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Remdesivir: From Ebola to COVID-19

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ABSTRACT

Human coronaviruses (HCoV) were discovered in the 1960s and were originally thought to cause only mild upper respiratory tract diseases in immunocompetent hosts. This view changed since the beginning of this century, with the 2002 SARS (severe acute respiratory syndrome) epidemic and the 2012 MERS (Middle East respiratory syndrome) outbreak, two zoonotic infections that resulted in mortality rates of approximately 10% and 35%, respectively. Despite the importance of these pathogens, no approved antiviral drugs for the treatment of human coronavirus infections became available. However, remdesivir, a nucleotide analogue prodrug originally developed for the treatment of Ebola virus, was found to inhibit the replication of a wide range of human and animal coronaviruses *in vitro* and in preclinical studies. It is therefore not surprising that when the highly pathogenic SARS-CoV-2 coronavirus emerged in late 2019 in China, causing global health concern due to the virus strong human-to-human transmission ability, remdesivir was one of the first clinical candidates that received attention. After *in vitro* studies had shown its antiviral activity against SARS-CoV-2, and a first patient was successfully treated with the drug in the USA, a number of trials on remdesivir were initiated. Several had encouraging results, particularly the ACTT-1 double blind, randomized, and placebo controlled trial that has shown shortening of the time to recovery in hospitalized patients treated with remdesivir. The results of other trials were instead negative. Here, we provide an overview of remdesivir discovery, molecular mechanism of action, and initial and current clinical studies on its efficacy.

1. Introduction

Coronaviruses (CoV) are members of the family *Coronaviridae* in the order *Nidovirales*, and comprise a large number of enveloped, positive-sense single-stranded RNA viruses causing a variety of diseases in animals and humans [1]. CoV have the largest identified RNA genomes (typically ranging from 27 to 32 kb) containing multiple open reading frames with an invariant gene order: a large replicase-transcriptase gene preceding structural (S-E-M-N) and accessory genes [1,2]. On the basis of their phylogenetic relationships and genomic structures, CoVs are subdivided in four genera: alpha-, beta-, gamma- and delta-coronavirus; among these, alpha- and beta-CoVs infect only mammals [1,2].

Human coronaviruses (HCoV) were discovered in the 1960s and were originally thought to cause only mild upper respiratory tract diseases (10–30% of all common colds) in immunocompetent hosts [2]. However, public awareness of HCoVs has changed considerably since the beginning of this century, with the SARS (Severe Acute Respiratory Syndrome) epidemic in 2002 and the MERS (Middle East Respiratory Syndrome) outbreak in 2012, two zoonotic infections that resulted in mortality rates of approximately 10% and 35%, respectively [1].

At the end of 2019 a novel HCoV, named SARS-CoV-2, emerged in China, causing a new disease referred to as COVID-19 (coronavirus disease 19) [3]. COVID-19 clinical features vary, ranging from an asymptomatic state to a state with respiratory symptoms that, in a subset of patients, may continue to deteriorate and may progress to interstitial pneumonia, acute respiratory distress syndrome (ARDS) often requiring mechanical respiration, multi organ dysfunction and, eventually, death [4]. This progression is associated with an extreme increase in the production of pro-inflammatory cytokines and chemokines [5]. SARS-CoV-2 turned

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out to spread more efficiently than SARS and MERS coronaviruses, making extremely difficult to contain it worldwide, and wreaking havoc on global public health and economy.

Given the dimension of the COVID-19 pandemic, in the past months considerable efforts have been directed towards the repurposing of FDA-approved drugs to eliminate extensive safety testing required for novel drugs. So far, only one clinically available antiviral drug has been approved by US health authorities for COVID-19: remdesivir.

1.1. The discovery of remdesivir

Remdesivir (GS-5734), an adenosine analogue prodrug (Fig. 1) developed by Gilead Sciences, emerged from a collaboration between Gilead Sciences, the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in the attempt to identify broad-spectrum small-molecule antiviral drugs effective against RNA viruses with global pandemic potential, including SARS and MERS coronaviruses and in particular Ebola virus (EBOV), driven by the EBOV outbreak in 2014 [6]. A library of approximately 1.000 molecules, mostly nucleoside analogues, was collected based on prior knowledge of therapeutic agents effective against RNA virus infections [7]. As nucleosides usually suffer from low cell permeability, the library was mostly composed of modified nucleosides such as monophosphate, ester, and phosphoramidate prodrugs, which are usually more permeable and, once inside the cell, are metabolized to liberate the active compound [8].

