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PHAMCOLOGICAL STUDY OF REMDESIVIR ON COVID-19

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ABSTRACT

Remdesivir (Veklury®), a nucleotide analogue prodrug with broad-spectrum antiviral activity, is approved for the treatment of coronavirus disease 2019 (COVID-19), the illness caused by severe acute respiratory syndrome coronavirus 2 infection. Unlike some antivirals, remdesivir has a low potential for drug-drug interactions. In the pivotal ACTT-1 trial in hospitalized patients with COVID-19, daily intravenous infusions of remdesivir significantly reduced time to recovery relative to placebo. Subsequent trials provided additional support for the efficacy of remdesivir in hospitalized patients with moderate or severe COVID-19, with a greater benefit seen in patients with minimal oxygen requirements at baseline. Clinical trials also demonstrated the efficacy of remdesivir in other patient populations, including outpatients at high risk for progression to severe COVID-19, as well as hospitalized paediatric patients. In terms of mortality, results were equivocal. However, remdesivir appeared to have a small mortality benefit in hospitalized patients who were not already being ventilated at baseline. Remdesivir was generally well tolerated in clinical trials, but pharmacovigilance data found an increased risk of hepatic, renal and cardiovascular adverse drug reactions in the real-world setting. In conclusion, remdesivir represents a useful treatment option for patients with COVID-19, particularly those who require supplemental oxygen.

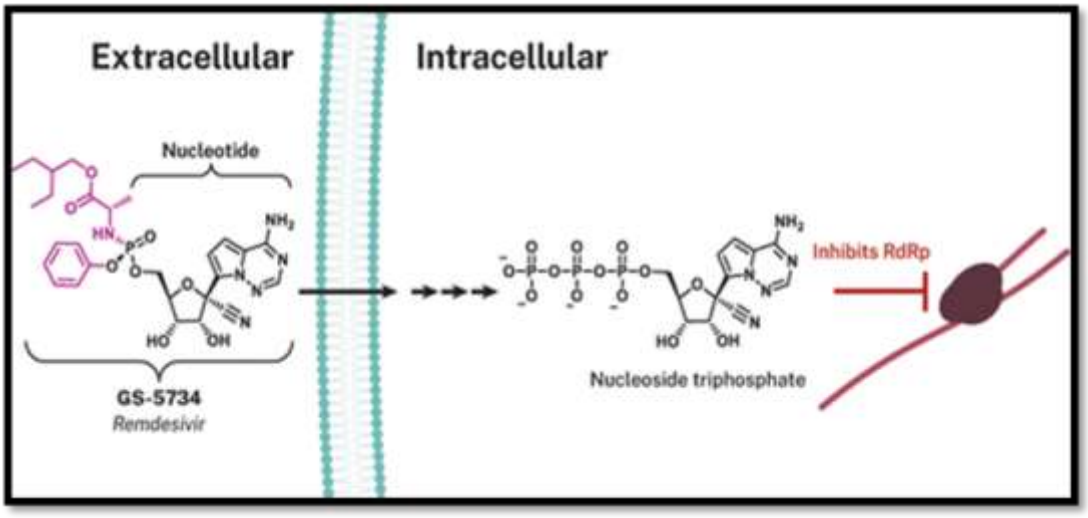

Fig.1.[1]

KEYWORD: Remdesivir, Coronavirus disease 2019, Severe acute respiratory syndrome, coronavirus 2, Antiviral, Variants.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), the illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, first appeared in China in December 2019 as an unusual idiopathic pneumonia. The global spread of COVID-19 was rapid, with the World Health Organization (WHO) declaring it a global pandemic in March 2020.[2,3] An urgent need to control the pandemic and ease the burden on healthcare systems, together with an ongoing improved understanding of the pathophysiology of COVID-19, led to the rapid development and authorization of numerous COVID-19 vaccines and novel therapeutics (some of which were repurposed).

