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Development of remdesivir repositioning as a nucleotide analog against COVID-19 RNA dependent RNA polymerase

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative representative of a severe respiratory illness resulted in widespread human infections and deaths in nearly all of the countries since late 2019. There is no therapeutic FDA-approved drug against SARS-CoV-2 infection, although a combination of anti-viral drugs is directly being practiced in some countries. A broad-spectrum of antiviral agents are being currently evaluated in clinical trials, and in this review, we specifically focus on the application of Remdesivir (RVD) as a potential anti-viral compound against Middle East respiratory syndrome (MERS) -CoV, SARS-CoV and SARS-CoV-2. First, we overview the general information about SARS-CoV-2, followed by application of RDV as a nucleotide analogue which can potentially inhibits RNA-dependent RNA polymerase of COVs. Afterwards, we discussed the kinetics of SARS- or MERS-CoV proliferation in animal models which is significantly different compared to that in humans. Finally, some ongoing challenges and future perspective on the application of RDV either alone or in combination with other anti-viral agents against CoVs infection were surveyed to determine the efficiency of RDV in preclinical trials. As a result, this paper provides crucial evidence of the potency of RDV to prevent SARS-CoV-2 infections.

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Introduction

The new coronavirus (CoV), known as Severe Acute Respiratory Syndrome (SARS)-CoV-2, which is currently spreading around the world, can lead to a respiratory illness that can be exacerbated (Liu et al., 2020; Novel, 2020; Xu et al., 2020). The disease known as CoV diseases-19 (COVID-19) appears to induce a mortality rate of less than 2%, which is lesser that of most epidemics that have ever become global headlines (Dong et al., 2020; Pan et al., 2020). CoVs are very similar to influenza viruses and show almost identical symptoms (Heymann & Shindo, 2020; Rothan & Byrareddy, 2020). Two recent outbreaks of the new CoV, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) have originated from animals (Gretebeck & Subbarao, 2015; Song et al., 2019; Yao et al., 2014). The diseases caused by these viruses were extremely fatal to humans, and very few cases were reported as mild or asymptomatic. However, it has been reported that the mortality rate of COVID-19 has been more than that of SARS and MERS (Mahase, 2020; Peeri et al., 2020).

In the past days, it has been repeatedly reported that a vaccine for the SARS-CoV-2 has been discovered, but vaccines, like other medicines, require a lengthy testing process to be approved for medical applications (Ahmed et al., 2020; Chen et al., 2020; Prompetchara et al., 2020). One strategy to date is the replication of a piece of virus RNA that could one day serve as a vaccine (Enayatkhani et al., 2020). Although, well-developed DNA sequencing devises has let to rapid genetic sequencing, vaccine development is costly, complex, and time intensive (Plotkin et al., 2017). This includes finding a viral sequence that, while providing memory to the immune system, does not lead to an acute inflammatory reaction. Achieving this goal requires laboratory experimentation on animal models before subjecting humans. In addition, once the vaccine is discovered, it is not possible to dispatch the sample quickly and easily worldwide. Therefore, pharmaceutical companies have generally found it more profitable to invest in drugs that are used for chronic medical conditions. The CoV, as in case of influenza, may undertake mutations and therefore would require continuous vaccine development.

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With Having a considerable number of people worldwide infected with COVID-19, scientists have identified a number of cases of broad-spectrum antiviral agents (BSAAs) that could serve as potential candidates for the treatment of the viral diseases (Andersen et al., 2020; Ianevski et al., 2018). Indeed, re-purposing of current approved anti-viral drugs could be a solution to treat new viral infections (Guo, 2020; Kouznetsova et al., 2014; Mercorelli et al., 2018; Xu et al., 2016).

