Antivirals for Respiratory Viral Infections: Problems and Prospects

Qiang Liu, MD[1,*]   Yuan-hong Zhou, PhD[1,*]   Feng Ye[1,*]   Zhan-qiu Yang, MD[2]

[1] The First College of Clinical Medical Science, China Three Gorges University/Yichang Central People's Hospital, Yichang, China
[2] State Key Laboratory of Virology, Institute of Medical Virology, School of Medicine, Wuhan University, Wuhan, China

Address for correspondence Zhan-Qiu Yang, MD, Institute of Medical Virology, School of Basic Medical Sciences, Wuhan University, 185 Donghu Road, Wuhan 430071, China (e-mail: zqyang@whu.edu.cn).

Abstract

Keywords
► respiratory virus
► antiviral
► entry
► replication
► pathway

In the past two decades, several newly emerging and reemerging viral respiratory pathogens including several influenza viruses (avian influenza and pandemic influenza), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV), have continued to challenge medical and public health systems. Thereafter, the development of cost-effective, broad-spectrum antiviral agents is the urgent mission of both virologists and pharmacologists. Current antiviral developments have focused targets on viral entry, replication, release, and intercellular pathways essential for viral life cycle. Here, we review the current literature on challenges and prospects in the development of these antivirals.

Respiratory infections are a major cause of morbidity and mortality for humans, and most of these infections are caused by respiratory viruses.[1] Between 1997 and 2015, several unprecedented epizootic avian influenza viruses (e.g., H5N1, H7N9, and H10N8) crossed the species barrier to cause human death.[2–6] From severe acute respiratory syndrome coronavirus (SARS-CoV) to recent Middle East respiratory syndrome coronavirus (MERS-CoV), coronaviruses also pose a major threat to medical and public health systems.[7] Moreover, human adenovirus-14, human metapneumovirus, enterovirus D68, bunyaviruses, and common respiratory viruses (respiratory syncytial virus, parainfluenza viruses, rhinoviruses, and human influenza) are all gangsters of respiratory disease.[1,8] The continual emergence of these new and reemerging viral threats emphasizes the unpredictability of these pathogens, and brings challenges to the development of control strategies.

Although vaccination remains a milestone for the prevention of viral infections, a homologous vaccine was not available during the first pandemic wave of most newly emerging viruses.[9,10] Furthermore, continuous antigenic drifting and antigenic shifting of virus can lead to the escape of recognition by immune system and inefficacy to protect the host from a novel antigenic variant.[11] Thus, antivirals with novel pharmacological activities are highly needed. Several antiviral agents have been developed. Some of the agents are undergoing clinical trials and some are at different stages of preclinical development, but the vast majority have not yet been approved for clinical use.[12,13] Several challenges have blocked the development of these antivirals as well as its application.[13] In this review, we focus on the problems and prospects of these antivirals which were developed to control the newly emerging and reemerging respiratory viruses with pandemic potential.

Diagnostics and Measurements of Virus in the Development of Antivirals

A respiratory viral infection is difficult to differentiate from other respiratory viral infections by clinical symptoms alone.[14,15] Thus, a rapid and reliable laboratory diagnostic result is required before clinical therapy. For the rapid viral antigen detection tests, traditional solid-phase immunoassay and immunofluorescence have a moderate sensitivity.[15,16] But their specificities to low prevalence disease are limited. The viral diagnostics were significantly promoted by the

---

* All three authors contributed equally to this work.

development of nucleic acids amplification techniques (NAAT).[10,15] This method enables the detection of low concentrations of viral nucleic acid.[15] Moreover, NAAT are the only available method for most newly emerging respiratory viruses as traditional tests lack efficient cell-culture system or antigen detection methods.[17] Currently, automated and multiplex NAAT have been developed to make it easier to obtain laboratory diagnostic results.[18] These techniques are anticipated to be used for the cost-effective assessment of the antivirals for respiratory virus.

The Targets of Antivirals in Viral Replication Cycle

A series of antivirals have been discovered and developed to control the respiratory virus infection. Some of these agents target the essential steps of viral infection, including viral attachment, endocytosis, replication, assembly, budding, and release (►Fig. 1). The others directly target the virus or the intracellular pathway essential for viral replication.

Viral Attachment Inhibitory

The attachment process is a critical step for viruses to gain entry into cells; blocking the interactions between viral-specific surface proteins and cell-receptors is a promising strategy in developing novel antivirals.[19] The sialidase and amphiregulin glycosaminoglycan recombinant construct DAS181 (Fludase®, Ansun Biopharma Inc., San Diego, CA), can hydrolyze both α2,3- and α2,6-linked sialic acid receptors on the respiratory epithelium cell surface.[20] Thus, it can efficiently block

the attachment of human and avian influenza virus.[21] DAS181 have been confirmed to have antiviral activity on H1N1, H3N2, and H5N1 both in vitro and in vivo.[21] Moreover, a phase II clinical trial confirmed DAS181 can significantly reduce viral load and viral shedding, compared with the placebo group ($p = 0.002$ and $p = 0.006$ for the multiple-dose and single-dose groups, respectively).[22] DAS181 is advancing in clinical development now, with prospects for treatment in human and avian influenza infections. N-(2-aminoethyl)-1-aziridine-ethanamine (NAAE) is a small molecule which inhibits interactions between the SARS-Cov spike protein and its cell surface receptor, angiotensin converting enzyme 2 (ACE2).[23] However, inhibition of ACE2 by NAAE also has a potential risk for hypertension.[24] Hence, if the viral receptor is also important to cell function, agents that utilize this mechanism might cause side effects to the host too.

