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Nucleoside analogs for management of respiratory virus infections: mechanism of action and clinical efficacy
Annelies Stevaert1, Elisabetta Groaz2,3 and Lieve Naesens1

The COVID-19 pandemic has accelerated the development of nucleoside analogs to treat respiratory virus infections, with remdesivir being the first compound to receive worldwide authorization and three other nucleoside analogs (i.e. favipiravir, molnupiravir, and bemnifosbuvir) in the pipeline. Here, we summarize the current knowledge concerning their clinical efficacy in suppressing the virus and reducing the need for hospitalization or respiratory support. We also mention trials of favipiravir and lumicitabine, for influenza and respiratory syncytial virus, respectively. Besides, we outline how nucleoside analogs interact with the polymerases of respiratory viruses, to cause lethal virus mutagenesis or disturbance of viral RNA synthesis. In this way, we aim to convey the key findings on this rapidly evolving class of respiratory virus medication.

Addresses
1 Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, KU Leuven, Herestraat 49 box 1043, B-3000 Leuven, Belgium
2 Rega Institute for Medical Research, Medicinal Chemistry, KU Leuven, Herestraat 49 box 1041, B-3000 Leuven, Belgium
3 Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy

Corresponding author: Lieve Naesens (lieve.naesens@kuleuven.be)

Introduction
The awareness that airborne viruses can be a global threat peaked after the emergence of the last influenza pandemic (2009) and the severe acute respiratory syndrome (SARS, 2003) and Middle East respiratory syndrome (MERS, 2012) coronaviruses (CoVs). Nonetheless, the COVID-19 pandemic hit the world unprepared, resulting in an estimated 18.2 million excess deaths over only two years [1]. For seasonal influenza, the excess mortality is rated at ∼469 000 deaths per year [2], while respiratory syncytial virus (RSV) causes each year up to 199 000 fatalities [3]. Even outside pandemics, the medical and socioeconomic burden of respiratory viruses is immense, considering the diversity in virus species and disease manifestations. Rhinoviruses and endemic human CoVs are the main cause of common cold, a self-limiting infection of the upper respiratory tract. For respiratory adenoviruses, RSV, metapneumovirus, and parainfluenza virus, the disease outcome depends on the person’s immune status and age, with infants and elderly being at higher risk. This is also true for SARS-CoV-2 and influenza virus, which can cause a life-threatening and highly inflammatory disease ('cytokine storm') when invading the lower respiratory tract [4]. This explains the use of dexamethasone for severe COVID-19 patients requiring respiratory support.

Vaccination against respiratory viruses is currently limited to influenza and COVID-19. Despite being clearly effective, these vaccines do not offer full protection, leaving frail persons at risk of severe infection requiring hospitalization. In these patients, antiviral drugs can be life-saving. Besides, these medicines may help to prevent virus spread in outbreak situations. In this concise review, we provide an update on nucleoside analogs that are already in clinical use or in the pipeline to treat respiratory virus infections (see Table 1 for a summary of selected trials).

Stricto sensu, the term nucleoside analog refers to a molecule composed of a nucleobase and sugar (e.g. ribose) moiety, while extension with a phosphate or phosphonate group yields a nucleotide analog. Since the anionic phosph(on)ate group diminishes cell permeability and the phosphate linkage is prone to esterase cleavage, prodrug concepts were developed in which the phosph(on)ate is masked by lipophilic moieties. To exert antiviral effect, nucleoside/nucleotide analogs require one, two, or three intracellular phosphorylations. For oral prodrugs, the activation process starts with cleavage to the free nucleoside/nucleotide, during absorption or first-pass. In most cases, the nucleoside-5′-triphosphate form is the active metabolite, since it binds to the viral polymerase and uses the mechanisms specified below to disturb viral nucleic acid synthesis. Alternatively, indirect inhibition of this process occurs when the drug targets a cellular enzyme of the purine or pyrimidine synthesis pathway, leading to depletion of one of the natural nucleoside-5′-triphosphates.
## Table 1