Drug re-purposing means that by examining existing drugs, they will find therapeutic effects on new diseases (Aggarwal et al., 2020; Khan, Jha, et al., 2020; Senathilake et al., 2020). BSAAs are small molecules that can inhibit different infections by blocking the viral replication (Pant et al., 2020; Xiong et al., 2020; Xu et al., 2020). These drugs block the virus or host-related factors and thus prevent the virus from proliferating, then lowering the level of the virus in the body to an extent that the immune system can inhibit their infection (Cui et al., 2020; Ji & Li, 2020). BSAA has received special attention with the emergence of numerous new viral diseases. Re-purposing existing drugs, or even rejected drugs, for viral diseases increases the possibility of market success as well as reducing the costs and time required to launch it. The benefit of drug re-purposing is that drug details, such as the stages of chemical synthesis, mass production processes, various stages of clinical trials and many more have been identified beforehand (Aanouz et al., 2020; Gupta et al., 2020).

There is currently no drug or vaccine to prevent the SARS-CoV-2 infection, but the use of widespread antivirals can be effective against the prophylaxis of this virus (Boopathi et al., 2020; Elmezayen et al., 2020). For example, chloroquine and remdesivir (RDV, GS-5734) are two drugs that in vitro studies have suggested that they can inhibit the viral replication (Wang et al., 2020). Teicoplanin, oritavancin, dalbavancin, and monensin antibiotics also prevent viral replication (Andersen et al., 2020). Currently, the BSAAs are treatment or pyrophylaxis candidates against SARS-CoV-2 (Senanayake, 2020).

Furthermore, a combination of BSAAs can be applied against a wider range of viruses, such as those that are not yet well recognized or drug-resistant viruses (Wang, Cao, et al., 2020). Potential clinical trials are currently being conducted on these drugs and their results will be published soon (Li & De Clercq, 2020; Senanayake, 2020). Perhaps in a near future, BSAAs will be used to treat COVID-19 patients. Although a number of BS have been reported to date, in this review we focused on the RDV as a potential compound that has been assessed on the animal models and reaches the clinical phase against MERS -CoV, SARS-CoV and SARS-CoV-2.

General information on SARS-CoV-2

CoVs are a large family of viruses ranging from the common cold virus to the cause of SARS (Cascella et al., 2020; Paules et al., 2020). The structure of CoV has a common RNA genome and is classified as enveloped viruses (Mackie, 2003). SARS-CoV-2 originating in China and the city of Wuhan is believed to be a member of the Corona family, which has infected many people to date (Lai et al., 2020). Despite the emergence of the virus in China, it is spreading rapidly to other parts of the world (Lai et al., 2020; Zhu et al., 2020). Similar cases have been reported in other countries such as Thailand, South Korea, Japan, Taiwan, Australia, and the United States, and even more recently in Iran (Velavan & Meyer, 2020; Zhu et al., 2020). The disease can be spread through close contact with the infected person, handling contaminated equipment and airborne outbreaks (Velavan & Meyer, 2020). Most people with hypertension, diabetes, respiratory problems, and weak immune systems are at the higher risk to infection and the likely death from it (Fang et al., 2020).

CoVs have four types of proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) (Hasan et al., 2020). S protein is attached to the virus membrane and play an important role in binding and entry into the host cell; hence, targeting this protein with various drugs and inhibitors is one potential approach to combat these types of viruses (Chatterjee, 2020; Lai & Cavanagh, 1997; Woo et al., 2010). SARS-CoV-2 has proteins on its surface that mediate viral infection by binding to angiotensin-converting enzyme 2 (ACE2) receptor (Batlle et al., 2020; Chen & Hao, 2020). Therefore, one promising way to stop infection by SARS-CoV-2 is to find a compound that blocks the receptor, and consequently prevent the infection by preventing the interaction of S protein with ACE2 receptor (Andersen et al., 2020; Joshi et al., 2020). The CoV also has 16 unstructured protein (Nsp 1-16) encoded by ORF1a/1b which act as important co-factors for activation of viral replication enzymes (Guo et al., 2020) (Figure 1).

