Antiviral drugs against SARS-CoV-2

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ABSTRACT

The use of antiviral drugs represents an important progress in the therapeutic management of COVID-19, leading to a substantial reduction of SARS-CoV-2-related complications and mortality. In immunocompetent host, peak viral replication occurs around the symptom's onset, and it prolongs for 5 to 7 days that is the window of opportunity for giving an antiviral. Accordingly, early and rapid diagnostic of the infection in the outpatient clinic is essential as well as the availability of oral agents that can be easily prescribe. Remdesivir has demonstrated its efficacy in hospitalized patients requiring oxygen support and in mild/moderate cases to avoid the hospitalization, however, the intravenous administration limits its use among outpatients. Molnupiravir and nirmatrelvir/ritonavir are potent oral antiviral agents. In the present review we discuss the potential targets against SARS-CoV-2, and an overview of the main characteristics and clinical results with the available antiviral agents for the treatment of SARS-CoV-2.

Keywords: oral antivirals, intravenous antivirals, remdesivir, molnupiravir, nirmatrelvir/ritonavir

BACKGROUND

Coronavirus disease 2019 (COVID-19) continues to represent a major health concern worldwide with over 612 million people infected, of whom more than six million have died. The spectrum of infection severity depends on virulence of SARS-CoV-2 variants and underlying host risk factors.

By early January 2022 the B.1.1.529 (omicron) variant of SARS-CoV-2 has widely spread, even among groups with high levels of preexisting immunity. As of September 2022, omicron variants have been divided into five subvariants: BA.1 to BA.5, which are also subdivided into diverse sublineages based on additional mutations that change the genomic viral profile. The initial omicron subvariants, BA.1 and BA.2, are being progressively displaced by BA.5 in many countries. A recent study showed that infection by BA.5 subvariant was less likely among persons with previous SARS-CoV-2 infection, especially due to BA.1 or BA.2 variants, than among uninfected persons [1].

Immune dysregulation related to underlying diseases contributes to COVID-19 severity and immunomodulatory therapy has demonstrated beneficial effect on patients' outcome. In addition, numerous studies have shown that immunocompromised patients have a risk of suboptimal humoral immune response to SARS-CoV-2 vaccines, resulting in increased likelihood for severe illness [2–4].

Therefore, antiviral drugs for COVID-19 may represent a milestone in controlling the progression of the disease into more severe form, particularly in high-risk individuals, including elderly and those with comorbidities such as cancer, cardiovascular disease, and immunosuppression. This review provides a clinical practice overview of potential targets and current available antiviral agents against SARS-CoV-2.

PHARMACEUTICAL TARGETS AND ANTIVIRALS ACTIVE AGAINST SARS-COV-2

Targets for antiviral drugs include molecules involved in life cycle and/or pathogenesis of SARS-CoV-2 that can be divided into two categories: host-derived and viral-derived targets. Different host receptors and enzymes are used by SARS-CoV-2 to entry and maturate in the host cell and represent potential therapeutic targets. The major entry receptor is the angiotensin-converting enzyme 2 (ACE2) that interacts with the S1 subunit of spike protein. In the presence of transmembrane serine protease 2 (TMPRSS2), the S1 and S2 subunits of the spike protein are cleaved and S2 becomes activated to

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Table 1	Characteristics of main antiviral drugs acting against host–derived targets.		
Drug class	Compound name	Mechanism of action	Current evidence of efficacy
Drugs targeting the ACE Inhibitor pathway	Telmisartan Soluble recombinant ACE2/APN01	Interfering virus and host ACE2 interaction	Most of the clinical and epidemiological studies failed to establish a link between the use of ACEI or ARB and severe COVID-19. No soluble recombinant ACE2/APN01 has entered clinical trial as a candidate for COVID-19 treatment.
Transmembrane serine protease 2 (TMPRSS2) inhibitors	Camostat mesylate	Serine protease inhibitor blocking the conformational changes in spike protein (S2 subdomain) involved in the fusion of the viral with the cellular membrane	A double blind randomized controlled trial with 137 patients showed that camostat mesylate do not affect time to clinical improvement, progression to ICU admission or mortality in hospitalized patients with COVID-19 [23].
Furin inhibitors	Decanoyl-RVKR-CMK a1-antitripsin PDX	Blocking of the proteolytic cleavage site between S1/S2 subunits of the S protein on the viral surface, required for virus cell entry	No molecule of this class has so far entered a clinical trial for treatment of COVID-19.
Cathepsin L inhibitors	Gallinamide Telocinobufagin	Inhibiting of the cleavage at the S1/S2 site of the S protein that enables fusion of viral and cellular membranes	Combination therapy with remdesivir and Gallinamide A or telocinobufagin and molnupiravir has been proposed to optimize the effectiveness of antivirals and to reduce the risk of selection of resistant variants. Clinical and preclinical evidence is still required to assess a possible benefit.
Phosphatidilinositol 3-phosphate 5-kinase (PIKfyve) inhibitors	Apilimod	Blocking of the receptor-mediated endocytosis and the release of the viral genome from a vacuolated endosome	Although apilimod has entered a clinical trial against COVID-19 (NCT04446377), the results have not been published yet.

