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DRUG DISCOVERY CASE HISTORY

An overview of the preclinical discovery and development of remdesivir for the treatment of coronavirus disease 2019 (COVID-19)

Pasquale Pagliano[1], Carmine Sellitto[2], Giuliana Scarpati[3], Tiziana Ascione[4], Valeria Conti[2], Gianluigi Franci[5], Ornella Piazza[3] and Amelia Filippelli[2]

[1]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", Unit of Infectious Diseases, University of Salerno, Baronissi, Italy; [2]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", Unit of Pharmacology, University of Salerno, Baronissi, Italy; [3]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", Unit of Anesthesiology, University of Salerno, Baronissi, Italy; [4]Department of Medicine, Service of Infectious Diseases, Cardarelli Hospital, Naples, Italy; [5]Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Unit of Microbiology, University of Salerno, Baronissi, Italy

Abstract

Introduction: Remdesivir (RDV) is an inhibitor of the viral RNA-dependent RNA polymerases that are active in some RNA viruses, including the Ebola virus and zoonotic coronaviruses. When severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) was identified as the etiologic agent of the coronavirus disease 2019 (COVID-19), several investigations have assessed the potential activity of RDV in inhibiting viral replication, giving rise to hope for an effective treatment.

Areas covered: In this review, the authors describe the main investigations leading to the discovery of RDV and its subsequent development as an antiviral agent, focusing on the main clinical trials investigating its efficacy in terms of symptom resolution and mortality reduction.

Expert opinion: RDV is the most widely investigated antiviral drug for the treatment of COVID-19. This attention on RDV activity against SARS-CoV-2 is justified by promising in vitro studies, which demonstrated that RDV was able to suppress viral replication without significant toxicity. Such activity was confirmed by an investigation in an animal model and by the results of preliminary clinical investigations. Nevertheless, the efficacy of RDV in reducing mortality has not been clearly demonstrated.

Keywords
Coronavirus; COVID-19; Dexamethasone; Remdesivir; RNA polymerase

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1. Introduction

The inhibition of the RNA-dependent RNA polymerase (RdRp) is a relevant therapeutic target for many viruses, such as the human immunodeficiency virus (HIV) and hepatitis C virus (HCV), or other less common viruses, such as the Ebola virus (EBOV) [1]. Remdesivir (RDV), formerly known as GS-5734, is a 1-cyano-substituted adenine C-nucleoside ribose analog that inhibits the RdRp of many viruses. In human macrophages, RDV exposure causes an accumulation of the drug, which is then rapidly converted into its active triphosphate metabolite. The metabolite's levels tend to remain high even after a long period, as demonstrated by the half-life of 24 h after RDV removal.

RDV is a broad-spectrum antiviral agent that is active against filoviruses (EBOV, Marburg virus) and some paramyxoviruses (respiratory syncytial virus, Nipah virus, and Hendra virus). No cytotoxicity was observed after exposure to RDV. Although *in vitro* data suggest that RDV is effective against EBOV, a recent large trial failed to demonstrate an actual advantage compared to supportive therapy or monoclonal antibody administration [2,3].

The activity of RDV against both epidemic and zoonotic coronaviruses has been shown by *in vitro* studies that demonstrated a dose-dependent inhibition without relevant cytotoxicity after exposure to concentrations of up to 10 μM. In a study of mice, the administration of RDV reduced the severe acute respiratory syndrome (SARS)-CoV-induced lung pathology. In this model, all aspects of lung pathology, such as denuding bronchiolitis, perivascular accumulation of inflammatory infiltrates, and intra-alveolar edema, were ameliorated, as compared to vehicle-treated animals [4,5]. In another mouse model of Middle East Respiratory Syndrome (MERS), a valuable activity was observed after the administration of RDV and interferon-b, but clinical evidence is currently lacking [6].

The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease 2019 (COVID-19), which is currently causing a pandemic that has affected millions of people and caused numerous deaths. After the administration of RDV to the first patient detected to be affected by COVID-19 in the United States, a rapid disappearance of symptoms was observed [7]. Further comparative studies have reported

CONTACT Pasquale Pagliano ppagliano@unisa.it Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", Unit of Infectious Diseases, University of Salerno, Baronissi, Italy

a low impact on mortality but a favorable effect in terms of symptom disappearance [8].

Here, we report the results of preclinical investigations on RDV for SARS-CoV-2 infection, evaluating both the results derived from *in vitro* models and clinical evidence.

2. Coronavirus and outbreaks

The four genera of coronaviruses are classified as *Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronovirus*. The alpha and beta coronaviruses are known to cause diseases in humans. In the past, human coronaviruses were found to be involved mainly in a mild, self-limiting upper respiratory tract infection resembling a flu-like syndrome. Starting in 2002, three outbreaks of severe respiratory diseases caused by coronaviruses have been reported, all of which were considered to have originated from a zoonotic spillover of a bat coronavirus due to its similarities in the spike proteins of the alpha and beta coronaviruses [9].