COVID-19 is a highly contagious respiratory illness that spreads mainly via exposure to airborne particles and droplets.[2] The symptoms of COVID-19 vary widely, but often include fever, cough, and breathing difficulties. The spectrum of its clinical presentation ranges from asymptomatic infection to severe life-threatening acute respiratory failure with multiple organ dysfunction. The risk of severe disease, hospitalization and death is higher in elderly patients, males, smokers, and those with certain underlying medical conditions.[3] Most patients with mild COVID-19 can safely treat their symptoms at home.[4] However, some patients with COVID-19, particularly high-risk patients, require additional treatments such as immunomodulatory agents and antiviral drugs.

Remdesivir (Veklury®) is a broad-spectrum antiviral drug with activity against viruses from several families, including coronaviruses. It was previously under development for the treatment of Ebola virus disease. Remdesivir is approved for the treatment of COVID-19 in multiple countries worldwide, including the USA and those of the EU. This article reviews the clinical efficacy and tolerability of remdesivir in this indication and summarizes its pharmacological properties.

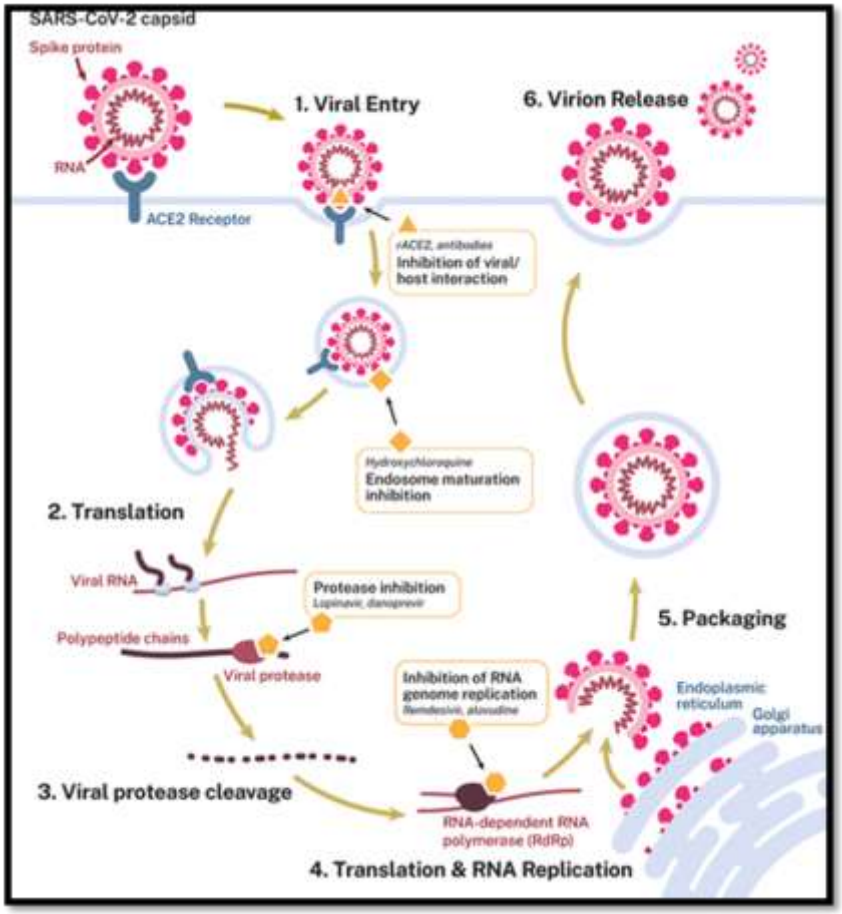

Fig.2.[1]

Figure 2. Life cycle of SARS-CoV-2 in host cells. SARS-CoV-2 primarily infects the respiratory tract (nasal epithelial cells, pneumocytes, and alveolar macrophages) and the gastrointestinal tract (enterocytes). The virus enters though direct interaction between the viral S protein and the cellular receptor angiotensin-converting enzyme 2 (ACE2). Following entry, the viral genome is released and translated into the viral replicase polyproteins PP1a and PP1ab, which are cleaved into functional proteins by viral proteases. Viral genome replication is mediated by the viral replication complex, including the RNA-dependent RNA polymerase (RdRp). Viral nucleocapsids are assembled from the packaged viral genomes and

translated viral structural proteins and released through exocytosis. Potential targets and postulated mechanism of action for antiviral interventions are shown: blocking virus/host cell interaction through the use of antibodies/nanobodies (and convalescent plasma therapy) or recombinant ACE2 protein; use of hydroxychloroquine (based on *in vitro* data) to inhibit endosome maturation; use of protease inhibitors to inhibit viral/endosome membrane fusion or viral polypeptide maturation; nucleoside/nucleotide analogues to inhibit viral genome replication.