Remdesivir is a phosphoramidate prodrug of the C-adenosine analog GS-441524 that is metabolized within cells into the alanine metabolite (GS-704277) and further processed into the monophosphate derivative and ultimately into the active nucleoside triphosphate (NTP) derivative, which is substrate-competitive with ATP for incorporation by the viral RNA-dependent RNA polymerase, resulting in inhibition of viral RNA synthesis (Fig. 2) [7,9,10].

Remdesivir has demonstrated broad-spectrum antiviral activity against RNA viruses belonging to different families, including *Paramyxoviridae* (respiratory syncytial virus, Nipah virus and Hendra virus), *Coronaviridae* (SARS-CoV, MERS-CoV and bat coronaviruses), as well as *Filoviridae* (Ebola virus) *in vitro* and in preclinical studies [6,11–14]. In particular, in nonhuman primates inoculated with MERS-CoV remdesivir treatment reduced lung virus levels and lung damage [15]. During the recent Ebola virus epidemic in the Democratic Republic of Congo, remdesivir was included in a randomized, controlled trial of selected therapeutics in EBOV patients (NCT02818582); since in the midstudy primary analysis, remdesivir treatment was found inferior to antibody-based therapy with respect to mortality, the remdesivir intervention arm was terminated [16]; however, this study provided an initial insight into the safety profile of the drug.

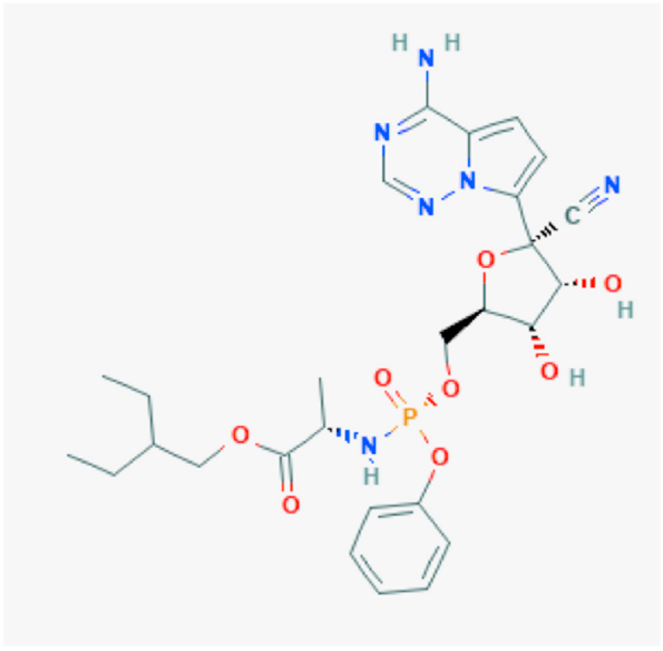

Fig. 1. Structure of remdesivir. Image from National Center for Biotechnology Information (2020). PubChem Compound Summary CID 121304016, Remdesivir. https://pubchem.ncbi.nlm.nih.gov/compound/Remdesivir.

1.2. Remdesivir and SARS-COV-2

SARS-CoV-2 is a positive-sense single-stranded RNA virus, sharing 79.6% sequence identity to SARS-CoV [17,18].

After virus entry into cells, the positive-sense RNA genome serves as a direct template for protein translation by the host ribosomes of two large open reading frames, ORF1a and ORF1b, into the polyproteins pp1b and pp1ab. Pp1b and pp1ab are co-translationally and post-translationally processed into different non-structural proteins (nsps) that form the multi-subunit replication/transcription machinery, containing several nsps, including the RNA-dependent RNA polymerase (RdRp), the helicase and the exonucleaseN proteins [1,20].