Soon after the isolation of the SARS-CoV outbreak that began in Guandong in 2002, SARS-CoV-like viruses were found in horseshoe bats, palm civets, and a raccoon dog from wild-animal markets in the same province. Evidence suggests that these animals could be a source of human infections [10,11]. Although zoonotic transmission has been reported as the origin of SARS-CoV, its further diffusion has been caused by inter-human contacts, with respiratory droplets being the main vehicle for intra-human spreading. Healthcare workers performing procedures on patients with SARS are considered to be at high risk of infection due to the high infectivity of the virus [12].

Fever was the main symptom reported in patients with SARS, and an evolution towards respiratory failure, associated with pulmonary ground-glass opacities, was the main finding observed in severe cases. As reported after December 2004, 8096 cases of SARS were diagnosed. It had a case fatality rate of 9.6%, with the highest mortality reported among elderly and immunocompromised patients. Currently, there are no available data supporting the use of any specific antiviral agent for SARS. Steroids have been administered as supportive care for patients with severe respiratory failure due to the possibility of immune-mediated lung damage, but their use has been reported to be associated with late viral clearance from the respiratory tract. In addition, antivirals such as ribavirin did not demonstrate any effect [13,14]. SARS has vanished, and no cases have been reported since December 2004.

Starting in 2012, a novel coronavirus was identified in Saudi Arabia as the etiologic agent of the disease called MERS. This coronavirus spilled over from bats to dromedary camels and was later transmitted to humans as well. Transmission to dromedary camels started more than 30 years before the virus was identified in humans, as assessed by a retrospective study identifying neutralizing antibodies against MERS-CoV (or a putative MERS-like-CoV) in sera obtained from camels in 1983–1997 [15]. Based on the latest data from the World Health Organization (WHO), 2566 laboratory-confirmed cases and 882 deaths have been reported starting in April 2012. Most of the cases originated from the Arabian Peninsula and surrounding regions, and many clusters were identified. Transmission in healthcare settings has been documented extensively [16]. Diabetes mellitus, chronic renal failure, and previous pulmonary diseases were commonly reported among symptomatic patients. The clinical manifestations of MERS can range from an asymptomatic infection to severe pneumonia, which rapidly evolves to acute respiratory distress syndrome and other life-threatening complications. Fever, cough, and shortness of breath are the main characteristics of MERS. In addition to the respiratory manifestations, gastrointestinal symptoms, including diarrhea, have also been reported. No effective treatment is available for patients with MERS, and only supportive care is recommended. Oxygen therapy must be administered to reach a target SpO_2 of ≥90% in non-pregnant adults and ≥ 92–95 % in pregnant patients. Intensive supportive care must be provided in severe cases [17,18].

The COVID-19 pandemic is a difficult challenge for the scientific community because of the high diffusion rate via respiratory droplets and contact with aerosol-infected surfaces. The disease was discovered after an outbreak reported among people working in a “wet market” in Wuhan, suggesting zoonotic spillover as the origin of this new infection. It is postulated that the novel coronavirus originated from bats and was transmitted to an intermediate host, probably pangolin, and then to humans [19]. COVID-19 is transmitted through personal contact with respiratory droplets, and intra-hospital outbreaks have been reported. Based on the last available data, over 163 million cases have been detected, causing over 3 million deaths with an ongoing high incidence in the poor areas of India and Brazil (checked on May 16th, 2021) (Tab. 1).

COVID-19 has characteristics that are rarely reported in cases of pneumonia caused by other viral agents, considering

Table 1. Outbreaks of severe respiratory diseases caused by coronaviruses originating from zoonotic spills

		symptoms	case/fatality rate	any specific antiviral agent	supportive care
SARS	horseshoe bats; palm civets; raccoon dog	fever; respiratory failure, pulmonary ground-glass opacities	9.6% [9–13],	none	steroids
MERS	Bats; dromedary camels	fever, cough, shortness of breath, diarrhoea; severe pneumonia to ARDS	43,1% [20]	none	Not specific
COVID-19	Bats; pangolin	cough, fever, dyspnea, loss of gustatory and olfactory functions, gastrointestinal symptoms, headache; neurologic manifestations [stroke, encephalitis, and peripheral nerve inflammation]; ARDS	1,8% [text] 7,2% [21]	Remdesevir; casirivimab + imdevimab, made by Regeneron; bamlanivimab + etesevimab, made by Eli Lilly	dexamethasone, tocilizumab

