*Teaser This communication focuses on the repurposing of clinically approved drugs and promising preclinical drug candidates for therapeutic development of host-based antiviral agents to control diseases caused by coronavirus and influenza virus.*



# Repurposing host-based therapeutics to control coronavirus and influenza virus

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**The development of highly effective antiviral agents has been a major objective in virology and pharmaceutics. Drug repositioning has emerged as a cost-effective and time-efficient alternative approach to traditional drug discovery and development. This new shift focuses on the repurposing of clinically approved drugs and promising preclinical drug candidates for the therapeutic development of host-based antiviral agents to control diseases caused by coronavirus and influenza virus. Host-based antiviral agents target host cellular machineries essential for viral infections or innate immune responses to interfere with viral pathogenesis. This review discusses current knowledge, prospective applications and challenges in the repurposing of clinically approved and preclinically studied drugs for newly indicated antiviral therapeutics.**

## Introduction

The repurposing of approved pharmaceutical drugs for additional applications is an efficient and alternative approach to advancing therapeutic development in a cost- and time-effective manner. Other advantages of repurposing drugs are the existence of clinical data and the availability of affordable drugs for patients. Viral infection is a major problem of morbidity and mortality in animals and humans worldwide. Only 12 therapeutic drugs have been approved by the FDA to treat viral infections since 2013 and, among these agents, ten are used to treat hepatitis C virus (HCV) and HIV, one is used to treat cytomegalovirus (CMV) and one is used to treat influenza virus (IFV). The limitations of available agents in controlling other viral infections and the emerging resistance to antiviral drugs underline the urgent need for effective drugs to manage viral infections.

Coronavirus (CoV) and IFV (see Glossary for full list of abbreviations) are two major respiratory pathogens causing significant morbidity and mortality in animals and humans worldwide. CoV is an enveloped positive-sense RNA virus classified in the *Coronaviridae* family, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus

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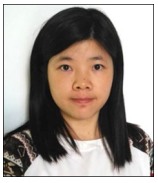

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**GLOSSARY**

**Abl** Abelson kinase
**BHH** Bromhexine hydrochloride
**CAPE** Caffeic acid phenethyl ester
**$CC_{50}$** The half maximal cytotoxic concentration
**CDK** Cyclin-dependent kinase
**$C_{max}$** The maximum serum concentration
**CML** Chronic myeloid leukemia
**CPZ** Chlorpromazine
**DAA** Direct-acting antiviral
**DAP** 14-Deoxy-11,12-dehydroandrographolide
**DENV** Dengue virus
**DFMO** Difluoromethylornithine
**DHODH** Dihydroorotate dehydrogenase
**EBOV** Ebola virus
**$EC_{50}$** The half maximal effective concentration
**ECD** Erdheim–Chester disease
**ERK** Extracellular signal related kinase
**EV-A71** Human enterovirus A71
**GCN2** General control non-derepressible-2
**GGA** Geranylgeranylacetone
**HA** Hemagglutinin
**HCoV-229E** Human coronavirus-229E
**4-HPR** N-(4-hydroxyphenyl) retinamide
**HRI** Heme-regulated inhibitor kinase
**HSV-1** Herpes simplex virus type 1
**HTA** Host-targeting antiviral
**THE cells** Human tracheal surface epithelial cells
**$IC_{50}$** Half-maximal inhibitory concentration
**IRNAR** Cell surface IFN receptor
**IFN** Interferon
**IFV-A** Influenza virus A
**INDO** Indomethacin
**IRES** Internal ribosome entry site
**ISG** IFN-stimulated gene product
**LANCL2** Lanthionine synthetase C-like 2
**Mek** Mitogen-activated protein kinase kinase
**MERS-CoV** Middle East respiratory syndrome coronavirus
**MHV** Mouse hepatitis virus
**MxA** Myxovirus resistance 1
**NA** Neuraminidase
**PatA** Pateamine A
**PERK** PKR-like endoplasmic reticulum-resident protein kinase
**PKR** Protein kinase R
**PRR** Pattern recognition receptor
**Raf** Rapidly accelerating fibrosarcoma kinase
**RdRp** Viral RNA-dependent RNA polymerase
**Saliphe** Saliphenylhalamide
**SARS-CoV** Severe acute respiratory syndrome coronavirus
**SG** Stress granule
**SNMC** Stronger neo-Minophagen C
**SSa** Saikosaponin A
**TMPRSS11D** Transmembrane protease serine 11D
**TMPRSS2** Transmembrane protease serine 2
**UPS** Ubiquitin-proteasome system
**v-ATPase** Vacuolar ATPase
**vRNP** Viral ribonucleoprotein complex
**VSV** Vesicular stomatitis virus

(MERS-CoV) and human coronavirus-229E (HCoV-229E). SARS and MERS have been well recognized globally owing to their outbreaks of severe infection [1]. However, the lack of effective therapeutic drugs to control SARS and MERS means that high morbidity and mortality rates have not reduced [2]. In addition to high morbidity and mortality, the emergence of new wide-host-range viral members, the potential domestic animal adaptation and the difficulty in identifying intermediate hosts are major concerns in the control of CoV infection. IFV is an enveloped negative-sense RNA virus associated with the *Orthomyxoviridae* family, consisting of IFV-A, -B and -C genera. IFV causes seasonal outbreaks of pandemic and zoonotic diseases [3]. Vaccination has been used to control epidemic IFV for decades. Antiviral agents have been developed to target IFV M2 ion channels (amantadine and rimantadine) and neuraminidases (zanamivir and oseltamivir); however, the use of these agents results in substantial drug resistance [4,5].

The direct-acting antivirals (DAAs), including vaccines and some therapeutic agents, are developed to directly target specific viral components. However, the concerns of these approaches include ineffective control of viral infections and resistance of DAAs that result from mutation-associated variations and evolved new viral variants. Thus, therapeutics targeting host cellular machineries, which are essential for viral infection, are considered in developing broad-spectrum agents to overcome viral variations and drug resistance. The host-targeted antivirals (HTAs) are designed to target specific steps during viral infection, including viral binding to host cells, viral entry into host cells, viral replication and viral budding. For example, an FDA-approved CCR5 receptor antagonist maraviroc (MVC) is effective to inhibit R5-tropic HIV-1 entry into cells [6]. Treatment with the humanized IgG4 monoclonal antibody ibalizuman, which blocks the entry of HIV-1 into cells by noncompetitive binding to CD4, showed that 43% and 50% of patients had a viral load <50 and 200 copies per milliliter, respectively, at week 25 [7]. However, it is unclear which side-effects result from long-term use of HTAs. In this review, we discuss the progress and challenges in the repurposing of clinically approved and preclinically promising therapeutic HTAs targeting viral entry, viral replication and innate immune responses for treating CoV and IFV infections.