A key component of this complex is the RdRp, also known as nsp12, which contains the canonical viral RdRp motifs in its C-terminal part and, employing a primer-dependent initiation mechanism, catalyzes the synthesis of viral RNA, thus playing a central role in SARS-CoV-2 replication and transcription (Fig. 3) [1,19,20]. However, in order to function effectively nsp12 requires accessory factors including nsp7 and nsp8 that increase RdRp template binding and processivity [20,21]. The replicase complex is quite unique to CoVs and it is used not only to transcribe full-length negative and positive strand RNAs, but also subgenomic negative strand RNAs, and a 3′-co-terminal set of nested subgenomic mRNAs [1,20–22]. It is likely that additional viral nsp subunits are necessary to carry out the full repertoire of transcription/replication activities; however, so far the nsp12-nsp7-nsp8 complex represents the minimal complex required for nucleotide polymerization [21,23]. The Cryo-EM 3D structure of the polymerase complexed with nsp7 and nsp8 has now been solved [21,23], and the site and mechanism of the binding of remdesivir have been elucidated [23,24]. The SARS-CoV-2 polymerase consists of a right hand RdRp domain, which retains the canonical structure of the viral polymerase family containing the conserved "palm, fingers, and thumb" subdomains, and is connected by an interface domain to a nidovirus-specific N-terminal extension domain that presents the architecture of a nidovirus RdRp-associated nucleotidyl-transferase (NiRAN), and is followed by an N-terminal beta-hairpin domain (Fig. 3A). The complex also contains one nsp7 and two nsp8 molecules, which dramatically increase the binding of the template-primer RNA to nsp12 [23,24]. The RdRp active site is configured as in other viral RNA polymerases (Fig. 3). In the current model, similarly to other viral RNA polymerases, the primer-template entry, the NTP entry, and the nascent-strand exit paths converge in a central cavity where different RdRp motifs mediate template-directed RNA synthesis (Fig. 3) [23]. The RdRp subdomains bind the template RNA and, after selection of the appropriate nucleoside triphosphate, bind the NTP forming a phosphodiester bond, thus extending the 3′-end of the nascent viral RNA chain with the incoming nucleotide [23,25].