Development of Remdesivir

Remdesivir (GS-5734) was developed by Gilead Sciences and emerged from a collaboration between Gilead, the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). They sought to identify therapeutic agents for treating RNA-based viruses that maintained global pandemic potential, such as those that indeed emerged following the initiation of the program, including EBOV and the Coronaviridae family viruses exemplified by Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS).

As a starting point for discovery, a library of ~1000 small molecules focused around nucleoside analogues was compiled, based on prior knowledge of effective antiviral compounds targeting RNA viruses. Nucleosides are poorly cell-permeable (and therefore can have a low hit rate in cell-based screens such as antiviral screens), so modified nucleosides such as monophosphate, ester, and phosphoramidate prodrugs composed a significant portion of the library. Such prodrugs are typically more permeable and metabolized to liberate the nucleoside or phosphorylated nucleoside within cells. While the data from the original full screen does not appear to have been disclosed, a 1′-CN modified adenosine C-nucleoside hit (GS-441524), along with a prodrug form of the monophosphate of GS-441524 (GS-5734, later renamed as remdesivir), was found to be highly potent. GS-441524 and its S-acyl-2-thioethyl monophosphate prodrug had previously been reported in 2012 as potent leads from a series of 10-substituted 4-aza-7,9-dideazaadenosine C-nucleosides, with broad activity against a panel of RNA viruses: yellow fever virus (YFV), Dengue virus type 2 (DENV-2), influenza A, parainfluenza 3, and SARS. (44) The primary assay used was the cytoprotection effect (CPE) assay, in which live virus is incubated with a target cell line and the antiviral activity is inferred by the ability of a test agent to rescue cell death, measured using a standard cell viability reagent. In a 2012 study, GS-5734 showed CPE activity against SARS

strain Toronto 2 (IC50 = 2.2 μM) without causing cytotoxicity toward the host Vero African green monkey kidney epithelial cells used in the CPE assay (note that different target cells were utilized in viral CPE assays).

The pharmacokinetics of remdesivir have been summarized in compassionate use documentation published by the European Medicines Agency (EMA, 2020). Remdesivir is administered via an intravenous injection (IV) with a loading dose on day 1 (200 mg in adults, adjusted for body weight in pediatric patients) followed by a daily maintenance dose (100 mg in adults) for up to 10 days. In nonhuman primates, daily administration of 10 mg/kg of remdesivir yielded a short plasma half-life of the prodrug (t1/2= 0.39 h), but sustained intracellular levels of the triphosphate form.[1]

Pharmacodynamic Properties of Remdesivir

Remdesivir is a prodrug of an adenosine nucleotide analogue. Upon distribution within host cells, remdesivir is metabolized by carboxyesterase 1 and/or cathepsin A to form a nucleoside monophosphate intermediate, which is then phosphorylated by cellular kinases to form GS-443902, a pharmacologically active nucleoside triphosphate metabolite (Sect. 3). Acting as an adenosine triphosphate (ATP) analogue, remdesivir triphosphate has favourable selectivity over the natural ATP substrate for incorporation into nascent viral RNA chains by the SARS-CoV-2 RNA-dependent RNA polymerase (also known as Nsp12). RNA synthesis is subsequently inhibited when remdesivir triphosphate has been incorporated into the chain and is present in the viral RNA template. Remdesivir residues embedded in the first RNA strand used as a template may also cause inhibition during synthesis of the second RNA strand (i.e. during transcription of viral genome synthesis), suggestive of a second mechanism of action.[2,3,4,5]

Pharmacokinetic Properties of Remdesivir

Remdesivir and metabolite GS-441524 plasma and lung concentrations over time data were analyzed by non-compartmental analysis using PKSolver to obtain pharmacokinetic parameters in the hamster model after inhalation of the formulations (Zhang et al., 2010). Due to the sparse sampling requirements with this animal model and to obtain lung concentrations over time a naïve pooled-data approach was used in which the noncompartmental analysis was fit to the data as if the average of the measured concentrations from the five animals at each time point were taken from a single subject. This

was based on the methods previously reported for estimating population kinetics from very small sample sizes.[6]