## Targeting viral entry

The entry of CoV and IFV into host cells relies on the binding of viral particles to cell-surface receptors and the endocytosis of virus–receptor complexes (Fig. 1). The endosomal pathway initiates the fusion of the viral envelope with the host cell membrane to deliver viral nucleocapsid into the cell. CoV also enters cells via a nonendosomal pathway. Thus, endosomal and nonendosomal pathways should be considered as targets in the development of therapeutic drugs to block viral entry into host cells, as depicted in Fig. 1 (promising drugs are listed in Table 1).

### *Targeting host proteases*

CoV entry requires host proteases to cleave the viral trimeric transmembrane spike (S) glycoprotein (Fig. 1). The cleavage of S involves the cysteine protease cathepsin L in the endosomal pathway or the host transmembrane serine protease 2 (TMPRSS2) in a nonendosomal pathway [8]. IFV entry requires an envelope fusion with the host membrane, via viral hemagglutinin (HA), followed by the proteolytic cleavage of HA by TMPRSS11D (also HAT) to initiate endocytosis [9]. Thus, these host proteases in endosomal and nonendosomal pathways can be targeted to block viral entry into host cells [10].

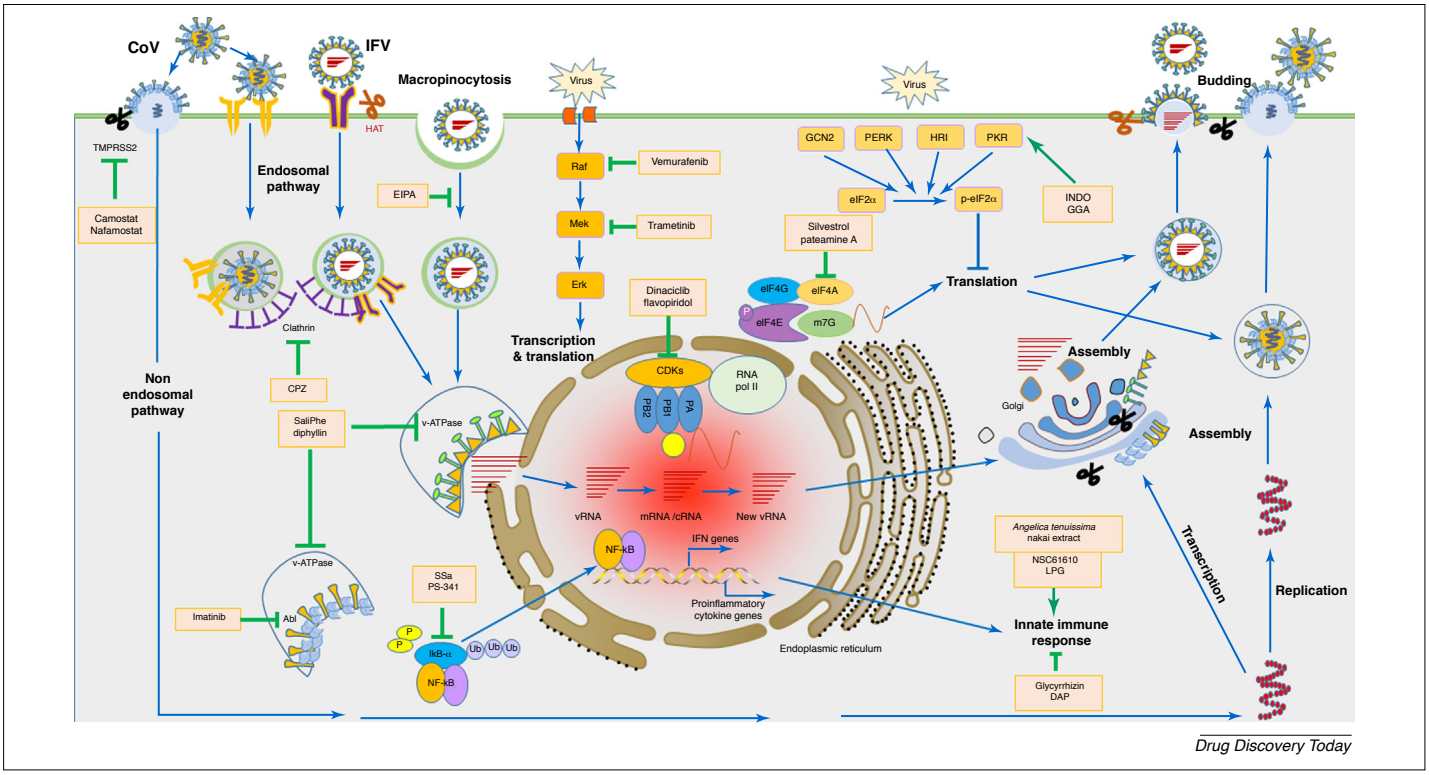


**FIGURE 1**

Scheme of targeting CoV and IFV infection by host-based repurposed drugs. CoV entry into cells relies on either a nonendosomal pathway or the endosomal pathway involving the host protease TMPRSS2 or the host adapter protein clathrin, respectively. IFV entry utilizes the endosomal pathway or the macropinocytic pathway. These pathways render the release of vRNA into the cytoplasm, followed by importing vRNA into the nucleus for viral replication. The host protein kinases Raf, Mek, Erk and CDKs have important roles in regulation of transcription and translation at various stages of viral replication. CoV and IFV utilize the host eIF-involved cap-dependent translational machinery to produce viral proteins. Activation of the host protein kinases PKR, PERK, HRI and GCN2 can phosphorylate the eIF2α to attenuate translation. Assembly of viral particles requires the host TMPRSS2 for cleavage of CoV spike and IFV HA in the Golgi apparatus to produce viral progeny to be released by budding. In addition, the NF-κB pathway is activated through inactivation of IκBa to induce proinflammatory cytokines, such as IFNs, for innate immune response to viral infection. These host-based pathways are targetable by repurposed agents to control viral infection. Representative repurposed drugs have a pink background. Green thick arrows indicate induction. Green thick stop signs indicate inhibition. Scissor signs indicate proteolytic cleavage.

