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Remdesivir for Severe Acute Respiratory Syndrome Coronavirus 2 causing COVID-19: an evaluation of the evidence

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Abstract

The novel coronavirus infection that initially found in Wuhan at the end of 2019 has attracted great attention. So far, the number of infectious cases has increased globally to more than 100 thousand and defined as a pandemic situation, but there are still no "specific drug" available. Relevant reports have pointed out the novel coronavirus has 80% homology with SARS. In the difficulty where new synthesized drug cannot be applied immediately to patients, “conventional drug in new use” has become a feasible solution. The first medication experience of the recovered patients in the US has led remdesivir to be the "specific drug". China has also taken immediate action to put remdesivir into clinical trials with the purpose of applying it into clinical therapeutics for Corona Virus Disease 2019 (COVID-19). We started from the structure, immunogenicity, and pathogenesis of coronavirus infections of the novel coronavirus. Further, we analyzed the pharmacological actions and previous trials of remdesivir to identify the feasibility of conducting experiments on COVID-19.

Keywords: severe acute respiratory syndrome coronavirus 2, Corona Virus Disease 2019, remdesivir, phase III clinical trial

Introduction

The novel coronavirus 2019 (2019-nCoV), officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a newly-emerged human infectious coronavirus. It initially originated in a seafood market in Wuhan, a city with a population of 11 million. Since

December 2019, it has spread rapidly in China in a short period of time. As of 17 March 2020, there have been 81116 confirmed cases and 3231 deaths. It has spread to other countries, such as Korea, Japan, Italy, Singapore, and Iran, with a total of 85296 cases confirmed. Due to it is a newly-emerged virus, researchers have taken quick actions to isolate the virus and perform gene sequencing, making identifying treatments possible. Even so, it takes time to develop new drugs and vaccines, as well as to explore biotherapeutics, thus it is unlikely to be applied to patients with urgent need. Therefore, "conventional drug in new use" becomes a viable solution. The SARS-CoV-2 is 80% homologous with the acute respiratory syndrome-associated coronavirus (SARS-CoV), which also broke out in China in 2002, and some enzymes are even more than 90% homologous.[1] Consequently, we are expecting to find drugs for the treatment of COVID-19 from the experience of SARS-CoV and Middle East Respiratory Syndrome (MERS-CoV). Some drugs, such as ribavirin, interferon, lopinavir, and corticosteroids, have been used in patients with SARS or MERS,[2] within the selection range of “conventional drug in new use”. Through clinical treatment of the COVID-19, it has been found that neuraminidase inhibitors (oseltamivir, peramivir, zanamivir), ganciclovir, acyclovir, ribavirin are ineffectual and not recommended for clinical application.[3] When we set our sights on the broad-spectrum antiviral drugs, we found that a drug unlisted, remdesivir, has demonstrated strength in trials related to MERS-CoV and Ebola virus infection. In the United States, the first patient with COVID-19 has shown significant improvement in clinical symptoms within 24 hours of treatment with remdesivir. This case has convinced the public that remdesivir could become a new "specific drug" for COVID-19. This article starts from the structure, immunogenicity, and pathogenesis of infection of the SARS-CoV-2, and then analyzes the feasibility of conducting trials and putting into clinical use of

COVID-19 from the pharmacological characteristics and successful cases of remdesivir.

1 Structure and immunogenicity of SARS-CoV-2

SARS-CoV-2 is an enveloped, single, and positive stranded RNA virus. The virus particles are round or oval in shape, with a diameter about 60~140nm. Based on sequence analysis, it shows that the novel coronavirus belongs to Beta coronavirus Lineage β, Sarbecovirus, where SARS-CoV and MERS-CoV are included. However, it forms a new clade different from SARS-CoV and MERS-CoV, and becomes the seventh member of the coronavirus family to infect humans.[4]

SARS-CoV-2 shows the typical beta coronavirus organization: 5' untranslated region (UTR), replication enzyme coding region, S gene, E gene, M gene, N gene, 3' UTR, and several unidentified nonstructural open reading frames (Figure 1).[4] The replication enzyme coding region mainly expresses and encodes two large genes: ORF1a and ORF1b, which encode 16 nonstructural proteins (nsp1 ~ nsp16) that are highly conserved throughout the coronavirus. S gene, M gene, E gene, and N gene respectively encode four main structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. The S protein is the receptor binding site, which is on the viral surface; The M protein shapes the virions, promotes membrane curvature, and is responsible for the transport of nutrients across cell membranes; the E protein plays a role in the assembly and release of virus, and is involved in viral pathogenesis; the N protein can bind virus RNA genome and maintain its stability.[5] Among them, S protein plays a key role in virus recognizing and binding to host cell surface receptors, and mediating the fusion of virus envelope and cell membrane.[6] Through the analysis of the whole genome sequence of SARS-CoV-2, it shares 40% sequence similarity with MERS-CoV and 80% sequence similarity

with SARS-CoV, indicating that SARS-CoV-2 is more compatible with SARS-CoV.[7] In addition, by performing systematic structural simulations and immunogenicity scans of the S proteins of all coronaviruses, as well as calculating the immunogenic distance between SARS-CoV-2 and other coronavirus subtypes, it can be concluded that the immunogenicity of the S protein of SARS-CoV-2 is closer to that of SARS-CoV.[8] It is known that SARS-CoV enters target cells by binding the S protein to the ACE2 receptor on the cell surface, which is triggered by the cell serine protease TMPRSS2.[9] In view of the 76% amino acid similarity between SARS-CoV-2 and SARS-CoV,[10] we can speculate that the novel coronavirus may have a similar function to SARS-CoV, which has been preliminary proved in bioinformatics prediction methods as well as in vitro tests.[9]