Remdesivir Mode of Action

Antiviral chemotherapeutic interventions often target specific viral enzymes or attack a weak point of viral replication within the host, such as targeting the divergent RNA-dependent RNA polymerase (RdRp; Figure 2). Nucleoside analogues represent a class of antiviral agents that has proven efficacious against several viruses, including hepatitis B and C as well as HIV. Generally, these fall into three general classes: mutagenic nucleosides, obligate chain terminators, or delayed chain terminators. Ribavirin, a mutagenic nucleoside, targets the viral reliance on an RdRp to catalyze the replication of the RNA genome from the original RNA template. In a seminal paper, Crotty et al. demonstrated that the RNA virus poliovirus exists on the edge of viability, due to the proportion of virus particles with deleterious mutations. Furthermore, treatment with concentrations of ribavirin that caused a 9.7-fold increase in mutations was sufficient to induce "error catastrophe," in effect lethally mutating the poliovirus, reducing infectivity by 99.3%. Obligate chain terminators, such as azidothymidine (AZT), lack the reactive 3′-hydroxyl group, which directly prevents additional DNA synthesis after incorporation. Lastly, delayed chain terminators, which include remdesivir, block transcription despite still possessing the 3′-hydroxyl and thus can still form a phosphodiester bond with the next incorporated nucleotide. However, evidence suggests that the 1′CN substituent of remdesivir sterically clashes with RdRp (residue S861) upon further chain elongation (remdesivir + three additional nucleotides), distorting the positioning of the RNA and hampering translocation to the remdesivir + fourth position.

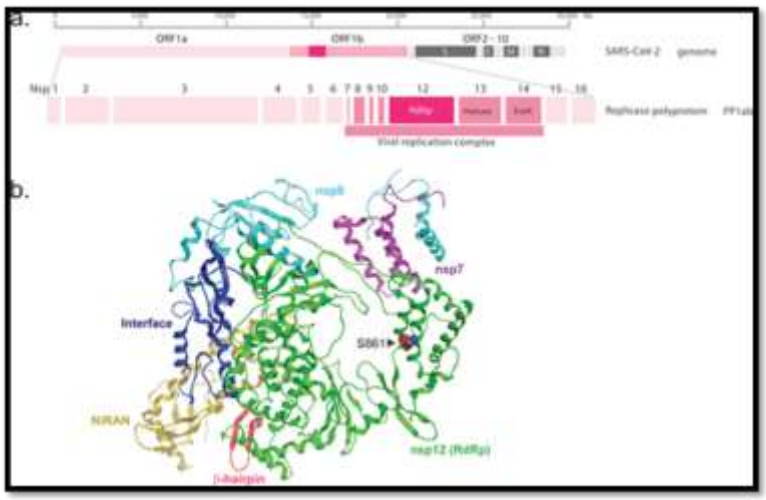

Fig.3[1]

Figure 2. SARS-CoV-2 genome and RNA-dependent RNA polymerase structure. (a) Representation of the SARS-CoV-2 RNA genome. As SARS-CoV-2 is a positive-sense RNA virus, the genome serves as a direct template for protein translation. Replication of the viral genome requires a functional viral replication complex, including an RNA-dependent RNA polymerase (RdRp). (b) Domain organization of the SARS-CoV-2 RdRp (encoded by nsp12) domains bound to cofactors nsp7 and dimers of nsp8, that serve as essential cofactors that increase polymerase activity. The rendering was based on the cryo-EM structure at a resolution of 2.9-Å, published by Gao et al, 2020 (PDB: 6M71). The nsp12 RdRp domain is shown in green, nsp7 in purple, nsp8 in cyan, nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain in yellow, interface in blue, and a newly identified β-hairpin domain is shown in red.Highlighted is RdRp residue S861, which is predicted to sterically interact with the 1′CN substituent of remdesivir inducing delayed chain termination.