### *Inhibition of nonendosomal TMPRSS2*

TMPRSS2 activates S glycoprotein of SARS-CoV and MERS-CoV for viral entry through the plasma membrane [11,12]. Camostat is a synthetic low-molecular-weight serine protease inhibitor and is used to treat human dyspepsia associated with mild pancreatic disease [13]. Inhibition of TMPRSS2 with camostat results in a 270-fold reduction of MERS-CoV production in the human bronchial submucosal gland-derived Calu-3 cells [11]. Camostat is also effective at protecting mice from a SARS-CoV infection [14]. The treatment of human tracheal epithelial (HTE) cells with camostat significantly reduces infection by IFV-A/H1N1 and A/H3N2 viruses [15]. Nafamostat is an FDA-approved serine protease inhibitor to treat pancreatitis and disseminated intravascular coagulation [16,17]. Treatment of the human airway epithelial Calu-3 cells with nafamostat blocks MERS-CoV infection by inhibiting TMPRSS2 and results in a significant reduction of viral production [18]. The $EC_{50}$ of camostat mesilate and nafamostat for IFV-A are 4.4 and 0.82 μM, respectively, in MDCK cells; and their $CC_{50}$ values are >1000 and 278 μM, respectively [19], indicating that these agents are effective at controlling viral infection and safe to host cells. A recent screening of the chemical library identified bromhexine hydrochloride (BHH) as a bioavailable TMPRSS2 inhibitor [20]. Because BHH has already been approved by the FDA as an ingredient in mucolytic cough suppressants, it is likely to be developed to treat CoV and IFV infection.

### *Potential targets*

Cathepsins have important roles in CoV entry. Cathepsin L and B can serve as targets for antiviral agents [21,22]. The vinylsulfone K11777 is an irreversible cysteine protease inhibitor used to control various parasitic infections, such as schistosomiasis [23]. K11777 has been reportedly effective in controlling cathepsin-mediated viral entry [14], including SARS-CoV, HCoV-229E, Nipah virus (paramyxovirus) and Ebola virus (filovirus). However, K11777 treatment fails to result in a statistically significant reduction of mortality induced by SARS-CoV in animals [14].

### *Targeting the endocytic pathway*

CoV and IFV enter host cells via pH- and receptor-mediated endocytosis, involving clathrin- and caveolae-dependent or -independent endocytic pathways [24–26]. IFV also utilizes macropinocytosis as an alternative entry pathway to the acidic late-endosomal compartment [27,28]. Chlorpromazine (CPZ), an inhibitor for clathrin-dependent endocytosis, is the first antipsy-

**TABLE 1**

**Targeting viral entry**

| Targets (for inhibition) | Drugs | FDA approved | $C_{max}$ (μM) | Primary indications | Virus | $IC_{50}/EC_{50}$ (μM) | Model system | $CC_{50}$ (μM) | Refs |
|---|---|---|---|---|---|---|---|---|---|
| TMPRSS2 | Camostat | Yes | 0.22[a] | Pancreatic disease | IFV-A | 4.4 | MDCK | >1000 | [11,13–15,19] |
| | Nafamostat | Yes | 0.173[b] | Pancreatitis and intravascular coagulation | SARS-CoV | – | Calu-3 | – | [16–19] |
| | | | | | IFV-A | 0.82 | MDCK | 278 | |
| | | | | | MERS-CoV | – | Calu-3 | – | |
| Clathrin-mediated endocytic pathway | CPZ | Yes | 0.14[c] | Schizophrenia | MERS-CoV | 4.9 | Huh 7 | 21.3 | [29–31] |
| | | | | | SARS-CoV | 8.8 | Vero | 24.3 | [29–34] |
| Macropinocytic endocytic pathway | EIPA | No | – | Cardiovascular disease | IFV-A | – | MDCK | – | [28,36] |
| v-ATPase | SaliPhe | No | – | Cancers | IFV-A | 0.03 | MDCK | 1.7 | [38,39] |
| | Diphyllin | No | – | Gastric adenocarcinoma | IFV-A | 0.08 | A549 | 1.1 | [38,39] |
| | Diphyllin-N[e] | | | | IFV-A | – | Mice | – | [38,40] |
| | | | | | IFV-A | 0.04–0.63[d] | MDCK | 3.48 | [42–44] |
| | | | | | Feline CoV | – | Mice | – | [45] |
| Abl | Imatinib | Yes | 4.76[f] | Cancers | MERS-CoV | 17.69 | Vero | >100 | [51–54] |
| | | | | | SARS-CoV | 9.82 | Vero | >100 | [51–54] |

Abbreviations: $C_{max}$, the maximum serum concentration; $IC_{50}/EC_{50}$, the half-maximal inhibitory concentration/the half-maximal effective concentration; $CC_{50}$, the half-maximal cytotoxic concentration.

[a] The recommended dosage is oral administration of 200 mg to patients three-times a day. Oral administration of 200 mg to adults results in the $C_{max}$ of 0.22 μM by 40 min (FDA Guide).

[b] Intravenous administration of a single dose of 40 mg results in the $C_{max}$ of 0.173 μM by 3.7 min [17].

[c] Intramuscular administration of 100 mg to patients results in the $C_{max}$ of 0.14 μM by 2 h [30].

[d] $IC_{50}$ values for H3N2 and H1N1 strains [44].

[e] Nanoparticle-packed diphyllin.

[f] Administration of 400 mg to patients results in the $C_{max}$ of 4.76 μM [53].

chotic drug for treating schizophrenia [29,30]. CPZ was identified by screening FDA-approved drugs effective at inhibiting MERS-CoV replication at low micromolar levels in Huh 7 cells. CPZ inhibits MERS-CoV replication at an early and a post-entry stage, indicating its ability to inhibit the early clathrin-mediated endocytosis and other machineries [31]. CPZ also inhibits SARS-CoV and animal coronaviruses with an $IC_{50}$ value of 8.8 μM in Vero cells [32–34]. Treatment of caveolin-1-negative HepG2 cells with CPZ significantly reduces the entry of SARS-CoV into cells [32]. These findings indicate that CoV mainly utilizes the clathrin-mediated endocytic pathway to enter cells. Amiloride, a potassium-sparing diuretic, is used to treat cardiovascular disease by blocking epithelial sodium channels within the distal tubule of the kidney [35]. Amiloride derivative 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA) is routinely used specifically to inhibit macropinocytic endocytosis [36]. A recent report revealed that a combination of EIPA with CPZ synergistically reduces the ability of IFV-A to infect the bovine kidney MDCK cells [28], indicating that clathrin-mediated endocytic and macropinocytic pathways are used by IFV to enter cells. Thus, combining complementary agents needs to be further developed to achieve an effective control of viral entry into host cells.