the high diffusion rate and the significant mortality in selected at-risk populations. While the general pulmonary symptoms are quite indistinguishable from other etiologies of viral pneumonia, many other aspects deserve particular attention. In addition to the common symptoms (cough, fever, and dyspnea), loss of gustatory and olfactory functions, gastrointestinal manifestations, and headache have also been reported in some cases. Neurological manifestations, including stroke, encephalitis, diffuse leukoencephalopathy, venous sinus thrombosis, meningitis, and peripheral nerve inflammation, have also been described [20–25]. All these characteristics should be considered when suspecting COVID-19 in patients presenting with worsening respiratory symptoms to provide supportive care and limit intra-hospital diffusion. Severe cases evolve toward acute respiratory distress syndrome and require ventilator support. In some cases, signs of neurological involvement can be referred to as late complications of COVID-19. About 50% of the patients had relevant comorbidities, and about 20% of the cases reported bacterial coinfections at the time of intensive care unit admission. Patients who survived from severe COVID-19 have reported alterations in lung function that require rehabilitative treatment. Interestingly, the clinical manifestations in children are usually mild because of a less pronounced inflammatory response. Several antiviral treatments have been investigated for COVID-19 therapy and prophylaxis, but none have been shown to improve survival, as assessed by large prospective comparative studies. The repositioning of previously discovered drugs has been a widely adopted tool to identify new treatments for formerly unknown or poorly investigated diseases. When the COVID-19 pandemic emerged, several known molecules with antiviral activity were proposed, and RDV was the most investigated drug, receiving recommendations regarding its use by many scientific societies' guidelines [26–30].

3. RDV development and *in vitro* activity

During the last two decades, the occurrence of outbreaks due to newly discovered viruses, such as novel coronaviruses, or threatened RNA viruses, such as EBOV, led to an increase in the research on new antiviral agents. Through collaborative research among Gilead, the U.S. Centers for Disease Control and Prevention, and the U.S. Army Medical Research Institute of Infectious Diseases, RDV (GS-5734) was developed.

Research on these new antiviral agents that led to the discovery of RDV started with the screening of approximately 1000 small molecules with some activity on RNA viruses through the RdRp. All the molecules were nucleotide analogs; among them, a 1-cyano-substituted adenine C-nucleoside ribose analog (NUC) was found to have potential antiviral activity by inhibiting viral RdRp [31]. NUC analogs acting against RNA viruses need to be converted into an active triphosphate form into the infected cells to interact with the RdRp and consequently inhibit viral RNA synthesis. RDV demonstrated its activity against a broad range of non-segmented negative-sense RNA virus members of families such as *Filoviridae, Paramyxoviridae, Pneumoviridae*, and *Corononaviridae*. On the other hand, no *in vitro* activity of RDV has been reported against other non-segmented negative-sense RNA viruses such as the Lassa virus (*Arenaviridae*) and the Crimean Congo hemorrhagic fever virus (*Bunyaviridae*). It is well reported that RDV is active against the Marburg virus and several variants of the EBOV, Nipah virus, and respiratory syncytial virus, as assessed by investigations in primary human macrophages and endothelial cells [32,33]. (Table 2).

RDV is a diastereomer monophosphophoramidate prodrug of the adenine nucleoside analog GS-441524 that acts as a substitute for adenosine, which is essential for DNA and RNA synthesis. To be active, RDV needs to be converted into the triphosphate form (RTP), which is recognized by the viral RNA polymerase enzyme and is incorporated into the growing RNA chain (Figure 1).

Once RDV has been incorporated into the RNA chain, the presence of carbon-nitrogen groups contributes to the

Table 2. Further in vitro, pre-clinical, and clinical studies are needed to definitively determine the effectiveness of remdesivir in treating COVID-19; moreover, mathematical modeling and computer simulations can provide unexpected insights

RDV	In vitro	In vivo	Mathematical model
	activity against: SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-229E	Mouse model: highest concentration in the liver and in the kidney of RDV and its metabolites rhesus macaque model: lower lung infiltrates, lower viral levels	application of remdesivir could lengthen SARS-CoV -2 infections, no clinical benefit

Table 3 *In vitro* studies and PK data.

Authors	Model	Aim	Results
Hanafin [43]	Pharmacokinetic (PK) analyses using in vivo plasma PK data based on a previous study conducted in female Ces1c-/- mice	defining PK profiles of remdesivir and its metabolites under current clinical treatment regimens (200 mg loading dose + 100 mg daily maintenance doses)	The mouse PK model was used to predict human PK parameters
Wang [40]	in vitro, Vero E6 cells (ATCC-1586)	antiviral efficiency of ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, remdesivir (GS-5734) and favipiravir (T-705)	EC90 value of remdesivir was 1.76 μM, working concentration achievable in nonhuman primate models.
Ko [44]	In vitro, human lung cells (Calu-3 from human lung adenocarcinoma)	antiviral efficiency of 24 FDA-approved drugs	Half maximal Inhibitory concentration (IC50) was 1,3 μM
Sheahan [4]	multiple in vitro systems, including primary human airway epithelial cell cultures	antiviral potency and breadth of activity of GS-5734	prodrug GS-5734 can inhibit SARS-CoV and MERS-CoV replication with submicromolar IC50 values.

