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ABOUT COVER

Editorial Board Member of *World Journal of Clinical Cases*, Fan-Bo Meng, MD, PhD, Chief Doctor, Deputy Director, Professor, Department of Cardiology, China-Japan Union Hospital of Jilin University, Changchun 130000, Jilin Province, China. mengfb@jlu.edu.cn

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The primary aim of *World Journal of Clinical Cases* (*WJCC*, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

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SYSTEMATIC REVIEWS

Controversies' clarification regarding ribavirin efficacy in measles and coronaviruses: Comprehensive therapeutic approach strictly tailored to COVID-19 disease stages

George D Liatsos

ORCID number: George D Liatsos 0000-0002-8203-2748.

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George D Liatsos, Department of Internal Medicine, "Hippokration" General Hospital, Athens 11527, Attiki, Greece

Corresponding author: George D Liatsos, MD, PhD, Chief Doctor, Department of Internal Medicine, "Hippokration" General Hospital, 114 Vass. Sophia's Ave, Athens 11527, Attiki, Greece. geoliatsos@yahoo.gr

Abstract

BACKGROUND

Ribavirin is a broad-spectrum nucleoside antiviral drug with multimodal mechanisms of action, which supports its longevity and quality as a clinical resource. It has been widely administered for measles and coronavirus infections. Despite the large amount of data concerning the use of ribavirin alone or in combination for measles, severe acute respiratory syndrome, Middle East respiratory syndrome, and coronavirus disease 2019 (COVID-19) outbreaks, the conclusions of these studies have been contradictory. Underlying reasons for these discrepancies include possible study design inaccuracies and failures and misinterpretations of data, and these potential confounds should be addressed.

AIM

To determine the confounding factors of ribavirin treatment studies and propose a therapeutic scheme for COVID-19.

METHODS

PubMed database was searched over a period of five decades utilizing the terms "ribavirin" alone or combined with other compounds in measles, severe acute respiratory syndrome, Middle East respiratory syndrome, and COVID-19 infections. The literature search was performed and described according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Articles were considered eligible when they reported on ribavirin dose regimens and/or specified outcomes concerning its efficacy and/or possible adverse-effects. *In vitro* and animal studies were also retrieved. A chapter on ribavirin's pharmacology was included as well.

RESULTS

In addition to the difficulties and pressures of an emerging pandemic, there is the burden of designing and conducting well-organized, double-blind, randomized

manuscript

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controlled trials. Many studies have succumbed to specific pitfalls, one of which was identified in naturally ribavirin-resistant Vero cell lines in *in vitro* studies. Other pitfalls include study design inconsistent with the well-established clinical course of disease; inappropriate pharmacology of applied treatments; and the misinterpretation of study results with misconceived generalizations. A comprehensive treatment for COVID-19 is proposed, documented by thorough, long-term investigation of ribavirin regimens in coronavirus infections.

CONCLUSION

A comprehensive treatment strictly tailored to distinct disease stages was proposed based upon studies on ribavirin and coronavirus infections.

Key Words: COVID-19; Ribavirin; Severe acute respiratory syndrome-associated coronavirus; Middle East respiratory syndrome coronavirus; Measles; Treatment

Core Tip: Documented by accumulated data from coronaviruses studies and considering six identified pitfalls to which most of the studies fall victim, the early antiviral treatment is crucial for reducing viral load, transmission, and preventing disease severity. In coronavirus disease 2019, initiation of interferon-β plus ribavirin plus lopinavir/ritonavir is beneficial when targeting selected patients early during Stage I, and is a regimen that can be administered while the patient is at home in quarantine. If disease progresses to Stages IIb-III, corticosteroids (mainly pulsed methylprednisolone) are effective, but if they fail or extrapulmonary systemic hyperinflammation syndrome develops, tocilizumab (or anakinra) should be co-administered.

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INTRODUCTION

Ribavirin (RBV) was synthesized in 1972 in an attempt to identify ribonucleosides with the potential to affect enzymatic processes common to all viruses[1]. It is a broad-spectrum antiviral agent that exerts inhibitory activity against deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses both in tissue cultures and in animal models. RBV has been used clinically for almost five decades in numerous viral infections but its efficacy has only been established for chronic hepatitis C virus (HCV) infection[2-4], chronic hepatitis E virus (HEV) infection in transplant recipients[5], respiratory syncytial virus infection in infants and immunocompromised elderly patients[6] and for some of the large group of hemorrhagic fever viruses, mainly for Lassa and Crimean-Congo hemorrhagic fever virus[7].

During the 2018 measles outbreak, a number of adult cases suffering measles pneumonitis were hospitalized and treated with RBV. Because of the lack of specific guidelines on severe measles disease treatment in adults, we reviewed the literature on RBV dosing regimens and outcomes in any infectious disease. The most amount of clinical data available was for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), where RBV was widely utilized. There were, however, conflicting data on its efficacy due to the lack of randomized controlled trials (RCTs) thus probably resulting in suboptimal targeting and efficacy. While preparing the measles/RBV study for publication, the new coronavirus disease-19 (COVID-19) outbreak emerged, prompting us to focus heavily on COVID-19 treatment with RBV alone or in combination with other compounds. These conclusions are based principally on data already available regarding other coronaviruses. Since then, COVID-19 has emerged as a global health issue with the highest priority.

MATERIALS AND METHODS

PubMed (https://pubmed.ncbi.nlm.nih.gov/) was queried with the following search term combinations: ("measles", or "SARS", or "MERS", or "COVID-19", or "viral infection") AND ("ribavirin treatment") between 1971 and October 15, 2020. *In vitro*, animal and clinical studies, reviews, and meta-analyses in English language only were considered for data extraction. Each coronavirus was searched separately with the general term "treatment", retrieving a large amount of results. Furthermore, all review articles referring to COVID-19 treatment were searched, regardless of whether "ribavirin" was included in key words. Because those two last searches retrieved a very large number of relevant articles, it was not possible to read them from beginning to end. In downloaded files, we applied the computer software order "find on page" to locate instantly specific words within article's body and to assess evidence of our interest. After an exhaustive work-up of the retrieved literature, we limited studies to those reporting on RBV treatment regimens in coronaviruses. All the references within each eligible article were also evaluated carefully and downloaded if relevant. We considered eligible those manuscripts referring to RBV treatment alone or in combination and/or those reporting on its dose regimens, adverse effects, or outcomes. The literature search was performed and described according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines. After a thorough and repetitive revision of all relevant literature throughout five decades of RBV utilization, we identified six specific pitfalls into which both *in vitro* and *in vivo* studies fell; these result in misinterpreted conclusions and contradictory outcomes and thus misleading the scientific community and creating misconceptions about the true efficacy of RBV.

RESULTS

Rbv clinical pharmacology

RBV inhibits some DNA[8-10] and several RNA viruses[11-31] (Table 1). The degree of inhibition varies with the virus, the cell line used (RBV-resistant cell lines), and the parameters of antiviral activity examined. The mechanisms of action of RBV comprise direct antiviral effects[32-50] and indirect, immunomodulatory effects[51-71] (Table 2). RBV is accumulated and concentrated intracellularly through specific transporter proteins, including the equilibrative nucleoside transporters (ENTs) 1 and 2, concentrative nucleoside transporter 2, and multidrug resistance proteins 4 and 5[72-75]. Adenosine kinase converts RBV to RBV monophosphate (RMP), and subsequent phosphorylation of RMP yields the di- and triphosphorylated nucleotides, with RBV triphosphate (RTP) being the predominant metabolite[76].

RBV may act by perturbing intracellular nucleoside triphosphate pools. RBV is a structural analogue of guanosine, and RMP acts as a potent competitive inhibitor of the enzyme inosine monophosphate dehydrogenase, leading to reduced guanosine monophosphate biosynthesis and depletion of the guanosine triphosphate (GTP) pool [51-52]. Guanosine monophosphate is converted to the guanine metabolites GTP and deoxy-GTP, which are essential precursors for RNA and DNA synthesis, respectively [53]. GTP depletion has a major impact on host cell and viral gene expression as well as on viral replication[54-58]. Another direct mechanism is interference with the formation of the 5′ cap structure of viral mRNA (capping activity) [39]. The 5′-end of most cellular RNAs and some viral RNAs contains a 7-methylguanosine cap structure essential for RNA stability and translation[39]. RBV has the potential to interact with enzymes responsible for "capping" cellular mRNAs and viral genomic RNAs[48]. Another direct mechanism is the inhibition of RNA dependent RNA polymerase (RdRp) through direct interaction with RTP[32]. RBV also increases viral mutation rates *via* its misincorporation into the genome, leading to population extinction[39].

RBV exerts important immunomodulatory effects that seem to be mediated by enhancing T helper (Th)1 over Th2 responses or upregulating the interferon (IFN)-stimulated response element[61-63]. Years before SARS emerged, the first coronavirus animal model for acute and chronic liver disease was induced by mouse hepatitis virus strain-3[67]. Viral infection of macrophages leads to a marked inflammatory response and is associated with a Th2 cellular immune response and production of non-neutralizing antibodies. In hepatocellular necrosis (viral, toxins, *etc*.) resident macrophages (Kupffer cells) are activated and release a number of inflammatory mediators. Inactivation of Kupffer cells prevents hepatic necrosis. RBV has minimal inhibitory effects on replication of mouse hepatitis virus strain-3 *in vitro* even at high

Table 1 Ribavirin's antiviral activity and clinical uses

Type of virus	Antiviral activities and clinical uses
DNA viruses	HSV1, 2[8]; CMV at higher concentrations[9]; Several adenovirus serotypes[10]
RNA viruses	HCV non-genotype 1: RBV + PEG-IFN; although, none of the drugs seems to have direct effect on virus. RBV enhances the pSTAT4 and IFN-γ response of NK cells to IFN-α stimulation[11]; however, in later studies, RBV was found to induce significantly more G-to-A and C-to-U transitions, a genetic signature that is indicative of RBV-induced mutagenesis[12,13]. HEV: In transplant recipients treated for HEV, RBV ensures a sustained virological response[14]. Pre-treatment HEV polymerase mutations and de novo mutations under ribavirin did not have a negative impact on HEV clearance[15]. RSV: RBV is a well-tolerated option to treat RSV infections in immunocompromised patients[16,17]. β-Coronaviruses, comprising MHV-3, SARS-CoV, MERS-CoV, and SARS-CoV-2 (see text for extensive data). Influenza virus (A and B)[18]; Paramyxoviruses[19]; Measles (see text); Mumps[20]; Parainfluenza types 1, 2, 3[21]; Rhinoviruses exhibit variable sensitivity[22], while combination treatment was effective in patients with hypogammaglobinemia[23]. HIV (*in vitro*)[24], WNV (flavivirus): RBV lowers RNA levels and reduces cytopathogenicity *in vitro*[25]. Poliovirus and coxsackie B virus are insensitive[9], Hemorrhagic fever viruses, including arenaviruses, bunyaviruses, hantaviruses, filoviridae (Marburg and Ebola viruses) and the Flaviviridae (yellow fever and dengue virus). RBV is effective against most of these major pathogens, except for Ebola, Marburg, yellow fever, dengue, and Machupo virus[26]. Arenaviruses: LCMV inhibition is also mediated through a decrease in GTP levels[27]. Crimean-Congo hemorrhagic fever: RBV is the only antiviral treatment with decreased fatality rates[28,29]. Lassa fever and Hantaan virus have been tested and showed potential susceptibility *in vitro* and/or in animal models[30,31]

DNA: Deoxyribonucleic acid; RNA: Ribonucleic Acid; CMV: Cytomegalovirus; GTP: Guanosine triphosphate; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HIV: Human immunodeficiency virus; HSV1,2: Herpes simplex virus 1 and 2; IFN: Interferon; LCMV: Lymphocytic choriomeningitis virus; MERS-CoV: Middle East respiratory syndrome; MHV-3: Mouse hepatitis virus strain-3; NK: Natural killer; PEG-IFN: Pegylated-interferon; RBV: Ribavirin; RSV: respiratory syncytial virus; SARS-CoV: Severe acute respiratory syndrome; SARS-CoV-2: Severe acute respiratory syndrome-2; WNV: West Nile virus.

concentrations. However, at concentrations achievable *in vivo*, RBV almost totally inhibits the production of proinflammatory mediators tumor necrosis factor alpha, interleukin (IL)-1, and procoagulant activity in macrophages *in vitro*[67]. Similar Th1 and Th2 responses have been associated with susceptibility/resistance in murine models of leishmaniasis, candidiasis, and listeriosis[77,78]. Therefore, the beneficial effect of RBV may be related to its ability to reduce markedly macrophage activation and diminish Th2 cytokine production while preserving Th1 cytokine production. The cellular mechanisms involved in enhancement of IFN signaling by RBV are mediated by stimulation of mammalian target of rapamycin, which interacts and activates p53, which in turn stimulates the transcription of IFN regulatory factor-9[70]. RBV may also stimulate extracellular signal-regulated kinase 1/2 pathway with subsequent enhanced antiviral response of IFN-a + RBV against HCV, suggesting that mammalian target of rapamycin signaling might interact with extracellular signal-regulated kinase 1/2 signaling in some way[71].

Oral RBV is rapidly absorbed and distributed, with a bioavailability of 40%-50% (± 22%)[79,80] compared with intravenous administration. Plasma protein binding is negligible, whereas the plasma elimination of RBV occurs in two phases; the first has a relatively short half-life of 2 h and the second has a much longer terminal half-life of 16–164 h or a mean half-life of 37 ± 14 h[80]. Due to large distribution volume and elimination dependent on renal function, RBV may require more than 4 wk to reach steady-state concentrations[79,81]. The active metabolite of the drug, RTP, concentrates in erythrocytes and leaches out slowly, with a half-life of 40 d. In nucleated cells, RMP is rapidly hydrolyzed to RBV by 5-nucleotidase or alkaline phosphatase. Recently, RTP was reported to be dephosphorylated intracellularly to RMP by inosine triphosphate pyrophosphatase (ITPase)[53]. As RBV and RMP, but not RTP, can be transported across the plasma membrane through transporters, it is not surprising that reduced ITPase activity is associated both with higher intracellular RTP levels[53] and lower plasma RBV concentrations[82]. Notably, ITPase gene variants associated with reduced enzymatic activity that are naturally occurring in approximately one-third of humans have been demonstrated to protect against RBV-induced hemolytic anemia during RBV therapy in combination with pegylated (peg)-IFN-α[83,84] for HCV. This improved efficacy was associated with a reduced relapse risk in spite of lower RBV plasma concentrations[82]. Unchanged RBV and its major metabolite are excreted in the urine. Urinary metabolites, however, may be up to 5-fold higher after oral *vs* intravenous administration, suggesting a major role for gastrointestinal or, more likely, hepatic metabolism when given orally[79].

RBV's principal toxicity is the development of a dose-dependent, reversible anemia. This anemia is due to a combination of shortened erythrocyte half-life because of hemolysis and bone marrow suppression. Hemolysis is hypothesized to be secondary to oxidative membrane stress induced by depletion of adenosine triphosphate in