### *Targeting endosomal membrane fusion*

Cellular vacuolar ATPase (v-ATPase), located at the intracellular organelles and cytoplasmic membranes, acidifies the late endosome by pumping protons across the endosomal membrane, which is a crucial step for CoV and IFV entry [37,38]. Blocking v-ATPase activity interferes with viral infection by preventing the pH-dependent membrane fusion of viral envelops with the endosomal membrane. Saliphenylhalamide (SaliPhe) is an anticancer compound capable of inhibiting v-ATPase [39]. SaliPhe inhibits acidification of endosomes and reduces production of progeny viruses at $IC_{50}$ values of 0.03 and 0.08 μM in IFV-A-infected MDCK and human lung adenocarcinoma epithelial A549 cells, respectively [38,40]. Administrating mice with 7 mg/kg SaliPhe three-times daily for 8 days resulted in a 62.5% survival rate from lethal IFV infection, and mice recovered by 15 days [38]. Packing SaliPhe with nanoparticles enhances the *in vitro* stability and antiviral activity with low cytotoxicity [41]. Diphyllin, a natural compound isolated from *Cleistanthus collinus*, has been identified as a novel v-ATPase inhibitor [42]. Diphyllin inhibits endosomal acidification in human osteoclasts and reduces v-ATPase expression in gastric adenocarcinoma cells [43]. Diphyllin has been shown to effectively inhibit IFV infection in MDCK cells with $IC_{50}$ values ranging from 0.04 to 0.63 μM [44]. Diphyllin also effectively inhibits the clinically isolated oseltamivir-resistant IFV-A/San Diego/21/2008 (H1N1) strain, which carries a drug-resistant mutation (H275Y) in the NA gene, and the amantadine-resistant A/PR/8/34 strain [44]. Diphyllin is well tolerated in mice [43]. Delivery of diphyllin by nanoparticles increases its antiviral effects on feline CoV and it is also well tolerated in mice [45]. Niclosamide, a salicylanilide, has been used to treat parasitic helminthic infestations in humans for >40 years [46]. Niclosamide acts as a proton carrier to target acidic endosomes and to neutralize the pH of coated vesicles or synthetic liposomes [47]. Niclosamide effectively inhibits IFV-A in A549 cells [47] and pH-dependent SARS-CoV infection [48]. Amiodarone, an antiarrhythmic agent, is used clinically to treat supraventricular and ventricular arrhythmias [49]. Amiodarone inhibits SARS-CoV infection at a post-endosomal stage, and the pretreatment with amiodarone results in a 10 000-fold reduction of viral production in Vero cells [50].

Imatinib is an inhibitor for the non-receptor tyrosine kinase Abelson (Abl), which is involved in the regulation of cellular pathways for cell migration, adhesion and actin reorganization [51]. Imatinib is used as an anticancer agent to reduce CML-related disease progression and death [52,53]. Imatinib is also an endosomal membrane fusion inhibitor capable of inhibiting SARS-CoV and MERS-CoV infection with $EC_{50}$ values of 9.82 and 17.69 μM, respectively [2,54]. Pretreatment with imatinib reduces 1000-fold MERS-CoV and SARS-CoV production in Vero cells and is safe to Vero cells ($CC_{50}$ value >100 μM) [54]. However, use of 400 mg imatinib daily in myeloid leukemia patients comes with the side-effects: dizziness, blurred vision or somnolence (FDA Guide). Administration of imatinib at 60 or 30 mg/kg/day resulted in a statistically significant reduction in the longevity of males and females, respectively.

### *Summary and perspectives*

TMPRSS2 plays an important part in nonendosomal pathways to support the entry of viruses into cells and the assembly of viral particles for CoV and IFV. The inhibition of the nonendosomal TMPRSS2 by camostat or nafamostat does result in suppression of CoV infection, but inhibition of the endosomal cathepsin by K11777 does not. Thus, TMPRSS2, but not cathepsins, is a promising target for antiviral therapeutic development. Camostat and nafamostat are FDA-approved drugs. Camostat has been shown to exhibit a potent antiviral activity *in vitro* and *in vivo*. Thus, it is logical to further determine the efficacy of camostat in the control of CoV and IFV in clinical studies. Endosomal acidification facilitates membrane fusion between endosomes and virions for viral entry. Imatinib, an FDA-approved agent, inhibits CoV fusion with the endosomal membrane *in vitro* [2,54]; thus, the *in vivo* efficacy of imatinib in the control of viral infection needs to be determined in an animal model. Treatment of animals with the v-ATPase inhibitors SaliPhe or diphyllin reduces acidification and suppresses viral infection; and packing with nanoparticles helps to increase the efficacy of these inhibitors in controlling viral infection with tolerable adverse effects in animals. Thus, these agents are promising in pursuing FDA approval for their clinical application to control viral infection.

## Targeting viral replication

Viral replication utilizes host cell machineries of transcription, translation, signaling pathways and the cell cycle. Accordingly, these cellular machineries become targets for the development of therapeutic drugs to block viral replication, as depicted in Fig. 1 and promising drugs are listed in Table 2.

### *Targeting transcription*

Viral replication requires pyrimidine nucleotides and continued biosynthesis of pyrimidine [55]. The pyrimidine nucleosides include uridine, cytidine and thymidine. Dihydroorotate dehydrogenase (DHODH) dehydrogenizes dihydroorotate to orotic acid, a key step in biosynthesis of *de novo* pyrimidine to generate uracil, which binds with a ribose sugar to form the ribonucleoside uridine for transcription [56]. The DHODH inhibitors teriflunomide (or its prodrug leflunomide) and brequinar are used as immunosuppressive agents to treat rheumatoid arthritis and multiple sclerosis patients [57]. Although these agents showed an ability to inhibit *de novo* pyrimidine synthesis that contributes to their broad-spectrum antiviral activity *in vitro*, their antiviral activity is ineffective *in vivo*, resulting from exogenously provided uridine and bypassing the inhibited biosynthesis of pyrimidine to sustain viral repli-