Although a recent research in China suggests bat as the potential source of the virus, there are ongoing researches to clarify the exact origin of the virus (Guo et al., 2020) (Figure 1). Researches into the origin of SARS outbreaks have led to the discovery of many bat viruses. SARS-CoV-2 belongs to this category of SARS-related viruses (Fung et al., 2020). The two genome sequences of *Rhinolophus sinicus* show 80% similarity with SARS-CoV-2. The third genome of the *Rhinolophus affinis* virus, RaTG13, resembles 96% similarity with SARS-CoV-2 (Andersen et al., 2020). To have better sense of this variation, it is similar to the rate of mutation observed over ten years in the human H3N2 influenza virus strain (Wang et al., 2020).

Remdesivir

RDV (GS-5734) as a nucleotide analogue was originally developed to treat Ebola (Tchesnokov et al., 2019). The laboratory assessments has shown that RDV is effective against SARS-CoV (Ju et al., 2020) and MERS-CoV (Gordon et al., 2020) viruses, therefore it can be used as a potential anti-viral agent against SARS-CoV-2 (Khan et al., 2020; Wang et al., 2020). The mechanism of RDV's anti-viral function is based on the blockage of viral RNA transcription as revealed in molecular examinations using different recombinant viral

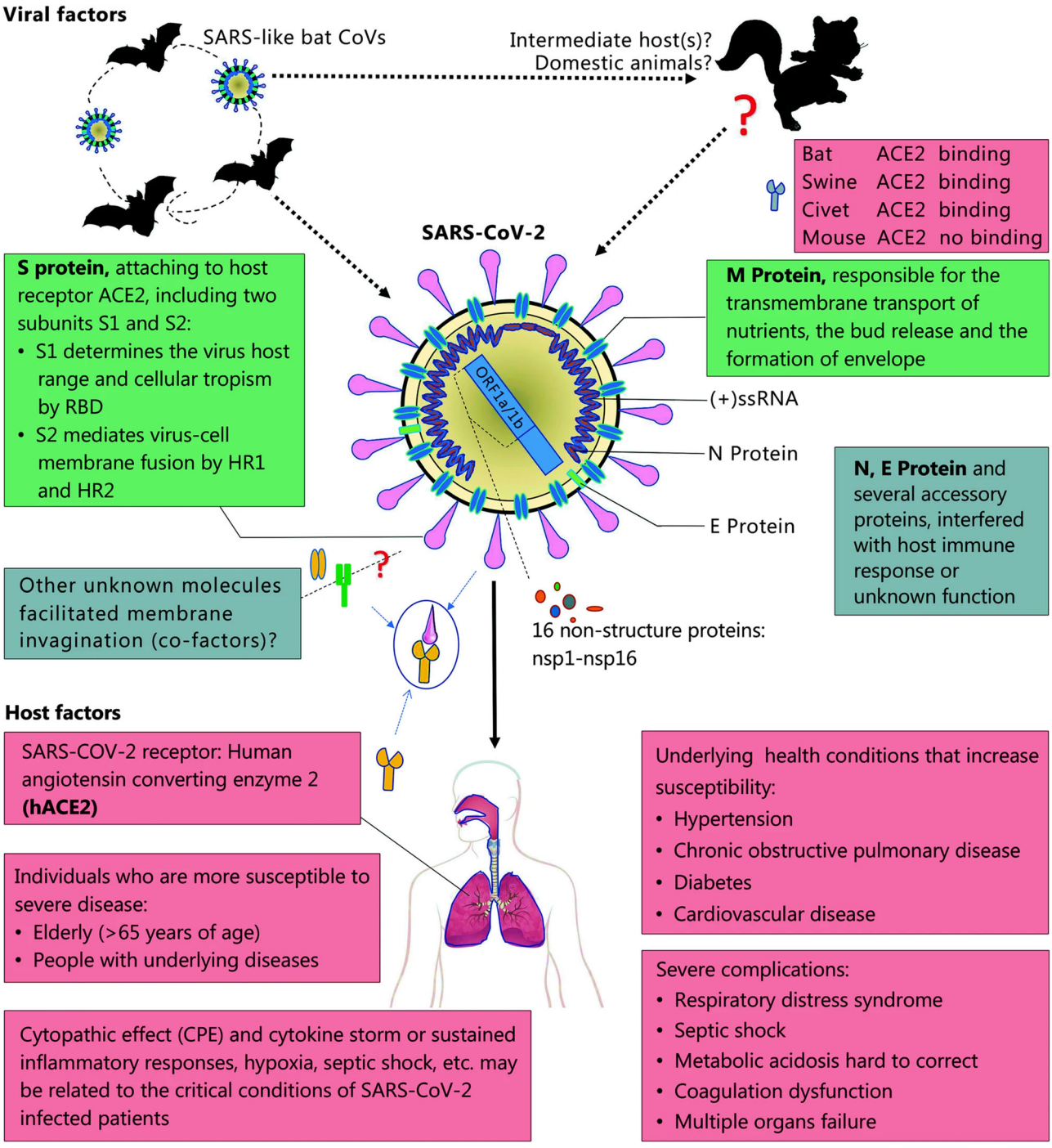

Figure 1. Some specifications such as origin, transmission and clinical symptoms of SARS-CoV-2. Reprinted with permission from Ref. (Guo et al., 2020).

