rhamnopyranoside], isolated from cassia trees, showed NA inhibitory activity (50% inhibitory concentration [IC_{50}] value of 187.40 μ M which can serve as a potential lead for synthesizing novel NA inhibitors (187).

Baloxavir binds to the endonuclease of both IAV and IBV and is a critical drug in fighting IAV infection. However, Baloxavir-treated subjects with resistant viruses carrying Ile38Thr and Glu23Lys substitutions in PA have been sequenced (209). The mutation at position 38 in the PA has been shown to have a major impact on viral susceptibility to the inhibitor and is therefore also the most well studied (194). In addition, the PA double mutant Glu23Arg Ile38Thr can severely compromise the utility of BXA (210). Currently, there are no drugs that target and inhibit PA-resistant mutants. Therefore, next generation drugs need to be developed that are also effective against mutant strains.

ANTIVIRALS IN CLINICAL DEVELOPMENT OR APPROVED FOR USE IN DIFFERENT PARTS OF THE WORLD

As discussed earlier, influenza viruses are resistant to currently used antiviral drugs (26, 220). Therefore, developing new influenza drugs to work against drug-resistant

strains is a public health need. An ideal influenza drug should have a broad range of activity against different strains, ease of administration, flexibility in the timing of administration during infection, improved effectiveness, and fewer chances of development of drug-resistant viral strains (221). Here, we summarize some promising antivirals either approved for use in different parts of the world or currently in various phases of clinical trials that can fulfill the need for new influenza drugs (Fig. 7 and Table 2).

NA Inhibitors

NAIs are the most prescribed antiviral drugs against influenza, with proven effectiveness in shortening the duration of clinical illness and virus clearance (28, 152, 161, 222). Therefore, different chemical approaches have been applied in search of novel neuraminidase inhibitors (187). Laninamivir is one such drug that inhibits NA activity and has been used in Japan for several years.

Laninamivir. Laninamivir octanoate, or CS-8958, is an octanoyl ester is a prodrug for Laninamivir. Upon administration, it changes to its active form in the respiratory tract, as shown in Fig. 7A. Laninamivir exhibits a broad inhibitory activity against influenza A and B viruses, including Oseltamivir-resistant A(H5N1) influenza viruses *in vitro* and *in vivo* (223). A clinical study investigating the pharmacokinetics of 40 mg of Laninamivir administered as an inhaled prodrug in healthy individuals indicates maintenance of 50% inhibitory concentrations in epithelial lining fluids for 10 days (224). Different mechanisms, such as increased lipophilicity and hydrolysis leading to the generation of a highly hydrophilic form, have been proposed for this long-lasting ability of the drug (225, 226). The clinical efficacy of Laninamivir was evaluated in a double-blind noninferiority trial in which 334 and 326 patients exhibiting febrile influenza symptoms were administered a single inhalation of 40 mg or 20 mg Laninamivir prodrug, respectively. As a control, 336 patients received an oral dosage of 75 mg of Oseltamivir twice daily for 5 days.

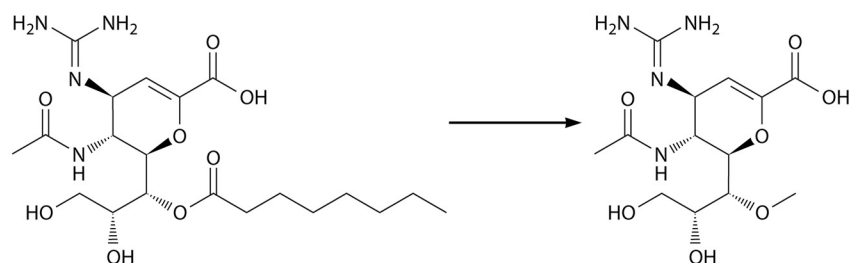
Laninamivir at a 40-mg dose reduced the number of patients shedding the viruses on day 3 and was also effective against Oseltamivir-resistant A(H1N1) and A(H1N1) pdm09 viruses (227). All three groups had similar time courses for the alleviation of influenza symptoms. The most common side effects included diarrhea, vomiting, and nausea. The incidence of these mild to moderate side effects was similar among the three groups. Mild to moderate dizziness was reported only by the patients receiving 40 mg or 20 mg of Laninamivir octanoate.

Furthermore, a safety evaluation study of Laninamivir octanoate hydrate treatment of 567 patients revealed that abnormal behavior/delirium and syncope were primarily associated with influenza infection and not due to the treatment. However, the incidence of adverse events and their potential causal relationship with Laninamivir are similar to those caused by other NAI treatments (228). Hence, patients need to be closely monitored for behavioral changes when taking this drug. A clinical trial with 803 patients evaluated the potential of Laninamivir octanoate as a preexposure prophylactic treatment strategy. The results indicated that a single administration of 40 mg or 20 mg of the drug effectively prevented the development of clinical influenza (229). Recently, a meta-analysis of nine studies involving treatment and three studies of prophylactic treatment revealed that during an A (H3N2) influenza infection, Laninamivir octanoate treatment was associated with a longer duration of fever than Oseltamivir treatment (230). Thus far, isolation of viruses with mutations causing resistance to Laninamivir has not been reported following treatment.

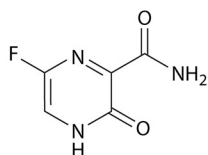
Viral Polymerase Complex Inhibitors

For nearly two decades, influenza antiviral therapy has been based mainly on adamantanes and NAIs. However, the emergence of resistant viral strains and suboptimal clinical effectiveness with these drugs warrant a search for alternative targets for drug development. Along with its indispensable role in the influenza virus replication cycle and highly conserved sequence among different influenza viruses, the polymerase complex seems

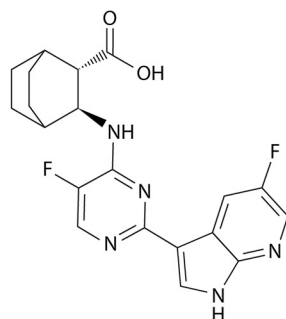
A. Laninamivir Prodrug (CS-8958)



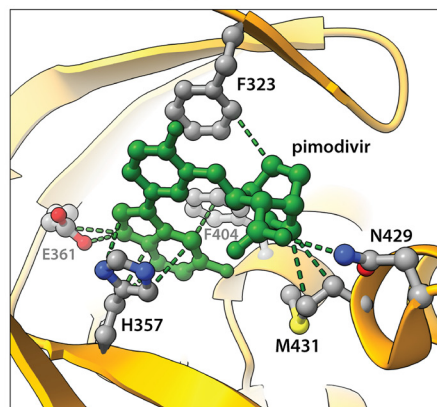
B. Favipiravir



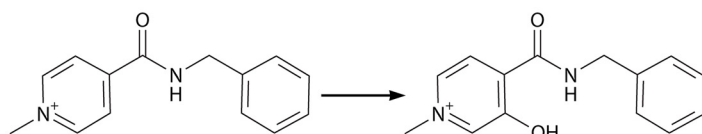
C. Pimodivir



D. Pimodivir bound to PB2

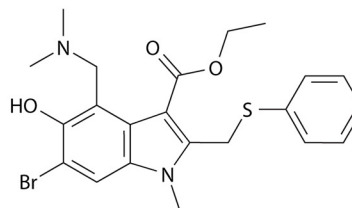


E. Enisamium

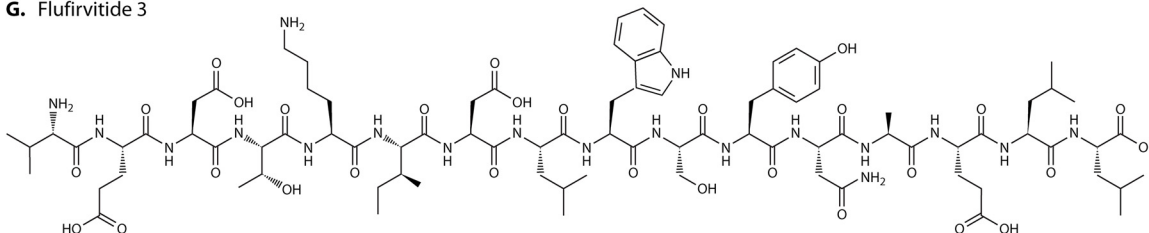


VR17-04 (active metabolite)

F. Arbidol



G. Flufirvitide 3



H-Val-Glu-Asp-Thr-Lys-Ile-Asp-Leu-Trp-Ser-Tyr-Asn-Ala-Glu-Leu-Leu-OH

FIG 7 Structures of influenza antivirals in clinical trials. (A) Laninamivir (IUPAC name, (2R,3R,4S)-3-acetamido-4-(diaminomethylideneamino)-2-[[1R,2R]-2,3-dihydroxy-1-methoxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid) and its prodrug (CS-8958). (B) Favipiravir (IUPAC name, 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide). (C) Pimodivir (IUPAC name, (2S,3S)-3-[[5-fluoro-2-(5-fluoro-1H-pyrrol[2,3-b]pyridin-3-yl)]pyrimidin-4-yl]amino]bicyclo[2.2.2]octane-2-carboxylic acid). (D) Polymerase basic protein 2 (PB2) wild type (PDB accession no. 7AS0) bound with pimodivir (JNJ-63623872). This structure depicts the strong binding mode of the antiviral pimodivir (green) in the binding pocket of PB2 (orange). Studying mutant variations of the residues in direct contact with pimodivir could be informative as to possible escape mutants for this investigational therapeutic. (E) Enisamium (IUPAC name, *N*-benzyl-1-methylpyridin-1-ium-4-carboxamide) and its active metabolite. (F) Arbidol (IUPAC name, ethyl 6-bromo-4-[[dimethylamino)methyl]-5-hydroxy-1-methyl-2-(phenylsulfanylmethyl)indole-3-carboxylate). (G) Flurifivir 3 (IUPAC name, (2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-4-amino-2-[(2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S,3S)-2-[[[(2S)-6-amino-2-[[[(2S,3R)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-2-amino-3-methylbutanoyl]amino]-4-carboxybutanoyl]amino]-3-carboxypropanoyl]amino]-3-hydroxybutanoyl]amino]hexanoyl]amino]-3-methylpentanoyl]amino]-3-carboxypropanoyl]amino]-4-methylpentanoyl]amino]-3-(1H-indol-3-yl)propanoyl]amino]-3-hydroxypropanoyl]amino]-3-(4-hydroxyphenyl)propanoyl]amino]-4-oxobutanoyl]amino]propanoyl]amino]-4-carboxybutanoyl]amino]-4-methylpentanoyl]amino]-4-methylpentanoic acid).

TABLE 2 Antivirals in clinical development or approved in different parts of the world

Target molecule	Nature of compound	Drug	Mode of action	Effectiveness	Side effect(s)	Reference(s)
NA Inhibitors	Chemical	Laninamivir	Inhibits neuraminidase activity	Broad range of influenza inhibitory activity, including influenza B- and Oseltamivir-resistant strains	Diarrhea, vomiting, nausea	224, 227
Viral polymerase inhibitors	Chemical	Favipiravir or T-705	Acts as purine nucleoside, can induce chain termination and lethal mutagenesis	Activity against novel and reemerging influenza virus	Teratogenicity, embryotoxicity, gastrointestinal symptoms, increased uric acid	234, 235
		Pimodivir or JNJ-63623872	Blocks cap binding functions of PB2 subunit of RNA polymerase	Discontinued, as it failed to demonstrate benefit over standard of care	Dose-related diarrhea, decreased neutrophils	243, 244
		AL794	Binds selectively to endonuclease domain of PA subunit	Discontinued due to failure to establish a single safe and effective dose and variability in drug exposure	Headache, dizziness	248, 249
		ZSP1273	Inhibits PB2 subunit of RNA polymerase	Results of clinical trials awaited for antiviral efficacy	Diarrhea, leucopenia, neutropenia	251
Ion channel inhibitor	Si-RNA	Enisamium iodide or Amizon	Inhibition of RNA synthesis	Faster patient recovery and reduced symptoms	Bitter taste in mouth and burning sensation in throat	256
		TG1000	Inhibition of cap-dependent endonuclease activity of PA	Results awaited from clinical trial	Results on safety awaited	257
		GP681	Inhibition of PA subunit	Clinical trial ongoing	Results on safety awaited	259
		AVI-700 or radavirsen	Targets both M1 and M2 translation	Well tolerated but clinical efficacy studies awaited	Headache and Proteinuria	279
HA inhibitors	Peptide Chemical	Flufirvitide 3	Broad spectrum entry inhibitor	Results not posted	Results not posted	268
		Arbidol or umifenovir	Inhibition of HA-induced membrane fusion	Reduction in time taken for resolution of clinical symptoms	Not known so far	269, 273
		MHAA4549A	Targets highly conserved epitope on HA and neutralizes virus	No significant difference in viral load	Not known	322
		MED18852	Broad-spectrum neutralizing activity	No significant advantage found in treated groups	Adverse side effects Including bronchitis	327
	MAB	VIS410	Broad-spectrum neutralizing activity	Provides therapeutic benefit with reduced viral load	Generally safe	330, 331

to be a target for antiviral development (188, 231, 232). Several new compounds targeting different subunits of influenza virus polymerase showed effectiveness. Here, we have discussed new compounds which target influenza polymerase complex.

Favipiravir. Favipiravir, also known as T-705, exhibits a broad-spectrum antiviral activity against influenza and flavi-, arena-, bunya-, noro-, and alpha-RNA viruses (233, 234). Favipiravir has anti-influenza activity against different virus subtypes, including A, B, and C. It acts as a purine nucleoside and can induce chain termination and lethal mutagenesis. Viral RNA polymerases recognize it as an alternative substrate, primarily as guanosine and secondarily as an adenine analog, resulting in errors during viral RNA synthesis (235, 236). A dose-finding, placebo-controlled, double-blind study with three groups of patients was conducted to assess the appropriate dose regimen and pharmacokinetics of Favipiravir for treating uncomplicated influenza. Two groups were treated for 4 days with either a high dose administered to 195 patients or a low dose of Favipiravir administered to 134 patients. Additionally, 201 patients served as a placebo control. Results from this study suggest that Favipiravir is well tolerated (237); however, the time to resolve symptoms was only 6.1 h shorter than the placebo (238). Recently, Wang et al. compared the effectiveness of the combination therapy of Favipiravir and Oseltamivir with the monotherapy of Oseltamivir in 128 and 40 critically ill influenza patients, respectively. Favipiravir and Oseltamivir therapy, compared to Oseltamivir therapy alone, accelerated clinical recovery. In addition, only 21.9%, compared to 67.5%, of patients demonstrated undetectable influenza viral RNA on day 10 (239). Although Favipiravir was found to be safe in clinical studies, due to concerns of teratogenicity and embryotoxicity, it was approved for conditional marketing in Japan for limited usage involving treatment for novel or reemerging influenza viruses (234).

Moreover, the usage of Favipiravir is completely restricted for pregnant women. Females of childbearing age are directed to avoid pregnancy during treatment and 7 days after the last dose to eliminate the drug from the system (233). Besides embryotoxicity, less severe side effects from treatment with Favipiravir include gastrointestinal symptoms and increased uric acid levels (240). Finally, although Favipiravir-resistant influenza viruses have been reported from an *in vitro* study (241), viruses with such mutations have not been isolated from the patients treated with this drug.

Pimodivir. Pimodivir is also known as VX-787 or JNJ-63623872. This orally administered nonnucleoside inhibitor suppresses the early stages of viral RNA transcription by blocking the cap-binding function of the PB2 subunit of RdRp (242). Pimodivir interacts with the m7 GTP guanine base by occupying the central cap-binding domain of PB2 (243). In a phase IIa study, 104 healthy subjects were challenged with A/Wisconsin/67/2005 A(H3N2) influenza virus, and 72 subjects were given Pimodivir at 100 mg, 400 mg, a loading dose of 900/600 mg, and a loading dose of 1,200/600 mg once daily for 5 days, and 32 subjects served as placebo controls. Interestingly, all the Pimodivir-treated groups showed a reduction in viral shedding and clinical symptoms compared to the control group (244). A double-blind, placebo-controlled phase IIb study assessing the clinical efficacy of Pimodivir reported dose-related diarrhea, nausea, and decreased neutrophils as a side effect (245). In this study consisting of 293 influenza patients between the ages of 18 to 64, patients received a twice-daily oral dose of Pimodivir alone (300 mg or 600 mg) or in combination with Oseltamivir (600 mg Pimodivir and 75 mg Oseltamivir) for 5 days. Pimodivir in combination with Oseltamivir resulted in lower viral titers and a shorter time to the resolution of symptoms. Another phase II clinical trial with a combination therapy of 600 mg Pimodivir with 75 mg Oseltamivir administered twice daily for 7 days was carried out to assess Pimodivir pharmacokinetics in influenza virus-infected 25 elderly patients between the ages of 65 to 85 years old compared to 38 nonelderly adults aged 18 to 65 years old. The primary outcome was a comparison of the time taken for the resolution of symptoms (246).

Interestingly, the two age groups of patients did not show any meaningful differences in pharmacokinetics or efficacy between them. The Pimodivir-Oseltamivir versus Oseltamivir groups did not show any differences in time to discharge. The viruses

isolated from Pimodivir-treated groups did not show any mutations in PB2. However, in other clinical studies, the emergence of mutations in the PB2 protein of the virus replicating in the presence of Pimodivir has been reported (244, 245). These include PB2 substitutions Met431Ile, Ser324Lys/Asn/Arg, Phe325Leu, Ser337Pro, Lys376Asn/Arg, Thr378Ser, and Asn510Lys, which decreases the susceptibility to the drug by several fold (245). Despite some promising initial results in the clinical setting, the clinical developmental program for Pimodivir was discontinued by Janssen, as the drug failed to demonstrate a benefit over standard of care (SOC) in influenza-infected patients (247).

AL-794. AL-794, an ester prodrug of ALS-033719, is orally active and inhibits both IAV and IAB viruses by binding selectively to the endonuclease domain of the PA subunit (240). In a double-blind phase I clinical study, the drug's pharmacokinetics, safety, and tolerability were evaluated in healthy individuals. The findings indicated that a twice-daily dosage of AL-794 up to 200 mg is well tolerated and generally achieved the expected efficacious levels of ALS-033719 in plasma (248). Further, to characterize the safety and antiviral activity of AL-794, a challenge study was conducted in which healthy subjects were intranasally inoculated with influenza A/Perth/16/2009 A(H3N2) virus and were subsequently treated with AL-794 50 mg and 150 mg twice daily for 5 days. AL-794 treatment reduced symptoms, viral load, and mucus weight, with a more significant decrease in these parameters at 150 mg without any safety concerns (249). The most common side effects associated with the oral administration of this drug were headache and dizziness (248). AL-794 was discontinued because, unlike another PA inhibitor, Baloxavir, a single dose that was effective and tolerated was not established in the clinical studies, along with significant variability in the exposure levels of drugs based on gender and prior food consumption (240).

ZSP1273. ZSP1273 is an orally administered small-molecule inhibitor that possesses anti-influenza activity because of its capability to inhibit the PB2 subunit of RdRp. A phase I clinical trial with 100 healthy individuals was conducted in China to assess the safety, tolerability, pharmacokinetics, and food-drug interactions of ZSP1273 (250). The study's findings revealed that the bioavailability of the drug was not affected by food intake, and high-fat diet had a limited effect on the drug's pharmacokinetics. The most common side effects were diarrhea, leukopenia, and neutropenia. The study recommended a dose of ≥ 200 mg for influenza patients (251). In addition, a phase III clinical study that compares the antiviral efficacy of ZSP1273 to placebo or Oseltamivir in uncomplicated IAV patients is expected to be completed by 2022 (252).

Enisamium iodide. Enisamium is an isonicotinic acid derivative, a low-molecular-weight compound known by the trade name Amizon or lab code FAV00A and is currently marketed in former Soviet Union countries and Mongolia as an antiviral agent against influenza and other viruses. A study by Boltz et al. showed a broad range of antiviral activity of Enisamium iodide against multiple strains of influenza A and influenza B viruses in primary normal human bronchial epithelial (NHBE) cells at 23- to 64-fold-lower doses than the cytotoxic concentration (253). In a follow-up study, the antiviral effects of the drug were reported in the ferret model of influenza infection. The authors also suggested that the mode of action of this compound is the inhibition of RNA synthesis of influenza A viruses (254). To evaluate the safety and clinical efficacy of Enisamium iodide, a randomized single-blind clinical study was conducted in patients aged 18 to 60 years with confirmed influenza and other respiratory viruses (255). The study reported faster patient recovery with reduced disease symptoms and viral shedding in the treatment group of 60 subjects than in the placebo group of 40 subjects (256). The adverse events included a bitter taste in the mouth, a burning sensation in the throat, and minor gastrointestinal side effects. Furthermore, the authors also confirmed that the mode of action of Enisamium iodide is through the inhibition of viral RNA polymerase by a hydroxylated metabolite of VRI7-04, as shown in Fig. 4D.

TG-1000. Developed by Tiagen Biotechnology company in Taiwan, TG-1000 is a novel influenza drug that shows inhibitory activity against IAV, IBV, and Oseltamivir-resistant viruses via inhibition of cap-dependent endonuclease activity of the PA subunit. The FDA

approved the investigational new drug application for TG-1000 in 2020. A phase II double-blind dose-ranging study to investigate the clinical efficacy and safety of TG-1000 is underway in adult patients with uncomplicated influenza (257).

GP681. GP681 is a prodrug that gives rise to GP1707007, a metabolite that inhibits the PA subunit of RdRp. GP681 was effective in preclinical studies; hence, it is being evaluated in clinical trials. A phase I clinical trial to assess the safety and tolerance of GP681 tablets in healthy adults has been completed (258). The results from a phase I clinical study are awaited. Currently, a phase II clinical trial to evaluate the safety and antiviral efficacy of GP681 tablets in the treatment of uncomplicated influenza is underway (259).

Antivirals Targeting the HA Protein

HA, the surface glycoprotein of the influenza virus, is a preferred target for developing antivirals, therapeutic antibodies, and vaccines. HA is translated from mRNA as a precursor protein HA0, which, upon proteolytic cleavage, gets converted to HA1 and HA2 subunits linked by a disulfide linkage (260, 261). Structurally, it possesses two distinct domains, a globular head that varies greatly among the different strains of influenza and a helix-rich stem region, which is conserved (261). Though the field of antiviral agents targeting HA is relatively recent compared to existing antivirals targeting NA, the developments in the area are promising, with increasing numbers of HA-targeting antivirals (161, 262–265). Here, we summarize small molecules and peptide inhibitors targeting the HA protein in clinical trials.