**TABLE 2**

**Targeting viral replication**

| Targets (for inhibition) | Drugs | FDA approved | $C_{max}$ (μM) | Primary indications | Virus | $IC_{50}/EC_{50}$ (μM) | Model system | $CC_{50}$ (μM) | Refs |
|---|---|---|---|---|---|---|---|---|---|
| eIF2α phosphorylation | INDO | Yes | 14.36[a] | Inflammation | Canine-CoV | 5 | A72 | >550 | [68–71] |
| | GGA | Yes | 0.75[b] | Ulcer | SARS-CoV | 50 | Vero | >400 | [68–71] |
| | | | | | Canine-CoV | – | Dog | – | [68–71] |
| | | | | | IFV-A | – | Mice | – | [75–77] |
| eIF4A | Silvestrol | No | 1.57[c] | Cancers | MERS-CoV | 0.001 | MRC-5 | >10 | [79–82] |
| | | | | | HCoV-229E | 0.003 | MRC-5 | >10 | [79–82] |
| B-Raf (V600E) | Vemurafenib | Yes | 126[d] | Orbital Erdheim-Chester disease | IFV-A | 0.22–3.8[e] | A549 | >25 | [88–90] |
| MEK1/2 | Trametinib | Yes | 0.042[f] | Cancers | IFV-A | 0.016 | A549 | >20 | [85,95–97] |
| | | | | | IFV-A | – | Mice | – | |
| CDK1/2/5/9 | Dinaciclib | No | – | Cancers | IFV-A | 0.02–0.21[g] | A549 | >100 | [5] |
| CDK1/2/4/6 | Flavopiridol | No | – | Cancers | IFV-A | 0.24–0.7[g] | A549 | >100 | [5] |

Abbreviations: $C_{max}$, the maximum serum concentration; $IC_{50}/EC_{50}$, the half-maximal inhibitory concentration/the half-maximal effective concentration; $CC_{50}$, the half-maximal cytotoxic concentration.

[a] The dosage for moderate-to-severe rheumatoid arthritis in patient is 25 mg two or three times a day (FDA Guidance). Oral administration to dogs results in the $C_{max}$ of 14.36 μM by 1.4 h [69].

[b] Oral administration of 50 mg results in the mean $C_{max}$ of 0.75 μM by 5.35 h [76].

[c] Intravenous injection of 5 mg/kg to mice results in the $C_{max}$ of 1.57 μM [81].

[d] Oral administration of 960 mg to patients twice a day results in the mean $C_{max}$ of 126 μM (FDA Guidance). Treatment of mice with 100 mg/kg results in the $C_{max}$ of 124 μM by 2 h [89].

[e] $IC_{50}$ values at low micromolar concentrations against virus strains of H7N7 and H7N9 [90].

[f] Oral administration of 2 mg to patients reaches the peak serum concentration by 1.5 h (FDA Guidance). Oral administration of 0.1 mg/kg to mice results in the $C_{max}$ of 0.042 μM by 4 h [95,96].

[g] $IC_{50}$ values for IFV-A strains H7N9, H1N1 and H3N2 [5].

cation [58,59]. Recent studies showed the small-molecule GSK983 was capable of inhibiting DHODH and effective in blocking replication of various viruses and arresting cell growth [60,61]. Supplying exogenous deoxycytidine to sustain cellular DNA synthesis, but not RNA, results in reducing GSK983 cytotoxicity without reducing GSK983-mediated inhibition of dengue virus (DENV) replication [61]. Thus, the combined use of deoxycytidine with GSK983 provides an intriguing strategy with reduced cytotoxicity to safely use DHODH inhibitors in controlling RNA viruses.

Gemcitabine, an FDA-approved anticancer agent, is a fluorouracil analog capable of inhibiting pyrimidine biosynthesis [62]. Although gemcitabine was expected to interfere with pyrimidine biosynthesis in viral replication [63], gemcitabine showed a limited antiviral ability [40]. Whether gemcitabine can be repurposed to control viral infection remains to be clarified.

### *Targeting translation*

The protein translation process consists of three steps: initiation, elongation and termination. Initiation of translation in eukaryotes is a rate-limiting step of protein synthesis involving the family of eukaryotic initiation factors (eIFs). eIF4F, consisting of eIF4A, eIF4E and eIF4G, recruits the small ribosome subunit and binds to the $m^7$GTP residue at the 5′-end of mRNAs for cap-dependent translation [64]. Many viruses utilize host eIFs to initiate translation [65]. Phosphorylation of eIF2 plays an important part in regulating initiation [66]. Thus, eIF and eIF phosphorylation could be targeted to develop antiviral agents.

### *Phosphorylation of eIF2α*

The dsRNA-activated protein kinase (PKR) and the PKR-like endoplasmic-reticulum-resident protein kinase (PERK) phosphorylate the α-subunit of the eIF2 (eIF2α) in response to stress, leading to the attenuation of protein synthesis [66,67]. Indomethacin (INDO), a cyclooxygenase-1/2 inhibitor, is routinely used in managing inflammation and pain in clinics [68,69]. INDO treatment of cells activates PKR in an interferon (IFN)- and dsRNA-independent manner, resulting in rapid (<5 min) phosphorylation of eIF2α [70] and termination of the viral translation in SARS-CoV-infected Vero cells with an $IC_{50}$ value of 50 μM [71]. INDO reduces the production of canine CoV in the canine adenocarcinoma A72 cells with an $IC_{50}$ value of 5 μM; 400 μM INDO can significantly reduce viral production (>1000-fold) in A72 cells ($CC_{50}$ > 550 μM), and oral administration of 1 mg/kg INDO daily for 4 days reduced production of canine CoV in dogs 1000-fold [71]. Similarly, the bioactive lipid *N*-(4-hydroxyphenyl) retinamide (4-HPR, fenretinide), which is used to control cancer [72], can reduce DENV replication by inducing eIF2α phosphorylation [73]. Oral administration of mice with 4-HPR protects 70% of mice, fully recovered, from DENV infection [74]. The safety and bioavailability of INDO and 4-HPR have been clinically determined; thus, these compounds are promising candidates ready for repurposing as antiviral agents. In addition, the antiulcer drug geranylgeranylacetone (GGA) has been reported to augment the expression of the PKR gene and promote eIF2α phosphorylation to counteract IFV-A infection [75,76]. GGA is given orally every 2 days with the daily doses of 100 mg/kg resulting in 75% reduction in mean tumor burden compared with the control mice group [77]. Mice treated orally with GGA at 150 mg/kg twice daily (at 12 h intervals) had completely eradicated virus without any notable replication within 3 days [76]. Accordingly, therapeutic induction of eIF2α phosphorylation is a promising approach to the development of broad-spectrum antiviral agents.