Previous studies have shown that 4 of the 5 key amino acids of the S protein on the surface of SARS-CoV-2 that binds to angiotensin-converting enzyme 2 (ACE2) receptor on the target cells have changed. It was suspected it may affect the affinity of the S protein to ACE2 receptor, and in turn affect the spread of the virus among the public.[10] However, through calculation methods of molecular structure simulation, the interaction between the S protein of SARS-CoV and the ACE2 receptor has perfectly maintained in a holistic manner.[11] At present, it has been proved that the binding affinity between the extracellular domain of the S protein of SARS-CoV-2 and ACE2 receptors is about 10-20 times higher than that of SARS-CoV, which may facilitate human-to-human transmission of SARS-CoV-2.[12]

2 The pathogenic mechanisms of COVID-19

COVID-19 is a respiratory syndrome caused by SARS-CoV-2 infection. In general, COVID-19 is an acute resolved disease, and the most common symptoms at onset are fever, dry

cough, and fatigue, partly with nausea, diarrhea, or other gastrointestinal symptoms. Compared with SARS and MERS, COVID-19 has milder clinical symptoms and lower fatality,[13, 14] but it can also be fatal. Severe patients may develop diffuse alveolar injury, progressive respiratory failure, and acute respiratory distress syndrome (ARDS) and so on.

Similar to SARS-CoV, the receptor binding domain (RBD) of S protein on the surface of SARS-CoV-2 binds to the ACE2 receptor on the cell surface to facilitate the virus entering the host cell; then the virus exposes its RNA, translates its RNA replicase, and forms an RNA replicase-transcriptase complex. Through transcription and replication, the complex forms RNA negative strands that will be translated for the structural proteins of the virus later. Then the structural proteins and RNA in the cytoplasm assemble into new viral particles, which are released from infected cells by exocytosis to infect other cells (Figure 2). Each infected cell produces thousands of novel viral particles that spread to bronchi, eventually reach the alveoli, and extrapulmonary organs, causing pneumonia and targeted organic infections. However, the ACE2 receptor is not only expressed in the respiratory organs. It has been reported that, by using the RNA-seq method to express ACE2 receptors in human tissues, the number of ACE2 receptors expressed in the gastrointestinal tract (high in esophagus, small intestine, and colon, but low in stomach), kidneys, and testes is nearly 100 times higher than that in the lung,[15] suggesting that these tissues may also be the target organs for SARS-CoV-2 invasion. It may explain why some patients with COVID-19 developed other system injuries clinically besides respiratory system injuries. Furthermore, it have been found that SARS-CoV-2 nucleic acid detection is positive in the feces of some patients, indicating that there may be live virus in the feces, and the digestive system may be a potential route for COVID-19.[16]

In COVID-19, in addition to the direct damages caused by the virus, the indirect immune injuries caused by the injured tissues also attract great concern, which may be related to the severity and fatality of the disease. Previous studies have shown that pulmonary inflammation and extensive lung injury in patients with SARS are associated with an increase in proinflammatory cytokines (such as IL-1β, IL-6, IL-12, IFN-γ, IP-10, and MCP-1) in serum.[17] And it has been reported that the MERS-CoV infection induced elevated proinflammatory cytokine concentrations (such as IFN-γ, TNF-α, IL-15, and IL-17) in serum.[18] We note that patients with COVID-19 also have high levels of IL-1β, IFN-γ, IP-10, and MCP-1 in their serum, leading to activation of the Th1 cell responses. Furthermore, the concentrations of GCSF, IP-10, MCP-1, MIP-1A, and TNF-α in ICU patients were higher than those in non-ICU patients, indicating that cytokine storms were associated with disease severity. Apart from this, SARS-CoV-2 infection also activates the secretion of cytokines (such as IL-4 and IL-10) in Th2 cell responses that suppress inflammation, which is different from SARS-CoV infection.[17] Further researches are needed to investigate the responses of Th1 and Th2 in SARS-CoV-2 infection to elucidate the pathogenesis of COVID-19.

Currently, the pathogenesis of COVID-19 is unclear. The first pathologic autopsy of a patient with COVID-19 demonstrated that the lungs of the patient reviews diffuse alveolar injury and pulmonary hyaline membrane formation, consistent with ARDS. The overall pathological manifestations of the lungs were similar to SARS and MERS. Flow cytometry signified that the number of $CD4^+$ and $CD8^+$ T lymphocytes in peripheral blood was greatly reduced, but their state was overactivated. Other than this, $CCR4^+$ and $CCR6^+$ Th17 lymphocytes with highly proinflammatory effects increased in $CD4^+$ T lymphocytes; $CD8^+$ T lymphocytes had a high concentration of cytotoxic granules, of which 31.6% were perforin positive, 64.2% were particle

lysin positive, and 30.5% were both particle lysin and perforin positive. It manifests that the severe immune injury in this patient may be closely linked to the overactivation of T lymphocytes characterized by the increase of Th17 lymphocytes and the high cytotoxicity of $CD8^+$ T lymphocytes.[19]