Flufirvitide 3. Flufirvitide 3 is a peptide composed of 16 amino acids derived from a specific region of HA2 called the fusion initiation region. It shows a broad-spectrum entry inhibition against different influenza subtypes, including IBV *in vitro* (266). A randomized placebo-controlled phase I dose-escalating study for evaluation of the safety profile of Flufirvitide 3 nasal spray was conducted in healthy subjects (267). Another phase I study for safety, tolerability, and pharmacokinetics of single and repeated doses of the dry powder form of Flufirvitide 3 was done in healthy adults (268). However, to our knowledge, the results of both these clinical trials have not been posted yet.

Arbidol. Arbidol (umifenovir or DB13609), an indole derivative molecule, is the first clinically used drug to inhibit HA-induced membrane fusion (262, 263, 269). This antiviral agent is believed to also act as an immunomodulator against some other viruses (270–272). Currently, it is licensed for over-the-counter use against both IAV and IBV infections in Russia and China. Arbidol functions as a molecular glue by attaching to the hydrophobic cavity in the HA trimer stem region. This binding interferes with the conformational changes, leading to the inhibition of membrane fusion and release of viral genome into the infected cells (269).

In a clinical study of 119 patients with influenza infection, 200 mg of Arbidol was administered orally for 5 days. The efficacy of Arbidol is observed mostly in the acute stage of the disease. Furthermore, the treatment effectively reduced the time taken to resolve all symptoms of the disease, severity of the disease, and duration of virus shedding (273). A phase III clinical trial to evaluate combination antiviral therapy of Oseltamivir and Arbidol versus Oseltamivir monotherapy for the treatment of severe influenza has been completed in China, but results are not yet available (274). In Russia, a phase IV clinical study evaluating Arbidol as a therapeutic and prophylactic agent against influenza has been completed; the results of the study results are not yet available (275).

Ion Channel Inhibitors

M1 and M2 proteins of the influenza virus are splice variant products of the same gene segment, and these are also a target for therapeutic interventions, including antisense strategies. As an antiviral agent against influenza viruses, small interfering RNA (siRNA) has demonstrated utility in preclinical studies (276–278). AVI-7100 is one such drug to be tested in clinical studies.

AVI-7100 (Radavirsen). AVI-7100, or Radavirsen, a phosphorodiamidate morpholino oligomer, targets M1 and M2 mRNA translation, as these two proteins share the same translation initiation start site. Further, the oligonucleotide is designed with a unique backbone structure for increased uptake in the infected cells. A phase I clinical trial of AVI-7100 was conducted in 56 healthy subjects between 18 and 60 years old, with intravenous administration of this molecule at different dosages. The findings suggest that a single injection of AVI-7100 up to 8 mg/kg and multiple dosages at 8 mg/kg once daily for 5 days are well tolerated and safe (279). However, the study also reported headache and proteinuria as the most common side effects in the placebo group (280). Further clinical studies are required to assess the therapeutic potential of AVI-7100 against influenza.

Passive Immunization

Treatment or protection against a disease can also be achieved through passive immunization where antibodies are transferred from immunized/infected and recovered individuals or recombinant monoclonal antibodies (mAbs) to naive individuals or patients. Convalescent plasma, hyperimmune sera, and lab-generated mAbs fall under the passive immunization strategy.

Convalescent plasma and intravenous immunoglobulins. Viral challenge studies in ferret and mouse models demonstrated the utility and efficacy of convalescent plasma and intravenous immunoglobulins against influenza (281, 282). Treatment of infected patients with passive transfer of antibodies reduced the severity and duration of the disease (283–285). During the 1918 influenza pandemic, convalescent human serum was administered to patients as a treatment against influenza. A meta-analysis by Luke et al. has reported that this mode of treatment during the pandemic results in a reduced risk of death. Interestingly, the timing of administration is crucial, as the positive outcome is associated with early administration of sera after the onset of symptoms (286). However, due to the safety issues related to the use of serum, the passive immunization approach did not get much attention until recently when hyperimmune sera were tested in clinical trials. The administration of hyperimmune intravenous globulin (hIVIG) fractionated from convalescent plasma of recovered patients of the 2009 A (H1N1)pdm09 pandemic to patients with severe influenza A(H1N1)pdm09 infection reduced mortality and virus load (287). However, the efficacy of hIVIG in hospitalized influenza patients from different geographical regions over five influenza seasons was not superior to placebo when it was coadministered with Oseltamivir (288). In another clinical trial with severe influenza A patients, the high-titered anti-influenza plasma has shown no significant benefit over the standard low-titered plasma as a treatment (289). Hence, further clinical studies on the passive transfer of sera/plasma in severe influenza patients are needed to assess the safety profile and therapeutic efficacy in reducing the viral titers and time to resolution of disease symptoms and pathology.

Monoclonal antibodies. The tremendous technological advancement during the past decade has facilitated the generation of recombinant human mAbs (290, 291). Intensive studies of human B cell repertoire upon influenza virus infection and vaccination have led to the isolation of several antiviral mAbs directed against different influenza viral proteins (290, 292–295). These antibodies are beneficial for treating hospitalized influenza patients without any added drug resistance concerns, as they target the highly conserved epitopes of viral proteins. Moreover, in recent years, the cost of these antibodies has also reduced significantly due to improved production methods (296). The advances in genetic immunization and vector-based delivery of these monoclonal antibodies can reduce the cost of production (297–299). Influenza infection or vaccination induces antibodies against 5 antigenic sites of HA protein (Ca1, Ca2, Cb, Sa, and Sb in cases of group 1A viruses and A, B, C, D, and E in cases of group 2 viruses). These antibodies are directed predominantly against the highly variable strain-specific HA head region. These antibodies may inhibit hemagglutination and neutralization of the virus. Antibodies against receptor-binding sites prevent infection and neutralize the virus. mAbs that react with multiple influenza A strains, including cross-subtypes (S139/1, C05, and F045-092) and cross-subclasses

against influenza B viruses (CR8033 and C12G6), were isolated (295, 300, 301). Receptor-binding antibodies drive the evolution of influenza viruses due to immune pressure. In addition to the antibodies against the head region, antibodies are also induced against the stem region of group 1 (CR8020, C179, F10, CR6261, 3.1, 3E1, and S9-3-3), group 2 (9H10), or against both group 1 and group 2 (31.a.83, 56.a.09, FI6v3, MEDI8852, and CR9114), which demonstrated broad neutralizing activity preventing membrane fusion (295, 302). These broadly neutralizing antibodies against HA stem can either function directly by preventing membrane fusion or indirectly engage in antiviral effector function by NK cells or macrophages via antibody-dependent cell-mediated cytotoxicity (ADCC) (303, 304). Apart from HA, NA is also a target for antibody responses and antibodies induced against the catalytic site. Other regions of NA can inhibit NA catalytic activity, thus blocking the release of progeny viruses from the infected cells. NA-specific MAbs, 228-1BO3, and several anti-N1 and -N2 antibodies, HCA2, 1GO1, 2E01, 1GO4, NA-45, and Z2B3, bind to the catalytic site, while CD6, NA-63, NA-80, and NA-22 bind outside the catalytic site and inhibit NA function (295, 305–308). In addition to the surface glycoproteins HA and NA, the ectodomain of matrix protein 2 (M2e) also is a target for antibody induction by infection (309). mAbs against M2e (14C2, C40, C40G1, L66, N547, Z3, Z3G1, 391, 472, 522, 602, 770, 934, and 1191) were generated in mice and tested for their activity *in vitro* and *in vivo* in mouse models (310–312). However, the MAbs against M2e have not yet been isolated from infected or vaccinated individuals. Influenza NP is a conserved internal protein that has been shown to activate CD8 T-cell responses, aiding in heterosubtypic protection and viral clearance (313–315). Following infection, antibodies against NP are found, and their role in conferring protection is not understood (316). Animals immunized with soluble NP protein or animals that received NP-specific antibodies were protected against viral challenge (317, 318). Mice transgenic for a human mAb against H5N1 NP isolated from a patient infected with H5N1 virus conferred protection against viral challenge (319). However, there are no reports on the isolation or function of human anti-NP mAbs. The mAbs directed against the HA stem region in clinical development are discussed here.

MHAA4549A. MHAA4549A is a human anti-influenza IgG1 mAb isolated and referred to as 39.29 earlier (292). This antibody targets a highly conserved epitope on the HA stalk region of the influenza virus and can neutralize influenza viruses from both groups 1 and 2 (320). Lim et al. conducted two phase I studies to evaluate the safety and pharmacokinetics of intravenously administered MHAA4549A (321). Results from the clinical trial suggest that the study drug is safe and well tolerated, with a serum half-life of 23 days. Additionally, both these studies reported mild headaches as the most common adverse side effect. The therapeutic potential of the MHAA4549A antibody was evaluated in a challenge study where 100 healthy subjects were inoculated with A/Wisconsin/67/2005 A(H3N2) virus followed by intravenous injection with different dosages of MHAA4549A (322). This placebo-controlled phase IIa study demonstrated that the highest tested dose of 3,600 mg of MHAA4549A significantly reduced virus burden and influenza symptoms.

Furthermore, in a phase IIb randomized, double-blind, placebo-controlled study, the safety and efficacy of MHAA4549A (3,600 mg and 8,400 mg) plus a standard dose of Oseltamivir were evaluated in hospitalized influenza patients with severe infection. The median time taken to the cessation of oxygen support to maintain a stable oxygen saturation of 95% and normalization of respiratory function were considered the study's primary outcome. However, an interim analysis showed no significant difference in the time taken for normalizing respiratory functions or virus load between placebo plus Oseltamivir and MHAA4549A plus Oseltamivir (323).

MED18852. MED18852 is a broad-spectrum human mAb with potent IAV-neutralizing activity. It was first isolated and characterized by Kallewaard et al. and has shown promising results in preclinical settings (324, 325). This has led to the evaluation of its therapeutic potential in both uncomplicated and severe influenza infection in the clinical setting. MED18852 has proven safe at a dose ranging from 250 mg to 3,000 mg in

healthy adults without any antibody detection against MED18852 posttreatment (326). A follow-up study assessed the safety profile and therapeutic potential of this antibody in patients with uncomplicated influenza (327). The treatment group comprised of 126 influenza A confirmed adults receiving a single intravenous injection of MED18852 at 3,000 mg, 3,000 mg of MED18852 in combination with Oseltamivir, 750 mg of MED18852 in combination with Oseltamivir, or a placebo combined with Oseltamivir. MED18852 treatment has resulted in more adverse effects than Oseltamivir alone, with bronchitis being the most common side effect. Moreover, there has been no significant difference in all treatment groups' viral titers and influenza symptoms. Therefore, a planned clinical trial to evaluate MED18852 efficiency in hospitalized influenza patients did not move forward (328).

VIS-410. VIS-410 is an engineered human mAb known to target unique epitopes on the influenza HA stem region and possesses broad neutralizing activity against different strains of IAV. In preclinical studies, VIS-410 protected the mice from the lethal challenge of A(H7N9) influenza virus (329). Following this, a phase I safety trial has been conducted in 41 healthy adults, revealing that VIS-410 is generally safe at dose level 2 to 50 mg/kg with mild to moderate adverse effects, including diarrhea (330). Furthermore, the study has reported that a single intravenous injection of VIS-410 has a mean half-life of 12.9 days, and the concentration in the serum and respiratory tract is proportional to the dosage used. Furthermore, Hershberger et al. also have shown in a randomized double-blind, placebo-controlled, phase II study that a dose ranging from 2,000 mg to 4,000 mg per individual is safe and well tolerated in 138 healthy adults with uncomplicated influenza (331). Finally, a phase IIA challenge study with A/California/07/2009 A(H1N1)pdm09 in 46 healthy adults has demonstrated that upon treatment with VIS-410 post-inoculation with the virus, there was 76% decrease in viral load, suggesting that VIS-410 provides therapeutic benefits (332).

Combination Therapies

Antivirals against influenza can also be used in different combinations for enhanced potency and reduced emergence of drug-resistant viruses. One of the first drug combinations against the influenza virus was Amantadine with interferon (333). Subsequently, a triple combination of antivirals, including interferon alpha 2, Ribavirin, and Rimantadine, was also tested against influenza virus (334). This combination of antiviral therapy can simultaneously act on different targets and can prove beneficial for treating more severe forms of influenza. In addition, as described in previous sections, several new drugs have been tried in combination with already-established antivirals like Oseltamivir. Currently, several clinical studies are ongoing to test the pharmacokinetic interactions and tolerability of several drug combinations, Flufenamic acid-Clarithromycin-Oseltamivir, Naproxen-Clarithromycin-Oseltamivir, and ADS-8902 (335–337).

Host Protein-Targeted Therapies

The study of the interaction between the influenza virus and various host proteins is another important area of active investigation. In recent years, considerable progress has been made in deciphering the role of host factors during the influenza virus life cycle (338). Genome-wide screens using siRNA or chemical inhibitors or clustered regularly interspaced short palindromic repeats (CRISPR), along with interactome and transcriptome data using different approaches, expanded our current knowledge (339–342). These host factors can also serve as potential targets for developing antivirals, as the virus is dependent on the host cellular machinery to complete its life cycle (342). Targeting the host factors for antiviral therapy has some added advantages, including the low likelihood of the emergence of resistance, as most of these factors are evolutionarily conserved. Moreover, it can also potentially apply to multiple respiratory viruses, as these viruses mainly utilize common molecular pathways (343, 344). However, the major drawback of host-targeted antivirals is the greater risk of host toxicity, as there are safety and tolerability concerns in targeting cellular functions; hence, careful safety studies are required (345).

GSK1325756 (Danirixin). GSK1325756 is a selective and reversible small molecule and a CXCR2 antagonist that decreases neutrophil activation and migration to the area of inflammation in preclinical studies. In the respiratory tract, there is neutrophil influx during infection, and excessive neutrophil influx correlates with severe influenza symptoms (346). The Danirixin dose-dependent inhibition of agonist-induced activation of neutrophils in healthy patients suggested that the inhibitor can be beneficial for controlling inflammation (347). Additionally, the phase I study demonstrated that oral Danirixin was generally well tolerated as a single dose (up to 400 mg) or repeated treatment (50 mg daily and 200 mg for 14 days). However, the pharmacokinetics of the study drug can be affected by diet, age, and other factors (347, 348). The efficacy and safety during a small phase IIb trial evaluated the safety and efficacy of two dosages of Danirixin in combination with Oseltamivir in 10 adults hospitalized with influenza (349). Danirixin exhibited a similar safety and tolerability profile as reported previously with no serious adverse effects. However, a conclusion on the efficacy is unavailable because of the small sample size consisting of four patients in each combo treatment group and two in the placebo-Oseltamivir group (349). Additionally, 45 patients with mild-to-moderate chronic obstructive pulmonary disease (COPD) demonstrated improvement in respiratory symptoms and health status when they received Danirixin along with the standard-of-care inhalation medications compared to 48 patients who received only the standard-of-care treatment. These data indicate potential benefits of treating influenza in patients with COPD (350).

Probenecid. Probenecid is an inhibitor of the host protein organic anion transporter (OAT3) required to support IAV replication (351). Probenecid is a good candidate to be repurposed for influenza treatment, as it is already used for treating hypouricemic disorder (gout), with established safety, pharmacokinetics, and interactions with other drugs (352). Several studies demonstrated that probenecid coadministration could significantly increase Oseltamivir metabolite concentration in plasma (353, 354). Alternate-day dosing of Oseltamivir in combination with Probenecid four times daily and the conventional Oseltamivir dosing had similar levels of plasma concentration (354). As Oseltamivir is considered a standard-of-care treatment and can be limiting in the event of an influenza pandemic, additional studies on alternative dosing strategies and pharmacokinetics are needed.

Celecoxib. It is an immunomodulator that inhibits the COX-2 enzyme. Celecoxib (200 mg daily) was used with Oseltamivir and compared with oseltamivir alone as a treatment for severe influenza A infection in phase III trial (355) from 2014 to 2017. The results demonstrated that the cotreatment of celecoxib with oseltamivir significantly reduced mortality and cytokines (interleukin 6 [IL-6] and IL-10) without increased adverse effects (356).

DAS-181 (Fludase). Fludase, or DAS-181, is a recombinant fusion protein that enzymatically cleaves sialic acid receptors responsible for the binding and entering of influenza virus in respiratory epithelial cells. The interaction results in the inhibition of entry of the virus in these cells (357). Preclinical studies of this drug against influenza demonstrated its potent antiviral activity (357, 358). A phase II clinical trial evaluated multiple doses of 30 mg and a 10-mg single dose of DAS-181 along with a placebo in 56, 69, and 52 influenza-infected patients, respectively (359). In addition, three doses of 10 mg of DAS-181-F2 compared to placebo significantly reduced influenza virus shedding (359). Additional phase I trials were conducted as dry powder administered at dosages ranging from 0.5 mg to 4.5 mg (formulations DAS-181-F03 and F-04) to target the upper respiratory tract and minimize adverse events and the increased dose to 20 mg for 3 days as a single dose to improve the effectiveness in healthy adults (360). The trials in healthy adults to evaluate the safety and systemic exposure demonstrated that 1- and 3-day treatments with 20 mg of DAS-181 daily were well tolerated with no severe side effects. However, adverse respiratory symptoms were recorded during the treatment longer than 7 days with the development of anti-DAS-181 antibodies in the subjects with respiratory symptoms.

Diltiazem. Diltiazem is a licensed calcium channel blocker known to relax blood vessels and is used to treat hypertension. It was identified in a screen of host factors with influenza-inhibitory activity (361). It was demonstrated *in vitro* and *in vivo* in a mouse model that Diltiazem modulates host antiviral genes *in vitro* and *in vivo* in a mouse model, and cotreatment with Oseltamivir enhanced the antiviral efficacy (362). The results lead to rapid authorization of a phase II ongoing clinical trial to assess the efficacy of the Diltiazem-Oseltamivir combination in treating severe influenza (363).

Host Innate Immune Activators

Strategies designed to modulate and boost the innate immune response against the virus are critical in reducing the impact of influenza. During the influenza infection, different innate immune sensors get activated eventually, leading to the establishment of the antiviral state (as described in an earlier section) (66). Lee et al. studied the role of TLRs in influenza A infection and reported that TLRs play an essential role in early influenza infection with the upregulation of TLR3 with other TLRs (364). Since these innate immune sensors are evolutionarily conserved, their ligands have potential as next-generation antivirals without the associated risk of developing drug resistance.

In most cases, coinfection with other respiratory viruses occurs with influenza infection (365, 366). Therefore, targeting influenza virus with the ligands of these pathogen sensors can also target several of these coinfections, ultimately leading to containing their infection. Figure 2 shows the synthetic ligands of PRRs with their downstream cellular signaling pathways.

RIG-I agonists. The cytosolic sensor RIG-I plays an indispensable role in recognizing 5'PPP-RNA and induction of the innate immune response (367). RIG-I ligand and 5'PPP-RNA activate the innate immune response and inhibit the replication of WT and drug-resistant influenza viruses *in vitro* and *in vivo* in animal models (368). This inhibition is independent of virus subtypes, drug sensitivity status, and virulence. Furthermore, the activation of the RIG-I pathway generates a broad-spectrum antiviral response as suggested by the replication inhibition of the Ebola virus in cell culture (369). Similarly, using the systems approach, Goulet et al. showed the antiviral potential of 5'PPP-RNA *in vitro* against several viruses along with the lethal challenge of A(H1N1)pdm09 influenza virus in a murine model (370–372).

Furthermore, the length, secondary structure, and sequence of 5'PPP-RNA modulate RIG-I-mediated protective antiviral response in cell cultures and murine models (373). In some cases, small-molecule agonists could also serve as antivirals for efficient control of virus infection by inducing tissue-wide innate immunity like the IRF3 activator, which induces innate immune gene expression (374). However, inefficient cytosolic delivery hindered the efficacy of RIG-I ligands. Recently, the clinical development of RIG-I agonists has advanced by developing novel polymeric carriers, which promote endosomal escape, leading to the enhanced activity and delivery of 5'PPP-RNA to the cytosol where RIG-I is located (375).

TLR3 agonists. Polyriboinosinic:polyribocytidylic acid [polyI:C] is a double-stranded RNA analog and one of the most used TLR3 agonists in experimental settings (376). Depending on the cellular localization, polyI:C activates different PRRs. For example, in endosomes, polyI:C activates TLR3 in the endosomes and RIG-I and MDA5 in cytosol (376–378). Prophylactic treatment of mice with polyI:C has shown immediate protection against the challenge with several influenza virus strains via upregulation of TLR3 in airways (379). Furthermore, intranasal delivery of polyI:C to aged mice upon challenge with influenza virus protected them without severe side effects (379). Nonavailability of a formulation that enhances the uptake of polyI:C and protects it from the RNases hindered the clinical development. PrEP-001 is a proprietary formulation of polyI:C which is in powder form and can be delivered intranasally, eliciting innate immunity. In a placebo-controlled study, 27 subjects received two doses of 6.4 mg of PrEP prior to challenge with 10 50% tissue culture infective doses (TCID₅₀) of A/Perth/16/2009 virus (380). PrEP-001 is safe and well tolerated with reduced symptom score, peak viral titers, and duration of A(H3N2)-IAV infection (381).

Type I interferons and IFN inducers. As interferons are the key contributors in the antiviral response against influenza, any defects in their production or regulation can have devastating effects. In humans, defective type I or type III IFN amplification causes the mild disease to intensify into a severe life-threatening disease (382). *In vitro* and *in vivo* studies have suggested the effectiveness of IFN treatment in influenza infection (383). The prophylactic use of oral interferon alpha at a low dose (150 IU) for prevention of acute respiratory viral infection (ARI) and influenza resulted in reduced disease severity in those who also received influenza vaccination. However, the low-dose IFN administration did not affect the overall incidence of ARI (384). Other immune molecules like Alfacon-1, known to be potent against different viruses, were tested against influenza in hospitalized patients. However, results from clinical trials are not published yet (385). Kagocel is an immunomodulator that induces type I and III interferons and exhibits broad-spectrum activity against influenza (Fig. 2). A recent study using murine lymphocytes from Peyer's patches suggested that PRRs mediate the action of this drug (386). In a clinical study, Kagocel administration increased cytokine levels in the plasma of patients with influenza infection (387). The drug has been evaluated for preventing ARI in young adult students over 18 years of age and adult and older adult (18 to 70 years) health care workers in Russia (388, 389).

Volatile anesthetics. A prospective blind cohort study of pediatric patients with upper respiratory tract infection who underwent anesthesia with halothane demonstrated significantly shorter and fewer clinical symptoms than untreated patients (390). In addition, halothane and other volatile anesthetics exhibited antiviral responses in murine and ferret models of influenza (391–393). The mechanism by which volatile anesthetics decrease the pathogenesis of influenza virus involves the modulation of the host Th1-adaptive immune response, specifically IFN- γ activation of CD8 T cells and monocyte recruitment (394). Although we do not normally anesthetize patients to treat viral infections, this therapy was again explored in COVID-19 patients on ventilators. Additionally, this therapeutic strategy may lead to the development of new approaches.