| Drug name | Study designa | Patient characteristics | Effect of drug treatment | NCT number | Reference |
|-----------|---------------|-------------------------|--------------------------|------------|-----------|
| Favipiravir | Phase-3 RCT (vs. placebo) | Otherwise healthy adults with uncomplicated influenza | Lower virus titers | NCT02026349 and NCT02008344 | [23] |
| Favipiravir | Phase-2 RCT (vs. SOC) | Hospitalized COVID-19 patients with mild-to-severe disease | Shorter duration of viral shedding | NCT04358549 | [25] |
| Favipiravir | Phase-3 RCT (vs. SOC) | Nonhospitalized patients with mild-to-moderate COVID-19 and at risk of severe disease | Shorter time to clinical improvement | NCT04501783 | [26] |
| Molnupiravir | Phase-3 RCT (vs. placebo) | Hospitalized COVID-19 patients | Lower rate of hospitalization or death, lower need for respiratory support | NCT04575597 | [41,74] |
| Molnupiravir | Phase-2/3 RCT (vs. placebo) | Nonhospitalized patients with mild-to-moderate COVID-19 and at risk of severe disease | No effect on time to recovery | NCT04575584 | [42] |
| Remdesivir | Phase-3 RCT (vs. placebo) | Hospitalized COVID-19 patients | Shorter time to recovery | NCT04280705 | [59,60] |
| Remdesivir | Phase-3 RCT (vs. SOC) | Hospitalized COVID-19 patients | Lower rate of clinical deterioration, lower need for respiratory support | NCT04330690 | [61] |
| Remdesivir | Phase-3 RCT (vs. placebo) | Nonhospitalized COVID-19 patients at risk of severe disease | Lower need for respiratory support | NCT04501952 | [62] |
| Bemnifosbuvir | Phase-3 RCT (vs. placebo) | Nonhospitalized COVID-19 patients with mild-to-moderate disease | Lower rate of hospitalization | NCT04889040 | Unpublished |
| Lumicitabinea | Phase-2a RCT (vs. placebo) | Healthy adults challenged with RSV | Lower virus titers, lower symptom scores | NCT02094365 | [69] |
| Galidesivira | Phase-1b RCT (vs. placebo) | Hospitalized patients with moderate-to-severe COVID-19 | Lower virus titers | NCT03891420 | [71] |

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*a No longer in development for the indicated virus infection.

b RCT, randomized controlled trial; SOC, standard-of-care.

[https://ateapharma.com/covid-19/bemnifosbuvir/](https://ateapharma.com/covid-19/bemnifosbuvir/)
Ribavirin
First reported in 1972, the broad antiviral agent ribavirin (Figure 1) is still in use as a last resort against viruses for which specific medication is lacking. It inhibits viral RNA synthesis via different indirect or direct mechanisms, not all covered here [5]. For influenza virus, direct inhibition of the viral RNA-dependent RNA polymerase (RdRp) requires high concentrations of the ribavirin-5′-triphosphate metabolite. This makes (GTP) guanosine-5′-triphosphate depletion, due to an inhibitory effect of ribavirin-5′-monophosphate (RBV-MP) on the cellular inosine-5′-monophosphate dehydrogenase enzyme, an equally plausible mechanism [6]. Ribavirin has reasonable in vitro activity against influenza virus and RSV, which both accumulate transition mutations when cultured under ribavirin [6,7]. Its poor efficacy against CoV [8] seems related to the ability of CoV nsp14 exonuclease (ExoN) to remove RBV-MP once it is incorporated in viral RNA [9].

The American Society of Transplantation recently recommended aerosolized or oral ribavirin for RSV-infected lung or other solid organ transplant recipients [10]. For infants, prophylaxis with the RSV-specific antibody palivizumab is advised. Early clinical trials did not support the use of ribavirin for influenza. Its efficacy against SARS-CoV-2 is the subject of ongoing trials. A study of 2003, conducted in SARS patients, indicated that the adverse effects of high-dose ribavirin, such as anemia, were too serious to justify its use [11]. The daily dose tested in this trial was ∼3-fold higher than that used for hepatitis C virus (HCV) infections.