polymerases (Jordan et al., 2018; Sarma et al., 2020; Tchesnokov et al., 2019; Warren et al., 2016).

Siegel et al. (2017) reported that GS-5734 can be used as a potential candidate for the treatment of Ebola and emerging CoV. Agostini et al. (2018) reported that CoV is susceptible to the RDV targeting the viral polymerase and the nsp14 exoribonuclease (ExoN). They compared the sensitivity of WT and ExoN (-) virus to RDV, which ExoN (-) virus showed a greater decrease in viral titer in the presence of GS-5734 relative to WT virus and the determined EC_{50} value for ExoN (-) virus was around 0.019 M, whereas the EC_{50} value for to the WT was determined to be 0.087 M (Figure 2A(i)). This increased inhibition of ExoN (-) virus by GS-5734 (Figure 2A(ii)) indicated that GS-5734 is integrated into viral genome and can be excluded by ExoN (Agostini et al., 2018). Also, it was shown that the type of CoV, concentration of antiviral drug, type of anti-viral drug, and incubation time can play an important role on the inhibition of virus infection (Figure 2B(i–iii).

Tchesnokov et al. (2019) declared that the significant inhibition of Ebola virus RNA polymerase can be attributed to the anti-viral effect of RDV. Brown et al. (2019) also reported that RDV stimulate its anti-viral effects through inhibition of RNA polymerase in human CoV OC43 (HCoV-OC43) (Figure 3(i–vi)).

Lo et al. (2019) also displayed that RDV prevent Nipah virus infection in monkeys. Furthermore, to assess the