MACHINE LEARNING AS A TOOL FOR DRUG DISCOVERY AND DEVELOPMENT

Machine learning (ML) has become increasingly popular to facilitate all stages of drug discovery and development, as it has the potential to reduce time and lower cost (395, 396). A typical application of ML is the prediction of antiviral resistance. For instance, we can use decision trees and neural networks to predict resistance to antivirals, such as the M2 ion channel inhibitor, adamantane class of drugs and the NA inhibitor, Oseltamivir (397). In addition, ML can predict the potential functional causality of putative targets based on known antiviral agents. Pang et al. (398) developed AVPIden, a two-stage classification tool for predicting antiviral peptides (AVP) and potential functional activities against eight viruses, including HIV, influenza A virus, and SARS-CoV.

Additionally, with the recent advances in natural language processing, ML algorithms such as BeFree (399) and DigSee (400) can quickly scan massive amounts of data to identify relevant gene-disease associations. Furthermore, ML can identify novel therapeutics by understanding fundamental biological mechanisms. For example, recent evidence suggests that viral-induced alternative splicing promotes influenza replication (401). Deep neural networks (DNNs) accurately predict alternate splicing signals based on genomic and cellular features (402). Another vital application of ML is to reduce the search space for druggable targets and improve the virtual screening efficiency of lead compounds. For example, support vector machine models identified five oseltamivir derivatives with potent inhibition targeting NA against H1N1 and H3N2 (403). Combined with molecular docking software, random-forest models were used to screen group 2 NAs (N2, N3, N6, N7, and N9), of the IAV(H7N9) (404) virus. In a recent study (405), SiMMap, a hierarchical clustering method (406), screened more than 200,000 public records of compounds to identify HA inhibitors for the influenza A virus. Multineural networks can identify compounds with structures similar to the currently used drugs (407, 408). In addition, ML can optimize the compound design for small-molecule and other

therapeutics. For example, AstraZeneca used deep reinforcement learning as a parameter tuning tool to find optimal values for *de novo* compound designs (409).

Moreover, ML methods can design small molecules based on multiscale behavior and interactions to selectively inhibit multiple influenza targets while mitigating interaction with host proteins to minimize adverse effects (410). Finally, ML can have application in drug repurposing for influenza (362, 411–417). With the integrated efforts to improve data quality and availability, ML is a promising approach to developing next-generation antivirals and therapeutics for infectious diseases.

CONCLUSIONS AND FUTURE PERSPECTIVES

Influenza, though a vaccine-preventable disease, remains a serious public health problem globally. Current FDA-approved therapeutics have a short treatment window. Influenza virus can overcome the effects of the antiviral agents and develop drug resistance, leading to epidemics and pandemics. Apart from developing new classes of direct-acting antiviral drugs that act on viral factors, targeting the essential host factors needed for viral entry, replication, and release is another important area for therapeutic interventions, especially when the circulating viruses are resistant to one or more currently approved antiviral drugs. Activating innate immune responses with the ligands of evolutionarily conserved pathogen sensors can be a pan-antiviral strategy against drug-resistant viruses. Treatment approaches like passive immunization using mAbs or polyclonal sera in combination with antiviral drugs can benefit severely ill influenza patients with limited treatment options. Developing potent antiviral therapeutics that target influenza is crucial for prophylactic and therapeutic interventions against seasonal and pandemic influenza viruses.

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REFERENCES

1. World Health Organization. 2022. Comparison of number of influenza detections by subtype. <https://app.powerbi.com/view?r=eyJrIjoiaZlTlxMzAwMzYtZW4NC00YTU2LWE3MTU0MTI0OGY1ZjQyMWVliwidCI6ImY2MTBjMGJ3LWJkMjQ0NGlzM004MTBjLTNkYzI4MGFmYjU5MCI6ImMiOjBh>. Retrieved 18 February 2022.
2. Centers for Disease Control and Prevention. 2022. Weekly U.S. influenza surveillance report. <https://www.cdc.gov/flu/weekly/index.htm>. Retrieved 18 February 2022.
3. Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, Cohen C, Gran JM, Schanzer D, Cowling BJ, Wu P, Kyncl J, Ang LW, Park M, Redlberger-Fritz M, Yu H, Espenhain L, Krishnan A, Emukule G, van Asten L, Pereira da Silva S, Aungkulanon S, Buchholz U, Widdowson M-A, Bresee JS, Global Seasonal Influenza-associated Mortality Collaborator Network. 2018. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* 391:1285–1300. [https://doi.org/10.1016/S0140-6736\(17\)33293-2](https://doi.org/10.1016/S0140-6736(17)33293-2).
4. Centers for Disease Control and Prevention. 2020. Past seasons estimated influenza disease burden. <https://www.cdc.gov/flu/about/burden/past-seasons.html?web=1&wdLOR=cEAB96504-2317-493E-BEF7-B9E799A85D55>. Retrieved 18 February 2022.
5. Centers for Disease Control and Prevention. 2021. People at higher risk of flu complications. <https://www.cdc.gov/flu/highrisk/index.htm>. Retrieved 18 February 2022.
6. Watanabe T. 2013. Renal complications of seasonal and pandemic influenza A virus infections. *Eur J Pediatr* 172:15–22. <https://doi.org/10.1007/s00431-012-1854-x>.
7. Tsai JP, Baker AJ. 2013. Influenza-associated neurological complications. *Neurocrit Care* 18:118–130. <https://doi.org/10.1007/s12028-012-9796-8>.
8. Zangiabadian M, Nejadghaderi SA, Mirsaeidi M, Hajikhani B, Goudarzi M, Goudarzi H, Mardani M, Nasiri MJ. 2020. Protective effect of influenza vaccination on cardiovascular diseases: a systematic review and meta-analysis. *Sci Rep* 10:20656. <https://doi.org/10.1038/s41598-020-77679-7>.

9. Husby A, Hansen JV, Fosbøl E, Thiesson EM, Madsen M, Thomsen RW, Sørensen HT, Andersen M, Wohlfahrt J, Gislason G, Torp-Pedersen C, Køber L, Hviid A. 2021. SARS-CoV-2 vaccination and myocarditis or myopericarditis: population based cohort study. *BMJ* 375:e068665. <https://doi.org/10.1136/bmj-2021-068665>.
10. Patone M, Mei XW, Handunnetthi L, Dixon S, Zaccardi F, Shankar-Hari M, Watkinson P, Khunti K, Harnden A, Coupland CAC, Channon KM, Mills NL, Sheikh A, Hippisley-Cox J. 2022. Risks of myocarditis, pericarditis, and cardiac arrhythmias associated with COVID-19 vaccination or SARS-CoV-2 infection. *Nat Med* 28:410–422. <https://doi.org/10.1038/s41591-021-01630-0>.
11. Sellers SA, Hagan RS, Hayden FG, Fischer WA, II. 2017. The hidden burden of influenza: a review of the extra-pulmonary complications of influenza infection. *Influenza Other Respir Viruses* 11:372–393. <https://doi.org/10.1111/irv.12470>.
12. Putri W, Muscatello DJ, Stockwell MS, Newall AT. 2018. Economic burden of seasonal influenza in the United States. *Vaccine* 36:3960–3966. <https://doi.org/10.1016/j.vaccine.2018.05.057>.
13. Kosik I, Yewdell JW. 2019. Influenza hemagglutinin and neuraminidase: Yin–Yang proteins coevolving to thwart immunity. *Viruses* 11:346. <https://doi.org/10.3390/v11040346>.
14. Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen L-M, Johnson A, Tao Y, Dreyfus C, Yu W, McBride R, Carney PJ, Gilbert AT, Chang J, Guo Z, Davis CT, Paulson JC, Stevens J, Rupprecht CE, Holmes EC, Wilson IA, Donis RO. 2013. New World bats harbor diverse influenza A viruses. *PLoS Pathog* 9:e1003657. <https://doi.org/10.1371/journal.ppat.1003657>.
15. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, Gubareva LV, Xu X, Bridges CB, Uyeki TM, Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 360:2605–2615. <https://doi.org/10.1056/NEJMoa0903810>.
16. Centers for Disease Control and Prevention. 2021. Past seasons' vaccine effectiveness estimates. <https://www.cdc.gov/flu/vaccines-work/past-seasons-estimates.html>. Retrieved 18 February 2022.
17. Tricco AC, Chit A, Soobiah C, Hallett D, Meier G, Chen MH, Tashkandi M, Bauch CT, Loeb M. 2013. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. *BMC Med* 11:153. <https://doi.org/10.1186/1741-7015-11-153>.
18. Skowronski DM, Chambers C, De Serres G, Sabaiduc S, Winter A-L, Dickinson JA, Gubbay JB, Drews SJ, Fonseca K, Charest H, Martineau C, Hickman R, Chan T, Jassem A, Petric M, Rose C, Bastien N, Li Y, Krajden M. 2019. Vaccine effectiveness against lineage-matched and -mismatched influenza B viruses across 8 seasons in Canada, 2010–2011 to 2017–2018. *Clin Infect Dis* 68:1754–1757. <https://doi.org/10.1093/cid/ciy876>.
19. Drori Y, Pando R, Seftly H, Rosenberg A, Mendelson E, Keinan-Boker L, Shohat T, Mandelboim M, Glatman-Freedman A, Israel Influenza Surveillance Network IISN. 2020. Influenza vaccine effectiveness against laboratory-confirmed influenza in a vaccine-mismatched influenza B-dominant season. *Vaccine* 38:8387–8395. <https://doi.org/10.1016/j.vaccine.2020.10.074>.
20. Kang EK, Eun BW, Kim NH, Lim JS, Lee JA, Kim DH. 2016. The priming effect of previous natural pandemic H1N1 infection on the immunogenicity to subsequent 2010–2011 influenza vaccination in children: a prospective cohort study. *BMC Infect Dis* 16:438. <https://doi.org/10.1186/s12879-016-1769-7>.
21. Liu F, Tzeng W-P, Horner L, Kamal RP, Tatum HR, Blanchard EG, Xu X, York I, Tumpey TM, Katz JM, Lu X, Levine MZ. 2018. Influence of immune priming and egg adaptation in the vaccine on antibody responses to circulating A(H1N1)pdm09 viruses after influenza vaccination in adults. *J Infect Dis* 218:1571–1581. <https://doi.org/10.1093/infdis/jiy376>.
22. Leibovici Weissman Y, Cooper L, Sternbach N, Ashkenazi-Hoffnung L, Yahav D. 2021. Clinical efficacy and safety of high dose trivalent influenza vaccine in adults and immunosuppressed populations—a systematic review and meta-analysis. *J Infect* 83:444–451. <https://doi.org/10.1016/j.jinf.2021.08.028>.
23. Wiggins KB, Smith MA, Schultz-Cherry S. 2021. The nature of immune responses to influenza vaccination in high-risk populations. *Viruses* 13:1109. <https://doi.org/10.3390/v13061109>.
24. Avey S, Mohanty S, Chawla DG, Meng H, Bandaranayake T, Ueda I, Zapata HJ, Park K, Blevins TP, Tsang S, Belshe RB, Kaech SM, Shaw AC, Kleinstein SH. 2020. Seasonal variability and shared molecular signatures of inactivated influenza vaccination in young and older adults. *J Immunol* 204:1661–1673. <https://doi.org/10.4049/jimmunol.1900922>.
25. Wen F, Guo J, Li Z, Huang S. 2018. Sex-specific patterns of gene expression following influenza vaccination. *Sci Rep* 8:13517. <https://doi.org/10.1038/s41598-018-31999-x>.
26. Palomba E, Castelli V, Renisi G, Bandera A, Lombardi A, Gori A. 2021. Antiviral treatments for influenza. *Semin Respir Crit Care Med* 42:859–872. <https://doi.org/10.1055/s-0041-1733830>.
27. Hayden FG, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, Ishida T, Sekino H, Yamada K, Portsmouth S, Kawaguchi K, Shishido T, Arai M, Tsuchiya K, Uehara T, Watanabe A, Baloxavir Marboxil Investigators Group. 2018. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. *N Engl J Med* 379:913–923. <https://doi.org/10.1056/NEJMoa1716197>.
28. Tejada S, Tejo AM, Peña-López Y, Forero CG, Corbella X, Rello J. 2021. Neuraminidase inhibitors and single dose baloxavir are effective and safe in uncomplicated influenza: a meta-analysis of randomized controlled trials. *Expert Rev Clin Pharmacol* 14:901–918. <https://doi.org/10.1080/17512433.2021.1917378>.
29. Centers for Disease Control and Prevention. 2022. Interim guidance on the use of antiviral medications for treatment of human infections with novel influenza A viruses associated with severe human disease. <https://www.cdc.gov/flu/avianflu/novel-av-treatment-guidance.htm>. Retrieved 15 February 2022.
30. Holmes EC, Hurt AC, Dobbie Z, Clinch B, Oxford JS, Piedra PA. 2021. Understanding the impact of resistance to influenza antivirals. *Clin Microbiol Rev* 34:e00224–20. <https://doi.org/10.1128/CMR.00224-20>.
31. Jackson GG, Muldoon RL, Akers LW. 1963. Serological evidence for prevention of influenzal infection in volunteers by an anti-influenzal drug adamantanamine hydrochloride. *Antimicrob Agents Chemother (Bethesda)* 161:703–707.
32. Abed Y, Goyette N, Boivin G. 2005. Generation and characterization of recombinant influenza A (H1N1) viruses harboring amantadine resistance mutations. *Antimicrob Agents Chemother* 49:556–559. <https://doi.org/10.1128/AAC.49.2.556-559.2005>.
33. Bright RA, Medina M-J, Xu X, Perez-Oroz G, Wallis TR, Davis XM, Povinelli L, Cox NJ, Klimov AI. 2005. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 366:1175–1181. [https://doi.org/10.1016/S0140-6736\(05\)67338-2](https://doi.org/10.1016/S0140-6736(05)67338-2).
34. Deyde VM, Xu X, Bright RA, Shaw M, Smith CB, Zhang Y, Shu Y, Gubareva LV, Cox NJ, Klimov AI. 2007. Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. *J Infect Dis* 196:249–257. <https://doi.org/10.1086/518936>.
35. Centers for Disease Control and Prevention. 2009. Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. *MMWR Morb Mortal Wkly Rep* 58:433–435.
36. Deyde VM, Sheu TG, Trujillo AA, Okomo-Adhiambo M, Garten R, Klimov AI, Gubareva LV. 2010. Detection of molecular markers of drug resistance in 2009 pandemic influenza A (H1N1) viruses by pyrosequencing. *Antimicrob Agents Chemother* 54:1102–1110. <https://doi.org/10.1128/AAC.01417-09>.
37. Garcia V, Aris-Brosou S. 2014. Comparative dynamics and distribution of influenza drug resistance acquisition to protein M2 and neuraminidase inhibitors. *Mol Biol Evol* 31:355–363. <https://doi.org/10.1093/molbev/mst04>.
38. Hutchinson EC, Charles PD, Hester SS, Thomas B, Trudgian D, Martínez-Alonso M, Fodor E. 2014. Conserved and host-specific features of influenza virion architecture. *Nat Commun* 5:4816. <https://doi.org/10.1038/ncomms5816>.
39. Noda T. 2011. Native morphology of influenza virions. *Front Microbiol* 2:269. <https://doi.org/10.3389/fmicb.2011.00269>.
40. Rossman JS, Jing X, Leser GP, Balannik V, Pinto LH, Lamb RA. 2010. Influenza virus M2 ion channel protein is necessary for filamentous virion formation. *J Virol* 84:5078–5088. <https://doi.org/10.1128/JVI.00119-10>.
41. Roberts PC, Lamb RA, Compans RW. 1998. The M1 and M2 proteins of influenza A virus are important determinants in filamentous particle formation. *Virology* 240:127–137. <https://doi.org/10.1006/viro.1997.8916>.
42. Kohno Y, Muraki Y, Matsuzaki Y, Takashita E, Sugawara K, Hongo S. 2009. Intracellular localization of influenza C virus NS2 protein (NEP) in infected cells and its incorporation into virions. *Arch Virol* 154:235–243. <https://doi.org/10.1007/s00705-008-0292-6>.
43. Ferhadian D, Contrant M, Printz-Schweiger A, Smyth RP, Paillart J-C, Marquet R. 2018. Structural and functional motifs in influenza virus RNAs. *Front Microbiol* 9:559. <https://doi.org/10.3389/fmicb.2018.00559>.
44. Bouvier NM, Palese P. 2008. The biology of influenza viruses. *Vaccine* 26 Suppl 4:D49–D53. <https://doi.org/10.1016/j.vaccine.2008.07.039>.

45. Dou D, Revol R, Östbye H, Wang H, Daniels R. 2018. Influenza A virus cell entry, replication, virion assembly and movement. *Front Immunol* 9: 1581. <https://doi.org/10.3389/fimmu.2018.01581>.
46. Moreira EA, Yamauchi Y, Matthias P. 2021. How influenza virus uses host cell pathways during uncoating. *Cells* 10:1722. <https://doi.org/10.3390/cells10071722>.
47. Richardson JC, Akkina RK. 1991. NS2 protein of influenza virus is found in purified virus and phosphorylated in infected cells. *Arch Virol* 116:69–80. <https://doi.org/10.1007/BF01319232>.
48. Thompson AJ, Paulson JC. 2021. Adaptation of influenza viruses to human airway receptors. *J Biol Chem* 296:100017. <https://doi.org/10.1074/jbc.REV120.013309>.
49. Sun X, Whittaker GR. 2013. Entry of influenza virus. *Adv Exp Med Biol* 790:72–82. https://doi.org/10.1007/978-1-4614-7651-1_4.
50. Sempere Borau M, Stertz S. 2021. Entry of influenza A virus into host cells—recent progress and remaining challenges. *Curr Opin Virol* 48: 23–29. <https://doi.org/10.1016/j.coviro.2021.03.001>.
51. Mukaigawa J, Nayak DP. 1991. Two signals mediate nuclear localization of influenza virus (A/WSN/33) polymerase basic protein 2. *J Virol* 65: 245–253. <https://doi.org/10.1128/JVI.65.1.245-253.1991>.
52. Wu WW, Sun YH, Panté N. 2007. Nuclear import of influenza A viral ribonucleoprotein complexes is mediated by two nuclear localization sequences on viral nucleoprotein. *Virol J* 4:49. <https://doi.org/10.1186/1743-422X-4-49>.
53. Ozawa M, Fujii K, Muramoto Y, Yamada S, Yamayoshi S, Takada A, Goto H, Horimoto T, Kawaoka Y. 2007. Contributions of two nuclear localization signals of influenza A virus nucleoprotein to viral replication. *J Virol* 81:30–41. <https://doi.org/10.1128/JVI.01434-06>.
54. Resa-INFANTE P, Jorba N, Zamarreño N, Fernández Y, Juárez S, Ortín J. 2008. The host-dependent interaction of alpha-importins with influenza PB2 polymerase subunit is required for virus RNA replication. *PLoS One* 3:e3904. <https://doi.org/10.1371/journal.pone.0003904>.
55. Stubbs TM, Te Velthuis AJ. 2014. The RNA-dependent RNA polymerase of the influenza A virus. *Future Virol* 9:863–876. <https://doi.org/10.2217/fvl.14.66>.
56. Fodor E, Te Velthuis AJW. 2020. Structure and function of the influenza virus transcription and replication machinery. *Cold Spring Harb Perspect Med* 10:a038398. <https://doi.org/10.1101/cshperspect.a038398>.
57. Eisfeld AJ, Kawakami E, Watanabe T, Neumann G, Kawaoka Y. 2011. RAB11A is essential for transport of the influenza virus genome to the plasma membrane. *J Virol* 85:6117–6126. <https://doi.org/10.1128/JVI.00378-11>.
58. Plotch SJ, Bouloy M, Ulmanen I, Krug RM. 1981. A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* 23:847–858. [https://doi.org/10.1016/0092-8674\(81\)90449-9](https://doi.org/10.1016/0092-8674(81)90449-9).
59. Engelhardt OG, Smith M, Fodor E. 2005. Association of the influenza A virus RNA-dependent RNA polymerase with cellular RNA polymerase II. *J Virol* 79:5812–5818. <https://doi.org/10.1128/JVI.79.9.5812-5818.2005>.
60. Dias A, Bouvier D, Crépin T, McCarthy AA, Hart DJ, Baudin F, Cusack S, Ruigrok RWH. 2009. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature* 458:914–918. <https://doi.org/10.1038/nature07745>.
61. Koppstein D, Ashour J, Bartel DP. 2015. Sequencing the cap-snatching repertoire of H1N1 influenza provides insight into the mechanism of viral transcription initiation. *Nucleic Acids Res* 43:5052–5064. <https://doi.org/10.1093/nar/gkv333>.
62. York A, Fodor E. 2013. Biogenesis, assembly, and export of viral messenger ribonucleoproteins in the influenza A virus infected cell. *RNA Biol* 10: 1274–1282. <https://doi.org/10.4161/rna.25356>.
63. Peacock TP, Sheppard CM, Staller E, Barclay WS. 2019. Host determinants of influenza RNA synthesis. *Annu Rev Virol* 6:215–233. <https://doi.org/10.1146/annurev-virology-092917-043339>.
64. Rossman JS, Lamb RA. 2011. Influenza virus assembly and budding. *Virol* 411:229–236. <https://doi.org/10.1016/j.virol.2010.12.003>.
65. Mifsud EJ, Kuba M, Barr IG. 2021. Innate immune responses to influenza virus infections in the upper respiratory tract. *Viruses* 13:2090. <https://doi.org/10.3390/v13102090>.
66. Ranjan P, Bowzard JB, Schwerzmann JW, Jeisy-Scott V, Fujita T, Sambhara S. 2009. Cytoplasmic nucleic acid sensors in antiviral immunity. *Trends Mol Med* 15:359–368. <https://doi.org/10.1016/j.molmed.2009.06.003>.
67. Aoshi T, Koyama S, Kobiyama K, Akira S, Ishii KJ. 2011. Innate and adaptive immune responses to viral infection and vaccination. *Curr Opin Virol* 1:226–232. <https://doi.org/10.1016/j.coviro.2011.07.002>.
68. Takeuchi O, Akira S. 2010. Pattern recognition receptors and inflammation. *Cell* 140:805–820. <https://doi.org/10.1016/j.cell.2010.01.022>.
69. Malik G, Zhou Y. 2020. Innate immune sensing of influenza A virus. *Viruses* 12:755. <https://doi.org/10.3390/v12070755>.
70. Tate MD, Mansell A. 2018. An update on the NLRP3 inflammasome and influenza: the road to redemption or perdition? *Curr Opin Immunol* 54: 80–85. <https://doi.org/10.1016/j.coi.2018.06.005>.
71. Lupfer C, Kanneganti TD. 2013. The expanding role of NLRs in antiviral immunity. *Immunol Rev* 255:13–24. <https://doi.org/10.1111/imr.12089>.
72. Biondo C, Lentini G, Beninati C, Teti G. 2019. The dual role of innate immunity during influenza. *Biomed J* 42:8–18. <https://doi.org/10.1016/j.bj.2018.12.009>.
73. Blasius AL, Beutler B. 2010. Intracellular Toll-like receptors. *Immunity* 32: 305–315. <https://doi.org/10.1016/j.immuni.2010.03.012>.
74. Chen X, Liu S, Goraya MU, Maarouf M, Huang S, Chen J-L. 2018. Host immune response to influenza A virus infection. *Front Immunol* 9:320. <https://doi.org/10.3389/fimmu.2018.00320>.
75. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. 2002. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol* 169:4697–4701. <https://doi.org/10.4049/jimmunol.169.9.4697>.
76. Lin KL, Suzuki Y, Nakano H, Ramsburg E, Gunn MD. 2008. CCR2+ monocyte-derived dendritic cells and exudate macrophages produce influenza-induced pulmonary immune pathology and mortality. *J Immunol* 180:2562–2572. <https://doi.org/10.4049/jimmunol.180.4.2562>.
77. Hao X, Kim TS, Braciale TJ. 2008. Differential response of respiratory dendritic cell subsets to influenza virus infection. *J Virol* 82:4908–4919. <https://doi.org/10.1128/JVI.02367-07>.
78. Chiu C, Openshaw PJ. 2015. Antiviral B cell and T cell immunity in the lungs. *Nat Immunol* 16:18–26. <https://doi.org/10.1038/ni.3056>.
79. Jung HE, Lee HK. 2020. Host protective immune responses against influenza A virus infection. *Viruses* 12:504. <https://doi.org/10.3390/v12050504>.
80. Ji ZX, Wang XQ, Liu XF. 2021. NS1: a Key protein in the “game” between influenza A virus and host in innate immunity. *Front Cell Infect Microbiol* 11:670177. <https://doi.org/10.3389/fcimb.2021.670177>.
81. Klemm C, Boergeling Y, Ludwig S, Ehrhardt C. 2018. Immunomodulatory nonstructural proteins of influenza A viruses. *Trends Microbiol* 26: 624–636. <https://doi.org/10.1016/j.tim.2017.12.006>.
82. Nogales A, Martinez-Sobrido L, Topham DJ, DeDiego ML. 2018. Modulation of innate immune responses by the influenza A NS1 and PA-X proteins. *Viruses* 10:708. <https://doi.org/10.3390/v10120708>.
83. Guo Z, Chen L-m, Zeng H, Gomez JA, Plowden J, Fujita T, Katz JM, Donis RO, Sambhara S. 2007. NS1 protein of influenza A virus inhibits the function of intracytoplasmic pathogen sensor, RIG-I. *Am J Respir Cell Mol Biol* 36:263–269. <https://doi.org/10.1165/rcmb.2006-0283RC>.
84. Kumari R, Guo Z, Kumar A, Wiens M, Gangappa S, Katz JM, Cox NJ, Lal RB, Sarkar D, Fisher PB, Garcia-Sastre A, Fujita T, Kumar V, Sambhara S, Ranjan P, Lal SK. 2020. Influenza virus NS1-C/EBPβ gene regulatory complex inhibits RIG-I transcription. *Antiviral Res* 176:104747. <https://doi.org/10.1016/j.antiviral.2020.104747>.
85. Hale BG, Randall RE, Ortín J, Jackson D. 2008. The multifunctional NS1 protein of influenza A viruses. *J Gen Virol* 89:2359–2376. <https://doi.org/10.1099/vir.0.2008/004606-0>.
86. Marión RM, Zürcher T, de la Luna S, Ortín J. 1997. Influenza virus NS1 protein interacts with viral transcription-replication complexes in vivo. *J Gen Virol* 78:2447–2451. <https://doi.org/10.1099/0022-1317-78-10-2447>.
87. Hayashi T, MacDonald LA, Takimoto T. 2015. Influenza A virus protein PA-X contributes to viral growth and suppression of the host antiviral and immune responses. *J Virol* 89:6442–6452. <https://doi.org/10.1128/JVI.00319-15>.
88. Glubokova EA, Leneva IA, Kartashova NP, Falynskova IN, Tikhov RM, Kuznetsov NY. 2021. Efficacy of (R)-6-adamantane-derivatives of 1,3-oxazinan-2-one and piperidine-2,4-dione in the treatment of mice infected by the A/California/04/2009 influenza virus. *Acta Naturae* 13:116–125. <https://doi.org/10.32607/actanaturae.11020>.
89. Food and Drug Administration. 2002. Drug approval package. https://www.accessdata.fda.gov/drugsatfda_docs/nda/99/187035S56_zantac.cfm. Retrieved 19 February 2022.
90. Jefferson TO, Demicheli V, Di Pietrantonj C, Jones M, Rivetti D. 2006. Neuraminidase inhibitors for preventing and treating influenza in healthy adults. *Cochrane Database Syst Rev* CD001265. <https://doi.org/10.1002/14651858.CD001265.pub2>.
91. Maugh TH, II. 1979. Panel urges wide use of antiviral drug: NIH group says amantadine should be used for both prevention and therapy of influenza A in the next epidemic. *Science* 206:1058–1060. <https://doi.org/10.1126/science.386515>.