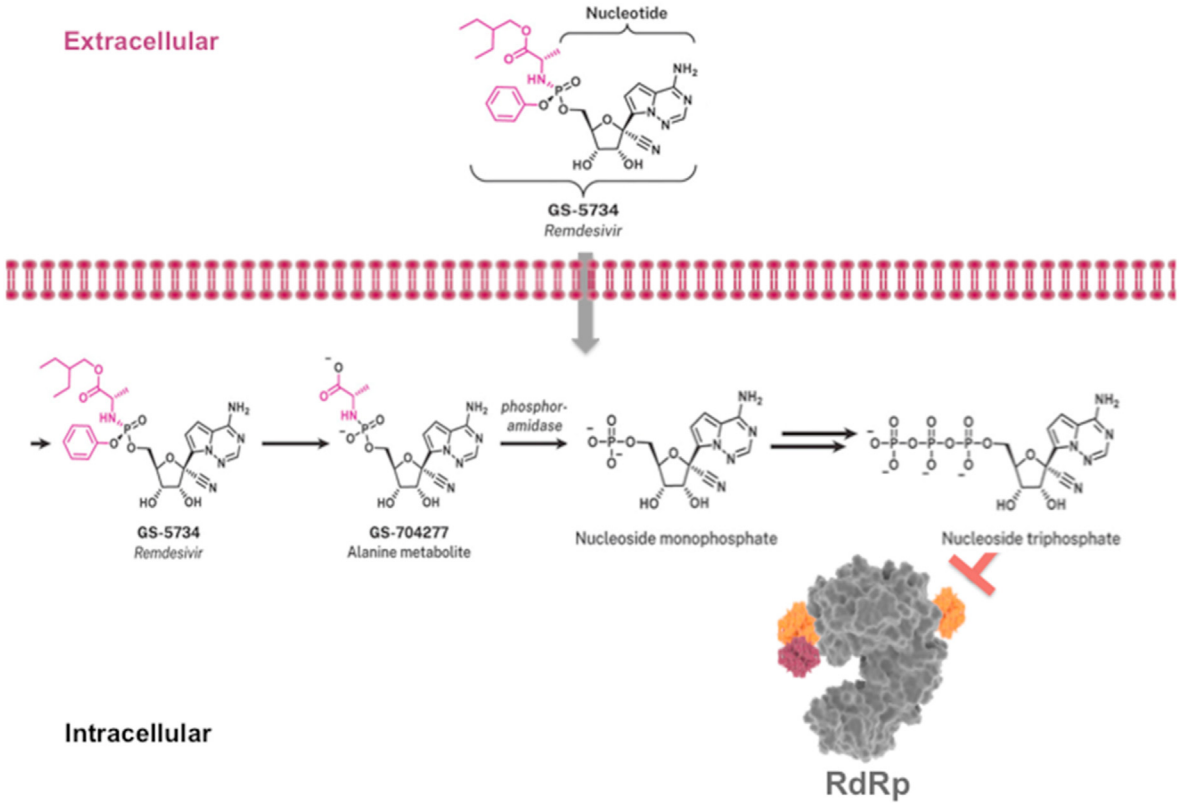

Fig. 2. Intracellular processing of remdesivir (GS-5734). Upon diffusion into the cell, remdesivir (GS-5734), the aryloxy phosphoramidate (purple) prodrug of GS-441524 monophosphate, is metabolized into the nucleoside monophosphate form, via a sequence of steps leading to the synthesis of the intermediate alanine metabolite GS-704277, which is further hydrolyzed by phosphoramidase-type enzymes to liberate the nucleoside monophosphate (modified from Eastman et al. [7]). The nucleoside monophosphate undergoes further phosphorylation events via the endogenous phosphorylation pathway, generating the active nucleoside triphosphate analogue form that can then be mis-integrated into viral RNA by the viral RNA-dependent RNA polymerase (RdRp), inducing delayed chain termination (for more details see Eastman et al. [7]). Nsp8 is shown in yellow and nsp7 in magenta.

As indicated above, the active trisphosphate form of remdesivir competes with ATP for binding to RdRp, and once incorporated, inhibits the RdRp by delayed chain termination. The active form of the drug was shown to display high selectivity over its natural ATP counterpart [10] and to adopt a not 'classic' chain termination mechanism, causing termination after a further incorporation of up to three nucleotides [10]. The current model suggests steric hindrance by remdesivir as a likely reason for termination, disturbing the positioning of the RNA and thus hampering the translocation to the next position [10,26].

Clearly, being distinct from the host cell transcriptional machinery, the viral RdRp is a primary target for anti-coronavirus drugs [10,24]. The development of nucleoside-based therapeutics for CoV infections has been hampered by the presence of an exoribonuclease (ExoN, nsp14) that acts as a "proofreading" enzyme correcting errors in the RNA sequence [27], thus potentially limiting the effects of analogues. However, the not 'classic' chain termination mechanism of remdesivir, has important implications for the proofreading activity of CoV polymerases, protecting the incorporated drug from ExoN-mediated excision [28].

1.3. Remdesivir in the treatment of COVID-19

Remdesivir was identified early in 2020 as a promising therapeutic candidate for COVID-19 because of its ability to inhibit SARS-CoV-2 *in vitro* [29]. Using qRT-PCR quantification of viral RNA in infected Vero E6 cells, Wang et al. found that remdesivir is a potent inhibitor of SARS-CoV-2 replication with an $EC_{50} = 0.77$ μM and a high selectivity index (SI > 129.87) [29]. In a later study, Pruijssers et al. confirmed that remdesivir potently inhibited SARS-CoV-2 replication in Calu3 human lung cells ($EC_{50} = 0.28$ μM) and in primary human airway epithelial cultures ($EC_{50} = 0.01$ μM) [30]. This study showed a lower potency of remdesivir in established human and monkey cell lines, due to their lower metabolic ability to activate the compound. Interestingly, a recent study on remdesivir metabolism evidences a differential expression of the prodrug bioactivating enzymes (CES1/CTSA/HINT1) in various tissues, with low expression in type II pneumocytes in the lung, and high expression in the GI tract, liver and kidneys, likely explaining the different range of EC_{50} values of the drug *in vitro* [31].

In addition to *in vitro* studies, treatment of mice infected with a chimeric SARS-CoV virus encoding the SARS-CoV-2 RdRp decreased viral loads in the lungs and improved pulmonary function as compared with vehicle-treated animals [30]. The efficacy of remdesivir was also demonstrated in a rhesus macaque model of SARS-CoV-2 infection [32]. In this study, differently from vehicle-treated macaques, animals treated with remdesivir did not show signs of respiratory disease; in addition, they showed reduced pulmonary infiltrates on radiographs and reduced virus titres in bronchoalveolar lavages 12 h after the first dose, indicating that treatment with remdesivir initiated early during infection had a clinical benefit in SARS-CoV-2 infected macaques [32].