#### ***eIF4A***

The helicase eIF4A unwinds mRNA 5′-untranslated region, facilitating the assembly of translation preinitiation complexes [78]. Silvestrol, a natural compound isolated from the plant *Aglaia foveolata*, is an inhibitor of eIF4A [79]. Silvestrol has been shown to control cancer cells *in vitro* and *in vivo* [80,81]. Silvestrol can inhibit the translation and replication of MERS-CoV and HCoV-229E in human embryonic lung fibroblast MRC-5 cells with $EC_{50}$ values of 0.001 and 0.003 μM, respectively [82]. CoV uses cap-independent *cis*-acting RNA internal ribosome entry site (IRES) elements with eIF4A for viral translation that can also be inhibited by silvestrol [83]. Interestingly, silvestrol exhibits a potent and modest ability to inhibit IFV-A infection in A549 cells and MDCK cells, respectively, but fails to inhibit viral infection in Vero cells [84]. The antiviral effect of silvestrol on IFV infection is reversible, where drug withdrawal results in rapid dissolution of stalled pre-initiation complexes (stress granules) and resumption of viral protein synthesis. Inhibition of IFV-A by silvestrol is associated with cytotoxicity [84]. By contrast, pateamine A irreversibly binds to eIF4A to result in an extended blockade of IFV-A replication after drug withdrawal, and it inhibits IFV-A replication in all A549, MDCK and Vero cells with minimal cytotoxicity [84]. These findings indicate the eIF4A is a promising target for therapeutic development to inhibit viral translation and replication by agents, such as silvestrol and pateamine A.

### *Targeting signaling pathways*

Kinase-involved signaling pathways are widely involved in the regulation of cellular machineries of metabolism, transcription and translation for cell proliferation, differentiation, death, among others. Studies have shown that the ERK pathway and cyclin-dependent kinases (CDKs) are essential for the viral replication of CoV and IFV [5,85]. Targeting protein kinases or signaling pathways becomes a focus in the therapeutic development for broad-spectrum antiviral agents.

#### ***The ERK pathway***

The ERK pathway mainly mediates intracellular signals from membrane-associated Ras to the cytoplasmic kinase cascade Raf, Mek and Erk [86]. The ERK pathway plays an important part in the regulation of gene transcription, protein translation, cell death and cell cycle machinery. Oncogenic mutation of the B-Raf gene (V600E) is frequently detectable in human cancers [87]. The B-Raf (V600E) inhibitor vemurafenib, which effectively inhibits the ERK pathway, was approved by the FDA to treat orbital Erdheim–Chester disease (ECD) [88,89]. Vemurafenib, at low micromolar levels, can inhibit IFV-A replication (1000-fold reduction) via suppression of viral translation [90]. Interestingly, vemurafenib also inhibits virus-induced apoptosis in A549 cells via the suppression of apoptosis-inducing cytokines [90]. Other Raf inhibitors, such as dabrafenib and sorafenib, were FDA-approved to treat cancer. These Raf inhibitors could be further studied to repurpose them for the control of viral infection.

It has been reported that the blockage of the ERK pathway limits the replication of CoV and IFV. Treatment with U0126, a specific

inhibitor of Mek1/2, suppresses early steps of the replication of mouse hepatitis virus (MHV) in various cell lines [91]. Treatment of cells with U0126 significantly reduces the replication of IFV-A, and nasal administration of U0126 protects mice from a lethal infection with IFV-A without developing any adverse effects [92]. Treatment of H1N1-infected mice with the Mek inhibitor CI-1040 reduces 80% viral production in the lung [93]; however, the low bioavailability of CI-1040 needs improving for it to be an effective antiviral agent [94]. The Mek1/2 inhibitor trametinib and the Erk1/2 inhibitor selumetinib, which are FDA-approved anticancer agents, can effectively inhibit MERS-CoV infection [85]. Trametinib also inhibits replication of various IFV strains (FPV/H7N7, SC35M/H7N7, PR8M/H1N1) *in vitro* and *in vivo* by interfering with the export of progeny vRNPs from the nucleus. Pretreatment with trametinib ($EC_{50}$ = 0.016 μM) blocks IFV-induced cytopathogenic effect and decreases viral production in A549 cells. Oral administration of 0.1 mg/kg trametinib to mice daily is effective in treating rheumatoid arthritis [95,96], and oral administration of 3 mg/kg trametinib daily for five consecutive days reduces IFV production [97]. These results indicate the application of targeting the ERK pathway to the development of broad-spectrum therapeutic antiviral agents.

### *CDKs*

CDKs are a family of protein kinases where activation is dependent on interaction with the cyclin family members to regulate the cell cycle, gene transcription, RNA processing and cell survival. CDKs, such as CDK1, CDK2, CDK9 and CDK13, are required for efficient replication of IFV [98]. Studies showed that cyclinT1/CDK9 interacts with viral RNA-dependent RNA polymerase (RdRp) and facilitates vRNP association with cellular RNA polymerase II (Pol II) for viral transcription and replication [99].

A recent HTS of a human kinase inhibitor library to target IFV-A (strain H7N9) in A549 cells revealed 273 structurally diverse, cell-permeable compounds with known bioactivity and safety profiles [5]. Among these compounds, dinaciclib (inhibitor of CDK1/2/5/9) and flavopiridol (inhibitor of CDK1/2/4/6) exhibited potent antiviral activity against various IFV-A strains without detectable cytotoxicity [5]. Dinaciclib and flavopiridol are currently undergoing clinical trials for human cancers [100,101]. SNS-032 (inhibitor of CDK2/7/9) was shown to inhibit viral gene expression [98,102]. SNS-032 treatment fully protects mice from H1N1 infection in contrast to >80% mortality of the untreated mice [98]. These results indicate that further development of CDK inhibitors should be highly promising to control IFV infection.

#### *Polyamine biosynthesis inhibitor*

Polyamines are small molecules, and each polyamine carries more than two amino groups [103]. Polyamines are involved in conformational changes of DNA and modulation of transcription, translation and signaling pathways in cells [104–107]. Polyamines were shown to regulate the transcription and translation of DNA and RNA viruses [108,109]. Difluoromethylornithine (DFMO, also eflornithine), a polyamine inhibitor, has been approved by the FDA to treat African trypanosomiasis and cancer [110,111]. DFMO has been shown to reduce viral production of MERS-CoV 30-fold in Vero cells [112]. DFMO needs to be further studied to clarify its *in vivo* toxicity and efficacy.