Several human monoclonal antibodies have been developed to block the viral attachment to cells. The human monoclonal antibody CR6261 can protect ferrets 100% from lethal challenges from the highly pathogenic avian H5N1 virus with both prophylactic and therapeutic administration, compared with 20% survival in the control group ($p < 0.001$).[25] Motavizumab and palivizumab have been confirmed to prevent participants from respiratory syncytial virus infection.[26,27] But an independent clinical trial showed that motavizumab treatment has no effect on the duration of hospitalization, severity of illness measures, or wheezing episodes at 12-month follow-up among children infected with respiratory syncytial virus, when compared with placebo treatment.[28] Despite the inconsistent clinical data to
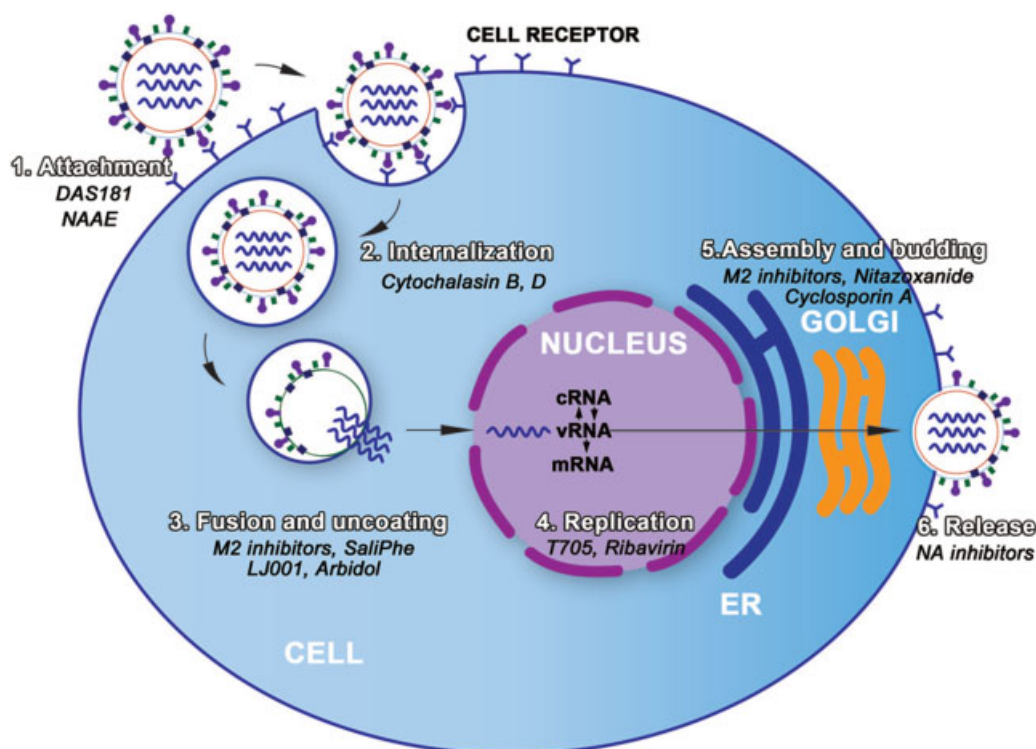
Fig. 1 The replication cycle of virus and agents for the treatment of respiratory viruses.

human monoclonal antibodies, the high costs of these biologics can also be a formidable problem to the therapy of these infections.[29]

Viral Endocytosis Inhibitor

Pharmacologic inhibition of the endocytosis process of viral envelope with cell membrane is also a promising strategy to develop antivirals.[30] The endocytosis process of respiratory virus can be subdivided into three steps: internalization, fusion, and uncoating.[31] And the inhibition of this process can be achieved through the following mechanisms: (1) blocking the pathways that facilitate viral internalization; (2) suppression of acidification in the endosome; (3) reducing membrane curvature and fluidity; (4) interfering with the conformational changes of viral protein required for fusion; and (5) viral uncoating inhibition. Numerous broad-spectrum antivirals have been developed for these purposes.[32]

Both dynamin-dependent and dynamin-independent pathways are considered as the routes of influenza cell internalization.[33] The inhibitors to these pathway including Cytochalasin B and D, jasplakinolide, and latruncul A are all specific inhibitors to the internalization of influenza.[34] There are accumulated evidence to confirm that activation of PI3K promotes virus internalization.[35] The interplay of influenza virus and receptor tyrosine kinases (RTKs) of host cell can promote this pathway and facilitate viral uptake.[36] However, one should be cautious about potential benefit of RTK inhibitors as potential antiviral agents, as a limited number of RTKs can be manipulated by chemical inhibitors or siRNAs at the same time.[36]