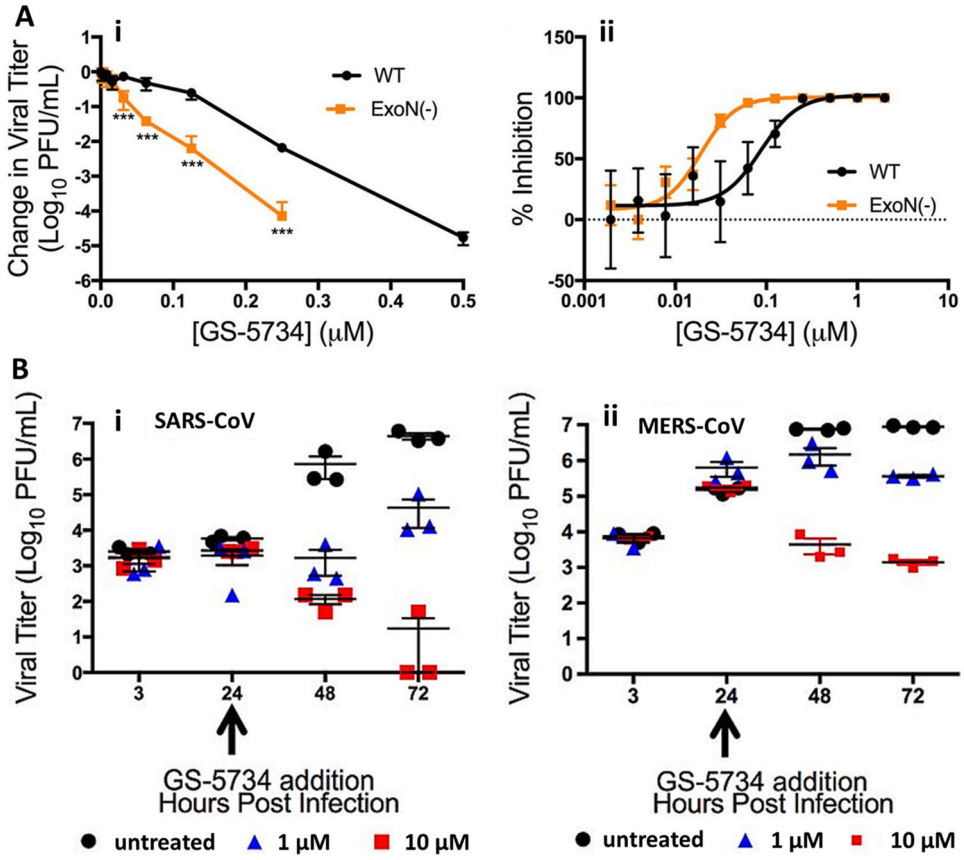

Figure 2. (A) ExoN (-) virus are more sensitive to anti-viral drug. (i) Viral titer of WT and ExoN (-) viruses, (ii) percentage of viral titer reduction. (B) The effect of different variations on the viral titer value. (i) The SARS-CoV titer against different concentrations of GS-5734 over time, (ii) The MERS-CoV titer against different concentrations of GS-5734 over time. Reprinted with permission from Ref. (Agostini et al., 2018).

reduction of MERS-CoV infection *in vivo*, Sheahan et al. (2020) demonstrated that RDV stimulate superior antiviral function against MERS-CoV in cell culture and animal models as compared with other conventional anti-viral agents (Figure 4). Furthermore, they found that RDV was the only therapeutic agent which remarkably decreased pulmonary infection.

Furthermore, Gordon et al. (2020) and de Wit et al. (2020) revealed that RDV derives anti-viral effects against MERS-CoV though inhibition of RNA polymerase. As with SARS-CoV investigations treated by RDV, similar outcomes were observed for MERS-CoV along with limited weight loss, increased pulmonary activity and decreased virus replication (Sheahan et al., 2017).

Kinetic of CoV proliferation

The kinetics of SARS- or MERS-CoV proliferation in animal models is significantly different compared to that in humans. In animal, SARS-CoV or MERS-CoV proliferation in the lung tissue reaches to the maximum at 2 dpi and mortality is stimulated at 7–10 dpi (Douglas et al., 2018; Sheahan et al., 2017). Thus, the therapeutic window to control infected animal model prior to the peak of CoV proliferation is less than 2 days. In human, MERS-CoV replication in the lung tissue reaches the maximum at 7–10 days after the onset of infections and the disease severity increases to death within ~21 days (Choi et al., 2016; Oh et al., 2016). Thus, the time for therapeutic handling is substantially divergent in humans and experimentally infected animal models. Although, applying RDV led to a reduction in MERS-CoV pathogenesis and pronounced decrease in viral dose, therapeutic handling did not thoroughly reduce infection. Furthermore, at high levels of CoV, RDV is unable to sustain viral viability and pulmonary cells functionality, despite of remarkable decrease in viral loads. These outcomes are the same as those reported for SARS-CoV, where therapeutic platforms were launched after the peak of virus titer and lung injury did not show any progress in resultant outcomes (Sheahan et al., 2017). Since SARS- and MERS-CoV infections are controlled by both CoVs and host immune system modulators, therefore early handling of antiviral therapeutics either solely or in combination with other therapeutic drugs, and based on the stage of the disorder progression, can inhibit virus proliferation and immunopathology, switch on repairing systems, or control the pulmonary homeostasis.