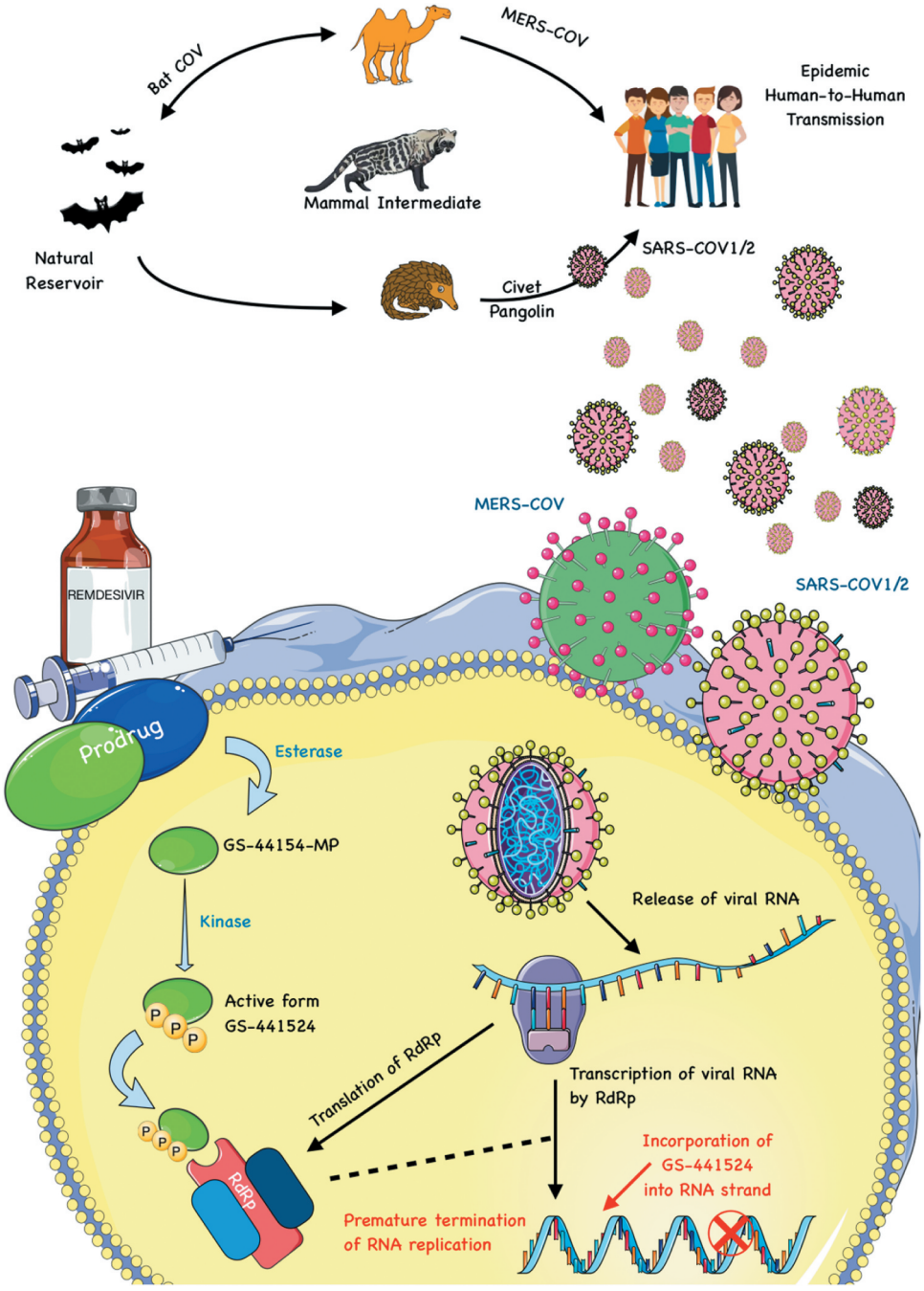

Figure 1 Lifecycle of a coronavirus as represented by the SARS-CoV-1/2 and MERS-CoV. SARS-CoV-2 enters target cells by binding with the S protein of the ACE2 receptor on the cell surface. Remdesivir specifically inhibits the activity of the viral RNA-dependent RNA-polymerase (RdRp), which is essential in viral replication. Upon entry into the cell, remdesivir is rapidly metabolized into nucleoside monophosphate (GS-441542 MP), which is then further processed into the active triphosphate form (GS-441524). GS-441524 is an adenosine triphosphate (ATP) analog and thus, it can be used as a substrate by the viral RdRp. GS-441524 outcompetes ATP for incorporation into the newly synthesised RNA strand, ultimately causing premature termination of the RNA product. The incorporation of GS-441524 causes delayed chain-termination downstream of this site.

distortion of the RNA's shape, which can receive only three additional nucleotides prior to being stopped. Thus, RDV inhibits viral replication [34]. In cellular models, RDV affects EBOV replication by targeting RdRp and finally inhibiting viral RNA synthesis. This activity against EBOV was also demonstrated in an *in vivo* model of EBOV-infected rhesus monkeys [35]. Another relevant aspect supporting RDV development as an antiviral agent is its selectivity for viral RNA polymerase, which makes it ineffective in inhibiting human RNA polymerase and human mitochondrial RNA polymerase. Based on current knowledge, NUCs are poor substrates for human polymerases, and incorporation into the growing viral RNA chain occurs at an increased rate, as compared to natural nucleotide pools [36,37].