For most enveloped viruses, the low-pH environment of endosome is not only triggers the fusion between endosomes and virions, but also causes dissociation of viral ribonucleoprotein complexes (vRNPs) from the capsid, which allows for its subsequent import into the nucleus and the start of viral replication.[37,38] Thus, blocking endosomal acidification can also interfere with viral infection. Saliphenylhalamide, concanamycin A, archazolid B, diphyllin, and bafilomycin A1 are all endosomal acidification inhibitors which may abrogate influenza viral infection through inhibiting cellular vacuolar ATPase (vATPase) or vacuolar-type proton (V-H$^+$) pump of endosome.[39–41] Among them, saliphenylhalamide (SaliPhe) can effectively inhibit several wild type of influenza viruses, including pandemic swine-origin influenza virus, highly pathogenic H5N1 avian influenza virus, and oseltamivir-resistant seasonal H1N1 influenza virus.[41] Moreover, it protected 62.5% of mice against a lethal challenge of a mouse-adapted influenza strain.[41] Thus, these endosomal acidification inhibitors with even better selective indices may become candidates for the treatment of viral infections in the future.

Membrane curvature and fluidity is critical for viral-endosomal membrane fusion.[42] LJ001 is a lipophilic thiazolidine derivative that inhibits membrane curvature and fluidity by O$_2$-mediated lipid oxidation, and LJ001 exhibits broad-spectrum antiviral activity against enveloped viruses, including influenza virus, bunyaviruses, Ebola and Rift Valley fever viruses, HIV-1, and so on.[43] LJ001 has led to a new class of membrane-targeted photosensitizers (oxazolidine-2,4-dithiones) with increased potencies in antiviral research.

Arbidol is another broad-spectrum antiviral with antifusion activity by stabilizing the surface glycoprotein of influenza virus (hemagglutinin, HA) in a low-pH form.[44] This agent has proved to be active against several subtypes of avian influenzas, such as H5N1, H9N2, H2N2, and H6N1 in vitro.[2,44–46] GS-5806 and MDT-637 are all small-molecule inhibitors that interfere with RSV fusion through interaction with the F protein of respiratory syncytial virus, and some of them are now in phase I development.[47,48] It should be noted that the entry of several highly pathogenic viruses (such as SARS-Cov and Ebola virus) into host cells depends on cathepsin L to facilitate the fusion of viral envelope and cellular membrane after viral attachment.[49] Thus, small molecules to prevent cathepsin L cleavage would be anticipated to ameliorate severe respiratory viral infection.[49]

There are also agents targeted to the uncoating process.[50] This process of influenza requires coordinated action of the viral proteins M2 and M1.[50] M2 is a tetramer presented in virions possessing selective H$^+$ ion-channel activity that allows for the uncoating of virus particles in the endosome.[50,51] There are two commercially available M2 inhibitors, amantadine and rimantadine, which have been widely used in the treatment of both human and avian influenza infections for many years. Unfortunately, most of the current human and avian influenza viruses are amantadine- or rimantadine-resistant due to drug overuse.[52–54] However, Wang et al offer a glimmer of hope to the development of M2 inhibitors.[55] They discovered a novel M2 proton channel inhibitor which is effective against both the wild-type and the most prevalent drug-resistant mutant (S31N) from influenza A virus.

Viral Replication Inhibitor

As RNA polymerase is essential for the replication of RNA viruses, an inhibitor specific for viral RNA polymerase would exhibit antiviral activity and does not affect mRNA synthesis or protein translation of the host cells.[56] Favipiravir (T705) is one of these agents; it can selectively inhibit the viral RNA polymerase of several influenza strains, respiratory syncytial virus, and Ebola virus.[57,58] Moreover, favipiravir was effective in protecting mice from the lethal infection of highly pathogenic influenza H5N1 virus.[59] It is now in phase II clinical trials in the United States. Novel compounds (such as THC19, ASN2, etc.) have been developed to inhibit the polymerase of influenza viruses.[60] Moreover, inhibitors such as BCX4430, ALS-8176, and JMN3–003 exhibit broad-spectrum antiviral activity against various respiratory viruses, including influenza viruses, respiratory syncytial virus, paramyxoviruses, coronaviruses, and bunyaviruses.[61–63] That makes them particularly attractive to researchers.

The target of ribavirin is inosine 5′-monophosphate (IMP) dehydrogenase involved in viral RNA synthesis and cellular biosynthesis of GTP.[64] Ribavirin exhibits efficient anti-H5N1 avian influenza activity in vitro with IC50 ranging from 6 to 22 μmol/L.[65] Treatment with ribavirin at 75 mg·kg^{-1}·d^{-1}

(po, bid × 8 d) protected 70% of mice from lethal H5N1 infection.[65,66] However, the clinical efficacy of ribavirin toward influenza was limited by the mode of delivery.[67] Aerosolized ribavirin exhibits effective inhibitory activity against both influenza viruses and respiratory syncytial viruses in clinical observations, while orally administered ribavirin did not.[67] Moreover, the clinical utility of ribavirin is restrained by the risk of hemolytic anemia and teratogenicity.[64] A prodrug of ribavirin, viramidine, is anticipated to solve these problems.[68] This prodrug has a similar antiviral activity as ribavirin but is less toxic. This drug is now in Phase III development for hepatitis C virus (HCV) treatment.[68]