Current challenges and future opportunities

Arising viral disorders have resulted in meaningful catastrophic pandemics. Genetic exploration in animal models have shown a pronounced mutation of viral genome in the case of CoV, and have even pointed out some viruses indistinguishable to ongoing and past pathogenic strains in

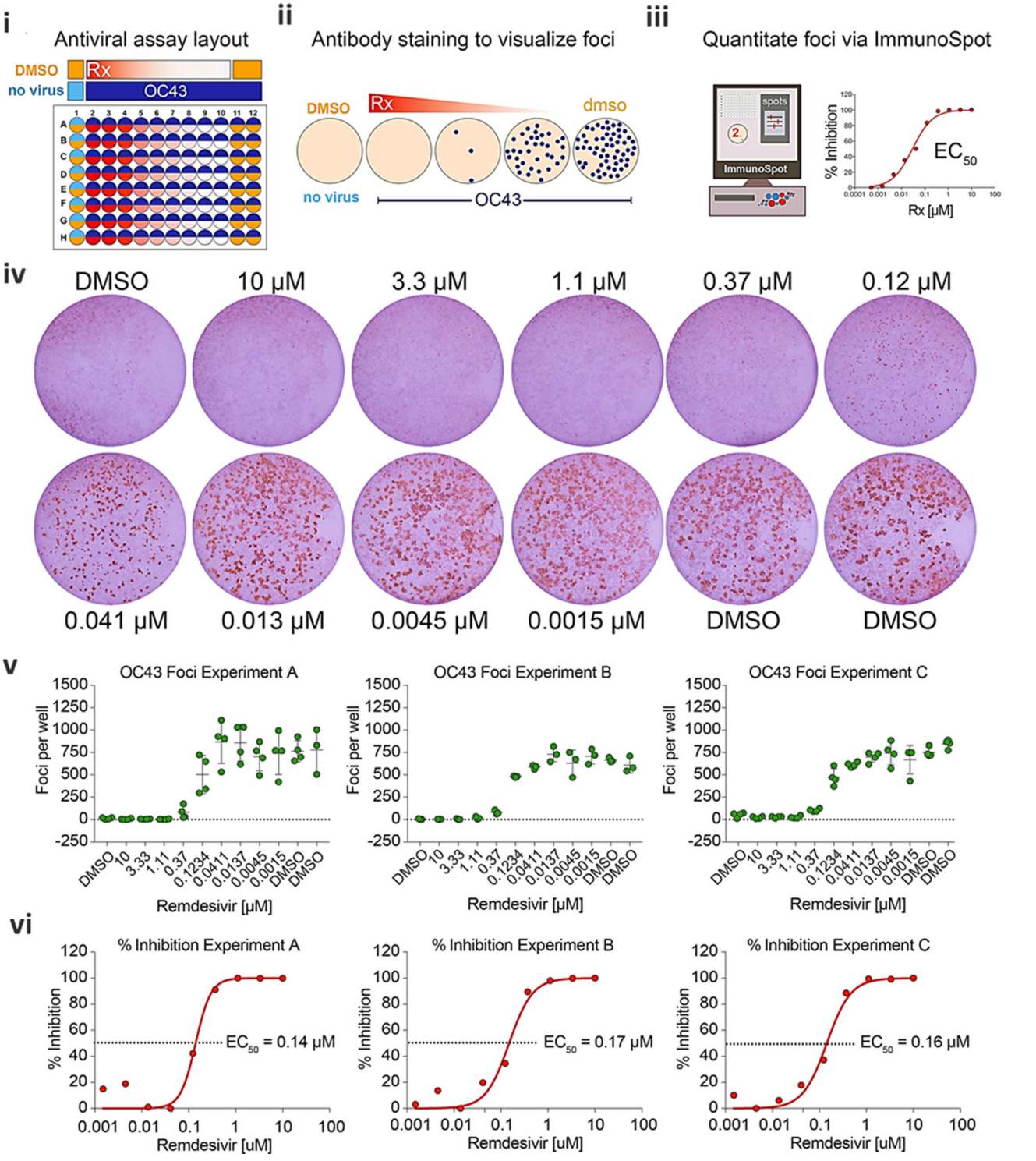

Figure 3. Anti-viral test. (i) HCoV-OC43 anti-viral assay plate layout in human hepatoma (Huh7) cells incubated with different agents, (ii) A decrease in viral foci assayed by antibody staining, (iii) percentage of inhibition, (iv) dose response of RDV, (v) the number of spots per well (A, B, C), (vi) EC_{50} values. Reprinted with permission form Ref. (Brown et al., 2019).

animals (Ge et al., 2013; Woo et al., 2006). Therefore, in the absence of FDA-approved therapeutics for reducing the human CoV infection, useful broad-spectrum therapeutic platforms against well-known epidemic and zoonotic strains probably pave a way for diminishing the current epidemic diseases.

In the case of CoV, patients were received off-label antiviral drugs as well as immunomodulators, either solely or combined, to reduce severe disease symptoms (Zumla et al., 2016). However, due to the lack of patient, therapeutic consistency, and verified standard efficiency measurement is a complicated process. Although interferon does not result in improved clinical consequence in MERS-CoV patients (Morra et al., 2018), a fixed dose combination of lopinavir/ritonavir-interferon β is efficient to reduce MERS-CoV infections (Arabi et al., 2018; Muralidharan et al., 2020). Sheahan et al. (2020)