We presume that the failure to develop a full adaptive immune response to COVID-19 could be due to: The progression of pneumonia was too rapid to allow the available establishment of adaptive immune responses. Likewise, the counts of peripheral $CD4^+$ and $CD8^+$ T lymphocytes were substantially reduced, leading to insufficient immune defenses. Furthermore, Peripheral T lymphocytes are in an over-activated state, manifested by increase of Th17 and high cytotoxicity of $CD8^+$ T lymphocytes, accounting for to a certain degree of immune injury in patients. This over activation not only failed to establish an immune response, but also caused tissue injuries, mostly manifested as severe injury in the lungs, and some patients died of multiple organ failure. This situation further accelerates the deterioration and shortens the course of the disease, hampering the establishment of fully adaptive immune response. The immunopathological injuries caused by the over activation also provides us with an idea for treating COVID-19, for example, we can probably apply the IL-17 inhibitor (secukinwmab) directed against Th17 cell activation, but it still need more exploration. Also, vaccines are also one of the solutions to make up for the lack of adaptive immune response.

The latest study terms that the changes of viral nucleic acid in patients with COVID-19 is similar to that in patients with influenza, but different from those with SARS. Viral load can be detected not only in symptomatic patients but also in asymptomatic patients, pointing out the potential for virus transmission in asymptomatic or mildly symptomatic patients. These findings

are coherent with reports evidencing that the virus transmission may have occurred early in infectious processes, illustrating that case detection and isolation may require a different strategy from that required to control SARS-CoV.[20]

3 Structure, pharmacokinetics, and RCTs of remdesivir

Remdesivir (GS-5734) is a nucleoside analogs drug (Figure 3B) with extensive antiviral activity and effective treatment of lethal Ebola and Nipah virus infections in nonhuman primates.[21] As an RNA-dependent RNA polymerase (RdRp) inhibitor, it can inhibit the replication of multiple coronaviruses in respiratory epithelial cells. A recent study reported that remdesivir competes with natural counterpart ATP. Once remdesivir added into the growing chain (i position), it cannot cause an immediate stop. On the contrary, it will continue to extend three more nucleotides down to stop the strand at (i + 3) position (Figure 2).[22]

In the Ces1c (-/-) mouse SARS model, the preventive treatment trial of remdesivir achieved satisfactory results. Administering 1 day after the onset of the disease, lung virus titers decreased significantly, with improvements on pulmonary function. Administering 2 days after the onset, the pulmonary virus titer can be obviously reduced, but the survival rate of mice is still relatively low. This study implied that when the pulmonary injuries reach the maximum, simply reducing the virus titer can no longer suppress the strong immune responses in mice, also showing that administering before the peak of virus replication can significantly improve symptoms of the infected mice.[23] In a rhesus monkey model infected with MERS-CoV, treating with remdesivir 24h before infection can completely prevent symptoms caused by MERS-CoV, strongly inhibit viral replications in the respiratory tract, and prevent the formation of pulmonary lesions. Administering remdesivir 12 hours after infection provides clear clinical benefits, reducing

clinical symptoms, lung virus replication, and lung lesions.[24]

Pharmacokinetic experiments in cynomolgus monkeys showed the first-pass effect of oral remdesivir resulted in a low bioavailability of the drug. Intramuscular injection of 3 mg/kg had a 50% survival rate compared with the control group. Administering intravenously at a dose of 10 mg/kg, remdesivir rapidly decomposed into the original drug (nucleoside phosphate) in rhesus monkeys. Within two hours, remdesivir quickly distributed in peripheral blood mononuclear cells (PBMCs), and soon afterwards activated to nucleoside triphosphate to reach a peak, with a survival rate of 100%.[25] As for pharmacokinetic studies in vivo, after the intravenous infusion of the remdesivir solution formulation at a single dose of 3 to 225 mg for two hours, it showed dose-linear pharmacokinetics. Intravenous infusion of 150 mg of a remdesivir solution repeated one hour per day showed a linear pharmacokinetics over a period of 14 days. After intravenously injecting 75 and 150 mg of remdesivir solution formulations over two hours, the pharmacokinetic profile was similar to that of a lyophilized formulation. Intravenous infusion of 75 mg of drug over 30 minutes provides similar levels of parent drug exposure to the same dose over two hours (Table 1). After the intravenous infusion, remdesivir will enter the cellular metabolism to form active GS-443902 (Figure 3C), but the frequencies of PBMCs exposure of GS-443902 is higher than those of intravenous infusion of remdesivir 150 mg within two hours. Studies in PBMCs show that the half-life of GS-443902 is more than 35h.[26] In the case of daily administration, the active substance of the drug GS-443902 will accumulate in vivo. As a result, in large-scale clinical trials, after the first dose of 200 mg is administered, the subsequent dose is adjusted to 100 mg to ensure the proper blood concentration in vivo.[27]