Dihydroorotate dehydrogenase (DHODH) is a key enzyme essential for replication of both viral RNA and DNA by the biosynthesis of de novo pyrimidine to generate uracil.[69] Inhibitors (such as compound A3, D282, etc.) targeting DHODH have been found to inhibit the replication of both RNA and DNA respiratory viruses (such as influenza virus and adenovirus).[70,71]

Proteases or proteinases of virus are also the attractive targets for the development of antiviral agents.[7,72] The papain-like protease (PLP) is the nsp3 protein which takes part in the formation of the ORF1a polyprotein during the SARS-CoV replication.[73] NSC158362, an nsp3-dependent protease inhibitor, has exhibited antireplication acitivity against SARS-CoV, but not for influenza virus in vitro.[74] Helicases of virus are proteins to unpackage the duplex oligonucleotides into single strands.[75] Bananin and SSYA10–001 are helicase inhibitors which have been confirmed to be efficient inhibitors against the SARS-CoV replication in vitro with IC50 values in the range 0.5 to 3 μM.[76,77]

Viral Assembly, Budding and Release Inhibitor

Since several respiratory viruses replicate their genome in the nucleus of infected cells, their newly synthesized vRNA, together with RNA polymerase, and nucleoprotein, in the form of vRNPs, have to export to the cytoplasm before assembly.[78,79] This process depends on a series of pathways, such as a Crm1-dependent pathway.[80] Leptomycin B is a potent inhibitor of Crm1-mediated export, and leptomycin B treatment can lead to a decrease in the export of influenza A vRNPs from the nucleus.[80] However, leptomycin B is toxic in vivo, which limits its further therapeutic use.[81] Verdinexor is a novel selective vRNP-exportation inhibitor targeted to the Crm-1 homolog exportin-1, and verdinexor effectively inhibits the replication of various influenza stains, including pandemic H1N1 influenza virus, highly pathogenic H5N1 and newly emerged H7N9 avian influenza viruses.[81] Moreover, both prophylactic and therapeutic administration of verdinexor can effectively protect mice from influenza infections.[81] Thus, verdinexor is a promising candidate which deserves further clinical investigation.

Nitazoxanide is an approved antiparasitic agent which can also prevent the HA transport of influenza virus to cell surface by interfering HA trafficking between endoplasmic reticulum and the Golgi complex.[82] Moreover, nitazoxanide is confirmed to be a broad-spectrum antiviral against both RNA and DNA viruses in vitro.[83] Currently, nitazoxanide is in clinical development for the treatment of influenza.

The influenza virus budding event is initiated by the deformation of the membrane caused by the clustering of HA and NA.[79] Then, M1 binds to the cytoplasmic tails of HA and NA and recruits the vRNPs and M2 to form the interior structure of the emerging virion at the site of virus budding.[79] Subsequently, M2 alters the membrane curvature at the neck of the budding virus to cause membrane scission and initiate the release of the progeny virion.[79] Thus, M2 inhibitors mentioned above may also inhibit the budding of influenza virus. Cyclophilin inhibitors such as Cyclosporin A can also inhibit the propagation of influenza virus by interfering the assembly or budding of influenza virus besides interfering with viral RNA synthesis in A549 cells.[84,85]

In the final release process of influenza virus, NA enables progeny virus to be cleaved from its receptor and spread to other cells.[86] Thus, the NA inhibitors can prevent the influenza from spreading further by blocking the release of progeny virion.[87] Four commercial NA inhibitors (oseltamivir, zanamivir, peramivir, and laninamivir) have been approved for use in humans.[2] Among these NA inhibitors, laninamivir has an extremely long persisting time in the lungs, increasing the prospect of the long-acting antiviral that can effectively prevent influenza infection with a single dose.[88] The emergence of NA-inhibitor-resistant influenza strains can be a substantial challenge to antiviral therapy in the future.[89] Although there is no direct evidence to confirm that the development of resistance have correlations to the wide use of oseltamivir, the resistant viruses would acquire more chances of transmissibility under drug-selection pressure compared with the drug-sensitive strains.[11]

Signal-Transduction Inhibitors

Aside from agents that directly target viral proteins, current antiviral strategies also focus on the intracellular pathways essential for viral replication.[90,91] The NF-κB, Raf/MEK/ERK, PI3K/Akt, or PKC signaling pathways might be promising targets for antiviral approaches.[90–93] Since these pathways are also critical for cytokine and interferon synthesis during viral infection, inhibitors targeting these cascades may inhibit not only viral replication, but also the subsequent inflammation of viral infection.[90,91] Moreover, compounds for these targets have an extremely low risk of emergence of drug-resistant variants.[90–93] Thus, inhibitors of intracellular pathways are promising candidates as treatment against respiratory viruses, and further development should be pursued.