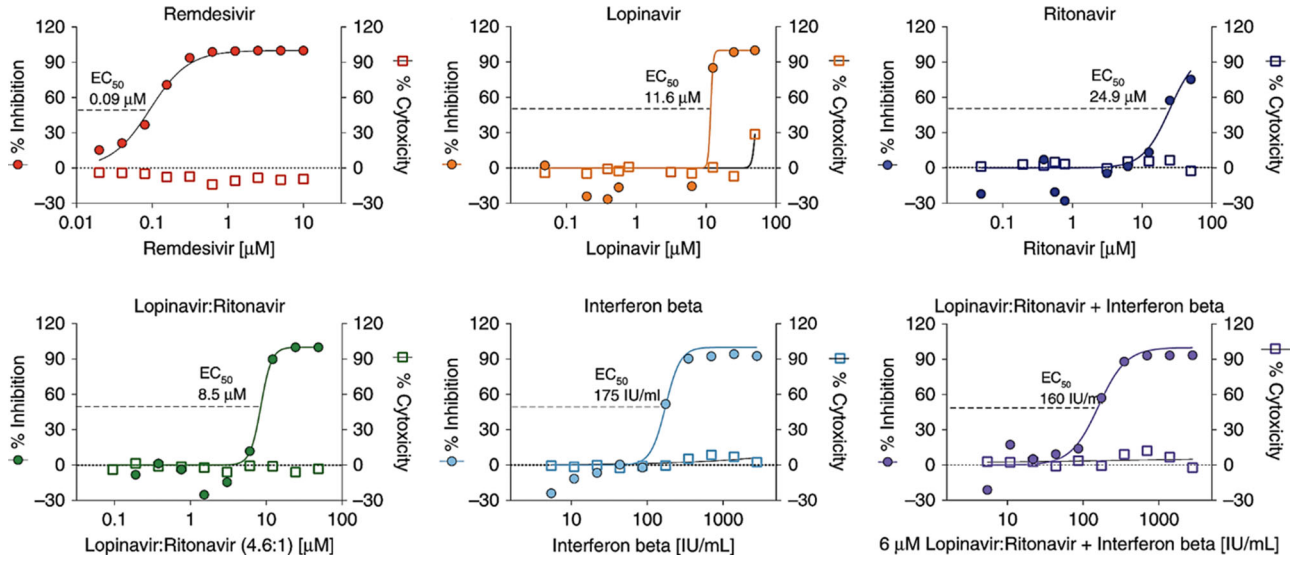

Figure 4. Percentage of inhibition of MERS-CoV replication as well as cytotoxicity stimulated by different anti-viral agents *in vitro*. The Figure was reprinted with permission from Ref. (Sheahan et al., 2020).

showed that RDV stimulated superior anti-viral function against MERS-CoV both *in vitro* and *in vivo* in comparison with lopinavir/ritonavir-interferon β.

Nucleoside/nucleotide analogues inhibit virus replication by blocking the activity of the polymerase enzyme in the virus (Chhikara et al., 2020; Menéndez-Arias et al., 2014). The usage of nucleoside/nucleotide analogues is a major step in the treatment of patients infected with CoVs due to the appropriate antiviral response (Chhikara et al., 2020). However, the application of these drugs may lead to genetic variation and subsequent mutation emergence. Hence, the safety of RDV and its broad-spectrum anti-viral activity should be considered before suggesting them as potential alternative candidates for clinical development.

Over the recent years, animal model progression of RDV seems to orient primarily on CoV respiratory infections (Sheahan et al., 2020). Clinical trials in selective patient populations with CoV or CoV-like diseases are needed to examine the efficacy of the developed drugs. Regarding safety data for RDV, some necessary studies in COVID-19 patients should be conducted to proceed the clinical trials. Studies over CoV-like diseases will probably require the enrollment of a large number of infected patients.