Intravenous infusions in previously phase I clinical trials have good safety and

pharmacokinetic properties. Also, no cytotoxicity, hepatorenal toxicity, or no serious adverse reactions related to metering have been observed in climbing experiments. Subjects were tolerant in studies that repeated 150 mg intravenously daily for 7 to 14 days. Remdesivir did not show any renal injuries in a multi-dose study.[26] Phase II clinical trials were conducted in Ebola virus-infected patients. In clinical trials of anti-Ebola drugs, the fatality rate of patients in the experimental group using remdesivir was 53%, and the efficacy was significantly worse than that of the two monoclonal antibodies MAb114 (fatality rate 35%) and REGN-EB3 (fatality rate 33%).[27] The 53% fatality rate was not significantly different from the average 50% fatality rate of Ebola virus infection, and as a result, phase II clinical trials were stopped. Nevertheless, in consideration of Ebola's high lethality and monoclonal antibodies with more obvious therapeutic effects, when there are merely 175 patients injected remdesivir, we cannot assume remdesivir of no avail. The small sample size is not enough to deny the effect of remdesivir. Moreover, receptors of Ebola virus are widely distributed in vivo, not only to the respiratory tract, but also to the digestive tract, urinary tract, and blood system, etc., causing mortally hemorrhagic fever; in addition, Ebola virus persists in the eyes and central nervous system for long[28]. Once remdesivir entering body, it will be quickly distributed to the testis, epididymis, eyes, and brain, but relatively less in eyes and brain.[29] All these indicate that the wide range of spread of Ebola virus in the high lethality tissues make remdesivir control Ebola ineffectively. The Wuhan Virus Research Institute conducted in vitro experiments on COVID-19 of remdesivir and found that remdesivir was the fastest-acting and most powerful antiviral agent. In the primary culture of human airway epithelial cells in vitro, SARS-CoV's IC_{50} = 0.069 μM, MERS-CoV's IC_{50} = 0.074 μM, and the dose-dependent effect on virus inhibition,[2] which is speculatively related to the fact that

remdesivir triphosphate cannot be removed by nsp14-ExoN.[30] It has been conjectured the loss of function of exonuclease may be involved with the three additional nucleotides added after the incorporation of remdesivir into the extended strand.[22]

In vitro and animal models, remdesivir has demonstrated activity against both SARS and MERS that also belong to coronaviruses, and theoretically provides support its effectiveness in treating COVID-19.

4 Successful cases of remdesivir in treating COVID-19

Presently, there have been successful cases of remdesivir in the treating COVID-19. *The New England Journal of Medicine* reported the entire course of rehabilitation of the first patient with COVID-19 in the United States. The patient once visited Wuhan but was neither directly exposed to Wuhan Seafood Market nor had direct contact with the diagnosed patients. He returned to Washington on 15 January 2020. On 19 January, due to cough and fever for four days, he went to the hospital for emergency treatment, and was then diagnosed with COVID-19. His condition was stable from the second to the fifth day of admission (the sixth to ninth day of onset). On the evening of the fifth day of admission, the blood oxygen saturation decreased to 90%. The condition continued to worsen, and chest radiographs on the sixth day of admission (tenth day of onset) showed typical characteristics of COVID-19. In view of the continuous aggravation of the patient's clinical symptoms, the physicians gave a chartered medication (Compassionate Use) to remdesivir on the evening of the 6th day of admission, and began to give intravenous to the patient on the evening of the seventh day of admission (the eleventh day of onset), without adverse reactions. Vancomycin was discontinued that night and cefepime was discontinued the following day. On the eighth day of admission (the twelfth day of onset), the patient's clinical symptoms

were improved, and the oxygen saturation increased to 94%. Although the patient was still hospitalized as of 30 January 2020, all symptoms had been resolved except for cough and occasional running nose.[31]

It is worth noting that from the data in the article, it can be found the viral load of patients has decreased before remdesivir injection (Table 2), which is not described in detail in the original report. It's known that the viral infection is self-limiting, and the patient is a mild to moderate infectious case with a controlled fever in time, thus it is possible that his recovery is related to the role of self-defense mechanisms and supportive treatment as well. It cannot be inferred that the improvement of patients' condition after taking the drug is definitely connected to remdesivir. Whether there is a link between the improvement of the symptoms and the drug is worth further consideration.

Clinical symptoms, especially respiratory symptoms, have been improved significantly within 24 hours, bringing hope for the treatment of patients with severe COVID-19. For COVID-19 no specific medication is available, remdesivir is expected to be a "specific drug". However, for the acute infectious diseases, reducing the number of viral copies in the body is the key point. Also, the efficacy of the drug should be focused on the pharmacokinetics and kinetics data of COVID-19 in the ongoing phase III clinical trials.

5 Feasibility of trials on remdesivir on COVID-19

The outbreak of SARS-CoV-2 in Wuhan constituted an epidemic threat in China. The World Health Organization announced it a public health emergency of international concern on 30 January 2020. During the outbreak, the number of confirmed cases in China showed an exponential growth. The people and the government of the country tried their best to fight the