Remdesivir (GS-5734), a prodrug, is metabolized within cells into an alanine metabolite (GS-704277), further processed into the monophosphate derivative and ultimately into the active nucleoside triphosphate derivative (Figure 3). Nucleotide analogues are not highly cell permeable, and once in the cell they require di- and then triphosphorylation to produce the nucleoside triphosphate (NTP) that can be utilized by the viral RNA-dependent polymerases for genome replication. As such, NTPs can then be misintegrated into viral RNA by the viral RNA-dependent RNA polymerase (RdRP; Figure 2). To address this, an approach to antiviral drug design can employ the utilization of phosphoramidate prodrugs (ProTides, inferred as prodrugs of nucleotides). Protides are composed of a nucleoside monophosphate capped with an aryl group and an amino acid ester (a phosphoramidate). Following diffusion into the cell, the prodrug is presumed to metabolize in a sequence of hydrolytic steps that starts with esterase-mediated ester hydrolysis to a carboxylate that cyclizes internally to the phosphonate ejecting the phenoxide; the resultant unstable cyclic anhydride is hydrolyzed open by water to the alanine metabolite GS-704277 whose P–N bond is hydrolyzed by a phosphoramidase-type enzyme (Figure 3). This final step liberates the nucleoside monophosphate, which is highly polar, and does not diffuse back across the cell membrane (essentially trapping it within the cell). Subsequent phosphorylation by host cell kinases convert the compound into the NTP analogue that can be used as a substrate by the viral RdRp enzyme. While the nucleoside analogue core of remdesivir, GS-441524, can diffuse into cells, the initial phosphorylation step for nucleosides is rate-limiting (slow), which is believed to account for the reduced antiviral activity of GS-441524 compared to remdesivir. This approach has been

successfully applied to a number of FDA-approved antiviral drugs including the Gilead products sofosbuvir (for treating HCV) and tenofovir alafenamide (first approved for treating HIV).

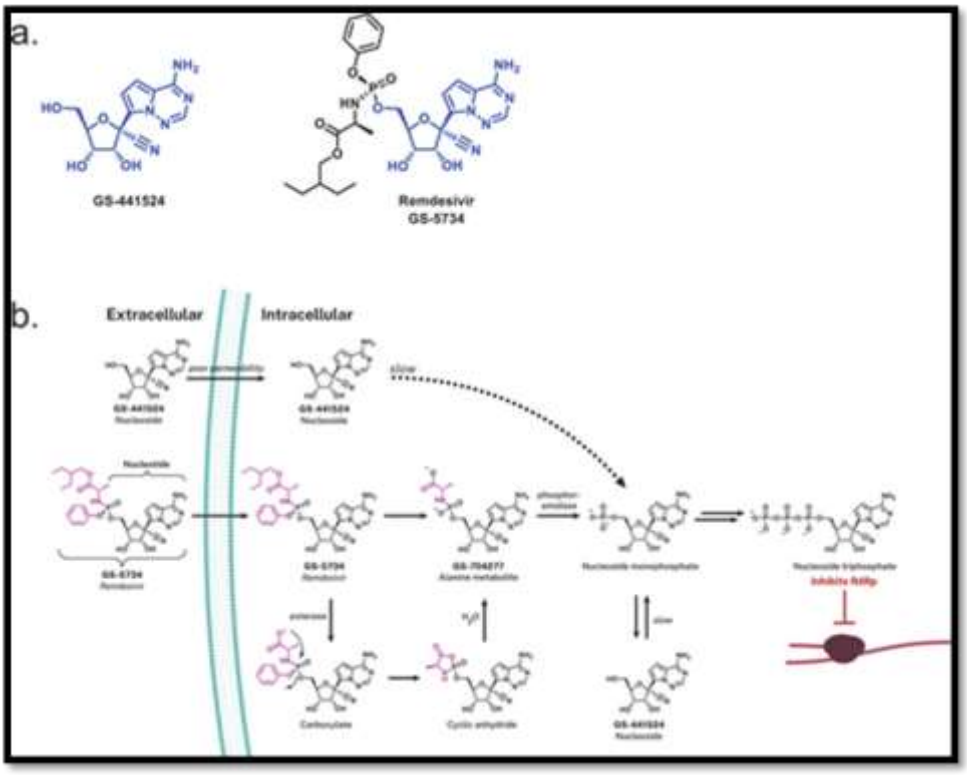

Fig.4[14,15,16]