#### *Summary*

Targeting the translation by INDO to induce phosphorylation of eIF2α has been shown to inhibit viral replication *in vitro* and safely protect animals from viral infection. INDO is FDA-approved to control inflammation and pain. Targeting signaling pathways involved in cell proliferation by blocking the ERK pathway with trametinib and inhibiting the CDK function with SNS-032 is also effective in controlling viral replication *in vitro* and protecting mice from viral infection. Trametinib is FDA-approved to treat cancer. Thus, INDO and trametinib should be clinically studied and efficiently repurposed to treat viral infection. Other agents, such as vemurafenib, GGA, dinaciclib, flavopiridol, DFMO and the combination of deoxycytidine with GSK983, need to be further studied to reveal their ability to control viral infection in animal models. Accordingly, agents targeting translation and proliferation signaling pathways are highly promising for potential repurposing as broad-spectrum antiviral agents.

## Modulating the innate immune response

The host innate immune system, capable of recruiting immune cells to pathogen-infected sites through cytokines and activating the adaptive immune system, is crucial for the control of pathogenic infection. IFNs and proinflammatory cytokines have important roles in modulating the innate immune response to control viral infection. To repurpose drugs that have been used to modulate the innate immune system (Fig. 1, Table 3), it is expected that therapeutic development for producing broad-spectrum antiviral agents will be accelerated.

#### *Type I IFN*

Viral infection generates highly conserved pathogen-associated molecular patterns that preferentially activate the host's pattern recognition receptors (PRRs) [113], resulting in the activation of transcription factors, the induction of type I IFN expression and the induction of canonical IKK for NF-κB activation, leading to the production of proinflammatory cytokines [114]. IFNs, produced from virus-infected cells, bind to the cell-surface IFN receptor (IFNAR) to induce the upregulation of hundreds of IFN-stimulated gene products (ISGs). ISGs have been shown to exhibit a wide range of antiviral abilities to degrade viral nucleic acids, inhibit viral gene expression and serve as PRRs to amplify IFN signals [113–115].

Traditional Chinese medicine and drugs have been used to control inflammation, cancer and pathogenic infection for thousands of years. Herbal medicines have been shown to augment type I IFN responses to counteract CoV and IFV infections *in vitro* and *in vivo*. YZH-106, a rupestonic acid derivative extracted from *Artemisia rupestris* L, can inhibit a broad spectrum of IFVs (IFV-A H1N1 and H3N2, IFV-B and oseltamivir-resistant and amantadine-resistant IFV strains) via the activation of the Heme oxygenase-1-mediated IFN response [116]. YZH-106 treatment reduces viral production in the lung of H1N1-infected mice but is unable to fully protect mice from lethal infection, possibly as a result of IFN suppression by the viral NS1 protein [116].

#### *Inflammation*

IFV-A and SARS-CoV infection induce inflammation *in vivo* [117,118]. Activation of NF-κB is a hallmark for detecting viral

**TABLE 3**

**Targeting the innate immune response**

| Targets (for modulation) | Drugs | FDA approved | $C_{max}$ (μg/ml) | Primary indications | Virus | $IC_{50}/EC_{50}$ (μg/ml) | Model system | $CC_{50}$ (μg/ml) | Refs |
|---|---|---|---|---|---|---|---|---|---|
| NF-κB pathway (inhibition) | SSa | No | – | Inflammation, immunomodulation | IFV-A | 1.55–1.73[a] | A549 | >5.9 | [121–123] |
| | PS-341 | Yes | 0.11[b] | | IFV-A | – | Mice | – | [121–123] |
| | | | | Mesothelioma | IFV-A | – | A549 | – | [125–127] |
| Proinflammatory genes (inhibition) | Glycyrrhizin | No | – | Oxidation, inflammation | SARS-CoV | 300 | Vero | >20 000 | [128–132] |
| Proinflammatory cytokines and chemokines (inhibition) | DAP | No | 26.9[c] | Inflammation | IFV-A | 5 | A549 | 77 | [134,135] |
| | Nitazoxanide | Yes | 10.6[d] | Parasite | IFV-A | 38 | MDCK | 243 | [134,135] |
| | | | | | IFV-A/B[e] | 0.2–1.5 | Multiple cells | – | [136–138] |
| | | | | | MERS-CoV | 0.92 | LLC-MK2 | – | [139] |
| LANCL2 pathway (induction) | NSC61610 | No | – | Inflammation | IFV-A | – | Mice | – | [141] |
| IFN-γ (induction) | LPG | No | – | Oxidation, inflammation | IFV-A | – | Mice | – | [142,143] |

Abbreviations: $C_{max}$, the maximum serum concentration; $IC_{50}/EC_{50}$, the half-maximal inhibitory concentration/the half-maximal effective concentration; $CC_{50}$, the half-maximal cytotoxic concentration.

[a] $IC_{50}$ values for IFV-A strains H1N1, H9N2 and H5N1 [123].

[b] The recommended starting dose of PS-341 for intravenous injection is 1.3 mg/m$^2$, the mean $C_{max}$ is 0.11 μg/ml (FDA Guidance).

[c] Intravenous administration of 320 mg to patients results in the $C_{max}$ of 26.9 μg/ml by 1 h [134].

[d] After oral administration of 500 mg to patients, the parental nitazoxanide is not detectable in the plasma. The $C_{max}$ of the metabolite tizoxanide is 10.6 μg/ml by 3 h (FDA Guidance). Oral nitazoxanide at 300 and 400 mg/kg/day for 14 days shows protoscolicidal effects in infected mice [137].