92. Hayden FG, Cote KM, Douglas RG, Jr. 1980. Plaque inhibition assay for drug susceptibility testing of influenza viruses. *Antimicrob Agents Chemother* 17:865–870. <https://doi.org/10.1128/AAC.17.5.865>.
93. Ohigashi Y, Ma C, Jing X, Balannick V, Pinto LH, Lamb RA. 2009. An amantadine-sensitive chimeric BM2 ion channel of influenza B virus has implications for the mechanism of drug inhibition. *Proc Natl Acad Sci U S A* 106:18775–18779. <https://doi.org/10.1073/pnas.0910584106>.
94. Wintermeyer SM, Nahata MC. 1995. Rimantadine: a clinical perspective. *Ann Pharmacother* 29:299–310. <https://doi.org/10.1177/106002809502900312>.
95. Hayden FG, Minocha A, Spyker DA, Hoffman HE. 1985. Comparative single-dose pharmacokinetics of amantadine hydrochloride and rimantadine hydrochloride in young and elderly adults. *Antimicrob Agents Chemother* 28:216–221. <https://doi.org/10.1128/AAC.28.2.216>.
96. Lamb RA, Zebedee SL, Richardson CD. 1985. Influenza virus M2 protein is an integral membrane protein expressed on the infected-cell surface. *Cell* 40:627–633. [https://doi.org/10.1016/0092-8674\(85\)90211-9](https://doi.org/10.1016/0092-8674(85)90211-9).
97. To J, Torres J. 2019. Viroporins in the influenza virus. *Cells* 8:654. <https://doi.org/10.3390/cells8070654>.
98. Betakova T. 2007. M2 protein-a proton channel of influenza A virus. *Curr Pharm Des* 13:3231–3235. <https://doi.org/10.2174/138161207782341295>.
99. Zebedee SL, Lamb RA. 1988. Influenza A virus M2 protein: monoclonal antibody restriction of virus growth and detection of M2 in virions. *J Virol* 62:2762–2772. <https://doi.org/10.1128/JVI.62.8.2762-2772.1988>.
100. Chen BJ, Leser GP, Jackson D, Lamb RA. 2008. The influenza virus M2 protein cytoplasmic tail interacts with the M1 protein and influences virus assembly at the site of virus budding. *J Virol* 82:10059–10070. <https://doi.org/10.1128/JVI.01184-08>.
101. Rossman JS, Jing X, Leser GP, Lamb RA. 2010. Influenza virus M2 protein mediates ESCRT-independent membrane scission. *Cell* 142:902–913. <https://doi.org/10.1016/j.cell.2010.08.029>.
102. Townsend JA, Sanders HM, Rolland AD, Park CK, Horton NC, Prell JS, Wang J, Marty MT. 2021. Influenza A M2 channel oligomerization is sensitive to its chemical environment. *Anal Chem* 93:16273–16281. <https://doi.org/10.1021/acs.analchem.1c04660>.
103. Thomaston JL, Woldeyes RA, Nakane T, Yamashita A, Tanaka T, Koiwai K, Brewster AS, Barad BA, Chen Y, Lemmin T, Uervirojnangkoorn M, Arima T, Kobayashi J, Masuda T, Suzuki M, Sugahara M, Sauter NK, Tanaka R, Nureki O, Tono K, Joti Y, Nango E, Iwata S, Yumoto F, Fraser JS, DeGrado WF. 2017. XFEL structures of the influenza M2 proton channel: room temperature water networks and insights into proton conduction. *Proc Natl Acad Sci U S A* 114:13357–13362. <https://doi.org/10.1073/pnas.1705624114>.
104. Wu Y, Canturk B, Jo H, Ma C, Gianti E, Klein ML, Pinto LH, Lamb RA, Fiorin G, Wang J, DeGrado WF. 2014. Flipping in the pore: discovery of dual inhibitors that bind in different orientations to the wild-type versus the amantadine-resistant S31N mutant of the influenza A virus M2 proton channel. *J Am Chem Soc* 136:17987–17995. <https://doi.org/10.1021/ja508461m>.
105. Williams JK, Tietze D, Wang J, Wu Y, DeGrado WF, Hong M. 2013. Drug-induced conformational and dynamical changes of the S31N mutant of the influenza M2 proton channel investigated by solid-state NMR. *J Am Chem Soc* 135:9885–9897. <https://doi.org/10.1021/ja4041412>.
106. Park EK, Castrucci MR, Portner A, Kawaoka Y. 1998. The M2 ectodomain is important for its incorporation into influenza A virions. *J Virol* 72:2449–2455. <https://doi.org/10.1128/JVI.72.3.2449-2455.1998>.
107. Wang C, Takeuchi K, Pinto LH, Lamb RA. 1993. Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block. *J Virol* 67:5585–5594. <https://doi.org/10.1128/JVI.67.9.5585-5594.1993>.
108. Stouffer AL, Acharya R, Salom D, Levine AS, Di Costanzo L, Soto CS, Tereshko V, Nanda V, Stayrook S, DeGrado WF. 2008. Structural basis for the function and inhibition of an influenza virus proton channel. *Nature* 451:596–599. <https://doi.org/10.1038/nature06528>.
109. Ito T, Gorman OT, Kawaoka Y, Bean WJ, Webster RG. 1991. Evolutionary analysis of the influenza A virus M gene with comparison of the M1 and M2 proteins. *J Virol* 65:5491–5498. <https://doi.org/10.1128/JVI.65.10.5491-5498.1991>.
110. Sakaguchi T, Tu Q, Pinto LH, Lamb RA. 1997. The active oligomeric state of the minimalist influenza virus M2 ion channel is a tetramer. *Proc Natl Acad Sci U S A* 94:5000–5005. <https://doi.org/10.1073/pnas.94.10.5000>.
111. Lin TI, Schroeder C. 2001. Definitive assignment of proton selectivity and attoampere unitary current to the M2 ion channel protein of influenza A virus. *J Virol* 75:3647–3656. <https://doi.org/10.1128/JVI.75.8.3647-3656.2001>.
112. Leidinger T, Wang J, Martinsson J, DeGrado WF, Arsköld SP. 2010. Proton and cation transport activity of the M2 proton channel from influenza A virus. *Proc Natl Acad Sci U S A* 107:15409–15414. <https://doi.org/10.1073/pnas.1009971107>.
113. Liang R, Swanson JMJ, Madsen JJ, Hong M, DeGrado WF, Voth GA. 2016. Acid activation mechanism of the influenza A M2 proton channel. *Proc Natl Acad Sci U S A* 113:E6955–e6964. <https://doi.org/10.1073/pnas.1615471113>.
114. Jallily PH, Duncan MC, Fedida D, Wang J, Tietjen I. 2020. Put a cork in it: plugging the M2 viral ion channel to sink influenza. *Antiviral Res* 178:104780. <https://doi.org/10.1016/j.antiviral.2020.104780>.
115. Sugrue RJ, Bahadur G, Zambon MC, Hall-Smith M, Douglas AR, Hay AJ. 1990. Specific structural alteration of the influenza haemagglutinin by amantadine. *EMBO J* 9:3469–3476. <https://doi.org/10.1002/j.1460-2075.1990.tb07555.x>.
116. Takeuchi K, Lamb RA. 1994. Influenza virus M2 protein ion channel activity stabilizes the native form of fowl plague virus hemagglutinin during intracellular transport. *J Virol* 68:911–919. <https://doi.org/10.1128/JVI.68.2.911-919.1994>.
117. Schnell JR, Chou JJ. 2008. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* 451:591–595. <https://doi.org/10.1038/nature06531>.
118. Jing X, Ma C, Ohigashi Y, Oliveira FA, Jardetzky TS, Pinto LH, Lamb RA. 2008. Functional studies indicate amantadine binds to the pore of the influenza A virus M2 proton-selective ion channel. *Proc Natl Acad Sci U S A* 105:10967–10972. <https://doi.org/10.1073/pnas.0804958105>.
119. Cady SD, Schmidt-Rohr K, Wang J, Soto CS, DeGrado WF, Hong M. 2010. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature* 463:689–692. <https://doi.org/10.1038/nature08722>.
120. Cady SD, Hong M. 2009. Effects of amantadine on the dynamics of membrane-bound influenza A M2 transmembrane peptide studied by NMR relaxation. *J Biomol NMR* 45:185–196. <https://doi.org/10.1007/s10858-009-9352-9>.
121. Hu F, Luo W, Cady SD, Hong M. 2011. Conformational plasticity of the influenza A M2 transmembrane helix in lipid bilayers under varying pH, drug binding, and membrane thickness. *Biochim Biophys Acta* 1808:415–423. <https://doi.org/10.1016/j.bbame.2010.09.014>.
122. Cady SD, Luo W, Hu F, Hong M. 2009. Structure and function of the influenza A M2 proton channel. *Biochemistry* 48:7356–7364. <https://doi.org/10.1021/bi9008837>.
123. Wang J, Ma C, Wu Y, Lamb RA, Pinto LH, DeGrado WF. 2011. Exploring organosilane amines as potent inhibitors and structural probes of influenza A virus M2 proton channel. *J Am Chem Soc* 133:13844–13847. <https://doi.org/10.1021/ja2050666>.
124. Thomaston JL, Polizzi NF, Konstantinidi A, Wang J, Kolocouris A, DeGrado WF. 2018. Inhibitors of the M2 proton channel engage and disrupt transmembrane networks of hydrogen-bonded waters. *J Am Chem Soc* 140:15219–15226. <https://doi.org/10.1021/jacs.8b06741>.
125. Cady SD, Wang J, Wu Y, DeGrado WF, Hong M. 2011. Specific binding of adamantane drugs and direction of their polar amines in the pore of the influenza M2 transmembrane domain in lipid bilayers and dodecylphosphocholine micelles determined by NMR spectroscopy. *J Am Chem Soc* 133:4274–4284. <https://doi.org/10.1021/ja102581n>.
126. Ma C, Soto CS, Ohigashi Y, Taylor A, Bournas V, Glawe B, Udo MK, DeGrado WF, Lamb RA, Pinto LH. 2008. Identification of the pore-lining residues of the BM2 ion channel protein of influenza B virus. *J Biol Chem* 283:15921–15931. <https://doi.org/10.1074/jbc.M710302200>.
127. Mould JA, Drury JE, Frings SM, Kaupp UB, Pekosz A, Lamb RA, Pinto LH. 2000. Permeation and activation of the M2 ion channel of influenza A virus. *J Biol Chem* 275:31038–31050. <https://doi.org/10.1074/jbc.M003663200>.
128. Wang C, Lamb RA, Pinto LH. 1995. Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. *Biophys J* 69:1363–1371. [https://doi.org/10.1016/S0006-3495\(95\)80003-2](https://doi.org/10.1016/S0006-3495(95)80003-2).
129. Shuck K, Lamb RA, Pinto LH. 2000. Analysis of the pore structure of the influenza A virus M2 ion channel by the substituted-cysteine accessibility method. *J Virol* 74:7755–7761. <https://doi.org/10.1128/jvi.74.17.7755-7761.2000>.
130. Pinto LH, Dieckmann GR, Gandhi CS, Papworth CG, Braman J, Shaughnessy MA, Lear JD, Lamb RA, DeGrado WF. 1997. A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. *Proc Natl Acad Sci U S A* 94:11301–11306. <https://doi.org/10.1073/pnas.94.21.11301>.
131. Nelson MI, Simonsen L, Viboud C, Miller MA, Holmes EC. 2009. The origin and global emergence of adamantane resistant A/H3N2 influenza viruses. *Virology* 388:270–278. <https://doi.org/10.1016/j.virol.2009.03.026>.
132. Bright RA, Shay DK, Shu B, Cox NJ, Klimov AI. 2006. Adamantane resistance among influenza A viruses isolated early during the 2005–2006 influenza season in the United States. *JAMA* 295:891–894. <https://doi.org/10.1001/jama.295.8.joc60020>.