Table 2 Ribavirin multimodal mechanisms of action with distinct examples

Type	Mechanism
Direct	
1	Inhibition of RdRp-RNA synthesis
	Direct interaction with RTP. RTP has been reported to competitively inhibit the influenza virus RNA polymerase, with respect to ATP and GTP, whereas RMP has no such observable effect[32]; however, for HCV, there are conflicting data regarding the impact of RBV on the RdRp, with reports of both no direct inhibition[33] as well as observations that RBV-containing RNA templates can cause a significant blockage of RNA elongation[34]
	Shown for Influenza A, La Crosse virus and for the key HIV polymerase, reverse transcriptase[24,32,35]
	Influenza virus: For cells treated with either RBV or methotrexate (a purine synthesis inhibitor that decreases intracellular concentrations of purines), the loss of polymerase activity at low concentrations of nucleotide is the culprit[36]
	Hantaan virus: The observed increase in RBV-5′-triphosphate supports the direct interaction and inhibition of the virus RdRp[37,38]
2	Increasing viral mutation rates through the misincorporation of RBV into the genome, leading to population extinction[39]
	RBV triphosphate is incorporated into the viral RNA by poliovirus polymerase, where it templates cytidine and uridine equally efficiently[40]. It has been suggested that RBV enhances viral mutagenesis, leading to error catastrophe by incorrect substitution of RTP for GTP[34,40,41] into viral RNA as most viral RdRps lack proofreading capability; although, this mechanism has been disputed for some viruses[42]. For example, for poliovirus, a 9.7-fold increase in mutagenesis following RBV treatment resulted in 99.3% loss in infectivity[40]. RTP is incorporated into the viral RNA by poliovirus by poliovirus polymerase, where it templates cytidine and uridine equally efficiently[40]. It has been suggested that RBV enhances viral mutagenesis, leading to error catastrophe by incorrect substitution of RTP for GTP[34,40,41] into viral RNA as most viral RdRps lack proofreading capability; although, this mechanism has been disputed for some viruses[42]. For example, for poliovirus, a 9.7-fold increase in mutagenesis following RBV treatment resulted in 99.3% loss in infectivity[40]. RTP is incorporated into the viral RNA by poliovirus polymerase, where it is mutagenic, since it templates cytidine and uridine equally efficiently[9]. Unlike GTP, RTP has ambiguous base-pairing capacity and can form two hydrogen bonds with uridine triphosphate or cytidine triphosphate with equal efficiency[40], leading to a subsequent increase in G-to-A and C-to-U single nucleotide variations throughout the entire HCV open reading frame[43]. Recent *in vivo* studies indicate that similar RBV-induced mutagenesis occurs in HEV[44]
	HCV: Initial studies showed no mutagenic effects, while in later results a mutagenic activity was indeed observed[45-47]
	LCMV: Along with the inhibition of IMPDH enzyme, a mutagenic activity also occurs[27]
3	Interference with formation of the 5′ cap structure of viral mRNA (capping activity)
	This is probably due to competitive inhibition of both guanyltransferase and methyltransferase capping enzymes
	mRNAs contain extensive modification on the 5′ end (known as the “five prime cap”), often utilizing guanine which is methylated in the 7-position, as this is essential for the stability and efficient translation of mRNA[39]. Thus, RNA capping has major secondary impact on the translation of both viral and host cell mRNAs. Interestingly, RTP reportedly acts as a competitive inhibitor for the capping of mRNAs, subsequently leading to impaired translation[48] by forming a covalent RMP-capping enzyme intermediate in place of the normally observed GMP-enzyme intermediate[49]
	Thus, virus which do not form capped mRNA are relative insensitive to RBV
	Mutants of Sindbis virus with an altered guanyltransferase demonstrate acquired resistance to RBV[50]
Indirect	
1	Inhibition of IMPDH by RBV-5′-monophosphate
	RBV is a structural analogue of guanosine and acts as a potent competitive inhibitor of the enzyme IMPDH by RMP, leading to reduced GMP biosynthesis by diminution of the conversion of inosine monophosphate to XMP and resulting in the depletion of intracellular GTP[51]; this process is reversible *in vitro* by the addition of exogenous guanosine[52]. XMP can then be aminated to GMP by the GMP synthase enzyme. GMP is further converted to guanine metabolites, such as GTP and dGTP, essential precursors for RNA and DNA synthesis, respectively. This inhibition of IMPDH may occur even at relatively low RBV concentrations (10 μmol/L)[53] and leads to marked changes in the balance of the GTP pool in cells, with subsequent major impact on the host cell and viral gene expression as well as on viral replication[54-58]. This effect may be reversible *in vitro* by the addition of exogenous guanosine[59]
	HEV replication *in vitro*: MPA (an IMPDH inhibitor) has the same antiviral effect as RBV, which can be neutralized by the addition of guanosine [60]
	HCV: RBV acts through the inhibition of IMPDH, since the addition of guanosine negates this effect[45]
	LCMV: Inhibition is also mediated through a decrease in GTP levels[27]
2	Immunomodulatory effects of RBV
	Initially, a possible effect on T-cell subset balance was suggested[40,41]. RBV was also shown to inhibit lymphocyte proliferation, possibly due to the depletion of GTP which is essential for proliferating T-cells[61,62]. *In vitro*, IL-2 and IL-4 production was affected at lower RBV concentrations than IFN-γ production, suggesting a differential effect on Th1 and Th2 lymphocytes[63]. RBV administration in mice infected with influenza virus significantly attenuated respiratory immune responses as well as secretory and total IgA mucosal responses[64]. Affects T-cell subset balance[61, 62]. Ameliorates spontaneous autoimmune disease in mice[65]. Inhibits lymphocyte proliferation due to depletion of GTP, which is essential for proliferating T-cells[61,66]. Enhances Th-1 over Th-2 responses or up-regulates the IFN-stimulated response element[63,67]. Clinical HCV studies have demonstrated that RBV monotherapy down-regulates the expression of IFN-stimulated genes in addition to reducing systemic concentrations of liver enzymes[68], and similarly lowered systemic concentrations of IP-10 (also known as CXCL10) associated with successful therapeutic outcome[69] in spite of only modest impact on viral levels

In an animal model[67] for acute and chronic liver disease induced by the first coronavirus, many years before SARS emerged, MHV-3 was examined. MHV-3 has been the main model in studies on coronavirus replication and pathogenesis. Resistance to MHV-3 is associated with predominant Th1 response, the production of IFNs, neutralizing antibodies, and cytotoxic T cells. Viral infection of macrophages leads to a marked inflammatory response, including sustained production of TNF, IL-1, and procoagulant Fg12 prothrombinase and is associated with a Th2 cellular immune response and production of non-neutralizing antibodies. In hepatocellular necrosis (viral, toxins, *etc.*) resident macrophages (Kupffer cells) are activated and release a number of inflammatory mediators, including TNF, IL-1, proteolytic and enzymes, and inactivation of these macrophages prevents hepatic necrosis. RBV has minimal inhibitory effects on replication of MHV-3 *in vitro*, even at high concentrations. However, at concentrations achievable *in vivo*, it almost totally inhibits the production of the proinflammatory mediators TNF, IL-1 and procoagulant activity in macrophages *in vitro*[67]. RBV diminishes IL-4 production both by the Th1/Th2 lines as well as by the MHV-3 specific Th2 cell line, while it has no effects on IFN-γ production by Th1 cells, thereby preventing the shift to a Th2 response. The beneficial effect of RBV may be related to its ability to markedly reduce macrophage activation, thereby inhibiting the production of proinflammatory mediators from virally-activated macrophages; in addition, it diminishes Th2 cytokine production, while preserving Th1 cytokine production. RBV activates p53 by stimulating the mTOR protein and promoting the interaction between mTOR and p53. Activated p53 stimulates the transcription of IFN regulatory factor 9 and subsequently enhances IFN signaling (plasmids of lentiviruses that express either scrambled sequence or short-hairpin RNA against mTOR[70]. Furthermore, RBV-induced activation of mTOR and p53 enhances IFN-dependent signaling for the IFN-α/RBV combination treatment [71]. RBV stimulates the ERK1/2 pathway and subsequently promotes p53 activity, which at least partly contributes to the enhanced antiviral response of IFN-α plus RBV against HCV[71]. Regarding which is the predominant RBV antiviral mechanism, it is likely that for most viruses, RBV does indeed exert pleiotropic effects. However, a trend in the last decade has been to explore mutagenesis as the primary antiviral effect against RNA viruses, while clinical studies could determine whether mutagenesis occurs *in vivo* and how to optimize this activity in a therapeutic context [26]

ATP: Adenosine triphosphate; dGTP: Deoxyguanosine triphosphate; GMP: Guanosine monophosphate; GTP: Guanosine triphosphate; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HIV: Human immunodeficiency virus; IFN: Interferon; IL: Interleukin; IMPDH: Inosine monophosphate dehydrogenase; IP-10: IFN-γ inducible protein 10; MHV-3: Mouse hepatitis virus strain-3; MPA: Mycophenolic acid; RBV: Ribavirin; RdRp: RNA-dependent RNA polymerase.

erythrocytes[84]. Hemolytic anemia usually occurs 10 d after therapy but may appear 3-5 d after RBV initiation; it is usually observed with doses of 1 g/d or higher in patients with chronic HCV[85] and HEV viremia[14]. Short-course RBV therapy of medium doses may not cause significant complications[86]. Other side effects include bradycardia[87,88], electrolyte disturbances (hypocalcemia, hypomagnesemia)[89], transaminitis[90], pancreatitis, metallic taste, headache, reduction in bone-mineral density, and central nervous system effects (mood changes, sleep disturbance)[91].

Rbv in measles

Measles is a worldwide and highly contagious (90%) viral illness caused by the measles virus, a single-stranded, negative-sense RNA virus in the genus *Morbillivirus* of the family *Paramixoviridae*. Diarrhea is the most common complication, and the majority of deaths are due to pneumonitis or encephalitis[92]. According to 2018 World Health Organization (WHO) reports, 82596 people in 47 of 53 European countries contracted measles, with the 2193 cases reported from Greece[93]. Nearly two-thirds of measles cases were hospitalized, with 72 deaths having occurred in Europe and 140000 globally, mostly among children under the age of 5 years.

RBV inhibits the replication of measles virus *in vitro*[94,95]. In a cohort of 93 severe measles hospitalized cases during the 2018 outbreak[96], our center treated 13 cases, seven of which were given RBV due to severe pneumonitis. In that cohort, RBV tended to be prescribed to those with numerically lower PaO_2/FiO_2 ratios, whereas others were managed conservatively[96]. RBV was commenced within 5 d from symptom onset at an oral dose of 2.4 g/d for 5-7 d. No patient required intubation, and all recovered completely. We selected high-dose levofloxacin (750 mg intravenously) as an antimicrobial agent based upon a unique animal-study in which levofloxacin was examined for its possible protective effect against Influenza virus-induced lung injury [97]. Levofloxacin exerted a substantial anti-oxidative effect by clearly suppressing the levels of oxidative and nitrative stress metabolites in bronchoalveolar lavage fluid. According to lung histology, levofloxacin significantly suppressed not only the inflammatory infiltration into alveoli and the bronchial pathway but also hemorrhage and necrosis. A double-blind, placebo-controlled trial of oral RBV showed a reduction in the severity duration of measles in children[98].

Given the high risk of measles-associated mortality among immunosuppressed individuals, some authors recommend RBV treatment in measles pneumonia/encephalitis. In a study of severe measles in immunocompromised patients with case fatality rate (CFR) of about 70% for oncology patients and 40% for human immunodeficiency virus-infected patients, the authors observed a rapid defervescence in those treated with RBV[99]. A unique RCT enrolled 100 patients (aged between 6 mo and 47 years) with measles confirmed by positive immunoglobulin M antibody detection to assess the possible beneficial effect of RBV on measles[100]. Fifty patients were treated with oral RBV 200 mg qid (in children, 20 mg/kg/d as syrup) for 7 d. Constitutional symptoms resolved much earlier in the treatment group than in the non-treatment group (3.2 ± 0.6 d *vs* 7.3 ± 0.8 d), and there were no complications or deaths.

Importantly, in the comparator group, almost 50% developed pneumonitis, 30% watery diarrhea, and 8% encephalitis, with an overall mortality of 16%.

The reported dosage regimen for RBV varies significantly among different viral infections and studies. According to 2018 European Association for the Study of the Liver recommendations, hepatitis C patients with decompensated cirrhosis without hepatocellular carcinoma can be treated with two antivirals, including RBV 1000 or 1200 mg in patients < 75 kg or > 75 kg, respectively, for 12 wk[101]. Immunocompromised patients with chronic HEV viremia were treated with RBV monotherapy at a median dose of 600 mg/d (equivalent to 8.1 mg/kg/d) adjusted to creatinine clearance for 3 mo resulting in 85% sustained viral response (SVR)[14]. In a large cohort, 255 solid organ transplant recipients with chronic HEV infection were treated with RBV monotherapy. After a first course of RBV, the SVR rate was 81.2%, which increased to 89.8% when some patients were offered a second course of RBV. Surprisingly, pre-treatment HEV polymerase mutations and *de novo* mutations under RBV did not have a negative impact on HEV clearance. Twenty patients had *de novo* mutations, 16 of whom were re-treated with RBV, and 12 achieved SVR[15].

One of our measles pneumonitis cases with decompensated liver cirrhosis of autoimmune etiology under maintenance immunosuppressive regimen was started on 2.4 g/d, a dose 2.4-fold to 4.0-fold higher than the regimens recommended in HCV decompensated cirrhosis and chronic HEV viremia, respectively. This high dose was based on findings from Forni *et al*[102], who treated severe measles pneumonitis cases (not cirrhosis) with intravenous RBV 35 mg/kg/d in three divided doses for the initial 2 d of therapy and 20 mg/kg/d for the remaining 5 d. Intravenous formulations of RBV were unavailable in our country, thus we administered orally 40 mg/kg/d as a loading dose for 3 d and then a maintenance dose of 20 mg/kg/d for the next 4 d, taking into account oral dose bioavailability of approximately 50% and the patient's liver cirrhosis. In one report, high-dose regimens administered for measles pneumonitis comprised a loading dose of 2 g intravenously and then 1 g qid as a maintenance dose in combination with high doses of vitamin A[103]. RBV was discontinued, however, due to transaminitis and acute kidney injury in two patients, both of whom fully recovered[103]. The same dosing regimen was applied to another patient [104], who despite requiring intubation, eventually fully recovered. In a measles outbreak at a pediatric oncologic unit[105], clinicians reported that early RBV treatment of 15 mg/kg orally within 24 h from rash onset resulted in a significantly better outcome (P = 0.009). Several immunocompromised cases have been treated with RBV. A 9-year-old boy with Hodgkin's disease and a 26-year-old human immunodeficient virus positive patient with measles pneumonitis both fully recovered with early initiation of RBV[106,107]. In contrast, two other immunosuppressed patients with measles pneumonitis and late introduction to RBV succumbed to their illness[108,109].

Taken together, these findings suggest that early administration of treatment (within the first 5-7 d from disease onset) of adequate dosing (40 mg/kg/d p.o.) and duration (2 or 3 wk) in severely immunosuppressed patients is essential for the best therapeutic outcome.

Rbv in SARS

On November 16, 2002, the first known case of atypical pneumonia was reported in Foshan City, Guangdong Province, China, but the cause was not identified until much later. The SARS coronavirus is a positive-sense, single-stranded RNA virus that was the causative pathogen of secondary cases elsewhere in the world. On July 5, 2003, the WHO announced that the epidemic of SARS had been contained worldwide but called for continued vigilance. A total of 8098 people worldwide became sick in 29 countries (mostly in China and other parts of Asia). Of these, 774 died, with a CFR 9.6%[110]. Some scattered cases were reported in China until May 2004.

SARS in vitro studies

To examine the *in vitro* efficacy of several compounds against coronaviruses, Vero and Vero E6 cell lines have been utilized almost exclusively. The easy propagation of coronaviruses in Vero cell lines may be related to the lack of a functional IFN system [111,112]. However, the IFN-dependent pathway function can be activated by exogenously provided IFN. Among the nine retrieved *in vitro* studies, only one utilized a cell line other than Vero or Vero E6; while in four studies, Vero cells were examined in combination with other cell lines (Table 3). Three studies concluded that RBV was inactive[112-114], and one demonstrated an inhibitory effect of RBV[115]. In three studies where Vero cell lines were co-examined with other cell lines, researchers concluded that RBV was less active[116] or ineffective (included the Caco2 cell line) [117]; in the third study, the authors concluded that no inosine monophosphate

Table 3 Severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus-2 *in vitro*, *in vivo* (animal), *in silico* study results focused on ribavirin treatment

Ref.	Isolates	Cell line	Compounds studied	Results (EC50 μg/mL INFs IU/mL, unless stated otherwise)	Researchers' conclusion	Comment
SARS						
Cinatl *et al* [113], 2003	FFM-1, FFM-2	Vero	6-azauridine	→ 16.8	Glycyrrhizin inhibits SARS-CoV replication; search for therapeutic compounds against SARS will be greatly facilitated by establishing growth of SARS-CoV in human cells	Utilization of a resistant to RBV cell line (Vero E6 cell line, Pitfall 1); however, authors noticed the need for growth in human cell lines
			Pyrazofurin	→ 4.2		
			MPA	→ > 50		
			RBV	→ > 1000		
			Glycyrrhizin	→ 300		
Tan *et al* [114], 2004	Singapore isolate	Vero E6	IFN-β1b	→ 10000	RBV is inactive against SARS-CoV. IFNs exhibit antiviral activity	Utilization of Vero E6 cell line, Pitfall 1
			IFN-αn3	→ 10000		
			RBV	→ 10000		
			IFN-β1b + RBV	→ No synergistic inhibitory effect		
Ströher *et al* [112], 2004	Tor2, Tor3 Tor7, Tor684	Vero E6	RBV	→ No SARS susceptibility up to concentrations of 2000 μg/mL	RBV alone is unlikely beneficial; combination with IFN-α2b should be evaluated	Utilization of Vero E6 cell line, Pitfall 1
			IFN-α2b	→ Substantial inhibitory effect at concentrations ≥ 1000		
Chu *et al* [87], 2004	HKU-39849	Fetal Rhesus Kidney-4	RBV	→ 50	Cytopathic effect of SARS was inhibited by Lop and RBV	First different cell line used than Vero → positive results
			Lop	→ 4		
Saijo *et al* [115], 2005	HKU-39849Frankfurt-1	Vero E6	Mizoribine	→ IC50 3.5	Mizoribine and RBV possess an inhibitory effect. Discrepancy between studies for RBV could be attributed to the duration of incubation times of the cells in the presence of RBV	First study with positive results for RBV in Vero cell lines. Totally correct conclusion for discrepancies between studies
			RBV	→ IC50 20		
Chen *et al* [116], 2004	10 SARS-CoV isolates	Vero E6, fRhk-4	IFN-α	→ 5000 (fRhk-4), 19.5 (Vero)	Pre-incubation 16 h with IFNs enhanced activity. RBV and Lop were less active in the Vero cell line. IFNs + RBV was the most effective combination	The study includes Pitfall 1, the rest are ok
			IFN-β1α	→ 2000 (fRhk-4), 10.6 (Vero)		
			RBV	→ 50-100 (fRhk-4), > 200 (Vero)		
			Lop			
			IFNs + RBV synergism	→ 2-4 (fRhk-4), 4-8 (Vero)		