Herbs

Herbs may also be a potential choice for patients hospitalized with respiratory viruses. Several Chinese herbal prescriptions were recommended and authorized by the Chinese government during the 2004 SARS-CoV, 2009 H1N1 influenza, and 2013 H7N9 avian influenza pandemics.[94,95] These herbal prescriptions commonly contain herbal medicines including *Isatis tinctoria* L., *Lonicera japonica* Thunb., *Saposhnikovia divaricata* (Turcz) Schischek, *Bupleurum chinense* DC., *Forsythia suspensa* (Thunb.) Vahl, and *Citrus reticulata*

Blanco,[94,95] which are considered to have heat-clearing and detoxifying properties according to the theoretical system of Traditional Chinese Medicine. The antiviral mechanism of these herbal medicines has not been fully elucidated; however, some data suggest that the active components exhibit actual antiviral activity.[96–99] Additionally, we have reported that extracts from Jiawei-Yupingfeng-Tang, a traditional Chinese herbal formula, exhibit antirespiratory viral activity.[100] We also have confirmed that a green tea–derived polyphenol, epigallocatechin gallate (EGCG) can inhibit influenza virus by its antioxidant activity.[101] These data suggest that herbal medicine may be a reservoir for antiviral development. Nevertheless, the current confirmation of herbal therapy is still insufficient owing to a lack of data from randomized and controlled clinical trials.

Conclusion

Persistent outbreaks of respiratory viral infections all over the world suggest that respiratory viral pathogens continue to be the most important threats to human health. Because of the the similarity of clinical symptoms to respiratory viral infections, rapid and sensitive diagnoses are essential for early viral identification and subsequently cost-effective monitoring of antivirals in clinical trials. The automated and multiplex NAAT may help to solve these problems, and this method is anticipated to generalize its applications in the future. There are still many challenges and unanswered questions about the development of antivirals. Several potent agents have been developed to target viral proteins or cellular factors essential for virus replication. However, they generally have yet to pass through clinical trials to show antiviral evidence in humans. On the other hand, the existing antiviral therapies also face the challenges of drug resistance and nonspecific side effects. Therefore, there is an urgent need for novel drugs and combination therapies. Efforts should be made to design and develop new antivirals that specifically target the basic steps of viral life cycle, including attachment, endocytosis, replication, assembly, budding, release, and cellular processes. The efficacy, viral resistance, mutual effect, and toxicity of combined usage of antivirals need to be clarified.

References

1 Jartti T, Jartti L, Ruuskanen O, Söderlund-Venermo M. New respiratory viral infections. Curr Opin Pulm Med 2012;18(3): 271–278

2 Liu Q, Liu DY, Yang ZQ. Characteristics of human infection with avian influenza viruses and development of new antiviral agents. Acta Pharmacol Sin 2013;34(10):1257–1269

3 Hirst M, Astell CR, Griffith M, et al. Novel avian influenza H7N3 strain outbreak, British Columbia. Emerg Infect Dis 2004;10(12): 2192–2195

4 Peiris M, Yam WC, Chan KH, Ghose P, Shortridge KF. Influenza A H9N2: aspects of laboratory diagnosis. J Clin Microbiol 1999; 37(10):3426–3427

5 Peiris M, Yuen KY, Leung CW, et al. Human infection with influenza H9N2. Lancet 1999;354(9182):916–917

6 Su S, Bi Y, Wong G, Gray GC, Gao GF, Li S. Epidemiology, evolution and recent outbreaks of avian influenza viruses in China: A Review. J Virol 2015;89(17):8671–8676

7 Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J 2014; 281(18):4085–4096

8 Osterhaus AD. New respiratory viruses of humans. Pediatr Infect Dis J 2008;27(10, Suppl):S71–S74

9 Houser K, Subbarao K. Influenza vaccines: challenges and solutions. Cell Host Microbe 2015;17(3):295–300

10 Knipe DM, Howley PM. Fields virology. 6th ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2013

11 Webster RG, Govorkova EA. Continuing challenges in influenza. Ann N Y Acad Sci 2014;1323:115–139

12 Simões EA, DeVincenzo JP, Boeckh M, et al. Challenges and opportunities in developing respiratory syncytial virus therapeutics. J Infect Dis 2015;211(Suppl 1):S1–S20

13 Zhu JD, Meng W, Wang XJ, Wang HC. Broad-spectrum antiviral agents. Front Microbiol 2015;6:517

14 Zambon MC, Stockton JD, Clewley JP, Fleming DM. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: an observational study. Lancet 2001; 358(9291):1410–1416

15 Henrickson KJ. Advances in the laboratory diagnosis of viral respiratory disease. Pediatr Infect Dis J 2004;23(1, Suppl)S6–S10

16 Principi N, Esposito S. Antigen-based assays for the identification of influenza virus and respiratory syncytial virus: why and how to use them in pediatric practice. Clin Lab Med 2009;29(4): 649–660

17 Pérez-Ruiz M, Pedrosa-Corral I, Sanbonmatsu-Gámez S, Navarro-Marí M. Laboratory detection of respiratory viruses by automated techniques. Open Virol J 2012;6:151–159

18 Dundas NE, Ziadie MS, Revell PA, et al. A lean laboratory: operational simplicity and cost effectiveness of the Luminex xTAG™ respiratory viral panel. J Mol Diagn 2011;13(2):175–179

19 Edinger TO, Pohl MO, Stertz S. Entry of influenza A virus: host factors and antiviral targets. J Gen Virol 2014;95(Pt 2):263–277

20 Hedlund M, Aschenbrenner LM, Jensen K, Larson JL, Fang F. Sialidase-based anti-influenza virus therapy protects against secondary pneumococcal infection. J Infect Dis 2010;201(7): 1007–1015

21 Nicholls JM, Moss RB, Haslam SM. The use of sialidase therapy for respiratory viral infections. Antiviral Res 2013;98(3):401–409