epidemic with soaring combat mood. The nation's enthusiasm to fight the epidemic provides the trials on COVID-19 a favorable environment. At the same time, Article 23 of China's new Drug Administration Law, which came into effect on 1 December 2019, has enabled the "Compassionate Use" to develop adaptively in China. Two clinical trials on remdesivir have passed the most stringent ethical review of the 74 projects. On 5 February 2020 the trial has officially launched with experimental drugs provided by Gilead Sciences for free in China by professor Chen Wang, an academician of Chinese Academy of Engineering, an internationally renowned respiratory expert who successfully suggested Chinese Government building "Fang Cang" hospitals to cure more than 10 thousand mild or prepatent COVID-19 patients[32]. Due to the large number of confirmed cases of COVID-19 in China with no effective drugs, it is easy to collect clinical samples for trials theoretically. However, the rigor of the included samples hindered recruitment. As public attach more attention on prevention and treatment, fewer patients meet stringent inclusion criteria, resulting in a slow recruitment process. Another reason is that there are plenty of drugs in the clinical trials, speeding up the patients' leaving hospital. Nevertheless, it has been reported that more severe patients have been recruited, which provides favorable conditions for the trial of the severe group, and as a result, at least, it can be rapidly applied to the clinical treatment of severe patients in the near future. The need of treatment on COVID-19 is urgent, so if the results of clinical trials prove it has the potential to benefit the treatment, according to China's "Compassionate Use", remdesivir will be more immediately used in patients with severe illness. Meanwhile, the opening of green channels under special circumstances to speed up the review and approval process of the drug approval center will undoubtedly help save the lives of critical patients and promote the developing of "specific drugs".

In the absence of clinical trial results, it is still difficult to put remdesivir into large scale clinical use[33]. With the political support, the rapid development of clinical trials on remdesivir is imperative.

A drug, GS-441524 (compound A, Figure 3A) for treating feline infectious peritonitis (FIP) caused by coronavirus infection in cats has been tested in cats. Its safety and effectiveness in treating FIP have been proven,[34] with FDA's approval. It can be seen from the structure that remdesivir is phosphorylated from GS-441524, with identical target RdRp (Figure2). It is noteworthy that though coronavirus reproduces more than 20 generations in GS-441524 yields resistance, the resistant virus is still sensitive to high concentrations of remdesivir and the fitness of the resistant virus has reduced to the same level as wild-type MERS-CoV,[35] which avoids resistant mutant coronaviruses from producing resistant supervirus. At the beginning of developing remdesivir, Gilead Science selected a large number of nucleosides or their prodrugs to conduct in vitro growth inhibition experiments on Ebola-infected human microvascular endothelial cells in the laboratory and found compound A showed inhibitory activity (EC_{50}=0.78 μM), and the compound A was GS-441524. Thereafter, on the basis of Compound A, after examining the activity and toxicity of compounds surrounding Compound A, modifying the prodrug, and optimizing amino acids and acyl groups, the cynomolgus monkey performs a pharmacokinetic test to select a structure such as GS-5734 (Figure 3B).[25] Although in phase II it was not as effective as competitive drugs and clinical trials were terminated, remdesivir showed good safety and pharmacokinetics in both phases I and II clinical trials. COVID-19 has once again brought remdesivir to the stage of clinical trials. Whether the results of phase III clinical trial will make its comeback to the stage is worthy of expectation.

Phase II clinical trials have demonstrated human tolerance to remdesivir. Of the 175 patients in the phase II clinical trial administering remdesivir, 9 were reported to have serious adverse reactions, 8 of whom were considered not related to drugs, and 1 with severe hypotension was thought to be drug-related, but still not confirmed.[27] The GS-441524, a drug used to treat FIP, has shown a high degree of safety in feline trials as well. The focal injection site reactions only showed in immediate pain with vocalization, occasional growling, and postural changes lasting for 30–60s. These initial reactions were relieved after the owners became more adept at administering the injection. Except for a cat with a slight increase in urea nitrogen and SDMA of the third round of treatment, no other symptoms of systemic poisoning were observed.[34] Relevant research signified that through a large number of synthesis and structure-activity analysis, the toxicity was greatly reduced after GS-411524 was synthesized into GS-5734 (remdesivir).[36] The safety of remdesivir in human is further speculated.

Coronaviruses must replicate nucleic acids to generate new progeny virus after entering human cells. SARS-CoV-2 is known to be single stranded RNA virus, so RdRp must be used to replicate nucleic acids. Remdesivir, a nucleotide analogues, act as RdRp inhibitor, can provide a scheme for blocking RNA replication. Related studies have found that it plays a role in the final stage of entering the cell, which is consistent with its expected mode of action. Wuhan Virus Research Institute carried out a vitro inhibition test and found that remdesivir can block virus infection at very low micromolar concentration of Vero E6 cells infected with virus, and the cell selectivity is high (EC_{50} = 0.77 μM, CC_{50} > 100 μM, SI > 129.87).[2] In an anti-Ebola infection experiment on cynomolgus monkeys, intravenous injection of 10 mg/kg of remdesivir, the drug can exist in the blood for a long time and can inhibit to Ebola virus with a percentage of 100.[25]

Wuhan Virus Research Institute's research that applied remdesivir to Vero E6 cells with an EC_{90}= 1.76 μM, lower than that of the monkey model, draw to a speculation that it could also play a role in SARS-CoV-2 infected monkeys. Based on the effectiveness in previous researches, although there are many unknowns and limits of remdesivir, the phase III clinical trials on SARS-CoV-2 are not only a fight against this epidemic, but also of strategic importance to reserve more effective antiviral drugs for the future.