RDV shows *in vitro* activity against a genetically diverse panel of human endemic and zoonotic coronaviruses, including SARS-CoV and MERS-CoV, and the causative

agents of the common cold, human coronavirus-OC43 and human coronavirus-229E [34]. It is relevant to highlight that coronaviruses have an enzyme that inactivates many analogs artificial nucleotides, though it is not able to remove RDV [38]. (Tab. 3)

As expected, inhibition of RNA chain growth is the main factor that enables RDV inhibition of SARS-CoV-2 replication. It was observed and demonstrated that RDV can determine a chain termination at position i+3. Indeed, the protein-RNA complex of SARS-CoV-2-RdRp expressed in insect cellular models highlighted the higher affinity of RDV for the enzyme task compared to ATP. This mechanism of action overlaps with the SARS-CoV-, MERS-CoV-, and SARS-CoV-2-RdRp complexes [32,39].

Following an *in vitro* study on infected Vero E6 cells, RDV was demonstrated to most actively inhibit SARS-CoV-2 among seven putative antiviral drugs with an IC50 of 770 nM and an IC90 of 1760 nM. The same study demonstrated that cytotoxic concentrations were achieved after concentrations of RDV above 100 mM were added to the experimental assay. RDV activity is measured through the quantification of viral copy numbers in the cell supernatant via quantitative real-time reverse transcription-polymerase chain reaction and visualization of viral nucleoprotein expression through immunofluorescence microscopy 48 h post-infection [40]. The same study suggested that chloroquine could also be evaluated in COVID-19 treatment, although its IC50 and IC90 were lower than those reported for RDV. However, it is important to note that mutations causing RDV resistance are also possible. Indeed, previous studies have reported that in murine hepatitis virus, an amino acid mutation alters the binding pocket of RDV and confers resistance to the treatment [41]. The main problem is that this region presents the homolog residues V557 and F480 in the SARS-CoV-2-RdRp complex, which can lead to the possibility of developing resistant or less effective mutants in the future (Tab. 3) [42,44].

4. In vivo activity of RDV against coronavirus

Pharmacokinetic studies in animal models have shown that RDV has excellent tissue and plasma concentrations, supporting the results of previous *in vivo* studies.

As previously assessed using a mouse model of SARS, the prophylactic administration of RDV ameliorated some characteristics of the disease compared to untreated controls, including virus titers in the lungs and viral antigen staining in lung sections. The same study demonstrated that many characteristic pathological findings, such as denuding bronchiolitis, perivascular accumulation of inflammatory infiltrates, and intra-alveolar edema associated with diffuse alveolar damage, had a lesser degree, as compared to untreated controls [4]. Moreover, a randomized controlled trial during the Ebola outbreak in 2019 demonstrated that the patient group receiving RDV combined with monoclonal antibodies had lower mortality compared to those receiving an intravenous administration of the triple monoclonal antibody, ZMapp [45].

Since the emergence of COVID-19, many experimental models have been investigated to assess the antiviral efficacy of repurposed drugs [30]. In a mouse model, the bolus administration of 20 mg/kg of RDV resulted in detectable blood levels of the drug up to 0.5 hours after dosing. Instead, its metabolites had different blood kinetics as the RTP was detectable up to 0.5 hours after dosing, while the monophosphate (RMP) and nucleotide (RT) forms were detectable up to 8 and 24 h after dosing, respectively, suggesting that daily dosing could be considered to achieve RDV therapeutic concentration [46]. The same model investigated the tissue concentrations of RDV and its metabolites (RMP, RTP, and RT) at different time points. RDV and its metabolites had the highest concentrations in the liver and kidneys, while RT and RTP had the highest concentrations in the lungs. RMP showed the highest concentration in the liver. Interestingly, the RTP:RMP ratio was 350-fold higher in the lungs than in the liver.

In a study on a rhesus macaque model of SARS-CoV-2 infection, the administration of RDV 12 h after SARS-CoV-2 infection resulted in lower lung infiltrates on X-ray and lower viral levels. Only one of the six RDV-treated animals showed mild respiratory symptoms, and all untreated animals appeared critically ill. Such a difference in terms of disease course was confirmed by radiographic examination, which demonstrated less pulmonary lobe involvement and infiltration in the animals receiving RDV. When the animals were euthanized, SARS-CoV-2 RNA was not detected in ten of the 36 specimens from the RDV-receiving animals and in three of the 36 specimens from the untreated controls, confirming the evidence of favorable lung concentrations of RDV and its metabolites. Histological examination revealed minimal pulmonary pathology in three of the six RDV-treated animals. Five of the six untreated animals demonstrated multifocal, mild-to-moderate interstitial pneumonia [47]. On the other hand, analysis of a mathematical model constructed on the data derived from the study by Williams et al. [47], which analyzed the kinetics of SARS-CoV-2 and RDV and compared the kinetics of this virus growth to those of influenza virus after oseltamivir administration, has questioned the ability of RDV to effectively inhibit SARS-CoV-2 replication [48].