22 Moss RB, Hansen C, Sanders RL, Hawley S, Li T, Steigbigel RT. A phase II study of DAS181, a novel host directed antiviral for the treatment of influenza infection. J Infect Dis 2012;206(12): 1844–1851

23 Huentelman MJ, Zubcevic J, Hernández Prada JA, et al. Structure-based discovery of a novel angiotensin-converting enzyme 2 inhibitor. Hypertension 2004;44(6):903–906

24 Danilczyk U, Penninger JM. Angiotensin-converting enzyme II in the heart and the kidney. Circ Res 2006;98(4):463–471

25 Friesen RH, Koudstaal W, Koldijk MH, et al. New class of monoclonal antibodies against severe influenza: prophylactic and therapeutic efficacy in ferrets. PLoS ONE 2010;5(2):e9106

26 Lagos R, DeVincenzo JP, Muñoz A, et al. Safety and antiviral activity of motavizumab, a respiratory syncytial virus (RSV)-specific humanized monoclonal antibody, when administered to RSV-infected children. Pediatr Infect Dis J 2009;28(9):835–837

27 Han YM, Seo HJ, Choi SH, et al. Effect of Prophylactic Palivizumab on Admission Due to Respiratory Syncytial Virus Infection in Former Very Low Birth Weight Infants with Bronchopulmonary Dysplasia. J Korean Med Sci 2015;30(7):924–931

28 Ramilo O, Lagos R, Sáez-Llorens X, et al; Motavizumab Study Group. Motavizumab treatment of infants hospitalized with respiratory syncytial virus infection does not decrease viral load or severity of illness. Pediatr Infect Dis J 2014;33(7):703–709

29 Hegele RG. Respiratory syncytial virus therapy and prophylaxis: have we finally turned the corner? Eur Respir J 2011;38(2): 246–247

30 Plemper RK. Cell entry of enveloped viruses. Curr Opin Virol 2011;1(2):92–100

31 Cosset FL, Lavillette D. Cell entry of enveloped viruses. Adv Genet 2011;73:121–183

32 Teissier E, Penin F, Pécheur EI. Targeting cell entry of enveloped viruses as an antiviral strategy. Molecules 2011;16(1):221–250

33 de Vries E, Tscherne DM, Wienholts MJ, et al. Dissection of the influenza A virus endocytic routes reveals macropinocytosis as an alternative entry pathway. PLoS Pathog 2011;7(3):e1001329

34 Sun X, Whittaker GR. Role of the actin cytoskeleton during influenza virus internalization into polarized epithelial cells. Cell Microbiol 2007;9(7):1672–1682

35 Ehrhardt C, Marjuki H, Wolff T, et al. Bivalent role of the phosphatidylinositol-3-kinase (PI3K) during influenza virus infection and host cell defence. Cell Microbiol 2006;8(8): 1336–1348

36 Eierhoff T, Hrincius ER, Rescher U, Ludwig S, Ehrhardt C. The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. PLoS Pathog 2010;6(9): e1001099

37 Li S, Sieben C, Ludwig K, et al. pH-Controlled two-step uncoating of influenza virus. Biophys J 2014;106(7):1447–1456

38 Garcia NK, Guttman M, Ebner JL, Lee KK. Dynamic changes during acid-induced activation of influenza hemagglutinin. Structure 2015;23(4):665–676

39 Ochiai H, Sakai S, Hirabayashi T, Shimizu Y, Terasawa K. Inhibitory effect of bafilomycin A1, a specific inhibitor of vacuolar-type proton pump, on the growth of influenza A and B viruses in MDCK cells. Antiviral Res 1995;27(4):425–430

40 Chen HW, Cheng JX, Liu MT, et al. Inhibitory and combinatorial effect of diphyllin, a v-ATPase blocker, on influenza viruses. Antiviral Res 2013;99(3):371–382

41 Müller KH, Kainov DE, El Bakkouri K, et al. The proton translocation domain of cellular vacuolar ATPase provides a target for the treatment of influenza A virus infections. Br J Pharmacol 2011; 164(2):344–357

42 Vigant F, Lee J, Hollmann A, et al. A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. PLoS Pathog 2013;9(4):e1003297

43 Wolf MC, Freiberg AN, Zhang T, et al. A broad-spectrum antiviral targeting entry of enveloped viruses. Proc Natl Acad Sci U S A 2010;107(7):3157–3162

44 Leneva IA, Russell RJ, Boriskin YS, Hay AJ. Characteristics of arbidol-resistant mutants of influenza virus: implications for the mechanism of anti-influenza action of arbidol. Antiviral Res 2009;81(2):132–140

45 Boriskin YS, Leneva IA, Pécheur EI, Polyak SJ. Arbidol: a broad-spectrum antiviral compound that blocks viral fusion. Curr Med Chem 2008;15(10):997–1005

46 Burtseva EI, Shevchenko ES, Beliakova NV, et al. [Monitoring of the sensitivity of epidemic influenza virus strains isolated in Russia to etiotropic chemical agents]. Vopr Virusol 2009;54(5): 24–28

47 DeVincenzo JP, Whitley RJ, Mackman RL, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N Engl J Med 2014;371(8):711–722