[e] Nitazoxanide is undergoing Phase III clinical development for the treatment of IFV-A and B strains [138].

infections [119]. NF-κB plays an important part in the regulation of genes involved in the inflammation counteracting viral infection [119]. However, IFV-A can take advantage of NF-κB activation, via the degradation of IκB, for viral replication [119]. Thus, NF-κB could be targetable for interfering with IFV replication [120]. Saikosaponin A (SSa), a lipophilic triterpene saponin derived from *Radix Bupleurum*, has anti-inflammatory and immunomodulatory properties [121,122]. SSa inhibits NF-κB activation and effectively attenuates IFV-A replication at $IC_{50}$ values of 1.55, 1.62 and 1.73 μg/ml for H1N1, H5N1 and H9N2 strains, respectively, in A549 cells. Daily subcutaneous injection with SSa at 50 mg/kg for six consecutive days beginning at 4 h post-infection protects mice from a lethal infection of IFV-A [123]. Treatment of mice with the NF-κB inhibitor caffeic acid phenethyl ester (CAPE) and/or parthenolide reduces the expression of proinflammatory cytokines in the lung and significantly increases animal survival (~17% to ~33–56%) from SARS-CoV infection [124]. The proteasome inhibitor bortezomib (PS-341), an anticancer drug, suppresses IκB degradation to result in blocking NF-κB activation [125,126]. It was shown that PS-341 at a noncytotoxic level of 0.05 μM can reduce IFV-A replication (up to three orders of magnitude) in A549 cells; however, 0.1 μM PS-341 becomes cytotoxic [127]. Thus, concerning the antiviral activity of PS-341 in association with cytotoxicity, PS-341-associated adverse effects need to be carefully determined for developing an optimal antiviral agent. In addition, stronger neo-Minophagen C (SNMC), a glycyrrhizin preparation, has been approved in Japan to treat chronic hepatic diseases [128]. Glycyrrhizin is an antioxidant, anti-inflammatory, immunomodulatory and antiviral agent [129,130]. Glycyrrhizin suppresses IFV-A replication, in part through interference with virus-induced proinflammatory gene expression [131]. Glycyrrhizin can suppress SARS-CoV infection at the early entry and the late replication stages in Vero cells with an $EC_{50}$ value of 300 μg/ml [132]. *Andrographis paniculata* is an anti-inflammatory, antipyretic, analgesic, antibacterial and hepatoprotective agent [133], and the major component 14-deoxy-11,12-dehydroandrographolide (DAP) suppressed gene expression of proinflammatory cytokines and chemokines, as well as IFV-A replication [134,135]. These results suggest that anti-inflammatory agents could be further developed for the therapeutic control of IFV-A and SARS-CoV infection. The broad-spectrum antiparasitic drug nitazoxanide is a safe drug for treating intestinal infection by *Cryptosporidium parvum* [136,137]. Nitazoxanide is currently undergoing studies to control viral infections. Nitazoxanide can interfere with post-translational modification of IFV hemagglutinin in human and animal cells with $IC_{50}$ values ranging from 0.2 to 1.5 μg/ml, and nitazoxanide is reportedly effective in controlling IFV-A and -B infection in a Phase III clinical study [138]. Nitazoxanide has also been shown to inhibit MERS-CoV infection of mice and LLC-MK2 cells with an $IC_{50}$ value of 0.92 μg/ml by suppressing expression of the viral N protein and proinflammatory cytokines, such as interleukin (IL)-6, in peripheral blood mononuclear cells [139]. Accordingly, nitazoxanide is regarded highly as a promising antiviral agent repurposed from an antiparasitic agent.

Lanthionine synthetase C like 2 (LANCL2) is a therapeutic target for treating inflammatory, chronic metabolic, immune-mediated and infectious diseases [140]. Oral administration of animals with 20 mg/kg NSC61610 daily for 12 days reduces mortality induced by pandemic IFV-A infection (H1N1/California/04/09) 30% by interfering with the trafficking of inflammatory tissue-damaging cells and increasing IL-10-producing $CD8^+$ T cells and regulatory macrophages in the lungs in a LANCL2-dependent manner [141]. *Ligustrum purpurascens* Y.C. Yang (Oleaceae) Ku Ding Cha tea is used as an anti-inflammatory, antioxidant and hepato-protectant agent [142]. The *Ligustrum purpurascens* extract phenylethanoid

glycoside (LPG) can inhibit IFV-A replication (A/FM/1/47 H1N1, FM1) *in vitro* and *in vivo* by inducing IFN-γ [143]. Oral administration of 900 mg/kg LPG daily for 5 days protects 30% of mice from a lethal infection of IFV-A (H1N1) [143].

### *Summary*

Pandemic and zoonotic IFV strains are serious concerns in public health, particularly in the population lacking preexisting immunity. Recent development of antiviral agents in modulating host immune responses has substantially advanced the fields of virology and pharmaceutics, as well as significantly contributed to healthcare advances in humans and animals. NSC61610 and LPG have been shown to effectively control IFV-A infection in animals by activating the immune response. These agents should be considered in clinical trials for treating viral infection. By contrast, SSa, CAPE, glycyrrhizin and DAP are undergoing *in vitro* and *in vivo* studies to reveal their ability to control viral infection. However, adverse effects of these immune-modulating agents need to be seriously clarified.

## Concluding remarks

Developing a new drug can take ~15 years and cost billions of US dollars. Approximately one-in-ten new promising preclinical drugs, identified from hundreds of compounds, might be approved for clinical use. Approximately 30% of new drugs fail to pass safety protocols in clinical trials. Repurposing FDA-approved drugs is a time-efficient, cost-effective and safe approach to developing therapeutic drugs for the new indication of treating viral infection. To repurpose an FDA-approved drug, such as anticancer and other agents, researchers still need to go through *in vitro*, *in vivo* and clinical studies to determine the value of a candidate drug for treating IFV and/or CoV diseases. *In vitro* studies can identify viral strains to be targeted, determine the host cell range susceptible to viral infection and reveal additional molecular targets specifically for controlling viral infection. The value of host molecular targets can be evaluated by gene knockdown, knockout or mutation via the methods of RNA interference or CRISPR/Cas9. *In vivo* studies will determine the maximal tolerable doses and adverse effects, efficacy in controlling viral infection and pharmacokinetics of drug concentrations in the plasma to reestablish an effective and safe regimen and protocol for treating IFV and/or CoV diseases. The new *in vitro* and *in vivo* results will be considered with existing data together to design clinical studies for viral diseases.

Inevitably, there are challenges to the repurposing of drugs for antiviral therapeutics. In general, viral infection is an acute pathogenesis that can require higher doses of a repurposed drug than doses used in treating originally targeted chronic diseases for an optimal outcome. Thus, the administration route and toxicity of a repurposed drug need to be carefully determined. Viral infection also results in changes in not only the host's immune system but also the function of the host's organs, which could interfere with pharmacological effects of a repurposed drug or cause additional side effects. Thus, it will be beneficial to study combination therapies capable of targeting viral components, modulating cellular machineries and alleviating adverse effects to achieve optimal outcomes of increased antiviral efficacy, reduced viral resistance and minimized toxicity and side effects.

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