Figure 3. Remdesivir and its intracellular conversion. (a) Chemical structures of GS-441524 that compose the nucleoside analogue core (blue) of remdesivir (GS-5734). (b) Intracellular processing of the prodrug remdesivir (GS-5734), the aryloxy phosphoramidate (purple) prodrug of GS-441524 monophosphate. Upon diffusion of remdesivir into the cell, it is metabolized into the nucleoside monophosphate form via a sequence of steps that are presumably initiated by esterase-mediated hydrolysis of the amino acid ester that liberates a carboxylate that cyclizes on to the phosphorus displacing the phenoxide. The unstable cyclic anhydride is hydrolyzed by water to the alanine metabolite GS-704277 whose P–N bond is hydrolyzed by phosphoramidase-type enzymes to liberate the nucleoside monophosphate or nucleotide analog. The artificial nucleoside monophosphate is routed to further phosphorylation events (hijacking the endogenous phosphorylation pathway) yielding the active nucleoside triphosphate analogue form that is utilized by the viral RNA-dependent RNA polymerase (RdRp). Utilization of the GS-441524 nucleoside triphosphate analogue by RdRp inhibits viral replication through inducing delayed chain termination.[7,8,9,10,11,12]

Antiviral Activity

Remdesivir has broad-spectrum antiviral activity against Ebola virus, Nipah virus, respiratory syncytial virus (RSV) and a number of coronaviruses, including Middle East respiratory syndrome coronavirus and SARS-CoV-2.[8] In vitro, remdesivir demonstrated antiviral activity against SARS-CoV-2 infection of primary human airway epithelial cells, inhibiting SARS-CoV-2 replication with a half-maximal effective concentration (EC50) of 9.9 nm. after 48 h of drug exposure.[6,7] Remdesivir also inhibited SARS-CoV-2 replication in the continuous human lung epithelial cell lines Calu-3 and A549-hACE2, with EC50 values of 280 nm. after 72 h of exposure and 115 nm. after 48 h of exposure, respectively. The antiviral activity of remdesivir was dose-dependently antagonized by chloroquine phosphate when the two drugs were co-incubated at clinically relevant concentrations in RSV-infected cells. Higher concentrations of chloroquine phosphate led to higher remdesivir EC50 values and reduced formation of remdesivir triphosphate. Therefore, coadministration of remdesivir and chloroquine phosphate or hydroxychloroquine sulphate is not recommended.[3,4,13]

CONCLUSION

COVID-19 is accelerating rapidly; until April 16, 2021, >139 million cases and 2.9 million deaths were confirmed globally. Additionally, hundreds of mutations were identified from viral isolates which resulted in the emergence of new variants that spread rapidly worldwide. Unfortunately, there is no fundamental therapy of COVID-19 till now. Remdesivir became the first FDA-approved antiviral agent for the treatment of hospitalized patients with COVID-19.

Despite conditional recommendation against its use, remdesivir could still be effective in early clinical improvement; reduction of early mortality and avoiding high-flow supplemental oxygen and invasive mechanical ventilation among hospitalised COVID-19 patients. Remdesivir was also well tolerated without significant SAEs compared to placebo, yet available evidence from clinical studies support the need to conduct close monitoring.

RESULTS

Treatment with remdesivir was associated with an increase in clinical recovery rate by 21% (RR 1.21; 95% CI 1.08–1.35) on day 7 and 29% (RR 1.29; 95% CI 1.22–1.37) on day 14. The likelihoods of requiring high-flow supplemental oxygen and invasive mechanical ventilation in the remdesivir group were lower than in the placebo group by 27% (RR 0.73; 95% CI 0.54–0.99) and 47% (RR 0.53; 95% CI 0.39–0.72), respectively. Remdesivir-treated

patients showed a 39% (RR 0.61; 95% CI 0.46–0.79) reduction in the risk of mortality on day 14 compared to the control group; however, there was no significant difference on day 28. Serious adverse effects (SAEs) were significantly less common in patients treated with remdesivir, with an absolute risk difference of 6% (RD −0.06; 95% CI −0.09 to −0.03).

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