Morgenstern *et al*[117], 2005	FFM1, 6109	Vero, CL14, CaCo2, PK-15, HPEK, MA-104,	RBV IFN-β + RBV Synergism	→ > 1000 (Vero), → 9.4 (MA-104), → 2.2 (PK-15), → 5.2-8.2 (human cell lines) → 10-fold lower RBV concentration,→ 50-2000-fold, lower IFN concentrations → Combination index 0.45	IFN-β + RBV combination inhibits SARS-CoV replication in drastically reduced concentrations	The study includes Pitfall 1, the rest are ok
Barnard *et al* [118], 2006	SARS Urbani strain	Vero 76, Vero E6 MA-104 CaCo2, BALB/c mice	RBV RBV RBV RBV	→ 270, EC90 = 560 → 1253 → EC90 = 225 → EC90 = 4100	RBV, like other IMPDH inhibitors, may enhance viral replication in lungs. Four days after cessation of therapy, RBV promoted pro-inflammatory cytokine production, although 3 d after RBV administration inhibited pro-inflammatory cytokine production in mice by significantly reducing IL-1α, IL-5, MCP-1 and GM-CSF. Authors concluded that their data do not support the use of RBV or other IMPDH inhibitors for SARS treatment	The only study with such high RBV's EC50 in human CaCo2. It is not explained why animals were treated only for 3 d with RBV showing good results and then it was ceased for 4 d, just to confirm the disease worsening with untoward outcomes. Also Pitfall 1 is included in the study
Shah *et al* [119], 2010	VSV, SeV	BHK21, BSRT7, HeLa, A549, 4T1, HEp2, Vero	RBV	Both viruses have the ability to initiate infection in all cell line tested. RBV effectively inhibited VSV in BSRT7, HeLa and HEp2 cells even at the lowest tested drug concentrations. However, RBV had a surprisingly mild effect on VSV in Vero and A549 cells even when used at 1000 μg/mL concentration with a somewhat intermediate effect in 4T1 cells. A similar pattern of RBV-resistance was shown for SeV in BHK21, Vero and A549, suggesting that cellular rather than virus-specific factors determine the dramatic differences in their response to RBV. RBV treatment even at 1000 μg/mL concentration did not produce any statistically significant decrease in cell viability in any of the tested cell lines. The development of cell-based resistance to RBV treatment *via* decreased RBV uptake can greatly limit RBV activity. RBV uptake was inhibited in most cell lines at both lower and higher NBMPR concentrations, a specific inhibitor of ENT *via* ENT1, 2, previously shown to be primarily responsible for RBV import into the cells. Dramatic variations were observed in the long-term accumulation of RBV in different cell types. Importantly, it correlated with the antiviral efficacy of RBV in the tested cell lines. All the three RBV-resistant cell lines, BHK21, A549, and especially Vero showed markedly decreased levels of RBV accumulation suggesting that such differences in the intracellular RBV metabolism may be responsible for the natural cell resistance to antiviral RBV treatment. Act-D an inhibitor of DNA-primed RNA synthesis, was able to revert the antiviral effect of RBV against several RNA viruses, with two proposed mechanisms (1) the stabilization of cellular GTP levels, and (2) inhibition of RTP production. ActD had a clear neutralizing effect on RBV in most cell lines. ActD treatment did not inhibit RBV uptake, demonstrating that the observed reversal of RBV antiviral action was not due to interference of ActD with RBV uptake. The observed resistance of VSV and SeV to RBV in Vero, BHK21, and A549 was not due to the generation of RBV-resistant mutants in these cells. Even when the cells were pretreated with RBV starting 24 h before infection, a little effect of RBV on viral replication in RBV-resistant cells was observed, ruling out any possibility of virus adaptation to RBV. In addition, when VSV was passed by 10 to 15 times in HeLa, BSRT7, and BHK21 cells in the presence of sub-inhibitory RBV concentrations, no viral adaptation to RBV was ever observed. RBV uptake in all tested cell lines after 15min treatment showed that no one of the tested cell lines was defective to RBV uptake. In long-term RBV accumulation in cells after 16 h or 24 h treatment four cell lines sensitive to RBV showed significantly higher levels of RBV accumulation compared to RBV-resistant BHK21, A549, and Vero. Vero cells had a particularly low accumulation which explains the highest resistance to RBV. This long-term accumulation is dependent on the cellular metabolism of RBV. Neutral RBV molecule can be transported freely in and out of a cell *via* ENTs but once it is phosphorylated, negative charged RMP, RDP, or RTP are trapped inside the cells. A good illustration of the difference between the RBV uptake and its long-term accumulation is RBV hyper-accumulation in erythrocytes resulting in hemolytic anemia in some RBV-treated patients. Similarly to nucleated cells, RBV is transported into erythrocytes *via* ENTs and converted into RMP, RDP, and RTP. However, unlike nucleated cells, erythrocytes lack the phosphatases needed to hydrolyze RMP/RDP/RTP into RBV. Exogenous guanosine had a clear (almost 100%) neutralizing effect on RBV in BHK21, A549 and Vero cells, which are already highly resistant to RBV. However, very little effect was observed on the RBV activities in RBV-sensitive cells, especially HeLa, 4T1, and HEp-2 cells. Unlike guanosine, ActD was able to effectively neutralize RBV in all tested cell lines. Authors hypothesized that RBV antiviral activity in these cell lines depends not only on the depletion of GTP pool (can be restored by guanosine) but also on the successful 5'-phosphorylation of RBV into RMP/RDP/RTP. At the same time they suggested that RBV acts in RBV-resistant cells types primarily *via* depletion of GTP pool due to insufficient amounts of phosphorylated RBV molecules in these cells, explaining why the effect of RBV can be completely reversed in these cell lines by guanosine		
Smith *et al* [120], 2013	MHV-A59 SARS-CoV (Urbani strain)	Murine astrocytoma DBT Vero E6	CoVs contain the largest known RNA genome and encode an array of 16 viral replicase proteins, including a 3' to 5' exoribonuclease domain, ExoN, within the non-structural protein 14 Nsp14. The exon is the first identified proofreading enzyme for an RNA virus and functions together with other CoV replicases to perform the crucial role of maintaining CoV replication fidelity. In DBT cells, MHV-ExoN+ viruses were resistant to 10 μM of RBV, while MHV-ExoN- virus titers decreased by~200-fold following treatment with same RBV concentrations, a surprising finding because at least 10-fold higher RBV concentrations are required to inhibit poliovirus and chikongunya viruses. Furthermore, they determined the sensitivity of ExoN + and ExoN-at a low multiplicity of infection. Unexpectedly, multi-cycle replication of ExoN-viruses in the presence of RBV was indistinguishable from single-cycle replication. Using qRT-PCR, researchers determined that ExoN-genomic RNA was dose-dependently reduced by RBV, while ExoN + RNA was unaffected. Extracellular addition of guanosine restored ExoN-titers, even in presence of RBV. These data indicate that the antiviral activity of RBV against MHV-ExoN-viruses is occurring, at least in part, through			

			decreasing viral RNA synthesis and inhibition of IMPDH, while the presence of ExoN activity is capable of preventing RBV inhibition of CoV replication. However, the increased sensitivity of MHV-ExoN-to RBV could result from the impairment of undefined functions of ExoN during replication, particularly during RNA synthesis			
MERS						
Chan *et al* [153], 2013	hCo-EMC	MDCK	1280 drugs screened		IFN-β1b and MPA should be considered in the treatment trials of MERS. IMPDH inhibitors inactive in Vero cell line	A combination of IFNs with RBV was not tested. Coexistence of Pitfall 1
			MPA	→ 0.17		
			RBV	→ 9.99-41.45		
			IFN-α2b	→ 6709.8		
			IFN-β1a	→ 480.5		
			IFN-β1b	→ 17.6		
		Vero	RBV, MPA	→ Inactive		
Falzarano *et al*[155], 2013	hCoV-EMC/2012	Vero, LLC-MK2	RBV	→ 41.45 (Vero), 16.33 (LLC)	Lower sensitivity to RBV for LLC than Vero cells. RBV + IFN-α2b, when combined, the inhibitory concentrations drops to ranges achievable in humans	Coexistence of Pitfall 1. Very significant outcomes for IFN + RBV combination
			IFN-α2b	→ 58.08 (Vero), 13.26 (LLC)		
			Combination	8-and 16-fold decrease in the inhibitory concentration as either treatment alone		
Falzarano *et al*[156], 2013	hCoV-EMC/2012	Rhesus macaque	IFN-α2b + RBV	Treated animals showed improved clinical parameters, no dyspnea, little evidence on X-ray. They also showed reduced systemic and local pro-inflammatory markers, significant reduction in viral genome copies in lung tissues and less severe histopathological changes compared to untreated	They suggested IFN-α2b + RBV should be considered for early intervention therapy in MERS. The hedgehog signaling pathway was identified as a putative contributor to decreased lung damage	Very significant results for the early IFN-α2b + RBV administration
Hart *et al* [154], 2014	Hu/Jordan-N3/2012 (Jordan strain)	Vero E6	MPA	→ IC50 = 2.87	INF-β and MPA or a combination should be considered for MERS-CoV infected patients	The study involves Pitfall 1
			RBV	→ > 250		
			IFN-β	→ IC50 = 1.37IFN-β antiviral activity was 16- 41-, 83-, and 117-fold higher than those of IFN-α2b, IFN-γ, IFN-type I and IFN-α2a, respectively		
Chan *et al* [157], 2015	EMC/2012	Common marmosets	MMF	→ A single dose did not improve and might have worsened MERS infection	IFN-β and Lop/r are effective against MERS infection in common marmosets. They concluded that potentially effective combinations that should be evaluated could be RBV and IFN- β1b and/or Lop/r. Although high doses of RBV are limited by side-effects, low-dose RBV combined with IFN- β1b and/or Lop/r may be synergistic	RBV was not tested, but surprisingly the authors discuss its combination with IFN-β1b and/or Lop/r as potentially effective
			Lop/r	→ Improved clinical, radiological, pathological features, and lowered lung and other tissue viral load		
			IFN-β1b	→ Less severe disease and lower tissue viral loads		
SARS–CoV–2						
Computational studies						
Kandeel *et al*	The first available crystal structure		20 drugs	Molecular modelling, virtual screening, docking, sequence comparison statistics and phylogenetics of the COVID-19 M-pro were investigated. Phylogenetic analysis		

[183], 2020	of COVID-19 proteins is the main protease, M-pro, and belongs to the translated NSPs together with the papain-like protease Pl-pro			showed a 96.08% identity between COVID-19 and SARS-CoV M-pros, while low identity of 51.61% was detected for COVID-19 and MERS-CoV. In the Schrodinger glide docking module, curcumin was found to be a strong inhibitor of SARS M-pro and the tested compounds' relative docking scores were calculated compared with the docking score for curcumin. RBV and telbivudine were ranked at the 2nd and 3rd positions respectively, where RBV was shown to form two hydrogen bonds with M-pro. Given the high similarity of SARS and COVID-19 M-pros, RBV as well as telbivudine might be of value in treating COVID-19		
Elfiky *et al* [184], 2020	NSPs such as RdRp (nsp12) are crucial enzyme in the life cycle of the RNA viruses. Docking experiments were performed using the optimized COVID-19 and SARS RdRps		Anti-polymerase drugs against HCV	The active site of RdRp is highly conserved, representing two successive aspartate residues protruding from a beta-turn structure, making them surface accessible through the nucleotide channel (which free nucleotides can pass through). Sofosbuvir and RBV are nucleotide derivatives competing with physiological nucleotides for the RdRp active site, and form 7 and 13 H-bonds respectively. Sofosbuvir, RBV and remdesivir can be used against the nCoV-2019, having promising results. GTP derivatives may be used as specific inhibitors against COVID-19		
In vitro studies						
Choy *et al* [187], 2020	BetaCoV/ Hong Kong VM20001061/2020	Vero E6	RBV Remdesivir Lop Favipiravir and others	→ CPE 500 mmol/L → CPE 25 μmol/L → CPE 25 μmol/L → CPE > 100 μmol/L	Remdesivir, Lop, and emetine inhibit SARS-CoV-2 replication. RBV and favipiravir showed no inhibition. Combinational therapy may provide better clinical benefits	Pitfall 1
Wang *et al* [186], 2020	nCoV-2019 BetaCoV/ Wuhan/ WIV04/2019	Vero E6	RBV Favipiravir Nafamostat Nitazoxanide Remdesivir Chloroquine	→ 109.5 μmol/L → 61.88 μmol/L → 22.5 μmol/L → 2.12 μmol/L → 0.77 μmol/L → 1.13 μmol/L	Remdesivir and chloroquine are highly effective in the control of 2019-nCoV	Pitfall 1

ActD: Actin-D; CoV: Coronavirus; COVID-19: Coronavirus disease 2019; ENT: Equilibrative nucleoside transport; GTP: Guanosine triphosphate; IFN: Interferon; IL: Interleukin; IMPDH: Inosine monophosphate dehydrogenase; Lop: Lopinavir; M-pro: Main protease; MERS: Middle East respiratory syndrome; MMF: Mycophenolate mofetil; MPA: Mycophenolic acid; nCoV-2019: Novel coronavirus 2019; NSP: Non-structural protein; RBV: Ribavirin; RdRp: RNA-dependent RNA polymerase; RMP: Ribavirin monophosphate; RTP: RBV triphosphate; SARS: Severe acute respiratory syndrome; SeV: Sendai virus; VSV: Vesicular stomatitis virus.

dehydrogenase inhibitor should be used for SARS treatment[118]. They emphasized, however, the need for growth in human cell lines[113]. The combination use of IFNs with RBV was considered the most effective[116,117]. In only one study where fetal rhesus kidney-4 cells were utilized did RBV exhibit an inhibitory effect against SARS-coronavirus (CoV)[87].

Several years after most of these *in vitro* studies were published, a very enlightening investigation was conducted to determine the existence of "natural" (without pre-exposure to drug) resistance to RBV in some cell-types[119]. Seven commonly used cell lines that support replication of both vesicular stomatitis virus and Sendai virus were compared regarding the antiviral activity of RBV. Decreased RBV uptake can greatly limit RBV activity. RBV uptake was inhibited in most cell lines by nitrobenzyl-

thioinosine, a specific inhibitor of ENT1 and ENT2. RBV-resistant cell lines baby hamster kidney 21, A549, and especially Vero showed markedly decreased levels of RBV accumulation. Exogenous guanosine resulted in a neutralizing effect on RBV in already resistant baby hamster kidney 21, A549, and Vero cells but had very small or intermediate effects in RBV-sensitive cells. Actin-D, an inhibitor of DNA-primed RNA synthesis, reverts the antiviral effect of RBV *via* the stabilization of cellular GTP levels and the inhibition of RTP production without inhibiting RBV uptake. Data strongly argue that the observed resistance of vesicular stomatitis virus and Sendai virus to RBV was not due to the generation of RBV-resistant mutants in these cells. RBV uptake in all tested cell lines after 15 min treatment, which determines the ability of cells to internalize RBV, showed no defective uptake. However, in long-term RBV accumulation analysis in cells with 16 h or 24 h treatment, four cell lines sensitive to RBV showed significantly higher levels of RBV accumulation compared to RBV-resistant cell lines, thereby explaining the highest resistance to RBV. This long-term accumulation is dependent on the cellular metabolism of RBV[120,121].

Neutral RBV molecules can be transported freely in and out of a cell *via* ENTs, but once they are phosphorylated, negatively charged RMP, ribavirin diphosphate (RDP), or RTP are trapped inside the cells. Similar to nucleated cells, RBV is transported into erythrocytes *via* ENTs and converted to RMP, RDP, and RTP. However, unlike nucleated cells, erythrocytes lack the phosphatases needed to hydrolyze RMP/RDP/RTP into RBV[73,76,122]. In a study of radiolabeled RBV after long-term administration, radioactivity was predominantly attributed to RMP and RTP[72]. Unlike guanosine, actin-D was able to neutralize effectively RBV in all tested cell lines. Therefore, RBV antiviral activity in RBV-sensitive cell lines depends not only on the depletion of the GTP pool (exogenous guanosine has a small effect on RBV activity) but also on the successful 5'-phosphorylation of RBV into RMP/RDP/RTP. At the same time, in RBV-resistant cells-types RBV acts primarily *via* depletion of the GTP pool due to insufficient amounts of phosphorylated RBV molecules in these cells.

Overall, the activity of RBV is naturally limited in many cell-types. Most *in vitro* studies have inferred RBV inefficiency by testing in cell lines (Vero) that are less likely to phosphorylate the compound[118] (Pitfall 1). When antiviral efficacy and potency are examined *in vitro*, multiple cell lines of different origin, including human, should be utilized.

SARS clinical studies

Some international societies have published recommendations or protocols for SARS management and treatment that included RBV[123] (Table 4). During the SARS outbreak, RBV monotherapy or in combination with other drugs was widely administered[124-143] (Table 5). The clinical progression of SARS was mostly uniform, with a tri-phasic pattern[124,131,141,144]. Week 1 was characterized by systemic symptoms that were largely related to the effect of viral replication and cytolysis and generally improved after a few days. In week 2, symptoms reoccur and oxygen desaturation may develop. Taken together, these findings suggest that the lung damage at this phase is related to immunopathological events as a result of an overexuberant host response rather than uncontrolled viral replication. A quarter of patients will progress to Phase III, characterized by acute respiratory distress syndrome (ARDS), necessitating ventilatory support. Examination of the sequential changes in viral load and disease progression suggested that the initial viral replicative phase peaks at around day 10. The key facet of management should include early institution of an effective antiviral agent to decrease the peak viral load and the associated immune-regulatory damage. This therapeutic window should be exploited as early as possible from disease onset and is limited to within the first 5-7 d (Pitfall 2).

An epidemiologic analysis[140] using an integrated database of 1755 cases in Hong Kong concluded that the timing of RBV administration did not seem to influence significantly clinical outcome, despite there being a clear trend in favor of earlier initiation (when RBV commenced on day 1 of symptom onset, CFR = 4.0%; after the first week, CFR = 12.5%; treatment not prescribed, CFR = 29.4%). Non-emergence of statistical significance was attributed to insufficient power to detect a difference because most patients were treated. There were 19 studies (Table 5) that referred to treatment with RBV and/or other drugs for SARS patients, and in four [124,128,137,139], treatment started too late. In the remaining studies, the time-gap between symptom onset and treatment initiation was not reported[89,126,134,142,145] at all or was unclear[12,131,133]. Notably, antivirals were started in Phase II[124] and even post-intubation[137], therefore conferring frustrating results.