133. Dong G, Peng C, Luo J, Wang C, Han L, Wu B, Ji G, He H. 2015. Adamantane-resistant influenza A viruses in the world (1902–2013): frequency and distribution of M2 gene mutations. *PLoS One* 10:e0119115. <https://doi.org/10.1371/journal.pone.0119115>.
134. Musharrafieh R, Lagarias PI, Ma C, Tan GS, Kolocouris A, Wang J. 2019. The L46P mutant confers a novel allosteric mechanism of resistance toward the influenza A virus M2 S31N proton channel blockers. *Mol Pharmacol* 96:148–157. <https://doi.org/10.1124/mol.119.116640>.
135. Astrahan P, Kass I, Cooper MA, Arkin IT. 2004. A novel method of resistance for influenza against a channel-blocking antiviral drug. *Proteins* 55: 251–257. <https://doi.org/10.1002/prot.20018>.
136. Heider H, Adamczyk B, Presber HW, Schroeder C, Feldblum R, Indulen MK. 1981. Occurrence of amantadine- and rimantadine-resistant influenza A virus strains during the 1980 epidemic. *Acta Virol* 25:395–400.
137. Sweet C, Hayden FG, Jakeman KJ, Grambas S, Hay AJ. 1991. Virulence of rimantadine-resistant human influenza A (H3N2) viruses in ferrets. *J Infect Dis* 164:969–972. <https://doi.org/10.1093/infdis/164.5.969>.
138. Cheung C-L, Rayner JM, Smith GJD, Wang P, Naipospos TSP, Zhang J, Yuen K-Y, Webster RG, Peiris JSM, Guan Y, Chen H. 2006. Distribution of amantadine-resistant H5N1 avian influenza variants in Asia. *J Infect Dis* 193:1626–1629. <https://doi.org/10.1086/504723>.
139. Musharrafieh R, Ma C, Wang J. 2020. Discovery of M2 channel blockers targeting the drug-resistant double mutants M2-S31N/L26I and M2-S31N/V27A from the influenza A viruses. *Eur J Pharm Sci* 141:105124. <https://doi.org/10.1016/j.ejps.2019.105124>.
140. Centers for Disease Control and Prevention. 2006. CDC recommends against the use of amantadine and rimantadine for the treatment or prophylaxis of influenza in the United States during the 2005–06 influenza season. <https://stacks.cdc.gov/view/cdc/25151>. Retrieved 21 February 2022.
141. Mandala VS, Loftis AR, Shcherbakov AA, Pentelute BL, Hong M. 2020. Atomic structures of closed and open influenza B M2 proton channel reveal the conduction mechanism. *Nat Struct Mol Biol* 27:160–167. <https://doi.org/10.1038/s41594-019-0371-2>.
142. Harris A, Cardone G, Winkler DC, Heymann JB, Brecher M, White JM, Steven AC. 2006. Influenza virus pleiomorphism characterized by cryoelectron tomography. *Proc Natl Acad Sci U S A* 103:19123–19127. <https://doi.org/10.1073/pnas.0607614103>.
143. Colman PM, Ward CW. 1985. Structure and diversity of influenza virus neuraminidase. *Curr Top Microbiol Immunol* 114:177–255. https://doi.org/10.1007/978-3-642-70227-3_5.
144. von Itzstein M. 2007. The war against influenza: discovery and development of sialidase inhibitors. *Nat Rev Drug Discov* 6:967–974. <https://doi.org/10.1038/nrd2400>.
145. von Itzstein M, Wu WY, Kok GB, Pegg MS, Dyason JC, Jin B, Van Phan T, Smythe ML, White HF, Oliver SW. 1993. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* 363:418–423. <https://doi.org/10.1038/363418a0>.
146. Taylor G. 1996. Sialidases: structures, biological significance and therapeutic potential. *Curr Opin Struct Biol* 6:830–837. [https://doi.org/10.1016/s0959-440x\(96\)80014-5](https://doi.org/10.1016/s0959-440x(96)80014-5).
147. Palese P, Compans RW. 1976. Inhibition of influenza virus replication in tissue culture by 2-deoxy-2,3-dehydro-N-trifluoroacetylneuraminic acid (FANA): mechanism of action. *J Gen Virol* 33:159–163. <https://doi.org/10.1099/0022-1317-33-1-159>.
148. Palese P, Schulman JL, Bodo G, Meindl P. 1974. Inhibition of influenza and parainfluenza virus replication in tissue culture by 2-deoxy-2,3-dehydro-N-trifluoroacetylneuraminic acid (FANA). *Virology* 59:490–498. [https://doi.org/10.1016/0042-6822\(74\)90458-9](https://doi.org/10.1016/0042-6822(74)90458-9).
149. Meindl P, Bodo G, Palese P, Schulman J, Tuppy H. 1974. Inhibition of neuraminidase activity by derivatives of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid. *Virology* 58:457–463. [https://doi.org/10.1016/0042-6822\(74\)90080-4](https://doi.org/10.1016/0042-6822(74)90080-4).
150. Varghese JN, Laver WG, Colman PM. 1983. Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 Å resolution. *Nature* 303: 35–40. <https://doi.org/10.1038/303035a0>.
151. Elliott M. 2001. Zanamivir: from drug design to the clinic. *Philos Trans R Soc Lond B Biol Sci* 356:1885–1893. <https://doi.org/10.1098/rstb.2001.1021>.
152. Bassetti M, Castaldo N, Camelutti A. 2019. Neuraminidase inhibitors as a strategy for influenza treatment: pros, cons and future perspectives. *Expert Opin Pharmacother* 20:1711–1718. <https://doi.org/10.1080/14656566.2019.1626824>.
153. Food and Drug Administration. 2012. Highlights of prescribing information (Tamiflu). https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021087s062lbl.pdf. Retrieved 21 February 2022.
154. Centers for Disease Control and Prevention. 2022. Influenza antiviral medications: summary for clinicians. <https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm>. Retrieved 21 February 2022.
155. Eiland LS, Eiland EH. 2007. Zanamivir for the prevention of influenza in adults and children age 5 years and older. *Ther Clin Risk Manag* 3: 461–465.
156. Bradley JS, Blumer JL, Romero JR, Michaels MG, Munoz FM, Kimberlin DW, Pahud B, DeBiasi RL, Yamamoto G, Roberts G, Hossain M, Shortino D, Yates PJ, Adams B, Peppercorn A. 2017. Intravenous zanamivir in hospitalized patients with influenza. *Pediatrics* 140:e20162727. <https://doi.org/10.1542/peds.2016-2727>.
157. European Medicines Agency. 2019. Dectova (zanamivir). An overview of Dectova and why it is authorised in the EU. https://www.ema.europa.eu/en/documents/overview/dectova-epar-medicine-overview_en.pdf. Retrieved 3 April 2022.
158. Colman PM, Varghese JN, Laver WG. 1983. Structure of the catalytic and antigenic sites in influenza virus neuraminidase. *Nature* 303:41–44. <https://doi.org/10.1038/303041a0>.
159. Stoll V, Stewart KD, Maring CJ, Muchmore S, Giranda V, Gu YG, Wang G, Chen Y, Sun M, Zhao C, Kennedy AL, Madigan DL, Xu Y, Saldivar A, Kati W, Laver G, Sowin T, Sham HL, Greer J, Kempf D. 2003. Influenza neuraminidase inhibitors: structure-based design of a novel inhibitor series. *Biochemistry* 42:718–727. <https://doi.org/10.1021/bi0205449>.
160. Burmeister WP, Ruigrok RW, Cusack S. 1992. The 2.2 Å resolution crystal structure of influenza B neuraminidase and its complex with sialic acid. *EMBO J* 11:49–56. <https://doi.org/10.1002/j.1460-2075.1992.tb05026.x>.
161. Bai Y, Jones JC, Wong SS, Zanin M. 2021. Antivirals targeting the surface glycoproteins of influenza virus: mechanisms of action and resistance. *Viruses* 13:624. <https://doi.org/10.3390/v13040624>.
162. McAuley JL, Gilbertson BP, Trifkovic S, Brown LE, McKimm-Breschkin JL. 2019. Influenza virus neuraminidase structure and functions. *Front Microbiol* 10:39. <https://doi.org/10.3389/fmicb.2019.00039>.
163. Russell RJ, Haire LF, Stevens DJ, Collins PJ, Lin YP, Blackburn GM, Hay AJ, Gamblin SJ, Skehel JJ. 2006. The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. *Nature* 443: 45–49. <https://doi.org/10.1038/nature05114>.
164. Shtyrya YA, Mochalova LV, Bovin NV. 2009. Influenza virus neuraminidase: structure and function. *Acta Naturae* 1:26–32. <https://doi.org/10.32607/20758251-2009-1-2-26-32>.
165. Colman PM. 1994. Influenza virus neuraminidase: structure, antibodies, and inhibitors. *Protein Sci* 3:1687–1696. <https://doi.org/10.1002/pro.5560031007>.
166. Crennell SJ, Garman EF, Laver WG, Vimr ER, Taylor GL. 1993. Crystal structure of a bacterial sialidase (from *Salmonella typhimurium* LT2) shows the same fold as an influenza virus neuraminidase. *Proc Natl Acad Sci U S A* 90:9852–9856. <https://doi.org/10.1073/pnas.90.21.9852>.
167. Bossart-Whitaker P, Carson M, Babu YS, Smith CD, Laver WG, Air GM. 1993. Three-dimensional structure of influenza A N9 neuraminidase and its complex with the inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid. *J Mol Biol* 232:1069–1083. <https://doi.org/10.1006/jmbi.1993.1461>.
168. Varghese JN, Epa VC, Colman PM. 1995. Three-dimensional structure of the complex of 4-guanidino-Neu5Ac2en and influenza virus neuraminidase. *Protein Sci* 4:1081–1087. <https://doi.org/10.1002/pro.5560040606>.
169. Mtambo SE, Amoako DG, Somboro AM, Agoni C, Lawal MM, Gumedé NS, Khan RB, Kumalo HM. 2021. Influenza viruses: harnessing the crucial role of the M2 ion-channel and neuraminidase toward inhibitor design. *Molecules* 26:880. <https://doi.org/10.3390/molecules26040880>.
170. Thorlund K, Awad T, Boivin G, Thabane L. 2011. Systematic review of influenza resistance to the neuraminidase inhibitors. *BMC Infect Dis* 11: 134. <https://doi.org/10.1186/1471-2334-11-134>.
171. McKimm-Breschkin JL. 2000. Resistance of influenza viruses to neuraminidase inhibitors—a review. *Antiviral Res* 47:1–17. [https://doi.org/10.1016/s0166-3542\(00\)00103-0](https://doi.org/10.1016/s0166-3542(00)00103-0).
172. de Jong MD, Tran TT, Truong HK, Vo MH, Smith GJD, Nguyen VC, Bach VC, Phan TQ, Do QH, Guan Y, Peiris JSM, Tran TH, Farrar J. 2005. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 353:2667–2672. <https://doi.org/10.1056/NEJMoa054512>.
173. Gubareva LV, Webster RG, Hayden FG. 2001. Comparison of the activities of zanamivir, oseltamivir, and RWJ-270201 against clinical isolates of influenza virus and neuraminidase inhibitor-resistant variants. *Antimicrob Agents Chemother* 45:3403–3408. <https://doi.org/10.1128/AAC.45.12.3403-3408.2001>.
174. Moscona A. 2009. Global transmission of oseltamivir-resistant influenza. *N Engl J Med* 360:953–956. <https://doi.org/10.1056/NEJMp0900648>.

175. Ives JAL, Carr JA, Mendel DB, Tai CY, Lambkin R, Kelly L, Oxford JS, Hayden FG, Roberts NA. 2002. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. *Antiviral Res* 55:307–317. [https://doi.org/10.1016/S0166-3542\(02\)00053-0](https://doi.org/10.1016/S0166-3542(02)00053-0).
176. Kohno S, Yen M-Y, Cheong H-J, Hirotsu N, Ishida T, Kadota J-I, Mizuguchi M, Kida H, Shimada J, S-021812 Clinical Study Group. 2011. Phase III randomized, double-blind study comparing single-dose intravenous peramivir with oral oseltamivir in patients with seasonal influenza virus infection. *Antimicrob Agents Chemother* 55:5267–5276. <https://doi.org/10.1128/AAC.00360-11>.
177. Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y, Hayden FG. 2001. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183:523–531. <https://doi.org/10.1086/318537>.
178. Wang MZ, Tai CY, Mendel DB. 2002. Mechanism by which mutations at his274 alter sensitivity of influenza A virus N1 neuraminidase to oseltamivir carboxylate and zanamivir. *Antimicrob Agents Chemother* 46:3809–3816. <https://doi.org/10.1128/AAC.46.12.3809-3816.2002>.
179. Hurt AC, Ernest J, Deng Y-M, Iannello P, Besselaar TG, Birch C, Buchy P, Chittaganpitch M, Chiu S-C, Dwyer D, Guigon A, Harrower B, Kei IP, Kok T, Lin C, McPhie K, Mohd A, Olveda R, Panayotou T, Rawlinson W, Scott L, Smith D, D'Souza H, Komadina N, Shaw R, Kelso A, Barr IG. 2009. Emergence and spread of oseltamivir-resistant A(H1N1) influenza viruses in Oceania, South East Asia and South Africa. *Antiviral Res* 83:90–93. <https://doi.org/10.1016/j.antiviral.2009.03.003>.
180. World Health Organization. 2020. Summary of neuraminidase (NA) amino acid substitutions associated with reduced inhibition by neuraminidase inhibitors (NAIs). [https://www.who.int/publications/m/item/summary-of-neuraminidase-\(na\)-amino-acid-substitutions-associated-with-reduced-inhibition-by-neuraminidase-inhibitors-\(nais\)](https://www.who.int/publications/m/item/summary-of-neuraminidase-(na)-amino-acid-substitutions-associated-with-reduced-inhibition-by-neuraminidase-inhibitors-(nais)). Retrieved 4 March 2022.
181. Kode SS, Pawar SD, Cherian SS, Tare DS, Bhoje D, Keng SS, Mullick J. 2019. Selection of avian influenza A (H9N2) virus with reduced susceptibility to neuraminidase inhibitors oseltamivir and zanamivir. *Virus Res* 265:122–126. <https://doi.org/10.1016/j.virusres.2019.03.019>.
182. Rameix-Welti MA, Agou F, Buchy P, Mardy S, Aubin JT, Véron M, van der Werf S, Naffakh N. 2006. Natural variation can significantly alter the sensitivity of influenza A (H5N1) viruses to oseltamivir. *Antimicrob Agents Chemother* 50:3809–3815. <https://doi.org/10.1128/AAC.00645-06>.
183. McKimm-Breschkin JL, Selleck PW, Usman TB, Johnson MA. 2007. Reduced sensitivity of influenza A (H5N1) to oseltamivir. *Emerg Infect Dis* 13:1354–1357. <https://doi.org/10.3201/eid1309.070164>.
184. Meijer A, Rebelo-de-Andrade H, Correia V, Besselaar T, Drager-Dayal R, Fry A, Gregory V, Gubareva L, Kageyama T, Lackenby A, Lo J, Odagiri T, Pereyaslov D, Siqueira MM, Takashita E, Tashiro M, Wang D, Wong S, Zhang W, Daniels RS, Hurt AC. 2014. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2012–2013. *Antiviral Res* 110:31–41. <https://doi.org/10.1016/j.antiviral.2014.07.001>.
185. Takashita E, Meijer A, Lackenby A, Gubareva L, Rebelo-de-Andrade H, Besselaar T, Fry A, Gregory V, Leang S-K, Huang W, Lo J, Pereyaslov D, Siqueira MM, Wang D, Mak GC, Zhang W, Daniels RS, Hurt AC, Tashiro M. 2015. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. *Antiviral Res* 117:27–38. <https://doi.org/10.1016/j.antiviral.2015.02.003>.
186. Takashita E, Daniels RS, Fujisaki S, Gregory V, Gubareva LV, Huang W, Hurt AC, Lackenby A, Nguyen HT, Pereyaslov D, Roe M, Samaan M, Subbarao K, Tse H, Wang D, Yen H-L, Zhang W, Meijer A. 2020. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2017–2018. *Antiviral Res* 175:104718. <https://doi.org/10.1016/j.antiviral.2020.104718>.
187. Mahal A, Duan M, Zinad DS, Mohapatra RK, Obaidullah AJ, Wei X, Pradhan MK, Das D, Kandi V, Zinad HS, Zhu Q. 2021. Recent progress in chemical approaches for the development of novel neuraminidase inhibitors. *RSC Adv* 11:1804–1840. <https://doi.org/10.1039/d0ra07283d>.
188. Hou L, Zhang Y, Ju H, Cherukupalli S, Jia R, Zhang J, Huang B, Loregian A, Liu X, Zhan P. 2022. Contemporary medicinal chemistry strategies for the discovery and optimization of influenza inhibitors targeting vRNP constituent proteins. *Acta Pharm Sin B* 12:1805–1824. <https://doi.org/10.1016/j.apsb.2021.11.018>.
189. Dufrasne F. 2021. Baloxavir marboxil: an original new drug against influenza. *Pharmaceuticals (Basel)* 15:28. <https://doi.org/10.3390/ph15010028>.
190. Noshi T, Kitano M, Taniguchi K, Yamamoto A, Omoto S, Baba K, Hashimoto T, Ishida K, Kushima Y, Hattori K, Kawai M, Yoshida R, Kobayashi M, Yoshinaga T, Sato A, Okamatsu M, Sakoda Y, Kida H, Shishido T, Naito A. 2018. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. *Antiviral Res* 160:109–117. <https://doi.org/10.1016/j.antiviral.2018.10.008>.
191. Mishin VP, Patel MC, Chesnokov A, De La Cruz J, Nguyen HT, Lollis L, Hodges E, Jang Y, Barnes J, Uyeki T, Davis CT, Wentworth DE, Gubareva LV. 2019. Susceptibility of influenza A, B, C, and D viruses to baloxavir. *Emerg Infect Dis* 25:1969–1972. <https://doi.org/10.3201/eid2510.190607>.
192. Ikematsu H, Hayden FG, Kawaguchi K, Kinoshita M, de Jong MD, Lee N, Takashima S, Noshi T, Tsuchiya K, Uehara T. 2020. Baloxavir marboxil for prophylaxis against influenza in household contacts. *N Engl J Med* 383:309–320. <https://doi.org/10.1056/NEJMoa1915341>.
193. Uyeki TM. 2020. Baloxavir for postexposure prophylaxis against influenza in households. *N Engl J Med* 383:389–390. <https://doi.org/10.1056/NEJMe2022702>.
194. Omoto S, Speranzini V, Hashimoto T, Noshi T, Yamaguchi H, Kawai M, Kawaguchi K, Uehara T, Shishido T, Naito A, Cusack S. 2018. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. *Sci Rep* 8:9633. <https://doi.org/10.1038/s41598-018-27890-4>.
195. Te Velhuis AJ, Fodor E. 2016. Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. *Nat Rev Microbiol* 14:479–493. <https://doi.org/10.1038/nrmicro.2016.87>.
196. Reich S, Guilligay D, Pflug A, Malet H, Berger I, Crépin T, Hart D, Lunardi T, Nanao M, Ruigrok RWH, Cusack S. 2014. Structural insight into cap-snatching and RNA synthesis by influenza polymerase. *Nature* 516:361–366. <https://doi.org/10.1038/nature14009>.
197. Carcelli M, Rogolino D, Bacchi A, Rispoli G, Fiscaro E, Compari C, Sechi M, Stevaert A, Naesens L. 2014. Metal-chelating 2-hydroxyphenyl amide pharmacophore for inhibition of influenza virus endonuclease. *Mol Pharm* 11:304–316. <https://doi.org/10.1021/mp400482a>.
198. Carcelli M, Rogolino D, Gatti A, De Luca L, Sechi M, Kumar G, White SW, Stevaert A, Naesens L. 2016. N-acylhydrazones inhibitors of influenza virus PA endonuclease with versatile metal binding modes. *Sci Rep* 6:31500. <https://doi.org/10.1038/srep31500>.
199. Checkmahomed L, M'hamdi Z, Carbonneau J, Venable M-C, Baz M, Abed Y, Boivin G. 2020. Impact of the baloxavir-resistant polymerase acid I38T substitution on the fitness of contemporary influenza A(H1N1)pdm09 and A(H3N2) strains. *J Infect Dis* 221:63–70. <https://doi.org/10.1093/infdis/jiz418>.
200. Takashita E, Morita H, Ogawa R, Nakamura K, Fujisaki S, Shirakura M, Kuwahara T, Kishida N, Watanabe S, Odagiri T. 2018. Susceptibility of influenza viruses to the novel cap-dependent endonuclease inhibitor baloxavir marboxil. *Front Microbiol* 9:3026. <https://doi.org/10.3389/fmicb.2018.03026>.
201. Uehara T, Hayden FG, Kawaguchi K, Omoto S, Hurt AC, De Jong MD, Hirotsu N, Sugaya N, Lee N, Baba K, Shishido T, Tsuchiya K, Portsmouth S, Kida H. 2020. Treatment-emergent influenza variant viruses with reduced baloxavir susceptibility: impact on clinical and virologic outcomes in uncomplicated influenza. *J Infect Dis* 221:346–355. <https://doi.org/10.1093/infdis/jiz244>.
202. Yoshino R, Yasuo N, Sekijima M. 2019. Molecular dynamics simulation reveals the mechanism by which the influenza cap-dependent endonuclease acquires resistance against baloxavir marboxil. *Sci Rep* 9:17464. <https://doi.org/10.1038/s41598-019-53945-1>.
203. Takashita E, Kawakami C, Morita H, Ogawa R, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Mitamura K, Abe T, Ichikawa M, Yamazaki M, Watanabe S, Odagiri T. 2019. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. *Euro Surveill* 24:1800698. <https://doi.org/10.2807/1560-7917.Es.2019.24.3.1800698>.
204. Imai M, Yamashita M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Kiso M, Murakami J, Yasuhara A, Takada K, Ito M, Nakajima N, Takahashi K, Lopes TJS, Dutta J, Khan Z, Kriti D, van Bakel H, Tokita A, Hagiwara H, Izumida N, Kuroki H, Nishino T, Wada N, Koga M, Adachi E, Jubishi D, Hasegawa H, Kawaoka Y. 2020. Influenza A variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets. *Nat Microbiol* 5:27–33. <https://doi.org/10.1038/s41564-019-0609-0>.

205. Takashita E, Kawakami C, Ogawa R, Morita H, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Ota A, Togashi H, Saito A, Mitamura K, Abe T, Ichikawa M, Yamazaki M, Watanabe S, Odagiri T. 2019. Influenza A(H3N2) virus exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019. *Euro Surveill* 24:1900170. <https://doi.org/10.2807/1560-7917.Es.2019.24.12.1900170>.
206. Takashita E, Abe T, Morita H, Nagata S, Fujisaki S, Miura H, Shirakura M, Kishida N, Nakamura K, Kuwahara T, Mitamura K, Ichikawa M, Yamazaki M, Watanabe S, Hasegawa H, Influenza Virus Surveillance Group of Japan. 2020. Influenza A(H1N1)pdm09 virus exhibiting reduced susceptibility to baloxavir due to a PA E23K substitution detected from a child without baloxavir treatment. *Antiviral Res* 180:104828. <https://doi.org/10.1016/j.antiviral.2020.104828>.
207. Takashita E, Ichikawa M, Morita H, Ogawa R, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Sugawara H, Sato A, Akimoto M, Mitamura K, Abe T, Yamazaki M, Watanabe S, Hasegawa H, Odagiri T. 2019. Human-to-human transmission of influenza A(H3N2) virus with reduced susceptibility to baloxavir, Japan, February 2019. *Emerg Infect Dis* 25:2108–2111. <https://doi.org/10.3201/eid2511.190757>.
208. Hirotsu N, Sakaguchi H, Sato C, Ishibashi T, Baba K, Omoto S, Shishido T, Tsuchiya K, Hayden FG, Uehara T, Watanabe A. 2020. Baloxavir marboxil in Japanese pediatric patients with influenza: safety and clinical and virologic outcomes. *Clin Infect Dis* 71:971–981. <https://doi.org/10.1093/cid/ciz908>.
209. Hashimoto T, Baba K, Inoue K, Okane M, Hata S, Shishido T, Naito A, Wildum S, Omoto S. 2021. Comprehensive assessment of amino acid substitutions in the trimeric RNA polymerase complex of influenza A virus detected in clinical trials of baloxavir marboxil. *Influenza Other Respir Viruses* 15:389–395. <https://doi.org/10.1111/irv.12821>.
210. Jones JC, Rovito SW, Penafior MK, Webby RJ, Govorkova EA. 2022. Influenza A virus polymerase acidic protein E23R substitution is a marker of reduced susceptibility to baloxavir. *Antiviral Res* 204:105369. <https://doi.org/10.1016/j.antiviral.2022.105369>.
211. World Health Organization. 2021. Summary of polymerase acidic (PA) protein amino acid substitutions analysed for their effects on baloxavir susceptibility. [https://cdn.who.int/media/docs/default-source/influenza/summary-of-polymerase-acidic-\(pa\)-protein-amino-acid-substitutions-analysed-for-their-effects-on-baloxavir-susceptibility.pdf](https://cdn.who.int/media/docs/default-source/influenza/summary-of-polymerase-acidic-(pa)-protein-amino-acid-substitutions-analysed-for-their-effects-on-baloxavir-susceptibility.pdf). Retrieved 4 April 2022.
212. Wang Y, Hu Y, Xu S, Zhang Y, Musharrafieh R, Hau RK, Ma C, Wang J. 2018. In vitro pharmacokinetic optimizations of AM2-S31N channel blockers led to the discovery of slow-binding inhibitors with potent antiviral activity against drug-resistant influenza A viruses. *J Med Chem* 61:1074–1085. <https://doi.org/10.1021/acs.jmedchem.7b01536>.
213. Cáceres CJ, Hu Y, Cárdenas-García S, Wu X, Tan H, Carnaccini S, Gay LC, Geiger G, Ma C, Zhang Q-Y, Rajao D, Perez DR, Wang J. 2021. Rational design of a deuterium-containing M2-S31N channel blocker UAWJ280 with in vivo antiviral efficacy against both oseltamivir sensitive and -resistant influenza A viruses. *Emerg Microbes Infect* 10:1832–1848. <https://doi.org/10.1080/22221751.2021.1972769>.
214. Wang J, Ma C, Fiorin G, Carnevale V, Wang T, Hu F, Lamb RA, Pinto LH, Hong M, Klein ML, DeGrado WF. 2011. Molecular dynamics simulation directed rational design of inhibitors targeting drug-resistant mutants of influenza A virus M2. *J Am Chem Soc* 133:12834–12841. <https://doi.org/10.1021/ja204969m>.
215. Hu Y, Musharrafieh R, Ma C, Zhang J, Smee DF, DeGrado WF, Wang J. 2017. An M2-V27A channel blocker demonstrates potent in vitro and in vivo antiviral activities against amantadine-sensitive and -resistant influenza A viruses. *Antiviral Res* 140:45–54. <https://doi.org/10.1016/j.antiviral.2017.01.006>.
216. Thomaston JL, Konstantinidi A, Liu L, Lambrinidis G, Tan J, Caffrey M, Wang J, DeGrado WF, Kolocouris A. 2020. X-ray crystal structures of the influenza M2 proton channel drug-resistant V27A mutant bound to a spiro-adamantyl amine inhibitor reveal the mechanism of adamantane resistance. *Biochemistry* 59:627–634. <https://doi.org/10.1021/acs.biochem.9b00971>.
217. Thomaston JL, Wu Y, Polizzi N, Liu L, Wang J, DeGrado WF. 2019. X-ray crystal structure of the influenza A M2 proton channel S31N mutant in two conformational states: an open and shut case. *J Am Chem Soc* 141:11481–11488. <https://doi.org/10.1021/jacs.9b02196>.
218. Hu Y, Hau RK, Wang Y, Tuohy P, Zhang Y, Xu S, Ma C, Wang J. 2018. Structure–property relationship studies of influenza A virus AM2-S31N proton channel blockers. *ACS Med Chem Lett* 9:1111–1116. <https://doi.org/10.1021/acsmedchemlett.8b00336>.
219. Musharrafieh R, Lagarias P, Ma C, Hau R, Romano A, Lambrinidis G, Kolocouris A, Wang J. 2020. Investigation of the drug resistance mechanism of M2-S31N channel blockers through biomolecular simulations and viral passage experiments. *ACS Pharmacol Transl Sci* 3:666–675. <https://doi.org/10.1021/acspsci.0c00018>.
220. Lampejo T. 2020. Influenza and antiviral resistance: an overview. *Eur J Clin Microbiol Infect Dis* 39:1201–1208. <https://doi.org/10.1007/s10096-020-03840-9>.
221. Behzadi MA, Leyva-Grado VH. 2019. Overview of current therapeutics and novel candidates against influenza, respiratory syncytial virus, and Middle East respiratory syndrome coronavirus infections. *Front Microbiol* 10:1327. <https://doi.org/10.3389/fmicb.2019.01327>.
222. Treanor JJ, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, Singh S, Kinnerley N, Ward P, Mills RG, US Oral Neuraminidase Study Group. 2009. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. *JAMA* 283:1016–1024. <https://doi.org/10.1001/jama.283.8.1016>.
223. Kiso M, Kubo S, Ozawa M, Le QM, Nidom CA, Yamashita M, Kawaoka Y. 2010. Efficacy of the new neuraminidase inhibitor CS-8958 against H5N1 influenza viruses. *PLoS Pathog* 6:e1000786. <https://doi.org/10.1371/journal.ppat.1000786>.
224. Ishizuka H, Yoshida S, Okabe H, Yoshihara K. 2010. Clinical pharmacokinetics of laninamivir, a novel long-acting neuraminidase inhibitor, after single and multiple inhaled doses of its prodrug, CS-8958, in healthy male volunteers. *J Clin Pharmacol* 50:1319–1329. <https://doi.org/10.1177/0091270009356297>.
225. Koyama K, Takahashi M, Oitate M, Nakai N, Takakusa H, Miura S-i, Okazaki O. 2009. CS-8958, a prodrug of the novel neuraminidase inhibitor R-125489, demonstrates a favorable long-retention profile in the mouse respiratory tract. *Antimicrob Agents Chemother* 53:4845–4851. <https://doi.org/10.1128/AAC.00731-09>.
226. Gubareva L, Mohan T. 2022. Antivirals targeting the neuraminidase. *Cold Spring Harb Perspect Med* 12:a038455. <https://doi.org/10.1101/cshperspect.a038455>.
227. Watanabe A, Chang SC, Kim MJ, Chu DW, Ohashi Y, MARVEL Study Group. 2010. Long-acting neuraminidase inhibitor laninamivir octanoate versus oseltamivir for treatment of influenza: a double-blind, randomized, noninferiority clinical trial. *Clin Infect Dis* 51:1167–1175. <https://doi.org/10.1086/656802>.
228. Nakano T, Okumura A, Tanabe T, Niwa S, Fukushima M, Yonemochi R, Eda H, Tsutsumi H. 2013. Safety evaluation of laninamivir octanoate hydrate through analysis of adverse events reported during early post-marketing phase vigilance. *Scand J Infect Dis* 45:469–477. <https://doi.org/10.3109/00365548.2012.763104>.
229. Kashiwagi S, Watanabe A, Ikematsu H, Uemori M, Awamura S, Laninamivir Prophylaxis Study Group. 2016. Long-acting neuraminidase inhibitor laninamivir octanoate as post-exposure prophylaxis for influenza. *Clin Infect Dis* 63:330–337. <https://doi.org/10.1093/cid/ciw255>.
230. Higashiguchi M, Matsumoto T, Fujii T. 2018. A meta-analysis of laninamivir octanoate for treatment and prophylaxis of influenza. *Antivir Ther* 23:157–165. <https://doi.org/10.3851/IMP3189>.
231. Massari S, Desantis J, Nizi MG, Cecchetti V, Tabarrini O. 2021. Inhibition of influenza virus polymerase by interfering with its protein–protein interactions. *ACS Infect Dis* 7:1332–1350. <https://doi.org/10.1021/acsfeddis.0c00552>.
232. Te Velthuis AJW, Grimes JM, Fodor E. 2021. Structural insights into RNA polymerases of negative-sense RNA viruses. *Nat Rev Microbiol* 19:303–318. <https://doi.org/10.1038/s41579-020-00501-8>.
233. Delang L, Abdelnabi R, Neyts J. 2018. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. *Antiviral Res* 153:85–94. <https://doi.org/10.1016/j.antiviral.2018.03.003>.
234. Shiraki K, Daikoku T. 2020. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. *Pharmacol Ther* 209:107512. <https://doi.org/10.1016/j.pharmthera.2020.107512>.
235. Furuta Y, Takahashi K, Kuno-Makawa M, Sangawa H, Uehara S, Kozaki K, Nomura N, Egawa H, Shiraki K. 2005. Mechanism of action of T-705 against influenza virus. *Antimicrob Agents Chemother* 49:981–986. <https://doi.org/10.1128/AAC.49.3.981-986.2005>.
236. Goldhill DH, Langat P, Xie H, Galiano M, Miah S, Kellam P, Zambon M, Lackenby A, Barclay WS. 2019. Determining the mutation bias of favipiravir in influenza virus using next-generation sequencing. *J Virol* 93:e01217-18. <https://doi.org/10.1128/JVI.01217-18>.