Conclusion

Strategic reservation for antiviral drugs will avoid the difficulty of medicine unavailable when an outbreak comes again. Remdesivir's situational and political superiority, as well as its previous research results and application effects make it imperative to carry out the clinical trials focusing on the SARS-CoV-2. Given that SARS-CoV-2 is an RNA virus that is easy to mutate, the rapid starting of clinical trials is undoubtedly a right choice to prevent the resistance mutation due to blind medication. It has been covered in the World Health Organization (WHO) Director-General's opening remarks at the media briefing on COVID-19 on 20 February 2020 that the two clinical trials on remdesivir of therapeutics prioritized by the WHO R&D Blueprint are expected preliminary results in three weeks. On February 24, the WHO cast a vote of confidence for Gilead Sciences' experimental antiviral drug, remdesivir, indicating that remdesivir has great potential and may be the best candidate for the treatment of COVID-19. Whatever the progress of the clinical trials is, we are expecting that the clinical trials of remdesivir, a starring drug, would bring outstanding breakthroughs to the treatment of COVID-19, or more promisingly, other virus infection in the future.

Contributors：

All authors contributed to the conception of the Review. YC Cao and QX Deng reviewed the literature and drafted the manuscript. SX Dai critically reviewed the manuscript. All authors contributed to the revision of the manuscript.

Conflicts of interest:

The authors report no conflicts

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Table 1 Drug concentrations in plasma and the concentration of pharmacologically active substances in PBMCs in healthy people

Table 2 Viral load in the first course of rehabilitation in the United States

Figure 1 The RNA diagram of SARS-CoV-2

The structure of spike protein is downloaded from RCSB PBD (http://www.rcsb.org/pdb/home/home.do, accessed Feb 25); The model that S protein receptor binding domain (RBD) bind to ACE2 is downloaded from National Microbiology Data Center (http://nmdc.cn/?from=groupmessage&isappinstalled=0#/, accessed Feb 25).

Figure 2 SARS-CoV-2 invasion process and how remdesivir works

1 SARS-CoV-2 enters target cells by binding the S protein to the ACE2 receptor on the cell surface; **2** Remdeivir, the nucleotide analogues, act as RdRp inhibitors ,can provide a scheme for blocking RNA replication; **3** Once remdesivir added into the growing chain (I position), is cannot cause an immediate stop. On the contrary, it will continue to extend three more nucleotides down to stop the strand at (i + 3) position; **4** Remdesivir triphosphate cannot be removed by nsp14-ExoN.

Figure 3 Structure of remdesivir and its precursors and metabolites

The original structure of the drug is derived from DRUGBANK (https://www.drugbank.ca, accessed Feb 25)

Reference

[1] Liu W, Morse J S, Lalonde T, et al. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV[J]. Chembiochem,2020.

[2] M W, R C, L Z, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro.[J]. Cell research,2020.

[3] H L, Ym W, Jy X, et al. [Potential antiviral therapeutics for 2019 Novel Coronavirus].[J]. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases,2020,43:E2.

[4] Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019[J]. The New England journal of medicine,2020,382(8):727-733.

[5] Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis[J]. Journal of medical virology,2020,92(4):418-423.

[6] Du L, He Y, Zhou Y, et al. The spike protein of SARS-CoV — a target for vaccine and therapeutic development[J]. Nature Reviews Microbiology,2009,7(3):226-236.

[7] Zhou P, Yang X, Wang X, et al. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin[J]. bioRxiv,2020:2020-2021.

[8] Qiu T, Mao T, Wang Y, et al. Identification of potential cross-protective epitope between 2019-nCoV and SARS virus[J]. Journal of Genetics and Genomics,2020.

[9] Hoffmann M, Kleine-Weber H, Kr眉ger N, et al. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells[J]. bioRxiv,2020:2020-2021.

[10] Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission[J]. Sci China Life Sci,2020.

[11] Zhou P, Yang X L, Wang X G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin[J]. Nature,2020,579(7798):270-273.

[12] Wrapp D, Wang N, Corbett K S, et al. Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation[J]. bioRxiv,2020:2020-2022.

[13] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China[J]. Lancet (London, England),2020,395(10223):497-506.

[14] Chan J F, Yuan S, Kok K, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster[J]. Lancet (London, England),2020,395(10223):514-523.

[15] Fan C, Li K, Ding Y, et al. ACE2 Expression in Kidney and Testis May Cause Kidney and Testis Damage After 2019-nCoV Infection[J]. medRxiv,2020:2020-2022.

[16] Zhang H, Kang Z, Gong H, et al. The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes[J]. bioRxiv,2020:2020-2021.

[17] Wong C K, Lam C W K, Wu A K L, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome[J]. Clinical & Experimental Immunology,2004,136(1):95-103.

[18] Mahallawi W H, Khabour O F, Zhang Q, et al. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile[J]. Cytokine,2018,104:8-13.

[19] Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome[J]. The Lancet. Respiratory medicine,2020:S2213-S2600.

[20] Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of

Infected Patients[J]. N Engl J Med,2020.

[21] Mk L, F F, Jm G, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge.[J]. Science translational medicine,2019,11(494).

[22] Cj G, Ep T, Jy F, et al. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus.[J]. The Journal of biological chemistry,2020.

[23] Tp S, Ac S, Sr L, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV.[J]. Nature communications,2020,11(1):222.

[24] de Wit E, Feldmann F, Cronin J, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection[J]. Proc Natl Acad Sci U S A,2020.

[25] Warren T, Jordan R, Lo M, et al. Nucleotide Prodrug GS-5734 Is a Broad-Spectrum Filovirus Inhibitor That Provides Complete Therapeutic Protection Against the Development of Ebola Virus Disease (EVD) in Infected Non-human Primates[J]. 2015,139(3):311-320.