Table 4 Treatment recommendations for viral hemorrhagic fevers and coronavirus outbreaks

Ref.	Number of patients/RBV commencement after symptom onset in d	Treatment protocol/dosing regimens	Outcomes	Authors' conclusions	Comments
Viral hemorrhagic fevers					
Borio *et al*[123], United States	Recommendations for viral hemorrhagic fevers	Intravenous: ld of 30 mg/kg (max of 2 g) once, followed by 16 mg/kg (max of 1 g per dose), qid × 4 d, followed by 8 mg/kg (max of 500 mg per dose) tid × 6 d	Peros: Ld 2000 mg → 1200 mg/d in two divided doses (if weight > 75 kg) or 1000 mg/d in two doses (400-600 mg) if weight ≤ 75 kg for 10 d	RBV is the only potentially effective drug available for selected hemorrhagic fevers	There seems to be a discrepancy between the iv and the oral posology
SARS-CoV					
Koren *et al*[91], Canada	Recommendations by the Canadian Society for Clinical Pharmacology	Recommended RBV dosage adjusted to Crcl: If Crcl > 60 mL/min → 400 mg tid iv × 3 d, then 1200 mg bid × 7 d	Adverse events: Dose-dependent anemia; electrolyte disturbances (hypocalcemia, hypomagnesemia) CNS effects; teratogenic potential	Until more information becomes available, RBV will continue to be recommended at least in a subset of sicker patients	Deals mostly with RBV adverse-effects
MERS-CoV					
Chong *et al*[158], Korea	Antivirals should be considered as soon as possible after diagnosis	High-dose: 2.0 g po Ld → 1.2 g tid po × 4 d → 600 mg tid po × 4-6 d (adjusted to Crcl). Intermediate-dose: 2.0 g po. Ld → 10 mg/kg po tid × 10 d. IFN-α2a 180 μg/wk sc × 2 wk. Lop/r 400/100 mg po bid × 10 d	No data available. Side-effects: RBV → hemolytic anemia. Peg-IFN → myeloid dysfunction	The Guidelines focus on antiviral drugs to achieve effective management of MERS treatment	OK
SARS-CoV-2					
National Health Commission of the People's Republic of China: the COVID-19 Diagnosis and Treatment Guide 7th Edition[188], China	RBV 500 mg iv bid or tid × 10 d Use in combination with Lop/r or IFNs	IFN-α 5 MU nebulization bid. Lop/r 400/100 mg bid 10 d. Chloroquine 500 mg po bid × 7 d. Umifenovir 200 mg po tid × 10 d	Lp/r: Monitor closely for nausea/vomiting. Chloroquine: Avoid in cardiovascular disease. Concurrent use of three or more antiviral agents is not recommended		OK

bid: Bis in die; CNS: Central nervous system; COVID-19: Coronavirus disease 2019; Crcl: Creatinine clearance; ENT: Equilibrative nucleoside transport; GTP: Guanosine triphosphate; IFN: Interferon; iv: Intravenous; ld: Loading dose; Lop/r: Lopinavir/ritonavir; MERS: Middle East respiratory syndrome; po: Per os; Peg: Pegylated; RBV: Ribavirin; RdRp: RNA-dependent RNA polymerase; sc: Subcutaneous; tid: Ter in die.

Besides Pitfall 2, after scrutinizing SARS studies, we noticed very wide fluctuations regarding RBV dosing regimens. In RBV monotherapy, the high-dose intravenous scheme for viral hemorrhagic fevers[123] is the most efficacious[127,128,134,138], but it is associated with the highest adverse-effects rates[89]. In some studies, RBV dosing was equal[126] or even lower[135] than that administered for chronic HCV infection with negative outcomes (Pitfall 3). In patients treated with RBV monotherapy [127,128,132,133] or in combination with low-dose steroids[133,138] during Phases II and III, treatment results were disappointing (Pitfall 4). In contrast, when RBV was combined with pulsed methylprednisolone[127,129,130,135,136,145] or with high-doses of hydrocortisone[132,133] after development of hypoxemia, results were far more promising. Indeed, a review study confirmed the success of RBV combination with pulsed methylprednisolone when dyspnea develops. In addition, they noted that combination with IFNs might be even more helpful[146]. Although another review failed to show statistical difference in several treatment combinations, there was a clear trend in favor of RBV + lopinavir/ritonavir (Lop/r) + steroids, IFN + steroids, and RBV + pulsed methylprednisolone[142].

A large meta-analysis[143] assessed the effectiveness of RBV and corticosteroids as the initial treatment within 2 d of admission for SARS compared with no treatment. Patients without treatment had a CFR of 23.3%, with RBV alone CFR was 8.9%, with steroids alone 29.4%, and with the combination 12.6%. Based on generalized propensity score weighting, the initial findings above were totally reversed as the model predicted that the overall CFR would have been highest (19.2%) if all patients

Table 5 Severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus-2 clinical studies focused on ribavirin treatment

Ref.	Total no patients/ Patients treated with RBV	Days from symptoms onset to RBV initiation, as mean	Dosing regimen/Duration	Other treatments	Outcome	Side effects	Authors' conclusions	Comments
SARS-CoV clinical studies								
Hsu *et al* [124], Singapore	20/14	10-14	20 mg/kg tid orally	Antibiotics, Oseltamivir	6 intubated, 3 died	No	No obvious response to RBV, some deteriorated in spite of its use	Too late RBV initiation when disease is already in Phase II (Pitfall 2)
Chiang *et al* [125], Taiwan	4	4-9	1 g/d orally	Levofloxacin,IVIG, If severe hypoxia developed → Mp 2 mg/kg/d	No mortality	No	Beneficial preliminary results. Despite early use of steroids in SARS may prolong its natural course, in rapid progression and severehypoxia it may prevent from further lung injury by cytokine storm	Despite the low administered RBV dosing (Pitfall 3), satisfactory outcome
Poutanen *et al*[126], Canada[1]	10/7	Unclear	2 g ld → 1 g qid × 4 d → 0.5 g tid × 4-6 d	Antibiotics, Oseltamivir No steroids	RBV → 1 died. 1 in ICU but improving and 5 recovered	No	Pts treated with RBV improved but due to an array of therapeutics. The effect of RBV is unclear	The time gap between illness onset and RBV initiation is not reported
Avendano *et al*[127], Canada	14	4.6 d stayed at home	2 g ld → 1 g qid × 4 d → 0.5 g tid × 4-6 d	Levofloxacin 8 pts received pulsed MP	All developed dyspnea, abnormal X-ray. None intubated. Full recovery	9 pts hemolysis (days 4-6), 2 pts transfuse. 8 pts discontinued RBV but 2 pts relapsed, restarted RBV→ Recovered	RBV was associated with hemolysis that might have increased morbidity in 9 pts. No death, No intubation. 3 pts with severe hypoxia treated with *iv* steroids	Very promising combination of RBV + Levofloxacin + Pulsed Mp when hypoxia occurred
Tsang *et al* [128], Hong Kong	10	9.6 ± 5.4 d	8 mg/kg tid iv or 1.2 g tid orally	Antibiotics Steroids iv in all	2 pts → died, 8 improved	No	Combination of RBV + high dose steroids coincided with clinical improvement	Late RBV administration (Pitfall 2)
Lee *et al* [129], Hong Kong	138	When fever persisted > 48 h or Leukopenia/ Thrombo-cytopenia occurred	1.2 g tid po. If worsening 0.4 g tid iv	Antibiotics, Oseltamivir, Ps 1 mg/kg. If worsening 2-3 Mp pulses 0.5 g iv daily	5 pts → died, 32 pts in the ICU. 19 pts intubated, 76 pts were discharged.	No	The similarity of disease imaging with BOOP and of histologic features with ARDS, prompted authors to use RBV + steroids. The majority of the cohort responded to the combination	CMR = 3.6%. The time-gap between the disease onset and the therapy initiation was not reported. Nevertheless, outcomes were satisfactory
Ho *et al* [130], Hong Kong	72	4d	8 mg/kg iv tid × 7 d → 1.2 g tid po, altogether 10-14 d	Antibiotics, Steroids in 3 different regimens: Hc or Mp at dosages similar to treatment of acute severe asthma or pulsed Mp as in ARDS	Day-21 as assessment for short-term outcome. 4/72 died, 12 admitted to ICU, 6 intubated	No	Initial use of pulsed Mp appears to be a more safe and efficacious steroid regimen when compared with regimens of lower dosages	CMR = 5.5% Satisfactory results for RBV + steroids when RBV early applied
Peiris *et al* [131], Hong Kong[1]	75	As soon as SARS diagnosis was established	8 mg/kg iv tid × 14 d	Antibiotics, Hc tailing regimen (200 mg iv tid × 10 d then tapered),	At day 21, 5 died (6.7%). Convalescence at home 27 pts, 43 pts remained in	No	Higher mortality than that reported from Lee *et al*[129] (6.7% *vs* 3.5%). The clinical progression, shifting	The time-gap from symptoms onset to treatment initiation is

				Mp pulses if worsening 0.5 g iv/d for 2-3 doses	hospital of whom 13 in ICU (17%) and totally 19 pts intubated		radiological findings, and the inverted V viral-load profile suggest that worsening in week 2 is related not to uncontrolled viral replication but rather to immunopathological damage	unclear
Peiris *et al* [132], Hong Kong	50 monitored for 12 d	6.7 d	8 mg/kg tid iv 7-10 d	Antibiotics, Hc 200 mg tid tailed off	6 pts received treatment before ICU admission all recovered. 31 uncomplicated pts recovered. From 19 complicated pts 1 died	No	Complicated cases were associated with underlying diseases and delayed use of RBV and steroid treatment. CMR = 2%	Ok
Booth *et al* [133], Canada	144/126	First 48 h of hospitalization	2 g ld → 1 g qid × 4 d → 0.5 g tid × 3 d	Antibiotics Ster 40%, Hc 20-50 mg/d × 10 d	103 pts discharged. 8 pts died (6 with DM, 1 with cancer)	49% decrease in Hb > 2 g/dL. 40% transaminitis. 14% bradycardia	Poor outcome was associated with RBV treated pts but it was not significant	Despite unclear time gap between disease onset and RBV initiation, it seems that RBV alone (no Mp pulses, low steroid regimen in only 40% of pts), might not exert a clear benefit (Pitfall 4)
Zhao *et al* [134], China[3]	190/40. pts allocated to 4 groups	Not reported	group A: 0.4-0.6 g/d iv	Antibiotics	2 pts died. 3 intubated. The rest followed group D → improved	No	Early use of high- dose steroids with quinolone + azi gave the best outcome. No advantage from RBV	Unclear time-gap, too low RBV dosing (Pitfall 3). RBV treatment alone (Pitfall 4)
So *et al*[135], Hong Kong	31 pts → 1 recovered on antibiotics	5.5 d	RBV 400 mg iv tid × 3 d then 1200 mg bid orally × 10-14 d	Broad-spectrum antibiotics, Mp 1 mg/kg tid × 5 d then 1 mg/kg bid × 5 d. When worsening pulsed Mp 0.5 g iv. Then Ps 0.5 mg/kg bid × 5 d orally	17 pts showed rapid response. 13 achieved improvement with step-up or pulsed MP. None intubated. No mortality	No	Protocol provided satisfactory outcomes	No mortality reported
Lau *et al* [136], Hong Kong	88 pts → 3 recovered on antibiotics/ 68	5.8 d	So *et al*[135] treatment protocol applied	So *et al*[135] treatment protocol applied	18 pts required ventilation. 30 pts needed Mp pulses. All-cause mortality for pts aged < 60 was 0% (0/76) and 3/12 (25%) in aged > 60. CXRs of all survivors were significantly clearer in discharge	No	The standard treatment protocol of RBV + steroids and pulsed Mp resulted in satisfactory outcomes	Total CMR = 3.4% Ok
Dwosh *et al* [137], Canada	15 pts, treatment data only for 1 case	Post-intubation 9 d	2 g ld iv → 1 g qid × 4 d → 0.5 g tid × 6 d	Mp 40 mg × 2	Successfully extubated	No	No treatment conclusions	Late RBV initiation (Pitfall 2)
Sung *et al* [138], Hong Kong	138/94	3 d (0-11) to admission. RBV started after 48 h	2.4 g ld orally → 1.2 g tid. If dyspnea → 400 mg tid iv	Antibiotics Ps 0.5-1 mg/kg. If dyspnea → Hc 100 mg tid. Mp pulses for 3 d (up to 3 g)	25/94 pts responded toRBV. Mp in 107 non-resp. → 88.8% success. 15 pts died (mortality 10.9%)	Modest degree of anemia in 59%	RBV's role is doubtful in treatment. Pulsed Mp associated with improvement	RBV alone or associated with low dose steroids seems insufficient for SARS Phase 2 (Pitfall 4).

								Possibly RBV is insufficient when applied in respiratory failure
Leong *et al* [139], Singapore	229/97 compared to a group of pts who did not receive RBV on day 6	6.4 d. Duration 5.6 d. Doctor-dependent RBV use	Oral 1.2 g tid iv 400 mg tid	Insufficient data	Mortality 10.3% *vs* 12.9% in control. HR of death for RBV 0.78 (P = 0.53). When adjusted for steroids HR = 1.03 (P = 0.93)	No difference in side effects	Use of RBV alone does not seem to confer any benefit	Late use of RBV (Pitfall 2), uneven groups, doctor-dependent use of RBV (Pitfall 5). RBV alone seems insufficient (Pitfall 4)
Leung *et al* [140], Hong Kong	1755/1467 met SARS criteria/ 1416 received RBV	On symptom onset: 25 pts. 1-3 d: 480 pts. 4-6 d: 499 pts. ≥ 7 d: 412 pts	Not reported	Not reported	302 died → mortality 17.2%. CFR of 25 pts: 4.0%, of 480 pts: 11.1%, of 499 pts: 10.0%, of 412: 12.5%, of 51 pts treatment not prescribed: 29.4%	No side-effects reported	The timing of RBV administration did not seem to statistically significantly influence outcome	Authors explain their finding that it possibly results from residual confounding or insufficient power to detect a difference given that most pts were treated (Pitfall 5)
Knowles *et al*[89], Canada	110 pts focused on RBV side-effects	Not reported	High-dose RBV(total > 20 g): 2 g ld → 1 g qid × 4 d → 0.5 g tid × 3 d; Low-dose RBV: 0.4 g iv tid × 4 d → 1.2 g po bid × 7 d	Antibiotics 50% steroids	61% hemolytic anemia. 28% transfused with ≥ 1 U of RBCs. A significant decrease (> 2 mg/dL) in Hb was seen at 6.8 d after RBV started, and reached a nadir at 13 d. Anemia associated with higher RBV doses (P = 0.005) and prolonged hospital stay (P = 0.001). 35/76 pts developed hypomagnesemia, 32/62 pts developed hypocalcemia. Teratogenic effect: it is recommended that 15 half-lives (6 mo) is required to complete washout after RBV discontinuation		In contrast to HK experience where RBV associated side effects have not been detailed, their comparable RBV doses suggest that associated side effects are frequent. The benefits of RBV use may not outweigh the risk of side effects with negative economic consequences on hospitals	No outcome results for the 110 pts were reported
Chan *et al* [141], Hong Kong[4]	75 pts compared with matched cohorts of 643 and 343 pts	As soon as SARS diagnosis established. Lop/r 5.5d and 1 d after RBV. Rescue therapy: 18 d	2.4 g oral ld → 1.2 po tid or 8 mg/kg tid × 10-14 d	Lop/r 400/100mg bid × 10-14 d. 1 group received it as initial treatment and a 2nd as rescue. In addition, tailing steroids regimen × 21 d and pulsed Mp	Lop/r as initial therapy CRF 2.3% *vs* 15.6%(P < 0.05), intubation rate 0% *vs* 11% (P < 0.05). As rescue no difference	No	Early Lop/r initiation in addition to standard treatment protocols (Ho, So) showed significantly beneficial outcomes	Combination of early RBV with Lop/r and steroid regimens with pulsed Mp when needed showed statistically significant results in intubation and mortalityrates. Ok
Chu *et al* [87], Hong Kong[5]	111pts historical controls compared to 41 pts treated with RBV + Lop/r	Once diagnosis was established for RBV. For Lop/r initial treatment group it was started at a median of 3.5 d while in the rescue group at 14 d	4 g oral ld → 1.2 g tid or 8 mg/kg iv tid × 14 d	Lop/r 400/100mg bid orally ´ 14 d. Tailing steroid regimen × 21 d and pulsed Mp	21-d adverse outcome (ARDS or death) was 28.8% for the historical control *vs* 2.4% in the initial treatment group (P < 0.001). No deaths in the treatment group	Mild gastrointestinal adverse-effects. Anemia (70%) → 2 pts transfused. 26.8% bradycardia	Apparent favorable clinical response to combination of Lop/r + RBV + steroids when needed	The second study showing statistically significant benefits from the combination of RBV+ Lop/r + ster when early applied in the disease course
Cheng *et al* [142], Hong Kong[6]	772	No data available	No data available	Steroids Lop/r	No data available	No	675 pts received RBV and 44 Lop/r. No obvious difference noted irrespective of treatment combination	In Table 2 of the article however, RBV + Lop/r + ster provided a CFR of 2.3%, IFN + ster 0%, RBV + pulsed Mp 5.9%