237. Clinicaltrials.gov. 2010. Dose-finding study of favipiravir in the treatment of uncomplicated influenza. <https://clinicaltrials.gov/ct2/show/NCT01068912>. Retrieved 19 February 2022.
238. McKimm-Breschkin JL, Jiang S, Hui DS, Beigel JH, Govorkova EA, Lee N. 2018. Prevention and treatment of respiratory viral infections: presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV Antiviral Group conference. *Antiviral Res* 149:118–142. <https://doi.org/10.1016/j.antiviral.2017.11.013>.
239. Wang Y, Fan G, Salam A, Horby P, Hayden FG, Chen C, Pan J, Zheng J, Lu B, Guo L, Wang C, Cao B. 2020. Comparative effectiveness of combined favipiravir and oseltamivir therapy versus oseltamivir monotherapy in critically ill patients with influenza virus infection. *J Infect Dis* 221:1688–1698. <https://doi.org/10.1093/infdis/jiz656>.
240. Mifsud EJ, Hayden FG, Hurt AC. 2019. Antivirals targeting the polymerase complex of influenza viruses. *Antiviral Res* 169:104545. <https://doi.org/10.1016/j.antiviral.2019.104545>.
241. Goldhill DH, Te Velthuis AJW, Fletcher RA, Langat P, Zambon M, Lackenby A, Barclay WS. 2018. The mechanism of resistance to favipiravir in influenza. *Proc Natl Acad Sci U S A* 115:11613–11618. <https://doi.org/10.1073/pnas.1811345115>.
242. Clark MP, Ledeboer MW, Davies I, Byrn RA, Jones SM, Perola E, Tsai A, Jacobs M, Nti-Addae K, Bandarage UK, Boyd MJ, Bethiel RS, Court JJ, Deng H, Duffy JP, Dorsch WA, Farmer LJ, Gao H, Gu W, Jackson K, Jacobs DH, Kennedy JM, Ledford B, Liang J, Maltais F, Murcko M, Wang T, Wannamaker MW, Bennett HB, Leeman JR, McNeil C, Taylor WP, Memmott C, Jiang M, Rijnbrand R, Bral C, Germann U, Nezami A, Zhang Y, Salituro FG, Bennani YL, Charifson PS. 2014. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. *J Med Chem* 57:6668–6678. <https://doi.org/10.1021/jm5007275>.
243. Byrn RA, Jones SM, Bennett HB, Bral C, Clark MP, Jacobs MD, Kwong AD, Ledeboer MW, Leeman JR, McNeil CF, Murcko MA, Nezami A, Perola E, Rijnbrand R, Saxena K, Tsai AW, Zhou Y, Charifson PS. 2015. Preclinical activity of VX-787, a first-in-class, orally bioavailable inhibitor of the influenza virus polymerase PB2 subunit. *Antimicrob Agents Chemother* 59:1569–1582. <https://doi.org/10.1128/AAC.04623-14>.
244. Trevejo JM, Asmal M, Vingerhoets J, Polo R, Robertson S, Jiang Y, Kieffer TL, Leopold L. 2018. Pimodivir treatment in adult volunteers experimentally inoculated with live influenza virus: a phase IIa, randomized, double-blind, placebo-controlled study. *Antivir Ther* 23:335–344. <https://doi.org/10.3851/IMP3212>.
245. Finberg RW, Lanno R, Anderson D, Fleischhackl R, van Duijnhoven W, Kauffman RS, Kosoglou T, Vingerhoets J, Leopold L. 2019. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. *J Infect Dis* 219:1026–1034. <https://doi.org/10.1093/infdis/jiy547>.
246. O'Neil B, Ison MG, Hallouin-Bernard MC, Nilsson AC, Torres A, Wilburn JM, van Duijnhoven W, Van Dromme I, Anderson D, Deleu S, Kosoglou T, Vingerhoets J, Rossenu S, Leopold L. 2020. A phase 2 study of pimodivir (JNJ-63623872) in combination with oseltamivir in elderly and nonelderly adults hospitalized with influenza A infection: OPAL study. *J Infect Dis* 226:109–118. <https://doi.org/10.1093/infdis/jiaa376>.
247. Janssen. 2020. Janssen to discontinue pimodivir influenza development program. <https://johnsonandjohnson.gcs-web.com/static-files/e175eddf-f6a7-47dd-9f72-34d25f69d13e>. Retrieved 19 February 2022.
248. Kakuda TN, Yogaratnam J, Rito J, Boyce M, Mitchell T, Gupta K, Symons JA, Chanda S, Van Remoortere P, Fry J. 2018. Phase I study on safety and pharmacokinetics of a novel influenza endonuclease inhibitor, AL-794 (JNJ-64155806), following single- and multiple-ascending doses in healthy adults. *Antivir Ther* 23:555–566. <https://doi.org/10.3851/IMP3244>.
249. Yogaratnam J, Rito J, Kakuda TN, Fennema H, Gupta K, Jekle CA, Mitchell T, Boyce M, Sahgal O, Balaratnam G, Chanda S, Van Remoortere P, Symons JA, Fry J. 2019. Antiviral activity, safety, and pharmacokinetics of AL-794, a novel oral influenza endonuclease inhibitor: results of an influenza human challenge study. *J Infect Dis* 219:177–185. <https://doi.org/10.1093/infdis/jiy410>.
250. Clinicaltrials.gov. 2018. The safety, tolerability and pharmacokinetic study of ZSP1273 in healthy volunteers. <https://clinicaltrials.gov/ct2/show/NCT03679143>. Retrieved 19 February 2022.
251. Hu Y, Li H, Wu M, Zhang H, Ding Y, Peng Y, Li X, Yu Z. 2021. Single and multiple dose pharmacokinetics and safety of ZSP1273, an RNA polymerase PB2 protein inhibitor of the influenza A virus: a phase 1 double-blind study in healthy subjects. *Expert Opin Invest Drugs* 30:1159–1167. <https://doi.org/10.1080/13543784.2021.1994944>.
252. Clinicaltrials.gov. 2020. A study of ZSP1273 tablets in patients with acute uncomplicated influenza A. <https://clinicaltrials.gov/ct2/show/NCT04683406>. Retrieved 28 December 2022.
253. Boltz D, Peng X, Muzzio M, Dash P, Thomas PG, Margitich V. 2018. Activity of enisamium, an isonicotinic acid derivative, against influenza viruses in differentiated normal human bronchial epithelial cells. *Antivir Chem Chemother* 26:2040206618811416. <https://doi.org/10.1177/2040206618811416>.
254. Cocking D, Cinatl J, Boltz DA, Peng X, Johnson W, Muzzio M, Syarkevych O, Kostyuk G, Goy A, Mueller L, Margitich VI. 2018. Antiviral effect of a derivative of isonicotinic acid enisamium iodide (FAV00A) against influenza virus. *Acta Virol* 62:191–195. https://doi.org/10.4149/av_2018_211.
255. Clinicaltrials.gov. 2020. Efficacy and safety of enisamium iodide for the treatment of acute respiratory viral infections, including influenza. <https://www.clinicaltrials.gov/ct2/show/NCT04682444>. Retrieved 19 February 2022.
256. Te Velthuis AJW, Zubkova TG, Shaw M, Mehle A, Boltz D, Gmeinwieser N, Stammer H, Milde J, Müller L, Margitich V. 2021. Enisamium reduces influenza virus shedding and improves patient recovery by inhibiting viral RNA polymerase activity. *Antimicrob Agents Chemother* 65:e02605-20. <https://doi.org/10.1128/AAC.02605-20>.
257. Clinicaltrials.gov. 2021. To evaluate the efficacy and safety of TG-1000 compared with placebo in adult patients with acute uncomplicated influenza virus infection. <https://clinicaltrials.gov/ct2/show/NCT04706468>. Retrieved 19 February 2022.
258. Clinicaltrials.gov. 2021. Evaluation the safety and tolerance of GP681 tablets in healthy subjects. <https://clinicaltrials.gov/ct2/show/NCT04729764>. Retrieved 19 February 2022.
259. Clinicaltrials.gov. 2021. A clinical study on the safety and efficacy of GP681 tablets in the treatment of uncomplicated acute influenza. <https://clinicaltrials.gov/ct2/show/NCT04736758>. Retrieved 19 February 2022.
260. Byrd-Leotis L, Galloway SE, Agbogu E, Steinhauer DA. 2015. Influenza hemagglutinin (HA) stem region mutations that stabilize or destabilize the structure of multiple HA subtypes. *J Virol* 89:4504–4516. <https://doi.org/10.1128/JVI.00057-15>.
261. Wilson IA, Skehel JJ, Wiley DC. 1981. Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3 Å resolution. *Nature* 289:366–373. <https://doi.org/10.1038/289366a0>.
262. Zhang Q, Liang T, Nandakumar KS, Liu S. 2021. Emerging and state of the art hemagglutinin-targeted influenza virus inhibitors. *Expert Opin Pharmacother* 22:715–728. <https://doi.org/10.1080/14656566.2020.1856814>.
263. Chen Z, Cui Q, Caffrey M, Rong L, Du R. 2021. Small molecule inhibitors of influenza virus entry. *Pharmaceuticals (Basel)* 14:587. <https://doi.org/10.3390/ph14060587>.
264. Li F, Ma C, Wang J. 2015. Inhibitors targeting the influenza virus hemagglutinin. *Curr Med Chem* 22:1361–1382. <https://doi.org/10.2174/0929867322666150227153919>.
265. van Dongen MJP, Kadam RU, Juraszek J, Lawson E, Brandenburg B, Schmitz F, Schepens WBG, Stoops B, van Diepen HA, Jongeneelen M, Tang C, Vermond J, van Eijgen-Obregoso Real A, Blokland S, Garg D, Yu W, Goutier V, Lancacker E, Klap JM, Peeters DCG, Wu J, Buyck C, Jonckers THM, Roymans D, Roevens P, Vogels R, Koudstaal W, Friesen RHE, Raboisson P, Dhanak D, Goudsmit J, Wilson IA. 2019. A small-molecule fusion inhibitor of influenza virus is orally active in mice. *Science* 363:eaar6221. <https://doi.org/10.1126/science.aar6221>.
266. Badani H, Garry RF, Wimley WC. 2014. Peptide entry inhibitors of enveloped viruses: the importance of interfacial hydrophobicity. *Biochim Biophys Acta* 1838:2180–2197. <https://doi.org/10.1016/j.bbame.2014.04.015>.
267. Clinicaltrials.gov. 2011. Safety study of flufiravide-3 nasal spray in healthy subjects. <https://clinicaltrials.gov/ct2/show/NCT01313962>. Retrieved 19 February 2022.
268. Clinicaltrials.gov. 2013. Safety, tolerability, and PK of escalating doses of flufiravide-3 dry powder for inhalation in healthy subjects. <https://clinicaltrials.gov/ct2/show/NCT01990846>. Retrieved 19 February 2022.
269. Kadam RU, Wilson IA. 2017. Structural basis of influenza virus fusion inhibition by the antiviral drug arbidol. *Proc Natl Acad Sci U S A* 114:206–214. <https://doi.org/10.1073/pnas.1617020114>.
270. Blaising J, Polyak SJ, Pêcheur EI. 2014. Arbidol as a broad-spectrum antiviral: an update. *Antiviral Res* 107:84–94. <https://doi.org/10.1016/j.antiviral.2014.04.006>.
271. Haviernik J, Štefánik M, Fojtiková M, Kali S, Tordo N, Rudolf I, Hubálek Z, Eyer L, Ruzek D. 2018. Arbidol (umifenovir): a broad-spectrum antiviral drug that inhibits medically important arthropod-borne flaviviruses. *Viruses* 10:184. <https://doi.org/10.3390/v10040184>.