These findings, along with the safety profile of remdesivir in the clinical trial assessment against EBOV [16], prompted the evaluation of remdesivir as a potential therapeutic drug for repurposing against the SARS-CoV-2 pandemic.

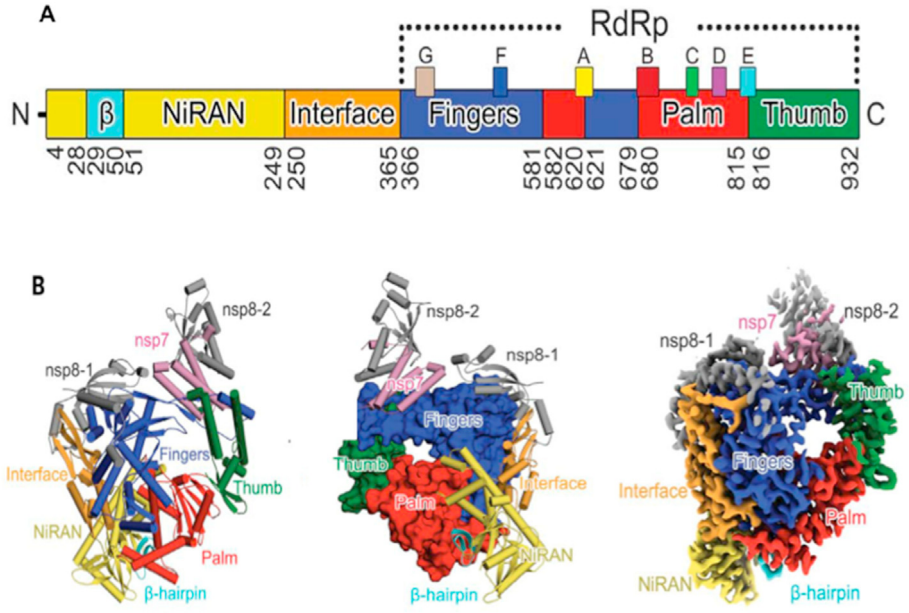

Fig. 3. Structure of the SARS-COV-2 RNA-dependent RNA polymerase (RdRp). The polymerase (nsp12) represented in complex with the non-structural proteins nsp7 and nsp8 (modified from Gao et al. [23]; Protein Data Bank accession number PDB 6M71). **A**, Organization of nsp12 domains. Domain borders are indicated with the amino acid numbers. The RdRp domain (residues S367 to F920, indicated by dots) is preceded by a nidovirus specific RdRp-associated nucleotidyltransferase domain (NiRAN, yellow). The RdRp and NiRAN domains are connected by an interface domain (orange). The polymerase domain is composed of three subdomains: fingers (blue), palm (red), and thumb (green), maintaining the viral polymerase family architecture. The colored rectangles in the RdRp domain represent the polymerase motifs. **B.** Ribbon structures of SARS-COV-2 virus nsp12 protein in three perpendicular views (domains are colored as in A). One nsp12 monomer is represented in complex with one nsp8 monomer (nsp8-1) and one nsp7-nsp8 pair (nsp8-2); nsp8 is shown in gray and nsp7 in pink. The RdRp active site is formed by motifs A to G and is configured as in other viral RNA polymerases: motif A contains the classical divalent cation-viral binding D618 residue; motif C contains 3 catalytic residues (759–761). In this structure, similarly to other viral RNA polymerases, the primer-template entry, the NTP entry, and the nascent-strand exit paths converge in a central cavity where the RdRp motifs described in A mediate template-directed RNA synthesis. The RNA template enters the active site through a groove clamped by motifs F and G in the fingers subdomain (see panel A), and the product-template hybrid leaves the active site through the RNA exit tunnel at the front side of the polymerase (for more details see Gao et al. [23]).