								and RBV + ster 7.7% compared to a 15.4% of supportive treatment
Lau *et al* [143], Hong Kong, Canada[7]	Integrated data base containing 1755 HK pts and 191 Toronto cases	Within 2 d from hospital admission	No data available	Data showed for HK pts crude CMR 23.3% in neither treatment, 29.4% in steroids, 8.9% in RBV and 12.6% for combination. For Toronto pts no treatment 20%, RBV 9.3% and RBV + ster 12.8%. Authors adjusted these results for propensity scores and balance was achieved among all pts characteristics. Side-effects not considered in this study		Estimated CFRs based on the generalized propensity score weighting, the model predicted that the overall CFR would have been highest if all pts in HK had been treated with RBV + steroids, whereas it would have been the lowest if none treated. Toronto results were consistent. The combination of RBV + ster has no therapeutic benefit		The generalized propensity score weighting model prediction reversed the initial finding for CFR 12.7% of the combination to 19.2% and of untreated from 23.3% to 15.4% (!!). Inconclusive study (Pitfall 5)
MERS clinical studies								
Omrani *et al* [161], Saudi Arabia[2]	44 with severe pneumonia 20 treated 24 control. Scores APACHE II: 27, and SOFA: 11	3 d from diagnosis	2 g ld → 1.2 g tid 4 d → 600 mg tid × 4-6 d. Dosing adjusted to Crcl. Orally RBV	Antibiotics, Oseltamivir, PegIFN-α2α sc 180 μg/wk for 2 wk. Hc 200 mg/d in pts with refractory septic shock	41/44 intubated. 14-d mortality: treat 6/20 *vs* control 17/24 (P = 0.004). 28-d: treat: 14/20 *vs* control 20/24 (P = 0.054)	RBV well tolerated. Hb drop in treat > control (P = 0.002). No differences in transfusions, no treatment discontinuation	Significant benefit in 14-d survival. The loss of difference in 28-d might be explained by high initial APACHE II and SOFA scores and several comorbidities	Surprisingly, statistically significant results despite that eligible patients had initially severe pneumonia (Phase 2) (Pitfall 4) without high dose steroids applied. Long- lasting IFNs (peg) might not be the best form for acute infections
Shalhoub *et al*[162], Saudi, Arabia[2]	32 pts were already under MERS pneumonia and some with respiratory failure	For IFNs: 1 d after MERS diagnosis. For RBV not reported	2 g ld orally → 600 mg bid	Antibiotics, IFN-α2a sc 180 μg/wk × 2 wk. IFN-β1a sc44 μg × 3 times/wk	Overall mortality: 22/32 (69%). IFN-α2a + RBV: 11/13 (85%). IFN-β1a + RBV: 7/11 (64%). Hemodialysis pts: 14/14 (100%)	No	IFN-α2a or IFN-β1a + RBV were ineffective against MERS mortality	Unknown time-gap between symptom onset and treatment initiation. Very low RBV dose applied (Pitfall 3). In specific cases with severe pneumonitis high-dose steroids and Mp pulses should have been used for better outcomes (Pitfall 4)
Al Ghamdi *et al*[171], Saudi, Arabia[2]	51 pts	No data reported	No data reported	Antibiotics, IFN-α, IFN-β, MMF, Hc in 5 pts	31 pts received antivirals (IFNs,RBV) in several combinations, 8 pts MMF all survived. (IFN-β and MMF were given to less severely pts). CMR = 37%	No	IFN-β and MMF were predictors of increased survival	No time gap from symptom onset reported. No dosing reported. Inconclusive study for RBV treatment
Choi *et al* [172], Korea[8]	186 pts	6 d (1-20 d) 14% of pts within 48 h	81% IFN-α + RBV + Lop/r, 12.7% IFN-α + RBV, 5.0% RBV + Lop/r, No dosing regimens reported		CMR = 20.4% lower than others ranging 36.5%-65%	No	Unable to assess the clinical impact of therapies as most pts received antivirals	No dosing regimens, not duration reported

Arabi *et al* [166], Saudi, Arabia[9]	309/151 pts critically ill received steroids	3 d from ICU admission	Antivirals: RBV, IFN, RBV + IFN, oseltamivir. The median of the maximum daily Hc-equivalent was 300 mg with a median duration of 7 d		CMR 74.2% *vs* 57.6% (no steroids). After adjustment for baseline and time-varying confounders the use of steroids was not associated with increased 90-d mortality but with delayed RNA clearance	No	Steroids were commonly used in critically ill patients with MERS. Pts given steroids were more likely to have 1 or more comorbidities than those who did not (P = 0.001)	No Mp pulses were administered. Maximum Hc doses reported (300 mg) are equivalent to only 60 mg of Mp. In addition, authors do not comment about the impact of the co-administered antivirals (Pitfall 5)
Habib *et al* [163], Saudi Arabia[2]	63/61pts presented with severe illness (pneumonia 87.3% and septicemia 11%)	No data reported	No data reported	No data reported	Overall CMR 25.4%. Treated 22.9%. Survivors were more likely to have had received IFN + RBV than those who died (P = 0.01)	No	CMR 25% comparable to that of Omrani 30%, lower than AlMekhlafi (74.2%), Khalid (55%), and Al-Tawgiq (100%). Unable to determine the combination efficacy in the absence of a reference group	No dosing regimen, no time-gap from onset. The severity in admission probably implies an advanced disease phase, where antivirals are less effective (Pitfall 4)
Arabi *et al* [166], Saudi, Arabia[2]	349/144 critically ill all ICU pts	2d from ICU admission but 9 d (6-12) from symptom onset	RBV: 2 g ld po → 1.2 g po tid × 4 d → 600 mg tid po × 4-6 d	Peg-IFN-α2b → 1.5 mcg/kg sc × 2 wk Per-IFN-α2a → 180 μg/wk × 2 wk Peg-IFN-β1a → 44 mg sc × 3/wk	Crude CMR was higher in antiviral treated group 73.6% *vs* 61.5% (P = 0.02). However, with a marginal structural model there was no significant difference in 90-d mortality (aOR: 1.03; 95%CI: 0.73-1.44, P = 0.87). Also, no significant difference in RNA clearance (aOR: 0.65; 95%CI: 0.3-1.44, P = 0.29)		During ICU stay RBV/IFN treated pts were more likely to receive steroids (59.7% *vs* 44.9%P = 0.006). Future studies should test the efficacy of newer antiviral interventions	Very late antiviral initiation. Possible higher needs for steroids in antiviral – treated group could imply more severely ill pts (Pitfalls 2, 5)
AlMekhlafi *et al*[167], Saudi, Arabia[2]	31 pts in ICU. 13 pts received RBV+ IFN-α2α	ICU pts	Not reported	Not reported	CMR 74.2%. Among 13 pts who were given antivirals, 9 died	No	All pts who received either oseltamivir or RBV + IFN-α2a had no favorable outcomes	Antivirals may have no efficacy in Phase II-III of MERS (Pitfall 4)
Khalid *et al* [168], Saudi, Arabia[2]	14 pts intubated 11 pts received RBV	6 d	Not reported	Antibiotics RBV + Peg-IFN-α2a, Mp 1 mg/kg/d × 7 d	9 pts died in the ICU, 5 discharged	No	MERS with ARDS has high mortality rates. The role of RBV + IFN warrants further evaluation	Antivirals may have no effect in Phase II-III of MERS-infected pts under mechanical ventilation (Pitfall 4)
Khalid *et al* [164], Saudi, Arabia[2]	6 pts, 3 cases 74-84 yr, 3 cases 17-54 yr	1st group 12-19 d; 2nd group 1-2 d	2 g ld → 1.2 g tid × 4 d → 0.6 g tid × 4-6 d	IFN-α2b sc 180 μg/wk × 2 wk. 1 case received pulsed Mp and recovered	1st group pts all died. 2nd group all recovered	No	Combination of RBV and IFN-α2b have a role in treatment of MERS if started early in disease course	Very late (12-19 d) antiviral initiation in 1st group when disease is already in the ARDS phase (Pitfall 4). 1 case was helped by Mp pulses
Al-Tawfiq *et al*[169], Saudi, Arabia	5/5	11-21 d (after admission)	2 g ld → 400 mg po tid	Antibiotics Oseltamivir IFN-α2b Mp 40 mg tid or Ps 40 mg/d	All died	No	All pts were already intubated when treatment started	Antivirals in Phase 2, very low RBV dosing, low ster dosing for Phase 2-3 (Pitfalls 2, 3, 4)

Park *et al* [159], Korea[2]	43 HCW with high-risk exposure to MERS pneumonia pts. 21 HCW with more severe exposure received PEP. 22 HCW no PEP	Within 36 h after unprotected exposure	RBV 2.0 g ld orally → 1.2 g tid × 4 d → 600 mg tid × 6-8 d	Lop/r 400/100 mg bid × 11-13 d	6/43 HCW exposed developed MERS infection. The attack rate was lower in the PEP *vs* no-PEP (0% *vs* 28.6% OR: 0.405 P = 0.009). No MERS infection in PEP group. Only PEP therapy reduced significantly the risk of MERS infection (OR: 0.714; P = 0.009)	Mild: diarrhea, nausea, anemia, stomatitis, leucopenia, hyperbilirubinemia. No PEP discontinuation. All normalized after completion of PEP	PEP therapy was associated with a 40% decrease in the risk of infection	The only study reporting results of PEP prophylaxis with the combination of Lop/r + RBV. Ok
COVID-19 clinical studies								
Tong *et al* [189], China[2]	115/44 pts Severe disease. 9 pts intubated 28 pts NINV	8 d from onset 4 d from diagnosis	500 mg iv bid	Antibiotics	Negative conversion time of SARS-CoV-2 test in RBV *vs* control (12.8 d *vs* 14.1 d, P = 0.314) CFR 17.1% *vs* 24.6% (P = 0.475)	No side effects. No difference in anemia	RBV administration was doctor-dependent and sometimes RBV was out of stock. In severe COVID-19 RBV is not associated with improved negative conversion time for SARS-CoV-2 test or improved mortality	Pitfall 2. Relatively moderate RBV dosing (Pitfall 3). Possibly not regular RBV administration (Pitfall 5)
Li *et al*[190], China[2]	151 pts, Number of pts treated with RBV was not specified. Moderate to critical disease	Not reported	500 mg iv bid or tid × 10 d	Umifenovir Lop/r, Traditional medicine, Peramivir, Oseltamivir, Penciclovir Ganciclovir	25 pts discharged 25 pts hospitalized 79 pts clinical improvement7 died (CFR = 4.6%)	The use of two-step clustering and subgroup analysis enabled an in-depth analysis of the effects of single or combined antiviral therapy. Following the antiviral therapy, there was indeed an improvement of severe patients' condition. Combination was superior to single or dual agents. A quadruple combinationof Umifenovir + RBV + Lop/r+ Lianhua Qingwen has been recommended for critically ill COVID-19 pts		Incomplete data (time-gap from symptom onset to treatment initiation) (Pitfall 5)
Yuan *et al* [191], China[2]	94 pts, 46 pts IFN-α + Lop/r.21 pts IFN-α + Lop/r + RBV. Median age 40 yr. 15 pts, 1 or 2 comorbiditie. Mild disease: 8 pts. Moderate: 75 pts. Critical: 11 pts	Hospitalized 7d after symptom onset	No data reported	No data reported	Significant correlation between the length of hospital stay and PCR negative conversion time in pts treated with IFN + Lop/r (P = 0.012) and with IFN + Lop/r + RBV (P = 0.0215). No death, no intubation, all recovered	No	These two regimens might be beneficial for COVID-19 treatment	Pitfall 2. No dosing regimens reported. Ok
Wu *et al* [192], China[9]	80/80 pts, 41 females, 46.1 yr. 77 pts mild to moderate symptoms. 3 pts severe. 38 pts chronic diseases	Not reported	Not reported. Duration 7 d	Moxifloxacin duration 7 d12 pts Mp to alleviate the shortness of breath	No death, no INV. 35 pts NINV. 55 pts abnormal chest CT. 3 pts transaminitis. 1 pt hemodialysis. As of writing, 21 pts discharged (stay 8 d)	No	Notably, infected patients may be falsely excluded based on 2 consecutively negative respiratory pathogenic PCR tests	Surprisingly, authors do not discuss at all the role of treatment administered (RBV + Mp + Moxi) (Pitfall 5)
Chen *et al* [193], China[2]	681 pts with severe disease/279 received RBV. 375 pts had comorbidities. Median 65 yr. 40-65 yr 46.1% of pts, > 65 yr 47.1% of pts	No time-gap between symptom onset and initiation of treatment, no dosing regimens reported, or drug combinations. 666 pts received antivirals, antibiotics (83.8%), IVIG (54.6%), and steroids (48.8%)			In a report from China overall mortality from COVID-19 was 2.3% while in critical cases 49%. In another from Italy CFR was 26% in ICU pts. Another study indicated a mortality of 15% while in ICU cases 38%. In this study CFR was 15.3%. 45.8% of the pts had preexisting cardiovascular disease, of which 23.4% died. In multivariate analysis, RBV and arbidol were positively associated with death, OR: 0.208 (95%CI: 0.07-0.618; P = 0.005). Of notice, RBV might have a beneficial effect in severe COVID-19 pts with cardiovascular diseases and cardiac injury by disease. Therefore, every drug regimen should include arbidol or RBV for severe cases			Impressive findings for both antivirals in reducing mortality in severe cases. The beneficial effect of RBV in cardiac injury is supported by another

								study which showed that RBV is mostly concentrated in heart and intestines
Peng *et al* [197], China[2]	75 pediatric pts. 8 most critical cases received RBV + IFN-α	4.9 d	10 mg/kg/d bid iv	IFN-α neb1-4 μg/kg/d bid. Antibiotics, arbidol 5 pts, oseltamivir 20 pts	All discharged. Length of hospital stay 10.6 d and SARS-CoV-2 clearance 6.4 d. The two most severe cases were treated with RBV	No	Severity in pediatric pts milder than adults. The efficacy of antiviral therapy in children remains to be evaluated	Ok
Huang *et al* [201], China[10]	101 pts, 33 pts RBV + IFN-α, 36 pts IFN-α + Lop/r, 32 pts RBV + Lop/r + IFN-α, Mild to moderate severity	4 d to enrollment	2.0 g ld iv → 400-600 mg tid depending on bw × 14 d	Lop/r 400/100 mg bid × 14 d IFN-α in h 5 MU bid × 14 d	SARS-CoV-2 time to negativity 12 d in group 2 *vs* 13 and 15 d in groups 1 and 3 (P = 0.23). Higher proportion of nucleic acid negativity in group 2 (61.1%) than (51.5% and 46.9%) in groups 1 and 3 in 14 d	GI side-effects mainly in the triple combination	No significant differences among the three regimens in terms of antiviral efficacy. Significant GI effects in the triple combination	Ok
Hung *et al* [202], China[11], Open-label Phase 2 trial	127/81 pts 81 RBV + Lop/r + IFN-β1b. 41 Lop/r (control). Median age 52 yr. Men 54%, 51 pts had underlying diseases. Mild to moderate COVID-19	Triple combination: 5 d, control: 4 d	400 mg bid × 14 d	Oral Lop/r 400/100 mg IFN-β1b 8MU on alternate day sc up to 3 doses (within 1st wk). Hc 50 mg tid in oxygen desaturation	Abnormal chest X-ray in 96 pts. 17 pts oxygen desaturation → 6 in ICU, 1 intubated (96 yr) but extubated after 10 d. No one succumbed. Time to negative swab from treatment initiation in combo 7 d *vs* 12 d in control (P = 0.001)	Mild and self-limiting. Diarrhea, nausea, transaminitis, all resolved within 3 d from treatment initiation	Time to NEWS2 0 in combo 4 d *vs* 8 d (P = 0.0001) in control and time to SOFA 0 in combo 3 d *vs* 8 d in control (P = 0.041). Hospital stay duration: combo 9 d *vs* 14.5 d in control (P = 0.016). In subgroup analysis when authors compared pts with early (< 7 d) treatment initiation in both groups, all comparisons where statistically very significant (P < 0.0001) including improvement in NEWS2 and SOFA scores, and time to negative viral loads	Early antiviral triple therapy is superior to lop/r in shortening shedding, alleviating symptoms and facilitating discharge of pts with mild to moderate COVID 19. Ok
Eslami *et al* [199], Iran[12]	62/27pts All treated with SOC: Lop/r + HCQ	Not reported (at admission)	RBV 600 mg bid × 14 d	Sof/ daclatasvir 400/60 mg qd. All treated with Lop/r 400/100 mg bid × 5 d and HCQ 400 mg single dose	Median stay 5 d for Sof/d *vs* 9 d for RBV. CFR 6% in Sof/d *vs* 33% in RBV. Relative risk of death for those treated with Sof/d0.17 (95%CI: 0.04-0.73; P = 0.02)	Mild adverse effects reported but no discontinuation was demanded	Given these encouraging initial results, further investigation in larger-scale trials seems warranted	Unclear time-gap from disease onset to RBV initiation. Low RBV dosing. Confusing study as both arms were concurrently treated with other anti-coronaviruses agents (Pitfalls 3, 5)
Kasgari *et al* [200], Iran[13]	48 pts moderate disease, 24 pts → Sof/d + RBV24 pts → SOC: Lop/r + HCQ + RBV depending on recommendations at the time of the study	Not reported	RBV 600 mg bid	The median duration of hospital stay, number of ICU admissions, and number of deaths: no statistically significant differences between the two groups. Only trends for recovery and lower deaths in the Sof/d + RBV arm				Very small number of participants, Pt 2 unclear, fell under Pt3. Confounding results as both arms were concurrently being treated with antivirals, even with RBV (Pitfalls 2, 3, 5)

Liu *et al* [198], China[14]	Enrolled studies with COVID-19 (n = 12), MERS (n = 2), SARS (n = 4) and influenza (n = 1)	Interventions in the studies RBV (n = 3), HCQ (n = 5), favipinavir (n = 3), IFN (n = 3), Lop/r (n = 2), umifenovir (n = 1)	This review did not find persuasive evidence of benefit for treatment using RBV in a population of pts with COVID-19 and results from studies evaluating SARS or MERS provided no support for a reduction in mortality with RBV treatment[85,119,171]	Only treatment with Lop/r for which authors found low-quality evidence for a decrease in hospital stay in ICU. To date, persuasive evidence of important benefit in COVID-19 does not exist for any antiviral although for each treatment evidence has not excluded important benefit	Very controversial conclusion (Pitfall 5)
Zhong *et al* [88], China[14]	COVID-19 = 7 studies. SARS = 9, MERS = 2, RBV = 4 studies, RBV + Lop/r + ster = 2, RBV + IFNs = 3, RBV + ster = 1	Compared with comparators, interventions notably reduce mortality (RR: 0.65, 95%CI: 0.44-0.96, I^2 = 81.3%). In subgroup analysis, the combination of RBV + ster remarkably decreased mortality (RR: 0.43, 95%CI: 0.27-0.68). Besides, Lop/r, RBV, RBV + IFN and combination of Lop/r + RBV + ster showed tendency of lower mortality. Interventions also remarkably ameliorated clinical and radiological improvement, without manifesting clear effect on virological eradication (except for Lop/r-based regimens), incidence of ARDS, intubation, and adverse effects	In conclusion, there was evidence of lower mortality, better clinical and radiological improvement in intervention group compared to control		A very large meta-analysis with remarkable conclusions for coronaviruses treatment. Ok