272. Perfetto B, Filosa R, De Gregorio V, Peduto A, La Gatta A, de Caprariis P, Tufano MA, Donnarumma G. 2014. In vitro antiviral and immunomodulatory activity of arbidol and structurally related derivatives in herpes simplex virus type 1-infected human keratinocytes (HaCat). *J Med Microbiol* 63:1474–1483. <https://doi.org/10.1099/jmm.0.076612-0>.
273. Kiselev OI, Maleev VV, Deeva EG, Leneva IA, Selkova EP, Osipova EA, Obukhov AA, Nadorov SA, Kulikova EV. 2015. Clinical efficacy of arbidol (umifenovir) in the therapy of influenza in adults: preliminary results of the multicenter double-blind randomized placebo-controlled study ARBITR. *Ter Arkh* 87:88–96. (In Russian.) <https://doi.org/10.17116/terarkh201587188-96>.
274. Clinicaltrials.gov. 2018. Severe influenza trial of ARbidol (STAR). <https://clinicaltrials.gov/ct2/show/NCT03787459>. Retrieved 19 February 2022.
275. Clinicaltrials.gov. 2012. A study of arbidol (umifenovir) for treatment and prophylaxis of influenza and common cold (ARBITR). <https://clinicaltrials.gov/ct2/show/NCT01651663>. Retrieved 20 February 2022.
276. Mizuta T, Fujiwara M, Abe T, Miyano-Kurosaki N, Yokota T, Shigeta S, Takaku H. 2000. Inhibitory effects of an antisense oligonucleotide in an experimentally infected mouse model of influenza A virus. *Biochem Biophys Res Commun* 279:158–161. <https://doi.org/10.1006/bbrc.2000.3924>.
277. Ge Q, Filip L, Bai A, Nguyen T, Eisen HN, Chen J. 2004. Inhibition of influenza virus production in virus-infected mice by RNA interference. *Proc Natl Acad Sci U S A* 101:8676–8681. <https://doi.org/10.1073/pnas.0402486101>.
278. Zhou H, Jin M, Yu Z, Xu X, Peng Y, Wu H, Liu J, Liu H, Cao S, Chen H. 2007. Effective small interfering RNAs targeting matrix and nucleocapsid protein gene inhibit influenza A virus replication in cells and mice. *Antiviral Res* 76:186–193. <https://doi.org/10.1016/j.antiviral.2007.07.002>.
279. Clinicaltrials.gov. 2012. Testing the AVI-7100 flu drug in healthy volunteers. <https://clinicaltrials.gov/ct2/show/NCT01747148>. Retrieved 19 February 2022.
280. Beigel JH, Voell J, Muñoz P, Kumar P, Brooks KM, Zhang J, Iversen P, Heald A, Wong M, Davey RT. 2018. Safety, tolerability, and pharmacokinetics of radavirsen (AVI-7100), an antisense oligonucleotide targeting influenza A M1/M2 translation. *Br J Clin Pharmacol* 84:25–34. <https://doi.org/10.1111/bcp.13405>.
281. Francis T, Magill TP. 1935. Immunological studies with the virus of influenza. *J Exp Med* 62:505–516. <https://doi.org/10.1084/jem.62.4.505>.
282. Rockman S, Lowther S, Camuglia S, Vandenberg K, Taylor S, Fabri L, Miescher S, Pearse M, Middleton D, Kent SJ, Maher D. 2017. Intravenous immunoglobulin protects against severe pandemic influenza infection. *EBioMedicine* 19:119–127. <https://doi.org/10.1016/j.ebiom.2017.04.010>.
283. Zhou B, Zhong N, Guan Y. 2007. Treatment with convalescent plasma for influenza A (H5N1) infection. *N Engl J Med* 357:1450–1451. <https://doi.org/10.1056/NEJMc070359>.
284. Wu X-X, Gao H-N, Wu H-B, Peng X-M, Ou H-L, Li L-J. 2015. Successful treatment of avian-origin influenza A (H7N9) infection using convalescent plasma. *Int J Infect Dis* 41:3–5. <https://doi.org/10.1016/j.ijid.2015.10.009>.
285. Kong LK, Zhou BP. 2006. Successful treatment of avian influenza with convalescent plasma. *Hong Kong Med J* 12:489.
286. Luke TC, Kilbane EM, Jackson LJ, Hoffman SL. 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med* 145:599–609. <https://doi.org/10.7326/0003-4819-145-8-200610170-00139>.
287. Hung IFN, To KKW, Lee C-K, Lee K-L, Yan W-W, Chan K, Chan W-M, Ngai C-W, Law K-I, Chow F-L, Liu R, Lai K-Y, Lau CCY, Liu S-H, Chan K-H, Lin C-K, Yuen K-Y. 2013. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. *Chest* 144:464–473. <https://doi.org/10.1378/chest.12-2907>.
288. Davey RT, Fernández-Cruz E, Markowitz N, Pett S, Babiker AG, Wentworth D, Khurana S, Engen N, Gordin F, Jain MK, Kan V, Polizzotto MN, Riska P, Ruxrungtham K, Temesgen Z, Lundgren J, Beigel JH, Lane HC, Neaton JD, Jr., INSIGHT FLU-IVIG Study Group. 2019. Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial. *Lancet Respir Med* 7:951–963. [https://doi.org/10.1016/S2213-2600\(19\)30253-X](https://doi.org/10.1016/S2213-2600(19)30253-X).
289. Beigel JH, Aga E, Elie-Turenne M-C, Cho J, Tebas P, Clark CL, Metcalf JP, Ozment C, Raviprakash K, Beeler J, Holley HP, Warner S, Chorley C, Lane HC, Hughes MD, Davey RT, Beigel JH, Aga E, Elie-Turenne M-C, Cho J, Tebas P, Clark CL, Metcalf JP, Ozment C, Raviprakash K, Beeler J, Holley HP, Warner S, Chorley C, Lane HC, Hughes MD, Davey RT, Barron M, Bastani A, Bauer P, Borkowsky W, Cairns C, Deville J, Elie M-C, Fichtenbaum C, Finberg R, Jain M, Kaufman D, Lin M, Lin J, Maves R, Morrow L, Nguyen M-H, Park P, Polk C, et al. 2019. Anti-influenza immune plasma for the treatment of patients with severe influenza A: a randomised, double-blind, phase 3 trial. *Lancet Respir Med* 7:941–950. [https://doi.org/10.1016/S2213-2600\(19\)30199-7](https://doi.org/10.1016/S2213-2600(19)30199-7).
290. Smith K, Garman L, Wrammert J, Zheng N-Y, Capra JD, Ahmed R, Wilson PC. 2009. Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat Protoc* 4:372–384. <https://doi.org/10.1038/nprot.2009.3>.
291. Dessain SK, Adekar SP, Stevens JB, Carpenter KA, Skorski ML, Barnoski BL, Goldsby RA, Weinberg RA. 2004. High efficiency creation of human monoclonal antibody-producing hybridomas. *J Immunol Methods* 291:109–122. <https://doi.org/10.1016/j.jim.2004.05.005>.
292. Nakamura G, Chai N, Park S, Chiang N, Lin Z, Chiu H, Fong R, Yan D, Kim J, Zhang J, Lee WP, Estevez A, Coons M, Xu M, Lupardus P, Balazs M, Swem LR. 2013. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza A antibodies. *Cell Host Microbe* 14:93–103. <https://doi.org/10.1016/j.chom.2013.06.004>.
293. Throsby M, van den Brink E, Jongeneelen M, Poon LLM, Alard P, Cornelissen L, Bakker A, Cox F, van Deventer E, Guan Y, Cinatl J, ter Meulen J, Lasters I, Carsetti R, Peiris M, de Kruijff J, Goudsmit J. 2008. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS One* 3:e3942. <https://doi.org/10.1371/journal.pone.0003942>.
294. Tharakaraman K, Subramanian V, Viswanathan K, Sloan S, Yen H-L, Barnard DL, Leung YHC, Szretter KJ, Koch TJ, Delaney JC, Babcock GJ, Wogan GN, Sasisekharan R, Shriver Z. 2015. A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proc Natl Acad Sci U S A* 112:10890–10895. <https://doi.org/10.1073/pnas.1502374112>.
295. Sun X, Ling Z, Yang Z, Sun B. 2022. Broad neutralizing antibody-based strategies to tackle influenza. *Curr Opin Virol* 53:101207. <https://doi.org/10.1016/j.coviro.2022.101207>.
296. Lu R-M, Hwang Y-C, Liu I-J, Lee C-C, Tsai H-Z, Li H-J, Wu H-C. 2020. Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci* 27:1. <https://doi.org/10.1186/s12929-019-0592-z>.
297. Wang X, Zhou P, Wu M, Yang K, Guo J, Wang X, Li J, Fang Z, Wang G, Xing M, Zhou D. 2020. Adenovirus delivery of encoded monoclonal antibody protects against different types of influenza virus infection. *NPJ Vaccines* 5:57. <https://doi.org/10.1038/s41541-020-0206-5>.
298. Tan TK, Gamlen TPE, Rijal P, Townsend AR, Gill DR, Hyde SC. 2020. Lung-targeting lentiviral vector for passive immunisation against influenza. *Thorax* 75:1112–1115. <https://doi.org/10.1136/thoraxjnl-2020-214656>.
299. Yamazaki T, Nagashima M, Ninomiya D, Aina A, Fujimoto A, Ichimonji I, Takagi H, Morita N, Murotani K, Hasegawa H, Chiba J, Akashi-Takamura S. 2018. Neutralizing antibodies induced by gene-based hydrodynamic injection have a therapeutic effect in lethal influenza infection. *Front Immunol* 9:47. <https://doi.org/10.3389/fimmu.2018.00047>.
300. Corti D, Suguitan AL, Pinna D, Silacci C, Fernandez-Rodriguez BM, Vanzetta F, Santos C, Luke CJ, Torres-Velez FJ, Temperton NJ, Weiss RA, Sallusto F, Subbarao K, Lanzavecchia A. 2010. Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine. *J Clin Invest* 120:1663–1673. <https://doi.org/10.1172/JCI41902>.
301. Guthmiller JJ, Utset HA, Wilson PC. 2021. B cell responses against influenza viruses: short-lived humoral immunity against a life-long threat. *Viruses* 13:965. <https://doi.org/10.3390/v13060965>.
302. Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, Silacci C, Fernandez-Rodriguez BM, Agatic G, Bianchi S, Giacchetto-Sasselli I, Calder L, Sallusto F, Collins P, Haire LF, Temperton N, Langedijk JPM, Skehel JJ, Lanzavecchia A. 2011. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science* 333:850–856. <https://doi.org/10.1126/science.1205669>.
303. DiLillo DJ, Tan GS, Palese P, Ravetch JV. 2014. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo. *Nat Med* 20:143–151. <https://doi.org/10.1038/nm.3443>.
304. Ye Z-W, Yuan S, Poon K-M, Wen L, Yang D, Sun Z, Li C, Hu M, Shuai H, Zhou J, Zhang M-Y, Zheng B-J, Chu H, Yuen K-Y. 2017. Antibody-dependent cell-mediated cytotoxicity epitopes on the hemagglutinin head region of pandemic H1N1 influenza virus play detrimental roles in H1N1-infected mice. *Front Immunol* 8:317. <https://doi.org/10.3389/fimmu.2017.00317>.
305. Zhu X, Turner HL, Lang S, McBride R, Bangaru S, Gilchuk IM, Yu W, Paulson JC, Crowe JE, Ward AB, Wilson IA. 2019. Structural basis of protection against H7N9 influenza virus by human anti-N9 neuraminidase

- antibodies. *Cell Host Microbe* 26:729–738.e724. <https://doi.org/10.1016/j.chom.2019.10.002>.
306. Chen Y-Q, Wohlbold TJ, Zheng N-Y, Huang M, Huang Y, Neu KE, Lee J, Wan H, Rojas KT, Kirkpatrick E, Henry C, Palm A-KE, Stamper CT, Lan LY-L, Topham DJ, Treanor J, Wrammert J, Ahmed R, Eichelberger MC, Georgiou G, Krammer F, Wilson PC. 2018. Influenza infection in humans induces broadly cross-reactive and protective neuraminidase-reactive antibodies. *Cell* 173:417–429.e410. <https://doi.org/10.1016/j.cell.2018.03.030>.
 307. Stadlbauer D, Zhu X, McMahon M, Turner JS, Wohlbold TJ, Schmitz AJ, Strohmeier S, Yu W, Nachbagauer R, Mudd PA, Wilson IA, Ellebedy AH, Krammer F. 2019. Broadly protective human antibodies that target the active site of influenza virus neuraminidase. *Science* 366:499–504. <https://doi.org/10.1126/science.aay0678>.
 308. Doyle TM, Hashem AM, Li C, Van Domselaar G, Larocque L, Wang J, Smith D, Cyr T, Farnsworth A, He R, Hurt AC, Brown EG, Li X. 2013. Universal anti-neuraminidase antibody inhibiting all influenza A subtypes. *Antiviral Res* 100:567–574. <https://doi.org/10.1016/j.antiviral.2013.09.018>.
 309. Tan MP, Tan WS, Mohamed Alitheen NB, Yap WB. 2021. M2e-based influenza vaccines with nucleoprotein: a review. *Vaccines (Basel)* 9:739. <https://doi.org/10.3390/vaccines9070739>.
 310. Bimler L, Ronzulli SL, Song AY, Johnson SK, Jones CA, Kim T, Le DT, Tompkins SM, Paust S. 2021. Matrix protein 2 extracellular domain-specific monoclonal antibodies are an effective and potentially universal treatment for influenza A. *J Virol* 95:e01027-20. <https://doi.org/10.1128/JVI.01027-20>.
 311. Wang R, Song A, Levin J, Dennis D, Zhang NJ, Yoshida H, Koriazova L, Madura L, Shapiro L, Matsumoto A, Yoshida H, Mikayama T, Kubo RT, Sarawar S, Cheroute H, Kato S. 2008. Therapeutic potential of a fully human monoclonal antibody against influenza A virus M2 protein. *Antiviral Res* 80:168–177. <https://doi.org/10.1016/j.antiviral.2008.06.002>.
 312. Zebedee SL, Lamb RA. 1989. Growth restriction of influenza A virus by M2 protein antibody is genetically linked to the M1 protein. *Proc Natl Acad Sci U S A* 86:1061–1065. <https://doi.org/10.1073/pnas.86.3.1061>.
 313. Sambhara S, Kurichh A, Miranda R, Tumpey T, Rowe T, Renshaw M, Arpino R, Tamane A, Kandil A, James O, Underdown B, Klein M, Katz J, Burt D. 2001. Heterosubtypic immunity against human influenza A viruses, including recently emerged avian H5 and H9 viruses, induced by FLU-ISCOM vaccine in mice requires both cytotoxic T-lymphocyte and macrophage function. *Cell Immunol* 211:143–153. <https://doi.org/10.1006/cimm.2001.1835>.
 314. Townsend AR, Skehel JJ. 1984. The influenza A virus nucleoprotein gene controls the induction of both subtype specific and cross-reactive cytotoxic T cells. *J Exp Med* 160:552–563. <https://doi.org/10.1084/jem.160.2.552>.
 315. Yewdell JW, Bennink JR, Smith GL, Moss B. 1985. Influenza A virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes. *Proc Natl Acad Sci U S A* 82:1785–1789. <https://doi.org/10.1073/pnas.82.6.1785>.
 316. Sueno N, Otsuki Y, Konno J, Yamane N, Odagiri T, Arikawa J, Ishida N. 1979. Anti-nucleoprotein antibody response in influenza A infection. *Tohoku J Exp Med* 128:241–249. <https://doi.org/10.1620/tjem.128.241>.
 317. LaMere MW, Lam H-T, Moquin A, Haynes L, Lund FE, Randall TD, Kaminski DA. 2011. Contributions of antinucleoprotein IgG to heterosubtypic immunity against influenza virus. *J Immunol* 186:4331–4339. <https://doi.org/10.4049/jimmunol.1003057>.
 318. Carragher DM, Kaminski DA, Moquin A, Hartson L, Randall TDA. 2008. novel role for non-neutralizing antibodies against nucleoprotein in facilitating resistance to influenza virus. *J Immunol* 181:4168–4176. <https://doi.org/10.4049/jimmunol.181.6.4168>.
 319. Fujimoto Y, Tomioka Y, Takakuwa H, Uechi G-I, Yabuta T, Ozaki K, Suyama H, Yamamoto S, Morimatsu M, Mai LQ, Yamashiro T, Ito T, Otsuki K, Ono E. 2016. Cross-protective potential of anti-nucleoprotein human monoclonal antibodies against lethal influenza A virus infection. *J Gen Virol* 97:2104–2116. <https://doi.org/10.1099/jgv.0.000518>.
 320. Chai N, Swem LR, Reichelt M, Chen-Harris H, Luis E, Park S, Fouts A, Lupardus P, Wu TD, Li O, McBride J, Lawrence M, Xu M, Tan M-W. 2016. Two escape mechanisms of influenza A Virus to a broadly neutralizing stalk-binding antibody. *PLoS Pathog* 12:e1005702. <https://doi.org/10.1371/journal.ppat.1005702>.
 321. Lim JJ, Deng R, Derby MA, Larouche R, Horn P, Anderson M, Maia M, Carrier S, Pelletier I, Burgess T, Kulkarni P, Newton E, Tavel JA. 2016. Two phase 1, randomized, double-blind, placebo-controlled, single-ascending-dose studies to investigate the safety, tolerability, and pharmacokinetics of an anti-influenza A virus monoclonal antibody, MHAA4549A, in healthy volunteers. *Antimicrob Agents Chemother* 60:5437–5444. <https://doi.org/10.1128/AAC.00607-16>.
 322. McBride JM, Lim JJ, Burgess T, Deng R, Derby MA, Maia M, Horn P, Siddiqui O, Sheinson D, Chen-Harris H, Newton EM, Fillos D, Nazzari D, Rosenberger CM, Ohlson MB, Lambkin-Williams R, Fathi H, Harris JM, Tavel JA. 2017. Phase 2 randomized trial of the safety and efficacy of MHAA4549A, a broadly neutralizing monoclonal antibody, in a human influenza A virus challenge model. *Antimicrob Agents Chemother* 61:e01154-17. <https://doi.org/10.1128/AAC.01154-17>.
 323. Lim JJ, Nilsson AC, Silverman M, Assy N, Kulkarni P, McBride JM, Deng R, Li C, Yang X, Nguyen A, Horn P, Maia M, Castro A, Peck MC, Galanter J, Chu T, Newton EM, Tavel JA. 2020. A phase 2 randomized, double-blind, placebo-controlled trial of MHAA4549A, a monoclonal antibody, plus oseltamivir in patients hospitalized with severe influenza A virus infection. *Antimicrob Agents Chemother* 64:e00352-20. <https://doi.org/10.1128/AAC.00352-20>.
 324. Paules CI, Lakdawala S, McAuliffe JM, Paskel M, Vogel L, Kallewaard NL, Zhu Q, Subbarao K. 2017. The hemagglutinin A stem antibody MEDI8852 prevents and controls disease and limits transmission of pandemic influenza viruses. *J Infect Dis* 216:356–365. <https://doi.org/10.1093/infdis/jix292>.
 325. Kallewaard NL, Corti D, Collins PJ, Neu U, McAuliffe JM, Benjamin E, Wachter-Rosati L, Palmer-Hill FJ, Yuan AQ, Walker PA, Vorlaender MK, Bianchi S, Guarino B, De Marco A, Vanzetta F, Agatic G, Foglierini M, Pinna D, Fernandez-Rodriguez B, Fruehwirth A, Silacci C, Ogorodowicz RW, Martin SR, Sallusto F, Suzich JA, Lanzavecchia A, Zhu Q, Gamblin SJ, Skehel JJ. 2016. Structure and function analysis of an antibody recognizing all influenza A subtypes. *Cell* 166:596–608. <https://doi.org/10.1016/j.cell.2016.05.073>.
 326. Mallory RM, Ali SO, Takas T, Kankam M, Dubovsky F, Tseng L. 2017. A phase 1 study to evaluate the safety and pharmacokinetics of MEDI8852, an anti-influenza A monoclonal antibody, in healthy adult volunteers. *Biologicals* 50:81–86. <https://doi.org/10.1016/j.biologics.2017.08.007>.
 327. Ali SO, Takas T, Nyborg A, Shoemaker K, Kallewaard NL, Chiong R, Dubovsky F, Mallory RM. 2018. Evaluation of MEDI8852, an Anti-influenza monoclonal antibody, in treating acute uncomplicated influenza. *Antimicrob Agents Chemother* 62:e00694-18. <https://doi.org/10.1128/AAC.00694-18>.
 328. Clinicaltrials.gov. 2017. Dose ranging study to evaluate the efficacy and safety of MEDI8852 in adults who are hospitalized with type A influenza. <https://clinicaltrials.gov/ct2/show/NCT03028909>. Retrieved 19 February 2022.
 329. Baranovich T, Jones JC, Russier M, Vogel P, Szretter KJ, Sloan SE, Seiler P, Trevejo JM, Webby RJ, Govorkova EA. 2016. The hemagglutinin stem-binding monoclonal antibody VIS410 controls influenza virus-induced acute respiratory distress syndrome. *Antimicrob Agents Chemother* 60:2118–2131. <https://doi.org/10.1128/AAC.02457-15>.
 330. Wollacott AM, Boni MF, Szretter KJ, Sloan SE, Yousofshahi M, Viswanathan K, Bedard S, Hay CA, Smith PF, Shriver Z, Trevejo JM. 2016. Safety and upper respiratory pharmacokinetics of the hemagglutinin stalk-binding antibody VIS410 support treatment and prophylaxis based on population modeling of seasonal influenza A outbreaks. *EBioMedicine* 5:147–155. <https://doi.org/10.1016/j.ebiom.2016.02.021>.
 331. Hershberger E, Sloan S, Narayan K, Hay CA, Smith P, Engler F, Jeeninga R, Smits S, Trevejo J, Shriver Z, Oldach D. 2019. 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* 40:574–582. <https://doi.org/10.1016/j.ebiom.2018.12.051>.
 332. Sloan SE, Szretter KJ, Sundaresh B, Narayan KM, Smith PF, Skurnik D, Bedard S, Trevejo JM, Oldach D, Shriver Z. 2020. Clinical and virological responses to a broad-spectrum human monoclonal antibody in an influenza virus challenge study. *Antiviral Res* 184:104763. <https://doi.org/10.1016/j.antiviral.2020.104763>.
 333. Lavrov SV, Eremkina EI, Orlova TG, Galegov GA, Soloviev VD, Zhdanov VM. 1968. Combined inhibition of influenza virus reproduction in cell culture using interferon and amantadine. *Nature* 217:856–857. <https://doi.org/10.1038/217856a0>.
 334. Hayden FG, Schlepshchkin AN, Pushkarskaya NL. 1984. Combined interferon- α 2, rimantadine hydrochloride, and ribavirin inhibition of influenza virus replication in vitro. *Antimicrob Agents Chemother* 25:53–57. <https://doi.org/10.1128/AAC.25.1.53>.
 335. Clinicaltrials.gov. 2017. Flufenamic acid for hospitalised influenza infection. <https://clinicaltrials.gov/ct2/show/NCT03238612>. Retrieved 19 February 2022.