On January 20, 2020, a 35-year-old man, later confirmed as the first positive case of COVID-19 in the USA, was admitted to urgent care clinic in Snohomish County, Washington and was given remdesivir under compassionate use access; the patient clinical condition improved the next day, but the interpretation of remdesivir impact was difficult, due to concurrent treatment with anti-inflammatory drugs and antibiotics [33]. Over the past months a series of studies, summarized by Eastman et al. [7], have been launched to investigate the effectiveness of remdesivir, alone or in combination with other drugs, against COVID-19, including the WHO SOLIDARITY trial, a four-arm trial comparing remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta-1a, and chloroquine or hydroxychloroquine (ISRCTN83971151), the open-label, randomized interventional DisCoVeRy trial (NCT04315948), and the multicenter retrospective REMDECO-19 trial (NCT04365725) in Europe. In the US the National Institute of Allergies and Infectious Diseases (NIAID) initiated on February 21, 2020 the Adaptive COVID-19 Treatment Trial (ACTT-1), a double-blind, randomized, placebo-controlled phase 3 trial to evaluate the safety and efficacy of remdesivir compared with a placebo-control (NCT04280705). However, up to now, these studies have produced conflicting results.

An early analysis of 53 people seriously ill with COVID-19 in the United States, Canada, Europe and Japan who were given remdesivir raised initial hopes, since 68% of the patients showed a clinical improvement when given the drug; however, the study did not include a randomized control group [34].

No significant benefit was instead found in a randomized placebo-controlled trial of intravenous remdesivir conducted in China starting with 236 patients with COVID-19 [35]; this study, though, could not exclude clinically meaningful differences following remdesivir treatment since the trial was halted early, due to the fact that the China outbreak subsided.

On April 29 Gilead Sciences released the results from a study of 397 people (NCT04292899) showing that remdesivir diminishes to a modest degree the time to recovery for people hospitalized with COVID-19, raising new hopes; however, since the study lacked a control group, it was impossible to conclude with any certainty whether the drug had worked. On the same day, NIAID announced preliminary results from the multinational ACTT-1 trial on intravenous remdesivir in 1062 adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement (NCT04283481) [36]. The final report of the study was published on October 8, and showed that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with COVID-19 and had evidence of lower respiratory tract infection [37]. Of the total 1062 patients who had undergone randomization 541 had been assigned to remdesivir and 521 to placebo; 15% were categorized as having mild-to-moderate disease, 85% as having severe disease. The primary outcome considered was the time to recovery, which was the first day, during the 28 days after enrollment, in which the patient met various criteria of evaluation, from total recovery to death. The study concluded that remdesivir was superior to placebo in shortening the time to recovery in the patients: a median of 10 days in hospital (95% confidence interval [CI], 9 to 11), as compared with 15 days (95% CI, 13 to 18) for those assigned to the placebo group (rate ratio for recovery: 1.29; 95% CI, 1.12 to 1.49; $P < 0.001$). A different open-label, randomized multi-center clinical trial (NCT04292899) comparing two remdesivir courses in patients with severe COVID-19 who did not require mechanical ventilation at baseline found that the outcomes of 5-day and 10-day regimens of remdesivir were not significantly different [38].

Based on these findings [36], on May 1, 2020 the Food and Drug Administration (FDA) made remdesivir (*trademark name* VEKLURY®) available under an emergency-use authorization (EUA) for the treatment of adults and children with severe COVID-19 disease in the United States [39].

However, the mortality rate recorded in the ACTT-1 study, even if lower in the patients treated with remdesivir, remained high: Kaplan-Meier estimates of mortality at day 15 after enrollment were 6.7% with remdesivir and 11.9% with placebo (hazard ratio, 0.55; 95% CI, 0.36 to 0.83); at day 29 they were 11.4% and 15.2% in the two groups respectively (hazard ratio, 0.73; 95% CI, 0.52 to 1.03). The differences in mortality between the two groups varied considerably according to baseline severity [37].