As the antiviral effects of RDV do not report a significant increase in the mortality of COVID-19 patients, other studies available as preprints have also investigated the effect of early coadministration of RDV and monoclonal antibodies [49]. In a mouse model of C57BL/6 mice lacking carboxyesterase 1c (Ces1c (–/–)), the administration of RDV from 12 h before to 12 h after infection reduced weight loss, which was the main marker of coronavirus infection in mice. No effect was obtained when RDV was administered for 24 h or more after infection. Instead, strategies of early RDV administration were able to reduce lung involvement when administered up to 24 h after the establishment of infection. The SARS-CoV-2 therapy efficacy of the combination of RDV/C144+C135 mAb against was also evaluated in the same study, demonstrating a slight improvement in body weight loss with RDV/mAb treatment compared to the groups receiving monotherapy, which was evident when therapy was administered up to 5 days after infection. Lung pathology confirmed this observation since lower diffuse alveolar damage was observed in the mice receiving a combination of monoclonal antibodies and RDV [49].

5. Pharmacokinetics of RDV and drug interactions

RDV can be administered through the parenteral route. Meanwhile, an oral administration is not feasible due to its complete metabolism after the first-pass effect, although some metabolites, such as GS-441524 exhibited favorable oral bioavailability and effective bioactivation in mice. Furthermore, preliminary investigations suggest that coadministration with CYP inhibitors, such as cobicistat, can make this drug suitable for oral use. The intramuscular administration of RDV is not currently adopted because it leads to variable levels of blood and tissue concentrations of the drug. Instead, intravenous administration of RDV is rapidly metabolized by carboxylesterase 1 to the intermediate alanine metabolite (GS-704277), which is in turn converted to GS-441524 (Figure 2). The active metabolite GS-441524 has a long half-life (>24 h) and reaches a steady-state by day 4, as demonstrated in a paper reporting two phase I studies on the pharmacokinetics of single escalating and multiple intravenous doses of RDV. GS-441524 is not a substrate for major CYP enzymes and does not undergo multiple hepatic passes. This metabolite is converted into its triphosphate form, which is incorporated into the growing viral RNA chain. All these characteristics make RDV suitable for daily intravenous administration of 100 mg, following a loading dose of 200 mg. Carboxylesterase 1 is highly expressed in the liver and has poor activity in the type II pneumocytes in the lung, resulting in GS-441524 blood concentration 1000-fold higher than RDV concentration after 7 days of administration. Protein binding of the different RDV metabolites differs. Although RDV has moderate protein binding with a relatively low free fraction (12.1%), its metabolites report very low protein binding in plasma and free fraction values ranging from 85% to 127%, as assessed by a report on the drug by the European Medicines Agency. RDV reaches the highest concentrations in the kidneys, liver, and arterial walls [50–53].

Metabolism of RDV is mainly mediated by cytochrome p450 enzymes, and the metabolites are eliminated by the renal route and through feces. The organic anion-transporting polypeptide 1B1 and P-glycoprotein proteins are substrates for RDV. In addition, RDV can inhibit the CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, OATP1B1, and OATP1B3, multidrug resistance-associated protein 4, and sodium-taurocholate cotransporting polypeptides [52].

RDV appears to be less prone to significant drug interactions than the other drugs proposed for COVID-19 treatment [54]. A mathematical drug-drug interaction liability prediction model, which was based on *in vitro* and phase I data, confirmed the low potential for drug-drug interactions of RDV. However, CYP3A induction caused by rifampicin exposure is expected to reduce RDV levels by 30%, and inhibition of CYP3A can increase blood levels by only 4%. Moreover, an