336. Clinicaltrials.gov. 2020. Efficacy of clarithromycin-naproxen-oseltamivir combination therapy vs. oseltamivir alone for hospitalised paediatric influenza patients. <https://clinicaltrials.gov/ct2/show/NCT04315194>. Retrieved 19 February 2022.
337. Clinicaltrials.gov. 2009. Oral triple combination antiviral drug therapy for treatment of influenza A in immunocompromised subjects (PO206). <https://clinicaltrials.gov/ct2/show/NCT00979251>. Retrieved 19 February 2022.
338. Long JS, Mistry B, Haslam SM, Barclay WS. 2019. Host and viral determinants of influenza A virus species specificity. *Nat Rev Microbiol* 17: 67–81. <https://doi.org/10.1038/s41579-018-0115-z>.
339. Shapira SD, Gat-Viks I, Shum BOV, Dricot A, de Grace MM, Wu L, Gupta PB, Hao T, Silver SJ, Root DE, Hill DE, Regev A, Hacohen N. 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell* 139:1255–1267. <https://doi.org/10.1016/j.cell.2009.12.018>.
340. Karlas A, Machuy N, Shin Y, Pleissner K-P, Artarini A, Heuer D, Becker D, Khalil H, Ogilvie LA, Hess S, Mäurer AP, Müller E, Wolff T, Rudel T, Meyer TF. 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 463:818–822. <https://doi.org/10.1038/nature08760>.
341. Han J, Perez JT, Chen C, Li Y, Benitez A, Kandasamy M, Lee Y, Andrade J, tenOever B, Manicassamy B. 2018. Genome-wide CRISPR/Cas9 screen identifies host factors essential for influenza virus replication. *Cell Rep* 23:596–607. <https://doi.org/10.1016/j.celrep.2018.03.045>.
342. Kumar N, Sharma S, Kumar R, Tripathi BN, Barua S, Ly H, Rouse BT. 2020. Host-directed antiviral therapy. *Clin Microbiol Rev* 33:e00168-19. <https://doi.org/10.1128/CMR.00168-19>.
343. Smith SB, Dampier W, Tozeren A, Brown JR, Magid-Slav M. 2012. Identification of common biological pathways and drug targets across multiple respiratory viruses based on human host gene expression analysis. *PLoS One* 7:e33174. <https://doi.org/10.1371/journal.pone.0033174>.
344. Pichlmair A, Kandasamy K, Alvisi G, Mulhern O, Sacco R, Habjan M, Binder M, Stefanovic A, Eberle C-A, Goncalves A, Bürckstümmer T, Müller AC, Fauster A, Holze C, Lindsten K, Goodbourn S, Kochs G, Weber F, Bartenschlager R, Bowie AG, Bennett KL, Colinge J, Superti-Furga G. 2012. Viral immune modulators perturb the human molecular network by common and unique strategies. *Nature* 487:486–490. <https://doi.org/10.1038/nature11289>.
345. Geraghty RJ, Aliota MT, Bonnac LF. 2021. Broad-spectrum antiviral strategies and nucleoside analogues. *Viruses* 13:667. <https://doi.org/10.3390/v13040667>.
346. Camp JV, Jonsson CB. 2017. A role for neutrophils in viral respiratory disease. *Front Immunol* 8:550. <https://doi.org/10.3389/fimmu.2017.00550>.
347. Miller BE, Mistry S, Smart K, Connolly P, Carpenter DC, Cooray H, Bloomer JC, Tal-Singer R, Lazaar AL. 2015. The pharmacokinetics and pharmacodynamics of danirixin (GSK1325756)—a selective CXCR2 antagonist—in healthy adult subjects. *BMC Pharmacol Toxicol* 16:18. <https://doi.org/10.1186/s40360-015-0017-x>.
348. Miller BE, Smart K, Mistry S, Ambrey CL, Bloomer JC, Connolly P, Sanderson D, Shreeves T, Smith R, Lazaar AL. 2014. The pharmacokinetics of conventional and bioenhanced tablet formulations of danirixin (GSK1325756) following oral administration in healthy, elderly, human volunteers. *Eur J Drug Metab Pharmacokinet* 39:173–181. <https://doi.org/10.1007/s13318-014-0179-8>.
349. Madan A, Chen S, Yates P, Washburn ML, Roberts G, Peat AJ, Tao Y, Parry MF, Barnum O, McClain MT, Roy-Ghanta S. 2019. Efficacy and safety of danirixin (GSK1325756) co-administered with standard-of-care antiviral (oseltamivir): a phase 2b, global, randomized study of adults hospitalized with influenza. *Open Forum Infect Dis* 6:ofz163. <https://doi.org/10.1093/ofid/ofz163>.
350. Lazaar AL, Miller BE, Tabberer M, Yonchuk J, Leidy N, Ambrey C, Bloomer J, Watz H, Tal-Singer R. 2018. Effect of the CXCR2 antagonist danirixin on symptoms and health status in COPD. *Eur Respir J* 52:1801020. <https://doi.org/10.1183/13993003.01020-2018>.
351. Perwitasari O, Yan X, Johnson S, White C, Brooks P, Tompkins SM, Tripp RA. 2013. Targeting organic anion transporter 3 with probenecid as a novel anti-influenza A virus strategy. *Antimicrob Agents Chemother* 57: 475–483. <https://doi.org/10.1128/AAC.01532-12>.
352. Aronson JK. 2016. Meyler's side effects of drugs: the international encyclopedia of adverse drug reactions and interactions, 16th ed, 936–939. Elsevier Science, USA. Retrieved 29 December 2022.
353. Rayner CR, Chanu P, Gieschke R, Boak LM, Jonsson EN. 2008. Population pharmacokinetics of oseltamivir when coadministered with probenecid. *J Clin Pharmacol* 48:935–947. <https://doi.org/10.1177/0091270008320317>.
354. Holodniy M, Penzak SR, Straight TM, Davey RT, Lee KK, Goetz MB, Raisch DW, Cunningham F, Lin ET, Olivo N, Deyton LR. 2008. Pharmacokinetics and tolerability of oseltamivir combined with probenecid. *Antimicrob Agents Chemother* 52:3013–3021. <https://doi.org/10.1128/AAC.00047-08>.
355. Clinicaltrials.gov. 2014. Treatment of severe influenza A infection. <https://clinicaltrials.gov/ct2/show/NCT02108366>.
356. Hung I, To K, Yuen K. 2019. Treatment of severe influenza A infection with Celecoxib: a double blind randomised controlled trial. https://rfs1.healthbureau.gov.hk/images/events/HRS2019/HRS2019_S17_powerpoint.pdf. Retrieved 29 December, 2022.
357. Triana-Baltzer GB, Babizki M, Chan MCW, Wong ACN, Aschenbrenner LM, Campbell ER, Li Q-X, Chan RWY, Peiris JSM, Nicholls JM, Fang F. 2010. DAS181, a sialidase fusion protein, protects human airway epithelium against influenza virus infection: an in vitro pharmacodynamic analysis. *J Antimicrob Chemother* 65:275–284. <https://doi.org/10.1093/jac/dkp421>.
358. Marjuki H, Mishin VP, Chesnokov AP, De La Cruz JA, Fry AM, Villanueva J, Gubareva LV. 2014. An investigational antiviral drug, DAS181, effectively inhibits replication of zoonotic influenza A virus subtype H7N9 and protects mice from lethality. *J Infect Dis* 210:435–440. <https://doi.org/10.1093/infdis/jiu105>.
359. Moss RB, Hansen C, Sanders RL, Hawley S, Li T, Steigbigel RT. 2012. A phase II study of DAS181, a novel host directed antiviral for the treatment of influenza infection. *J Infect Dis* 206:1844–1851. <https://doi.org/10.1093/infdis/jis622>.
360. Zenilman JM, Fuchs EJ, Hendrix CW, Radebaugh C, Jurao R, Nayak SU, Hamilton RG, McLeod Griffiss J. 2015. Phase 1 clinical trials of DAS181, an inhaled sialidase, in healthy adults. *Antiviral Res* 123:114–119. <https://doi.org/10.1016/j.antiviral.2015.09.008>.
361. Pizzorno A, Terrier O, Nicolas de Lamballerie C, Julien T, Padey B, Traversier A, Roche M, Hamelin M-E, Rhéaume C, Croze S, Escuret V, Poissy J, Lina B, Legras-Lachuer C, Textoris J, Boivin G, Rosa-Calatrava M. 2019. Repurposing of drugs as novel influenza inhibitors from clinical gene expression infection signatures. *Front Immunol* 10:60. <https://doi.org/10.3389/fimmu.2019.00060>.
362. Pizzorno A, Padey B, Terrier O, Rosa-Calatrava M. 2019. Drug repurposing approaches for the treatment of influenza viral infection: reviving old drugs to fight against a long-lived enemy. *Front Immunol* 10:531. <https://doi.org/10.3389/fimmu.2019.00531>.
363. Clinicaltrials.gov. 2017. Efficiency of antagonist drugs of the cellular transcriptomic signature of influenza A virus infection (FLUNEXT). <https://clinicaltrials.gov/ct2/show/NCT03212716>. Retrieved 21 February 2022.
364. Lee N, Wong CK, Hui DSC, Lee SKW, Wong RYK, Ngai KLK, Chan MCW, Chu YJ, Ho AWY, Lui GCY, Wong BCK, Wong SH, Yip SP, Chan PKS. 2013. Role of human Toll-like receptors in naturally occurring influenza A infections. *Influenza Other Respir Viruses* 7:666–675. <https://doi.org/10.1111/irv.12109>.
365. Nickbakhsh S, Mair C, Matthews L, Reeve R, Johnson PCD, Thorburn F, von Wissmann B, Reynolds A, McMenamin J, Gunson RN, Murcia PR. 2019. Virus–virus interactions impact the population dynamics of influenza and the common cold. *Proc Natl Acad Sci U S A* 116:27142–27150. <https://doi.org/10.1073/pnas.1911083116>.
366. Meskill SD, O'Bryen SC. 2020. Respiratory virus co-infection in acute respiratory infections in children. *Curr Infect Dis Rep* 22:3. <https://doi.org/10.1007/s11908-020-0711-8>.
367. Hornung V, Ellegast J, Kim S, Brzózka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann K-K, Schlee M, Endres S, Hartmann G. 2006. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 314:994–997. <https://doi.org/10.1126/science.1132505>.
368. Ranjan P, Jayashankar L, Deyde V, Zeng H, Davis WG, Pearce MB, Bowzard JB, Hoelscher MA, Jeisy-Scott V, Wiens ME, Gangappa S, Gubareva L, García-Sastre A, Katz JM, Tumpey TM, Fujita T, Sambhara S. 2010. 5'PPP-RNA induced RIG-I activation inhibits drug-resistant avian H5N1 as well as 1918 and 2009 pandemic influenza virus replication. *Virology* 407:102. <https://doi.org/10.1186/1743-422X-7-102>.
369. Spiropoulou CF, Ranjan P, Pearce MB, Sealy TK, Albariño CG, Gangappa S, Fujita T, Rollin PE, Nichol ST, Ksiazek TG, Sambhara S. 2009. RIG-I activation inhibits ebolavirus replication. *Virology* 392:11–15. <https://doi.org/10.1016/j.viro.2009.06.032>.
370. Singh N, Ranjan P, Cao W, Patel J, Gangappa S, Davidson BA, Sullivan JM, Prasad PN, Knight PR, Sambhara S. 2020. A dual-functioning 5'-PPP-NS1shRNA that activates a RIG-I antiviral pathway and suppresses

- influenza NS1. *Mol Ther Nucleic Acids* 19:1413–1422. <https://doi.org/10.1016/j.omtn.2020.01.025>.
371. Chakravarthy KV, Bonoio AC, Davis WG, Ranjan P, Ding H, Hu R, Bowzard JB, Bergey EJ, Katz JM, Knight PR, Sambhara S, Prasad PN. 2010. Gold nanorod delivery of an ssRNA immune activator inhibits pandemic H1N1 influenza viral replication. *Proc Natl Acad Sci U S A* 107:10172–10177. <https://doi.org/10.1073/pnas.0914561107>.
 372. Goulet M-L, Olgner D, Xu Z, Paz S, Belgnaoui SM, Lafferty EI, Janelle V, Arguello M, Paquet M, Ghneim K, Richards S, Smith A, Wilkinson P, Cameron M, Kalinke U, Qureshi S, Lamarre A, Haddad EK, Sekaly RP, Peri S, Balachandran S, Lin R, Hiscott J. 2013. Systems analysis of a RIG-I agonist inducing broad spectrum inhibition of virus infectivity. *PLoS Pathog* 9:e1003298. <https://doi.org/10.1371/journal.ppat.1003298>.
 373. Chiang C, Beljanski V, Yin K, Olgner D, Ben Yebdri F, Steel C, Goulet M-L, DeFilippis VR, Streblow DN, Haddad EK, Trautmann L, Ross T, Lin R, Hiscott J. 2015. Sequence-specific modifications enhance the broad-spectrum antiviral response activated by RIG-I agonists. *J Virol* 89: 8011–8025. <https://doi.org/10.1128/JVI.00845-15>.
 374. Pattabhi S, Wilkins CR, Dong R, Knoll ML, Posakony J, Kaiser S, Mire CE, Wang ML, Ireton RC, Geisbert TW, Bedard KM, Iadonato SP, Loo Y-M, Gale M. 2015. Targeting innate immunity for antiviral therapy through small molecule agonists of the RLR pathway. *J Virol* 90:2372–2387. <https://doi.org/10.1128/JVI.02202-15>.
 375. Jacobson ME, Becker KW, Palmer CR, Pastora LE, Fletcher RB, Collins KA, Fedorova O, Duval CL, Pyle AM, Wilson JT. 2020. Structural optimization of polymeric carriers to enhance the immunostimulatory activity of molecularly defined RIG-I agonists. *ACS Cent Sci* 6:2008–2022. <https://doi.org/10.1021/acscentsci.0c00568>.
 376. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413:732–738. <https://doi.org/10.1038/35099560>.
 377. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh C-S, Reis e Sousa C, Matsuura Y, Fujita T, Akira S. 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 441:101–105. <https://doi.org/10.1038/nature04734>.
 378. Gitlin L, Barchet W, Gilfillan S, Cella M, Beutler B, Flavell RA, Diamond MS, Colonna M. 2006. Essential role of mda-5 in type I IFN responses to polyribonucleic:polyribocytidylic acid and encephalomyocarditis picornavirus. *Proc Natl Acad Sci U S A* 103:8459–8464. <https://doi.org/10.1073/pnas.0603082103>.
 379. Zhao J, Wohlford-Lenane C, Zhao J, Fleming E, Lane TE, McCray PB, Perlman S. 2012. Intranasal treatment with poly(I:C) protects aged mice from lethal respiratory virus infections. *J Virol* 86:11416–11424. <https://doi.org/10.1128/JVI.01410-12>.
 380. Clinicaltrials.gov. 2017. Study examining PrEP-001 in healthy subjects. <https://clinicaltrials.gov/ct2/show/NCT03220048>. Retrieved 20 February 2022.
 381. Malcolm BA, Aerts CA, Dubois KJ, Geurts FJ, Marien K, Rusch S, Van Dijk AH, Verloes R, Vingerhoets J. 2018. PrEP-001 prophylactic effect against rhinovirus and influenza virus-results of 2 randomized trials. *Antiviral Res* 153:70–77. <https://doi.org/10.1016/j.antiviral.2018.03.005>.
 382. Ciancanelli MJ, Huang SXL, Luthra P, Garner H, Itan Y, Volpi S, Lafaille FG, Trouillet C, Schmolke M, Albrecht R, Israelsson E, Lim HK, Casadio M, Hermesh T, Lorenzo L, Leung LW, Pedergnana V, Boisson B, Okada S, Picard C, Ringuier B, Troussier F, Chaussabel D, Abel L, Pelletier I, Notarangelo LD, García-Sastre A, Basler CF, Geissmann F, Zhang S-Y, Snoeck H-W, Casanova J-L. 2015. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science* 348: 448–453. <https://doi.org/10.1126/science.aaa1578>.
 383. Haasbach E, Droebner K, Vogel AB, Planz O. 2011. Low-dose interferon type I treatment is effective against H5N1 and swine-origin H1N1 influenza A viruses in vitro and in vivo. *J Interferon Cytokine Res* 31:515–525. <https://doi.org/10.1089/jir.2010.0071>.
 384. Bennett AL, Smith DW, Cummins MJ, Jacoby PA, Cummins JM, Beilharz MW. 2013. Low-dose oral interferon alpha as prophylaxis against viral respiratory illness: a double-blind, parallel controlled trial during an influenza pandemic year. *Influenza Other Respir Viruses* 7:854–862. <https://doi.org/10.1111/irv.12094>.
 385. Clinicaltrials.gov. 2010. Safety and efficacy study of interferon to treat patients hospitalized for influenza. <https://clinicaltrials.gov/ct2/show/NCT01227798>. Retrieved 21 February 2022.
 386. Andreev-Andrievskiy AA, Zinovkin RA, Mashkin MA, Frolova OY, Kazaisvili YG, Scherbakova VS, Rudoy BA, Nesterenko VG. 2021. Gene expression pattern of Peyer's patch lymphocytes exposed to kagocel suggests pattern-recognition receptors mediate its action. *Front Pharmacol* 12:679511. <https://doi.org/10.3389/fphar.2021.679511>.
 387. Popov AF, Simakova AI, Dmitrenko KA, Shchelkanov MY. 2017. Time course of changes in cytokines (IFN- γ , IFN- α , IL-18, TNF- α) in the treatment of moderate influenza A (H1N1) pdm09 (2013–2016) with oseltamivir (Tamiflu) and umifenovir (Arbidol) alone and in combination with Kagocel. *Ter Arkh* 89:66–70. (In Russian.) <https://doi.org/10.17116/terarkh2017891066-70>.
 388. Clinicaltrials.gov. 2020. Kagocel for the prevention of ARVI and influenza in adults health care workers. <https://clinicaltrials.gov/ct2/show/NCT04611061>. Retrieved 20 February 2022.
 389. Clinicaltrials.gov. 2020. Kagocel for the prevention of ARVI and influenza in young people. <https://clinicaltrials.gov/ct2/show/NCT04626622>. Retrieved 21 February 2022.
 390. Tait AR, Knight PR. 1987. The effects of general anesthesia on upper respiratory tract infections in children. *Anesthesiology* 67:930–935. <https://doi.org/10.1097/0000542-198712000-00009>.
 391. Tait AR, Du Boulay PM, Knight PR. 1988. Alterations in the course of and histopathologic response to influenza virus infections produced by enflurane, halothane, and diethyl ether anesthesia in ferrets. *Anesth Analg* 67: 671–676.
 392. Penna AM, Johnson KJ, Camilleri J, Knight PR. 1990. Alterations in influenza A virus specific immune injury in mice anesthetized with halothane or ketamine. *Intervirology* 31:188–196. <https://doi.org/10.1159/000150153>.
 393. Knight PR, Bedows E, Nahrwold ML, Maassab HF, Smitka CW, Busch MT. 1983. Alterations in influenza virus pulmonary pathology induced by diethyl ether, halothane, enflurane, and pentobarbital anesthesia in mice. *Anesthesiology* 58:209–215. <https://doi.org/10.1097/0000542-198303000-00001>.
 394. MacDonald BA, Chakravarthy KV, Davidson BA, Mullan BA, Alluri R, Hakansson AP, Knight PR. 2015. Halothane modulates the type I interferon response to influenza and minimizes the risk of secondary bacterial pneumonia through maintenance of neutrophil recruitment in an animal model. *Anesthesiology* 123:590–602. <https://doi.org/10.1097/ALN.0000000000000766>.
 395. Serafim MSM, dos Santos Júnior VS, Gertrudes JC, Maltarollo VG, Honório KM. 2021. Machine learning techniques applied to the drug design and discovery of new antivirals: a brief look over the past decade. *Expert Opin Drug Discov* 16:961–975. <https://doi.org/10.1080/17460441.2021.1918098>.
 396. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, Li B, Madabhushi A, Shah P, Spitzer M, Zhao S. 2019. Applications of machine learning in drug discovery and development. *Nat Rev Drug Discov* 18: 463–477. <https://doi.org/10.1038/s41573-019-0024-5>.
 397. Shaltout N, Rafea A, Moustafa A, Moustafa M, ElHefnawi M. 2016. Optimizing the detection of antiviral-resistant influenza-A strains using machine learning. In proceedings of the World Congress on Engineering and Computer Science. https://www.iaeng.org/publication/WCECS2016/WCECS2016_pp527-532.pdf. Retrieved 29 December 2022.
 398. Pang Y, Yao L, Zhong J-H, Wang Z, Lee T-Y. 2021. AVPidn: a new scheme for identification and functional prediction of antiviral peptides based on machine learning approaches. *Brief Bioinform* 22:bbab263. <https://doi.org/10.1093/bib/bbab263>.
 399. Bravo A, Piñero J, Queralt-Rosinach N, Rautschka M, Furlong LI. 2015. Extraction of relations between genes and diseases from text and large-scale data analysis: implications for translational research. *BMC Bioinformatics* 16:55. <https://doi.org/10.1186/s12859-015-0472-9>.
 400. Kim J, Kim J-J, Lee H. 2017. An analysis of disease-gene relationship from Medline abstracts by DigSee. *Sci Rep* 7:40154. <https://doi.org/10.1038/srep40154>.
 401. Thompson MG, Dittmar M, Mallory MJ, Bhat P, Ferretti MB, Fontoura BM, Cherry S, Lynch KW. 2020. Viral-induced alternative splicing of host genes promotes influenza replication. *Elife* 9:e55500. <https://doi.org/10.7554/eLife.55500>.
 402. Leung MKK, Xiong HY, Lee LJ, Frey BJ. 2014. Deep learning of the tissue-regulated splicing code. *Bioinformatics* 30:i121–i129. <https://doi.org/10.1093/bioinformatics/btu277>.
 403. Lian W, Fang J, Li C, Pang X, Liu A-L, Du G-H. 2016. Discovery of influenza A virus neuraminidase inhibitors using support vector machine and naïve Bayesian models. *Mol Divers* 20:439–451. <https://doi.org/10.1007/s11030-015-9641-z>.
 404. Zhang L, Ai H-X, Li S-M, Qi M-Y, Zhao J, Zhao Q, Liu H-S. 2017. Virtual screening approach to identifying influenza virus neuraminidase

- inhibitors using molecular docking combined with machine-learning-based scoring function. *Oncotarget* 8:83142–83154. <https://doi.org/10.18632/oncotarget.20915>.
405. Chang Y-J, Yeh C-Y, Cheng J-C, Huang Y-Q, Hsu K-C, Lin Y-F, Lu C-H. 2021. Potent sialic acid inhibitors that target influenza A virus hemagglutinin. *Sci Rep* 11:8637. <https://doi.org/10.1038/s41598-021-87845-0>.
 406. Chen Y-F, Hsu K-C, Lin S-R, Wang W-C, Huang Y-C, Yang J-M. 2010. SiM-Map: a web server for inferring site-moiety map to recognize interaction preferences between protein pockets and compound moieties. *Nucleic Acids Res* 38:W424–W430. <https://doi.org/10.1093/nar/gkq480>.
 407. Ma J, Sheridan RP, Liaw A, Dahl GE, Svetnik V. 2015. Deep neural nets as a method for quantitative structure–activity relationships. *J Chem Inf Model* 55:263–274. <https://doi.org/10.1021/ci500747n>.
 408. Ramsundar B, Liu B, Wu Z, Verras A, Tudor M, Sheridan RP, Pande V. 2017. Is multitask deep learning practical for pharma? *J Chem Inf Model* 57:2068–2076. <https://doi.org/10.1021/acs.jcim.7b00146>.
 409. Olivecrona M, Blaschke T, Engkvist O, Chen H. 2017. Molecular de-novo design through deep reinforcement learning. *J Cheminform* 9:48. <https://doi.org/10.1186/s13321-017-0235-x>.
 410. Overhoff B, Falls Z, Mangione W, Samudrala R. 2021. A deep-learning proteomic-scale approach for drug design. *Pharmaceuticals (Basel)* 14: 1277. <https://doi.org/10.3390/ph14121277>.
 411. Mangione W, Falls Z, Chopra G, Samudrala R. 2020. cando.py: open source software for predictive bioanalytics of large scale drug–protein–disease data. *J Chem Inf Model* 60:4131–4136. <https://doi.org/10.1021/acs.jcim.0c00110>.
 412. Schuler J, Falls Z, Mangione W, Hudson ML, Bruggemann L, Samudrala R. 2020. Evaluating performance of drug repurposing technologies. *Drug Discov Today* 27:49–64. <https://doi.org/10.1016/j.drudis.2021.08.002>.
 413. Mangione W, Falls Z, Melendy T, Chopra G, Samudrala R. 2020. Shotgun drug repurposing biotechnology to tackle epidemics and pandemics. *Drug Discov Today* 25:1126–1128. <https://doi.org/10.1016/j.drudis.2020.05.002>.
 414. Mangione W, Falls Z, Samudrala R. 2022. Optimal COVID-19 therapeutic candidate discovery using the CANDO platform. *Front Pharmacol* 13: 970494. <https://doi.org/10.3389/fphar.2022.970494>.
 415. Falls Z, Mangione W, Schuler J, Samudrala R. 2019. Exploration of interaction scoring criteria in the CANDO platform. *BMC Res Notes* 12:318. <https://doi.org/10.1186/s13104-019-4356-3>.
 416. Sethi G, Chopra G, Samudrala R. 2015. Multiscale modelling of relationships between protein classes and drug behavior across all diseases using the CANDO platform. *Mini Rev Med Chem* 15:705–717. <https://doi.org/10.2174/1389557515666150219145148>.
 417. Minie M, Chopra G, Sethi G, Horst J, White G, Roy A, Hatti K, Samudrala R. 2014. CANDO and the infinite drug discovery frontier. *Drug Discov Today* 19:1353–1363. <https://doi.org/10.1016/j.drudis.2014.06.018>.
 418. Moscona A. 2005. Neuraminidase inhibitors for influenza. *N Engl J Med* 353:1363–1373. <https://doi.org/10.1056/NEJMr050740>.
 419. Świerczyńska M, Mirowska-Guzel DM, Pindelska E. 2022. Antiviral drugs in influenza. *Int J Environ Res Public Health* 19:3018. <https://doi.org/10.3390/ijerph19053018>.
 420. Phanich J, Rungrotmongkol T, Kungwan N, Hannongbua S. 2016. Role of R292K mutation in influenza H7N9 neuraminidase toward oseltamivir susceptibility: MD and MM/PB(GB)SA study. *J Comput Aided Mol Des* 30: 917–926. <https://doi.org/10.1007/s10822-016-9981-5>.
 421. Cass LM, Efthymiopoulos C, Bye A. 1999. Pharmacokinetics of zanamivir after intravenous, oral, inhaled or intranasal administration to healthy volunteers. *Clin Pharmacokinet* 36(Suppl 1):1–11. <https://doi.org/10.2165/00003088-199936001-00001>.
 422. Koshimichi H, Ishibashi T, Kawaguchi N, Sato C, Kawasaki A, Wajima T. 2018. Safety, tolerability, and pharmacokinetics of the novel anti-influenza agent baloxavir marboxil in healthy adults: phase I study findings. *Clin Drug Invest* 38:1189–1196. <https://doi.org/10.1007/s40261-018-0710-9>.
 423. Abraham GM, Morton JB, Saravolatz LD. 2020. Baloxavir: a novel antiviral agent in the treatment of influenza. *Clin Infect Dis* 71:1790–1794. <https://doi.org/10.1093/cid/ciaa107>.

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