Encouraging results for the survival of remdesivir-treated patients came from a recent study that included a comparative analysis of the Phase 3 SIMPLE-Severe trial and a real-world retrospective cohort analysis of patients with severe COVID-19 (NCT04292899 and EUPAS34303) [40]. The authors showed that, by day 14 after enrollment, remdesivir was associated with both an improvement in clinical recovery, and a 62% reduction in the risk of mortality compared with standard-of-care treatment [7.6% of patients in the remdesivir-cohort had died versus 12.5% in the non-remdesivir-cohort (adjusted odds ratio 0.38, 95% CI: 0.22–0.68, $p = 0.001$)]. Unfortunately, this analysis had several limitations linked to the nature of the trial, and requires confirmation. It should also be mentioned that in a different study remdesivir was reported to be less effective in patients hospitalized with moderate COVID-19 pneumonia (pulmonary infiltrates and room-air oxygen saturation >94%) [41]. In an open-label study conducted at 105 hospitals in the United States, Europe, and Asia (NCT04292730), investigators enrolled 584 patients with confirmed COVID-19 infection, and moderate pneumonia; the patients were randomized 1:1:1 to a 10-day ($n = 193$) or 5-day ($n = 191$) course of remdesivir, or of standard care ($n = 200$). The results indicated that patients randomized to a 10-day course of the drug did not have a statistically significant difference in clinical status compared with standard care patients at 11 days after initiation of treatment; patients randomized to a 5-day course of remdesivir had a statistically significant difference in clinical status compared with those assigned to standard care, but the difference was modest and of uncertain clinical importance [41].

The effects of remdesivir use in patients with mild infection is still unexplored. However, an interesting new approach for the treatment of patients in the early stage of COVID-19 infection is a randomized, blinded, placebo-controlled, single- and multiple-dose clinical study, now in Phase 1b/2a, aimed at evaluating the safety, efficacy, and pharmacokinetics of remdesivir administered by inhalation (NCT04539262). Administering remdesivir by inhalation may also decrease adverse effects, as intravenous remdesivir can cause gastrointestinal symptoms (e.g., nausea, vomiting), elevated transaminase levels, and an increase in prothrombin time (https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/remdesivir/).

2. Conclusions and perspectives

As this article was about to be submitted, on October 15, 2020, the interim results of the WHO SOLIDARITY trial mentioned above have been made public in preprint form [42]. They described a global, open-label, multicentric randomized trial on more than 11.000 adults on remdesivir and a number of other drugs repurposed for COVID-19, and concluded that remdesivir had little or no effect on mortality and duration of hospital stay. Although it will be necessary to wait for the appearance of the publication of the final results of the trial, these interim negative results may increase uncertainty in the medical community. The fact that there are now several randomized clinical trials of remdesivir in hospitalized patients with differing results raises the question of whether the drug is less efficacious than initially hoped or whether the discrepancies are artifacts of study design choices.

The reasons for the discrepancies observed are probably manifold, one factor of prime importance being the different designs of the trials [43]. In particular, the timing of treatment with remdesivir during the course of the disease appears to be an important variable for clinical improvement; therefore, understanding the dynamic of SARS-CoV-2 infection is essential for the selection of the optimal patient population and optimal duration of therapy in order to maximize the drug potential. One additional point which should be considered to explain the discrepancies of the results on the efficacy of remdesivir is the possibility of mutations in the viral RNA polymerase that would make it remdesivir-resistant, as previously described for other coronaviruses introducing specific mutations in highly conserved residues within the fingers domain of the RdRp right-hand structure [28,44].

As it is becoming clear from the results described in this as well as in other contributions in this Special Issue, no drug tested so far, including remdesivir, appears to be a game changer. However, until an effective vaccine will be available, drugs that have shown some efficacy remain of critical importance in the direct fight against the SARS-CoV-2 virus. Future strategies are needed to evaluate whether cocktails of remdesivir with current and novel antiviral agents acting on different viral or cellular targets, and/or in combination with other therapeutic approaches will increase its efficacy. Currently the NIAID ACTT-2 trial (NCT04401579) is evaluating the activity of remdesivir in combination with modifiers of the immune response (e.g., the Janus kinase inhibitor baricitinib), while the ACTT-3 trial (NCT04492475) is aimed at evaluating the combination of interferon beta-1a and remdesivir compared to remdesivir alone.

Finally, following the EUA, on October 22, 2020, the FDA has approved VEKLURY for use in adults and pediatric patients 12 years of age and older requiring hospitalization [45], indicating that the drug had cleared more rigorous regulatory hurdles involving a more thorough review of clinical data and manufacturing quality since it was given emergency authorization in May 2020. Remdesivir is now the first antiviral drug approved to treat COVID-19.

Conflict of Interest

All authors declare no conflict of interest

Acknowledgments

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