Favipiravir
Favipiravir (T-705) requires conversion to its ribosyl-5′-triphosphate (FPV-RTP) metabolite to interfere with viral RNA synthesis [12]. Its poorly efficient phosphorylation [13] partially explains why this drug requires high dosing. Alike ribavirin, favipiravir carries a rotating carboxamide moiety (Figure 2), generating a pseudobase that mimics guanine but also adenine. As a result, virus exposed to favipiravir accumulates G→A and C→U mutations in its genome, as first demonstrated in influenza virus-infected cell cultures [14] and later confirmed in SARS-CoV-2-infected cells [15] and hamsters [16]. The antiviral effect of favipiravir thus seems to rely on ‘error catastrophe’: virus mutagenesis leading to the formation of noninfectious particles. Alternatively, the compound may cause termination of viral RNA synthesis, as evident from studies with FPV-RTP and influenza virus RdRp, yielding an IC₅₀ value of ∼3 µM [6,17]. The consecutive incorporation of two FPV-RTP molecules is key to obtain the delayed chain-terminating effect (Figure 2) [17,18] and induces influenza virus RdRp backtracking, as evident from recent biochemical and (cryo-EM) cryogenic electron microscopy studies with the 6-defluorinated analog of FPV-RTP [19]. In the stalled complex, the pseudobase interacts with PB1-Lys229 [19], rationalizing why this residue is associated with viral resistance to favipiravir [20]. Nevertheless, double FPV-RTP incorporation is fairly unlikely to occur in infected cells, considering that the inhibitor needs to compete with high concentrations of the natural (NTPs) nucleoside-5′-triphosphates. The more likely effect, insertion of a single FPV-RTP molecule, permits further extension and, due to its ambiguous base pairing with C and U, can result in transition mutations [17,18].

Regarding CoV polymerase, SARS-CoV RdRp [= non-structural protein 12 (nsp12) complexed to nsp7 and nsp8] was shown to readily incorporate FPV-RTP as a GTP analog, with elongation to full-length products [15]. Apparently, its incorporation as an ATP analog (i.e. opposite to uracil) causes some stalling but still allows chain extension. On the other hand, another study observed inefficient incorporation of FPV-RTP by SARS-CoV-2 nsp12, in line with cryo-EM evidence that its β-phosphate is not optimally oriented in the catalytic site.
On a general note, conducting these biochemical experiments is complicated by the fact that the fluorine atom of favipiravir renders its ribosylated forms chemically unstable. Favipiravir exhibits broad in vitro and in vivo activity against many RNA viruses but particularly influenza-A and -B viruses. Thus far, only one study succeeded in selecting resistant influenza virus in cell culture, implying a high barrier for drug resistance. This drug is approved in Japan for oral use against influenza viruses unresponsive to the common drugs. In two phase-3 trials in adults with uncomplicated influenza, favipiravir was shown to reduce virus titers, whereas the alleviation of symptoms was less consistent. Owing to possible teratogenic effects, it is not recommended for pregnant and lactating women, while men should adhere to contraceptive methods when taking the drug.

Soon after the appearance of SARS-CoV-2, favipiravir was proposed as a candidate drug, although its in vitro activity against this virus is weak when compared with influenza virus. Accordingly, it likely requires relatively high dosing for COVID-19. In a phase-2 trial in patients hospitalized with mild-to-severe COVID-19, the favipiravir group showed more rapid viral clearance than those receiving standard-of-care. In a phase-3 trial in patients with mild-to-moderate COVID-19 pneumonia, favipiravir shortened the time to clinical improvement by about four days. The conclusion that favipiravir shortens the duration of SARS-CoV-2 shedding and symptoms was also reached in a meta-analysis of 157 reported studies. The positive outcome is limited by the drug’s adverse effects (e.g. hyperuricemia) and teratogenic potential. Also, early initiation after symptom onset seems needed to maximize the effect.