fuse the viral and host cells lipid bilayers, releasing the viral ribonucleoprotein complex into the cell [5]. An alternative entry pathway, in the absence of TMPRSS2, is via endocytosis. Once within the endosome, catepsin-L play the role of cleaving S1–S1 subunits leading to membrane fusion and releasing the viral RNA into the host cell cytoplasm. During the maturation of the new virions, in Golgi apparatus of the host cells, the S1-S2 subunits should also be cleaved and the main enzymes doing it are furins [6]. As SARS-CoV-2 replication occurs on host membranes, vesicular trafficking machinery is essential for the development of new virions and represents an additional target. The main drugs under development targeting host mechanisms implicated in viral cycle are shown in table 1, however, no one has achieved advanced clinical development yet.

The second group of antiviral drugs include those compounds that target proteins involved in life cycle and/or pathogenesis of SARS-CoV-2: 1) RNA-dependent RNA polymerase (RdRP) inhibitors, 2) viral protease inhibitors, and 3) maturation inhibitors. The main pharmacological characteristics of these molecules are shown in table 2.

Remdesivir is an RNA-dependent RNA polymerase (RdRp) inhibitor approved by the US FDA for the treatment of COV-ID-19 infection in hospitalized patients in October 2020. Because of its membrane-permeable backbone, remdesivir can easily reach the cytoplasm, where is converted to remdesivir monophosphate and remdesivir triphosphate. The final molecule is an analog of adenosine, and it is incorporated by the RdRP complex into the nascent RNA strands, resulting in termination of RNA synthesis and efficiently stopping viral replication. A recent metanalysis by Lee et al. analyzed the benefits of remdesivir in the treatment of hospitalized patients with COVID-19 [7]. Eight randomized trials with 10,751 participants were considered. The risk ratio of mortality comparing remdesivir vs control was 0.77 (95% CI, 0.5-1.19) in the patients who did not require supplemental oxygen; 0.89 (95% CI, 0.79-0.99) for nonventilated patients requiring oxygen; and 1.08 (95% CI, 0.88-1.31) in the setting of mechanical ventilation. Several observational and retrospective studies have supported these results and have suggested that early administration has been associated with significant reductions in mortality [8,9]. Currently, remdesivir is included as an effective drug in guidelines of different medical societies for hospitalized patients requiring oxygen support. For patients requiring invasive mechanical ventilation, clinical trials do not support the use of remdesivir, however a recent large retrospective analysis shows a signifi-

Table 2	Main pharmacological characteristics of compounds targeting proteins involved in life cycle and/or pathogenesis of SARS-CoV-2.

Compound name	Drug class	Route of administration	Dosing regimens	Most common adverse reactions
Remdesivir	Nucleoside analog	Intravenous	200 mg on Day 1, then 100 mg once daily	Diarrhea, rash, renal impairment, hypotension
Molnupiravir	Nucleoside analog	Oral	800 mg twice daily	Diarrhea, nausea, dizziness, embryo-fetal toxicity bone and cartilage toxicity, hypersensitivity to ingredients Male contraception is required during treatment and 3 months after the last dose
Nirmatrelvir/ritonavir	Protease inhibitor (nirmatrelvir)	Oral	300 mg nirmatrelvir plus 100 mg ritonavir twice daily	Diarrhea, dysgeusia, hypertension, myalgia, hypersensitivity to ingredients; hepatotoxicity

cant reduction of mortality also in this population [10]. In the opinion of the authors, the window of opportunity to use an antiviral drug in patients with severe COVID-19 does not depend on the oxygen support required but on the presence of active viral replication. For SARS-CoV-2, the window of viral replication is 5 to 7 days from symptoms onset in mild-moderate cases, but it can be longer for patients with severe COVID-19 and in immunosuppressed patients. Accordingly, in patients requiring hospitalization an antiviral should always be considered within the first 7 days disregarding the oxygen support. For patients with more than 7 days from symptoms onset, the decision should be based on the presence of active viral replication. Unfortunately, detection of genomic RNA by real-time PCR is not correlated with active viral replication and should not be used as a qualitative test. The evaluation of cycle threshold (Ct) as a surrogate marker has been proposed and a meta-analysis showed a higher mortality rate among hospitalized patients with a Ct <25 [11]. However, the validity of Ct in an heterogenous sample like respiratory secretions is controversial. Alternative, subgenomic RNA detection (qualitative measurement) and antigen test detecting nucleocapsid protein have shown a good correlation with active viral replication and should be considered to guide the antiviral prescription in patients with severe COVID-19 and more than 7 days from symptoms onset [12,13].