[1]Prospective.
[2]Retrospective.
[3]Prospective randomized.
[4]Multicenter retrospective matched cohort.
[5]Open non-randomized prospective.
[6]Review.
[7]Systematic review.
[8]Retrospective observational.
[9]Multicenter retrospective.
[10]Randomized, open-label, prospective trial.
[11]Multicenter randomized prospective.
[12]Open label parallel trial.
[13]Randomized controlled trial.
[14]Meta-analysis.

aOR: Adjusted odds ratio; ARDS: Acute respiratory distress syndrome; bid: Bis in die; bw: Body weight; CI: Confidence interval; COVID-19: Coronavirus disease 2019; CFR: Case fatality rate; Crcl: Creatinine clearance; GI: Gastrointestinal; Hb: Hemoglobin; HCQ: Hydroxychloroquine; HR: Hazard ratio; ICU: Intensive care unit; IFN: Interferon; IVIG: Intravenous immunoglobulin; neb: Nebulizer; Lop/r: Lopinavir/ritonavir; MERS: Middle East respiratory syndrome; MMF: Mycophenolate mofetil; OR: Odds ratio; pts: Patients; RR: Relative risk; SARS: Severe acute respiratory syndrome; RBV: Ribavirin; Sof: Sofosbuvir; ster: Steroids; tid: Ter in die.

had been treated within 2 d of admission, whereas it would have been lowest (15.4%) if no treatment applied. As they underlined, the main design analyses were a snapshot of treatment or not within first 2 d of admission. However, they arbitrarily concluded that clinicians should not use RBV and corticosteroids to treat SARS as they provide no benefit in terms of survival (Pitfall 5). This generalization was based on their initial condition of 2 d snapshot findings, and they missed the care during the long intermediate period extending to the final outcome. Initiation of corticosteroids early during Phase I of viral replication may suppress the immune response and allow a higher peak viral load[146] (Pitfall 6). In contrast, two studies[87,140] showed statistically significant superiority in intubation and mortality rates of RBV combination with Lop/r when applied early in the disease course pulsed methylprednisolone was added when necessary. In the second one, CFR was 2.3% in the combination group *vs*

15.6% in standard of care (SOC) group ($P < 0.05$). In the second one, there were no deaths in the combination group, and likelihood of ARDS development was much higher in SOC (28.8% *vs* 2.4%, $P < 0.001$). IFN regimens were not widely utilized in the SARS outbreak, except for a prospective study with a limited number of cases that was inconclusive for IFN efficacy[134]. Another preliminary study concluded that the use of IFN alfacon-1 + corticosteroids was associated with reduced desaturation and more rapid resolution of imaging abnormalities[147].

In conclusion, Severe acute respiratory syndrome-associated coronavirus (SARS-CoV) treatment with RBV alone or in combination with low-dose steroids in Phase II of the disease is probably ineffective (Pitfall 4), while early initiation of RBV + Lop/r combination decreases the viral load and significantly lowers the need for steroid use, intubation, and finally mortality. When ARDS develops (Phase III), pulsed methyl-prednisolone should be administered in addition to adequate ventilation.

MERS outbreak

Since September 2012, WHO has been notified of 2519 Laboratory-confirmed cases of MERS in 27 countries, including 866 associated deaths (CFR = 34.3%) globally[148]. A majority of cases were reported from Saudi Arabia. By the end of January 2020, confirmed Middle East respiratory syndrome coronavirus (MERS-CoV) cases have occurred every year, mostly in the Middle East. MERS β-coronavirus is closely related to two Asian bat β-coronavirus (HKU4 and HKU5) in lineage C. In contrast to SARS-CoV, which uses angiotensin-converting enzyme 2 to gain entry into cells, MERS uses dipeptidyl peptidase 4 as a functional receptor[149]. MERS-CoV *in vivo* targets type II alveolar cells, Clara cells, and endothelial cells but not ACE-2-expressing ciliated epithelial cells infected by SARS-CoV. MERS-CoV, unlike SARS-CoV, can also infect and replicate in human monocyte-derived macrophages[150]. This increases the expression of major histocompatibility complex class I and co-stimulatory molecules, leading to a more exaggerated activation of the immune response. These differences in receptor usage and susceptibility to type I and type III IFN may account for the differences in disease patterns, organ tropism, and virus shedding[150-152].

MERS in vitro studies

A chemical library of 1280 known drugs against influenza A was assessed for possible anti-MERS-CoV activity[153]. In the Madin-Darby canine kidney cell line, mycophenolic acid (MPA), RBV, and IFNs were active against MERS-CoV, while in Vero cells, RBV and MPA were inactive (Pitfall 1). Scientists concluded that IFN-β1b and MPA should be considered for treatment trials. Similar were the findings in another *in vitro* study[154] that also fell into Pitfall 1. When both Vero and LLC-MK2 cell lines were utilized, authors concluded that the latter was more sensitive to RBV, and when combined with IFN-α2b, inhibitory RBV concentrations were achievable in humans[155]. Additionally, two animal studies with MERS-infected rhesus macaques [156] and common marmosets[157]were performed. In the former, scientists concluded that IFN-α2b + RBV should be considered for early intervention therapy in MERS and in the latter that low dose RBV combined with IFN-β1b and/or Lop/r may have synergistic effects.

MERS clinical studies

Physicians have published recommendations for the antiviral treatment of MERS-CoV infection and propose the combination of IFN-α2a + RBV + Lop/r[158], and the combination of RBV + Lop/r for post-exposure prophylaxis[159]. Early drug administration is essential in MERS as there is a more rapid progression to death than SARS [160]. In MERS-CoV published studies (Table 5), antiviral treatment was commenced very late in the disease course in patients with severe pneumonia and respiratory failure[161-164] (Pitfall 2), or in patients already in the intensive care unit (ICU) or intubated[165-170] (Pitfalls 2, 4). Characteristically, in a case-series of five patients who were intubated when antivirals (IFN-α2b + RBV) and low-dose steroids were initiated, mortality was 100%[169]. The majority of MERS clinical studies fell under Pitfall 2, probably because the disease progression is much more rapid than SARS-CoV. However, in a study by Omrani *et al*[161], when antivirals were initiated 3 d after symptom onset, some statistically significant findings and trends were provided. Forty-four MERS patients (73% men, mean 65.5-year-old) with a median of three comorbidities and with severe pneumonia [APACHE II score of 27, Sequential Organ Failure Assessment (SOFA) score of 11] were treated with the antiviral combination of Peg-IFN-α2a + RBV. Fourteen-day mortality was 6/20 *vs* 17/24 in the comparator group ($P = 0.004$). Nevertheless, 28-d mortality did not show any significant difference

(CRF 70% *vs* 83.3%, P = 0.054), probably due to the small number of cases, the high initial APACHE II and SOFA scores, and the comorbidities but mostly due to the fact that antiviral treatment is ineffective in Phases II-III of the disease. Moreover, they did not administer immunosuppressive/immunomodulatory therapy for ARDS (such as pulsed methylprednisolone) (Pitfall 4). A retrospective study[162] aimed to find potent efficacy of IFN-a2a or IFN-b1a in combination with RBV in MERS pneumonia but concluded that there was lack of efficacy (Pitfalls 2, 3, 4 present in this study). In a retrospective study[171] in which 31 patients received a number of different antiviral combinations (overall CFR of 37%), authors concluded that only any IFN (mainly IFN-β, P = 0.009) and mycophenolate mofetil treatment (P = 0.019) were predictors of increased survival in the univariate analysis. In a Korean[172] retrospective observational study, the lowest ever CFR of 20.4% was reported. The triple combination of IFN + RBV + Lop/r was administered in 112 pts and accounted for a CFR of 17.9%, while combination therapy IFN + RBV provided only 5.6% (1/18). Researchers attributed this low CFR to the application of aggressive treatment measures, including antiviral agents early from disease onset (median 6 d). Another study confirmed these findings [163] in patients with pneumonia and/or sepsis. The combination of IFN-α + RBV resulted in a relatively satisfactory CFR (22.9%), whereas patients who survived were more likely to have had received the combination therapy than patients who died (P = 0.01). No steroid use was reported in this study too (Pitfall 4). Researchers in a multicenter study[165] with ICU patients deduced that the use of steroids was not associated with increased 90 d mortality but with delayed RNA clearance. Nevertheless, the patients who received steroids had one or more comorbidities (*e.g.*, diabetes, chronic pulmonary, and cardiac diseases) compared to those who did not receive steroids (P = 0.001). Furthermore, they utilized a maximum hydrocortisone equivalent of 300 mg/d, which corresponds to 60 mg of methylprednisolone, far from pulsed methylprednisolone dosing (Pitfall 3).

In a retrospective study of critically ill patients, the association of RBV + IFN was evaluated[166]. Using a marginal structural model, RBV + IFN was not associated with changes in 90 d mortality or with more rapid MERS-CoV RNA clearance. This large study, however, also fell under specific pitfalls. The time-gap from symptom onset to treatment initiation was 9 d (Pitfall 2). Furthermore, patients under antiviral treatment received statistically significant (P = 0.006) more steroids compared than the rest, an intervention implying that those were more severe patients than the former group. Steroid dosing regimen and time of steroid administration was not reported (Pitfalls 3, 4). In a number of ICU and intubated patients suffering MERS infection with very high CFRs (74.2%[167] and 64.3%[168]), the authors concluded that RBV + IFN combination had no favorable outcome in patients with ARDS, a finding consistent with Pitfall 4. In a small case-series, RBV + IFN-α2b was administered within 1-2 d of admission to three patients, all of whom survived. In contrast, three other patients who received therapy 12–19 d after admission did not survive[164]. Clinicians inferred that combination treatment has a role in MERS infection only when given early in the disease course.

Coronaviruses have been shown to suppress IFN response in hosts[149]. A subdued IFN response diminishes antigen presentation and reduces antiviral adaptive Th-1 immune response[173,174]. Therefore, recombinant IFNs have been identified as a treatment modality for MERS for their ability to augment host response. Type I (α, β) [112,121,175,176], type II (γ)[177,178], and type III (λ) IFNs[177] exhibit activity against SARS-CoV. IFN-β is the most potent[112,117,179] when compared with IFN-α and -γ. MERS-CoV is 50-100 times more sensitive to IFN-α than SARS-CoV in Vero cells[180]. As viruses that cause lysis of their target cells are most effectively inhibited by IFNs in uninfected cells, IFNs have their highest utility in prophylaxis or early post-exposure. IFNs display synergistic characteristics. When administered together, IFN-β and IFN-γ inhibited SARS-CoV plaque formation 30-fold and inhibited replication by 3000-fold [176,178]. The combination of IFN-α2b and RBV was effective in reducing MERS-CoV replication in Vero and LLC-MK2 cells[155]. The biological plausibility of the combination was studied *via* microarray, which showed that RBV and IFN-α targeted MERS-CoV genes were involved in pathogen recognition, cytokine release, and immune responses[181]. On the other hand, lopinavir was found to inhibit MERS-CoV *in vitro* in Vero E6 and in Huh7 cells, at a mean half maximal effective concentration of 8.0 mmol/L in a screen of 348 Food and Drug Administration-approved drugs for anti-MERS-CoV activity[182]. Taking into account all these data and considering the MERS clinical studies with the least pitfalls and the lowest CFRs, we conclude that the combination of RBV with IFNs (mainly PEG-IFN-α2α)[161,163,164,172] or triple combination with Lop/r[172] is the best antiviral early treatment in MERS-CoV infection. The role of steroids as immunomodu-latory/immunosuppressive therapy in

Phases II-III (ARDS) was not thoroughly studied in MERS, and patients were managed at this Phase solely by mechanical ventilation or extracorporeal membrane oxygenation.

COVID-19 outbreak

A novel, very contagious coronavirus (nCoV-19) was first identified in humans in December 2019 in Wuhan, China, and it quickly spread globally. Data provided by the WHO Health Emergency Dashboard report almost 50 million confirmed cases of COVID-19, including 1.24 million deaths, with confirmed cases in 219 countries (accessed November 08, 2020) https://www.who.int/emergencies/diseases/novel-coronavirus 2019?gclid=Cj0KCQjw2or8BRCNARIsAC_ppyblMJawKCnLtU9F6oYuWCpdraGvdC7QvkrQKqF5 LpbNX5G7kBUefqYaAkB3EALw_wcB. nCoV-19 virus was subsequently termed the SARS-CoV-2 virus, as it is very similar to SARS-CoV. Indeed, in phylogenetic analysis there is 96.08% identity between COVID-19 and SARS-CoV main protease, called 3-C-like protease (M-pro), while identity is only 51.61% between COVID-19 and MERS-CoV M-pros.

In computational (in silico) studies (Table 3), molecular modelling, virtual screening, docking, and sequence comparison statistics of the COVID-19 M-pro were investigated. In the Schrodinger glide docking module, RBV and telbivudine were ranked at the 2nd and 3rd positions in SARS-CoV-2 M-pro inhibition, respectively where RBV was shown to form two hydrogen bonds with M-pro[183]. In another in silico study, scientists used the optimized COVID-19 and SARS RdRps, and found that sofosbuvir and RBV compete with physiological nucleotides for the RdRp active site and form seven and 13 H-bonds, respectively, suggesting that they can be used against COVID-19 with promising results[184]. In a transcriptosome-based drug repositioning study, scientists using bronchoalveolar lavage fluid transcriptome data of eight COVID-19 patients and 20 healthy controls and found that the endocytosis and lysosome pathways are highly involved in the disease and that the regulation of genes involved in neutrophil degranulation was disrupted[185]. The principle of transcriptome-based drug screening identifies drugs that are capable of restoring virus-induced gene expression dysregulation rather than directly targeting viral or human proteins. They identified a total of 1569 differentially expressed genes, consisting of 872 genes with upregulated expression and 697 genes with downregulated expression. Two Food and Drug Administration-approved antiviral drugs (saquinavir and RBV) were identified in the coexpression-based drug enrichment analysis for the prevention and treatment of COVID-19 pneumonia. In contrast, in two *in vitro* studies utilizing nCoV-19 isolates, RBV did not exert any inhibitory effects (half maximal effective concentration 109.5-500 μmol/L). However, in both studies[186,187] Vero cell lines were utilized (Pitfall 1), which are naturally RBV-resistant, as they are inefficient at converting RBV into its mono- and triphosphate forms[119].

A literature search retrieved 137 articles, 12 of which reported on RBV monotherapy or in combinations in COVID-19 and were considered eligible (Table 5) (accessed November 14, 2020). RBV was also included in the Chinese treatment guide[188]. There were four randomized open-labeled prospective studies, six retrospective studies, and two meta-analyses. In one study[189], researchers compared retrospectively 71 patients with severe COVID-19 treated with SOC and 44 patients treated with RBV. SOC applied in both groups included ventilation, corticosteroids, and intravenous immunoglobulin. Despite the obvious Pitfall 2 (median time from symptom onset was 8 d), the relatively low (500 mg bis in die) RBV monotherapy dosing (Pitfall 3), and the unclear corticosteroid background treatment, there was a trend in favor of RBV in the negative conversion time of reverse transcription polymerase chain reaction (12.8 d *vs* 14.1 d), in intubation rates (4.5% *vs* 9.9%), and in CFR (17.1% *vs* 24.6%). Because those findings were statistically insignificant, authors extrapolated their results to conclude RBV insufficiency for severe COVID-19, despite the serious study design issues (small number of patients, doctor-dependent RBV administration, and irregular administration-sometimes RBV was out of stock) (Pitfall 5).

In another retrospective study[190], 151 moderately to critically ill patients were recruited, and combination treatment was superior to single or dual antiviral agents. Use of a quadruple combination therapy (RBV + Lop/r + umifenovir + Lianhua Qingwen) significantly improved severe COVID-19 patients. Yuan *et al*[191] aimed to evaluate the correlation between viral clearance and blood biochemical index of 94 patients suffering predominantly moderate COVID-19 who were hospitalized 7 d after symptom onset (Pitfall 2). Correlation analysis indicated that the duration of hospital stay was significantly correlated with polymerase chain reaction negative conversion times in IFN-α + RBV + Lop/r group (P = 0.0215) as well as in IFN-α + Lop/r group (P

= 0.012). In addition, there were no intubations or deaths, and all were discharged. Authors inferred that these two therapeutic regimens are beneficial for the treatment of COVID-19 infected patients. Wu *et al*[192] described 80 imported cases in Jiangsu Province (China) with mild to moderate symptoms and compared them with those from Wuhan. They presumed that the cases in Jiangsu exhibited mild or moderate symptoms and no obvious gender susceptibility. As of their writing, no one was intubated or died, and 21 patients were discharged. However, they did not mention at all the possible therapeutic role of treatments applied (RBV to all patients, methylprednisolone in 12 patients at an appropriate dose to alleviate the shortness of breath, and moxifloxacin). The largest retrospective study enrolled 681 patients with severe COVID-19[193] in order to clarify their epidemiological, clinical, and therapeutic features. Their median age was 65 years, and 55.1% had comorbidities, with incomplete data regarding the time-gap from symptom onset to treatment initiation, the dosing regimens, and possible drug-combinations administered. Overall mortality of this cohort was 15.3%; this was similar to CFR in another study in which higher CFR (38%) was calculated for ICU patients[194] and comparable (26%) with another report from Italy[195]. Out of 681 patients, 45.8% had cardiovascular disease, and 23.4% of them succumbed. In multivariate analysis, RBV was independently associated with predicting the risk of death in COVID-19 patients [odds ratio: 0.477 (0.232–0.982); P = 0.044] as well as arbidol. Multivariable logistic regression in patients combined with COVID-19 and cardiovascular disease performed to evaluate the efficiency of the intervention showed that RBV was also significantly effective in these patients in two different models applied [odds ratio: 0.208 (0.070–0.618); P = 0.005], in accordance with the findings of the total cohort. The authors inferred that RBV and arbidol were effective in patients with severe COVID-19, especially in the subgroup of those with cardiovascular comorbidities and cardiovascular injury by SARS-CoV-2. The latter would not be surprising, taking into account another report[196] in which scientists compared the pharmacokinetic profiles and tissue distribution of antiviral drugs and concluded that RBV is highly concentrated in the heart and the intestines. Other clinicians treated six critical and two severe out of 75 pediatric patients[197] with IFN-α nebulizer and intravenous RBV with favorable outcomes.