To solve the drawback of favipiravir’s low potency, more potent analogs are desirable. Its simple pyrazinecarboxamide structure seems to tolerate some variations. Alternatively, prodrug strategies may help to bypass inefficient steps in the metabolic activation pathway from favipiravir to FPV-RTP. For T-1105 (the favipiravir analog lacking the 6-fluorine), conversion of the 5′-mono- into 5′-diphosphate form proved to be the rate-limiting step in cell culture models.

**Molnupiravir**

Molnupiravir (EIDD-2801, MK-4482) is the oral 5′-iso-propyl ester prodrug of N4-hydroxycytidine (NHC, EIDD-1931) (Figure 2), with NHC-5′-triphosphate (NHC-TP) being the active metabolite. NHC is a broad anti-RNA virus nucleoside analog that was first identified in a HCV replicon study. The promising activity of oral NHC/molnupiravir in animal models for...
influenza virus [31], RSV [32], or CoV [33–36] infection, supports its broad development for respiratory virus infections. All these viruses accumulate mutations in their RNA genome, when cultured under NHC. In MERS-CoV-infected mice, the molnupiravir-driven mutagenesis correlated with the reduction in viral load [33]. The mutagenic effect of NHC (already observed in bacteria in the 1970s) is explained by its tautomerizing base that mimics cytosine and uracil [37]. Biochemical studies with NHC-TP and SARS-CoV-2 RdRp (= nsp12–nsp7–nsp8) indicate that RNA mutagenesis occurs in two steps [38,39] (Figure 2). First, the compound is incorporated without stalling RNA extension, and occurs opposite to guanine but also, to a lesser extent, opposite to adenine. Second, once present in the RNA template, NHC directs incorporation of either the correct or incorrect nucleotide, resulting in mutated RNA products. Cryo-EM analysis confirmed that the NHC base stably pairs with guanine and adenine, in the RdRp active site of SARS-CoV-2 nsp12 [39]. Furthermore, NHC appears to resist excision by CoV nsp14 ExoN [40]. This proofreading activity is characteristic of CoVs but lacking in other RNA viruses.

The clinical development of molnupiravir accelerated in the past two years. In the MOVe-OUT phase-3 trial in nonhospitalized unvaccinated adults with mild-to-moderate COVID-19, the drug was found to significantly reduce the risk of hospital admission or death, compared with placebo [41]. Since no safety issues arose, molnupiravir received authorization in the United Kingdom for the treatment of outpatients with COVID-19, as well as Emergency Use Authorization by the (FDA) Food and Drug Administration. More clinical data are pending. In a trial in hospitalized COVID-19 patients, oral molnupiravir did not shorten the time to recovery [42]. Hence, the drug seems to be most effective when initiated early in outpatients with mild-to-moderate COVID-19. It has a favorable pharmacokinetic and safety profile [43] but is not recommended during pregnancy or breastfeeding, due to reproductive toxicity observed in animals exposed to high doses. Cell culture findings indicate that prolonged exposure to NHC is devoid of mitochondrial toxicity [44] but may give mutations in cellular DNA [45].

**Remdesivir**

Remdesivir is the arylxophosphoramidate prodrug of the 5'-monophosphate form of GS-441524, a 1'-cyano-substituted C-nucleoside analog. In enzymatic assays with SARS-CoV-2 RdRp, the 5'-triphosphate of GS-441524 (RDV-TP) causes delayed stalling or chain termination, depending on the method used (Figure 3). Being efficiently incorporated as an ATP analog [46–49], RDV-TP initially allows chain elongation via its 3'-hydroxyl group. However, RNA extension is stalled after addition of three nucleotides [47,48,50], due to steric clash between the 1'-cyano group of the incorporated GS-441524 and RdRp active site [48,50,51]. RdRp stalling can be overcome at relatively high yet physiologically relevant concentrations of the natural NTPs [48,50,52]. In addition, RDV may act during the next round of RNA synthesis, by compromising the efficiency of UTP incorporation opposite to template RDV [53]. Moreover, although the relation between the RdRp stalling effect and potential recognition by CoV nsp14 ExoN remains to be clarified, the fivefold higher antiviral activity of remdesivir against an ExoN-deficient CoV [54] suggests that the drug is somehow affected by this mechanism of excision.