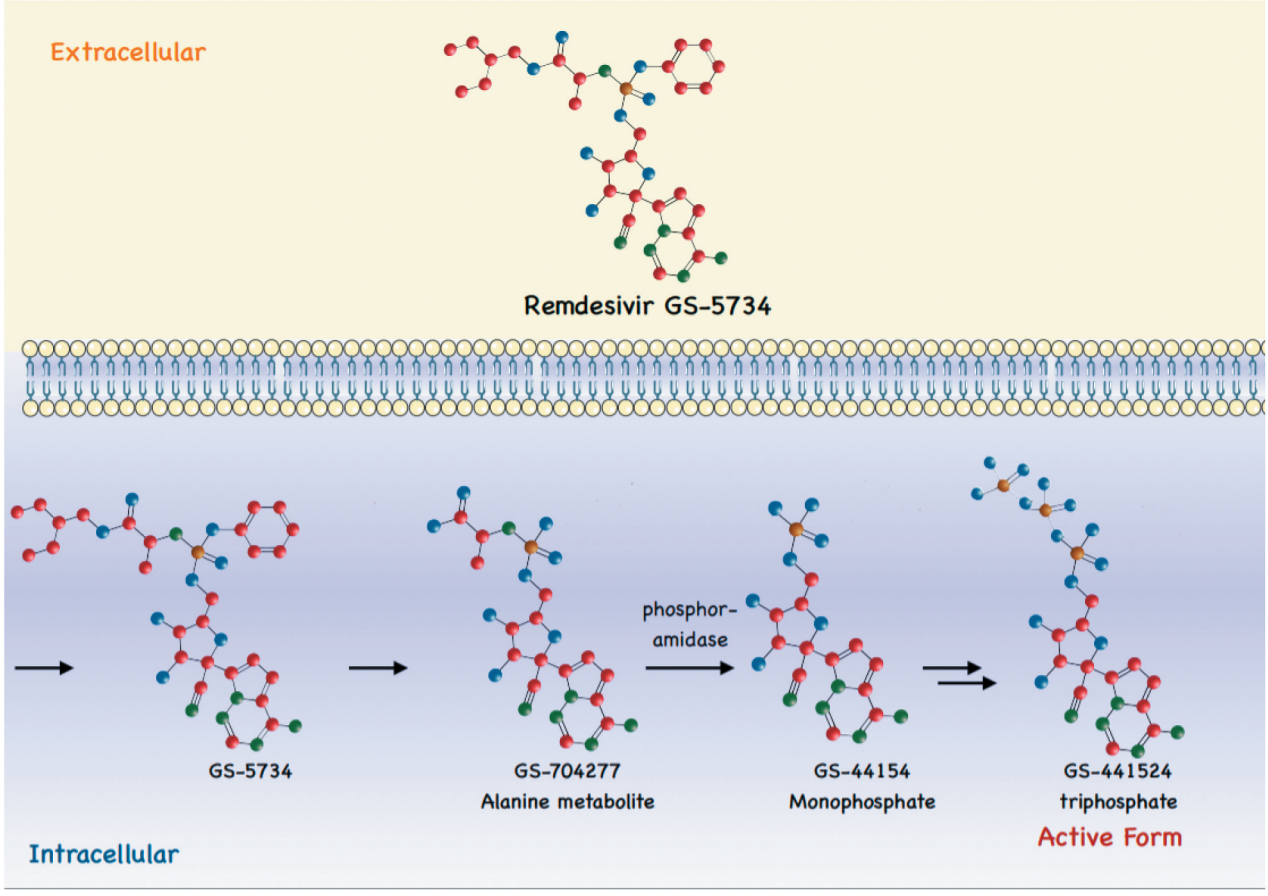

Figure 2 After entering the cell, the prodrug GS-5734 is metabolized into the nucleoside monophosphate form (GS-44154), through the synthesis of the intermediate metabolite GS-704277. GS-44154 undergoes further phosphorylation via the endogenous phosphorylation pathway, generating the analog form of the active nucleoside triphosphate GS-441524.

antagonistic effect of chloroquine on the intracellular activation and antiviral activity of RDV has been reported, making co-administration of these two drugs unsuitable, based on *in vitro* data [55]. Another relevant aspect in terms of drug interactions can be reported in selected populations, such as those receiving antiepileptic drugs (carbamazepine, phenobarbital, and phenytoin), which induce CYP 3A4 and theoretically reduce RDV levels [53].

6. Experience with RDV for COVID-19 treatment

A number of pharmacological agents have been proposed for the treatment of COVID-19, but, currently, only a few supportive treatments have demonstrated sufficient efficacy. RDV was administered to the first patient with COVID-19 in the United States, and its effectiveness was established based on the rapid symptom and viral load reduction. It was approved for COVID-19 treatment after a multicenter study evaluating the efficacy of a 100-mg dose of intravenously administered RDV demonstrated that a 5-day course of treatment resulted in an improvement in the time to recovery. Such promising results have not been validated by current investigations [8,56,57].

A recent meta-analysis involving randomized controlled trials (RCTs) published in relevant databases/websites (up to September 2020) and selected English-language publications failed to demonstrate RDV's favorable effect on mortality reduction. This study identified five comparative trials addressing RDV efficacy; all but one were multinational trials. The manufacturer funded two trials (both open-label trials). All trials evaluated the effect of daily RDV administration at a dosage of 100 mg over a period of 5–10 days. A cumulative analysis assessed that the studies comparing RDV treatment to standard of care did not demonstrate an advantage in terms of mortality, considering either a 5-day or a 10-day course of treatment. Similar discouraging results were reported by another study investigating both randomized trials and observational studies on RDV, raising questions on the efficacy of RDV for COVID-19 treatment, at least following the current schedule of treatment [58,59].