[26] Appendix 4. Summaries of evidence from selected experimental therapeutics, as of October 2018[J].(https://www.who.int/ebola/drc-2018/summaries-of-evidence-experimental-therapeutics.pdf?ua=1 accessed in 17 March,2020)

[27] S M, Le D, Rt D, et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics.[J]. The New England journal of medicine,2019,381(24):2293-2303.

[28] L B, Ds C, Km J, et al. The Pathogenesis of Ebola Virus Disease.[J]. Annual review of pathology,2017,12:387-418.

[29] Tk W, R J, Mk L, et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys.[J]. Nature,2016,531(7594):381-385.

[30] Jordan P C, Stevens S K, Deval J. Nucleosides for the treatment of respiratory RNA virus infections[J]. Antivir Chem Chemother,2018,26:1631083325.

[31] Kim J Y, Choe P G, Oh Y, et al. The First Case of 2019 Novel Coronavirus Pneumonia Imported into Korea from Wuhan, China: Implication for Infection Prevention and Control Measures[J]. J Korean Med Sci,2020,35(5):e61.

[32] Chen S, Yang J, Yang W, et al. COVID-19 control in China during mass population movements at New Year[J]. The Lancet,2020,395(10226):764-766.

[33] Ja A, Ah A, Za M. Remdesivir as a possible therapeutic option for the COVID-19.[J]. Travel medicine and infectious disease,2020:101615.

[34] Pedersen N C, Perron M, Bannasch M, et al. Efficacy and safety of the nucleoside analog GS-441524 for treatment of cats with naturally occurring feline infectious peritonitis[J]. J Feline Med Surg,2019,21(4):271-281.

[35] Ml A, El A, Ac S, et al. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease.[J]. mBio,2018,9(2).

[36] A C, Ol S, T B, et al. Synthesis and antiviral activity of a series of 1'-substituted 4-aza-7,9-dideazaadenosine C-nucleosides.[J]. Bioorganic & medicinal chemistry letters,2012,22(8):2705-2707.

Table 1 Drug concentrations in plasma and the concentration of pharmacologically active substances in PBMC in healthy people

Time (min)	75mg		150mg
	30	120	120
Drug in plasma (μ M)	0.09	0.13	0.23
GS-443902 in PBMC(μ M)	0.031	0.014	0.023

Table 2 Viral load in the first course of rehabilitation in the United States

	4^{th} day onset	7^{th} day onset	11^{th} day onset
Nasopharyngeal swab	Ct*，18-20	Ct，23-24	Ct，33-34
Oropharyngeal swab	Ct，21-22	Ct，32-33	Ct，36-40

（*：Higher Ct means lower viral load）

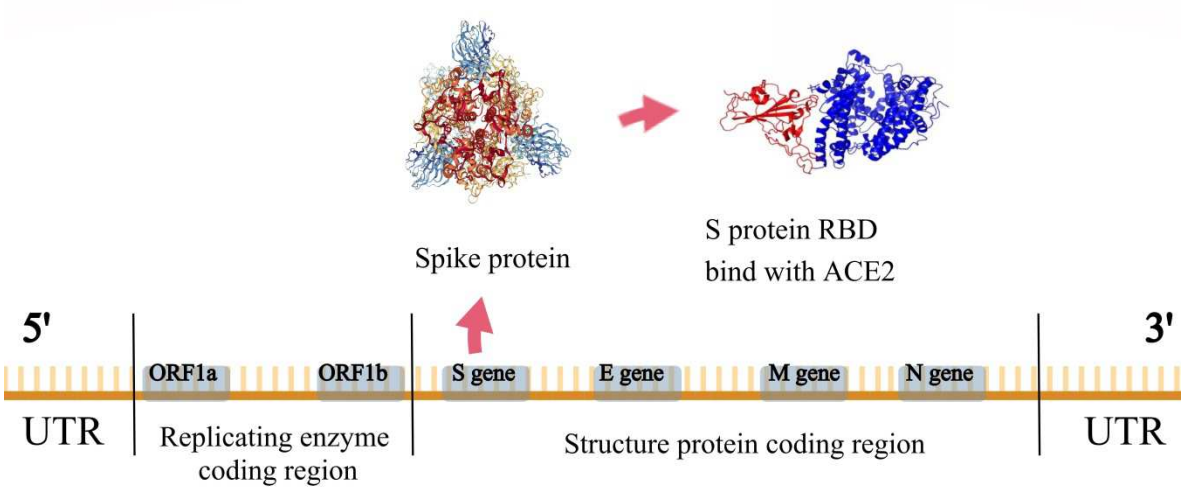

Figure 1 The RNA diagram of SARS-CoV-2

The structure of spike protein is downloaded from RCSB PBD (http://www.rcsb.org/pdb/home/home.do, accessed Feb 25); The model that S protein receptor binding domain (RBD) bind to ACE2 is downloaded from National Microbiology Data Center (http://nmdc.cn/?from=groupmessage&isappinstalled=0#/, accessed Feb 25).