The duration of remdesivir treatment is 5 days, and longer treatment has not demonstrated additional benefit in general population. However, the experience in immunosuppressed patients is scarce, and failure after 5 days of treatment is not uncommon with potential to develop resistance [14]. In the future is necessary to evaluate the need of longer courses in this population. Remdesivir is contraindicated in patients with alanine aminotransferase (ALT) levels >5-times the upper limit of normality or severe hepatic dysfunction, and in patients with severe renal impairment (estimated glomerular filtration ≤ 30 mL/min). However, some clinical reports have demonstrated good tolerability an no nephrotoxicity in patients with eGF ≤30 mL/min. A clinical trial in this population is ongoing

and results will be available in the future.

Remdesivir has also been evaluated in non-hospitalized mild-moderate COVID-19 to avoid the progression of the infection. The PINETREE study is a randomized, double-blind, placebo-controlled trial involving non-hospitalized patients with COVID-19 at high risk of severe disease [15]. Among 562 patients included most subjects were unvaccinated adults with comorbid medical conditions (diabetes, obesity, and hypertension). The study demonstrated that 279 patients who received a 3-day course of intravenous remdesivir within 7 days of symptoms onset, had a relative reduction of 87% in the need of hospital admission or death compared to placebo. In addition, remdesivir reduced the risk of COVID-19 related medically visits or all-cause mortality by day 28. Nevertheless, it should be noticed that only 5% of patients in Remdesivir group and 3.2% in placebo group were immunocompromised. The need to administer remdesivir through the intravenous route has limited its use in the outpatient settings. An orally bioavailable formulation of remdesivir is currently under development and may have the potential to maximize its availability and prevent progression to severe disease.

Molnupiravir is a small-molecule prodrug of β-d-N4-hydroxycytidine (NHC), a ribonucleoside analog which is finally incorporated to viral RNA by RdRp. Molnupiravir prompts an accumulation of errors in the replicating virus, until the virus can no longer survive. It was originally discovered for Venezuelan equine encephalitis virus (VEEV) infection but was later found to have antiviral activity against several respiratory viruses, including influenza virus and SARS-CoV-2. In a phase-3 study of 1433 patients with mild-to-moderate COVID-19 and at least one risk factor for severe illness, treatment with molnupiravir within 5 days of symptoms onset reduced the chances of hospitalization and death by 30% compared with placebo [16]. This reduction rate is lower than the ones reported with remdesivir or nirmatrelvir/ritonavir in similar clinical trials. Accordingly, molnupiravir has been approved for use in patients with mild to moderate COVID-19 who are at high risk of progression to severe disease, and for whom alterative antiviral

therapies are not accessible or contraindicated. In addition, recent real-world data originating from a large cohort during Omicron BA.2 dominance confirms that molnupiravir reduces the risk of progression and mechanical ventilation [17]. Another recent real-world study conducted in Poland during the dominance of the Omicron variant evidenced that administration of molnupiravir in hospitalized patients within five days from symptoms onset resulted in reduced mortality and less frequent use of oxygen supplementation [18].

The first SARS-CoV-2 protease inhibitor with clinical evidenced of efficacy in preventing a progression to severe disease is nirmatrelvir/ritonavir. Nirmatrelvir inhibits Mpro (3CL protease), the main protease of SARS-CoV-2, which catalyzes the cleavage of viral polyproteins into nonstructural proteins that are essential for viral replication. It is combined with ritonavir, a potent cytochrome P450-3A4 inhibitor which prolongs nirmatrelvir half-life, supporting twice-daily administration. Nirmatrelvir/ritonavir has been authorized for treating mild-to-moderate COVID-19 in adults and children aged 12 years and older weighting at least 40 kg, who are at high risk for progression to severe COVID-19, including hospitalization and death. The EPIC-HR trial is a randomized, double-blind study of non-hospitalized adults with COVID-19 who were at high risk of progression to severe disease [19]. In the final analysis, 5/697 (0.7%) of patients who received nirmatrelvir/ritonavir within 5 days from symptoms onset were hospitalized up to day 28 post-randomization, compared to 6.5% of patients who received placebo, resulting in a relative risk reduction of 89% (p<0.0001). A population-based study on real world data from Israel shows that nirmatrelvir/ritonavir and adequate vaccination against SARS-CoV2 were associated with significant decrease in the rate of severe COVID-19 or mortality, compared to those not treated, and/or not vaccinated [20]. Of the 180,351 patients included, 4,737 patients were treated with nirmatrelvir/ritonavir, and 135,482 (75.1%) had adequate vaccination status. Nirmatrelvir/ritonavir appeared to be more effective in older patients, immunosuppressed patients, and those with underlying cardiovascular or neurological disease. In addition, a recent real-world retrospective study involving a large inpatients cohort during Omicron BA.2 variant domination confirmed high efficacy of nirmatrelvir/ritonavir in shortening viral replication, reducing disease progression, and preventing death [21]. It should be noted that patients from this cohort were correctly vaccinated and developed COVID-19 at least one month after vaccination. Recently, concerns have been raised about a rebound phenomenon with nirmatrelvir/ritonavir, whereby patients develop symptoms of COVID-19 after taking the drug [20]. A recent study found that rebound occurred in 0.8% of patients, resulted in mild symptoms without requiring additional COVID-19 therapy [22].