48 Douglas JL, Panis ML, Ho E, et al. Small molecules VP-14637 and JNJ-2408068 inhibit respiratory syncytial virus fusion by similar mechanisms. Antimicrob Agents Chemother 2005;49(6): 2460–2466

49 Elshabrawy HA, Fan J, Haddad CS, et al. Identification of a broad-spectrum antiviral small molecule against severe acute respiratory syndrome coronavirus and Ebola, Hendra, and Nipah viruses by using a novel high-throughput screening assay. J Virol 2014; 88(8):4353–4365

50 Gu R, Liu LA, Wei D. Drug inhibition and proton conduction mechanisms of influenza a M2 proton channel. Adv Exp Med Biol 2015;827:205–226

51 Wei C, Pohorille A. M2 proton channel: toward a model of a primitive proton pump. Orig Life Evol Biosph 2015;45(1–2): 241–248

52 Ilyushina NA, Govorkova EA, Webster RG. Detection of amantadine-resistant variants among avian influenza viruses isolated in North America and Asia. Virology 2005;341(1):102–106

53 Huang Y, Hu B, Wen X, et al. Evolution analysis of the matrix (M) protein genes of 17 H9N2 chicken influenza viruses isolated in northern China during 1998-2008. Virus Genes 2009;38(3): 398–403

54 Centers for Disease Control and Prevention (CDC). Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. MMWR Morb Mortal Wkly Rep 2009;58(16): 433–435

55 Wang J, Ma C, Wang J, et al. Discovery of novel dual inhibitors of the wild-type and the most prevalent drug-resistant mutant, S31N, of the M2 proton channel from influenza A virus. J Med Chem 2013;56(7):2804–2812

56 Rodriguez-Frandsen A, Alfonso R, Nieto A. Influenza virus polymerase: Functions on host range, inhibition of cellular response to infection and pathogenicity. Virus Res 2015; 209:23–38

57 Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res 2013;100(2):446–454

58 Nagata T, Lefor AK, Hasegawa M, Ishii M. Favipiravir: a new medication for the Ebola virus disease pandemic. Disaster Med Public Health Prep 2015;9(1):79–81

59 Kiso M, Takahashi K, Sakai-Tagawa Y, et al. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. Proc Natl Acad Sci U S A 2010;107(2):882–887

60 Yamada K, Koyama H, Hagiwara K, et al. Identification of a novel compound with antiviral activity against influenza A virus depending on PA subunit of viral RNA polymerase. Microbes Infect 2012;14(9):740–747

61 Warren TK, Wells J, Panchal RG, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 2014;508(7496):402–405

62 Wang G, Deval J, Hong J, et al. Discovery of 4′-chloromethyl-2′-deoxy-3′,5′-di-O-isobutyryl-2′-fluorocytidine (ALS-8176), a first-in-class RSV polymerase inhibitor for treatment of human respiratory syncytial virus infection. J Med Chem 2015;58(4): 1862–1878

63 Krumm SA, Ndungu JM, Yoon JJ, et al. Potent host-directed small-molecule inhibitors of myxovirus RNA-dependent RNA-polymerases. PLoS ONE 2011;6(5):e20069

64 Brochot E, François C, Castelain S, et al. A new tool to study ribavirin-induced haemolysis. Antivir Ther 2012;17(7):1311–1317

65 Sidwell RW, Bailey KW, Wong MH, Barnard DL, Smee DF. In vitro and in vivo influenza virus-inhibitory effects of viramidine. Antiviral Res 2005;68(1):10–17

66 Ilyushina NA, Hay A, Yilmaz N, Boon AC, Webster RG, Govorkova EA. Oseltamivir-ribavirin combination therapy for highly pathogenic H5N1 influenza virus infection in mice. Antimicrob Agents Chemother 2008;52(11):3889–3897

67 Smith CB, Charette RP, Fox JP, Cooney MK, Hall CE. Lack of effect of oral ribavirin in naturally occurring influenza A virus (H1N1) infection. J Infect Dis 1980;141(5):548–554

68 Gish RG. Treating HCV with ribavirin analogues and ribavirin-like molecules. J Antimicrob Chemother 2006;57(1):8–13

69 Evans DR, Guy HI. Mammalian pyrimidine biosynthesis: fresh insights into an ancient pathway. J Biol Chem 2004;279(32):33035–33038

70 Hoffmann HH, Kunz A, Simon VA, Palese P, Shaw ML. Broad-spectrum antiviral that interferes with de novo pyrimidine biosynthesis. Proc Natl Acad Sci U S A 2011;108(14):5777–5782

71 Smee DF, Hurst BL, Day CW. D282, a non-nucleoside inhibitor of influenza virus infection that interferes with de novo pyrimidine biosynthesis. Antivir Chem Chemother 2012;22(6):263–272

72 Graham AC, Temple RM, Obar JJ. Mast cells and influenza a virus: association with allergic responses and beyond. Front Immunol 2015;6:238

73 Frieman M, Basu D, Matthews K, et al. Yeast based small molecule screen for inhibitors of SARS-CoV. PLoS ONE 2011;6(12):e28479

74 Adedeji AO, Sarafianos SG. Antiviral drugs specific for coronaviruses in preclinical development. Curr Opin Virol 2014;8:45–53

75 Valiente-Echeverría F, Hermoso MA, Soto-Rifo R. RNA helicase DDX3: at the crossroad of viral replication and antiviral immunity. Rev Med Virol 2015;25(5):286–299