Different data favoring RDV use come from a systematic review and meta-analysis that evaluated the preliminary results of the WHO SOLIDARITY therapeutics trial and the final results of the National Institute of Allergy and Infectious Diseases trial. Analysis of results derived from RCTs highlighted an improvement in both 14-day and 28-day alive discharge rates (8.6% and 5.9%, respectively), but only the former reached statistical significance. Similar results were obtained after the mortality rate was evaluated, and a non-randomized study of intervention was analyzed. No improvement was observed in terms of outcome after RDV was administered during a 10-day period [60].

7. Expert opinion

The current COVID-19 pandemic is plaguing the world due to its high burden of deaths. No novel antiviral drugs are currently suitable for its treatment, and repurposed drugs have been investigated to hasten the discovery of therapeutic solutions. Currently, only massive vaccination programs can be considered to reduce the disease's considerable burden [61,62].

Since the granting of an emergency use authorization for RDV in May 2020, several comparative trials have been investigating its efficacy. Following this, in October 2020, the US Food and Drug Administration approved RDV (Veklury; Gilead Sciences) for use in COVID-19 treatment of adult and pediatric patients aged 12 years and older and weighing at least 40 kg requiring hospitalization [63]. Following approval by the Food and Drug Administration on November 20, 2020, the WHO issued a conditional recommendation against the use of RDV in hospitalized patients, regardless of disease severity, as large investigations did not demonstrate an improvement in terms of survival and other outcomes after RDV administration [64,65].

This drug was developed following the search for novel molecules that are active against RNA viruses, such as HIV and HCV, by targeting the RdRp. RDV is a prodrug that needs to be converted into its triphosphate form to be incorporated into the growing chain of viral RNA. The large number of studies on RDV was justified by the results of preliminary *in vitro* investigations on VERO E6 cells, which demonstrated that RDV reported the highest activity against SARS-CoV-2 with the lowest toxicity. RDV has the great advantage of being a selective inhibitor of SARS-CoV-2 RNA-polymerase, without relevant activity against human cellular and mitochondrial RNA polymerase.

This promising aspect of RDV activity reported in the *in vitro* model was confirmed in an *in vivo* model of Rhesus macaques, where RDV administration resulted in a low degree of pulmonary lesions, as assessed by X-ray examination, and a lower SARS-CoV-2 RNA concentration. However, the application of the *in vivo* model addressing RDV efficacy was questioned using a mathematical model constructed by applying the data derived from the study by Williamson et al. [45].

An excellent profile of tolerability and few drug interactions make RDV a suitable drug for use in humans. However, many concerns arise about its routine use, mainly due to its low efficacy in reducing the SARS-CoV-2 viral load, which finally results in a limited ability to reduce the overall morbidity and mortality of COVID-19. When the efficacy of RDV was assessed in RCTs, the real advantage of RDV was questioned. A meta-analysis including five RCTs investigating over 7000 COVID-19 patients failed to demonstrate that RDV administration was associated with an improvement in survival. The quality assessment revealed that the risk of bias was high for four RCTs, questioning the real value of this evidence. Other meta-analyses demonstrated that RDV improved clinical outcomes but did not reduce mortality in comparison to untreated patients [58,66,67]. These conflicting results are attributable to many factors (i.e., steroid use, age, immuncompromission, and timing of administration), which can influence the effects of antiviral treatments, as we have observed in the other etiologies of viral pneumonia. For example, ribavirin, which is effective in the treatment of infant respiratory syncytial virus bronchiolitis, does not provide a clear advantage when it is administered for RSV pneumonia in adults [68].

In conclusion, treatment of COVID-19 cannot be based only on RDV administration due to important considerations, including its low antiviral efficacy, confirmed by the mathematical model, and the lack of solid data assessing RDV efficacy derived from high-quality RCTs. However, the data demonstrating efficacy in terms of reduction of the time to clinical improvement suggest new well-structured studies to assess its role in the reduction of the time of hospitalization and costs associated with COVID-19.

Abbreviations

COVID-19: coronavirus disease 19
RDV: Remdesivir
RdRp: RNA-dependent RNA polymerase
HIV: Human Immunodeficiency Virus
HCV: Hepatitis C Virus
EBOV: Ebola virus
SARS-CoV: Severe Acute Respiratory Syndrome
MERS: Middle East Respiratory Syndrome
BatCoV: bat coronavirus
WHO: World Health Organization
ARDS: acute respiratory distress syndrome
CDC: Centers for Disease Control and Prevention
NUC: 1′-cyano-substituted adenine C-nucleoside ribose analogue
CN: carbon-nitrogen
qRT-PCR: quantitative real-time RT-PCR
NP: virus nucleoprotein
RTP: triphosphate form
RMP: monophosphate form
RT: nucleotide form
CES1: carboxylesterase 1
PKs: pharmacokinetics
EMA: European Medicines Agency
MRP4: multidrug resistance-associated protein 4
FDA: Food and Drug Administration

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Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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