After remdesivir was discovered as a broad anti-RNA virus agent, it was first developed to treat Ebola virus infection. With regard to respiratory viruses, it has cell culture activity against CoVs and RSV, but not influenza virus [55]. The EC50 value depends on whether remdesivir or GS-441524 is tested, and on the cell line used. Remdesivir was developed to bypass the first inefficient phosphorylation of GS-441524, but unfortunately requires intravenous administration. This inconvenience might be solved by an inhaled remdesivir formulation [56] or oral prodrug of GS-441524 [57]. Validation of these concepts in mice is hindered by the rapid metabolic inactivation of RDV in this species [58].

Remdesivir was the first nucleoside analog to receive wide approval or Emergency Use Authorization for COVID-19. In the pivotal ACTT-1 trial in hospitalized patients [59], it shortened the median recovery time to 10 days, compared with 15 days in the placebo group. The drug also reduces the rate of clinical deterioration and dependence on respiratory support [60,61]. Earlier treatment seems even better, since a study in nonhospitalized COVID-19 patients with high risk for complications [62] showed that a three-day course with intravenous remdesivir lowered the risk for hospitalization or death by 87%, compared with placebo. The safety profile is acceptable.

**Bemnifosbuvir**

Bemnifosbuvir (AT-527, RO7496998) is an orally administered dual prodrug of 2'-fluoro-2'-C-methyl-guanosine-5'-monophosphate, bearing an arylxophosphoramidate part and N6-methylated nucleobase (Figure 3). The two 2'-substituents render the 5'-triphosphate form (encoded AT-9010) an immediate chain terminator toward SARS-CoV-2 RdRp, and weak excision by nsp14 ExoN reinforces the inhibitory effect [63]. In a cryo-EM study revealing the binding mode of AT-9010 in the RdRp active site of SARS-CoV-2 nsp12, an additional AT-9010 molecule was found to occupy a so-far unknown cavity in the ‘nidovirus RdRp-associated nucleotidyl transferase’ (NiRAN) domain of nsp12 [63]. In enzymatic assays, AT-9010 [63] but also RDV-TP [64] proved able to inhibit NiRAN-mediated nucleotidyl
transferase reactions, suggesting that these nucleoside analogs might halt CoV RNA synthesis based on more than a single mechanism. Guanosine analogs may be better at combining these two activities, considering that the NiRAN-binding site appears to be GTP-specific [63,65].

Bemnifosbuvir recently completed a phase-3 trial (ClinicalTrials.gov identifier: NCT04889040) in outpatients with mild-to-moderate COVID-19. A study in nonhuman primates showed that oral lumicitabine is converted to ALS-8112, which enters the respiratory tract to give high levels of ALS-8112-TP [68]. This active metabolite has a long intracellular half-life of \( \sim 29 \) h. In a placebo-controlled trial in healthy adults challenged with RSV, oral lumicitabine reduced the duration of virus shedding and disease parameters [69]. Nevertheless, its clinical development was halted in 2019.

**Lumicitabine**

Lumicitabine (ALS-8176, ALS-008176, JNJ-64041575) is an oral 3',5'-diester prodrug of 2'-fluoro-4'-chloromethyl-cytidine (ALS-8112) (Figure 1). This nucleoside analog inhibits the replication of RSV, metapneumovirus, and parainfluenza virus, but not of influenza virus and rhinovirus [66]. The 5'-triphosphate form (ALS-8112-TP) is efficiently recognized by RSV RdRp to cause immediate chain termination, with the 4'-chloromethyl being a key substituent for polymerase selectivity [66]. Also, the related compound 4'-fluorouridine possesses favorable selectivity for inhibition of RSV and SARS-CoV-2 [67].