76 Tanner JA, Zheng BJ, Zhou J, et al. The adamantane-derived bananins are potent inhibitors of the helicase activities and replication of SARS coronavirus. Chem Biol 2005;12(3):303–311

77 Adedeji AO, Singh K, Kassim A, et al. Evaluation of SSYA10-001 as a replication inhibitor of severe acute respiratory syndrome, mouse hepatitis, and Middle East respiratory syndrome coronaviruses. Antimicrob Agents Chemother 2014;58(8):4894–4898

78 Liu G, Xiang Y, Guo C, Pei Y, Wang Y, Kitazato K. Cofilin-1 is involved in regulation of actin reorganization during influenza A virus assembly and budding. Biochem Biophys Res Commun 2014;453(4):821–825

79 Rossman JS, Lamb RA. Influenza virus assembly and budding. Virology 2011;411(2):229–236

80 Chase GP, Rameix-Welti MA, Zvirbliene A, et al. Influenza virus ribonucleoprotein complexes gain preferential access to cellular export machinery through chromatin targeting. PLoS Pathog 2011;7(9):e1002187

81 Perwitasari O, Johnson S, Yan X, et al. Verdinexor, a novel selective inhibitor of nuclear export, reduces influenza a virus replication in vitro and in vivo. J Virol 2014;88(17):10228–10243

82 Rossignol JF, La Frazia S, Chiappa L, Ciucci A, Santoro MG. Thiazolides, a new class of anti-influenza molecules targeting viral hemagglutinin at the post-translational level. J Biol Chem 2009;284(43):29798–29808

83 Rossignol JF. Thiazolides: a new class of antiviral drugs. Expert Opin Drug Metab Toxicol 2009;5(6):667–674

84 de Wilde AH, Li Y, van der Meer Y, et al. Cyclophilin inhibitors block arterivirus replication by interfering with viral RNA synthesis. J Virol 2013;87(3):1454–1464

85 Hamamoto I, Harazaki K, Inase N, Takaku H, Tashiro M, Yamamoto N. Cyclosporin A inhibits the propagation of influenza virus by interfering with a late event in the virus life cycle. Jpn J Infect Dis 2013;66(4):276–283

86 Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D. Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part II: Future compounds against influenza virus. J Prev Med Hyg 2014;55(4):109–129

87 Varghese JN, Smith PW, Sollis SL, et al. Drug design against a shifting target: a structural basis for resistance to inhibitors in a variant of influenza virus neuraminidase. Structure 1998;6(6):735–746

88 Yamashita M, Tomozawa T, Kakuta M, Tokumitsu A, Nasu H, Kubo S. CS-8958, a prodrug of the new neuraminidase inhibitor R-125489, shows long-acting anti-influenza virus activity. Antimicrob Agents Chemother 2009;53(1):186–192

89 Dharan NJ, Gubareva LV, Meyer JJ, et al; Oseltamivir-Resistance Working Group. Infections with oseltamivir-resistant influenza A (H1N1) virus in the United States. JAMA 2009;301(10):1034–1041

90 Planz O. Development of cellular signaling pathway inhibitors as new antivirals against influenza. Antiviral Res 2013;98(3):457–468

91 Ludwig S. Targeting cell signalling pathways to fight the flu: towards a paradigm change in anti-influenza therapy. J Antimicrob Chemother 2009;64(1):1–4

92 Droebner K, Pleschka S, Ludwig S, Planz O. Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo. Antiviral Res 2011;92(2):195–203

93 Mazur I, Wurzer WJ, Ehrhardt C, et al. Acetylsalicylic acid (ASA) blocks influenza virus propagation via its NF-kappaB-inhibiting activity. Cell Microbiol 2007;9(7):1683–1694

94 Guidelines for Management of Avian H7N9 Influenza Infection. 2013http://www.moh.gov.cn/ewebeditor/uploadfile/2013/04/20130410212136993.doc

95 Guidelines for Management of Pandemic. (H1N1) 2009 Influenza. 2009http://www.moh.gov.cn/mohwsyjbgs/s9990/200910/43111.shtml

96 Shang X, Pan H, Li M, Miao X, Ding H. Lonicera japonica Thunb.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. J Ethnopharmacol 2011;138(1):1–21

97 WHO monographs on selected medicinal plants. 1999http://apps.who.int/medicinedocs/en/d/Js2200e/

98 Li C, Dai Y, Zhang SX, et al. Quinoid glycosides from Forsythia suspensa. Phytochemistry 2014;104:105–113

99 Xu JJ, Wu X, Li MM, et al. Antiviral activity of polymethoxylated flavones from "Guangchenpi", the edible and medicinal pericarps of citrus reticulata 'Chachi'. J Agric Food Chem 2014;62(10):2182–2189

100 Liu Q, Lu L, Hua M, et al. Jiawei-Yupingfeng-Tang, a Chinese herbal formula, inhibits respiratory viral infections in vitro and in vivo. J Ethnopharmacol 2013;150(2):521–528

101 Ling JX, Wei F, Li N, et al. Amelioration of influenza virus-induced reactive oxygen species formation by epigallocatechin gallate derived from green tea. Acta Pharmacol Sin 2012;33(12):1533–1541