There is currently no approved antiviral drug against SARS-CoV-2 to treat hospitalized patients. Moreover, clinical trials over COVID-19 patients seems to be complicated due to several factors such as inability to apply a placebo, underlying diseases, and evaluating anti-viral drug efficiency. If the synergistic activity of RDV and other anti-viral agents in cell cultures is approved by the current Phase 3 clinical trials in patients with SARS-CoV-2, the outcome may propose a way for developing and performing clinical trials of the relative integration to RDV monotherapy and other anti-viral drug monotherapy for treating patients hospitalized with COVID-19.

Ongoing and future perspectives are trying to determine the resistance of different CoVs to RDV both *in vitro* and *in vivo*, and to elucidate whether the mutational strains behave in a same way as wild types.

Conclusion and perspective

Due to the emergence of a new respiratory infections such as the SARS-CoV-2, progression of animal studies and subsequent preclinical and clinical trials are required to explore the activity of RDV. Some preclinical explorations are ongoing to examine the potential of the RDV against the SARS-CoV-2. Given application history of RDV in treating a wide range of infections, as well as the outcomes from clinical trials in patients with SARS-and MERS-CoV, reinforces the rationale for additional trials of RDV against a wider range of infectious including COVID-19. The ongoing studies in SARS-CoV-2 supported by the WHO is expected to furnish potential data corresponding to RDV application in treating COVID-19.

Other similar investigations may be envisioned with respect to the increasing identifications of the importance of CoVs as a driving force of COVID-19. Apart from the potential progression of RDV for treating SARS- and MERS-CoVs, the emergence of other viral illnesses may pave the way for clinical trials of RDV derivatives.

Disclosure statement

The authors declare no conflict of interest.

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