A study in nonhuman primates showed that oral lumicitabine is converted to ALS-8112, which enters the respiratory tract to give high levels of ALS-8112-TP [68]. This active metabolite has a long intracellular half-life of \( \sim 29 \) h. In a placebo-controlled trial in healthy adults challenged with RSV, oral lumicitabine reduced the duration of virus shedding and disease parameters [69]. Nevertheless, its clinical development was halted in 2019.

**Galidesivir**

Galidesivir (BCX4430, immucillin A, Figure 1) has a particularly broad anti-RNA virus spectrum that covers Ebola virus and flaviviruses, besides influenza virus, RSV, CoV, and rhinovirus [70]. Although the inhibitory mechanism toward the RdRp enzymes of these viruses has not yet been revealed, experiments with HCV RdRp support the qualification of BCX4430-5'-triphosphate as a delayed nonobligate chain terminator acting as an analog of ATP [70]. X-ray crystallographic or cryo-EM data are still lacking but may help to explain how the iminosugar moiety precisely interacts with viral RdRp enzymes to stop RNA synthesis.
In dose-ranging studies in healthy individuals, galidesivir proved to be safe and generally well tolerated [71]. A trial conducted in 2020 to evaluate intravenous galidesivir in hospitalized COVID-19, demonstrated dose-dependent reduction in the SARS-CoV-2 viral load in the airways. At the time of writing, galidesivir was however no longer under development for COVID-19 [71].

**Brincidofovir**

Although the focus of this review lies on RNA viruses, respiratory infections can also be caused by DNA viruses, specifically some types of adenovirus (Ad). Ads have broad tropism for diverse organs, not limited to the respiratory tract, and can cause severe disseminated disease in immunocompromised individuals. They are inhibited by brincidofovir (CMX001, Figure 1), a lipid-conjugated oral prodrug of the acyclic nucleoside phosphonate cidofovir. Compared with intravenous cidofovir, oral brincidofovir attains higher intracellular concentrations of cidofovir diphasphate (CDV-pp). In enzymatic assays with Ad5 polymerase, CDV-pp acts as a (dCTP) 2′-deoxyctydine-5′-triphosphate-competitive non-obligate chain terminator, while high concentrations cause direct arrest of Ad DNA synthesis [72].

Brincidofovir is currently authorized to treat potential outbreaks of smallpox, and passed phase-2/3 trials for Ad infections. Preemptive brincidofovir appeared superior to cidofovir in controlling Ad viremia in pediatric hematopoietic stem cell transplant recipients [73]. Brincidofovir lacks the nephrotoxicity of cidofovir but is associated with gastrointestinal side effects.

**Conclusion and outlook**

In recent years, the development of nucleoside analogs against respiratory viruses has shown clear progress for coronavirus and influenza virus, however, the field has been evolving more slowly in the case of RSV (along with the related metapneumovirus and parainfluenza virus), adenovirus, and particularly rhinovirus. For all these viruses, the acute nature of the airway infection urges antiviral drug initiation as soon as possible after symptom onset. Accordingly, the FDA recently expanded its approval of remdesivir to include not only hospitalized COVID-19 patients, but also outpatients risking severe disease. Besides, nucleoside analogs might help to limit the spread of respiratory viruses within the community, but their utility for this purpose has not yet been demonstrated. To accomplish this wider use, oral drugs appear more suitable than drugs that require injection or inhalation.

One of the main assets of nucleoside analogs is the potential to cover diverse viruses and allow for a tunable antiviral profile by introducing minor chemical modifications. They can act by diverse biochemical mechanisms, not strictly limited to immediate or delayed chain termination and virus mutagenesis. This versatility implicates that discovering novel nucleoside analog leads is still possible, although this may require extensive medicinal chemistry efforts to generate different combinations of modified sugar and base moieties.

The dependence on metabolic activation may represent a hurdle, since poor phosphorylation to the active 5′-triphosphate form can render a nucleoside analog virtually inactive. Overcoming this bottleneck requires better understanding of which prodrug concepts are superior at enhancing drug disposition in the respiratory tract. Therefore, the design of nucleoside analogs to treat respiratory virus infections is, more than ever, alive and kicking.

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**Conflict of interest statement**

None.

**Data Availability**

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