In one meta-analysis[198], it was concluded that, except for Lop/r for which they found low quality evidence in decreasing ICU stay, there was no persuasive evidence demonstrating important benefit for any antiviral in COVID-19. Notwithstanding, they came to the contradictory conclusion that evidence had not excluded the important benefit of each treatment. On the contrary, another meta-analysis[88] that evaluated the efficacy and safety of antiviral therapeutic options in coronaviruses infections, scientists inferred that therapeutic interventions notably reduce mortality [relative risk (RR): 0.65, 95% confidence interval (CI): 0.44-0.96, I^2 = 81.3%]. In a subgroup analysis, the combination of RBV and corticosteroids remarkably decreased mortality (RR: 0.43, 95%CI: 0.27-0.68). Besides, Lop/r IFN + RBV, RBV, and combination of Lop/r + RBV + corticosteroids showed a tendency to lower mortality, remarkably improve clinical manifestations (RR: 1.52, 95%CI: 1.05-2.19), and improve radiographical findings (RR: 1.62, 95%CI: 1.11-2.36, I^2 = 11.0%), even without manifesting clear effects on virological eradication, incidence of ARDS, intubation, and adverse events. The Lop/r-based combination showed superior virological eradication and radiographical improvement with reduction in the rate of ARDS. Conversely, RBV might cause more safety concerns, especially bradycardia.

Of the four RCTs of COVID-19 therapy that included RBV, two of them were based on sofosbuvir/daclatasvir (Sof/d) combination. The first one[199] compared Sof/d with RBV orally, with both arms being treated with SOC (Lop/r and hydroxychloroquine). Although timing of treatment initiation from symptom onset was unknown and there was biased low RBV oral dosing (1.200 mg) and confounding "background" antiviral SOC, researchers deduced that the duration of stay and mortality were lower in the Sof/d group. The relative risk of death for patients treated with Sof/d was 0.17 (95%CI: 0.04–0.73; P = 0.02). In the other RCT[200], researchers evaluated the efficacy of Sof/d in combination with RBV for hospitalized COVID-19 patients with moderate disease compared with SOC. Although it fell under Pitfalls 2, 3, same as the previous one, it was even more confounding as the SOC group could have also received RBV according to the given recommendations at the time of the study. The authors deduced only trends for recovery and lower deaths in the Sof/d + RBV arm. Huang *et al*[201] conducted an open-label RCT to evaluate the efficacy of three antiviral combination regimens. The results indicated that there were no significant differences among the three regimens in terms of antiviral effectiveness. Furthermore, the combination of RBV and Lop/r was associated with a significant increase in gastrointestinal adverse events, suggesting that RBV and LPV/r should not

be co-administered to COVID-19 patients simultaneously. No mortality was recorded in that study. Finally, Hung *et al*[202] published a multicenter, open-label, phase 2 RCT trial to assess the efficacy and safety of combined IFN-β1b + Lop/r + RBV for treating patients with COVID-19. In total, 127 patients were recruited; 81 were assigned in the combined treatment group and 41 were in the control group and received Lop/r monotherapy. For the primary endpoint, which was time from start of treatment to negative nasopharyngeal swab, the combination group had a significantly shorter median time [7 d *vs* 12 d, hazard ratio (HR): 4.37 (1.86–10.24); $P = 0.0010$]; clinical improvement was better in the combination group, with a significantly shorter time to complete alleviation of symptoms, defined as a National Early Warning Score 2 of 0 [4 d *vs* 8 d, HR: 3.92 (1.66–9.23); $P = 0.0001$] and SOFA score of 0 [3.0 d *vs* 8.0 d, HR: 1.89 (1.03–3.49); $P = 0.041$]. The significantly better clinical and virological response was also reflected in the shorter median hospital stay in the combination group [9.0 d *vs* 14.5 d, HR: 2.72 (1.2–6.13); $P = 0.016$]. Eight patients were given stress doses of corticosteroids in the second week from symptom onset. Of the 127 patients, 17 developed oxygen desaturation and required oxygen treatment; six were admitted to the ICU, of whom five required noninvasive ventilation, and a 96-year-old female patient required intubation but was successfully extubated. There were no reported deaths during this study. Interestingly, post-hoc subgroup comparison of the 76 patients who started early treatment less than 7 d after symptoms onset showed better clinical outcomes (time to National Early Warning Score 2 of 0: 4 d *vs* 8 d; $P = 0.0001$; time to SOFA score of 0: 3 d *vs* 7 d; $P = 0.001$), shorter duration of hospital stay (8 d *vs* 15 d; $P = 0.003$), and better virological outcomes (time to negative viral loads all specimens 7 d *vs* 13 d; $P = 0.0001$) in the combination group (52 patients) than in the control group across all measured variables except stool samples. Scientists concluded that the early triple antiviral therapy was safe and superior to Lop/r alone in alleviating symptoms and shortened the duration of viral shedding and hospital stay in patients with mild to moderate COVID-19.

DISCUSSION

Under the aggravating pressure of an emerging pandemic it is burdensome to design and conduct well-organized, double-blind, RCTs. These difficulties may explain why the majority of published studies in SARS, MERS, and COVID-19 outbreaks were uniformly inconclusive. We have identified five specific pitfalls to which many studies fall victim. These include use of inappropriate cell lines (Pitfall 1), not fully understanding the clinical course of the disease (Pitfall 2), and incorrect pharmacology of applied treatments (Pitfalls 3, 4). Another pitfall is the misinterpretation of study results with generalizations that are disrespectful to study design conditions and the primary end-point (Pitfall 5). Other important confounding factors were the concurrent administration of multiple therapies and the absence of a control group in some of them. In Table 6, studies of all three coronaviruses that produced statistically significant results are summarized.

Siddiqi *et al*[203] proposed a clinical-therapeutic staging classification of COVID-19. In stage I, infection occurs at the time of inoculation, and there is early establishment of disease. During this period, SARS-CoV-2 multiplies and establishes residence in the host, primarily focusing on the respiratory system. In stage II of established pulmonary disease, viral multiplication and localized inflammation in the lung are the norm, while patients develop a viral pneumonia and possibly hypoxia. Imaging reveals bilateral infiltrates or ground glass opacities. In early stage IIa (without significant hypoxia), the use of corticosteroids may be avoided. However, if hypoxia ensues (stage IIb), it is likely that patients will progress to requiring mechanical ventilation, and in that situation, the use of anti-inflammatory therapy, such as corticosteroids, may be useful and can be judiciously employed[203]. A minority of COVID-19 patients will transition into the most severe stage of the illness (stage III), which manifests as an extrapulmonary systemic hyperinflammation syndrome with elevated markers of systemic inflammation. COVID-19 infection results in a decrease in helper, suppressor, and regulatory T cell counts[204]. Inflammatory cytokines and biomarkers such as IL-2, IL-6, IL-7, granulocyte colony-stimulating factor, macrophage inflammatory protein 1a, tumor necrosis factor alpha, C-reactive protein, ferritin, and D-dimer are significantly elevated in patients with more severe disease[205]. A form akin to secondary hemophagocytic lymphohistiocytosis and systemic organ involvement may occur in this advanced stage[206]. Tailored therapy in stage III hinges on the use of immunomodulatory agents to reduce systemic inflammation

Table 6 Studies of all coronavirus outbreaks with statistically significant findings

Regimen tested *vs* control, Type of study	Severity or disease stage when applied	Significant findings and other very important notes	Outbreak applied
IFN-β + RBV + Lop/r (gr 1) *vs* Lop/r (gr 2), Randomized, Prospective, Open-label Phase 2 [202]	Mild to moderate; No mortality	(1) Shorter time from start of treatment to neg nasopharyngeal swab in group 1 [7 d *vs* 12 d; HR: 4.37 (1.86-10.24); $P = 0.001$]. (2) Time to NEWS2 score 0: [4 d *vs* 8 d; HR: 3.92 (1.66-9.23) $P < 0.0001$] time to SOFA score 0: [3 d *vs* 8 d; HR: 1.89 (1.03-3.49); $P = 0.041$] time to neg viral loads (all specimens): (8 d *vs* 13 d; $P = 0.001$). (3) Duration of hospital stay: (9 d *vs* 14.5 d; $P = 0.016$). (4) In subgroups when treatment started < 7 d of symptom onset time to NEWS2 score 0: (4 d *vs* 8 d; $P < 0.0001$) time to SOFA score 0: (3 d *vs* 7 d; $P = 0.001$) time to neg viral loads (all specimens): (7 d *vs* 13 d; $P < 0.0001$) Duration of hospital stay: (8 d *vs* 15 d; $P = 0.003$]. And (5) Insignificant differences between groups in adverse-effects	COVID-19
RBV + steroids, Retrospective, Multicenter[131]	Moderate to severe 2nd wk Phase 2 all had pneumonia	(1) Time from symptom onset to treatment applied 5.7 d in those uncomplicated *vs* 7.7 d in those who needed ventilatory support ($P = 0.03$); (2) Response to treatment in early initiation 28/31 *vs* 11/19 in late initiation ($P = 0.02$). Final outcome 31/31 improved/recovered *vs* 10/19 in late applied (complicated) ($P = 0.0001$); and (3) Risk factor for complicated outcome was associated with delay starting of treatment	SARS
RBV + Lop/r + steroids *vs* RBV + steroids (historical), Open-label, Prospective, Non-randomized[87]	Mild to moderate initiation 3.5 d after symptom onset	(1) Development of ARDS or death within 21 d: 1/41 *vs* 32/111 ($P < 0.001$); (2) Independent risk factor predicting adverse outcome for the treatment group: aOR 0.07 [(0.01-0.55); $P = 0.011$]; and (3) Significant lower adverse outcome for those treated early ($P < 0.001$)	SARS
Peg-IFN-α2α + RBV *vs* SOC, retrospective[161]	Severely ill with pneumonia	(1) 14-d mortality in treatment gr 6/20 *vs* 17/24 in control ($P = 0.004$); (2) 28-d mortality in treated 14/20 *vs* 20/14 in control ($P = 0.054$); Loss of difference in 28-d might be explained by high initial APACHE II and SOFA scores and several comorbidities	MERS
RBV + Lop/r + steroids *vs* RBV + steroids (SOC), Multicenter retrospective matched-cohort (with 643 pts)[140]	Mild to moderate Initiation of RBV 4.5 d and of Lop/r 5.5 d	(1) Less proportion and dose of pulsed Mp in treated gr ($P < 0.05$); (2) Intubation rate in treated 0% *vs* 11% (7.7-15.3) in control ($P < 0.05$); and (3) CFR 0% (0-6.8) in treated *vs* 15.6% (9.8-22.8) in control ($P < 0.05$)	SARS
IFN-α + RBV + Lop/r and IFN-α + Lop/r *vs* SOC, Retrospective[191]	Moderate, hospitalized 7d after symptom onset	Significant correlation of PCR-negative conversion time and length of hospital stay (days) in IFN + lopinavir/ritonavir combined with RBV treatment group ($P = 0.0215$) and IFN + lopinavir/ritonavir treatment group ($P = 0.012$)	COVID-19
Several antiviral combinations Retrospective[190]	Severe	(1) The use of two-step clustering and subgroup analyses enabled an in-depth analysis of the effects of single and combination drug therapies. Improvement rate was highest (84.9%) in the group combination of RBV + Lop/r + Umifenovir + Lianhua Qingwen ($P < 0.001$); (2) Antiviral combination was superior to single or dual agents	COVID-19
IFN + RBV *vs* SOC, Retrospective[163]	Severe	Patients who survived were more likely to have received IFN + RBV than those who died ($P = 0.01$)	MERS
RBV + pulsed steroids (PS) (equivalent to Mp > 500 mg/d *vs* RBV + non-PS (NPS), Multicentre, Retrospective[129]	Severe pneumonia (Phase 2)	(1) Overall trend for chest radiograph scores significantly lower in the PS group than NPS ($P = 0.026$); (2) The radiographic scores were significantly lower in days 14 and 21 in PS compared to NPS ($P = 0.04$ and $P = 0.04$); and (3) No significant difference between the PS and NPS groups in the need of ICU, mechanical ventilation and mortality	SARS
Steroids *vs* no-steroids, Multicentre, Retrospective[165]	Critically ill pts all in ICU	(1) In marginal structural modelling, steroid therapy was not significantly associated with 90-d mortality but with a delay in MERS RNA clearance ($P = 0.005$); (2) However pts given steroids were more likely to have one or more comorbidities than without steroids ($P = 0.001$)	MERS
4 different treatment groups, Prospective, randomized[133]	Moderate	(1) High-dose steroids with a quinolone + azithromycin resulted in significant resolution of pyrexia ($P < 0.001$), pulmonary infiltrates ($P < 0.001$), and respiratory improvement ($P < 0.001$); (2) No particular advantage in using ribavirin was seen (not significant)	SARS
Sofosbuvir/daclatasvir *vs* RBV SOC: Lop/r + HCQ, Open-label, Parallel trial[199]	Severe	(1) Duration of hospital stay 5 d in Sof/d *vs* 9 d in RBV arm ($P < 0.01$); (2) Relative risk of ICU admission 0.36 (0.16–0.81) in Sof/d *vs* 2.8 (1.2–6.4) in RBV arm ($P = 0.01$); and (3) Relative risk of death 0.17 (0.04–0.73) in Sof/d *vs* 5.8 (1.4–25) ($P = 0.02$)	COVID-19
Multivariate analysis of several treatments, Retrospective[171]	Unclear	(1) IFNs (mainly IFN-β) and MMF were predictors of increased survival in univariate analysis ($P = 0.009$ and $P = 0.019$, respectively)	MERS
RBV + steroids within the first 2 d of admission *vs* no treatment within first 2 d, Retrospective[142]	All cases	The generalized propensity score weighting model predicted that the overall CFR would be the highest (19.2%) if all patients treated with RBV + steroids within 2 d of admission compared with those receiving neither treatment within 2 d of admission (15.4%) with and the difference was marginally statistically significant	SARS
Several therapies evaluated, Retrospective[193]	Moderate to severe	(1) In multivariate analysis for predicting the risk of death in RBV treated was OR 0.477 (0.232-0.982) $P = 0.044$ and of arbidol 0.28 (pneumonia onset) (0.126-0.625) $P = 0.002$; (2) in multivariate analysis of parameters associated with death in pts with cardiovascular	COVID-19

	disease and cardiac injury from the disease, RBV had an OR 0.208 (0.070-0.618) $P = 0.005$ and arbidol $P = 0.006$	
Several therapies evaluated, Meta-analysis[88]	(1) Anti-coronavirus interventions significantly reduced mortality RR 0.65 (0.44-0.96; $I^2 = 81.3\%$), remarkably ameliorate clinical improvement RR 1.62 (1.11-2.36; $I^2 = 11\%$) without manifesting clear effect on virological eradication, incidence of ARDS, intubation and adverse effects; (2) The combination of RBV + steroids remarkably decreased mortality RR 0.43 (0.27-0.68); (3) The combination of RBV + Lop/r + steroids showed tendency of lower mortality whereas the combination of IFN + steroids demonstrated higher mortality tendency; and (4) The Lop/r-based combination showed superior virological eradication and radiographic improvement with reduced rate of ARDS	COVID-19, SARS, MERS
Treatment side-effects		
RBV[88]	RBV can induce more bradycardia, anemia, and transaminitis	COVID-19, SARS, MERS
IFN-α + RBV[161]	Reduction in Hb 4.32 g/L *vs* 2.14 g/L ($P = 0.002$)	MERS
RBV[89]	Hemolytic anemia was significantly associated with high-dose RBV ($P = 0.005$) and prolonged hospital stay ($P = 0.001$). Also hypocalcemia, hypomagnesemia	SARS
Antiviral combinations[201]	Gastrointestinal side-effects (vomiting, diarrhea) more significant ($P < 0.01$) in the combination of IFN-α + RBV + Lop/r than in IFN-α + RBV and the IFN-α + Lop/r groups. The combination of RBV + Lop/r should not co-administered to COVID-19 pts simultaneously	COVID-19

ARDS: Acute respiratory distress syndrome; CFR: Case fatality rate; HCQ: Hydroxychloroquine; HR: Hazard ratio; ICU: Intensive care unit; IFN: Interferon; Lop/r: Lopinavir/ritonavir; MERS: Middle East respiratory syndrome; MMF: Mycophenolate mofetil; OR: Odds ratio; RBV: Ribavirin; RR: Relative risk.

before it overwhelmingly results in multiorgan dysfunction. The use of corticosteroids may be justified in concert with the use of cytokine inhibitors such as tocilizumab (IL-6 inhibitor) or anakinra (IL-1 receptor antagonist)[206].