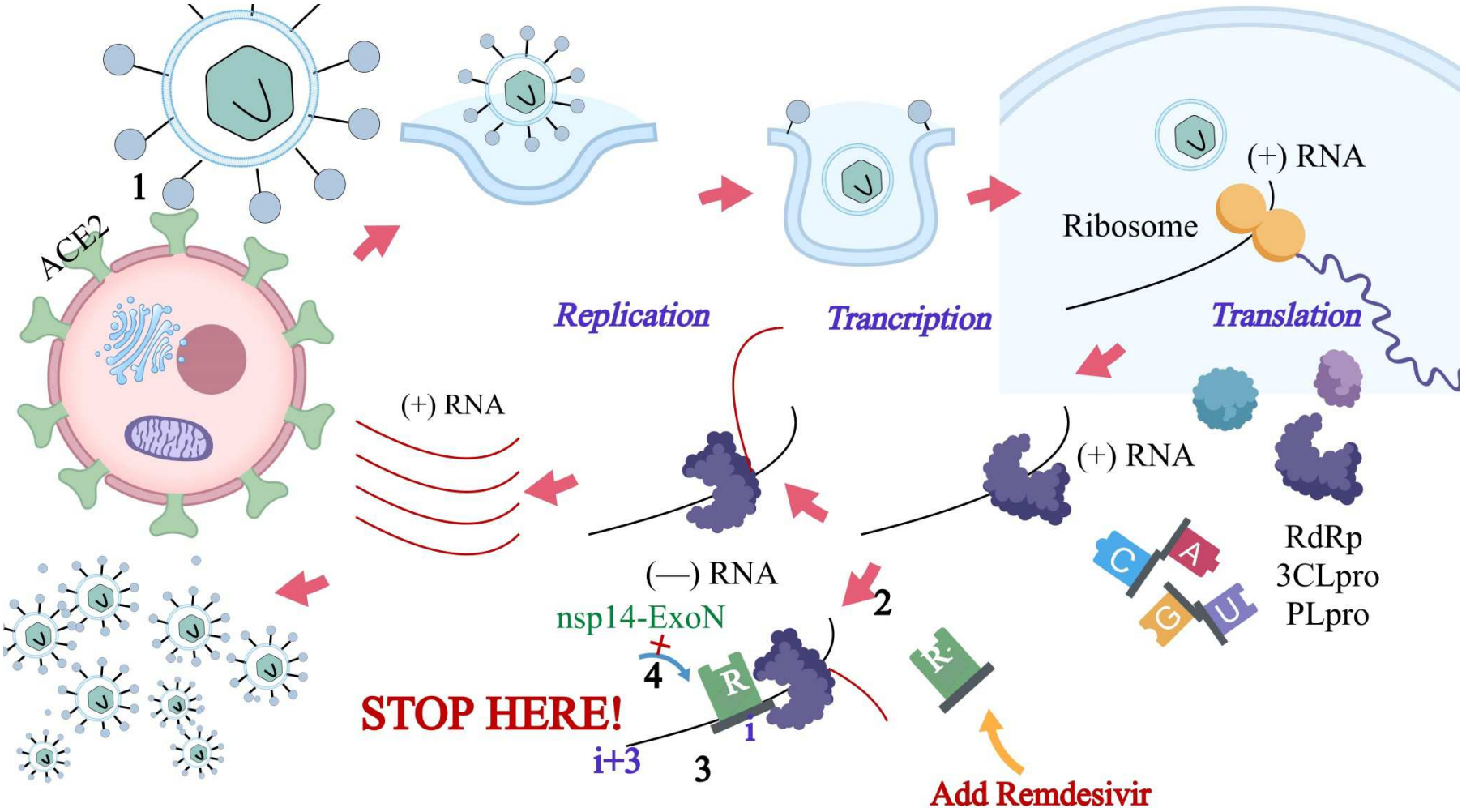

Figure 2 SARS-CoV-2 invasion process and how remdesivir works

1 SARS-CoV-2 enters target cells by binding the S protein to the ACE2 receptor on the cell surface; **2** Remdeivir, the nucleotide analogues, act as RdRp inhibitors ,can provide a scheme for blocking RNA replication; **3**Once remdesivir added into the growing chain (i position), is cannot cause an immediate stop. On the contrary, it will continue to extend three more nucleotides down to stop the strand at (i + 3) position; **4** Remdesivir triphosphate cannot be removed by nsp14-ExoN.

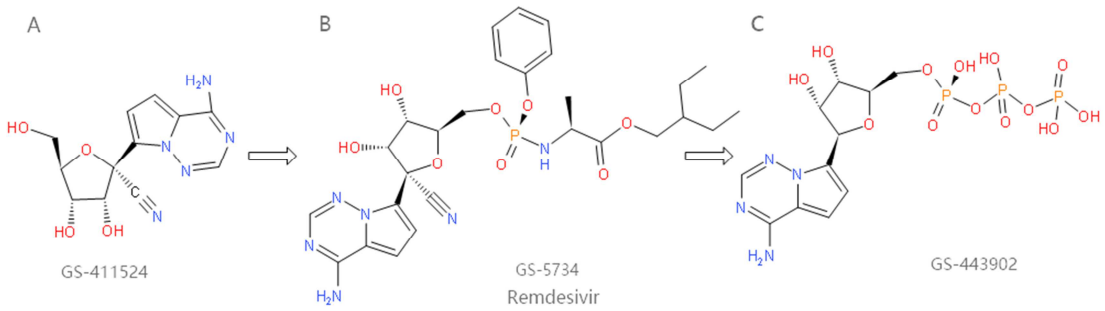

Figure 3 Structure of remdesivir and its precursors and metabolites

The original structure of the drug is derived from DRUGBANK (https://www.drugbank.ca, accessed Feb 25)