Ritonavir has significant and complex drug-drug interactions. Concomitant medications, including over-the-counter medicines, herbals, or recreational drugs, must be reviewed for their potential interactions prior to prescribing nirmatrelvir/ritonavir. Consultation with pharmacologist, COVID-19 guidelines, drug-drug interaction specialized websites, or the drug fact sheet is mandatory for providers prescribing this medication.

CHALLENGES IN CLINICAL PRACTICE

Early administration. The main challenge of antiviral therapies is that they should be administered while the viral replication occurs. This window in the case of SARS-CoV-2 are the first 5 days from symptoms onset in immunocompetent host. Early treatment, by reducing progression to hospitalization, might reduce long-term morbidity and mortality and reduce the burden on healthcare resources. For an early-intervention strategy to work, it is mandatory to accurately identify patients at greater risk of clinical deterioration. Phenotyping studies, incorporating both clinical features and immunological biomarkers and potentially using machine-learning techniques, may allow a better identification of patients requiring early antiviral therapy.

Vulnerable populations. Even with widespread COVID-19 vaccination uptake, specific risk groups remain particularly vulnerable to severe infection. Age ≥ 65 years, obesity, diabetes mellitus, chronic kidney disease, cardiovascular and neurological diseases but most specially the presence of 2 or more of these co-morbidities and being vaccinated >6 months prior to the acute episode are the most relevant risk factors associated with complications among vaccinated population and real-world experience with antivirals has demonstrated a beneficial effect in this population. In contrast, the evidence among patients with immunosuppression (e.g. patients under B cell-depleting therapies, active chemotherapy, or organ transplant) is scarce. These patients have a significantly longer viral shedding with consequences for the management of the patient (delay in chemotherapy), need of prolonged isolation periods, and risk of host evolving viruses leading to the emergence of new variants. It is of utmost important to investigate the efficacy of antivirals in this population and to define the most adequate duration of therapy and even the potential need to combine different antiviral strategies to shorten the viral shedding minimizing the risk of selecting resistant strains.

Pregnant women. Pregnant women are at a greater risk of severe COVID-19. While molnupiravir is not recommended in pregnancy, no data are available with remdesivir and nirmatrelvir/ritonavir safety profile in pregnancy or breastfeeding. These drugs may be considered in pregnant women with mild-to-moderate COVID-19 if one or more additional risk factors are present (e.g., body mass index >25, chronic kidney disease, diabetes mellitus, or cardiovascular disease).

Pediatric population. Remdesivir is approved for patients 28 days and older and weighing at least 3 kg with mild to moderate COVID-19 and at high risk for progression to severe COVID-19, including hospitalization or death. The use of oral antivirals for COVID-19 treatment in the pediatric group may be limited. Molnupiravir is contradicted in children due to potential bone and cartilage toxicity. The use of nirmatrelvir/

ritonavir is not authorized in pediatric patients younger than 12 years of age or weighing less than 40 kg. The clinical trial involving non-hospitalized children with COVID-19 aged 6–12 who are at risk of progression to severe disease is ongoing.

CONCLUSION

The introduction of COVID-19 vaccines has significantly reduced the incidence, the hospitalization rate and the mortality of SARS-CoV-2 infection. However, the emergence of new variants that escape from vaccine immunity, maintains the virus circulation and the risk of infecting vulnerable population that will require hospitalization. The availability of antivirals that have demonstrated a great reduction of the hospitalization rates among patients with mild-moderate COVID-19, particularly among vulnerable population, is one of the most important achievements in the COVID-19 management. Remdesivir, and nirmatrelvir/ritonavir have demonstrated the highest risk reduction and considering the oral formulation of nirmatrelvir/ritonavir, this is the first choice to treat COVID-19 in the community. In the future, it is necessary to enlarge the experience with antivirals in immunosuppressed patients, and to define the duration of therapy or even the need of combine different agents to faster reduce the viral load avoiding the selection of resistant strains.

CONFLICT OF INTEREST

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