The clinical progression of SARS was mostly uniform[131] and very similar to that of COVID-19, with a tri-phasic pattern. Week 1 was characterized by systemic symptoms that generally improve after a few days. The increasing viral load during this phase suggests that the symptoms are largely related to the effect of viral replication and cytolysis. As the disease progresses into week 2, the patients frequently experience recurrence of fever, onset of diarrhea, and oxygen desaturation with shifting radiographic shadows. If viral induced damage was the primary pathological mechanism, such a flitting pattern of radiological change is difficult to explain. The timing of the immunoglobulin G seroconversion, which starts on day 10, seems to correlate with falls in viral load, which occur between days 10 and 15. There is a progressive decrease in rates of viral shedding from nasopharynx, stool, and urine from days 10 to 21 after symptom onset. The severe clinical worsening that may occur at this time cannot be explained by uncontrolled viral replication. The lung damage is related to immunopathological events as a result of an overexuberant host response, rather than uncontrolled viral replication[129,131]. Twenty percent of patients may progress to Phase III, characterized by ARDS, necessitating ventilatory support. Inevitably, several patients will develop nosocomial sepsis during this phase of end-organ damage and severe lymphopenia. Interestingly, in terms of pathogenesis, in pulmonary reovirus infection in athymic mice, a lower plaque-forming value of 10^6 is associated with pathological changes of bronchiolitis obliterans organizing pneumonia (BOOP), whereas a higher inoculum of 10^7 is associated with ARDS[207]. Thus, rapid reduction in viral load is critical for the development of a more severe disease stage.

However, there is a critical difference between COVID-19 and the other two coronaviruses. The viral loads of SARS and MERS peak at around days 7–10 after symptom onset[131,142], implying that there is a therapeutic window that could be exploited[87]. In contrast, the viral load of COVID-19, as detected in posterior oropharyngeal saliva samples, is highest at presentation with higher viral loads in the nose than in the throat[208,209], or it peaks at around 5-6 d after symptom onset in throat swab and sputum samples[40]. After the first week, COVID-19 viral load gradually declines[210]. This suggests there is very short time-interval after symptom onset during which a viable antiviral therapy will be beneficial. Targeting selected patients during stage I not only will minimize contagiousness but may prevent progression to higher disease severity. SARS-CoV-2 is highly contagious, as the basic reproduction number (R0) is approximately 2-3.5[211,212]. Thus, except for protecting from disease progression, the reduced viral load would also translate to reduced virus

shedding, thereby reducing the risk of secondary transmission and thus acting as a prophylaxis.

For SARS, scientists concluded that in order to lessen the risk of progression to the ARDS phase, an effective antiviral was necessary to reduce the viral load and decrease the initial cytolytic damage in Phase I, which in turn may result in decreased immunopathological damage during Phase II[131]. Therefore, an antiviral therapy in stage I of COVID-19 may have the greatest benefit. The doses of RBV used in reports from Hong Kong[129,131] were associated with a reversed V-shaped curve of viral load, excluding the absence of antiviral activity. The characteristic finding on computed tomography mimicked that in BOOP. In addition, the similarity of the histologic features to those of early ARDS in post-mortem studies prompted physicians to use corticosteroids in combination with RBV for the treatment of SARS[129], as corticosteroid therapy had been used with some success in BOOP[144]. Corticosteroids may decrease the release of macrophage-derived inflammatory cytokines[213]. In a Canadian SARS outbreak, RBV was administered early with steroids, and no conclusive results of efficacy could be established despite viral and symptom flare-up in a portion of patients after treatment cessation[126,133]. High dose methylprednisolone should be avoided in the early phase of SARS and SARS-CoV-2 as viral clearance by host immunity might be hampered.

It is more than clear since the SARS outbreak that an efficient antiviral treatment, administered as early as possible following COVID-19 onset, is the critical step to reduce viral load and restrain disease progression to stages II and III. In contrast to SARS, in which the time from symptom onset to highest viral loads may exceed 1 wk, the viral load in SARS-CoV-2 peaks with presentation or within the first few days. That is the reason why prompt antiviral initiation is crucial. If disease progresses to stage IIb and stage III, antivirals probably have no beneficial effect. At the stage of aberrant immunopathological damage in lungs and extrapulmonary systemic hyperinflammation syndrome, with cytokine storm and possible occurrence of secondary hemophagocytic lymphohistiocytosis, an effective immunosuppressive/immunomodulator treatment is needed.

Several studies in coronavirus diseases have shown that the combination of RBV with steroids and especially pulsed methylprednisolone when hypoxia develops or in worsening pulmonary infiltrates can yield improvement in imaging findings and final outcome. It should be kept in mind that corticosteroids may delay viral clearance, prolonging infections while reducing the symptomatic inflammatory cytokines [214-216]. Although initiating steroids early can prevent the cytokine storm and lung damage, starting too early might inhibit antibody production, thus prolonging the natural course of the disease[125]. Hemophagocytosis has been attributed to cytokine dysregulation, and intervention with steroids might modulate this cytokine response and prevent a fatal outcome, as it has been proposed for other causes of ARDS[217]. In a retrospective cohort[207] of patients with severe pneumonia COVID-19 and subsequent ARDS, the administration of methylprednisolone appeared to reduce the risk of death in patients with ARDS (HR: 0.38; 95%CI: 0.20-0.72; $P = 0.003$). Those who received methylprednisolone treatment were much more likely to develop ARDS, likely owing to confounding by indication (*e.g.*, sicker patients were more likely to be given methylprednisolone). These concerns may be avoided if corticosteroids are applied during the proper time window of the disease; not during the early phase, but when hypoxia and apparent imaging findings are established. Finally, specific immunomodulatory compounds (tocilizumab and anakinra) are beneficial in stages IIb and III. In COVID-19 pneumonia with ARDS characterized by hemophagocytic lymphohistiocytosis, the response to tocilizumab was rapid, sustained, and associated with significant clinical improvement[218].

Accumulated data from all coronavirus outbreaks converge to the conclusion that antiviral combination therapy is more beneficial than antiviral monotherapy. The checkerboard assay demonstrated synergism between lopinavir and RBV at a low viral inoculum[87]. RBV combined with Lop/r seems to improve the clinical efficacy of SARS[161]. Use of IFNs was not considered by most clinicians during the SARS epidemic because of their known proinflammatory activity, which may potentiate the inflammatory damage initiated by the viral infection[219]. Early administration of IFN protected mice from lethal MERS-CoV infection, while late administration of exogenous IFN promoted the proinflammatory cytokine response and inhibited the optimal virus-specific T cell response[220,221]. IFN-β1a or leukocytic IFN-α with RBV appeared to be the most effective combination[116]. RBV concentrations inhibiting virus production in combination with IFN-β were at least 10-fold lower compared to monotherapy, with highly synergistic antiviral effects of combination treatment[117]. In animal studies, researchers concluded that potentially effective combinations were

RBV with IFN-β1b and/or Lop/r[157]. Moreover, when both IFN-α2b and RBV were applied as a combination, significant synergism was observed, with 8-fold reduction in half maximal inhibitory concentration of IFN-α2b and 16-fold reduction in that of RBV [108], while the combination improved MERS infection in rhesus macaque[156]. IFN-β combined with Lop/r had better efficacy to treat MERS-CoV[140].

RBV is a broad-spectrum nucleoside that is phosphorylated in virus-infected cells, and its product acts as a competitive inhibitor of virus synthetase, interfering with early viral transcription events and hindering the synthesis of ribonucleoproteins and virus replication and spread[222]. RBV's multiple mechanisms of action likely support its longevity and quality as a clinical resource. The risk of RBV-associated anemia—although substantial and in need of careful monitoring—might not hinder the use of RBV for patients with severe coronavirus infections, especially if a survival benefit can be confirmed.

CONCLUSION

COVID-19 behaves like a bipolar disease. On the one hand it is a mild, self-limiting viral respiratory tract infection, and the majority of patients recover with no sequelae. On the other hand, it is a severe pneumonitis with a deadly systematic auto-inflammatory disease component. With respect to the most significant studies and to those that fell under the least serious pitfalls analyzed in this article, we conclude by presenting below a scheme of treatment modalities tailored to COVID-19 disease stages, which could, if timely applied, be beneficial.

Suggestion 1 (Stage I treatment)

IFN-β + RBV + Lop/r should be commenced early after disease onset at doses as reported by Hung *et al*[202]. Concerns: IFN-β should not exceed the first week of the disease due to its proinflammatory activity; the other antivirals should not be administered for more than 7-10 d and unequivocally when severe hypoxia develops due to the loss of their benefit and subsequent prevalence of their side-effects. Rationale: The effective reduction of viral load and subsequent deterrence of disease progress; the reduction of virus shedding, thus reducing the risk of secondary transmission, therefore acting as a prophylaxis. Implementation: Certainly, this antiviral combination would not be initiated in most COVID-19 cases, as the majority of patients have a benign, self-limited illness. This approach targets selected patients, as specific age groups present severe morbidity and mortality. According to Centers for Disease Control and Prevention COVID-19 data tracker (https://www.cdc.gov/covid-data-tracker/index.html#demographics), CFR by age groups are: 18-49 (0.5%-3.1%); 50-64, 15.2%; 65-74, 21%; 75-84, 26.6%; and 85+, 32.2%. Therefore, all patients older than 50 years should be treated. For younger patients (18-years-old to 49-years-old), stage I treatment should be applied only to adults with certain underlying medical conditions (*e.g.*, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, obesity, smoking, chronic kidney disease, cancer, and immunosuppression in solid organ transplant recipients, https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-increased-risk.html) that are independent risk factors for severe illness and negative COVID-19 outcome. The remarkable advantage of this therapeutic regimen is that it can be applied on an outpatient basis with the patient at home in quarantine.

Suggestion 2

Corticosteroids and tocilizumab or anakinra should be used for severe pneumonitis and for stage III of systemic hyperinflammation syndrome with cytokine storm and possible occurrence of secondary hemophagocytic lymphohistiocytosis. If not initiated early, corticosteroids have been shown to be beneficial in reducing intubation and mortality rates in all coronaviruses infections. The combination of corticosteroids with tocilizumab showed superior survival outcome when compared with SOC treatment and treatment with corticosteroids alone or in combination with anakinra. Furthermore, corticosteroid use either alone or in combination with tocilizumab or anakinra was associated with reduced hospital mortality for patients with cytokine storm compared with patients receiving SOC treatment[223]. Proposal A: Dexamethasone 6 mg/d for 10 d in those with respiratory failure or those intubated [224]. Proposal B: High-dose steroids with pulsed methylprednisolone being the most tested and effective in SARS and MERS studies at a dose of 0.5-1.0 g/d for 2-3 d or in a total of 3 g. It has been shown that pulsed methylprednisolone not only restricts

radiological abnormalities and restores respiratory failure but may also restrain the cytokine storm. Proposal C: Immediate treatment with methylprednisolone 250 mg intravenously on day 1, followed by methylprednisolone 80 mg intravenously on days 2–5. In cases with lack of clinical improvement or worsening in respiratory status, escalation of immunosuppressive treatment with a monoclonal antibody directed against the IL-6 receptor and tocilizumab should follow between day 2 and day 5 (single-dose tocilizumab, 8 mg/kg intravenous, max 800 mg)[225,226].

Suggestion 3

Regarding measles pneumonitis, RBV seems an adequate treatment at a dose of 600 mg qid for 5-7 d. In case of malignancies, especially hematological and severely immunosuppressed patients, a longer regimen of 2-3 wk may be required.

ARTICLE HIGHLIGHTS

Research background

Ribavirin is a broad-spectrum nucleoside antiviral drug that despite it has been widely used clinically for almost five decades, evidence regarding its efficacy in viral infections remains conflicting. Ribavirin use has only been established in chronic hepatitis C virus infection, chronic hepatitis E virus infection in transplant recipients, *respiratory syncytial virus* in children, and some of the viral hemorrhagic fever viruses. Ribavirin was widely utilized alone, or in combination with other compounds in severe acute respiratory syndrome (SARS), Middle-East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19) outbreaks. Despite the large amount of data however, the conclusions of all three coronaviruses studies concerning ribavirin efficacy have been contradictory. The present review article aims to clarify the underlying reasons for these discrepancies including possible study design inaccuracies and failures, misinterpretations of data, and to address these potential confounds. Moreover, the possible role of ribavirin in COVID-19 therapeutic schemes is thoroughly studied.

Research motivation

COVID-19 pandemic has emerged as a global health issue with the highest significance and is currently the number one priority for scientists worldwide. During the 2018 measles outbreak, we hospitalized a number of adult cases suffering measles pneumonitis and treated them with Ribavirin (RBV). Because of the lack of specific guidelines on severe measles disease treatment in adults, we reviewed the literature on RBV dosing regimens and outcomes in any infectious disease. The most amount of clinical data available was for SARS and MERS, where RBV was widely utilized. While preparing the measles/RBV study for publication, the new COVID-19 outbreak emerged, prompting us to focus heavily on COVID-19 treatment with RBV alone or in combination with other compounds.

Research objectives

To shed light in and clarify the confounding factors of ribavirin treatment studies regarding SARS, MERS, and COVID 19 and to propose a therapeutic scheme for COVID-19 that would be tailored to its distinct disease stages.

Research methods

A meticulous electronic search of PubMed database was performed covering a period of over five decades up to October 15, 2020 using the terms “ribavirin”, “treatment” in combination with “measles”, “SARS”, “MERS”, and “COVID-19”. All review articles referring to COVID-19 treatment were searched and studied, regardless of whether “ribavirin” was included in key words. *In vitro*, animal and clinical studies, reviews, and meta-analyses in English language only were considered for data extraction. The citations in each article were reviewed to locate additional references that were not retrieved during the initial search. Eligible to be included in the review were those studies referring to RBV treatment alone or in combination and/or those reporting on its dose regimens, adverse effects, or outcomes. The literature search was performed and described with respect to PRISMA guidelines.

Research results

A total of 32 severe acute respiratory syndrome-associated coronavirus studies, 18 Middle East respiratory syndrome coronavirus studies, and 17 severe acute respiratory syndrome-2 associated coronavirus studies were considered eligible to be included in this review. The burden of designing and conducting well-organized, double-blind, randomized controlled trials under the difficulties and pressures of an emerging pandemic is obvious. Hence, many of those studies succumbed to specific pitfalls that resulted in conflicting evidence regarding the clinical efficacy of ribavirin for coronaviruses infections. We detected six pitfalls that were carefully identified and described in this review and comprise: utilization of naturally ribavirin-resistant Vero cell lines in *in vitro* studies; study design inconsistent with the well-established clinical course of disease (*i.e.*, antiviral administration late in the disease course or early use of corticosteroids); inappropriate pharmacology of applied treatments (*i.e.* dosing regimens, treatment duration); and misinterpretation of study results with misconceived generalizations. Considering all those studies with their pitfalls and mostly taking into account those with statistically significant outcomes, we concluded to a comprehensive treatment for COVID-19 documented by thorough, long-term investigation of ribavirin regimens in coronavirus infections which is strictly tailored to distinct disease stages.

Research conclusions

COVID-19 behaves like a bipolar disease being an asymptomatic or mild, self-limiting viral respiratory tract infection with the majority of patients recovering without sequelae on the one hand, and on the other it may progress to a severe pneumonitis with a deadly systematic auto-inflammatory disease component. Documented by accumulated data from the three coronaviruses studies and considering the six identified pitfalls to which most of the studies fall victim, the early antiviral treatment is crucial for reducing viral load, transmission, and preventing disease progression to severity. Interferon-β + Ribavirin + Lopinavir/ritonavir should be commenced as early as possible after disease onset resulting in an effective reduction of viral load and subsequent deterrence of disease progress, reduction of virus shedding, and thus reducing the risk of secondary transmission, therefore acting as a prophylaxis. This approach could target selected patients, as specific age groups (older than 50 years) present severe morbidity and mortality, as well as younger patients with well-recognized independent risk factors for severe illness and increased mortality. The remarkable advantage of this therapeutic regimen is that it can be applied on an outpatient basis with the patient at home in quarantine. On the other hand, corticosteroids and anti-interleukin monoclonal antibodies (tocilizumab or anakinra) should be used for severe pneumonitis and for systemic hyperinflammation syndrome with cytokine storm and possible occurrence of secondary hemophagocytic lymphohistiocytosis. Corticosteroids in COVID-19 comprise dexamethasone 6 mg/d as previously shown to reduce mortality; methylprednisolone 250 mg intravenously on day 1, 80 mg on days 2–5 and escalation of immunosuppressive treatment with a monoclonal antibody directed against the interleukin-6 receptor (tocilizumab) when respiratory status worsens; a third corticosteroid treatment proposal being the most tested and effective in SARS and MERS studies includes high-dose steroids with pulsed methylprednisolone at a dose of 0.5-1. 0 g/d for 2-3 d or for a total of 3 g. Finally, regarding measles pneumonitis, ribavirin seems an adequate treatment at a dose of 600 mg for 5-7 d, but in cases of hematological malignancies, or severely immunosuppressed patients, a longer regimen of 2-3 wk may be required.

Research perspectives

It is of paramount importance to confirm the efficacy of the early triple antiviral combination in reducing viral load, transmission, and preventing disease progress to severity by conducting Phase III randomized controlled trials, as this early triple antiviral combination efficacy has already been determined for COVID 19 in a Phase II clinical trial with statistically significant outcomes. It would be of great interest also to perform clinical studies to determine the impact of pulsed methylprednisolone on COVID 19 pneumonitis and/or on cytokine storm compared with the already approved approaches such as administration of dexamethasone 6 mg/ d. COVID 19 is the pinpoint of interest of all scientists worldwide.

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